

**EFFICACY OF COLONY COUNTS AS CONTAMINANT INDICATOR IN ASSESSING
SAFE CONSUMPTION STATUS OF KUNUN-ZAKI (LOCAL JUICE), SOLD WITHIN
NASARAWA STATE UNIVERSITY, KEFFI, NIGERIA.**¹ Patrick, N. Okechukwu² Lemuel M. Samuel² Nwokocha A. Chukwuemeka¹ Adewumi k Liyah³ Ekwerekwu I. John Paul¹ Consultancy Department, Bioresources Development Center, FCT, Abuja, Nigeria² Department of Biological Science, Nasarawa State University Keffi, Nigeria.³ Medical Department, National Biotechnology Development Agency, FCT, Abuja Nigeriap.nwokocha@yahoo.comnwokochaandrew4@gmail.com**ABSTRACT**

The study seeks to investigate colony counts as indicator for biological contaminants of Kunun-zaki, a household-name for a local juice consumed domestically or commercialized for income earning in the Middle Belt and Northern part of Nigeria. The investigation focus on identifying bacterial contamination in the finished product, at what number they are found and to enlighten the general public on the probable infectious potentials of such bacteria contaminations, so as to alleviate medical and social burdens of food borne infections. Thirty (30) samples of Kunun-zaki were collected from ten (10) different sales locations grouped into A-J in Nasarawa State University, Keffi. The pH of samples ranged between 3.79-4.39 and total bacterial counts ranged between 17-105CFu/ml. The pathogenic bacteria recovered were streptococcus species, Staphylococcus species, Escherichia coli and Klebsiella species, while the lactobacilli species was recovered as normal flora for fermentation. Findings have shown that, the types and density of bacteria recovered from the Kunun-zaki calls for measures to be taken by regulatory authorities to standardize the process and handling of the product for safe human consumption at homes and or for commercial purposes which may endanger the highly susceptible populace.

Keywords:

Colony count, contaminant indicator, Kunun-zaki, Safe consumption Status

INTRODUCTION

, The study area, Nasarawa State University Keffi, is located in Nasarawa State of Nigeria and lies within 8.8423°N and 7.9067°E of 8.5705°N and 8.3088°E location of the state. Kunun – zaki is an indigenous traditional fermented non – alcoholic beverage that is widely consumed in Middle Belt and Northern Nigeria for its thirst quenching properties. Though consumed throughout the year, it is extensively consumed during the dry season, the drink is produced from cereals such as; millet, sorghum, guinea-corn and maize (Abegaz, 2007), but for this work, the red guinea corn was used, More often than not, due to it's short shelf life, Kunun -zaki would have to be consumed within 18-32 hours of production else it gets fermented further, giving high alcoholic content and a sour taste (Adeyemi and Umar, 1994). The cereals and additives used in Kunun -zaki production are locally sourced as they are grown throughout the savannah belt of West Africa. Packaging materials are also cheap and easily available. Kunun – zaki is rich in carbohydrate, vitamins and minerals but low in proteins (Ayo and Okaka, 1998). The high water content coupled with crude method of production and packaging under improper sanitary conditions may predispose Kunun-zaki to bacteriological microbial contamination leading to probable incidence of diarrhea and abdominal pains even though, no clinical or laboratory findings are provided (Elmahmood and Doughari, 2007). Therefore, there is need to comply with personnel and general hygiene standards for food/beverage preparations, since the local non-alcoholic drink has neither standard procedure nor inspection by regulatory bodies.

Generally speaking, foods that we eat are rarely if ever sterile, they contain microbes whose composition depends on which organism' gains access and how fast or slow they grow, survive and interact in food over

time. The microorganisms present may originate from the natural micro flora of raw materials and distribution, the numerical balance between various types will be determined by the properties of the food, its storage, environment and properties of the organisms themselves and the effects of processing (Adam and Moss, 1999). Bacteria grow faster in the pH range of 6.0 – 8.0, yeast 4.5 – 6.0, firmament fungi 3.5 – 4.0. Although there are some exceptional cases. Adebayo (1999) equally mentioned that microbial growth can occur over a temperature range from about -8°C to 100°C , even though, no single organism is capable of growth over the whole of this range; hence bacteria are normally limited to a temperature span of around 35°C , mold rather less at about 30°C and most organisms that are food born pathogen can survive optimal temperature of 37°C . Bacteria have an optimum temperature of 37°C and some are capable of growing over a range of neutral pH of 5 – 8.5 while fungi prefer slightly acidic conditions and grow best at pH around 5 although can grow at either end of the pH range (Adebayo, 1999). Biological contaminants such as bacteria, virus, fungi, protozoa and helminthes constitutes the major cause of food borne disease with varying degrees of severity ranging from mild indisposition to chronic or life threatening illness or both. In developing countries, such contaminants are responsible for food borne diseases such as cholera, campylobacteriosis, *Escherichia coli*, gastroenteritis, salmonellosis, shigellosis, typhoid fever, brucellosis, amoebiasis and poliomyelitis (Edema *et al*, 2005).

Aerobic colony counts, coliform and enterococci enumeration are useful and most often used means of passing overall situation in the environments of food service establishment (Collins, 1964, Moyo and Baudi, 2004). The presence of indicator organisms, which are capable of fermenting lactose with the production of acid and gas at $35-37^{\circ}\text{C}$ and 44°C in less than 48 hours, are able to produce indole in peptone water containing tryptophan, they are unable to use sodium citrates as its sole source of carbon and incapable for producing acetylmethyl carbinol and they give a positive result in the methyl red test. Example; *Escherichia coli* (Cheesbrengh, 1985). The high bacteria counts in food stuffs, food contact surface equipment and utensils provide a direct and relevant measure of cleaning efficiency and hygiene (Clark, 1965, Mayo and Baudi, 2004).

Ogbonna *et al* (1993), carried out studies on microorganisms associated with locally prepared burukutu, pito and palin wine locally prepared alcoholic drinks in barakiladi, Plateau State. The pH values were taken as well as the nutrients composition of the three (3) brews were determine and each of the brews were plated out on malt extract agar (MEA). Yeast starch agar, potato dextrose agar (PDA) and nutrient agar (NA). The resultant plates were incubated at 25°C for four days and re-examined a week later for the pressure of additional microorganism. The micro flora of the water samples used for the preparation of the three drinks, were also investigated for comparison. The physiochemical features of burukutu are as follows: 1.040 specific gravity 18%, total, solid, 5.04%, alcohol, 5.2 pH and dark brown color. Microbes isolated from burukutu includes; *aspergillus flavus*, *aspergillus fumigates*, *Aspergillus niger*, *Aspergillus oryzae*, *Aspergillus terreus*, *cladosporium herbarium*, *Curvularia lunata*, *Fusarium oxysporum*, *Rhizopus oryzae*, *Rhizopus stolonifer*, *Candida tropicalis*, *Candida utilis*, *Saccharomyces cerevisiae*, *Saccharomyces uranum*, *Schizosaccaromyces pompe beer*, *lactobacillus brevis*, *Lacto bacillus platarun*, *Streptococcus* species, *staphylococcus* species, *Ascuris lumbricoides*, *Ancylostoma duodenali* and nematode ova and larvae were isolate from water used for burukutu production. This implies that some of the isolate from burukutu are likely to have originated from the water samples used from the preparation of the drinks. Ogbonna *et al*, {1983}, Also reported that some burukutu consumers have often complained about having running stomach after taking the beverage.

Sathe *et al* (1981) highlighted the significant of rheological characteristics in processing quality control, season, evaluation and structural analysis of kunun-zaki. Increasing temperatures reduced viscosity but did not alter the rheological characteristics of the products. The time of shear (up to one hour) did not appreciably alter the viscosity, substantial nutrient losses occurs during the various steps, milling and sieving are the processing step of kunun-zaki. According to Hamad and fields (1979), steeping, milling and sieving are the processing steps during which considerable nutrient losses take place. Much of the proteins in cereals grains are located in the testa and germ which are usually sifted off during processing.

Efforts are currently underway in Africa to modify the processing of kunun-zaki with a view to enhancing its nutritive value, shelf-life and possible therapeutic qualities (Akoma *et al*, 2006). Kunun zaki and other indigenous Nigeria non-alcoholic beverages, such as kunun-aya and kunun-tsami have been reported to be of high nutritional values because of the raw materials from which they are made. Spices are usually added in small quantities to improve taste and flavor (Adeyemi and Umar, 1994). The increase in acidity (Lactic acid) of Kunun-zaki during production has been attributed to the dominance of *lactobacillus leichmannii* and

Lactobacillus fermentum during the fermentation process (Efiuvwevwere and Akoma, 1995). In the production of Kunun-zaki, two methods are employed, firstly, the cereal could be steeped in water for 24 hours, wet milled and sieved. The sediment obtained is divided into two unequal portions; one portion is cooked and then mixed with the uncooked portion (being the source of inoculums) and allowed to ferment for 8-10 hours (Efiuvwevwere and Akoma, 1995). In the second method, a portion of the cereal is malted, dried grounded and then mixed with the uncooked portion. The mixture is then added to the cooked portion and stirred vigorously and allowed to ferment, the hydrolytic enzymes (amylase) in the malted cereal aids in digesting the thick slurry thereby converting the complex carbohydrate to simple sugars and final product is sieved and packaged for consumption (Akoma *et al.*, 2002). Foods fermented with lactic acid bacteria have long been held in special favour as safe and nutritious food, they may elicit positive effect on health and well being (Kaplan and Hutkins, 2000). Over the last 30 years, intensified efforts to identify and characterized lactic bacteria have revealed their many important roles in food, including acid production, texture development, flavor generation, preservation and synthesis of B-vitamin. Selected members of the lactic acid bacteria have now been implicated through clinical studies to provide resistance to entire pathogens, stimulate the immune system and help maintain a balanced gastrointestinal micro flora (Kullen and Klanhammer, 1990).

OBJECTIVES

The work objectively seek to enlighten the highly susceptible populace on the potential dangers of bacteria contaminations in locally processed Kunun-Zaki, so as to assuage medical and social burdens of food borne infection via: Determining the bacteriological load in Kunun-zaki drink, determine the pH of Kunun-zaki drink and by isolation and identification of some bacteria found in kunun zaki.

METHODOLOGY

30 Samples of processed Kunun-zaki were collected from 10 different sales points and grouped into A-J within the month of July 2018 in Nasarawa State University Campus Keffi. All samples were packaged in 500ml sterile bottles and immediately transferred to the Microbiology laboratory for analysis. The pH of the various samples were immediately determined using 3505 pH meter model, these were done by dipping the electrodes into each samples and the reading taken after 2-3 minutes. Determination of Total Count of Bacteria was carried out on agar plates of nutrient agar (NA) using the pour plate method and sodium chloride as diluents. The samples were serially diluted and 1ml dilution of the 9th test tube of every sample was used to inoculate each of the plate. The culture plates were then incubated at 37°C for 24 – 48 hours and colonies were counted on a Gallen Kamp colony counter and results recorded as the colony count. Isolation and Identification of Discrete colonies of the organism (for bacteria) were selected and sub-cultured from mixed culture of the plates to respective MacConkey agar plates, blood agar plate and chocolate agar plate, incubated at 37°C for 18-24 hours. The bacteria isolate were then identified following standard microbiological procedures as described by Cheesbrengh (1985).

RESULTS AND DISCUSSION**TABLE: PH VALUES AND TOTAL BACTERIA COUNTS (cfu/ml) FOR KUNUN-ZAKI.**

Hawker	pH	Sample	Nutrient agar (cfu/ml)
A	4.39	A1	21X10 ⁹
B	4.30	B1	17X10 ⁹
C	3.98	C1	105X10 ⁹
D	4.15	D1	52X10 ⁹
E	3.80	E1	44X10 ⁹
F	4.16	F1	30X10 ⁹
G	4.23	G1	19X10 ⁹
H	3.79	H1	49X10 ⁹
I	4.15	I1	25X10 ⁹
J	4.00	J1	47X10 ⁹

Key CFU/ml = colony forming unit per milliliter

TABLE 2: BACTERIAL ISOLATES PRESENT IN EACH SAMPLE

	Staph sp	Strep sp	E.Coli	Kleb. sp	Lact sp	Total No of bacteria isolates	% of bacteria isolates
A	-ve	-ve	+ve	-ve	+ve	2	6.45
B	-ve	+ve	+ve	-ve	+ve	4	12.90
C	-ve	+ve	+ve	-ve	+ve	3	9.68
D	-ve	-ve	+ve	-ve	+ve	2	6.45
E	-ve	+ve	+ve	-ve	+ve	3	9.68
F	-ve	+ve	+ve	-ve	+ve	3	9.68
G	+ve	-ve	+ve	-ve	+ve	3	9.68
H	+ve	-ve	+ve	-ve	+ve	3	9.68
I	+ve	+ve	+ve	-ve	+ve	4	12.90
J	+ve	+ve	+ve	-ve	+ve	4	12.90
						31	100

KEY; Staph-Staphylococcus , Strep-Streptococcus,E-Escherichia, Kleb-klebsiella, Lacto-Lactobacilli, Sp – species
%- percentage, No – Number, +ve - Positive, -ve – Negative.

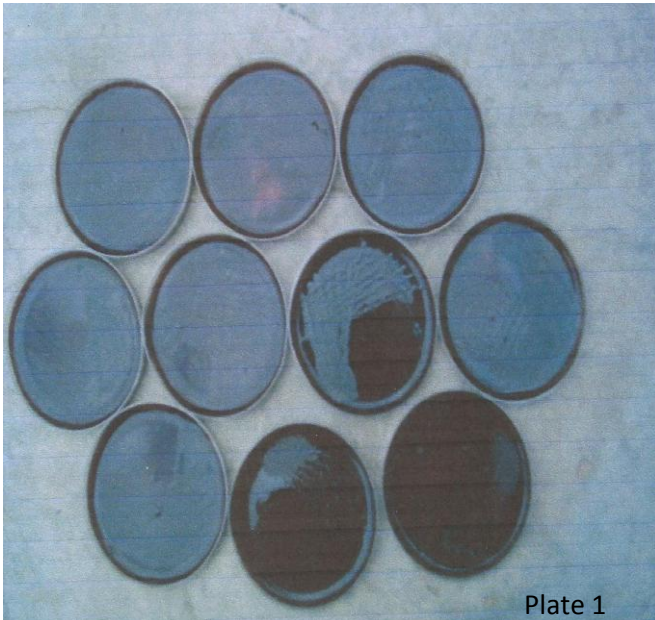


Plate 1: Chocolate Agar

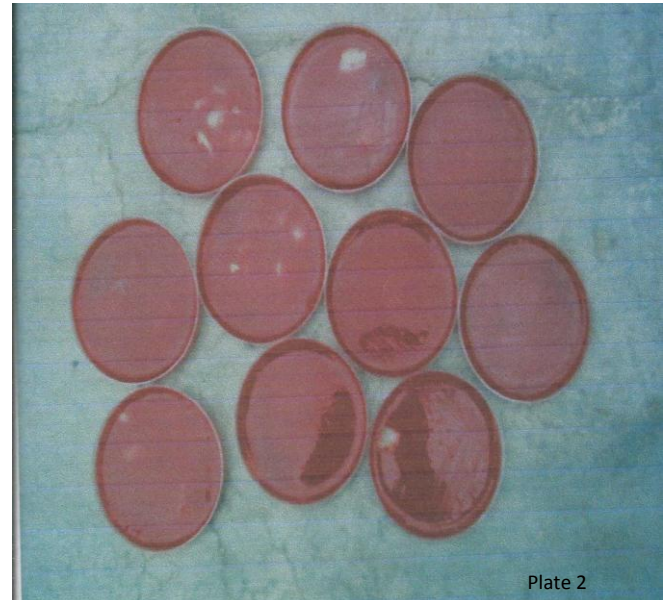


Plate 2: Blood agar

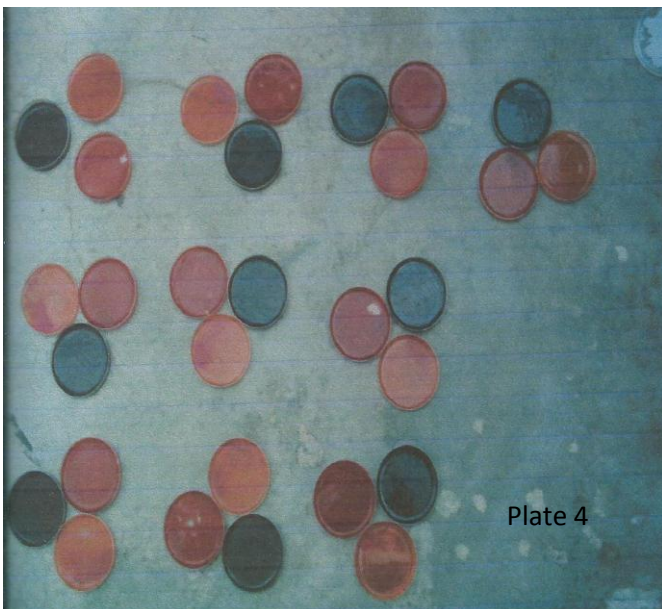
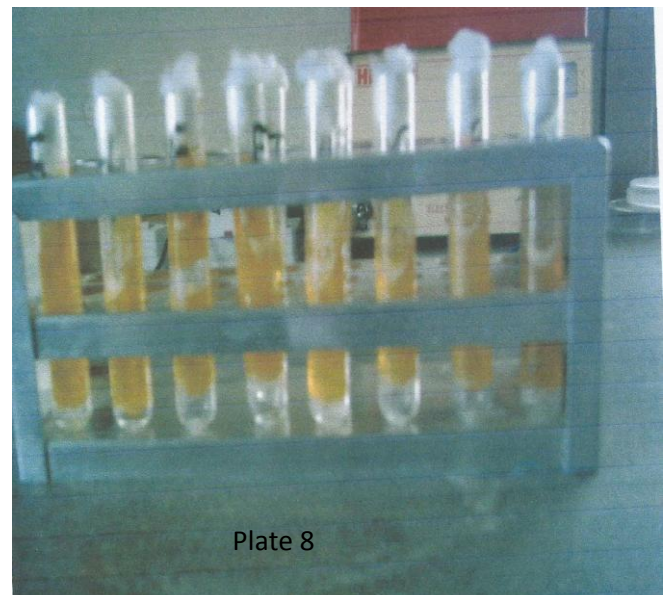


Plate 4: Three replicate of bacteria growth on MacConkey Agar, Blood Agar and Chocolate Agar
Plate 8 Triple Sugar test for Escherichia coli



The pH values of the Kunun-zaki and the total bacteria count are shown on table 1. Result of pH determinations shows that all the samples were acidic in nature. Samples collected from hawker H had the lowest pH of 3.79

and the one collected from hawkers A had the highest pH of 4.39. Sample A1, B1, D1, E1, F1, G1, H1, I1, J1, had bacterial counts range of $21-52 \times 10^9$ CFU/ml on nutrient agar and sample C the highest count on nutrient agar 105×10^9 CFU/ml. Sample C1 had highest number of count on nutrient agar with density of 105×10^9 CFU/ml, closely followed by sample D1, 52×10^9 CFU/ml and sample B1 with lowest bacterial population of 17×10^9 CFU/ml on nutrient agar. Table 2. These shows bacteria isolate present in each sample, the number of bacteria isolated and percentage of the isolators'. Sample A, *Escherichia coli* and *Lactobacilli* species are present with total number of two (2) bacteria and 6.45%. Sample B, *Streptococcus* species, *Escherichia coli*, *Klebsiella* species and *Lactobacilli* species are present with total number of four (4) bacteria and with 12.90%. Sample C, *Streptococcus* species, *Escherichia coli* and *Lactobacilli* species present with total number of bacteria three (3) and 6.5%. Sample D, only *Escherichia coli* and *Lactobacilli* species are present with total number of two (2) bacteria and 6.45%. Sample E, *Streptococcus* species, *Escherichia coli* and *Lactobacilli* species are present with total number of three (3) bacteria and 9.68%. Sample F, *Streptococcus* species, *Escherichia coli* and *Lactobacilli* species are present with total number of three (3) bacteria with 9.68%. Sample G, *Staphylococcus* species, *Escherichia coli* and *Lactobacilli* species are present with three (3) total number of bacteria and 9.68%. Sample I, *Staphylococcus* species, *Escherichia coli* and *Lactobacilli* species present with total number of four (4) bacteria with 12.90%. And sample J, with *Staphylococcus* species, *Streptococcus* species, *Escherichia coli* and *Lactobacilli* species are present with total number of four (4) bacteria with 12.90%. The table shows the kind of bacteria each sample carries and contamination is determined by their numbers in which they are found in a particular sample. Kunun-zaki has high moisture content, the water proportion varies from 55-98%, while remainder being mostly additives. All samples were acidic in nature (pH. 3.79-4.39), this level of acidity have been described by several researchers including Efiuwevwe and Akoma *et al* (2006) who attributed these to the presence of certain species of lactic acid bacteria, namely *Lactobacilli leichmannii* and *Lactobacilli fermentum* during the fermentation process. Similar local drinks with acidic pH values have been reported for Zobo drink and Orange juice products (Lateef *et al*, 2004), as well as burukutu and pito (Kolawole *et al*, 2007). Although these classes of beverages are acidic in nature, the acidity tends to increase with increase in fermentation period resulting into spoilage. The main component of cereals from which kunun-zaki is made are carbohydrate, proteins, vitamins and minerals and the chief product of fermentation is lactic acid which causes decrease in pH values and an increase in acidity. Samples acidity may result from effect of spoilage before the purchase of Kunun-zaki which invariably may lead to production of certain metabolites that could bring about reduction in pH of the products.

The pH of Kunun-zaki is usually too low to allow the growth of pathogenic microorganism, but the presence of *Escherichia coli* and *Staphylococcus* species, could be a matter of serious concern (Alice, 1976). They might have gained entrance through poor hygiene of the producer, *Staphylococcus* species is a normal flora of the skin upper respiratory tract and intestinal tract and common etiological agents of septic arthritis (Alice, 1979) while, *Escherichia coli* is an important member of the coliform group, is part of the normal microbial flora of the intestinal tract of humans and animals, they can also be found in water, soil and vegetation (Cheesbrough, 1985). With the normal habitat, positive, confirms *Staphylococcus* species and if no bubbles, it is negative, confirming *Streptococcus* species.

Contamination may be due to poor hygiene and water used. *Escherichia coli* and cause gastroenteritis, diarrhea and urinary tract infections (Pelczar *et al*, 1993). The presence of *Escherichia coli* indicates that there is acid gas production as confirm using triple sugar test (TSI) diluted and solidified slanty (slope) and a straight wire loop was used to inoculate in the test tubes by stabbing and zig-zag streaking and incubated for 18-24 hours at 30°C which produce bubbles indication of gas production which turn into yellow. *Streptococcus* species are widely distributed in nature, being found in water, dust, vegetation, milk and milk product as normal habitat (Cheesbrough, 1985). The catalase test is carried out on *Staphylococcus* species and *Streptococcus* species using hydrogen peroxide to differentiate them, the colony is dipped and bubbles were produced depicting positive reaction, which may mean that contamination by these pathogens could have occurred during sieving and packaging processes, as most of the people involved in the production, packaging and hawking do not take necessary precautions and as such contamination could be very prominent due to poor hygiene (Cheesbrough, 1985).

Lactobacilli species can be found in mouth, intestinal tract and in breast milk, they rarely cause disease. Lactobacilli species are lactic acid producers, so they are normal flora of any fermentation process depending on the species and duration of fermentation. The whole sample A-J have Lactobacilli species, this may be because they are involved in fermentation process. *Escherichia coli* presence may be due to less heat applied since Kunun-zaki is not boiled; the water is boiled to a certain high degrees and poured into the slurry mixture. The absence of *Klebsiella* species, *staphylococcus* species and *streptococcus* species may be due to the species which have been revealed to have more potent components that are capable of destroying microbes (Ayo *et al*, 2003).

The high colony count in sample C1 table 1, may be an indication of spoilage, poor hygiene and or poor quality of cereals which could stem from harmful storage practices and usage during the Kunun-zaki processing. To test for significant effect of the pH on bacteria count, the pH and the bacteria colony were put into regression analysis using software econometric view to run it. The result shows that the equation is satisfactory because the variables are positive, that is when the variables increase by 1% the pH will increase by 1. At 5% level of significant, the standard error reveals that, the variables are not statistically significant. The F-test shows the joint effect of the independent variable on the dependent variable. Since the F(calculated) is less than the F (tabulated). That is, ($t_{cal} < 5.12_{tab}$), the null hypothesis is accepted; meaning that pH values does not have an effect on the bacteria count.

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CONCLUSION

In conclusion, the colony counts are an indication of spoilage as a consequent of either poor hygiene or poor quality of the cereals and water used. The extent of organic and inorganic contamination of Kunun-zaki beverage has not been evaluated. Further studies are therefore recommended as these equally affect the health and well being of the populace. In order to avoid consumption risk, efforts should therefore be made to improve the quality, production techniques and shelf life of these indigenous exotic beverages, so that large scale production for export outside continent can be done. Improving the production technique can be done through the following ways:

- To safe guard public health, Government and regulatory authorities should intervene by setting standards in acquisition of raw materials; contamination could be from storage sources.
- Producers and hawkers of Kunun-zaki should be encouraged to utilize the technical assistance of National Agency of Food, Drugs administration and Control (NAFDAC) as local body towards attaining quality standard and packaging.
- Prompt and proper refrigeration of the drinks to avoid multiplication of bacteria to be done by the kunun-zaki producers and consumers.
- Provision of good sanitary facilities including potable water, distilled water, proper cleaning and maintenance of equipment used for processing; and
- Health Education of individual and food handler on personal hygiene and proper handling of food.

The afore listed could be achieved through involvement of the local government health workers, state ministry of health and federal ministry of health as well as local agencies such as National Agency of Food, Drugs Administration and Control (NAFDAC), Standard Organization of Nigeria (SON) Consumer Protection Council (CPC) and International Agencies such as World Health Organization (WHO).

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