VOL. 22 NO. 2
ISSN: 2010-1086
SEPTEMBER, 2021

African Scholar Publications & Research International

African Scholar Journal of African Sustainable Development (JASD-2)

Microbiological Quality Assessment of Kunun-Zaki Sold in Federal Polytechnic, Mubi Campus. Adamawa State Nigeria

***Wafari[1], U. U and Ta'awu[1], K. G**

Department of Food Science and Technology, Federal Polytechnic, Mubi.

Abstract

Kunun zaki is a traditional fermented non-alcoholic drink that has been widely accepted as a nutritional drink by Nigerians particularly in the northern region. Kunun zaki is served as a food complement, to quench taste and as a refresher. This study evaluated the microbiological quality of kunun zaki drinks sold in Federal Polytechnic Mubi, campus. Triplicate samples were obtained in 12 different locations. Standard microbiological procedures were employed. The results showed that the mean aerobic colony count ranged from (4.123 ± 0.11log$_{10}$ CFU/mL to 4.897 ± 0.020log$_{10}$ CFU/mL). Mean ACC of kunun zaki from these points were significantly different from each other ($p<0.05$). The mean staphylococcus aureus count ranged between (3.460 ± 0.15log$_{10}$ CFU/mL to 4.742 ± 0.06log$_{10}$ CFU/mL) and were statistically different ($p<0.05$). E.coli counts ranged between 3.504 ± 0.035log$_{10}$ CFU/mL to 4.783±0.02log$_{10}$ CFU/mL. All the twelve samples were higher than the standard values of 2log$_{10}$ CFU/mL. The fungal counts ranged between 2.13 ± 0.07log$_{10}$ CFU/mL to 2.93 ± 0.04log$_{10}$ CFU/mL, and the counts was statistically significant ($p < 0.05$). Eight (8) fungal genera with 11 species were observed. The pH value ranged between 3.537 ± 0.152 to 4.313 ± 0.103 which suggest acidity. It is recommended that further research be conducted in other part of Nigeria using other pathogens, other types of foods/or beverages

and other food service establishment to establish a comprehensive profile of microbial risks/or safety of various food products. To mitigate the effects of the natural presence of microbes in the food chain, the application of Good Hygiene Practices (GHP) and Good Manufacturing Practices (GMP) allows a minimization in the contamination to satisfactory levels.

***Keywords**: kunun zaki, Microbiological, quality, pH, fermented, assessment.*

Introduction

Kunu-zaki is the traditional Hausa name of an indigenous non-alcoholic fermented beverage, which is produced and commonly consumed by adults and children in Nigeria particularly, in the northern region as a refreshing drink, an appetizer, food complement and to quench thirst (Oranusi, Umoh & Kwagga, 2003). Similarly, it is used as an alternative for or to complement soft drinks and wines at traditional, religious, marriage, funeral and other social gathering, markets, offices, and schools (Abegaz, 2007).

Kunun-zaki is a major source of calories and an excellent source of energy in form of carbohydrates. Sopade and Kassum (1992) reported that Kunun-zaki has about 9.5% dry matter, 90.5% moisture, 12.2% carbohydrate, 0.3% protein, 1.0% fat and 1.5% ash and pH range of 4.0 to 5.0 with different types of amino acids. It provides source of income and a means of poverty alleviation and contributes to variety in the diet and the food security of millions peoples (Ajao & Yakubu, 2011)

Kunun-zaki is prepared from any of the following cereal grain: guinea-corn (sorghum bicolour), millet (pennisetum typhoides), maize (zea mays), rice (oryza sativa), or wheat (triticum aestivum) (Akoma et al., 2006). Usually, the production of Kunun-zaki involves sorting, cleaning, washing and steeping of whole grains for 10 to 24 hours (Oluwajoba et al., 2013), wet milling with spices and sweet potato, gelling of about three-quarters of the mixture in boiled water, addition of about one-quarter fresh ungelled part of the

mixture and allowing for overnight fermentation (Oluwalana & Adedeji, 2013). The Kunun-zaki is ready for consumption after filtration (Akoma et al. 2013).
The short keeping quality of 24hrs for kunun zaki, the unhealthy condition of local production, pitching with mixed culture from fresh wet milled mixture, and the variable taste and flavour from different producers are the limiting factor for the mass production of kunun zaki (Akoma et al., 2012).
There are lot of worry about the unhygienic condition of street vended kunun-zaki. This because well and untreated borehole are the main sources of water for kunun zaki production in many localities in Mubi, some of these waters are prone to contamination from various sources; animals are usually reared in households where kunun zaki is produced, cereals and spices used are highly prone to environmental contamination (Inabo et al., 2000) and the ready to drink kunun zaki are commonly packaged and sold in used plastic containers. All these will predispose the kunun zaki to microbial contamination which will negatively affect the quality and safety of the finished product.
The quality and the safety of the kunun zaki drinks therefore depend on the raw materials, the hygiene of the personnel, water, packaging materials and the production environment.
A lot of foodborne diseases are caused by the ingestion of food contaminated with pathogenic bacteria (Lawal, 2012). The high moisture contents in addition to crude methods of processing and packaging under improper sanitary condition expose kunun zaki to microbial contamination. According to Umar et. al. (2014), local beverages such as kunun zaki could act as vehicle for zoonotic and foodborne infections which include *staphylococcosis*, *brucellosis*, *shigellosis* and *listeriosis*. Therefore, the aim of this study is to carry out the microbiological analysis of kunun zaki sold in Federal Polytechnic Mubi campus, Adamawa State, Nigeria.

Material and Methods

Study Area

The study area was the food vending sits and the provision shops within the Federal Polytechnic Mubi campus, Adamawa State Nigeria. The institution was established by decree No.33 of 1979 constitution. Approximately seven thousand students are enrolled every session; most of them stay in boarding house near the campus. Their time is mostly spent doing academic work in the campus. Hence, they usually buy the kunun zaki from these points/vendors.

Recently, the number of shops and food vendors around the Polytechnic campus is increasing due to the increasing numbers of students. The food stall and the shops are usually helpful from the consumer perspective as they provide convenient and affordable beverages such as kunun zaki, although not all of them meet the hygiene standard set by the regulatory authority.

Sample Collection

Three samples of freshly prepared kunun zaki were purchased from each of the twelve (12) different points and shops within the Federal Polytechnic, Mubi campus between the month of January and March, 2020. The samples were immediately transported to the laboratory for microbiological analysis.

Media Preparation and Microbial Analysis

All media were prepared in accordance with the manufacturer's instruction.

Microbiological Analysis

Aerobic Colony Count (ACC)

Total aerobic mesophilic bacterial count was determined according to Maturin and Peele (2001). Twenty five gram (25 g) of each food type was added into 225 mL of two separate flasks containing each, sterile 0.1 % (w/v) peptone water (Oxoid CM 009) and shaken for 2-3 minutes using shaker (STUART, UK) to prepare initial (10^{-1}) homogenate dilution. Further dilution was made through transfer of 1 mL of the 10^{-1} food homogenate into test tube containing 9 mL of sterilized 0.1 % (w/v) peptone water to prepare 10^{-2} dilution. The process was repeated to prepare 10^{-3} and 10^{-4} serial dilution in similar manner 1mL of the last three dilutions (10^{-2} , 10^{-3} and 10^{-4}) was dispensed to sterilized petri plate in triplicate. Then sterile molten plate count agar (GREEN STAR) was added to each petri plate. The plates were then incubated at 37 $^{\circ}$C for 48hrs. Then colonies were counted using colony counter (STUART SCIENTIFIC, UK) all plate were counted but those showing colony counts between 30 and 300 were selected and their colony forming unit per gram of food item ($CFUg^{-1}$) were calculated by multiplying by the dilution factors.

***Staphylococcus aureus* Count**

S. aureus were isolated from the samples (25 g) homogenized in 225 mL of 0.1 % sterile peptone with (Oxoid, CM0009). Further dilution of 10^{-2}, 10^{-3} and 10^{-4}

were made as mentioned above from the food homogenate. One mL of each 10^{-2} 10^{-3}, and 10^{-4} serial dilution were dispensed onto sterilized petri plate in triplicate. Sterile molten mannitol salt agar (B LULUX) was poured to each petri plate. The plate were then incubated at 37 ºC for 48 hrs, yellow to orange colonies surrounded by yellow zone due to mannitol fermentation were counted using colony counter (STUART SCIENTIFIC, UK) and recorded as colony forming unit per gram (CFU/g) of the food samples. Six of these yellow colonies were purified and transferred to nutrient agar slant for further biochemical test. All biochemical tests were performed after Gram staining according to (Willey, Sherwood & Woolverton, 2017). These include catalase test, sugar fermentation and coagulase test.

E.coli Count

One millilitre (1 mL) of each serial dilution from 10^{-2}, 10^{-3} and 10^{-4} were dispensed onto sterilized petri plate in triplicate. Sterile molten violet red bile agar (VRBA) was poured to each petri plate. The plates were then incubated at 37 °C for 48 hrs. Pink to red-purple colonies of diameter 0.5 mm or more with or without halo of precipitation was counted as positive. Biochemical test such as IMVIC test was used to characterize the colonies.

Identification of Isolates

The isolates were purified by sub culturing and characterized based on colonial morphology, cellular morphology, staining and biochemical reaction and identified using Bergey's manual of determinative Bacteriology (Holt *et al*., 1994). The fungi were characterized based on colonial morphology and cellular morphology was identified as described by Cooper (1992)

Fungal Plating and Incubation

This was carried out according to the procedure outlined for plating by Kortei et al. (2018) with media and process modifications as follows: aliquots of one milliliter (1 ml) of each test sample were added to 9 ml of sterile distilled water and agitated vigorously and was used as the stock solution. The samples were serially diluted 10^{-2} up to 10^{-4} and then plated on Dichloran Rose Bengal Chloramphenicol (DRBC) agars. The media was prepared according to the manufacturer's specifications. It was followed by incubation at 37°C for 7 days.

After 1 week, observable molds and yeast appeared for counting and identification.

Fungal Identification and Enumeration

Lacto Phenol Cotton Blue Teased Mount Procedure for Identification

A drop of lactophenol cotton blue dye was placed on the slide, and a sterile iron needle was used to transfer a tiny piece of a colony into Lacto Phenol Cotton Blue Dye on the slide. The colony was then teased into very tiny pieces using an iron needle. The slide was covered with a coverslip with a magnification ×400 used. The identification of the fungi was done macroscopically (texture and colour in the plate) and microscopically by observation of their cultural and morphological features under the microscope. Molds and yeast that appeared were identified by their cultural and morphological characteristics using standard identification manuals (Moss, 1989; Samson et al., 2000).

Determination of pH

The pH of samples was determined directly with a bench pH meter (Jenway 3510) after calibration using standard buffers 4.0 and 7.0 pH.

Data analysis

Procedures for microbial counts and pH were carried out in triplicates, and data collected were subjected to a single-factor analysis of variance (ANOVA). Differences among means were separated using Tukey's test and significances were accepted at a 5% level (p<0.05) using Minitab version 19.0 for windows (Minitab Inc.). The analysis was done using the mean counts expressed in the standard forms which were transformed into logarithmic values and results reported as means ± standard deviation.

Results and Discussion

Table 1: Loads of Aerobic Colony Count (ACC), *S. aureus, E. coli,* fungal counts and pH of the kunun zaki samples from the respective pointe/vendors. Mean (Log_{10}CFU/ mL ± SD) were used.

Samples	ACC	*S. aureus*	*E. coli*	Fungal counts	pH
P1	4.579 ± 0.02^{c}	3.935 ± 0.06^{d}	3.504 ± 0.04^{j}	2.93 ± 0.04^{a}	4.31 ± 0.10^{a}
P2	4.435 ± 0.05^{e}	3.742 ± 0.13^{e}	4.324 ± 0.08^{d}	2.39 ± 0.13^{d}	3.68 ± 0.16^{e}
P3	4.505 ± 0.03^{d}	3.460 ± 0.15^{f}	4.023 ± 0.08^{i}	2.13 ± 0.07^{e}	3.54 ± 0.15^{h}
P4	4.166 ± 0.06^{g}	4.535 ± 0.08^{a}	4.783 ± 0.02^{a}	2.15 ± 0.21^{e}	4.12 ± 0.10^{b}
P5	4.897 ± 0.02^{a}	4.628 ± 0.05^{a}	4.277 ± 0.05^{d}	2.81 ± 0.05^{b}	3.58 ± 0.37^{g}

P6	4.731 ± 0.04^{b}	4.208 ± 0.04^{b}	4.165 ± 0.05^{g}	2.39 ± 0.13^{d}	4.10 ± 0.10^{c}
P7	4.163 ± 0.10^{g}	4.072 ± 0.05^{c}	4.236 ± 0.06^{e}	2.54 ± 0.09^{c}	3.60 ± 0.10^{g}
P8	4.206 ± 0.04^{g}	4.611 ± 0.05^{a}	4.087 ± 0.08^{h}	2.51 ± 0.05^{c}	3.73 ± 0.06^{e}
P9	4.123 ± 0.11^{g}	4.594 ± 0.04^{a}	4.183 ± 0.06^{g}	2.65 ± 0.07^{b}	3.73 ± 0.06^{e}
P10	4.418 ± 0.05^{e}	4.596 ± 0.06^{a}	4.367 ± 0.04^{c}	2.39 ± 0.11^{d}	4.03 ± 0.15^{d}
P11	4.290 ± 0.07^{f}	4.742 ± 0.06^{a}	4.191 ± 0.07^{f}	2.74 ± 0.06^{b}	3.77 ± 0.15^{e}
P12	4.531 ± 0.03^{d}	4.583 ± 0.02^{a}	4.423 ± 0.06^{b}	2.81 ± 0.05^{b}	3.63 ± 0.15^{f}

Note: Means in a column with the same superscript letters are not statistically different ($p>0.05$). (N=3)

The microbiological analysis in table 1 reveals that the aerobic colony count for kunun zaki from the twelve different vendors ranged from (4.123 ± 0.11$\log_{10}$ CFU/mL to 4.897 ± 0.020$\log_{10}$ CFU/mL). Some ACC of kunun zaki from these vendors were significantly different from each other ($p<0.05$). However, four of the kunun zaki samples had comparable mean ACC between (4.123 ± 0.11$\log_{10}$ CFU/mL to 4.206 ± 0.04$\log_{10}$ CFU/mL, ($p<0.05$). Similarly, samples from vendor 3 and 12 had respective mean ACC of 4.505 ± 0.57$\log_{10}$ CFU/mL and 4.531 ± 0.03$\log_{10}$ CFU/g, $p<0.05$). Also, samples from vendor 2 and 10, had related mean ACC of (4.435 ± 0.02$\log_{10}$ CFU/mL and 4.419 ± 0.05$\log_{10}$ CFU/mL, $p<0.05$) respectively. The relationships that exist from these samples of kunun with similar mean APC that did not statistically differ from each other might be they may have common source of contamination, raw materials, water and the production environment.

The mean *staphylococcus aureus* count ranged between (3.460 ± 0.15$\log_{10}$ CFU/mL to 4.742 ± 0.06$\log_{10}$ CFU/mL). Some kunun zaki samples had mean *staphylococcus aureus* count that statistically differs significantly from each other ($p<0.05$). However, kunun zaki from seven vendors had comparable mean *staphylococcus aureus* count between (4.532 ± 0.079$\log_{10}$ CFU/mL to 4.742 ± 0.063$\log_{10}$ CFU/mL, $p>0.05$) that statistically did not differs from each other.

The result from table 1 showed that the highest mean *E. coli* count was 4.783± 0.022$\log_{10}$ CFU/mL and the lowest was 3.504±0.04$\log_{10}$ CFU/mL, the mean *E. coli* from these vendors were significantly different from each other ($p<0.05$), except two of the kunun zaki samples that have similar mean *E. coli* count of (4.165 ± 0.05$\log_{10}$ CFU/mL and 4.183$\log_{10}$ CFU/ml, $p>0.05$).

Results of the different fungal counts of kunun zaki from the different locations are represented in Table 1.The fungal counts ranged between 2.13 ± 0.07$\log_{10}$ CFU/mL to 2.93 ± 0.04$\log_{10}$ CFU/mL. Count of kunun zaki from the different

locations was statistically significant ($p < 0.05$). However, counts from points/vendor (P2, P6, & P10), (P3 & P4), (P7 & P8), and (P5, P9, P11, & P12) were not statistically significant and the counts were all comparable ($p > 0.05$). There was an observed low fungal count generally. The range of counts was within the acceptable to borderline range of microbiological counts for ready-to-eat foods 2-4$\log_{10}$ CFU/mL or /gm. as prescribed by the International Commission for Microbiological Specification of Foods (International Commission for Microbiological Specification of Foods [ICMSF], 1998).

A total of eleven (11) fungal species belonging to eight (8) fungal genera were isolated; *Aspergillus (A. niger, A. fumigatus, A. parasiticus), Rhizopus (R. stolonifer), Mucor (M. racemosus), Fusarium (F. oxysporum), Penicillium (P. digitatum, P. verucosum), Cladosporium (C. cladosporoides), Curvularia (C. lunata),* and *Rhodotorula* sp. were recorded.

The pH is used to test the acidity and alkalinity of the kunun zaki samples. The result showed that the highest mean pH value obtained was (4.313 ± 0.102) and the lowest was (3.537 ± 0.152). The mean pH values determined from kunun zaki from the respective locations were significantly different from each other ($p<0.05$) except four of the kunun zaki from points/vendor (P2, P8, P9, and P11) with comparable pH values between 3.683 ± 0.161 and 3.767 ± 0.153 that statistically were not significant from each other ($p>0.05$)

Discussion

Consumption of kunun zaki is greatly patronized, especially by majority of staffs and students in Federal Polytechnic, Mubi campus. One of the main reasons for such high patronage of the product is the well reported nutritional and health benefits, however, it is also well reported that the main ingredients of preparing kunun zaki are potential sources for the transmission of pathogenic bacteria (Umar et al., 2014). From this study, all the samples examined from the various shops/vendors were contaminated with aerobic mesophiles.

From the results, aerobic colony counts (ACC) of 4.123 ± 0.11$\log_{10}$ CFU/mL to 4.731 ± $\log_{10}$CFU/mL were recorded. ACC of samples from all the shops/vendors were within the standard requirement of less than 5$\log_{10}$ CFU/mL for beverages (Public Health Laboratory Service [PHLS], 2000). ACC for various kunun zaki have been reported by some researchers. Aboh and Oladosu (2014) reported ACC ranging from <1 to 8.04$\log_{10}$CFU/mL in ten kunun zaki samples from Abuja Municipal Area Council (AMAC) in the

Federal Capital Territory, Nigeria. Ajao and Yakubu (2011) observed ACC between 4.69 to 6.86$\log_{10}$ CFU/mL from ten kunun zaki in Illorin metropolis, Kwara State, Nigeria. Also, Makut et. al. (2013) reported ACC from kunun zaki in Keffi, Nasarawa State, Nigeria ranging from 8.56 to 8.96$\log_{10}$ CFU/mL. Similarly, Akoma et. al. (2014) and Olaoye et. al. (2017) reported ACC of 4.77 ± 0.09 to 6.63 ± 0.19$\log_{10}$ CFU/mL and 3.6 to 5.8$\log_{10}$ CFU/mL from kunun zaki respectively. The AAC results of this work generally appear relatively lower than most of the research findings as well as the standard values of <5$\log_{10}$ CFU/mL (PHLS, 2000). High ACC may indicate poor handling, inappropriate processing or a general lack of hygiene, indicating that the vendors/shops selling kunun zaki involved in this study probably employed some level of proper handling and hygiene practice.

Staphylococcus aureus is a pathogen known to be carried by food handlers (Beuchat, 1998). The presence of *S. aureus* in kunun zaki indicates poor hygiene practices and levels higher than 4$\log_{10}$ CFU/mL are potentially hazardous. From this study, *S. aureus* counts ranged from 3.460±0.15$\log_{10}$ CFU/mL to 4.742 ± 0.06$\log_{10}$ CFU/mL. Samples from points/vendor P1, P2, and P3 were within the standard values of <4$\log_{10}$ CFU/mL (PHLS, 2000) while samples from the remaining nine points were all above the standard values Table 1. Various researchers have reported the isolation of *S. aureus* from kunun zaki. Ajao and yakubu (2014) reported the *S. aureus* counts between 1.97$\log_{10}$ CFU/mL to 4.0$\log_{10}$ CFU/mL. Also, Olaoye et. al. (2017) and Orutugu et. al. (2015) reported the mean *S. aureus* counts ranging from 3.1log CFU/mL to 4.4$\log_{10}$ CFU/mL and 1.875±0.350$\log_{10}$ CFU/mL to 2.498 ± 0.301$\log_{10}$ CFU/mL respectively.

E. coli is an organism that is part of the normal microbial flora of the intestinal tract of humans and warm-blooded animals, therefore, their presence in kunu zaki can be an indication of poor hygiene and sanitation or inadequate heat treatment and levels above 2$\log_{10}$CFU/mL are unsatisfactory. From this study, *E.coli* counts ranged between 3.504 ± 0.035$\log_{10}$ CFU/mL to 4.783±0.02$\log_{10}$ CFU/mL. All the twelve samples Table 1, were higher than the standard values of 2$\log_{10}$ CFU/mL (PHLS, 2000 and New South Wales, 2009). Many researchers have documented the isolation of *E. coli* from kunun zaki.

Ajao and Yakubu (2011) reported the *E. coli* counts ranging from 0.32 to 3.43$\log_{10}$ CFU/mL from ten kunun zaki in Illorin metropolis, Kwara State, Nigeria. Also Orutugu et. al. (2015) reported total coliform counts between

2.8±0.585 to 3.079 ± 0.132$\log_{10}$ CFU/mL from kunun zaki in Yenagoa metropolis, Bayelsa State, Nigeria. In addition, Olaoye et. al. (2017) reported coliforms count ranging from 2.7 to 3.4$\log_{10}$ CFU/mL. The *E.coli* counts results of this work are higher than the research findings and the standard values of 2$\log_{10}$ CFU/ml (PHLS, 2000). Holding kunun zaki at ambient temperature for sale could be risky. The Standard Organisation of Nigeria (SON) stated that coliforms bacteria and pathogenic microorganisms should not be present in beverages (Standard Organisation of Nigeria [SON], 1985). High *E. coli* counts may indicate poor hygiene and sanitation or inadequate heat treatment. The plausible reason to explain the high level of *E. coli* in this study could be from one or all of these; poor personal hygiene of the kunun zaki handlers, low level of sanitary condition of the environment from which these kunun were prepared, processed control not fully achieved, possible raw materials contamination or post processing contamination with fecal related bio burden.
The fungal counts recorded in this work were comparable with the one reported by Aboagye et al. (2020) ranging from 2.098 to 4.23 $\log_{10}$ CFU/mL as counts of "asaana" a beverage of maize from Ghana. Minamor, Mensah, et. al. (2017) recorded fungal counts of <4$\log_{10}$CFU/mL in "pito" a cereal beverage of sorghum was found to be within the permissible limits.
However, Oriola et. al. (2017) reported higher fungal counts of range of 5.53 to 6.65 $\log_{10}$ CFU/mL from "Otika" a Nigerian cereal beverage of sorghum. Also, Popoola (2019) reported a range between 5.11 to 5.23$\log_{10}$ CFU/mL in "akamu" samples obtained from different cereals. High colony counts >4$\log_{10}$ are an indication of spoilage as a result of either poor hygiene or poor quality of cereals and water used in the preparation of the beverage.

Mossel et. al. (1986) highlighted that variation in fungal counts could be attributed to differences in compliance with Good Manufacturing Practices (GMP) conditions during the growing, processing, or storage of the raw material of the food. Lastly, the high incidence of particular fungal specie may indicate the presence of Mycotoxins. A consumer must be made aware the consequences of drinking kunun zaki not hygienically prepared and also stored for longer periods as may contain greater fungal counts; hence, its safety is doubtful.
The range of counts recorded in this work was, however, within the acceptable range of microbiological counts for ready-to-eat foods as prescribed by the International Commission for Microbiological Specification of Foods

(International Commission for Microbiological Specification of Foods [ICMSF], 1998).

Fungal species, many types of microorganisms, molds, bacteria, and yeasts are established in the naturally fermented starters. This work was in agreement with the results of Fadahunsi et al. (2013), they also identified the fungi *Saccharomyces cerevisiae, Candida krusei,* and *Aspergillus niger* in the both fresh and stored samples of burukutu and pito. The genera *Aspergillus, Fusarium,* and *Penicillium* are often associated with contamination of agricultural products from the field, during storage and transportation. Fungal contamination on grain during storage and transportation occurs frequently in the global trade of cereals. Wheat, barley, com, and other cereals are regulated for their mold (physical) and mycotoxin contaminations by the quarantine service of export and import harbours. Similarly, fungi isolated by Elmahmood and Doughari (2007) included *Penicillium digitatum, Aspergillus fumigatus, Rhizopus nigricans, and Mucor sitophila* from Kunun zaki. The incidence of these fungal species has been linked to the spoilage of beverages as explained by Kolawole et. al. (2007). Anupma and Tamang (2020) also isolated filamentous molds belonging to seven genera, *Mucor, Aspergillus, Penicillium, Bjerkandera, Rhizopus, Trametes,* and *Cladosporium* from amylase and alcohol-producing starters in India.

The presence of pathogenic microorganisms and mycotoxins in beverages constitutes a Public Health hazard for consumers and economic loss for the producers (Granados-Chinchilla et al., 2018) as many of these fungi are in the spoilage category and the presence of toxigenic fungi is indicators of a possible Mycotoxins contamination risk (Anjorin et al., 2013). It is of paramount importance that these fermented products be prepared under good sanitary and hygienic conditions. To mitigate the effects of the natural presence of microbes in the food chain, the application of Good Hygiene Practices (GHP) and Good Manufacturing Practices (GMP) allows a minimization in the contamination to satisfactory levels.

pH is a measure of the relative acidity of a solution and is defined as the negative logarithm of the hydrogen ion concentration (Willey, Sherwood & Woolverton, 2017).

The pH scale extends from pH 0.0 to pH 14.0, and each pH unit represents a tenfold change in hydrogen ion concentration.

Each bacterial and fungal species has a definite pH growth range and pH growth optimum. Acidophiles have their growth optimum between pH 0 and 5.5; neutrophiles, between pH 5.5 and 8.0; and alkaliphiles (alkalophiles), between pH 8.0 and 11.5. Low pH can be used to hinder microbial spoilage of food.

Cereals are prone to infections by a wide range of plant pathogens. The national and international trade of cereal is mostly connected to increased bacterial and fungal infection and cross-contamination hazards (Waage et al., 2006). Bacterial and Fungal growth is influenced by favourable environmental conditions such as pH, temperature, moisture, and light. The pH of a medium is positively correlated with the growth of most bacteria and fungi. All samples analysed were acidic (pH 3.537 ± 0.152 to 4.313 ± 0.102) as referenced from the pH scale.

From this study, the result showed that the highest mean pH value obtained was (4.313 ± 0.102) and the lowest was (3.537 ± 0.152), which indicates that the predominant organisms responsible for the fermentation and contamination of these kunun zaki were acidophiles.

Various researchers have reported the pH of kunun zaki. Egbere et. al. (2017) reported mean pH values from ten kunun zaki sold within Jos metropolis, Plateau State Nigeria with pH range between 3.95 to 4.24, also Akoma et. al. (2014) reported mean pH value ranging from 3.36±0.01 to 4.20±0.06, Olaoye et.. al.. (2017) reported pH range between 4.32 to 4.62 for inoculated samples of kunun aya and 5.62 for uninoculated kunun aya with lactobacillus plantarum as starter culture. Most of the pH from these research findings is comparable with pH values obtained from this study for the fermented kunu zaki. However, Olaoye et. al. (2017) reported a higher pH values ranging from 4.32 to 5.62 for kunun aya. Nonetheless, a much higher pH range was reported by Yamanaka (2003) as 7–9 for optimum growth of fungi. The acidity of this beverage tended to be directly proportional to the storage period (increase with an increase in the fermentation period) resulting in spoilage. Consequently, the low pH values may have inhibited the growth of some fungal species isolated.

Conclusion

The aerobic colony count (ACC) of the various kunun-zaki analysed from these shops/vendors ranged between 4.123 ± 0.11$\log_{10}$ CFU/mL to 4.731 ± 0.06$\log_{10}$ CFU/mL which were within the standard of UK public Health Laboratory Service (PHLS, 2000) acceptable reference of less than 5$\log_{10}$ CFU/mL for

beverages. The *S. aureus* counts ranged from 3.460±0.15$\log_{10}$ CFU/mL to 4.742 ± 0.06$\log_{10}$ CFU/mL. Only three samples were within the acceptable reference standard value of less than 4$\log_{10}$CFU/mL (PHLS, 2000) while the remaining nine samples were above the reference standard values of <4$\log_{10}$ CFU/mL. The *E. coli* counts ranged between 3.504 ± 0.035$\log_{10}$ CFU/mL to 4.783±0.02$\log_{10}$ CFU/mL which were higher than the standard value of 2$\log_{10}$CFU/mL (PHLS, 2000) and (New South Wale [NSW], 2009).

The fungal count ranged between (2.13 ± 0.07 to 2.93 ± 0.04$\log_{10}$CFU/mL). Eight (8) fungal genera with 11 species represented by *Aspergillus (A. niger, A. fumigatus, A. parasiticus), Rhizopus (R. stolonifer), Mucor (M. racemosus), Fusarium (F. oxysporum), Penicillium (P. digitatum, P. verucosum), Cladosporium (C. cladosporoides), Curvularia (C. lunata),* and *Rhodotorula* sp as fungal contaminant of kunun zaki beverages.

The pH value ranged between 3.537 ± 0.152 to 4.313 ± 0.103 which suggest acidity.

Recommendation

It is recommended that further research be conducted in other part of Nigeria using other pathogens, other types of foods/or beverages and other food service establishment to establish a comprehensive profile of microbial risks/or safety of various food products.

It is of paramount importance that these fermented products be prepared under good sanitary and hygienic conditions.

To mitigate the effects of the natural presence of microbes in the food chain, the application of Good Hygiene Practices (GHP) and Good Manufacturing Practices (GMP) allows a minimization in the contamination to satisfactory levels.

REFERENCES

Abegaz, K. (2007). Isolation, characterization, and identification of lactic acid bacteria involved in traditional fermentation of borde, an Ethopian cereal beverage. *African Journal of Biotechnology. 6*(12), 1469-1478

Aboagye, G., Gbolonyo-Cass, S., Kortei, N. K., & Annan, T. (2020). Microbial evaluation and some proposed good manufacturing practices of locally prepared malted corn drink ("asaana") and *Hibiscus sabdarifa* calyxes extract ("sobolo") beverages sold at a university cafeteria in Ghana. *Scientific African, 8,* e00330. https://doi.org/10.1016/j.sciaf.2020.e00330

Ajao, A. T. & Yakubu, S. E. (2011). Microbiological risk assessment and antimicrobial activities of azadirachta indica stem extract against sanitary indicator bacteria associated with kunu samples. *Global Jornal of Science Frontier Research*, 118.

Akoma, O., Agarry, O. & Nkama, I. (2013). A study on the production and consumption of kunun zaki. A cereal based ethnic fermented beverage of northern Nigeria. *British Journal of Applied Science and technology, 3*, 1220-1227

Akoma, O., Agarry, O., & Nkama, I.. (2012). The microbiological quality of freeze-dried kunun zaki during production and storage. *Internal journal of biology and pharmacy*, 1, 1397-1410

Akoma, O., Daniel, A. A., Ajewole, A. E, & Nwodo, P. G. (2014). Quality characteristics of kunun zaki (A Nigerian fermented cereal beverage) sold within Bida metropolis. *Global Advanced Research Journal of Agricultural Science*, *3*(9), 298-303

Akoma, O., Jiya, E. A., Akumak, D. D., & Mshill, E. (2006). The influence of malting on the nutritional characteristicsof Kunun zaki. *African Journal of Biotechnology*, *5*(10), 996-1000.

Anjorin, T. S., Salako, E. A., & Makun, H. A. (2013). Control of toxigenic fungi and mycotoxins with phytochemicals: Potentials and challenges. In *Mycotoxin and food safety in developing countries* (p. 181– 202). https://doi.org/10.5772/53477

Anupma, A., & Tamang, J. P. (2020). Diversity of filamentous fungi isolated from some amylase and alcohol-producing starters of India. *Frontiers in Microbiology*, *11*, 905. https://doi.org/10.3389/ fmicb.2020.00905

Beuchat, L. R. (1998). *Surface decontamination of fruits and vegetables eaten raw: a review* (WHO/FSF/FOS/98.2); WHO, Gevena.

Cooper, B.H. (1995). Taxonomy, Classification and Nomenclature of Fungi. Manual of Clinical Microbiology, *American Society of Microbiology*, Washington D.C.4:10-12.

Ebere, O. J., Idoko, B. O., Danladi, M. M. A., Yakubu, D., & Solomon, S. (2017). Simultaneous saccharification of and fermentation in the production of kunun zaki (A Nigerian non-alcoholic beverage) bt selected lactic acid bacteria. *Internal Journal of Food Science and Nutrition. 2*(1), 72-76

Elmahmood, A., & Doughari, J. (2007). Microbial quality assessment of kunun-zaki beverage sold in Girei town of Adamawa State, Nigeria. *African Journal of Food Science*, *1*(1), 11–15.

Fadahunsi, I., Ogunbanwo, S., & Fawole, A. (2013). Microbiological and nutritional assessment of burukutu and pito (indigenously fermented alcoholic beverages in West Africa) during storage. *Natural Sciences*, *11*(4), 98–103.

Granados-Chinchilla, F., Redondo-Solano, M., & Jaikel-Víquez, D. (2018). Mycotoxin contamination of beverages obtained from tropical crops. *Beverages*, *4*(4), 83

Holt, J.G., Kreig, N.R., Sneath, P.H.A., Staley, J.T, & Williams (1994) Bergey's Manual of Determinative Bacteriology 9th edition, The William and Wilkins Co. Baltimore, USA.

ICMSF (1998). *International Commission on Microbiological Specification of Food Microorganisms. Microbiological analysis principles and specific application*.

Inabo, H. H., Ogbadu, L. J., Umuh, V. J., & Ameh, J. B. (2000). Microbiological quality of selected marketed condiments. *Namoda Techscope Journal*. 4, 20-30

Kolawole, O., Kayode, R., & Akinduyo, B. (2007). Proximate and microbial analyses of burukutu and pito produced in Ilorin, Nigeria. *African Journal of Biotechnology*, *6*(5), 587.

Kortei, N. K., Odamtten, G. T., Obodai, M., & Wiafe-Kwagyan, M. (2018). Mycofloral profile and the radiation sensitivity (D10 values) of solar dried and gamma irradiated *Pleurotus*

ostreatus (Jacq. Ex. Fr.) Kummer fruitbodies stored in two different packaging materials. *Food Science & Nutrition, 6*(1), 180–188.

Lawal, A. O. (2012). Microbial quality of kununzaki beveragesold in Ile-Ife, Osun State. *Journal of Food Technology,* 10(1), 4-7

Makut, M. D., Nyam, M. A., Obiekezie, S. O., & Abubakar, A. E. (2013). Antibiogram of bacteria isolated from kunun zaki drink sold in Keffi metropolis. *African Journal of Infectious Disease, 9*(3), 71-76

Maturin, L. & Peeler, J. T. (2001) *Aerobic Plate Count, Chapter 3, rev*. Jan. 2001. In FDA Bacteriological Analytical Manual, 8th ed., Rev. A. AOAC International, Gaithersburg, MD

Minamor, A. A., Mensah, A. L., Laryea, E. N., Afutu, E., Patience, B., & Quarcoo, T. (2017). Microbiological quality of a locally brewed alcoholic beverage (PITO) sold in Prampram within the Greater Accra Region, Ghana. *Microbiology Research Journal International*, *18*(5), 1–10. https://doi.org/10.9734/MRJI/2017/31623

Moss, M. (1989). Samson RA, Van Reenen-Hoekstra ES, Introduction to Food-borne Fungi, Edit. 3, Centraalbureau voor Schimmelcultures, PO Box 273, 3740 AG Baarn, The Netherlands (1988), p. 299, ISBN 90-70351-16-1. Price Hfl. 42.50: Elsevier.

Mossel, D. A. A., Beckers, H. J., King, A. D., Anderson, C. B., Moberg, L. J., Davenport, R. R., & Seiler, D. A. L. (1986). Significance of fungal populations on foods (baseline counts). A. D. King Jr In *Methods for the mycological examination of food* (pp. 177–206). New york: Plenum Press.

New South Wales Food Authority. (2009). Microbiological Quality Guide for Ready-To-Eat Foods. A Guide to Interpreting Microbiological Quality Results.

Ndulaka, J.C., Obasi, N. E., & Omeire, G. C. (2014). Production and evaluation of reconstituted kunun zaki. *Nigerian Food Journal, 32*(2), 66-72

Olaoye, O. A., Ndife., J. & Raymond, V. I. (2017). Use of lactobacillus plantarum as starter culture and its influence on the physicochemical, microbiological, and sensory characteristics of kunun aya produced from sorghum and tigernut. *Journal of Food Quality* 2017

Olowajoba, S. O., Akinyosoye, F. A, & Oyetayo. (2013). Comparative sensory and proximate evaluation of spontaneously fermenting kunun zaki made from germinated and ungerminated composite cereal grains. *Food Science and Nutrition,* 1,336-344

Oluwalana., I. B., & Adedeji, T. O. (2013). Nutritional composition of non-alcoholic beverage spiced with Zingiber Officinale extract produced from sorghum bicolor stem sheath, *International Journal of Food Science, Nutrition and Engineering*, 3, 21-27

Oranusi, S. U., Umoh, V. J, & Kwaga, J. K.P. (2002). Hazards and critical control pointsof kunun zaki, a non alcoholicbeverage in Northern Nigeria. *Food Microbiology*, 20, 127-132

Oriola, O., Boboye, B., & Adetuyi, F. (2017). Bacterial and fungal communities associated with the production of a Nigerian fermented beverage, "Otika". *Jordan Journal of Biological Sciences*, *10*(2).127–133.

Orutugu, L. A., Izah, S. C., & Aseibai, E. R. (2015). Microbiology quality of kunun zaki drinks sold in some major market of Yenagoa. *Nigerian Continental Journal of Biomedical Science. 9*(1), 9-16.

Popoola, O. (2019). Microbiological quality of some selected Akamu samples sold in some areas of Kano metropolis (a case study of Hotoro, Tarauni and Mariri). *Research Journal of Food Science and Quality Control*, *5*(1), 8–11.

Samson, R. A, Hoekstra, E. S, Flemming, L., Filtenborg, O., & Frisvad, J. C. (2000). Methods for the detection, isolation and characterization of food –borne fungi. R. A. Samson E. S. Hoekstra J. C. Frisvad & O. Filtenborg (Eds.), *Introduction to food- and airborne fungi*, (283–297). Utrecht: Centraalbureau voor Schimmelcultures.

Sapode, P. A, & Kassum, A. L. (1992). Rheological characterization of Nigerian liquid and semi-solid foods. Kunun-zaki and kunun gyada. *Nigerian Food Journal*, 10, 23-33

Standard Organisation of Nigeria (SON). (1985). Nigerian Industrial Standards: Revised Standard for soft drinks. Nigeria, ministry of industry, Lagos, 1-6

Umar, G. A., Tukur, S.I., Akensire, U. A., Adamu, Z. E., Bello, O. A., Shawulu, A. H. B, & Audu, M. (2014). Microflora of kunun zaki and sobo drinks in relation to public health in Jalingo metropolis, North-Eastern Nigeria. *International Journal of Food Research, 1*, 16-21.

Waage, J., Woodhall, J. W., Bishop, S. J., Smith, J., Jones, D. J., & Spence, N. J. (2006*). Foresight 1461 infectious diseases: Preparing for the future*. Retrieved from www.dti.gov.uk

Willey, J. M., Sherwood, L. M, & Woolverton, C. J. (2017). *Prescott's Microbiology*, Tenth Edition. McGraw Hill

Yamanaka, T. (2003). The effect of pH on the growth of saprotrophic and ectomycorrhizal ammonia fungi in vitro. *Mycologia*, *95*(4), 584–589.