Microbial Quality of Millet-Based Kunu (Gruel) Made with Aqueous Tamarind (*Tamarindus indica*)

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Citation

Abstract
Microbial quality of millet-based Kunu (gruel) made with aqueous tamarind in Wukari, Taraba State was investigated. The tamarind fruits (800g) were sorted, cleaned, peeled to expose the orange pulp. The pulp was scraped from the seeds with a clean knife, blended and sieved to get a fine powder (250g). The millet grains (500g) were washed, steeped in 1000ml of clean water (1:2, w/v) for 24h at 30-32°C and the water decanted off. The grains were washed again and wet-milled with dried ginger. The tamarind powder was mixed with 500ml of clean water, allowed to stay for about 5mins and then further decanted to separate the tamarind solution from the fine particles. The tamarind solution was further mixed with the millet paste to make it less thick. Boiling water (200ml) was then added to the mixture and stirred gradually and then allowed to cool off. Exactly 0.1 ml of the prepared kunu was then transferred to a conical flask, which was serially diluted. The media used were nutrient Agar and Sabaroud Dextrose agar. Isolation and identification of bacteria and fungi were done using standard techniques. The bacterial count at the various sampling sites ranged between $1.64 \times 10^8$ – $2.68 \times 10^9$ cfu/ml. The bacterial species isolated include *Bacillus* species, *Lactobacillus*, *Escherichia coli* and *Pseudomonas aeruginosa*. While the fungal species isolated include *Mucor circinelloides* and *Aspergillus* species. Some of these isolates are pathogens and may pose public health hazards. Hence, measures need to be taken in the processing and handling kunu before consumption.

1. Introduction

Food is an essential substance for life of both plants and animals. Its importance to life cannot be over-emphasized. Every country of world depends on different indigenous recipes of food for survival. For Nigeria, they employ an indigenous technology to prepare some of these foods such as Tuwo, Amala, Zobo and kunu (gruel) among others. Kunu (gruel) is a vital non-alcoholic beverage mostly prepared and consumed in the Northern parts of Nigeria. Depending on the available cereal, grains such as millet (*Pennisetum typhoideum*), sorghum (*Sorghum vulgare*), maize (*Zea mays*), rice (*Oriza sativa*), acha (*Djita exilis*) and or wheat are commonly used for the traditional production of kunu [1, 2, 8].

Millets are a group of highly variable small-seeded grasses, widely grown around the world as cereal crops or grains for fodder and human food. Millets are important crops in
the semi-arid tropics of Asia and Africa (especially in India, Mali, Nigeria, and Niger). About 97% of millet production globally is in developing countries [14]. The crop is favored due to its productivity and short growing season under dry, high-temperature conditions. Millets are indigenous to many parts of the world. Millets have been important food staples in human history, particularly in Asia and Africa. They have been in cultivation in East Asia for the last 10,000 years [13].

The varieties of the drink made from millet is a milky light brown colour. Sometimes, additives such as tamarind (Tamarindus indica), ginger, black pepper, garlic, red pepper and clover are added as flavor, taste improver and preservatives to increase their shelf-life. Depending on the brewer’s or consumer’s choice, sugar, could sometimes be added as sweetener or natural honey with small quantity of dried pellets of sweet potatoes, malted rice, malted sorghum and cadaba farinose crude extract could also be used to sweetened it. The millet grains could be used singly or combined with sorghum in the ratio of 1:2 (w/w) [2].

Tamarind (Tamarindus indica) (Awin-Yoruba, Tsamiya - Hausa) is a woody plant that occurs in the rain forest region of West Africa. Its fruit is most valued for its high ascorbic acid content, minerals and sugar. However, it has been earlier shown that the fruit could be processed into beverages, soft drinks, alcoholic drinks, syrup/concentrate and jams [2]. Since the fruit is high in ascorbic acid and most people (children and adult) enjoy it when incorporated into beverage kunu as it increases the nutritional value, taste and shelf-life. The use of tamarind as a sweetener in kunu could also be a good source of natural sweetener for people allergic to artificial sweeteners. The effect of ginger and garlic separately were compared to the combined effect of ginger and garlic in reducing the microbial population. Of all the treatments, garlic (2g in 200mls or 0.01%w/v) was most effective in reducing the microbial populations.

According to [19], kunu contains 0.3% protein, 1.0% fat, 1.52% ash and 12.2% carbohydrate. [17] re-confirmed that kunu is rich in carbohydrates, vitamins, and minerals but low in protein. In addition, [17], reported the composition of kunu per 100ml to be 8.9mg of ascorbic acid (vitamin C), 20.2g of carbohydrates and 7.2g of protein. Kunu is now widely accepted in most parts of Nigeria, due to its refreshing qualities [18]. People from all walks of life are now being served kunu both at occasions, festivals, home and other public places; as appetizers, refreshments and follow-up food for infants. It serves as a major source of protein and nourishments for many Nigerians especially the rural populace who could not afford dairy drinks. It was reported that, Nigerian locally brewed fermented cereals like ogi, burukutu, fura and kunu among others, are particularly important drinks used for weaning of infants and also dietary supplements for adults [18].

The short shelf-life of these beverage, are however a major problem faced by their producers and consumers, as it has relatively short shelf-life storage. Kunu can undergo spoilage as a result of some factors such as microorganisms present in the drink that helps in the fermentation process. The short shelf-life of kunu could be linked to the presence of lactic acid bacteria such as coliforms, molds, yeasts, Lactobacillus species, Streptococcus species and Leuconostoc species which are the major candidate organisms causing spoilage of the beverage [21]. Other organisms such as Staphylococcus speciee, Bacillus species, Pseudomonas species, Penicillium species, Aspergillus species, Trichoderma species, and Candida species present were known to cause spoilage of kunu beverage and other food drinks [16]. Also activities of the natural food enzymes could also contribute in the spoilage. Other factors include time, temperature, light insects, rodents or pests presents in the environment during the preparation. All these and much more if not properly manage could contribute to the spoilage of kunu has a very high moisture content and total solids which may encourage growth of strains to hazardous levels during storage at ambient temperature [18]. Food pathogens such as Escherichia coli have been implicated in food poisoning resulting from their consumption. The effect of storage and the time lag during which these drinks could lose their nutritional properties varies. Hence, the need to know the more appropriate methods of storage (whether ambient or refrigerated), to reduce the incidence of certain diseases is important [20]. Studies conducted by [1] revealed that the product has a shelf life of 24 hrs at ambient temperature, which was extended to days by pasteurization at 60°C for 1 hours and storage under refrigeration conditions. According to [6], the shelf life based on sensory overall acceptability and microbial quality of the samples varied with treatments but combination treatment with 2g in 200mls (0.01%w/v) ginger and garlic extended the shelf life by approximately four (4) days whereas other treated samples showed marginal enhanced shelf life of 2days. However, untreated kunu sample exhibited remarkably high microbial load and was virtually unacceptable after 24h of production. The result shows the potential of the combination treatment of ginger and garlic as antimicrobials and in extending the shelf life [6].

Several studies have being conducted in different locations on the microbial assessment of locally brewed beverages (such as kunu) in Nigeria and abroad. Diverse microbial genera: Lactobacillus, Bacillus, Staphylococcus, Aspergillus, Penicillium, Fusarium and Saccharomyces were isolated from kunu samples [6]. From their studies, [7] recovered Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes and some fungal species which include Rhizopus nigricans, Penicillium digitatum, Aspergillus fumigatus and Monilia sitophila. In a separate study of freshly prepared kunu produced from millet and analyzed the microbiological quality. Bacterial identification revealed the presence of Bacillus sp and E. coli, Lactobacillus, Pseudomonas sp and Streptococcus sp in the kunu. The fungal isolates revealed the presence of Saccharomyces cerevisiae, Aspergillus and Geotrichum sp
A further research on kunu by Mbachu, et al., 2015, revealed the presence of the following bacteria: *Staphylococcus* species, *Streptococcus* species, *E. coli*, *Pseudomonas* species and *Bacillus* species in fresh kunu. The study further reported that *Streptococcus* sp. had the highest occurrence of 43.31% with *E. coli* having the least occurrence of 5%. The fungal isolates of the study include the presence of *Penicillium* species, *Aspergillus* species and *Fusarium* species. And that *Penicillium* sp. had the highest occurrence of 34.6% and yeast having the least occurrence of 7.7% [15]. The aim of this study is to determine the bacteria and fungi associated with millet-based kunu (gruel) made with aqueous tamarind.

### 2. Materials and Methods

Millet, tamarind and dried ginger were obtained from Wukari market, Taraba state. They were aseptically collected using clean polythene bags and transported immediately to the laboratory for analysis.

#### 2.1. Sterilization of Materials

The glass wares (Test tubes, pipettes, conical flasks, beakers, petri-dishes and universal bottles) were washed with soapy water and rinsed with distilled water; they were allowed to dry and wrapped with kraft paper and further sterilized in a hot air oven at 180°C for 1 hour and stored at 4°C. The media used was also sterilized.

#### 2.2. Processing of Tamarin Fruit

Exactly eight hundred gram (800 g) of the tamarind fruits was sorted and cleaned. The shells of the fruits were peeled to expose the orange pulp. The pulp was subsequently scrape from the seeds with a knife. The separated pulp was then milled and sieved to get a fine powder of about 250g.

#### 2.3. Production of Tamarin Based Kunun-gero

The kunu drink was produced as described by [17], with some modifications. The millet (500g) was washed with clean water and steeped in 1000ml of clean water (1:2, w/v) for 24h at 30-32°C. The water in the steeped grains was decanted off and the grains were further washed with more clean water before wet milling with dried ginger. Again, 1000 ml of clean water was added to form a wet paste.

The fine pulp powder of the tamarind was mixed with 500ml of clean water, mixed thoroughly and was allowed to stay for about 5mins. The mixture was then further decanted to separate the water from the fine particles. The tamarind solution was then further mixed with the millet paste to make it less thick. Boiling water (200ml) was then added to the mixture and stirred gradually. And then allowed to cool off.

### 2.4. Sample Preparation and Analysis for Bacteria

Exactly 1ml of the kunu sample was mixed with distilled water in a conical flask. Six-fold (10⁻⁶) serial dilution of the fresh was done in test tubes. Exactly 0.5ml was obtained using sterile syringe from the 10⁻² test tube and inoculated onto already prepared nutrient agar plates by spread plate and pour plate methods of inoculation. The inoculated plates were incubated at 37°C for 24h. Bacterial colonies that emerge on the plates were counted and recorded as colony forming units per milliliter (cfu/ml) of the sample. The colonies were also sub-cultured repeatedly on fresh plates to obtain pure isolates. The pure bacterial isolates were Gram-stained and subjected to different biochemical tests as described by [23]. The bacterial isolates were identified by comparing their characteristics with those of known taxa using the schemes of [5] and [4]. An aliquot (0.1ml) of the prepared sterile distilled water used for the sample analysis from each of the conical flask was inoculated onto a freshly prepared nutrient agar media and incubated accordingly for 24 h at 37°C as control. The pure colonies of bacteria were maintained on slopes of nutrient agar (NA) slant and stored in a refrigerator at 8°C. See Table 2

#### 2.5. Sample Preparation and Analysis for Fungi

The kunu was mixed with distilled water in a conical flask. Serial dilution of the fresh sample and the digested slurry sample were carried out up to 10⁶ tubes. Exactly 0.5ml was obtained using sterile syringe from the 10⁻² tube and inoculated onto already prepared Saboraud Dextrose Agar (SDA) and was kept at a room temperature in the dark for about 4 to 10 days. After which distinct growth colonies were sub-cultured in the middle of fresh plates of SDA, so the growth spread-out. Incubations were also done for another 4 to 10 days. Identification was done using the lactophenol cotton blue and slide cultures techniques [12, 3]. See Table 3

### 3. Results

This section presented results showing the bacterial load (Table 1), Bacterial isolates from the kunu sample (Table 2) and the fungal isolates from the kunu sample (Table 3)

#### Table 1. Showing bacterial mean load.

<table>
<thead>
<tr>
<th>Plates</th>
<th>Mean Bacterial load (CFU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2.1×10⁷</td>
</tr>
<tr>
<td>B</td>
<td>2.68×10⁷</td>
</tr>
<tr>
<td>C</td>
<td>1.64×10⁸</td>
</tr>
</tbody>
</table>
### Table 2. Showing bacterial isolates from the kunun-gero.

<table>
<thead>
<tr>
<th>Plates</th>
<th>G/R</th>
<th>Colonial Characteristics</th>
<th>CT</th>
<th>UR</th>
<th>CI</th>
<th>IN</th>
<th>MT</th>
<th>H₂S</th>
<th>GP</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>+Rods</td>
<td>Small raised colonies with rough edges, opaque and creamy in colours.</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>A</td>
<td>-Rods</td>
<td>Large circular and transparent colonies that are milky in colours</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B</td>
<td>+Rods</td>
<td>Large circular rough edged</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>B</td>
<td>-Rods</td>
<td>Small circular raised and transparent colonies with creamy colours</td>
<td>NA</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C</td>
<td>+Rods</td>
<td>Large raised colonies, opaque and creamy in nature</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C</td>
<td>-Rods</td>
<td>Small circular colonies, transparent and creamy in nature swarmed all over the plate</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Key: MR= Methyl Red LT= Lactose CT= Catalase IN= Indole SS= Spore staining VP = Voges Proskauer G/R= Gram reaction UR= Urease MT= Motility S/S= Sampling site GL= Glucose GP= Gas Production CT= Citrate SC= Sucrose ++= Positive - = Negative

### Table 2. Continued.

<table>
<thead>
<tr>
<th>Plates</th>
<th>G/R</th>
<th>Colonial Characteristics</th>
<th>LT</th>
<th>SC</th>
<th>GL</th>
<th>MR</th>
<th>VP</th>
<th>SS</th>
<th>Organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>+Rods</td>
<td>Small raised colonies with rough edges, opaque and creamy in colours.</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>Bacillus species</td>
</tr>
<tr>
<td>A</td>
<td>-Rods</td>
<td>Large circular and transparent colonies that are milky in colours</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Lactobacillus</td>
</tr>
<tr>
<td>B</td>
<td>+Rods</td>
<td>Large circular rough edged</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>Bacillus species</td>
</tr>
<tr>
<td>B</td>
<td>-Rods</td>
<td>Small circular raised and transparent colonies with creamy colours</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>Escherichia coli</td>
</tr>
<tr>
<td>C</td>
<td>+Rods</td>
<td>Large raised colonies, opaque and creamy in nature</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>Bacillus species</td>
</tr>
<tr>
<td>C</td>
<td>-Rods</td>
<td>Small circular colonies, transparent and creamy in nature swarmed all over the plate</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Pseudomonas aeruginosa</td>
</tr>
</tbody>
</table>

### Table 3. Showing fungal isolates from kunun-gero.

<table>
<thead>
<tr>
<th>S/S</th>
<th>Media used</th>
<th>Colour of a special hyphae</th>
<th>Colour of substrate hyphae</th>
<th>Nature of hyphae</th>
<th>Shape and type of asexual structure</th>
<th>Presence of special structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>SDA</td>
<td>Black</td>
<td>Brown</td>
<td>Non septate</td>
<td>Globose black conidiophore</td>
<td>Round column-like present</td>
</tr>
<tr>
<td>B</td>
<td>SDA</td>
<td>Cotton grey</td>
<td>White or bloused</td>
<td>Non septate</td>
<td>Globose black conidiophore</td>
<td>Branched sporangiophore</td>
</tr>
<tr>
<td>C</td>
<td>SDA</td>
<td>Grayish green</td>
<td>Grayish e</td>
<td>Septate</td>
<td>Globose black conidiophore</td>
<td>Foot cell present</td>
</tr>
</tbody>
</table>

### Table 3. Continued.

<table>
<thead>
<tr>
<th>S/S</th>
<th>Media used</th>
<th>Appearance of sporangiophore / conidiophore</th>
<th>Characteristics of spore head</th>
<th>Maximum days</th>
<th>Organism Isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>SDA</td>
<td>Non septate</td>
<td>Multinucleate</td>
<td>4</td>
<td>Aspergillus niger</td>
</tr>
<tr>
<td>B</td>
<td>SDA</td>
<td>Ellipsoidal sporangiophore</td>
<td>Multinucleate vesicle</td>
<td>4</td>
<td>Aspergillus niger</td>
</tr>
<tr>
<td>C</td>
<td>SDA</td>
<td>Long erect and non-septate</td>
<td>Radiating sterigma</td>
<td>4</td>
<td>Aspergillus fumigatus</td>
</tr>
<tr>
<td>D</td>
<td>SDA</td>
<td>Long erect and non-septate</td>
<td>Radiating sterigma</td>
<td>4</td>
<td>Aspergillus fumigatus</td>
</tr>
</tbody>
</table>

### 4. Discussion

The bacterial species isolated from the kunu sample include Bacillus species, Lactobacillus, Escherichia coli and Pseudomonas aeruginosa (See Table 2). Bacillus species were the most isolated, while Lactobacillus, Escherichia coli and Pseudomonas aeruginosa were least isolated. These results falls in alignment with several other studies conducted in several parts of the world [6, 7, 11, 15, 22, 24, 25, 26]. However, it could be observed that some microorganisms isolated from these studies were not isolated in this study. This is could most likely be linked to the methods of processing of raw materials, source of raw materials and locations.

And the fungal species isolated from the kunu sample include Aspergillus niger, Mucor circinelloides and Aspergillus fumigatus. The Aspergillus species were the most isolated while Mucor circinelloides were the least isolated.

These results falls in alignment with several other studies conducted in several parts of the world [6, 7, 11, 15, 22, 24, 25, 26]. However, it could be observed that some microorganisms isolated from these studies were not isolated in this study. This is could most likely be linked to the methods of processing of raw materials, source of raw materials and locations.

### 5. Conclusion

The importance of food in the growth and development of humans and animals cannot be over-emphasized. However, care need to be taken in the processing of foods; from the stage of collection to the final stage. The need to analyse these critical control points is important. The types and number of microorganisms isolated from the drink calls for a swift measures to be taken by regulatory authorities to observe critical control points in the processing and handling.
of the product before consumption or being sold to unsuspecting public. As some of these isolates are pathogens. Hence may pose some public health hazards. Therefore, to

discountenance, public health awareness need to be made to

the community on the dangers of consuming such drinks. And there is need to scientifically evaluate this study further to get more conclusive evidences and results.

References


