Comparative Studies on the Prevalence of Salmonella Species in Two Homemade Fermented Beverages (Zobo and Kunun-Zaki) Sold At Samaru, Zaria, Kaduna, Nigeria

Umar, M.[1, 3], Mohammed, I. B.[2], Abdulkarim, I. M.[1], Yusuf, G.[1], Yaya, A.A.[1] and Leo, G.[1]

[1]Department of Science Laboratory Technology, Nigerian Institute of Leather and Science Technology, Zaria, Kaduna state, Nigeria.
[2]Department of Industrial Chemical Processing Technology, Nigerian Institute of Leather and Science Technology, Zaria, Kaduna state, Nigeria.
[3]Department of Microbiology, Ahmadu Bello University, Zaria, Kaduna state, Nigeria.

Abstract- A survey of the comparative studies on the prevalence of Salmonella species in two home-made beverages sold at Samaru, Zaria was carried out. Ten (10) samples of freshly formulated Kunun-Zaki and Zobo were obtained from five (5) different sales locations in Samaru, Zaria, and analyzed using the standard plate counts, to determine their respective bacteriological quality. The samples were subjected to physicochemical analysis where parameters such as pH, temperature, turbidity and lactic acid contents were analyzed using standard methods. The results obtained show that the total bacterial counts of Kunun-Zaki and Zobo in the five (5) different locations range from 9.37 X 103 to 2.66 X 105 cfu/ml. Zobo was found to record more microbial counts beyond the safe limit given by National Administration for food Drugs and Control (NAFDAC) than Kunun-Zaki. The common isolated bacteria were Salmonella species and other members of the family Enterobacteriaceae respectively. The temperature, lactic acid content and pH of the beverages were found to influence the microbial counts. The isolates were found to be sensitive to Chloramphenicol (80%), Streptomycin (60%), Septrin (60%), and Amoxicillin (60%) but resistant to Penicillin (50%) and Erythromycin (60%). The high bacterial loads in most of the samples can be attributed to the poor hygienic practices of the handlers and possible contamination from the utensils and water that were used for processing of the beverages. The presence of Salmonella species could be a matter of serious concern as these bacterial species involve in a serious health implications causing food-borne diseases.

Index Terms- Beverages, Characterization, Isolation, Physicochemical, Susceptibility

I. INTRODUCTION

In every society, drinks of indigenous origins are produced in different ways and served sometimes at occasions (Akema *et al.,* 2006). Some of these drinks are prepared by fermentation, which is a widely practiced ancient technology and these fermented foods are essential part of diet in all regions of the world (Abegaz, 2002). Non-alcoholic beverages such as Zoborodo (Zobo) drink and Kunun-Zaki drink of recent are among the popular traditional food drinks which are very important in the dietary pattern of people in the Northern part of Nigeria (Terna *et al*., 2002a). The non-alcoholic nature of Zobo drink and Kunun-Zaki drink makes them to be readily consumed by Christians and Muslims alike as a substitute for alcoholic ones (Abegaz, 2002).

Kunun-Zaki is a non-alcoholic beverage produced by fermentation of several cereals such as millet, sorghum, maize and rice (Ahmed *et al*., 2003). Kunun zaki (in *Hausa*) is a traditional fermented non-alcoholic beverage consumed by a large population of the people in Northern Nigeria (Adesuyitan and Adelekun*,* 2008). Preparation protocol varies among people and can generally be produced from either the following substrates; millet (*Pennisetum typhoideum*), maize (*Zea mays*) sorghum (*Sorghum typhoideum* or *Sorghum bicolar*). Spices such as ginger, black pepper, red pepper, cloves and sugar are commonly added as flavor to enhance acceptability and as a taste improver (Ahmed *et al.,* 2003), and the beverage is maintained at the pH of 4.5-5.0. This slightly acidic pH is effective in keeping the microbial population low (Adeyemi and Umar, 1994).

Zobo is an indigenous drink obtained from the infusion of dry calyces of Roselle plant (*Hibiscus sabdariffa*). The drink enjoys patronage mostly in the Northern Part of Nigeria, where it has become a household name since it is affordable among the low income earners. Other people use it during social occasions such as birthdays, marriage, thanksgiving, weddings and naming ceremonies as a refreshing drink. It is also prepared in small quantity for family use as a beverage to quench thirst and as a source of energy, or in large quantities by many people and sold in market places, home and campuses as a local soft drink. Zobo is thus a drink available in many local stores where it is marketed in various forms and patronized by a variety of people. Since its preparation does not require special procedures, it is produced in many neighborhoods, often where strict sanitary conditions are not observed. The water used is often obtained from the local water sources often boreholes and in some cases well water is used for preparation. It is these conditions that result in the microbial contamination of the product, coupled with contamination that may result from handling (Nduka and Yakubu, 1990). Some of its nutritive elements are various amino acids, proteins, carbohydrate, vitamins, and fats among others (Lydia *et al*., 1988; Ahmed *et al*., 2003 and Abegaz, 2002).

Food is a substrate for the growth and proliferation of microbes, Kunun-Zaki and Zobo being foods cannot be exceptions. The type of microorganism present in food and the environmental conditions are also very important in the consideration of food quality. However, the food determines what microbe can or cannot grow. Nutrients of a food play an important role in the kinds and number of microorganisms that grow on it because of the great organic compound in food and the numerous kinds of microorganisms that can decompose them. Many chemical changes are possible and many kinds of products are yielded. However, like any other non-alcoholic locally, brewed drinks, the presence of microbial contaminant is not always underestimated due to the unhygienic condition of preparation, hence, microbial isolates reportedly associated with these drinks include, *Lactobacillus* species, *Leuconostoc* species, *Bacillus* species, *Staphylococcus* species, *Candida* species and *Saccharomyces* species. Others include *Salmonella*, Enterobacteria species and *Clostridium* species (Terna *et al.,* 2002a).

Food spoilage is a major concern throughout the world. It can occur at any point in the food production process, growth, harvesting, transport, storage or final preparation. Spoilage can also occur if foods are not stored properly. When fungi grow in foods, especially cereals and grains, they produce important diseases causing chemicals, including aflatoxins and fumonisins (carcinogens) to contaminate the food. Microorganisms usually grow rapidly and can make an attractive and appealing food becomes sour, off-flavour and poisonous. This leads to visible changes which include changes in color, change in texture and change in smell (Adeyemi and Umar, 1994).

Kunun-Zaki and Zobo are liable to microbial spoilage if not adequately stored and could act as an important medium for the transmission of pathogenic microorganisms. Many organisms can use the carbohydrate content for their fermentation processes producing undesirable changes in them. The sugar used as a sweetening agent could also contribute to these changes. The microorganisms can be in dormant or semi-metabolic changes, but others are of public health significance since the microbes may be potential pathogens or can produce toxins in food, which can cause illness to consumers (Nduka and Yakubu, 1990). The most common food poisoning in the United State is the consumption of an enterotoxin produced by certain strains of Staphylococcal intoxication, which stands worldwide out as one of the main foodborne diseases with a frequency in incidents of microbiological origin second only to Salmonellosis (Lydia *et al*, 1988) currently, more than one-third of the reported cases of food poising in the United State is caused by Salmonellae. According to the Centers for Disease Control and prevention (CDC, 2008), more than 40, 000 cases of Salmonellosis are reported each year, but because most cases go unreported and it is estimated that between 400,000 and 4,000,000 may occur annually (Norcross and Post, 2000). They also reported that about 18000 hospitalizations in developing countries such as Nigeria and 500 deaths are associated with salmonellosis.

Salmonellosis is a very important public health hazard often resulting in high global mortality rate. In Samaru Local Government Zaria, community, it is second most prevalent diseases after Malaria ones (Amusa *et al.*, 2005).

Salmonellosis is a classic food-borne disease relative importance of *S. enteritidis*. Salmonellosis not only infects humans but also many food animals such as poultry, pigs and cattle from which humans can be directly infected (CDC, 2007). Some ingredients, such as sweetening agents and spices have been used for long, as food additives to provide distinctive flavours for foods and beverages around the world (Norcross *et al.*, 2000). However, the spices have been found to have contaminant and from investigation, ginger was found to be the most contaminated and red pepper the least, with total aerobic plate counts of 9.5×10^6 g^{-1} respectively. Sugar also a food additive available in the market containing from very few to several organisms per gram, most bacteria spores cannot grow on them, but when moisture has been absorbed there is a change for microbial spoilage and is thus a source of contamination in food. Ice is contaminated with microorganism and when used to maintain coolness in food acts as a source of contamination upon melting. The water used in food processing is also a source of contamination of the food as natural flora, but also microorganism from soil and possibly from animals or sewage. Food can also be contaminated from air and packaging from contamination with dust or other sources of microorganisms like equipment during handling (Terna *et al.*, 2002a).

Global estimates of salmonellosis ranges from 200 million cases to 1.3billion cases with an estimated annual death toll of 3million people. The serovars responsible for enteric fever *typhi* and *paratyphi* account for 6-20 million eases and 200,000 deaths annually, especially in the tropics including Nigeria (CDC, 2007).

In general, infections caused by Salmonella causes great economic losses in both developed and developing countries. In developed countries like America Salmonellosis is also considered to be the most important problem threatening public health (CDC, 2007). This problem has been estimated to cost the American government nearly one billion dollars annually. In many developing countries, typhoid fever remains an important public health problem, with 16.6m cases and 600.000 deaths annually. For Salmonella to cause infection in the host, it has to overcome the host immune factors which include gastric acidity, normal intestinal micro-flora and local intestinal immunity. Where the organism is able to overcome the host factors enteric fever may result (CDC, 2007).

The severity of salmonellosis, which may result in death for some individuals means that manufacturers need to detect contamination before food is released for sale. The result of fermented drinks being associated with salmonella outbreak, which must be swallowed in order to set up an infection, is still a matter for debate (Ahmed *et al.,* 2003).

There is no regulation on the preparation of these foods despite the wide patronage of the people. The near breakdown of basic infrastructure such as potable water and light often results in poor preparation and storage conditions in the processing of these drinks. It is imperative that a study that focuses on the microbial quality of these drinks might help to determine their safety for consumption in our immediate environment and the nation at large. It might also provide decision makers with information that will help to initiate necessary standards for these and other indigenous drinks that have found their ways to the Nigerian markets. It might also help Nigerians in making choices

as to the drinks they patronize. The study is however not intended to put out local producers of these drinks by revealing their unhygienic practices, but to help them with knowledge of their products and ultimately lead to improvement in their processing methods.

The aim of this research work is to compare the prevalence of Salmonella species in two home-made beverages (Zobo and Kunun-Zaki) marketed by various retail outlets in Samaru, Zaria, Kaduna state, with view to determine the physicochemical property of the drinks, and to determine and compare the bacteriological density or microbial loads of the two drinks with standard given by the National Agency Food and Drugs and Administration Control (NAFDAC). The survey was designed to isolate and biochemically characterize Salmonella species, and to subject the isolates to antibiotic susceptibility test.

II. MATERIALS AND METHODS

A total of ten (10) samples, 5 of each of Zobo and Kunun Zaki were aseptically and randomly collected by direct purchase from the retail points in various locations within Samaru, Zaria, Kaduna state, Nigeria. The samples were kept in a sterile and non-pyrogenic polythene bag and immediately transported to the Veterinary Medicine Laboratory, Ahmadu Bello University, Zaria for analysis.

A. Physicochemical analysis of Zobo and Kunun Zaki

The physicochemical qualities of the samples were determined using the methods described by Ademoranti (1996) and Sadiq and Malami (2009). The parameters determined include: pH, temperature, Lactic acid contents and turbidity of the two beverages.

B. Determination of pH

This was determined using pH meter 3015, Jenway®, U. K. Ten milliliter of the sample was placed in a beaker. A buffer solution was used to zero and calibrate the pH meter. Then the electrode of the pH meter was inserted into each of the samples and the pH readings were taken.

C. Determination of temperature

The temperature was determined at the point of sample collection. This was done by dipping the bulb of mercury-in-glass thermometer into the beverages and the readings were recorded.

D. Determination of turbidity

The beverage samples were subjected to Atomic absorption spectrophotometry. This was determined using Vanado-molybdophosphoric acid colorimetric method. The dilutions of the stock solution of the beverages were used for the preparation of standard calibration solutions. Then $100cm^3$ of the samples were digested with concentrated HCl and HNO_3 in a ratio of 3:1, filtered and diluted to $250cm^3$ with distilled water. A blank solution was prepared by treating $100cm^3$ of distilled water in the same manner. The intensity of the yellow colour was measured using spectrophotometer at 490nm as adopted by Rabah and Ibrahim (2010).

E. Determination of lactic acid

A mass of 10g was weighed out of the beverages and added into a conical flask. About 30 ml of distilled water was added to the sample and mix well by shaking. This was followed by the addition of 5 drops of Phenolpthalein indicator. The burette was filled with 0.1 M Sodium hydroxide solution. The solution was titrated until it retains a very slight pink tinge after mixing. After which the volume of sodium hydroxide used was recorded. The titration for each sample was repeated three times, and the percentage of lactic acid in the sample was calculated using the equation below:

$$\% \, acid = \frac{(ml\ of\ NaOH\ used) * (conc.of\ NaOH) * 0.09 * 100}{wt.of\ sample\ used}$$

2.3   Sample processing

The collected samples were diluted serially on arrival to the laboratory. The samples were mixed gently, and a quantity of 1ml was aspirated using a sterile pipette and dispensed into a test tube containing 9ml buffered peptone water. The solution was mixed to make a dilution of 1:10 or 10^{-1}. A volume of 1ml from the dilution of 1:10 was aspirated and transferred into another test tube containing 9ml of buffered peptone water using a different sterile syringe to make a dilution of 1:100 or 10^{-2}. The procedure was repeated for the third test tube containing 9ml buffered peptone water to make a dilution of 1:1000 or 10^{-3} as adopted by APHA (1999).

2.4   Mesophilic aerobic plate count

An aliquot of 0.1ml of the dilution from each diluent was transferred into each of the correspondingly marked duplicate Petri dishes. A quantity of about 15ml of an already prepared molten Salmonella-shigella agar (SSA) was poured into each correspondingly marked Petri dish within 15 minutes from the time of original dilution. The molten Salmonella-shigella agar (SSA) was mixed with the inoculums uniformly, after which it was allowed to solidify. The prepared Petri dishes were incubated in inverted position at 30^oC for 72 hours. Following incubation, the number of colonies was counted using colony counting chamber, and the colony forming unit per ml was calculated. The colonies formed on Salmonella-Shigella agar (SSA) plates were determined by multiplying the number of colonies with a reciprocal of the dilution factor divided by the volume of the inoculum to quantify the total number of the organisms using the equation:

$$CFU/ML = \frac{No.of\ colonies \times \frac{1}{DF.}}{volume\ of\ inoculum} \quad (Where\ DF = Dilution\ factor)$$

2.5   Biochemical characterization of the isolated salmonella species

The following biochemical tests were employed to further characterize the suspected Salmonella isolates.

A. Indole Test

This used to determine the ability of an organism to split amino acid tryptophan to form compound indole. The tryptophan was inoculated with an emulsified isolated colony of the test organism in tryptophan broth. The set up was incubated at a temperature of 37^oC for 24hrs after which a 0.5ml of Kovac's reagent was added to the broth culture. The Indole reagent retained its yellow colour indicating a negative test. While a positive reaction was determined by the development of a red

colour on the reagent layer floating above the broth within one minute (Cheesbrough, 2000 and Umar *et al.,* 2015).

B. Citrate Utilization Test

Inoculation needle was used to inoculate Simmon's citrate agar slant prepared in a bijou bottle, and incubated at 37^oC for 24 to 72 hours. The development of a deep blue colour indicated positive reaction, while negative results indicated no change in colour (Cheesbrough, 2000).

C. Methyl Red-Voges Proskauer Test

About 5ml of MR-VP broth were inoculated and incubated for 48 hours at $37°C$, after the period of incubation, 1ml of the broth was transferred to a small serological test tube and 2 drops of methyl red was added. Following the addition of the indicator, a red colour signifies positive methyl red test. While a yellow colour signifies a negative test. A heavy inoculum of the test organism was inoculated into Voges-Proskauer medium contained in different test tubes. The tubes were incubated at 37^oC for 48 hours. After which 0.5ml of alpha naphthol was added, followed by 0.5ml of 40% KOH. It was then agitated and allowed to stand for 30 minutes; a red to pink colour signifies a positive test (Cheesbrough, 2000; and Umar *et al.,* 2015).

D. Triple Sugar Iron Test

The triple sugar iron was made in such a way that there are slant and the butts. With a sterilized straight inoculation needle, the isolate colony was picked from the solid media and stabbed the TSI up to the butt and then streaked the surface of the agar slant. The cap was left loose and incubated at a temperature of 37^oC for 24hours. After the incubation, black precipitates were observed on the TSI, and also reddish or yellow colourations signifies acidic or basic utilization. The black of the butt indicate hydrogen sulphide is produced (H_2S), whereas gap spacing of the medium signifies gas formation (Cheesbrough, 2000). The development of black precipitate and gas at the butt indicated the presence of Salmonella species.

E. Urease Utilization Test

The inoculation needle was used to inoculate a urease agar heavily over the entire surface of the slant. The cap of the bottle was loosened and it was incubated overnight at 35^oC - 37^oC. Urease-positive cultures produced an alkaline reaction in the medium (pinkish-red color). While, urease negative test retained the color of the medium which is a pale yellowish-pink (Cheesbrough, 2000).

F. Motility Test

Inoculated needle was used to inoculate a motility agar by stabbing about two thirds down and they pull the needle up in the same path. It was incubated at 37^oC for 24-48 hours. The appearance of cloudy tube with organisms spread over the top of the media indicated motile, while non-motile organisms grew along the streak line only, the media showed no cloudy appearance (Cheesbrough, 2000).

2.6   Antibiotic Susceptibility Test

The Kirby-Bauer method was employed for the antibiotic susceptibility testing. Susceptibility to antibiotic agents was determined by using the disk diffusion method. From the nutrient agar plate, bacterial colonies were transferred into McCartney bottles containing sterile normal saline to obtain bacterial density of 3×108 organism per milliliter as determined by McFarland standard scale number 1. The culture was streaked uniformly onto freshly prepared Nutrient agar plates using disposable sterile swabs. The plates were allowed to dry briefly, and then the discs of multiple antimicrobials were mounted on the surface of the streaked inoculum. The plates were incubated at $37°C$ for 24 hours. The culture plates were examined for evidence of inhibition. A meter rule was used to measure the zones of growth inhibition (Cheesbrough, 2000, Bauer *et al.,* 2006 and Umar *et al.,* 2015). The isolates were recorded as sensitive or resistant to the antibiotics by comparing with value recommended standard charts given by NCCLS (1990 and 2008).

III.   RESULTS

Table 1 shows the physicochemical analysis of Zobo and Kunun-Zaki. Physicochemical parameters such as pH, Temperature, Turbidity and lactic acid content were tested. Predominant pH in all the samples was said to be acidic with a temperature range of $18\text{-}24^oC$. Zobo showed high lactic acid content compared to Kunun-Zaki.

Table 2 shows the comparison of microbial loads between the isolates and NAFDAC standard (1×10^5 cfu/ml). The average microbial load of the beverages that is Kunun-Zaki and Zobo range from 10^3 to 10^4 cfu/ml, with samples Z_1 and Z_5 accounting for counts greater than 10^5 cfu/ml.

Table 3 shows the biochemical characterization of the salmonella isolates in relation to NCCLS guidelines.

Table 4 shows the antibiotic sensitivity profile of the isolated salmonella species from Zobo samples.

Table 5 shows the antibiotic sensitivity profile of the isolated salmonella species from Kunun-Zaki samples.

Table 1: Physicochemical Analysis of Zobo and Kunun-Zaki

Samples	pH Analysis	Temperature Analysis (^oC)	Turbidity (FAU)	Lactic Acid (%)
K_1	2.19	24	14801	0.002
K_2	2.29	18	11440	0.002
K_3	2.24	24	15808	0.002
K_4	2.47	20	6897	0.002
K_5	2.23	23	18840	0.002

Z_1	3.95	24	4428	0.005
Z_2	2.27	23	306	0.005
Z_3	2.47	19	3375	0.005
Z_4	4.00	24	4329	0.005
Z_5	3.77	24	6147	0.005

Key: FAU=Formazin attination unit, K=Kunun-Zaki, Z=Zobo

Table 2: Comparison between the microbial loads and NAFDAC Standard

Samples	Average Microbial Load (cfu/ml)	NAFDAC Standard (cfu/ml)	Remark
K_1	1.96×10^4	10^5	Safe
K_2	5.45×10^4	10^5	Safe
K_3	9.37×10^3	10^5	Safe
K_4	6.5×10^4	10^5	Safe
K_5	4.93×10^4	10^5	Safe
Z_1	2.66×10^5	10^5	Not Safe
Z_2	9.7×10^3	10^5	Safe
Z_3	6.5×10^4	10^5	Safe
Z_4	1.0×10^4	10^5	Safe
Z_5	1.18×10^5	10^5	Not Safe

Key: K=Kunun-Zaki, Z=Zobo

Table 3: Biochemical characterization of the Salmonella species isolated from both Zobo and Kunun-Zaki

Samples	Indole	MR	Voges Proskauer	Citrate	TSI	Urease	Motility	Inference
K1	+	+	−	+	K/A	−	Non motile	Salmonella is suspected
K2	−	+	−	+	K/A (Weak)	−	Motile	Salmonella species Isolated
K3	+	+	−	+	A/A + Gas	+	Non motile	Salmonella is suspected
K4	−	−	+	+	K/A (Weak)	−	Motile	Salmonella is suspected
K5	−	+	−	+	K/A (Weak)	−	Motile	Salmonella species Isolated
Z1	−	+	−	+	K/A (Weak)	−	Motile	Salmonella species Isolated
Z2	−	+	−	+	A/A + Gas	+	Non motile	Salmonella is suspected
Z3	−	+	−	+	K/A (Weak)	−	Motile	Salmonella species Isolated
Z4	+	−	+	+	K/A	−	Non motile	Salmonella is suspected
Z5	+	+	−	+	K/A (Weak)	−	Motile	Salmonella is suspected

Key: MR= Methyl Red   **(NCCLS, 2008).**

Table 4: Antibiotic sensitivity profile of Salmonella species isolated from Zobo

Antibiotics	Highly sensitive	Intermediate sensitive	Resistant
Amoxicillin	3 (60%)	1 (20%)	1 (20%)
Penicillin	0(0%)	1 (20%)	4 (80%)
Augmentin	0(0%)	4 (80%)	1 (20%)
Ciprofloxacin	1 (20%)	3 (60%)	1(20%)
Septrin	3 (60%)	0(0%)	2 (40%)
Chloramphenicol	4 (80%)	0(0%)	1 (20%)
Erythromycin	1 (20%)	1 (20%)	3 (60%)
Streptomycin	3 (60%)	0(0%)	2 (40%)
Tetracycline	2 (40%)	2 (40%)	1 (20%)
Pefloxacin	1 (20%)	3 (60%)	1 (20%)

Table 5: Antibiotic Sensitivity Profile of Salmonella species isolated from Kunun-Zaki

Antibiotics	Highly sensitive	Intermediate sensitive	Resistant
Amoxicillin	2(40%)	2 (40%)	1 (20%)
Penicillin	0(0%)	2 (40%)	3 (60%)
Augmentin	0(0%)	2 (40%)	3 (60%)
Ciprofloxacin	0(0%)	0(0%)	5 (100%)
Septrin	0(0%)	2 (40%)	3 (60%)
Chloramphenicol	3 (60%)	2 (40%)	0(0%)
Erythromycin	0(0%)	2 (40%)	3(60%)
Streptomycin	5 (100%)	0(0%)	0(0%)
Tetracycline	3 (60%)	0(0%)	2 (40%)
Pefloxacin	5 (100%)	0(0%)	0(0%)

IV. DISCUSSIONS

Based on the findings of the research study, the physicochemical analysis of the local beverages analyzed showed that, all the samples are acidic with a pH range of $2.19 - 4.00$ and lactic acid ranged from $0.002 - 0.005\%$ (Table 1). The high acidic content of Kunun Zaki and Zobo may be the factor that inhibits the growth of many spoiling microbes. The findings concurs with the work of Terna *et al* (2002b) and Adeyemi and Umar (1994), who reported pH of $4.5-5.0$ and documented that this pH ($4.5-5.0$) is effective in keeping the population of microbial contamination low, making the risk of Kunun-Zaki, borne infection or intoxication low.

The microbial loads of Kunun-Zaki and Zobo were determined. Highest microbial load was recorded in the Zobo with bacterial count ranging between $9.7 \times10^3 - 2.66 \times10^5$ cfu/ml. Least microbial load was recorded in the Kunun-Zaki with bacterial count ranging between $9.37 \times10^3 - 6.5 \times10^4$ cfu/ml. Comparing the findings with the standard given by National Administration for food Drugs and Control (NAFDAC) which stated that fermented foods and beverages with mesophilic aerobic count above 1×10^5 cfu/ml are not fit for human consumption (NAFDAC, 2004). Therefore, all the analyzed beverages (Kunun-Zaki and Zobo) were found to fall within the safe range, except samples Z_1 and Z_5 (Table 2). This may be attributed to the temperature of the beverages i.e. 19-24°C. This conformed to the work of Ahmed *et al.* (2003), who reported that Zobo can be stored for $45-90$ days at ambient temperature of

18°C without change in colour and flavour. The presence of salmonella beyond the safe limit in the study agrees with the findings of Amusa *et al.* (2005), who reported that, despite the popularity of this local drink, it has been associated with some peculiar problems such as non-consistency in quality and short shelf-life. The studies of Terna *et al*, (2002a) and Umoh *et al*., (2006) showed that locally produced Kunun-Zaki may contain various undesirable contaminants which may affect its quality and can pose danger to consumers. Also the inconsistency in the quality of Kunun-Zaki drink may not be unconnected with the traditional technique of its production, whereas Akema *et al.,* (2006) expressed that, most of the quality deterioration of the beverages and slow chemical changes occur during storage. These are responsible for changes in nutritional value, flavor, color and taste of the products.

Based on the biochemical characterization of the isolates, after preliminary identification by microscopy and cultural appearance, the predominant isolates subjected to biochemical tests were found to be Methyl Red positive and Citrate positive. Some 6 (60%) of the isolates are motile while some 4 (40%) are non-motile (Table 3). This showed that majority of the isolates were confirmed to be salmonella species, which was known to be motile. The contamination may have developed as a result of poor handling, processing and storage as well as faecal contamination of human and domestic pet(s) origin. This is in line with the findings of LeJeune and Hancock (2001) who reported dogs and cats are potential sources of several zoonotic diseases, including salmonellosis. Flies also may spread the Salmonella, especially by cross contamination from fecal matter

to food and water. While, Rita *et al* (2006) opined that although ~95% of cases of non-typhoidal salmonellosis in humans are associated with foodborne contamination, an unknown proportion of these cases are the result of contact with infected pets and contaminated pet food products.

Based on the antimicrobial sensitivity profile of the isolated Salmonella species from Kunun-Zaki and Zobo, the isolates were found to be highly sensitive 5 (100%) to Pefloxacin and Streptomycin; followed by Chloramphenicol 4 (80%); and Tetracycline 3 (60%). The isolates were found to be relatively resistant to Ciprofloxacin 5 (100%) and Penicillin 4 (80%).

V. CONCLUSION

Based on the findings of the research study, Kunun-Zaki and Zobo have appreciable counts of microbial loads, but they are found to be within normal/safe range given by National Administration for food Drugs and Control (NAFDAC), Nigeria. The predominant isolates are salmonella species. However, pH and temperature are some of the factors that influence microbial loads of Kunun-Zaki and Zobo. Antibiotics such as Streptomycin, Pefloxacin are highly active against the isolates, followed by Chloramphenicol and Tetracycline. The isolates were found to be resistant to Ciprofloxacin and Penicillin.

Examination of foods for human consumption is important to ensure the safety of the food consumed and also its wholesomeness. To reduce contamination with microorganisms to a minimum and obtain a good keeping quality of the product, the raw materials used for the preparation should be examined for sterility. The equipment contacting the food should be adequately cleaned, sanitized and tested. The preservation process should be routinely checked; packaging and storage should be supervised.

It is also recommended that the producers of local fermented beverages should practice personal hygiene, so that fermented beverages such as Kunun Zaki and Zobo may be free from pathogenic germs and safe for human consumption.

REFERENCES

[1] Abegaz, K., Beyene, F., Langsrud, T. and Narvhus, J.A. (2002). Indigenous processing method and Raw materials of Borde,an Ethiopian Traditional fermented Beverage. The Journal of Food Technology in Africa. 7(2); 59-64

[2] Adesuyitan, J. A. and Adelekun, O. E. (2008). Evaluating the quality characteristics of Kunu produced from dry-milled sorghum. African Journal of Biotechnology. 7(13): 2244-2247.

[3] Ademoranti, C.M.A. (1996). Standard methods for Water Effluent Analysis. 1st edition, Fokudex Press Ltd, Ibadan, Nigeria, pp111-117

[4] Adeyemi, I. A. and Umar, S. (1994). Effect of method of Manufacture on the quality characteristics of Kunun-Zaki. Nigeria Food Journal, 12: 34-40

[5] Ahmed, E. U., Musa N. and Ngoddy, P. O. (2003). Sensory Attributes of Extruded Cereal-Legume Blends for Instant Kunun-Zaki" Beverage Analogue. Proceeding of the 27th Annual NIFIST Conference. pg 84

[6] Akema, O., Jiya, E. A., Akumka, D. D. and Mshilia, E. (2006). Influence of Malting on the Nutritional Characteristics of Kunun-Zaki. African Journal of Biotechnology 5(10): 996-1000.

[7] Amusa, N. A., Ashaye, O. A., Aiyegbayo, A. A., Oladapo, M. O. O. and Afolabi, O. O. (2005). Microbiological and Nutritional Quality of Hawked sorrel Drinks (Zoborodo) (The Nigerian Locally Brewed Soft Drinks) Widely Consumed and Notable Drinks in Nigeria. Journal of Food, Agriculture and Environment, 3(3-4); 47-50.

[8] APHA (1999). American Public Health Association, American Water Works Association, Water Environment Federation, pp1-46.

[9] Bauer, A. W., Kirby, W. H. M., Sherris, J. C., and Turck, M. (2006). Antibiotic Susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology, 45 493-496.

[10] Centers for Disease control and prevention CDC (2008). Preliminary Food Net Report.

[11] Centers for Disease Control and Prevention CDC (2007). Preliminary food net Data on the incident of infection with pathogens Transmitted commonly theory foods. MMWR. Morb. Wkly Rep; 58(13); 333-336

[12] Cheesbrough, M. (2000). District Laboratory Practice in Tropical Countries. Cambridge University Press, UK; 2: 13—77.

[13] LeJeune J. T, Hancock D. D. (2001). Public health concerns associated with feeding raw meats diets to dogs. J Am Vet Med Assoc; 219:1222–5.

[14] Lydia, B., Norgatta, M., Pilar, O. and Manual, N. (1988). A Quantitative Study of Enterotxin Production by Sheep Milk Staphylococci. Journal of Applied Environmental Microbiology, 54(2): 566-569

[15] NAFDAC (2004). National Agency for Food and Drug Agency. Beverage and Water quality standard for consumption. Annual Report. In: WHO (2004). Sanitation and Health programme. Managing water in the home.

[16] National Committee for Clinical Laboratory Standards (NCCLS), (1990). Approved standard M7-A2: Standard Methods for Dilution Antimicrobial Tests with Bacteria that Grow Aerobically, 2nd ed. National Committee for Clinical Laboratory Standards, Villanova, Pa.

[17] National Committee for Clinical Laboratory, (2008). Performance Standard for Antimicrobial Disk Susceptibility Test for Bacteria. 6th Edition. NCCLS

[18] Nduka, U. and Yakubu, I. (1990). Public Health, Food and Industrial Microbiology. Uniben Press. Pp. 17-19.

[19] Norcross, M. and Post, A. R. (2000). Problems of Antibiotics Resistant Salmonella that caused Complication in Medical Treatment. Journal of Animal Science, 68(3): 863-869

[20] Rabah, A. B. and Ibrahim, M. L. (2010). Physico-Chemical and Microbiological Characterization of Soils Laden with Tannery Effluents in Sokoto, Nigeria. Nigerian Journal of Basic and Applied Science, 18(1): 65-71

[21] Rita, F., Richard, R., and Scott Weese, J. (2006). Human Health Implications of Salmonella-Contaminated Natural Pet Treats and Raw Pet Food. Clinical Infectious Diseases; 42:686–9. Retrieved from: http://cid.oxfordjournals.org/

[22] Sadiq, S. I. and Malami, S. (2009). Comparative study of the effects of tannery effluents on two major streams in Sokoto State, Nigeria. Nig. J. Basic Appl. Sci. 17(1): 48-52.

[23] Terna A. and Dookeran, Muyanja B. (2002a). Salmonella Infection in Emergency Medicine. A brief review Immunol Cell Biol. 85(2); 112-118.

[24] Terna, Ayo and Dookeran, Muyanja B. (2002b) Role of Non-Host environments in the life style of salmonella and Escherichia coli. Applied and Environmental Microbiology, 69 (7): 3687-3694.

[25] Umar, M., Akafyi, D. E., Abdulkarim, I. M., Yaya, A. A. and Danasabe, Y. J. (2015). Biochemical Characterization and Antibiogram Pattern of Streptococcus mutans isolated from Dental Unit, Sick-Bay, Ahmadu Bello University, Zaria, Nigeria. International Journal of Biological and Biomedical Sciences, 4 (10), PP. 063-066. Retrieved in October 2015 from: http://www.wrpjournals.com/journals/V/IJBBS

[26] Umoh, V. J., Agbogu, V. N., Okuofu, C.A. , Smith, S. I. and Ameh, J.B. (2006). Study of the bacteriological and physiochemical indicators of

pollution of surface waters in Zaria Nigerian African Journal of Biotechnology.5:1-5

AUTHORS

First Author – Umar Mustapha completed B.Sc. Microbiology from Bayero University, Kano in 2006 and pursuing M.Sc. in Medical Microbiology from Ahmadu Bello University, Zaria. He is a research fellow heading Microbiology Unit at the Nigerian Institute of Leather and Science Technology, Zaria, Kaduna State, Nigeria since June 2013. Email: mustapha4mina@yahoo.com

Second Author – Mohammed Ibrahim Balarabe completed B.Eng Chemical Engineering from Ahmadu Bello University, Zaria, and pursuing MSc. in Chemical Engineering. He has been heading Department of Industrial Chemical Processing Technology, Nigerian Institute of Leather and Science Technology, Zaria, Kaduna state, Nigeria, since 2014. Email: balarabeibrahim02@gmail.com

Third Author – Abdulkarim Ismail Muhammad completed B.Sc. Biochemistry from Usmanu Danfodio University, Sokoto. He is a lecturer at Biochemistry Unit, department of Science Laboratory Technology, the Nigerian Institute of Leather and Science Technology, Zaria, Kaduna State, Nigeria. Email: ismailalab@yahoo.co.uk

Fourth Author – Garba Yusuf completed B.Sc. Biochemistry and M.Sc. Biochemistry. He is heading Biochemistry Unit at the Nigerian Institute of Leather and Science Technology, Zaria, Kaduna State, Nigeria since 2015. Email: yusufgarba371@gmail.com

Fifth Author – Yaya Aisha Abdulkadir completed B.Sc. Applied Biology from Bayero University, Kano in 2006 and pursuing M.Sc. in Biological Sciences from Ahmadu Bello University, Zaria. She is a lecturer at the Nigerian Institute of Leather and Science Technology, Zaria, Kaduna State, Nigeria since 2015. Email: aishayaya1919@gmail.com

Sixth Author – Leo Godiya completed Higher National Diploma in Science Laboratory Technology (Microbiology option) in 2015 from Nigerian Institute of Leather and Science Technology, Zaria, Kaduna State, Nigeria. Email: godileo2016@gmail.com
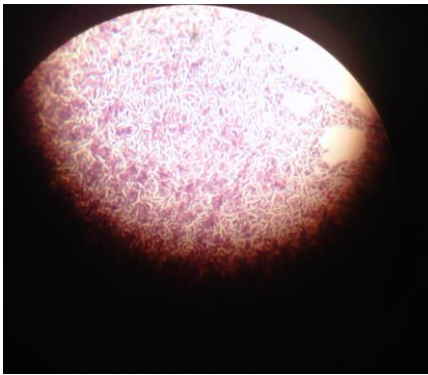
Plate 1(a) Morphological appearance of the isolated Salmonella species observed under microscope
Plate 1(b) Plate 2. Antibiotic susceptibility test multidisc setup against the isolated Salmonella species