MICROBIOLOGICAL ANALYSIS OF KUNUN-ZAKI: A FERMENTED MILLET DRINK

Hilda A. **Emmanuel-Akerele**[1]*, Etin-Osa **Edobor**[2]
[1]Department of Biological Sciences, Anchor University Lagos.
[2]Department of Microbiology, University of Benin, Edo State.
*E-mail: joellenemmanuel@gmail.com

Abstract
Microbiological and physicochemical analyses were carried out on samples of fermented millet drink Kunun Zaki. Samples were obtained from Ikpoba Hill Market and Aduwawa Quarters. The microbiological analysis was carried out using the standard plate count technique to determine the total microbial population. The mean count of bacteria and fungi was 2.57x107 cfu/ml and 0.98x107cfu/ml respectively. Microorganisms identified were Lactobacillus sp, Bacillius sp, Staphylococcus aureus, Streptococcus sp, Escherichia coli, Pseudomonas sp, Mucor sp and Fusarium sp. Staphylococcus shows the highest incidence while Escherichia and Pseudomonas shows lowest incidence. The mean value of the pH and titrable acidity was 4.26±0.09 and 2.73±0.08ml 0.1M NaOH respectively. The moisture content was high with a mean of 85.90±0.95 and mean solid content of 14.1±0.95. This study has shown that kunun-zaki sold in Ikpoba Hill Market and Aduwawa Quarters is highly contaminated with microorganisms. Practices of good hygiene are therefore necessary in an environment where kunun-zaki is produced.

Keywords: Fermented millet, kunun-zaki, microbiological and physicochemical

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1. INTRODUCTION

Millet is one of the main cereals staples of West Africa. There are various species of millets found and they include Bulrush millet (Nigeria millet), *Pennisetum typhoideun* in America, popularly known as Pearl millet, *Pennisetum mericanum*, foxtail millet (Setaria italic) and finger millet (*Eleusine coracana*). The grain had its origin in Central America and West Asia (Efiuvwevwere and Akoma, 1995). They are widely grown in Ghana, Cameroon and throughout the Savannah zone of Nigeria such as Bauchi, Sokoto, Katsina and Kano States (Ònuorah *et al.*, 1987). The quality of millet determines its use, if the grain is to be used as seed for planting, it should be pure, have a good yield and be free from disease and insect pest. If the grains are to be eaten by man or livestock, a high protein content is desirable and it must taste good (Oranusi *et al.*, 2003). Kunun-zaki is an indigenous fermented non-alcoholic beverage that is widely consumed for its thirst quenching properties. Though consumed throughout the year, it is extensively consumed during the dry season. The drink can also be produced from fermented sorghum, guinea-corn and maize (Amusa and Odunbaku, 2008).

It is a popular drink with characteristic sweet-sour taste and fermented cereal drink, it is consumed both in rural and urban areas of Northern Nigeria and enhances lactation in nursing mothers, increase libido, sustain erection and increase sperm count (Amusa and Ashaye, 2009). Other food products derived from these cereals include; malted alcohol known as 'Oyokpo' 'pito' or 'burukutu' (Ekanem *et al.*, 2018; Innocent *et al.*, 2011). Organisms usually associated with millets grains include *Aspergillus sp, Penicillim sp.* as well bacteria like *Bacillus sp, Staphylococus aureus and Lactobacillus sp.* (Elmahmood and Doughari, 2007).

Like other grains, maize and millet contain essential nutrients such as vitamins A, B and C, minerals like potassium, zinc, anti-diabetic,

anti-diuretic and anti-cancerous compound which are useful in treatment of diseases like diabetics, cancer and urogenital tract infections (Amusa and Odunbaku, 2008). This study seeks to give an in-depth focus into the sources of microorganisms that could contaminate Kunun-zaki and also identify practices that would aim at reducing the microbial load of the beverage.

2. MATERIALS AND METHODS

Sample Collection

Forty samples of kunun zaki drinks were purchased from different sales outlets in Ikpoba hill market and Aduwawa quarters in Benin City, Edo State. These samples were placed in sterile bags and transported in a cold pack to the laboratory for analysis within one hour of collection.

Preparation of Media and Samples

1 ml of every Kunun zaki beverage was placed into 9 ml of distilled water and serial dilution was carried out up to 10^{-10} dilution. The culture media used for microbiological analyses which include nutrient agar, MacConkey agar, potato dextrose agar, eosin methylene blue agar and mannitol salt agar, were prepared according to manufacturers' instruction (Gadage *et al.*, 2004).

Isolation of Microorganisms

1 ml from the dilutions were inoculated on Nutrient agar, MacConkey agar, potato dextrose agar for the enumeration of total microorganism, coliform count and enumeration of fungi respectively. After inoculation, Petri dishes containing Nutrient agar, MacConkey agar, Eosin methylene blue and Mannitol salt agar were incubated at 37^{o}C for 24 h, while inoculated plates containing Potato dextrose agar was incubated at 28^{o}C for 5-7 days (Gadage *et al.*, 2004).

Identification and Characterization of isolates

The isolated organisms were characterized and identified based on their cultural, morphological, and biochemical tests (Buchanan and Gibbson, 1974; Gadage *et al.*, 2004).

Physicochemical analysis

pH of the Kunun zaki drinks was measured by dipping the pH electrode into 10 ml of the beverage placed in a beaker, and the reading were recorded. Total titrable acidity was measured as percentage lactic acid by adding 3 drops of phenolphthalein indicator into 10ml of the drink placed in a conical flask and thoroughly shaken. The mixture was then titrated against 0.1 M NaOH (Sodiun hydroxide) to a pink color end point and the titre value was calculated. Moisture and solid content were determined by methods described by Ceese (1995).

3. RESULTS AND DISCUSSION

The results of this study showed that the mean total viable bacterial and fungal count was $2.51x10^{7}$ cfu/ml and $0.98x10^{7}$ cfu/ml respectively as shown in Table 1.

Table 2 shows the cultural, morphological and biochemical characteristics of the isolates. The bacteria isolated were *Lactobacillus, Bacillus sp, Escherichia coli, Staphylococcus aureus, Streptococcus sp and Pseudomonas sp.*

Table 3 shows the cultural and morphological characteristics of the fungi isolated and they are *Aspergillus sp, Penicillum sp, Rhizopus sp, Mucor sp and Fusarium sp.*

The pH value of the samples ranged from of 4.21 to 4.30, while the titrable acidity shows the presence of organic acids and it ranged from 2.67 to 2.82ml.

Table 1: Mean Total Viable Counts of Both Bacterial and Fungal Isolates (cfu/ml).

SAMPLE	BACTERIA COUNT	FUNGAL COUNT
A_1	$1.8x10^{7}$	$6.00x10^{11}$
A_2	$2.56x10^{7}$	$12.0x10^{9}$
A_3	$2.00x10^{9}$	$7.00x10^{7}$
A_4	$1.15x10^{9}$	$6.50x10^{9}$
A_5	$2.08x10^{7}$	$12.0x10^{7}$
B_1	$1.96x10^{7}$	$5.50x10^{9}$
B_2	$5.60x10^{11}$	$20.0x10^{7}$
B_3	$2.36x10^{9}$	$10.5x10^{9}$
B_4	$1.34x10^{9}$	$7.5x10^{7}$
B_5	$8.70x10^{9}$	$15.0x10^{7}$
C_1	$1.72x10^{7}$	$4x10^{11}$
C_2	$1.28x10^{9}$	$11.0x10^{7}$
C_3	$1.05x10^{9}$	$6.00x10^{7}$
C_4	$2.51x10^{9}$	$9.61x10^{7}$
C_5	$1.47x10^{9}$	$14.0x10^{9}$
Total Mean count	**$2.51x10^{7}$**	**$0.98x10^{7}$**

Table 2: Characteristics of Bacterial Isolated from the Kunun-Zaki Sample

Characteristics	Description					
Cultural						
Color	Cream	Cream	Cream	Yellow	Cream	Florescent green
Surface appearance	Mucoid	Rough	Dry	Mucoid	Smooth	Rough
Elevation opacity	Umbonated translucent	Slightly convex opaque	Convex opaque	Flat opaque	Convex semi-transparent	Convex opaque
Morphological						
Gram stain	+	+	-	+	+	-
Cell type	Rods	Rods	Rods	Cocci	Cocci	Rods
Cell arrangement	Single	Chains	Single	Cluster	Single	Chains
Motility	-	+		-	+	+
Biochemical						
Catalase	-	+	+	+	-	-
Coagulase	-	-	+	+	-	-
Oxidase	-	-	-	-	-	+
Urease	-	-	-	-	-	-
Citrate	+	-	-	+	-	+
Indole	-	-	+	-	-	-
Sugar fermentation						
Glucose	AG	A	AG	A	A	-
Lactose	A	-	AG	A	A	-
Manitol	A	A	A	A	A	-
Bacteria isolated	*Lactobacillus sp*	*Bacillus sp*	*Escherichia coli*	*Staphylococcus aureus*	*Streptococcus sp*	*Pseudomonas sp*

Table 3: Cultural and Morphological Characteristics of the Fungal Isolate

Isolates	Physical Appearance	Microscopic Observation	Fungi isolated
F_1	Black powdery threads	Septated mycelium conidiophore septate and arising from foot cell. Bear sterigmata conidia in chains and black coloration	*Aspergillus sp*
F_2	Dirty blue powdery growth, hair-line	Septated branched mycelium, septated aerial conidiophores with brush-like spore bearing head with sterigmata bore in clusters	*Penicillium sp*
F_3	White thread with surface colored black	Non-septated with aerial sporangiophore. Round columella smooth spores. No stolons and rhizoids	*Mucor sp*
F_4	Pure white thick and abundant cotton mycelium	Non-septated with stolons and rhizoids. Sporangiophores arising at the nodes. Sporangia are usually black	*Rhizopus sp*
F_5	Cotton-like growth with white coloration	Separated with large canoe-shaped microconida on branched conidiophores	*Fusarium sp*

Table 4: pH and Titratable Acidity

Sample	pH	Titratable acidity (ML 0.1M NaOH)
A_1	4.22	2.90
A_2	4.21	2.80
A_3	4.22	2.84
A_4	4.31	2.80
A_5	4.20	2.80
Mean value	**4.23**	**2.83**
B_1	4.30	2.75
B_2	4.27	2.65
B_3	4.21	2.70
B_4	4.98	2.66
B_5	4.27	2.60
Mean value	**4.21**	**2.67**
C_1	4.33	2.68
C_2	4.31	2.68
C_3	4.33	2.66
C_4	4.27	2.72
C_5	4.30	2.70
Mean value	**4.33**	**2.70**
Total mean	**4.26±0.09**	**2.73±0.08**

Table 5: Percentage of Moisture and Solid content present in the Sample

Sample	Moisture content (%)	Solid content (%)
A_1	86.47	13.53
A_2	85.80	14.20
A_3	85.85	14.15
A_4	85.83	14.17
A_5	85.89	14.11
Mean value	**85.97**	**14.03**
B_1	87.22	12.78

B_2	86.83	13.17
B_3	86.70	13.30
B_4	86.86	13.14
B_5	87.08	12.92
Mean value	**86.94**	**13.06**
C_1	84.99	15.01
C_2	83.90	16.10
C_3	85.25	14.75
C_4	85.27	14.73
C_5	84.51	15.49
Mean value	**84.78**	**15.22**
Total mean	**85.90±0.95**	**14.10±0.95**

The total mean of the percentage of moisture as shown in Table 5 was 85.90±0.95 and solid content was 14.1±0.95.

In many earlier reports, the pH was 4.3 (Ekanem *et al.*, 2018); 3.80 and 3.99 reported by Innocent *et al.* (2011), 2.42 to 3.83 recorded by Otaru *et al.* (2013), 5.25 to 5.65 reported by Amusa and Ashaye, (2009). The acidity of the kunu drinks may be due to the presence of some bacteria which help in acid fermentation of the kunu products (Ekanem *et al.*, 2018). The results of the investigation showed that the samples of Kunun-zaki contained a fairly large microbial population. The high microbial densities could be related to the fact that usually a heterogeneous population of microorganism are usually involved in the fermentation process and also that foodstuffs are also susceptible to microbial contamination during the processing and storage. Most especially in cases of fungal contamination. The microorganisms isolated from the samples include *Lactobacillus sp, Staphylococcus aureus, Bacillus sp, Streptococcus sp, Escherichia coli and Pseudomonas sp, Aspergillus, Mucor, Rhizopus, Pencillium* and *Fusarium.* The results correlate with that of Amusa and Odunbaku (2008); Oyenuga *et al.*, (2003); Elmahomood and Doughari (2007). Sources of the organisms may be traced to the cereals as had been reported by Efiuvwevwere and Akoma (1995). The presence of *Lactobacillus* indicates that kunun-zaki is a lactic acid bacteria fermented beverage and it is not unexpected because they help in fermentation process. *Lactobacillus* do not readily cause food poisoning, instead they tend to dominate by preventing other pathogenic microorganisms from surviving in the beverage, and their ability to produce lactic acid reduces the pH of food medium. Most food poisoning organisms cannot tolerate low pH, therefore the isolation of *Lactobacillus* favors this fact. Efiuvwevwere and Akoma (1995) reported the presence of some bacteria including *Lactobacillus spp* in kunun drink.

The presence of *Staphylococus aureus* indicate contamination from handlers as *Staphylococcus aureus* is a normal flora of the skin, nose, throat, palms, hairs and mucus membrane and a common etiological agent of septic arthritis (Charles *et al.*, 2005; Emmanuel-Akerele and Uchendu, 2021). The contamination could have been contracted by sneezing or by picking of the nostrils by food handlers. *Staphylococus aureus* can also produce Staphylococcal bacteraemia and abscesses in cell during food infection. *Escherichia coli* in food is an indication of faecal contamination of product. However, *E. coli* is a normal floral of the intestinal tract of man, presence of it in excess could lead to gastroenteritis and bacterial diarrhea disease (Emmanuel-Akerele and Uchendu, 2021). *Streptococcus* sp. may also

have been enumerated from the beverage as a result of the handlers, since it is also normal flora of the throat and the buccal activity. The presence of *Bacillus* could render a beverage unsuitable for human consumption (Innocent *et al.*, 2011). It is possible that the contamination by this pathogen may have occurred during sieving and packaging, as most of the people involved in the production, packaging and hawking do not take necessary precautions, and so such contamination could be very prominent. *Bacillus* is a spore former and as such the spores were easily distributed and was able to withstand high temperature and pH to fully germinate (Otaru *et al.*, 2013). The organism has the potential of causing an array of infections. The presence of *Pseudomonas* is not of great significance due to their low population. They could have occurred due to environmental contamination.

The fungal isolates present could be traced right to when the grains were either being harvested or stored. The presence of *Aspergillus, Penicillium,* and *Fusarium* in the kunun-zaki samples might not be too surprising as they are known as common spoilage

condition during the preparation and finally germinate in the finished product. The presence of these fungi such as *Aspergillus, Mucor, Fusarium* and *Rhizopus* is associated with spoilage of the beverage (Oyenuga *et al.*, 2003). Some of these fungal species elicit some toxins which are very hazardous. One of such is *Aspergillus* which produce aflatoxins that are quite harmful and as such their occurrence is undesirable.

organism of carbohydrate foods as well as storage microflora of many cereals including sorghum (Ekanem et al., 2018; Omonigho and Osubor, 2002). The fungal may produce spores attached to the grains and overcome adverse

The acidity level of kunun-zaki drinks have been described by several researchers including Efiuvwevwere and Akoma (1995) and Amusa and Ashaye (2009), who attributed these to the presence of lactic acid bacteria. The acidity tends to increase with increase in fermentation period resulting into spoilage. Consequently, the low pH value may have encouraged the growth of fungi and this could be responsible for the species of microorganism isolated. The pH brought about a corresponding increase in the titratable acidity and sour taste flavour of the kunun-zaki drink. The moisture and solid content of the analyzed Kunun-Zaki had overall mean of 85.90±0.95 and 14.1± 0.95 respectively. The presence of all these organisms are of great public health concern and in situations where the beverage is contaminated, quick medical care should be sought to avoid food poisoning.

4. CONCLUSION

Since there are no routine hygiene standard techniques for preparation of kunun-zaki such food will always contain an unusual large population of fermentative beneficial organisms and some pathogenic microorganisms. This study has shown that the preparation procedure for kunun-zaki does not completely eliminate microorganisms from the finished products. Storage of the product at room temperature allowed for proliferation of microorganisms and this tends to utilize the kunun-zaki constituents resulting in significant changes in the physicochemical composition of the product. The isolation of pathogens as *Staphylococcus, Streptococci* and *Aspergillus* could be indicative of health hazards, though this is not to cause extreme worries because their population has been inhibited to an extent by acid produced by the lactic acid bacteria. In order to reduce the rate of contamination and gently enhance the microbiology qualities of the product the following measures should be adhered to; educate producers and hawkers of

the product on good sanitary practice during the preparing and sale of the product; advocate the use of boiled water in washing utensils; treated municipal water should be used during processing and dilution of the processed drinks to avoid contamination with entero-pathogenic bacteria; the processing environment should be hygienic and the packaging materials should be sterilized.

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