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Effect of fungi isolates on proximate composition of natural and pure-culture fermentation of African yam bean (*Sphenostylis sternocarpa* Harms.) seeds.

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Abstract

Cream colored variety of African yam bean (*Sphenostylis sternocarpa* Harms) seeds were natural fermentation for 72h, 96h and 120 hours respectively at 28^0C . Microbiological analysis was carried out on the raw and fermented AYB to determine the types of fungi present in the samples. The fungi isolated from the fermented sample were *Saccharomyces cerevisiae, Candida mycoderm and Aspergillus niger. Aspergillus niger* was the only mould isolated from the raw seeds which disappeared after dehulling. Each of the fungi encountered from naturally fermented samples was used to ferment the seeds at 30^0C for 72 h after autoclaving the seeds at 121^0C for 15 minutes. The result of proximate composition showed that processing significantly ($p \leq 0.05$) affected the composition of the seeds. The highest protein content (44.22± 0.07%) was observed in sample fermented at 120 hours. Protein enrichment was highest in sample fermented with *Candida mycoderm* (29.35± 0.46%) and the least was observed in sample fermented with *Saccharomyces cerevisiae* (24.73± 0.06%).

Keywords: African yam bean (*sphenostylis sternocarpa* Harms), monofungi fermentation, proximate composition

Introduction

Fermentation is identified as an economic processing method that could be used in the homes to improve the nutritional quality of plant foods (Olowoniyan, 1994) The acceptability of such fermented products is important in practical nutrition (Aderiye *et al.,*1991). It is known that the nutritional advantages, the relative cost, availability and organoleptic acceptability of a food are the major factors which determine its consumption by the targeted group. 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 1994). It has been used quite extensively in various parts of the world, especially in the orient. It is probably the oldest method of processing legumes. 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 (Kingsley, 1995). In Nigeria, fermented foods play a major role in the diet of the people (Steinkraus, 1995) 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 macropyhlla),* 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 (Fagbemi, 2004) "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 flavour.
Therefore this work aims at isolating and characterizing the fungi associated with fermentation of African yam bean Seeds and assessing the effect of fermentation on the level of nutritrient content of the seeds.

Materials and Methods

Collection and processing of African yam bean seeds
Raw cream coloured seeds of African yam bean (plate 1) were harvested from Ighoba farm in Akure, Nigeria and processed into various forms.

The raw seeds samples were sorted ,cleaned and were fermented as described by Ikemefuna (1998) in fig (1) which involves hand sorting of the seeds, washing, soaking in water for 12 hours, dehulling, washing, boiling for 2hours, draining, wrapping in plantain leaves and jute sacks, fermentation for 24 hours, 72 hours, 96 hours and 120 hours.

After dehulling another portion of the seeds, 50g of it was wrapped in aluminium foil and autoclaved at 121^0C for 15 minutes. Then 5ml of cell suspension of each of the previously inoculated isolates was poured into the sterile seeds after cooling to 45^0C.It was then incubated at 30^0C for 72 hours.

Another portion of the seeds which serves as control was autoclaved without any inoculation.

Preparation and sterilization of material used

All glass wares were washed with detergent, rinsed with clean tap water, air-dried and then oven sterilized at 160^0C for 2 hours. Inoculating loop, used were usually flamed to red hot, dipped into 70 % ethanol, reflamed and allowed to cool before used. Laboratory benches were also swabbed with cotton wool moisten with 70 % ethanol before and after investigation.Laboratory coat was washed with detergent, rinsed with clean tap water. The hands were also washed with detergent, rinsed dried and cleaned with 70 % ethanol before and after every inoculation.

Inoculating chamber was swarbed with 70% ethanol; thereafter the UV light was on for 2 hours before and after inoculation.

Potato dextrose agar was prepared by dissolving 39g in 1000ml of distilled water in a conical flask. Thereafter, each of the mixtures was placed on hot plates for 20 minutes to ensure proper dissolution of the agar. Physiological saline was prepared by dissolving 0.85g of sodium chloride in 100ml of distilled water. These were autoclaved at 121^0C for 15 minutes. The method used for isolation and identification of microorganisms was as described by Olutiola *et al., (*1991)

Source of isolates

The yeast used for part of investigation were obtained from naturally fermented African yam bean seeds; they were isolated, purified, identified and stored in agar slant. The cells of the isolates were harvested with sterile physiological saline (10ml, 0.85 % NaCl). The cells were counted using a counting chamber and adjusted to 10^7 – 10^8 cells per ml with sterile water.

Sterilization of African yam bean samples

A fifty-gram (50g) portion of each dehulled African Yam bean seeds were wrapped in aluminium foil and sterilized in autoclave at 121^0C for 15 minutes according to Abu *et al.,* (1998).

Solid substrate fermentation

Fifty grammes of each of the sterile seed sample were aseptically mixed with 5ml of the cell suspension in a flask and corked with sterile cotton plug and foil paper. The flasks were then incubated at 30^0C for 72 hours (Abu *et al.,* 1998). microbial analysis was then carried out. After 72 hours of incubation, the fermented samples were oven dried at 55^0C for 18 hours, milled and analyzed (proximate) control experiment was set up by incubating the seeds without inoculum at 30^0C for 72 hours.

Proximate analysis

The proximate analysis (Moisture, crude Protein, ash, Lipid, crude fiber and carbohydrate contents) of the fermented and unfermented African yam bean seeds was performed using the standard method of AOAC, (2005).

Statistical analysis

Statistical analysis was performed using SPSS Statistic 17.0 (SPSS, Inc., Chicago, IL). Analysis of variance was applied, and every sample was analysed in triplicate to identify statistically significant differences using Duncan's multiple range test ($P< 0.05$).

Results and Discussion

Microorganisms isolated from Raw and processed African yam bean seeds.

African yam bean seeds fermented naturally were found to contain two types of yeast. Mould was isolated from raw samples. The yeast encountered during natural fermentation includes *Saccharomyces cerevisiae and Candida mycoderm.* Raw seeds were found to contain *Aspergillus niger.*

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 hour of fermentation. *Saccharomyces cerevisiae* was present until the end of fermentation (120 h) while *Candida mycoderm* was not isolated at 120 hour of fermentation

The total viable count of yeast from processed samples.

The changes in yeast population during natural fermentation of African Yam Bean seeds at different time interval are shown on Fig 2. The yeast count was lower than the total bacteria count. Yeast count ranged from 2.0×10^2 cfu/g at zero hour and increased to 6.0×10^3 cfu/g at 24 hours, 6.7×10^3 cfu/g at 48 hours, 6.8×10^3 cfu/g at 72 hours and decreased to 3×10^3cfu/g and 3×10^2 cfu/g at 96 and 120 hours respectively. *Saccharomyces cerevisiae* was not isolated at zero hour but the count ranged from 0 to 4×10^3 after 24 hours, 4.9×10^3 cfu/g at 48 hours, and increased to 6.3×10^3 cfu/g at 72 hours which in turn decreased to 3×10^3 cfu/g at 96 hours and further decreased to 3×10^2 cfu/g at 120 hours.

The result showed that *Saccharomyces cerevisiae* count was high (6.3×10^3 cfu/g) at 72 hours of fermentation. *Candida mycoderma* count increased from 2.0×10^2 cfu/g at zero hour and increased slightly to 2.0×10^3 cfu/g at 24 hours. The count remain unchanged till 72 hours of fermentation while *Candida mycoderma* was not isolated at 96 and 120 hours of fermentation.

Changes In the proximate composition of the samples

The crude protein content of the raw and autoclaved African Yam bean seeds was 22.17 0± 24 and 22.12 ± 0.02% respectively. Fermentation significantly increased ($p \leq 0.05$) the crude protein content of the samples during natural fermentation. The highest crude protein content was recorded in sample fermented at 120 h (44.22± 0.07%). The crude protein content of the monofungi fermented samples showed that protein enrichment was highest in the sample fermented with *Candida mycoderm* (29.35± 0.46%) as shown on Table 1

The ash content of the raw African Yam bean seeds was 4.14± 0.06%. Fermentation reduced the ash content of the seeds. Natural fermentation of the sample significantly reduced the crude fibre content ($p \leq 0.05$).

Natural and monocultural fermentation decrease lipid content of the sample with increase in fermentation time. There was also reduction in the percentage lipid content of the sample. Also, monofungi fermentation of the samples decreases the lipid content.

The carbohydrate content otherwise known as nitrogen free extracts of raw sample (54.53 ±1.22 %) was significant decreased ($p \leq 0.05$) natural and monofungi fermentation. Sample fermented with *Saccharomyces cerevisiae* had the highest carbohydrate content (23.62± 0.30%).

The results of the fungi counts of the fungi involved in the fermentation of African yam bean seeds are shown Table 2 and Figure 2. The progressive increases in the counts of fermenting seeds has been similarly reported (Fagbemi, and Atum, 2001) on melon seeds who reported a range of less than 30cfu/g on the 1st day to 6.8 x 10^{11} cfu/g on the 7th day. Different results were reported on locust bean and melon seeds respectively (Barber and Achinewhu, 1992). The bacteria and yeast counts decreased after the 72h. This may be due to increased acidity after the 72h of fermentation (Alabama and Legged, 1998). Similar results were obtained during the fermentation of *Hura crepitans* seeds; reduced oxygen tension obtainable in solid substrate fermentation was also reported to reduce mould growth (Fagbemi and Atum, 2001).

The results of the tests carried out on microorganisms isolated from the onset of the African yam bean seeds during fermentation is in line with the findings of Onifade *et al. (*2003*)* who reported the isolation of *Saccharomyces* sp. and *Aspergillus niger* among other fungi from coconut. The fermenting microorganisms might have, started fermentation by hydrolysing available carbohydrate. The result obtained for the raw seeds of African yam bean is similar to the result of Jeff-Agboola and Oguntuase (2006) who observed similar trend on the raw crude protein on the raw crude protein of soybean seeds.

The increase in percentage crude protein of African yam bean seeds after fermenting for 72 to 120h is also similar to those reported for other tempeh and tempeh-like products and Onifade and Jeff-Agboola (2003) suggested that the increase in protein may be due to biomas accumulation. The biomas could equally have contributed to the protein content (Achinewhu and Isichei, 1990, Aderiye, *et al.*, 1991). This increase also suggested that there was proteolysis during the fermentation. African yam bean seeds may be effectively supplementing the protein requirement of man (Fagbemi, 2004). The crude protein of African yam bean seeds comparable with that of non or low oil seeds like pigeon pea, cajanus cajan (22.4%), sesame seeds (20.1%), kidney bean (22.1%) and it is better than the crude protein of many cereal grains (Fagbemi, 2004). The crude protein obtained from raw African yam bean seeds is comparable with the 21% reported by (Achinewhu and Isichei, 1990) thus suggested that African yam bean seed is a protein food. The result obtained in this work revealed that fungi plays an important role in protein hydrolysis to peptides and amino acid, providing free glutamate as well as other amino acid that could function directly in taste, or eventually serve as precursors for aroma active molecules (Fagbemi, 2004).

Ami (2001) reported that the highest protein enrichment has compared to other fungi in their work could be attributed to the great enzymatic activities of the fungi. The reduction in the crude fat content of the fermented seeds may be due to breaking down and leaching of the fat content and other metabolic activities taking place during fermentation.

Processing affected the crude fat content of the seed. There was reduction in the fat content after natural fermentation to 2.15 at 72 hours of fermentation, to 1.26 at 96 hours and 0.99 at 120 hours of fermentation. There were no significant different in sample fermented naturally at 96 and 120 hours and sample fermented with *Saccharomyces sereviseae and Candida mycoderma* ($P < 0.05$). There were also reductions in the fat content of samples fermented the isolates.

Reduction in the fibre content of autoclaved and fermented seeds may be due to the dissolution effect on the fibre as well as enzymatic degradation of the fibrous material during fermentation (Ikemefuna, 1998). Reduction in the ash content of all the samples may be due to boiling loss and leaching of the soluble inorganic salt during fermentation (Onifade and Jeff-Agboola, 2003) reported similar observations, on African oil bean seed. Fermentation reduced the carbohydrate content of the seeds respectively. These results are consistent with the observation of Kingsley, (1995) on African oil bean seeds. The reduction may be due to the utilization of some of the sugars by fermenting microorganisms for growth and other metabolic activities. Usually the carbohydrates are hydrolysed to glucose and then used as source of carbon and energy for microbial growth (Onifade and Jeff-Agboola, 2003).

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Conflict of Interests

The authors declare no conflict of interests

Tables, Figures and Charts

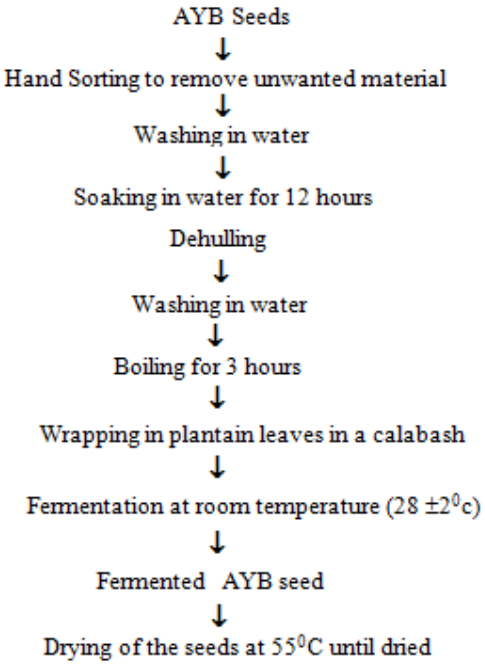

Fig 1: Flowchart for processing of African yam bean seed into fermented seed (Source Ikemefuna (1998)

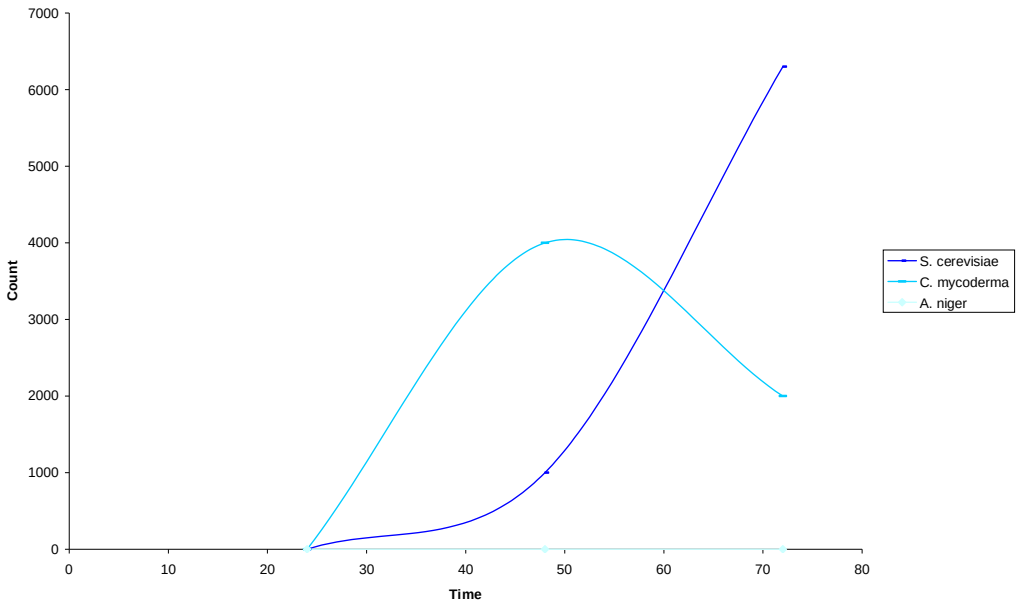

Fig. 2: fungi counts of the fungi involved in the fermentation of Afrcan yam bean seeds

Table 1: Proximate composition of raw fermented and monofungi fermented samples

Samples	Moisture Content	Crude Protein	Ash Content	Crude fibre	Lipid content	NFE
			(%)			
RS	11.03± 0.06[a]	22.17± 0.24[e]	4.14± 0.06[e]	5.06± 0.04[h]	4.17± 0.03[f]	54.53 ±1.22[f]
AS	50.05± 0.02j	22.12± 0.02e	4.02± 0.002de	4.10± 0.02a	4.14± 0.03f	15.58± 0.09d
NFS_{72h}	48.61± 0.006[i]	24.81 ±0.06[b]	5.40± 0.02[b]	1.82 ±0.01[c]	2.15± 0.02[c]	17.20± 0.07[e]
NFS_{96h}	42.09± 0.004[e]	30.79± 0.34[d]	7.39± 0.14[c]	1.26 ±0.08[b]	1.26± 0.07[a]	17.17± 0.36[e]
NFS_{120h}	32.01± 0.50[b]	44.22 ±0.07[b]	7.86± 0.009[d]	1.07± 0.006[a]	0.99 ±0.03[a]	13.85± 0.40[c]
FS_5	43.22 ±0.11[f]	24.73± 0.06[b]	2.47 ±0.5[b]	3.19± 0.05[e]	2.76± 0.01[d]	23.62± 0.30[c]
FS_6	44.23± 0.17[g]	29.35± 0.46[c]	3.73± 0.02[d]	2.07± 0.03[d]	1.77± 0.40[b]	18.85±0.35[a]

Values are mean of three determinations. Values in the same column having different superscripts are significantly different ($p \leq 0.05$)

RS - Raw Sample, AS - Autoclaved Sample, NFS_{72h} - Naturally Fermented Sample for 72 hours, NFS_{96h} - Naturally Fermented Sample for 96 hours, NFS_{120h} - Naturally Fermented Sample for 120 hours, FS_5 – Sample Fermented with *Saccharomyces cerevisiae,* FS_6 - Sample Fermented with *Candida mycoderm*

Table 2: Total microbial load (cfu/g) of raw, naturally fermented, and monofungi- fermented African yam bean samples.

Samples	Yeast	Mould	*S. cerevisiae*	*C. mycoderma*
	Total Viable Count (cfu/g)			
RS	$2 X10^2$	$4 X10^2$	0	$2X10^2$
24 h	$6X10^3$	0	$4X10^3$	$2X10^3$
48h	$6.7X10^3$	0	$4.9X10^3$	$2X10^3$
72h	$6.8X10^3$	0	$6.3X10^3$	$2X10^3$
96h	$3X10^3$	0	$3X10^3$	0
120h	$3X10^2$	0	$3X10^2$	0

Values are mean of three determinations. Values in the same column having different superscripts are significantly different ($p \leq 0.05$)

RS - Raw Sample, AS - Autoclaved Sample, NFS_{72h} - Naturally Fermented Sample for 72 hours, NFS_{96h} - Naturally Fermented Sample for 96 hours, NFS_{120h} - Naturally Fermented Sample for 120 hours, FS_5 – Sample Fermented with *Saccharomyces cerevisiae,* FS_6 - Sample Fermented with *Candida mycoderm*

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