



BEST JOURNAL 13(3): 135 - 140

Date received: October 5, 2016

Date accepted: November 10, 2016

Printed in Nigeria



MICROBIOLOGICAL QUALITY ASSESSMENT OF 'KUNU' SOLD WITHIN ILORIN METROPOLIS, NIGERIA

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ABSTRACT

Many individuals prefer locally processed foods to modern day contemporary processed foods. "Kunu" is a popular cereal based non-alcoholic beverage in Nigeria and it is widely consumed especially in the Northern parts of the country. Hence, this research focused on assessing the quality of these locally produced "kunu" drinks. Freshly prepared samples of "Kunu" were purchased from local vendors and hawkers within Ilorin metropolis, Kwara State, Nigeria and analyzed for their microbiological and physicochemical quality. The pH of the samples ranged from 2.66 to 4.06. The ~~total~~ bacterial count ranged from 1.5×10^4 to 7.4×10^4 cfu/ml; the total coliform count ranged from 1.0×10^2 to 8.0×10^3 cfu/ml; the faecal coliform count ranged from 0 to 3.0×10^3 cfu/ml; and the ~~total~~ fungal count ranged from 3.2×10^4 to 2.7×10^5 cfu/ml. The presence of high microbial load was an indication of poor hygiene and/or poor quality cereals and water used in the preparation. The bacteria isolated were: *Listeria* sp., *Corynebacterium cystitidis*, *Aeromonas* sp., *Corynebacterium amycolatum*, *Corynebacterium* sp., *Corynebacterium pseudotuberculosis*, *Listeria innocua*, *Kurthia* sp., and *Corynebacterium ulcerans* while the fungi were: *Mucor* sp., *Geotrichum candidum*, *Saccharomyces cerevisiae*, *Saccharomyces* sp., *Debaryomyces hansenii*, and *Aspergillus* sp. It is concluded from this study that "kunu" contains an array of microorganisms and stringent hygiene should be ensured at all time during and after its production. It is recommended that good quality grain should be used in the production of "kunu", the production utensils should be devoid of microorganisms, sterile plastic bottles should be used for its packaging, the "kunu" should be pasteurized, and it should be stored under refrigerated condition and consumed within 24 hours of production.

Key words: Consumption, Investigation, Local beverage, Microbial quality, Physicochemical parameters

INTRODUCTION

Many individuals prefer locally processed food over the modern day contemporary processed foods. "Kunu" is a popular cereal based non-alcoholic beverage. It is can be consumed by people whose religion restricts alcohol consumption. It is a popular indigenous drink consumed throughout Nigeria, mostly in the North. (Adeyemi and Umar, 1994).

It is a staple beverage drink that is relatively cheap and nutritious when compared to carbonated drinks (Adejuyitan *et al.*, 2008). Its cheapness is owed to the ready availability of cereals since it grows throughout the savannah belt of West Africa (Elmahmood and Doughari, 2007). Depending on the cereal availability, grains such as millet (*Pennisetum typhoideum*), sorghum (*Sorghum vulgare*), maize (*Zea mays*), rice (*Oryza sativa*) and acha (*Dijita pexilis*) are commonly used for the traditional production of "kunu" (Ahmed *et al.*, 2003). Spices such as ginger, black pepper, garlic, red pepper and clove are commonly added as flavour and taste improver. Sugar is also added to act as a sweetener. The grains are used singly or combined; for instance sorghum/millet in the ratio 1:2 (w/w) (Ahmed *et al.*, 2003). It has been reported that "kunu" is rich in

carbohydrates, vitamins, and minerals but low in protein.

"Kunu" has relatively short shelf-life. Studies conducted by Adeyemi and Umar (1994) revealed that the product has a shelf life of 24 hours at ambient temperature, which could be extended to days by pasteurization at 60°C for 1 hour and storage under refrigeration conditions.

Preparation of this drink takes a minimum of 2-3 days. The processes involved include soaking of the millet; intermittent changing of the steeping water; grinding and blending of the grain with other ingredients such as sweet potato, peeled ginger, and clove; sieving of the slurry in cloth bag; allowing the filtrate to settle; decanting of the supernatant from the sediment; dividing the sediment into 2 parts; adding hot water to one part and thoroughly mixed; and finally adding this to the second cold part (Ogbonna *et al.*, 2011; Ikpoh *et al.*, 2013).

"Kunu" can undergo spoilage as a result of some factors such as microorganisms present in the drink. Studies have shown that "kunu" contains lactic acid bacteria such as *Lactobacillus* spp., *Streptococcus* spp. and *Leuconostoc* spp. and these organisms could cause the spoilage of the beverage.



Other organisms such as *Staphylococcus* spp., *Bacillus* spp., *Pseudomonas* spp., *Penicillium* spp., *Aspergillus* spp., *Trichoderma* spp., and *Candida* spp. if present in large quantity could cause the spoilage of beverage (Kolawole *et al.*, 2007).

Due to the popularity of "kunu" within our society, it is necessary to verify the safety of consuming this food drink. Hence, this research was conducted to determine the bacterial and fungal loads and their species present in some "kunu" samples and also to determine some physicochemical quality of the "kunu" samples.

MATERIALS AND METHODS

Collection of samples

The "kunu" samples were purchased from different sellers within Ilorin metropolis. These samples have been packaged in plastic polyvinyl chloride (PVC) bottles.

Determination of microbial counts

The "kunu" sample was diluted up to 10^{-3} . The bacterial and fungal counts were determined using nutrient agar and potato dextrose agar (PDA) respectively. Pour plate and spread plate techniques were used for the bacterial and fungal counts respectively. The PDA was supplemented with streptomycin before pouring (Adelekan *et al.*, 2013; Adesokan *et al.*, 2013).

The total coliform and faecal coliform counts were enumerated using MacConkey agar and eosin methylene blue agar respectively as the choice media. This was done according to Willey *et al.* (2011) and Sule *et al.* (2015).

Isolation, characterization and identification of microbial isolates

The bacteria and fungi obtained from the samples were subcultured according to Fawole and Oso, (2007). Pure bacterial colonies were characterized based on their colonial, cellular, and biochemical characteristics and then identified by making reference to Cowan and Steel, (1985). Similarly, the fungal isolates were characterized based on their macroscopic and microscopic characteristics (Fawole and Oso, 2007) and identified by making reference to Onions *et al.* (1981) as well as and Samson and Van Reenen-Hoesktra (1988).

Determination of pH

The hydrogen-ion concentration (pH) of each the "kunu" sample was measured using a Denver pH

meter which was standardized with buffers of pH 4, 7 and 9 prior to use (Fawole and Oso, 2007).

Determination of moisture Content

A clean crucible was weighed to obtain w_1 , then 30ml some amount of "kunu" added to it and weighed to obtain (w_2). The crucible and its content were dried in an oven at 105°C for 3 hours until constant weight was obtain (w_3) (Ogbonna *et al.*, 2011).

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

Determination of ash content

The ash content was determined by burning the dried components obtained from moisture content determination above (w_3) in a furnace at 600°C for 2hours. After ashing, the weight (w_4) was obtained (Ogbonna *et al.*, 2011).

$$\text{Ash content (\%)} = \frac{W_3 - W_4}{W_2 - W_1} \times 100\%$$

Statistical analyses

The mean of each data was determined using SPSS statistical package and Duncan's multiple range test was used to determine if there is significant difference between the mean at 5% confidence level (SPSS, 2010).

Results

The bacterial counts, total coliform, and faecal coliform count of the "kunu" ranged from 1.5×10^4 to 7.4×10^4 cfu/ml, 1.0×10^2 to 8.0×10^3 cfu/ml, and 0 to 3.0×10^3 cfu/ml respectively. Similarly, the fungal counts ranged from 3.2×10^4 to 2.7×10^5 cfu/ml (Table 1).

Based on the characterization, the nine bacterial species identified were: *Listeria* sp., *Corynebacterium cystitidis*, *Aeromonas* sp., *Corynebacterium amycolatum*, *Corynebacterium* sp., *Corynebacterium pseudotuberculosis*, *Listeria innocua*, *Kurthia* sp., and *Corynebacterium ulcerans* (Table 2) while the fungal species identified were *Mucor* sp., *Geotrichum candidum*, *Saccharomyces cerevisiae*, *Saccharomyces* sp., *Debaryomyces hansenii*, and *Aspergillus* sp. (Table 3). The occurrence of the bacterial and fungal isolates in the "kunu" were as presented in Tables 4 and 5 respectively..

The result of the physicochemical analysis revealed that all the samples have an acidic pH in the range of 2.66 to 4.06 (Table 6). The moisture and ash content of the "kunu" sample had a range of 79.3-92.9%, and 6.9-19.8% respectively (Table 6).



Table 1: Bacterial and fungal counts of the “kunu” samples

Sampling points	Bacterial count (cfu/ml) x 10 ³	Faecal coliform (cfu/ml) x 10 ²	Total coliform (cfu/ml) x 10 ²	Fungal count (cfu/ml) x 10 ⁴
Unilorin motion ground I	56 ^{cd} ±3.0	2.0 ^a ±0.0	10 ^b ±1.0	24 ^c ±4.0
Unilorin car park I	62 ^d ±2.0	0 ^a ±0.0	1.0 ^a ±0.0	3.2 ^a ±0.1
Unity road, Ilorin I	74 ^e ±4.0	60 ^b ±0.0	13 ^b ±3.0	5.7 ^a ±0.2
Obo road, Ilorin	40 ^b ±3.0	20 ^e ±3.0	30 ^d ±4.0	4.0 ^a ±0.2
Unity road, Ilorin II	59 ^d ±4.0	0 ^a ±0.0	15 ^{bc} ±2.0	27 ^c ±3.0
A-Division	50 ^c ±5.0	15 ^d ±3.0	40 ^e ±4.0	5.0 ^a ±0.2
Bekandims	15 ^a ±1.0	10 ^c ±1.0	50 ^f ±5.0	10 ^b ±1.0
Unilorin car park II	50 ^c ±3.0	30 ^f ±5.0	80 ^g ±6.0	13 ^b ±1.0
Unilorin motion ground II	60 ^d ±5.0	0 ^a ±0.0	20 ^c ±3.0	11 ^b ±2.0
Oja-Oba, Ilorin	40 ^b ±3.0	0 ^a ±0.0	13 ^b ±1.0	3.5 ^a ±0.1

All values are mean of 3 replicate, Mean values followed by different alphabets for the same parameter are significantly different at $\alpha = 0.05$ based on Duncan's multiple range test

Table 2: Cultural, morphological and biochemical characteristics of bacterial isolates

Bacterial isolates	Colonia	Colonia shape	Gram's reaction	Cell's shape	H ₂ S	Motility	Catalase	Coagulase	Indole	Oxidase	Methyl red	Voges Proskauer	Citrate	Urease	Starch hydrolysis	TSI	NO ₃ reduction	Maltose	Sucrose	Lactose	Mannitol	Glucose	Fructose	OF	Probable identity
1	Cr	Ir	+	R	-	+	+	-	+	+	+	+	+	+	-	kk	-	A	A	-	A	A	A	F	<i>Listeria</i> sp.
2	Cr	Ci	+	R	-	-	+	-	+	-	+	-	+	+	+	AK	-	A	A	-	-	A	A	F	<i>Corynebacterium cystitidis</i>
3	Cr	Ci	-	R	-	+	+	-	+	-	+	+	-	+	+	kk	-	A	A	-	A	A	A	-	<i>Aeromonas</i> sp.
4	Cr	Ir	+	R	-	-	+	-	+	-	+	+	+	+	+	kk	-	A	A	-	-	A	A	-	<i>Corynebacterium amycolatum</i>
5	Cr	Ci	+	R	-	-	+	-	+	+	+	+	+	+	-	kk	-	A	A	-	A	A	A	F	<i>Corynebacterium</i> Sp.
6	Cr	Ci	+	R	-	-	+	-	+	-	+	+	-	+	+	K A	-	A	A	-	A	A	A	F	<i>Corynebacterium pseudotuberculosis</i>
7	Cr	Ci	+	R	-	+	+	-	+	-	+	+	+	+	+	kk	-	A	A	-	-	A	A	F	<i>Listeria innocua</i>
8	Cr	Ci	+	R	-	+	+	-	-	+	-	+	+	+	-	A A	-	A	AG	A	AG	A	A	-	<i>Kurthia</i> sp.
9	Cr	Ci	+	R	-	-	+	-	-	-	-	+	+	+	+	kk	-	A	A	A	-	AG	A	F	<i>Corynebacterium ulcerans</i>

Key: Cr = cream; Ci = circular; Ir = irregular; R = Rod; F = Fermentative; - = negative reaction; + = positive reaction; OF = Oxidation-Fermentation; AK = Acid/Alkaline reaction; AA = Acid/Acid reaction; KK = Alkaline/Alkaline reaction; AG = Acid and gas; A = Acid only



Table 3: Macroscopic and microscopic features of fungal isolates

Fungal isolates	Features		Fungi identified
	Macroscopic	Microscopic	
F ₁	Colony initially white with black spores; it turned black with time	Presence of sporangium borne on the sporangiophore; absence of rhizoid	<i>Mucor</i> sp.
F ₂	Filamentous colony with green centre surrounded by whitish hyphal edge; reverse of plate black	Presence of rectangular shaped arthrospores which are in chains	<i>Geotrichum candidum</i>
F ₃	The fungus was creamish colony; yeastlike; non-filamentous	Budding cells observed; unicellular cells; cells are ellipsoidal in shape	<i>Saccharomyces cerevisiae</i>
F ₄	A filamentous colony with shades of green on potato dextrose agar	Hyphae septate and hyaline; conidiophores terminated into vesicle; presence of foot cell	<i>Aspergillus</i> sp.
F ₅	Colony yeastlike, viscous, brownish cream; reverse of plate pale yellow	Cell globose, unilateral budding; cell appeared cluster or singly	<i>Debaryomyces hansenii</i>
F ₆	Colony yeastlike, smooth, dull, tarnish cream	Unilateral budding cells; unicellular cells; cells are elongated in shape	<i>Saccharomyces</i> sp.

Table 4: Occurrence of bacterial isolates in the "kunu" samples

S/N	Bacterial isolates	Sampling locations									
		A	B	C	D	E	F	G	H	I	J
1	<i>Listeria</i> sp	+	-	-	+	-	-	+	+	-	-
2	<i>Corynebacterium cystitidis</i>	+	+	+	-	+	-	+	+	+	-
3	<i>Aeromonas</i> sp.	-	-	+	-	-	-	-	-	-	-
4	<i>Corynebacterium amycolatum</i>	+	+	-	+	+	-	-	-	-	+
5	<i>Corynebacterium</i> sp.	-	-	+	-	-	+	-	-	-	-
6	<i>Corynebacterium pseudotuberculosis</i>	-	-	-	-	+	-	+	+	-	+
7	<i>Listeria innocua</i>	-	-	-	-	-	-	+	-	-	-
8	<i>Kurthia</i> sp.	-	-	-	-	-	-	-	+	-	-
9	<i>Corynebacterium ulcerans</i>	-	-	-	-	-	-	+	-	+	+

Key: +, isolated; -, not isolated; A, Unilorin car park I; B, Unilorin motion ground I; C, Unity road Ilorin I; D, Obo road Ilorin; E, Unity road Ilorin II ; F, A-division; G, Bekandims; H, Unilorin car park II; I, Unilorin motion ground II; J, Oja-Oba

Table 5: Occurrence of fungal isolates in the "kunu" samples

S/N	Fungal isolates	Sampling locations									
		A	B	C	D	E	F	G	H	I	J
1	<i>Mucor</i> sp.	+	+	-	-	-	-	+	-	-	+
2	<i>Geotrichum candidum</i>	-	+	-	-	-	+	+	-	+	+
3	<i>Saccharomyces cerevisiae</i>	-	+	+	+	+	-	+	+	+	+
4	<i>Aspergillus</i> sp.	-	-	-	-	-	-	+	+	-	+
5	<i>Debaryozoma hansenii</i>	+	+	+	+	+	+	+	+	+	-
6	<i>Saccharomyces</i> sp.	-	+	-	-	-	+	-	-	+	-

Key: +, isolated; -, not isolated; A, Unilorin car park I; B, Unilorin motion ground I; C, Unity road Ilorin I; D, Obo road Ilorin; E, Unity road Ilorin II ; F, A-division; G, Bekandims; H, Unilorin car park II; I, Unilorin motion ground II; J, Oja-Oba Ilorin



Table 6: Physicochemical characteristics of the "Kunu" samples

Sampling points (Locations)	pH	Moisture content (%)	Ash content (%)
Unilorin motion ground I	3.38 ^d ±0.03	90.2 ^b ±4.0	9.6 ^b ±0.6
Unilorin car park I	3.31 ^c ±0.02	88.8 ^b ±2.0	11.0 ^b ±2.0
Unity road, Ilorin I	4.06 ^a ±0.03	82.9 ^a ±4.0	16.7 ^c ±1.1
Obo road, Ilorin	3.91 ^f ±0.03	79.3 ^a ±2.0	19.8 ^d ±1.0
Unity road, Ilorin II	3.84 ^e ±0.04	82.3 ^a ±0.8	17.7 ^c ±0.8
A-Division	4.06 ^a ±0.02	92.5 ^b ±5.0	7.5 ^a ±1.0
Bekandims	2.66 ^a ±0.03	80.1 ^a ±0.9	19.8 ^d ±1.0
Unilorin car park II	3.25 ^b ±0.03	92.0 ^b ±3.0	7.6 ^a ±0.4
Unilorin motion ground II	3.20 ^b ±0.02	92.9 ^b ±0.9	6.9 ^a ±0.3
Oja-Oba, Ilorin	4.01 ^a ±0.04	89.9 ^b ±0.7	9.8 ^b ±0.5

All values are mean of 3 replicate

Mean values followed by different alphabets for the same parameter are significantly different at $\alpha = 0.05$ based on Duncan's multiple range test

DISCUSSION

The high counts of bacteria and fungi obtained from "kunu" in this study could have detrimental effect on the shelf life of this product. Moreover, some of these organisms are pathogenic and could affect the health of the consumers. A similar study by Elmahmood and Doughari, (2007) also showed a high bacterial counts in the range of 1.0×10^2 - 1.0×10^5 cfu/ml. In addition, Ogbonna *et al.* (2011) got total heterotrophic bacterial count ranging from $1.8 - 6.1 \times 10^3$ cfu/ml in their "kunu" samples.

Ikpoh *et al.* (2013) isolated bacteria such as *Staphylococcus aureus*, *Streptococcus* sp., *Pseudomonas* sp., *Bacillus* sp. as well as fungi such as *Penicillium* sp., *Fusarium* sp., *Aspergillus* sp., *Candida albicans*, and *Rhizopus nigricans* from "kunu" beverage locally prepared and hawked in Calabar, Cross river, Nigeria. Furthermore, Ogbonna *et al.* (2011) was able to isolate pathogens such as *E. coli*, *S. aureus*, *Salmonella* sp., and *Shigella* sp. from their samples of "kunu zaki" in Maiduguri, Nigeria. Kolawole *et al.* (2007) isolated *Aspergillus* sp., *Fusarium* sp., *Penicillium* sp., *Rhizopus nigricans* from the "burukutu" and "pito" produced within Ilorin, Nigeria. Ugwuanyi *et al.* (2015) isolated bacteria such as *E. coli*, *Lactobacillus*, *Pseudomonas* sp. as well as fungi such as *Saccharomyces cerevisiae*, *Aspergillus* sp., and *Geotrichum* spp. from "kunu" samples. Isolation of *E. coli* in "kunu" samples in this study is an indication of faecal contamination.

The genera of bacteria isolated in this study: *Corynebacterium*, *Listeria*, and *Aeromonas* could come from soil and water. The contaminants might come from utensils used in the production of "kunu", environment, as well as from the food handlers.

Ugwuanyi *et al.* (2015) reported the isolation of fungi such as *S. cerevisiae*, *Aspergillus* sp., and *Geotrichum* sp. from "kunu zaki". In this present study, the fungi isolated also included *S. cerevisiae*, *Aspergillus* sp., *Geotrichum candidum* as well as *Debaryomyces hansenii* and *Mucor* sp. *Saccharomyces* and *Debaryomyces* could make the

"kunu" to have sour taste due to alcohol production during fermentation while some species of *Aspergillus* are known to produce mycotoxin (Onions *et al.*, 1981). All the "kunu" used in this study were acidic in their pH. In a similar study, Ikpoh *et al.* (2013) got a pH range of 3.34 – 4.12 from "kunu" beverage hawked in Calabar, Cross river, Nigeria. The level of acidity of "kunu" has been reported by several researchers including Efiuvwever and Akoma (1995), and Akoma *et al.* (2006) who attributed this to the presence of certain species of lactic acid bacteria namely *Lactobacillus leichmanni* and *Lactobacillus fermentum* during the fermentation process of "kunu".

"Kunu" is a water based product and it will not be surprising when very high moisture content is obtained in this product. Ogbonna *et al.* (2011) obtained moisture content in the range of 85.4 – 89.8% in "kunu zaki" samples. The high moisture and total solid content of "Kunu" may encourage growth of some microorganisms to hazardous level during storage at ambient temperature (Olasupo *et al.*, 2002).

CONCLUSION

It can be concluded from this study that a number of microorganisms found in the "kunu" samples are not desirable. Hence, efforts should be made to ensure aseptic technique at all points in the production of "kunu" and its preservation in Ilorin, Kwara State, Nigeria.

Recommendation

The following recommendations were made in order to improve the quality of "kunu": stringent sanitation and monitoring of handling of the product should be encouraged; adoption of aseptic techniques during production; sterile packaging; and pasteurization should be encouraged by cooking the "kunu" at 60 °C for 30 minutes; use of good quality water and grain during production; storage under refrigeration temperature; and thorough washing of the plastic bottle containers.



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