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Article

Assessment of Microbiological Safety and Organoleptic Properties of Tiger-nut *(Cyperus esculentus)* Beverage Processed locally and Sold in Uyo Metropolis of Akwa Ibom State, Nigeria

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Abstract: Tiger-nut beverage is a one of the widely consumed non- alcoholic beverages in Nigeria with no standardized method of preparation. A total of 15 ready-to-drink tiger-nut beverages were purchased from five (5) locations within Uyo metropolis these include Ibom State secretariat, Akpan Adem market, Etaha Itam market, Akpan Ekpo market (Uniuyo), and Ibom plaza. Each sample was collected randomly from three (3) different vendors within the same location and a control sample was prepared in the laboratory. Microbiological analysis of the samples revealed that, the heterotrophic Bacterial, Coliform, Salmonella/Shigella and Fungal loads of the samples ranged from $1.1x10^3$- $4.3x10^5$ CFU/ml, 0 - $3.3x10^3$ CFU/ml, 0 - $2.1x10^2$ CFU/ml and $1.0x10^2$ - $3.7x10^4$ CFU/ml respectively. A total of 9 bacterial species including *Citrobacter* sp., *Bacillus cereus, Micrococus Sp., Kiebsiella sp., Shigella sp., E.coli, Pseudomonas sp., Staphylococcus aureus and Lactobacillus sp.* and 6 fungal species including *Rhyzopus oryzae, Aspergillus niger, Penicillium sp, Saccharomyces cerevisae, Aspergillus paraciticus* and *Candida pseudotropicalis* which were associated with the samples. Of these, *Citrobacter* sp, *Pseudomonas* sp and *S. cerevisiae* were the most encountered 9 (56.25%) and was followed by *Klebsiella sp., Lactobacillus sp.*

and *A. paraciticus* 8(50%). Mean sensory results revealed that the sample from Akpan Ekpo Market University of Uyo competed favorably with the control (laboratory prepared) in terms of overall acceptability the tiger-nut beverage. Most of the microbial species associated with the samples are known etiologic agent of human diseases and posing a health threat to the consumers, thus adoption of proper sanitary and good manufacturing practices by the producers of tiger-nut beverage should be encouraged.

Keywords: Tiger-nut, Bacteria, Fungi, Contamination, Coliform, Public health

1. Introduction

Tiger-nut (*Cyperus esculentum*) is a perennial grass-like plant with spheroid tubers, pale yellow cream kernel surrounded by a fibrous sheath. It is also known as yellow nut sedge, earth or ground almonds, "souchet" in French, "ermandeln" in German and "chufa" in Spanish (TTSL, 2005). Grossman and Thomas (1998) reported that chufa came to Spain from Africa. Tiger-nut is found wild and cultivated in Africa, South America, Europe and Asia. Tiger-nuts grow in the wild, along rivers and are cultivated on a small scale by rural farmers mostly in the Northern States of Nigeria. It is locally called "aya" in Hausa; "aki awusa" in Igbo; "Imumu" in Yoruba and "isip isong" in Efik/Ibibio. Tiger-nuts are edible, sweet, nutty, flavoured tubers which contain protein, carbohydrate, sugars, and lots of oil and fiber (FAO, 1979). Grossman and Thomas (1998) showed that tiger-nuts have been cultivated for food and drink for men and planted for hogs for many years in Spain and that the lovely milky preparation is served in health Spars, Pubs, and Restaurants as a refreshing beverage (competing successfully with other soft drinks/beverages). Despite these potentials, tiger-nuts has been a neglected crop in Nigeria.

The search for lesser known and underutilized crops, many of which are potentially valuable as human and animal foods has been intensified to maintain a balance between population growth and agricultural productivity, particularly in the tropical and sub-tropical areas in the world (Odoemelan, 2003; Ntukidem *et al*., 2019). Tiger-nut was found to be a cosmopolitan perennial crop of the same genus as the papyrus plant. The drink is cheap as its raw materials and additives used in its production are easily and locally sourced. The packaging materials are also cheap and available. Furthermore, the methods of production are simple and cheap as no elaborate equipment and expertise are required. The preparation of this beverage has become a technology in many homes particularly in rural communities and more recently in the urban areas where more women have been engaged in its commercial production.

However, the high water content coupled with its crude methods of production and packaging may predispose tiger-nut beverage to microbial contamination and subsequent deterioration caused by

spoilage microorganisms (Abaejoh *et al.,* 2006). In terms of spoilage, consumption of these contaminated beverages may lead to food borne disease (Balewu and Adedunni, 2014)

In Uyo and other major cities across Nigeria, tiger-nut beverages are sold on the streets usually in pre-used pet bottles like Coca cola, Pepsi cola, Lacasera or bottle water containers etc. which may also serve as source of contamination. This poor manufacturing process, coupled with the lack of functional regulation and high consumption of the beverage has prompted the researchers to assess the microbiological safety and organoleptic properties of locally produced Tiger-nut beverage sold within Uyo, Akwa Ibom State.

2. Material and Methods

2.1. Study Area

Uyo metropolis is the capital city of Akwa Ibom state in the South region of Nigeria. The city is located on Latitude 5º2'20.2668"N and longitude 7º54'34.0920"E and bounded by some parts of the adjourning local Government areas including Itu, Uruan, Ibesikpo Asutan and Nsit Ibom. Uyo has a population of about 304,000, a land area of 95 square kilometers and an average altitude of 6.5 (AKSG, 2008). The city has experienced influx of people, high traffic congestion and intense commercial activities since its creation on the 23rd of September 1987. Being a state capital, the area is defined by large structures, institutions and a great inflow of people who migrate into it. The major water sources in the area are borehole and Pipe borne water supplied by the water corporation of the state.

2.2. Sample Collection

Samples of tiger-nut beverages were purchased randomly from vendors in populated areas in Uyo metropolis, namely; State secretariat, Akpan Adem market, Etaha Itam market, Akpan Ekpo market (University of Uyo), and Ibom plaza. Samples were purchased from three (3) different vendors at each of these locations. The samples were placed in iced-packed container and transported to the Postgraduate Microbiology Laboratory, University of Uyo, Uyo for examination.

2.3. Tiger-nut Beverage Extract

Tiger nuts were sorted out to remove broken, rotten, stones, pebbles, and other dirt materials before rinsing in water to remove adhering soils. One kilogram (1kg) of the fresh-sorted tiger-nuts was blended several times into slurry with water (6L) in a Q-link auto-clean blender. The slurry was pressed using muslin cloth to extract the beverage. The extract was pasteurized at 72^{O} C for 15 Sec. It was

homogenized using improvised equipment; Q-link auto-clean blender, bottled when hot and rapidly cooled (Udeozor and Awonorin, 2014; Ntukidem *et al*., 2019).

2.4. Methods of Analysis

2.4.1. Microbiological Analysis

2.4.1.1.Enumeration of microbial loads

10ml of each sample were subjected to tenfold serial dilution as described by Harrigan and McCane (1990). Precisely 1ml of the desired diluents (10^{-3} ml) was pipetted using micropipette from the appropriate dilutions and was plated out in duplicates using the pour plate technique (Cappuccino and Sherman, 2002). Preparation and sterilization of all microbiological culture media used were carried out according to their manufacturer's instruction. The total heterotrophic bacterial load, total fungal, coliform and Salmonella loads of the samples were determined using Nutrient Agar (Lab. Tech. India), Sabouraud dextrose agar (Lab. Tech. India), MacConkey agar (Oxiod, CM 115, India) and Salmonella/Shigella agar (Oxiod, UK) respectively. Bacterial culture plates were incubated at 37°C for 48 hours while fungal plates were incubated at room temperature (28 ± 2^{o}C) for 3-5 days. The emerging colonies were enumerated using the Quebec colony counter and recorded as colony forming unit per milliliter (CFU/ml).

2.4.1.2. Maintenance of Pure Microbial Isolates

The emerging colonies after the incubation period were discretely isolated and sub-cultured repeatedly on freshly prepared Nutrient agar for bacteria and Sabouraud Dextrose agar (SDA) for fungi to obtain pure isolates. The pure isolates were maintained on agar slants bottle and stored at 4°C for further use.

2.4.1.3. Characterization of Bacterial Isolates

The bacterial isolates were characterized based on their cultural and morphological characteristics as well as their responses to standard biochemical test as described by Cheesbrough (2006). Twenty four (24) hours old cultures of bacteria obtained were subjected to Gram's staining and several biochemical test such as Catalase test, Citrate Utilization test, Oxidase test, Motility test, Urease test, Starch hydrolysis, Methyl red and Vogues Proskauer test, Indole test, as well as sugar fermentation test as described in Bergey's manual of determinative bacteriology (Brenner *et al.,* 2005) for identification.

2.4.1.4. Characterization and Identification of Fungal Isolates

Yeast isolates were characterized on the basis of their morphological and biochemical characteristics as presented by (Barnett, *et al.,* 2005: Kurtzman and Fell 2006), while mold isolates were

characterized on the basis of their cultural attributes, and identified by consulting various taxonomic books and monographs available on various groups of fungi (Aneja, 2003).

2.4.2. Determination of pH

Digital pH Meter produced by HANNA Instruments Model STD218F was used in this study. The glass electrode was thoroughly wetted with distilled water. The pH meter was switched on and was standardized. This was done by connecting glass electrode to the pH meter and inserting the electrode into the buffer solution. About 20ml of the sample was poured into a 50ml beaker, the pH of the samples was tested by inserting the electrode into it. This was then allowed to stabilize and the readings were recorded.

2.4.3. Sensory Evaluation

Sensory evaluation was carried out on the tiger-nut beverages purchased from different vendors in Uyo metropolis, using a nine (9) point hedonic Scale for scoring; Where 1 = dislike extremely, 5 = neither like nor dislike, and 9 = like extremely (Ihekoronye and Ngoddy, 1985). The samples were presented to a twenty (20) semi-trained panelists of the Department of Food Science and Technology who were requested to assess the samples for Colour, Flavor, Taste, mouthfeel and General acceptability. All the samples were presented at the same time and the identities of the samples were not revealed to the panelists. Each panelist was provided with sufficient privacy to ensure that his/her result would be arrived at independently and without being influenced by other panelists. The panelists were asked to rinse their mouths with water after assessing each sample.

2.4.4. Statistical Analysis

Statistical Package for Social Science (SPSS, version 20) was used for the statistical analysis. The differences between samples in each parameter tested was done using One Way Analysis of Variance (ANOVA) and New Duncan's Multiple Range Test as a post-hoc test when the analysis of variance indicates significant difference in their means. A significant level of $P<0.05$ was used throughout the study.

3. Results and Discussion

3.1. Microbial Analysis

Microbial loads in tiger-nut beverages from the different locations in Uyo Metropolis are presented in Table 1. The analyses revealed that the samples had varying microbial loads. The mean heterotrophic bacterial load, coliform load and Salmonella count ranged from $1.1x10^3$- $4.3x10^5$ CFU/ml, 0 - $3.3x10^3$ CFU/ml and 0 - $2.1x10^2$ CFU/ml respectively while the fungal load ranged from $1.0x10^2$ -

$3.7x10^4$ CFU/ml. These bacterial loads obtained in this study were however slightly lower than range of values ($0.22x10^5$ to $14.4x10^5$ CFU/ml) reported by (Umar and Raubilu, 2014) and $3.0x10^3$-$2.2x10^7$CFU/ml reported by (Ogodo *et al.*, 2016). The low microbial counts observed in the control sample may be attributed to good sanitary conditions adopted during and after processing of the tiger-nut beverage. Of the entire tiger nut beverages purchased from the vendors, the sample from Akpan Ekpo market (University of Uyo) had the least microbial load followed by those from State Secretariat while samples from Ibom Plaza had the highest microbial load. The low microbial counts observed from samples collected from Akpan Ekpo Market, may be attributed to the fact that, it was a purchased from the university community with scientific and hygienic conscious people. The high microbial counts from the other vended samples may be attributed to environmental factors such as exposure of sample to soil, air and other contaminants, type of water used as well as personal hygiene of the processors/handlers (Kawo and Abdulmunin 2009).

Table 1: Microbiological Loads of Tiger-nut Milk sample sold Within Uyo Metropolis

Location	Sample Code	TBC CFU/ml	Average TBC CFU/ml	TFC CFU/ml	Average TFC CFU/ml	TCC CFU/ml	Average TCC CFU/ml	TSC CFU/ml	Average TSC CFU/ml	pH
State Secretariat	SS 1	$2.1x10^5$		$2.3x10^4$		$1.4x10^3$		$1.6x10^2$		2.93
	SS 2	$1.9x10^5$		$3.0x10^4$		$1.7x10^3$		$1.3x10^2$		3.12
	SS 3	$2.0x10^5$	$2x10^5$	$2.5x10^4$	$2.7x10^4$	$1.6x10^3$	$1.6x10^3$	$1.7x10^2$	$1.5x10^2$	2.91
Ibom Plaza	IP 1	$4.3x10^5$		$3.6x10^4$		$3.6x10^3$		$1.7x10^2$		3.94
	IP 2	$3.8x10^5$	$4.3x10^5$	$3.2x10^4$	$3.6x10^4$	$2.9x10^3$	$3.1x10^3$	$1.6x10^2$	$1.8x10^2$	3.82
	IP 3	$4.7x10^5$		$4.1x10^4$		$2.8x10^3$		$2.0x10^2$		4.22
Akpan Ekpo Market	AE1	$1.6x10^4$		$1.8x10^4$		$1.6x10^2$		$1.1x10^2$		2.57
	AE2	$1.1x10^4$	$1.2x10^4$	$1.7x10^4$	$1.6x10^4$	$1.5x10^2$	$1.6x10^2$	$1.0x10^2$	$1.0x10^2$	2.73
	AE3	$1.0x10^4$		$1.4x10^4$		$1.8x10^2$		$1.0x10^2$		2.43
Itam Market	IM1	$3.9x10^5$		$4.2x10^4$		$3.8x10^3$		$2.1x10^2$		4.21
	IM2	$4.6x10^5$	$4.1x10^5$	$3.6x10^4$	$3.7x10^4$	$3.2x10^3$	$3.3x10^3$	$2.3x10^2$	$2.1x10^2$	4.12
	IM3	$3.9x10^5$		$3.4x10^4$		$2.9x10^3$		$1.9x10^2$		3.90
Akpan Andem Market	AA1	$3.2x10^5$		$3.7x10^4$		$3.0x10^3$		$1.8x10^2$		4.21
	AA2	$2.9x10^5$	$3.1x10^5$	$4.1x10^4$	$3.7x10^4$	$3.4x10^3$	$3.2x10^3$	$2.1x10^2$	$2.1x10^2$	3.83
	AA3	$3.1x10^5$		$3.2x10^4$		$3.1x10^3$		$2.3x10^2$		3.91
Laboratory Sample	CTL	$1.1x10^3$	$1.1x10^3$	$1.0x10^2$	$1.0x10^2$	NG	NG	NG	NG	2.04

SS 1-3 = State Secretariat, IP 1-3 =Ibom Plaza, AE 1-3 = Akpan Ekpo Market Uniuyo, IM 1-3 = Itam Market, AA 1-3 = Akpan Andem Market, CTL = Laboratory Prepared Control, TBC= Total Bacterial Counts, TFC= Total Fungal Counts, TCC= Total Coliform Counts, TSC= Total Salmonella Counts

The biochemical characterization and identification of the isolates revealed the presence of eight bacterial isolates (Table 2) including *Citrobacter sp., Bacillus cereus, Micrococcus Sp., Kiebsiella sp., Shigella sp., E. coli, Lactobacillus sp., Pseudomonas* sp., *and Staphylococcus aureus* and six (6) fungal species (*Rhyzopus oryzae, Aspergillus niger, Penicillium sp., Saccharomyces cerevisae, Aspergillus paraciticus* and *Candida pseudotropicalis* (Table 3). Similar bacterial species have also been reported on Kunu-zaki Samples by (Edem *et al.,* 2017; Gyar *et al.,* 2014; Ofundje *et; al.,* 2016; Ayandele, 2015). Ibrahim *et al.,* (2016), who reported that *E. coli, Staphylococcus sp. Bacillus sp.* and *Kiebsiella* sp were associated with tiger-nut beverage prepared with tiger-nut tubers with additives such as coconut, date, cinnamon and ginger. Several other authors have reported the presence of *Staphylococcus, E. coli* and *Shigella* sp. (Ogodo *et al.,* 2016), *Staphylococcus aureus* and *E. coli* (Musa and Hamza, 2013) in comparative studies of locally prepared 'kunun aya' (Tiger-nut beverage) consumed by students of Kaduna State University, Kaduna State.

Table 2: Morphological and Biochemical Characteristics of the Bacterial Isolates Associated with the Samples

Cell shape	Gram reaction	Catalase test	Methyl red test	Motility test	Citrate test	Oxidase test	Spore test	Indole test	Urease test	Glucose	Lactose	Maltose	Manitol	Sucrose	Galactose	V P test	Coagolsase test	Probable Organisms
Rod	-	+	+	+	+	-	-	-	+	AO	AG	OO	OO	AO	AG	-	-	*Citrobacter sp*
Rod	+	-	+	-	-	-	-	-	-	AG	AO	AO	AG	OO	OO	-	-	*Shigella sp.*
Cocci	+	+	+	-	+	-	-	-	+	OO	OO	AG	AO	AO	OO	+	+	*Staphylococcus aureus*
Rod	-	+	+	-	-	-	+	+	-	AG	AG	AG	OO	AG	AO	-	-	*E coli*
Rod	-	+	-	-	+	+	+	-	-	AO	AG	OO	AG	AG	AG	-	-	*Pseudomonas sp*
Rod	+	+	-	+	+	-	+	-	-	AO	AG	AO	AO	AO	OO	+	-	*Bacillus cereus*
Cocci	+	-	-	-	+	-	+	+	-	OO	OO	AO	OO	OO	AO	+	+	*Micrococcus sp*
Rod	-	+	-	-	+	-	-	-	-	OO	AG	AO	OO	OO	AO	+	-	*Kiebsiella sp.*
Rod	+	+	-	-	+	-	-	-	-	AG	OO	AO	OO	AO	AO	+	+	*Lactobacillus sp.*

Key: + = Positive reaction, - = Negative reaction, AG = Acid and Gas Production, AO = Acid and no gas production, OO = No acid production and no acid formation

The occurrence and distribution of the microbial isolates in the samples as presented in Table 4 revealed that, sample IM1 (from Itam market) had the highest microbial diversity by harboring 9 (64.3%) of microbial species associated with the samples. This was followed by IP3 (Ibom Plaza 3) and AA 2 (Akpan Andem market 2), which harbored 8 (57.1%) microbial species. The control samples had the least microbial diversity with only 4 (28.6%) of the microbial species being detected. Of the fifteen (15)

microbial species associated with the samples, *Citrobacter* sp., *Pseudomonas* sp. and *S. cerevisiae* were the most encountered by being present in 9 (56.25%) of the samples. This was followed by *Klebsiella sp.* and *A. paraciticus and Lactobacillus sp.* with 8 (50%) respectively.

Table 3: Cultural and Morphological Characteristics of Fungi Isolates from Tiger-nut beverages

Cultural characteristics	**Morphological Characteristics**	**Identification/Probable Organisms**
White-cotton like fluffy mass mycelium	Umbrella-like columnella, coenocytic sporagiophores, non-septate hyphae	*Rhyzopus oryzae*
Dark brown mycelium	Dark brown conidia, long conidiospore, covered glucose vesicles, biseriate Rhialides borne on brown metulae	*Aspergillus niger*
Blue green mycelium	Filamentous, blue green mycelium, cylindrical shape, broomlike appearance, globose conidia, dene conidiospores, elliposoidal, smooth hyaline	*Penicillium sp.*
White-cream, yeast-like mycelium	White-cream smooth glabrous colonies, blastoconidia, large globose, ellipsoidal budding	*Saccharomyces cerevisiae*
Large light green colony with milky background	Filamentous, erect fort cell, globose conidia, to sub-globose, metulae phialides	*Aspergillus paraciticus*
Creamy white mycelium smooth unextensive colony	Pseudo-hyphae, creamy white mycelium, septate well developed pseudo-mycelium, blasioconidia chlomydospores	*Candida pseudotropicalis*

Table 4: Distribution of the Microbial isolates in the samples

Samples	SS1	SS2	SS3	IP1	IP2	IP3	AE1	AE2	AE3	IM1	IM2	IM3	AA1	AA2	AA3	CTL	Distribution (%)
Citrobacter sp.	+	+	-	+	+	-	-	-	+	+	-	+	+	-	+	-	9 (56.25)
Shigella sp.	-	-	+	+	+	-	+	-	-	-	+	-	-	+	-	-	6 (37.5)
Staphylococcus aureus	-	+	-	-	-	+	-	+	-	+	-	+	+	-	+	-	7 (43.75)
E.coli	-	-	+	-	+	+	+	-	-	-	+	-	-	+	-	-	6 (37.5)
Pseudomonas sp.	+	+	-	+	-	+	-	-	+	+	-	+	-	+	-	+	9 (56.25)
Bacillus cereus	-	-	-	-	+	-	+	+	-	+	+	-	+	-	-	+	7 (43.75)
Micrococcus sp.	+	-	-	+	-	+	-	-	-	-	+	-	-	-	+	-	5 (31.25)
Klebsiella sp.	-	-	+	-	-	+	-	+	-	+	-	+	+	+	+	-	8 (50)
Lactobacillus sp.	-	+	+	-	+	-	+	+	-	-	-	+	-	+	+	-	8 (50)
Rhyzopus oryzae	-	-	+	+	+	-	-	-	-	+	+	+	-	+	-	-	7 (43.75)
Aspergillus niger	-	-	+	-	-	+	+	-	-	-	+	-	+	-	+	-	6 (37.5)
Penicillium sp.	+	-	-	-	+	-	-	+	-	+	-	-	+	+	-	-	6 (37.5)
Saccharomyces cerevisiae	+	+	-	+	-	+	+	-	+	-	-	+	-	-	+	+	9 (56.25)
Aspergillus paraciticus	-	+	-	-	-	+	-	-	-	+	+	-	+	+	+	+	8 (50)
Canida pseudotropicalis	-	+	-	-	+	-	-	-	-	+	-	+	-	+	-	-	5 (31.25)
Occurrence (%)	5 (35.7)	7 (50)	6 (42.9)	6 (42.9)	8 (57.1)	8 (57.1)	6 (42.9)	5 (35.7)	3 (21.4)	9 (64.3)	7 (50)	8 (57.1)	7 (50)	9 (64.3)	8 (57.1)	4 (28.6)	

+ = Present, - = Absent, SS 1-3 = State Secretariat, IP 1-3 =Ibom Plaza, AE 1-3 = Akpan Ekpo Market Uniuyo, IM 1-3 = Itam Market, AA 1-3 = Akpan Andem Market, CTL = Laboratory Prepared control

The result revealed that the tiger-nut beverage samples were not only contaminated with spoilage microorganism but also with known pathogenic bacterial species. The presence of *E. coli, Staphylococcus* and *Shigella sp.* in the beverage is of public health concern as they are considered as the leading cause of food-borne toxicosis outbreak worldwide (Karagozlu *et al.,* 2007). The occurrence of *Citrobacter sp.* and *Shigella sp.* in tiger-nut beverage has been reported by other researchers and are considered to be detrimental to the health of consumers (Ogodo *et al.,* 2018). *E. coli* in the tiger-nut beverage sample is an indication of faecal and environmental contaminations and has been reported to case gastroenteritis, diarrhea and urinary tract infection if ingested by humans. *Bacillus species* and *S. aureus* are common contaminants of food especially from food handlers, environment and post processing contaminations (Ogodo *et al.,* 2016).

Moreover *S. aureus* is a normal flora of the skin, nose, mucus membrane etc. and is implicated as the causative agent of septic arthritis (Taiwo *et al.,* 2017). *Pseudomonas species* has been implicated in the spoilage of beverage and food (Mbachu *et al.,* 2014). According to Adesaiyun *et al.,* (1983) the presence of *Bacillus cereus, Staphylococcus* and *E.coli* in beverages such as *kunun-aya,* can render them unsuitable for human consumption as well as serve as a medium of disease transmission. Contamination of the commercial tiger-nut milk samples with these organisms could have occurred from water, equipment during processing and storage or through the handlers or from the environment as a result of poor sanitation and hygiene. Also, these organisms when present in food cause spoilage and this could account for the short-shelf life of the tiger-nut beverage (Bolarinwa *et al.,* 2009).

Saccharomyces cerevisiae has equally been implicated in food spoilage due to its fermentative ability, osmophilic nature, tolerance of acid, tolerance of alcohol and ability to grow at low temperature (Badaua *et al.,* 2006). The presence of *Saccharomyces cerevisae* and *Rhyzopus oryzae* in tiger-nut soymilk drink has also reported by Awonorin (2014). *Saccharomyces cerevisiae* are harmless organisms, they have an extensive history of use in the area of food processing especially in bread making and as a fermenter of alcoholic beverages (Battock and Azam-Ali, 1998). On the other hand, *Rhyzopus oryzae* is the most common etiologic agent of mucomycosis, also referred to as *Zygomycosis* (Julie *et al.,* 2000).

A. niger and *A. paraciticus* are known to produce aflatoxin, ochratoxins which are carcinogenic and are capable of causing kidney and liver disorders, invasive and non-invasive aspergillosis, allergic and sinusitis (Benneth and Klich, 2003; Sumson *et al.,* 2014). *Penicillium* species produce citromycetin which is known to cause allergy asthma and some respiratory problems. *Candida species* causes *Candidiasis* and oval thrust.

The presence of microorganism especially in higher thresholds in the commercial samples can be traced to improper hygienic conditions in terms of personal hygiene, handling storage, packaging (with pre-used empty plastic containers), vending/dispensing conditions as good hygiene practices were not implemented by vendors/hawkers (Ukapbi and Ukenye 2015; Nyarko *et al.,* 2011).

3.2. Sensory Evaluation

Appearance is a visual perceptual property corresponding in humans to the categories called red, blue, brown, green and others (Berlin and Kay, 1969). It is derived from the spectrum of light interacting in the eye with the spectral sensitivities of the light receptors (Fairchild, 2005). Result of this study indicated that, there was no significant difference ($P<0.05$) in the appearance between samples SS, AE, IM, AA and the control, but there was a significant difference ($P<0.05$) in sample IP. The appearance of the control was closely followed by that of AE. The appearance of SS, IM and AA were fairly liked while that of IP was least preferred of all.

Taste as a sensory attribute defined as a sense that distinguishes the sweet, sour, salty, and bitter qualities of dissolved substances in contact with the taste buds on the tongue. Results presented in Table 5 indicate that, there was no significant ($P<0.05$) difference between Samples SS, IP and IM but there was a significant ($P<0.05$) difference between samples AE, AA and the control sample. The taste preference for control sample was the highest followed by samples AE and AA. Samples SS, IP, IM were the least preferred of all the samples. The control sample was most preferred by the panelist in term of taste this may be due to the addition of ginger and date palm used as a sweetener, which enhanced its taste of the beverage.

Flavor is a sensation caused by properties of any substance taken into the mouth, which stimulates one or both of the senses of taste and smell and/or also the general, pain, tactical and temperature receptors in the mouth. Results of this study indicated that, there was no significant ($P<0.05$) difference in the flavor between samples SS, IP, AE, and IM, but there was a significant ($P<0.05$) difference in the flavor between samples SS, IP, AE, IM with samples AA and the control. Results further indicated that, the flavors for samples AA and CTL (control) were the most preferred followed by that of AE, whereas sample IP was the least preferred.

Mouthfeel is another sensory property that is important in food processing and evaluation. There was no significant ($P<0.05$) difference between samples SS, IP, AE and IM but there was a significant ($P<0.05$) difference with samples AA and the control when compared with others. In terms of after taste all the samples showed no significant ($P<0.05$) difference.

All the parameters tested were very important in that, they stimulate the consumer's likeness and acceptance for the tiger-nut beverage. Indeed, even if a product is appealing and meets nutrients requirements without good product taste, the product is likely not to be acceptable. From the results obtained, laboratory prepared (control) sample was most preferred by the panelists followed by sample obtained from Akpan Ekpo market University of Uyo.

Table 5: Sensory evaluation of tiger-nut milk from different vendors in Uyo

Locations	Appearance	Taste	Flavour	Mouth-feel	Aftertaste	General Acceptability
SS	6.9±0.02^{e}	6.5±0.02^{e}	6.7±0.01^{d}	6.4±0.01^{d}	6.4±0.01^{cb}	6.8±0.20^{b}
IP	6.2±0.01^{e}	6.7±0.01^{d}	6.5±0.01^{e}	6.3±0.01^{e}	6.5±0.01^{b}	6.7±0.20^{b}
AE	7.1±0.01^{b}	7.0±0.01^{b}	6.9±0.01^{c}	6.5±0.01^{c}	6.8±0.01^{a}	7.0±1.00^{ab}
IM	6.7±0.01^{e}	6.9±0.01^{c}	6.7±0.01^{d}	6.4±0.01^{d}	6.2±0.01^{c}	6.8±0.01^{b}
AA	6.8±0.01^{d}	7.0±0.01^{b}	7.1±0.01^{b}	7.1±0.01^{b}	6.3±0.20^{b}	6.8±0.01^{b}
CTL	7.5±0.01^{d}	8.1±0.01^{a}	7.5±0.01^{a}	7.5±0.01^{a}	6.5±0.26^{b}	7.7±0.20^{a}

Key:
SS = State Secretariat, IP =Ibom Plaza, AE = Akpan Ekpo Market Uniuyo, IM = Itam Market, AA = Akpan Andem Market, CTL = Laboratory Prepared control

4. Conclusion and Recommendation

The result obtained in this study has revealed high microbial loads and sensory properties among the locally prepared tiger-nut beverage sold within Uyo metropolis. The presence of known etiologic agents of human infections in the samples is a pointer to the health risk associated with the consumption of poorly prepared or contaminated tiger-nut beverage. The taste, flavor and mouth-feel of the samples varied. Based on the result of this study, adequate campaign should be organized for the major stakeholders (vendors/hawkers/processors and the consumers) of ready-to-eat foods such as tiger-nut beverage on good manufacturing practices as well as the health risk associated with consumption of microbes contaminated foods.

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