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MICROBIAL QUALITY ASSESSMENT OF KUNUN ZAKI BEVERAGE SOLD IN PORT HARCOURT, RIVERS STATE, NIGERIA

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ABSTRACT

The microbial quality assessment of kunun zaki beverage sold in Port Harcourt, Rivers State, Nigeria was evaluated. The pH of the samples ranged from 3.44-4.34, while the colony forming units per milliliter (cfu/ml) of kunun zaki ranged from 0.2 x 10^4 -9.2 x 10^4 cfu/ml. Microbial identification revealed the presence of *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pyogenes*, *Rhizopus nigricans*, *Penicillium digitatum*, *Aspergillus fumigatus* and *Monilia stiophila*. The presence of the various kinds of microorganisms in the beverage is not desirable; hence, the need to maintain adequate hygienic conditions during its preparation and processing in order to get rid of microbial contamination and to improve its shelf-life as it is very short. Its short shelf-life is a major problem that the producers and consumers are facing and as a result, it is recommended that chemical preservative be used to increase the shelf-life. Furthermore, the types and density of the microorganisms recovered from this study calls for urgent measures to be taken in order to ensure a good hygiene before the beverage is sold to the general public as this beverage is consumed by so many people in the country.

KEYWORDS: Kunun zaki; Microorganisms; Beverage; Cereal; Drinks.

1. INTRODUCTION

Kunun zaki is an indigenous and traditional fermented non-alcoholic beverage drink found in Nigeria. It is widely consumed for its thirst and used during festivities, weddings and in naming ceremonies. The drink is taken as an appetizer and has thirst quenching properties. It is found particularly in the Northern part of Nigeria and also other parts both in the rural and urban regions of the country. Kunun zaki is used as food and fortunately for the people of Nigeria, kunun plant is cheap as it is widely grown throughout the savannah region of Nigeria, such as, Katsina, Sokoto, Kano and Bauchi States. Although kunun zaki is called a beverage, it can also be consumed at anytime of the day by both children and adults (Amusa and Ashaye, 2006; Umaru *et al*., 2014; Mbachu *et al*., 2014).

Aside from kunun zaki, there are other types of kunun that are consumed in Nigeria. They are kunun gyada, kunun akamu, kunun tsamiya, kunun baule, kunun jiko, kunun amshau and kunun gayamba. Notwithstanding, kunun zaki is the most preferred and commonly consumed. Depending on the availability of cereals, kunun can be made from sorghum, guinea corn, maize, rice or millet seeds. For instance, according to Odunfa and Adeyeye (1985), the indigenous processing of kunun involves the steeping of grains, wet milling with spices (ginger and cloves pepper), wet sieving and partial gelatinization of the slurry, followed by the addition of sugar and bottling. The processed kunun is normally packed for sale either in plastic bottles or in large containers which is then distributed under ambient temperature or cooled in a refrigerator. According to Lichtenwalner and his colleagues, kunun zaki processed from sorghum grains contains protein, fat, ash, carbohydrate and array of amino acid (11.6 %, 3.3 %, 1.9 % and 76.8 %), respectively (Lichtenwalner *et al*., 1979).

In Nigeria, kunun zaki is usually sold by hawkers in parks (motor parks), market places, military barracks, Churches (after service) and in school premises during recess. Even if the government has tried to have control over the processing of hawked food, it has not been possible. Most of the kunun vendors or hawkers have been found to lack the adequate knowledge of food processing and handling practices, and as a result, the beverage will presumably be contaminated with chemicals and microorganisms. According to researchers, food pathogen such as *Escherichia coli* has been implicated in food poisoning resulting from their consumption (WHO, 2006; Mbachu *et al*., 2014).

Researchers have reported that a number of lactic acid bacteria, mold, yeasts and coliforms are found in food spoilage as they use the carbohydrate content of the foods for undesirable fermentation process (Odunfa, 1988; Amusa *et al*., 2005). A slight fermentation often occurs during the processing of kunun (steeping of the grains in water over 8-48 hours period) and this involves mainly the lactic acid bacteria and yeasts (Odunfa and Adeyeye, 1985).

This research was therefore conducted to investigate and assess the microbial quality of kunun zaki beverage sold in Port Harcourt, Rivers State, Nigeria.

2. MATERIALS AND METHODS

Study area

The samples of freshly prepared kunun zaki were collected from Mile 1 market in Port Harcourt, Rivers State, Nigeria. The samples were packaged in 200 ml sterile plastic bottles and immediately transferred into the microbiology laboratory for analyses.

Sample collection and preparation

The samples collected were stored under different conditions, such as refrigerator temperature (-40 ^{O}C) and room temperature (28±2^{O}C). The storage was carried out immediately to avoid further fermentation processes and contamination outside the area of production. The samples were monitored for microbial quality and pH changes.

Sample preparation for total heterotrophic count (THC)

In the laboratory, 1 ml each of the kunun sample was placed into 9 ml of sterile peptone water giving a 1 in 10 (10^{-1}) dilution, thereafter, another tube, giving 10^{-2} dilution and up to 10^{-4} dilution. An aliquot (0.1 ml) of the last two dilutions were placed on the surface of a well dried nutrient agar plate using a pipette. Thereafter, the inoculated plates were spread evenly with the aid of a glass spreader and later incubated in the inverted position at 37 ^{O}C for 24 hours.

Viable count

After incubation, the plates were carefully examined and colonies counted and representative colony types were picked and sub-cultured on nutrient agar using the streaking techniques.

Isolation of pure cultures

Distinct colonies on MacConkey Agar and Nutrient Agar, were isolated, counted and Gram stained (Carpenter, 1977).

Identification of the isolates

The following steps were taken for the identification.

- **Cultural characteristics**

The cultural characteristics of the isolates were observed after 24 and 48 hours of incubation at 37 ^{O}C on MacConkey Agar and Nutrient Agar. Features such as nature and pattern of growth, shapes of colony, color of colony, elevated surface area, edge and odor were carefully noted.

- **Gram staining**

The method used was that described by Cheesbrough (2002). The principle is based on the ability of microorganisms to retain the basic dye – crystal violet after decoloration with alcohol. The alcohol decolorizes Gram negative bacteria and thus become red or pink after being counter stained with a red dye. The Gram positive bacteria are not decolorized, but retain the violet or purple color of the primary stain.

- **Procedure**

A loop full of the culture was emulsified in a drop of sterile distilled water on a clean grease free glass slide. The emulsion was evenly distributed on the surface of the slide as to obtain a thin smear. The smear was allowed to air-dry before it was heat-fixed by passing over blue Bunsen flame. It was then stained with crystal violet solution, which was allowed to stay for one minute before it was rinsed off with tap water. Gram's iodine was applied to it and it was left for 1 minute. Then, the iodine was rinsed gently with tap water. After that, the smear was decolorized with 95 % alcohol till the blue color no more dripped out (about 5-15 seconds). The slide was then rinsed with tap water and counter stained with a contrasting dye called safranin. Finally, it was observed under the oil immersion objective (x100) for Gram positive and Gram negative bacteria.

Biochemical tests

- **Motility test**

This test was usually done to differentiate motile organisms from the non motile ones. The method used was the hanging drop method, as described by Kirk *et al*., (1975). A little of petroleum jelly was rubbed around a plain slide instead of the hanging drop slides. By means of pipette, a drop of sterile distilled water was placed on a cover slip, then a wire loop was sterilized and used to pick a colony from pure culture and transferred to the cover slip. It was mixed properly and the slide placed over a cover slip in a way that the centre of the depression lies over the drop. The slide was quickly inverted and viewed under the microscope, using x40 magnification.

- **Catalase test**

This is based on the liberation of free oxygen as gas bubbles indicating the presence of catalase, an enzyme that catalyses the breakdown of hydrogen peroxide to water and oxygen. In this test, 2 drops of 3 % hydrogen peroxide were placed on a clean grease free glass slide and test isolate was transferred to one of the drops of hydrogen peroxide on the slide, while the other was used as control.

- **Coagulase test**

This was used as one of the confirmatory tests for the identification of *Staphylococcus aureus*. It was used to differentiate pathogenic *Staphylococcus aureus*, which produces the enzyme, coagulase, from the other species of Staphylococcus. This test was carried out on a slide using human plasm. Two drops of distilled water were placed on a clean grease free glass slide. The isolates were emulsified in one of the drops. A loopful of fresh human plasma was transferred to the two drops on the slide. Clumping was observed on the drop containing the isolate, while no clumping was seen on the isolate free drop. Clumping indicated a positive reaction.

3. RESULTS

The mean pH values of the kunun zaki were within 3.44-4.34, which means that the beverage drink is acidic in nature. This also, reflected on the microbial load that was as well very low (Table 3.1).

Table 3.1: Mean pH values and total bacteria counts (cfu/ml) for fresh kunun zaki.

Vendors	pH	Samples	Nutrient Agar (cfu/ml)	MacConkey Agar (cfu/ml)
A	4.34	A1	4.0×10^4	-
		A2	3.1×10^4	-
		A3	3.6×10^4	-
B	3.75	B1	2.5×10^4	2.0×10^4
		B2	4.6×10^4	2.6×10^4
		B3	3.1×10^4	1.8×10^4
C	3.51	C1	1.9×10^4	1.3×10^4
		C2	3.2×10^4	0.9×10^4
		C3	3.4×10^4	0.5×10^4
D	4.15	D1	1.5×10^4	8.9×10^4
		D2	1.26×10^4	7.4×10^4
		D3	8.6×10^4	5.8×10^4
E	3.44	E1	5.0×10^4	2.1×10^4
		E2	9.2×10^4	1.7×10^4
		E3	7.5×10^4	1.3×10^4
F	4.10	F1	8.0×10^4	0.5×10^4
		F2	5.9×10^4	2.0×10^4
		F3	1.27×10^4	1.3×10^4
G	3.48	G1	1.84×10^4	5.0×10^4
		G2	1.15×10^4	1.3×10^4
		G3	1.46×10^4	2.0×10^4
H	3.47	H1	1.60×10^4	1.0×10^4
		H2	1.48×10^4	2.3×10^4
		H3	1.75×10^4	0.3×10^4
I	3.69	I1	1.23×10^4	1.6×10^4
		I2	1.76×10^4	0.7×10^4
		I3	1.08×10^4	0.2×10^4

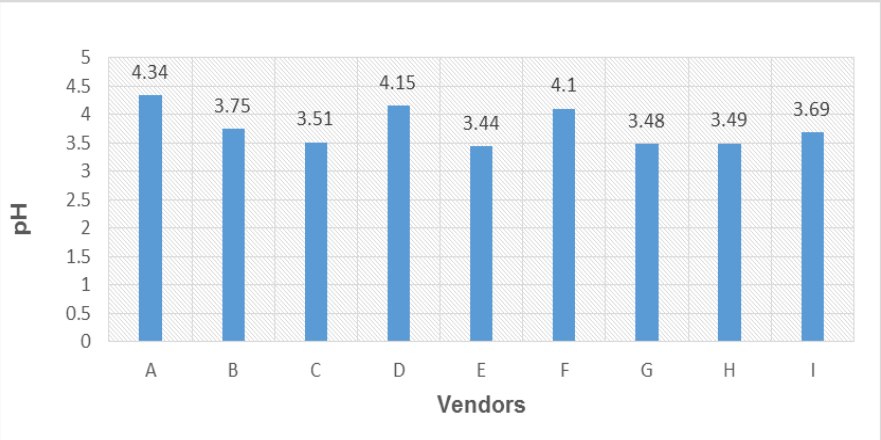

Figure 1: The mean pH of the fresh kunun zaki from different vendors.

Table 2: Microorganisms isolated from the kunun zaki samples.

Organisms	Characteristics	Sample vendors								
		A	B	C	D	E	F	G	H	I
		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 ferment 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
E. coli	Large, circular, low convex colourless on BA, G+ cocci in chains	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
Strep. pyogenes	Small dry shiny mucoid colonies on BA, G+ cocci in chains	x - -	- - -	- - -	x - -	- - -	- - -	- - -	- - -	- - -
P. digitatum	Green/blck mycelia, spores on flask-shaped sterigmata	x x x	x x -	- - -	x - -	- - -	x - -	x - -	- - -	- - -
M. sitophila	Red mycelia, floccose and salmon-coloured spores	- - -	x - -	- - -	- x x	- - -	- x x	- - -	- - -	x x x
R. nigiricans	White and cottony mycelia, floccose white to gray spores	- - -	- - -	x x x	- - -	- - -	- - -	x - -	- - -	- - -
A. fumigatus	Bluish green floccose matted mycelia, conidiophores-bearing phialides	- - -	- - -	- - -	- - -	- x x	x - -	- - -	x - -	- - -

Key note: x = presence; - = Absent; MC = MacConkey; A-I = Vendors; 1-3 = Samples

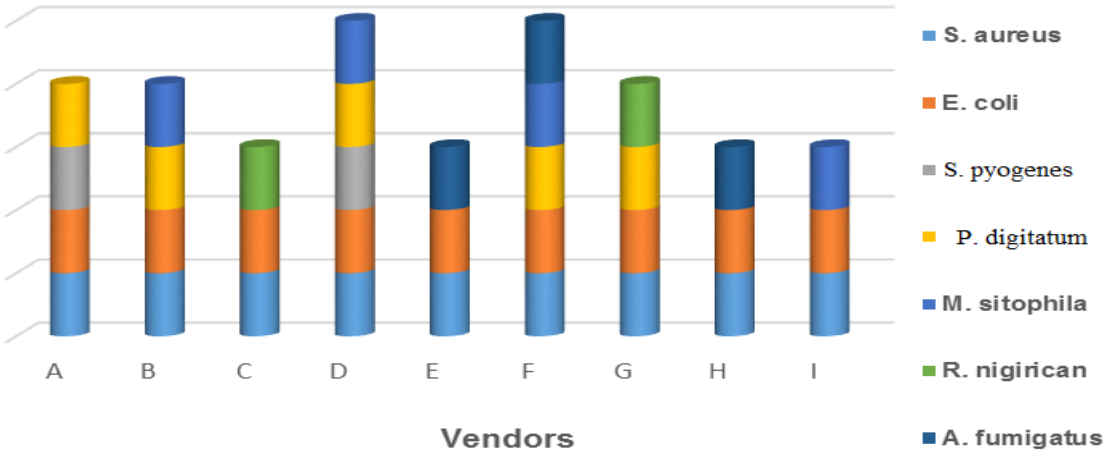

Figure 2: Microorganisms isolated from kunun zaki sold by different vendors (A-I).

4. DISCUSSION

According to this study, the microorganisms associated with kunun beverage samples include *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pyogenes*, *Rhizopus nigricans*, *Penicillium digitatum*, *Aspergillus fumigatus* and *Monilia stiophila*. This is in accordance with the report by Mbachu and his colleagues. They as well reported the presence of some microorganisms such as, *Staphylococcus spp*., *Streptococcus spp*., *Bacillus spp*., *E.coli* and others (Mbachu, 2014). The presence of microbes in this study is indicative of contamination during the processing of the beverage; even if some of these organisms, such as *Rhizopus nigricans* may not be harmful to the human body. Nonetheless, the presence of *E. coli* is indicative of fecal contamination; including other members of coliform. *E coli* has often been reported to be implicated in food poisoning (gastroenteritis) outbreaks and its presence in drinks, beverages and water, constitute a serious public health threat (CDC, 2002; WHO, 2006). Hence, the need for urgent and effective intervention as this beverage is commonly prepared by illiterates and the poor, and consumed by the general public.

Since the sources of water used in many parts of Rivers State are well, river/stream, borehole or tap, the presence of microorganisms, such as *E.coli*, *Streptococcus spp*. and *Staphylococcus spp*. in the hawked kunun drinks are not unexpected. For instance, in a work in South-Western Nigeria by Amusa and Ashaye (2006), the presence of *Coliforms*, which include *E. coli*, was reported. They stated that it was as a result of the use of contaminated water, containers/bottles and dirty environment.

More so, the presence of *Staphylococcus aureus* in this study might be contaminants from handlers or those who process/prepare or sell the beverage. This also poses a serious public health concern as *Staphylococcus aureus* has also been implicated in food poisoning. In 1988, Odunfa reported that the organism levels of 10^3 ml are considered potentially hazardous to consumers and according to the results of this study, kunun sold in Nigeria may be considered hazardous to the people of Nigeria. This is because many Nigerians rely on the beverage as an alternative to the bottled drinks, which costs are expensive.

Aspergillus and *Penicillium*, which were among the microbes identified might be the ones involved in the fermentation and the nutritional improvement of the kunun zaki. These organisms were also among the ones isolated by Essien and his co-researchers (2009). In order to avoid contamination with pathogenic microorganisms during the preparation of the beverage, it is therefore recommended that treated water be used for the processing of the beverage and in its dilution. It is also suggested that health education training be organized often on the importance of cleanness of the environment where the beverage is processed/prepared and the bottle/containers used in the selling of the beverage.

5. CONCLUSION

The presence of microorganisms, especially *E. coli*, *Staphylococcus aureus* and *Streptococcus pyogenes* was indicative of poor hygiene and/or poor quality cereals and water used in the preparation of kunun zaki. Therefore, it is advised that the preparation of the beverage be done under good hygiene; monitoring the microbial standard of the local ones sold to the general public. Finally, it would as well be helpful if an effort is made to improve the quality and production techniques of kunun zaki so that large scale production for export outside the continent can be made. For instance, the use of chemical preservatives would be useful.

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