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Bacteriological quality of kunu-zaki sold on the streets of owerri metropolis, Nigeria

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Abstract

Kunu-zaki is a nourishing non-alcoholic beverage widely consumed in Nigeria. There is no standardized method for its preparation thus production practices differ amongst retailers. This study was undertaken to evaluate the bacteriological quality of kunu drink retailed in major markets of Owerri metropolis, Nigeria. Triplicate samples were obtained from four markets in Owerri and a control sample prepared in the laboratory. Kunu drink was analysed using the standard pour plate procedure. The results obtained showed that total heterotrophic bacteria count, total coliform count and total *Salmonella Shigella* count ranged from 1.4 x 10^3 – 4.5 x 10^4 cfu / ml, 1.2 x 10^3 – 3.8 x 10^4 cfu / ml to 0.6 x 10^3 – 3.1 x 10^4 respectively. A total of 9 bacteria genera including *Staphylococcus* specie, *E. coli*, *Enterobacter* specie, *Proteus* specie, *Citrobacter* specie, *Serratia* specie, *Lactobacillus* specie, *Salmonella* specie and *Streptococcus* specie were isolated with the highest percentage frequency of occurrence recorded for *Staphylococcus* sp. (16.66%) indicating possible low hygiene of the kunu zaki producers. The bacteria genera isolated from kunu zaki sold in Owerri and their number constitute main concerns for public health as these can cause a variety of infections or food intoxications. Thus, there is a need to establish a system of monitoring of street vended kunu zaki to make sure that it is safe for consumption.

Keywords: *kunu-zaki, bacteria contamination, coliform, public health*

1. Introduction

Kunu is an indigenous non-alcoholic fermented beverage consumed widely especially in the northern regions of Nigeria (Amusa and Odunbaku, 2009). It is consumed as a breakfast snack, used as weaning product and as a food supplement / appetizer (Oranusi et al., 2003). Kunu-zaki is prepared traditionally using millet, maize, wheat or sorghum (Gaffa *et al.*, 2002). Apart from these cereals, kunu has been shown to be produced from tigernuts (Belewu and Abodunrin, 2006), guinea corn or rice (Umaru et al., 2014). The appearance of kunu is milky cream and is usually consumed within few hours after its production (Adeleke and Abiodun, 2010). Because kunu is prepared in the traditional method, the ingredient concentrations are neither quantified nor standardized (Aboh and Oladosu, 2014). 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., 2014, the processes involved in the preparation of kunu-zaki include:

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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 (*Afromoniummelegueta*), red pepper (*Capsicum* species), black pepper (*Piper guinense*) and Kakandoru or Eru are usually utilized (Adelekan et al., 2013). Nutritionally, kunu is made up of 87-92% moisture, 3.19-7.86% crude protein, 0.37-0.75% crude fat, 0.93-1.20% ash and 2.69-5.84% carbohydrate as reported by Gaffa et al., 2002. 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 (Ofudje et al., 2016).

Because of the traditional method employed in the preparation of kunu, the unregulation / unstandardization of ingredients used and possible low sanitary procedures employed in its preparation, kunu may provide an ideal environment for the growth of microorganisms and its consumption leading to food borne intoxication / poisoning. Various studies have investigated the microbial composition of kunu zaki in various parts of Nigeria with varying results. Osuntogun and Aboabo, 2006, reported that kunu contains lactic acid bacteria (LAB) including *Lactobacillus* spp., *Streptococcus* spp. and *Leuconostoc* spp. which can cause food borne diseases. Thus, this study was undertaken to investigate the microbial quality of kunu zaki sold in Owerri metropolis, the diversity of bacteria contaminants and their numbers. Findings from this study highlight the safety or otherwise of street vended kunu zaki, informing public policy on the consumption of such products.

2. Materials and Methods

2.1. Sample Collection

Kunu zaki drinks were purchased from four different markets in Owerri metropolis. These include: Ama-Hausa Area 1 (Sample A), Ama-Hausa Area II (Sample B), Obinze Barrack Market (Sample C) and Relief Market (Sample D). A home-made sample (Sample E) was used as control. The samples were collected in sterile sample bottles and aseptically transported to the laboratory for immediate analysis.

2.2. Total Bacteria Count

Total bacteria count was evaluated according to the method of Edem and Elijah, 2016. Briefly, the samples were subjected to 10-fold serial dilutions. Aliquots (0.1 ml) of appropriate dilutions for each sample were plated by the pour plate method in triplicates onto nutrient agar, MacConkey agar and *Salmonella Shigella* agar for total heterotrophic bacteria count, total coliform count and total *Salmonella Shigella* count respectively. These were incubated at 30 ^{0}C for 24 hours. Resulting colonies were counted with the aid of a colony counter and the colonial morphologies noted.

2.3. Identification of Bacterial Isolates

Resulting bacteria isolates were transferred onto fresh nutrient agar medium and incubated at 30 °C for 24 hours. Pure colonies of bacteria were maintained on nutrient agar slant and stored at 4 °C until needed. The identification of the bacteria isolates was by morphological examination under the microscope and biochemical tests following the methods of (Harrigan and McCane, 1976). The isolates were identified by referring to the Bergey´s Manual of Systematic Bacteriology (Holt et al., 2000).

3. Results and Discussion

3.1. Bacterial Load of Kunu-zaki

The results of the bacterial load of kunu zaki are presented in Table 1 below. Sample A has a total heterotrophic bacteria count of 4.5 x 10^4 cfu / ml; total coliform count of 3.8 x 10^4 cfu / ml and a total *Salmonella-Shigella* count of 3.1 x 10^4 cfu / ml. Sample B has a total heterotrophic bacteria count of 3.2 x 10^4 cfu / ml, total coliform count of 3.0 x 10^4 cfu / ml and a total *Salmonella-Shigella* count of 2.8 x 10^4 cfu / ml. Similarly, Sample C has a total heterotrophic bacteria count of 3.2 x 10^4 cfu / ml; total coliform count of 3.0 x 10^4 cfu / ml and a total *Salmonella-Shigella* count of 2.8 x 10^4 cfu / ml. Sample D has a total heterotrophic bacteria count of 2.4 x 10^4 cfu / ml; total coliform count of 2.2 x 10^4 cfu / ml and a total *Salmonella-Shigella* count of 2.0 x 10^4 cfu / ml. Finally, Sample E has a total heterotrophic bacteria count of 1.3 x 10^3 cfu / ml; total coliform count of 1.2 x 10^3 cfu / ml and a total *Salmonella-Shigella* count of 0.6 x 10^3 cfu / ml.

Table 1: Bacterial load of kunu samples

Samples	Total heterotrophic bacteria count (cfu/ml)	Total coliform count (cfu/ml)	Total *Salmonella-Shigella* count (cfu/ml)
Sample A	4.5×10^4	3.8×10^4	3.1×10^4
Sample B	3.2×10^4	3.0×10^4	2.8×10^4
Sample C	3.2×10^4	3.0×10^4	2.8×10^4
Sample D	2.4×10^4	2.2×10^4	2.0×10^4
Sample E	1.4×10^3	1.2×10^3	0.6×10^3

The results show moderate levels of contamination of street vended kunu drinks. These results are lower than the reports of Amusa and Odunbaku, 2009, who reported total viable counts of 2.2-8.8 x 10^6 in street vended kunu in South-Western Nigeria. Similarly, the coliform counts are lesser than that of 2.2-5.6 x 10^6 reported by Amusa and Odunbaku, 2009, but higher than 0.5-3.0 x 10^3 reported by Etang et al., 2017, in locally produced kunu drinks sold in Calabar, Southern Nigeria. Contamination of kunu drink by coliforms indicates possible faecal contamination of the drink, most likely due to the use of unclean water in the fermentation process. Overall, Samples A, B and C have very high microbial loads and the constant consumption of kunu drinks from these markets can possibly lead to a case of food poisoning. Sample D had a relatively lesser microbial load indicating better sanitary practices. Sample E which was prepared in the laboratory under controlled environment had the least microbial load indicating that proper control of the fermentation process, use of potable water and proper hygiene will result in the production of kunu drinks with less microbial load.

3.2. Bacterial Diversity of Kunu-zaki

A total of thirty bacteria isolates were obtained from kunu-zaki. Biochemical characterization of these isolates indicates the presence of nine (9) genera including; *Staphylococcus* specie, *E. coli*, *Enterobacter* specie, *Proteus* specie, *Citrobacter* specie, *Serratia* specie, *Lactobacillus* specie, *Salmonella* specie and *Streptococcus* specie as shown in Table 2 below.

Table 2: Biochemical characterization of isolates from kunu

S. No.	Gram reac-tion	Oxidase	Glucose	Sugar fermen-tation	Gas	H_2S	Indole	Citrate	Urease	Motility	Catalase	Probable organism
A1	+ve	+	–	+	–	–	–	+	–	–	+	*Staphylococcus* sp.
A2	–ve	–	+	+	+	+	–	+	–	+	–	*Citrobacter* sp.
A3	–ve	–	+	–	–	–	–	+	–	+	–	*Serratia* sp.
A4	+ve	+	–	+	–	–	–	+	–	–	+	*Staphylococcus* sp.
A5	–ve	–	+	+	+	–	+	–	–	+	+	*E. coli*
A6	–ve	–	+	+	+	–	–	+	–	+	+	*Enterobacter* sp.
A7	–ve	–	+	–	–	+	+	–	+	+	+	*Proteus* sp.
A8	+ve	–	+	–	–	–	–	–	–	–	–	*Streptococcus* sp.
B9	–ve	–	+	+	+	–	+	–	–	+	+	*E. coli*
B10	–ve	–	+	–	+	+	–	–	–	+	+	*Salmonella* sp.
B11	+ve	–	+	+	+	–	–	+	–	–	+	*Lactobacillus* sp.

Table 2 (Cont.)

S. No.	Gram Reaction	Oxidase	Glucose	Sugar fermentation	Gas	H_2S	Indole	Citrate	Urease	Motility	Catalase	Probable organism
B12	–ve	–	+	+	+	+	–	+	–	+	–	*Citrobacter* sp.
B13	–ve	–	+	–	–	–	–	+	–	+	–	*Serratia* sp.
B14	+ve	+	–	+	–	–	–	+	–	–	+	*Staphylococcus* sp.
C15	–ve	+	+	–	–	+	+	–	+	+	+	*Proteus* sp.
C16	+ve	–	+	–	–	–	–	–	–	–	–	*Streptococcus* sp.
C17	–ve	–	+	+	+	–	+	–	–	+	+	*E. coli*
C18	+ve	–	+	+	+	–	–	+	–	–	+	*Lactobacillus* sp.
C19	+ve	+	–	+	–	–	–	+	–	–	+	*Staphylococcus* sp.
C20	–ve	–	+	+	+	+	–	+	–	+	–	*Citrobacter* sp.
D21	–ve	–	+	+	+	–	–	+	–	+	+	*Enterobacter* sp.
D22	–ve	–	+	–	+	+	–	–	–	+	+	*Salmonella* sp.
D23	–ve	–	+	–	–	–	–	+	–	+	–	*Serratia* sp.
D24	+ve	–	+	–	–	–	–	–	–	–	–	*Streptococcus* sp.
D25	+ve	–	+	+	+	–	–	+	–	+	+	*Lactobacillus* sp.
D26	+ve	+	–	+	–	–	–	+	–	–	+	*Staphylococcus* sp.
E27	–ve	–	+	+	+	–	+	–	–	+	+	*E. coli*
E28	–ve	–	+	+	+	+	–	+	–	+	–	*Citrobacter* sp.
E29	–ve	–	+	–	–	+	+	–	+	+	+	*Proteus* sp.
E30	–ve	–	+	–	+	+	–	–	–	+	+	*Salmonella* sp.

From the table above, Sample A had the highest bacterial diversity with 8 isolates, followed by Samples B, C and D having 6 isolates each. Sample E which was prepared in the laboratory had the lowest number of isolates (4). Overall, the percentage occurrence of the 9 bacteria genera is as follows; *Staphylococcus* sp. (16.66%), *E. coli* (13.33%), *Enterobacter* sp. (6.66%), *Proteus* sp. (10.00%), *Citrobacter* sp. (13.33%), *Serraria* sp. (10.00%), *Lactobacillus* sp. (10.00%), *Salmonella* sp. (10.00%) and *Streptococcus* sp. (10.00%). This result agrees with the findings of Edem et al., 2017. who isolated *Shigella* sp., *Salmonella* sp., *E. coli, Klebsiella* sp., *Lactobacillus* sp., *Citrobacter* sp., *Staphylococcus aureus, Streptococcus* sp. amongst others from kunu zaki sold in Eiyenkorin, Kwara State, Nigeria. The organisms isolated from kunu zaki retailed in Owerri metropolis are of public health concern as these bacteria genera are known to cause a variety of human illnesses and have been implicated in cases of food intoxication. Finally, the presence of *E. coli* and *Salmonella* sp. indicates possible faecal contamination and unhygienic preparation process of the drink. This is because part of the preparation process of kunu is cooking which should eliminate most of the pathogens isolated in this work, thus indicating that the contamination recorded was after the cooking process when the drink must have cooled down.

4. Conclusion

The results of this study showed that the bacterial load of locally prepared kunu zaki drink in Owerri metropolis is high due to possible use of contaminated water in its preparation and improper handling. The most predominant bacterial contaminant is *Staphylococcus* sp. with a percentage occurrence of 16.66%, followed by *E. coli* and *Citrobacter* sp. at 13.33%. Of all the samples analysed, the lowest microbial load was recorded for

Sample E, which was prepared under controlled environment in the laboratory. This result indicates that with correct control of the fermentation process, the spate of microbial contamination of street vended kunu zaki drink will be drastically reduced.

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