ISSN No:-2456-2165

Physicochemical Characteristics and Bacteria Quality of Kunu (A Non-Alcoholic Beverage) Consumed in Yola, Adamawa State Capital, Nigeria

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Abstract:- The physicochemical properties (pH, Protein content, Carbohydrate, Total Soluble Solid and Titratable Acidity) as well as the bacteria quality of 15 different kunu samples consisting of 5 each of kunu-zaki, kunu-aya and kunu-tsamiya were determined . From the study, the pH range of kunu-zaki was 5.0 to 6.9, that of kunu-aya was 3.0 to 4.2 and kunu-tsamiya was 2.0 to 2.1 respectively. The % protein content of kunu-zaki ranged from 2.9 to 3.6, that of kunu-aya 3.7 to 4.1 and kunu-tsamiya 2.7 to 3.2 respectively while the % Carbohydrate content of kunu-zaki ranged from 3.3 to 4.5, that of kunu-aya 2.6 to 5.4 and kunu-stamiya 2.6 to 5.6. Similarly, the Total Soluble Solid (Brix) of kunu-zaki ranged from 3.8 to 5.2, kunu-aya 2.5 to 6.3 and kunu-tsamiya 4.0 to 9.3 respectively. Also, Titratable Acidity of kunu-zaki ranged from 0.243 to 0.594, kunu-aya 0.035 to 0.045 and finally kunu-tsamiya 0.245 to 0.873. The bacteria load was highest for kunu-zaki with 9.6×10^9 while the least was from kunu-tsamiya with 1.3×10^9. The bacteria isolated from the samples were *E.coli, Staphylococcus aureus, Bacillus* sp , *Enterococcus* sp and *Pseudomonas* sp. *E. coli* and *Bacillus* sp were isolated from kunu-zaki, kunu-aya and kunu-tsamiya while *Enterococcus* sp was isolated only from kunu-aya and kunu-tsamiya. Similarly, *Staphylococcus aureus* was isolated from kunu-aya and kunu-zaki respesctively while *Pseudomonas* sp was isolated from kunu-zaki and kunu-tsamiya. *Enterococcus* sp had the highest percentage occurrence of (55%) in kunu-tsamiya, followed by *E. coli* and *Bacillus* sp with (40%) each in kunu-aya and kunu-zaki as well as (30%) each from the same samples. *Enterococcus* sp, *E. coli* and *S. aureus* had (20%) each from kunu-aya, kunu-tsamiya and kunu-zaki respectively. Similarly, *Bacillus* sp had (15%) from kunu-tsamiya, *S. aureus* had (10%) from kunu-aya while *Pseudomonas* sp had(10%) each from kunu-tsamiya and kunu-zaki respectively. The presence of potential diseases causing bacteria in all the samples is a clear indication that the hygiene practices are not usually observed during production and handling processes and thus not fit for human consumption.

Keywords:- *Beverages, Kunu-Zaki, Kunu-Aya, Kunu-Tsamiya and Physicochemical.*

I. INTRODUCTION

Kunu is a nourishing non-alcoholic beverage widely consumed in Nigeria. There is no standardized method for its preparation thus production practices differ amongst retailers (Anumudu and Anumudu, 2019). All cereals-based non-alcoholic beverages are generally known as Kunu in Hausa and are common in almost all the Northern States of Nigeria (Terna *et al*., 2002). It is prepared from grain such as Sorghum, Millet, Maize or Wheat (Gaffa *et al.*, 2002). Apart from these cereals, kunu has shown to be produced from tigernuts as well as Guinea corn and rice (Umaru *et al.*, 2014). Because kunu is prepared in traditional method, the ingredient concentrations are neither quantified nor standardized (Aboh and Oladosu, 2014). 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 (Ekanem *et al.*,2018). As earlier mentioned, kunu is prepared using cereals, although other minor ingredients are included and the differences are due to the ingredients used in the preparation. Thus, in the traditional processing of kunu-zaki, sweet potatoes (*Ipomoea batates*), ginger (*Zingiber officinale*), cloves (*Eugenia aromatic*), water and black pepper (*Piper guinese*) are often used as additional ingredients to the basic ingredient; millet (Bede *et al.*, 2015). Similarly, in the production of kunu-aya, dates, coconut, ginger and water are often used as additional ingredients to the basic ingredient; tiger nuts (Zakari, 2021). Kunu-tsamiya is produced using ingredients such as ginger, tamarind, cloves, sugar and water in addition to the basic ingredient; millet (Sani, 2022).

ISSN No:-2456-2165

Kunu contains lactic acid bacteria (LAB) including *Lactobacillus* species, *Streptococcus* species and *Leuconastic* species which can cause food borne diseases (Osuntogun and Aboaba, 2004). Bacteria species such as *Staphylococcus*, *Pseudomonas*, *Bacillus* and fungi species such as *Penicillium*, *Aspergillus*, *Trichoderma* and yeasts have been isolated from processed kunu (Osuntoki and Korie, 2010). The presence of these organisms in small number could render a beverage unsuitable for human consumption (Amusa and Odunbako, 2009).

Kunu is acceptable to people of all works of life in Nigeria and is being served at home and public places as food appetizer, refreshing drink and complementary food for infants. Kunu is prepared from different cereals and carries different names partly as a result of the nature of the additional ingredients added to the cereals during preparation. Consequently, the aim of this work is to determine the physicochemical characteristics and the bacteria quality of Kunu-zaki, Kunu-aya and Kunu-tsamiya consumed in Yola town, the capital city of Adamawa state, Nigeria.

II. METHODOLOGY

- *Sample Collection*

A total of 15 samples consisting five each of kunu-zaki, kunu-aya and kunu-tsamiya were purchased in sterile bottle containers at Jimeta Modern Market, Yola, Adamawa State, Nigeria. The samples were labelled and held at 4^0C by placing in refrigerated coolers and were conveyed in an ice pack cooler and transported to the Laboratory for analysis.

- *Physicochemical Analysis of the Kunu Samples*

The protein content was determined using formol titration method as described by Egan *et al* (1981) and reported by (Tyokusa and Owuama 2018) while the carbohydrate content was determined as reported by (Terna *et al*., 2007). The total soluble solids (TSS) were determined using refractometer (REF 503) (Cheesbrough, 1987). The pH of each sample was measured with a pH metre (pHep HANNA instruments). The titratable acidity (TTA) was determined by titration of 10ml of the sample against 0.1N NaOH to phenolphthalein end point (Bede *et al*., 2015).

- *Isolation of Bacteria from the Samples*

The bacteria load of the kunu samples were determined following the method of (Chengula *et al.*, 2014) and (Ogodo *et al.*, 2016) and the bacteria isolates were identified using colony morphology, microscopic examination and biochemical characteristics as described by (Cheesbrough, 2000 and 2002).

III. RESULTS

- *Results of the Physicochemical Characteristics of the Samples*

Table 1 The pH, Protein Content, Carbohydrate Content, Total Soluble Solids (TSS) and Titratable Acidity (TA) of the Samples

Samples	pH	Protein content (%)	Carbohydrate (%)	TSS (Brix)	TTA (%)
KZ1	6.8	3.5	4.1	3.9	0.594
KZ2	6.9	3.4	4.0	4.6	0.468
KZ3	5.0	3.6	4.3	4.0	0.243
KZ4	5.1	2.9	5.3	3.8	0.360
KZ5	6.0	3.0	4.5	5.2	0.594
KA1	3.1	3.9	2.3	6.3	0.035
KA2	3.0	4.0	4.1	5.0	0.037
KA3	4.0	3.8	3.3	4.3	0.045
KA4	4.2	4.1	2.6	2.5	0.036
KA5	3.1	3.7	3.4	3.4	0.039
KT1	2.0	3.2	2.6	4.8	0.425
KT2	2.1	2.9	3.0	5.3	0.873
KT3	2.1	2.7	4.5	4.0	0.245
KT4	2.0	3.0	3.3	6.5	0.523
KT5	2.0	2.8	5.6	9.3	0.361

Key: KZ= Kunu-zaki, KA= Kunu-aya and KT= Kunu-tsamiya

➢ *Results of the Bacteria Analysis of the Samples*

Table 2 Total Bacteria Count of the Samples

Samples	Bacterial count (CFU/ml)
KZ1	2.1×10^9
KZ2	9.6×10^9
KZ3	3.6×10^9
KZ4	4.7×10^9
KZ5	5.0×10^9
KA1	2.0×10^9
KA2	8.2×10^9
KA3	9.5×10^9
KA4	8.1×10^9
KA5	3.2×10^9
KT1	6.3×10^9
KT2	5.2×10^9
KT3	3.5×10^9
KT4	2.1×10^9
KT5	1.3×10^9

Key: KZ= Kunu-zaki, KA= Kunu-aya and KT= Kunu-tsamiya

Table 3 Distribution of Bacteria Isolates

S/N	Bacterial isolates	KZ	KA	KT
1.	*E.coli*	+	+	+
2.	*Bacillus* sp.	+	+	+
3	*Staphylococcus aureus*	+	+	-
4.	*Enterococcus sp*	-	+	+
5.	*Pseudomonas* sp	+	-	+

KEY: KZ= Kunu- zaki, KT= Kunu- tsamiya, KA = Kunu- aya, + = present, - = absent

➢ *Percentage (%) Occurrence of the Bacteria Isolates from the Samples*

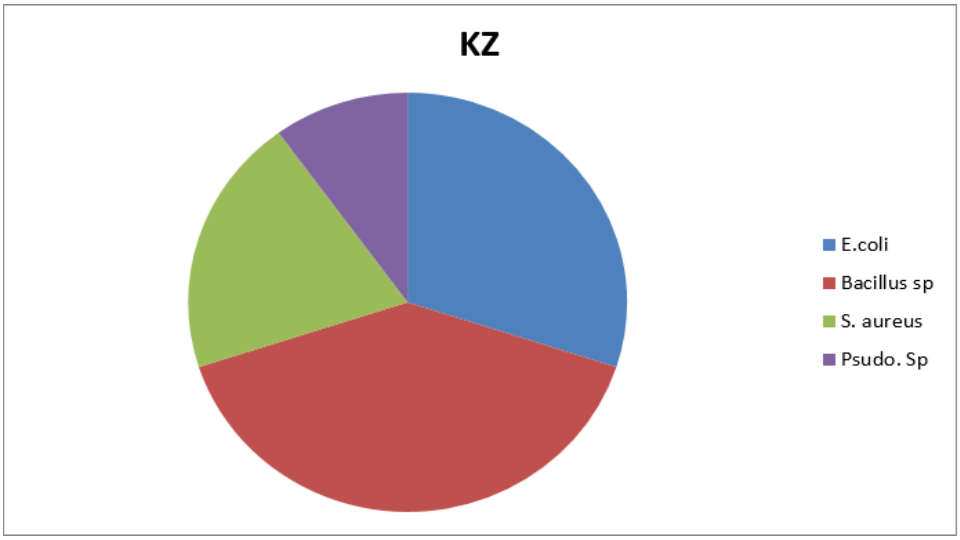

Fig 1 Percentage (%) Occurrence of Bacteria Isolates in Kunu-zaki Samples

ISSN No:-2456-2165

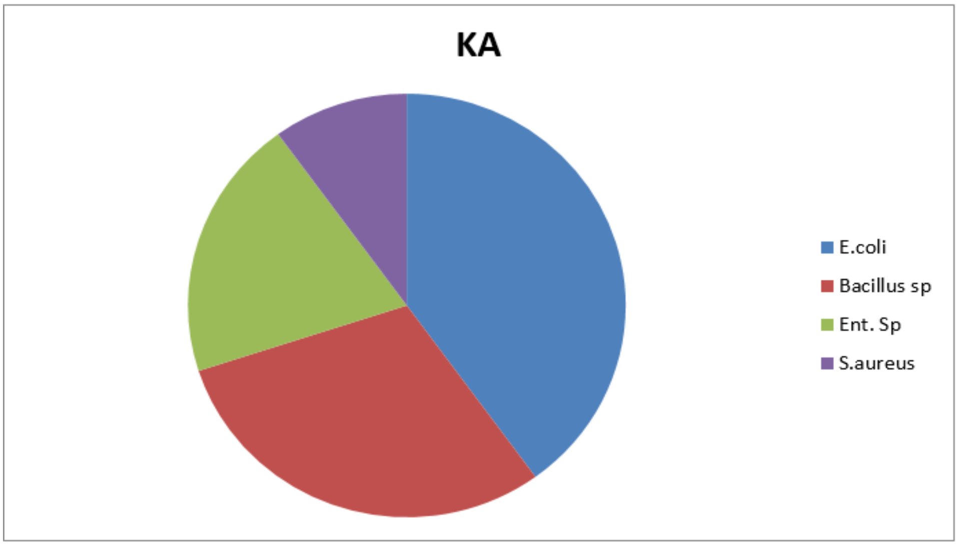

Fig 2 Percentage (%) Occurrence of Bacteria Isolates in Kunu-aya Samples

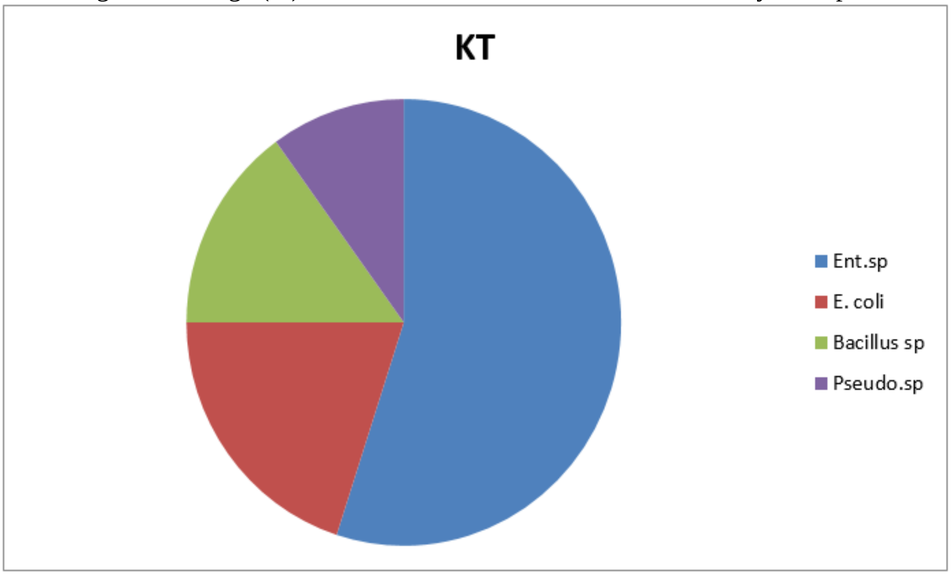

Fig 3 Percentage (%) Occurrence of Bacteria Isolates in Kunu-tsamiya Samples

IV. DISCUSSION

The physicochemical characteristics of the samples studied were pH, protein content, carbohydrate content, total soluble solids and titratable acidity (Table 1). The pH range of the samples is from 2.0 to 6.9. Generally, kunun-zaki samples had higher pH values, 6.8, 6.9. 5.0. 5.1 and 6.0, followed by kunun-aya with 3.1, 3.0, 4.0, 4.2 and 3.1 and lastly kunun-tsamiya with 2.0, 2.1, 2.1 2.0 and 2.0. Similar pH results from kunu have been reported by some researchers. Terna *et al* (2002), reported the range of 3.12 to 5.46 while Bede *et al* (2015), reported a pH range of 4.0 to 4.6. Also Nduka *et al* (2018), reported a pH range of 5.37 to 5.76.

The acidic pH of kunu-aya and kunu-tsamiya are largely due to the presence of *Enterococcus* sp, a lactic acid bacterium (Table 3). Lactic acid bacteria are a group of Gram positive, non- spore forming, facultative anaerobes, catalase negative cocci or rods which produce lactic acid as the major end product of fermentation of carbohydrates (Axelson, 1998). It is likely that the lactic acid bacteria present in these kunu samples converted the carbohydrates into lactic acid thus making the samples more acidic. The extreme acidic pH values of kunun-tsamiya could be due to the addition of the ingredient called tamarind fruit extract. Tamarind fruits contain acid called tartaric acid (Muzaffar and Kumar, 2017).

From the study, the % protein content of kunun-zaki ranged from 2.9 to 3.6, that of kunun-aya 3.7 to 4.1 and kunun-tsamiya 2.7 to 3.2. This is similar to Terna *et al* (2002), that reported % protein content of 2.5 to 4.0 from different types of kunu made from Maize, Millet, Sorghum and Rice. Ibegbulem and Chikezie (2014), also reported a protein content of 10.6g/l and 3.0g/l of kunu-zaki produced from sprouted guinea corn and unsprouted guinea corn. Also, Noah and Yusuf (2020), reported % protein content of 4.21, 4.39, 4.86, 4.87 and 8.86 obtained from five samples of kunu-zaki produced from sorghum and date fruit. Similarly, Musa and Hamza (2013), reported a % protein content of 2.9, 3.2 and 2.6 from three samples of kunu-aya.

The % carbohydrate of the kunu samples from this study ranged from 2.3 to 5.6. Terna *et al* (2002), reported a range of 2.6 to 7.9 from different types of kunun-zaki. Similarly, Nduka *et al* (2018), reported a range of 5.54 to 7.74 while Noah and Yusuf (2020), however reported higher values in the range of 10.25 to 13.57.

The Total Soluble Solids (TSS) values of the samples from the study range from 2.5 to 9.3. Generally, kunu-tsamiya samples have higher TSS values followed by kunu-aya and lastly kunu-zaki. Abiodun *et al* (2017), reported TSS of 6.15, 6.42, 6.44, 6.95, 7.45 and 7.85 from kunu-zaki samples sweetened with black velvet tamarind. Total Soluble Solids of 9.0,9.50, 10.05 and 9.05 from 100% kunu made from millet, 90% kunu +10% raw walnut juice, 90% kunu + 10% roasted walnut juice, 90% kunu + 10% boiled walnut juice respectively have been reported (Arise *et al*., 2023). Similarly, Ibrahim *et al* (2012), reported TSS of different kunu samples made from sorghum as 4.95, 4.65, 5.45, 6.05, 7.05 and 8.25 respectively.

Also from this study, the titratable acidity values of the samples ranged from 0.035 to 0.873. Bede *et al* (2015) however, reported a range of 0.387 to 0.460 from 4 different samples of kunu-zaki. Relatedly, Nduka *et al* (2018), reported a range of 0.23 to 0.28 from kunu-zaki samples while Terna *et al* (2002), reported a range of 0.02 to 0.11from kunu samples.

Table (2) contained the results of the total bacteria counts of the samples which ranged from $2.1x10^9$ to $9.6x10^9$ colony forming unit per milliliter (cfu/ml) for kunu-zaki, $2.0x10^9$ to $9.5x10^9$ cfu/ml for kunu-aya and $1.3x10^9$ to $6.3x10^9$ cfu/ml for kunu-tsamiya respectively. This is an indication that the kunu-zaki samples were more contaminated followed by kunu-aya and lastly kunu-tsamiya. The source of contamination could be from the water used in the preparation, the cereals used or other ingredients added such as tamarind fruits, potatoes etc. Furthermore, the hygiene of the producers or the vendors leaves much to be desired as it is mostly produced and handled by the locals who have limited knowledge about basic hygiene. According to Makut *et al* (2013), the high microbial counts of kunu may to a large extent be attributed to lack of effective precautions on hygiene practice in handling procedures during processing of the beverage. The high bacteria count in kunu-aya agreed with the findings of Aleru *et al* (2017), who reported a total bacteria count within the range of $1.08x10^4$ to $9.8x10^4$ cfu/ml on Nutrient agar and $0.2x10^4$ to $8.9x10^4$ cfu/ml on MacConkey agar from nine (9) different kunu zaki samples sold in Port Harcourt, Rivers state, Nigeria. Anumudu and Anumudu (2019), reported a total heterotrophic bacteria count in the range of $1.4x10^4$ to $4.5x10^4$ cfu/ml from six (6) different samples of kunu zaki. The bacteria counts of the Kunun-zaki sold in ten different locations in Keffi metropolis, Nigeria, range from 9.1×10^8 to 2.6×10^8 cfu/mL (Makut *et al*., 2013). The bacteria count in kunu-aya from this study is similar to some reports. Umar *et al* (2014), reported a total bacteria count range of $0.22x10^5$ to $14.40x10^5$ for four (4) different samples of kunu-aya sold at Umaru Musa Yaradua University, Katsina campus. Similarly, the bacteria counts of the 25 samples of kunu-aya consumed by students of Nassarawa state University, Keffi, Nigeria revealed the total viable counts which ranged from 1.2 to 12.0×10^4cfu/ml (Opeyemi and Obuneme, 2020). Wakil *et al* (2004), reported a total bacteria count of 10^7 cfu/ml in kunu tsamiya after 72 hr of fermentation.

From this study, a total of five bacteria species were isolated from the samples; *E. coli*, *S. aureus*. *Enterococcus* sp, *Bacillus* sp, and *Pseudomonas* sp. (Table 3). *E. coli* and *Bacillus* sp were isolated in all the samples (kunu-zaki, kunu-aya and kunu-tsamiya). *Enterococcus* sp was isolated in kunu-aya and kunu-tsamia only. Similarly, *S.aureus* was isolated in kunu-zaki and kunu-aya only, just like *Pseudomonas* sp was isolated in kunu-tsamiya and kunu-zaki only. The presence of *E. coli* which is a coliform in all the samples is an indication of human contamination from the water and containers used in the preparation of the beverages. Amusa and Ashaye (2009), reported that, the presence of coliforms such as *Esherichia coli* in hawked Kunun-zaki was as a result of contaminated water, containers, as well as dirty environment where the kunun-zaki were being processed and hawked. All the bacteria isolates from this study except *Enterococcus* sp are potential pathogens. *E. coli* is a food borne pathogen that causes foodborne illness (Kornacki and Marth, 1982) while *S. aureus* is associated with food poisoning (Jonathan Gotfried, 2023). *Bacillus* sp is associated with self-limited food poisoning (Carmelita *et al*., 2014) while *Psudomonas* sp causes pneumonia, endocarditis, urinary tract infection etc (Iglewski, 1996).

The isolation of different bacteria species from different samples of kunu is in agreement with some researchers. Four species of bacteria namely, *Escherichia coli, Enterobacter aerogenes, Staphylococcus aureus* and *Streptococcus* sp were isolated from kunu-zaki (Makut *et al*,.2013). Okechukwu *et al* (2021), however, isolated two species of bacteria namely *Streptococcus* sp and *Bacillus subtilis* from kunu zaki. Also, Ogbonna *et al* (2011), isolated four bacteria species namely *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* sp and *Shigella* sp from kunu-zaki. In a similar development, a total of nine (9) bacteria genera including *Staphylococcus*, *Escherichia*, *Enterobacter, Proteus, Citrobacter*, *Serratia*, *Lactobacillus*, *Salmonella* and *Streptococcus* were isolated from kunu-zaki (Anumudu and Anumudu, 2019).

From the study also, four (4) bacteria species were isolated from kunu-aya which were *E. coli*, *S. aureus*. *Entreococcus* sp and *Bacillus* sp. Akubuenyi and Sylvanus (2022), isolated three (3) genera of bacteria; *Staphylococcus*, *Streptococcus* and *Micrococcus* from kunu-aya. Agbo and Tahir (2018), however, isolated six (6) bacteria species from kunu-aya namely *Staphylococcus aureus, Bacillus subtilis, Escherichia coli Streptococcus* sp, *Salmonella* sp and *Shigella* sp. Similarly, Opeyemi and Obuneme (2020), reported the isolation of five species of bacteria from kunu aya namely *E. coli*, *S. aureus Salmonella* sp, *Klebsiella* sp and *Proteus* sp.

Four (4) bacteria species were isolated from samples of kunu-tsamiya from the study. These includes *Enterococcus* sp, *E. coli*, *Bacillus* sp and *Pseudomonas* sp. Wakil *et al* (2004), also reported the isolation of six (6) genera of bacteria, namely *Bacillus*, *Klebsiella*, *Pediococcus*, *Corynebacterium* and *Escherichia* from kunu-tsamiya.

The percentage occurrence of the bacteria isolated from the samples are presented in figures 1, 2 and 3 respectively. *Bacillus* sp (40%), *E. coli* (30%), *S. aureus* (20%) and *Psudomonas* sp (10%) were isolated from kunu-zaki. Similarly, *E. coli* (40%), *Bacillus* sp (30%), *Enterococcus* sp (20%) and *S. aureus* (10%) were isolated from kunu-aya while *Enterooccus* sp (55%), *E.coli* (20%), *Bacillus* sp (15%) and *Psudomonas* (10%) were isolated from kunu-tsamiya respectively. *Enterococcus* sp have the highest percentage occurrence of (55%) in kunu-tsamiya, followed by *E. coli* and *Bacillu*s sp with (40%) each in kunu-aya and kunu-zaki as well as (30%) each from the same samples. *Enterococcu*s sp, *E. coli* and *S. aureus* have (20%) each from kunu-aya, kunu-tsamiya and kunu-zaki respectively. Similarly, *Bacillus* sp have (15%) from kunu-tsamiya, *S. aureus* have (10%) from kunu-aya while *Pseudomonas* sp have (10%) each from kunu-tsamiya and kunu-zaki respectively. Similar results are reported from some researchers. Akubuenyi and Sylvanus (2022). isolated *Staphylococcus* with (50%), (16.7%) *Streptococcus*, (16.7%) and *Micrococcus* (16.7%) from kunu-aya. According to Makut *et al* (2013), the most predominant bacteria isolates from kunu- zaki in terms of occurrence was *Escherichia coli* (100%) followed by *Enterobacter aerogenes* (70%), *Staphylococcus aureus* (30%) and *Streptococcus* sp. (10%).

V. CONCLUSION

Although, both samples are kunu, the physicochemical properties of kunu-zaki samples are closely related just like those of kunu-aya and kunu-stamiya but differs among the different samples of the kunu samples. The presence of potential diseases causing bacteria in all the samples is a clear indication that the hygiene practices are not usually observed during production and handling processes and thus not fit for human consumption. I therefore, recommend that, regulatory agencies should from time to time organize training for producers/hawkers of these beverages to educate them on the proper hygiene to reduce the microbial contamination and the health hazards associated with it.

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