Bacteriological and nutritional assessment of tiger nut milk (kunu-aya) consumed by students of Nasarawa State University, Keffi Nigeria

Adesakin Funmilola Opeyemi and Obiekezie Smart Obuneme *

Department of Microbiology, Nasarawa State University, Keffi, Nigeria.

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Abstract

Tiger nut milk is a locally prepared indigenous non-alcoholic beverage that is widely produced and consumed in Nigeria most especially in the Northern part of Nigeria where it is referred to as kunu-aya. Twenty five (25) kunun-aya samples were obtained as freshly formulated beverages from five (5) different locations within Nasarawa State University, Keffi, Nigeria. The samples were separately subjected to nutritional assessment (proximate analysis, minerals analysis and determination of vitamins content) and bacteriological assessment. The bacteria loads were determined in terms of total bacteria counts using standard methods involving pour plates. The antibiotic susceptibility pattern of the bacteria isolates was determined using the Kirby-Bauer disc diffusion method. The bacteria counts of the samples from all the locations revealed the total viable counts which ranged from $1.2 - 12.0 \times 10^4 \text{ cfu/ml}$, total coliform $(0.8-6.6 \times 10^4 \text{ cfu/ml})$, total fecal count $(1.0-11.0 \times 10^4 \text{ cfu/ml})$ and total staphylococcus count $(1.2-7.0 \times 10^4 \text{ cfu/ml})$ Five species of bacteria were isolated and identified by standard microbiological methods and these were *Escherichia coli*, *Staphylococcus aureus*, *Salmonella spp.*, *Klebsiella spp* and *Proteus spp*. Result of the proximate composition showed that moisture content ranged from 79.50-81.30%, crude protein( 6.84-7.1%), ash (1.87-2.01%),crude fat( 4.66-5.62%), Crude fiber( 0.70-1.24%) Carbohydrate (2.0-4.50%). Energy values ranged from 77-97(cal). The mineral [mg/l] analysis was done using atomic absorption spectrophotometric method and the result revealed Ca (5.13-8.7), K (0.42-0.53), Na (0.23-0.64), Mg [1.14-2.6], Fe (0.61-0.86). These results provided information about the nutritional value and confirm that tiger milk is an interesting healthy beverage but its contamination with potentially pathogenic bacteria poses a great threat to the consumer.

Keywords: Bacteriological assessment; Nutritional assessment; Kunnu-Aya; Keffi

1. Introduction

Food is essential for continuity of life; so the demand for food cannot be overemphasized. Tiger- nut milk is an example of food that originates from plant known as tiger nut. Tiger nut (Cyperus esculent var.savitus) is a grass-like plant which has been found to be a cosmopolitan perennial crop of the same genus as the papyrus plant [1]. Tiger nut has been cultivated over a long period of time in South Europe and West Africa for its small tuberous rhizomes, which can be eaten raw, roasted, dried, baked or processed into Tiger-nut milk [2] but it has spread to other part of the country like Ghana, Nigeria and Sierra Leone [3].

In Spain the tubers are consumed as a drink locally called “horchata de chufa’.Three varieties of tiger-nut are available in the Nigeria market these are yellow, brown and black, while the two most readily available ones are the yellow and brown varieties. The yellow variety is mostly preferred because of its bigger size and attractive colour. According to [4], the yellow variety yields more milk upon extraction, contains lower fat, more protein and less anti-nutritional factors especially polyphenols.
The usage of tiger nut for milk production was introduced by the Arabs [5]. Tiger-nut milk is one of the indigenous locally fermented, non-alcoholics beverage drinks that are widely consumed most especially in the North due to its thirst-quenching and nutritive properties. Although it is being consumed throughout the year, it is more extensively consumed during the dry season. It is prepared locally by washing the nut to remove soil and dirt, after washing, the nuts are then soaked for about 4 to 8 hours, it can then be grinded with coconut, date fruit or pineapple into a mash. Three litres of water can be added to 1kg of tiger-nuts and the mixture left to macerate for few minutes after which it is pressed and sieved to obtain the milk [6]. Based on individual differences it could be served alone or alongside with either sugar or honey. It is usually served cold and packaged in used and unsterilized plastic bottle for sale. Tiger nut milk has a poor shelf life, it should be consumed within 2-24hours if kept between 40-100°C [7]. However, the shelf life can be increased by pasteurization, irradiation or refrigeration at 4±2 °C [8].

Tiger nut has been reported as a very healthy crop because it helps in preventing heart attacks, thrombosis, cancer especially colon cancer due to its high level of soluble sugar [9]. The nut was found to be rich in myristic acid, oleic acid and linoleic acid [10]. Tiger nut has also been reported to contain adequate level of calcium and potassium for the development of teeth and bone in infants [11].

Tiger nut milk is nutritious because the tiger nut tuber is rich in fats, proteins, carbohydrates as well as minerals [19]. Tiger nut milk has also been recommended for diabetic patients because it does not contain sodium, lactose, casein, gluten and cholesterol[12] According to[13] tiger nut milk has the ability to reduce body cholesterol by increasing the amount of high density lipoprotein (HDL) and lowering low density lipoprotein (LDL) thereby reducing the risk of atherosclerosis. In addition tiger nut milk contains more iron, magnesium and carbohydrates than cow's milk [14]. Also the use of tiger nut products (such as biscuits, flour, milk etc.) should be recommended in children because it helps to reduce protein-calorie malnutrition, since it is rich in nutritional content [15].

However the water content coupled with the crude method of preparation predisposes tiger nut milk to microbial contamination as it has not been possible to have control over the processing of hawked food in developing countries like Nigeria and most vendors lack adequate knowledge of safe food processing and handling practices.

The consumption of local beverage is of public health significant because food borne intoxication is common in man and consumption of local beverages may be the common source of infection.

According to [16], local drinks may serve as vehicles for food borne diseases or pathogens such as Staphylococcosis, Salmonellosis, Brucellosis, Tubercullosis, Shigellosis, Listerrosis and Eschericia coli infection. The spices which are usually added to kunu aya are of agricultural origin and may contain high level of impurities which may be a source of contaminant and pathogenic microorganisms [17].

Tiger nut milk can also undergo spoilage as a result of some factors such as microorganism which are always in the beverage and these microorganisms make use of the carbohydrate content for fermentation process and example of this is lactic acid bacteria such as Lactobacillus specie and Streptococcus specie. Other activities that can cause spoilage of this beverage include natural food enzymes, insects, rodents, or pest present in the environment during preparation [18].

In most Nigerian Universities (especially in the North), the sales and consumption of tiger nut milk is high due to the high cost of other non-alcoholic beverages. It is against this background that this research will be conducted with the aim of investigating the bacteriological and nutritional qualities of tiger nut milk consumed by Students of Nasarawa State University.

2. Material and methods

2.1. Study area

This work was carried out in Nasarawa State University, Keffi, located at 8.85°North latitude, 7.78°East latitude and 338meters elevation above sea level. It is 53km away from Abuja (Capital of Nigeria) in the Guinea Savannah region of Nigeria [19].

2.2. Sample collection

Freshly prepared tiger nut milk was purchased randomly from 5 different sales points within the University for five consecutive days in duplicate. The samples was labelled as(A,B,C,D,E) and transported on ice parked cooler immediately
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to Microbiology Laboratory Unit of Nasarawa State University and Biotechnology Unit of Sheda Science and Technology Complex on Abuja-Lokoja Road for bacteriological and nutritional assessment respectively.

2.3. Determination of pH of the sample
The pH of the various samples was determined immediately using sterile probe of the pH meter.

2.4. Determination of total bacterial load
1 ml of tiger nut milk was added to 9 mls of sterile normal saline test tubes, shaken and from which 10-fold serial dilution was carried out to achieve 10⁸ dilution according to [20]. After carrying out the 10-fold serial dilution, 0.2 ml of the appropriate diluent was spread on sterile MacConkey agar, Mannitol Salt agar, Nutrient agar, Eosin Methylene Blue and Deoxycolate Citrate agar. The cultured plates were incubated at 37°C for 24 hours. After 24 hours of incubation, the colonies in each plate was counted and expressed as colony forming unit per millimeter (cfu/ml). Hence the number of bacterial present in 1ml of the sample was calculated as shown below;

No of bacterial /ml = number of colonies on plate × reciprocal of the dilution of the sample/ 0.2

2.5. Isolation and identification of bacterial isolated from the sample
Streaking method was employed to isolate discrete colonies of bacteria from the mixed culture. MacConkey agar, nutrient agar, Eosin Methylene Blue, Mannitol Salt agar and deoxycolate Citrate were employed for the isolation of bacteria for the purposes of identification. McConkey agar was used to isolate lactose fermenting gram negative bacteria, Eosin Methylene Blue was used for the selective isolation of enteric coliforms, Mannitol Salt agar was for the selective isolation of salt-tolerant bacteria and deoxycolate citrate agar for the isolation of coliforms. All plates were incubated at 37 °C for 24 hours. Identification of bacteria isolates was based on standard cultural, morphological and biochemical methods [21].

2.6. Antibiotic susceptibility test
The isolates were screened for antimicrobial susceptibility using the Kirgy-Bauer agar disc diffusion method [22]. The colonies of each organism was transferred into sterile Mueller-Hilton broth and incubated at 37°C for 24 hrs. The overnight culture was adjusted to the turbidity equivalent to 0.5 Mcfarland standard by adding 0.85% sterile normal saline to the overnight culture. The adjusted inocula was subcultured on the surface of Mueller-Hilton agar (MHA) and the antibiotic was aseptically placed at the center of the MHA plate and incubated at 37°C for 24 hours.

The zone of inhibition of the bacteria isolates were measured using a transparent ruler and the measured value was compared with the reference standard for susceptibility breaking point of antibiotics as described by Clinical and Laboratory Standard Institute [22].

2.7. Proximate analysis of tiger nut milk
The proximate analysis of tiger nut milk was carried out using standard procedures as described by [23]. The proximate analysis include the following: percentage moisture, percentage ash, percentage crude fibre, percentage crude fat, percentage crude protein, carbohydrate determination and Total Energy. The Food total energy was estimated using the modified Atwater factor (4 x protein + 4 x carbohydrate + 9 x fat) [24].

2.8. Determination of mineral composition and vitamin content of tiger nut milk
The following mineral content calcium, magnesium, potassium, sodium, iron and copper was determined by the atomic absorption spectrophotometric method as described by [23] while the Vitamin A and Vitamin C content was determined as described by [25].

3. Results and discussion
Samples A (collected from Faculty of Natural and Applied Science) had total viable counts which ranged from 5.0-6.8 x 10⁸ cfu/ml, total coliform counts also ranged from 3.3 – 6.6 x 10⁸ cfu/ml, total fecal counts ranged from 1.0 – 8.0 x cfu/ml and total staphylococcus count ranged from 2.8 – 5.6 x 10⁵ cfu/ml. Samples B (collected from Faculty of Business and Administration) had total viable counts ranged from 1.4 – 2.5 x 10⁸ cfu/ml, total coliform counts ranged from 2.0 – 4.0 x 10⁵ cfu/ml, total fecal counts of 1.3 – 4.2 x 10⁴ cfu/ml, and total staphylococcus counts ranged from 4.8 – 7.0 x 10⁵ cfu/ml. Samples C (collected from Convocation Square) had total viable counts ranged from 3.2 – 9.5 x 10⁴ cfu/ml,
total coliform counts ranged from $2.0 - 4.0 \times 10^4$ cfu/ml, total fecal counts ranged from $5.5 - 8.5 \times 10^4$ cfu/ml and total staphylococcus counts ranged from $2.5 - 5.4 \times 10^4$ cfu/ml. Samples D (collected from Post graduate School) had total viable counts ranging from $5.6 - 12.0 \times 10^4$ cfu/ml, total coliform count ranged from $1.0 - 5.0 \times 10^4$ cfu/ml, total fecal counts ranged from $5.5 - 11.0 \times 10^4$ cfu/ml and total staphylococcus counts ranged from $3.0 - 6.5 \times 10^4$ cfu/ml. Samples E (collected from Female Hostel) had total viable counts ranged from $5.6 - 12.0 \times 10^4$ cfu/ml, total coliform counts ranged from $1.0 - 5.0 \times 10^4$ cfu/ml, total fecal counts ranged from $5.5 - 11.0 \times 10^4$ cfu/ml and total Staphylococcus counts ranged from $3.0 - 6.5 \times 10^4$ cfu/ml (Table I).

The bacteria isolates from the samples of tiger nut milk sold at different sales point in Nasarawa State University revealed the following which include E. coli, S. aureus, Proteus spp, klebsiella spp, and Salmonella spp (Table II).

The percentage occurrence of bacteria isolates from tiger nut milk sold at different sales point showed that E. coli had the highest percentage which was 76%, Klebsiella spp had (36%), Proteus spp. recorded (20%), while Salmonella spp. was (36%) and S. aureus had (25%)(Table III).

The E. coli isolates were susceptible to Septrin, Chloramphenicol, Sparfloxacin, Ciprofloxacin, Amoxicillin, Augmentin, Gentamycin, Pefloxacin, Tarivid, Streptomycin. Klebsiella spp on the other hand were more susceptible to Chloramphenicol, Ciprofloxacin, Amoxicillin, Gentamycin, Pefloxacin, Tarivid, and Streptomycin and less susceptible to Septrin and Sparfloxacin but completely resistant to Augmentin. S. aureus were susceptible to Septrin, Chloramphenicol, Sparfloxacin, Ciprofloxacin, Amoxicillin, Gentamycin, Pefloxacin, Tarivid, Streptomycin and completely resistant to Augmentin. Proteus spp were susceptible to Septrin, Chloramphenicol, Ciprofloxacin, Amoxicillin, Gentamycin, Pefloxacin, Streptomycin, Tarivid and Augmentin and completely resistant to Sparfloxacin. Salmonella spp. were highly susceptible to Septrin, Ciprofloxacin, Amoxicillin, Tarivid, Sparfloxacin, Augmentin, Pefloxacin, Chloramphenicol, Gentamycin and Streptomycin. (Table IV)

The proximate composition of tiger nut milk in % revealed that sample A contains 79.50 moisture, 5.62 crude fats, 2.01 ash, 1.24 crude fibre, 7.11 crude protein, 4.50 carbohydrate and has 97 total energy. Sample B had 83.70 moisture, 4.66 crude fat, 1.98 ash, 0.7 crude fibre, 6.84 crude protein, 2.0 carbohydrate and 77 has total energy. Sample C had 81.30 moisture, 6.00 crude fat, 1.87 ash, 1.01 crude fibre, 6.90 crude protein, 3.0 carbohydrate and 94 has total energy (Table V).

The mineral (mg/l) and vitamin composition of tiger nut milk samples showed that sample A had 6.36 calcium, 0.53 potassium, 0.23 sodium, 1.32 magnesium, 0.61 iron, 1.23 IU/l of vitamin A, and 1.23 mg/l of vitamin C. sample B had 5.13 calcium, 10.42 potassium, 0.64 sodium, 2.61 magnesium, 0.86 iron, 1.78 IU/l of vitamin A, and 5.21 (mg/l) of vitamin C (Table VI).

Table 1 Total bacterial load (cfu/ml) of Kunu aya sold in Nasarawa State University (x10^4)

<table>
<thead>
<tr>
<th></th>
<th>Total viable counts</th>
<th>Total counts</th>
<th>coliform counts</th>
<th>Total fecal counts</th>
<th>Total Staphylococcus counts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample A</td>
<td>5.0-6.8</td>
<td>3.3-6.6</td>
<td>1.0-8.0</td>
<td>2.8-5.6</td>
<td></td>
</tr>
<tr>
<td>Sample B</td>
<td>1.4-2.5</td>
<td>2.0-4.0</td>
<td>1.3-4.2</td>
<td>4.8-7.0</td>
<td></td>
</tr>
<tr>
<td>Sample C</td>
<td>3.2-9.5</td>
<td>2.0-4.0</td>
<td>5.5-8.5</td>
<td>2.5-5.4</td>
<td></td>
</tr>
<tr>
<td>Sample D</td>
<td>5.6-12.0</td>
<td>1.0-5.0</td>
<td>5.5-11.0</td>
<td>3.0-6.5</td>
<td></td>
</tr>
<tr>
<td>Sample E</td>
<td>1.2-2.5</td>
<td>0.8-2.0</td>
<td>1.1-1.8</td>
<td>1.0-2.0</td>
<td></td>
</tr>
</tbody>
</table>

Key: A=Faculty of Natural and Applied Science, B=Faculty of Business Administration, C=Convocation Square, D=Postgraduate School, E=Female Hostel
Table 2 Cultural, Morphological and Biochemical Characteristics of the Bacterial Isolates

<table>
<thead>
<tr>
<th>S/N</th>
<th>Cultural</th>
<th>Morphology</th>
<th>Biochemical Characteristics</th>
<th>Carbohydrate Utilization</th>
<th>Probable Isolate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>G.S</td>
<td>IN</td>
<td>CA</td>
</tr>
<tr>
<td>1.</td>
<td>Circular</td>
<td>0.4mm</td>
<td>Smooth</td>
<td>Greenish metallic sheen on EMB, Pinkish on MAC</td>
<td>Rod</td>
</tr>
<tr>
<td>2.</td>
<td>Circular</td>
<td>2mm</td>
<td>Smooth</td>
<td>Pinkish on MAC, purple on EMB</td>
<td>Rod</td>
</tr>
<tr>
<td>3.</td>
<td>Circular</td>
<td>0.3mm</td>
<td>Smooth</td>
<td>-</td>
<td>Cocci (cluster)</td>
</tr>
<tr>
<td>4.</td>
<td>Irregular</td>
<td>Rough</td>
<td>Swarms on MAC</td>
<td>Rod</td>
<td>-</td>
</tr>
<tr>
<td>5.</td>
<td>Circular</td>
<td>2-3mm</td>
<td>Smooth</td>
<td>Red with black dot on DCA</td>
<td>Rod</td>
</tr>
</tbody>
</table>

Table 3 Number and Percentage Occurrence of Bacteria Isolates from Kunnu Aya sold in Nasarawa State University.

<table>
<thead>
<tr>
<th>Isolate (%)</th>
<th>No. of sample</th>
<th>E. coli (%)</th>
<th>Salmonella spp (%)</th>
<th>Staphylococcus aureus (%)</th>
<th>Klebsiella spp (%)</th>
<th>Proteus spp (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Point A</td>
<td>5</td>
<td>5 (100%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Point B</td>
<td>5</td>
<td>4 (80%)</td>
<td>0 (0%)</td>
<td>3 (60%)</td>
<td>4 (80%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Point C</td>
<td>5</td>
<td>3 (60%)</td>
<td>2 (40%)</td>
<td>0 (0%)</td>
<td>3 (60%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Point D</td>
<td>5</td>
<td>2 (40%)</td>
<td>4 (80%)</td>
<td>5 (100%)</td>
<td>2 (40%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Point E</td>
<td>5</td>
<td>5 (100%)</td>
<td>3 (60%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>5 (100%)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>25</td>
<td>19 (76%)</td>
<td>9 (36%)</td>
<td>8 (25%)</td>
<td>9 (36%)</td>
<td>5 (20%)</td>
</tr>
</tbody>
</table>

Keys: A=Natural and Applied Science, B=Faculty of Business Administration, C=Convoation Square, D=Postgraduate School, E=Female Hostel

Table 4 Antibiotic Susceptibility of Bacterial Isolates from Kunun Aya sold in Nasarawa State University.

<table>
<thead>
<tr>
<th>Isolation (n)</th>
<th>SX (30 µg)</th>
<th>CH (30 µg)</th>
<th>SP (10 µg)</th>
<th>CPX (10µg)</th>
<th>AM (30µg)</th>
<th>AU (25µg)</th>
<th>CN (10µg)</th>
<th>PEF (10µg)</th>
<th>OFX (30µg)</th>
<th>S (30µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli (19)</td>
<td>19 (100.0)</td>
<td>9 (47.4)</td>
<td>19 (100)</td>
<td>19 (100)</td>
<td>16 (84.2)</td>
<td>10 (52.6)</td>
<td>16 (84.2)</td>
<td>10</td>
<td>19 (100)</td>
<td>19(100)</td>
</tr>
<tr>
<td>S. aureus (8)</td>
<td>6 (75.0)</td>
<td>6 (75.0)</td>
<td>6 (75.0)</td>
<td>6 (75.0)</td>
<td>4 (50)</td>
<td>0 (0)</td>
<td>5 (62.5)</td>
<td>6 (75)</td>
<td>5 (62.5)</td>
<td>6 (75)</td>
</tr>
<tr>
<td>Proteus spp (5)</td>
<td>5 (100.0)</td>
<td>5 (100.0)</td>
<td>0 (0)</td>
<td>2 (40)</td>
<td>3 (60)</td>
<td>2 (40)</td>
<td>4 (100)</td>
<td>3 (60)</td>
<td>5 (100)</td>
<td>3 (60)</td>
</tr>
<tr>
<td>Salmonella spp (9)</td>
<td>9 (100.0)</td>
<td>9 (100.0)</td>
<td>9 (100.0)</td>
<td>9 (100.0)</td>
<td>6 (66.7)</td>
<td>9 (100)</td>
<td>9 (100)</td>
<td>9 (100)</td>
<td>9 (100)</td>
<td>9 (100)</td>
</tr>
<tr>
<td>Klebsiella spp (9)</td>
<td>3 (33.3)</td>
<td>6 (66.6)</td>
<td>3 (33.3)</td>
<td>9 (100)</td>
<td>5 (55.6)</td>
<td>0 (0)</td>
<td>4 (44)</td>
<td>6 (66.6)</td>
<td>6 (66.6)</td>
<td>4 (44)</td>
</tr>
</tbody>
</table>

Key: SX – Septrin, CH – Chloramphenicol, SP – Sparfloxaciln, CPX – Ciprofloxaciln, AM – Amoxacillin, AU – Augmentin, CN – Gentamycin, PEF – Pefloxaciln, OFX – Tarivid, S - Streptomycin
Table 5 Proximate Composition of Tiger nut milk in %

<table>
<thead>
<tr>
<th>Samples</th>
<th>Moisture Content</th>
<th>Crude fat</th>
<th>Ash</th>
<th>Crude fibre</th>
<th>Crude protein</th>
<th>Carbohydrate</th>
<th>Total Energy</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample A</td>
<td>79.50</td>
<td>5.62</td>
<td>2.01</td>
<td>1.24</td>
<td>7.11</td>
<td>4.50</td>
<td>5.4</td>
<td>97</td>
</tr>
<tr>
<td>Sample B</td>
<td>83.70</td>
<td>4.66</td>
<td>1.98</td>
<td>0.70</td>
<td>6.84</td>
<td>2.0</td>
<td>7.6</td>
<td>77</td>
</tr>
<tr>
<td>Sample C</td>
<td>81.30</td>
<td>6.00</td>
<td>1.87</td>
<td>1.01</td>
<td>6.90</td>
<td>3.0</td>
<td>5.97</td>
<td>94</td>
</tr>
</tbody>
</table>

Keys: A = Natural and Applied Science, B = Faculty of Business Administration, C = Convocation Square

Table 6 Mineral and Vitamin Composition of tiger nut milk

<table>
<thead>
<tr>
<th></th>
<th>Ca (mg/L)</th>
<th>K (mg/L)</th>
<th>Cu (mg/L)</th>
<th>Na (mg/L)</th>
<th>Mg (mg/L)</th>
<th>Fe (mg/L)</th>
<th>Vit. A (IU/L)</th>
<th>Vit. C (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample A</td>
<td>6.36</td>
<td>0.53</td>
<td>-</td>
<td>0.23</td>
<td>1.32</td>
<td>0.61</td>
<td>1.23</td>
<td>1.23</td>
</tr>
<tr>
<td>Sample B</td>
<td>5.13</td>
<td>0.42</td>
<td>-</td>
<td>0.64</td>
<td>2.61</td>
<td>0.86</td>
<td>1.78</td>
<td>5.21</td>
</tr>
<tr>
<td>Sample C</td>
<td>8.7</td>
<td>0.74</td>
<td>-</td>
<td>0.49</td>
<td>1.14</td>
<td>0.74</td>
<td>1.52</td>
<td>3.24</td>
</tr>
</tbody>
</table>

4. Discussion

The relative bacteria count recorded were indicative of high level of bacterial contamination. Kunnu aya sold at the different locations of Nasarawa State University is indicative that the beverages sold are grossly contaminate. This may be due to the fact that similar handling procedures are employed during processing and marketing of the beverage. The high microbial counts may to a large extent be attributed to lack of effective precautions on hygiene practice in handling procedures during processing of the beverage. This is in accordance with the report of [26] which has earlier reported this high bacterial count to be as a result crude processing method, non-aseptic handling, water and utensils used during preparation.

The percentage occurrence of Escherichia coli (76%), Staphylococcus aureus (25%), Klebsiella spp. (36%), Salmonella spp. (36%), and Proteus spp. (20%) in the samples analyzed is a pointer to the fact that kunu aya drink sold in the different locations in Nasarawa State University is contaminated with potentially pathogenic bacteria and this may have come from water used for domestic purpose, equipment used for processing or through the handlers or from the environment as a result of poor sanitation and hygiene and even the usage of unsterilized bottle in the packaging of kunnu aya could serve as a means of contaminant. Additionally spices are usually added in small quantities to improve taste and flavor and these are agricultural commodities which may have a high level of microbial impurities [17].

The presence of Eschericia coli, Salmonella spp., Klebsiella spp., Proteus spp. in these samples is an indication of fecal contamination of these products which might be as a result of poor personal hygiene of the producers of this beverage or poor sewage drainage system.

The antibiotic susceptibility test of the isolates showed that the E. coli isolates were susceptible to Septrin, Chloramphenicol, Sparfloxacin, Ciprofloxacin, Amoxacillin, Augmentin, Gentamycin, Pefloxacin, Tarivid, Streptomycin, Klebsiella spp on the other hand were more susceptible to Chloramphenicol, Ciprofloxacin, Amoxacillin, Gentamycin, Pefloxacin, Tarivid, and Streptomycin and less susceptible to Septrin and Sparfloxacin but completely resistant to Augmentin. S. aureus were susceptible to Septrin, Chloramphenicol, Sparfloxacin, Ciprofloxacin, Amoxacillin, Gentamycin, Pefloxacin, Tarivid, Streptomycin and completely resistant to Augmentin. Proteus spp. were susceptible to Septrin, Chloramphenicol, Ciprofloxacin, Amoxicillin, Gentamycin, Pefloxacin, Streptomycin, Tarivid and Augmentin and completely resistant to Sparfloxacin. Salmonella spp. were highly susceptible to Septrin, Ciprofloxacin, Amoxacillin, Tarivid, Sparfloxacin, Augmentin, Pefloxacin, Chloramphenicol, Gentamycin and Streptomycin.

All the samples were found to be acidic (3.9 - 4.6). This level of acidity of kunun aya has been recorded by several researchers including [2], [27] and [9] who attributed this to the presence of certain species of lactic acid bacteria namely Lactobacillus leichmanni and Lactobacillus fermentum during the fermentation process, the acidic nature of this beverage
may also be due to the fact that kunun aya might have started undergoing spoilage even before the time of purchase and such may lead to certain metabolites and could bring about reduction in pH of the product[2]

According to [2] the low pH of kunun aya is not supposed to permit the growth of pathogenic microorganisms, but the presence of Escherichia coli, Staphylococcus aureus, Klebsiella spp., Proteus spp. and Salmonella spp. as seen in this study is a great concern and the presence of these bacteria is in agreement with [28] and [26], who have also isolated some of these contaminants from kunu aya.

The high moisture content reported in this research is in accordance with the report of [19] who had earlier reported that kunun-aya has high moisture content which could affect stability and safety with respect to microbial growth and proliferation, hence the need for cold storage for shelf-life extension.

The result of the proximate composition revealed an appreciable level of crude protein which is in accordance with the report of [27] which conducted a comparative research between kunun-aya and other beverages of cereal origin precisely millet and sorghum and he recorded the crude protein content of kunun-aya, millet and sorghum as 7.20, 2.10 and 1.50 respectively, he then concluded that kunun-aya should be used as a weaning food instead of kunun from cereal to alleviating protein-calorie malnutrition in children.

The mineral content of kunun aya in this study revealed high level of calcium, this relative high content of calcium in kunun aya indicates that it will promote strong teeth and bone development in children and adults. Potassium is an essential nutrient with important role in the synthesis of amino acids and proteins [29] the potassium content obtained in this study is in accordance with the report of [9] who reported that kunun-aya has an appreciable level of potassium. A low sodium content has recorded in this which makes kunun-aya a good beverage for diabetic and hypertensive patient[12] Iron is an important element that supplies the red blood with haemoglobin and prevents anaemia and other related diseases in pregnant women, nursing mothers, infants and elderly people [30], this study revealed an appreciable level of iron which is in accordance with the report of [27].Despite the nutritious value of kunnu aya as revealed in this research, the presence of fecal coliforms is of public health concern because teeming populace especially students rely on this drinks as cheap alternative to the bottled soft drinks.

5. Conclusion

Kunu aya is a nutritive beverage and could serve as a good substitute to the expensive beverage but the levels of microbial contamination observed in all the samples analyzed is a major health concern, therefore there is need to maintain adequate hygienic conditions during processing and preparation of this beverage to eliminate these microbial contaminants and to improve on the quality of the final product to permit Kunun aya to serve as a perfect substitute it’s ought to be.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no conflict of interest.

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