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Assessment of the Microbiological Quality of Kunu Zaki Sold at Gariki, Enugu State, Nigeria

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Abstract: Freshly prepared kunu zaki produced from pearl millet was randomly collected from six different hawkers in Gariki Enugu and was analyzed to determine the microbiological quality. Kunu zaki stored under room and refrigeration temperature was serially diluted in sterile test tubes for 3 days daily and 10^{-2} dilution was used for each day. The media used for inoculation was MacConkey agar, Nutrient agar, Sabround Dextrose agar and CLED agar. Bacterial identification revealed the presence of *Bacillus sp* and *E. coli* in fresh kunu. *Lactobacillus*, *Pseudomonas sp, Bacillus sp* and *E.coli* were found in kunu zaki stored under refrigeration temperature. The above mentioned organisms including *streptococcus sp* were found in kunu stored at room temperature. *E.coli* had the highest occurrence of 30.8% and *Streptococcus sp* had the least occurrence of 7.7%. Fungal isolates revealed the presence of *Sacchromyces cerevisiae, Aspergillus* and *Geotrichum spp,* in fresh kunu zaki with the emergence of *Torula* yeast after 2-3 days storage at room and refrigeration temperatures. *Geotrichum spp* had the highest occurrence of 54.5% and *Sacchromyces cerevisiae* had the least occurrence of 29.1%. Kunu drink should be consumed within 24 hours of preparation or preserved using chemical preservatives rather than refrigeration.

Key words: Kunu zaki · Media · Fungal isolates · Agar and yeast

INTRODUCTION

Food is any substance, usually composed of carbohydrates, fats, proteins and water, which can be eaten or drink by animals including humans for nutrition or pleasure [1]. In many developing countries like Nigeria, people depend mostly on indigenous technology for food preparations especially food of plant origin, some of these foods that originates from plant includes an alcoholic beverages made mostly from cereal grains. In addition to filling a basic human need, beverages form part of the culture of human society [2].

Kunu is an important non-alcoholic fermented beverages widely consumed in Northern parts of Nigeria especially during the dry season [3, 4]. Kunu is cheap and the cereals used in its preparation are widely grown throughout the savanna region of Nigeria such as Bauchi, Kano, Sokoto and Katsina States. It is prepared from sorghum, millet, maize or wheat. Studies have shown that kunu contains 0.33% protein, 1.0% fat, 1.52 ash and 12.2% carbohydrate but according to research carried out by Onyeleke and Shittu [5] reported that kunu contains 8.9mg of ascorbic acid (vitamin C), 20.2g of carbohydrates and 7.2g of protein per 100ml of kunu.

Kunu according to Jango-cohen [6] used to be consumed mainly in the Northern parts is now widely acceptable in almost all parts of Nigeria, owing to its refreshing qualities. 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 can also be consumed in the morning as breakfast by adult and children, serve as appetizer to entertain visitors in rural and urban centers and is commonly served at social gatherings [7]. It forms the major source of protein for many Nigerians especially the rural populace who could not afford imported milk products. Fermented cereals like ogi, burukutu, fura, kunu etc are particularly important as weaning food for infants and as dietary staples for adults.

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Although there are various types of kunu processed and consumed in Nigeria including kunu zaki, kunu gyada, kunu akamu, kunu tsamiya, kunu baule, kunu jiko, ashamu and kunugayamba. However, kunu zaki is the most commonly consumed. Kunu processing is mostly done by women with simple house hold equipment utensil. Depending on ceral availability, sorghum, maize, millet, guinea corn or rice are commonly used for kunu preparation [8].

Kunu zaki is one of the types of kunu (a non-alcoholic beverage) that is very popular in the northern part of Nigeria but it is being consumed now in all parts of the country. Kunu zaki is produced from millet and sorghum grains but in this work millet was used to produce kunu zaki because of its availability. The process of manufacture involves wet milling of millet grains with spices (ginger, clove, black pepper, ehuru) and sweet potato (Ipomea botatas) but sweet potato is optional. Wet sieving and partial gelatinization of the slurry, sugar addition, bottling, pasteurization and immediate cooling [9].

Unlike other traditional cereal based alcoholic beverage, information is very scanty on the manufacturing process, quality and archeological characteristics of "kunu zaki". In its traditional manufacture, the basic processes are not standardized and levels of ingredients Such as spices and sweetener are not quantified. Furthermore, a wide variation exists in the method of preparation depending on taste and cultural habits, which partly explains the lack of consistency in the product quality.

It has been found that "kunu zaki" contains all the essential nutrients; carbohydrates, fat, protein, mineral and vitamins. It is mainly used in homes more especially in Northern part of Nigeria. It is also that to obtain "kunu zaki" with a desirable flavour and taste, the optimal enzymes acting should be achieved, this can also include the malting of millet grain before producing "kunu zaki". Malting period is necessary during production of kunu zaki beverage because it helps to break down the higher molecular weight of polysaccharides to smaller molecular weight compounds for easy digestibility. It is desirable, however, to improve the storage stability of kunu zaki, by reducing the initial microbial load through controlled fermentation, use of treated (potable) water for preparing the slurry or addition of chemical preservative such as sodium benzoate etc [10].

The short shelf-life of this beverage is however a major problem faced by their producers and consumers. Also in developing nations like Nigeria, it has not been possible to have control over the processing of hawked foods, because most of the vendors lack the adequate knowledge of food processing and handling practices. A large number of lactic acid bacteria, *coliforms*, *E.coli*, molds and yeast have been reportedly implicated in food spoilage as they use the carbohydrate content of the foods for undesirable fermentation processes. Brief fermentation usually occurs during kunu processing (steeping of the grains in water over an 8-48 hours period) and is known to involve mainly lactic acid bacteria and yeast [11].

In most Nigeria cities (Northern part of Nigeria), the sales and consumption of this locally made beverage is high due to the high cost of other non-alcoholic drinks. This drink is usually hawked in the motor parks, military barracks, Hausa people residing areas (in the East like Gariki, Mammy areas etc.), school premises and market places.

Aim and Objectives of the Study:

- To determine the microbial quality of kunu zaki sold at Gariki, Enugu, Enugu State Nigeria.
- To isolate the microorganisms involve in the fermentation of fresh kunu zaki produced from millet.
- To isolate microorganisms in kunu zaki stored at refrigeration temperature for 72 hours.

Millets are a group of highly variable small-seeded grasses, widely grown around the world as cereal crops or grains for both human food and fodder. They do not form a taxonomic group, but rather a functional or agronomic one. MacDonough *et al.,* [12] pointed out that millet are important crops in the semi-arid tropics of Asia and Africa (especially in India, Nigeria and Niger), with 97% of millet production in developing countries. The crop is favoured due to its productivity and short grooming season under dry, high temperature conditions.

MATERIAL AND METHODS

Methods

Sterilization of Materials: The glasswares (Test tubes, pipettes, conical flasks, beakers, petri-dishes and universal bottles) were washed with soapy water and rinsed with distilled water; they were allowed to dry and wrapped with kraft paper and further sterilized in a hot air oven at 180°C for 1hour and stored at 4°C. The media used was also sterilized.

Collection of Soup Samples: Six bottles of kunu zaki produced from pearl millet were purchased from different hawkers at Garki market, Enugu with sterile sample bottles and sent immediately to the laboratory for analysis.

Preparation of Samples and Media: The samples were serially diluted and the media for culturing were aseptically prepared according to the manufacturer's instruction and autoclaved at 121°C for 15 minutes.

Plating Technique (Pour Plating): The pour plating technique was used. 0.1ml of the solution was aseptically pipette from each 10^{-2} serial diluted sample in the test tube and put into petri-dishes. Cooled nutrient agar, MacConkey agar, sabourand Dextrose agar and CLED agar were poured into the plates and stirred in clockwise and anticlockwise direction for homogenous mixture and allowed to solidify and labeled. The plates were inverted and incubated at 28°C for 24 hours for bacteria then at 37°C for five days for fungi.

Bacterial Counting and Incubating: After incubation, the number of these colonies on the petri-dishes were counted, using the colony chamber and sub-cultured in order to get a pure colony. The average total and differential standard plate counts (SPC) where taken colonies were selected, characterized and identified using various morphological and biochemical tests such as Gram stain, citrate, lactophenol cotton blue test, oxidase test and sugar fermentation test.

Gram Staining: Smears were separately formed from each representative colony. Each smear was heat-fixed other at the slide containing the heat-fixed smear was laid on a staining rack and flooded with crystal violet to act for 30 seconds. Then excess stain was drained and the residual stain on the smear was washed with excess Lugol's iodine which is allowed to react for 30 seconds while the slide is laid across the staining ract, the iodine was carefully rinsed out with distilled water. Then the smear was rinsed briefly with 50:50 acetone-alcohol until blue colour ceases to come off. This is a decolourizing step which if prolonged will interfere with the result. Thus, the slide is quickly rinsed out with distilled water to avoid excess decolourization.

The slide was again laid across the staining rack and flooded with 1% aqueous the function of safranin is it is a counter stain and it gives gram negative bacterial led or pink colour for one minute (60 seconds) after which the stain was washed off with distilled water. The back of the slide was dried between folds of filter paper. Then place or drop of immersion oil on the smear and examine under the immersion objective microscope. The whole procedure takes five minutes on completion.

Identification of Fungi: 1ml of the 10^{-2} diluted Kunu zaki was taken from the sample and cultured on sabourand dextrose agar (SDA) in a plate. The plates were swirled clockwise and anticlockwise direction for homogenous mixture. Allowed to solidify, inverted and incubated at 37°C for 5 days. The colonies were examined microscopically using lactophenol cotton blue and the microscopic appearances of the fungal organisms were recorded.

Biochemical Tests for Identification of Isolates

Catalase Test: A small amount of culture colonies was picked from the culture using a clean platinum wireloop. This was inserted into a solution of hydrogen peroxide (H_2O_2) on slide and mixed together, production of gas bubbles indicates a positive reaction, but if there is no bubbles of gas it indicates negative reaction.

Indole Test: Isolate was inoculated in a bottle containing 3ml of sterile peptone water. It was incubated at 30°C for up to 48 hours. The indole was tested by adding 0.5ml of Kovac's reagent with gentle shake. It was observed for 10 minutes. Red surface layer indicates positive indole test while no red surface layer indicates a positive oxidase. No blue purple colour indicates a negative oxidase.

Oxidase Test: A piece of filter paper was placed in a clean petridish and drops of freshly prepared oxidase reagent was added. A piece of glass rod was used to collect the isolate and the isolate was smeared on the filter paper. It was observed after 10 seconds – deep or dark purple colour indicates a positive oxidase. No colour change indicates a negative oxidase.

Citrate Test: Simmon's citrate agar was prepared according to manufacturer's instruction and autoclaved at temperature of 121°C for 15 minutes. Slopes of the medium were poured into test tubes and the isolates introduced using the swab method. The tubes were incubated at 30°C for 48 hours after which it was observed. A bright blue colour indicates a positive citrate test while a green colour (no colour change) indicates a negative citrate test.

Sugar Fermentation Test: Fermentation was carried out by using 3g of the following sugars glucose, lactose, sucrose and fructose in medium which comprises of 1.0% peptone and 1.0% Nacl respectively. Three (3) drops of

0.01% of phenol red which series as indicator were added to the medium and 10mls were discharged into test tubes and sterilize at 110°C for 15 minutes.

After sterilization, it was inoculated with isolate from subculture and Durhan tubes were inverted position in each of the tubes for detection of gas production. The tubes were pluged with non absorbent cotton wool and were sealed with aluminum foil and was incubated at 30°C for 24 hours to serve as a control. Acid production from orange (Alkaline) to yellow (acid) in the fluid. Gas production was indicated by the presence of air space at the bottom of the inverted Durham tube.

RESULTS AND DISCUSSION

Nine microbial isolates including five species of bacteria and four species of fungi were isolated from the Kunu zaki samples. The bacterial isolates include *Bacillus spp., Lactibacillus spp., Pseudomonas sp, Streptococcus spp* and *Escherichia coli,* while fungi isolates were the species of *S. cerevisisas, Geotrichum spp, Aspergillus spp* and Torula yeast. Tables II to VI shows the colony count of bacteria isolated from kunu zaki stored at room and refrigeration temperature. The results indicate that fresh kunu zaki presented a low bacterial count after 24 hrs of incubation. All the isolates mentioned above in addition to streptococcus were found in kunu zaki after 2-3 days of storage at room temperature. Olasupo *et al.,* [13] reported the isolation of *Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Klebsiella spp* and *Enterococcus faecalis* from kunu zaki drink.

The fungal isolates revealed the presence of *S.cerevisiae* and *Geotrichum candidum* in fresh kunu zaki. It also showed the presence of torula yeast and Aspergillus sp in kun zaki drink after 2-3 days storage at room and refrigeration temperatures. Lichtenuralner *et al.*, [14] reported that the yeast *Saccharomyces cerevisae, 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 kunu zaki). These organisms can cause the spoilage of the beverage if not eliminated during the heating process.

Table 1: Total Plate Count of Viable Organisms (10^{-2}) of Fresh Kunu zaki

Samples	Total plate count
1	12
2	14
3	12
4	15
5	14
6	13

Table 2: Total Plate Count of viable Bacteria Organisms of Kunu zaki at Room Temperature and Refrigeration Temperature

Days	Samples for Room Temp.	Total Plate at Room Temp	Sample for Refrigeration Temp.	Total plate count at Refrigeration Temp.
1	1	36	4	29
2	2	89	5	40
3	3	261	6	73

Table 3: Total Plate Count of Fungi Isolates at Room Temperature and Refrigeration Temperature

Days	Samples for Room Temp.	Total Plate at Room Temp	Sample for Refrigeration Temp.	Total plate count at Refrigeration Temp.
1	1	107	4	63
2	2	138	5	82
3	3	151	6	97

Table 4: Standard Plate Count for Kunu at Room Temperature

Days	A	B	C	D	E
1	-	10	8	-	18
2	25	35	-	29	-
3	70	-	90	-	101
Average	31.67	15	32.67	9.67	39.67

CODE:
A - Cream colonies on Nutrient
B-Yellow colonies on CLED
C-Pink colonies on MacConkey
D-Small colonies yellow on CLED
E-Big cream colonies on Nutrient

Table 5: Standard Plate Count for Kunu at Refrigeration Temperature

Days	A	B	C	D
1	5	7	8	9
2	-	10	14	16
3	20	25	-	28
Average	8.33	14	7.3	17.67

Organism Code:

A - Cream colonies on Nutrient
B-Pink colonies on MacConkey
C-Yellow colonies on CLED
D-Big cream colonies on Nutrient

Table 6: Bacterial Mean Count and Percentage Distribution of Kunu zaki at Room and Refrigeration temperature

Code	Mean Bacterial count per ml at Room Temp.	% Distribution at Room Temp	Code	Mean Bacterial count per ml at Refrig. Temp.	% Distribution at Refrig. Temp.
A	32	24.6	A	8	17
B	15	11.5	B	14	30
C	33	25.4	C	7	15
D	10	7.7	D	18	38
E	40	30.8			
Total	130	100	Total	47	100

Organism Code:

Room temperature	Refrigeration temperature
A-*Bacillus*	A-*Bacillus*
B-*Lactobacillus*	B-*Pseudomonas spp*
C-*Pseudomonas spp*	C-*Lactobacillus*
D-*Streptococcus spp*	D-*E. coli*
E-*E. coli*	

Table 7: Standard Plate Count for Fungi Isolate at Room Temperature

Days	A	B
1	50	57
2	70	68
3	60	91
Average	90	108

Organism Code:

A-Big Cream colonies on SDA
B-Brownish colonies on SDA

Table 8: Standard Plate Count for Fungi Isolate at Refrigeration Temperature

Days	A	B	C
1	30	-	31
2	-	40	42
3	40	57	-
Average	70	97	73

Code:

A-Small cream colonies on SDA
B-Big cream colonies on SDA
C-Round black colonies on SDA

Table 9: Mean Count and Percentage Distribution of Kunu zaki at Room and Refrigeration Temperature for Fungi Isolates

Code	Mean Bacterial count per ml at Room Temp.	% Distribution at Room Temp	Code	Mean Bacterial count per ml at Refrig. Temp.	% Distribution at Refrig. Temp.
A	90	45.5	A	23	29.1
B	108	54.5	B	32	40.5
C	24	30.3			
Total	198	100	Total	89	100

Organism Code:

Room Temperature	Referigeration Temperature
A-*S. Cerevisvae*	A-*S. Cerevisvae*
B-*Geotrichum candidum*	B-*Torala Yeast*
C - *Aspergillus*	

Table 10: Morphological characteristics and gram reactions of bacteria isolated under room temperature and refrigeration temperature

Colony code	Colonial characteristics after incubation	Gram's reaction	Presumptive identification
A.	Cream with irregular shape on nutrient agar and light yellow coloured colonies on CLED	Gram positive rods	*Bacillus spp.*
B	Light yellow coloured colonies on CLED and circular cream coloured colonies on nutrient agar	Gram positive cocci	*Lactobacillus*
C	Yellow coloured colonies ranged 0.1-0.4µm in size on cysteine lactose electrolyte deficient (CLED)	-verod	*Pseudomonas spp.*
D	Small cream coloured colonies in chain on nutrient agar	Gram positive cocci	*Streptococcus spp*
E	Smooth pinkish coloured colonies on MacConkey agar and cream colour with opague on on CLED	Gram negative rods	*Escherichia coli*

Key: A-*Bacillus spp*
B-*Lactobacillus spp*
C-*Pseudomonas spp*
D-*Streptococcus spp*
E-*Escherichia coli*

Table 11: Biochemical Test identification of Bacteria Isolated at room Temperature and Refrigeration Temperature

Code	Indole	Catalylase	Oxidase	Citrate	Glucose	Fluctose	Sucrose	Lactose	Indentified organism
A	+	+	+	+	AG	AG	AG	A	*Bacillus*
B	-	-	-	+	AG	AG	A	AG	*Lactobacillus*
C	-	-	+	-	A	A	A	A	*Pseudomonas*
D	-	+	+	-	AG	A	AG	A	*Streptococcus spp*
E	+	+	-	+	A	AG	A	AG	*E. coli*

KEY
A = Acid
G = Gas
+ = Positive
- = Negative
AG = Acid/Gas

Part of kunu zaki 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 zaki suggests that it must have been contaminated after the cooking process and after the drink had cooled down. The presence of *E.coli* in kunu zaki indicates the 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. Reports indicate that toxin produced by Bacillus sp cause pneumonia and bronchopneumonia. Beside, *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.

CONCLUSION

It was observed that the bacterial and fungal load in kunu zaki samples increased with the storage conditions as well as storage period. The bacterial count in kunu zaki 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, from the result obtained in this work, it showed that the kunu zaki has a shelf-life of 24 hours at ambient temperature, which can be extended to 8 days by pasteurization at 60°C for 1 hour and storage under refrigeration temperature.

Recommendations: During the production of kunu zaki contamination could come from the syrup, fermentation vessels, storage containers, sieves used for filtration, hands of the handlers and even the polythene bags or bottles in which it was packaged for sale, therefore there is need for high degree of sanitation during the processing of the beverage to eliminate these microbial contaminants and to improve on the quality of the final product and also there is the need to employ adequate preservative measures to improve the shelf-life of the beverage.

Apart from sanitary measures, there is the need for closer monitoring of the microbial standard of the local beverage "kunu zaki" 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.

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