Bacteriological and Proximate Analysis of Soymilk and Kunnu Beverages Commonly Sold in Polytechnic Ede Environ in Osun – State, Nigeria

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Abstract:
Kunun and Soymilk are fermented non alcoholic beverages widely consumed in Nigeria. This study evaluated and compared bacterial contamination of these commonly sold products in Federal Polytechnic Ede environment as well as their proximate analysis. Samples of these beverages were obtained in three different locations within the Polytechnic environment and Standard microbial analytical procedure was employed for the work. Results showed that the total bacteria count in kunnu ranged from 3.5 x 10³ cfu/ml to 6.4 x 10⁴ cfu/ml while total bacterial count in soymilk ranged from 4.4 x 10⁴ cfu/ml to 9.9 x 10⁵ cfu/ml. Total coliform bacterial count in kunnu ranged from 1.4 x 10⁴ cfu/ml to 1.8 x 10⁵ cfu/ml while coliform count in soymilk ranged from 1.2 x 10⁴ cfu/ml to 2.3 x 10⁵ cfu/ml. The bacterial species recovered from these beverages included Staphylococcus aureus, Escherichia coli, Klebsiella sp, Bacillus sp, Proteus sp and Streptococcus sp. The proximate parameters determined included percentage protein, moisture, fat, ash, carbohydrate and dry matter. The values obtained from kunnu ranged from 4.34 to 6.56 percentage protein, 82.40 to 91.56 moisture, 0.45 to 0.82 ash, 1.43 to 1.58 fat, 10.82 to 19.90 carbohydrate and 22.42 to 28.45 percentage dry matter. The soymilk analysis showed the percentage protein range of 5.63 to 6.68, moisture ranged from 77.50 to 87.56, fat ranged from 1.34 to 1.56, ash ranged from 1.34 to 1.55, carbohydrate ranged from 1.20 to 2.15 and percentage dry matter ranged from 8.44 to 12.46. Adequate sanitary measures should be employed during and after the production of these beverages to reduce the bacteria load, since results of this study showed that kunnu and soymilk prepared and sold in all the locations in this environment were grossly contaminated with potential pathogenic bacteria. The producers of these beverages should be enlightened on how to process in a very good hygienic environment so as to reduce the level of contamination. Proper refrigeration will also go a long way to improve the shelf life of the products.

Keywords: Bacteria, Contamination, Proximate analysis, Soymilk, Kunnu

1. Introduction
The diets of people in many developing countries comprise mainly starchy roots, cereals and legumes. Unfortunately, animal sources of protein which are used to complement the starchy diets are expensive and at times out of reach for low income family. Kunun is a fermented non-alcoholic beverage drink sold in several public places including markets, offices, schools, motor parks and as drinks during festivities, weddings and naming ceremonies (Umaru et al., 2013). It is an appetizer, foodcomplement and refresher to quench thirst which must be consumed within few hours (Adelekan et al., 2013; Amusa and Odunbaku, 2009; Amusa and Ashaye, 2009; Oguntayo-inboet al., 2011). Kunun is milky cream in appearance and are consumed within few hours of its production (Adeleke and Abiodun, 2010). Unlike zobo that is produced from only one plant, Kunun can be prepared from several independent plants including sorghum (Sorghum bicolor), millet (Pennisetum typhoides), maize (Zea mays), rice (Oryza sativa), wheat (Triticumaestivum) andacha (Digitalis exilis) (Umur et al., 2014; Adelekanet al., 2013; Essienet al., 2009; Gaffaet al., 2002). There are different types of Kunnu depending on the feedstock used for processing. This include Kunun-zaki, Kunun-gyada, Kunun-akamu, Kunun-tsamiya, Kunun-baule, Kunun-jiko and Kunun-gayamba (Amusa and Odunbaku, 2009;
Essienet al., 2009). Of these, kunun-zaki is the most widely produced and consumed beverage(Ayo et al., 2010; Amusa and Ashaye, 2009; Gaffa et al., 2002).

Soybean (Glycine max) is a high-protein legume grown as food for both humans and livestock. It is the most valuable crops in the world not only as the staple food in most Asian countries, providing a good source of protein for human diet, but also as an oil seed crop, feed for livestock and aquaculture, and biofuel feedstock(Adelekan et al., 2013; Inoguchi et al., 2011). Presently, soybean is incorporated into so many food formulation of both children and adults to enhance nutritional value of foods, in preparations such as “dawadawa”, alle, moi-moi, akara, soy-ogi and most recently as soymilk. Other commercial uses include animal feed production and the vegetable oil industry (Inoguchi et al., 2011; Akinyele, 2000).

Studies have shown that the safety and quality of most indigenous drink such as kunnu and soymilk could not be ascertained as the traditional method involved in the production of these drinks create room for possibilities of contamination at various stages of unit operations thereby making these products to be carriers of potential pathogens(Inoguchi et al., 2011). In addition to poor handling and unhygienic practices of local producers of soymilk products in particular, the nutrient composition makes it an excellent bacteriological medium which have been implicated in the occurrence and prevalence rate of diseases such as typhoid fever and dysentery among soymilk consumers. It is on this note that the bacteriological examination of these products commonly sold in this community is examined to determine the level of bacterial contamination and how safe the products are especially to students who are the primary consumers in this community.

2. Materials and Methods

2.1. Sample Collection

The samples used for the study were purchased randomly from vendors in the Polytechnic mini market (location 1), Rector’s market (location 2) and Timi market (location 3). They were transported in an ice packed containers to the microbiology laboratory of the SLT department in the Federal Polytechnic Ede, Osun State. Samples were preserved at 4°C for further analysis.

2.2. Isolation of Bacteria Isolates from Samples

Serial dilution of each sample was carried out and third fold of each dilution was plated out using the pour plate technique. 0.1ml of each sample was pipetted and dispensed separately into Petri dishes in duplicate. The prepared media (Nutrient agar and Eosin Methylene blue agar) were poured aseptically on each plate containing the inoculum. The plates were swirled gently for even distribution and allowed to solidify before incubating at 37°C for 24 - 48hrs. The colonies were observed and counted to determine the colony forming unit per ml (cfu/ml). Pure culture of isolates were obtained by streaking distinct colony of each representative colonies on freshly prepared media. The plates were then incubated at 37°C for 24 - 48hrs.

2.3. Characterization and Identification of Isolates

The isolates were identified using the cultural characteristics such as; colour, size, shape and elevation. The isolates were further identified using the Gram staining procedures as described by Cheesbrough, 2004.

2.4. Proximate Analysis of Samples

The proximate analysis was carried out on the samples using the method of Association of Analytical Chemists (AOAC, 2005). The analysis includes Moisture content, crude protein, crude fiber, Ash content and Carbohydrate.

3. Results and Discussion

The average bacterial count in soymilk and kunnu at the three different locations used in this study is as revealed in Table 1. Soymilk samples collected from Timi market (location 3) had the lowest bacterial load while that of Rector’s market (location 2) had the highest. Similar results were obtained from kunnu samples. This could be as a result of heavy human and vehicular movement along this area especially when students are in session.

The average coliform count from all the samples is as shown in Table 2. For Soymilk samples, Timi market (location three) samples had the least coliform count while Polytechnic market (location one) samples had the highest. In kunnu samples, Timi market (location 3) samples had the lowest coliform count while Rector’s market (location 2) samples had the highest.

Similar bacterial species were recovered from the two samples. This includes Bacillus sp, Proteus sp, Staphylococcus sp and Staphylococcus aureus. The coliform bacteria identified from the samples were mainly Escherichia coli and Klebsiella sp. The bacterial load in each sample at different locations is as compared in fig 1. Soymilk tends to have a higher bacterial load on the average when compared with Kunnu beverage. This could probably be as a result of the low pH of kunnu which is usually too low for the growth of pathogenic microorganisms (Adebayo et al., 2010). The presence of some of these micro-organisms in kunnu and soymilk drinks may as a result of contaminations through human handling, the use of contaminated containers, washing with polluted water, unhygienic environment, nutritional composition, or substrate contamination (Akinyele, 2000). Amusa and Odunbaku (2009) had also reported that water and crude method of production and packaging under unhygienic conditions predisposes Kunnu zaki to microbial contamination. The presence
of some of these pathogens (E. coli, S. aureus and streptococcus sp) even in small numbers could render a beverage unsuitable for human consumption (Umar et al., 2014).

The proximate analysis of the samples is as shown in Table 3. Kunnu and Soymilk drink are fermented non-alcoholic beverages with short shelf-life (Ogbonna et al., 2011). The high moisture content (77 - 91%) of kunnu and (77-88%) of soymilk as well as the poor sanitary practices associated with their production could account for easy spoilage of the products. This is a serious concern for the producers and the consumers of these products (Akoma et al., 2006).

The percentage of ash content of these beverages was found to be within the range of 1.3 to 1.5. These values were higher than 0.2% obtained by Otaru et al., 2013 and 0.3 to 0.72% obtained by Essien et al., 2009. These results however agree with 1 to 2.0% recorded by Amusa and Ashaye, 2009, and 1.00 to 2.00% obtained by Ogbonna et al., 2011.

The results for the analysis of protein content revealed a range of 5.65% - 6.66% on the average in soymilk drink and 4.41% - 6.46% in kunnu drink as compared to some findings (Adebayo et al., 2010; Essien et al., 2009). Essien et al., 2009 also reported that loss of protein during the processing of the drinks may be responsible for the low protein content observed especially in kunnu drinks.

| Samples  | Plate 1 (cfu/ml) | Plate 2 (cfu/ml) | Average (cfu/ml) |
|----------|------------------|------------------|-----------------|
| SB1L1    | 5.8 x 10^4       | 6.6 x 10^4       | 6.2 x 10^4      |
| SB1L2    | 10.6 x 10^4      | 9.2 x 10^4       | 9.9 x 10^4      |
| SB1L3    | 5.1 x 10^4       | 3.7 x 10^4       | 4.4 x 10^4      |
| KB1L1    | 4.0 x 10^4       | 5.4 x 10^4       | 4.7 x 10^4      |
| KB1L2    | 5.6 x 10^4       | 7.2 x 10^4       | 6.4 x 10^4      |
| KB1L3    | 3.8 x 10^4       | 3.2 x 10^4       | 3.5 x 10^4      |

Table 1: Bacterial Load in Soymilk and Kunnu

Key:
SB1L1 = Soymilk bought at location 1
SB1L2 = Soymilk bought at location 2
SB1L3 = Soymilk bought at location 3
KB1L1 = Kunnu bought at location 1
KB1L2 = Kunnu bought at location 2
KB1L3 = Kunnu bought at location 3

| Samples  | Plate 1 (Cfu/ML) | Plate 2 (Cfu/ML) | Average Coliform Count (Cfu/ML) |
|----------|------------------|------------------|-------------------------------|
| SB1L1    | 2.0 x 10^4       | 2.6 x 10^4       | 2.3 x 10^4                   |
| SB1L2    | 2.1 x 10^4       | 1.5 x 10^4       | 1.7 x 10^4                   |
| SB1L3    | 1.4 x 10^4       | 1.0 x 10^4       | 1.2 x 10^4                   |
| KB1L1    | 1.3 x 10^4       | 1.9 x 10^4       | 1.6 x 10^4                   |
| KB1L2    | 1.6 x 10^4       | 2.0 x 10^4       | 1.8 x 10^4                   |
| KB1L3    | 1.2 x 10^4       | 1.6 x 10^4       | 1.4 x 10^4                   |

Table 2: Coliform Bacterial Count in the Samples

Figure 1: Chart Showing Bacterial Load of Each Sample in the Three Different Locations under Study
In this study, the bacterial load of kunnu and soymilk drink were compared in which kunnu had a low bacterial count than soymilk. Majority of the microorganisms from these drinks are potential pathogens which are of serious health concern. Most of these pathogens would have been introduced during processing and handling which can be controlled through improved hygiene conditions of both the processors and handlers. The shelf life of the products can also be improved by proper refrigeration.

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### Table 3: Proximate Analysis of the Beverages

| Sample Code | Protein% | Moisture% | Fat%  | Ash%  | CHO%  | Dry Matter% |
|-------------|----------|-----------|-------|-------|-------|-------------|
| SB1L1       | 6.66     | 77.50     | 3.45  | 1.55  | 2.15  | 8.50        |
| SB2L1       | 6.66     | 77.55     | 3.45  | 1.55  | 2.08  | 8.48        |
| SB3L1       | 6.65     | 77.58     | 3.46  | 1.45  | 1.88  | 8.44        |
| KB1L1       | 5.68     | 87.56     | 3.34  | 1.45  | 1.97  | 12.44       |
| KB2L1       | 5.66     | 87.56     | 3.34  | 1.46  | 1.98  | -           |
| KB3L1       | 5.66     | 87.56     | 3.35  | 1.46  | 1.99  | 12.46       |

4. Conclusion and Recommendation

This study, the bacterial load of kunnu and soymilk drink were compared in which kunnu had a low bacterial count than soymilk. Majority of the microorganisms from these drinks are potential pathogens which are of serious health concern. Most of these pathogens would have been introduced during processing and handling which can be controlled through improved hygiene conditions of both the processors and handlers. The shelf life of the products can also be improved by proper refrigeration.

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