American Journal of Food Science and Health
Vol. 6, No. 2, 2020, pp. 74-79
http://www.aiscience.org/journal/ajfsh
ISSN: 2381-7216 (Print); ISSN: 2381-7224 (Online)

Microbial Assessment of Different Formulations of "Kunu": An Indigenous Non-alcoholic Beverage, Vended in Awka, Anambra State, Nigeria

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Abstract

Different formulations of "kunu", a traditional non-alcoholic beverage sold in Awka metropolis were analyzed. Five (5) "kunu" samples were obtained as freshly formulated beverages from five different locations. The total heterotrophic bacterial count ranged from 1.7 x 10^6cfu/ml to 3.2 x 10^6cfu/ml; the total coliform count ranged from 1.1 x 10^6cfu/ml to 1.6 x 10^6cfu/ml; the total fungal count ranged from 1.3 x 10^6cfu/ml to 3.3 x 10^6cfu/ml. Of the five varieties of "kunu" samples, Tiger nut showed the highest number of bacteria with 3.2 x 10^6 while Wheat showed the least bacterial count with 1.7 x 10^6. This could be attributed to the difference in the composition of the raw materials. Also, maize variety has the highest coliform count with 1.6 x 10^6 while Millet has the least coliform count with 1.1 x 10^6. Tiger nut also has the highest fungal count with 3.3 x 10^6 while Maize has the least fungal count with 1.3 x 10^6. The microorganisms isolated included bacteria: *E. coli* (30%), *Staphylococcus aureus* (10%), *Salmonella* spp (10%), *Pseudomonas* spp (20%) and *Lactobacillus* spp (30%). The fungal isolates include *Penicillium* spp, *Aspergillus* spp, *Candida albicans*, *Rhizopus stolonifer,* and *Saccharomyces* spp. The presence of high microbial load was an indication of poor hygiene and/or poor quality cereals and water used in the preparation. The results of this study showed that "kunu" prepared and sold in all the locations in Awka metropolis were grossly contaminated with potential pathogenic bacteria. Thus, there is a need to establish a system of monitoring of street vended "kunu" to make sure that it is safe for consumption.

Keywords

Non-Alcoholic Beverages, Microorganisms, Kunu, Awka Metropolis, Hawked Foods

Received: May 8, 2020 / Accepted: May 28, 2020 / Published online: June 18, 2020

1. Introduction

"Kunu" is a non-alcoholic beverage drink sold in several public places including markets, offices, schools, motor parks and as drinks during festivities, weddings and naming ceremonies [1]. "kunu" is an appetizer, food complement and refresher to quench thirst [2-5]. "kunu" is an indigenous non-alcoholic fermented beverage consumed widely especially in the northern regions of Nigeria [3]. It is consumed as a breakfast snack, used as weaning product and as a food supplement/appetizer [5]. Unlike zobo that is produced from only one plant, "kunu" can be prepared from several independent plants including sorghum (*Sorghum bicolor*), millet (*Penisetum typhoides*), maize (*Zea mays*), rice (*Oryza sativa*), wheat (*Triticum aessitivum*) acha (*Digitalis exilis*) [6, 7]. There are different types of "kunu" depending on the feedstock used for processing. This include "kunu", "kunugyada", "kunuakamu", "kunutsamiya", "kunubaule",

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"kunujiko" and "kunugayamba". Of these, "kunuzaki" is the most widely produced and consumed [7-9].

In spite of the benefits derived from this drink ("kunu"), there have been reports of microbial contamination which may cause spoilage. Some of the microorganisms implicated in the spoilage of "kunu" include lactic acid bacteria (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 [10]. Poor hygiene and preparation practices in which the production process is subjected can also introduce microbial pathogens in foods and have been implicated in causing food-borne illnesses [3]. In developing countries like Nigeria, it has not been possible to have safety control over the processing of hawked foods, because most of the vendors lack the adequate knowledge of food processing and handling practices as well as lack of portable water, proper storage and waste disposal facilities at preparation and service points have resulted in poor unsanitary conditions and thus served as potential microbial contaminants and increased risk to public health [11, 12]. The short shelf-life of these beverages are however a major problem faced by their producers and consumers.

A large number of lactic acid bacteria, coliforms, molds and yeasts cause spoilage in these drinks thereby producing undesirable changes. Food pathogens such as *Escherichia coli* have been implicated in food poisoning resulting from their consumption. The effect of storage on each drink varies and the time lag during which the drinks lose their nutritional properties vary, hence there is a need to know the more appropriate method of storage, whether ambient or refrigerated, to reduce the incidence of certain diseases [13].

This study was carried out in Awka, Anambra State. Anambra State is in the South East geo-political Zone of Nigeria. It lies between latitude 6°06'N and 6°15'N and longitudes 7°05'E and 7°15'E. The physical size and structure of Awka capital city makes it suitable to play several roles and serve several functions in the socio-economic and socio-political development of Anambra State especially its administrative functions as the capital city of the State [14]. Awka as an administrative, commercial and educational town is a rapidly growing urban center with a large percentage of migrant settlers. There is also an increase in the number of industries, commercial and recreational centres in the town. The National Population Commission (1991) gave the population of the town as approximately 60,000. The population when projected to 1996 was about 70,000, 2000 was 77,000 and 2005 was about 84,000 at a growth rate of 2.83 percent per annum [14].

This work is aimed at assessing the microbial load in "kunu" and identifying the various bacteria and fungi associated with different compositions of Kunu sold in Awka Metropolis. The objectives are, to isolate and enumerate microorganisms (bacteria and fungi) from "kunu", obtained from Awka metropolis in Anambra State, to identify and characterize the isolated organisms, and to compare the microbial load of different formulations of "kunu" sold among different sellers in Awka. metropolis.

2. Materials and Methods

2.1. Sample Collection

Two samples each of five different formulations of hawked "kunu" drinks were purchased from different locations in Awka metropolis. The formulations are;

Table 1. Sample Design.

Sample	Formulation (Raw-Material) And Location
A	- Guinea corn (*Sorghum bicolor*) from Temp Site.
B	- Millet (*Pennisetum typhoides*) from Aroma.
C	- Maize (*Zea mays*) from Eke Awka.
D	- Tiger nut (*Cyperus esculentus*) from Ifite.
E	- Wheat (*Triticum aessitivum*) from Amansea.

The samples were properly labeled, placed in plastic containers and transported to the Microbiology laboratory of Nnamdi Azikiwe University, Awka, immediately for analyses.

2.2. Isolation of Microorganisms from the "kunu" Samples

Ten (10) ml of each "kunu" samples 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 was carried out in clean sterile test tubes containing 9ml of sterile distilled water. 0.1ml of desired dilutions (10^{-3} - 10^{-5}) was spread-plated in triplicates onto nutrient agar, MaCconkey agar, Sabouroud dextrose agar. The nutrient agar and MaCconkey agar plates were incubated at 37°C for 24hrs for bacterial and coliform counts, while the Sabouroud dextrose agar plates were incubated at 35°C for 48-72 hours for fungi count. Pure isolates of bacterial and fungal isolates were obtained and stored onto Nutrient Agar and SDA slants respectively. The stock cultures was then preserved in a refrigerator 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 millilitre (CFU/ml) for bacteria and spore forming unit per milliliter (sfu ml^{-1}) for fungi of "kunu" drinks.

Distinct colonies were subcultured into another nutrient agar using sterile wire loop to streak on freshly prepared nutrient agar. The colonies were later subcultured on the appropriate

agar slants. They were incubated for 18 - 24hrs at 35°C and then stored at 4°C in refrigerator [15].

2.3. Identification and Characterization of Isolates

Bacterial isolates were characterized and identified by observation of colonial, and morphological characteristics, Gram reaction and biochemical tests. The various biochemical tests used for identification include the citrate utilization, catalase, methyl red, Voges-Proskauer, coagulase, triple sugar iron, oxidase motility and indole - ornithine tests [15]. Fungal isolates were characterized and identified based on colonial appearance and microscopic characteristics by wet mount preparation using Lactophenol in cotton blue. This was accomplished using appropriate identification scheme for yeast and molds respectively [16]. Motility test, Indole test/Methyl-Red test, Catalase test, Coagulase test, Citrate utilization test, Urease test, Oxidase test, Voges-Proskauer (V.P) test [17] and, Sugar fermentation test [18]. Germ tube test and Lactophenol cotton-blue staining technique were used for the Fungal identification [17].

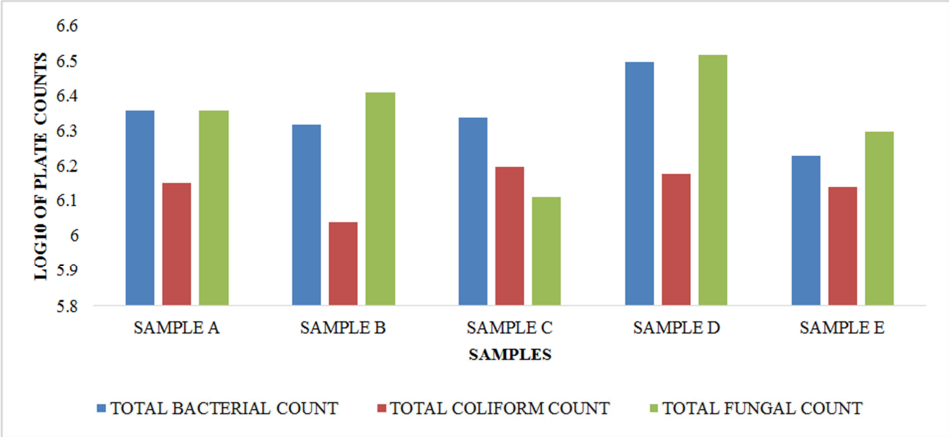

Figure 1. Total Bacterial, Coliform and Fungal counts.

3. Results and Discussion

Table 2. Total Bacterial Count of the samples.

Sample	Total Bacterial Count (CFU/ml)	Mean Bacterial Count (CFU/ml)	Log_{10} of Mean Bacterial Count
A	9.5×10^4 4.5×10^6	2.3×10^6	6.36
B	7.0×10^4 4.2×10^6	2.1×10^6	6.32
C	6.9×10^4 4.3×10^6	2.2×10^6	6.34
D	7.7×10^4 6.3×10^6	3.2×10^6	6.50
E	6.0×10^4 3.3×10^6	1.7×10^6	6.23

Table 3. Total Coliform Count of the samples.

Sample	Total Coliform Count (CFU/ml)	Mean Coliform Count (CFU/ml)	Log_{10} of Mean Coliform Count
A	3.5×10^4 2.8×10^6	1.4×10^6	6.15
B	4.0×10^4 2.1×10^6	1.1×10^6	6.04
C	5.2×10^4 3.2×10^6	1.6×10^6	6.20
D	4.1×10^4 3.0×10^6	1.5×10^6	6.18
E	5.6×10^4 3.5×10^6	1.4×10^6	6.14

Table 4. Total Fungal Count of the samples.

Sample	Total Fungal Count (SFU/ml)	Mean Fungal Count (SFU/ml)	Log_{10} of Mean Fungal Count
A	5.7×10^4 5.3×10^6	2.3×10^6	6.36
B	7.2×10^4 5.2×10^6	2.6×10^6	6.41
C	5.0×10^4 2.6×10^6	1.3×10^6	6.11
D	9.3×10^4 6.5×10^6	3.3×10^6	6.52
E	4.5×10^4 4.0×10^6	2.0×10^6	6.30

Table 5. Morphological/Cultural Characteristics of Bacterial Isolates.

Sample	Isolate	Colour	Shape	Margin	Elevation
A	SPE 1	Cream	Circular	Entire	Flat
	SPE 2	Cream	Circular	Entire	Flat
B	CED 1	Cream	Circular	Entire	Flat
	CED 2	Cream	Circular	Entire	Flat
C	BRK 1	Green	Irregular	Lobate	Flat
	BRK 2	Green	Irregular	Lobate	Flat
D	TAM 1	Cream	Circular	Entire	Flat
	TAM 2	Cream	Circular	Entire	Flat
E	SWT 1	Cream	Circular	Entire	Flat
	SWT 1	Cream	Circular	Entire	Flat

Key: Sample A = Guinea corn (*Sorghum bicolor*) from Temp Site
Sample B = Millet (*Pennisetumtyphoides*) from Aroma.
Sample C = Maize (*Zea mays*) from Eke Awka.
Sample D = Tiger nut (*Cyperusesculentus*) from Ifite.
Sample E = Wheat (*Triticumaessitivum*) from Amansea.

Table 6. Morphological/Cultural Characteristics of Mold Isolates.

Sample	Isolate	Colour	Texture
A	PRA 1	White	Cottony
B	ZED 1	Black	Filamentous
	ZED 2	Black	Filamentous
C	BAK 1	White	Fluffy
E	KRA 1	Dark brown	Fluffy

Key: Sample A = Guinea corn (*Sorghum bicolor*) from Temp Site
Sample B = Millet (*Pennisetumtyphoides*) from Aroma.
Sample C = Maize (*Zea mays*) from Eke Awka.
Sample E = Wheat (*Triticumaessitivum*) from Amansea.

Table 7. Morphological/Cultural Characteristics of Yeast Isolates.

Sample	Isolate	Colour	Shape	Margin	Elevation
A	TES 1	Cream	Irregular	Entire	Flat
B	ARO 1	Cream	Irregular	Entire	Flat
C	IFI 1	Cream	Irregular	Entire	Flat

Key: Sample A = Guinea corn (*Sorghum bicolor*) from Temp Site
Sample B = Millet (*Penisetumtyphoides*) from Aroma.
Sample C = Maize (*Zea mays*) from Eke Awka.

Table 8. Biochemical Characteristics of Yeast Isolates.

Sample	Isolate	Gram Staining	Germ Tube Test	Sugar Fermentation					Probable Organism
				Gluc	Malt	Lact	Sucr	Fruc	
A	TES 1	+	-	AG	AG	-	AG	-	*Saccharomyces*spp
B	ARO 1	+	+	AG	AG	-	-	-	*Candida albicans*
C	IFI 1	+	+	AG	AG	-	AG	-	*Candida albicans*

Key: Sample A = Guinea corn (*Sorghum bicolor*) from Temp Site
Sample B = Millet (*Penisetumtyphoides*) from Aroma.
Sample C = Maize (*Zea mays*) from Eke Awka.
AG = Acid and Gas Production

Table 9. Microscopy of Mold isolates.

Sample	Isolate	Hyphae	Type of Asexual Spores	Presence of Rhizoid/ Stolon	Probable Organism
A	PRA 1	Septate	Conidiospores	Stolon	*Aspergillus*spp
B	ZED 1	Aseptate	Sporangiospore	Rhizoid	*Rhizopusstolonifer*
	ZED 2	Aseptate	Sporangiospore	Rhizoid	*Rhizopusstolonifer*
C	BAK 1	Septate	Conidiospores	Stolon	*Penicillum*spp
E	KRA 1	Septate	Conidiospores	Stolon	*Aspergillus*spp

Key: Sample A = Guinea corn (*Sorghum bicolor*) from Temp Site
Sample B = Millet (*Penisetum typhoides*) from Aroma.
Sample C = Maize (*Zea mays*) from Eke Awka.
Sample E = Wheat (*Triticum aessitivum*) from Amansea.

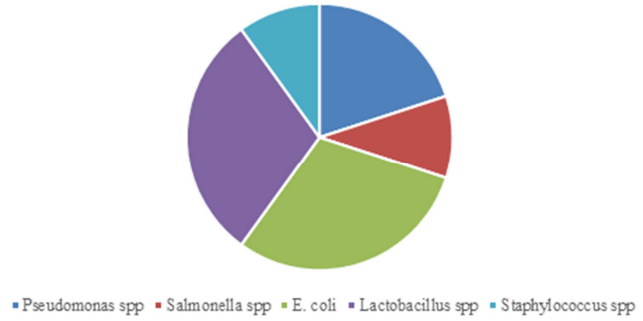

Figure 2. Frequency of Occurrence of the Bacterial Isolates.

This study provides an interesting documentation of the occurrence of microorganisms in different formulations of "kunu" samples from five markets in Awka metropolis. Of the five varieties of "kunu" samples, Tiger nut showed the highest number of bacteria with 3.2 x 10^6 while Wheat showed the least bacterial count with 1.7 x $10^{6.}$ This could be attributed to the difference in the composition of the raw materials. Also, maize variety has the highest coliform count with 1.6 x 10^6 while Millet has the least coliform count with 1.1 x 10^6. Tiger nut also has the highest fungal count with 3.3 x 10^6 while Maize has the least fungal count with 1.3 x 10^6. The microbes found associated with the different formulations of "kunu" samples comprises of bacteria which includes, *Lactobacillus* spp,, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella* spp. The isolated molds are *Aspergillus* spp, *Rhizopus stolonifer* and *Penicillium* spp and the yeasts isolated from the samples include *Candida albicans* and *Saccharomyces* spp. The presence of some of these organisms are not surprising as most of them are known to thrive in medium rich in fermentable substrates such as sugars which often led to the production of acids after fermentation. [19] reported that *Lactobacillus plantarum* was the predominant organism in the fermentation responsible for lactic acid production while, *Streptococcus lactis and Micrococcus acidiophilis* are known to be involved in fermentation of agricultural produce. The presence and the activities of these fermenters might be responsible for the souring of taste usually observed if not consumed within six hours of processing. *Staphylococcus* and *Pseudomonas* species were possible contaminants from handlers and utensils used especially after the processing, as they are mesophiles though some *Pseudomonas* are spoilage

organisms at refrigerated temperatures. The presence of *Pseudomonas* spp in the marketed "kunu" drink might be responsible for changes in taste which normally occurs if not consumed within few hours of production. The ropiness associated with the drink has been associated with the presence of both *Pseudomonas* spp. and *Bacillus subtilis* [20]. Some of these associated microbes have been implicated in food poisoning outbreak of some food materials. The presence of *Escherichiacoli* in food is an indication of faecal contamination of product. However, Odunfa [21] reported that *Staphylococcus* spp levels of 10^8m/L is considered potentially hazardous to consumers. According to him, the presence of *E. coli* in water indicates faecal contamination and most of the coliforms found associated with the hawked "kunu" are known to be causative agents of food borne gastroenteritis and bacterial diarrhoea diseases [18]. The presence of *Aspergillus*spp**,** *Penicillium* spp and *Rhizopus stolonifer* in the "kunu" samples might not be too surprising as they are known common spoilage organisms of carbohydrate foods as well as storage micro flora of many cereals including sorghum. These fungi also have high survival rate of their spores hence their presence in these food drinks could have emanated from the air as air spores since most of the hawked "kunu" drinks are not usually well covered. The presence of *S. cerevisae and Candida* spp in the "kunu" samples is probably because they played a significant role in the flavor development [19, 22], had earlier reported the association of *Penicillium spp*, *Aspergillus spp Lactobacillus spp* and *Streptococcus sp* from "kunu" drink samples. However, [23] and [24] isolated *Saccharomyces cerevisiae***,** *Aspergillus niger***,** *Aspergillus flavus***,** *Escherichia coli***,** *Bacillus subtilis* and *Klebsiella* sp from fermented zobo drinks. The presence of coliform bacterial in the hawked "kunu" drinks in Nigeria is a source of concern because the teaming populace relies on these drinks as alternative to the bottled soft drinks whose price is becoming unaffordable. It is therefore suggested that "kunu"n drinks should be properly processed to avoid microbialcontamination. While treated municipal water or clean water should be used during for processing and dilution of the processed drinks to avoid contamination with enteropathogenicbacteria. Since spices have been reported to inhibit microbial growth. [19] had reported that fortification of zobo drinks with pineapple juice and lemon grass greatly enhanced the inhibition of the growth of contaminating pathogenic bacteria like *P. aeruginosa.* [23] reported that addition of some spices to groundnut products reduced the microbial load significantly. Hence the addition of spices to the processed "kunu" drinks is highly advocated. The processing environment should be hygienic, while the packaging materials should be sterilize and additives such as sugar, ginger and other coloring materials should be sterilized. Health education training should be organized regularly for the processors by the health workers on the importance of cleanness of their environment.

4. Conclusion

From the research, it could be seen that the probable organisms associated with "kunu" were *Escherichia coli, Staphylococcus aureus, Streptococcus* spp*, Lactobacillus* spp, *Candida albicans and Aspergillus* spp. and the action or presence of these organisms in "kunu" renders it unfit for human consumption. The microbial content of these hawked "kunu" drinks was high and were contaminated with microorganisms which are potentially pathogenic to man. The presence of these isolated organisms in "kunu" samples analyzed could serve as an indicator for the need to promote awareness about possible health hazards that could arise due to handling and processing. If properly processed and packaged, it will not only alleviate the longing for fluid intake in warm tropical climates but would also provide a cheaper and more nutritive drink than the sugar laden fizzy (soft) drinks in the market. Hence, regulatory agencies should intervene by setting standards in acquisition of raw material, production techniques as well as health status of personnel involved in the production process.

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