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Investigation On Hygiene Indicator Analysis (Total Coliforms, *E. coli*) of Kunun Aya Sold By Different Vendors Along Zoo Road, Kano Municipal, Kano State, Nigeria

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ABSTRACT
Kunun Aya is a common non-alcoholic beverage that is widely consumed in Nigeria owing to its high nutritional value and low cost. Poor hygiene during the production, packaging, storage, and sales of the beverage may expose it to microbiological contamination. This work evaluated the microbiological quality of Kunun Aya beverages sold by various vendors in Zoo Road, Kano Municipal Area, Nigeria, using total coliforms and *Escherichia coli* as indicators of hygiene. Twenty (20) Kunun Aya samples were collected aseptically and subjected to standard microbiological procedures. Total coliforms were determined by the MPN technique, while *Escherichia coli* was identified by isolation on EMB and confirmed through Gram staining. The results indicated a coliform count range of 0 to 1100 MPN/100 mL. Eighteen (90%) samples tested positive for total coliforms, but only two (10%) of the samples conformed to the accepted standard. Isolation of *E. coli* was positive in 8 (40%) samples, suggesting the potential presence of feces. Comparative analysis with WHO and NAFDAC standards revealed that most of the samples did not meet the standards. This study indicates that a considerable amount of Kunun Aya produced in the studied area presents various hazards to public health. Hygienic improvement, consumption of clean drinking water, microbial monitoring, and stringent regulation are therefore required for ensuring the microbiological safety of locally produced drinks.

Keywords: Kunun Aya, total coliforms, Escherichia coli, hygiene indicator organisms, MPN, food

INTRODUCTION

Food safety is an essential concern for human health worldwide, especially in developing countries where street-vended foods and drinks are an essential source of nutrients Okwelle *et al*., 2020. Locally manufactured non-alcoholic drinks such as *kunun aya*, popularly known as tiger nut milk, are popular in Nigeria for their affordability, nutritional value, and acceptability. *Kunun aya* is traditionally made from tiger nuts, water, and sometimes sweeteners. It is usually processed at informal production sites with little or no regulatory control (Okwelle *et al*., 2020; Sadiq & Atteh, 2023).

Although *kunun aya* is nutrient-rich, it is highly susceptible to microbial contamination due to its water activity, pH, and unhygienic production, storage, and vending practices. It has been observed in previous research works that the use of contaminated water, personal hygiene of vendors, utensil cleanliness, and exposure to ambient temperatures during hawking are some of the factors responsible for microbial growth in locally manufactured drinks (Eruteya *et al*., 2024)

Hygiene indicator organisms, such as total coliforms and *Escherichia coli*, have been used as criteria for evaluating the sanitary standard of food products, including drinks and beverages. While total coliforms point to environmental contamination, including unhygienic practices, *Escherichia coli* is more specific to faecal contamination (Ezemba *et al*., 2021). The presence of *Escherichia coli* in ready-to-drink beverages such as *Kunun Aya* is totally unacceptable, posing a critical health hazard to consumers.

Usually, street-vended drinks are produced with poor hygienic practices, which may include the use of contaminated water, poor utensils, unclean personal hygiene on the part of the vendors, and poor control of temperatures. This exposes them to contamination by microorganisms, especially coliform bacteria, which may include total coliforms and *Escherichia coli* (Tersoo-Abiem and Orinya, 2024).

Total coliform bacteria are widely employed as indicators for general sanitation, while the presence of *E. coli* is suggested to indicate faecal contaminants with the possibility of the presence of intestinal pathogens. The isolation of the microorganisms in the beverages is inferred to suggest the inadequacy of the hygiene, followed by potential risks to the consumers (Asante-Poku *et al.*, 2024).

Even with the huge and widespread consumption of this local beverage in Kano, there is little to no data on the microbiological and hygiene quality on vendor sold kunun aya. The justification of this study is assess the hygiene practices of vendors and the potential health risk posed to consumers with an objective to evaluate the microbiological quality of the drink using coliform and *E. coli* as indicators, with the hypothesis being that majority of these vendors and their products would not meet the NAFDAC and WHO microbiological standards for ready-to-eat beverages.

MATERIALS AND METHODS

Study Area

This study was carried out in Zoo Road, Kano Municipal Area, Kano State, Nigeria, chosen due to high patronage and wide sale of traditionally prepared beverages, mainly *Kunun Aya*. There are many street vendors in the selected location with different levels of hygienic measures, thus making it fit for the analysis of the microbiological quality of the beverage.

Study Design
An experimental cross-sectional laboratory-based study design was used for the assessment of the hygienic quality of *Kunun Aya* sold by various vendors. This research aimed at establishing the presence of hygiene indicator organisms such as total coliforms and Escherichia coli as a measure of hygiene quality and potential faecal contamination.

Sample Collection
Twenty (20) samples of *Kunun Aya* were obtained from twenty different vendors using sterile sample containers. Samples were taken at different time intervals to reduce the chances of overcrowding and cross-contamination while collecting samples. These samples were taken in an ice-packed cooler and transferred to the laboratory for analysis within six hours of taking the samples.

Determination of Total Coliform Bacteria
Multiple-Tube Fermentation Method
The detection of total coliform bacteria was determined by the three-tube Most Probable Number (MPN) technique. Lactose broth medium was used in the analysis for the detection of lactose-fermenting coliforms.

Preparation of Lactose Broth Medium
Lactose broth medium was prepared following the manufacturer's instructions. An appropriate quantity of lactose broth medium was dissolved in distilled water and placed in test tubes along with an inverted Durham tube. The test tubes were plugged with cotton wool and aluminum foil before sterilizing. Sterilization of the media was done in an autoclave at 121°C for 15 minutes. (Ezemba *et al.,* 2021).

Preparation of Serial Dilutions
Each of the *Kunun Aya* samples was serially diluted aseptically using sterile peptone water as the diluting medium. 1 ml of each sample was taken and added to 9 ml of sterile peptone water to make a 10^{-1} dilution. The same procedure was done again to make 10^{-2} and 10^{-3} dilutions. (Orinya, 2024)

Inoculation of Lactose Broth Tubes
The dilutions obtained above were inoculated aseptically into lactose broth using the three-tube MPN method. Three (3) lactose broth tubes were inoculated with 1 ml of the 10^{-1} dilution, 3 other tubes with 1 ml of the 10^{-2} dilution, and three more tubes with 1 ml of the 10^{-3} dilution. Inoculation was carried out with sterile pipettes to avoid contamination from the environment, and the Durham tubes were made sure to be fully submerged in the medium. (Orinya, 2024)

Incubation and Examination
The inoculated lactose broth samples were incubated at 37°C for 24-48 hours. Following incubation, samples were observed for coliform presence. Positive results were determined by the color change of the medium from purple to yellow due to acidification and the presence of bubbles in the Durham tube. Color change or absence of bubble formation in the medium showed that the test was negative. (Tersoo-Abiem and Orinya, 2024)

Most Probable Number (MPN) Determination
The number of positive tubes in each dilution was noted and compared against the MPN index table. The estimated concentration of total coliform bacteria was stated in terms of most probable number per 100 ml (MPN/100 ml) of *Kunun Aya.* (Tersoo-Abiem and Orinya, 2024)

Isolation and Identification of *Escherichia Coli*
Preparation of Eosin Methylene Blue (EMB) Agar Medium
One liter (1000 ml) of distilled water was measured and placed in a sterile conical flask. Fifty-two grams (52 g) of EMB agar powder was added slowly and stirred until fully dissolved.

Inoculation and Incubation on EMB Agar
Those samples that gave a positive result in lactose broth were streaked on EMB agar plates using an inoculating loop. The inoculated plates were incubated at 37°C for 24 hours. After the incubation period, the colonies were examined based on cultural characteristics. Colonies with a metallic green sheen are presumed to be *E. coli*.

Identification of E. coli
Presumptive colonies of *E. coli* were identified through Gram staining. The characteristic features of *E. coli* include gram-negative short rods. The presence of E. coli was noted based on cultural characteristics in EMB agar and the Gram reaction.

Microscopic Examination
The stained smears were viewed under a light microscope using an oil immersion objective lens (100x). Gram-positive bacteria showed a violet color due to crystal violet retained by the cells, whereas Gram-negative bacteria were pink-red in color. Gram-negative rod-shaped bacteria consistent with *E. coli* were recorded.

Comparison with WHO and NAFDAC Standards
Microbiological quality assessment of the *Kunun Aya* samples was done by comparison of the results with microbiological standards set by the World Health Organization (WHO) and the National Agency for Food and Drug Administration and Control (NAFDAC). Based on both sets of standards, ready-to-drink beverages must not contain any detectable levels of total coliforms or *E. coli* in 100 mL of samples. The detection of either bacterium was considered indicative of poor hygiene and potential contamination of the beverage, making it microbiologically unsuitable for consumption by humans (NAFDAC, 2020; WHO, 2022).

Data Analysis
The analysis of total coliform bacteria was carried out using the three-tube most probable number (MPN) method, which was presented in terms of MPN/100 mL. *Escherichia coli* was distinguished by metallic green colonies in EMB agar and the Gram staining technique. The MPN data was classified according to the risk levels by WHO in no risk (0), low risk (1-10), moderate risk (11-100), high risk (101-1000), and very high risk (more than 1000 MPN/100 mL). The findings were analyzed in relation to WHO and NAFDAC microbiological criteria for compliance purposes. Descriptive statistics such as frequencies, percentages, and mean MPN were calculated, while tables were used to represent the findings.

RESULTS AND DISCUSSION
Total Coliform Count Using MPN Method
The total coliform count of *kunun-aya* samples was determined using the multiple-tube fermentation technique (three-tube method) with lactose broth.
The results are presented in Table 1. The results show that 18 (90%) of the samples contained total coliforms, indicating poor hygiene practices during production. The presence of

high-risk and very high-risk samples suggests significant contamination that poses potential health risks to consumers.

Table 1: MPN Values of Total Coliforms in Kunun Aya Samples

S/N	Sample identity	10-1	10-2	10-3	Ratio	MPN/100ml
1.	Sample 1	3	2	2	3:2:2	210
2.	Sample 2	3	2	2	3:2:2	210
3.	Sample 3	3	3	3	3:3:3	1100
4.	Sample 4	3	3	3	3:3:3	1100
5.	Sample 5	3	2	2	3:2:2	210
6.	Sample 6	3	1	1	3:1:1	75
7.	Sample 7	3	2	0	3:2:0	93
8.	Sample 8	3	1	0	3:1:0	43
9.	Sample 9	3	3	3	3:3:3	1100
10.	Sample 10	3	2	2	3:2:2	210
11.	Sample 11	3	0	0	3:0:0	23
12.	Sample 12	2	2	1	2:2:1	28
13.	Sample 13	1	1	0	1:1:0	7.4
14.	Sample 14	2	0	0	2:0:0	9.2
15.	Sample 15	1	1	1	1:1:1	11
16.	Sample 16	1	0	1	1:0:1	7.2
17.	Sample 17	1	0	0	1:0:0	3.6
18.	Sample 18	0	0	0	0:0:0	0.0
19.	Sample 19	2	0	0	2:0:0	9.2
20.	Sample 20	0	0	0	0:0:0	0.0

Table 2: Detection of *Escherichia coli* in each Sample

S/N	Sample identity	Cultural characteristics	Morphological characteristics	Confirmation
1.	Sample 1	Greenish colonies on E.M.B agar plate	Gram-negative bacteria	*Escherichia coli*
2.	Sample 2	No growth of *Escherichia coli* detected after incubation	Absent	No *Escherichia coli*
3.	Sample 3	Greenish colonies on E.M.B agar plate	Gram-negative bacteria	*Escherichia coli*
4.	Sample 4	Greenish colonies on E.M.B agar plate	Gram-negative bacteria	*Escherichia coli*
5.	Sample 5	Greenish colonies on E.M.B agar plate	Gram-negative bacteria	*Escherichia coli*
6.	Sample 6	No growth of *Escherichia coli* detected after incubation	Absent	No *Escherichia coli*
7.	Sample 7	Greenish colonies on E.M.B agar plate	Gram-negative bacteria	*Escherichia coli*
8.	Sample 8	No growth of *Escherichia coli* detected after incubation	Absent	No *Escherichia coli*
9.	Sample 9	Greenish colonies on E.M.B agar plate	Gram-negative bacteria	*Escherichia coli*
10.	Sample 10	Greenish colonies on E.M.B agar plate	Gram-negative bacteria	*Escherichia coli*
11.	Sample 11	No growth of *Escherichia coli* detected after incubation	Absent	*No Escherichia coli*
12.	Sample 12	Greenish colonies on E.M.B agar plate	Gram-negative bacteria	*Escherichia coli*
13.	Sample 13	No growth of *Escherichia coli* detected after incubation	Absent	No *Escherichia coli*
14.	Sample 14	No growth of *Escherichia coli* detected after incubation	Absent	No *Escherichia coli*
15.	Sample 15	No growth of *Escherichia coli* detected after incubation	Absent	No *Escherichia coli*
16.	Sample 16	No growth of *Escherichia coli* detected after incubation	Absent	No *Escherichia coli*
17.	Sample 17	No growth of *Escherichia coli* detected after incubation	Absent	No *Escherichia coli*
18.	Sample 18	No growth of Escherichia coli detected after incubation	Absent	No *Escherichia coli*
19.	Sample 19	No growth of Escherichia coli detected after incubation	Absent	No *Escherichia coli*
20.	Sample 20	No growth of *Escherichia coli* detected after incubation	Absent	No *Escherichia coli*

Table 2 shows that the presence of E. coli in 8 (40%) of the samples indicates faecal contamination, which is a serious public health concern. This suggests the use of contaminated water or poor hygiene practices by vendors. Such beverages are unsafe for human consumption and may transmit enteric pathogens.

TABLE 3: Comparison of Results of the presence of total coliform with WHO and NAFDAC Standards

S/N	Sample Identity	MPN/100ML	WHO /NAFDAC Standard
1.	Sample 1	210	High risk
2.	Sample 2	210	High risk
3.	Sample 3	1100	Very high risk
4.	Sample 4	1100	Very high risk
5.	Sample 5	210	High risk
6.	Sample 6	75	Moderate risk
7.	Sample 7	93	Moderate risk

S/N	Sample Identity	MPN/100ML	WHO /NAFDAC Standard
8.	Sample 8	43	Moderate risk
9.	Sample 9	1100	Very high risk
10.	Sample 10	210	High risk
11.	Sample 11	23	Moderate risk
12.	Sample 12	28	Moderate risk
13.	Sample 13	7.4	Low risk
14.	Sample 14	9.2	Low risk
15.	Sample 15	11	Moderate risk
16.	Sample 16	7.2	Low risk
17.	Sample 17	3.6	Low risk
18.	Sample 18	0.0	No risk
19.	Sample 19	9.2	Low risk
20.	Sample 20	0.0	No risk

The presence of total coliform will be categorised under various risk categories using the criteria provided by the World Health Organization, as indicated below: 0 (no risk), 1-10 (low risk), 11-100 (moderate risk), 101-1000 (high risk), and above 1000 (very high risk).

Only 2 (10%) of the samples met the WHO/NAFDAC standard of zero total coliforms per 100 mL. The remaining 18 (90%) samples were deemed unacceptable for human consumption, indicating widespread contamination and poor hygiene practices among vendors.

Table 4: Comparison of Results of the presence of *Coli* with WHO and NAFDAC Standards

S/N	Sample Identity	Presence/Absence of *E.coli*	WHO/NAFDAC Standard
1.	Sample 1	Present	Unacceptable
2.	Sample 2	Absent	Acceptable
3.	Sample 3	Present	Unacceptable
4.	Sample 4	Present	Unacceptable
5.	Sample 5	Present	Unacceptable
6.	Sample 6	Absent	Acceptable
7.	Sample 7	Present	Unacceptable
8.	Sample 8	Absent	Acceptable
9.	Sample 9	Present	Unacceptable
10.	Sample 10	Present	Unacceptable
11.	Sample 11	Absent	Acceptable
12.	Sample 12	Present	Unacceptable
13.	Sample 13	Absent	Acceptable
14.	Sample 14	Absent	Acceptable
15.	Sample 15	Absent	Acceptable
16.	Sample 16	Absent	Acceptable
17.	Sample 17	Absent	Acceptable
18.	Sample 18	Absent	Acceptable
19.	Sample 19	Absent	Acceptable
20.	Sample 20	Absent	Acceptable

The presence of E. coli in 8 (40%) of the samples indicates faecal contamination, which is a serious public health concern. According to WHO and NAFDAC standards, any detectable E. coli in ready-to-drink beverages renders them unacceptable for human consumption. This highlights the urgent need for improved hygiene practices and regulatory enforcement

Discussion

The results obtained in the study showed a significant presence of microorganism contamination in *Kunun Aya* from various vendors within the Kano municipal area. In this study, total coliform was found in 90% of the samples tested. As an indicator of poor sanitation, total coliform bacteria are commonly used in assessing the quality of food and beverages.

Escherichia coli was present in 40% of the samples tested. This is of concern since the bacteria could indicate faecal contamination. The presence of *E. coli* in ready-to-eat drinks could suggest contamination from a poor water source, poor personal hygiene of food handlers, contaminated equipment, or processing environments. As recommended by the World Health Organization (WHO), *Escherichia coli* should not be present in 100 mL of water or ready-to-drink beverages; hence, the samples that had *E. coli* failed to meet the recommended microbiological safety standards (WHO, 2022).

There was a correlation between the levels of total coliform counts and the presence *of E. coli.* Where there were extremely high levels of total coliform counts (1100 MPN/100 mL), there was always an isolation of *E. coli;* but where there were low coliform counts or none, there was no isolation of *E. coli.* There were some samples that had moderate total coliform counts but lacked *E. coli* isolations, thus implying that the contamination was environmentally based. The results support the application of total coliforms as a broad indicator of the hygienic state of the sample, while *E. coli* is the faecal-specific one.

The results agree with earlier studies showing higher microbial loads in locally prepared beverages due to poor

sanitation, lack of hygiene, and contaminated water used in preparation (Adeleye et al., 2024; Tersoo-Abiem & Orinya, 2024). Likewise, it has been seen that drinks that are sold in the open environment are more likely to get contaminated with microorganisms compared to the drinks that are properly packaged.

In general, the high incidence of total coliforms and the isolation of *E. coli* in a considerable number of samples point towards poor food handling practices by the *Kunun Aya* vendors. The study shows that there is a requirement for better hygienic measures during the manufacturing process, clean drinking water, cleaning of the machinery used in production, proper packaging and storage of the products, and regular microbiological testing to reduce contamination risks to the consumers.

CONCLUSION

This study has shown that *Kunun Aya* produced in the Kano Municipal Area is associated with various degrees of microbial contamination. While some samples were of acceptable microbial quality, the presence of total coliform in 90% of the samples and *E. coli* in 40% of the samples shows poor hygiene practices among some producers.

The isolation of *E. coli* raises a doubt of faecal contamination and is a potential health hazard for the consumer. It is therefore important to improve on the methods of production and hygiene as well as the testing of microbes in *Kunun Aya.* Based on the findings of this study, the vendors should use only treated and microbiologically safe water during the preparation of *Kunun Aya*. Food handlers should receive regular training on food safety practices, including proper hand washing, personal hygiene, and safe handling procedures. Preparation equipment, storage containers, and packaging materials should be properly washed and disinfected before use. Regulatory agencies, including NAFDAC and local health authorities, should strengthen monitoring and routine inspection of locally produced beverages. Microbiological testing should be encouraged before commercial distribution of ready-to-drink beverages to detect contamination early. Consumers should be educated on the risks associated with purchasing beverages produced under poor sanitary conditions.

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