Acta Scientific Microbiology (ISSN: 2581-3226)

Volume 7 Issue 6 June 2024

Research Article

Microbial and Sensory Evaluation of Kunnu Blend from Millet, Sorghum and Maize

Adeoye AO[1]*, Adegbola GM[1], Arotiowa CA[2], Onifade AO[2], Akanfe JC[1] and Ojo MA[1]

[1]*Department of Food Science, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria*

[2]*Department of Food Engineering, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria*

***Corresponding Author:** Adeoye AO, Department of Food Science, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria.

DOI: 10.31080/ASMI.2024.07.1385

Received: February 12, 2024

Published: May 29, 2024

Abstract

Kunnu, a non-alcoholic beverage, has gained wide acceptance in many part of Nigeria, owing to its pleasing sweet taste. This study examine the use of three different grains to produce kunnu as a blend and separately.

The physicochemical microbial and sensory properties ware evaluated. The physicochemical analysis showed low pH value between 3.81- 4.04, Total soluble solid was 15, crude protein ranged between 0.45 - 0.67, ash content of between 0.0013 - 0.004, total titratable acidity ranged between 2.06 - 2.16. Total viable count was between 74x106 - 84x106, yeast count was between 65x106-75x106, while mould count was between 29x106- 80x106.

Among the organisms isolated ware: E. coli, and staphylococcus aureus; an indication of contamination, other isolate ware: Pseudomonas aeruginosa, Lactobacillus spp, Bacillus aureus. In kunnu samples A and B; there ware presence of Pseudomonas aeruginosa, in sample C; Lactobacillus spp. was isolated, while Bacillus aureus, Staphylococcus aureus, E coli was isolated from sample D. Sample A has the highest acceptability while sample C has the lowest value.

The use of different grains blend was prove feasible and indicated the augmentation of one grain for another in time of scarcity is therefore feasible. Training of food vendors to prevent food poisoning is important.

Keywords: Kunnu; Blend; Millet; Grains; Isolation

Introduction

Kunnu is a cereal food drink, a popular refreshing non-alcoholic beverage in Nigeria, particularly, in the North and South West [1]. It is a watery gruel cereal based non-alcoholic fermented beverage that is widely consumed in Nigeria due to its refreshing qualities. Millet (*Pennisetum glaucum*), sorghum (*Sorghum bicolor*) and maize (*Zea mays*) grains are the three principal cereals for the production of Kunnu in the past, however the use of different starchy food material have been involved in kunnu production, this resulted to different kunnu product named according to raw material used; thus we have kunnu-aya, kunnu-zaki and other such blends [2]. Kunnu contains a high proportion of moisture and carbohydrate; a minimal protein composition with different mineral contents.

In addition to the use of millet, sorghum, maize as major raw materials for production of kunnu, fortification is commonly done with the use of other cereals and starchy ingredients. On record, sweet potatoes, cocoyam, tiger nut, and other cereal like rice have been incorporated to the production of kunnu [4-6]. Supplementation of the grains with soy milk, groundnut or other protein rich plant product has been reported [7]. The additions of spice like ginger, cloves, black pepper is common practice. Kunnu is commonly used as a weaning food in some part of northern Nigeria, likewise, it is generally consumed and acceptable by both young and old people. Nowadays, kunnu is a commodity product in most market in Nigeria and beyond. It is used as breakfast food, or as refreshment, commonly available in the market place chilled as refreshing drinks during the hot afternoon [8,9].

In most cultures and localities, the production of non-alcoholic and alcoholic drinks depends on the availability of suitable raw materials, especially in Africa and Asia where locally made foods and drinks are held in high esteem because they are important source of carbohydrate and protein. Example is the production of Ogi from maize, burukutu from guinea corn, so is Kunnu produced from cereals [8]. It is a simulated product of "malt" drink made from millet and sorghum though sometimes maize could be used. It is a popular dessert drink, indigenous to the Northern part of Nigeria. But with increase in urbanization and socialization, its popularity and acceptance among people from other ethnic groups continued to increase by the day [10].

Products from kunnu vary depending on raw materials used, peoples' taste and cultural preference. Kunnu as one of the common traditional products popular among people in Northern Nigeria, the product is gaining acceptability across the country. Increasing knowledge of its value in comparison with other beverages in terms of its natural sour, sweet taste, addition of spices that are medicinal has increased its consumption [10,11]. Study by some research works show that Kunnu is rich in carbohydrate, B-Vitamins and minerals but low in protein, hence, blend of other protein rich additives such as soy milk, tiger-nut are incorporated to improve the nutrient [5,7].

Generally in the production of Kunnu, different methods are employed. In one of the methods, the cereal to be used (maize, millet or sorghum) is steeped in water for 24hrs, wet milled and sieved. The slurry/sediment obtained is divided into two unequal portions; a portion of the divided slurry is cooked and then mixed with the other portion which is uncooked, this uncooked portion serves as the source of inoculum and is allowed to ferment for 8-10hrs [12,13]. In another method of production, the cereal grain is malted, dried, ground, and then mixed with the uncooked portion and stirred vigorously and allowed to ferment. The hydrolytic enzyme (amylase) in the malted cereal aids in digesting the thick slurry thereby converting the complex carbohydrate into simple sugars (the process of hydrolysis that is caused by the enzyme) [14]. After it has undergone hydrolysis, the final product gotten from it is usually sweet. Kunnu is of great importance nutritionally to humans especially people suffering from diabetic ailments, according to research conducted by Awogbenga, on the glycemic effect of Kunnu on the blood glucose in non-diabetic persons, high energy drinks (glucose) suggesting that it would be beneficial to diabetic patients [15,16].

Materials and Methods

Materials

Millet, maize and Sorghum, samples with other ingredients such as spices (ginger, alligator pepper, black pepper etc.) were purchased from Wazobia Market Ogbomoso. Equipment and apparatus were obtained from the Food Processing Laboratory (Owodunni Processing Laboratory) of the department of Food science and Engineering Laboratory of Ladoke Akintola University of Technology, Ogbomoso, Oyo state. Sorghum, maize and millet were used to prepare the Kunnu seperately, after which the three samples were blend together for Kunnu production as the common grain for kunnu production [17].

Production of Kunnu from millet, maize, sorghum and blend

The Kunnu from the appropriate grains (Millet, Maize, and Sorghum, blend) four production samples from millet, maize and sorghum respectively was prepared separately, and equal proportion of each of the three grains were blend for kunnu production. As shown in Figure 1 bellow, each batch of grain sample was first sorted to remove extraneous materials such as stones, metallic objects, chaff etc. [18]. The grain in each batch was malted after tempering. The same processing method was adopted for Sorghum, Millet, Maize, and blend kunnu production. The grains in the blend were in ratio 50:50:50, of 600g, were then soaked in 1000 ml of water for a 24 h before milling of the soaked grain together with ginger to form a smooth paste. This paste was then divided into two parts of 1:3 v/v. the lager part was placed in a stainless vessel and homogenized. Double volume of boiling water was poured on it to gelatinize, then it was stirred to give a thick mixture, the other portion of the paste was then added to this mixture and it was stirred for about 5 min. Stirring resulted to a smooth Kunnu paste. Liquefaction and saccharification of the mash was gotten and the smooth paste was then cooled to 45^0 C it was subsequently bottled and preserved under refrigeration [19,20].

Culture media and preservation

The media used are nutrient agar, potato dextrose agar, yeast extract agar, MRS agar and Mac-Conkey agar. The media was prepared according to manufacturer's instruction. Distinct plate colonies was picked on the plates and inoculated on an already pre-

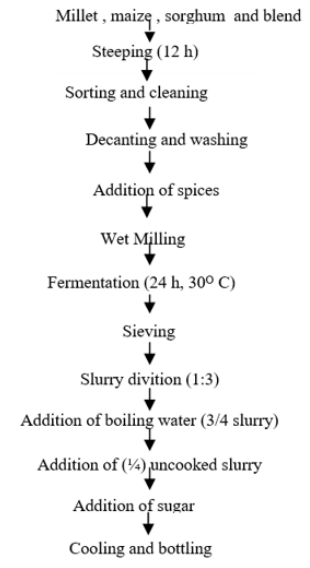

Figure 1: Flow chart for kunnu production.

pared sterilized nutrient agar, potato dextrose agar, yeast extract agar, MRS agar and Mac-Conkey agar slant was incubated at 37^{0} C for 24hrs.Slants with visible growth were then preserved in the refrigerator at 4^{0} C

Isolation procedure

One ml of each Kunnu sample was serially diluted. 1ml of an appropriate dilution was inoculated on nutrient agar, potato dextrose agar, yeast extract agar, MRS agar and Mac-Conkey agar and the plates were incubated for 24hrs, wire loop was used to pick the isolate from the plate and was streaked on freshly prepared sterile nutrient agar, potato dextrose agar, yeast extract agar, MRS agar and Mac- Conkey agar plates, then incubated for 24hrs at 37^{0} C in order to get pure cultures. Pure cultures were then stored in the refrigerator at 4^{0} C. The routine laboratory method of [16] was to characterize different isolates. The isolates were identified using their microscopic, cultural and physiological and biochemical characteristics [22].

Biochemical characterization of isolates

Various tests were carried out on bacteria isolates for possible identification. A fresh 24-28h old culture was used for every biochemical test. All the sterilization was done by autoclaving at 121^{0} C for 15minutes and incubation was done at 37^{0} C unless otherwise stated. Characterization of the bacteria isolates to identify the organisms present, colonial characteristic of the bacteria isolates were determined using parameters such as size, elevation, pigment, surface, opacity, edge and shape. Cellular and biochemical characteristics of the isolates were determined according to Adeoye., *et al.* (2018). Identification of the bacterial isolates was carried out by standard microbiological methods. The characterizations of the isolates were based on gram staining, morphological and cultural characteristics couple with relevant biochemical test [24,25].

Physicochemical analysis

Physicochemical analysis carried out include: Moisture content analysis (Total Solid analysis) Ash content analysis, Protein analysis according to [26].

Sensory evaluation of the samples

The sensory evaluation was carried out by 18 panellists. The panellists assessed the product as regards peculiar attributes of kunnu. The attribute were measured using the 7 point hedonic scale. The scale ranged from like extremely (1) to dislike extremely (9). analysis of variance was computed for each sensory attribute. panellist drawn from Ladoke Akintola University of Technology, based on their familiarity with the product was used for the evaluation. The parameters that were evaluated include Colour, Taste, Flavour, Sweetness, Smoothness and Overall acceptability. The sample was served in a transparent plastic cup at room temperature (25^{0} C), the sample was presented to the panelists at random and one at a time [27].

Results and Discussion

The result of physicochemical analysis of kunnu sample: A, B, C, and D were presented in Table 1 as shown bellow. Sample A is produced from millet; with a pH of 4.04 as the highest pH, the lowest was 3.82 from sample B, the total soluble solid was 15 g from all the samples, the highest crude protein was 0.67 from millet; the lowest was 0.45from sample C. for the ash content; the lowest was 0.002 from sample B, the highest was from sample D; which was the blend of the three grains (ABC) [11]. The lowest total titratable acidity was obtained in sample C, while the highest was obtained from sample B. The physicochemical characteristics of Kunnu samples as shown, the pH ranged between 3.81 -4.04, The pH increased due to the fermentation process involved, malting of the grain improve the level of fermentable sugar which resulted to high acidity by the relevant micro-organism. These observations appear to be in agreement with the previous work of [28] who reported

the production of acid from sugar by various metabolisms from microorganism such as lactic acid bacteria, acetic and butyric acid bacteria [3,11].

The crude protein (c. p.) content ranged between 0.48% - 0.67%. Sample A had the highest protein content while sample B had the lowest protein content. The protein content of Kunnu enhances its value and makes it more nutritious than any of the carbonated soft drinks which seem to containe no protein. The total solid of all the samples were uniform (15%), the same value for total solid may be due to the fact that the similar production method was followed for the four samples. The moisture content of all the samples were also deduced to be 85 % a uniform production process been the reason and kunnu been a beverage is water based food product. The ash content ranged between 0.0013% - 0.004% sample D had the highest ash content while sample B had the lowest ash content, the significant of ash is that it aid assimilation of nutrients and aid digestion. The Total Titratable acidity of the samples ranged between 2.06% - 2.16%. The variation may be traced to the presence of some bacteria which help in fermentation of Kunnu [29].

S/no	Sample	pH	TSS	C.P.	Ash	TTA
1	A	4.04	15	0.67	0.002	2.10
2	B	3.81	15	0.48	0.0013	2.16
3	C	3.90	15	0.45	0.003	2.06
4	D	3.82	15	0.53	0.004	2.08

Table 1: Physicochemical characteristics of kunnu beverages.

KEY. TSS= Total Soluble Solid, C.P =Crude protein (%), TTA= Total titratable Acid,

A represent Kunnu produced from Millet.

B represent Kunnu produced from sorghum.

C represent Kunnu produced from Maize.

D represent Kunnu produced from Millet, Maize and Sorghum blend.

The microbial count results for various Kunnu samples are shown in Table 2. The total viable count ranged between 74 x 10^6- 84 x 10^6, sample B (Maize) had the highest total viable count while sample D had the lowest total viable count. The yeast count of the samples ranged between 65 x 10^6- 75 x 10^6, sample B (Maize) had the highest yeast count while sample D had the lowest yeast count and the total mould count ranged between 29 x 10^6- 80 x 10^6, sample B had the highest mould count while samples A and D had the lowest mould count. In kunnu samples A and B; there is presence of *Pseudomonas aeruginosa,* in sample C; *Lactobacillus spp*. was isolated, while *Bacillus aureus, Staphylococcus aureus, E coli* was isolated from sample D kunnu. The distribution of the isolates in the kunnu samples is shown in Table 3.

Sample	TVC	Yeast	Mould
A	75 x 10^6	68 x 10^6	29 x 10^6
B	79x 10^6	73 x 10^6	38 x 10^6
C	84 x 10^6	75 x 10^6	80 x 10^6
D	74 x 10^6	65 x 10^6	29 x 10^6

Table 2: Microbial counts of the samples.

A represent Kunnu produced from Millet.

B represent Kunnu produced from Sorghum.

C represent Kunnu produced from maize.

D represent Kunnu produced from Millet, Maize and Sorghum blend.

The presence of some of the bacteria may be due to contamination from the substrate, the environment where the samples were produced and the personal hygien of the personnel, state of the processing of the sample and water source [19,22,23]. Therefore it can be inferred from the study that Kunnu produced at the household level in different locations may be contaminated by microorganisms mostly during the processing stage due to many factors such as personal hygiene and source of raw materials, of which source water and equipment used in the production are inclusive factors [5,30]. The microbial isolates as obtained in this study is commonly implicated in many previous work, [5] similarly obtained these organisms in kunnu product.

The phenotypic and biochemical characteristics which aided the identification of bacteria is shown in Table 4. Five species of bacteria were isolated and identified from fermented "Kunnu" as shown as probable organisms isolated. The micro-organisms isolated were identified as *Bacillus aureus, Staphylococcus aureus, Lactobacillus spp, E coli and Pseudomonas aeruginosa.*

The changes that occurred in the sensory attributes of "Kunnu" samples are shown in Table 5 with respect to appearance, flavor,

Isolates	Cultural characteristics	Gram staining Reaction	Motility test	Spore staining	Capsule staining	Catalase test	Methyred test	Starch hydrolysis	Citrate utilization	O_{2} reaction	Lactose reaction	Glucose reaction	Sucrose reaction	Maltose reaction	Fructose reaction	Identification
D1	Raised, dull translucent cream, lobate	Gram +, chain rod	-	+	+	+	+	+	+	AE	-	AG	A	AG	AG	*Bacillus cereus*
D2	Raise, opaque, smooth, creamy white, entire.	Gram+, cocci	-	-	-	+	-	+	+	FAN	AG	A	A	A	+	*Staph. aeurus*
D3	Raised, translucent, rough, creamy Lobate.	Gram- single rod	-	-	-	-	-	-	-	AN	A	A	-	-	-	E. *coli*
B1	Raised, opaque, smooth, creamy white, lobate	Gram +, cluster rod	-	-	-	-	-	-	-	FAN	A	A	A	A	AG	Lactobacillus spp
A1	Raised, translucent, smooth,Yellowish cream, entire.	Gram +, rod chain.	-	-	-	+	-	+	+	AE	-	A	-	-	-	*Pseudomonas auruginosa*
C1	Raised, translucent, smooth, Yellowish cream, entire.	Gram +, rod chain.	-	-	-	+	-	+	+	AE	-	A	-	-	-	*Pseudomonas auruginosa*

Table 3: Cultural Identification.

KEY= -, negative; +, positive; AE, Aerobic; AN, Anaerobic; A, Acid production; FAN, Facultative anaerobic, AG, Acid and gas production

Bacteria isolates	A	B	C	D
Staphylococcus aureus	-	-	-	+
Bacillus cereus	-	-	-	+
E coli	-	-	-	+
Lactobacillus spp.	-	-	+	-
Pseudomonas aeruginosa	+	+	-	-

Table 4: Types of bacteria isolated from kunnu.

+ = Detected. = Not Detected. A represent Kunnu produced from Millet. B represent Kunnu produced from Sorghum. C represent Kunnu produced from Maize. D represent Kunnu produced from Millet, Maize and Sorghum.

sweetness and overall acceptability. It was inferred from the sensory evaluation that Sample A (Kunnu produced from Millet) gives the most pleasant taste and it is widely accepted by all the taste panelist, this is followed by the Kunnu produced from Sorghum follows in acceptability but the colour of this Kunnu had lower acceptability was not as favorable as Millet, while Kunnu produced from Maize was the least acceptable, however on the overall, there assessment showed that all the product are above average acceptability. The level of sensory attributes was as a result of sour taste, low level of acidity present in the samples analyzed, which gives kunnu characteristic appealing taste. All the samples remain acceptable based on sensory assessment while there microbiological result was of great concerned [27,32].

Samples	Appearance	Flavour	Sweetness	Overall acceptability
A	7.06^{a}	6.56^{a}	6.61^{a}	6.61^{a}
B	6.39^{b}	6.44^{a}	6.39^{a}	6.22^{ab}
C	6.22^{b}	5.72^{b}	6.61^{a}	5.94^{b}
D	6.22^{b}	6.06^{ab}	6.33^{a}	6.44^{ab}

Table 5: Sensory evaluation for Kunnu samples.

A represent Kunnu produced from Millet, B represent Kunnu produced from Sorghum, C represent Kunnu produced from Maize, D represent Kunnu produced from Millet, Maize and Sorghum.

Conclusion

This research study has shown the use of different grains for production of kunnu, and possibility of blend of grains. As kunnu is one of the locally made beverages that people valued and highly patronized as a commodity in the local market, with increasing demand, not only for its low price compared to other beverage, but also for the spices addition that are beneficial to health. Application of blend of grains as demonstrated shows that there will always be alternative raw materials that can be used for the production in situation of scarcity of particular grain. Moreover, microbial isolated indicated different type of microorganisms involved in fermentation. The isolate showed there is need for caution, the presence of human pathogenic organisms such as *E. coli*, *Staph. aureous* as an indication organisms which calls for increase cleanliness and improve personal hygiene on the part of kunnu producer. In addition, due to the relatively low temperature of processing of kunnu, it may be concluded that every stage is sensitive and cleanliness and good personal hygiene must be observed to prevent food contamination.

Acknowledgement

We acknowledged the use of laboratory facilities at the Department of Food Science and Engineering, LAUTECH, Ogbomoso.

Conflict of Interest

There is no conflict of interest in what so ever.

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