ECRONICON OPEN ACCESS

EC NUTRITION
Research Article

Microbial Quality Evaluation of Tiger Nut Beverage (*Kunun Aya*) Processed Sold in University of Maiduguri

Badau MH[1]*, Bilyaminu D[1], Ogori AF[2], Charles B[1] and Ogori Joeguluba[3]

[1]*Department of Food Science and Technology, University of Maiduguri, Maiduguri, Nigeria*

[2]*Department of Home Science, Federal University Gashua, Gashua, Nigeria*

[3]*Department of Agricultural Education, Federal College of Education, Kontagora, Nigeria*

***Corresponding Author**: Badau MH, Department of Food Science and Technology, University of Maiduguri, Maiduguri, Nigeria.

Received: December 15, 2017; **Published:** February 26, 2018

Abstract

The study determined the microbiological quality of tiger nut beverages (*Kunun Aya*). It is a non-alcoholic drinks often processed and consumed within the University of Maiduguri Campus. A total of twelve (12) samples were collected and analyzed from different points (four cardinal points) within the university campus. Bacteria, mould and yeast were enumerated and identified. Serial dilutions were carried on the collected samples, and a dilution factor of 10^4 was used for each sample. Total aerobic bacterial count, coliforms count, yeast count, staphylococcal count and mould count were determined using pour plate techniques. The results obtained show high bacterial count which ranged between 9.92 x 10^4 to 3.13 x 10^4 CFU/ml. Total coliforms count ranged from 1.56 x 10^4 to 6.0 x 10^4 CFU/ml. Total staphylococcal count ranged from 2.23 x 10^4 to 1.56 x 10^4 CFU/ml. Total mould count ranged from 2.60 x 10^4 to 1.56 x 10^4 and total yeast count ranged from 2.6 x 10^4 to l.56 x 10^4 CFU/ml. A conventional processing technique if fully utilized for this product would minimize contamination and spoilage; and present acceptable wholesome product to its consumers, preferably for exportation.

Keywords: *Tiger nut; beverages; Drinks; Bacterial count; Nutrients; Ingredients; Spoilage*

Introduction

Beverage is any food intended to be taken in liquid form which includes juices, coffee, tea, soft and alcoholic drinks etc. Several kinds of beverages are consumed not for food value but rather for thirst quenching properties and for their stimulating effects [1,2]. Imitation milk extract of tiger nut which is equivalent to *Kunun Aya* is rich in nutrients such as protein, carbohydrate and vitamins as reported by Temple., *et al.* [3] and Abaejoh., *et al* [4].

Despite the nutritive value of tiger nut, its production and utilization in Nigeria has been hampered due to deterioration caused by spoilage micro-organisms [4]. Recently, there was an awareness to increase utilization of tiger nut beverages [5]. But effective control measure to present wholesome products proofs abortive due to inadequate storage. A study conducted by Chukwu., *et al.* [6] indicates many group of micro-organisms (*Aspergillus niger, Aspergillus flavus, Penicillium citrinum, Coliforms, Staphylococcus aureus, Bacillus, Fusarium solani,* and *Candida pseudotropicalis*) associate with tiger nuts and its products. Also large numbers of lactic acid bacteria and coliforms have been reportedly implicated in good spoilage processes [7,8]. The consumption of contaminated food could have harmful effects such as food infection or intoxication. According to WHO [9], food borne illness are diseases, usually infectious or toxic in nature, caused by agents that enter the body systems through ingestion. Food borne illness is a major international health problem that also affects nation economy [10]. The present study is therefore become an important one, aimed at enumeration of bacteria, yeast and fungi associate with tiger nut beverages (*Kunun Aya*).

The essential components of any beverage are its water content and other components such as stimulants, colouring and flavouring ingredients [11]. These beverages are classified as: carbonated non-alcoholic carbonated mildly alcoholic and noncarbonated non-alcoholic beverages [4].

Tiger-nut beverage is a non-alcoholic drink obtained from tiger nut tuber, which is consumed widely in Africa [4]. Tiger nut (*Cyperus esculentus*) belongs to the family of *Cyperaceae* [12]. It is a tuber that grows freely and is consumed widely in Nigeria and in other part of West and East Africa [4]. Tiger nut beverages are popularly known in the northern part of Nigeria as *Kunun Aya*. It is one of the indigenous locally fermented non-alcoholic beverages drinks that is widely consumed for its thirst -quenching and nutritive properties. Even though it is consumed throughout the year, it is extensively consumed during the dry season [13].

There are three varieties of tiger nut: black, brown and yellow. All 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 colour. The yellow variety also yields more milk upon extraction, contains lower fat and more protein, and possesses less anti-nutritional factors such as polyphenols [13,14]. Tiger nut beverages are whitish and considered by many to be very refreshing especially when chilled. It is prepared mostly for domestic consumption. Tiger nuts have long been recognized for their health benefits. It was reported that the proportion of its dietary fibre could be effective in treatment and prevention of many disease including colon cancer, coronary heart disease, obesity, diabetes and gastro intestinal diseases [14-16]. Temple., *et al.* [15] further reported that tiger nut is rich in protein (7%) and carbohydrate. These carbohydrates include reducing sugar (7.4%), soluble polysaccharide (7.4%) and starch (86.4%). Its biological value is slightly higher as reported by Ojobe and Tempo [11] than many other nutritious foods proposed by FAO/WHO [17]. The amino acid content of tiger nut is essentially found within the range needed by adult [18].

Therefore, there is a need to evaluate the microbiological quality of tiger nut beverages sold within the University of Maiduguri, in order to ascertain its safety on the health of its consumers. This is because production and consumption of food hawk in Northern Nigeria especially in schools and colleges where commercial activities are intense, usually end up not meeting the right needs of individuals. There are also many concerns about sanitation of places where foods are sold or street vended foods such as *Kunun Aya*. Similarly, identifying the right need and negative hindrance of presenting this product in its wholesomeness, would be a way forward for its large scale commercialization.

Materials and Methods

Study Area

The study was conducted within the University of Maiduguri. Apparatus and reagent used were obtained from the Department of Food Science and Technology, University of Maiduguri.

Samples and sampling method

Sample of *Kunun Aya* (Figure 1) was procured from four (4) cardinal point of the University of Maiduguri, Borno State. Each sample collected from each of the cardinal point was collected from three (3) different processors and times. Sample of *Kunun Aya* was collected in a sterile container (plastic) and appropriately labelled at the point of collection as A, B, and C respectively. The sample was carried out immediately to the laboratory of the Department of Microbiology, University of Maiduguri where the analysis was carried out as described by Jideani and Jideani [7].

Figure 1: *Kunun Aya.*

Sterilization of glass wares and preparation of culture media

All glass ware were washed with clean water. They were dried and sterilized in an analytical oven, at a temperature of 160^{0}C for 1 hour. Each of the media used were separately prepared by using 28g of nutrient agar, 48.5g of MacConkey agar, 15g of Peptone water, 17g of Corn meal agar, 39g of potato dextrose agar and 108g of Mannitol Salt Agar (MSA) in 100 ml of distilled water each. They were autoclave at a temperature of 121^{0}C for 15 minutes before being used [7,19].

Microbiological analysis and examination

A sample for the serial dilution was prepared by introducing 1 ml of *Kunun Aya* into a bijou bottle containing 9 ml of sterilized distilled water to form a stock solution. This was further continued until a required serial dilution was made as described by Bristone., *et al* [20].

Pour plate method was used for plating the samples using the prepared media. The plates were then incubated at 35^0C for 24 hours and were observed for microbial growth. After observing the microbial growth for cultural characteristics, colonies of the microbial growth were smeared on a microbial slides and later gram stained. These were then observed microscopically for the morphological characteristics of those microorganisms isolated. Motility test was further performed on those pure cultures using agar with concentration of 0.2 - 0.5% (w/v). A diffused growth at the place of inoculation was considered as positive and restricted growth as negative. Catalase test was carried out using pure culture and was emulsified with a loop full of freshly prepared 3% hydrogen peroxide (H_2O_2) on a slide. Coagulase test was done by slide method. A drop of normal saline (0.85%) was placed on portions of 18-hour old culture of the test organism and also a drop of rabbit plasma was added to one of the suspensions and the other one was kept without rabbit plasma to serve as a control. These were then stirred for about 0.5 seconds and observed. Oxidase test was carried out using filter paper and plate method. A portion of the culture plate containing filter paper and a drop of 1% aqueous solution of freshly prepared oxidase reagent was used. A loop full of the test organism was added using glass rod and the result was observed within 10 second. Citrate utilization test was carried out using Simon's citrate agar method. A slope and a butt were prepared in a bijou bottle and it was stab inoculated the test organism for 48 hour and later observed for citrate utilization. Urease test was carried out using urease broth. After preparation, 5 ml potion of the broth was dispensed into sterile bijou bottle to obtain a slope. The slope surface was inoculated by streaking with a loop full of peptone water broth culture. It was incubated at 35°C for 24 hours before and was observed. Methyl Red and Voges-Proskaur (MRVP) test was carried out using glucose-phosphate and 40% KOH. A bijou bottle containing prepared MRVP broth was inoculated with a loop full of test organism. It was incubated for 24 day. This culture was divided into two portions. One was tested with 5 drops of methyl red and the other 1 ml of 40% potassium hydroxide and 3 drops of alpha-naphthol solution. The result was then observed. The fungal isolates were stained with lactophenol cotton blue stain and were observed microscopically for morphological characteristics and for identification [7,19].

Results and Discussion

Table 1 shows the microbiological load count of *Kunun Aya* obtained from the four (4) cardinal points of the University of Maiduguri. The total bacteria count ranged from 3.13 x 10^4 to 9.93 x 10^4 CFU/ml. Total Coliforms count ranged from 1.56 x 10^4 to 6.0 x 10^4 CFU/ml. Total Staphylococcal count ranged from 1.43 x 10^4 to 2.23 x 10^4 CFU/ml. Total mould count ranged from l.43 x 10^4 to 2.60 x 10^4 CFU/ml. Total yeast count ranged from 2.60 x 10^4 to 1.43 x 10^4 CFU/ml. Table 2 shows the percentage occurrence of microorganisms isolated from Kunun Aya drink The occurrence of *Staphylococcus aureus* in *Kunun Aya* was 37.5%, *Salmonella* spp 25.05%, *E. coli* 50.0%, *Shigella* spp 37.0%, *Pseudomonas* spp 37.0%, *Candida albicans* 12.0% *Saccharomyces cerevisiae* 37.5% and *Rhizopus oryzae* 25.0% occurrence respectively.

Sample	TBC	TCC	TSTC	TMC	TYC
S.E					
A1	8.20×10^4	4.25×10^4	2.23×10^4	2.06×10^4	2.00×10^4
A2	7.30×10^4	3.56×10^4	1.43×10^4	2.03×10^4	1.83×10^4
A3	2.26×10^4	3.00×10^4	2.10×10^4	1.80×10^4	2.00×10^4
S.W					
B1	4.93×10^4	2.73×10^4	1.90×10^4	2.06×10^4	2.60×10^4
B2	6.03×10^4	3.56×10^4	1.43×10^4	2.03×10^4	1.56×10^4
B3	9.20×10^4	6.60×10^4	2.20×10^4	2.20×10^4	1.83×10^4
N.E					
C1	9.93×10^4	3.80×10^4	1.86×10^4	1.63×10^4	1.56×10^4
C2	8.26×10^4	3.80×10^4	2.00×10^4	2.03×10^4	1.96×10^4
C3	5.73×10^4	3.13×10^4	2.00×10^4	2.00×10^4	1.60×10^4
N.W					
D1	5.86×10^4	2.20×10^4	2.20×10^4	1.43×10^4	2.20×10^4
D2	3.20×10^4	1.56×10^4	1.53×10^4	1.73×10^4	1.56×10^4
D3	3.13×10^4	1.83×10^4	1.80×10^4	1.60×10^4	1.83×10^4

Table 1: *Microbiological count of kununaya obtained from the four (4) cardinal point of the University of Maiduguri. Each value is a mean of triplicate determination.*

Note: TBC: Total Bacterial Count; TTC: Total Coliforms Count; TSTC: Total Staphylococcal Count; TMC: Total Mould Count; TYC: Total Yeast Count

S.E: South East; S W: South West; NE: North East; NW: North West

Isolates	Sample location				
	Southeast	Southwest	Northeast	Northwest	% Occurrence
S. aureus	+	+	+	-	37.5
Salmonella	+	+	-	-	25.0
E. coli	+	+	+	+	50.0
Shigella	+	+	+	+	37.0
Pseudomonas	+	+	-	+	37.0
Candida albicans	+	-	-	-	12.5
Saccharomyces cerevisiae	+	+	+	+	37.5
Rhizopus oryzae	-	-	+	+	25.0

Table 2: *Percentage occurrence of microbial Isolates in Kunun Aya drinks.*

Samples from the northeast showed the highest count of microbial growth. The high microbial count observed could be as a result of non-aseptic handling, processing method, utensils and water used during preparation. Samples from the northwest showed the least microbial count. The presence of some of these microorganisms may be due to prolonged storage of the product at ambient temperature which is a factor that may result in spoilage [21]. All the bacterial counts observed were indicators of contamination while coliform bacteria in the drink are considered as an indication of bacteria pollution of human or animal origin perhaps introduced during processing [22]. The bacteria identified may pose a special health risk on susceptible populations such as infants, young children and people with compromised immune systems [23]. The official limit recommended for microbial contamination of beverages or sorrel drinks requires the complete absence of *E. coli, Pseudomonas, Salmonella* and *Staphylococcus aureus* as reported by Okereke., *et al* [24]. The total aerobic bacterial count must not be more than 10^2 CFU/ml and total fungi count not more than 10^2 CFU/ml as reported by NAFDAC (2007). Tropical climates are characterized by ambient temperatures frequently above 30^0C rapid increase of microorganisms may likely occur in beverages [21].

We identified *Staphylococcus aureus, E. coli, Salmonella, Shigella* and *Pseudomonas* in all samples we collected which agrees with the findings of Taiwo., *et al* [25]. We also identified *Candida albicans, Saccharomyces cerevisiae* and *Rhizopus oryzae* which correspond with the findings of Udeozor and Awonorin [26]. Similar result but with higher microbial growth was also reported by Elmahmood and Doughari [27] and Musa and Hamza [28]. The isolation of fungi from these drinks may be linked to contamination through air/dust, packaging material or processing environment. Other sources of contamination of *Kunun Aya* is mostly through water, handling and storage practices, and the underground tuber (tiger nut) itself. Growth of fungi can occur over a wide range of temperature and pH and some of these fungi can produce mycotoxins which can cause mycotoxicosis in humans as reported by Umaru., *et al* [29].

Conclusion

In this study, the types of microorganisms encountered are significant in public health. The most important pathogenic ones isolated are *Salmonella, Shigella, Staphylococcus aureus, E. coli* and *Candida albicans*. *Saccharomyces cerevisiae* and *Rhizopus oryzae* are also isolated but have no history of health implication. Consumption of *Kunun Aya* from those processors and other sources from the study area, pose threat to consumers. However, conventional processing and storage systems if fully utilized would go a long way toward presenting wholesome product (*Kunun Aya*) to its consumers, since these organisms are easy to be controlled during processing.

Bibliography

1. Abegaz K. "Isolation, characterization and identification of lactic acid bacteria involved in traditional fermentation of bread, an Ethiopian cereal beverage". *African Journal of Biotechnology* 6.12 (2007): 1469-1478.

2. Ihekoronye AI and Ngoddy PO. "Integrated food science and technology for the tropics". Macmillan Education L.T.D., London and Oxford (1985): 312-317.

3. Temple VJ., *et al*. "Chemical analysis of tigernut (Cyperus esculentus)". *Journal of Science Food Agriculture* 50.2 (1990): 262-263.

4. Abaejoh R., *et al*. "Characteristics of tigernut (Cyperus esculentus) tubers and their performance in the production of a milky drink". *Journal of Food Processing and Preservation* 30.2 (2006): 145-163.

5. Balewu MA and Adedunni AO. "Preparation of kunu from exploited rich food source tiger nut (*cyperus esculentus*)". *Pakistan Journal of Nutrition* 7 (2014): 109-111.

6. Chukwu MO., *et al*. "Study on fungi and physiochemical and proximate composition of dry and fresh tiger nut (cyperus esculentus)". *International Research Journal of Biotechnology* 4.1 (2013): 11-14.

7. Jideani VA and Jideani IA. "Laboratory manual of food bacteriology, Kaduna, Nigeria". Amana Printing and Advertising Ltd (2006): 1-205.

8. Amusa NA and Odunbaku OA. "Microbiological and Nutritional Quality of Hawked Kunun (A Sorghum Based Non-Alcoholic Beverage) Widely Consumed in Nigeria". *Pakistan Journal of Nutrition* 8.1 (2009): 20-25.

9. WHO. "Foodborne disease outbreaks: Guidelines for investigation and control". Publications of the World Health Organization can be obtained from WHO Press, World Health Organization, Geneva (2008).

10. CFS. "Microbiological Guidelines for food for ready-to-eat food in general and specific food items" (2014).

11. Ojebo TO and Tempo VJ. "Amino acid composition of tigernut tubers (Cyperus esculentus)". *Journal of Science Food Agriculture* 50 (1993): 262-263.

12. Uva RH., *et al*. "Weed of the north east". Comelll University Press, Ithaca New York (1997): 397.

13. Okafor TS and Nwanchukwu. "Phytochemical screening of tiger nut (cyperus esculentus) of three different varieties". *Journal of Biological Sciences* 81 (2003): 115-120.

14. Bamishaiye EI and Bamishaiye OM. "Tiger nut: as a plant, its derivatives and benefits". *African Journal of Food, Agriculture, Nutrition and Development* 11.5 (2011): 5157-5169.

15. Temple VJ. "Effect of processing treatment on the quality of tiger nut milk". *Pakistan Journal of Nutrition* 10.1 (1989): 95-100.

16. Bristone C., *et al*. "Production and evaluation of yoghurt from mixtures of cow milk, milk extract from soybean and tiger nut". *World Journal of Dairy and Food Sciences* 10.2 (2015): 159-169.

17. FAO/WHO. "Milk and Milk product. Joint FAO/WHO Food standards programme". Codex Alimentations compassion (2002): 42-50.

18. Bosch L., *et al*. "RP-HPLC determination of tigernut and Orgeat amino acid contents". *Food Science Technology International* 11.1 (2005): 33-40.

19. Cheesbrough M. "District laboratory practice in tropical countries, part 2". U.S.A: Cambridge University Press (2006): 1-247.

20. Bristone C., *et al*. "Microbial ecology and effectiveness of different starter cultures in yoghurts produced from whole cow milk and soybeans". *African Journal of Food Science and Technology* 7.5 (2016): 107-117.

21. Bolarinwa IF., *et al*. "Some Physiochemical Properties of flour obtained from fermentation of tiger-nut (Cyperus esculentus). sourced from a market in Ogbomoso, Nigeria". *African Journal of Food Science* 3.2 (2009): 51-55.

22. Okonko IO., *et al*. "Microbiological and physicochemical analysis of different water samples used for domestic purposes in Abeokuta and Ojota, Lagos State, Nigeria". *African Journal of Biotechnology* 7.5 (2008): 617-621.

23. Obire O., *et al*. "Bacteriological water quality of elechi creek in Port Harcourt, Nigeria". *Journal of Applied Science and Environmental Management* 9.1 (2005): 79-84.

24. Okereke CN., *et al*. "Physicochemical and medicinal uses of Hibiscus sabdariffa". *International Journal of Herbal Medicine* 2.6 (2015): 16-19.

25. Taiwo AA., *et al*. "Comparative study of nutritive composition and microbial level of Kunun sold in three campuses in Abeokuta". *Sky Journal of Food Science* 6.1 (2017): 7-13.

26. Udeozor LO and Awonorin SO. "Comparative microbial analysis and storage of tiger nut soy milk extract". *Austin Journal of Nutrition and Food Sciences* 2.5 (2014): 1-6.

27. Elmahmood AM and Doughari JH. "Microbial quality assessment of kunun-zaki beverage sold in Girei town of Adamawa State, Nigeria". *African Journal of Food Science* (2007): 11-15.

28. Musa AA and Hamza A. "Comparative Analysis Of Locally Prepared 'Kunun Aya' (Tiger-Nut Milk) Consumed By Students Of Kaduna State University, Kaduna-Nigeria". *Science World Journal* 8.2 (2013): 2013.

29. Umar ZD., *et al*. "Study on bacteriological quality of kunun aya (Tigernut Juice) Sold At Umaru Musa Yar'adua University (Umyu) Campus, Katsina". *International Journal of Environment* 3.2 (2014): 87-97.

Volume 13 Issue 3 March 2018