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ORIGINAL ARTICLE

MICROBIAL QUALITY ASSESSMENT OF KUNU BEVERAGE LOCALLY PREPARED AND HAWKED IN CALABAR, CROSS RIVER STATE, NIGERIA

Ikpoh IS[1], Lennox JA[1], Ekpo IA[2], Agbo BE[1], Henshaw EE[1], Udoekong NS[3]
1- Department of Microbiology, University of Calabar, Calabar, Cross River State, Nigeria
2- Department of Genetics and Biotechnology, University of Calabar, Calabar, Cross River State, Nigeria
3- Science Technology Department, Akwa Ibom State Polytechnic, Ikot Osurua, Ikot Ekpene, Akwa Ibom State, Nigeria

ABSTRACT: Ten kunu samples were obtained as freshly formulated beverages from five different local vendors or hawkers in Calabar, Cross River State and analyzed to determine the microbiological quality. The pH of the samples ranged from 3.34-4.12. The total heterotrophic bacteria ranged from 1.8-6.1x10^3 cfu/ml; the total coliform count ranged from 1.0-5.0x10^3cfu/ml; the total fungi count ranged from 4.2-9.0x10^3 cfu/ml. the presence of high microbial load was an indication of poor hygiene and/or poor quality cereals and water used in the preparation. The microorganisms isolated included bacteria: *E.coli* (33.3%), *Staphylococcus aureus* (26.7%), *Streptococcus sp* (23.3%), *Pseudomonas sp* (10%) and *Bacillus sp* (6.7%). Fungi: *Penicillium sp* (22.2%), *Fusarium sp* (18.6%), *Aspergillus sp* (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 refrigerated. The types and density of microorganisms recovered from the drink requires urgent measures to be taken by regulatory authorities in the processing and handling of the product before being sold to the unsuspecting general public.

KEYWORDS: Kunu, Locally, Microbial Quality Assessment, Hawked

INTRODUCTION

Kunu is a popular cereal based, non-alcoholic beverage (Adejuyitan *et al*., 2008). Non alcoholic beverages are beverages that contain no alcohol. There 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 (Wikipedia, 2009; Elmahmood *et al*., 2007). Though consumed throughout the year, it is extensively consumed during the dry season (Adeyemi and Umar, 1997; Elmahmood *et al*., 2007). It is a staple beverage drink that is relatively cheap and nutritious when compared to carbonated drinks (Adejuyitan *et al*., 2008). Its cheapness is owed to the ready availability of cereals and additives locally sourced as they grow throughout the savannah belt of West Africa (Elmahmood, 2007). Depending on the cereal availability, grains such as 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 *et al*., 2003; Gaffa *et al*., 2004). The varieties of the drink made from sorghum is a milky light brown colour, while is made from millet and maize is whitish in colour (Wikipedia, 2009). Species such as ginger, black pepper, garlic, red pepper and clover are commonly added as flavor and taste improver. 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 grain were used singly or combined; sorghum/millet was the most common combination in the ratio 1:2 (w/w) (Ahmed et al., 2003). Ayo and 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 and Umar (1994) revealed that the product has a shelf life of 24 hrs at ambient temperature, which was extended to days

Corresponding Author: Ikpoh IS, Department of Microbiology, University of Calabar, Calabar, Cross River State, Nigeria. **E-mail:** Agborreagan@yahoo.com; **Phone No.** 08063430125

by pasteurization at 60^{0}C for 1 hours and storage under refrigeration conditions. Kunu can undergo spoilage as a result of some factors such as microorganisms present in the drink that helps in the fermentation process studies have shown that kunu contains lactic acid bacteria such as *Lactobacillus sp, Streptococcus sp* and *Leuconostoc sp* and these organisms could cause the spoilage of the beverage. Other organisms such as *Staphylococcus sp, Bacillus sp, Pseudomonas sp, Penicillium sp, Aspergillus sp, Trichoderma sp,* and *Candida sp* present in the food drink if in large quantity could cause the spoilage of kunu beverage (Osuntoki and Korie, 2009). Also activities of the natural food enzymes could also contribute in the spoilage. Other factors include insects, rodents or pests presents in the environment during the preparation, temperature, time and light. All these and much more if not properly manage could contribute to the spoilage of 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 *et al.*, 2002). This study is designed to assess the microbial quality of this popular beverage produced locally and hawked in Calabar.

MATERIALS AND METHODS

2.1. Collection of Samples

Ten samples of freshly prepared kunu were collected from each of 5 different hawkers/vendors in Bogobiri, Calabar. 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. These 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 were then incubated at 37^{0}C of 24-48 hours 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 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^{0}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^{0}C for 24 hours. Pure colonies of bacteria were maintained on slopes of nutrient agar (NA) slant and stored in a refrigerator at 8^{0}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^{0}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 as described by Bergey's Manuel of Determinative Bacteriology, 9th edition (1994).

RESULTS AND DISCUSSION

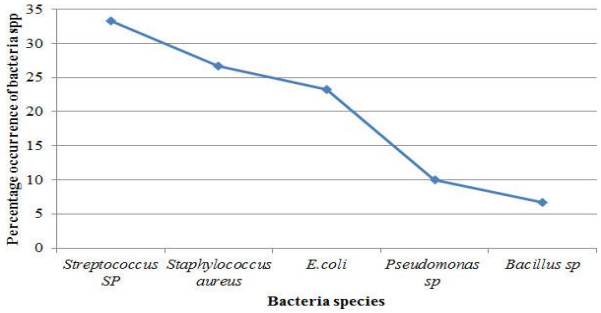

Figure 1: Percentage occurrence of bacteria species

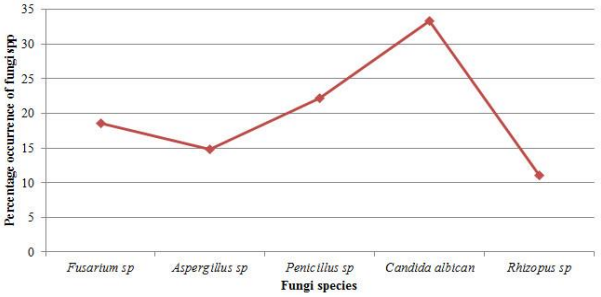

Figure 2: Percentage occurrence of fungi species

The result 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 samples were culture on lactobacillus agar and *lactobacillus sp* grew on the plates. The level of acidity of kunu has been reported by several researchers including Efiuvwevere and Akoma (1995) and Akoma *et al*., 2006, who attributed these to the presence of certain species of lactic acids bacteria namly *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 to spoilage. The pH of kunu is usually too low to allow the growth of pathogenic microorganism, but the presence of *E. coli, Pseudomonas sp, Staphylococcus aureus, Streptococcus sp* and *Bacillus sp* 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). E. coli 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 *et al*., 1993). *Streptococci sp* are normal flora of the throat and the buccal cavity. Umar *et al*., 2004 reported the presence of organisms like *Bacillus cereus, Staphylococcus aureus* and *E.coli*. The presence of these pathogens even in small numbers could render a beverage unsuitable for human consumption (PHLS, 1996). 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 sp, Fusarium sp, Penicillium sp, Rhizopus nigricans*. The presence of these fungi moulds species is associated with spoilage of the beverages (Kolawole *et al*., 2007). The NAFDAC set down maximum level of microbial load of finished cereals products (e.g Kunu) as aerobic mesophilic bacteria 104/g, moulds 5x1021g, coliform 501g and E. coli 01g above which the drink is unfit human consumption. This study showed a high prevalence of these contaminants and spoilage organisms which is a cause of great concern.

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