INTRODUCTION
Fermentation process serves as a mean of providing a source of nourishment for large rural populations. Fermentation enhances the nutrient content of foods through the synthesis of proteins, vitamin, and essential amino acids (1).

Akamu is a porridge prepared from fermented maize. It is a popular breakfast cereal and infant weaning food among the Igbo – speaking people of Nigeria. Akamu is similar to ogi, a lactic acid fermented food made from maize, sorghum or millet which may the fortified with legumes (2-4). Akamu is prepared by soaking clean maize grains in water for 2-3 days. The grains are washed and ground to a paste. The paste is sieved to smooth slurry which is allowed to settle and the supernatant decanted. The slurry is mixed with hot water with stirring until it forms a get which serves as food.

Fermented foods have become a major source of diarrhoeal diseases (5, 6). Moterjemi et al., (7) reported that maize porridge samples prepared for infants in Ghana were contaminated with pathogenic bacteria including Aeromonas, Bacillus cereus, Salmonella, Staphylococcus aureus and Vibrio cholerae. This present investigation was aimed at assessing the microbiological and some physicochemical properties of akamu and the association of some pathogenic microorganisms with the food.
Studies on Akamu, a traditional fermented maize food

MATERIALS AND METHODS

Traditional preparation of Akamu: Five kilograms of maize (Zea mays) was cleaned and steeped in 6 L of tap water in a plastic container for three days. The water was decanted and the grains were wet – milled before sieving with muslin cloth. The pomace was discarded and the starch suspension was allowed to sediment during which fermentation occurred by the natural flora of the grains.

Isolation of microorganisms

At 0 h and then at 24 h intervals, 1 g sample was aseptically withdrawn and used for microbiological analysis. The sample was homogenized in 10 mL of sterile distilled water using a sterile mortar and pestle. Then 1 mL of the pulp was added into a test tube containing 9 mL 0.1% sterile peptone water diluents and mixed. Then 0.1 mL was aseptically withdrawn with a sterile pipette and inoculated onto MRS agar (Oxoid Ltd., UK) for the isolation of lactic acid bacteria, Plate Count Agar (Difco) plates for total aerobic mesophilic bacteria, Mac Conkey agar (Oxoid) for Enterobacteriaceae and Sabouraud Dextrose agar containing 0.1% chloramphenicol for yeasts and mould counts. The plates were incubated at 30 °C for 24–72 h and the number of colony forming units per gram (CFU/g) was determined. The morphological, physiological and biochemical characterization of bacterial isolates were carried out as described by Holt et al., (8). Carbohydrate fermentation patterns of the lactic acid bacteria (LAB) isolates were determined by using API 50 CHL test kit. On-line software of Bio Merieux (www.apiweb.biomerieux.com) was used to identify the isolates. Yeasts and fungi were identified based on the taxonomic schemes described by Pitt and Hocking (9). Qualitative test for starch hydrolysis by the isolates was done by surface – plating starch (Merck) agar plates with the isolates. The plates were flooded with 5 mL iodine solution and visualized for zones of clearing around the colonies after 2 d incubation.

Chemical analyses

Sample (100 g) was treated with 5% NaOH, 8% urea and 0.05 % sodium dodecyl sulphate and incubated at 32°C for 16 h. After incubation, the sample was centrifuged at 2515xg and the supernatant was used for protein assay. Protein content was determined by the method of Lowry et al., (10) using bovine serum albumin (Sigma-Aldrich) as a standard. The pH was determined using a glass electrode pH meter (PYE Unicam, England). Reducing sugar was assayed by a modification of the dinitrosalicylic acid (DNS) method of Miller (11): DNS (10 g) was dissolved in 200 mL of 0.2 M NaOH. Potassium sodium tartrate (300 g) was dissolved in 800 mL of distilled water. The two solutions were mixed and stored in an air tight dark bottle. An aliquot (4 mL) of this reagent was added to tubes containing 1 mL of glucose solution and to distilled water blanks. The tubes were placed in boiling water for 10 minutes and cooled to room temperature. The solutions were read in a Spectrum Lab 23A spectrophotometer at 540 nm. The readings were used to draw a standard curve for micrograms glucose equivalents per mL against absorbance. Titratable acidity was determined using the standard method described by Amoa – Awua et al; (12). A 10 g sample was blended with 100 mL of distilled water and filtered through two successive Whatman No 1 filter papers. Then 25 mL of the filtrate was titrated with 0.25M NaOH and 1% phenolphthalein as an indicator. The titratable acidity was calculated as percent lactic acid in the sample using the relationship given by Kimayo et al., (13). Starch content was determined by anthrone method of Clegg (14).

RESULTS AND DISCUSSION

The microbial composition of akamu sample is shown in table 1. Within the first 48 h of fermentation, the organisms isolated included Lactobacillus delbrueckii, Lactobacillus plantarum, Lactobacillus fermentum, Lactobacillus amylovorus, Pseudomonas aeruginosa, Pseudomonas alkaligenes, Bacillus cereus, Bacillus licheniformis, Bacillus subtilis, Candida utilis, Candida tropicalis, Saccharomyces cerevisiae, Aspergillus oryzae, Aspergillus niger, Penicillium citrinum, Rhizopus microsporus and Rhizopus oligosporus. The presence of these organisms in significant numbers at this time indicated that they constituted part of the maize microflora. Mucor circinelloides and Rhodotorula glutinis were the only organisms isolated beyond 48 h.

Bacillus subtilis, Bacillus cereus and Bacillus licheniformis showed saccharolytic activities and these organisms persisted towards the end of the fermentation indicating that they continued the saacharification of maize starch to release sugars. The majority of lactic acid bacteria isolated from akamu belonged to the genera Lactobacillus. These organisms arose early in the fermentation, increasing rapidly from 1.6 x107 cfu/g to 7.1 x 108 cfu/g after 72 h. The decrease in sugar concentrations could be attributed largely to the activities of these organisms which metabolized and converted sugars into organic acids during maize fermentation (15). Giraud et al., (16) demonstrated amylase ability of Lactobacillus plantarum but all the lactic acid bacteria isolated from akamu were unable to hydrolyze starch in pure culture. Lactobacilli are the most important organisms that produced acidity in maize fermentation (17).These organisms have also been implicated as partly responsible for initiating acidification in fermenting maize dough (18). This work confirms this suggestion because lactobacilli had the highest population

<table>
<thead>
<tr>
<th>TABLE 1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fermentation Time (h)</strong></td>
</tr>
<tr>
<td>-------------------------------</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>24</td>
</tr>
<tr>
<td>48</td>
</tr>
<tr>
<td>72</td>
</tr>
</tbody>
</table>
counts towards the end of the fermentation when pH was 3.9 and titratable acidity was 0.79. This highly acidic condition which developed at the latter part of the fermentation may be responsible for the dominance of lactobacilli towards the end of the fermentation.

Enterobacteriaceae showed a slight decrease during the early stages of the fermentation (table 1). The Enterobacteriaceae isolated were identified as Escherichia coli, Proteus sp, Serratia sp. and Enterobacter aerogenes. E coli and E. aerogenes are generally considered as indicators of faecal contamination. Their presence may be due to the contamination of water samples used to prepare the akamu sample.

The yeast, Saccharomyces proliferated rapidly during fermentation and then fell in counts disappearing towards the end of the fermentation.

**FIGURE 1**
Changes in pH during the fermentation of Akamu

![pH Graph](image)

**FIGURE 2**
Changes in titratable acidity during the fermentation of Akamu

![Titratable Acidity Graph](image)
end of the fermentation at which time other yeasts namely Candida spp. became predominant. The role of Saccharomyces spp. is not easy to ascertain especially as ethanol, the man product of its fermentation of carbohydrates has not been detected in fermented maize. However Saccharomyces rouxii (19) and Saccharomyces cerevisiae (20) have been implicated as being partly responsible for the organoleptic properties of fermented maize. All the yeast strains isolated did not show amylolytic activity in pure culture.

The moulds Aspergillus oryzae, Aspergillus niger, Rhizopus microsporus and Rhizopus oligosporus were able to degrade starch in pure culture whereas Penicillium citrinum was unable to do so. These organisms decreased in population as the fermentation progressed and their population reduced significantly after 24 h (table 1). Ekundayo (21) reported that Penicillium sp and Aspergillus sp. were responsible for the conversion of starch to sugars during the steeping of maize for the production of pito. Rhizopus microsporus is mycotoxigenic and was found in 18% of maize samples (9).

The results of pH development and titratable acidity of akamu during fermentation are shown in figures 1 and 2. The pH decreased from 6.6 at the start of the fermentation to 3.9 after 72 h. Titratable acidity increased from 0.48 at 0 h to 0.79 after 72 h. The acidity of ogi was attributed to the presence of lactic acid, acetic acid and butyric acid (20). Banigo and Muller (2) detected the presence formic acid in ogi. Production of acid during natural fermentation of maize led to a significant reduction of pH which determined the shelf-life of the products as well as the destruction of enteric pathogens (22).

Changes in starch, protein and total reducing sugar concentrations of akamu are shown in table 2. Starch consisted 68.1% of unfermented maize but this decreased to 37.4% after 72 h. The total reducing sugar increased from 5.3g/100 g to 17.6 g/100 g. The sugar production may be as a result of starch degradation by the fermenting microflora. The protein content increased significantly ($p \leq 0.01$) from 12.8 g/100 g to 18.5 g/100 g after 72 h. The increase in protein may be due to microbial growth with a resultant increase in microbial biomass during the fermentation process. Some microbial strains have been found useful for protein enrichment of foods (23,24). Since no external source of nitrogen was applied in this study, this could be due to conversion of some of the plant proteins or other nitrogenous compounds into microbial protein.

Several authors have highlighted the importance of adequate nutritional quality and hygiene during the preparation of foods and also the link between infection and nutrition (5). Oyelana and Coker (25) reported the presence of pathogenic bacteria such as Staphylococcus aureus and mycotoxigenic fungi such as Aspergillus flavus and Fusarium oxysporum from fermented maize samples. Fermented foods have many advantages such as improving the nutritional value and safety of foods against food – borne pathogens. Fermentation is protective against food-borne diseases and it is usually recommended as a cheaper way of preparing foods especially in the developing countries (26). However the occurrence of bacterial pathogens in fermented foods suggests a need for caution in the use of these foods for infant feeding. Challenge tests have shown the possibility of pathogens to survive and grow in fermented foods (27, 28). Akamu is a traditional food for weaning infants who are fed at least once a day as a supplement to breast milk. From this study, Akamu could pose a health risk to consumer based on the types and numbers of pathogenic microorganisms isolated.

### TABLE 2

<table>
<thead>
<tr>
<th>Component (g/100 g)</th>
<th>Non fermented</th>
<th>Fermented</th>
<th>P (t-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>12.8±0.57</td>
<td>18.5±0.42</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Starch</td>
<td>68.1±12.73</td>
<td>37.4±2.83</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Reducing sugar</td>
<td>5.3±0.71</td>
<td>17.6±0.99</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

### RESUMEN

Se estudiaron las propiedades microbiológicas y fisiocquimicas del akamu, un alimento fermentado a base de maíz. Se midió la población microbiológica, el pH, la acidez por titulación, azúcar y almidón durante la fermentación. La microflora inicial consistía en una mezcla heterogénea de microorganismos denominados Lactocabillus delbrueckii, Lactocabillus plantarum, Lactocabillus fermentum, Lactocabillus amylolovor, Pseudonomas aeruginosa, Pseudonomas alkaligenes, Bacillus cereus, Bacillus licheniformis, Bacillus subtilis, Candida utilis, Candida tropicivalls, Saccharomyces cerevisiae,Aspergillus oryzae, Aspergillus niger, Penicillium citrinum, Rhizopus microsporus y Rhizopus oligosporus. El cabe de 24 horas, las bacterias lactobacillus y mesofílicas aeróbicas formaron una parte importante de la microflora total. Las bacterias lactobacilli aumentaron a 1.6 x107 cfu/g a las 24 horas y al cabo de 72 horas a 7.1 x 10 8 cfu/g. El total de bacterias mesofílicas aeróbicas aumentaron desde 2.5 x 109 en 24 horas y a 4.2 x 10 8 a las 72 horas. El recuento de enterobacteria disminuyó de 6.3 x 103 en 24 horas a 2.5x 102 cfu/g a las 72 horas, aunque este nivel permaneció significativamente alto para un producto alimentario final. Las levaduras aumentaron en forma significativa y alcanzaron un rango de 6.8 x 105 a las 72 horas. Los recuentos de hongos disminuyeron desde 6.3 x 103 cfu/g a las 24 horas a un rango de 1.3 x 102 cfu/g a las 72 horas. Los hongos fueron responsables de la actividad amilolítica en el cultivo puro. La fermentación provocó una disminución general en el pH desde 6.6 a 3.9 a las 72 horas y la acidez por titulación aumentó a 0.48 a 0.79 a las 72 horas. La concentración de almidón disminuyó desde 68 g/100 g a 37.4 g/100 g. Las concentraciones de proteínas y azúcar reducida aumentaron desde 12.8 g/100 g a 18.5 g/100 g y desde 5.3 g/100 g a 17.6 g/100 g respectivamente. Los tipos y cantidades de microorganismos aislados del akamu suponen un riesgo sanitario para los consumidores especialmente dado que este producto es utilizado como una formula láctea para lactantes.

Palabras clave: Akamu, recuento bacteriano y hongos, proteína, azúcares reductoras.
REFERENCES


