MICROBIOLOGICAL AND BIOCHEMICAL ANALYSIS OF SOYMILK PRODUCED AND SOLD WITHIN CALABAR METROPOLIS

ABSTRACT

This study was aimed to evaluate the microbiological and biochemical content of soymilk produced and sold locally within Calabar metropolis. Samples of soymilk were obtained from five areas in Calabar metropolis namely: Bogobiri, Watt market, Akim market, Army barracks and Marian market. Standard microbiological techniques including the pour plate method and relevant biochemical tests were used to isolate, characterize and identify the found microorganisms. The probable bacterial isolates were identified as: Pseudomonas, Bacillus and Klebsiella species while the fungi species were that of Yeast and Aspergillus. There was no significant difference in bacterial and fungal count across the locations sampled (P>0.05). The mean bacterial counts were highest in samples from Army market 6.90±0.01x10^8 cfu/ml and lowest in Marian market 4.80±0.04x10^8 cfu/ml while the mean fungal count was highest in Army market 6.50±0.17x10^8 cfu/ml and lowest in Akim Barracks 3.50±0.22x10^8 cfu/ml. Proximate composition analysis of the foods sampled revealed high carbohydrate content (62.20±0.21% - 71.20±0.07%), lipid content (11.80±0.03% - 14.20±0.22%) and fibre content (10.00±0.12% - 14.00±0.21%) while the range for other proximate composition indices such as ash (2.00±0.01% - 3.10±0.02%), moisture (16.00±0.15% - 12.00±0.12%), and protein content (14.00±0.25% - 14.51±0.30%) were relatively low. Elemental analysis of the soymilk samples showed relatively good amounts of calcium, zinc, iron and sodium which were within the permissible limits set by WHO guidelines for food standard. Lead, Mercury and Cadmium were not detected indicating that the source of water was portable and the soil or cultivation was devoid of harmful/toxic
metals. Samples from Marian market however had the highest nutritional and mineral composition.

**Keywords:** Microbiological, Biochemical, Soymilk, Composition, Characterize.

1.0 Introduction

Soymilk is a healthy satisfying beverage obtained from the water extract of soybeans. It has a smooth creamy texture with an off-white emulsion-like suspension containing water soluble vitamins, proteins and carbohydrates [1]. Soymilk is a substrate used for the production of a number of fermented products such as fermented beverages, culture drinks, yogurt-like products, frozen deserts, cheese substitutes, tofu, sauces and other typical Asian products. Different edible microorganisms (bacteria, yeast and fungi) and their enzymes particularly glycosidases, amylases, proteases and lipases are used in the preparation of these fermented (cereal) foods to yield nontoxic products, with flavours, aromas and textures pleasant and attractive to the human consumer [2]. Soy-based foods are rich in high quality proteins, essential amino acids, minerals, vitamins (especially A and B) and vegetable oil etc. However, consumption of soymilk is hindered due to the presence of unpleasant off-flavours carried over from soybean as well as various oligosaccharides mainly raffinose and stachyose that may cause a gastrointestinal discomfort known as flatulence to consumers [3]. Microorganisms found in soymilk contribute to its spoilage. The spoilage rate is increased by storage temperature that favours the growth and proliferation of microorganisms liable to use soymilk as substrate. Generally, results show that the spoilage rate is lower at refrigeration temperature than room storage [4, 5].
The consumption of locally produced foods and drinks are becoming very popular, with acceptability cutting across the various multi-ethnic groups and socioeconomic classes [6]. They have economic potentials especially now that emphasis is on development of local foods. The high cost and scarcity of milk supplies in developing countries has led to the development of alternative milk supplies from vegetable sources such as soymilk [2]. This is a vital substitute when solving malnutrition problems in developing countries like Nigeria [3]. Many women in Nigeria have learnt the skills of producing soymilk commercially as a strategy for poverty alleviation. These beverages are however prone to microbial spoilage if not properly processed, handled and stored. Bacteria such as *Lactobacillus lactis* have been found growing in soymilk [7]. This study was therefore, undertaken to evaluate the microbiological, proximate and mineral content of soymilk sold within Calabar metropolis in order to ascertain its safety level and quality.

2.0 Materials and methods

2.1 Collection and preparation of samples

Two liquid soymilk samples each were purchased from hawkers in five areas in Calabar metropolis namely: Watt market, Akim market, Army Barracks, Bogobiri, Marian market. The samples were properly labeled, and transported to the microbiology and chemistry laboratory of the University of Calabar for microbiological, proximate and mineral content analysis respectively.

2.2 Microbiological analysis
A tenfold serial dilution of the soymilk samples were carried out in duplicates using the pour plate method on Nutrient agar and MacConkey agar for total bacterial count while Sabouraud dextrose agar (SDA) was used for total fungal count [8].

2.2.1 Determination of total bacterial count

The pour plate method of Frazier and Westhoff [9] was used where Nutrient agar was inoculated with serial dilutions of the samples and incubated at 37°C for 24-48 hours after which the colonies were counted.

2.2.2 Determination of coliform count

MacConkey agar was inoculated with serial dilutions of the sample by the pour plate method and incubated at 37°C for 24-48hr. The different representative colonies from MacConkey agar plates were then picked and inoculated on Eosin-methylene blue (EMB) agar at 37°C for 24 hr. Strains that exhibit green metallic sheen on EMB agar were identified as *E. coli* [8].

2.2.3 Determination of fungi counts

Fungi counts were determined on Sabouraud dextrose agar plates (SDA). The SDA media were modified with streptomycin to inhibit bacterial contamination. One ml of the diluted samples were poured into an empty, sterile 9 ml petri dish followed by pouring SDA into the prepared plates and incubated for 3-5 days at 37°C. Colonies were then counted and recorded after incubation period. SDA slants were prepared for further identification. Fungi were then identified by cultural and morphological characteristics (using a microscope) based on mycelium structure, branch conditions, conidiophore presence, shape etc. [8].

2.2.4 Purification, characterization and identification of isolates
Following enumeration, repeated sub-culturing was carried out on nutrient agar plates for bacteria growth and SDA plates for fungi growth. Pure isolates were stocked in nutrient agar slants for bacteria and SDA slants for fungi and stored in the refrigerator at 4°C for further studies. Characterization and identification of bacteria isolates were carried out according to Medical microbiology laboratory procedures [10], and *Bergey’s Manual of Determinative Bacteriology* [11], fungi isolates were identified according to [10; 12; 13].

### 2.3 Proximate composition

Biochemical analysis of the soymilk samples was carried out for pH, ash, moisture, crude fibre, lipid, protein, carbohydrate, total titratable acidity and mineral content according to the method described by AOAC [14].

### 2.4 Statistical analysis

Data were expressed as means ± standard deviation of triplicate determinations. Analysis was done by one-way analysis of variance (ANOVA) using the randomized complete block design and the least significant difference test was used to assess the difference between the blocks. A significant difference was considered at the level of 5% probability [15].

### 3.0 Results

#### 3.1 Microbiological analysis

The bacterial species contained in the soymilk samples were *Pseudomonas*, *Bacillus* and *Klebsiella* species as presented in Table 1 while the fungal species isolated were species of Yeast and *Aspergillus*. The microbial load of soymilk did not differ significantly across the sampling locations ($P >0.05$).
TABLE 1

Microscopic and biochemical characteristics of bacterial isolates

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Gram’s reaction</th>
<th>Morphology</th>
<th>Catalase</th>
<th>Oxidase</th>
<th>Indole</th>
<th>Ornithine</th>
<th>Motility</th>
<th>Glucose</th>
<th>Sucrose</th>
<th>Lactose</th>
<th>Acid</th>
<th>Gas</th>
<th>Citrate</th>
<th>Urease</th>
<th>Methyl red</th>
<th>Voges Proskauer</th>
<th>Probable org.</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>-</td>
<td>Rods</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>Pseudomonas</td>
</tr>
<tr>
<td>S2</td>
<td>-</td>
<td>Rods</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>Pseudomonas</td>
</tr>
<tr>
<td>S3</td>
<td>-</td>
<td>Rods</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>Bacillus spp</td>
</tr>
<tr>
<td>S4</td>
<td>-</td>
<td>Rods</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>Klebsiella</td>
</tr>
</tbody>
</table>

Legend: - represents a negative reaction; + represents a positive reaction

Samples S1 – S4 are codes for the isolates obtained from soymilk samples

The mean bacterial counts were highest in samples from Army market $6.90 \pm 0.01 \times 10^8$ cfu/ml and lowest in Marian market $4.80 \pm 0.04 \times 10^8$ cfu/ml while the mean fungal count was highest in Army market $6.50 \pm 0.17 \times 10^8$ cfu/ml and lowest in Akim Barracks $3.50 \pm 0.22 \times 10^8$ cfu/ml (Table 2).

TABLE 2

Mean microbial count of soymilk samples

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Locations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bogobiri</td>
</tr>
<tr>
<td>Bacterial count in $10^8$ cfu/ml</td>
<td>$6.44^{ab} \pm 0.02$</td>
</tr>
</tbody>
</table>
Fungal count in $10^8$ cfu/ml

|          | 5.00±0.32 | 6.30±0.36 | 3.50±0.22 | 6.50±0.17 | 4.80±0.18 |

Values are means ± SD for n = 3; LSD = 11.53. Values are means of three triplicates. Values after ± represents standard deviation. LSD = 9.25. Means followed by the same letter within the same row are not significantly (P<0.05) different according to LSD test.

3.2 Proximate composition of cereal foods and drinks

The proximate composition of soymilk samples showed that they contained a high carbohydrate and protein content and a low ash and moisture (Table 3). The pH ranged from 5.45±0.05% in Akim market to 6.63±0.01% in Marian market. Ash content range was from 1.6±0.023% in Watt market to 3.1±0.02% in Marian market, moisture content ranged from 10.00±0.04% in Akim market to 16.00±0.15% in Bogobiri, crude fibre content ranged from 10.00±0.08% in Bogobiri and Army Barracks to 16.00±0.04% in Watt market, fat content ranged from 11.80±0.03% in Bogobiri to 16.00±0.04% in Watt market while the carbohydrate content ranged from 41.32±0.04% in Watt market to 53.37±0.04% in Army barracks. The total titratable acidity range was lowest in Marian market (0.35±0.24%) and highest in Watt and Akim markets (0.75±0.05%).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Bogobiri</th>
<th>Watt Market</th>
<th>Akim Market</th>
<th>Army Market</th>
<th>Marian market</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>5.83±0.05</td>
<td>6.45±0.05</td>
<td>5.45±0.03</td>
<td>6.27±0.02</td>
<td>6.63±0.01</td>
</tr>
<tr>
<td>Ash Content</td>
<td>2.00±0.01</td>
<td>1.60±0.03</td>
<td>2.00±0.03</td>
<td>2.00±0.05</td>
<td>3.10±0.02</td>
</tr>
</tbody>
</table>

TABLE 3

Proximate composition of soymilk drinks (in %) sold in selected locations within Calabar metropolis.
3.3 Mineral content

The result of the mineral composition of soymilk samples (Table 4) showed that: Army market samples had the highest level of Fe, Mn and Cu (51.62 mg/l, 0.62 mg/l and 0.42 mg/l respectively). Akim market samples had the highest level of Na (35.62 mg/l), Watt market samples had the highest amount of Zn (44.22 mg/l) while Marian market samples had the highest level of Ca (22.61 mg/l). Ca and Cu levels were lowest in Bogobiri (8.95 mg/l and 0.05 mg/l respectively), Mn and Zn were lowest in Akim market samples (0.18 mg/l and 17.88 mg/l respectively), while Watt market samples were also least in Fe and Na content (23.54 mg/l and 20.32 mg/l respectively).

<table>
<thead>
<tr>
<th>Locations</th>
<th>Ca</th>
<th>Fe</th>
<th>Mg</th>
<th>Mn</th>
<th>Zn</th>
<th>Pb</th>
<th>Cu</th>
<th>Hg</th>
<th>Cd</th>
<th>Na</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bogobiri</td>
<td>8.95±0.01</td>
<td>41.2±0.01</td>
<td>21.1±0.01</td>
<td>0.37±0.02</td>
<td>30.2±0.03</td>
<td>ND</td>
<td>0.05±0.01</td>
<td>ND</td>
<td>ND</td>
<td>22.61±0.01</td>
</tr>
<tr>
<td>Watt Market</td>
<td>19.66±0.02</td>
<td>23.54±0.03</td>
<td>16.5±0.01</td>
<td>0.42±0.01</td>
<td>44.22±0.04</td>
<td>ND</td>
<td>0.06±0.01</td>
<td>ND</td>
<td>ND</td>
<td>20.32±0.03</td>
</tr>
</tbody>
</table>

Values are means of three triplicates. Values after ± represents standard deviation. LSD = 5.14. Means followed by the same letter within the same row are not significantly (P>0.05) different according to LSD test.

**Table 4**

Mineral composition of Soymilk drinks in mg/l sold locally in selected locations in Calabar metropolis.
<table>
<thead>
<tr>
<th></th>
<th>22.11&lt;sub&gt;bc±0.01&lt;/sub&gt;</th>
<th>27.88&lt;sub&gt;ab±0.02&lt;/sub&gt;</th>
<th>14.23&lt;sub&gt;de±0.04&lt;/sub&gt;</th>
<th>0.18&lt;sub&gt;f±0.03&lt;/sub&gt;</th>
<th>17.88&lt;sub&gt;cd±0.04&lt;/sub&gt;</th>
<th>ND</th>
<th>0.11&lt;sub&gt;g±0.03&lt;/sub&gt;</th>
<th>ND</th>
<th>ND</th>
<th>35.62&lt;sub&gt;a±0.02&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Akim market</td>
<td>14.22&lt;sub&gt;de±0.01&lt;/sub&gt;</td>
<td>51.62&lt;sub&gt;a±0.02&lt;/sub&gt;</td>
<td>21.42&lt;sub&gt;cd±0.01&lt;/sub&gt;</td>
<td>0.62&lt;sub&gt;f±0.04&lt;/sub&gt;</td>
<td>33.12&lt;sub&gt;b±0.03&lt;/sub&gt;</td>
<td>ND</td>
<td>0.41&lt;sub&gt;g±0.02&lt;/sub&gt;</td>
<td>ND</td>
<td>ND</td>
<td>27.44&lt;sub&gt;bc±0.01&lt;/sub&gt;</td>
</tr>
<tr>
<td>Army Barracks</td>
<td>22.61&lt;sub&gt;d±0.01&lt;/sub&gt;</td>
<td>34.73&lt;sub&gt;ab±0.03ab&lt;/sub&gt;</td>
<td>17.34&lt;sub&gt;de±0.01&lt;/sub&gt;</td>
<td>0.56&lt;sub&gt;f±0.02&lt;/sub&gt;</td>
<td>41.00&lt;sub&gt;a±0.01&lt;/sub&gt;</td>
<td>ND</td>
<td>0.24&lt;sub&gt;g±0.02&lt;/sub&gt;</td>
<td>ND</td>
<td>ND</td>
<td>32.43&lt;sub&gt;bc±0.03&lt;/sub&gt;</td>
</tr>
</tbody>
</table>

Values are means of three triplicates. Values after ± represents standard deviation. LSD = 9.25. Means followed by the same letter within the same column are not significantly (P>0.05) different according to LSD test.

4.0 Discussion

The bacterial count was very high compared to that reported in other investigations [16; 17]. Klebsiella isolated from this study is an important member of the coliform group. It is part of the normal flora of the intestine of human and vertebrates and can therefore cause gastroenteritis, diarrhea and urinary tract infections [18]. Its presence is not surprising since other authors had reported post processing contamination from producers, water, utensils and animal in the environment [19]. Bacillus sp. isolated has also been implicated in food poisoning especially in cereals that have been cooked and stored at warm temperature [20]. Reports available indicate that the toxin produced by Bacillus sp. can cause pneumonia and bronchopneumonia [8]. Besides, Bacillus cereus is known to produce heat resistant spores that cannot be eliminated by boiling. Pseudomonas and Klebsiella species with frequencies of 15%, and 10% respectively have all been implicated in the spoilage of food and beverages [21]. Aspergillus has been identified as a common spoilage organism of carbohydrate foods and storage micro flora of many cereals [22]. Yeast has been implicated in the secondary role of converting starch to ethanol and CO₂ thereby releasing esters and phenolic flavours into the food. Therefore, the high
microbial load obtained from soymilk sampled in this study may indicate high rate of laxity in hygiene especially during the processing and storage of these foods.

The pH range of the soymilk samples were not significantly different (p>0.05) and were comparable to pH values of (6.2%) reported by [4]. Low pH values are advantageous as they discourage the growth of pathogens which may cause gastrointestinal disorders. The moisture content range in this study was lower than that reported by [16] of 79.40% - 91.00% and [17] 68.50% - 91.49%. Lower moisture values may be due to loss of water by evaporation. This implies that the hawked soymilk will have a longer shelf life as high moisture favours microbial growth and hence spoilage [23]. The fibre content of samples from Army barracks, Bogobiri and Watt market (Table 3) were considerably higher than 0.29% - 0.90% recorded by [16], 0.01% by [5] and 0.10% - 0.35% by [17]. Fibre helps in the maintenance of human health and has been known to reduce cholesterol levels in the body. Emphasis has been placed on the importance of keeping fibre intakes low in the nutrition of infants and pre-school children [24] since it leads to reduced digestibility, reduced vitamin and mineral availability, lowers calorie density [25], and has local effects on the intestinal mucosa of infants. The ash content in this study was higher than that reported by [17] of 0.80% - 1.00%. This may be due to the differences in particle size of the flour before sieving. The smaller the particle size of the flour, the more endosperms that will be exposed and as such more minerals will be extracted from soymilk. It further confirms its high mineral content as shown in Table 4. This agrees with the findings that plants that contain a large quantity of ash also provide an appreciable quantity of minerals required by the body [26]. The fat content of Watt market and Marian market samples (Table 3) were close to that obtained in high level (17.00%) and Wadata (18.00%) of Markurdi metropolis [17] and 19.89% reported by [27]. The protein content range of 10.68±0.30% - 19.08±0.40% in this study was quite higher
than the values 6.78% - 7.76% obtained by [17] and 3.40% - 3.50% obtained by [16]. This may be attributed to the varying degree of heat treatments which may have resulted in the destruction or inactivation of some amino acids for example cysteine. Differences in nutrient content observed in this study as compared to reports by other researchers may be due to differences in cereal varieties used in production, differences in variety of bean seeds used and the mode or technique used in processing and preparing them.

Soymilk sampled for this study contained a high amount of calcium, iron, magnesium, sodium, and zinc, manganese and copper were present in small amounts while mercury, cadmium and lead were not detected. Recommended permissible limit of Zinc, copper, lead, and Cadmium are 99.0 mg/kg, 40.0 mg/kg, 0.30 mg/kg and 0.20 mg/kg respectively as set by [28]. The concentrations of zinc, copper, lead and cadmium found in soymilk samples were within these permissible limits set by FAO/WHO guidelines for food standard [28]. These toxic metals are carcinogenic affecting the kidney and liver and may be due to the water used for its preparation or from the soil used to plant the grains especially if phosphate fertilizer is used. Therefore, their absence shows that the drinks are not toxic but are safe for drinking. The various elements present in soymilk when analyzed statistically showed a high significant difference (p<0.05).

These mineral elements which are essential micro-nutrients required for the growth of bones and teeth, activation of enzymes, formation of red blood cells, melanin pigment formation, growth of bones and teeth, maintaining osmotic pressure, water balance and intracellular fluids in the body. Toxic metals like lead, mercury and cadmium were not identified in this work. Their carcinogenic effects often affect the kidney and liver. This may imply that these drinks were free from toxic metals and therefore not toxic to the body.
4.0 Conclusion

The result of this study carried out in Calabar metropolis revealed that soymilk samples sold in Army Barracks were found to be highly contaminated with microorganisms some of which were of fecal origin. Samples from Akim market were preferably lower in microbial content and as such less likely to undergo spoilage quickly. Samples from Marian market were observed to have the highest nutritional value and mineral composition. Most locally hawked soymilk in Calabar metropolis have been shown to contain little amounts of food spoilage organisms, coliforms, and fecal contaminants. Proximate composition of some of these foods/drinks revealed high carbohydrate, lipid and fibre content while other proximate composition indices such as ash content, moisture, and protein were relatively low. Soymilk had good amounts of calcium, zinc, iron and sodium while lead, mercury and cadmium were not detected indicating that the source of water was portable and the soil or cultivation was devoid of harmful/toxic metals.

It is therefore recommended that these foods should be properly processed with clean treated municipal water in order to avoid contamination with enteropathogenic bacteria. Also, health education training should be organized for the local people on the importance of cleanliness of their environment and the use of sterilized packaging materials for processing and packaging of these products, as well as the risk accrued to public health when these recommendations are not met by local producers.

REFERENCES


