Storage Effect on Microbial Load of Kunu Commonly Consumed by Tertiary Institution Students in Ekiti and Ondo States under Various Preservative Regimes

¹Oyarekua Mojisola, ²Ojo Olabimpe Iyabo, ³Ojo Oluwaseun Adedayo and ²Adeniran Ogunbiyi

¹Provost College of Education Ikere Ekiti, Ekiti State, Nigeria. ²Department of Chemistry, College of Education, Ikere Ekiti, Ekiti State, Nigeria. ³Department of Microbiology, Federal University of Technology, Akure, Ondo State, Nigeria. Corresponding Author: Oyarekua Mojisola

Abstract: Humans are exposed to toxic substances and microorganism through different sources but the most important are foods and drinks. Exposure to toxic substances or microorganisms may bring about harmful effects. Previous studies on the assessment of quality of Kunu drinks commonly consumed in Nigeria especially by the students were focused on the nutritional properties while less attention has been paid to the microbiological quality of kunu drinks hawked and consumed by students within Tertiary Institution campuses especially in Ekiti and Ondo States. Given the peculiar nature of the feeding habits of the students who eats junk foods, any drink that quench thirst and also energy given. Hence this study was designed to investigate the microbiological quality of freshly processed laboratory and hawked kunu drinks commonly consumed by students in College of Education Ikere Ekiti, Ekiti State, under different preservative regimes. The result obtained from this study showed that both hawked and laboratory prepared kunu within the study area were contaminated and contained different pathogenic microorganisms

Bacillus, cereus, Bacillus Subtilis Micrococcus lactis, Micrococcusluteus, Staphylococcusepidemidis and E-coli were identified to be associated with both hawked and laboratory processed kunu samples. In both locally prepared hawked and laboratory processed kunu samples under the preservatives regime of ginger, garlic, mixture of garlic and ginger, sodium benzoate, the one with sugar alone without preservatives stored for 3 days at room and refrigerating temperature, the result of the occurrence of microbial isolates revealed that E-colihas the least percentage occurrence while staphylococcus has the highest values. The result showed that sodium benzoate is the most active preservatives with minimum bacterial and fungi counts while ginger is the least active with the highest bacterial and fungi count. This article concludes that preservatives and also that the preservatives sodium benzoate is more efficient than that of natural preservatives and also that the preservation should not be longer than 24 hours. The differences in the statistical analysis of the samples data were considered significant at P<0.05.

Key Words: Kunu, Microbial load, bacterial and fungi count, students.

Date of Submission: 02-12-2019	Date of Acceptance: 18-12-2019

I. Introduction

The effect of economic crisis cannotbe underestimated on beverages production and health care delivery system especially in Nigeria, thereby causing majority of the populace, especially the tertiary institution students in Ekiti and Ondo States to look for an alternative and affordable natural and locally prepared beverage like 'kunu'.

This local indigenous Nigerian drinks is a non-alcoholic drink which is made from cereal grains such as maize, sorghum, and millet etc. (Ikpoh et al., 2013). Garlic, ginger which are added to enhance its flavor and also serve as preservatives. Honey or sugar is also added to serve as sweetener. (Gaffa et al., 2002).

'Kunu' drink is becoming popular in urban cities because they are cheaper than all these carbonated soft drinks like Coke, and Fanta which may believe to be associated with diabetics (Otaru et al., 2002).

The high demand for 'kunu' drinks among student is believe to be due to the presence of carbohydrate which serve as source of energy for their academic stress, also protein although in low content and vitamins.(Waki, 2004). Artisans, traders, market women, commercial drivers, students and children have developed great interest in 'kunu 'and frequently drinking it during tropical heat and stress.

Therefore, 'kunu' is also consumed for their thirst quenching property and their stimulating effect (Osuntogun and Aboaba, 2004).

'Kunu', when exposed to the air without refrigerating for two or three days, its in nutritive quality which may lead to spoilage of the 'kunu' product due to enzyme actions, poor handling and consequence fermentation of the carbohydrate contents (Elmahrnood, and Doughari, 2007)

According to health record, millet contains polynutrient called lignin which has cancer fighting properties and is beneficial in the treatment of heart diseases and also reduces the risks associated with diabetics(Umaru et al., 2014). Ginger and garlic (preservatives) contents in 'kunu' help to lower cholesterol level and prevent formation of blood clots, chronic inflammatory disease such as arthritis and as well as negative cognitive functioning.

Although no matter how nutritive or therapeutic the drink may be, storage effect, which involve microbial activities is a unique factor which may negatively affect and reduce the nutrients of the beverage(Amusa and Ashaye, 2009). However, the incidence of food and drink related diseases are becoming alarming among tertiary institution students in Ekiti and Ondo States. Previous studies on Kunu in Ondo and Ekiti State, southern part of Nigerians, focused mostly on the nutritive aspect of it with only sparse scientific information available on level of microbial loads intake from the local beverages especially 'kunu' drinks that are commonly consumed by the students nowadays. Hence the choice of using College of Education Ikere -Ekiti and Federal University of Technology Akure, Ondo State with some reported cases of drink related diseases among student according to the response through interview method designed to get information from the respondent's. Such diseases as upper respiratory tract infection, abdominal ailment and Alzheimer (mental disorder) falls into those category listed by Agency for toxic substance and disease registry (2011) were indicated as common ailments experienced by the respondents which justified this study.

The aim of this study was to investigate the microbial loads of locally and laboratory processed 'kunu' subjected to different preservative regimes.

II. Materials And Method

Hawked Kunu drinks samples prepared with same method according to the hawkers through interrogation were purchased from two different locations where students regularly bought their kunu drinks within Federal University of Technology, Akure and College of Education Ikere-Ekiti campuses. Samples of kunu drinks purchased were properly labelled in sterile plastic containers. The samples were brought to the laboratory on ice for microbiological analysis.

LABORATORY PREPARATION OF KUNU

The raw materials for the preparation of the Kunu were purchased from a major market in Ondo State, South Western Nigeria. These materials were *sorghum bicolor*, *pear millet*, gallic (*Allium sativum*), sugar and Ginger (*Boesebergia rotunda*). Sands and other solid impurities were removed through physical sorting, from the *sorghum bicolor* and *pear millet*. Each was then soaked separately for about 24 h after which the ingredients like ginger and gallicwere added. It was ground very well in a hygienic way and sieved with a very clean and white cloth. The filtrate was fermented for 24 hours, during which the slurry was allowed to settle and sediment. The supernatant liquid was decanted and the residue was mixed with water and divided into two. Half of the residue was boiled and the second half was poured into it to produce 'Kunu'. After this, water was added to meet the generally acceptable consistency and texture i.e., not too watery and not too thick. Sugar was added to taste some. The final product subsequently served as the stock for further experiments.

The following preservatives were used, Sodium benzoate, gallic, ginger, mixture of gallic and ginger. The samples were prepared, one set for storage at room temperature and the other, at low temperature, in the refrigerator. Each experiment and the control, which had no preservative was in duplicate.

Preparation of preservatives

Sodium benzoate: Two grams of Sodium benzoate was weighed and dissolved in 2 ml of distilled water. The Sodium benzoate solution was added to a 100 ml bottle of 'Kunu' sample and was mixed thoroughly.

Gallic: Some pieces of gallic weregroundinto fine powder and mixed with little water and put into a sterile beaker. With a sterile syringe, 5 ml was removed and added into a 100 ml bottle of 'Kunu' sample and shaken thoroughly.

Ginger: Some pieces of ginger were ground into fine powder and mixed with little water and put into a sterile beaker. With a sterile syringe, 5 ml was removed and added into a 100 ml bottle of 'Kunu' sample and shaken thoroughly.

Mixture of Gallic and Ginger: Some pieces of gallic and ginger of equal weights were ground into fine powder and mixed with little water and put into a sterile beaker. With a sterile syringe, 5 ml was removed and added into a 100 ml bottle of 'Kunu' sample and shaken thoroughly.

Isolation of bacteria and fungi and Determination of aerobic plate Count

Samples were serially diluted with sterile distilled water aseptically before inoculation. The media used for bacteria isolation were Nutrient Agar, Mannitol Salt Agar, and MacConkey agar while Potato dextrose agar was used for fungal isolation. The pour plate method was used for the isolation of bacteria and fungi.

Plates containing Nutrient Agar, Mannitol salt agar, and MacConkey agar were incubated for 24 hour at 37°C, while PDA plates were incubated at 30°C for 96 hr. Pure isolates of bacterial and fungal isolates were obtained and stored onto Nutrient Agar and PDA respectively. The stock cultures were then preserved in a refigerator at 4°C and used for further analyses of the organisms. The average microbial loads of the samples obtained from the different locations were expressed as Colony Forming Units per milliliter {CFU/mL} of Kunu drinks (Cheesbrough, 2004).

Identification of bacteria and fungi isolates.

The identification of the bacterial isolates was accomplished by the observation of colonial characteristics, Gram reaction and biochemical tests (Mbachu et al., 2014). Fungal isolates were identified using colonial appearance and microscopic characteristics.

Total Bacteria Count (TBC)

Standard microbiological methods were deployed in the TBC analysis. In each case, about 10 ml of the sample was aseptically introduced into 90 ml of sterile normal saline and thereafter mixed properly by serial dilution to a concentration of 10^4 - $10^50.1$ ml of the diluted sample was then used for the inoculation of freshly prepared media using spread-plate approach and thereafter incubated for 36 hours at a temperature of 37'C. Colonies were counted via digital colony counter[AOAC, 1990].

Fungi Count (FC)

The method described by Ikpoh et al (2013). was adopted. Briefly pour prate method was used in plating on sabourand dextrose agar. Dilution was achieved by diluting 1 ml of kunu sample with 9 ml of water. 0.1 ml was thereafter plated out into molten sabourand dextrose agar plate in triplicates and was spined gently. The content was allowed to solidify and incubation was done at 28°C for 72 hours [Innocent et al., 2001]. Total bacterial and fungi count for all samples was recorded for each day.

Statistical Analysis

All samples were in triplicate. The statistical analysis were conducted using one way ANOVA procedures. Statistical differences in samples were tested for at p<0.05 Duncan's New Multiple Range Test (DNMRT) was used to separate the mean values.

All analysis were done with spss (11.0) software.

III. Result And Discussion

Table 1 shows the mean and standard deviation values of the bacterial count in 'Kunu' stored for three days under various preservatives regimes.

The result of the bacterial counts of 'Kunu' under different preservatives regimes as reported in Table 1 varied from a low $1.33\pm0.58\times10^2$ cfµ/ml in laboratory processed 'kunu' stored for two days with garlic preservatives at refrigerating temperature (D₂FT) to a very high 697.33±7.37x10² cfµ/ml observed in the same 'kunu' sample stored for two days with garlic and ginger preservatives under room temperature.

It was observed that all the kunu samples preserved with sodium benzoate both at room temperature and refrigerating temperature contains moderated bacterial count.

This result compares well with the value of bacterial count for 'kunu' preserved with sodium benzoate in a research study carried out on microbial load and keeping quality 'kunu' under various preservatives regimes by Fapohunda and Adeware (2012). The result indicated that 'kunu' samples sweetened alone with sugar without any preservatives contained the least average bacterial count in all the investigated samples, in day (0) the day of preparation and increases gradually to a very high level of 84.00 4.58x10²CFU/mL at the third day of preparation at room temperature.

The report generally shows that locally and laboratory prepared kunu samples spiced and preserved with the combination of garlic and ginger has the highest bacterial count and this can be detrimental to human health. This result is similar to the result of a research carried out on Microbiological analyses of hawked kunu drinks within LAUTECH Campus, Ogbomoso, Oyo State, Nigeria by (Ayandele, 2015).

Table 2 showed the reports of Fungi count of the kunu samples. The table of result indicated that the fungi count ranged from low $3.00 \ 1.00 \times 10^2 \text{CFU/mL}$ in kunu sample (DIFT) preserved with sodium benzoate at room temperature to $7.67 \ 2.31 \times 10^2 \text{ CFU/mL}$ and the one (DIFT) preserved with garlic and ginger at refrigerating temperature while kunu samples stored for three days spiced and preserved with mixture of ginger

and garlic under room temperature has the highest fungi count of $7.67 \ 2.31 \times 10^2 \text{CFU/mL}$.the result compares well with the result of the workdone on microbial analysis of kunu in Ogun State by (Ofudje, 2016).

In a null shell, the research result indicated that the most active preservative against the fungi which has the minimum fungi count is Sodium benzoate. Ginger has the least reactive ability on fungi.

Preservatives	D0	D1RT	D1FT	D2RT	D2FT	D3RT	D3FT
SUGAR	12.00 ± 2.65	8.67 ± 1.53	8.33 ± 3.06	71.67 ± 2.89	1.33 ± 0.58	$84.00{\pm}4.58$	22.00±2.65
GARLIC	$17.33{\pm}3.06$	$14.67{\pm}2.52$	$10.67{\pm}1.53$	109.00 ± 9.54	0.33 ± 0.58	$502.33{\pm}~5.13$	$14.67{\pm}0.58$
GIINGER	30.00 ± 2.65	49.33 ± 3.21	30.00 ± 3.61	$28.33{\pm}2.08$	94.67 ± 3.79	$59.67{\pm}2.52$	102.33 ± 4.04
GARLIC AND GINGER	37.67±3.21	$12.33{\pm}0.58$	3.00 ± 1.00	697.33 ± 7.37	540.67 ± 27.65	327.00 ± 4.58	395.33 ± 4.16
SODIUM BENZOATE	$44.33{\pm}3.51$	$16.33{\pm}2.08$	19.00 ± 4.36	27.67±3.06	11.33 ± 1.53	$62.33{\pm}2.08$	$73.67{\pm}5.51$
LOCALLY PREPARED	30.00±2.00	37.33 ± 2.08	37.00 ± 3.00	35.00 ± 2.65	43.33 ± 3.06	323.00 ± 7.94	4.33±1.53

TABLE 1: AVERAGE BACTERIAL COUNT (x10 ² CFU	/mL)
--	------

Do - Day of preparation

D1RT - Day 1 Room Temperature

D1FT - Day 1 Refrigerating Temperature

D2RT - Day 2 Room Temperature

D2FT - Day 2 Refrigerating Temperature

D3RT - Day 3 Room Temperature

D3FT - Day 3 Refrigerating Temperature

-		I / BIUIGE		ouri (m	o er er mil	,	
Preservatives	D0	D1RT	D1FT	D2RT	D2FT	D3RT	D3FT
SUGAR	4.67 ± 3.06	5.33 ± 2.52	4.67 ± 3.06	4.67 ± 3.06	5.67 ± 0.58	4.00 ± 2.00	4.33±.08
GARLIC	5.33 ± 3.51	4.00 ± 1.00	5.33 ± 2.52	6.33 ± 1.53	6.33 ± 3.06	$4.67{\pm}0.58$	5.33 ± 2.52
GIINGER	4.33±1.53	4.00 ± 1.00	5.00 ± 1.00	7.00 ± 1.73	4.67 ± 2.08	7.00 ± 1.00	$5.67{\pm}2.08$
GARLIC AND GINGER	5.33±2.52	4.00 ± 2.00	3.00 ± 1.00	6.00 ± 1.00	7.33 ± 1.15	7.67 ± 2.31	6.00 ± 3.46
					3.67	7.33	
SODIUM BENZOATE	5.00 ± 2.65	4.00 ± 2.00	5.00 ± 2.65	5.00 ± 2.65	± 1.53	± 2.08	5.00 ± 1.73
LOCALLY PREPARED	5.67 ± 2.52	5.00 ± 1.00	$6.67{\pm}0.58$	4.67 ± 1.15	6.00 ± 2.00	5.33 ± 2.52	$5.67{\pm}1.53$
LOCALLY PREPARED	$5.67{\pm}2.52$	5.00±1.00	$6.67{\pm}0.58$	$4.67{\pm}~1.15$	6.00±2.00	5.33 ± 2.52	5.67 ± 1.53

TABLE 2: AVERAGE FUNGI COUNT (x10²CFU/mL)

Do - Day of preparation

D1RT - Day 1 Room Temperature

- D1FT Day 1 Refrigerating Temperature
- D2RT Day 2 Room Temperature
- D2FT Day 2 Refrigerating Temperature
- D3RT Day 3 Room Temperature
- D3FT Day 3 Refrigerating Temperature

Table 3,4 and 5 showed the occurrence of microbial isolates in kunu on day 0, first day of preparation at room temperature and n refrigerating temperature respectively while table 6,7,8 and 9 showed the occurrence of microbial isolates on the second day of storage at room temperature, second day of storage at refrigerating temperature, third day at room temperature and the fourth day of storage at refrigerating temperature and room temperature respectively Bacillus, cereus, *Bacillus Subtilis Micrococcus lactis, Micrococcus luteus, Staphylococcus epidemidis* and *E-coli* are the main microorganism that occur in the kunu sample E-coli has the least percentage occurrence of microbial isolates in kunu at the day of preparation (0) day, while *staphylococcus epidemidis* has the highest occurrence (61%).

On the first day of storage at room temperature *E-coli* and *Bacillus cereus* has the least occurrence of 17% while *Staphylococcusepidemics* still has the highest percentage of occurrence. E-coli, Bacillus subtillis micrococcus lactis recorded the least percentage occurrence of (17%) while the highest percentage occurrence was found in *Staphylococcus epidemics* again on the same first day of storage at refrigerating temperature. The result compares well with the research result on work done on Microbial Quality of Kunu Drink Sold in Calabar, Cross River State, Nigeria (Mbachu et al., 2014).

At room temperature on the second day of storage, E-coli has the least percentage of occurrence while *Staplylococcus epidemics* and *Bacillus subtillis* has the highest percentage occurrence (67%).

E-coli also has the least percentage occurrence (0%) while *Bacillus cereus* has the highest occurrence at the refrigerating temperature on the second day of preparation. The result compares well with the work done on Bacteriological assessment of 'kunu' a sold in Jos, Plateau State by Wonang, et al., (2001).

On the third day of preparation, at room temperature, *Micrococcus lactis* has the least percentage occurrence (17%) while staphylococcus has the highest percentage (83%).

At the refrigerating temperature, E-coli has the least percentage occurrence (0%) while staphylococcus epidemics has the highest (83%) as indicated through the result analysis

Storage Effect on Microbial Load of Kunu Commonly Consumed by Tertiary Institution Students In

The result is similar to the research done on the isolation of S. aureus, *proteus. sp, streptococcus sp, Bacillus sp, E-coli* and yeast from fresh and fermented milky product by Amech and Abubakar (2002). The differences in the statistical analysis of the samples data were considered significant at P<0.05.

TABLE 3 OCCURRENCE OF MICROBIAL ISOLATES IN KUNU ON THE (0) OF PREPARATION

Isolate	Sample						Percentage Occurence
	Sugar Only	Garlic Only	Ginger Only	Garlic And Ginger	Sodium Benzoate	Locally Prepared	
Bacillus cereus	-	+	-	-	-	-	17
Bacillussubtills	-	+	+	-	-	-	33
Micrococcus lactis	+	-	-	-	+	-	33
Micrococcus luteus	+	-	-	+	-	+	50
Staphylococcus <u>epidemidis</u>	-	-	+	+	+	+	667
E.coli	-	-	-	-	-	-	0

Keys: + = Present, - = Absent

TABLE 4OCCURRENCE OF MICROBIAL ISOLATES IN KUNU ON THE FIRST DAY (1) OF PREPARATION AT ROOM TEMPERATURE

Isolate	Sample						Percentage Occurence
	Sugar Only	Garlic Only	Ginger Only	Garlic And Ginger	Sodium Benzoate	Locally Prepared	
Bacillus cereus	-	-	-	-	-	+	17
Bacillussubtills	-	+	-	+	-	-	33
Micrococcus lactis	+	+	-	-	-	+	50
Micrococcus luteus	-	-	+	-	+	-	33
Staphylococcus epidemidis	+	-	+	+	+	-	37
E.coli	-	-	-	-	-	+	17

Keys: + = Present, - = Absent

TABLE 5 OCCURRENCE OF MICROBIAL ISOLATES IN KUNU ON THE FIRST DAY (1) OFPREPARATION AT REFRIGERATING TEMPERATURE

Isolate	Sample									
	Sugar Only	Garlic Only	Ginger Only	Garlic And Ginger	Sodium Benzoate	Locally Prepared				
Bacillus Cereus	+	-	+	-	-	-	17			
Bacillussubtills	-	-	-	-	-	+	33			
Micrococcus Lactis	-	-	-	+	-	-	17			
Micrococcus Luteus	-	+	-	-	+	-	33			
Staphylococcus Epidemidis	-	-	+	+	+	+	67			
E.Coli	-	-	-	+	-	-	17			

Keys: + = Present, - = Absent

TABLE 6 OCCURRENCE OF MICROBIAL ISOLATES IN KUNU ON THE SECONDT DAY (2) OF PREPARATION AT ROOM TEMPERATURE

Isolate	Sample						Percentage Occurence
	Sugar Only	Garlic Only	Ginger Only	Garlic And Ginger	Sodium Benzoate	Locally Prepared	
Bacillus Cereus	-	-	+	-	-	-	17
Bacillus Subtills	+	+	-	+	-	+	67
Micrococcus Lactis	+	-	-	-	+	-	33
Micrococcus Luteus	-	-	+	-	+	-	33
Staphylococcus Epidemidis	-	+	+	+	-	+	67
E.Coli	-	-	-	-	-	-	0

Keys: + = Present, - = Absent

TABLE 7 OCCURRENCE OF MICROBIAL ISOLATES IN KUNU ON THE SECOND DAY (2) OFPREPARATION AT REFRIGERATING TEMPERATURE

Isolate	Sample						Percentage Occurence
	Sugar	Garlic	Ginger	Garlic And	Sodium	Locally	
	Only	Only	Only	Ginger	Benzoate	Prepared	
Bacillus Cereus	+	+	+	-	+	-	33
Bacillus Subtills	-	+	-	-	-	+	67
Micrococcus Lactis	-	-	-	+	-	-	17
Micrococcus Luteus	+	-	-	-	-	+	33
Staphylococcus Epidemidis	-	-	+	+	+	-	50
E.Coli	-	-	-	-	-	-	0

Keys: + = Present, - = Absent

TABLE 8 OCCURRENCE OF MICROBIAL ISOLATES IN KUNU ON THE THIRD DAY (3) OFPREPARATION AT ROOM TEMPERATURE

Isolate	Sample						Percentage Occurence
	Sugar Only	Garlic Only	Ginger Only	Garlic And Ginger	Sodium Benzoate	Locally Prepared	
Bacillus Cereus	+	+	-	-	+		50
Bacillus Subtills	-	+	+	+	+	-	67
Micrococcus Lactis	-	-	-	-	+	-	17
Micrococcus Luteus	+	-	-	+	-	+	50
Staphylococcus Epidemidis	+	+	+	-	+	+	83
E.Coli	-	+	-	+	-	-	33

Keys: + = Present, - = Absent

TABLE 9 OCCURRENCE OF MICROBIAL ISOLATES IN KUNU ON THE THIRD DAY (3) OF PREPARATION AT REFRIGERATING TEMPERATURE

Isolate	Sample						Percentage Occurence
	Sugar Only	Garlic Only	Ginger Only	Garlic And Ginger	Sodium Benzoate	Locally Prepared	
Bacillus Cereus	-	+	-	+	-	-	33
Bacillus Subtills	-	-	+	+	+	-	80
Micrococcus Lactis	+	-	-	-	-	+	33
Micrococcus Luteus	-	-	+	-	+	+	50
Staphylococcus Epidemidis	+	+	+	+	+	-	33
Ê.Coli	-	-	-	-	-	-	0

Summarv.

IV. Summary And Conclusion

Comparative study of locally and laboratory prepared kunu drink commonly consumed among the tertiary institution students in Ondo and Ekiti were examined for microbial load under various preservative regimes (garlic, ginger, mixed ginger and garlic and sodium benzoate).

Bacillus, cereus, Bacillus Subtilis micrococcus lactis, micrococcus luteus, staphylococcus epidemidis and E-coli were identified as microorganism in which staphylococcusepidemics has the highest percentage occurrence of microbial isolates while E-coli has the least percentage occurrence. Bacillus subtillis which is one of the microorganisms causing neurogenerative diseases like Alzheimer (i.e. mental disorder), parlarosin (i.e. lack of control over movement, poor balance and co-ordinatioon) and also Huntington (progressive loss of motion co-ordination) which can seriously affect the cognitive functioning of the student adversely were identified in the sample. The percentage occurrence of Bacillus of increased from low 17% in the day of preparation to high level of 33% in the first day of preparation at refrigerating temperature and to a vary high level of 67% on the second day of preparation at refrigerating temperature. It was later increased to 80% at the third day even at refrigerating temperature.

Bacillus cereus and micrococcus luteus causes pneumonia fever and arthritis respectively while E-coli which the least percentage occurrence can cause kidney infections, brain damage, high blood pressure and death etc. Kunu preparation involves cooking, a process that would eliminate all the isolates reported in this work except the heat-resistant spore former (Bacillus sp.). The presence of these organisms in Kunu thus suggests that it must have been contaminated after cooking process and after the drink had cooled down. Contamination of Kunu could come from the syrup, fermentation vessels, storage containers, sieves used for filtration, hands of the handlers and even the polythene bags or bottles in which it was packaged for sale (Wonang et al., 2001). This was also observed in the laboratory prepared drinks which were also contaminated. Therefore, there is need for high degree of sanitation during the processing of these beverages.

The occurrence of *E. coli* in kunu is an indication of faecal and environmental contamination and a signal for the presence of other enteric pathogens. Therefore, their presence may be linked to faecal, environmental and human contamination (Ameh and Abubakar 2002) which may occur probably through the use of water. Bacillus sp. has been implicated in food especially in cereals that have been cooked and stored at warm temperature (Wonang et al., 2001). These Bacillus species can produce toxin that cause pneumonia and bronchopneumonia, and besides Bacillus cereus is known to produce heat-resistant spores that cannot be eliminated by boiling. The isolation of yeasts from these drinks may be linked to contamination through air/dust, contaminated packaging material or poor hygiene and sanitation of the processing environment. Yeasts can grow at a wide range of temperature and pH and some of these fungi can produce rnycotoxins which can cause mycotoxicosis in humans (Umaru et al., 2014). Microorganisms obtained from this study showed some level of resistance to commonly used antimicrobial agents and this may be as a result of use and misuse of drugs in the society (Danladi 2014).

V. Conclusion

Based on the finding if this research work, the microbial content of these hawked marketed kunu drinks was higher and are contaminated with microorganisms which may be potentially pathogenic to human beings. The presence of some of thismicroorganisms like Bacillus subtillis at high level is toxic and injurious to the health and could have long term effect on the cognitive functioning of humans.

VI. Recommendation

To safe guard this undesirable effects the attention of the local state and Federal Ministry of Health in Nigeria should be drawn to presence of toxic microorganisms in different locally prepared beverages e.g. kunu based on the specific evidence provided by this study and also serve as part of reference for enacting regulators and laws that control inputs of preservatives in drinks by manufacturing companies and the local hawkers. More emphasis should also be made on environmental sanitation for the hawker or producers of kunu beverages.

References

- Amusa N.A, Ashaye O.A. (2009). Effects of processing on nutritional, microbiological and sensory properties of kunu-zaki (A Sorghum based non-alcoholic beverage) widely consumed in Nigeria Par. H. Nutr., 8(3) 288-292.
- [2]. Amusa N.A, Odunbaku O.A. (2009) Microbiological and Nutritional Quality of Hawked Kunu (A Sorghum based Non-Alcoholic Beverage) widely consumed in Nigeria Par. H. Nutr. 2009, 8(1) 20-25.
- [3]. Association of Official Chemists (AOAC), 1990. Official method of analysis 15 edition, AOAC Arlington, USA.
- [4]. Ayandele A.A. (2015). Microbiological Analysis of Hawked Kunu and Sono Drinks within LAUTECH Campus, Ogbomoso, Oyo State, Nigeria. Journal of Environment Science.
- [5]. Chapman AC. Some derivatives of humulena. J. Chem. Soc. London. 1982; 1330-1306.
- [6]. Cheesbrough, M. (2000). District Laboratory Practice in Tropical Countries, Part 2. Cambridge University Press, Cambridge, UK; 434pp.
- [7]. Elmahrnood, A M. and Doughari, J. H. (2007). Microbial Quality Assessment of Kunu- zaki Bevcreages Sold in Griei Town 0(' Adamawa State, Nigeria. African Journal of food Science; 011-015.
- [8]. Fapohunda S.O. and Adeware A. (2012). Microbial Load and Keeping Quality of Kunu under various preservative Regimes. Journal of Nurt. & Food Science.
- [9]. Gaffa, T.; Jodeani, I. A. and Nkama, I. (2002). Traditional Production, Consumption and Storage of Kunu: A Non- Alcoholic Cereal Beverage. Plant Foods for Human Nutrition, 57 {1}: 73-81.
- [10]. Innocent OO, Miriam YO, Blessed K, James TW. Microbial evaluation and proximate composition of Kunu zaki, an indigenous fermented food drink consumed predominantly in Northern Nigeria. Internet Journal of Food Safety 2011; 13:93-97.
- [11]. Mbachu, A.E., Etok, C.A, Agu, K.C., Okafor, 0.1., Awah, N.S., Chidi-Onuorah, L.C., Ekwuemc, VC. Ok pala, J., Ogbue, M.O. and lkele, M.O. {2014}. Microbial Quality of Kunu Drink Sold in Calabar, Cross River State, Nigeria. Journal of Global Biosciences, 3(2): 511-515.
- [12]. Ofudje, E.A, Okon U.E., Oduleye O.S. and Williams O.D. (2016). Proximate, Mineral Contents and Microbial Analysis of Kunu-Zaki (A Non-Alconolic Local Beverage) in Ogun State, Nigeria. *Journal of Advances in Biology & Biotechnology* 7(1): 1-8.
- [13]. Otaru AJ, Ameh C.U, Okafor J.O, Odigure J. O, Abdulkareem AS. Development, carbonation and characterization of local mille beverage (Kunu). International Journal of Computational Engineering search. 2013;3(4):80-86.
- [14]. Umaru, G. A, Tukur, I. S., Akensire, U. A, Adarnu, Z., Bello, O. A, Shawulu A H. B., Audu, M., Sunkani, J. B., Adarnu, S. G. and Adarnu, N. B. (2014). Microflora of Kunun-zaki and Zobo Drinks in Relation to Public Health in Jalingo Metropolis, NorthEastern Nigeria. International journal of food research, I: 16-21.
- [15]. Wakil S. Isolation and screening of antimicrobial producing lactic acid. University of Ibadan, Nigeria; 2004.
- [16]. Wonang, D. L. Amienyo. C.A. Ekelerne, O.P. and Dazol, D.G. {2001}. Bacteriological assessment of "kunu" a local beverage sold in Jos, Plateau State. Journal of Environmental Sciences; 4(1): 5-7.

OYAREKUA MOJISOLA. "Storage Effect on Microbial Load of Kunu Commonly Consumed by Tertiary Institution Students In Ekiti and Ondo States Under Various Preservative Regimes." IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT) 13.12 (2019): 19-25.