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Comparative Study on the Bacteriological Quality of Kunun-Aya Sold in Wukari, Nigeria

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Abstract: *This study was carried out to ascertain the bacteria quality of kunun-aya from Wukari metropolis. A total of nine (9) samples, three from each of the wards (Avyi, Hospital and Puje) in Wukari and laboratory prepared sample (control) were evaluated for bacterial load and presence of bacteria using standard microbiological techniques. The results show that the pH of the kunun-aya samples were within the acidic range of 2.03 to 2.16. The total bacterial count ranged from 1.3 x 10^7 cfu/ml to 2.2 x 10^7 cfu/ml while control was 3.0 x 10^3cfu/ml. Total coliform and staphylococcal counts ranged from 1.0 x 10^3 cfu/ml to 7.0 x 10^3cfu/ml and 4.0 x 10^4 cfu/ml to 8.0 x 10^4 cfu/ml with control having < 10 colonies respectively. Bacteria isolated and their distributions show that Klebsiella species was present in samples AVY1, AVY2, HSP1, HSP2, PUJ2 and PUJ3, Bacillus species was present in samples AVY1, HSP1, HSP2, PUJ2 and control, Staphylococcus aureus was isolated from all the samples except HSP, PUJ2 and control, Citrobacter species, Salmonella species, Shigella species and Micrococcus species were only isolated from samples HSP1, PUJ1, PUJ3 and control respectively, Proteus species was isolated from PUJ1 and PUJ3, Pseudomonas species was present in AVY3 and PUJ1, Escherichia coli, was present in AVY1, HSP1, HSP2 and PUJ1 while Enterococcus species was isolated from HSP1, PUJ2 and control. The occurrence of these isolates shows that Staphylococcus aureus was the highest, 7 (70%), followed by Klebsiella species and Bacillus species having occurrences of 6 (60%) each, Escherichia coli, 4 (40%), Enterococcus species, 3 (30%), Proteus species and Pseudomonas species, 2 (20%) each while Citrobacter species, Salmonella species, Shigella species and Micrococcus species have the least occurrences of 1 (10%) each. The high bacteria count and the presence of potential pathogenic bacteria in some of the samples is an indication that they were contaminated and this can potentially pose a health hazard to the consumers. Hence, the need for producers and food vendors of kunun-aya to take hygienic measures in preparation and storage of the product to avoid outbreak of diseases associated with the organisms encountered in this study.*

Keywords: *Kunun-aya, bacterial quality, coliform count, staphylococcal count*

1. INTRODUCTION

Kunun-aya (tiger-nut milk) is a traditional fermented spicy non-alcoholic tiger-nut beverage that is indigenous and widely consumed in the Northern parts of Nigeria for its nutritional and medicinal properties especially during dry season [1-3]. It is a very refreshing and high nutritive, energy drink produced from tiger nut (*Cyperus esculentus*). Coconut, pineapple and date fruits may be added to flavour the tiger-nut milk [3]. Locally, kunun-aya is prepared by soaking washed tiger-nuts in water for 2-8 h, grinding the soaked nuts and mixing with water in the ratio of 3litres of water for 1 kg of tiger-nuts. The mixture is allowed for a period of time to macerate and then sieved. This is followed by addition of sugar (depending on quantity and taste) and then a final sieving for the pure drink [2, 4]. However, variations exist in procedure depending on taste and cultural habits, leading to differences in organoleptic properties of the milk [5].

Tiger-nut milk is refreshing and have many health benefits. Report indicates that it contributes to the reduction in Low Density Lipoprotein (LDL), and increases, High Density Lipoprotein (HDL) cholesterol [6, 7]. [8], reported that tiger-nut milk are found to be good in preventing arteriosclerosis,

and can help prevent heart problems, thrombosis as well as activate blood circulation. Also, tiger-nut milk is suitable for gluten and lactose-intolerant patients as well as individuals with digestion and diarrhea problems due to its ability to provide catalase, lipase, amylase and stimulant which helps in digestion [7, 9, 10]. Tiger nut milk (kunun-aya) is also found to be rich in phosphorus, calcium, magnesium, iron as well as in vitamin C and E and free from lactose and gluten [6].

Tiger-nut (*Cyperus esculentus*) belongs to the family, *Cyperaccae*, a tuber that grows freely which is widely consumed (raw or fried) in many parts of East and West Africa including Nigeria as source of energy, carbohydrate and protein [9, 11]. It is rich in vitamins, minerals, digestive enzymes such as amylase, catalase and lipase as well as in phytochemicals and atinutritional factors such as tannins, phytic acids, saponins, glycosides, steroids etc. [3, 8]. However, tiger-nut lack sodium, lactose, gluten and cholesterol, a property that makes it suitable for lactose and gluten intolerance patients as well as hypertensive individuals [3, 7]. Report also indicates that tiger-nut contain higher essential amino acids than those proposed in the protein to satisfy adult needs [12]. Also, tiger nuts are reported to be aphrodisiac and carminative, promoting urine production and menstruation [13].

Kunun-aya has a very short shelf-life at the prevailing weather conditions of tropical Africa and Nigeria and therefore should be consumed within 2-4 hours after production. However, shelf life of the drink can be extended by pasteurization and addition of ginger, garlic and citric acid [3, 14, 15]. Its production in Nigeria has been hampered due to the presence of microorganisms that deteriorate the milk and cause spoilage as a result of unhygienic preparation, use of contaminated raw materials and crude utensils [11, 16]. A range of microorganisms such as *Bacillus subtilis, Staphylococcus aureus, Aspergillus flavus, Aspergillus niger, Fusarium solani, Saccharomyces cerevisiae, S. fubiligera* and *Candida pseudotropicalis* have been associated with exposed tiger nut [7, 17]. In a reports by [18] on microbiological safety of tiger-nut in the cape coast metropolis of Ghana, *E. coli* and *Bacillus* spp (18.9%), *Enterococcus* spp (16.2%), *S. aureus* and *P. aeruginosa* (13.5%), *Streptococcus* spp (10.8%) and *Enterobacter cloacae* (8.1%) were isolated. [2], isolated *Staphylococcus aureus*, *E. coli* and *Streptococcus* species from kunun-aya samples. The presence of some of these potential pathogenic organisms in some of the commercial samples could be a matter of serious public health concern. Therefore, the aim of this study is to evaluate the bacteriological quality of commercial kunun-aya sold in Wukari metropolis and compare them to the quality of laboratory prepared sample.

2. MATERIALS AND METHODS

Sample Collection

A total of nine (9) freshly prepared samples of kunun-aya were purchased from three (3) wards of Wukari metropolis (Avyi, Puie and Hospital). Three samples were collected from each of these wards. A control sample was also prepared in the laboratory using dry tiger-nuts, which are bought from the market and transported to the Federal University Wukari biology laboratory for analysis.

Preparation of Laboratory-Based Kunun-Aya

Kunun-aya was prepared in the laboratory following the method described by [19]. Exactly 200g of dry tiger-nuts was weighted using G&G electronic scale weighing balance (T1000Y, Holland). Foreign materials, bad/cracked nuts and seeds which may alter the taste and quality of the extracted drink were removed. It was then washed with clean water and soaked in 300 ml of Sterile distilled water at room temperature for three (3) days. This was then washed with Sterile distilled water, boiled in 0.2 (w/v) solution of sodium bicarbonate for 30 minutes, and dried in Thermostat oven (DHG-9101-ISA, U.S.A) at 75^{O}C. It was then mixed with Sterile distilled water in a 1:4 ratio and milled using electrical grinding machine. The homogeneous slurry sample was filtered using a sieve.

Determination of Bacteria Load and Isolation of bacteria from Kunu-aya

The bacteria load of the kunun samples were determined following the method of [20] as described by [21]. Serial dilutions of the various kunun-aya samples were made up to 10^{-7} with sterile normal saline. 0.1ml of each dilution was evenly spread on nutrient agar and incubated at 37^{0}C for 24 hours. Plates were screened for discrete colonies after incubation period and the actual numbers of bacteria (total bacteria count) were estimated as colony forming unit per ml (cfu/ml) of the sample. Total coliform count (TCC) and total staphylococcal count (TSC) were performed in similar manner using Mac Conkey agar and Mannitol Salt Agar (MSA) mediums respectively. In each case plating was

done in triplicates and counts taken from plates that had less than 300 colonies. Bacteria load was estimated using standard method of the International Commission on Microbiology Specification for Food (ICMSF) [22]. The colonies were examined closely and distinct colonies were taken and sub-cultured in fresh sterile medium and incubated at 37^0C for 24 h to obtain pure cultures. The pure cultures were characterized and identified as previously described by [23], and [24] with reference to [25].

3. RESULTS

Table 1 presented the pH of the kunun-aya samples. The result showed that all the samples were within the acidic range with sample AVY2 having the highest value (2.16) and sample PUJ3 having the lowest value (2.03) while the laboratory control sample showed the value of (2.04).

The enumeration of the organisms in the various samples showed that the total bacterial count was observed to be highest in sample AVY1 (2.0×10^8 cfu/ml), followed by sample HSP2 (2.2×10^7 cfu/ml), HSP1 (2.1×10^7 cfu/ml), PUJ2 (1.9×10^7 cfu/ml), AVY3, PUJ1 and PUJ3 having (1.5×10^7 cfu/ml) each. Sample AVY2 and HSP3 showed a count of 1.3×10^7 cfu/ml and 1.2×10^7 cfu/ml respectively, while the laboratory prepared sample presented the least count of 3.0×10^3 cfu/ml. The total coliform count ranged from 1.0×10^3 to 7.0×10^3 cfu/ml with control having < 10 colonies while the staphylococcal count ranged from 4.0×10^4 to 8.0×10^4 cfu/ml and <10 colonies in control (Table 2).

Table 3 presents the bacteria isolated from the various samples and their distributions. The result shows that *Klebsiella* species was present in samples AVY1, AVY2, HSP1, HSP2, PUJ2 and PUJ3, *Bacillus* species was present in samples AVY1, HSP1, HSP2, PUJ2 and control, *Staphylococcus aureus* was isolated from all the samples except PUJ2 and control, *Citrobacter* species, *Salmonella* species, *Shigella* species and *Micrococcus* species were only isolated from samples HSP1, PUJ1, PUJ3 and control respectively, *Proteus* species was isolated from PUJ1 and PUJ3, *Pseudomonas* species was present in AVY3 and PUJ1, *Escherichia coli*, was present in AVY1, HSP1, HSP2 and PUJ1 while *Enterococcus* species was isolated from HSP1, PUJ2 and control.

Table 4 showed the percentage occurrence of the isolates. The result showed that *Staphylococcus aureus* was the highest, 7(70%), followed by *Klebsiella* species and *Bacillus* species having occurrence, 6 (60%), *Escherichia coli*, 4 (40%), *Enterococcus* species, 2 (20%) and *Proteus* species and *Pseudomonas* species, 2 (20%) while *Citrobacter* species, *Salmonella* species, *Shigella* species and *Micrococcus* species having the least value, 1 (10%).

Table 1. *pH values of kunun-aya samples.*

S/N	Samples	pH
1	AVY1	2.05
2	AVY2	2.16
3	AVY3	2.10
4	HSP1	2.09
5	HSP2	2.07
6	HSP3	2.09
7	PUJ1	2.04
8	PUJ2	2.11
9	PUJ3	2.03
10	CTL	2.04

AVY1-3=Avyi ward samples, HSP1-3=Hospital ward samples, PUJ1-3=Puje ward samples, CTL= control.

Table 2. *Bacterial load of kunun-aya samples*

S/N	Samples	Total bacterial count (cfu/ml)	Total coliform count (cfu/ml)	Total staphylococcal count (cfu/ml)
1	AVY1	2.0×10^7	1.1×10^3	8.0×10^4
2	AVY2	1.2×10^7	5.0×10^3	5.0×10^4
3	AVY3	1.5×10^7	6.0×10^3	5.0×10^4
4	HSP1	2.1×10^7	4.1×10^3	7.0×10^4
5	HSP2	2.2×10^7	7.0×10^3	6.0×10^4
6	HSP3	1.3×10^7	4.0×10^3	4.0×10^4
7	PUJ1	1.5×10^7	1.0×10^3	6.0×10^4
8	PUJ2	1.9×10^7	6.0×10^3	5.0×10^4
9	PUJ3	1.5×10^7	5.0×10^3	4.0×10^4
10	CTL	3.0×10^3	<10	<10

AVY1-3=Avyi ward samples, HSP1-3=Hospital ward samples, PUJ1-3=Puje ward samples, CTL= control.

Table 3. *Distribution of the Isolates in the Samples*

Samples	AVY1	AVY2	AVY3	HSP1	HSP2	HSP3	PUJ1	PUJ2	PUJ3	CTL
Klebsiella species	+	+	-	+	+	-	-	+	+	-
Bacillus species	+	-	-	+	+	+	-	+	-	+
Staphylococcus aureus	+	+	+	-	+	+	+	-	+	-
Citrobacter species	-	-	-	+	-	-	-	-	-	-
Salmonella species	-	-	-	-	-	-	+	-	-	-
Proteus species	-	-	-	-	-	-	+	-	+	-
Escherichia coli	+	-	-	+	+	-	+	-	-	-
Pseudomonas species	-	-	+	-	-	-	+	-	-	-
Shigella species	-	-	-	-	-	-	-	-	+	-
Micrococcus species	-	-	-	-	-	-	-	-	-	+
Enterococcus species	-	-	-	+	-	-	-	+	-	-

AVY1-3=Avyi ward samples, HSP1-3=Hospital ward samples, PUJ1-3=Puje ward samples, CTL= control.

Table 4. *Percentage occurrence of the isolates in the samples.*

Organisms	Frequency	Percentage (%)
Klebsiella species	6	60
Bacillus species	6	60
Staphylococcus aureus	7	70
Citrobacter species	1	10
Salmonella species	1	10
Proteus species	2	20
Escherichia coli	4	40
Pseudomonas species	2	20
Shigella species	1	10
Micrococcus species	1	10
Enterococcus species	2	20

4. DISCUSSION

In the present study, all the samples were found to be within the acidic pH range of 2.03 to 2.16. Similar observations have been reported by previous researchers [2, 14, 26, 27] and are attributed to the presence of certain species of lactic acid bacteria leading to production of lactic acid during the fermentation processes. However, the pH range observed in this study is lower than the report of [28] and [29] who reported 3.0-3.14 and 5.50 respectively for locally produced kunun-aya. Also, [30] reported pH range of 2.64-5.0 in kunun-zaki. The low pH values observed in the study is desirable as reports have shown that low pH values give advantage to fermentative organisms over spoilage organisms [31].

The present study revealed that most of the samples purchased contained high bacterial count ranging from 3.0×10^3cfu/ml (control) to 2.2 x 10^7cfu/ml (HSP2) which is in agreement with the findings of [30] who reported bacterial count ranging between 5.1×10^3 cfu/ml to 2.0×10^8 cfu/ml in kunun-zaki sample. Similarly, [32] and [33] reported a total bacterial count ranging from 5.0×10^4 cfu/ml to 2.0×10^6cfu/ml and 1.0×10^2 cfu/ml to 8.9×10^4 cfu/ml respectively in kunun-zaki. Also, [34] reported a relatively high count bacterial load of 2.3×10^8 cfu/ml in kunun-aya sample. The coliform count and staphylococcal counts in the present study ranged from <10 colonies to 7.0×10^3 cfu/ml and <10 colonies to 8.0×10^4 cfu/ml respectively. The high bacterial count observed in the commercial samples could be attributed to environmental factors (exposure of the samples to soil, air etc.), type of water used in processing as well as personal hygiene of the handlers [35, 36]. It could also be due to microorganisms inherent to the tiger-nut from which the kunun-aya was obtained which later multiply, milling method and milling machine [37, 38]. The low counts observed in the laboratory prepared kunun-aya is an indication that it was aseptically and hygienically prepared and shows a mark of quality product.

In the present study, the bacteria isolated from the commercial and laboratory prepared kunun-aya are *Klebsiella* species, *Bacillus* species, *Staphylococcus aureus*, *Citrobacter* species, *Salmonella* species, *Proteus* species, *Escherichia coli*, *Pseudomonas* species, *Shigella* species, *Micrococcus* species and *Enterococcus* species. Similar organisms have been reported on kunun-zaki samples [30, 39]. Also,

[2] isolated, *Staphylococcus aureus*, *E. coli* and *Strptococcus* species from kunun-aya samples. The presence of some of these potential pathogenic organisms in some of the commercial samples could be a matter of serious public health concern. *Bacillus* species and *Staphylococcus aureus* are common contaminants of food especially from food handlers, environment and post process contaminations [21]. Moreover, *S. aureus* is a normal flora of the skin, nose, mucus membrane etc. and is implicated as cause of septic arthritis [34]. Also, *Staphylococcus aureus* and *Bacillus* causes staphylococcal and *Bacillus* food borne intoxication respectively. Some strains of *Escherichia coli* such as EHEC causes haemorrhegic diarrhea which can result to serious health conditions as kidney failure and even death. *Salmonella* and *Shigella* species cause salmonellosis and shigellosis when consumed through food [40-43]. Moreover, according to the report of [44] on kunun-aya, the presence of *Bacillus cereus, S. aureus* and *E. coli* in beverages can render them unsuitable for human consumption and as well serve as a medium of disease transmission. Contamination of commercial kunun-aya samples with these organisms could have occurred from water and equipment during processing and storage or through the handlers or from the environment as a result of poor sanitation and hygiene. Also, these organisms when present in food causes spoilage and this could account for the short shelf of kunun-aya [45].

In the present study, the occurrences of the isolates showed that *Staphylococcus aureus* was the highest, 7 (70%), followed by *Klebsiella* species and *Bacillus* species having occurrence, 6 (60%), *Escherichia coli*, 4 (40%), *Enterococcus* species, 2 (20%) and *Proteus* species and *Pseudomonas* species, 2 (20%) while *Citrobacter* species, *Salmonella* species, *Shigella* species and *Micrococcus* species having the least value, 1 (10%). This observation is comparable to the report of [39] who reported 72% (*S. aureus*), 60% (*E. coli*), 56% (*Bacillus* species) and 28% for *Salmonella* spp. from kunun-zaki. The high rate of *S. aureus* (70%) and *Bacillus* species (60%) is of public health concern because the organisms are implicated as causes of food borne intoxication [43]. The high occurrence of *Klebsiella* species (60%), *E. coli* (40%) and *Enterococcus* species (20%) is an indication of feacal contamination. Hence, the need for hygienic measures to be taken during processing of the drink.

5. Conclusion

The present study has shown that commercial kunun-aya samples under study have high bacteria load. It has also shown that most of the samples were contaminated with potentially pathogenic microorganisms which are of public health importance. Hence, the need to employs standard hygienic measures during preparation and storage of kunun-aya, to avoid outbreak of diseases associated with some of the organisms isolated in this study.

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