Microbial Assessment of Kunu Sold In Awka Metropolis

Abstract

Kunu is a non-alcoholic fermented Nigerian beverage made from cereals locally. Due to its cost, nutritional value and high contents of nutrients, has been accepted generally. Hence, the reason for its nutritional and safety evaluation is very much essential. The basis of this research is to determine the microbiological quality of kunu-zaki drinks hawked and sold in Awka metropolis. Three samples of kunu-zaki beverages were obtained from three different market locations in Awka, Anambra state namely; Amansea, Eke-Akwa and Amawbia respectively. The pH of the samples ranged from 3.8-4.5. These samples were tested for both bacterial and fungal contamination. The results show that for the total viable colony counts from the Kunu samples vary from 3.5×10^4 to 4.0×10^5 for bacteria, 5.4×10^5 to 8.0×10^5 for fungi for the three samples analyzed. Some of the isolates identified include; *Staphylococcus* sp, Lactob*acillus* sp, *Bacillus* sp and *Escherichia coli*, for bacteria and *Saccharomyces* sp, *Aspergillus* sp, and *Penicillium* sp. for fungi isolates. This research reveals that kunu drinks hawked in some part of Awka metropolis contain different pathogenic microorganisms which contaminates the product, therefore making it unfit for human consumption. Hence, good hygiene and sanitary measures should be employed during the production, processing and packaging of the locally fermented beverage.

Keywords: Kunu, fermented beverage, bacteria, fungi, hygiene.

Introduction

Kunu, a locally non-alcoholic fermented beverage that was consumed by the northern part of Nigeria has become generally accepted in all the parts of the country for its nourishing and nutritive values. Kunu is produced from cereals example; guinea corn (*Sorghum bicolor*), millet (*Penisetum typhoides*), maize (*Zea mays*), rice (*Oryza sativa*) or wheat (*Triticum aestivum*). There are various types of kunu which are processed and consumed in Nigeria. They include: kunuzaki, kunugyada, kunuakamu, kunutsamiya, kunubaule, kunujiko, kunuamshau, kunugayamba. However, kunuzaki is mostly consumed in every part of Nigeria.

Kunu-zaki is prepared traditionally using millet, maize, wheat or sorghum. It contains 11.6% of protein, 3.3% of fats, 1.9% of ash and 76.8% of carbohydrates and arrays of amino acid [1]. Apart from these cereals, kunu has been shown to be produced from tigernuts [2], guinea corn or rice [3]. The appearance of kunu is milky cream and is usually consumed within few hours after its production [4]. Because kunu is prepared in the traditional method, the ingredient concentrations are neither quantified nor standardized [5]. The production procedure varies depending on household, taste and cultural habits of the consumers. This leads to variation in the taste, quality and specifications of the product. According to Umaru *et al*., [3], the processes involved in the preparation of kunu-zaki include: steeping the whole grains in water for 6-24 hours, followed by wet milling usually with spices and sweet potato, gelatinization of a portion of the mixture in hot water and then pitching with about one quarter fresh (ungelled part of the mixture. Spices such as ginger (*Zingiber officinales*),

aligator pepper (*Afromonium melegueta*), red pepper (*Capsicum* species), black pepper (*Piper guinense*) and Kakandoru or Eruare usually utilized [6]. Apart from the nutritional benefits of kunu consumption, the drink has been shown to have other benefits including reduction in blood cholesterol, lowering the risks of diabetes, and prevention of blood clot formation [7].

Aside these health benefits of kunu, there are several reports of microbial spoilage of this drink. Some of these pathogenic microbes include; lactic acid bacteria (LAB) such as *Lactobacillus* spp., *Streptococcus* spp. and *Leuconostoc* spp and food-borne microorganisms such as *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella spp* e.t.c. Also, activities of the natural food enzymes could also contribute in the spoilage. Other factors include insects, rodents or pests present in the environment during the preparation, temperature and light. All these and much more if not properly managed could contribute to the spoilage of kunu-zaki. The presences of the amount of sanitary indicator organisms in foods are of importance in the assessment of the quality and safety of foods. Even though, epidemiological evidence on outbreaks of food borne disease as a result of taking kunu-zaki is scarce, there are indications that it could still be contaminated to unsafe level at the point of consumption with air flora and other microorganisms from handlers, equipment serving containers, raw materials and lack of portable water. In-adequate hygiene practices in the production process can introduce many microbial pathogens in foods, which results to food-borne diseases. For developing countries like Nigeria, a safety control measure over the processing of hawked foods is still on the decline. This is because most sellers lack portable water, proper storage and waste disposal facilities as well as adequate information good manufacturing and handling practices. Thus resulting to microbial contamination and increase in health hazards. Nevertheless, simple practical but effective measures should be applied during the production of kunu-zaki by the producers, these include: cleaning of the food processing environment, use of potable water for processing, washing of utensils, hand washing by producers (before, during and after the production). More so, the clean containers should be used for packaging, local pasteurization using pressure pots should be used to eliminate the all contamination encountered during the production. A lot of research and reviews have been done on the microbiological and nutritive quality of kunu drinks, not much have been done on the microbiological and safety quailty of kunu drinks hawked in Awka metropolis, Anambra State. Hence this study tends to analyse the microbial composition and microbial quality of kunu beverages hawked in Awka Metropolis as it will be used as a reference and comparative indices to other literatures.

Materials and Methods

Study Location: Awka is a city in Anambra State, South-East, Nigeria. Its area is approximately 120 km2. The city's population is about 301,657. It lies below 300meters above sea in a valley on the plains of the Mamu River.

***Sterilization of Materials*:** The glass-wares (Test tubes, pipettes, conical flasks, beakers, petri-dishes and universal bottles) were washed with soapy water and rinsed with distilled water; they were allowed to dry and wrapped with kraft paper and further sterilized in a hot air oven at 180°C for 1hour and stored at 4°C.The media used was also sterilized.

Collection of Samples

Samples of freshly prepared kunu-zaki were collected from different hawkers at 3 different market locations (Amansea, Eke-Akwa and Amawbia), all within Awka metropolis. The samples were labelled and transferred immediately to the microbiology laboratory of Nnamdi Azikiwe University, Awka in their original package and the contents aseptically withdrawn from the bottles for pH analysis, isolation and enumeration of micro organisms

Preparation of Media: The three media (Nutrient agar {NA}; MacConkey agar {MAC}; Potato Dextrose Agar {PDA}) used were prepared according to the manufactures' procedures (Mereck).

Preparation of Nutrient Agar (NA): Twenty eight grams (28.0g) of nutrient agar powder was dissolved in 1000 ml of distilled water. The medium was gently heated to dissolve and completely sterilize by autoclaving at 15psi (121°C) for 15minutes.The autoclaved media was allowed to cool before pouring 15ml each onto sterile Petri dishes and allowed to gel.

Preparation of MacConkey agar (MAC) Agar: This was done by suspending 47grams of the powder in 1000ml of distilled water. The suspended was mixed very well and heated with frequent agitation to dissolve the powder completely. The suspended media was sterilized by autoclaving at 121°C and 15psi, for 15minutes. The autoclaved media was allowed to cool before pouring 15ml each onto sterile Petri dishes and allowed to gel.

Preparation of Potato Dextrose Agar (PDA): This was done by suspending 39g of the media in 1000ml of distilled water. The suspended was mixed very well and heated with frequent agitation to dissolve the powder completely. The suspended media was sterilized by autoclaving at 121°C and 15psi, for 15minutes. The autoclaved media was allowed to cool before pouring 15ml each onto sterile Petri dishes and allowed to gel.

Microbiological Analysis: One millilitre of each sampled kunu drink was sterile diluted in 9ml of sterile distilled water in sterile test tubes. From the appropriate dilution, 0.1ml was inoculated separately on to MacConkey agar, Nutrient Agar and Potato Dextrose Agar plates and spread evenly using sterile bent glass rod.

Each experiment was carried out in duplicates and mean standard value of the colony forming units (cfu/ml) on the plates was used. The inoculated MacConkey agar, Nutrient agar and Potato Dextrose agar plates were incubated at 30ºC and 35ºC for 24 and 48 hours respectively. the colonies on the plates were counted and recorded as colony forming unit per millilitre (cfu/ml) and coliform respectively after the period of incubation [8].

Each of the bacterial colonies on the agar plates was sub-cultured and pure culture obtained for further experiments. Characterization and identification of the Isolates were carried out using these tests: microscopic techniques, Gram staining, spore staining, catalase, coagulase, oxidase, citrate utilization, indole, methyl red, urease, Voges-Proskauer and sugar fermentation as described by [9;10]. The identities of the isolates were determined by comparing their characteristics with those of known taxa as described by Bergey's manual of Determinative Bacteriology [11and10] 18-24 hrs cultures were used for the experiment. Fungal identification and enumeration was based on their colony elevation, colour, texture, shape and arrangement of conidia (spherical or elliptical, unicellular or multicellular), branched or unbranched mycelia, presence or absence of cross walls (whether septate or non-septate) and others. They were enumerated by reference as illustrated manual on identification of some seed borne fungi [12] and illustrated genera of imperfect fungi [13].

Biochemical Tests

The entire biochemical test listed below was done according to the method described by Cheesbrough, [10].

Catalase Test

Catalase is an enzyme that catalyzes the reaction by which hydrogen peroxide (H_2O_2) is decomposed to water and oxygen. It was used to differentiate Staphylococcus (catalase positive) from Streptococcus (catalase negative).

A loopful inoculation of a 24 hour culture of the isolate was placed on a clean grease-free glass slide using a sterile wire loop. It was smeared using a drop of normal saline. A drop of hydrogen peroxide (H_2O_2) was placed on the smear. Catalase positive organisms produced effervescence reaction immediately.

Coagulase Test (Bound Coagulase)

A drop of normal saline was placed on two separate slides. Colony of the test organism was emulsified in each of the drops to make two suspensions and mixed gently. No plasma (human plasma) was added to the second suspension. This is used to differentiate any granular appearance of the organism from true coagulase clumping. Clumping of the organisms within 10 seconds indicated positive result. No clumping within 10 seconds indicated no bound coagulase ie negative coagulase.

Motility Test

10ml of nutrient agar (LabM) was poured into each of the test tubes aseptically. The test tubes were clogged and sealed using cotton wool and slanted to solidify. The test organisms were each inoculated into the nutrient agar slant using a straight wire loop and incubated for

24 hours. Growth along the stab is negative. Growth moving into the medium away from the stab is positive.

Indole Production Test

The test organisms were each inoculated into test tubes containing 5ml each of sterile peptone water. The test tubes were clogged using cotton wool and incubated at 37^0C for 48 hours. Kovacs reagent (0.5ml) was thereafter added and the culture shaken for 1min. a red color in the reagent layer indicated the presence of indole. A yellow color in the reagent layer indicated the absence of indole.

Methyl Red Test

The test organisms were introduced into different test tubes containing 5ml of sterile peptone water. The test organisms were incubated at 37^0C for 48 hours. 0.5ml or 5 drops of methyl red (indicator) was added to the culture. Then, it was mixed gently to disperse the methyl red and was allowed to settle. Red coloration is positive while a yellow coloration is negative.

Voges-Proskauer Test

The test organisms were inoculated into 5ml of sterile peptone water in corresponding test tubes. The test tubes were clogged using cotton wool and incubated at 37^0C at 48 hours. 0.5ml or 5 drops of alpha-naphthol (Braitt's reagent A) and 0.5ml or 5 drops of potassium hydroxide (Braitts reagents B) were added to the tubes containing the test organisms. The culture were shaken gently and allowed to settle for 15 minutes for color development. A positive reaction for Voges-Proskauer was indicated by red coloration. A negative reaction for Voges-Proskauer was indicated by yellow coloration.

Citrate Utilization Test

The test organisms were introduced into prepared slopes of Simmons citrate medium in test tubes using a sterile wire loop the slope was inoculated with the test organism aseptically, and then it was covered with cotton wool lightly. The test tubes containing the test organisms were incubated for 48 hours at a temperature of 35 – 37^0C. Bright blue color indicated positive citrate test. Green color indicated negative citrate test.

Sugar Fermentation Test

0.5g of sugar (glucose, lactose and sucrose) each was mixed with 100ml of peptone water and 3-5 drops of pH indicator, bromothymol blue was added. 5ml of medium each were dispensed in test tubes with the Durham tubes. The whole set up was sterilized at 115^0C for 10 minutes and was allowed to cool. Thereafter, the test organisms was introduced into the medium and incubated at 37^0C for 24 hours. Then the culture was examined for evidence of acid or gas production which is an indication that the organism fermented the sugar. Gas production was indicated by bubbles in the Durham tubes while acid production was indicated by a change in color of the medium.

Gram Staining

A thin film of the bacterial isolate was made on a clean grease free slide. It was fixed by passing the slide over a flame 3 times. The fixed smear was flooded with 0.5% aqueous solution of crystal violet and left for thirty (30) seconds. The stain was washed off in clean running water and then the smear was covered with Lugols iodine for 30 – 60 seconds. The iodine was washed off in clean running water. The smear was decolorized rapidly with acetone-alcohol for 2-5 seconds, and then the slide was washed in clean running water. The smear was then counterstained with 0.5% safranin solution, left for 30 seconds and washed off with clean running water. The slide was then air dried and examined microscopically under the oil immersion lens (100x) without a cover slip for either a Gram- positive or Gram-negative bacteria.

.**Spore Staining Test**

Bacteria film was made on a slide, dried and heat fixed with minimal flaming. The slide was placed on the rim of beaker of boiling water, with the bacterial film uppermost. The film was flooded with 0.5% aqueous solution of malachite green when large droplets have condensed on the underside of the slide and left to act for one minute while the water continued to boil. The slide was washed with cold water and treated with 0.5% of safranin solution and left for 30 seconds. The slide was washed, dried and examined microscopically under the oil immersion lens (100x). Red color indicated a negative result.

Urea Test

The test organisms were inoculated onto test tubes with solidified urea medium. Urea medium was dispensed into six test tubes and after inoculation with the organisms; the test tubes were sealed with cotton wool and incubated at 24 hours at 37^0C. Positive result was indicated by pinkish colour. Negative result was indicated by yellow colour.

Results and Discussion

The pH was acidic and ranges from 3.8-4.5 for all the kunu samples (Table 1). These results conform to the values obtained from previous researchers [14,15,16,17] that values of 4.3; 2.42-3.83; 3.80-3.99; 5.25 to 5.65 were observed in various kunu drinks respectively. Ekanem *et al* [14], noted that bacteria which help in acid fermentation of the kunu products might be cause of acidity. Various researchers have also attributed this to the presence of fermentative microorganisms in kunu which causes spoilage of the beverage by fermentation of its carbohydrate content and producing undesirable changes in them, altering their aroma and taste thus making them unpalatable for human consumption.

The colony counts of bacteria isolated from the kunu samples are shown in Table 2. The counts ranged from 1.08×10^4 to 4.0×10^5 and 5.0×10^5 to 8.0×10^5 for both bacteria and fungi respectively. The total viable count was highest in Amansea, 1.28×10^4 CFU/ml and lowest in Eke-Awka, 1.08×10^4 CFU/ml, for Mac-conkey agar while nutrient agar 4.0×10^5 CFU/ml was observed in kunu drink from Eke-Awka. After 24hr incubation, the result shows that fresh kunu have a high bacteria and count. Efiuwevwere and Akoma, [18] gave a similar report in kunu zaki produced/hawked in Jos metropolis . Ekanem *et al* [14] reported colony

counts of mos 0.5×105to 3.2 ×105 for total coliform bacteria, 2.5×105 to 4.2×105 for total heterotrophic bacteria and 5.2×105 to 8.0×105 for total heterotrophic fungi for the three kunu drinks obtained from different market at Ikot Ekpene, Akwa Ibom State.

Therefore, contamination leads to high colony count which result of spoilage. This cause might be due to poor hygiene, low quality; contaminated cereals that are unfit for human consumption are being used.

Table 2 also show Total number of fifteen isolates were obtained from all the samples which were subjected to biochemical tests for characterization and identification.

In sample 1 and sample 3, six isolates were obtained respectively while, three isolate was isolated from sample 2. Sample 1A, 1B, 2A and 3C stained positive with cocci shape. It showed negative reaction for, indole production, Voges-Proskauer, methyl red, spore staining, urea, citrate utilization tests, positive reaction to catalase, coagulase, motility tests. The result of the tests carried out showed that *Staphylococcus* sp was the most dominant organism.

Sample 3A stained negative, rod shaped, negative reaction for motility, spore staining ,citrate, indole production tests, positive reaction for catalase, Voges-Proskauer, coagulase tests and produced acid . The results gotten from the tests indicated that *Lactobacillus* species was the probable organism.

Sample 3A stained positive, rod shaped, negative reaction for motility, citrate Voges-Proskauer, spore staining, indole production tests, positive reaction for catalase, , coagulase tests and produced acids. The results gotten from the tests indicated that B*acillus* species was the probable organism

Sample 3B stained negative, rod shaped, negative reaction for citrate, coagulase, spore staining, Voges-Proskauer and methyl red tests and positive reaction for motility, catalase, indole production tests. The results obtained from these tests indicated that *Escherichia coli* was the probable organism.

The bacterial isolates isolated from these kunu samples include; Staphylococcus sp., Bacillus sp., Lactobacillus sp. *Escherichia coli* and (Table 3a and Table 3b) while the fungal isolates were the species of *Saccharomyces, Aspergillus and Penicillium* species. This work is similar to that observed in the work of [14], who isolated ten microbial isolates including six species of bacteria and four species of fungi were isolated and identified from the kunu samples. The bacterial isolates include; Lactobacillus sp., Staphylococcus sp., Streptococcus sp., Salmonella sp., Escherichia coli and Pseudomonas sp while the fungal isolates were the species of Fusarium, Aspergillus, Penicillium and Saccharomyces sp.

Table 4 shows the frequency and percentage occurrences of isolate in each sample. Staphylococcus sp, Lactobacillus sp. and Saccharomyces sp. have a 100% frequency in all the samples analysed. The percentage occurrences in each sample are 75%, 50% and 100% for samples A, B and C respectively.

A similar work was reported by Osuntogun and Abiola [19], who isolated lactic acid bacteria (*Lactobacilli*) which have the capacity of fermenting carbohydrates into lactic acid, hence reducing the pH. This work is in agreement with the work of Olasupo *et al.* [20] Reported the

isolation of *Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Klebsiellasp. and Enterococcus faecalis* from kunu drink. Akinrele *et al.* [21] reported that the yeasts *Saccharomyces cerevisiae, Candida mycoderma* and molds Cephalosporium, Fusarium, Aspergillus and Penicillium are the major organisms responsible for the fermentation and nutritional improvement of Nigerian locally cereal based fermented drinks (ogi and kunu-zaki). This view is supported by the reports of [22 and 23], who noted that lactic acid bacterium (LAB) such as Lactobacillus spp., Streptococcus spp. and Leuconostoc spp as well as other food-borne microbial pathogens such as *Escherichia coli, Staphylococcus aureus, Bacillus cereus* and Salmonella spp. among others was observed in the spoilage of kunu.

Many researchers have vetted this to the presence of fermentative microbes present in kunu which causes contamination of the drink due to fermentation of its carbohydrate content, hence producing changes in them. This change has a negative in the taste and aroma of the drink, thereby making it unfit for human consumption.

However, the poor hygienic practices of kunu producers and vendors, introduce high microbial loads in the drink if not killed during the boiling process [14].

Amusa and Odunbaku [24], noted that poor hygiene and preparation practices during production process contaminates the foods which might cause food-borne illnesses.

The study pointed out that some Kunu drinks sold within the study area were contaminated isolation of different pathogenic microorganisms can serve as sources of infections to human.

Table 1: pH and temperature results of Kunu Drinks Sold at Awka Metropolis at Three Different Locations

LOCATIONS	pH	Temp
LOCATION A	4.0	$20^{o}C$

LOCATION B	3.8	$20^{o}C$
LOCATION C	4.5	$23^{o}C$

Where; Market Location; A =Amansea, B=Eke-Akwa and C=Amawbia,

Table 2: Total Microbial Count of Kunu Drinks Sold at Awka Metropolis at Three Different Locations

LOCATIONS	KUNU MAC (cfu/ml)	NA(cfu/ml)	PDA(cfu/ml)	Total No MOS
LOCATION A	1.28×10^{4}	2.0×10^{5}	5.0×10^{5}	2
LOCATION B	1.08×10^{4}	4.0×10^{5}	8.0×10^{5}	1
LOCATION C	2.3×10^{5}	3.0×10^{5}	5.0×10^{5}	3

Where; Market Location; A =Amansea, B=Eke-Akwa and C=Amawbia,

MAC = MacConkey Agar, NA = Nutrient Agar, PDA= Potato Dextrose Agar; Cfu/ml = Colony forming unit per millimetres

Table 3a: Morphological and Biochemical Characteristics of the Bacterial isolated from Kunu Beverages.

isolated Strain	morphology	Gram's	Catalase	Oxidase	Motility	Citrase	Spore Stain	Indole	Methyl Red	VoguesProskauer	H_2S Production	Urease	Glucose	Sucrose	Lactose	Mannitol	Probable organism
1.	Cocci	+	+	-	+	-	-	-	-	-	+	-	+	+	-	+	*Staphylococcus spp*
2.	Rod	-	+	-	-	-	-	-	+	+		-	A	-	-	A	*Lactobacillus spp*
3.	Rod	+	+	+	-	+	+	-	-	-	+	-	A	-	A	A	*Bacillus spp*
4.	Rod	-	+	-	+	-	-	+	-	-		-	A/G	A/G	A/G	-	*E.coli*

Key: A/G=Acid and Gas production. ***A= Acid production.***

Staph.sp = *Staphylococcus* specie; *Lactobacillus* specie; *Bacillus*. sp = *Bacillus* specie;

Lact. sp = *E. coli* = *Escherichia coli*,

.

Table 3b: Morphological and Cultural Characteristics of Fungi Isolates

Fungal Isolates	Cultural characteristics	Morphological characteristics	Microscopy	Identification
1C2B3D	Colourless	Nil	Egg shaped	*Saccharomyces sp*
1D	Yellowish-green mycelium	Conidia in long chains, branched cells	Branched smooth conidiophores, brush-like conidia head	*Penicillium* sp
3E	Woolly white, black domains	Conidia in chains	Non-septate hyphae with branches	*Aspergillus* sp

Table 4: Frequency and Percentage Occurrence of the Isolates (Bacterial) in the sample each

Sample	Staph	Lacto.sp	Bacillus.sp	E.colis	Sacc.	Aspe	Pen	Total

								(%)
A	+	+	+	+	+	+	-	75
B	+	+	+	-	+	-	-	50
C	+	+	+	+	+	+	+	100

Key: **Where; Sample***; A* =Amansea, B=Eke-Akwa and C=Amawbia,

Then Staph.sp=Staphlocooccus; Lact. sp = Lactobacillus specie; Bacillus. sp = Bacillus specie; E. coli = Escherichia coli; Sacc.= Saccharomyces sp.; Aspe= Aspergillus sp.; Pen= Penicillium sp.

Conclusion and Recommendation

From the result obtained, it could be seen that the probable organisms associated with kunu-zaki were *Staphylococcus* sp, *Lactobacillus* sp., *Aspergillus* sp. *and Penicillium* sp. Despite the nutritional values of Kunu-zaki, the presence of these organisms rendered it unfit for human consumption. As a result of this, safety and public health of the consumers need to be safe guarded. Good standard practices should be set in acquisition of raw materials, production and packaging of this beverage. Proper personal hygiene should be observed by the handlers to ensure their body secretions not to get in contact with the beverage during processing. Again, Addition of spices to the kunu drink has been shown to inhibit microbial growth. Processed kunu-zaki beverage should be stored properly and refrigerated.
Finally, Producers and vendors of kunu-zaki should be encouraged to utilize the technical assistance of National Agency of Food and Drugs Administration and Control (NAFDAC) towards attaining quality standards.

COMPETING INTERESTS: Authors have declared that no competing interests exist.

ACKNOWLEDGEMENT : The authors appreciate **Late Associate Professor. Archibong, Etim, Joseph** for his supervision and mentorship during the research period. We also appreciate Chychy Gilgal Laboratories and Consultancy Services Anambra State, Nigeria, for providing necessary assistance for the realization of this research work.

References

1. GaffaT, Jideani IA, Nkama I. Nutrient and sensory qualities of kunun-zaki from different saccharifying agents. Inter. J. Food Sci. Nutrition 2002; 53: 109-115.

2. Belewu MA, Abodunrin OA. Preparation of kunu from unexploited rich food source: Tigernut (*Cyperusesculentus*). World Journal of Dairy and Food Sciences 2006;1 (1), 19-21.

3. Umaru GA, Tukur IS, Akensire UA, Adamu Z, Bello OA, Shawulu AHB, Sunkani JB, Adamu SG, Adamu NB. (2014). Micro flora of ofkunu-zaki and sobo drinks in relation to public health in Jalingo metropolis, North-Eastern Nigeria. International Journal of Food Research 2014;1, 16-21.

4. Adeleke RO, Abiodun OA. (2010).Physico-chemical Properties of Commercial Local Beverages in Osun State, Nigeria. Pakistan Journal of Nutrition 2010*;* 9 (9), 853-855.

5. Aboh MI, Oladosu P. Microbiological assessment of kunu-zaki marketed in Abuja Municipal area council (AMAC) in the Federal Capital Territory (FCT). Nigeria. African Journal of Microbiology Research 2014;8 (15), 1633-1637.
6. Adelekan AO, Alamu AE, Arisa NU, Adebayo YO, Dosa AS. Nutritional, Microbiological and Sensory Characteristics of Malted Soy Kunu-Zaki: An Improved Traditional Beverage. Advances in Microbiology 2013;3, 389-397.

7. Ofudje EA, Okon UE, Oduleye OS. Williams OD. Proximate, mineral contents and microbial analysis of kunu-zaki (A non-alcoholic local beverage) in Ogun State, Nigeria. Journal of Advances in Biology and Biotechnology *2016;*7 (1), 1-8.

8. Harrigan, WF; McCance, ME. Laboratory methods in food and dairy microbiology. Academic Press Ind. Ltd, London, 1996;pp: 25-29, 157,285-303.

9. Onyeagba A. Laboratory guide for microbiology. Crystal Publishers, Owerri, Imo State 2004.

10. Cheesbrough M. District Laboratory Practice in Tropical Countries, Part 2. Cambridge University Press, 2006; 52-70.

11. Holt JG, Kreig NR, Sneath,PHA, Stanley JT, William ST. Bergey's manual of Determinative Bacteriology. Baltimore, USA: William and Wikins 2004;213.

12. Kulwant SJC. An illustrated manual on identification of some Seed borne Aspergilli, Fusaria, Penicillia and their Mycotoxins. Hellerup, Denmark. Danish Government Institute of Seed Pathology for Developing Countries and Department of Biotechnology, The technical university of Denmark 2001.

13. Barnet HL, Hunter BB. Illustrated Genera of Imperfect Fungi. 3rd Edn., Burges Publishing Company, Minneapolis, 2002; 331.

14. Ekanem JO, Mensah BJ, Marcus NS, Ukpe BA. Microbial Quality and Proximate Composition of Kunu Drinks Produced and Sold in Ikot Ekpene Metropolis, Akwa Ibom State, Nigeria. J. Appl. Sci. Environ. Manage. 2018;22 (11) 1713–1718. Full-text Available Online at https://www.ajol.info/index.php/jasem ,http://ww.bioline.org.br/ja

15. Otaru AJ, Ameh CU, Okafor JO, Odigure JO, Abdulkareem AS. Development, carbonation and characterization of local millet beverage (Kunu). Inter. J. Computational Engineer. Res. 2013;3(4):80-86.

16. Innocent OO, Mariam YO, Blessed K, James TW. Microbial evaluation and proximate composition of kunuzaki, an indigenous fermented food drink consumed predominantly in Northern Nigeria. Internet J. Food Safety, 2011;13:93- 97.

17. Amusa NA, Ashaye OA. Effects of processing on nutritional, microbiological and sensory properties of kunu-zaki (A sorghum based non alcoholic beverage) widely consumed in Nigeria. Par. J. Nutr. 2009; 8(3):288-292.

18. Efiucwere BJO, Akoma O. The Microbiology of Kunu- Zaki a cereal Beverage from northern Nigeria during the fermentation (production) process. J. Microbial. Biotechnol. 2015;11:491-493.

19. Osuntogu B, Aboaba OO. Microbiological and physicochemical evaluation of some nonalcoholic beverages. Pakistan J. Nutrition, 2004;3:188-192.

20. Olasupo NA, Smith SI, Akinside KA. Examination of the microbial status of selected indigenous fermented foods in Nigeria. J. Food Safety 2002; 22 (2): 85.

21. Akinrele IA, Adeyinka O. Edwards CA. The development and production of Soy-ogi, a corn based complete protein food. FIIRO Res.1980;Report No. 42.

22. Bibek R. Fundamental Food Microbiology, 2nd Edition. The CRC Press ltd, Washington DC. 2001;56-90.

23. Amusa NA, Ashaye OA. Aiyegbayo AA, Oladapo MO, Oni MO, Afolabi OO. Microbiological and nutritional quality of hawked sorrel drinks (Soborodo) (the Nigerian locally brewed soft drinks) widely consumed and notable drinks in Nigeria. Int. J. Food Agric. Environ. 2005;3: 47-50.

24. Amusa NA; Odunbaku OA. Microbiological and Nutritional Quality of Hawked Kunu (A Sorghum Based Non-Alcoholic Beverage) Widely Consumed in Nigeria. Pakistan Journal of Nutrition 2009;8 (1): 20-25.