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(RESEARCH ARTICLE)

Assessment of the microbiological quality of tiger nut (*Cyperus esculentus*) drink sold in Ignatius Ajuru University of Education, Port Harcourt, Nigeria.

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Abstract

This research work assessed the microbiological quality of tiger nut juice (*Cyperus esculentus L.*) sold at Ignatius Ajuru University of Education, Port Harcourt. A total of six samples were purchased from different vendors selling in the University main campus. Microbiological analysis was done using nutrient agar, MacConkey agar and Potato dextrose agar media to culture, enumerate and isolate organisms from the samples. The total aerobic heterotrophic bacterial count, coliform count, and fungal count, were estimated by the pour plate techniques. The results revealed that total heterotrophic bacterial count ranged 1.11×10^5 to 2.40×10^5 cfu/ml, while the total coliform count ranged from 1.54×10^5 to 3.10×10^5 cfu/ml. The total staphylococcus and yeast count ranged from 1.44×10^5 to 2.61×10^5 cfu/ml. The high coliform count and the isolation of pathogenic bacteria in some samples indicate that the Tiger nut drink was contaminated and a potential health hazard to the unsuspecting consumers. The producers and vendors of tiger nut drink should be enlightened on the consequences of poor hygiene and the need to adopt good manufacturing practices in the preparation and storage of the drink to prevent the outbreak of epidemics.

Keywords: Tiger nut juice; Microbial quality; Deterioration; Consumers health.

1. Introduction

Tiger nut *(Cyperus esculentus L.*) grows freely as a tuber and is largely eaten in Nigeria and in various parts of West and East Africa. It is one of the best nutritional crops used to supplement diets with its high iron and calcium contents for growth and deve1opmcnt of the body (1) and FAO (2) reported that tiger nut tubers are rich in starch (20.30% of DW) and fat (20-28% DW) with small protein content which is about twice the quantity in cassava. Chandrasekara and kumar (3) states that tiger nuts could serve as an alternative to cassava in the baking industry due to the nutritional and therapeutic composition.

In Port Harcourt and many other parts of Nigeria, tiger nuts are sold on the streets by local women who carry it on open basins and exposed wheelbarrows. In Northern Nigeria, tiger nut drink is usually called “Kunun aya”. It is one of the indigenous, locally fermented, non-alcoholic beverage drinks that is consumed to quench thirst and for its nutritive value. It is eaten throughout the year, especially during the dry season and more among the male folk due to its believed aphrodisiac property. The children also enjoy the juice due to its sweet nutty flavor (4). The shelf life of tiger nut is short, sometime less than 24 hours and depending on the storage conditions. The short shelf life of raw tiger nut milk hinders widespread consumption of the beverage due to the deteriorating effects of some microorganisms in the milk (5).

According to Ayeh-Kumi (6), the various microbial contaminants can be contracted before, during or after harvest. Some of the different microbial species identified with tiger nuts include *Aspergillus flavus, Aspergilius* niger,

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Sacchromyces cerevisiae., Saccharomyes fubiligera Fuscirium solani, Candida pseudotropicalis Bacillus subtilis, and *Staphylococcus aureus.* The various microorganisms that find their way into foods are either introduced from the soil were they are grown, or during harvest, packaging, storage and handling (7). The occurrence of microorganism in high numbers in the tiger nut drinks could be traced to poor personal hygiene, handling, storage, vending and dispensing conditions by the hawkers (7,8). Also, the use of bare hands, rusted cups, contaminated water during washing and regular sprinkling to wet the nuts surfaces for freshness could be responsible for the microbial contamination.

The growing interest and increase in the production and consumption of tiger nut drink in Port Harcourt, Nigeria has made it a great concern to examine its microbial quality. It has therefore become necessary to ensure the safety of tiger nut drink because of the health of its numerous consumers.

2. Material and methods

2.1. Study Area

This study was carried out in the main campus of the Ignatius Ajuru University of Education, Rumuolumeni, in Obio/Akpor Local Government Area of Rivers State.

2.2. Sample Collection and Treatment

Prepared ready-to-drink samples of tiger nut juice was procured from three different sellers at three different positions within the University community. Each set of sample collected from three different vendours was kept in a sterile nylon container, labeled A, B, and C, respectively and immediately taken to the laboratory for analysis. The flow chart of the traditional method of preparation of tiger nut juice is shown in figure 1 below.

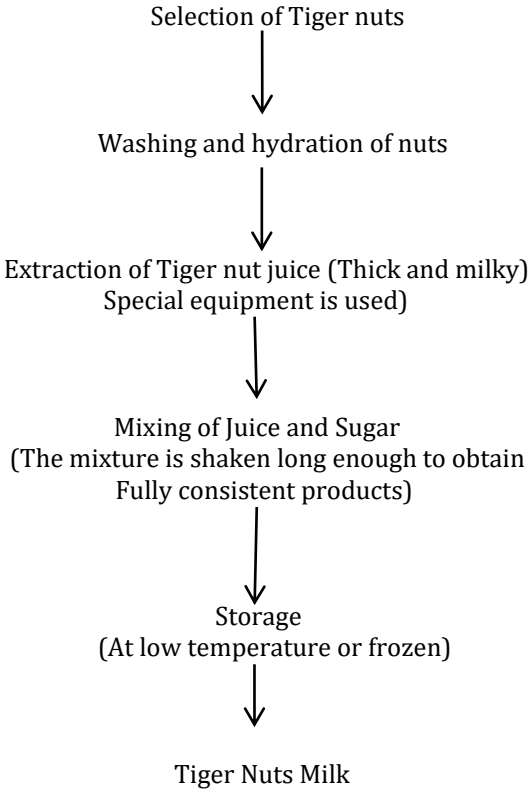

Figure 1 Flow chart of preparation of tiger nut juice

2.3. Microbiological Analysis

Each set of samples for the serial dilution were prepared by introducing 1ml of tiger nut juice into a test tube containing 9ml of sterilized distilled water to form a stock solution. The test tubes were labeled 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and $l0^{-5}$ for sample A, B and C respectively. Using a pipette, 1ml of the tiger nut juice (A, B, C) were introduced into the first test tube 10^{-1} for each set of sample, and dilution was continued until the required serial dilution was made as

described by (9). The total microbial count was determined by using the pour plate method. Dilution series of each were prepared from the liquid sample homogenate. 0.1ml aliquot of 10^{-4} and $l0^{-5}$ dilution of sample A, B and C was inoculated into the prepared agar plates and incubated at 37°C for 24 hours. Microbial growth was counted in the plates after 24 and 48 hours incubation period. Identification and characterization of the bacteria isolates was done their staining reactions and biochemical tests.

3. Results

The colony count of bacterial growth the tiger nut drink is presented in table 1 below. The total heterotrophic bacteria count ranged from 1.11 X105 to 2.40 X105 cfu/ml. The total coliform count ranged from 1.54 x 105 to 3.1 x 105 cfu/ml, while total staphylococcal count ranged from 1.41 x 104 to 2.11 x 104 cfu/ml. For mould and yeast, the total count ranged from 1.54 x 105 to 2.11 x 105 cfu/ml and 1.42x 104 to 2.61 x 104 cfu/ml respectively.

Table 1 Microbial count of tiger nut juice from sample A, B and C

Samples	colony count (cfu/ml)				
	THBC	TCC	TSC	TMC	TYC
A1	1.76 $X10^5$	3.00 $X10^5$	1.86 $X10^5$	2.23 $X10^5$	2.00 $X10^5$
A2	1.6 $X10^5$	1.56 $X10^5$	2.10 $X10^5$	1.56 $X10^5$	1.43 $X10^5$
B1	1.11 $X10^5$	2.20 $X10^5$	1.86 $X10^5$	1.80 $X10^5$	1.96 $X10^5$
B2	1.80 $X10^5$	1.83 $X10^5$	1.90 $X10^5$	1.63$X10^5$	2.60 $X10^5$
C1	2.40 $X10^5$	2.73 $X10^5$	1.80 $X10^5$	1.73 $X10^5$	1.56 $X10^5$
C2	2.32 $X10^5$	1.80 $X10^5$	1.43 $X10^5$	1.73 $X10^5$	1.83 $X10^5$

Note: THBC: Total heterotrophic bacterial Count;: TCC: Total coliform count; TSC; Total staphylococcal count; TMC: Total mould count; TYC: Total yeast count.

The percentage occurrence of the different organisms isolated from samples of tiger nut drink is shown in table 2. The percentage of *Escheriachia coli* 50.0%, *Staphylococcus aureus* was 30.5%, *Pseudomonas spp* 30.0%, *Salmonella spp* 22.05%, *Shigella spp* 30.0%, *Bacillus subtilis* 15.5%, *Klebsiella spp* 32.0%, *Streptococcus fecalis* 8.0%, *Candida albicans* 5.0%, *Fusarium Solani* 10.0%, *Aspergillus niger* 22.5%, *Aspergillus flavus* 7.5%, *Rhizopus oryzae* 20.0% *Saccharomyces cerevisiae* 31.5%.

Table 2 Prevalence of microorganisms in tiger nut juice from different samples

ISOLATES	Samples			
	Sample A	Sample B	Sample C	% occurrence
Staphylococcus aureus	+	+	+	30.5%
Salmonella spp	+	+	-	22.5%
Escherichia coli	+	+	+	50.0%
Shigella spp	+	+	+	30.0%
Pseudomonas spp	+	+	-	30.0%
Klebsiella spp	+	-	-	32.0%
Bacillus subtilis	+	+	-	15.5%
Streptococcus fecalis	-	-	+	8.5%
Candida albicans	+	-	-	5.5%
Aspergillus niger	+	+	-	22.5%
Asergillus flavus	-	-	+	7.5%
Fusarium solani	+	-	+	10.0%
saccharomyces cerevisiae	+	+	+	31.5%
Rhizopus oryzae	-	-	+	20.0%

Table 3 Characterization and identification of bacteria isolates in tiger nut juice from sample A

Isolates	Grams stain	Spore test	Motility test	Coagulase test	Catalase test	Oxidase test	Citrate test	Urease test	Indole test	Methy red	Voges proskauer	Starch hydrolysis	Hyrdogen sulphide	Glucose	Sucrose	Lactose	ORGANISMS IDENTIFIED
1	-	-	-	-	+	-	-	-	+	+	-	-	-	AG	A	A	*Escherichia coli*
2	-	-	-	-	+	-	-	-	-	+	+	-	-	AG	A	A	*Klebsiella spp.*
3	-	-	-	+	+	-	+	-	-	-	+	-	+	AG	A	A	*Staphylococcus aureus*
4	-	-	+	-	+	+	+	-	-	-	-	-	-	-	AG	AG	*Pseudomonas spp.*
5	-	-	+	-	+	-	+	-	-	+	-	-	+	AG	AG	AG	*Salmonella spp.*
6	-	-	-	-	+	-	-	-	-	+	-	-	-	A	A	-	*Shigella spp.*
7	+	+	-	-	+	-	+	-	-	-	+	+	-	A	A	A	*Bacillus spp.*

Table 4 Characterization and identification of bacteria isolates in tiger nut juice from sample B

Isolates	Grams stain	Spore test	Motility test	Coagulase test	Catalase test	Oxidase test	Citrate test	Urease test	Indole test	Methy red	Voges proskauer	Starch hydrolysis	Hyrdogen sulphide	Glucose	Sucrose	Lactose	ORGANISMS IDENTIFIED
1	-	-	-	-	+	-	-	-	+	+	-	-	-	AG	A	A	*Escherichia coli*
2	-	-	-	-	+	-	-	-	-	+	+	-	-	AG	A	A	*Klebsiella spp.*
3	-	-	-	+	+	-	+	-	-	-	+	-	+	AG	A	A	*Staphylococcus aureus*
4	-	-	+	-	+	+	+	-	-	-	-	-	-	-	AG	AG	*Pseudomonas spp.*
5	-	-	+	-	+	-	+	-	-	+	-	-	+	AG	AG	AG	*Salmonella spp.*
6	-	-	-	-	+	-	-	-	-	+	-	-	-	A	A	-	*Shigella spp.*
7	+	+	-	-	+	-	+	-	-	-	+	+	-	A	A	A	*Bacillus spp.*

Table 5 Characterization and identification of bacteria isolates in tiger nut juice from sample C

Isolates	Grams stain	Spore test	Motility test	Coagulase test	Catalase test	Oxidase test	Citrate test	Urease test	Indole test	Methy red	Voges proskauer	Starch hydrolysis	Hyrdogen sulphide	Glucose	Sucrose	Lactose	ORGANISMS IDENTIFIED
1	-	-	-	-	+	-	-	-	+	+	-	-	-	AG	A	A	*Escherichia coli*
2	-	-	-	-	+	-	-	-	-	+	+	-	-	AG	A	A	*Klebsiella spp.*
3	-	-	-	+	+	-	+	-	-	-	+	-	+	AG	A	A	*Staphylococcus aureus*
4	-	-	+	-	+	+	+	-	-	-	-	-	-	-	AG	AG	*Pseudomonas spp.*
5	-	-	+	-	+	-	+	-	-	+	-	-	+	AG	AG	AG	*Salmonella spp.*
6	-	-	-	-	+	-	-	-	-	+	-	-	-	A	A	-	*Shigella spp.*
7	+	+	-	-	+	-	+	-	-	-	+	+	-	A	A	A	*Bacillus spp.*

Table 6 Characteristics and identification of fungi in tiger nut juice sample A

Isolates No	Colony morphology	Mycelia/cell structure	Formation of spore	probable organism identified
1	Greenish mass with white edge, powdery and head structures	Non-septate	Conidiospore	Aspergillus flavus
2	Black mass, powdery with pin head structures	Non-septate	Conidiospore	Aspergillus niger
3	Pinkish-white cottony, fluffy colony	Septate	Conidiospore	Fusarium spp.
4	Whitish, pin head structures	Non-septate	Sporangiospere	Rhizopus spp.
5	White, circular with dome shape with entire edge	Budding and oval cells	Ascopore	Saharomyces spp.
6	Creamy-white, smooth colony	Budding cell with hyphae (Blastoconidia)		Candida spp.

Table 7 Characteristics and identification of fungi in tiger nut juice sample B

Isolates No	Colony morphology	Mycelia/cell structure	Formation of spore	probable organism identified
1	Greenish mass with white edge, powdery and head structures	Non-septate	Conidiospore	Aspergillus flavus
2	Black mass, powdery with pin head structures	Non-septate	Conidiospore	Aspergillus niger
3	Pinkish-white cottony, fluffy colony	Septate	Conidiospore	Fusarium spp.
4	Whitish, pin head structures	Non-septate	Sporangiospere	Rhizopus spp.
5	White, circular with dome shape with entire edge	Budding and oval cells	Ascopore	Saharomyces spp.
6	Creamy-white, smooth colony	Budding cell with hyphae (Blastoconidia)		Candida spp.

Table 8 Characteristics and identification of fungi in tiger nut juice from sample C

Isolates No	Morphology of colony	Mycelia/cell structure	Formation of spore	probable organism identified
1	Greenish mass with white edge, powdery and head structures	Non-septate	Conidiospore	Aspergillus flavus
2	Black mass, powdery with pin head structures	Non-septate	Conidiospore	Aspergillus niger
3	Pinkish-white cottony, fluffy colony	Septate	Conidiospore	Fusarium spp.
4	Whitish, pin head structures	Non-septate	Sporangiospere	Rhizopus spp.
5	White, circular with dome shape with entire edge	Budding and oval cells	Ascopore	Saharomyces spp.
6	Creamy-white, smooth colony	Budding cell with hyphae (Blastoconidia)		Candida spp.

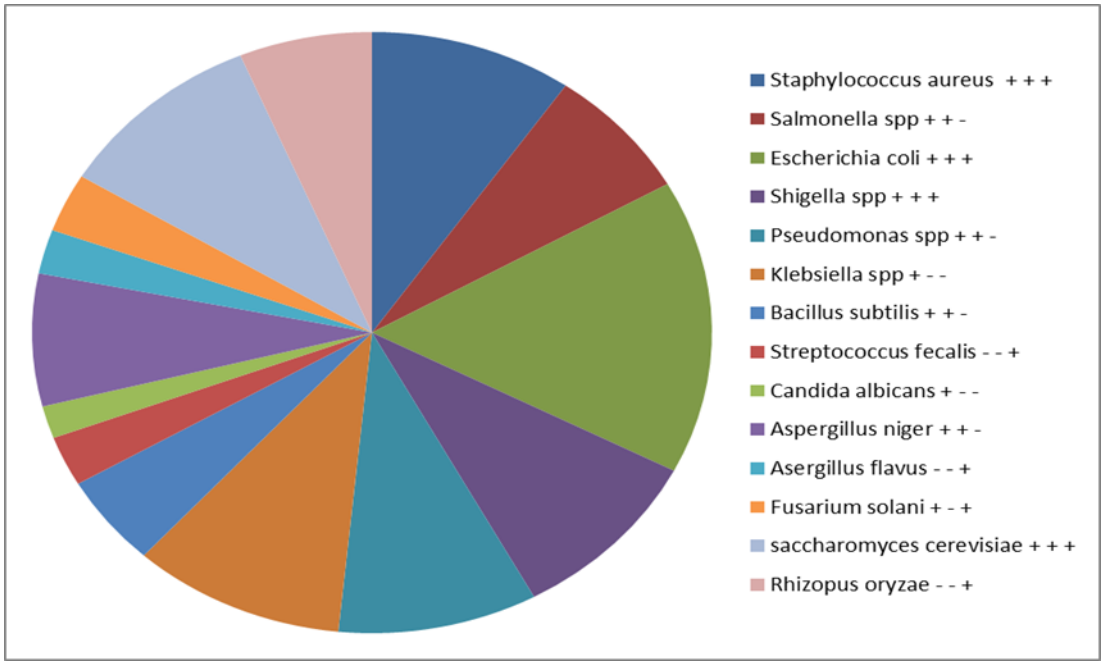

Figure 1 Percentage occurrence of microorganisms in samples of tiger nut juice.

4. Discussion

The local tiger nut fruit drink prepared and sold by vendors in the study area showed growth of different species of microorganisms. The major species of heterotrophic bacteria identified in all the samples include; *Staphylococcus aureus, Escherichia coli, Salmonella spp, Shigella spp, Bacillus subtilis, klebsiella spp, Streptococcus fecalis and Pseudomonas spp.* This is similar to the findings of Taiwo (10). The yeast and mould isolated from samples are *Candida albicans, Saccharomyces cerevisiae, Fusarium solani Aspergillus flavus, Aspergillus niger and Rhizopus oryzae*. Udeozor and Awonorin (11) also reported the identification of similar species of yeast and moulds in their investigation of the quality of tiger nut juice. The observed high microbial count recorded in this study agrees with the report of other workers (12, 13).

According to Okereke (14), the recommended official limit for microbial contamination of beverages or sorrel drinks requires the total absence of pathogenic bacteria such as; *Escherichia coli, Pseudomonas sp, Salmonella sp and Staphylococcus aureus.* Tropical climates encourages rapid growth of microorganisms in beverages and the growth of fungi may lead to the production of mycotoxins which can result in the development of mycotoxicosis in the affected individuals (15).

The proliferation of these microorganisms in high numbers in tiger nut juice may be linked to the poor hygienic environments in the processing of the drink with respect to personal hygiene, handling of products, utensils and water used during preparation, storage, vending and dispensing of the finished product. The effect of non-compliance by food producers, vendors or hawkers to the principles of good manufacturing practice in promoting the growth of undesirable microorganisms in local fruit drinks is well documented (7, 8). The usual practice of prolonged storage of the product at ambient temperature enhances the growth of microorganisms which may result in the deterioration or spoilage of the juice. .

The identification of fecal coliform bacteria in the drink is an indication of bacteria contamination from human or animal sources that may have been introduced during processing of the tiger nut. The presence of *E. coli* and other pathogenic bacteria in the drink portends great danger to the health of unsusceptible consumers such as infants, young students and adults with compromised immune systems.

5. Conclusion

Tiger nut drink has rich nutrient content making it nutritionally good for consumption. Analysis showed that all the three different samples contained high microbial load, which was probably due to poor handling of materials for

preparation of the juice and the use of unhygienic processing method. The most important pathogenic ones isolated are *Salmonella spp, Shigella spp, Staphylococcus aureus, Escherichia coli, Candida albicans. Saccharaomyces cerevisiae* and *Rhizopus oryzae.* These microorganisms are significant in public health because of the teaming number of people, especially students that rely on the drink as cheap alternative to the branded commercially bottled soft drinks.

Since most of the local producers and vendors lack good knowledge of food safety measures and the implications of their crude practices in terms of health hazards to the consumers there is need for continuous enlightenment programmes. The adoption of conventional processing and storage systems when fully embraced would help to reduce or eliminate the prevalence of pathogenic microorganisms in the processed fruit drink.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no conflict of interest.

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