



MICROBIAL ASSESSMENT OF KUNU (LOCAL BEVERAGE) SAMPLES FROM OLUODE MARKET IN OSOGBO, OSUN STATE OF NIGERIA.

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Abstract: Ten kunu samples were obtained as freshly formulated beverages from five different local vendors or hawkers from Oluode market, in Osogbo, Osun State and analyzed to determine the microbiological quality using the standard methods of analysis. The pH of the samples ranged 3.3-4.1, the total heterotrophic bacteria ranged $1.8-6.1 \times 10^3$ cfu/ml; the total coliform count ranged $1.0-5.0 \times 10^3$ cfu/ml; the total fungi count ranged $4.2-9.0 \times 10^3$ cfu/ml. The presence of high microbial load was an indication of poor hygiene or poor quality cereals and water used in the preparation. The microorganisms isolated included bacteria: *Staphylococcus aureus* (26.7%), *Escherichia coli* (24.0%), *Streptococcus spp*(23.3%), *Pseudomonas spp*(10%) and *Bacillus spp*(6.7%). Fungi: *Penicillium spp*(22.2%), *Fusarium spp*(18.6%), *Aspergillus spp*(14.8%), *Candida albicans* (33.3%), *Rhizopus nigricans*. The result showed that kunu should be consumed within 24 hours of preparation or preserved using chemicals, preservatives or refrigeration. The types and density of microorganisms recovered from this study suggests that urgent hygienic measures should be taken by kunu producers in the area. Regulatory agencies also need to carry out periodic inspections of kunu production premises to avoid outbreak of foodborne infections through consumption of kunu.

KEYWORDS: Kunu, Local, Microbial Quality Assessment, Hawked.

1.0 INTRODUCTION

Kunu is a popular cereal based, non-alcoholic beverage (Adejuyitan, Adelokun, Olaniyan & Popoola, 2008). Non alcoholic beverages are beverages that contain no alcohol. They are often consumed by children, people, whose religion restricts alcohol consumption, recovering alcoholics and anyone wishing to enjoy flavor drinks without alcohol. They are often available as alternative beverages (Wikipedia, 2009). Kunu is a popular local or indigenous drink consumed throughout Nigeria, mostly in the north for its thirst quenching properties (Elmahmood & Doughari, 2007). Though consumed throughout the year, it is extensively consumed during the dry season (Adeyemi and Umar, 1994). It

is a staple beverage drink that is relatively cheap and nutritious when compared to carbonated drinks (Adejuyitan *et al.*, 2008). Its cheapness is due to the readily availability of cereals and additives locally sourced as they grow throughout the savannah belt of millet (*Pennisetum typhoideum*), sorghum (*Sorghum vulgare*), maize (*Zea mays*), rice (*Orza sativa*) and acha (*Dijitap exilis*) are commonly used for the traditional production of kunu (Ahmed, Musa & Ngoddy, 2003; Gaffa, Jideani & Nkama, 2002)

The varieties of the drink made from sorghum include a milky light brown colour, while the one made from millet and maize is whitish in colour (Wikipedia, 2009). Spices such as ginger, black pepper, garlic, red pepper and clover are commonly

added as flavor and taste improper. Sugar is also added to act as a sweetener. It is also sweetened with honey together with small quantity of sweet potatoes, malted rice, malted sorghum and cadaba farinose crude extract. The grains were used singly or combined; sorghum/millet was the most common combination in the ratio 1:2 (w/w) (Ahmed *et al.*, 2003). Ayo & Okeke (2008) have reported that kunu is rich in carbohydrates, vitamins, and minerals but low in protein. This drink is however still produced at village technology level. Kunu has relatively short shelf-life storage. Studies conducted by Adeyemi & Umar (1994) revealed that the product has a shelf life of 24 hrs at ambient temperature, which was extended to days by pasteurization at 60°C for 1 hour and storage under refrigeration conditions.

Kunu can undergo spoilage as a result of some factors such as microorganisms present in the drink that help in the fermentation process. Studies have shown that kunu contains lactic acid bacteria such as *Lactobacillus spp.*, *Streptococcus spp.* and *Leuconostoc spp.* and these organisms could cause the spoilage of the beverage. Other organisms such as *Staphylococcus spp.*, *Bacillus spp.*, *Pseudomonas spp.*, *Penicillium spp.*, *Aspergillus spp.*, *Trichoderma spp.*, and *Candida spp.* present in the food drink if in large quantity could cause the spoilage of kunu beverage (Osuntoki & Korie, 2009). Also activities of the natural food enzymes could also contribute to the spoilage. Other factors include insects, rodents or pests present in the environment during the preparation, temperature, time and light. All these and much more if not properly managed could contribute to the spoilage. Kunu has a very high moisture content and total solids which may encourage growth of strains to hazardous levels during storage at ambient temperature (Olasupo, Smith & Akinside, 2002). The aim of this work is to assess the quality of kunu sold to people at Oluode market in Osogbo, Osun State to determine whether the organisms found are dangerous to health and recommend the best hygienic condition for its preparation.

2.0 MATERIALS AND METHODS

2.1 Collection of Samples

3.0 RESULTS

The results of percentage occurrence of bacteria and fungi species are presented in tables 3.1 and 3.2 respectively below.

TABLE 3.1

BACTERIA	RATE OF OCCURENCE (%)
<i>Streptococcus spp.</i>	34
<i>Staphylococcus spp.</i>	26
<i>Esherichia coli</i>	24
<i>Pseudomonas spp.</i>	10
<i>Bacillus spp.</i>	6

Ten samples of freshly prepared kunu were collected from each of 5 different hawkers/vendors in Oluode market, Igbona, Osogbo. The samples were packaged in 500ml sterile plastic containers and immediately transferred to the laboratory for isolation of microorganisms and enumeration of bacteria.

2.2 Determination of Total Bacteria Count

This was carried out using standard microbiological techniques. The media used provide a favorable environment for the growth of bacteria. The samples were first serially diluted to 10 fold dilution and 0.1ml of appropriate dilution was used to inoculate each of the plates and were then incubated at 37°C for 24-48 hours. They were then counted after incubation period. The mean of duplicate results were then recorded and the colony counted.

2.3 Determination of Fungi Count

Plating was done on Sabourand Dextrose Agar using pour plate method. Serial dilution was carried out using 1ml each of kunu samples to 9ml of water to reduce the microbial load. After dilution, 0.1ml was then plated out into molten Sabourand Dextrose Agar plate in triplicates. The plates were swirled gently and allowed to solidify. They were then incubated at room temperature 28°C for 48-96 hours.

2.4 Purification and Maintenance of Microbial Isolates

The bacteria isolates were transferred onto fresh nutrient agar medium and incubated at 37°C for 24 hours. Pure colonies of bacteria were maintained on slopes of nutrient agar (NA) slant and stored in a refrigerator at 8°C. They were brought out when needed for studies.

2.5 Characterization and Identification of the Isolates

Standard inocula were prepared from the preserved stock culture by taking a loopful of the isolates and aseptically inoculating onto sterile nutrient agar (NA) plates. The plates were incubated at 28°C for 24 hours. The characterization of the isolates were performed, by employing Gram staining reaction, Oxidase test, Catalase, Citrate test, Urease test, Coagulase test, TSI (Triple Sugar Iron agar) test, MIO (Motility Indole Ornithine) test and Methyl Red and Voges proskauer test.

TABLE 3.2

FUNGI	RATE OF OCCURENCE (%)
<i>Fusarium spp.</i>	18
<i>Aspergillus spp.</i>	15
<i>Penicillus spp.</i>	22
<i>Candida albican</i>	34
<i>Rhizopus spp.</i>	11

The results of pH, total heterotrophic, total coliform count and total fungi count are presented in table 3.3 below.

TABLE 3.3

LOCATION	pH	TOTAL BACTERIA (CFU/ML)	HETEROTROPHIC	TOTAL COUNT (CFU/ML)	COLIFORM	TOTAL FUNGI COUNT (CFU/ML)
A	4.1	3.5		1.4		4.5
B	3.8	2.5		5.0		5.2
C	3.5	2.7		1.2		6.7
D	3.6	1.8		1.0		4.8
E	3.9	3.7		1.5		7.8
F	3.7	6.1		2.8		8.5
G	4.0	4.6		1.4		6.5
H	3.4	2.1		4.1		4.4
I	3.3	5.6		4.3		9.0
J	3.8	4.2		1.3		4.2

4.0 DISCUSSION

The results of the analysis revealed that all samples used have an acidic pH range of 3.34-4.15, however to further ascertain the acidic nature of the samples, they were cultured on lactobacillus agar and *Lactobacillus spp* grew on the plates. The level of acidity of kunu has been reported by several researchers including Efiuvwevere & Akoma (1995) and Akoma, Jiya, Akumka & Mshelia, 2006, who attributed these to the presence of certain species of lactic acid bacteria namely *Lactobacillus leichmanni* and *Lactobacillus fermentum* during the fermentation process. Although the beverage is acidic in nature, the acidity tends to increase with increase in fermentation period resulting into spoilage. The pH of kunu is usually too low to allow the growth of pathogenic microorganism, but the presence of *Escherichiacoli*, *Pseudomonas spp.*, *Staphylococcus aureus*, *Streptococcus spp.* and *Bacillus spp.* could be a matter of serious concern. The organisms however were isolated from the kunu beverage. *Staphylococcus aureus* is a normal flora of the skin, nose, throat, palms, hair and mucus membrane and a common etiological agent of septic arthritis (Alice, 1976).

Escherichiacoli is an important member of the coliform group. It is part of the normal flora of the intestine of human and vertebrates therefore can cause gastroenteritis, diarrhea and urinary tract infection (Pelczar, Charre & Noel, 1993). *Streptococci spp* are normal flora of the throat and the buccal cavity. Gaffaet *al.*, 2002 reported the presence of organisms like *Bacillus cereus*, *Staphylococcus*

aureus and *Escherichia coli*. The presence of these pathogens even in small numbers could render a beverage unsuitable for human consumption. The fungi isolation and identification revealed the presence of yeast and moulds. The yeast contributes to the taste, aroma and flavor of the drink; making it a beneficial organism whereas the presence of moulds even in small quantity contributes to the spoilage and contamination of the kunu beverage. The moulds isolated include *Aspergillus spp.*, *Fusarium spp.*, *Penicillium spp.*, *Rhizopus nigricans*.

The presence of these fungi moulds species is associated with spoilage of the beverages (Kolawole, Kayode & Akinduyo, 2007). The NAFDAC set the maximum level of microbial load of finished cereal products (e.g kunu) as aerobic mesophilic bacteria 104/g, moulds 5x10²g, coliform 501g and *E. coli* 01g above which the drink is unfit for human consumption. This study showed a high prevalence of these contaminants and spoilage organisms which calls for great concern.

5.0 CONCLUSION

In conclusion, the possible sources of contaminating organisms with these products (kunu) could be traced to the use of well and stream water for processing. The contaminating organisms could also be through air and the bottles used for the sale of the products. Moreover, normal flora of the processors could also serve as contaminants.

6.0 RECOMMENDATION

This is a source of concern in Nigeria because the teeming populace relies on these drinks

as alternatives to the bottled canned drinks whose price is becoming unfordable. It is therefore suggested that kunu drinks should be properly processed to avoid microbial contamination. While treated municipal water or clean water should be used during processing and dilution of the processed drinks to avoid contamination with enteropathogenic bacteria. Health education training should be organized regularly for the processors by the health workers on importance of cleanness of their environment. Also the use of sterilized packaging materials should be encouraged.

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