MICROBIOLOGICAL AND PHYSICO-CHEMICAL ANALYSIS OF SOYMIK AND SOYFOUR SOLD IN UYO METROPOLIS, NIGERIA

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ABSTRACT

Ten samples each of unbranded soymilk and soy flour sold in Uyo metropolis were subjected to microbiological and physico-chemical studies. The microorganisms isolated from the milk and flour and their percentage occurrence were Staphylococcus epidermidis (14.4%, 11.8%), Salmonella sp. (13.2%, 10.5%), Rhizopus sp. (13.2%, 16.4%), Staphylococcus aureus (12.6%, 12.5%), Aspergillus flavus (12.0%, 16.4%), Bacillus sp. (12.0%, 10.5%), Streptococcus sp. (11.4%, 10.5%) and Lactobacillus sp. (11.4%, 11.2%) respectively. The total microbial counts varied with samples while the statistical analysis showed no significant difference at 1.0% level between the microbial load of the soymilk and that of the soy flour. The result of the physico-chemical analysis also showed variation in percentage composition of crude protein, crude fat, total sugar, ash content, pH and mineral element. The sensory analysis, determined by a 10 point scoring method for judging quality factors in soy products, indicated high beany flavour in both the soymilk and soy flour. The analysis also indicated high sweetness in 60%, and sourness in 40% of the soymilk samples. There was no significant difference in colour intensity of the soymilk obtained from different purchase points. Similarly, the colour of soy flour showed no variation as all the samples were predominantly light brown.

KEY WORDS: Soymilk, Soy flour, Microorganisms, Contamination, Spoilage.

INTRODUCTION

Soybean (Glycine max L. Merril) is widely consumed in Nigeria either in the form of soymilk, soy flour, soy awadawa or soybean cake. It is a high and cheap source of protein and a good substitute for dairy milk (Uweagbute, 1992). Soymilk is sometimes recommended by physicians as a substitute for dairy milk for lactose intolerant individuals because it does not contain lactose and is easily digested in the digestive system (Good-enough and Kleyn, 1978).

Soymilk and soy flour marketed in Uyo metropolis are produced locally by small scale industrialists or purchased in bulk from other states and re-packaged for retail trade. The production and/or re-packaging processes could be carried out under doubtful hygienic environment without strict adherence to aseptic and other quality standards prescribed for foods and similar products. These products may consequently be susceptible to microbial contamination or may act as vehicle for transmission of diseases. Hence, it is necessary to ascertain the microbiological quality and physico-chemical status of soymilk and soy flour marketed in Uyo metropolis.

Whereas studies on nutritional values, chemical composition and sensory evaluation of soymilk products in Nigeria have received considerable attention, (Ikenebomeh and Omogbai, 2000; Lienert, 1977; Eka, 1978), there seems to be a dearth of information on the microbiological stability and physico-chemical status of soymilk and soy flour similarly produced and marketed. This study is therefore aimed at isolating, characterizing and identifying microorganisms associated with the contamination and possible spoilage of soymilk and soybean flour, and to evaluate the physico-chemical parameters which may enhance or inhibit microbial proliferation in these products.

MATERIALS AND METHODS

Sample collection

In order to have a good representation of Uyo metropolis, triplicate samples each of 10 different unbranded soymilk and soy flour were purchased wholly from hawkers, open markets and three major supermarkets in Uyo municipality. The samples were designated according to the purchase points as follows: Open markets (1-4), hawkers (5-7), and supermarkets (8-10). All the samples were transported in ice-packed containers to the microbiology laboratory, University of Uyo, where microbiological and physio-chemical
studies were carried out within 4 hours of sample collection.

Preparation of samples for analysis
The soybean flour was prepared for analysis by mixing 5.0 grams of each sample separately in 50.0 ml of sterile distilled water and filtered through Whatman No1 filter paper. The filtrate were for analysis. Twenty millilitres each of the soymilk samples were allowed to attain laboratory conditions for 3 hours prior to analysis. All samples were prepared in triplicates.

Microbiological analysis
Microbial load of the soymilk and soyaflour was carried out using the pour plate technique as described by Macigan et al., (1997), on serially diluted samples. Viable bacteria were primarily enumerated on nutrient agar (Oxoid). Coliform, staphylococcal, fungal and other fastidious organisms were enumerated using MacConkey (Oxoid), mannitol salt, potato dextrose and Saimonella/Shigella agar respectively. Except for the PDA plates which were incubated at 25°C for 72 hours, other plates were incubated at 37°C for 24 hours. Acceptable plate counts were those that had between 30-300 cfu/ml.

Pure cultures were obtained by repeated subculturing on appropriate media, preserved on agar slants of the same media, and characterized using standard microbiological techniques (Holt et al., 1994; Mactaddin, 1980; Hartman, 1985). The characterization process involved colonial morphology, macroscopic/microscopic appearance, enzyme production and biochemical properties.

PHYSICO-CHEMICAL ANALYSIS
Measurements of pH were taken with portable pH meter with glass combination electrode (Griffin, England) at 4°C. The procedures were as follows: For soyaflour, 10.0 grams of the samples from each location were mixed with 90.0 ml of sterile distilled water in 250 ml conical flasks, shaken and allowed to stand for 1 hour. The temperature of the mixture was then measured with a Digitron thermometer (model 275-k). The pH values were obtained by inserting an electrode of the pH meter after standardizing each of the soyaflour-distilled water mixture. Soymilk from different locations were similarly treated, but in this case, 20.0 ml of the sample were used without mixing with sterile-distilled water. The temperature of 4°C for soymilk was obtained by putting the samples in the refrigerator for 10 minutes. The pH values were read on the meter and recorded. All measurements were done in triplicates and average calculated.

Total solids and moisture content were determined by oven drying at 100°C to constant dry weight as described by Osborne and Voogt (1978). Anthrone method (AOAC 1970) was used for the determination of total sugar. In this method the concentration of total sugar was read off from a standard glucose curve at 620 nm. Crude fat content was determined using a soxhlet extraction method described by AOAC (1970) and crude protein by the micro-Kjeldahl method (AOAC, 1984). The conversion factor (6.33) used was the value recommended for milk product (Davis and McLachlan, 1974).

The ash content was determined by igniting the samples in a muffle furnace at 550°C (Pearson 1976), while the crude fibre was quantified by separate exhaustive extraction technique (AOAC, 1984). The amount of crude fibre was calculated as the difference in subtraction of weight of the ash from the increase in weight on paper due to the insoluble material, (Akpan et al., 1999). Viscosities were measured at 4°C using the Capford viscometer (model 300) with spindle speed of 100 rpm. Mineral elements such as calcium and phosphorus were determined according to AOAC (1994), while the titratable acidity was calculated as:

\[
\text{Volume of 0.1m NaOH x 0.009 x 100} \\
\text{Volume or weight of sample used in titration (ml or g).}
\]

SENSORY EVALUATION OF THE SOY PRODUCTS
The sensory attributes determined were colour, flavour and taste. Evaluation was based on the use of a 10 point scoring method for judging quality factors, degree of colour intensity and general acceptability of soy products, adopted from Lamb score card (Amerine et al., 1965). According to this method, 10.0 ml each of the soymilk samples were measured into 50 ml glass beakers and served to the panelists, while 5.0 grams each of the soyaflour samples were served in sterile glass petri dishes.

The colour, flavour and taste panelists comprised an equal number of males and females within 18-30 years, who regularly took soymilk and soyaflour. Samples were presented to the panelists and all attributes for each product were scored on a single score card. All tests were conducted in a well ventilated room with florescent lighting. Attributes with highest mean score were selected and recorded as most acceptable.

STATISTICAL ANALYSIS
Student paired t-test at 1.0% level was
### TABLE 1: Incidence of different types of microorganisms isolated from locally produced soymilk.

<table>
<thead>
<tr>
<th>Sample Code</th>
<th>VIABLE COUNT (cfu/ml) *</th>
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<tbody>
<tr>
<td></td>
<td>LS</td>
</tr>
<tr>
<td>1.</td>
<td>2.4 X 10^4</td>
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<td>± 0.2</td>
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<tr>
<td>2.</td>
<td>1.7 X 10^4</td>
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<td>± 0.3</td>
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<td>3.</td>
<td>3.0 X 10^4</td>
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<td></td>
<td>± 0.1</td>
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<tr>
<td>4.</td>
<td>3.4 X 10^4</td>
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<tr>
<td></td>
<td>± 0.1</td>
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<tr>
<td>5.</td>
<td>2.0 X 10^3</td>
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<td>± 0.4</td>
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<td>6.</td>
<td>1.5 X 10^3</td>
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<tr>
<td>7.</td>
<td>2.4 X 10^3</td>
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<td>± 0.3</td>
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<td>8.</td>
<td>3.2 X 10^3</td>
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<td></td>
<td>± 0.4</td>
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<td>9.</td>
<td>3.0 X 10^3</td>
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<td></td>
<td>± 0.2</td>
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<tr>
<td>10.</td>
<td>2.8 X 10^3</td>
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<td>± 0.3</td>
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</tbody>
</table>

* Values are mean ± standard deviation of three replications.

LS: *Lactobacillus* sp;
SS: *Salmonella* sp;
SA: *Staphylococcus aureus*;
SE: *Staphylococcus epidermidis*;
ST: *Streptococcus* sp;
BS: *Bacillus* sp;
AF: *Aspergillus flavus*;
RS: *Rhizopus* sp;

employed to determine the significant difference in the frequency of occurrence of the microbial genera in the two products. The mean microbial count for each product was used in this analysis.

**RESULTS**

Based on cultural morphology, macroscopic/microscopic appearance, biochemical characteristics and with the aid of identification scheme of Holt et al., (1984), the organisms were identified as *Lactobacillus* sp., *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus* sp., *Bacillus* sp., *Aspergillus flavus* and *Rhizopus* sp. The incidence of the different types of microorganisms isolated from the soymilk and soyflour are shown in Tables 1 and 2.

The percentage occurrence of the isolates is presented in Table 3. The result shows specifically that bacteria species occurred more than fungal species in both soymilk and soyflour. The total percentage occurrence of fungi was 25.2% and 32.8% in soymilk and soyflour respectively, while that of bacterial species was 75.0% and 67.0% in soymilk and soyflour respectively. A total of 319 microbial isolates were obtained from both the milk and the flour, with higher incidence of 167 isolates occurring in soymilk and 152 isolates in soyflour.
mg/100 ml, and phosphorus 111.31 ± 0.23 mg/100 ml.

Similarly, average protein in soyflour from supermarkets was the highest 4.95 ± 0.99%; but least calcium and phosphorus contents, 38.9 ± 0.21 mg/100 ml and 100.32 ± 0.41 mg/100 ml respectively. The protein contents in a soyflour from hawkers and open markets were 4.05 ± 1.06 mg/100 ml and 3.95 ± 0.68 mg/100 ml respectively, while phosphorus content was 143.83 ± 0.01 mg/100 ml in soyflour from hawkers and 135.34 ± 0.80 mg/100 ml from open markets. The highest calcium content 64.38 ± 0.31 mg/100 ml was recorded is soyflour from hawkers while that from open market was 44.61 ± 0.31 mg/100 ml.

Sensory investigations of the soy products indicated beany flavour which was objectionable to many people. The mean panel scores for beanniness in both soymilk and soyflour were higher than the zero mean recommended for soy products. Similarly, there

| TABLE 2: Incidence of different types of microorganisms isolated from locally produced soyflour. |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Sample Code    | Viable Count (cfu/ml) a |
| LS | SS | SA | SE | ST | BS | AF | RS |
| 1. | 2.0 × 10^3 | 0.8 × 10^2 | 1.0 × 10^2 | 1.6 × 10^3 | 1.4 × 10^3 | 0 | 3.0 × 10^3 | 2.6 × 10^3 |
| 2. | 1.1 × 10^3 | 1.4 × 10^2 | 2.5 × 10^2 | 1.4 × 10^3 | 1.0 × 10^3 | 2.0 × 10^3 | 0 | 2.4 × 10^3 | 1.0 × 10^3 |
| 3. | 2.4 × 10^4 | 1.0 × 10^3 | 2.0 × 10^3 | 2.0 × 10^3 | 0 | 2.0 × 10^3 | 0 | 2.6 × 10^3 | 3.4 × 10^3 |
| 4. | 2.0 × 10^3 | 2.0 × 10^3 | 1.4 × 10^3 | 1.8 × 10^3 | 2.8 × 10^3 | 2.4 × 10^3 | 2.8 × 10^3 | 2.0 × 10^3 |
| 5. | 1.6 × 10^3 | 0.4 × 10^2 | 1.6 × 10^3 | 1.0 × 10^3 | 1.0 × 10^3 | 1.7 × 10^3 | 0 | 1.0 × 10^3 | 2.5 × 10^3 |
| 6. | 1.0 × 10^3 | 2.0 × 10^3 | 1.8 × 10^3 | 2.0 × 10^3 | 0 | 2.0 × 10^3 | 0 | 2.4 × 10^3 | 3.0 × 10^3 |
| 7. | 1.8 × 10^3 | 2.1 × 10^3 | 2.0 × 10^3 | 2.0 × 10^3 | 1.6 × 10^3 | 2.0 × 10^3 | 0 | 2.0 × 10^3 | 4.0 × 10^2 |
| 8. | 2.8 × 10^3 | 1.0 × 10^3 | 3.0 × 10^2 | 2.4 × 10^3 | 1.8 × 10^3 | 1.0 × 10^3 | 3.4 × 10^3 | 3.0 × 10^3 |
| 9. | 1.7 × 10^3 | 1.4 × 10^3 | 3.4 × 10^2 | 3.0 × 10^2 | 2.8 × 10^3 | 0 | 3.0 × 10^2 | 1.5 × 10^2 |
| 10. | 2.1 × 10^3 | 2.0 × 10^3 | 1.0 × 10^3 | 2.0 × 10^3 | 2.0 × 10^3 | 1.1 × 10^3 | 0.8 × 10^2 | 2.0 × 10^2 |

a Values are mean ± standard deviation of three replications

LS: Lactobacillus sp;
SS: Salmonella sp;
SA: Staphylococcus aureus;
SE: Streptococcus epidermidis;
ST: Streptococcus sp;
BS: Bacillus sp;
AF: Aspergillus flavus;
RS: Rhizopus sp.

| TABLE 3: Percentage occurrence of microorganisms isolated from soymilk and soyflour |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Organism                        | No. of Isolate | Percentage Occurrence | Soymilk | Soyflour | Soymilk | Soyflour |
| Staphylococcus epidermidis      | 24.0            | 18.0             | 14.4             | 11.8            |
| Salmonella sp                   | 22.0            | 16.0             | 13.2             | 10.5            |
| Bacillus sp                     | 22.0            | 25.0             | 13.2             | 16.4            |
| Staphylococcus aureus           | 21.0            | 19.0             | 12.6             | 12.5            |
| Aspergillus flavus              | 20.0            | 25.0             | 12.0             | 16.4            |
| Streptococcus sp                | 20.0            | 16.0             | 12.0             | 10.5            |
| Lactobacillus sp                | 19.0            | 15.0             | 11.4             | 11.2            |
| TOTAL                           | 167             | 152              | 100              | 100             |
TABLE 4: Physico-chemical properties of locally produced soy milk.

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<th>PARAMETERS</th>
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<th>10</th>
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<td>C</td>
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<td>C</td>
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<td>C, C</td>
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<td>B</td>
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<td>B</td>
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<td>B</td>
<td>B, B</td>
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<tr>
<td>Taste</td>
<td>S</td>
<td>SR</td>
<td>SR</td>
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<td>Viscosity (cp)</td>
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<td>340</td>
<td>360</td>
<td>340</td>
<td>360</td>
<td>380</td>
<td>360</td>
<td>370</td>
<td>360</td>
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<td>Moisture content (%)</td>
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<td>86.5</td>
<td>86.9</td>
<td>85.9</td>
<td>87.26</td>
<td>86.4</td>
<td>80.6</td>
<td>84.6</td>
<td>88.42</td>
<td>86.4</td>
<td>±0.18, ±0.37, ±0.41, ±0.32, ±0.36, ±0.34, ±0.28, ±0.30, ±0.24, ±0.31</td>
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<td>Total solid (%)</td>
<td>11.3</td>
<td>12.64</td>
<td>13.40</td>
<td>13.10</td>
<td>11.63</td>
<td>12.58</td>
<td>18.43</td>
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<tr>
<td>Crude protein (%)</td>
<td>7.80</td>
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<td>7.68</td>
<td>4.23</td>
<td>6.53</td>
<td>5.61</td>
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<td>Crude fat (%)</td>
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<td>Carbohydrate (%)</td>
<td>64.1</td>
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<td>63.10</td>
<td>70.0</td>
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<td>Ash content (%)</td>
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<td>1.40</td>
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<td>1.24</td>
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<td>1.73</td>
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<tr>
<td>Total Sugar (mg/100ml)</td>
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<td>1100</td>
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<td>1250</td>
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<td>1250</td>
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<td>Titrable acidity %</td>
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<td>1.36</td>
<td>0.90</td>
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<tr>
<td>Calcium (mg/100ml)</td>
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<td>24.00</td>
<td>52.40</td>
<td>78.40</td>
<td>48.60</td>
<td>64.6</td>
<td>70.0</td>
<td>27.45</td>
<td>36.20</td>
<td>60.45</td>
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<td>Phosphorus content (mg/100ml)</td>
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</tbody>
</table>

* Values are mean ± standard deviation of three replications.

C: Creamy; S: Sweet; B: Beany; SR: Sour.

was a high mean score in the general acceptability of the colour of the soy products. Soy milk samples from 6 purchase points exhibited high sweetness while samples from 4 purchase points were sour. Although the sour taste appeared to have masked to some extent the sweetness in sample from the 4 locations, the sweetness was still slightly perceptible.

**DISCUSSION**

Food borne diseases are becoming highly prevalent amongst Nigerian population. This may be attributed to the consumption of food products which, may have been grossly contaminated by pathogenic microorganisms or contain lethal microbial toxins liberated into the foods during microbial metabolism (Frazier and Westhoff, 1978). In this study, soy milk and soy flour marketed in Uyo metropolis were found to contain high microbial counts of contaminating microorganisms. This may be due to contamination of the products during processing, exposure of the products to unhygienic environment before packaging or the use of contaminated package. The presence of Staphylococcus aureus is a source of concern because of the ability of this organism to grow, multiply in foods and produce enterotoxin at room temperature, which may be liberated into the foods (Atanda and Akano, 1997). This organism is also associated with urinary tract infections (Ebie et al., 2001). Similarly, the presence of Aspergillus flavus, a toxigenic mould, in these soy products poses a great risk to human health because this organism is capable of producing aflatoxin at room temperature which may be liberated into the foods. Some food poisoning outbreaks have been traced to contamination of food products by Aspergillus flavus (Uriah and Ugbadu, 1980). The mycotoxicogenic potential of this mould has also been reported by Davis (1981).

The presence of coliform bacteria, especially *E. coli* and *Salmonella* species in the
TABLE 5: Physico-chemical properties of locally produced soy flour

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>SAMPLE CODE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour</td>
<td>LB</td>
</tr>
<tr>
<td>Flavour</td>
<td>B</td>
</tr>
<tr>
<td>Taste</td>
<td>R</td>
</tr>
<tr>
<td>PH</td>
<td>5.0</td>
</tr>
<tr>
<td>Viscosity (g/l)</td>
<td>ND</td>
</tr>
<tr>
<td>Moisture content (%)</td>
<td>5.50</td>
</tr>
<tr>
<td>Total solid (%)</td>
<td>ND</td>
</tr>
<tr>
<td>Crude protein (%)</td>
<td>4.86</td>
</tr>
<tr>
<td>Crude fat (%)</td>
<td>1.41</td>
</tr>
<tr>
<td>Crude fibre (%)</td>
<td>1.22</td>
</tr>
<tr>
<td>Carbohydrate (%)</td>
<td>60.0</td>
</tr>
<tr>
<td>Ash content (%)</td>
<td>1.82</td>
</tr>
<tr>
<td>Total Sugar (mg/100ml)</td>
<td>325</td>
</tr>
<tr>
<td>Titratable acidity %</td>
<td>0.92</td>
</tr>
<tr>
<td>Calcium content (mg/100ml)</td>
<td>40.0</td>
</tr>
<tr>
<td>Phosphorus content (mg/100ml)</td>
<td>9.50</td>
</tr>
</tbody>
</table>

Values are mean ± standard deviation of three replications.

LB: Light Brown; R: Roasty; B: Beany; ND: Not done.

Food is of concern because E. coli has been associated with gastroenteritis (Atanda and Akpan, 1999) and urinary tract infections (Omonigho et al., 2001), while Salmonella species are known to be responsible for certain food born infections including Salmonellosis and Typhoid fever. The presence of E. coli in particular is an indication of faecal contamination (Edema et al., 2001).

None of the products contained any preservative. This may probably account for the rapid growth and replication of spoilage microorganisms in the products. The level of occurrence of bacterial species which was higher in soymilk, could be due to the higher water activity (aw) value in the milk which would favour bacterial growth and metabolism. The heat treatment given to the bottled soymilk was probably insufficient to kill substantial number of microorganisms in the product or to denature the microbial enzymes which were not desirable in the milk. Also, improper or non-sterilization of the bottles before they were filled with the soymilk could encourage microbial contamination of the product.

The presence of Staphylococcus epidermidis and Streptococcus species in the products indicate inadequate precautions taken either during processing of raw material or during packaging and subsequent distribution of the finished products.

The carbohydrate content in the soymilk was higher (63.01%) than in soy flour (31.58%). This high level was expected because some soymilk producers add certain carbohydrates such as sugar as sweeteners.

The presence of high counts of food spoilage bacteria and toxigenic mould in soymilk and soy flour strongly suggests high level of contamination by these microorganisms. This poses serious health risk when such contaminated products are consumed. Producers of soymilk and soy flour should adhere strictly to good manufacturing practice (GMP). Proper handling and storage are also necessary because unhygienic handling and
poor storage methods are known to predispose them to microbial contamination. The National Agency for Food, Drug Administration and Control (NAFDAC) should, as a matter of urgency, set up adequate microbial and physico-chemical standards for these products, and ensure that producers adhere strictly to these regulations.

REFERENCES


