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Nutritional and Microbial Quality of Tiger Nuts (*Cyperus Esculetus*) Milk (Kunun-Aya)

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Abstract

This study evaluates the Nutritional and Microbial Quality of Tiger nuts milk. The raw materials were tiger nuts, coconut, date, cinnamon and ginger.The Standard microbiological methods were used to isolate, characterize and identify microbial isolates. The bacterial count was 8.2x10^3 cfu/ml , the coliform count was 3.5 x10^4 cfu/ml while the fungal load was 9.2x10^5 cfu/ml. The organisms isolated and identified from the analysed tiger nuts milk were Staphyloccus aureus, Escherichia coli and Yeast spp respectively. The proximate analysis revealed ; ash (65.5%), moisture (70.3%), crude fibre (9.5%), fat (1.20%), protein (10.1%), carbohydrate (56.7%)and the pH value was 3.5. The tiger nuts milk were evaluated by panelist using a 9-point hedonic scale and statistical analysis carried out on their responses. The sensory scores; the Appearance was 4.35, Colour 4.24, Aroma 4.59, Taste 4.35, Mouth feel 4.35 and Acceptability 4.41. All the sensory attributes were like much and extremely liked by the panelist.

Keywords: Nutritional, Microbial, Quality, Tiger Nuts (*Cyperus Esculetus*), Milk (Kunun-Aya).

Introduction

The world's population coupled with high cost of animal protein has necessitated the search for underutilized cheap crops which can be easily processed and serve as a source of dietary protein and energy for human. Tiger nuts milk is a refreshing high nutritive, energy drink produced mainly from tiger nut. Sometimes, fruits such as coconut,

ginger, cloves, and dates are added to impart flavour into the milk. Tiger-nut (*Cyperus esculentus*) belonging to the family, *Cyperaceae* is a good source of energy, fat, starch, fibre, glucose and protein. Is also rich in vitamins, minerals and some digestive enzymes such as lipase and amylase Rowland *et. al*, (2017). Tiger nut (*Cyperus esculentus*) belongs to the Division–Magnoliophyta, Class–Liliopsida, Order– cyperales and Family–Cyperaceae, Species- *Cyperus esculentus* and was found to be a cosmopolitan, perennial crop of the same genus as the papyrus plant Ibrahim *et. al*,(2016). The plant was introduced by the Arabs, first in the Valencia region. It is native to most of the Western Hemisphere as well as Southern Europe, Africa, Madagascar, the Middle East and the Indian Subcontinent (Gambo and Da'u, 2014). The tuber is known by various names in Nigeria, as "Aya" in Hausa, "Imumu" in Yoruba and "Aki Hausa" in Igbo. Tiger nuts can be eaten raw, roasted, dried, or baked Ibrahim *et. al*,(2016). It can also be used for preparation of "kunu aya" (a local beverage in Nigeria) (Musa and Hamza, 2013). Tiger nut milk or "kunu aya" is mostly consumed in the afternoon to cool the body from the hot weather, it is cheap and popular; available, affordable, drink of both the poor and the rich Ibrahim *et. al,* (2016).

The milk is underutilized due to its short shelf life and lack of information on its nutritional potentials Ibrahim *et. al*,(2016). 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 (Gambo and Da'u,2014). Recently, there is awareness for increased utilization of tiger nut (Gambo and Da'u, 2014).Tiger-nut is also an excellent source of some useful minerals such as iron and calcium which are essential for body growth and development Ibrahim *et. al,* (2016).They also contain other mineral elements such as phosphorus, potassium sodium, magnesium, zinc and traces of copper and vitamins E and C (Musa and Hamza, 2013). It is believed that they help to prevent heart attacks, thrombosis and cancers, especially of the colon (Musa and Hamza, 2013). They are thought to be beneficial to diabetic patients (if sugar-free) and those seeking to reduce cholesterol or lose weight (Musa and Hamza, 2013).

Spices have been used for thousands of centuries by many cultures to enhance the flavor, taste and aroma of foods. Early cultures also recognized the value of using spices in preserving foods and for their medicinal value Ene-Obong *et. al*,

(2015). The beneficial health effects of spices have been well documented Rowland *et. al*,(2017). Many spices have been reported to have antimicrobial properties, cholesterol lowering effects, anti-diabetic and anti inflammatory properties Rowland *et. al*, (2017). Spices such as garlic, ginger and pepper are good sources of nutrients, minerals and photochemical and could therefore serve as nutritional supplements Rowland *et. al*, (2017). Recently, milk sources from plants such as tiger-nut are gaining strong interest from the researchers as well as increasing acceptability from consumers not only because they provide refreshment but also because of their nutritional and medicinal values. However, tiger-nut milk (kunun-aya) must be consumed within 2-4 hours at 40°C–100°C due to its poor shelf life Rowland *et.al*,(2017). Some researchers have reported the ability of pasteurization and addition of citric acid, ginger and garlic to extend the shelf-life of tiger-nut milk by minimum of 2-3 days Nwobosi *et. al*, (2013). The shelf-life of food products is an integral part of food safety. Several factors encourage, prevent or limit the growth of microorganisms in foods; the most important are water availability, pH, and temperature (Okorie *et. al*,2014) Microbial quality of beverage drink, dairy products and other food is determined by significant total microbial count and presence of pathogenic microorganisms.

Microbial quality determination is completely used to reflect hygienic practice in food production. Milk analogue can serve as a good medium for the growth of many microorganisms especially bacterial pathogens; therefore, its quality control is considered essential to the health and welfare of a community. As reported by Okorie *et. al*,(2014), the threat posed by diseases spread through contaminated food is well known and the epidemiological impact of such diseases is considerable. The presence of these pathogenic microorganisms in beverage milk drink developed from under-utilized crops has emerged as a major public health concern especially for consumers. Bacterial contamination of milk analogue can originate from different sources: bad water, air, preparation equipment, unhygienic of the handler, poor post-pasteurization handling such as bottling and storage systems, among others.

METHODOLOGY

Sample Collection

Big yellow tiger nut (the most commonly used for preparation of tiger nut milk) was obtained from Muda lawal Market in Bauchi, Bauchi State, Nigeria. The

nuts were taken to the laboratory in a clean polythene bag for processing and analysis.

Three varieties (black, brown and yellow) are cultivated in the country and among these, only two varieties; (yellow and brown) are readily available in the markets. The yellow variety is preferred to all other varieties because of its inherent properties like its bigger size and attractive colour. The yellow variety also yields more milk upon extraction, contains lower fat and more protein and possesses less anti-nutritional factors especially polyphenols (Okafor and Nwachukwu, 2013).

Samples Preparation

Tiger nuts were sorted out to remove broken, rotten, stones, pebbles, and other dirt materials before rinsing in water to remove adhering soils. Other ingredients used in the milk preparation (coconut, date, cinnamon and ginger) were also processed before use. The shell of the coconut was removed using knife and the water was discarded, the coconut flesh was cut into smaller pieces. The seed of the date was removed and discarded. These entire ingredients were thoroughly washed in warm water. (Ayo and Okaka, 2013)

Sensory evaluations

The samples were served at 40ºC to members of a taste panel consisting of 17 students of the Federal Polytechnic Bauchi. The preference test was conducted using a nine point Hedonic scale of 1= dislike extremely; 2= dislike much; 3= dislike moderately; 4= dislike slightly; 5= indifferent; 6= like slightly; 7= like moderately; 8= like much; 9= like extremely to determine relative preferences for the various sensory attributes of appearance, colour, aroma, taste, mouth feel and overall acceptability Bonsi *et. al*, (2014).

Statistical Analysis

The data obtained was statistically analyzed (analysis of variance) using Statistical Package for Social Science (SPSS version 20). Amankwah *et. al*, (2009)

Media Used

The media used in this work were; Nutrient agar, MacConkey agar and Sabouroud dextrose agar. All the media were product of Diagnostic Laboratory

Ltd, USA. They were prepared according to the manufacturer's instructions, sterilized by autoclaving at 121ºC for15minute.

Microbiological Analysis

Ten (10) ml of the tiger nuts milk sample was aseptically transferred into 90ml of sterile distilled water in a 100ml conical flask. The samples were vortexed to homogenize and allowed to stand for 10 minutes. From the initial dilution, 10-fold serial dilutions were carried out in clean sterile test tubes containing 9ml of sterile distilled water

Plating Procedures

0.1ml of desired dilutions 10-3 -10-5 were spread plated in triplicates onto nutrient agar, MacConkey agar and Sabouroud dextrose agar. The nutrient agar and MacConkey agar plates were then incubated at 37ºC for 24 hrs for bacterial and coli form counts, while the Sabouroud dextrose agar plates was incubated at 35ºC for 48-72 hours for fungi count.

Purification of Isolates

Following enumeration of total bacterial, coli form and fungal counts, colonies were picked at random and sub-cultured for purification onto nutrient agar (bacteria), MacConkey agar (coliform) and Sabouroud dextrose agar (fungi). Purified isolates were stocked in appropriate media for further studies.

Identification and Characterization of Isolates

Purified isolates were characterized by gram morphology and biochemical test using Bergey's manual of determinative bacteriology (Holt *et al.,* 2017; Cheesbrough *et al.,* 2017), while fungi isolates were identified by cultural and morphological characteristics as described by (Barnett and Hinter, 2017).

RESULTS

Table 1 shows the proximate composition of Tiger nuts milk.The moisture content (55.3 %) contributes significantly to the microbial flora of the food drink sample. The protein content (10.1%) was observed to be high. The disparities observed in the proximate composition indices compared could be due to the non-standardized method of preparation of the beverage by the local people. The ash content(65.5%) of the product, gives an idea of the mineral

content. The carbohydrate content (56.7%) was within the recommended range. This implies that the product would supply the needed energy demands.
Table 2 shows the Bacteria counts 8.2 x 10^3 (cf/ml), coliform counts 3.5 x 10^4 (cf/ml) and the yeast/mould counts 9.2 x 10^5(cf/ml) respectively.
Table 3 shows the different bacterial and yeast isolates indentified in the analyzed sample.
Table 4 shows the overall acceptability expresses how the panelists generally accept the product.

Table 1: Proximate composition of tiger nut milk

Sample	Protein (%)	Fat (%)	Ash (%)	Crude fibre (%)	Moisture (%)	Total Carbohydrate (%)
	10.1	1.20	65.6	9.5	70.3	56.7

Table 2: Microbiological Composition of tiger nuts milk

Parameters	Count (cfu/g)
Total Bacterial count	8.2 x 10^3
Coliform count	3. 5x 10^4
Total fungal count	9.2 x 10^5

Key: TBC=Total Bacteria Count; CC= Coliform Count; FC=Fungal Count

Table 3: **Cultural, Morphological and Biochemical Characterization of the Isolate**

Organisms	Colony colour	Shape	Gram Reaction	Catalase	Coagulase	Indole
Staph. aureus	White to yellow	cocci	+	+	+	-
E. coli	Grayish white	rod	-	+	-	+
Yeast spp	Cream to yellow	Oval, some with bud	+	ND	ND	ND

Key: + = Positive, - = Negative, ND = Not done.

Table 4: Mean Sensory Score of Tiger nuts milk

Descriptive Statistics

	N	Sum	Mean	Std. Deviation	Variance
1. Appearance	17	74	4.35	.147	.368
2. Colour	17	72	4.24	.202	.691
3. Aroma	17	78	4.59	.123	.257
4. Taste	17	74	4.35	.191	.618
5. Mouth feel	17	74	4.35	.191	.618
6. Acceptability	17	75	4.41	.123	.257
Valid N (listwise)	17				

DISCUSSION.

A higher microbial count was observed in the analysed tiger nut milk, this was not surprising as the tiger nut milk production systems are sometimes done under unhygienic conditions Ayandele *et.al*, (2015), with no authorized agency to monitor their microbial quality and safety. The occurrence of *Staphylococcus aureus* , *Escherichia coli* and *Yeast* spp in the analysed sample was not surprising as it corroborates with the study of Innocent *et. al*, (2011); who reported the isolation of *S. aureus, S. pyogenes, P. aeruginosa, A. flavus, Rhizopus* spp*, Bacillus subtilis, Proteus* spp*, and S. cerevisiae* in hawked kunu and zobo drinks sold within LAUTECH campus, Ogbomoso. Similar study by (Ameh and Abubakar, 2002) reported the isolation of *Escherichia coli*, *Staphylococcus aureus, Salmonella* spp and *Shigella* spp in kunu-zaki sold in Maiduguri Metropolis. The organisms isolated from the analysed tiger nut milk sample is known to be important food borne pathogens. *Yeast* spp had the highest frequency occurrence (9.2×10^5 cf/ml) compared to other fungi *Penicillium* spp*, Saccharomyces cerevisiae,* and *Fusarium* spp in the kunu drinks may be linked to contamination through air or dust, contaminated packaging material or poor hygiene and sanitation of the processing environment Ashiru *et. al*, (2003).

Their presence is worrisome as some of these fungi can produce mycotoxins which can cause mycotoxicosis in humans Umaru *et. al*, (2014).

Staphylococcus aureus also had the high occurrence of (8.2×10^3 cf/ml) and are however known to produce intoxication risk (Ameh and Abubakar, 2002) and this makes their presence in the analysed tiger nut milk worrisome.

Staphylococcus aureus is found on the skin as normal flora of humans, but in situations where there is laxity in hygiene, their high load on products handled by man becomes unsurprising(Jablonski and Bohach, 1997) noted that dissemination of *Staphylococcus aureus* from humans to food can occur by direct contact, indirectly by skin fragments or through respiratory tract droplet nuclei. *Escherichia coli* had a lowest occurrence (3.5×10^4 cf/ml), compared to other bacteria isolates identified in the analysed tiger nut milk. Their presence is an indication of fecal and environmental contamination and a signal of the presence of other enteric pathogens which may have occurred probably through the use of contaminated water, utensils, Oranusi *et. al*, (2003).

The sample was found to be acidic (pH 3.5). This level of acidity of Tiger nut milk has been described by several researchers including (Efiuvwevwere and Akoma, 2013) and Akoma *et. al*, (2013), who attributed this to the presence of certain species of lactic acid bacteria namely *Lactobacillus leichmanni* and *Lactobacillus fermentum* during the fermentation process. Similar high acidic pH values have been reported for zobo and for orange juice products Bolarinwa *et. al*, (2013) as well as burukutu and pito (Kolawole and Afonja, 2007). Although, these classes of beverages are acidic in nature, the acidity tends to increase with increase in fermentation period resulting into spoilage. Consequently, the low pH values may have encouraged the growth of fungi and this could be responsible for the species of microorganisms isolated. The acidic nature of the sample may also be due to the fact that the tiger nut milk might have started undergoing spoilage even before the time of purchase, and such may lead to production of certain metabolites and could bring about reduction in pH of the product. The pH of tiger nut milk is usually too low to allow the growth of pathogenic microorganisms. Zaika *et.al*, (2013); (Adegoke and Shridhar, 2013), the addition of spices to the processed tiger nut milk is highly advocated.

The moisture content (55.3%) results agree well with the observations of Umaru *et .al*, (2014) that analyzed kunu made from maize but slightly lower than that reported by (Amusa and Ashaya, 2009). Moisture content contributes significantly to the microbial flora of the food drink samples. The protein content value of the analyzed tiger nut milk was observed to be high (10.1 %) .This value was found to be higher than that obtained for wet milled kunu by Presscott *et. al*,(2002). The percentage of crude fat(1.20%) obtained in this work is comparable to that obtained by Innocent *et. al*, (2011) but quite higher than

that reported by Akoma *et.al*,(2013). The disparities observed in the proximate composition indices of the analyzed tiger nut milk sample compared to those from other authors could be due to the non-standardized method of preparation of the beverage by the local people. The ash content in tiger nut milk was found to be 65.5%, the ash content of the product, gives an idea of the mineral content. Carbohydrate content of tiger nut milk was 56.7%, the carbohydrate content for the product was within the recommended range . This implies that the product would supply the needed energy demands. These results are in agreement with the work of (Moreno and Clemente, 2008).

Table 4 shows the mean sensory scores of tiger nut milk made from tiger nuts. The appearance of the tiger nut milk had a mean score of 4.35, colour 4.24, aroma 4.59, taste 4.35, mouth feel 4.35 and acceptability 4.41. All the sensory attributes were like much and extremely liked by the panelist. The overall acceptability expresses how the panelists generally accept the product. It was observed that complementary food produced from tiger nuts milk was accepted with the mean value of 4.41. The mean scores from sensory evaluation showed that the tiger nut milk sample was accepted. This result provide a basis for the development of an acceptable complementary local beverage that can provide the required protein energy levels that are essential basic nutrients to enable the accomplishment of a day work. Similar findings were reported by Bonsi *et. al*, (2014) and (Ojure and Quadri, 2012).

CONCLUSION

The study has revealed that the microbial quality of tiger nut milk is of low standard and unfit for human consumption. Therefore kunu producers and hawkers/sellers should maintain adequate hygienic conditions during processing and preparation of these beverages so as to curb or reduce potential public health risk associated with these foodborne pathogens.

RECOMENDATIONS

- This is therefore recommended that improved quality and production technologies of these indigenous exotic beverages should be employed so that large scale production for consumption by different staffs and students would be enhanced.

- Also producers and hawkers should embrace good sanitary practices during the preparation and sale of the products, since spices have been reported to inhibit microbial growth.

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