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Microbiological assessment of *kunun-zaki* marketedin Abuja Municipal Area Council (AMAC) in The Federal Capital Territory (FCT), Nigeria

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Forty-one (29) *kunun-zaki* samples were obtained as freshly formulated beverages from different local hawkers in 10 different locations in Abuja Municipal Area Council (AMAC) of the Federal Capital Territory (FCT) Abuja, Nigeria. The samples were evaluated for bacterial loads, isolation and identification of microorganisms present using spread plate agar dilution method. Bacterial loads ranged from 0.0 to 2.0 × 10^8 CFU/mL. The pH ranged between 2.64 to 5.0. The microorganisms isolated were identified by biochemical tests and microscopic analysis. The organisms isolated from the samples include *Staphylococcus aureus*, *Klebsiella* spp., *Eschericha coli*, *Citrobacter* spp., *Salmonella typhi*, *Shigella* spp., *Candida albicans*, *Lactobacillus* spp., *Aspergillus fumigatus*, *Aspergillus niger*, *Penicillium* spp. and *Saccharomyces cerevisiae.* The high bacterial load of most of the samples can be attributed to the poor hygienic practices of the handlers and possible contamination from the utensils and water that were used for processing of the beverage. The presence of these organisms could be a matter of serious concern as these organisms are involved in some health implications causing various diseases.

Key words: *Kunun-zaki*, spread plate, microbial contamination, Abuja.

INTRODUCTION

Kunun-zaki is a traditional fermented non-alcoholic beverage widely consumed in Northern Nigeria. It is widely consumed for its thirst quenching properties most especially during the dry season (Elmahmood and Doughari, 2007). It can be produced from millet, sorghum or maize. It has immense social, economic, nutritional and medicinal benefits to numerous consumers. *Kunun-zaki*, like other locally made drinks is widely consumed in Nigeria. In most Nigerian cities, the sales and consumption of this locally made beverage is high due to the high cost of other non-alcoholic drinks. Due to the non-alcoholic nature of this drink, it is widely accepted and consumed by both Muslims and Christians alike as a substitute for alcoholic drinks. The drink is usually sold at the motor parks, school premises and market places and even served during social gatherings (Abegaz, 2007).

Production methods are crude, ingredient concentrations are neither quantified nor standardized, instead preparation is largely a matter of family tradition (Onuorah et al., 1987). Significant variations exist in the production

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procedures depending on its taste and cultural habits of its consumers leading to differences in quality and stability. The high water content (about 85%) coupled with crude methods of production and packaging under inadequate sanitary conditions predisposes *kunun-zaki* to microbial contamination (Elmahmood and Doughari, 2007; Ayo et al., 2010). Osuntogun and Aboaba (2004) reported that *kunun-zaki* is prone to microbial deterioration if not adequately stored. *Kunun zaki* has been reported to have a shelf life of about 24 h (Adeyemi and Umar, 1999). The occurrence of wide genera of micro-organisms could be attributed to the unhygienic conditions of preparation and the use of contaminated raw materials and utensils (Ayo et al., 2004). The micro flora of the finished product depends on the processing and storage conditions. High temperature and lack of refrigeration facilities in most developing countries have led to the inability to produce and store fresh *kunun-zaki*.

This study was designed to assess the microbial quality of this indigenous beverage in the Abuja Municipal Area Council (AMAC) region of The FCT, Abuja, Nigeria and possibly highlight the risks involved in the consumption by the general public as well as the possible source of contamination.

MATERIALS AND METHODS

Collection of samples

Samples of freshly prepared *kunun-zaki* were collected from different hawkers from 10 different locations within the Abuja Municipal Area Council (AMAC) FCT, Abuja, Nigeria. The samples were labeled and transferred within 3 h of collection to the microbiology laboratory in their original package and the contents aseptically withdrawn from the bottles for isolation of micro-organisms and enumeration of bacteria.

Determination of pH of the samples

The pH of the various samples was within 3h of collection determined using sterile probes (disinfected with alcohol) of the pH meter (Corning 35).

Determination of total count of bacteria

Bacterial total count was carried out on plates of Nutrient agar (NA, Oxoid), using the spread plate method. The samples were serially diluted up to 10^6 ml and 0.1 ml of appropriate dilution was used to inoculate each of the plates in duplicates. The culture plates were then incubated at 37ºC for 48 h and colonies counted manually. The mean of triplicate results was recorded as the colony count (Oshoma et al., 2009).

Isolation and Identification

Discrete colonies of the organisms isolated (for bacteria) were selected and sub cultured from the plates to respective nutrient agar plates and incubated at 37ºC for 24 h. The bacterial isolates were 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 fungal isolates were surface-spread in duplicates on Sabouraud Dextrose Agar (SDA, Oxoid) plates and incubated at room temperature (25 to 27ºC) for 48 to- 72 h. The colonies were screened and identified based on the taxonomic schemes and descriptions by Ainsworth et al. (1973) and Mislivec et al. (1992).

RESULTS

Mean pH and total viable counts

The mean pH values and level of microbial contamination is shown in Table 1. The *kunun-zaki* samples had a pH range of 2.64 to 5.0. All samples were acidic in nature. Sample Kw3 had the lowest pH of 2.64 while sample Kw1 had the highest pH of 5.0. The bacteria count ranged from 0.0 to 2.0×10^8 cfu/ml. Samples A had highest bacteria count while samples E had the lowest bacteria count.

Table 2 shows the different microorganisms isolated from the *kunun-zaki* samples. *Saccharomyces cerevisiae* dominated the organisms as it was isolated from most of the samples (34.15%). This was closely followed by *Klebsiella* sp. isolated from nine samples (21.95%). *Staphylococcus aureus* was isolated from six samples (14.64%) and *Aspergillus niger* from five samples (12.2%). Other microbes isolated from the *kunun-zaki* samples include *Escherichia coli Candida albicans* and *Aspergillus fumigatus* isolated from two samples each (4.88%). *Citrobacter* spp*., Salmonella typhi, Shigella* spp., *Lactobacillus fermentum* and *Penicillium* spp*.* were isolated from one sample each (1.44%).

Cultural characteristics and biochemical Identification of isolated strains

A total of 22 bacterial types were isolated. Non-red colonies from MacConkey plates that grew with golden yellow on mannitol salt agar and were Gram positive, coagulase-positive and catalase-positive were taken as *Staphylococcus aureus*; non-red colonies from MacConkey plates that were Gram-negative, indole-negative, methyl red-positive, Voges-Proskauer-negative, citrate-negative, acidic butt, alkaline slant with no blackening on TSI slant were taken as *Shigella* spp., non-red colonies from MacConkey plates that were Gram negative, indole-negative, methyl red-positive, Voges-Proskauer-negative, citrate-positive, acidic butt and alkaline slant with blackening on TSI slant, urease-negative and colourless colonies with black center on SS agar were taken as *Salmonella* spp., Red colonies from MacConkey plates that grew with greenish metallic sheen on eosin methylene blue agar and were Gram negative, indole-positive, methyl red-positive, Voges-Proskauer-negative and citrate-negative were taken as *E. coli*; red mucoid colonies from MacConkey plates that were Gram negative, indole-negative, methyl red-negative, Voges-Proskauer-positive and citrate-positive were taken as *Klebsiella* spp.; non spore forming Gram positive,

Table 1. Mean pH values and Total viable counts (cfu/ml) for fresh *kunun-zaki*.

Location	Sampling no.	Average pH	Total viable count (cfu/mL)
Idu	Id1	3.55 ± 0.0	$8.4 \times 10^5 \pm 0.0$
	Id2	3.24 ± 0.04	$8.4 \times 10^7 \pm 0.0$
	Id3	3.31 ± 0.03	$5.0 \times 10^6 \pm 0.0$
	Id4	3.13 ± 0.01	$2.2 \times 10^5 \pm 0.58$
Lugbe	Lu1	3.55 ± 0.0	$3.6 \times 10^3 \pm 0.0$
	Lu2	3.02 ± 0.02	$2.9 \times 10^6 \pm 0.33$
	Lu3	3.52 ± 0.04	$2.0 \times 10^8 \pm 0.0$
Garki	Gk1	3.40 ± 0.01	$1.0 \times 10^5 \pm 0.0$
	Gk2	3.98 ± 0.01	$6.0 \times 10^5 \pm 0.0$
	Gk3	3.92 ± 0.06	$2.0 \times 10^5 \pm 0.0$
	Gk4	4.33 ± 0.17	$4.3 \times 10^5 \pm 0.33$
	Gk5	4.02 ± 0.07	$2.0 \times 10^5 \pm 0.0$
Wuse	Ws1	3.64 ± 0.0	$2. \times 10^30 \pm 0.0$
	Ws2	4.20 ± 0.01	$1.2 \times 10^7 \pm 0.58$
	Ws3	3.49 ± 0.01	$8.5 \times 10^6 \pm 0.0$
	Ws4	4.22 ± 0.03	$8.4 \times 10^5 \pm 0.0$
	Ws5	3.40 ± 0.025	$6.4 \times 10^6 \pm 0.58$
Asokoro	As1	4.85 ± 0.02	$3.6 \times 10^2 \pm 0.0$
	As2	2.94 ± 0.06	$1.1 \times 10^8 \pm 0.33$
	As3	3.90 ± 0.01	$7.3 \times 10^5 \pm 0.33$
	As4	3.32 ± 0.04	$2.1 \times 10^6 \pm 0.58$
Mararaba	Mb1	4.50 ± 0.0	$5.1 \pm 0.33 \times 10^2$
	Mb2	3.17 ± 0.01	0 ± 0.0
	Mb3	3.11 ± 0.03	0 ± 0.0
	Mb4	3.13 ± 0.1	$1.0 \times 10^3 \pm 0.0$
	Mb5	3.10 ± 0.01	0 ± 0.0
Kubwa	Kw1	5.0 ± 0.0	$3.0 \times 10^3 \pm 0.0$
	Kw2	3.78 ± 0.06	$1.0 \times 10^8 \pm 0.33$
	Kw3	2.64 ± 0.17	$5.0 \times 10^6 \pm 0.0$
	Kw4	3.14 ± 0.01	$1.1 \times 10^7 \pm 0.0$
Maitama	Mt1	4.55 ±0.01	$1.2 \times 10^3 \pm 0.0$
	Mt2	3.08 ± 0.03	$6.1 \times 10^7 \pm 0.0$
	Mt3	3.56 ± 0.08	$2.0 \times 10^6 \pm 0.0$
	Mt4	3.19 ± 0.03	$1.2 \times 10^5 \pm 0.0$
Jikwoi	Jw1	4.26 ± 0.0	$3.7 \pm 0.0 \times 10^3$
	Jw2	3.60 ± 0.01	$2.2 \pm 0.33 \times 10^7$
	Jw3	3.59 ± 0.05	$8.4 \pm 0.0 \times 10^5$
Karmo	Kr1	3.60 ± 0.0	$5.5 \pm 0.0 \times 10^3$
	Kr2	2.73 ± 0.056	$1.0 \pm 1.0 \times 10^7$
	Kr3	2.96 ± 0.04	$5.0 \pm 0.58 \times 10^6$
	Kr4	4.03 ± 0.01	$4.0 \pm 0.0 \times 10^7$

Table 2. Presence of Bacterial and Fungal Flora in *Kunu zaki* samples.

No. of sample	*Klebsiella* spp.	*Escherica coli*	*Staphylococcus aureus*	*Citrobacter* spp.	*Salmonella typhi*	*Shigella* spp.	*Candida albicans*	*Aspergillus Niger*	*Aspergillus fumigatus*	*Saccharomyces cerevisiea*	*Lactobacillus* spp.	*Penicillum* spp.
41	9	2	6	1	1	1	2	5	2	14	1	1
%	22.0	4.9	14.6	2.4	2.4	2.4	4.9	12.20	4.9	34.2	2.4	2.4

filamentous bacilli, catalase negative, glucose and mannitol fermenter were taken as *Lactobacillus* sp.; round, smooth , opaque colonies with regular margins from blood agar plates, Gram negative motile rods, indole-negative, methyl red-positive, Voges-Proskauer-negative and citrate-positive, urease positive, H_2S production on TSI agar slants, glucose, maltose and mannitol fermenter were taken as *Citrobacter* spp.; moist, creamy Gram positive colonies, germ tube test positive, urease negative, true and pseudo hyphae present, positive for assimilation of glucose, maltose, sucrose, galactose and negative lactose assimilation, glucose, maltose, galactose fermenter were taken as *C. albicans*. White to cream, smooth, glabrous yeast-like colonies on SDA plates, with large globose to ellipsoidal budding yeast-like cells or blastoconidia were taken as *S. cerevisiae* A compact white or yellow basal felt covered by a dense layer of dark-brown to black conidial heads on Czapek Dox agar, large, biserate dark brown to black conidia heads, globose, becoming radiate with the phialides borne on septate metulae which are twice as long as phialides were taken as *A. niger*. Blue-green surface pigmentation with suede like surface consisting of dense conidiophores on Czapek Dox agar, uniseriate and columnar conidial heads with the phialides limited to the upper two thirds of the vesicle and curving to be roughly parallel to each other were taken as *A. fumigatus*. Fast growing colonies in shades of green mostly consisting of a dense felt of conidiophores, globose, ellipsoidal, cylindrical, hyaline, smooth walled were taken as *Penicillum* spp.

DISCUSSION

All *kunun-zaki* samples were acidic in nature with pH range of 2.64 to 5.0. Various researchers have attributed this to the presence of fermentative microorganisms in *kunun-zaki* which cause spoilage of the beverage by fermentation of its carbohydrate content producing undesirable changes in them, altering their aroma and taste and thus making them unpalatable for human consumption. Osuntogun and Aboaba (2004) isolated lactic acid bacteria such as *Lactobacilli*, *Leuconostoc* and *Streptococcus* which were reported to possess the ability to ferment carbohydrates to produce lactic acid thus lowering the pH. *Lactobacilli* have also been isolated from other indigenous non- alcoholic beverage like *zoborodo*. The high bacteria load (5.1×10^2 to 2.0×10^8) of all the *kunun-zaki* samples can be attributed to the poor hygiene practices of the handlers and possible contamination from the utensils and water used for processing the beverage as well as the packages used in its distribution. The presence of *S. aureus, E. coli, Klebsiella* sp*, E. coli, S. typhi* and *Shigella* spp*.* could be a matter of serious concern, since these organisms are involved in some health implications.

Klebsiella is a gram negative bacilli belonging to the family Enterobacterioceae. It is usually associated with faecal contamination. Being an enteric bacterium its presence indicates poor hygiene practices among handlers. Due to the significance of the faecal-oral route transmission for many bacterial food-borne diseases, basic hygiene measures assume a decisive importance in food safety management (Uzeh et al., 2006).

S. aureus is a normal flora of the skin, nose, mucous membrane, throat, palms, hairs and a common etiological agent of septic arthritis. It is an ubiquitous microorganism that can enter foods from many sources such as handlers with acute pyogenic infections or healthy carriers who harbour the organism in their nose or throat. It is commonly implicated in water and food contamination. The detection of *S. aureus* is of serious public health importance because of its ability to cause a wide range of infections especially food-borne intoxication. This organism was equally isolated by Olasupo et al*.* (2002) from wara and *kunun-zaki*, a cereal based, non-alcoholic beverage.

E. coli is an important member of the coliform group. It is part of the normal flora of the human intestine. Some strains can cause gastroenteritis, diarrhoae and urinary tract infection. The presence of this organism in *kunun-zaki* is an indication of faecal contamination.

S. typhi an enteric bacteria is the causative agent of typhoid fever. The increased frequency of food-borne *Salmonella* has been causing recurring outbreaks, sometime with fatal infections which has been linked to the unsanitary practices of food and beverages processes leading to contamination of foods by *Salmonella* (Radji et al., 2010). The routine

detection of Salmonella in the environment including in foods and beverages is a necessary component of public health programs. The presence of *L. fermentum* and *S. cerevisiae* isolates in the samples analyzed is not as surprising as these organisms have been reported to thrive in medium rich in fermentable substrates. *Lactobacillus is* not usually pathogenic and is a known intestinal flora in humans. It has been reported to possess beneficial properties. These include colon cancer prevention, immune system enhancement and allergy reduction, owing to their ability to antagonize the activities of some food spoilage pathogenic bacteria like *S. aureus* and *E. coli* (Osuntogun and Aboaba, 2004). *S. cerevisiae* has 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, 2006).

Aspergillus and *Penicillium* species have also been implicated in food spoilage especially those with carbohydrate substrate. They are storage microflora of many cereals. Their growth can result in production and accumulation of mycotoxins which are of public health and economic importance (Rhodes and Flecher, 1966).

Conclusion

The presence of these isolated organisms in *kunun-zaki* samples analyzed could serve as indicator for the need to promote awareness about the possible health hazards that could arise due to handling and processing of the beverage. The range of microorganism isolated pose serious threat to food safety and hence the need to ensure microbial safety during the production and distribution of this drink that is widely consumed in most parts of Northern Nigeria.

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