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Assessment of the Microbial and Physico-Chemical Composition of Tigernut Subjected to Different Fermentation Methods

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Abstract: This research investigate the microbial, physical and proximate composition of tiger nut subjected to different fermentation methods such as traditional, back slope and control. Cleaned tiger nut were fermented for the period of four days in which samples were taken daily from each of the fermented groups to determine the total viable count of microorganisms associated with raw and fermented tiger nut samples, the p^H and total titratable acidity. The result of total viable count reveal that back slope fermented milled sample has the highest microbial count of $4.16x10^5$ cfu/g while control fermented sample show the least value ($2.30x10^5$ cfu/g). All the fermented samples show increment in lactic acid bacterial count throughout fermentation period. Traditional fermented whole sample had the highest fungal count of $4.40x10^5$ sfu/g while control fermented sample showed the least value ($9.33x10^4$ sfu/g). The overall microorganisms isolated and identified from raw and fermented tiger nut were *Streptococcus lactis*, *Lactobacillus plantarum*, *Staphylococcus aureus*, *Bacillus subtilis*, *Micrococcus luteus*, *Bacillus cereus*, *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus flavus* and *Penicillum italicum*. There was significant decrease in pH value while total titratable acidity (TTA) increased in the fermented samples. Fermented whole sample had the highest protein value of 7.65% while raw sample had the least (4.2%) back slope fermented sample had the lowest moisture content (3.5%). There was no significant difference in the value of fat in all the samples. Back slope fermented sample had the highest value in ash (2.5%) and carbohydrate (65.0%) while the least value was recorded in the control fermented sample ash (0.5%) and carbohydrate (45.0%). Traditional and back slope fermentation was the best methods that may increase the proximate composition of tiger nut.

Key words: Microbial, proximate, fermentation and tigernut

INTRODUCTION

Tigernut (*Cyperus esculentus*) is an edible perennial grass-like plant native to the Old World and is a lesser-known vegetable that produces sweet nut-like tubers known as "earth almonds" (Coskuner *et al*., 2002). Tiger nut is also known by various other names as chufa (in Spanish), earth nut, yellow nut sedge, groundnut, rush nut and edible galingale (Oderinde and Tairu, 1998).

In Nigeria, tiger nut is available in fresh, semi-dried and dried form in the markets where it is sold locally and consumed even uncooked. Tigernut is under-utilized due to lack of information on their nutritional potential (Rita, 2009). A lot of people eat the tigernut without knowing the nutritional benefits and products that can be obtained from it like tigernut oil and milk.

C. esculentus had been reported to be a "health" food, since its consumption can help prevent heart disease and thrombosis and is said to activate blood circulation (Chukwuma *et al*., 2010). It was also found to assist in reducing the risk of colon cancer (Adejuyitan *et al*., 2009). This tuber is rich in energy content (starch, fat, sugar and protein), minerals (mainly phosphorus and potassium) and vitamins E and C (Belewu and Belewu, 2007) thus making this tuber also suitable for diabetics and for those intent on losing weight (Borges *et al*., 2008).

The annual value of tiger nut production is close to 3.3 million euros (CRDO, 2012). In recent years, the popularity of "horchata" has been extended to other countries, such as the United Kingdom, France, Portugal, Argentina and United States of America (Pascual *et al*., 2000; Rubert *et al*., 2011).

To alleviate the problem of food shortage, malnutrition and wastage during harvesting, the development of new processing methods is imperative. Therefore, the purpose of this research work is to study the effect of different fermentation methods on microbial and physico-chemical composition of raw and fermented tiger nut.

MATERIALS AND METHODS

Sample collection: Yellow varieties of tiger nut were obtained from King's market in Akure, Ondo State, Nigeria. It was taken to the laboratory in a clean polythene bag for processing and analysis.

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Preparation and fermentation of tigernut: The sorted and washed nut were divided into six portions designated A to F. Each of the portion contain 500 g of cleaned tigernut. Part A was analyzed raw and this serves as control. Part B was fermented whole i.e., submerged in 1500 ml of portable water in a cleaned container that was covered for four days at 25°C and allowed to ferment with indigenous micro flora (spontaneous). C was milled and subjected to spontaneous fermentation. Part D and E were fermented by addition of the steep water from the previously fermented culture used as starter culture (back slope) but part E was milled before fermentation. while F was allowed to undergo control fermentation, in which pure culture of *Lactobaccilus plantarum* isolated in part B was used to inoculate the sixth part F. The fermented nuts were dried in oven at 50°C for 24 h and dry milled to powder using attrition mill. The milled samples were packaged in polythene prior to analysis.

Microbiological analysis: Plate count agar (PCA), De Mann Rogosa and Sharpe agar (MRS), Nutrient agar (NA) and Potato Dextrose agar (PDA) were used for total viable bacteria count, lactic acid bacteria, microbial culturing of bacteria and mould respectively. All media were prepared according to the manufacturer's specification and sterilized at 121°C for 15 min. 1ml from appropriate dilutions (10^4) was pipetted from raw and fermented samples, prepared by serial dilution, was plated on sterile molten agar, swirled and allowed to set. NA and PCA plates were incubated aerobically at 37°C for 24 h, while MRS agar was incubated at 37°C for 48 h anaerobically and PDA at 30°C for 2-5 days. After incubation, colonies on each plate were counted, streaked out repeatedly until pure cultures of each was obtained and maintained on appropriate agar slants at 4°C (Fawole and Osho, 2007).

Characterization of isolates: Bacteria isolates were characterized and identified on the basis of their cultural, morphological, physiological and biochemical properties using Bergeys Manual of Systematic Bacteriology (Holt *et al*., 2000). The fungi isolates were identified according to the methods of Barnett *et al*. (2000).

Physicochemical analysis: pH, ten milliliters of sample supernatant was titrated against O.IM NaOH with phenolphthalein as indicator pH was determined using the method of Kirk and Sawyer (1991).

Total titratable acidity (TTA): Total titratable acidity was determined as described by Nielsen (2002).

Proximate analysis of raw and fermented tigernut: The proximate composition (moisture, ash, fat, protein, fibre and carbohydrate) of the samples were determined using the method of A.O.A.C (2012).

Statistical analysis: The data obtained during the experiment was analyzed using Analysis of Variance (ANOVA) to determine significance difference between the means and was expressed as mean±standard deviation (SD). The level of significance was determined at $p \leq 0.05$ using SPSS Version 17.0.

RESULTS AND DISCUSSION

The result obtained for total viable count of fermented sample show an increase when compared to raw sample and it ranged from $4.16x10^5$ to $2.30x10^5$ cfu/g for fermented sample and $4.20x10^5$ cfu/g for raw sample. Fungal count in fermented sample ranging from $4.43x10^5$ sfu/g to $9.33x10^4$ sfu/g. The number of bacteria isolated from control fermented sample was found to be less when compared to raw and traditional fermented samples. These may be due to the effect of sterilization on control fermented sample. High temperature is known to inhibit microbial growth (Arotupin, 1999).

The overall microorganisms isolated and identified from the raw and fermented tigernut tuber are: *Streptococcus lactis*, *Lactobacillus plantarum*, *Staphylococcus aureus*, *Bacillus subtilis*, *Micrococcus luteus*, *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus flavus* and *Penicillum italicum*. These organisms have been found to be responsible for fermentation of most legumes and cereal (Tucker, 2003). Also, these microorganisms have been isolated in various investigations of fermented products such as alcoholic beverages, burukutu, fufu (Ogbona *et al*., 1983). The results also show that *Lactobacillus plantarum* and *Bacillus subtilis* were predominant in all the samples which were isolated from raw and fermented sample. This may be due to the fact that these organisms can invade and proliferate in many kind of food materials. *Bacillus subtilis* are known to ferment most sugars, hence involve in fermentation (Prescott *et al*., 1999). *Lactobacillus plantarum* isolated from most samples belong to the group of lactic acid bacteria which are highly responsible for fermentation process (Wakil *et al*., 2014).

The presence of *Aspergillus niger*, *Aspergillus funmigatus*, *Aspergillus flavus and Penicillum italicum* in this research was in line with the study conducted by Chukwu *et al*. (2013) which discovered the above fungi being associated with fresh and dry tiger nut.

The pH and total titratable acidity of unfermented and fermented tiger nut is as shown in Table 2. There was an indication that the value of pH decreased from 4.10 to 3.50 and total titratable acidity (TTA) increased throughout the period of fermentation (0.55-3.70%). The decreased in pH value with increase in TTA may be due to the increase in activities of the microorganisms resulting in the production of organic acids from

Table 1: Morphological and biochemical characteristics of bacteria isolated from Raw and fermented tigernut

Lab. Ref. number of isolates	D1	D2	D3	D4	D5	D6
Cultural characteristics						
Colour	White	White	Deep yellow	Creamy	Creamy	Dirty white
Shape	Circular	Irregular	Circular	Irregular	Circular	Circular
Edge	Lobate	Rhizoid	Entire	Rhizoid	Entire	Entire
Elevation	Raised	Flat	Raised	Flat	Raised	Raised
Surface	Smooth	Rough	Smooth	Rough	Rough	smooth
Biochemical test						
Gram's reaction and shape	+ve cocci	+ve long rod	+ve cocci	+ve Rod	+ve Rod	+ve cocci
Catalase	+	+	+	+	+	–
Motility	–	+	–	+	–	–
Spore	–	+	–	+	–	–
Coagulase	+	–	–	–	–	–
Citrate utilization	–	+	–	+	+	+
Urea	–	–	–	–	+	–
Oxidase	–	–	–	–	–	–
Starch hydrolysis	+	+	–	+	+	+
Glucose	AG	AG	–	AG	AG	AG
Lactose	A	A	L	AG	AG	A
Sucrose	A	L	–	–	A	L
Maltose	A	L	L	L	A	A
Mannitol	AG	AG	–	–	A	A
Arabinose	AG	A	–	–	AG	L
Inositol	AG	A		L	A	AG
Probable organism	*Staphylococcus aureus*	*Bacillus subtilis*	*M. luteus*	*Bacillus cereus*	*L. plantarum*	*S. lactis*

Keys: +ve or + = positive reaction, A = Acid only, L = Late fermenter, AG = Acid and Gas, –ve = negative reaction, W = weak reaction

Table 2: Microscopic identification of fungi isolated from raw and fermented tigernut

Cultural Characteristics	Microscopic Characteristics	Isolates
Black spores spread on the plate	Conidiophores are upright, radiating from the entire surface; conidia are one celled and vesicle globose	*Aspergillus niger*
Brown spore spread on the media	Conidiophores upright, terminating in clavate swelling bearing philides at the apex	*Aspergillus fumigatus*
Yellowish green in appearance	Conidia are one-celled, globose in dry biaseptal chain. Conidiophores are upright, radiating from the entire surface	*A. flavus*
Light blue	Septate mycelium bearing single conidiophores, which are branched near the apex and ending in phialides that carried conidia	*Penicillium italicum*

Table 3: Microbial succession of fermented Tigernut samples in days

Isolates	TFM 1 2 3 4	TFW 1 2 3 4	BFM 1 2 3 4	BFW 1 2 3 4	CF 1 2 3 4
Streptococcus lactics	+ + + +	+ + + +	+ + + +	+ + + +	+ + + -
Lactobacillus plantarum	+ + + +	+ + + +	+ + + +	+ + + +	+ + + +
Staphylococcus aureus	+ + - -	+ - - -	- - - -	- - - -	- - - -
Bacillus cereus	- - - -	+ - - -	+ - - -	+ - - + - -	+ - - -
Bacillus subtilis	+ + + +	+ + + +	+ + + +	+ + + +	+ + - -
Micrococcus luteus	+ - - -	+ + - -	+ - - -	- - - -	+ - - -
Aspergillus niger	+ - - -	+ - - -	+ - - -	+ - - -	+ - - -
Aspergillus fumigatus	- - - -	- - - -	+ - - -	+ - - -	- - - -
Penicillum italicum	- - - -	- - - -	+ - - -	+ - - -	- - - -

RAW : Raw, TFM: Traditional fermented milled, TFW: Traditional fermented whole, BFM: Backslope fermented milled, BFW: Backslope fermented whole, CF: controlled fermented sample. 1-4 represent fermentation from first to fourth days

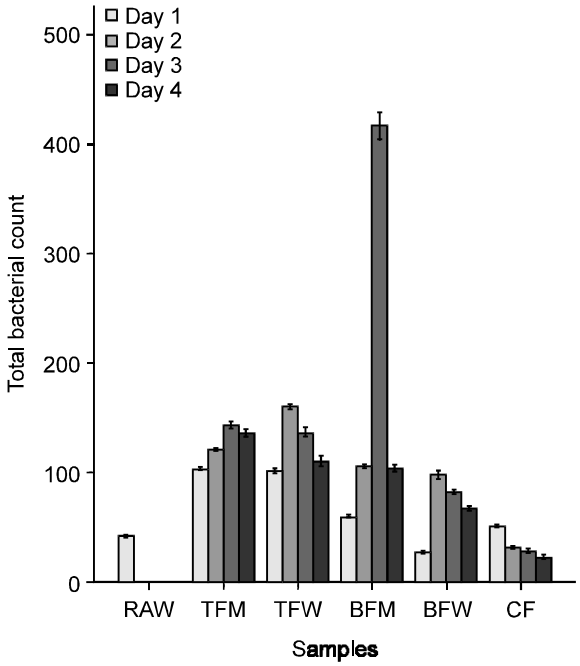

Fig. 1: Total bacterial count on raw and fermented tiger nuts (10^4 cfu/g)

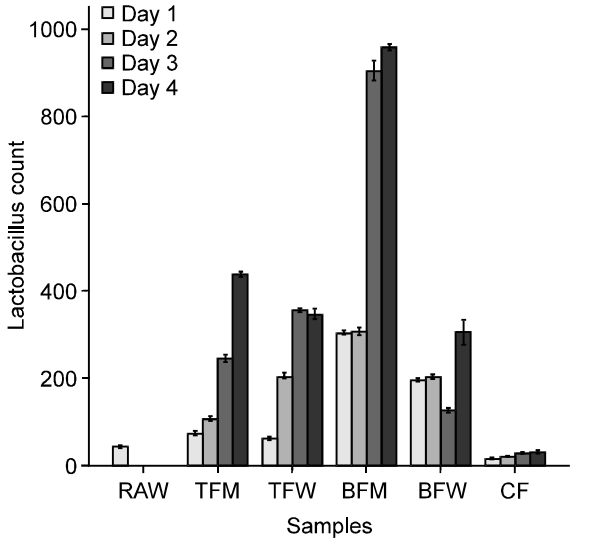

Fig. 2: Total lactic acid bacteria count on raw and fermented tiger nuts (10^4 cfu/g)

available nutrient and sugar present throughout the fermentation period due to the utilization of sugars by fermenting microorganisms for growth and metabolism (Okafor *et al*., 2003). Fermentation of food depends on the nature and quality of food itself, the changes that occur as a result of microbial activities and the interaction that occur between the products of these activities and constituents of food (FAO, 1998; Muhammed *et al*., 1991).

The proximate analysis result of raw and fermented tiger nut in Fig. 4 reveals that traditional fermented milled sample had the highest moisture content which may encourage microbial proliferation and food spoilage (Ajayi and Oyetayo, 2009). While back slope fermented whole sample had low moisture content which has significant importance of increasing the self-life of food (Ihekoronye and Ngoddy, 1985). The crude protein value of traditional fermented whole sample (7.65%) was higher than other fermented sample (Fig. 5). This valve obtained is within the range (7.15-9.70%) reported by Oladele and Aina (2007). This may also be due to the fact that microorganisms responsible for fermentation must have secreted extracellular enzymes which increases the protein content (Anyika, 2006) while raw sample showed the least protein content of 4.00%. Figure 6 shows the result of crude fibre with the values ranging between 2.00-13.50%. Control fermented sample had the highest value of 13.50% while the least value was recorded in back slope fermented whole (2.00%). This increase in fibre content is in agreement with Agbabiaka *et al*. (2012). High fibre content in food help in empty bowel and reduces the risk of constipation. There is no significant difference in the value of fat recorded in raw and control fermented samples. The carbohydrate value was higher in back slope fermented whole (70.46%) while control fermented samples show the least value of 49.01%.

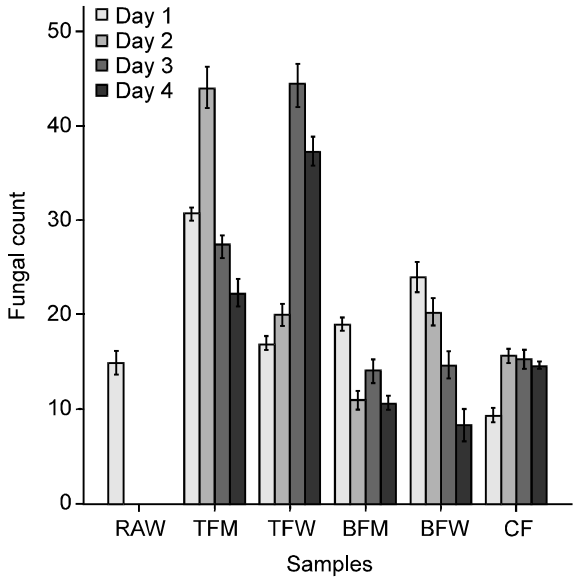

Fig. 3: Total fungal count on raw and fermented tiger nuts (10^4 cfu/g)

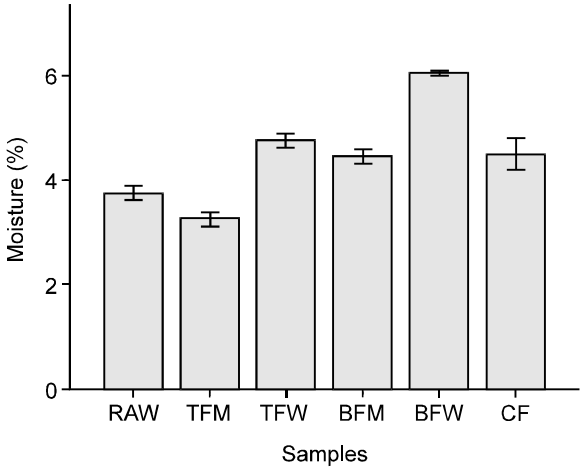

Fig. 4: Percentage moisture content of raw and fermented tigernut.
Key: RAW: raw, TFM: traditional fermented milled, TFW: traditional fermented whole, BFM: back slope fermented milled, BFW: back slope fermented whole, CF: controlled fermented sample

This may be due to the ability of the fermented organisms to utilize carbohydrate Adejuyitan *et al.* (2009). It was reported that reduction in carbohydrate content during fermentation attributed to breaking down of carbohydrate to soluble sugar during fermentation process. The result of Ash content was shown in Fig. 8. There was reduction in value of ash recorded in back slope fermented whole, traditional fermented milled and traditional fermented whole sample when compared with the raw sample. This may be due to leaching of

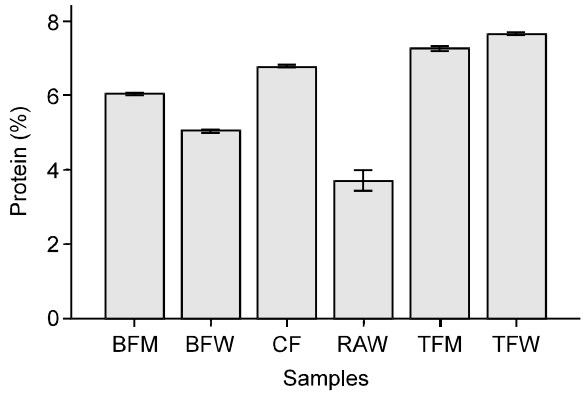

Fig. 5: Percentage protein content of raw and fermented tigernut.
For Key: (See Fig. 4)

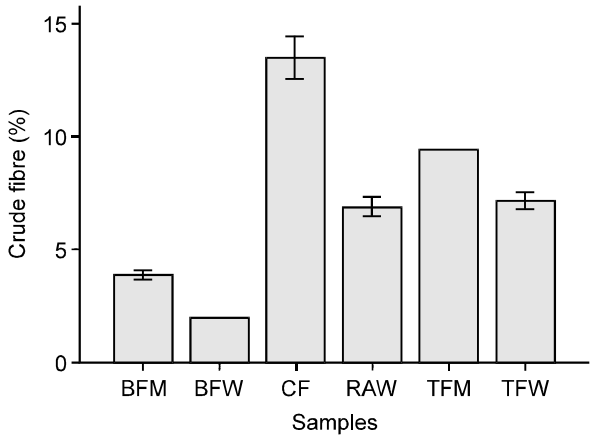

Fig. 6: Percentage crude fibre content of raw and fermented tigernut.
For Key: (See Fig. 4)

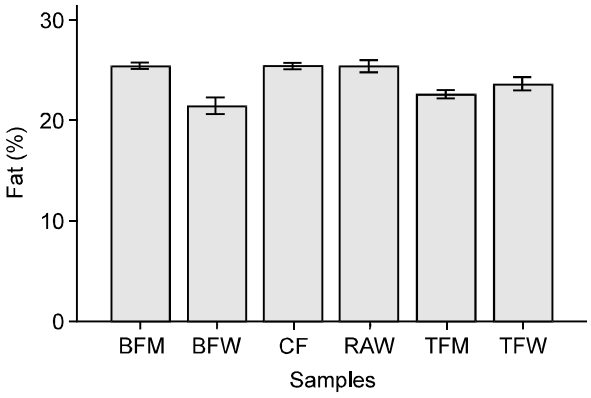

Fig. 7: Percentage fat content of raw and fermented tigernut.
For Key: (See Fig. 4)

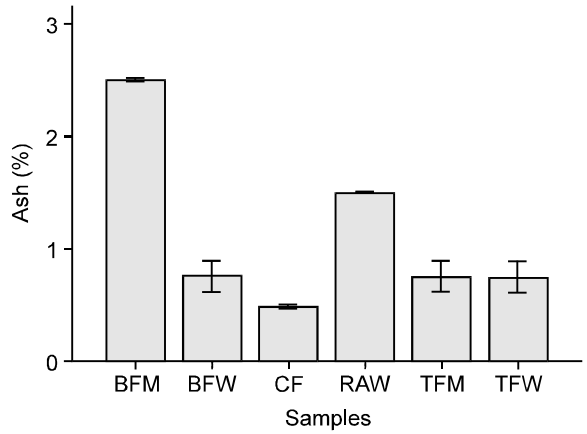

Fig. 8: Percentage ash content of raw and fermented tigernut.
For Key: (See Fig. 4)

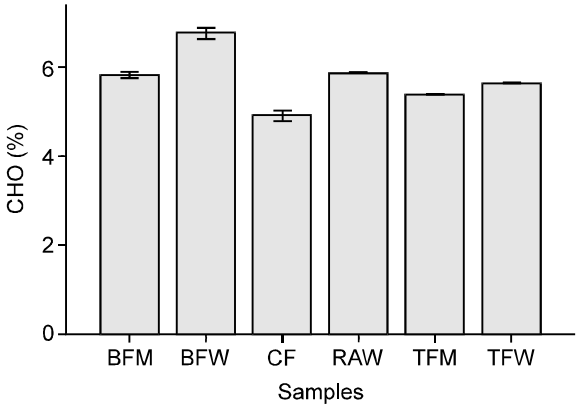

Fig. 9: Percentage carbohydrate content of raw and fermented tigernut.
For Key: (See Fig. 4)

some minerals into soaking water. Obizoba and Atii (1991) have made a similar observation for Sorghum soaked in water.

Conclusion: The isolation of different species of microorganisms reported in this research study give opportunities for the development of starter culture from locally available plants which may be useful for food and industrial microbiologists. Back slope and spontaneous fermentation are the best methods that may improve the nutritional composition of tiger nut while control fermentation was found to be the best method that may reduce the microbial load of a food sample.

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