MICROBIAL QUALITY OF *KUNUN-ZAKI* SOLD IN EIYENKORIN, KWARA STATE, NIGERIA

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ABSRACT

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Kunun-zaki, a staple non-alcoholic beverage plays a vital role in the dietary pattern of the people in Nigeria. However, a survey on the sanitary conditions of its production in Eiyenkorin, Kwara State, Nigeria, was not satisfactory, hence the need to assess the microbial quality of kunun-zaki beverage in relation to public health significance in Eiyenkorin, Kwara State, Nigeria. Kunun-zaki drink was purchased from vendors in different locations in Eiyenkorin. The samples were evaluated for microbial analyses and pH using standard procedures. Bacterial and fungal counts ranged from $1.6\pm0.32 \times 10^6$ to $5.7\pm0.01 \times 10^6$ cfu ml^{-1} and $1.0\pm0.10 \times 10^6$ to $10.0\pm0.50 \times 10^6$ cfu ml^{-1} respectively. The result showed that the pH of kunun-zaki samples were acidic with values ranging from 2.61 ± 0.21 to 5.01 ± 0.12. Bacterial species isolated include Shigella sp., Salmonella typhi, Escherichia coli, Klebsiella sp., Lactobacillus sp., Citrobacter sp., Pseudomonas sp, Bacillus cereus. Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus sp. and Lactobacillus plantarium while the fungal species isolated were Aspergillus niger, Aspergillus parasiticus, Rhizopus sp., Saccharomyces cerevisiae, Candida albicans, and Penicillium sp. Percentage occurrences of bacterial and fungal isolates were 4.1% for Salmonella typhi and Bacillus cereus, 18.0% for Streptococcus sp., 15.0% for Saccharomyces cerevisiae and 25.0% for Penicillium sp. The results showed that kunun-zaki drinks sold in this community were contaminated with pathogenic microbes as a result of poor hygienic practices during production. Some of this microbes are of public health importance and are capable of causing diverse food related illnesses or infections if ingested.

Keywords*: Kunun-zaki, non-alcoholic beverage, bacterial count, fungal count, Salmonella typhi, Escherichia coli*

INTRODUCTION

Kunun, also called *kunun-zaki* (in Hausa) is a popular indigenous non-alcoholic fermented beverage that is widely consumed in Nigeria. Gaffa *et al.* (2002) reported that *Kunun* is prepared traditionally from millet, maize, sorghum or wheat. However, Belewu and Abodunrin (2006) have demonstrated the production of *Kunun* using tigernut. Studies have also shown that *kunun* can be produced from guinea corn or rice (Umaru *et al.,* 2014). *Kunun* is considered as an appetizer, a food complement and a refreshing drink to quench thirst (Oranusi *et al.,* 2003). It is a staple beverage that is relatively cheap when compared to carbonated drinks (Elmahood and Doughari, 2007). Ikpoh, *et al.* (2013) reported that its affordability is owed to the ready availability of cereals and locally sourced additives throughout the savannah belt of West Africa.

Studies have shown that *kunun* plays a vital role in the dietary pattern of the people in developing countries like Nigeria (Ogbonna *et al.,* 2011). Mbachu *et al.* (2014) reported that *kunun* zaki contains 0.3% protein, 1.0% fat, 1.52% ash, 12.2% carbohydrate and 8.9 mg of vitamin C per 100 ml. More so, some studies have demonstrated the health benefits of *kunun* to include but not limited to reduction of risk associated with diabetes, lowering of blood cholesterol, prevention of formation of blood clot and helps to fight against cancer (Ofudje *et al.,* 2016).

Traditionally, the production procedure of *kunun* varies depending on the taste and cultural habits of the consumers, thus leading to variation in quality and stability. Aboh and Oladosu (2014) reported that production methods of *kunun-zaki* are crude, ingredient concentrations are neither quantified nor standardized, but instead preparation is largely a matter of family tradition. There is possibility of contamination at various stages of unit operations. The production is usually carried out under unhygienic conditions which may predisposed it to many pathogens of public health importance. Ayandele (2015) noted that the spices usually added being agricultural commodities may contain high level of microbial impurities, which can serve as a source of contamination. Umaru *et al.* (2014) reported that locally prepared beverages may serve as vehicles for zoonotic and food-borne diseases or pathogens; such as Staphylococcosis, Salmonellosis, Brucellosis, Tuberculosis, Listeriosis, Shigellosis, *E. coli*, etc. Thus, this study was undertaken to assess the microbial quality of *kunun* drink prepared and sold in Eiyenkorin, Kwara State, Nigeria. Findings from this study can assist government regulatory agencies in monitoring the quality of *kunun* and other local beverages.

MATERIALS AND METHOD

Sample collection

Kunun-zaki drink were purchased randomly from vendors in populated areas in Eiyenkorin, such as main market, Jebba road, Ogbomoso road, schools, motor parks and Balah road. They were transported in a cooler

(Thermocool) containing ice blocks to the Microbiology laboratory, Crown-Hill University, Eiyenkorin, Kwara State, Nigeria.

Total bacterial count

Total bacterial count was determined using the method of Edem and Elijah (2016). The samples were allowed to assume room temperature before serially diluted to 10 fold dilution. Aliquots (0.1 ml) of appropriate dilutions of *kunun* samples were pour plated in triplicates on plate count agar (PCA, Oxoid, UK) and MacConkey agar (Oxoid, CM 115, India) and incubated at 30°C for 24 - 48 hours for total bacterial and coliform count respectively. Distinct colonies were counted after incubation.

Fungal count

Plating was done on Sabourand dextrose agar using pour plate method. Serial dilution was carried out by adding 1 ml of *kunun-zaki* to 9 ml of water to reduce the microbial load. After dilution, 0.1 ml of the sample was then plated out on molten Sabourand dextrose agar plate in triplicates. The plates were swirled gently and allowed to solidify. They were then incubated at room temperature (28 °C) for 48 - 96 hours. Distinct colonies were counted after incubation.

pH determination

About 100 ml of sample was poured into a beaker and thoroughly mixed. pH was measured using a pH meter (Model Voltcraft pH-100 ATC, USA).

Purification and maintenance of microbial isolates

Bacteria isolates were transferred onto fresh nutrient agar medium and incubated at 37 °C for 24 hours. Pure colonies of bacteria were maintained on nutrient agar slant and stored at 4 °C until needed.

Characterization and identification of the isolates

Standard inocula were prepared from the preserved stock culture by taking a loopful of the isolates and aseptically inoculating onto sterile nutrient agar plates. The plates were incubated at 28°C for 24 h. Morphological and biochemical characterization was carried out using standard methods (Harrigan and McCane, 1976), while the isolates were identified by reference to Bergey´s Manual of Systematic Bacteriology (Holt *et al.*, 2000). Fungal isolates were identified using colonial appearance and microscopic characteristics (Chessbrough, 1984; Barnett and Hunter, 1987).

RESULTS AND DISCUSSION

The result of microbial count of *kunun* drink is presented in Table 1. The result revealed high bacterial count in *kunun* drink with values ranging from $1.6\pm0.32 \times 10^6$ - $5.7\pm0.01 \times 10^6$ cfu ml^{-1}. These values are however lower than the values ($0.0 - 2.0 \times 10^8$ cfu ml^{-1}) reported by Aboh and Oladosu (2014) in *kunun* drink. Similarly, fungi count ranged from $1\pm0.10 \times 10^6$ and $10\pm0.50 \times 10^6$ $cfuml^{-1}$. These values are however higher than the recommended standard of 10^2 $cfuml^{-1}$ (FDA, 2013). The high microbial load in this study may be attributed to poor sanitary conditions during production of the *kunun* drink. The pH (Table 1) of *kunun-zaki* samples ranged from 2.61 ± 0.21 to 5.01 ± 0.12, with the mean pH of 3.81 ± 0.40. The values are within the range of values (2.64 - 5.0) reported by Aboh and Oladosu (2014) in *kunun-zaki* marketed in Abuja municipality, Nigeria. The result revealed that *kunun-zaki* samples were acidic yet contaminated with some pathogenic microorganisms which is an indication of possible poor hygienic practices in the handling, processing and storage of the products.

The biochemical characterization and percentage occurrences of bacterial species isolated from *kunun* drink are presented in Table 2. The result showed that 12 bacterial isolates were identified from *kunun-zaki* beverage and they include; *Shigella* sp., *Salmonella typhi, Escherichia coli, Klebsiella* sp., *Lactobacillus sp., Citrobacter* sp, *Pseudomonas* sp., *Bacillus cereus, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus* sp. and *Lactobacillus plantarum*. Some of these organisms such as *E. coli* and *Staphylococcus aureus* (Adebayo *et al.*, 2010), *Shigella* sp. and *Salmonella typhi* (Ogbonna *et al.*, 2011); *Pseudomonas* sp. and *Candida albicans* (Ikpoh *et al.*, 2013); *Klebsiella* sp. and *Lactobacillus* sp (Aboh and Oladosu, 2014); *Pseudomonas aeruginosa, Staphylococcus aureus* and *Bacillus cereus* (Mbachu *et al.*, 2014); *Streptococcus* sp, *Escherichia coli* and *Staphylococcus aureus* (Umaru *et al.*, 2014); *Pseudomonas aeruginosa* (Ayandele, 2015), *Streptococcus* sp, *Escherichia coli* and *Staphylococcus aureus* (Ofudje *et al.*, 2016) had been isolated previously from *kunun- zaki*.

The percentage occurrence (Table 2) indicated that *Streptococcus* sp. (18.0%) predominated *kunun* drink while the least percentage occurrence (4.6%) was for *Pseudomonas aeruginosa.* The results revealed that *kunun* drink samples were contaminated with pathogenic bacteria. The presence of *E.coli, Salmonella typhi, Staphylococcus aureus, Streptococcus* sp. and *Shigella* sp. in the beverage is of public health significance as they are considered the leading cause of food-borne toxicosis outbreak worldwide (Karagozlu *et al.*, 2007). The occurrence of *Shigella sp.* and *Citrobacter sp.* in *kunun* drink has been reported by other researchers and are considered to be detrimental to the health of consumers (Arlington, 2007). The presence of *E. coli* (6.4%) in *kunun* is an indication of faecal and environmental contaminations (Umaru *et al.*, 2014; Aboh and Oladosu, 2014), probably through the use of water or directly during handling. *E. coli* is capable of causing gastroenteritis, diarrhea, and urinary tract infection if ingested by humans.

Table 1: Microbial counts and pH of *Kunun-zaki* obtained from Eiyenkorin, Kwara State, Nigeria

Bacterial count (x 10^6 cfuml^{-1})	Fungal count (x 10^6 cfuml^{-1})	pH
1.9± 0.20	2.0± 0.12	4.94 ±0.21
1.7 ±0.10	3.0 ±0.30	5.00 ±0.23
2.1± 0.00	8.0± 1.10	2.73 ± 0.31
4.3± 1.10	7.0± 1.20	3.52± 0.40
3.2 ±0.50	3.0± 0.00	4.01 ±0.20
2.7 ±2.00	5.0 ±0.40	4.21 ±0.00
5.1 ±0.50	10.0 ±0.50	2.60 ±0.30
5.4 ±0.20	9.0 ±1.00	2.65 ±0.25
1.8 ±0.30	6.0 ±0.00	3.95 ±0.42
2.7 ±0.00	9.0 ±0.40	2.72 ±0.17
5.7 ±0.01	1.0 ±0.10	2.90 ±0.10
4.0 ±0.00	5.0 ±0.20	3.94 ±0.30
1.7 ±0.05	4.0 ±0.00	4.21 ±0.21
1.6 ±0.32	8.0 ±0.10	3.64 ±0.20
2.9 ±0.10	7.0 ±0.20	4.92 ±0.45
3.9 ±0.00	2.0 ±0.32	5.01 ±0.12
4.2 ±0.50	6.0 ±0.40	4.30 ±0.33
4.8 ±0.00	8.0± 0.40	2.66 ±1.10
2.8 ±0.50	9.0 ±0.30	2.61 ±0.21
3.6 ±0.33	7.0 ±0.20	3.12 ±0.10

Table 2: Characterization and percentage occurrence of bacterial species isolated from *Kunun-zaki* sold in Eiyenkorin, Kwara State, Nigeria

	Isolate																		
Cell shape	Gram reaction	Catalase test	Methyl red test	Motility test	Citrate test	Oxidase test	Spore test	Indole test	Urease test	Glucose	Lactose	Maltose	Manitol	Sucrose	Galactose	V.P. test	Coagulase test	% occurrence	Probable Identity
Rod	+	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	5.2	*Shigella* sp.
Rod	-	+	+	-	-	-	-	-	-	+	-	+	+	-	-	-	-	4.1	*Salmonella* sp.
Rod	-	+	+	-	-	-	+	+	-	+	+	-	+	+	-	-	-	6.4	*E.coli*
Rod	-	+	-	-	+	-	-	-	-	-	+	-	+	+	-	+	-	6.9	*Klebsiella* sp.
Rod	+	-	-	-	+	-	-	-	-	+	+	-	+	+	+	-	-	8.1	*Lactobacillus* sp.
Rod	-	+	+	+	+	-	-	-	+	+	-	+	+	-	-	-	-	4.6	*Citrobacter* sp.
Rod	-	+	-	-	+	+	+	-	-	-	-	-	+	-	-	-	-	5.8	*Pseudomonas* sp.
Rod	+	+	-	+	+	-	+	-	-	+	-	+	-	-	-	+	-	4.1	*Bacillus cereus*
Rod	-	+	-	+	+	+	+	-	-	-	-	-	+	-	-	-	-	4.6	*Pseudomonas aeruginosa*
Cocci	+	+	+	-	+	-	-	-	+	+	+	+	+	+	+	+	+	11.1	*S. aureus*
Cocci	+	-	+	-	+	+	-	-	-	+	+	+	-	+	+	-	-	18.0	*Streptococcus* sp.
Rod	+	-	-	-	-	-	-	-	-	+	+	+	+	+	+	-	-	5.2	*Lactobacillus plantarum*

+ = positive result; - = negative result

Umaru *et al*. (2014) reported that the presence of *Bacillus* sp. in *kunun* may arise from the starter culture used as well as other ingredients and food contact surfaces. *Bacillus cereus* has been involved in food poisoning especially in cereals that have been cooked and stored at warm temperature (Wonang *et al.,* 2001). Mbachu *et al.* (2014) reported that toxins produced by *Bacillus* sp. caused pneumonia and bronchopneumonia. The occurrence of *Klebsiella* sp. was also reported by Aboh and Oladosu (2014) in *kunun-zaki*, indicating poor handling practices among handlers. The presence of *Streptococcus* and *Pseudomonas* species has also been implicated in the spoilage of beverages and foods (Mbachu *et al.,* 2014). Their presence in *kunun* drink is of public health significance, due to their ability to cause infections such as food-borne intoxication.

The presence of *Saccharomyces cerevisiae* has equally been implicated in food spoilage due to its fermentative ability, osmophilic nature, tolerance of acid, tolerance of alcohol and ability to grow at low temperature (Badua *et al.,* 2006). Cultural and morphological characteristics of fungi isolated from *kunun-zaki* sold at Eiyenkorin, Kwara State, Nigeria is presented in Table 3.The result showed that fungal species isolated in *kunun* include *Aspergillus*

niger, Aspergillus parasiticus, Rhizopus sp., *Saccharomyces cerevisiae* and *Penicillium* sp. (Table 3).The result of percentage occurrence revealed that *kunun* drink was predominated by *Penicillium* sp. (25.0%). This was followed by *Rhizopus* sp. (22.5%). The least percentage occurrence was recorded by *Saccharomyces cerevisiae* (15.0%). Ofudje *et al.* (2016) reported that food contaminated with fungi usually result in the production of undesirable odour, changes in colour and taste of the product.

Table 3: Cultural and morphological characteristics of fungi isolated from *Kunun-zaki* sold at Eiyenkorin, Kwara State, Nigeria

Cultural Characteristics	Morphological characteristics	Identification	Percentage occurrence (%)
Dark-brown mycelium	Dark-brown conidia, long conidiophores, covered globose vesicles, biseriate phialides borne on brown metulae	*Aspergillus niger*	17.5
Green mycelium	Green mycelium, cylindrical shape, globose, dense conidiophores, ellipsoidal, smooth, hyaline	*Penicillium* sp.	25.0
White-cream, yeast-like mycelium	White-cream smooth glabrous colonies, blastoconidia, large globose, ellipsoidal budding	*Saccharomyces cerevisiae*	15.0
Yellowish oil green to cedar green mycelium	Biseriate phialides, globose conidia to sub-globose, vesicles globose to pyriform, metulae phialides	*Aspergillus parasiticus*	20.0
White-cotton like fluffy mass mycelium	Umbrella-like columella, coenocytic twin sporagiophores, non-septate hyphae	*Rhizopus* sp.	22.5

CONCLUSION

The result of this study revealed that *kunun-zaki* sold in Eiyenkorin, Kwara State, Nigeria was contaminated with pathogenic microorganisms. The presence of *Shigella* sp., *Salmonella typhi, Escherichia coli, Klebsiella* sp., *Citrobacter* sp., *Candida albicans, Pseudomonas* sp., *Bacillus cereus, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus* sp. detected in the *kunu* drink could constitute serious threat to food safety. Ingestion of some of these organisms is capable of causing human illnesses such as pneumonia, food-borne intoxification, diarrhea, gastroenteritis, among others. Therefore, there is need to maintain adequate sanitary conditions during the preparation, and handling of *kunun* drink to prevent contamination. There is also need to enlighten the public on the possible health hazards associated with poor unhygienic processing and handling of *kunun-zaki*. Finally, regulatory agencies should encourage good manufacturing practices and ensure compliance with approved standards.

REFERENCES

Aboh, M. I. and Oladosu, P. 2014. Microbiological assessment of kunu-zaki marketed in Abuja Municipal area council (AMAC) in the Federal Capital Territory (FCT), Nigeria. *African Journal of Microbiology Research*, 8(15):1633-1637.

Adelekan, A. O., Alamu, A. E., Arisa, N. U., Adebayo, Y. O. and Dosa, A. S. 2013. Nutritional, microbiological and sensory characteristics of malted soy-kunuzaki: An improved traditional beverage. *Advances in Microbiology*, 3:389-397.

Arlington, V. A. 2007. Response to the questions posed by the food safety and inspection service regarding consumer guidelines for the safe cooking of poultry products. *Journal of Food Protection*; 70(1):251-260.

Ayandele, A. A. 2015. Microbiological Analyses of hawked kunu and sobo drinks within LAUTECH campus, Ogbomoso, Oyo State, Nigeria. *IOSR Journal of Environmental Science, Toxicology and Food Technology.* 9(10): 52-56.

Badua, M. H., Jideani, I. A. and Nkama, I. 2006. Production, acceptability and microbiological evaluation of weaning food formulations. *Journal of Tropical Pediatrics,* 52 (3): 166-172.

Barnett, H. L. and Hunter, B. B. 1987. Illustrated genera of imperfect fungi. Macmillian Publishing Company, New York.

Belewu, M. A. and Abodunrin, O. A. 2006. Preparation of kunu from unexploited rich food source: Tigernut (*Cyperus esculentus*). *World Journal of Dairy and Food Sciences*, 1(1): 19-21.

Chessbrough, M. 1984. Medical Laboratory Manual for Tropical Countries. 2nd Ed. ELBS Publication, UK. 11: 60-68.

Edem, V. E. and Elijah, A. I. 2016.Development of starter culture for the production of coconut yoghurt.*Annals. Food Science and Technology*, 17(1):41-49.

Elmahmood, A. M. and Doughari, J. H. 2007. Microbial quality assessment of kunu-zaki beverages sold in Griei town of Adamawa State, Nigeria. *African Journal of Food Science*, 011-015.

Food and Drug Administration (FDA) 2013.Revised guidelines for the assessment of microbiological quality of processed foods.FDA circular No 2013-010.

Gaffa, T., Jideani, I. A. and Nkama, I. 2002. Nutrients and sensory quality of kunu-zaki from different saccharifying agents.*International Journal of Food Science and Nutrition,* 53: 109-115.

Harrigan, W. F. and McCane, M. E. 1976. Laboratory methods in food and dairy microbiology. Academic Press, London. pp 240-249.

Holt, J. G., Kreig, N. G., Peter, H. A., Sneath, S. T. and Williams, S. T. 2000. Bergeys Manual of Systematic Bacteriology, 9th Edition. Lippincott Williams and Wilkins Publisher, Baltimore. Pp. 175-201, 527-528.

Ikpoh, I. S., Lennox, J. A., Ekpo, I. A., Agbo, B. E., Henshaw, E. E. and Udoekong, N. S. 2013. Microbial quality assessment of kunu beverage locally prepared and hawked in Calabar, Cross River State, Nigeria. *Global Journal of Biodiversity Science and Management,* 3(1):58-61.

Karagozlu N., Karagozlu C. and Ergonul B. 2007. Survival characteristics of *E. coli* O157:H7, *S. typhimurium* and *S. aureus* during kefir fermentation. *Czech Journal of Food Sciences,* 25(4): 202-207.

Mbachu, A. E., Etok, C. A., Agu, K. C., Okafor, O. I., Awah, N. S., Chidi-Onuorah, L. C., Ekwueme, V. C., Okpala, J., Ogbue, M. O. and Ikele, M. O. 2014. Microbial quality of kunu drink sold in Calabar, Cross River State, Nigeria. *Journal of Global Biosciences,* 3(2):511-515.

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-alcoholic local beverage) in Ogun State, Nigeria.*Journal of Advances is Biology and Biotechnology,* 7(1):1-8.

Ogbonna, I. O., Opobiyi, M. Y., Katuka, B. and Waba, J. T. 2011.Microbial evaluation and proximate composition of kunu-zaki, an indigenous fermented food drink consumed predominantly in Northern Nigeria. *Internet Journal of Food Safety,* 13: 93-97.

Oranusi, S. U., Umoh, V. J. and Kwaga, J. K. P. 2003. Hazards and critical control points of kunu-zaki, a non – alcoholic beverage in Northern Nigeria.*Food Microbiology,* 20: 127-132.

Umaru, G. A., Tukur, I. S., Akensire, U. A., Adamu, Z., Bello, O. A., Shawulu, A. H. B., Sunkani, J. B., Adamu, S. G. and Adamu, N. B. 2014. Micro flora of ofkunu-zaki and sobo drinks in relation to public health in Jalingo metropolis, North-Eastern Nigeria. *International Journal of Food Research,* 1: 16-21.

Wonang, D. L., Amienyo, C. A., Ekeleme, 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.