Journal of Global Biosciences ISSN 2320-1355
Vol. 3(2), 2014, pp. 511-515 http://mutagens.co.in
Date of Online: 16, April- 2014

MICROBIAL QUALITY OF KUNU DRINK SOLD IN CALABAR, CROSS RIVER STATE, NIGERIA

Mbachu A.E.[1], C.A.Etok[2] , K.C. Agu[3], O.I. Okafor[4] , N.S. Awah[5], L.C. Chidi-Onuorah[6], V.C. Ekwueme[7], J. Okpala[8], M.O. Ogbue[9], and M.O. Ikele[10]

[134567&10]Department of Applied Microbiology and Brewing, Faculty of Biosciences, Nnamdi Azikiwe University, P.M.B. 5025, Awka, Anambra State, Nigeria.
[2]Department of Microbiology, University of Uyo, P.M.B. 1017, Uyo, Akwa Ibom State, Nigeria.
[8]Department of Microbiology, Anambra State University, P.M.B. 02, Uli, Nigeria.
[9]Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, P.M.B. 5025, Awka, Anambra State, Nigeria.

Abstract

Kunu drink sold in Calabar was analysed to determine the microbiological quality. Colony forming units per millilitre of kunu ranged from 2.1 x 10^3 to 6.1 x 10^3, 2.1 x 10^3 to 6.5 x $10^3$and 2.3 x 10^3 to too numerous to count (TNC) for fresh kunu sample, kunu stored at refrigeration temperature (2-3 days) and kunu stored at room temperature (2-3 days) respectively. Bacterial identification revealed the presence of *Staphylococcus* sp., *Streptococcus* sp., and *Bacillus* sp. in fresh kunu. *Staphylococcus* sp., *Streptococcus* sp., *Bacillus* sp. and *Pseudomonas* sp. were found in kunu stored under refrigeration conditions. The above mentioned organisms including *E. coli* were found in kunu stored at room temperature. *Streptococcus* sp. had the highest occurrence of 43.31% and *E. coli* having the least occurrence of 5%. Fungal isolates revealed the presence of *Penicillium*sp., *Aspergillus*sp. and *Fusarium*sp. in fresh kunu, with the emergence of yeast after 2-3 days storage at room and refrigeration temperatures. *Penicillium*sp. had the highest occurrence of 34.6% and yeast having the least occurrence of 7.7%. From the data obtained, it was recommended that kunu drink should be consumed within 24 hr of preparation or preserved using chemical preservatives rather than the refrigerator.

Key words: Kunu drink, Microbial quality, Bogobiri area, Calabar, Room temperature, Refrigeration temperature.

INTRODUCTION

Kunu is an important non-alcoholic beverage mostly found in Northern Nigeria. It is prepared from sorghum, millet, maize or wheat [1]. Studies have shown that kunu contains 0.3% protein, 1.0% fat, 1.52% ash and 12.2% carbohydrate [2]. Oyeleke and Shittu [3] reported 8.9mg of ascorbic acid (vitamin C), 20.2g of carbohydrates and 7.2g of protein per 100ml of kunu.

Kunu which used to be consumed mainly in the Northern parts of Nigeria is now widely acceptable in almost all parts of Nigeria, owing to its refreshing qualities [4]. It is acceptable to people of all works of life and is being served at home and public places as food appetizer, refreshing drink and complementary food for infants. It forms the major source of protein for many Nigerians especially the rural populace who could not afford imported milk products. Olasupo*et al.* [5] reported that fermented cereals like ogi, burukutu, fura, kunu, etc. are particularly important as weaning foods for infants and as dietary staples for adults. The short shelf-life of these beverages are however a major problem faced by their producers and consumers. A large number of lactic acid bacteria, coliforms, molds and yeasts cause spoilage in these drinks thereby producing undesirable changes [6]. Food pathogens such as *Escherichia coli* have been implicated in food poisoning resulting from their consumption.

The effect of storage on each drink varies and the time lag during which the drinks lose their nutritional properties vary, hence there is a need to know the more appropriate method of storage, whether ambient or refrigerated, to reduce the incidence of certain diseases [7].

The present study was therefore undertaken to determine the microbiological quality of kunu drink sold in Calabar and to ascertain the microbial load in the kunu stored at room and refrigeration temperatures.

MATERIALS AND METHODS

Sample collection

Ten freshly prepared kunu samples were purchased from five different hawkers along Bogobiri area, Calabar ($4^{o}57'$N $8^{o}19'$E/4.950^{o}N 8.317^{o}E), Nigeria. They were aseptically collected using sterile bottles and transported immediately to the laboratory for analysis.

Storage condition

The kunu samples contained in 10 sterile containers were divided into two. Five were stored at ambient temperature while the remaining five at refrigeration temperature for 3 days. In both cases, the samples were analysed daily for microbial load within 3 days of storage.

Isolation of bacteria and fungi

Each sample was serially diluted with sterile distilled water before inoculation. The media used for bacteria isolation was nutrient agar and MacConkey agar while Sabouraud dextrose agar (SDA) was used for fungal isolation. The pour plate method was used for the isolation of bacteria and fungi [8]. Plates containing nutrient and MacConkeyagar were incubated for 24 hr at 37^{o}C while SDA plates were incubated at 30^{o}C for 96 hr. Both the bacterial and fungal isolates were purified by repeated subculturing onto nutrient and SDA respectively. Subsequently, the stock cultures of the bacterial and fungal isolates were prepared by inoculating onto nutrient and SDA slants in McCartney bottles. The stock cultures were preserved in a refrigerator at 4^{o}C and used for further identification of the organisms.

Identification of bacteria and fungi isolates

Identification of the bacterial isolates was accomplished by the observation of colonial characteristics, Gram reaction and biochemical tests [9]. Fungal isolates were identified using colonial appearance and microscopic characteristics [10 and 11].

RESULTS AND DISCUSSION

Nine microbial isolates including five species of bacteria and four species of fungi were isolated from the kunu samples. The bacterial isolates include*Staphylococcus* sp., *Streptococcus* sp., *Bacillus* sp., *Escherichia coli* and *Pseudomonas* sp., while the fungal isolates were the species of *Fusarium, Aspergillus, Penicillium*and yeast.

Colony counts of bacteria isolated from fresh kunu samples are presented(Table 1). The count ranged from $2.1 \times 10^3 - 6.1 \times 10^3$cfu/ml. Tables 2 and 3 shows the colony count of bacteria isolated from kunu stored at room and refrigeration temperatures. The count ranged from 2.3×10^3cfu/ml – TNC and $2.1 \times 10^3 - 6.5 \times 10^3$ cfu/ml for room and refrigeration temperatures respectively. The results indicate that fresh kunu presented a low bacterial count after 24 hr of incubation. However, it was observed that the bacterial load in kunu samples increased with storage conditions as well as storage period. The bacterial count in kunu samples stored at room temperature was higher than that stored at refrigeration temperature. Thus, refrigeration storage hindered microbial growth while room temperature storage encourages microbial growth and proliferation. Adeyemi and Umar [12] reported that kunu has a shelf-life of 24 hr at ambient temperature, which can be extended to 8 days by pasteurization at 60^{o}C for 1 hr and storage under refrigeration temperature.

Bacterial identification revealed the presence of *Streptococcus* sp., *Staphylococcus* sp. and *Bacillus* sp. in fresh kunu. The study also revealed the presence of *Streptococcus* sp., *Staphylococcus* sp., *Bacillus* sp. and *Pseudomonas* sp. in kunu after 2-3 days of storage at refrigeration temperature. All the isolates mentioned above in addition to *Escherichia coli* were found in kunu after 2-3 days of storage at room temperature. Olasupo*et al.* [5] reported the isolation of *Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Klebsiella*sp. and *Enterococcus faecalis*from kunu drink.

The fungal isolates revealed the presence of *Fusarium*sp., *Penicillium*sp. and *Aspergillus*sp. in fresh kunu. It also showed the presence of *Fusarium*sp., *Penicillium*sp., *Aspergillus*sp. and yeast in kunu drink after 2-3 days storage at room and refrigeration temperatures. Akinrele*et al.* [13] reported that the yeasts *Saccharomyces cerevisiae, Candida mycoderma*and molds*Cephalosporium, Fusarium, Aspergillus* and *Penicillium*are the major organisms responsible for the fermentation and nutritional

improvement of cereal based fermented foods (ogi and kunun-zaki). These organisms can cause the spoilage of the beverage if not eliminated during the heating process.

The bacterial and fungal isolates,withtheir frequency and percentage occurrences are presented(Tables 4 and 5) respectively. The result showed that *Streptococcus* sp. had the highest occurrence of 43.3%, followed by *Bacillus* sp. (23.3%), *Staphylococcus* sp. (21.7%), *Pseudomonas* sp. (6.7%) and *Escherichia coli* (5%). Among the fungal isolates, *Penicillium*sp. had the highest occurrence of 34.6%, followed by *Fusarium*sp. (30.8%), *Aspergillus*sp. (26.9%) and yeast with the least occurrence of 7.7%.

Part of "kunu'' preparation involves cooking, a process that would eliminate all the isolates reported in this work except the heat-resistant spore former (*Bacillus* sp.). The presence of these organisms in kunu thus suggests that it must have been contaminated after the cooking process and after the drink had cooled down. Contamination could come from the syrup, fermentation vessels, storage containers, sieves used for filtration, hands of the handlers and even the polyethene bags or bottles in which it was packaged for sale [14]. There is therefore the need for high degree of sanitation during the processing of the beverage.

The presence of *E. coli* in kunu indicates faecal contamination and may have serious health implications. *Bacillus* sp. has also been implicated in food poisoning especially in cereals that have been cooked and stored at warm temperature [14]. Reports indicate that toxin produced by *Bacillus* sp. cause pneumonia and bronchopneumonia [9]. Besides, *Bacillus cereus* is known to produce heat-resistant spores that cannot be eliminated by boiling. *Streptococcus*, *Pseudomonas* and *Klebsiella*species have been implicated in the spoilage of food and beverages [15]. Their presence in kunu is undesirable. There is then the need to maintain adequate hygienic conditions during processing and preparation of the beverage to eliminate these microbial contaminants and to improve on the quality of the final product. There is also the need toemploy adequate preservative measures to improve the shelf-life of the beverage.

Table 1: Average bacterial count in nutrient agar of fresh kunu drink (x 10^3 cfu/ml).

Samples	Average bacterial count
A	4.0
B	3.2
C	5.4
D	3.4
E	2.3
F	2.1
G	5.2
H	4.3
I	5.2
J	6.1

Table 2: Average bacterial count in nutrient agar of kunu drink stored at room temperature (x 10^3cfu/ml).

Samples	1st day	2nd day	3rd day
A	4.0	6.5	TNC
B	3.2	4.4	TNC
C	5.4	5.8	TNC
D	3.4	5.7	TNC
E	2.3	4.2	TNC

TNC = Too numerous to count

Table 3: Average bacterial count in nutrient agar of kunu drink stored at refrigeration temperature (x 10^3 cfu/ml).

Samples	1st day	2nd day	3rd day
F	2.1	3.2	4.2
G	5.2	5.3	5.9
H	4.3	4.7	5.0
I	5.2	5.6	6.1
J	5.1	6.2	6.5

Table 4: Frequency and percentage (%) occurrence of bacterial isolates.

Bacterial isolates	Frequency (F)	% occurrence
Staphylococcus sp.	13	21.7
Streptococcus sp.	26	43.3
Bacillus sp.	14	23.3
Escherichia coli	3	5.0
Pseudomonas sp.	4	6.7

Table 5: Frequency and percentage occurrence of fungal isolates

Fungal isolates	Frequency (F)	% occurrence
*Fusarium*sp.	8	30.8
*Aspergillus*sp.	7	26.9
*Penicillium*sp.	9	34.6
Yeast	2	7.7

CONCLUSIONS AND ACKNOWLEDGEMENT

Apart from sanitary measures, there is the need for closer monitoring of the microbial standard of the local beverage "kunu'' sold to the public by both the State and Federal Ministries of Health as a way of combating or reducing the health hazards that its consumption may cause.

The authors acknowledge with gratitude the effort of Mr.Umoh, the chief technologist of the Department of Microbiology, University of Calabar, Nigeria, for his assistance in identifying the organisms.

REFERENCES

1. Gaffa, T., Jideani, I.A. and Nkama, I. Nutrient and sensory qualities of kunun-zaki from different saccharifying agents. *International J. Food Science Nutrition* 53: 109-115. 2002.
2. Sapade, P.A. and Kassum, A.L. Rheological characterization of Nigerian liquid and semi liquid foods, kununzaki and kunungyada. *Nigerian Food Journal* 10: 23-33. 1992.
3 Oyeleke, S.B. and Shittu, A. Quality assessment of kunun-zaki sold in Minna. *Proceedings of the Biotechnology Society of Nigeria* (*10th Annual Conference*) 2nd to 5th April, Minna, Niger State. 1997.
4. Edward, E.B., Abasiekong, S.F. and Chiemeka, I. Kunun-zaki and Tsamiya: Some non-alcoholic beverages from sorghum grains. Chemical analysis for nutrients contents of fresh and ageing samples. *Nigerian J. Biotechnol.*5: 21-22. 1988.
5. Olasupo, N.A., Smith, S.I. and Akinside, K.A. Examination of the microbial status of selected indigenous fermented foods in Nigeria. *J. Food Safety.* 22 (2): 85. 2002.
6. Uche, S.N., Charity, U.O. and Aminat, O.S. Effect of storage conditions on the physicochemical properties and microbial load of kunu, soymilk, yoghurt and zobo drinks. *International J. Food Nutrition and Safety* 2(1): 17. 2012.

7. Tamine, A.Y. and Robinson, R.K. *Yoghurt Science and Technology* 1st ed., Pergamon Press, New York. PP. 431. 1989.
8. Fawole, M.A., Oso, B.A. Laboratory Manual of Microbiology. Spectrum books Ltd., Ibadan. PP. 74-121. 1988.
9. Chessbrough, M. Medical Laboratory Manual for Tropical Countries. 2nd Ed. ELBS Publication, UK. 11: 60-68. 1984.
10. Barnett, H.L. and Hunter, B.B. Illustrated genera of imperfect fungi. Macmillian Publishing Company, New York. 1987.
11. Efiuvwevwere, B.J.O. Microbial spoilage agents of tropical and assorted fruits and vegetables: an Illustrative Reference Book. Paragraphics, Port Harcourt, Nigeria. 2000.
12. Adeyemi, I.A. and Umar, S. Effect of method of manufacture on quality characteristics of kunu-zaki, a milk based beverage. *Nigerian Food Journal.*12: 34-41. 1994.
13. Akinrele, I.A., Adeyinka, O. and Edwards, C.C.A. The development and production of soy-ogi, a corn based complete protein food. FIIRO*Research Report No. 42.*1980.
14. Wonang, D.L., Amienyo, C.A., Ekeleme, O.P. and Dazol, D.G. Bacteriological assessment of "kunu" a local beverage sold in Jos, Plateau State. *J. Environ. Sciences* 4(1): 5-7. 2001.
15. Nester, E.W., Robert, C.E., Lidisfrom, M.E. and Pesrau, N.N. Microbiology 3rd Ed. *Saunders* College Publishing, Japan. PP. 115-116. 1984.