Journal homepage: www.fia.usv.ro/fiajournal
Journal of Faculty of Food Engineering,
Ştefan cel Mare University of Suceava, Romania
Volume XVII, Issue 2 - 2018, pag. 131 - 140

CYPERUS ESCULENTUS: PHYSICAL PROPERTIES OF TUBERS AND MICROBIOLOGICAL CHANGES DURING NATURAL FERMENTATION IN NIGERIA

***Olayinka O. ELUTADE[1], Olubukola M. OYAWOYE[2], Ediga B. AGBO[3]**
[1]Faculty of Sciences, Bowen University,
Iwo, Nigeria, olayinkaelutade@yahoo.com
[2]Faculty of Science, Adeleke University,
Ede, Nigeria, oyawoyeom@yahoo.com
[3]School of Science, Abubakar Tafawa Balewa University,
Bauchi, Nigeria, ediga_agbo@yahoo.com
**Corresponding author*
Received 18th December 2017, Accepted 27th June 2018

Abstract: *This work reports the comparison in the physical properties of tiger nut tubers (Cyperus esculentus), a crop extensively cultivated in Northern Nigeria as well as in the biochemical changes which occur during the spontaneous fermentation of the soaked tubers in water for tiger nut beverage production. Both the brown and yellow tuber varieties of C. esculentus were used in this study. For each variety, the length and width of 100 randomly selected tubers alongside the weight of 1000 tubers were determined. Furthermore, 10g of each variety was steeped in sterile distilled water for periods of time that ranged from 0-96 hours in order to highlight the bacterial and fungal diversities occuring among the tubers. Results of the physical properties revealed that the yellow variety tubers were highly significantly longer in length, bigger in size, have higher length/width ratio, and heavier in weight than the brown variety tubers. When steeped in water, the mean range count of log 2.763 – 10.20 CFU/g for lactic acid bacteria and log 2.073 – 7.73 for yeast/mold count in the brown tubers were significantly higher than the range of log 2.51 -6.14 and 1.56 – 8.17 obtained for the same parameters, respectively, in the yellow tubers. The dominant microorganisms during the steeping process, regardless of the variety, included Bacillus species, Gram-negative group Enterobacteriaceae, as well as yeasts, moulds, and acetic acid bacteria. These array of microorganisms are for assessment and proper selection of desirable strains, as potential starter cultures, towards tiger nut beverage production.*

Keywords*: tigernuts, brown variety, yellow variety, tuber length/width, Lactic acid bacteria, microbial profile*

1. Introduction

Cyperus esculentus is an erect perennial weed plant which occurs in the tropics, subtropics, Mediterranean and warm temperate regions [1,2] and produces edible tubers. In Nigeria, the tubers are commonly known as “Ayaya” in Hausa, “Ofio” in Yoruba, and “Akiausa” in Igbo and are cultivated extensively in the Northern part of the country; three varieties (black, brown and yellow) are cultivated and of these, only two varieties, yellow and brown, are readily available in the local market [3]. The tubers are widely consumed raw or unprocessed. They could be dried, mixed with groundnut or soaked in water for varied time-lengths of about 3

days [4]. They are particularly savoured for the production of non-alcoholic dairy-like beverage with a worldwide acceptance and which is non-indigenous to Nigeria. In Spain, the beverage is known as "Horchata de chufa" particularly in the Valencia region [5].

In recent times, the beverage has increasingly become popular in many local social functions as substitute to industrially-produced conventional carbonated drinks, perhaps probably due to its low price, and wide acceptance by the people.

Traditionally, in Nigeria, production of the consumed beverage extracts is by spontaneous fermentation. The extracts are prepared by steeping the tubers in water for different periods of time. This may range from a few hours to a full day; or for about three to four days, after which the harvested tubers could be consumed directly or sold in the public or hawked as delicacies. Information is not available on the effects of periods of steeping in water in terms of the microbial diversity and biochemical properties of the resultant extracts. Such information is therefore necessary in the quest to transform the local process into a conventional technological process.

This research aims to determine and compare the physical characteristics of the two varieties of tiger nut tubers, locally available in the market, and the biochemical changes produced during the natural fermentation of the steeped tubers

2. Materials and methods

2.1. Collection of Samples:

The tiger nuts used in the study were the yellow and brown varieties, identified locally as the 'Big' and 'small' varieties. These were obtained directly from local markets in Biliri (Gombe State) and labeled "BBF" and "VSF," respectively. A weight of five kilogrammes (5kg) of each variety was purchased. These were brought into the laboratory, and were separately sorted as described by [6], in order to remove foreign materials, broken and damaged tubers. The selected tubers were then used in every experimental procedure in the course of the study.

2.2. Physical Properties of Tubers

2.2.1. Estimation of the length and width of tubers

A vernier caliper was used to measure the length and width of 100 randomly selected tubers from each variety [7]. The obtained values were then appropriately noted and recorded.

2.2.2. Weight of 1000 tubers

The weights of randomly selected 1000 tubers were measured collectively in grammes using a pre-weighed large beaker and the obtained values recorded [7]. This procedure was repeated in six replicates for each variety to measure the quality of the tubers in terms of filling.

2.3. Isolation of microbial species associated with soaked tiger nut tubers

The microbial analytical methods described by [8] and [9] were utilized for the study. A weight of 10g of each variety of tiger nut tubers was thoroughly washed in three changes of sterile distilled water. These were then steeped in 90ml of sterile distilled water, contained in plastic bowls with covers, to make 10^{-1} dilution, and the bowls covered. At regular intervals, that is, 0 hour, 24 hours, 48 hours, 72 hours, and 96 hours, 1ml of the steeping water was aseptically serially diluted and plated using the pour-plate technique on solid medium. Based on the results of some preliminary trial experiments, diluents values 10^{-1}, 10^{-3}, 10^{-5}, 10^{-7}, and 10^{-9} of the steeping water were pour-plated on solid medium at 0 hour, 24 hours, 48 hours, 72 hours, and 96

hours, respectively. The experiments were triplicated.

The solid media used for enumeration were Nutrient agar, for aerobic colony count; Eosin methylene blue agar, for Enterobacteriaceace count; Mann Rogosa Sharpe agar for Lactic acid bacterial count; and Potato dextrose agar, for yeast and mould count. Inoculation into each media plate was done in three replicates, except for the lactic bacterial count where dual replicates were used. The plates for bacterial isolation were incubated at 37°C for 24 hours while plates for fungi were incubated at 25°C for 48 hours. The viable cells obtained on the inoculated media plates after incubation were counted using digital colony counter (Gallenkemp, England). The average colony counts obtained were expressed as colony forming units per gram (Cfu/g). Pure bacterial and yeasts cultures obtained from the study were identified to conventional biochemical identification keys. Moulds were identified by [10] methods.

2.4. Statistical Analyses

The various experimental data obtained were subjected to statistical analysis using the Genstat Release 7.22 DE, version 2008 (VSN, International Limited). The unpaired Students' T-test was used to analyze the physical properties of tiger nut tubers. All other analyses were by two-way analysis of variance (ANOVA). Probability values ($P \leq 0.05$) were considered as statistically significant.

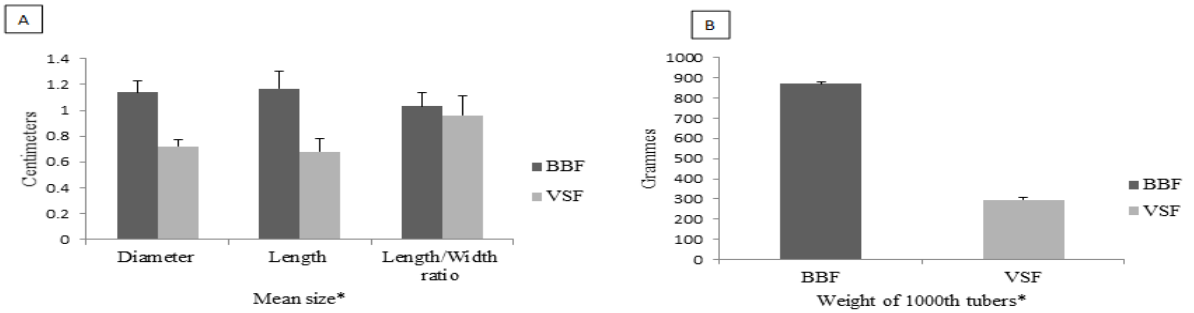
3. Results and discussion

3.1. Physical properties

The yellow tubers were highly significantly longer in length, bigger in size, have higher length/width ratio, and heavier in weight than the brown coloured tubers (Figure 1). Several authors, [11-13] have reported the yellow variety is preferred to all the other varieties in Nigeria, because of its inherent properties like its bigger size, attractive colour and fleshier body. These attributes may have been responsible for this variety's preference by the consumers. The length, length/width ratio, and unit weight of tiger nut tuber are as high heritable traits in tiger nut plants and soil texture has significant effect on the average tuber size [14]. Tubers grown in sandy soil have been significantly observed to be larger in diameter than those grown in clay [15, 14]. It is worth noting that the ratio values of length and length/width recorded for both the brown and yellow tuber were 0.68±0.10cm, 0.96±0.15cm and 1.17±0.13, 1.03±0.11, respectively. These values are different from the tubers of 'Gegant Africana,' 'Liagueta Alboraia' and 'Ametilla Bonrepos' cultivars, selectively cultivated in Spain, and described by [14] to have a length and length/width ratio values of 1.73cm, 1.62; 1.52cm, 1.68; and 1.24cm, 1.16, respectively.

3.2 Microbial species and pH changes associated with soaked tiger nut tubers

The aerobic colony count on nutrient agar of tiger nut tubers soaked in water at ambient temperature is shown in Figure 2. Generally, aerobic plate count in the yellow and brown tubers increased with time and varied between 2.647 to 9.54 log CFU/g, and 2.53 to 8.933 log CFU/g, respectively (Figure 2a). Statistically, the mean values obtained at each of the sampled periods were significantly different in the yellow tubers; whereas in the brown tubers, the mean values were significantly different at 0 hour, 24 hours, 48 hours and 72 hours. The Enterobacteriaceace count on eosin methylene blue (EMB) agar of tubers soaked in water at ambient temperature is

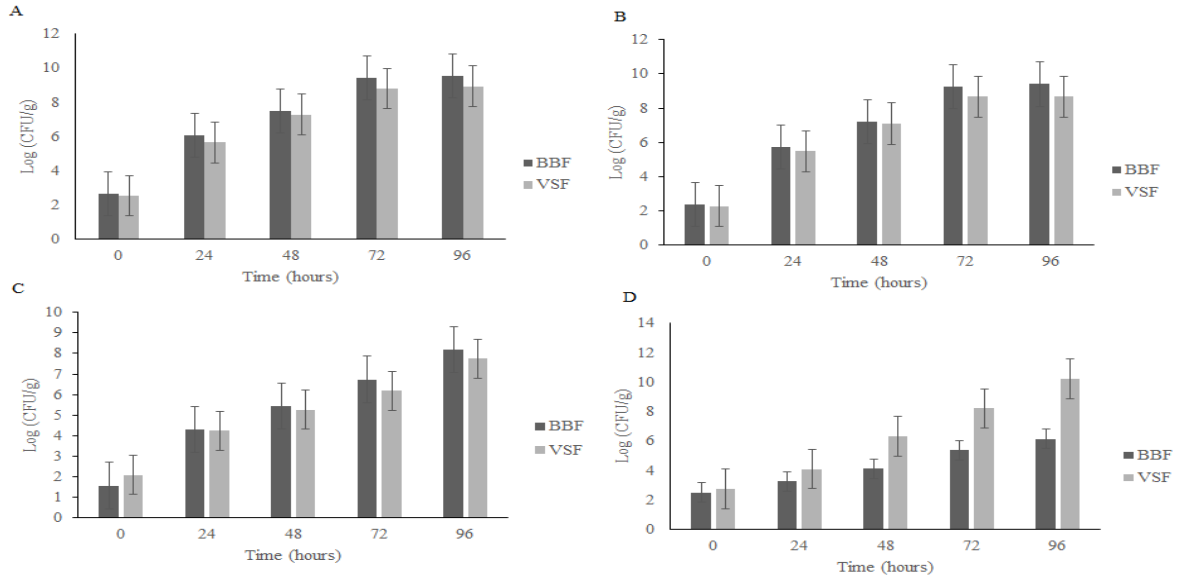


KEY: VSF-Brown variety **BBF- Yellow variety**

Fig. 1: Physical properties of tiger nut tubers* (A) and Weight of 1000th tubers÷

*Each parameter is a mean of 100 replicates ÷Mean of six replicates

shown in Figure 2b. Generally, the Enterobacteriaceace counts in the yellow and brown tubers increased with steeping time and varied between 2.357 to 9.40 log CFU/g, and 2.257 to 8.673 log CFU/g, respectively. The mean values obtained at each of the sampled periods were significantly different in the yellow tubers; whereas in the brown tubers, the mean values were highly significantly different at 0 hour, 24 hours, 48 hours and 72 hours. The fungal and yeasts, on potato dextrose agar (PDA), from tubers soaked in water at ambient temperature is shown in Figure 2c. Generally, the yeast and mold counts increased with steeping time, and the mean counts in the yellow and brown tubers ranged from 1.5667 to 8.1733 log CFU/g, and 2.0733 to 7.7367 log CFU/g, respectively. At the onset of the soaking process (0 hour), the yeast and mold counts from the brown tubers (2.0733 log CFU/g) were higher than the value (1.5667 log CFU/g) obtained in the yellow. Statistically, the mean fungal counts recorded at each of the sampling points, in the different varieties, were significant



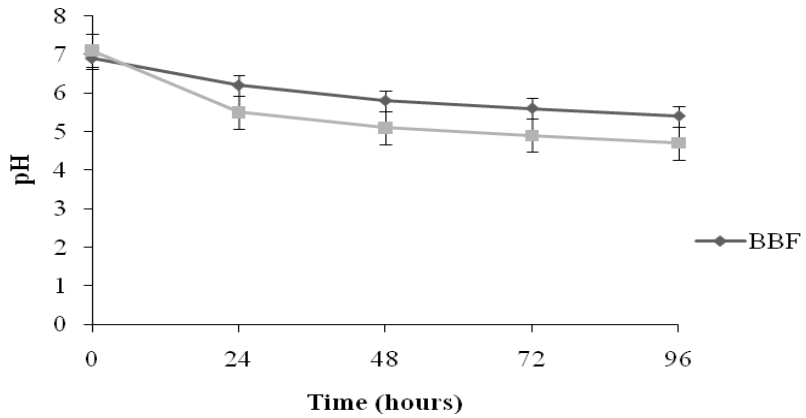
KEY: VSF-Brown variety; BBF- Yellow variety

Fig. 2: Aerobic colony counts (A), Enterobacteriaceace counts (B), Yeasts and mould count (C) and Lactic acid bacterial counts (D) from varieties of tiger nut tubers steeped in water

The mean Lactic acid bacterial (LAB) count in the soaked yellow and brown tubers is expressed in Figure 2d. Mean counts ranging from 2.510 to 6.143 log CFU/g and 2.763 to 10.205 log CFU/g in the yellow and brown tubers, respectively, were obtained. The counts obtained increased concomitantly with time. Statistically, the mean LAB counts obtained at each of the sampling periods in the different tubers were significant.

The change in pH, during the soaking of tiger nut tubes in water at ambient temperature, is shown in Figure 3. The pH fell from 6.86 - 4.73 and 7.13 – 4.82 within 72 hours of soaking in the yellow and brown tubers, respectively. Within 24 hours of soaking, there was a steep fall from 7.14 – 5.40 in the brown tubers, and a gradual fall from 6.86 – 5.69 in the yellow tubers. Between 72 hours and 96 hours of soaking, the two types of tubers experienced a further fall in pH values, i.e. 4.13 and 4.30 in yellow and brown tubers, respectively.

Cyperus esculentus tubers are underground stems which are harvested when ploughed from the soil. Bacteria have been reported to be the most dominant group of microorganisms in soils, while fungi are more numerous in the surface areas of well-aerated, cultivated soils [16]. It is also envisaged that the field-soil or storage conditions, or the combination of both, may have been responsible for the higher yeast and fungal count in the brown than yellow tuber, observed at the onset (0 hour) of soaking in water.



KEY: VSF-Brown variety BBF- Yellow variety

Fig. 3: Changes in pH during soaking of tiger nut tubers in water at ambient temperature

The identities and distribution of microorganisms obtained from tiger nut tubers soaked in water is shown in Table 1. *Bacillus* species, *Lactobacillus plantarum*, non-lactose fermenters such as *Serratia marscens* and *Providencia rettgeri* and the lactose-fermentors, like. *Klebsiella pneumonia*, were the dormant bacteria isolated from the tubers at the onset of the soaking process. These mentioned organisms are all bacilli (rod-shaped). [16] have reported that bacilli are very common in soils, and some of them like *Bacillus* species can persist in unfavourable conditions via formation of resistance structures called endospores which can withstand prolonged desiccation and high temperature. Similarly, aerobic epiphytic microflora found present at the start of fermentation on raw plant material, have been known to include *Bacillus* species, Pseudomonadaceae, fungi, Enterobacteriaceae, lactic acid bacteria, and yeasts [17].

Furthermore, all microorganisms of the *Bacillus* species are known to generate fermentable reducing sugars from raw plant materials via their ability to synthesize extracellular alpha-amylase enzymes that degrade starch molecules [18, 19] into products which are likely to stimulate the growth of other organisms such as lactic acid bacteria. [20] and [17] have explained that most lactic acid bacteria lack the ability to hydrolyse starch or are deficient in the use of amylolytic enzymes; consequently, the generation of fermentable carbohydrates by the *Bacillus* species helps to ensure the initiation of lactic acid fermentation. *Bacillus* species are also associated with the fermentation of cereals such as for 'burukutu' and 'Ogi', and during the soaking of cassava tubers in water for the production of 'lafun' [21, 22].

The lactic acid bacterium, *Lactobacillus plantarum*, was persistently commonly isolated from the two varieties of tubers throughout the period of soaking. Since, it was isolated from the onset of the soaking process; it may belong to the epiphytic microflora group of these tubers. *L. plantarum* has also been isolated as part of the microflora in 'kocho', a fermented, acidic, starchy, and staple food in Ethiopia; prepared from the plant, ensette (*Ensette ventricosum*) [23]. Similarly, [24] have isolated *L. plantarum* during the retting of cassava tubers in water, in the traditional production of the fermented food 'fufu' (cassava flour),

popularly consumed in the West African sub-region.

L. plantarum, *L. acidophilus* and *L. fermentum* come from the environment, and are associated with plant material [25]. *L. plantarum* and *L. fermentum* are heterofermenters which produce equimolar amounts of lactic acid, ethanol/acetic acid, and carbon dioxide from glucose [26]. Heterofermentative lactic acid bacteria contribute to flavor development [17]. *L. acidophilus* is a homofermenter which produces only lactic acid from fermentable carbohydrates [17].

Lactic acid bacteria are Gram-positive organisms which grow readily in most food substrates and can lower the pH rapidly to a point where competing organisms are no longer able to grow [27]. Hence, the rapid fall in pH from 7.10 – 5.50 in the brown tubers and 6.90 – 6.20 in the yellow tubers, within 24 hours of soaking in water, may be attributable to the metabolic activities of lactic acid bacteria. Lactic acid bacteria are important microorganisms for biotechnological applications

The spectrum of Enterobacteriaceae lactose-fermentor organisms obtained from yellow and brown tubers during the soaking process were the same, and these organisms were *Klebsiella pneumonia, K. oxytoca, Enterobacter aerogenes, E. cloacae* and *Citrobacter diversus*, (Table 1). The increased dominance in the isolation of these organisms, particularly observed from 24 hours of sampling, could have stemmed from the conducive near-neutral pH of 6.20 and 5.50 observed in the yellow and brown tubers, respectively, during the soaking process. In [17] it have reported that members of Enterobacteriaceae, particularly *Enterobacter* and *Klebsiella* species dominate the early stages of fermentation because of their high specific growth rates at neutral pH. *Klebsiella* species are known to contribute to the decay of cassava roots during the traditional submerged fermentation of cassava to 'fufu' and 'lafun' production [22]. It is envisaged that the *Klebsiella* species, along with the other lactose-fermenting organisms obtained in this study would have performed similar role that is of decaying of tubers.

Table 1

Identity and distribution of microorganisms isolated from tiger nut tubers steeped in water

Species identified	Number of isolates		Fermentation hours				
			0	**24**	**48**	**72**	**96**
Yellow variety							
Bacillus substilis	2	1	1	0	0	0	
B. megaterium	1		1	0	0	0	0
Lactobacillus plantarum	9	3	2	2	1	1	
L. acidophilus	3		0	1	0	0	2
L. fermentum	3		0	0	1	2	0
Klebsiella pneumoniae	7		2	2	2	0	1
Serratia marscens	1		1	0	0	0	0
Pseudomonas aeruginosa	1	1	0	0	0	0	
Enterobacter aerogenes	1		0	1	0	0	0
E. cloacae	1		0	1	0	0	0
Acetobacter species	5		0	0	2	2	1
Citrobacter diversus	2		0	0	1	1	0
Klebsiella oxytoca	2		0	0	0	1	1
Acinetobacter species	3		0	0	0	1	2
Aspergillus flavus	5	1	1	1	1	1	
Aspergillus niger	5	1	1	1	1	1	
Aspergillus species	5		1	1	1	1	1
Fusarium species	5	1	1	1	1	1	
Candida krusei	5		1	1	1	1	1
Candida glabrata	1	0	0	0	0	1	
Candida zeylanoides	2		1	1	0	0	0
Saccharomyces cerevisae	4	0	1	1	1	1	
Rhodotorula mucilaginosa	2	0	0	1	1	0	
Cryptococcus laurenti	1		1	0	0	0	0
Total	76		16	15	15	15	15
Brown variety							
Bacillus substilis	1	1	0	0	0	0	
Lactobacillus plantarum	12	3	3	2	2	2	
L. acidophilus	3		0	0	1	1	1
Klebsiella pneumoniae	10		2	3	2	2	1
Providencia rettgeri	1		1	0	0	0	0
Serratia marscens	1		1	0	0	0	0
Enterobacter aerogenes	2		0	1	1	0	0
Citrobacter diversus	3		0	1	1	1	0
Acetobacter species	2		0	0	1	0	1
Enterobacter cloacae	1		0	0	1	0	0
Klebsiella oxytoca	1		0	0	0	1	0
Acinetobacter species	3		0	0	0	1	2
Aspergillus flavus	5	1	1	1	1	1	
Aspergillus niger	5	1	1	1	1	1	
Aspergillus species	5		1	1	1	1	1
Candida krusei	5		1	1	1	1	1
Candida glabrata	1	0	0	0	1	0	
Candida zeylanoides	3		2	1	0	0	0
Saccharomyces cerevisae	4	0	1	1	1	1	
Rhodotorula mucilaginosa	2	0	0	1	0	1	
Total	70		14	14	15	14	13

Enterobacteriaceae count obtained from the two varieties of tubers, as soaking time progressed, may be attributable to favourable conditions, such as the near-neutral pH and availability of suitable substrates, for microbial activity.

The acetic acid bacteria, *Acetinobacter* and *Acetobacter* species were the dominant microorganisms that were increasingly isolated from 48 hours of soaking the yellow and brown tubers in water. Acetic acid bacteria, as reported by [28] and [29], belong to the family Acetobacteriaceae, and are obligate aerobic Gram-negative organisms. They are widely distributed in the natural habitat and have been isolated from alcoholic beverages, vinegar, fruits, flowers, honey, bees, sugar cane, juices, soil and water [30]. The observation that the isolated acetic acid bacterium became increasingly obvious only after 48 hours of soaking, suggest that this bacterium could have resulted from the accumulates of metabolites in the steeping water. [31]) and [32] reported that acetic acid bacteria can use a variety of substrates such as glucose, ethanol, lactic acid or glycerol as energy sources.

Acetic acid bacteria are known to play a fundamental role in the development of chocolate flavor precursors in the spontaneous fermentation of cocoa beans [9]. The dominance of Acetic acid bacteria population in cocoa fermented in Loh-Djibouna, Cote D'Ivoire, has been reported by [33].

The yeast species *Candida krusei*, *C. zeylanoides*, *Cryptococcus laurenti* and *C. krusei, C. zeylanoides* isolated from yellow and brown tubers, respectively, at the onset (0 hour) of the soaking process probably belong to the epiphytic flora of the tubers (Table 1). In the spontaneous fermentation of cocoa beans, in the Dominican Republic, [9] reported the presence of *Candida zeylanoides* among the yeast species isolated, and which produced pectinolytic enzymes that resulted in mucilage breakdown in the cocoa beans, and hence, release of fermentable carbohydrate substrates. It may probably be that the yeast species forming the epiphytic flora on the tiger nut tubers in this study could have probably produced similar pectinolytic activity. Nevertheless, majority of epiphytic yeasts in plant fermented foods are known not to ferment sugar, but rather use pectin as a carbon source [34, 17].

Yeast species of *Sacchromyces cerevisae* was continuously isolated from 24 hours until the end of the fermentation study (96 hours) in both varieties of tubers. In pickled vegetable, *S. cerevisae* is regarded as fermentable yeast because it is able to remove fermentable substrates, and consequently, preserve the pickled vegetable [17]. Similarly, *Candida glabrata* and *Rhodotorula mucilaginosa* were among the dominant yeast isolates obtained from the varieties of tubers between 24 hours and 96 hours of fermentation in water. *Rhodotorula* species have also been associated with fermenting 'kocho', a staple acidic, starchy food in Ethiopia, though the role of these yeast species in the fermentation process, was not be specified [23]. The study [17] reported the existence of two major groups of yeast, based on their roles in naturally fermented products of plant origin; fermentative yeasts and oxidative yeasts. The authors explained that fermentative yeasts metabolize fermentable substrates, and are important in preservation, while oxidative yeasts are associated with deterioration via breakdown of organic acids, which lead to increase in pH and growth of deteriogens, acid-sensitive organisms. The role of the yeast species obtained in the present study was not assessed. Generally, yeast species ferment

simple sugars to ethanol and carbon dioxide [9].

4. Conclusion

In Nigeria, the yellow tubers of *C. esculentus* significantly differ from the brown variety in length, width, length/width ratio and weight. During steeping, changes in APC, *Enterobacteriaceae* count and yeast and mould count were significantly higher in the yellow tuber variety than in the brown one. Nevertheless, the dominant microbial species associated with the natural fermentation of these tubers are similar, and present potentials of being used as starter cultures for not only the commercial production of the beverage, but perhaps also in the formulation and development of derived fermented beverages.

5. Acknowledgements:

We are grateful to the laboratory staff in Bowen University, Iwo and Abubakar Tafawa Balewa University, Bauchi, where this research work was carried out.

6. References

[1] KAY D., Crop and product digest Number 2-Root crops, 2nd edition Tropical Development and Research Institute, New Zealand. Pp 308, (1987)
[2] DJOMDI R., NDJOUENKEU R., Soaking behavior and milky extraction performance of tigernut (*Cyperus esculentus*) tubers *Journal of Food Engineering* 78: 546-550, (2007)
[3] OLADELE A., AINA J., Chemical composition and functional properties of flour produced from two varieties of tigernut (*Cyperus esculentus*) *African Journal of Biotechnology* 6 (21): 2473-2476, (2007)
[4] TEMPLE V., OJOBE T., KAPU M., Chemical analysis of tigernut (*Cyperus esculentus*) *Journal of the Science of Food and Agriculture* 50 (2): 261-263, (1990)
[5] PASCUAL B., MUROTO V., LOPEZ-GULAMA S., SANBAUTISTA A., ALAGARDA J., LOPEZ G,. Morphological and productive characteristics of nine "chufa" (*Cyperus esculentus* L-var sartivus Boeck) clones. *Acta-Horticulturae* **1** (614): 85-88, [2003]
[6] TURHAN M., SAYAR S., GUNASEKARAN S., Application of peleg model to study water absorption in chickpea during soaking. *Journal of Food Engineering* 53: Pp 153-159, [2002]
[7] MAZIYA-DIXON B., KING J., OKOORUWA A., Physical, chemical and water absorption characteristics of tropical maize hybrids *African Crop Science Journal* **8** (4): 419-428, [2000]
[8] BADAU M., Microorganisms associated with pearl millet cultivars at various malting stages. *Inter Journal of Food Safety* 8: 66-72, [2006]
[9] LAGUNES G., LOISEAU G., PAREDES J., BAREL M., GUIRAUD J., Study of the microflora and biochemistry of cocoa fermentation in the Dominican Republic. *International Journal of Food Microbiology* 114: 124-130, [2007]
[10] BROWN E., Benson's microbiology applications, 9th edition. Mc Graw-Hill, New York. Pp 57-62, 249- 263, (2005)
[11] OKAFOR J., MORDI J., OZUMBA A., SOLOMON H., OLATUNJI O., Preliminary studies on the characterization of contaminants in tiger nuts (yellow variety). In: Proceedings of 27th Annual Nigerian Institute of Food Science and Technology (NIFST) conference. 13-17 October, 2003. Pp 210-211, [2003]
[12] BELEWU M., ABODUNRIN O., Preparation of kunu from unexploited rich food; tigernut (*Cyperus esculentus) World Journal of Diary and Food Sciences* 1: 19-21, [2006]
[13] ADEJUYITAN J., OTUNOLA E., AKANDE E., BOLARINWA I., OLADOKUN F., Some physiochemical properties of flour obtained from the fermentation of tigernut (*Cyperus esculentus)* sourced from a market in Ogbomosho, Nigeria *African Journal of Food Science* 3 (2): 51-55, [2009]
[14] PASCUAL B., MAROTO V., LOPEZ-GULAMA S, SANBAUTISTA A., ALAGARDA J., Chufa (*Cyperus esculentus* L-var sartivus Boeck): An unconventional crop, Studies related to applications and cultivation *Economic Botany* 54 (4): 439-448, [2000]
[15] PASCUAL B., MAROTO J., Influencia de la textura en la cuantia y calidad de los tuberculos de las chufas (*Cyperus esculentus* L.). *Agricultura* 592:873-875, [1981]
[16] RANGASWAMI G., BAGYARAJ D., Agricultural Microbiology 2nd edition Prentice-Hall of India Private Limited, New Dehli, India. Pp 205-208, [2004]

[17] NOUT M., ROMBOUTS F., Fermentative preservation of plant foods *Journal of Applied Bacteriology Symposium Supplement* 73: 136S-147S, [1992]
[18] NJOKU H., OKEMADU C., Biochemical changes during the natural fermentation of the African oil bean (*Pentaclethra macrophylla*) for the production of ugba. *Journal of the Science of Food and Agriculture* 49: 457-465, [1989]
[19] ELIANA DE OLIVEIRA S., MERIE-LESLIS L.,. Effect of medium composition on formation of amylase by *Bacillus* species *Brazilian archives of Biology and Technology* 46: 129-134, [2003]
[20] SANTOS E., MARTINS M., Effect of medium composition on formation of amylase by *Bacillus* species *Brazilian archives of Biology and Technology* 46 (1) : 129-134, [2003]
[21] ACHI O., Microbiology of 'obiolor' a Nigerian fermented non-alcoholic beverage *Journal of Applied Bacteriology* 69: 321-325, [1990]
[22] OYEWOLE B., Cassava processing in Africa. In National Research Council *Applications of Biotechnology to Traditional Fermented Foods*, National Academy Press, Washington D.C., USA, Pp 89- 92, [1992]
[23] GASHE B., Kocho fermentation *Journal of Applied Bacteriology* 62: 473-477, [1987]
[24] BRAUMAN A., KELEKE S., MALONGA M., MIAMBI E., AMPE F., Microbiological and biochemical characterization of cassava retting, a traditional lactic acid fermentation for foo-foo (cassava flour) production *Applied and Environmental Microbiology* 62 (8): 2854-2858, [1996]
[25] NOUT M., SAKAR P., Lactic acid fermentation in tropical climates *Antonie van Leeuwenhoek* 76: 395-401, [1999]
[26] ADAMS M., MOSS M., Food Microbiology The Royal Society of Chemistry, Cambridge, UK. Pp 316-317 [2008]
[27] STEINKRAUS K., Lactic acid fermentations In: Applications of Biotechnology to Traditional Fermented Foods, National Research Council, National Academy Press, Washington D.C., USA, Pp 43- 51, [1992]
[28} MATSUSHITA K., TOYAMA H., ADACHI O., Respiratory chains and biogenetics of acetic acid bacteria; in rose and AH (ed.), *Advances in Microbial Physiology* Academic Press, Pp 247-301, [1994]
[29] ADACHI O., MOONMANGMEE D., TOYAMA H., YAMADA M., SHINAGAWA E., MATSUSHITA K., New developments in oxidative fermentation *Applied Microbiol. Biotechnol.* 60: 643-653, [2003]
[30] YAMADA Y., HOSONO R., LISDYANTI P., WIDYASTUTI Y., SAONO S., UCHIMURA T. and KOMAGATA K., Identification of acetic acid bacteria isolated from Indonesian sources, especially of isolates classified in the genus *Glucobacter*, *Journal Gen. Appl. Microbiology* 45: 23-28, [1999]
[31] DE LEY J., GILLIS M., SWINGS J., Bergey's Manual of Systematic Bacteriology, Williams and Wilkins Baltimore, Baltimore Pp 267-278, [1984]
[32] RASOOLI I., SHARAFI S., BEHESHTI-MAAL K., Isolation, characterization and optimization of indigenous acetic acid bacteria and evaluation of their preservation methods *Iranian Journal of Microbiology* 2 (1): 41-48, [2010]
[33] KOUSAME L., DOUE G., ADOM N., OUATTARA H., NIAMKE S., Biochemical characterization of microbial populations involved in Loh-Djiboua cocoa's fermentation in Cote D'Ivoire *Food Fermentation and Safety* 14 (2): 196-205, [2015]
[34] DOBOLYI C., KECSKES M., Ecophysiological characteristics of the epiphytic yeast populations of some agricultural plants *Zentralblatt fur Mikrobiologie* 145: 334, (1990)