Journal of Food Technology 10 (1): 4-7, 2012
ISSN: 1684-8462

Microbial Quality of Kunnu-Zaki Beverage Sold in Ile-Ife, Osun State

O.A. Lawal
Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria

Abstract: Fermented foods are estimated to constitute about a quarter of the foods consumed worldwide. These foods help in improving nutritional value and safety against bacterial pathogens. Pathogens have however been isolated from some fermented beverages and so, the microbial quality of Kunnu-zaki beverage produced and sold in some locations in Ile-Ife was determined. Five samples of kunnu were collected in duplicate from five different locations. Viable count, coliform count, isolation and characterization of isolates in the sample were done. Antibiotics susceptibility of the isolates was done thereafter. The kunnu samples were serially diluted and these were inoculated into nutrient agar, lactose and MacConkey broth for viable and coliform count, respectively. Isolation and characterization were done using various selective and differential media. The second sample has the highest viable count with 1.79×10^{5} cfu mL^{-1}. All the samples collected were positive for coliform count. Nine different enteric bacteria were isolated with 46.2% of the bacteria being coliform bacteria. The organisms isolated include *Escherichia coli*, *Citrobacter freundi*, *Shigella sonnei*, *Salmonella* sp. and *Proteus mirabilis*. Antibiotics sensitivity profile revealed that the most susceptible antibiotics to all the isolates was ofloxacin with 92.3% of the isolates sensitive to it while the least susceptible antibiotics was amoxicillin having 15.4% of the isolates sensitive to the antibiotics. *Citrobacter freundii* isolated from the first kunnu sample was resistant to all the antibiotics used while *Providential alcalifaciens* isolated from the third kunnu sample was sensitive to all the antibiotics.

Key words: Kunnu-zaki, viable count, coliform bacteria, antibiotics sensitivity, alcohalic beverages, Nigeria

INTRODUCTION

In Nigeria, one of the common traditional non-alcoholic beverages is Kunnu-zaki, it is made mainly from millet. It is of low viscosity and has a sweet-sour taste, milky cream appearance and is popular with people in northern Nigeria (Adeyemi and Umar, 1994). The drink is produced from fermented millet, sorghum, guinea corn and maize in decreasing order of preference. In some culture, the grains are used in a composite form, especially millet, guinea-corn and sorghum in a ratio of 1:2 w/w (Abegaz, 2007). It is generally consumed on its own by adults as a thirst quencher or serves as refreshment in some communities and sometimes it is used as a weaning drink for infants. However, since this drink is produced from cereals, its protein is incomplete and needs to be supplemented. To make up for amino balance, millet protein should be supplemented with legume protein. Soybean is a good substitute since it is a good source of protein (about 40%) (Elmahmood and Doughari, 2007).

Being a thirst quenching beverage, Kunun-zaki has high moisture content. The proportion of water varies from 55-98%, the remainder being mostly additives. Kunnu-zaki is acidic in nature. This level of acidity of Kunun-zaki has been described by several researchers including Efiuvwevwere and Akoma (1995) and Akoma *et al*. (2006) who attributed these to the presence of certain species of lactic acid bacteria, namely *Lactobacillus leichmannii* and *Lactobacillus fermentum* during the fermentation process. The acidic nature of the samples may also be due to the fact that the Kunun-zaki might have started undergoing spoilage even before the time of purchase and such may lead to production of certain metabolites that could bring about reduction in pH of the product. The pH of Kunnu-zaki is usually too low to allow the growth of pathogenic microorganisms but the presence of *E. coli* and other coliform organisms such as *Salmonella* sp. and *Citrobacter freundii* could be a matter of serious concern. A lot of foodborne diseases are caused by the ingestion of food contaminated with pathogens bacteria. A cardinal symptom of foodborne diseases is diarrhea. It is estimated that 1400 million episodes of diarrhea occur annually in children under 5 years and up to 70% of these episodes are due to pathogens transmitted through food (Gadaga *et al*., 2004). The method of production is crude not standardized with levels of ingredients not quantified. The procedure involves steeping the cereals in local household utensils such as buckets, calabashes and earthenware vessels. This is then followed by grinding of the steeped grains into a mush which is then mixed with spices (clove, red or black pepper and ginger) and then divided into two

unequal portions. One portion is gelatinized with hot water and the other portion mixed with liquefying agents (sweet potato paste, malted rice and extracts of *Cadaba farinose* stem). The two portions are then mixed together at 70-75°C and the mixture left at room temperature for chance fermentation for 18-24 h.

This is filtered first using a piece of muslin cloth and then a sieve. After filtration, honey or sugar is then added to the filtrate to taste and is now ready for consumption (Onuorah *et al*., 1987; Akoma *et al*., 2006).

Spices are usually added in small quantities to improve taste and flavour because these are agricultural commodities, they may contain a high level of microbial impurities (Adeyemi and Umar, 1994). Bibek (2001) reported these spices as possible sources of spoilage and pathogenic microorganisms. The high water content coupled with crude methods of production and packaging under improper sanitary conditions equally predisposes Kunnu-zaki to microbial contamination.

MATERIALS AND METHODS

Collection of samples: Two samples of freshly prepared Kunun-zaki were collected from each of five different locations between the months of October and December, 2007 in Ile-Ife, Ife Central local Government of Osun State, Nigeria. The fresh samples were inside 500 mL sterile plastic bottles (as packaged by the seller) and immediately transferred to the microbiology laboratory for the laboratory analysis.

Determination of total count of bacteria: This was carried out on agar plates of Nutrient Agar (NA), MacConkey Agar (MCA) and Eosin Methylene Blue Agar (EMB) all of oxoid grade using the pour plate method. The samples were serially diluted and 1 mL of appropriate dilution was used to inoculate each of the plates in duplicates. The culture plates were then incubated at 37°C for 24-48 h and colonies counted. The mean of duplicates results were then recorded as the colony count.

Determination of coliform count: The serially diluted samples were also inoculated into lactose and MacConkey broth for coliform count. Varying amounts of the sample are added to lactose broth tubes containing inverted Durham tubes to indicate production of gas and these were incubated for 48 h at 37°C. The positive ones were plated into differential and selective media. And characterization was done thereafter.

Isolation and identification: Discrete colonies of the organism were selected and sub cultured from the mixed cultures of the plates to respective NA plates and incubated at 37°C for 24 h. Each pure isolates was processed by the method described by Cowan and Steel (1985). Preliminary identification of isolates was from gram's reaction and morphological characteristics. Further characterization was carried out with various biochemical tests. These tests include spore stain, motility, sulphide production, catalase, coagsulase, lactose fermentation using Triple Sugar Iron (TSI) agar, carbohydrate test, citrate utilization and indole production. All media were prepared according to manufacturer's specification and sterilized at 121°C for 15 min.

RESULTS AND DISCUSSION

Samples were collected in duplicates and an average of the viable count of the bacteria was calculated. The first sample source has 1.57×10^5 cfu mL^{-1} as the total viable count; 1.79×10^5 cfu mL^{-1} as viable counts for the second sample.

The 1.55×10^5 cfu mL^{-1} was the average counts for the third samples; 1.72×10^5 and 5.0×10^4 cfu mL^{-1} for the fourth and fifth samples, respectively (Table 1). Table 2 shows the coliform counts obtained from the duplicates of five samples. Most probable number recorded against all the samples were much. The sample with the highest most probable no is the first sample sources with 1100 per 100 mL and the least is the second and fourth sample with 42 per 100 mL.

Table 1: The total bacterial counts (cfu mL^{-1}) of Kunnu-zaki from different sources

Samples	Counts (cfu mL^{-1})	Average (cfu mL^{-1})
SA 1a	8.40×10^4	1.57×10^5
SA 1b	1.37×10^5	
SA 2a	8.80×10^4	1.79×10^5
SA 2b	2.70×10^5	
SA 3a	1.29×10^5	1.55×10^5
SA 3b	1.80×10^5	
SA 4a	1.84×10^5	1.72×10^5
SA 4b	1.60×10^5	
SA 5a	5.50×10^4	5.0×10^4
SA 5b	4.50×10^4	

Table 2: Coliform counts using MPN Method

Quantity of the samples/samples	10 mL	1 mL	0.1 mL	Total (MPN per 100 mL)
SA 1a	3	3	3	1100
SA 1b	3	3	1	460
SA 2a	3	2	2	210
SA 2b	2	2	3	42
SA 3a	3	3	1	460
SA 3b	3	3	3	1100
SA 4a	2	2	3	42
SA 4b	3	3	3	1100
SA 5a	3	2	2	210
SA 5b	3	3	1	460

Duplicate samples were collected from five different locations and nine different enteric bacteria were isolated for the Kunnu-zaki samples collected.

The 23.1% of the organisms isolated were *Serratia marcescens* followed by *Escherichia coli* and *Edwardsiella* sp. with 15.4%. Among these organism coliforms took 46.2% of the total organisms isolated and the rest were non coliforms as shown in Table 3. Antibiotics sensitivity profile revealed that the most sensitive antibiotics to all the isolates was ofloxacin with 92.3% of the isolates sensitive to it, this is followed by nalidixic acid with 61.5% of the isolates sensitive to it while the least susceptible antibiotics was amoxicillin having 15.4% of the isolates sensitive to the antibiotics.

Citrobacter freundii isolated from the first kunnu sample was resistant to all the antibiotics used while *Providential alcalifaciens* isolated from the third kunnu sample was sensitive to all the antibiotics as shown in Table 4.

Garaga *et al.* (2004) reported that pathogens have been isolated from some fermented foods and some laboratory tests have shown the possibility of pathogens to survive and grow in some fermented foods. He said post processing contamination is often cited as the major cause of food poisoning, this agrees with the observation made as regards the source of Kunnu collected for this study which is not really hygenic.

Table 3: The organism isolated in the kunnu samples and their frequency

Organisms	Frequency (%)
Escherichia coli	2 (15.4)
Shigella sonnei	1 (7.7)
Providential alcalifaciens	1 (7.7)
Edwardsiella sp.	2 (15.4)
Salmonella sp.	1 (7.7)
Serratia marcescens	3 (23.1)
Proteus mirabilis	1 (7.7)
Proteus vulgaris	1 (7.7)
Citrobacter freundii	1 (7.7)
Total	13

Gadaga *et al.* (2004) also said some pathogens such as *Escherichia coli* are reported to develop acid tolerance. It was equally said by them that *Escherichia coli*, *Salmonella* sp., *Klebsiella* and *Shigella* sp. are most commonly encountered pathogens in African fermented food.

This tally with the types of organism isolated in this study. The occurrence of bacterial pathogens in fermented foods suggests a need for caution in the use of these foods for infant feeding.

Byaruhanga *et al.* (1999) reported that gram negative bacteria are more susceptible to the low pH in fermented food while the gram positive bacteria may be more resistant and so the presence of enteric bacteria in the sample is a thing of concern.

The presence of coliform bacteria such as *Escherichia coli* may be as a result of acid tolerance response mechanism they often develop. The same researchers however reported that pathogenic *E. coli* and *Shigella* sp. strains used were more tolerant to the low pH in some fermented foods such as sour porridge and survived longer under those conditions (Tamime and O'Connor, 1999).

Nyatoti *et al.* (1997) reported that out of 12 samples of naturally soured milk used as weaning foods, two contained enteropathogenic *E. coli*. This is also recorded in this study.

The total bacterial counts obtained in this study fall within the range of 5.0×10^4 and 1.79×10^5 cfu mL^{-1}. Earlier research by Hatcher *et al.* (1992) also reported similar abnormally high bacterial populations in orange juices. This high colony counts is an indication of contamination as a consequent of either poor hygiene or poor quality of cereals and water used. The most probable number of coliform count of the samples ranges from 42-1100 MPN per 100 mL.

Table 4: Antibiotic sensitivity pattern of isolate from kunnu-zaki samples

Isolates	NAL	OFL	AUG	TET	AMX	COT	NIT	GEN	Percentage
SA 1ai	S	S	R	R	R	R	S	R	37.5
SA 1aii	S	S	R	R	R	R	R	S	37.5
SA 1b	R	R	R	R	R	R	R	R	0.0
SA 2a	S	S	S	S	R	S	S	S	87.5
SA 2bi	S	S	S	S	R	S	R	S	75.0
SA 2bii	S	S	R	R	R	R	R	R	25.0
SA 3a	S	S	S	S	S	S	S	S	100.0
SA 3b	R	S	R	S	R	R	R	S	37.5
SA 4ai	R	S	R	R	R	R	S	R	25.0
SA 4aii	S	S	R	S	R	R	R	R	37.5
SA 4b	R	S	S	R	S	R	R	R	37.5
SA 5a	R	S	R	R	R	R	S	R	25.0
SA 5b	S	S	R	R	R	S	S	S	62.5
Percentage	61.5	92.3	30.8	38.5	15.4	30.8	46.2	46.2	-

NAL: Nalidixic Acid; AMX: Amoxicillin; AUG: Augmentin; NIT: Nitrofurantoin; COT: Cotrimaxozole; TET: Tetracycline; OFL: Ofloxacin; GEN: Gentamycin; S: Sensitivity; R: Resistant

Eight different Enteric bacteria were isolated from the samples of Kunnu-zaki collected for analysis from five different locations. This must have resulted from the poor handling by the producers of this local beverage. The presence of coliform indicates feacal contamination making these drinks unsafe for consumption.

Many native African beverages are little known outside the parent continent. This is mostly due to African methods of processing and preservation of their indigenous beverages. This makes many people to prefer imported and exotic beverages because of their sure microbial quality, attractive forms, long shelf life, ease of transportation and other forms of utility which consumers associate with them (Achi, 2005).

A concerted effort should therefore be made to improve the microbial quality and production techniques of these indigenous exotic beverages so that large scale production for export outside the continent can be carried out. In Nigeria there is presently no industry producing a local beverage like Kunun-zaki even though it is widely believed to be of immense social, economic and medicinal importance to its numerous consumers (Akoma *et al*., 2006).

To safeguard public health, governments and regulatory authorities should intervene by setting standards in acquisition of raw materials, production procedures and techniques as well as health status of personnel.

CONCLUSION

The study revealed that most of the places where this common local non alcoholic beverages were produced were not hygienic thereby posing a serious threat to the health of every individual consuming this food.

REFERENCES

Abegaz, K., 2007. Isolation, characterization and identification of lactic acid bacteria involved in traditional fermentation of borde, an acid bacterium involved in traditional fermentation of borde, an Ethiopian cereal beverage. Afr. J. Biotechnol., 6: 1469-1478.

Achi, O.K., 2005. The potential for upgrading traditional fermented foods through. Afr. J. Biotechnol., 4: 375-380.

Adeyemi, I.A. and S. Umar, 1994. Effect of method of manufacture on quality characteristics of kunun zaki, a millet based beverage. Nig. Food J., 12: 944-947.

Akoma, O., E.A. Jiya, D.D. Akumka and E. Mshelia, 2006. Influence of malting on the nutritional characteristics of Kunun-zaki. Afr. J. Biotechnol., 5: 996-1000.

Bibek, R., 2001. Fundamental of Food Microbiology. 2nd Edn., The CRC press Ltd., Washington, USA., pp: 56-90.

Byaruhanga, Y.B., B.H. Bester and T.G. Watson, 1999. Growth and survival of Bacillus cereus in mageu, a sour maize beverage. World J. Microbiol. Biotechnol., 15: 329-333.

Cowan, S.T. and K.J. Steel, 1985. Manual for the Identification of Medical Bacterial. 4th Edn., Cambridge University Press, London, Pages: 217.

Efiuvwevwere, B.J.O. and O. Akoma, 1995. The Microbiology of Kunun-zaki, a cereal beverage from Northern Nigeria during the fermentation (production) process. World J. Microbiol. Biotechnol., 11: 491-493.

Elmahmood, A.M. and J.H. Doughari, 2007. Microbial quality Assessment of Kunnu-Zaki beverages sold in Girei town of Adamawa State, Nigeria. Afr. J. Food Sci., 1: 11-15.

Gadaga, T.H., L.K. Nyanga and A.N. Mutukumira, 2004. The occurrence, growth and control of pathogens in African fermented foods. Afr. J. Food Agric. Nutr. Dev., 4: 5358-5374.

Hatcher, W.S., J.L. Weihe, D.F. Splittstoesser, E.C. Hill and M.E. Parish, 1992. Fruit Beverages. In: Compendium of Methods for the Microbiological Examination of Foods, Vanderzant, C. and D.F. Splittstoesser (Eds.). American Public Health Association, Washington, USA.

Nyatoti, V.N., S. Mtero and G. Rukure, 1997. Pathogenic *Escherichia coli* in traditional African weaning foods. Food Control, 8: 51-54.

Onuorah, S.I., A.A. Adesiyun and J.O. Adeyeye, 1987. Survival and multiplication of *Staphylococcus aureus* and *Escherichia coli* in a Nigeria cereal drink (*Kunun zaki*): Effect of spices and temperature. J. Food Agric., 1: 169-173.

Tamime, A.Y. and T.P. O'Connor, 1999. Kishk-A dried fermented milk/cereal mixture. Int. Dairy J., 5: 109-128.