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Full Length Research Paper

Evaluating microbial safety of *Kunun-Zaki* beverages in Girei Town: A comprehensive study in Adamawa State, Nigeria

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Thirty *Kunun- zaki* samples were obtained as freshly formulated beverages from 10 different local hawkers in Girei town, Adamawa State, Nigeria and screened for microbial contamination. The pH of the samples ranged between 3.44 - 4.34 and total bacterial count ranged between 1.0 x 10^3 - 1.8 x 10^4 cells/ml. The presence of high microbial loads was indication of poor hygiene and/or poor quality cereals and water used in the preparations. The microorganisms recovered were *Escherichia coli, Staphylococcus aureus, Streptococuus pyogenes*, *Rhizopus nigricans*, *Penicillium digitatum, Asper-gillus fumigatus* and *Monilia sitophila.* The types and density of microorganisms recovered from calls for urgent measures to be taken by regulatory authorities in the processing and handling of the product before being sold to the unsuspecting general public.

Key words: Microbial quality, *Kunun-zaki*, microbial load, microbial contamination.

INTRODUCTION

Kunun-zaki is an indigenous fermented non-alcoholic beverage that is widely consumed for its thirst quenching properties. Though consumed throughout the year, it is extensively consumed during the dry season. The drink is produced from fermented millet, sorghum, guinea-corn and maize in decreasing order of preference. In some cultures, the grains are used in a composite from espe-cially millet, guinea-corn and sorghum in a ratio of 1:2 w/w (Abegaz, 2007). It is sweetened with honey and sug-ar together with small quantities of sweet potatoes and spices (ginger, black pepper or clove). The method of production is crude, not standardized with levels of ingre-dients not quantified and largely a family art. The proce-dure involves steeping the cereals in local household utensils such as buckets, calabashes and earthenware vessels. This is then followed by grinding of the steeped grains into a mush which is then mixed with spices (clo-ve, red or black pepper and ginger). This is then divided into two unequal portions, one portion is gelatinized with hot water and the other portion mixed with liquefying agents (sweet potato paste, malted rice and extracts of *Cadaba farinose* stem). The two portions are then mixed together at 70 - 75^{o}C and the mixture left at room tempe-rature for chance fermentation for 18 - 24 h. This is then filtered first using a piece of muslin cloth and then a sie-ve. After filtration, honey or sugar is then added to the filtrate to taste and is now ready for consumption (Onuo-rah et al., 1987; Akoma et al., 2006). Significant varia-tions exist in the procedures depending on taste and cul-tural habits leading to differences in quality and stability. While some cultures prefer *Kunun-zaki* with much pep-per or sweet taste, others prefer it with no pepper or sug-ar (Adeyemi and Umar, 1994) .It is usually packaged and sold in 1litre and 500 ml plastic bottles or even tied in small polyethene bags. *Kunun-zaki* must be consumed within 18 - 36 h of production due to its poor keeping qua-lity. The drink is very cheap because the cereals and ad-ditives used in its production are locally sourced as they are grown throughout the savannah belt of West Africa. Packaging materials are also cheap and easily available. Ayo and Okaka (1998) have reported that *Kunun-zaki* is rich in carbohydrates, vitamins and minerals but low in proteins. Furthermore, the methods of production are simple and cheap as no elaborate equipment and exper-tise required (Agboola, 1987).

The high water content coupled with crude methods of production and packaging under improper sanitary condi-tions predisposes *Kunun- zaki* to microbial contamination. This study was designed to assess the microbial quality of this immensely popular beverage and possibly high-light the risks involved for the consuming general public.

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Table 1. Mean pH values and Total bacteria counts (cfu/ml) for fresh kunun—zaki.

Vendor	pH	Samples	Nutrient Agar	MacConkey Agar	Mannitol salt Agar
A	4.34	A_1	4.0×10^4	-	1.8×10^4
		A_2	3.1×10^4	-	0.2×10^4
		A_3	3.6×10^4	-	0.8×10^4
B	3.75	B_1	2.5×10^4	2.0×10^4	0.4×10^4
		B_2	4.6×10^4	2.6×10^4	1.0×10^4
		B_3	3.1×10^4	1.8×10^4	0.6×10^4
C	3.51	C_1	1.9×10^4	1.3×10^4	0.1×10^4
		C_2	3.2×10^4	0.9×10^4	0.3×10^4
		C_3	3.4×10^4	0.5×10^4	0.5×10^4
D	4.15	D_1	1.56×10^4	8.9×10^4	2.8×10^4
		D_2	1.26×10^4	7.4×10^4	4.0×10^4
		D_3	8.6×10^4	5.8×10^4	3.6×10^4
E	3.44	E_1	5.0×10^4	2.1×10^4	4.6×10^4
		E_2	9.2×10^4	1.7×10^4	5.1×10^4
		E_3	7.5×10^4	1.3×10^4	3.7×10^4
G	4.10	G_1	8.0×10^4	0.5×10^4	1.76×10^4
		G_2	5.9×10^4	2.0×10^4	8.8×10^4
		G_3	1.27×10^4	1.3×10^4	1.19×10^4
H	3.48	H_1	1.84×10^4	5.0×10^4	1.03×10^4
		H_2	1.15×10^4	1.3×10^4	9.6×10^4
		H_3	1.46×10^4	2.0×10^4	1.23×10^4
I	3.49	I_1	1.60×10^4	1.0×10^4	2.6×10^4
		I_2	1.48×10^4	2.3×10^4	4.2×10^4
		I_3	1.75×10^4	0.3×10^4	6.5×10^4
J	3.69	J_1	1.23×10^4	1.6×10^4	3.9×10^4
		J_2	1.76×10^4	0.7×10^4	5.3×10^4
		J_3	1.08×10^4	0.2×10^4	2.6×10^4

Key: **-** no growth

MATERIALS AND METHODS

Collection of samples

Three samples of freshly prepared *Kunun-zaki* were collected from each of 10 different hawkers between the months of February and July, 2005 in Girei town, Girei local Government of Adamawa State, Nigeria. The samples were packaged in 500 ml sterile plastic bottles and immediately transferred to the microbiology laboratory for isolation of microorganisms and enumeration of bacteria.

Determination of pH of the samples

The pH of the various samples was immediately determined using sterile probes of the pH meter (Corning 35).

Determination of total count of bacteria

This was carried out on agar plates of Nutrient agar (NA), Mac-Conkey agar (MCA) and Mannitol Salt agar (MSA) all of Oxoid gra-de, using the pour plate method. The samples were serially diluted and 1ml of appropriate dilution was used to inoculate each of the plates in triplicates. The culture plates were then incubated at 37ºC for 24 - 48 h and colonies counted on a Gallenkamp colony counter.

The mean of triplicate results were then recorded as the colony count (Lateef et al., 2004).

Isolation and Identification

Discrete colonies of the organisms (for bacteria) were selected and sub cultured from the mixed cultures of the plates to respective NA plates and incubated at 37ºC for 24 h. The bacterial isolates were then identified following standard microbiological procedures as described by Buchanan and Gibbons (1974) and Cheesbrough (2002).

For the filamentous fungi, appropriate spore dilutions (1.0×10^7 spores/ml) of the *Kunun- zaki* samples were surface-spread in triplicates on Potato Dextrose Agar (PDA, Oxoid) plates and incubated at room temperature (30 - 32ºC) for 48 - 72 h. After incubation, the colonies were screened and identified based on the taxonomic schemes and descriptions by Ainsworth et al. (1973) and Mislivec et al. (1992).

RESULTS

The mean pH values of the *Kunun-zaki* and the extent of microbial contamination are shown in Table 1. Results of

Table 2. Microorganisms isolated from *kunun-zaki* samples.

Organisms	Characteristics	Samples from vendors																											
		A			B			C			D			E			F			G			H			I			J		
		1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
S. aureus	Slightly raised golden yellow G+ colonies that ferments Manitol	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-
E. coli	Large, circular, low cobnvex colorless opaqueG+, LF colonies on MC	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Str. pyogenes	Small dry shiny mucoid colonies on BA, G+e cocci in chains	x	-	-	-	-	-	-	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P. digitatum	Grren/black mycelia, spores on flask-shaped sterigmata	x	x	x	-	x	x	-	-	-	x	-	-	-	-	-	X	-	-	x	-	-	-	-	-	-	-	-	-	-	-
M. sitophila	Red mycelia, floccose and salmon-colored spores	-	-	-	x	-	-	-	-	-	-	x	x	-	-	-	-	X	X	-	-	-	-	-	-	X	x	x	-	-	-
R. nigricans	White and cottony mycelia, floccose white to gray spores	-	-	-	-	-	-	x	x	x	-	-	-	-	-	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-
A. fumigatus	Bluish green floccose matted mycelia, conidiophore-bearing phialides (flask-shaped) that produce spores	-	-	-	-	-	-	-	-	-	-	-	-	x	x	-	x	-	-	-	-	-	X	-	-	-	-	-	-	-	-

Note: x = presence; - = Absence; G+ = Gram positive; LF = lactors fermenting; MC = MacCkonkey agar; BA = Blood agar; A-J = vendors; 1-3 = samples

pH determination showed that all the samples were acidic in nature. Samples collected from vendor E had the lowest pH of 3.44 and those collected from vendor A had the highest pH of 4.34. Samples A1 - A3 (from vendor A) had bacterial counts (cfu/ml) range of 3.1 - 4.0 x 10^4 cfu/ml on NA, 2.0 x 10^2 - 1.8 x 10^4 cfu/ml in MSA but none on MCA. Samples B1 - B3 (from vendor B) contained bacteria ranging from 2.5 - 4.6 x 10^4 cfu/ml on NA, 1.8 - 2.6 x 10^4 cfu/ml on MCA and 4.0 x 10^2 - 1.0 x 10^4 cfu/ml on MSA. These results follow similar trend for all the other samples. Sample E (E1 - E3) had highest number of counts on NA (5.0 - 9.2 x 10^4 cfu/ml), closely followed by sample F (F1 - F3) with a cell density of 4.4 - 6.2 x 10^4 cfu/ml and sample J (J1 - J3) with the lowest number of cells ranging from 1.08 - 1.74 x 10^4 cfu/ml. On MCA, sample D (D1 - D3) had highest number of cells ranging from 5.8 - 8.9 x 10^4 cfu/ml and sample J (J1 - J3) with lowest bacterial population of 2.0 x 10^3 - 1.6 x 10^4 cfu/ml. On MSA, sample from vendor I (I1 - I3) had the highest bacterial cell density of 2.6 - 6.5 x 10^4 cfu/ml, while sample C (C1 - C3) had the lowest cell density of 1.0 x 10^3-5.0 x 10^3 cfu/ml.

Table 2 shows the microbial isolates obtained from the various *Kunun-zaki* samples collected. *Staphylococcus aureus* and *Escherichia coli* were isolated from samples A1 - J3, while samples A1 and D1 contained *Streptococcus pyogenes* and *Penicillium digitatum* was isolated from each of samples A1 - A3, B2 - B3, D1, E1, G2, I1 and J1 - J3. Also *Monilia sitophila* was isolated from samples B1, D2 - D3, F1 - F2, and I1 - 13; *R. nigricans* was isolated from samples C1-C3 and G1; while *Aspergillus fumigatus* was isolated from samples E1 - E2, F1, and H1.

DISCUSSION

Being a thirst quenching beverage, *Kunun-zaki* has high moisture content. The proportion of water varies from 55 - 98%, the remainder being mostly additives (Giese, 1995). All the samples were acidic in nature (pH 3.34 - 4.42). This level of acidity of *Kunun-zaki* have been described by several researchers including Efiuvwevwere and Akoma (1995) and Akoma et al. (2006) who attributed these to the presence of certain species of lactic acid bacteria, namely *Lactobacillus leichmannii* and *Lactobacillus fermentum* during the fermentation process. In this study however, attention was directed at isolating pathogenic bacteria. Similar local drinks with acidic pH values have been reported for *Zobo* and for orange juice products (Lateef et al., 2004) as well as *burukutu* and *pito* (Kolawole et al., 2007). Although these classes of beverages are acidic in nature, the acidity tends to increase with increase in fermentation period resulting into spoilage. Consequently, the low pH values may have encouraged the growth of fungi and this could be responsible for the species of microorganisms isolated.

The main components of cereals from which *Kunun-zaki* is made are carbohydrates, proteins, vitamins and minerals, and the chief product of fermentation is lactic acid and this leads to a decrease in pH values and an increase in acidity (Nkama, 1993). The acidic nature of the samples may also be due to the fact that the *Kunun-zaki* might have started undergoing spoilage even before the time of purchase, and such may lead to production of certain metabolites that could bring about reduction in pH of the product.

Kunun-zaki and other indigenous Nigerian nonalcoholic beverages, such as *Kunun-aya* and *Simi* have been reported to contain high nutritional values because of the raw materials from which they are made. Spices are usu-ally added in small quantities to improve taste and flavour and as these are agricultural commodities, which may contain a high level of microbial impurities (Adeyemi and Umar, 1994). These can be sources of spoilage and pat-hogenic microorganisms (Bibek, 2001). The pH of *Kunun-zaki* is usually too low to allow the growth of pathogenic microorganisms, but the presence of *E. coli, S. aureus* and *Streptococcus* spp. could be a matter of serious concern. *S. aureus* is a normal flora of the skin, nose, throat, palms, hairs and mucus membrane and a common etiological agent of septic arthritis (Alice, 1976). *E. coli* is an important member of the coliform group it is part of the normal flora of the intestine of human and vertebrates. Some strains of *E. coli* can cause gastroenteritis, diarrhea and urinary tract infections (Pelczar et al., 1993). The Streptococci are normal flora of the throat and the buccal cavity. In their own study on two hundred and forty samples of *Kunun-zaki*, Umar et al (2004) reported the presence of organisms like *Bacillus cereus, S. aureus* and *E. coli.* The presence of these pathogens even in small numbers could render a beverage unsuitable for human consumption (PHLS, 2000). It is possible that contamination by these pathogens could have occurred during sieving and packaging, as most of the people involved in the production, packaging and hawking do not take necessary precautions, and as such contamination could be very prominent. Contamination of food items by specific species of microorganisms is largely due to the presence of these organisms and their entrance into the food or beverage as a result of poor hygiene and sanitation (Bibek, 2001). The dominance of *Lactobacillus leimannii* and *L. fermentum* in their own samples led Akoma et al. (2006) to conclude that Kunun-zaki is a lactic acid bacteria fermented beverage. Fungi isolated are *P. digitatum, A. fumigatus, R. nigricans* and *M. sitophila.* The presence of these fungal species is associated with spoilage of the beverages (Kolawole et al., 2007).

The total bacterial counts in the various *Kunun-zaki* samples ranged from 1.0 x 10^2 -8.9 x 10^4 cfu/ml as shown in Table 1. Sample A1-A3 obtained from vendor A had a bacterial count of 3.1 x 10^4 cfu/ml on NA, a range of 2.0 x 10^2 - 1.8 x 10^4 cfu/ml on MSA corresponding with pH 4.34. There was no growth on MCA which may be

due to relative absence of coliforms in this sample A .The total bacterial counts obtained in this study fall within then ranged between 1.0 x 10^2 - 1.0 x 10^5 cfu/ml. Earlier works by Hatcher et al. (1992) also reported similar abnormally high bacterial populations in orange juices (Hatcher et al., 1992). This high colony counts is an indication of spoilage as a consequent of either poor hygiene or poor quality of cereals and water used.

Many native African beverages are little known outside the parent continent. A concerted effort should therefore be made to improve in the quality and production techniques of these indigenous exotic beverages so that large scale production for export outside the continent can be carried out. Many people now prefer imported and exotic beverages because of their attractive forms, long shelf life, ease of transportation and other forms of utility which consumers associate with them (Achi, 2005). As of now, there are no industries involved in production of *Kunun-zaki*. Kunun-zaki is widely believed to be of immense social, economic and medicinal importance to its numerous consumers (Akoma et al., 2006). To safeguard public health, Governments and regulatory authorities should intervene by setting standards in acquisition of raw materials, production procedures and techniques as well as health status of personnel. Producers and vendors of *Kunun-zaki* should be encouraged to utilize the technical assistance of National Agency of Food, Drugs Administration and Control (NAFDAC) towards attaining quality standards. Reports have indicated that most NAFDAC approved sachet water produced by small scale industries in Nigeria have attained acceptable microbiological standards (Lateef and Yusuf, 2002). The extent of organic and inorganic contaminants of *Kunun-zaki* beverage has not been evaluated. Further studies are therefore recommended as these too equally affect the health and well being of the general public.

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