MICROBIAL EVALUATION OF KUNUN-ZAKI PREPARED AND SOLD IN THE BAUCHI METROPOLIS.

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ABSTRACT

Freshly prepared kunun-zaki samples were collected from ten different locations in the Bauchi metropolis and were analyzed for the presence of some microorganisms frequently implicated in foodborne disease outbreak using standard microbiological methods. Their microbial loads were determined using standard methods. The bacteria load in all the samples were higher compared to fungal load. The bacterial counts ranged from 1.9 x 10^4 to 8.6 x 10^6 cfu/ml while fungal counts ranged from 1.3 x 10^4 to 4.5 x 10^4 cfu/ml. The bacterial isolates were: Escherichia coli, Staphylococcus aureus, Streptococcus spp. and Lactobacillus plantarum while Candida albicans and Saccharomyces cerevisiae were the fungal isolates. The results of this study showed that kunun-zaki prepared and sold in all the locations in the Bauchi metropolis were grossly contaminated with potential pathogenic bacteria and antibiotic resistant bacteria (Escherichia coli and Staphylococcus aureus). This may lead to difficulty in antibiotic chemotherapy among those that consume kunun-zaki in Bauchi metropolis.

Introduction

Kunun-zaki drink is a locally prepared indigenous non-alcoholic beverage (Maji *et al*., 2011) which is widely produced and consumed in large quantity in Nigeria, especially the northern part of the country (Amusa and Ashaye, 2009). This local beverage is consumed in both wet and dry season but more in the dry season because of its optimal thirst quenching abilities. Kunu is consumed any time and by both adults and children as breakfast drink, food complement, as a refreshing drink usually used to entertain visitors. It is used to stimulate appetite and is commonly served at social gatherings and according to Onuorah *et al.,* (1987), the non-alcoholic nature of the drink makes it to be readily consumed by Christians and Muslims alike as a substitute for alcoholic ones.

The drink is mostly consumed within 20-35 h of its production due to its poor keeping quality (Akoma *et al*., 2012). This drink is not expensive because the grains and other ingredients used for its production are locally sourced and are mostly grown within the savannah region and almost throughout the year. The packaging materials are also available, cheap and easily affordable. No elaborate equipment and expertise are required for its production (Akoma *et al*., 2013; Oluwalana and Adedeji, 2013).

It is also very nutritious and of medicinal value. Kunun-zaki has been reported to be rich in vitamins, minerals, carbohydrates and proteins (Essien *et al*., 2009; Adebayo *et al*., 2010; Folasade and Oyenike, 2012; Oluwajoba *et al*., 2013). Kunu zaki processed from Sorghum grain has been reported to contain 11.6% protein, 3.3% fat, 1.9%ash and 76.8%

carbohydrate and arrays of amino acid. The different types of kunu in Bauchi are: kunu zaki, kunu gyade, kunun tsamiya, kunun baule, kunun jiko amshar and kunu gayamba as they are called in different tribes of Bauchi state.
The drink is produced from fermented millet, sorghum (guinea corn) and maize in decreasing order of preference. In some culture, the grains are used in composite form especially millet and sorghum (guinea-corn) in a ratio of 1:2 w/w (Abega, 2007). It is sweetened with honey and sugar together with small quantities of sweet potatoes and spices (Ginger or black pepper).
Its processing is mostly done by women using simple household equipment and utensils. The processed kunun zaki is usually packaged and sold in 50ml to 1 litre plastic bottles and at times tied in some disposable polythene bags or in bulk inside large containers and distributed under ambient temperature or cooled in a refrigerator where available.
In developing nations like Nigeria, it has not been possible to have control over the processing and sales of this beverage.
Kunu zaki has high moisture content, its method of production is crude and is packaged under unhygienic condition. It is also highly consumed due to the cost of other non-alcoholic drinks. The drink is hawked in the motor parks, school premises and in market places where it is exposed to contamination. These and other factors predispose kunu zaki to microbial contamination. This research was conducted to evaluate the microbiological qualities of this hawked non-alcoholic drink called kunu-zaki in the Bauchi metropolis.

Materials and Methods

Sample Collection

Different locations (5) where Kunun-zaki is mainly prepared and sold in the Bauchi metropolis were randomly selected for the purpose of sample collection. Samples of the Kunun-zaki were aseptically collected in triplicates into sterile corked plastic tubes from the five different locations . The samples were labeled appropriately, placed into separate plastic bags and conveyed to the Microbiology laboratory of the Federal Polytechnic Bauchi for analysis. The locations were: Central market, Gudun Sayawa, Gwallameji, Muda Lawal, Rafin-zurfi, Railway Market, Wunti-Dada, Wunti Market, Yelwa-Makaranta and Yelwa-Tudu.

Determination of pH

The pH of each sample was determined using the pH meter. The electrode of the pH meter was sterilized using a standard buffer solution. The electrode of the pH meter was inserted into 9ml of the kunu -zaki sample taken in a beaker and the reading displayed on the pH meter was recorded. This was repeated for each sample of kunu zaki collected.

Isolation and Enumeration of Bacteria and Fungi (Microbiological Analyses).

Kunu-zaki samples collected (approximately 50ml) each contained in pre-autoclaved containers were used for the isolation and enumeration of the microorganisms. In each isolation protocol, the sample was shaken and 1ml aseptically introduced into 9ml of sterile normal saline and was homogenized by shaking followed by further decimal

dilutions up to 10^{-6} concentrations. 0.1ml of diluted sample was used to inoculate freshly prepared media by spread-plate method. The media employed for the isolation and enumeration of the organisms were: Baird Parker Medium (BPM) (Lab M. Ltd, Bury Lancashire, United Kingdom) for *S. aureus;* Eosin Methylene Blue Agar (EMBA) (Himedia Laboratories Pot Ltd, India) for *E. coli;* de Man, Rogosa and Sharpe (MRS) media for Lactobacillus and Deoxycholate Citrate Agar (DCA) (Park Scientific Limited, Moultan Park, Northampton) for *Salmonella* and *Shigella spp*. Nutrient Agar (NA) (Biotech Lab. Ipswich, UK) was used for total viable count. Media were sterilized by autoclaving at 121ºC for 15min except DCA which involved only boiling over gauze. Sabouraud Dextrode Agar (SDA), with Chloramphenicol (250mg/100mL^{-1}) was used for fungi while for yeast the medium was adjusted to pH 3.5 with tartaric acid. All plates were incubated for 48hours at 37ºC except for SDA that were incubated at 28ºC for 7 days.

Pure cultures of each isolate were obtained by streaking the specific colonies on suitable media and incubated appropriately. These were then maintained in agar slants in McCarthney bottles and were used for identification purposes.

Total Viable Counts

Nutrient Agar (NA) (Biotech Lab. Ipswich, UK) was inoculated with a 0.1ml of appropriately diluted kunu-zaki by spread-plate technique and incubated at 37ºC for 24 h. In all cases of colony counts, the resulting colonies following inoculation and incubation were counted using digital illuminated colony counter (Labtech, New Delhi, India). Colonies were counted and multiplied by the dilution factor.

Biochemical Identification of the Isolates

Pure cultures of each of the isolates were identified based on the cultural, morphological characteristics and biochemical methods (Baron and Sydney 1990; Adegoke, *et al*, 1993; Bergyey's Manual of Determinative Bacteriology (Holt, 1994) and Cheesbrough, 2006).

Fungi were identified with reference to Frazier and Westhoff (1978), while the yeasts were identified using the methods of Lodder (1970)and Beech *at al*, (1986). The identity of the microbes were further confirmed by comparison with existing cultures already identified by the Mycological Institute, Kew, London obtained from the Institute of Agricultural Research and Training, Moor plantation Ibadan, Nigeria.

The biochemical tests carried out for the identification of bacterial isolates were: citrate utilization, indole, methyl-red, spore stain test, Voges-proskauer, triple sugar iron (TSI), urease, oxidase, coagulase and catalase tests (Washinton *et al*., 2006 and Cheesbrough, 2006).

Results

The pH of the sample was in the range of 4.02 to 4.73 as shown in table I. The bacteria load of the samples was as presented in table 2. As can be seen, the bacteria load in all the samples were higher compared to fungal load. The bacterial counts ranged from 1.9×10^4 to 8.6×10^6 cfu/ml while fungal counts ranged from 1.3×10^4 to 4.5×10^4 cfu/ml. The

morphological and biochemical characteristics of bacteria isolated were as shown in the table 3. Four probable bacteria genera were isolated, *Escherichia coli, Staphylococcus species, Streptococcus species, and Lactobacillus sp*. All were found in the entire sample *Saccharomyces cerevisiae and Candida* was the only fungi isolated.

Table 1: **The pH of the kunu-zaki Sample**

Sample site	**Mean pH value**
Central market	4.34
Gudun Sayawa	3.75
Gwallameji	3.51
Muda lawal market	4.15
Raffin zurfi	3.89
Railway market	3.45
Wunti dada	4.5
Wunti market	3.11
Yelwa makaranta	5.0
Yelwa tudu	4.0

Table 2: Bacteria and Fungi load in cfu/ml of sample

Samples sites	Mean bacteria Load (cfu/ml)	Mean fungal Load (cfu/ml)
Central market	4.0×10^5	3.1×10^4
Gudun Sayawa	6.1×10^4	2.3×10^4
Gwallameji	5.6×10^4	1.3×10^4
Muda lawal market	3.5×10^4	2.0×10^4
Raffin-zurfi	4.6×10^4	2.6×10^4
Railway market	3.1×10^4	1.8×10^4
Wunti dada	1.9×10^4	1.3×10^4
Wunti market	3.2×10^4	3.4×10^4
Yelwa makaranta	4.8×10^4	3.5×10^4
Yelwa tudu	8.6×10^6	4.5×10^4

Table 3: Morphological and Biochemical characteristic of Bacteria Isolates

Colonial/cell morphology	**Biochemical Tests**	Probable organism

	Coag	Mot	Gram rxn	Cat	Spst	Glu	Lac	Malt	Suc	Man	
Large, circular, low colonies colourless, lactose fermenting colonies on Mc Conkey	-	+	-	+	-	A	A	A	-	A	*Echericheria coli*
Large creamy white smooth opaque colonies	+	+	-	+	-	A -	A	A	A	A	*Staphylococcus sp*
Cocci in clusters and single	-	-	+	-	-	A	A	-	-	A	*Streptococcus sp*
Milky white colour entire edge cocci in pairs & chains	+	-	-	-	-	A	A	A	A	-	*Lactobacillus sp*

Key

A = Acid
Coag = Coagulase
Mot = Motility
Gram rxn = Gram reaction
Cat = catalase
Glu = Glucose
Lac = Lactose
Malt = Maltose
Suc = Sucrose
Man = mannitol
Spst = Spore stain

Table 4: Morphological and cultural Characteristics of fungi isolates

Colour of Aerial hyphae	Nature of hyphae	Presence of specific structure	Characteristics	Probable fungi
White to cream	Pseudohyphae	Pseudomycelum	Round, oval on the pseudomycelium	*Candida sp*
Colorless	nil	nil	Egg shaped	*S. cerevisiae*

Discussion

Kunu zaki has high moisture content. The proportion of water varies from 55 to 98%, the remainder being mostly additives (Cyrose, 1995). All the samples were acidic in nature (pH 3.34-4.72). This level of acidity of Kunu Zaki has been reported by several researchers including Efiuwevwere and Akoma, 1995) and Akoma *et al.,* (2001) who attributed these to the presence of certain species of lactic acid bacteria namely*:* *Lactobacillus*, *Leichmannii* and *lactobacillus* fermentation during the fermentation process.

In this study however, attention was directed at isolating pathogenic bacteria. Similar local drinks with acidic pH involved 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 the classes of beverages are acidic in nature, the acidity tends to increase with increase in fermentation period resulting to spoilage consequently, the low pH value may be encourage the growth of fungi and this could be reason for the 2 species of fungi isolated.

The pH of kunu 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, palm, hairs and mucus membrane and a common etiological agent of septic arthritis (Alice, 1990). *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* cause gastroenteritis, diarrhea and urinary tract infection (Pelezar *et al* 1993). The *Streptococcus* is a normal flora of the throat and the buccal cavity. In their own study, on two hundred and forty samples of kunu zaki (Umar *et al* (2004) reported that presence of these pathogens (*E. coli*, *S. aureus and streptococcus spp)* 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, package and hawking do not have necessary precaution and such contamination could be prevented.

The total bacteria counts obtained in this study fall within the ranges of 1.9×10^4 - 8.6×10^6 cfu/ml as shown in table 1. Efiucwere, and Akoma, 1995 also reported similar abnormality of high bacterial populations in kunu zaki prepared and sold in Jos methropolis (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.

Conclusion

From the result obtained, it could be seen that the probabte organisms associated with kunun-zaki were *Escherichia coli Staphylococcus species, Streptococcus species Lactobacillus species Candida species and Aspergillums species* and the action or presence of this organisms in kunun –zaki rendered it unfit for human consumption.

Recommendations

To safe guard public health, government and regulatory authorities should intervene by setting standards in acquisition of raw material, production techniques as well as health status of personnel involved in the production process.

Treated water or clean water should be used during processing and in dilution of the processed drinks to avoid contamination with pathogenic microorganisms.

The packaging materials should be sterilized.

Health education training should be organized regularly for those involved in the producrion of kunu zaki by the health workers on the importance of cleanness of their environment.

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