**Original Research Paper**

**The impact of traditional steeping processes on the nutritional-microbiological-sensory quality of akamu (pap)**

**ABSTRACT**

Akamu (Pap) is a well-known fermented and healthy food commonly prepared by different households in Nigeria. Different traditional steeping methods have been utilized by different households to process Akamu. This research two traditional steeping methods (continuous and discontinuous) employed in the processing of Akamu. Two (2) kilograms of corn were bought from Eke Awka market in Anambra State, Nigeria and were soaked in portable water using two different clean containers labelled samples A and B. Sample A was allowed to ferment continuously for three days without changing water and the water from sample B was changed every 12 hours until the third day. Samples of the steep water were taken on the first and last day of the experiment for microbiological analysis. Both samples were processed (wet milled and sieved) and the resulting samples of Akamu were used to access their nutritional (Proximate, Minerals and Vitamins) and sensory evaluations. T-test was used to compare sample means and statistical significance was placed at p < 0.05. Microbiological analysis showed higher bacterial count of 5.6 x 106from sample A than sample B (3.45 x 105). Fungal (Yeast) count of 2.3 x 104 was obtained only from Sample A. Organisms present in both samples on the first day of the experiment were *Streptococcus, Clostridium, Bacillus* species and Yeasts but were succeeded by *Lactobacillus* and *Saccharomyces* species in sample A on day 3 while sample B retained most of the initial isolates but with the addition of Lactobacillus spp. No yeast was found in Sample B. There was a clear difference in the microbial succession of both samples and there most of the mean differences in the nutritive compositions were statistically significant. Sample A was moderately higher in macro mineral content while sample B was higher in soluble vitamin content. The Proximate analysis of both samples was approximately the same and sample A was found to be more acceptable in the sensory evaluation. Understanding of these differences will guide the choice of the method of processing Akamu in different households.

**Keywords: Akamu, Steeping, Nutrition, Sensory evaluation, Quality**

**Introduction**

One of the popular indigenous cereal-based fermented foods in Nigeria is Akamu. Akamu is a traditional porridge produced from maize, sorghum or millet grains majorly used as powerful weaning diet for children as well as dietary staple for adults in West Africa (Adebukunola et al., 2015). Akamu has a distinct aroma, sour taste and fine texture. Its colour depends on the type of raw materials used for the processing and can either be consumed as porridge (pap) or as a gel-like product (agidi) (Olorunjuwon et al., 2018). Maize is an essential staple food that is valued for its high nutritional content, including macronutrients such as starch, fibre, protein, and fat, as well as micronutrients like B-complex vitamins, ß-carotene, magnesium, zinc, phosphorus, and copper (Shi et al., 2017). While maize is known for its nutritional significance, there is a need to better understand the effects of different processing methods on its micronutrient and phytochemical contents (Suri and Tanumihardjo, 2016). Steeping of maize grain is a process that involves soaking the kernels in water for a certain period of time to soften them and release starch and various value-added products such as starch, sweeteners, oil, gluten, and feed (Okeke et al., 2018).

Fermentation, also known as steeping, is a traditional method of processing corn that involves soaking the grains in water for a certain period of time, usually ranging from 12 to 72 hours, depending on the desired product. Fermentation is recognized as a natural way to preserve and safeguard foods and beverages, enhancing the nutritional value, improving the digestibility, destroying undesirable components, and inhibiting undesirable microorganisms (Marshall and Mejia, 2012). Fermentation of food typically involves the application of microorganisms that produces certain enzymes which changes the chemical attributes of the food from its original form. In developed countries, most fermented foods are produced under controlled conditions while in developing countries such as Nigeria; such foods are processed under uncontrolled conditions, using village art methods and age-old techniques (Badmos et al., 2014). During fermentation, microorganisms, such as bacteria, yeast and molds, break down the complex carbohydrates, proteins and lipids in corn, producing organic acids, alcohols, carbon dioxide and other metabolites. Fermentation can have various effects on the nutritional profile of corn, such as increasing the content of soluble dietary fibre, reducing the content of anti-nutritional factors, enhancing the availability of minerals, modifying the amino acid composition, and increasing the content of some vitamins, such as B-complex and vitamin C (Badmos et al., 2014).

During the fermentation, a given microorganism, or groups of them, initiates the growth and becomes established during a specific period of time; afterward, the growth decreases due to the accumulation of toxic end-products or other inhibitory factors. Culture-independent approaches have shown that microbial diversity in maize fermented product microbiomes is highly underestimated (Greppi et al., 2013**)**.

The mixture of microorganisms that carries out the fermentation leads to a product with very variable quality and sensory characteristics. On the other hand, the geographical isolation among the different fermented maize products provides significantly different microbial communities so that each maize fermented product can be considered as unique (Chaves-López et al., 2016).

The most common microorganisms found in fermented maize products- Lactic Acid Bacteria frequently produce enzymes able to breakdown polysaccharides or other molecules with high molecular weight, as well as organic acids and some compounds able to kill or reduce the microbial populations, such as bacteriocins and hydrogen peroxide (Mokoena et al., 2016). They are also able to increase the content of free amino acids and B group vitamins, improving the availability of iron, zinc, and calcium by breaking down antinutritional compounds (Blandino et al., 2003); in addition, they produce gas and other volatile compounds (VOCs) contributing to the sensory properties of the product.

Yeasts, besides providing growth factors such as vitamins and soluble nitrogen to Lactic Acid Bacteria, also produce several extracellular enzymes (lipases, esterases, amylases, and phytases), some of which participate in the formation of fermented maize flavour and aroma (Omemu et al., 2007**)**. On the other hand, the aerobic spore-forming bacteria (Bacillus spp.) secrete a wide range of degradative enzymes, such as amylases and proteases (Almeida et al., 2007)., and can also produce antimicrobial compounds such as bacilysin, which is able to inhibit molds and bacteria; and iturin and chloromethane, which inhibit bacteria, thus playing an important role in the fermented maize product development.

This study tried to evaluate the impact of steeping processes on the microbiological, nutritional and sensory profile of maize after steeping using different traditional steeping methods.

**MATERIALS AND METHODS**

**Study Area**

This study was carried out at the Laboratory Unit of Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, Awka, Anambra State, located on the South – Eastern part of Nigeria, latitude 6o14o34”N/6.242889oN and longitude 7o07o06”E/7.118289oE with a land mass of approximately 4.99km (equivalent to 499 hectares) containing about 40,000 students both undergraduate and post-graduates.

**Sample collection**

Two kilogram of Maize grains (yellow maize) was purchased from Eke Awka market Awka in Anambra State. The maize grains were sorted, washed thoroughly and steeped using two traditional methods (continuous steeping; this involved steeping of maize without changing the steep water for 72 hours) and intermittent steeping; this involved steeping of maize for 72 hours and removing and replacing the steep water every 12 hours).

An aliquot of the fermentate was aseptically collected from the steeping vessels using sterile containers. Two samples were collected from the first day to the final day of steeping for microbiological analysis. After the last day of steeping, the steeped grains were removed from the steeping vessels, wet milled, wet sieved using muslin cloth and allowed to settle and dewatered and bagged. The *Akamu* samples were collected for proximate, macro mineral and water-soluble vitamins analysis.

**Sample Preparation and Standardization of Inoculum**

Five millilitres (5 ml) of steep water collected from the two traditional steeping processes were added to 45ml of water in a conical flask. Then 1ml of the sample suspension was diluted using a tenfold serial dilution prior to the inoculation on the nutrient Agar and Sabouraud Dextrose Agar.

**Microbial Isolation**

A Tenfold serial dilution was carried out for the enumeration of microorganisms present by spread plate method. Sabouraud dextrose Agar (SDA) was supplemented with Chloramphenicol to inhibit the growth of commensal bacteria and was utilized to enumerate fungi while nutrient agar supplemented with Nystatin were utilized for bacteria growth. Aliquot of 0.2ml of the samples was inoculated in the respective media and a sterile bent glass rod was used to spread the sample on the agar. For Bacteria and Yeast total viable count, culture was incubated for 24-48 hours at 37oC.

The total viable count was obtained using TVC= N xD/V where TVC= Total Viable Count, N= mean colony, V= Volume of sample inoculated and D=Dilution Factor.

**Purification of Isolates**

Colonies obtained was purified by subculturing into sterile agar plates and incubated for 24hrs at 37oC. The purified isolates were stored in agar slants for further analysis.

**Characterization and Identification of Isolates**

 The isolates were sub-cultured and the pure culture were identified by colonial characteristics, Gram’s reaction, biochemical tests such as Catalase tests, Motility test, Citrate test fermentation and Hemolysis test. Anaerobic cultivation, growth on peptone water and sugar fermentation was used as basic distinctive tests for Anaerobes. (Chesbrough, 2006).Both microscopic and macroscopic techniques were employed for the identification of the organisms.

.

**Morphological Characterization**

Morphological characteristics of bacterial and fungal colonies were done based on the shape of the colonies, elevation, optical characteristics, margin opacity and pigmentation. The yeast colonies were described based on their shape, colour or pigmentation both forward and reverse, size.

**Preparation of Akamu**

Akamu was prepared from same varieties using different methods. In the first method, the maize varieties were steeped continuously for three days, wet milled, sieved and fermented separately at 30±2°C by the maize natural microflora, while also in the second method, the maize varieties were steeped for three days but the steep water was removed every 12 hours, wet milled, sieved and fermented separately at 30±2°C by the maize natural microflora. The softened corn is washed and ground in a mechanized mill. The ground materials were rinsed with water and passed through a sleeve (Muslin cloth) to remove parts of the hull. The filtrate pure starch is placed in pots of water to settle and cover it up completely. The sediment was placed in a cheese cloth and squeezed to remove excess water.

WHOLE YELLOW MAIZE GRAIN

CLEANING /SORTING

COLLECTION OF SAMPLES FOR MICROBIOLOGICAL ANALYSIS AFTER THE FIRST DAY AND THE LAST DAY OF STEEPING

STEEPING CONTINOUSLY FOR 3 DAYS AND INTERMITENT REMOVAL Of STEEPWATER

WET MILLING

WET SIEVING

FERMENTING AND SETTLING FOR (24H)

SEDIMENTATION/ DECANTATION

BAGGING/ DEWATERING

**Figure 1: Flow chart for the production of Akamu**

**NUTRIENT CONTENT DETERMINATION**

**Determination of** **Moisture**

This was done by the gravimetric method described by the [AOAC (2015).](https://scialert.net/fulltext/?doi=ijb.2014.37.41) A measured weight of the sample (5.0 g) was weighed into a previously weighed moisture dish. The sample in the dish was dried in the oven at 105°C for 3 hours, cooled in a desiccator and weighed. It was returned to the oven for further drying, cooling and weighing repeatedly at hourly interval until there was no further diminutions in the weight (that is, constant weight was obtained). The weight of moisture lost was calculated and expressed as a percentage of the weight of sample analyzed. It was given by the expression below:

Moisture content (%) = M2 – M3 × 100

 M2 – M1

Where:

M1 = Mass of empty moisture dish

M2 = Mass of empty dish + Sample before drying

M3 = Mass of dish + Sample dried to constant weight

**Determination of crude protein**

This was done using Kjeldahl method according to AOAC (2015). One gram of the sample was prepared into a micro kjeldahl flask. Twenty-five millilitres of sulphuric acid (H2SO4), one gram of cupric acid (CuSO4) and ten grams of sodium sulphate (Na2SO4) was added into the micro kjeldahl flask containing the sample. The flask was heated at an inclined angle (60o). Anti-bumping agent was added to avoid frothing. It was heated gently at first at 70oC then increased continuously until the liquid changed to bluish green and was free from brown or black colour. The flask was allowed to cool and the content was diluted with 200 mL of distilled water and 60 mL of 40/50% NaOH. The flask was connected to a distillation apparatus incorporating a head fitting and condenser. In a 250 mL conical flask, 4% boric acid was prepared and 2 drops of screened methyl red indicator was added to it. The mixture was boiled at 80-90oC allowing the distillate (ammonia gas) to get trapped into the boric acid until the content in the conical flask reached 200 ml. In a burette, prepared 0.1N H2SO4 was poured and titrated against the content in the conical flask till a light pink colour was obtained.

**Calculation:**

|  |  |
| --- | --- |
| Final reading (cm3/ml) |  -------- |
| Initial reading (cm3/ml) |  -------- |
| Volume of titrant (Tv) |  ------- |

% Nitrogen = Tv × 0.0014 g × 100

 Weight of the sample

% Protein = % Nitrogen × protein factor

**3.5.3 Determination of total ash content**

This was done by the furnace’s incineration gravimetric method according to AOAC (2015). Exactly 10 g of the sample was measured into a previously weighed porcelain crucible. The sample was burnt to ashes in a muffle furnace at 550°C for three hours. When it has become completely ashed or turned to grey, it was cooled in desiccator and weighed. The weight of ash obtained was determined by difference and calculated as a percentage of the weight of sample analyzed thus:

Ash (%) = M2 – M1 × 100

 Mass of sample

Where:

M1 = Mass (g) of empty crucible

M2 = Mass of crucible + Ash

**Determination of crude fibre content**

This was done by the method described by the [AOAC (2015).](https://scialert.net/fulltext/?doi=ijb.2014.37.41) Two grams of the defatted sampled was weighed into a conical flask. In the conical flask, 200 mL of 1.25% or 0.127N H2SO4 was added and boiled on a heating mantle at 80oC for 30 minutes. The solution was filtered while hot with muslin cloth and the residue was further washed with boiled water. Using 200 mL of 1.25% OR 0.313M NaOH, the residue was transferred into the conical flask and boiled at 80oC for 30 minutes. A filter paper was weighed and recorded (M1). The mixture was filtered into the already weighed filter paper, after filtration the paper and the residue were placed in a Petri dish and dried in an oven at 80oC until it dried. After drying, it was cooled in a desiccator, weighed and recorded (M2). In an already washed, dried, cooled and weighed crucible (M4), the paper containing the residue was transferred. The crucible was placed in a muffle furnace and allowed to burn for 5 hours at 600oC. After which it was cooled and weighed as M5.

**Calculation:**

 % Fibre = M7 × 100

 M

M3 = M2 – M1

M6 = M5 – M4

M7 = M3 – M6

M = mass of sample

**3.5.5 Determination of crude fat content**

This was determined by Soxhlet extraction method described by AOAC (2015). Five grams of sample was wrapped in a porous paper (Whatman filter paper) and put in a thimble. The thimble was put in a Soxhlet reflux flask and mounted into a weighted extraction flask containing 250 ml of petroleum ether. The upper of the reflux flask was connected to a water condenser. The solvent (petroleum ether) was heated, boiled vaporized and condensed into the reflux flask. Soon the sample in the thimble was covered with the solvent until the reflux flask filled up and siphoned over, carrying its oil extract down to the boiling flask. This process was allowed to go on repeatedly for 4 hours before the defatted sample was removed, the solvent recovered and the oil extract was left in the flask. The flask (containing the oil extract) was dried in the oven at 60°C for 30 min to remove any residual solvent. It was cooled in desiccator and weighed. The weight of oil (fat) extract was determined by difference and calculated as a percentage of the weight of sample analyzed thus:

Fat (%) = M2 – M1 × 100

 Mass of sample

Where,

M1 = Mass (g) of empty extraction flask

M2= Mass of flask + oil (fat) extract

**Determination of carbohydrate content.**

The carbohydrate content was determined by difference. That was by deducting the mean values of other parameters that were determined from 100.

Calculation:

% Carbohydrate =100 - (% Mc + %Cp + % Fat + %Crude fibre + % Ash)

Mc =moisture content

Cp = crude protein

%fat= fat

**Determination of vitamin using Uv-spectrophotometer**

**Procedure for Water Soluble Vitamins:**

**Vitamin B1 (Thiamine hydrochloride)**

Five millilitres of the standard and sample was taken in marked test tubes. In each test tube, 5 ml NH4OH (0.1M) and 0.5 ml 4-Amino phenol solution added and mixed well, then kept for 5 minute added 10 ml chloroform and separate of chloroform layer. The absorbance recorded chloroform layer at 430 nm against blank.

**Vitamin B2 (Riboflavin)**

Five millilitre of the standard and sample solution was taken in marked test tubes. In each test tube, 2 ml hydrochloric acid (1 M), 2 ml glacial acetic acid, 2 ml hydrogen peroxide, 2 ml potassium permagnate (15% w/v) and 2 ml phosphate buffer (pH 6.8) added and mixed well, and absorbance recorded at 444 nm against blank.

**Vitamin B3 (Nicotinamide)**

Two millilitres of the standard, sample and blank solution was taken in marked test tubes. In each test tube, 5 ml sulphanilic buffer (pH 4.5), 5 ml water and 2 ml cyanogen bromide solution (10% w/v) added and mixed well, and absorbance recorded at 450 nm against blank and recorded an interval of 2 minutes.

**Vitamin B5 (Pantothenic acid)**

**Hydrolysis of standard and sample**

Five millilitres of standard and sample solution was taken into 50 ml volumetric flask. In each volumetric flask, 2 ml hydrochloric acid (1 M) added and mixed well, then heat 5 hours at 690C ±10C for affecting the hydrolysis and cool at room temperature. After that 2 ml of hydroxylamine reagent (7.5% in 0.1M sodium hydroxide), 5 ml sodium hydroxide (1M) and kept for 5 minute now adjust pH 2.7 ± 0.1 with 1 M hydrochloric acid and make up the volume with water.

**Procedure**

Five millilitres of the standard and sample hydrolysis solution were taken in marked test tubes. In each test tube, 1 ml of 1% ferric chloride solution (in water) was added and mixed well to remove the air bubbles and absorbance was recorded at 500 nm against blank.

**Vitamin B6 (Pyridoxine hydrochloride)**

Two millilitres of the standard and sample solution were taken in marked test tubes. In each test tube, 1ml of ammonium buffer (in water), 1 ml of 20% sodium acetate (in water), 1ml of 5% boric acid (in water) and 1 ml dye (2,6- di-cholroquininechorimide) solution added and mixed well. It was absorbance recorded at 650 nm against blank.

**Vitamin B7 (Biotin)**

Weigh accurately equivalent to 500 mcg of vitamin B7 of sample was taken into 100 ml volumetric flask and 10 ml of dimethyl sulfoxide was added to dissolve. Heat the flask on water bath at 600o to 700oC for 5 minutes. The volume was made up to the mark with dilute water. Filter and absorbance recorded at 294 nm against blank of sample as well as standard.

**Folic Acid (Vitamin B9)**

Two millilitres of the standard and sample solution was taken in marked test tubes. In each test tube, 2 ml of 0.02% potassium permanganate solution, 2 ml 2 % sodium nitrate solution, 2 ml 4 M hydrochloric acid solution, 1 ml 5 % ammonium sulphamate solution and 1 ml dye solution (0.1 % N, N diethyl aniline dye solution in iso propyl alcohol) added and mixed well, then kept for 15 minutes at room temperature. It was absorbance recorded at 535 nm against blank.

**Vitamin B12 (Cyanocobalamin)**

Weigh accurately equivalent to 1 mcg of vitamin B12 of sample was taken into 25 ml volumetric flask and 10 ml of water was added to dissolve. 1.25 gm of dibasic sodium phosphate, 1.1 gm of anhydrous citric acid and 1.0 gm of sodium metabisulphate was added. The volume was made up to the mark with water. The solution was autoclaved at 1210 C for 10 minutes. Filter and absorbance recorded at 530 nm against blank of sample as well as standard.

**Vitamin C (Ascorbic Acid)**

Ten gram of the sample was extracted with 50mL EDTA/TCA extracting solution for 1 hour and filtered through the Whatman filter paper into a 50mL volumetric flask and made up to the mark with the extracting solution. 20 ml of the extract was pipetted into a 250 ml conical flask and 10ml of 10% KI and 50 ml of water were added. This was titrated against 0.01 N CuSO4 solution to a dark end point and Ascorbic acid was calculated as below:

**Vitamin C mg/100 = 20 x (V1-V2) x C**

 **Weight of sample**

**Sensory Evaluation**

Akamu was prepared by separately heating the slurry of the fermented Akamu sample in boiling water under constant stirring using a clean stirrer to form a thick paste. Sensory evaluation of the various Akamu samples was done by a 10-man panel who are familiar with the product. The evaluated parameters were appearance, colour, aroma, taste, texture and overall acceptance. The ratings were presented on 9 – point Hedonic scale (Peryam and Pilgrim, 1957) ranging from 9 = like extremely to 1 = dislike extremely. The average scores of the products tasted gave an idea of how each product was received by the tasters:

 - 9 = Like extremely

 - 8 = Like very much

 - 7 = Like moderately

 - 6 = Like slightly

 - 5 = Neither like nor dislike

 - 4 = Dislike slightly

 - 3 = Dislike moderately

 - 2 = Dislike very much

 - 1 = Dislike extremely

**Statistical Analysis**

Statistical analysis was done using excel and data were presented in tables. Frequencies were obtained and values tested using chi-square. The mean values were presented using standard deviation. T-test was used to compare the mean of quantitative variables and statistical significance was placed at p < 0.05.

**RESULTS**

On the first day of steeping, an average colony counts for Sample A and Sample B were almost identical, which was 4.4 x 105Cfu/ml for Sample A and 4.35 x 105Cfu/ml for Sample B). The total viable counts also reflected almost matching values, which equalled 2.2 x 106Cfu/ml for Sample A, and 2.17 x 106Cfu/ml for the Sample B (Table 1).

On the final day of steeping, Sample A portrayed a significant increase in mean bacterial colony count at 1.12 x 106Cfu/ml and a total viable count of 5.6 x 106Cfu/ml. In contrast, Sample B showed the mean bacterial colony count of 6.9 x 104Cfu/ml and a total viable count of 3.45 x 105Cfu/ml (Table 2).

Interestingly, no yeast growth was observed on the first day. On the last day of fermentation, however, yeast growth was observed in Sample A with a mean count of 4.6 x 104Cfu/ml and a total viable count of 2.3 x 105Cfu/ml (Table 3).

A total of four (4) bacterial species and yeast were identified. They include Bacillus species, Lactobacillus species, Streptococcus species, Clostridium species and Saccharomyces species (Table 4).

Table 5 shows the presence (+) or absence (-) of five different microorganisms in Samples A and B on day 1 and day 3 of the fermentation process. *Streptococcus* species, *Clostridium* species and *Bacillus* species were present on day 1 in both samples. While sample B retained most of the initial organisms, sample B retained only Bacillus species in addition to new sets of organisms – *Lactobacillus* and Streptomyces on day 3. Sample B added only Lactobacillus, but Streptomyces was completely absent on day 3.

**8: Proximate Composition of Akamu Samples.**

 The proximate compositions of the two samples were very similar. However, there are a few slight differences. Sample B has a slightly higher moisture content, crude fibre content, and ash content than Sample A. Sample A, on the other hand, has a slightly higher crude protein and carbohydrate content than Sample B. The mean differences in all the proximate composition of the samples were all statistically significant.

**The macro mineral content of the Akamu samples**.

 The content of calcium, magnesium, phosphorus, potassium, and sodium in milligrams per 100 grams (mg/100g) of akamu was shown in table 7. The macro mineral contents of the two samples differed with different contents examined. Sample A had slightly higher calcium content than Sample B. Sample B, on the other hand, had slightly higher magnesium, phosphorus, and potassium content than Sample A. The mean differences in all the macro mineral content were all statistically significant.

**Water Soluble Vitamin Content of the Akamu Samples**

Listed in the table are the milligram content for each of the mentioned vitamins per 100g for both Sample A and Sample B. Sample A had generally higher contents of Vitamin B2, B5, B9, and B12. Sample B had slightly higher contents of Vitamin C, B1, B7. Both samples had similar contents for Vitamins B3 and B6. The calculated mean differences in all the water-soluble vitamin content were all statistically significant with the exception of Vitamin B6.

Table 8.0 presents the results of a sensory evaluation comparing akamu samples prepared using two different steeping methods: continuous steeping (Sample A) and intermittent steeping (Sample B). The evaluation employed a 9-point hedonic scale, where 1 represents dislike extremely and 9 represented like-extremely. The table showed the average scores for each sensory attribute. Based on the sensory evaluation, panellists generally favoured Akamu produced from continuous steeping (Sample A) over Akamu from intermittent steeping (Sample B). This preference was driven mainly by the higher scores for appearance, colour, and aroma in Sample A. Taste and texture did not show a significant difference. 43.9, 40.3

**Table 1: Bacterial colony count on the first day of steeping.**

|  |  |  |
| --- | --- | --- |
| Samples | Mean colony count(Cfu/ml) | Total viable count (Cfu/ml) |
| Sample A | 4.4 x 105 | 2.2 x 106 |
| Sample B | 4.35 x 105 | 2.17 x 106 |

**Table 2: Bacterial colony count on the last day of steeping**

|  |  |  |
| --- | --- | --- |
| Samples  | Mean colony count(CFU/ml) | Total viable count (CFU/ml) |
| Sample A | 1.12 x 106 | 5.6 x 106 |
| Sample B | 6.9 x 104 | 3.45 x 105 |

**Table 3: Yeast colony count on the last day of steeping**

|  |  |  |
| --- | --- | --- |
| Samples | Mean colony count (CFU/ml) | Total Viable count (CFU/ml) |
| Sample A | 4.6 x 104 | 2.3 x 105 |

**Table 4: Characteristics of Microbial Isolates**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Characteristics  | Isolate 1 | SWIIsolate 2 | Isolate 3 | Isolate 4 | Isolate 5 |
| Morphology  | Small, circular, lobate and cream  | Small, circular, entire and grey  | Small, circular, undulate and grey  | Small, circular, entire and white  | Medium, circular, entire and white |
| Microscopic morphology  | Rod | Rod | Rod  | Cocci  | Oval |
| Gram reaction  | + | + | + | + | **+** |
| Catalase test | + | - | + | - | **+** |
| Citrate test | + | - | - | - | + |
| Motility test | Motile | Non – motile | Non – motile | Non motile | Non motile |
| Haemolysis test  | Gamma | Gamma | Gamma | Alpha | Gamma |
| Peptone water | Facultative Anaerobe | ObligateAnaerobe | FacultativeAnaerobe | Facultative Anaerobe | Facultative anaerobe  |
| Glucose test | + | + | + | + | **+** |
| Galactose test  | + | - | + | + | **+** |
| Mannitol test  | + | + | + | + | **+** |
| Fructose test | - | + | + | + | **+** |
| Lactose test  | - | - | + | + | **-** |
| Sorbitol test | - | - | - | - | **-** |
| Maltose test | + | + | + | + | **+** |
| Dextrose test | + | + | + | + | **+** |
| Sucrose | + | + | + | + | **+** |
| Probable Organisms | *Bacillus Species* | *Clostridium species* | *Lactobacillus Species* | *Streptococcus species* | *Sacharomyces species* |

**Table 5: The occurrence of succession of microorganisms during the fermentation of Samples A and B**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Isolates  | Day 1 |  |  | Day 3 |
|  | Sample A | Sample B | Sample A | Sample B |
| *Streptococcus species**Clostridium species**Bacillus species**Lactobacillus species**Saccharomyces species* | **+****+****+****-****-** | **+****+****+****-****-** | **-****-****+****+****+** | **+****+****+****+****-** |

**Table 6: The Proximate Composition of Akamu Samples**

|  |  |  |  |
| --- | --- | --- | --- |
| Parameters | Sample A | Sample B | p-value |
| Moisture Content % | 8.36±0.0404 | 8.54±0.0458 | 0.0068 |
| Crude Protein % | 7.82±0.0252 | 7.53±0.0265 | 0.0002 |
| Fats and Oil % | 3.93±0.0208 | 3.84±0.0173 | 0.0045 |
| Crude Fibre % | 5.64±0.0321 | 6.57±0.0265 | <0.00001 |
| Ash % | 2.63±0.02 | 2.45±0.0252 | <0.001 |
| Carbohydrate % | 71.61±0.0833 | 71.06±0.0379 | 0.009 |

Values are means of triplicate results.

**Table 7: Macro Mineral Content of the Akamu Samples**

|  |  |  |  |
| --- | --- | --- | --- |
| Parameters  | Sample A | Sample B | p-value |
| Calcium mg/100g | 100.897±0.4637 | 97.82±0.1756 | 0.008 |
| Magnesium mg/100g | 214.17±1.193 | 204.80±0.8 | <0.001 |
| Phosphorus mg/100g | 171.6±1.0583 | 178.22±1.0243 | 0.0016 |
| Potassium mg/100g | 13.73±0.0379 | 13.89±0.0611 | 0.031 |
| Sodium mg/100g | 1.90±0.03 | 1.83±0.0153 | 0.036 |

Values are means of triplicate results.

**Table 8: Water soluble Vitamin content of the Akamu Samples**

|  |  |  |  |
| --- | --- | --- | --- |
| Parameters  | Sample A | Sample B | p-value |
| Vitamin C mg/100g | 0.56±0.00 | 0.58±0.00 | - |
| Vitamin B1 mg/100g | 3.13±0.0361 | 3.46±0.0115 | 0.00195 |
| Vitamin B2 mg/100g | 0.69±0.0115 | 0.59±0.0153 | 0.00116 |
| Vitamin B3 mg/100g | 1.27±0.00 | 1.25±0.00 | - |
| Vitamin B5 mg/100g | 2.11±0.0153 | 2.00±0.00 | 0.00639 |
| Vitamin B6 mg/100g | 0.72±0.0208 | 0.703±0.0058 | 0.3454 |
| Vitamin B7 mg/100g | 0.19±0.0115 | 0.227±0.0153 | 0.0016 |
| Vitamin B9 mg/100g | 2.34±0.00 | 1.78±0.00 | - |
| Vitamin B12 mg/100g | 1.97±0.0208 | 1.91±0.0153 | 0.0001 |

Values are means of triplicate results. Note: the t-test of the samples with standard deviation of 0.00 could not produce any value, hence no result.

**Table 9: The Sensory Evaluation of the Akamu Samples**

|  |  |  |  |
| --- | --- | --- | --- |
| Parameters  | Sample A(Average 9-point hedonic scale scores) | Sample B (Average 9-point hedonic scale scores) | p-value  |
| Appearance  | 7.2 | 6.8 | 0.35 |
| Colour | 7.5 | 6.6 |
| Aroma  | 7.4 | 5.6 |
| Taste  | 7.0 | 6.8 |
| Texture  | 7.1 | 7.2 |
| General Acceptance | 7.7 | 7.3 |

**Sample A = Akamu obtained from continuous steeping for 3 days, Sample B = Akamu obtained from intermittent steeping for 3 days.**

**DISCUSSION**

 Fermented foods have unique functional properties imparting some health benefits to consumers due to presence of functional microorganisms, which possess probiotics properties, antimicrobial, antioxidant, peptide production, etc. Health benefits of some global fermented foods are synthesis of nutrients, prevention of cardiovascular disease, prevention of cancer, gastrointestinal disorders, allergic reactions, diabetes, among others (Marco *et al*., 2017). Probiotics are defined as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host (Hill *et al*., 2014). Probiotic organisms used in foods must have the ability to resist gastric juices, exposure to bile, and be able to proliferate and colonize the digestive tract (Saad *et al*., 2013). The most commonly used probiotic bacteria belong to the heterogeneous group of LAB (*Lactobacillus, Enterococcus*) and to the genus *Bifidobacterium*, however, yeasts and other microbes have also been developed as potential probiotics during recent years (Ouwehand *et al*., 2002).

The microbiological and nutritional profile of Akamu gave insight into the influence of both continuous and intermittent steeping on Akamu during the steeping and fermentation. The microbiological evaluation depicted several distinct differences between steep water collected using the two steeping techniques. The continuous steeping regimen produced a higher bacterial colony count and total viable count as compared to the intermittent steeping. This holds in view that a constant environment during continuous steeping favoured the proliferation of microbes (Nguyen *et al*., 2020). Still, neither of the samples showed *Lactobacillus species* and yeast growth after the first day of steeping, suggesting that bacterial populations were more vigorous in the initial hours of steeping. On the last day of fermentation, however, fungal colony growth was observed in Sample A. The Final-day yeast growth of Sample A showed that the population bloomed during the last days of fermentation with a mean count of 4.6 x 104Cfu/ml and a total viable count of 2.3 x 105Cfu/ml.

During fermentation, continuous steeping in Sample A always biased the weight towards *Bacillus species*, as other species, like *Streptococcus* and *Clostridium*, were outcompeted. *Bacillus* species displayed a nested succession pattern while other isolates showed a turnover succession pattern. This was not the case in Sample B with intermittent steeping, in which the *Streptococcus* *species*, *Clostridium* *species and* *Bacillus* *species* and displayed a nested succession pattern. *Lactobacillus species* appeared on the third day of steeping thus displaying a turnover succession pattern. Studies carried out by Akinleye *et al*. (2014) have reported that the microbial succession during fermentation/process determines the quality of the final product obtained. Also, the work of Anumudu *et al*. (2018) disclosed that constant conditions favour the growth of micro-organisms; this could be the reason why, in this work, a continuous steeping process had higher microbial counts.

 This result indicates that the steeping method could be a parameter that controls the microbial community during fermentation and, therefore, the flavour and health benefits. Significant alterations were observed between the microbial successions during the fermentations of the continuously and intermittently steeped samples. This suggests that the steeping method influences the dynamics of the microbial community as was statistically analyzed in the result.

The proximate composition structure of the two Akamu samples was very close, irrespective of the steeping method. There were slight variations: Sample B had higher moisture content, crude fibre, and ash content, while Sample A contained a bit more protein and carbohydrates. Obi and Okoronkwo, (2022) in their study, indicated an influence of different fermentation methods on the proximate composition of fermented foods. Their results showed variations similar to our study for moisture, protein, and fibre, proving the effect of fermentation methods on the nutritional content.

 Macro-mineral composition—calcium, magnesium, phosphorus, potassium, and sodium—also showed minor differences. Sample A was high in calcium and magnesium, while Sample B was slightly high in phosphorus and potassium. This could be due to the difference in the leaching of these nutrients during steeping.

Akamu seems to be an average source of some B vitamins, in particular, thiamin, riboflavin, pantothenic acid, and folate. Regardless of the steeping process, the contents do not vary much. Compared to Sample A, Sample B showed a remarkably higher level of vitamin B7 (biotin) and as such may be influenced significantly by the steeping method. Further investigation will, however, be required to establish that. Akamu, however, forms no significant source for both vitamin C and vitamin B12.

 Generally, Akamu made with continuous steeping, Sample A, had an overall higher consumer preference score compared to that made by intermittent steeping, Sample B. This was informed by the high scores for appearance, colour and aroma for sample A. There were, however, no differences recorded in taste and texture. This suggests that continuous steeping probably enhances the formation of desirable sensory characteristics under fermentation.

The results from the sensory evaluation indicated that Sample A had better scores regarding the appearance, colour, aroma, and flavour, where it could be preferred. In line with this, sensory evaluation results of Olaitan *et al*. (2018) showed a slight preference for the continuously steeped Akamu (Sample A) in terms of appearance, colour, aroma, and taste. The observed preference for Sample A could be due to **Fermentation process:** Continuous steeping might create a more consistent and controlled fermentation environment, leading to the development of preferred sensory attributes like aroma and colour.

 The overall results from the sensory score from continuous steeped Akamu suggest that continuous steeping selected for better sensory qualities: flavour and overall acceptability because more uniform microbial activity and fermentation process will be selected to increase the flavour quality of Akamu. However, texture and general acceptance gave almost similar scores in both samples, denoting that no steeping route had critical damaging incidences on these parameters. Generally, the two samples gave relatively high scores for the general acceptance.

It is well established that LAB frequently produce enzymes able to breakdown polysaccharides or other molecules with high molecular weight, as well as organic acids and some compounds able to kill or reduce the microbial populations, such as bacteriocins and hydrogen peroxide (Mokoena *et al*., 2016). They are also able to increase the content of free amino acids and B group vitamins, improving the availability of iron, zinc, and calcium by breaking down antinutritional compounds (Blandino *et al.,* 2003); in addition, they produce gas and other volatile compounds (VOCs) contributing to the sensory properties of the product.

Yeasts are groups of unicellular microorganisms, most of which belong to the fungi division of Ascomyota and fungi imperfecti (Ogu *et al.,* 2022) Yeasts, besides providing growth factors such as vitamins and soluble nitrogen to LAB, also produce several extracellular enzymes (lipases, esterases, amylases, and phytases), some of which participate in the formation of fermented maize flavor and aroma (Omemu *et al*., 2007). Yeasts produce a wide variety of VOCs, such as alcohols, esters, aldehydes, and ketones, that enrich the sensory characteristics of the maize fermented product and also contribute to reducing mold growth and spore germination, as in the case of ethyl acetate (Fredlund *et al*., 2004).

Recently, studies of Ponomarova *et al*., (2017), combining metabolomics and genetics, evidenced that yeasts enable the growth of LAB through endogenous, multi-component cross-feeding in a readily established community. On the other hand, the aerobic spore-forming bacteria (Bacillus spp.) secrete a wide range of degradative enzymes, such as amylases and proteases (Almeida *et al*., 2007), and can also produce antimicrobial compounds such as bacilysin, which is able to inhibit molds and bacteria; and iturin and chloromethane, which inhibit bacteria (Phister *et al*., 2004), thus playing an important role in the fermented maize product development. During food fermentation, metabolites produced by the desirable fermenting organisms, such as lactic acid, acetic acid, carbon dioxide, ethanol, hydrogen peroxide, bacteriocins and antimicrobial peptides, acting alone or in combination, inhibit the growth of spoilage and pathogenic organisms, there by achieving an extension in the shelf life of susceptible products (Hukins, 2006).

During maize fermentation, as occurring in other uninoculated fermentations, the competition among species for substrates, acid tolerance, syntrophic interactions, and other physiological properties of microbial populations causes fast variations in the microbiota structure. Microbiological studies have revealed that during spontaneous fermentation, bacteria and yeasts secrete a diverse array of metabolites that are available for all the community members. Thus, the interactions of the different microorganisms play a significant role during maize fermentation and participate in the changes of the nutritional, rheological, and sensorial treats through modification of the maize composition.

**Conclusion**

The steeping method used in the preparation of Akamu significantly alters the microbial population dynamics, nutrient composition, and sensory attributes of Akamu. The continuous steeping of Akamu seems to increase the population of microorganisms, especially bacteria, and also, hence, the product's sensory characteristics, as against long pauses in between steeping. There were differences in the bacterial population between the two samples. Sample A seems to show more clearly the settling-in pattern of succession of the samples over time relative to Sample B. Yeast growth was only apparent in Sample A at the end of fermentation.

The proximate, macro minerals and water-soluble vitamins of the resultant Akamu samples were similar. The mean general acceptability rating was higher for Akamu prepared by the continuous steeping method (Sample A).

 The results showed that the continuously steeping method is more preferred for akamu production. It yields a product with comparable nutritional quality and slightly better sensory characteristics; moreover, it is generally more acceptable to consumers as opposed to other methods. The steep water should not be changed during the production of Akamu because it has better nutritional and better sensory properties.

**Recommendation**

It is recommended that further studies on the microbial communities in those steeping methods and detailed nutritional composition will help to make better recommendation to the public.

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

Option 2:

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc. have been used during the writing or editing of manuscripts. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology

Details of the AI usage are given below:

1.

2.

3.

**REFERENCES**

[1] Adebukunola, M. M., Omemu, A. M., and Mobolaji, O. B. (2015). Consumer's knowledge, attitude, usage and storage pattern of ogi—A fermented cereal gruel in Southwest, Nigeria. *Food and Public Health*, 5:77-83.

[2] Akinleye, O. M., Fajolu, I. O., Fasure, A. K., Osanyinpeju, O. S., Aboderin, A. O., and Salami, O. O. (2014). Evaluation of microorganisms at different stages of production of ogi in Alimosho Community, Area Southwest, Lagos, Nigeria. *American Journal of Research Communication,* 2:215-230.

[3] Almeida, E. G., Rachid, C. C., and Schwan, R. F. (2007). Microbial population present in fermented beverage ‘cauim’ produced by Brazilian Amerindians. *International Journal of Food Microbiology,* 120:146–151. https://doi.org/10.1016/j.ijfoodmicro.2007.07.025

[4] Anumudu, C. K., Omeje, F. I., & Obinwa, G. N. (2018). Microbial succession pattern in Ogi fermentation. International Journal of Advanced Research in Biological Sciences, 5:*149*-157. <https://doi.org/10.22192/ijarbs.2018.05.07.019>.

[5[ Association of Official Analytical Chemists (AOAC). (2015). Official methods of analysis (15th ed. pp. 278–280).

[6] Badmos, A. H., Kayode, R. M., Amali, H. E., Ahmed, O. O., and Adeyemi, K. O. (2014). Fermentation and West African food culture. *Global Journal of Bio-Science and Biotechnology,* 3:128-132.

[7] Blandino, A., Al-Aseeri, M. E., Pandiella, S. S., Cantero, D., and Webb, C. (2003). Cereal-based fermented foods and beverages. *Food Research International, 36*:527–543. https://doi.org/10.1016/S0963-9969(03)00009-7

[8] Chaves-López, C., Serio, A., Delgado-Ospina, J., Rossi, C., Grande-Tovar, C. D., and Paparella, A. (2016). Exploring the bacterial microbiota of Colombian fermented maize dough “Masa Agria” Frontiers in Microbiology, 7, Article 1168.https://doi.org/10.3389/fmicb.2016.01168

[9] Chesbrough, M. (2006). District Laboratory Practice in tropical countries (Part 2, 2nd ed). Cambridge University Press. Pp.45-71.

 [10] Fredlund, E., Druvefors, U. Ä., Olstorpe, M. N., Passoth, V., & Schnürer, J. (2004). Influence of ethyl acetate production and ploidy on the anti-mould activity of *Pichia anomala*. *FEMS Microbiology Letters, 238*:133–137. <https://doi.org/10.1016/j.femsle.2004.07.045>.

[11] Greppi, A., Rantsiou, K., Padonou, W., Hounhouigan, J., Jespersen, L., Jakobsen, M., and Cocolin, L. (2013). Determination of yeast diversity in ogi, mawè, gowé and tchoukoutou by using culture-dependent and-independent methods. *International Journal of Food Microbiology,* 165:84–88.https://doi.org/10.1016/j.ijfoodmicro.2013.05.005

[12] Hill, C., Guarner, F., Reid, G., Gibson, G., Merenstein, D., Pot, B., Morelli, L., Canani, R. B., Flint, H., Salminen, S., Calder, P., and Sanders, M. (2014). Expert consensus document: The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nature Reviews Gastroenterology and Hepatology,* 11:506-514. <https://doi.org/10.1038/nrgastro.2014.66>

[13] Hukins, W. R. (2006). Microbiology and technology of fermented foods. John Wiley & Sons.

[14] Marshall, E., and Mejia, D. (2012). Traditional fermented food and beverages for improved livelihoods. Food and Agriculture Organization of the United Nations: Rome, Italy, 1-79.

[15] Mokoena, M. P., Mutanda, T., and Olaniran, A. O. (2016). Perspectives on the probiotic potential of lactic acid bacteria from African traditional fermented foods and beverages. *Food and Nutrition Research*, 60, 29630.

[16] [Jen Nguyen](https://pubmed.ncbi.nlm.nih.gov/?term=%22Nguyen%20J%22%5BAuthor%5D), J., [Juanita Lara-Gutiérrez](https://pubmed.ncbi.nlm.nih.gov/?term=%22Lara-Guti%C3%A9rrez%20J%22%5BAuthor%5D), J. and [Roman Stocker](https://pubmed.ncbi.nlm.nih.gov/?term=%22Stocker%20R%22%5BAuthor%5D), R. (2020). Environmental fluctuations and their effects on microbial communities, populations and individuals. FEMS Microbiology Reviews, 45(4): fuaa068. doi: [10.1093/femsre/fuaa068](https://doi.org/10.1093/femsre/fuaa068).

[17] Li, C., Song, W., Luo, Y., Gao, S., Zhang, R., Shi, Z., Wang, X., Wang, R., Wang, F., Wang, J., Zhao, Y., Su, A., Wang, S., Li, X., Luo, M., Wang, S., Zhang, Y., Ge, J., Tan, X., and Zhao, J. (2019). The HuangZaoSi maize genome provides insights into genomic variation and improvement history of maize. *Molecular Plant*, 12:402–409. <https://doi.org/10.1016/j.molp.2019.02.009>

[18] Marco, M.L., Heeney, D., Binda, S., Cifelli, C.J., [Cotter, P.D.,](https://www.sciencedirect.com/author/7006716039/paul-d-cotter) [Foligné, B.](https://www.sciencedirect.com/author/6507229883/benoit-foligne), Gänzle, M.,  Kort, R., Pasin, G., Pihlanto, A., Smid, E.J. and Hutkins, R. (2017). Health benefits of fermented foods: microbiota and beyond. *Current Opinion in Biotechnology*, 44: 94-102.

[19] Obi, C. N., & Okoronkwo, W. O. (2022). Production, microbiological and proximate analysis of Akamu produced from different varieties of maize. Nigerian Journal of Microbiology, 36:6001–6012.

[20] Olaitan, A. B., Adebowale, A. A., and Oyewole, O.B. (2018). Sensory evaluation of ogi produced from maize incorporated with Bambara groundnut and *Aframomum melegueta,* 6:901 – 908.

[21] Ogu, C.T., Umeh, S.O., Nwiyi, I.U., Ikele, M.O., Okonkwo, I.F. and Agwuna, L. Application of Palm Wine Yeast in Beer Brewing. *Journal of Advances in Microbiology*, 22(1):67-75.

[22] Omemu, A. M., Oyewole, O. B., and Bankole, M. O. (2007). Significance of yeasts in the fermentation of maize for ogi production. *Food Microbiology*, 24:571–576.

[23] Okeke, C., Ezekiel, C. N., Sulyok, M., Ogunremi, O. R., Ezeamagu, C. O., Šarkanj, B., Warth, B., and Krska, R. (2018). Traditional processing impacts mycotoxin levels and nutritional value of ogi – A maize-based complementary food. *Food Control,* 86:224–233. <https://doi.org/10.1016/j.foodcont.2017.11.021>

[24] Olorunjuwon, O. B., Temitope, K. B., Olumayowa, T. A., and Yisau, O. A. (2018). Comparative evaluation of microbiological and nutritional qualities of various cereal-based paps (Ogi) in Ondo State, Nigeria. *International Journal of Environment, Agriculture and Biotechnology*, 3:2456-1878.

[25] Ouwehand, A. C., Salminen, S., and Isolauri, E. (2002). Probiotics: an overview of beneficial effects. *Antonie VanLeeuwen* 82:279–289. doi: 10.1023/A:1020620607611.

[26] Peryam, D.R. and Pilgrim, F.J. (1957). Hedonic scale method of measuring food preferences. *Food Technology* (September 1957), 9-14.

[27] Phister, T. G., O’Sullivan, D. J., and McKay, L. L. (2004). Identification of bacilysin, chlorotetaine, and iturin A produced by *Bacillus* sp. strain CS93 isolated from pozol, a Mexican fermented maize dough. *Applied and Environmental Microbiology,* 70:631–634. https://doi.org/10.1128/AEM.70.11.631-634.2004.

[28] Ponomarova, O., Gabrielli, N., Sévin, D. C., Mülleder, M., Zirngibl, K., Bulyha, K., Andrejev, S., Kafkia, E., Typas, A., Sauer, U., and Ralser, M. (2017). Yeast creates a niche for symbiotic lactic acid bacteria through nitrogen overflow. *Cell Systems,* 5:345–357. https://doi.org/10.1016/j.cels.2017.08.002

[29] Saad, N., Delattre, C., Urdaci, M., Schmitter, J. M., and Bressollier, P. (2013). An overview of the last advances in probiotic and prebiotic field. *LWT Food Sci. Technol.* 50:1–16. doi:10.1016/j.lwt.2012.05.014.

[30] Shi, C., Zhang, Y., Lu, Z., and Wang, Y. (2017). Solid-state fermentation of corn-soybean meal mixed feed with *Bacillus subtilis* and *Enterococcus faecium* for degrading antinutritional factors and enhancing nutritional value. *Journal of Animal Science and Biotechnology,* 8:1:50. <http://doi.org/10.1186/s40104-017-0184-2>

[31] Suri, D. J., and Tanumihardjo, S. (2016). Effects of different processing methods on the micronutrient and phytochemical contents of maize: From A to Z. *Comprehensive Reviews in Food Science and Food Safety,* 15:912–926. <http://doi.org/10.1111/1541-4337.12216>.