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Microbial Quality and Proximate Composition of Kunu Drinks Produced and Sold in Ikot Ekpene Metropolis, Akwa Ibom State, Nigeria

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ABSTRACT: Kunu is a non-alcoholic Nigerian beverage produced locally from cereals and has been widely accepted as a nutritional drink due to its cost hence, the need for evaluation of its nutritional and safety status. In this study, proximate composition and microbial quality of Kunu drinks sold in Ikot Ekpene metropolis were evaluated. The results show that Kunu consists of 68.5% moisture, 9.33% crude protein, 5.5% crude fat, 3.14% ash and 49.2% carbohydrate while the calorie value was 283.67kcal. The Colony counts from theKunu samples ranged from $0.5\times10^5$to 3.2×10^5 for total coliform bacteria, 2.5×10^5 to 4.2×10^5 for total heterotrophic bacteria and 5.2×10^5 to 8.0×10^5 for total heterotrophic fungi for the three samples analyzed. Bacterial isolates identification revealed the presence of *Bacillus* sp, *Pseudomonas aeruginosa*, *Escherichia coli* and *Streptococcus* sp, while the fungal isolates include; *Aspergillus flavus*, *Penicillium* sp, *Fusarium* sp and *Saccharomyces cerevisiae*. The data revealed that all the Kunu drinks sold within the study area were contaminated and also contained different pathogenic microorganisms which can serve as sources of infections to human. Therefore, proper hygienic and sanitary measures need to be enforced during processing and packaging of this local beverage drink.

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Kunu, a non-alcoholic beverage used to be consumed mainly in the Northern parts of Nigeria is now widely acceptable in almost all parts of Nigeria, owing to its refreshing and nutritive qualities as well as it being cheaper as compared to carbonated drinks (Gaffa *et al*., 2002; Adejuyitan *et al*., 2008). Kunu is a popular cereal based non-alcoholic beverage that is prepared from either guinea corn (*Sorghum bicolor*), millet (*Penisetum typhoides*), maize (*Zea mays*), rice (*Oryza sativa*) or wheat (*Triticum aestivum*). Traditionally, the production involves steeping of the whole grains for 6-24 hours, wet milling with spices and sweet potato, gelling of about three-quarter of the mixture in hot water, pitching with about one- quarter fresh (ungelled) part of the mixture and then allowing to ferment overnight and the supernatant is ready for consumption (Adejuyitan *et al*., 2008; Elmahmood, 2007 and Oranusi, 2003).It is also very nutritious and of medicinal value. Kunu has been reported to be high in demand and rich in vitamins, minerals, carbohydrates and proteins (Essien *et al*., 2009; Adebayo *et al*., 2010; Folasade and Oyenike, 2012; Oluwajoba *et al*., 2013).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 (Bibek, 2001; Amusa *et al*., 2005). 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 (Amusa and Odunbaku, 2009). 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 (Omemu and Aderoju, 2008; Sperber, 2003).Though there are a lot of literatures on the microbiological and nutritive quality of kunu drinks, there is scarce or no information on the safety and nutritional status of kunu drinks sold in Ikot Ekpene

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where this study was carried out. Hence, the study intends to evaluate the proximate composition and microbial quality of kunu drinks sold in Ikot Ekpene Metropolis as this will serve as a comparative indices as to what is obtainable in other parts of the country and help take holistic and enduring decision towards the production of this food drink.

MATERIALS AND METHODS

Study Location: Ikot Ekpene is a city in Akwa Ibom State, South-South, Nigeria. Its area is approximately 125 km2. The city's population is about 254,806. It is situated at 5.18° North latitude, 7.71° East longitude and 159 meters elevation above the sea level.

*Sample Collection***:** Samples of freshly prepared Kunu were collected from different hawkers from 3 different locations (Ator market, Whites market and Oil market) within Ikot Ekpene metropolis. The samples were labeled P, Q and R for Ator market, Whites market and Oil market respectively for ease of identification. They were transferred within an hour of collection to the laboratory in their original package and the contents aseptically withdrawn from the bottles for isolation, enumeration and identification of microorganisms. The remaining samples were used for proximate analysis.

*Preparation of Media***:** The three media (Nutrient Agar, NA; MacConkey agar, MAC; Potato Dextrose Agar, PDA) used were prepared according to the manufactures' procedures (Titan Biotech Ltd. BHIWADI-301019, Rajasthan, India)**.**

*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 milliliter of each sampled kunu drink was put in 9ml of sterile distilled water in sterile test tubes, shaken and then serially diluted. 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 to get a mean standard value of the colony forming units (cfu/ml) on the plates. The inoculated MacConkey agar, Nutrient agar and Potato Dextrose agar plates were incubated at 30ºC and 35ºC for 24 and 48 hours respectively. After the period of incubation, the colonies on the plates were counted and recorded as colony forming unit per milliliter (cfu/ml) and coliform respectively (Harrigan and McCance, 1976). Each of the bacterial colonies on the agar plates was sub-cultured and the pure culture obtained. Isolates were identified by carrying out tests which include Gram staining, spore staining and biochemical tests such as catalase, coagulase, oxidase, citrate utilization, indole, methyl red, urease, Voges-Proskauer and sugar fermentation (Onyeagba, 2004; Cheesbrough, 2006).

The bacteria isolates were characterized using microscopic techniques and biochemical tests. 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 (Holt *et al*., 1994) and Cheesbrough, (2006). The cultures used for biochemical test were between 18 to 24 hours old.

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 to illustrated manual on identification of some seed borne fungi (Kulwant *et al*., 1991) and illustrated genera of imperfect fungi (Barnet and Hunter1972).

*Proximate Analysis of Kunu Samples: Moisture Determination***:** Ten ml of sample was measured in a clean crucible using sensitive balance. The crucible with the sample was placed in an air-dry oven at 105°C and left to stay overnight. Then crucible was transferred to oven again and weighted after 2 hours,

this was repeated until constant weight was obtained. The moisture content (MC) may be calculated as follows:

$$\text{MC (\%)} = \frac{(W_2 - W_1) - (W_3 - W_1)}{(W_2 - W_1)} \text{x100}$$

Where;W_1 = weight of empty crucible; W_2 = weight of crucible + sample; W_3 = weight of crucible +dry sample

Determination of Total Ash: The ash content was determined from the loss in weight that occurred during incineration of the evaporated sample at a temperature high enough to allow all organic matter to be burnt off without allowing appreciable decomposition of the ash constituents. Ashing was carried out in a muffle furnace subjected to heat at 550°C for 6 h (AOAC, 2005).

Determination of Fat: This was carried out using the method of AOAC (2005). Clean and dried thimble were weighed (W_1) and 5 g oven dried sample will be added and re-weighed (W_2). Round bottom flask was filled with petroleum ether (40-60°C) up to ¾ of the flask. Soxhlet extractor was fixed with a reflux condenser to adjust the heat source so that the solvent boiled gently, the sample was put in the thimble and inserted into the soxhlet apparatus and extraction under reflux was carried out with petroleum ether for 6 h. After the barrel of the extractor will be emptied, the condenser was removed and the thimble removed, taken into the oven at 100°C for 1 h and later cooled in the desiccator and weighed again (W_3).

$$\text{Fat (\%)} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Estimation of Crude fibre: About 2g of the sample was defatted with petroleum ether for 2 hours. It was then boiled under reflux for some minutes with 200ml of a solution containing 1.25g of H_2SO_4 per 100ml solution. The solution was filtered through a cloth on fluted funnel and washed with boiling water until they were no longer acidic. The residue was transferred to a beaker and boiled for another 30 minutes with 200ml of a solution containing 1.25g of NaOH per 100ml. The final residue was then filtered and washed with boiling water several times until it was no longer basic. The residue was finally washed twice with methanol and quantitatively transferred into a pre-weighed crucible and dried at 105°C (1a). The furnace was incinerated at 550oc and was allowed to stand at this temperature for 2 hours. It was then cooled in a dessicator and weighed as 1_o(AOAC, 2005). The crude fibre is calculated as

$$\text{CF (\%)} = \frac{1_a - 1_o}{\text{Weight of sample taken}} \text{x100}$$

Where; 1_a = weight of empty crucible; 1_o = weight of crucible and its content after incineration

Determination of crude protein: One gram of the sample was introduced into micro Kjeldahl digestion flask and one tablet of Selenium catalyst was added. The mixture was digested on an electro thermal heater until a clear solution was obtained. The flask was allowed to cool after which the solution was diluted with distilled water to 50 and 5 of this was transferred into the distillation apparatus, 5 of 2% boric acid was added into a 100 capacity conical flask (the receiver flask) and four drops of methyl red indicator were added. A 50% of NaOH was continually added to the digested sample until the solution turned cloudy which indicated that the solution had become alkaline. Distillation was carried out in the boric acid solution in the receiver flask with the delivery tube below the acid level. As the distillation was going on, the pink colour solution of the receiver flask turned blue indicating the presence of ammonia. Distillation was continued until the content of the flask was about 50 after which the delivery of the condenser was rinsed with distilled water. The resulting solution in the conical flask was then titrated with 0.1 M HCl and the protein content calculated (Pearson, 1970; AOAC., 2005).

*Determination of Carbohydrate:*This was determined as the difference obtained after subtracting total organic nitrogen (protein), Lipid, Ash, Moisture and Fibre from the total dry matter (AOAC., 2005).
*Calculation:*Carbohydrate % = 100 – value of moisture, protein, lipid, ash and fibre

i.e CHO = (M+P+L+A+F)

Determination of Caloric Value (Energy Level): The caloric value was obtained by multiplying the value of the crude protein, lipid and carbohydrate by 4:9:4 kcal respectively and taking the sum of the product.

RESULTS AND DISCUSSION

Parameters obtained for the proximate analysis are presented in Table 1. The pH was 4.3 which indicate an acidic pH. These values are within the range of 3.80 and 3.99 reported by Innocent *et al.* (2011), 2.42 to 3.83 recorded by Otaru *et al.* (2013), 5.25 to 5.65 reported by Amusa and Ashaye, (2009). The acidity of the kunu drinks may be due to the presence of some bacteria which help in acid fermentation of the kunu products.

Moisture content was 68 5%. The percentage ash content was 3.14. These values were higher than 0.2% obtained by Otaru *et al.* (2013), but the results however agree with 2.00 to 3.00% obtained by Innocent *et al.* (2011). The percentage content of crude fat, crude fibre, crude protein and carbohydrate were 5.5, 1.0, 9.3 and 49.20 respectively. Essien *et al.* (2011) reported that loss of protein during the processing of the drinks may be responsible for the low protein content observed.

Table 1: Proximate Composition of Kunu Sample

Parameter (%)	Kunu
pH	4.3
Moisture content	68.50
Ash content	3.141
Crude fat content	5.505
Crude fibre content	1.001
Crude protein	9.333
Carbohydrate	49.200
Calorie value (kcal)	283.677

Hamad and Fields (1979) opined that high value of protein content in cereals is often found in the germ and testa which are often sifted off during the preparation of kunu product. The results of the protein content analysis in this work were found to be higher than the values recorded by Essien *et al.* (2011). The calorie level was 283.6kcal which reveals that the kunu sample analyzed was rich in carbohydrate and energy.Colony counts of bacteria isolated from fresh kunu samples are presented (Table 2**).** The counts ranged from 0.5×10^5 to 3.2×10^5 for total coliform bacteria, 2.5×10^5 to 4.2×10^5 for total heterotrophic bacteria and 5.2×10^5 to 8.0×10^5 for total heterotrophic fungi. The results indicate that fresh kunu presented a high bacteria count after 24 hr of incubation. Efiuwevwere and Akoma, (1995) also reported similar abnormality of high bacterial populations in kunuzaki prepared and sold in Jos metropolis (Hatchers *et al.,* 1992). The high colony count is an indication of spoilage as a consequence of either poor hygiene or poor quality of cereals and the water used. 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 (Table 3) while the fungal isolates were the species of *Fusarium, Aspergillus, Penicillium* and *Saccharomyces* sp (Table 4).

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

Locations	Kunu		
	MAC (cfu/ml)	NA (cfu/ml)	PDA (cfu/ml)
Location P	2.3×10^5	2.5×10^5	5.2×10^5
Location Q	0.5×10^5	4.2×10^5	8.0×10^5
Location R	3.2×10^5	3.7×10^5	5.3×10^5

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

Table 3: Morphological and Biochemical Characteristics of the Bacterial Isolates

Isolated Strain	Morphology	Gram's Reaction	Catalase	Oxidase	Motility	Citrate	Spore Stain	Indole	MethylRed	VogesProskauer	H_2S Production	Urease	Glucose	Sucrose	Lactose	Mannitol	Probable Organism
A	R	-	+	-	-	-	-	-	+	+		-	A	-	-	A	*Lactobacillus sp*
B	R	-	+	-	+	-	-	-	+	-	+	-	A	-	-	A	*Salmonella sp*
C	C	+	-	+	-	+	-	-	-	-	+	-	A	-	A	A	*Streptococcus sp*
D	R	-	+	+	+	-	-	-	-	-	+	-	+	+	-	-	*Pseudomonas sp*
E	C	+	+	-	+	-	-	-	-	-	+	-	+	+	-	+	*Staphylococcus sp*
F	R	-	+	-	+	-	-	+	-	-		-	A/G	-	A/G	-	*E. coli*

Key: A/G=Acid and Gas production; A= Acid production, R = Rod, C = Cocci

All the Kunu samples were acidic in nature with pH range of 3.20 to 4.85. Various researchers have 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. Osuntogun and Abiola (2004) isolated lactic acid bacteria such as *Lactobacilli*, *Leuconostoc* and *Streptococcus* which were reported to possess the ability to ferment carbohydrates to produce lactic acid thus lowering the pH. *Lactobacilli* have also been isolated from other indigenous non-alcoholic beverages. Olasupo *et al.* (2002) reported the isolation of *Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Klebsiella*sp. and *Enterococcus faecalis* from kunu drink. Akinrele *et al.* (1980) 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

cereal based fermented foods (ogi and kunun-zaki). These organisms can cause the spoilage of the beverage if not eliminated during the heating process. The high bacterial and fungal loads in all the Kunu samples can be attributed to the poor hygienic practices of the handlers and possible contamination from utensils and water used for processing the beverage as well as packages used in its distribution. The bacterial isolates with their frequency and percentage occurrences in each sample is as presented (Table 5). The result showed that *Escherichia coli, Staphylococcus* sp *and Salmonella* sp had a hundred percent frequency in all the three samples analyzed .the percentage occurrence in each of the samples are; Sample A (67.0%), Sample B (100%) and Sample C (50%).

Table 4: Morphological and Cultural Characteristics of Fungi Isolates

ISOLATES	CULTURAL CHARACTERISTICS	MORPHOLOGJCAL FEATURES	MICROSCOPY	IDENTITY
F1	Yellowish-green mycelium	Conidia in long chains, branched cells	Branched smooth conidiophores, brush-like conidia head	*Penicillium* sp
F2	Wooly white, black domains	Conidia in chains	Non-septate hyphae with many branches	*Aspergillus* sp
F3	White cottony With felty colony	Macro-conidia in with light periphery in chains.	Septate hyphae with branched conidiophhore	*Fusarium* sp
F4	Colourless	Nil	Egg shaped	*Saccharomyces* sp

Table 5: Percentage Occurrence of the Isolates in each Sample

Sample	*E. coli*	*Staph.*	*Pseud.*	*Salm*	*Lact*	*Strept*.	Total (%)
P	+	+	-	+	+	-	67.0
Q	+	+	+	+	+	+	100.0
R	+	+	-	+	-	-	50.0

Key: E. coli = *Escherichia coli*, *Staph*.sp = *Staphylococcus* specie; *Pseu*. sp = *Pseudomonas* specie, Lact. sp = *Lactobacillus* specie

Conclusion: Proximate analysis of the kunu samples revealed moderate acidity in pH for the entire sample tested. The result suggests that they are of good nutritive value and could serve as source of protein and energy to. 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. 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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