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ASSESSMENT OF MICROBIOLOGICAL QUALITY OF COMMERCIALLY PREPARED READY TO DRINK TIGERNUTS SOLD IN OWERRI, IMO STATE.

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ABSTRACT

Assessment of microbiological quality of commercially prepared ready to drink tiger-nuts sold in Owerri, Imo State was carried out using serial dilution and pour plate technique. Tiger-nut were washed, blended and sieved to liquid extract. Aliquots of serially diluted tigernuts were inoculated unto a freshly prepared nutrient agar, MacConkey agar for bacteria isolates and incubate for 37^0C for 24hrs while Sabouraud dextrose agar for fungi isolates was incubated for 25^0C for 72hrs. Identification of pure culture was done using morphological characteristics and biochemical test. The total bacteria and fungi count showed the occurrence of bacteria isolated indicating that *Bacillus* sp (42.8%) was higher followed by *Klebsiella* sp (28.6%) and *Staphylococcus aureus* (28.6%). While fungi occurrence showed that *Rhizopus* sp (40%) was higher followed by *Sacharomyces* sp (33.3%), *Aspergillus* sp (13.4%) and *Penicillium* sp (13.3%). Therefore, the microbial status of the samples showed that bacteria and fungi species affect the quality of tiger nut. It will be necessary, however, to determine the effects of storage in different packaging materials at refrigeration and ambient temperatures on the microbiological status of tigernut milk extract to ascertain its shelf-life

Keywords: Microbiological quality, commercial food preparation, hygiene, food safety, tigernut.

INTRODUCTION

Tiger nut "*Cyperus esculentus lativum*" is an underutilized tuber of family Cyperaceae, which produces rhizomes from the base of the tuber that is somewhat spherical (Devries and Feuke, 2019). It is a tuber that grow freely and is consumed widely in Nigeria, other parts of west Africa, east Africa, parts of Europe particularly Spain as well as in the Arabian Peninsula (Abaejoh *et al.,* 2016). In many thousand years ago, tiger nut, in Spanish called chufa, was cultivated in region of chufa between Sudan and Egypt

on the borders of the Nile River. There are documents that certify this product over 400 years ago. Proof of this is that on many occasion archeologists found earthen jars containing tiger nut in graves of pharaohs. (Obadina *et al.,* 2018) previously, it was cultivated in the ancient Mesopotamia between the rivers Tigris and Euphrates. At the same time historical Persian and Arab documents mentioned the nutritive, digestive and disinfective value of tiger nut. During the era the tiger nut milk was classified as medicinal drink due to it's been highly energetic and diuretic, rich in mineral, predominantly phosphorus and potassium and also vitamins C and E (Abaejoh *et al.,* 2016). It was in the 8th century that Arab traders introduced the cultivation of tiger nut in the Mediterranean region of Valencia (Spain), for elaboration of tiger nut milk (leche de chufa), to know the tiger nut cultivation as it arrived to our days. It has been reported that grainy sandy group and mild temperatures are special for the cultivation growth of earth tuber (Abaejoh *et al.,* 2016). Tiger nuts tubers appear somewhat long or round in shape with a dimension of 8mm to 16mm, smaller size however, are not used for human consumption. When hydrated, it is slightly harder (nut texture), but with a rather more intense and concentrated taste. The cultivation time is April to November (Osagie *et al.,* 2016). Being cultivated through continuance irrigation, tiger nut has to be properly dried before storage. The drying process is completely natural, (i.e. sun drying) and the process can take up to one month. The dehydrating process ensures longer shelf life, preventing rot or any other bacterial infection securing their quality and nutritional level. Unfortunately, the dehydration process make the tiger nut skin wrinkled, a situation that limits its acceptability to some people (Belewu and Abodunrin, 2016). It is known in Nigeria as "Aya" in Hausa, "Ofio" in Yoruba and "Akiausa" in Igbo where these varieties (black, brown and yellow) are cultivated (Umerie *et al.,* 2017). Among these, the yellow variety is preferred over others because of its inherent properties such as large size, attractive color and fleshier nature. It also yield more milk upon extraction, contains lower fat and higher protein and less anti nutritional factors especially polyphenol (Okafor *et al.,* 2013). Recently, there is awareness for increased utilization of tigernut (Belewu and Abodunrin, 2016; Belewu and Belewu, 2017). As food, tiger nut can be eaten as snack which can be prepared by soaking in water for few minutes. It can also be eaten roasted, dried, baked and can be made into a refreshing beverage called "Horchata De Chufas" or tigernut milk. It also finds uses as a flavouring agent for ice cream and biscuits

(Cantalejo, 2017), as well as in making oil, soap, starch and flour. The tiger nut milk compared with any other soft drink is not just a refreshing drink but also very healthy. It contributes to the reduction in the in cholesterol by diminishing the 'bad' cholesterol low density Lipoprotein (LDL), and increasing the 'good' cholesterol, high density Lipoprotein (HDL) (Belewu and Abodunrin, 2016). Its content of vitamin E also collaborates against the cholesterol because it has an antioxidant effect over fats, which are ideal for coronary heart disease (Chukwuma *et al.,* 2010).

AIM OF STUDY

This study was aimed to determine the microbiological analysis of commercially prepared tiger nuts sold in Owerri, Imo State.

Specific Objectives

1. To isolate the microorganisms from the tiger nut.
2. To identify the microorganisms using morphological and biochemical tests
3. To determine the organism's frequency of occurrences

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MATERIAL AND METHODS

STUDY AREA

Owerri is the capital of Imo state in Nigeria, set in the heart of Igboland, it is approximately 100 square kilometers (40 sq mi) in area. Owerri is made up of three Local Government area, namely Owerri west, Owerri North and Owerri Municipal. Owerri has an estimated population of about 750,000 as of 2006 and is approximately 100 square kilometers (40 sq mi) in area it has a population of 215,038 is located at Latitude: 5° 29' 1.07" N and Longitude: 7° 01' 59.70"E. Owerri is bordered by the Otamiri River to the east and the Nworie River to the south.

SAMPLE COLLECTION

A total of (6) six fresh ready to drink Tiger nuts, comprising of three (3) yellow and three (3) brown varieties were purchased from the local Vendors at Relief market in Owerri, Imo State, Nigeria.

MICROBIAL ANALYSIS

INOCULATION OF SAMPLE

Ten-Fold serial dilution method was prepared by diluting One milliliter (1ml) of each sample was serially transferred into nine milliliters (9ml) of the sterile diluent (peptone water) with a sterile pipette and shaken vigorously. Serial dilution was continued until (10^{-10}) dilution was obtained. Aliquot portion (0.1ml) of 10^{-2}, 10^{-4} and 10^{-6} dilutions were inoculated onto freshly prepared, surface-dried Nutrient agar (NA) and MacConkey agar (MCA) respectively and incubated for 24-48h at 37^0C for bacteria and 25^0C for 72hrs unto sabouraud dextrose agar for fungi isolates. Growth of microorganisms was formed in colonies. These colonies were then streaked onto their primary media and incubated at the optimum temperature for growth for another 24-48 hours on nutrient agar for bacteria and sabouraud dextrose agar for to obtain a pure culture.

CHARACTERIZATION AND IDENTIFICATION OF BACTERIAL AND FUNGI ISOLATES:

Bacterial and fungal isolates were characterized and identified based on colonial morphological, Gram stating, lactophenol cotton staining and biochemical test such as catalase test, coagulase test, indole test, oxidase test, Citrate, methyl red, motility, and sugar fermentation test) to know the genera using the method as described by (Cheesbrough, 2016).

RESULTS

Table 1: Microbial count in cfu/ml of various tigernut milk extract. The total heterotrophic bacterial count on nutrient agar showed that bacterial growth was highest in sample TY2 ($5.4x10^4$ cfu/ml) and lowest in TB1 ($1.4x10^4$ cfu/ml), the coliform count on MacConkey showed that sample TB1 was higher ($1.76x10^6$) and lower in TY1 ($1.6x10^6$) while fungi count was higher in TY3 ($1.74x10^5$) and lower in $2.3x10^5$. This indicated that contamination existed in the product samples.

TABLE 1: MICROBIAL COUNT IN CFU/ML OF VARIOUS TIGERNUT MILK EXTRACT

SAMPLE	Counts on Nutrient Agar (10^{-2})cfu/ml	Count on MacConkey Agar (10^{-4}) cfu/ml	Count of Sabouraud Dextrose Agar (10^{-3}) cfu/ml
TY1	NG	$1.6x10^6$	$7.6x10^5$
TY2	$5.4x10^4$	NG	$2.3x10^5$
TY3	NG	$1.21x10^6$	$1.74x10^5$
TB1	$1.4x10^4$	$1.76x10^6$	$3.6x10^5$
TB2	$2.4x10^4$	$2.6x10^6$	$5.8x10^5$
TB3	$2.2x10^4$	$2.8x10^6$	$7.2x10^5$

Keys: NG: No Growth, TY = Tigernut Yellow, TB = Tigernut Brown

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TABLE 2a COLONIAL MORPHOLOGICAL CHARACTERISTICS OF BACTERIA ISOLATED FROM READY TO DRINK TIGERNUTS

Samples	Colour	Surface	Shape	Arrangement	Suspected organism
TY2, TB1, TB3, TY3	Yellow	Glassy	Round	Cocci in clusters	*Staphylococcus sp*
TB2, TY2, TY1, TB3	Translucent	Slimy	Raised growth	Short rods	*Klebsiella sp*
TY & TB				Rod	*Bacillus sp*

Keys: TY = Tigernut Yellow, TB = Tigernut Brown

TABLE 2b BIOCHEMICAL CHARACTERISTICS OF BACTERIA ISOLATES FROM READY TO DRINK TIGERNUTS

	BIOCHEMICAL TEST								SUGAR FERMENTATION TEST						
S/N	Gram stain	Catalase	Coagulase	Citrate	Indole	Methyl red	Motility	Oxidase	Glucose	Fructose	Lactose	Sucrose	Mannitol	Sorbitol	Suspected organism
1	+	+	+	+	-	+	-	+	A	A	A	A	A	A	*Staphylococcus sp*
2	-	+	-	+	-	-	+	-	A/G	A/G	A/G	A/G	A/G		*Klebsiella*
3	+	+	+	+	-		-	-	A	A	A	A	A	A	*Bacillus sp*

Key: - = Negative, + =Positive, A/G = Acid and Gas production, A = Acid production.

TABLE 3: SHOWS THE MICROSCOPIC AND MORPHOLOGICAL CHARACTERISTICS OF FUNGAL COLONY

Sample	Microscopic characteristics	Morphological characteristics	Suspected organism
TB 2 & TB3	Upright conidiophores with striped smooth wall. Conidia are one celled vesicle septate and collumella	White base with black conidiophores which make it appear black in colour	*Aspergillus spp*
TY & TB	Wooly white colony with orange spots, rapidly filling the plate and Produces spores, rhizoid and umbonate	Non septate hyphae, sporran- giospores are ovoid in shape and are directly opposite the branched Rhizoid	*Rhizopus sp*
TY, TB2, TB3	Dense white budding colony separated by a brown line	Non septate hyphae with large sporangia heads having numerous sporangiospores	*Saccharomyces sp*
TB1 & TY3	Septate conidiophores and branched to give rise to flask shaped sterigmata	Initially white, but later became blue green and powdery on aging	*Penicillium sp*

Keys: TY = Tigernut Yellow, TB = Tigernut Brown

The occurrence of bacterial showed that *Bacillus sp* (42.8%) was higher followed by *Klebsiella sp* (28.6%) and *Staphylococcus aureus* (28.6%). While fungi occurrence showed that *Rhizopus sp* (40%) was higher followed by *Sacharomyces sp* (33.3%), *Aspergillus sp* (13.4%) and *Penicillium sp* (13.3%) as showed in table 4 &5.

TABLE 4: DISTRIBUTION OF BACTERIA ISOLATED FROM READY TO DRINK TIGERNUTS

Isolates	**Samples**						**Frequency**	
	TY1	**TY2**	**TY 3**	**TB1**	**TB2**	**TB3**	**Occurrence**	**% Percentage**
Bacillus subtilis	+	+	+	+	+	+	6	42.8
Klebsiella sp	+	+	-	-	+	+	4	28.6
Staphylococcus aureus	-	+	+	+	-	+	4	28.6
Total	2	3	2	2	2	3	14	100

Keys: + Positive; - = Negative; TY = Tigernut Yellow, TB = Tigernut Brown

$$\textbf{Percentage} = \frac{\textbf{No of Occurrence}}{\textbf{Total Number}} \textbf{ x 100}$$

TABLE 5: DISRIBUTION OF FUNGI ISOLATED FROM READY TO DRINK TIGERNUTS

ISOLATES	Samples						Frequency	
	TY1	TY2	TY3	TB1	TB2	TB3	Occurrence	% Percentage
Penicillium sp	-	-	+	+	-	-	2	13.3
Aspergillus sp	-	-	-	-	+	+	2	13.4
Rhizopus sp	+	+	+	+	+	+	6	40
Saccharomyces spp	+	+	+	-	+	+	5	33.3
Total	2	2	3	2	3	3	15	100

Keys: + Positive; - = Negative ; TY = Tigernut Yellow, TB = Tigernut Brown

$$Percentage = \frac{No\ of\ Occurrence}{Total\ Number}\ x\ 100$$

DISCUSSION, CONCLUSION AND RECOMMENDATION

Discussion

A total of 7 different genera of microorganisms were isolated from the ready to drink tigernut samples as shown in (Table 5a & 5b). These included *Bacillus* spp, *Klebsiella sp* and *Staphylococcus aureus,* was isolated for bacteria while fungi isolates include *Saccharomyces* spp, *Penicillium* spp, *Aspergillus sp* and *Rhizopus* spp.

A wide variety of microorganisms, some of which can bring about the spoilage of the milk samples on prolonged and/ or unprotected storage *(e.g., Staphylococcus aureus, Bacillus* spp, *Penicillium spp,* and *Aspergillus flavus)* were isolated from the samples which could be due to the source of the raw materials (purchased from the

open market under conditions that allow the organisms in/on them to thrive). The exteriors of harvested grains, legumes, nuts and other food substrates retain some of the natural micro flora they had while growing on the field in addition to contamination from soil, insects, and other sources (Edema and Omemu, 2014) of the microorganisms but then, there was a possibility of re-contamination during packaging and handling. Microorganisms detected in samples were very significant to note that spore-forming bacteria *(Bacillus*) and moulds were found associated with the milk samples on storage. Bacteria re-contaminating pasteurized milk originate primarily from water and air in the filling equipment or immediate surroundings and can be resident for prolonged periods of time (Eneroth *et al.,* 2010).

Bacillus are spore-forming bacteria that are commonly found in soil, water (through soil-water contamination) and on vegetables (Edema and Omemu, 2014). The presence of these bacteria and moulds in food samples in this work may be unavoidable because the spores of some strains of these organisms are resistant to pasteurization temperature. Furthermore, the oxygen level during processing may be sufficiently low to permit the growth of microbes.

High bacterial and fungal counts may be attributed to the fact that the samples were held at temperatures lower than 46°C for more than 4 hours. Previous studies had revealed that viable counts on foods prepared in advance and kept at ambient temperatures (20 to 46°C) for a long period of time (4 hours or more) reached critical levels (Ali and Spencer, 2016; Mosupye and Von Holy, 2019).

Staphylococcus aureus is a common environmental bacterium and could have been introduced after processing through cross-contamination. *Staphylococcus aureus* is known to produce an enterotoxin of importance in food-borne illness. The processing utensils might have been kept on the open table, where they can be readily contaminated with food-poisoning organisms from raw food and from environment (Adrian, 2016).

Milk products are, however, easily perishable because contaminating bacteria may multiply rapidly and render it unfit for human consumption (Ukwuru *et al.,* 2011).

The level of contamination was not critical to the microbiological status of the refrigerated tiger-nut milk samples after production. Presence of yeasts in the tiger-nut milk was expected as yeasts are inherent in tiger-nuts, although the level of yeast cells (cfu/g) in the raw material was determined. The presence of these microbes may not be harmful to consumers as some of them assist in the enzymatic breakdown of food and some

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synthesize useful vitamins, but on prolonged storage these microbes can bring about the microbial spoilage of the beverage. Pelczar *et al.,* (2016) had earlier documented that *Bacillus cereus* is among the microorganisms responsible for the spoilage of tofu (a soymilk product).

Conclusion

The samples' microbiological state revealed that different types of fungi and bacteria have an impact on the tiger nut's quality. To sum up, the discovery of these bacteria in tiger-nut beverage suggests that despite its high nutritious content, the food item might also act as a medium for the growth and persistence of microbiological growth. The HACCP system (Hazard Analysis Critical Control Point), which is indicated on the flow chart of the processing of the natural tiger-nut beverages, may be used to control the presence of these various kinds of microorganisms.

Recommendation

To assess the shelf-life of tigernut drink, it will be imperative to ascertain the impact of storing the product in various materials under refrigeration and at room temperature on its microbiological condition. It is also advised against storing ready to drink tiger nuts at room temperature for a short period of time following processing. Then I suggest, the comparation the results of the microbiological analysis to established food safety standards to determine if the products meet acceptable microbiological standards for ready to drink beverages.

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