Abstract: Two hundred samples of freshly formulated Kunun-zaki, a locally fermented non-alcoholic cereal beverages were obtained from local hawkers in five (5) different locations in Chikun Local Government of Kaduna State, Nigeria and screened for enteric bacteria. They were analyzed using the Spread Plate Method. The pH of the samples ranged between 3.00 -7.50 and total bacterial count ranged between $2.0 \times 10^4$ – $9.0 \times 10^5$ cfu/ml. The microorganisms recovered were *Esherichia coli*, *Proteus vulgaris*, *Proteus mirabilis* and *Citrobacter freundii*. The frequency of the occurrence of the isolated bacteria were: *Citrobacter freundii* 14 (58%), *Esherichia coli* 3 (13%), *Proteus mirabilis* 5 (21%), and *Proteus vulgaris* 2 (8%). The percentage of contamination in each location is as follows: Television 20%, Sabon-Tasha 15%, Mararaban-rido 12.5%, Kujama 7.5%, and Narayi 5%. There was a significant difference in the rate of contamination ($P > 0.05$). The presence of these bacteria may be a serious health concern as these organisms are involved in causing various diseases. Therefore, hygienic measures should be taken in processing and handling of the product being sold to general public.

Keywords: Kunun-Zaki, Total Bacterial Counts, Beverage, Contamination by Enteric Bacteria
were well labeled and were immediately taking to the Microbiology laboratory of Kaduna State University (KASU) for processing.

2.2. pH Determination of the Samples

The pH of the samples were determined using a pH meter (WPA pH Meter, India) after standardization with pH 4, and 7 buffers (BDH England).

2.3. Determination of Total Bacteria Count

Nutrient Agar (NA) (Biotech Lab. Ipswich, UK) was inoculated with 0.1ml of appropriately diluted kunu-zaki by spread-plating technique and incubated at 37°C for 24hours. Colonies were counted and multiplied by the dilution factor.

2.4. Isolation of Bacteria from Hawked Kunu-zaki

Discrete colonies of bacteria were selected and sub cultured from mixed cultures of the plates of Salmonella Shigella Agar (SSA) respective. The plates were incubated at 37°C for 24hours. The bacterial isolates were then identified following standard microbiological procedures based on cultural, morphological and biochemical characteristics as described by [6].

3. Results

The mean pH, pH range, total bacteria count and the percentage of microbial contamination are shown in Table 1. Result of pH determination showed that pH of 7.5 was the highest and pH 3.0 was the lowest, obtained in Sabon-Tasha and Kujama respectively. The organisms recovered is shown in Table 2. The percentage occurrence of enteric bacteria isolated from Kunun-zaki is shown in Table 3.

4. Discussion

In this work, the highest pH of kunun-zaki was 7.5 and the lowest pH was 3.00, obtained from Sabon-Tasha and Kujama respectively. The pH range of the “kunun-zaki” in decreasing order are as follows: Sabon-tasha 3.01 to 7.5, Television 3.06 to 7.0, Narayi 3.11 to 6.5, Marara-rido 3.0 to 6.4, and Kujama 3.0 to 6.0. 3.95 to 4.08 were however obtained by [14]. Our values were higher than the values 4.12 to 5.10, 4.70 to 5.75 and 5.25 to 5.65 obtained by [2], [3], [4]. The acidic nature of the “kunun-zaki” is as a result of fermentation process which led to the production of lactic acid by bacteria. That increased the flavor and lower the pH of the kunu. The low pH reduce the activities of pathogens [13], [15]. Statistically there is no significant differences in the pH values in all locations (P>0.05).

The total bacteria count (TBC) in Table 1 showed a decrease in the order of the bacteria count. Sabon-Tasha had the highest TBC of 3×10^5 to 9.0×10^5 cfu/mls followed by Kujama with 2.0×10^4 to 9.0×10^4 cfu/mls, Television with 3.0×10^4 to 2.0×10^5 cfu/mls, Narayi with 8.0×10^4 to 1.8×10^5 cfu/mls and Mararaban-rido had the lowest 6.4×10^4 to 1.2×10^5 cfu/mls. This are all lower compared to the 7.33×10^6 cfu/mls to 81.67×10^6 cfu/mls obtained by [4]. But however, it fell within the range of findings by [1], [8], [9], and [11]. Who reported bacteria count of 5.1×10^2 to 2.0×10^8 cfu/mls, 1.0×10^7 to 8.9×10^7 cfu/mls, 3.5×10^7 to 8.4×10^7 cfu/mls and 5.0×10^4 to 1.79×10^5 cfu/mls, and. The high bacteria count therefore might be as a result of unclean water used in the preparation of the kunu, poor hygienic practice, exposing the kunun-zaki to dust and flies. Improper hand washing after going to the toilet during the preparation of the kunu is a common practice, and might be a source of contamination.

| Locations          | Mean pH | pH range       | Total Bacteria count (cfu/ml) | Percentage (%) of positive samples | Percentage (%) of negative Samples |
|--------------------|---------|----------------|-------------------------------|-----------------------------------|------------------------------------|
| Mararaban-rido     | 3.80    | 3.00 - 4.60    | 6.4×10^4-1.2×10^5             | 12.5                              | 87.5                               |
| Sabo-tasha         | 3.85    | 3.01 - 7.50    | 3.0×10^4-9.0×10^4             | 15.0                              | 85.0                               |
| Narayi             | 4.03    | 3.11 - 6.50    | 8.0×10^4-1.8×10^5             | 5.00                              | 95.0                               |
| Television         | 3.88    | 3.06 - 7.00    | 3.0×10^4-2.0×10^5             | 20.00                             | 80.0                               |
| Kujama             | 3.94    | 3.00 - 6.00    | 2.0×10^4-9.0×10^5             | 7.50                              | 92.5                               |

Table 1. pH range, total bacterial count (Cfu/ml) base on locations.

| Organisms          | Cultural characteristic | Urease | Oxidase | Citrate | Indole | Motility | Methyl red | TSI |
|--------------------|-------------------------|--------|---------|---------|--------|----------|------------|-----|
| Citrobacter freundii | Translucent with gray shining surface | +      | -       | +       | -      | +        | +          | Y   |
| Proteus mirabilis   | Transparent with black center  | +      | -       | -       | +      | +        | +          | R   |
| Proteus vulgaris    | Transparent with black center.  | +      | -       | -       | +      | +        | +          | R   |
| Escherichia coli    | Smooth pink and rosy      | +      | -       | +       | -      | -        | +          | Y   |

Table 2. Cultural and biochemical characteristic of enteric bacteria isolated from kunun-zaki.

| Organisms          | Frequency | Percentage (%) |
|--------------------|-----------|----------------|
| Citrobacter freundii | 14        | 58             |
| Escherichia coli    | 3         | 13             |
| Proteus mirabilis   | 5         | 21             |
| Proteus vulgaris    | 2         | 8              |

Table 3. Percentage occurrence of enteric bacteria isolated from kunun-zaki.
kunu can also lead to contamination [5], [9], [10]. The percentage of contamination in increasing order showed that Television had highest percentage of 20%, Sabon-Tasha 15%, Mararaban-rido 12.5%, Kujama 7.5% and the lowest was Narayi with 5.0%. These show improvement in hygiene practice among kunun-zaki producers and sellers and less contamination of the product [13]. There was a significant difference in rate of contamination at 95% confidence interval when the P-value is 5%.

Table 2 shows the organisms recovered in all of the samples. They include the following: C. freundii, E. coli, P. mirabilis and P. vulgaris.

Table 3 showed the percentage occurrence of enteric bacteria out of the two hundred (200) samples examined. The rate of contamination by bacteria was as follow: Citrobacter freundii 14 (58%), Proteus mirabilis 5 (21%), Escherichia coli 3 (13%) and Proteus vulgaris 2 (8%). All the organisms isolated from this study are important members of the coliform group. They are part of the normal flora of humans. Their habitat include soil, water, sewage, food, animal and human intestine. Some strains can cause gastroenteritis, diarrhoea and urinary tract infection. There presences are indicator of faecal contamination of the kunun-zaki. These organisms are opportunistic pathogens that can cause disease in immunosuppressed people, they cause diseases such as respiratory and urinary tract infections (UTI), diarrhea, gastroenteritis, wound, etc. The contamination may be as a result of cross-contamination unnoticed or through poor hygienic practice by the food handlers [1], [5], and [6].

5. Conclusion

It is now shown that opportunistic pathogens such as C. freundii, E. coli, P. mirabilis and P. vulgaris are present in commercially sold “kunun-zaki” in Chikun Local Government of Kaduna State. Their presence is as a result of faecal contamination of the product, and their presence does not cause disease in healthy human except in immunosuppressed and debilitated people. Since kunun-zaki can serve as vehicle for transmission of pathogens, their presence is not a surprising thing.

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