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Quality assessment of the 'Kunun-zaki' soft drink based on bacteria and fungi content

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Abstract

Abstract. *The purpose of the study was to made quality assessment of the 'Kunun-zaki', popular Nigerian soft drink based on bacteria and fungi content. Isolation of coliform of bacteria and fungi in Kunun-zaki was conducted in the month of May, 2019.Ten Kunun-zaki samples were obtained as freshly formulated beverages from 5 different sells point in Adamawa State University, Mubi metropolis, Adamawa State Nigeria and screened for microbial and fungi contamination. The media used for inoculation was Peptone water, Eosin methylene blue agar (EMB), Nutrient agar, Lauryl sulphate tryptonse broth (LST), Brilliant-green bile lactose broth (BGBLB).Microorganisms isolated were Escherichia coli, Staphyloccus aureus, Streptococuus pyogenes, Penicillium digitatum, Monilia sitophila, Rhizopus nigricans, Aspergillus fumigatus. The pH samples ranged between 2.34-4.99 and the estimated number of total coliform bacterial count ranged between* $2.0x10^3$-$2.9x10^4$ *cells/ml. The presences of high microbial loads were indication of poor hygiene and /or poor quality cereals and water used in the preparations. The types and density of microorganisms isolated from the samples examined need urgent measured. Producers should be given awareness to be taken in the processing and handling of the product before being sold to the unsuspecting general public in the school management. Kunu drink should be consumed within 24 hours of preparation or preserved using chemical preservatives rather than refrigeration. The present study concluded that the Kunun-Zaki sold in Adamawa State University was of low*

quality. Hence it was recommended that quality assurance programme should be started to ensure that good quality of Kunun-Zaki and Kunun-Zaki products are produced and consumed in the Campus.

Kewords: *beverage, isolation, microbial contamination, coliform bacteria, quality assessment*

Introduction

"Kunun-zaki" is an indigenous fermented non-alcoholic and non-carbonated beverage which is widely produced and consumed especially in the northern Nigeria. It is consumed by all age groups as a thirst quencher or as refreshment in some communities. It is used for entertainment at homes and during ceremonies and festive periods like Christmas and Sallah and sometimes as a weaning drink for infants.It is estimated that over 76% of northerner's drink kunun-zaki on regular basis. The production of kunun-zaki varies amongst people and can generally be produced from either fermented millet (*Pennisetum typhoideum*), maize (*Zea mays*) or sorghum (*Sorghum bicolor*) Millet is however the most common substrate. (Maji and Chigozie, 2011).

The traditional process for the production of kunun-zaki involves the steeping of millet grains, wet milling with spices (ginger, cloves and pepper), wet sieving and partial gelatinization of the slurry, followed by the addition of sugar and bottling. The presence of pathogens even in small numbers could render kunun-zaki unsuitable for human consumption. Contamination by pathogens could occur during production, hawking and packaging of the products and poor hygiene practice by the processors. (Amanabo et al., 2013).

Fungi, gram-positive and gram-negative bacteria had been isolated from sampled Kunun-zaki (Adeleke et al., 2011). It had been discovered that microorganisms especially *E. coli* can grow and survive during the storage of Kunun-zaki at different temperature. It is possible that the high counts of spoilage and pathogenic microorganisms in Kunun-zaki could be reduced if starter cultures are employed in its fermentation process

as done in the developed world (Agarry et al., 2010).

Agarry et al.,(2010) studied the microbiology of the fermentation process of Kunun-zaki and reported that *Lactobacillus fermentum* and *Lactobacillus leichmannii* were dominant at the end of the fermentation period.Lactic acid bacterial fermentation is used to improve sensory and nutritional properties of foods and to produce products of high and consistent qualities, the fermentation process has to be controlled using tailor- made starter culture.

The above arguments motivated the conduct of this study, which purpose was to made quality assessment of the 'Kunun-zaki', as a popular Nigerian soft drink based on bacteria and fungi content.

Material and methods

Study Area

The study was carried out in Adamawa State University, Mubi located in Mubi North Local Government Area (LGA) of Adamawa State, Nigeria. Mubi is situated in the northern part of Nigeria (i.e Northern Nigeria) between latitude 10^{o} 16' 8' North and longitude 13^{o} 16' 14'' East. Mubi has a semi urban area with some rural areas around it such as Madanya, Vimtim, Muchalla, Muva, Digil etc. The climate is tropical with average temperature of about 32.9^{o}C in dry season and relative humidity ranging from 10-45%, the mean annual rainfall is about 1050mm which usually starts around May and lasts for 6 months (Adebayo and Tukur, 2004).

Samples collection

Ten plastic bottles of Kunun-zaki samples were obtained as freshly formulated beverages from 5 different sells points: Faculty of Education, Science Complex, Up Commercial, Barde Hall and Large Hall in Adamawa State University, Mubi metropolis, Adamawa State, Nigeria.

Preparation of food homogenate and culture media

***Preparation of food homogenate*:** Using a sterilize pipette, 10ml of sterilize peptone water was pipetted into a sterilized test tube and 15ml of the sample (kunun zaki) was then added onto it. It was then shaken gently for uniform mixing. Peptone water was prepared by weighing 1.0g of peptone powder and dissolved into 1 liter of distilled water in a clean conical flask. This was warmed on a Bunsen flame for uniform dissolution it was then dispensed into test tube

and later sterilized at 121°C for 15minutes using a autoclave. (Agarry and Nkama, 2012).

***Isolation and identification of bacteria isolated from samples*:** McConkey agar, Brilliant Green Bile-Lactose Broth, Eosin Methylene Blue, Nutrient Agar and Lauryl Sulphate Tryptonse Broth were employed for the isolation of bacteria for the purposes of identification. All plates were incubated at 37°C for 24h. Identification of bacterial isolates was based on the standard cultural and morphological methods (Agarry et al., 2010 and Cheesbrough, 2006).

Samples pH determination

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

Dilution and inoculation

Three test tubes each containing 9ml of peptone water was used. Using a sterilized pipette 1ml of the food homogenate was pipetted into the first test tube containing 9ml of sterilize pipette water that is 1:10 dilution, this was then shaken gently for uniform mixing. From the first test tube; 1.0ml was transferred using a sterile pipette to 2nd test tube that is (1:100) it was then shaken for uniform mixing. From the 2nd test tube, 1.0ml was transfer with a sterile pipette to 3rd that is 1:1000 dilutions. It was shaken for uniform mixing. (Adejuyitan et al., 2011). Nine test tube of lauryl sulphate tryptonse broth containing inverted Durham tube was inoculated with 1.0ml of the food homogenate from the test tube containing 1:10 dilution tubes. Another 3 test tubes of lauryl sulphate broth containing inverted Durham test tube was also inoculated and incubated at 37°C for 24h (Agarry and Nkama, 2012).

Presumptive and confirmatory test

Test tube showing gas production after 24h was recorded as positive and calculated from most probable number (MPN). A loop full from each gas positive tube of Lauryl sulphate tryptonse broth (LST) was transferred to a separate test tube of brilliant green bile lactose broth (BGLB) containing inverted Durham tubes and was incubated at 37°C for 48h. The formation of gas confirms the presence of coliform bacteria. The analysis was completed by inoculating a gas inverted Durham tube, a gas form confirm the presence of coliform bacteria.

Coliform differentiation using EMB agar

Streaking plate method: a loop full of gas positive tube of BGBL was streaked onto EMB. The plate was then incubated at 37°C for 24 hours in an inverted position. *E. coli* was found growing on the plate, it was recognized by its morphological colony character which forms a small raised, flat dry colony with green metallic sheen appearances while *A. aero* genes was large moist convex colony with a dark centre.

Determination of Bacteria isolates

Streaking plate method: a loop full of a gas positive tube of Lauryl sulphate tryptonse broth (LST) was streaked onto nutrient agar plate. Wire loop was sterilized over the Bunsen flame cooled and parallel streak was made from the main inoculum. The plate was then incubated at 37°C for 24h. It was then observed for any growth on the medium after 24 hour. A dry smear of microorganisms was made on a clean slide. The smear was flame on a Bunsen burner to heat fixed the microorganism to the slide. Then crystal violet was used to stain for 1minute and excess dye was washed away from the slide and shake off excess water. It was flooded with iodine solution for 1minute and excess iodine was washed and allow drying. It was lastly counterstain with safranine for 1minute and then washed with distilled water and allowed to air dried. The prepared slide was mounted on a microscope and observed under the oil immersion objective lens and the morphological characteristics were noted.

Fungal identification: Fungal identification and enumeration was based on their colony elevation, colour, texture, shape and arrangement of conidia (spherical or elliptical, unicellular or multicellular), branched or unbranched mycelia, presence or absence of cross walls (whether septate or non-septate) and others.

Results

The pH values of the kunun zaki were within 2.99-5.11, which showed that all the samples were acidic in nature (Table 1). Samples collected from vendor at Large Hall (LH) had the lowest pH of 2.99 and those collected from vendor at Faculty of Education had the highest pH of 5.11. The acidic nature of the "kunun-zaki" is as a result of fermentation process which led to the production of lactic acid by bacteria which increased the flavor and lower the pH of the kunun- zaki and the low pH reduce the activities of pathogens. The results of these study also showed that serial dilution 10^{-1} to 10^{-3} has high coliform

bacteria count than that of serial dilution 10^{-5} which has the least in the confirmatory test. (Table 2). Percentage occurrence of microbial isolates from kunun- zaki drink obtained from the five sales point (two bottles each) showed that *Staphlococcus aureus and Escherichia coli* occurred highest in all samples at different locations while *Streptococcus pyogenes* has the least (Table 3) while the morphological and cultural characteristic of the bacteria showns golden yellow, colorless opaque and dry shiny mucoid colonies and the fungi shows green/black mycelia, red mycelia, white cottony mycelia and bluish- green floccose matted mycelia (Table 4).

Discussion

The pH values of the samples in these study ranged between 2.99-5.11. The acidic nature of the "kunun-zaki" is as a result of fermentation process which led to the production of lactic acid by bacteria. In this work, the highest pH of kunun-zaki was obtained from Faculty of Education(5.11) and the lowest (2.99) was Behind Large Hall (LH), respectively. Our values were a little lower than that obtained by Katuka et al., (2017) and and the estimated number of total coliform bacterial count ranged between $2.0x10^3$-$2.9x10^4$ cells/ml which is a little higher than that obtained by Elhmamood and Doughari (2007) which is $1.0x10^3$-$1.8x10^4$ cells/ml but similar to that isolation by Aboh and Aladosu (2014). It could be as a result of well construction defects such as insufficient well casing depth, improper sealing of the space between the well casing and the borehole, corroded or cracked well casing, and poor well seals or caps can allow sewage, surface water, or insects to carry coliform bacteria into the well. In this study *Staphylococcus aureus*, *E.coli* and *S. pyogenes* was found in all kunun-zaki samples obtained from various location of the Adamawa State University which is in agreement with Ekanem et al., (2018) who conducted a study on microbial quality and proximate composition of kunu drinks produced and sold in Ikot Ekpene Metropolis, Akwa Ibom State, Nigeria. This could be attributed to the poor hygienic practices of the handlers and possible contamination from utensils and water used for processing the beverage as well as packages used in its distribution.

Staphylococcus aureus is widely found in respiratory tract, nose and skin. Therefore, food that are exposed or frequently touched could be contaminated by it. This result is also supported by a study conducted by Aly et al., (2014) where *Staphylococcus aureus* count got higher due to frequently exposure. In

another study by Belickova et al., (2011), *S. aureus* count was reported in yogurt milk and strawberry flavored yogurt milk. The presence of *S. aureus, E.coli* and *S. pyogenes* could be a matter of serious concern, since these organisms are involved in some health implications.

Aspergillus, Penicillium and *Rhizopus* species have also been isolated in this study which is found to cause food spoilage especially those with carbohydrate substrate as it was also reported by (Rhodes and Flecher, 2010). 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.

Conclusion

The presence of microbial 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. There is therefore need for surveillance by Public Health officials to ensure safety of the Kunun-zaki being sold in Adamawa State University, Mubi for public consumption. There is need to also ensure that the water used for production especially post-heating processing of the Kunun-zaki is safe and free from microbial contaminants.

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Appendix

Table 1. pH values of the samples collected from the various sales point

Sales points	Behind Science Complex	Behind Large Hall	Barde Hostel (256)	Up commercial	Faculty of Education
pH values	3.00	2.99	4.33	3.70	5.11

Table 2. Comfirmatory test for coliform bacteria

Specimens	10-1	10-2	10-3	10-4	10-5	Frequency occurance, %
Behind Science Complex	+	+	+	+	_	+ = 4

Behind Large Hall	+	+	+	_	_	+ = 3
Barde Hostel (256)	+	+	+	+	_	+ = 4
Up commercial	+	+	+	+	_	+ = 4
Faculty of Education	+	+	+	+	+	+ = 5
						Sum=20%

+ = present; - = absent

Table 3. Percentage occurrence of bacteria isolated from Kunun-zaki drinks

Samples	*E. coli*, %	*Staphyloccus aureus*, %	*Streptococcus pyogenes*, %	*Penicillium digitatum*, %	*Moniliasitophila*, %	*Rhizopus nigricans*, %	*Aspergillus fumigatus*, %
Behind Science Complex	99.9	99.9	0	6	4	3	1
Behind Large Hall	99.8	99.9	1	3	7	2	4
Barde Hostel (256)	99.9	99.8	1	4	3	1	2
Up Commer-cial	99.9	99.9	2	3	1	2	2
Faculty of Education	99.9	99.9	2	8	8	4	3
Total	599.1	599.1	8	29	27	16	12

Table 4. Morphological and cultural characteristics of bacteria and fungi isolates

Organisms	Characteristics
S. aureuns	Slightly raised golden yellow G+ colonies.

E. coli	Large, circular, colorless opaque G+, LF colonies on EMB
Str. pyogenes	Small dry shiny mucoid colonies on BA, G+ cocci in chains.
P. digitatum	Green/black mycelia, spores on flask-shaped sterigmata.
M. sitophila	Red mycelia, floccose and salmon-colored spores.
R. nigricans	White and cottony mycelia, floccose white to gray spores.
A. fumigatus	Bluish green floccose matted mycelia, conidiophore-bearing phialides (flask-shaped) that produce spores.