Microflora of Kunun-Zaki and Sobo drinks in relation to public health in Jalingo Metropolis, North-Eastern India

ABSTRACT

A study was conducted to access the occurrence of microflora in *kunun-zaki* and *sobo*, two non-alcoholic locally produced beverage drinks in Jalingo Metropolis, North-eastern India. One hundred and fifty *kunu-zaki* and 100 *sobo* were collected and analyzed. Of these samples, 98% (of the *kunu-zaki*) and 95% (of the *sobo*) drinks showed growth presumed to be pathogenic bacteria. The occurrence of *Staphylococci* spp. is significantly higher (P<0.05) in both *kunun-zaki* and *sobo* drinks (64.0 and 48.0%, respectively) and followed by *Streptococci* spp. (16.7 and 20.0%, respectively). Similarly, occurrence was observed in the case of *Bacillus* spp. (8.9 and 4.0%, respectively), *Escherichia coli* (6.0% and 3.0, respectively) and yeast (2.7 and 0.0%, respectively). However, *sobo* drinks recorded higher occurrence of *Salmonella* sp. than *kunun-zaki* (7.3%). There was no significant difference (P>0.05) between the isolation of *Shigella* spp. in the two drinks. No yeast was isolated from all the *sobo* drinks examined. The presence of these bacteria is of public health importance since most of them are implicated with foodborne gastroenteritis in humans. Greater percentage of the bacteria were isolated in samples from Jalingo main market, followed by those obtained from ceremonies and motor parks. The least occurrence was in samples obtained from government ministries and schools. The contamination was mainly post processing due to environmental, faecal and human handlers as a result of unhygienic practices during packaging and sales. Proper hygienic and sanitary measures are recommended during processing to prevent contamination of these drinks.

INTRODUCTION

Food borne intoxication is common in man (USFDA, 2008) and local beverage, and alcoholic drinks may be common sources of infection. The consumption of these
local drinks is of public health significance. Hence, local drinks may serve as vehicles for zoonotic and food-borne diseases or pathogens such as Staphylococcosis, Salmonellosis, Brucellosis, Tuberculosis, Shigellosis, Listeriosis, *Escherichia coli* infection, etc. The current food safety challenges rise slowly over several years and requires strategic efforts to be controlled (Sperber, 2003). In the United States, the Center for Disease Control and
Prevention (CDC) has developed the FoodNet and PulseNet programs, which is a surveillance system that tracks foodborne illness and an electronic system that stores the DNA fingerprints of pathogens, respectively (Sperber, 2003).

Kunun-zaki is a non-alcoholic beverage drink produced and widely consumed by adults and infants in the savannah region of India as a refreshing drink, an appetizer, a food complement and to quench thirst (Oranusi et al., 2003). *Kunun-zaki* is prepared from either guinea corn (*Sorghum bicolor*), millet (*Penisetum typhoides*), maize (*Zea mays*), rice (*Oryza sativa*), or wheat (*Triticum aesstivum*). Traditionally, the production involves steeping of the whole grains for 6-24 hour, wet milling with spices and sweep potato, gelling of about three-quarter (3/4) of the mixture in hot water, pitching with about one-quarter (1/4) fresh (ungelled) part of the mixture and then allowing to ferment overnight (Oranusi et al., 2003). Thereafter, the supernatant is ready for consumption.

Sobo drink is also a non-alcoholic beverage drink prepared from dried calyces of Roselle (*Hibiscus sabdariffa*). The production involves the boiling of the calyces in water for 1-2 hours, cooling and then sieving. This is followed by the addition of ginger, flavor and sugar; and finally chilling in a refrigerator before consumption.

These products are being produced on daily basis for sales in markets, offices, schools, motor parks and as drinks during festivities, weddings and naming ceremonies. They serve as compliments or supplements to certain drinks which are exorbitant to the local populations. The production procedures and sales of these products are carried out under unhygienic conditions which may predisposed them to many pathogens of public health importance. For example, the productions are carried out with no precautions against cross contamination from producers and handlers and also done in an open environment. Apart from that, there is no single regulatory agency that monitors the production system of these drinks in spite of the public health dangers associated with their consumption. In a study on safety and quality evaluation of street foods sold in Zaria, India, Umoh and Odoba (1999) found the mean aerobic counts for *kunu* ranged from 3.67±0.67 to 4.29 ± 1.14 $\log_{10}$/g with those sold by mobile food sellers having a significantly higher mean (4.29±1.14 $\log_{10}$/g) than that sold by stationary food sellers (3.67±0.4 $\log_{10}$/g) with *Bacillus cereus* and *Staphylococcus aureus* being the major bacteria isolated. Also, Oranusi et al. (2003) worked on the hazards and critical control points of *kunu-zaki*, in Northern India and discovered that *S. aureus* contamination in all the samples after pitching increased to 2.90 $\log_{10}$ cfu ml^{-1} while *Bacillus* counts increased from 1.69 to 4.36 $\log_{10}$cfu ml^{-1}. Several foods and water were extensively studied to determined their microbial quality and safety in some parts of the world including India (Ameh and Abubakar, 2002; Okolocha et al., 2006; Oranusi et al., 2006; Oranusi et al., 2007; Kokkinakis et al., 2008; Abdalla and Hussain, 2010), but however, literature is scarce on foods generally and *kunu-zaki* and *sobo* drinks in particular in the study area. This study therefore, was aimed at providing preliminary information on the

occurrence of some microflora and their public health implications in *kunun-zaki* and *sobo* drinks sold and consume in Jalingo Metropolis, Taraba State, North-Eastern-India.

MATERIALS AND METHODS

The study area

The study was carried out in Jalingo Metropolis, Taraba State. Jalingo is the capital of Taraba State which was created in 1991 by Federal Military Government of India. It is located between latitude 6° 30 and 9° 36 North and longitude 9° 10 and 11° 50' East. It is bounded in the North by Lau Local Government Area (LGA), to the South by Ardo-kola LGA to the East by Yorro LGA. and West by Ardo-kola LGA. It is located on the Guinea savannah region and has two seasons; the dry season, which start from October to March and the rainy season which start from April and ends in September (TSMISD, 2004).

Sample collection

The sampling sites and sources were selected based on the availability of the samples and also the human population who normally purchases and consumes these products. The populated areas selected were; Jalingo main market, schools, motor parks and government ministries. *Kunun-zaki* and *sobo* drinks meant for sales and human consumption were purchased randomly from vendors in these areas. The samples were purchased without any information on the purpose. Also, samples were collected during ceremonies and occasions like weddings, birthday parties and naming ceremonies. One hundred and fifty samples (that is, 30 from each location) of *kunun-zaki* and 100 samples of *sobo* drinks (that is, 20 from each location) were collected. All the samples were placed on ice and transported to the Microbiology Laboratory of College of Agriculture, Jalingo, for analyses.

Microbial analyses: Microbial analyses of the *kunu-zaki* and *sobo* drinks was performed using the pour plating and streaking techniques as described by Bharath et al. (2003) and and Eaton et al. (2005). One mililitre of each of the samples was inoculated into 9 ml each of Tryptone Soya Broth (TSB) for enrichment in test tubes. The test tubes were then incubated at 37°C for 24 h. The inoculated broth were plated onto Baird Parker supplemented with egg yolk and Potassium Tellurite for the isolation of *Staphylococci* and yeast, Salmonella-Shigella agar for *Salmonella* and *Shigella*, Blood agar for *Streptococci*, Eosin Methylene Blue (EMB) agar for *Escherichia* and MacConkey agar for others (Egwu et al., 1995; Roberts and Greenwood, 2003; Oranusi et al., 2007; Kishere, 2012). All the plates were incubated overnight at 37°C for 24 h. Isolates showing growth were identified using biochemical tests, namely; Gram staining, catalase, coagulase, motility, Indole, Triptone sugar Iron, etc. as described by Ameh and Abubakar (2002), Bharat et al. (2003) and Eaton et al. (2005).

Table 1. Microflora occurrence in *kunun-zaki* and *sobo* beverage drinks in Jaingo Metrpolis.

Bacteria species	*Kunun-zaki*	*Sobo*
Bacillus	13(8.9)^{b}	4(4.0)^{a}
Eschericia	9(6.0)^{b}	3(3.0)^{b}
Proteus	10(6.7)^{b}	6(6.0)^{b}
Salmonella	11(7.3)^{c}	8(8.0)^{c}
Shigella	5(3.3)^{c}	3(3.0)^{c}
Staphylococci	96(64.0)^{f}	48(48.0)^{g}
Streptococci	25(16.7)^{e}	20(20.0)^{e}
Yeast	4(2.7)^{f}	
Negative samples	3(2.0)	
Total number of samples (n)	**150**	

Values in parenthesis represents percentage occurrences; values in the same row with same superscript differ significanty (P<0.05).

Data analyses

Simple percentages were calculated to determine the occurrence of bacteria in the two drinks and also in the five different locations. Chi-square test at 5% level of confidence was also used to compare the occurrence of the bacteria between the two drinks and the five different locations. P<0.05 was considered statistically significant.

RESULTS

The results obtained from the study revealed that 153 (98%) of the *kunu-zaki* and 95 (95%) of the *sobo* drinks analyzed showed growth presumed to be pathogenic bacteria. The occurrence of *Staphylococci* spp. was significantly higher (P<0.05) in both *kunun-zaki* and *sobo* drinks (64.0 and 48.0% respectively) and followed by Streptococci species (16.7 and 20.0%, respectively) (Table 1). Similarly, the occurrence of *Bacillus* spp. (8.9%), *Escherichia coli* (6.0%) and yeast (2.7%) in *kunun-zaki* were significantly higher (P<0.05) than the occurrence in *sobo* drinks with 4.0, 3.0 and 0.0%, respectively (Table 1). However, *sobo* drinks recorded higher occurrence of *Salmonella* species than *kunun-zaki* (7.3%). There was no significant difference

(P>0.05) between the isolation of *Shigella* spp. in *kunu-zaki* (3.3%) and *sobo* (3.0%) drinks. No yeast was isolated from all the *sobo* drinks examined.

Tables 2 and 3 show the occurrence of bacteria in the two drinks from different locations. Greater percentages of the bacteria were isolated in samples from Jalingo main market, followed by those obtained from ceremonies and motor parks. The least occurrence was in samples obtained from government ministries and schools. For instance, the occurrence of *Staphylococci* spp. was 76.7 and 55% in both *kunun-zaki* and *sobo* drinks, while that of *Streptococci* was 23.3 and 20%, respectively. On the other hand, 6.7% of the *E. coli* was isolated in *kunn-zaki* from all the sources except samples from government ministries which had only 3.3%, while the two *E. coli* isolated from *sobo* were in samples obtained from ceremonies and Jalingo main market.

DISCUSSION

Kunu-zaki and *sobo* drinks are two non-alcoholic beverage drinks prepare and consume in large quantities in Jalingo Metropolis and environs. The drinks are well accepted by all religious and ethnic groups in these areas, and hence been produced as supplements and complements to soft drinks during occasions like weddings, naming ceremonies, *Sallah* (Muslims Eid- el-Fitr), Christmas and during condolences. The production systems are sometimes done under unhygienic conditions (Oranusi et al., 2003) with no authority to monitor their microbial quality and safety. In this study, the two products were examined for the presence of microflora which can probably be transmitted to human beings.

The overall results revealed that both *kunun-zaki* and *sobo* drinks are highly contaminated with bacteria which may be potentially pathogenic to human beings. The occurrence of *Staphylococci* spp. (64.0 and 48.0%), *Streptococci* (16.7 and 20.0%), *Salmonella* (7.3 and 8.0%), *E. coli* (6.0 and 3.0%) and *Shigella* spp. (3.3 and 3.0%) in *kunun-zaki* and *sobo* drinks was statistically significant (P<0.05) from public health point of view. These organisms are considered the leading cause of food borne toxicosis outbreaks worldwide (Tsegaye and Ashenafi, 2005; Karagozlu et al., 2007; Arlington, 2007). Although *kunun-zaki* samples were more contaminated than the samples of *sobo* drink, the occurrence of the pathogens in *sobo* was also significant (P<0.05). The results obtained from this study are quite higher than those reported by Umoh and Odoba (1999) and Oranusi et al. (2003). The reason could be that Oranusi et al. used only three producers around Zaria to determine the hazard analysis and critical control points (HACCP) of the *kunu-zaki* while Umoh and Odoba analyzed different types of street foods with only 10 *kunun-zaki* samples among them. Therefore the sample size in these studies were small when compared to the present study coupled also with the fact that they used the total bacterial and coliform counts rather than the isolation procedures used in the present study. Interestingly, this study appears similar to the one reported by Ameh and Abubakar (2002) in Maiduguri who isolated *S. aureus* (32.0%), *Proteus* spp. (22.5%), *Streptococcus* spp. (18.7%), *Baccillus* spp. (10.0%), *E. coli* (8.8%) and Yeast (5.0%) from fresh milk and fermented milk product (*Nono*). Fermented products are generally considered to be safe, owing to its acidic nature, process failure and contaminated raw materials which eventually results to their being involved in foodborne illness (El-Kholy et al., 2014). From the investigations, the temperatures at which these drinks are subjected to were more than 70°C. This was

Table 2. Microfora occurrence in *kunun-zaki* drinks according to the locations in Jalingo Metropolis (n-30 for each location).

Bacteria species	Location				
	Ceremonies	Jalingo main market	Government ministries	Motor parks	Schools
Baccillus	1(3.3)	5(16.7)	0(0.0)	4(13.3)	3(10.0)
Escherichia	2(6.7)	2(6.7)	1(3.3)	2(6.7)	1(3.3)
Proteus	0(0.0)	5(16.7)	2(6.7)	1(3.3)	2(6.7)
Samonella	3(10.0)	4(13.3)	0(0.0)	1(3.3)	1(3.3)
Shigella	0(0.0)	2(6.7)	1(3.3)	0(0.0)	0(0.0)
Staphylococci	22(73.3)	23(76.7)	18(26.0)	19(63.3)	14(46.6)
Streptococci	8(26.7)	7(23.3)	4(13.3)	4(13.3)	2(6.7)
Yeast	2(6.7)	2(6.7)	0(0.0)	0(0.0)	0(0.0)
Nagative sampes	2(2.7)	1(3.3)	0(0.0)	0(0.0)	0(0.0)

Numbers in parentesis indicates percentages (%).

Table 3. Microfora occurrence in *sobo* drinks according to the locations in Jalingo Metropolis (n=20 for each location).

Bacteria psecies	Location				
	Ceremonies	Jalingo main market	Government ministries	Motor parks	Schools
Baccillus	2(10)	0(0)			
Escherichia	1(5)	1(5)			
Proteus	2(10)	2(10)			
Samonella	1(5)	2(10)			
Shigella	0(0)	1(5)			
Staphylococci	7(35)	11(55)			
Streptococci	5(25)	4(20)			
Yeast	0(0)	0(0)			
Nagative sampes	2(10)	2(10)			

Numbers in parenteses indicates percentages (%).

enough to eliminate most of the organism isolated. The contamination was thus post processing during handing and packaging rather than during processing. Factors such as poor hygiene, dirty environment, improperly washed food contact surfaces and equipment, the presence of animals in the cooking environment and source of water may have contributed to the contamination of these drinks (Oranusi et al., 2007; Umaru et al., 2014). This suggestion is also supported by Oranusi et al. (2003) who conducted a study on the hazards and critical control points of *kunun-zaki* in Northern India. Their study revealed the presence of total viable bacteria count, coliform, *Staphylococci*, *B. cereus, Salmonella* and *Shigella*, with the count increasing with time from the point of gelling to when the *kunun-zaki* was ready for consumption.

The occurrence of *E. coli* is an indication of faecal and environmental contamination and a signal for the presence of other enteric pathogens. Therefore, there occurrence may be linked to faecal, environmental and human contaminations (Ameh and Abubakar, 2002) which may occur probably through the use of water or directly during handing of these products. The prevalence of *E. coli* in the two drinks was 6.0 and 3.0%, respectively. This fail to agree with the WHO (1996) standards which suggested that water that contain >10 coliforms/100ml or one *E. coli*/100ml with or without other coliforms is unsatisfactory for human consumption. Furthermore, most of the households in the metropolis depend on the commercial water vendors popularly known as *Yan-Garuwa* for their source of water. These vendors sourced the waters from streams and rivers in which no standard precaution against faecal conta-mination is observed, hence hygienic and sanitary measures are compromised (Umaru et al., 2014). This may probably be the reason for the isolation of *E. coli* in these drinks.

The occurrence of *Bacillus* spp. is also of concern to public health and may arise from the raw starter culture used in the preparation of the *kunun-zaki* (Umoh and Odoba, 1999) and to a larger extent other ingredients and food contact surfaces. This explained why the occurrence is higher in *kunun-zaki* than *sobo* drinks which do not required any starter culture for its preparation.

Although, HACCP, total coliform counts (TCC) and total aerobic counts (TAC) which are regarded as gold standard for determining the microbial quality of foods and water (Bharath et al., 2003; Sperber and Richard, 2010) was not conducted, rather direct pour plating and streaking was used (Downes and Ito, 2001; Wehr and Frank, 2004). The isolation of these bacteria poses risk to human life.

The isolation of yeasts from *kunu-zaki* may be linked to contamination through air/dust, contaminated packaging material or poor hygiene and sanitation of the processing environment (El-Kholy et al., 2014). Yeasts can grow at a
wide range of temperature and pH and may result in change in colour, appearance, taste, flavours and odours (ICMSF, 2005). More importantly is the fact that yeast can produce mycotoxins which can cause mycotoxicosis in humans.

With reference to the locations of the samples, samples obtained from markets, motor parks and during ceremonies were more contaminated than those obtained from government ministries and schools. This could be due to the fact that these areas are highly populated with human beings and sanitary and hygienic measures are not properly practiced to limit or stop the spread of these organisms to the food chain.

Conclusion

Based on the findings of the study conducted, *kunun-zaki* and *sobo* drinks in Jalingo Metropolis are highly contaminated with microflora which may be potentially pathogenic to human beings. Most of the contaminations are post processing from environment, food contact surfaces and human handlers.

RECOMMENDATIONS

- Proper hygienic and sanitary measures should be practiced during processing and handling of these drinks to limit or avoid introduction of bacteria.
- Water intended for the preparation of these drinks should be potable or chemically treated to destroy all the potentially pathogenic bacteria.
- Quality control measures (although may be difficult), should be adopted to ensure that these drinks are safe and free of any pathogen before human consumption.
- Regulatory agencies like National Agency for Foods, Drugs Administration and Control (NAFDAC) and public health agencies should be mandated to regulate the sales and distribution of these drinks so as to prevent foodborne diseases outbreaks.

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