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Protein-enriched Akamu: Nutritional sensory and microbial quality evaluation

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Abstract

Mitigating Protein Energy Malnutrition (PEM) is paramount to cohort the infant mortality rate caused by inadequate nutrition. This study examines the effect of enriching maize-based starch gruel "Akamu" with Bambara groundnut flour. Yellow maize was steeped for 72 hours, with the steep water changed every 24 hours. Then, wet milled, its starch separated with a cheesecloth, oven-dried and milled to produce akamu flour. Bambara groundnut was steeped, boiled, dried and milled into flour and used to enrich (10%, 20% and 30%) the akamu. The steep water was evaluated for microbial analysis, while the sample blends were examined for proximate and sensory attributes. The microbes of steep water decreased in coliform count and pH as fermentation progressed. However, Lactobacillus spp and fungi increased from 0.93x103 and 3.63x103 to 8.42x103 and 6.62x103cfu/mL, respectively. Crude protein increased significantly from 8.64 % to 15.54 %, while carbohydrates reduced to 60.20 %. The 100% maize (control) akamu showed the highest acceptability of 6.58, while 30% Bambara groundnut was the least (3.38). Hence, this study helped to establish the nutritional improvement of akamu, a cereal starch gruel with Bambara groundnut aimed at addressing malnutrition.

Keywords: Protein-enriched; Gruel; Malnutrition; Fermentation; Bambara groundnut

1. Introduction

Malnutrition, primarily caused by inadequate feeding habits, is a significant public health issue in Nigeria, affecting infants and young children aged 6 months to two years. The occurrence of wasting, underweight, and stunting in Nigeria hit 7%, 23%, and 37%, respectively, according to a 2020 Nigeria Demographic and Health Survey study. Just 29% of newborns under the age of six months are exclusively breastfed, and 11% of children aged six to twenty-three months receive a minimum tolerable diet [1]. Furthermore, in Nigeria, the probability of a newborn dying before the age of five years is thirty times higher than in developed countries [2]. Malnutrition could be caused by various reasons, including poor dietary quality, low food intake, severe and recurring infectious infections, or a mix of all three [2]. Therefore, the need to consciously improve the staple or complementary meals like Akamu in Nigeria is paramount. This improvement could be achieved by supplementing maize with nutrient-rich legumes, such as Bambara groundnut, thereby mitigating nutritional deficiency and enhancing the overall quality of basic diets.

"Akamu" is a cereal-based fermented non-alcoholic starch gruel mostly consumed in Nigeria as a staple food. It is a good choice for patients who need soft, easily digestible foods and for newborns, serving as complementary foods [4]. Akamu (Igbo) is also known as Ogi (Yoruba) or commonly called pap and is primarily produced from cereals such as maize (*Zea mays*), millet (*Pennisetum glaucum*), or Guinea corn (*Sorghum* spp.). These cereals are high in carbohydrates but low in protein content of which the available protein composition is low in lysine and tryptophan amino acids [4]. These amino acids are essential for the growth of infants. Fortunately, legumes like Bambara groundnut are abundant in these amino acids [5].

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Bambara groundnut (*Vigna subterranea*) is known for its excellent nutritional content, availability, and cultivation tolerance. It has a commendable high protein content of 18.8% [6]. Since animal protein sources are getting more expensive, as well as other health risks associated with them, the nutritional content of Bambara groundnut provides a low-cost source of high-quality protein. As a result, combining legumes and cereals ensures that a meal has all the essential amino acids, thereby increasing the nutritional composition of that food [4]. This is the foundation of food supplementation, which aims to improve the nutritional value of foods while keeping sensory acceptance in mind.

In Africa, Bambara groundnut has the potential to enrich traditional complementary diets such as Akamu. Since akamu is a fermented food, if consumed fresh, it also supplies live bacteria known as probiotics to balance the gut microbiota, which may enhance immunity and so shield the body from infectious diseases like Covid-19 and other illnesses [7]. Recently, there has been an increased interest in fermented foods due to their health benefits and other advantages, ranging from boosting digestibility, unique flavour, and nutrient availability to food stability and storage. This fermentation is accomplished with the assistance of microorganisms.

The total number of microorganisms present in a particular environment is referred to as microbial profile [9]. Microbial profiles provide solutions for determining the composition of bacteria, Archaea, and fungi in a given media or sample [10]. Understanding the microbial profile of steep water during the processing of akamu could give room for improvement and modification of the food product. For instance, one could look at growing the akamu sector into an industrial production scale, such that microbial inoculation could be applied to reduce the fermentation time and enhance the molecular breakdown for easy digestibility. Many bacteria, mould, and yeast strains have been identified during akamu fermentation, including *Lactobacillus, Candida*, *Corynebacterium, Aspergillus, Penicillium, Zygosaccharomyces, Fusarium, Saccharomyces and Cephalosporium* [7]. Further investigation will contribute to the knowledge gap on the impact of enriching maize with bambara groundnut in the production of akamu. As a result, the objective of the study was to determine the microbial profile of the steep water during maize fermentation, as well as the proximate composition and sensory characteristics of maize-based gruel "Akamu" protein enriched with Bambara groundnut,

2. Material and methods

The yellow maize and Bambara groundnut were purchased from a local market in Enugu State and transported to the laboratory for analysis.

2.1. Sample preparation

2.1.1. Preparation of Bambara Groundnut Flour

Bambara groundnut was sorted by removing all unwholesome nuts and debris. The accepted Bambara groundnut (1 kg) was washed and soaked in water (2 L) for 1 h before boiling for 20 min at 100°C. The seed coats were manually dehulled and dried in an oven drier at 60 °C for 36 h. Then, the cotyledons were milled (Romer serial II mill, Romer Labs, USA) into flour. The resulting bambara groundnut flour was sieved using a 250 μm mesh size. After this, it was sealed tightly until ready for use [11].

2.1.2. Preparation of Akamu Flour

Yellow maize (2kg) was sorted, cleaned, and steeped in distilled water (4 L), at ambient temperature (28 ± 2 °C) for 72 hours, with the steep water changed every 24 hours. The softened corn was washed and wet-milled in a motorized mill (Model- Labmill- 257; Gibbons, Essex, UK). To separate the starch, the slurry was washed through a 200 μm sieve (muslin cloth). The starch in the filtrate was allowed for 3 hours to settle, afterwards, its supernatant was decanted [12]. The sediment was further squeezed in a cheesecloth to remove excess water and then dried for 36 hours at 55°C in an oven drier (Model- Labmill- 257; Gibbons, Essex, UK) [5]. Finally, it was milled, sieved and tightly stored for laboratory analysis.

2.1.3. Sample formulation and preparation

The akamu samples were prepared with various proportions of maize and Bambara groundnut flour. Five different samples were reached as follows: A: 100% maize (control); B: 90% maize with 10% Bambara; C: 80% maize with 20% Bambara; D: 70% maize with 30% Bambara, E: 100% Bambara. Flour samples of A-E were analyzed for proximate composition, while prepared akamu samples of A-D were evaluated for sensory features. Since sample E was 100% Bambara groundnut and could not be prepared into a gruel.

Akamu gruel was prepared with some modifications; 50 g of the flour samples were mixed with 50 g of distilled water to form a light thick consistency. Then, boil water with continuous stirring until it thickens [13].

2.1.4. Microbial Profile of the Steep Water During Maize Fermentation

The steep water from the maize was collected using a sterile beaker every 24 hours before changing the steep water. Then microbiologically with some modifications [7] [14]. The pH values of the samples were also determined. Total viable counts were cultured on Nutrient agar (NA), EMB agar (Eosin Methylene Blue) and Sabouraud agar for *Lactobacillus* spp, coliform and fungi, respectively. All media were prepared in line with the manufacturer's instructions and sterilized at 121 °C for 15 min. Serial dilution (0.5 mL, tenfold) was pour-plated on sterile molten agar and gently rotated both clockwise and anticlockwise to ensure even distribution before setting. The petri dish contents were allowed to harden. The NA and EMB plates were incubated aerobically at 37°C for 24 hours, while Sabouraud agar was incubated anaerobically at 28°C for 72 hours. A colony counter was used to count the number of microbial colonies. Plates with between 30-300 colonies were counted. Plates with less than 30 colony-forming units were too few to be reliable while plates with more than 300 colonies were too numerous to count, and plates with congested colony units were discarded.

Colony Forming Unit (CFU) per milliliter (CFU/mL) was calculated using the formula:

$$\text{CFU/mL} = \frac{\text{no of colonies dilution factor}}{\text{volume of culture plate}}$$

Selected counts were examined microscopically and macroscopically for distinguishing characteristics in line with their biochemical test [10].

2.1.5. Proximate Composition

The AOAC (2000) procedure was used to evaluate crude protein, ash, fat, moisture, and crude fibre contents. Moisture content was determined by loading known weights of samples into crucibles and drying at 105 °C temperatures. The weights of the dried samples were recorded after being cooled in desiccators. The samples were then returned to the oven, which was repeated until constant weights were obtained. Protein was calculated by multiplying total nitrogen content by 6.26 using the standard micro Kjeldahl method. The ash content was calculated by transferring already ignited samples over a low flame to char the organic matter. The crucible lids were removed and placed in a muffle furnace at 600 °C for 6 hours or until they completely turned into ash. The first and last weights were used to calculate the ash percentage. The Soxhlet extraction method was used to determine crude fat. Loss in the ignited samples above was used to calculate crude fiber. Carbohydrate was calculated by difference as % CHO = 100 - (the sum of moisture, ash, fat, protein, and crude fibre percentages).

2.1.6. Determination of total energy

The total energy values of the samples were calculated using the technique described by [15]. It is an indirect method that involves multiplying the proportions of dietary classes; carbohydrates, proteins, and fats, by energy factors of 4 kcal/g, 4 kcal/g, and 9 kcal/g, respectively.

$$\text{Calorific Value (Kcal/100 g)} = (\%\text{Protein} \times 4) + (\%\text{Carbohydrate} \times 4) + (\%\text{Fat} \times 9)]$$

2.1.7. Sensory Evaluation

The methods described by [16] [17] with some modifications were employed for the sensory evaluation of prepared 'akamu' samples from the different blends. Untrained panelists (50) were told to evaluate the prepared 'akamu' samples and to rinse their mouths after each one. They were asked to rate the samples based on the following criteria: appearance, taste, consistency and overall acceptability. Questionnaires were given to each panelist, and the sensory scores were calculated using a 7-point hedonic scale, where 1 meant extremely dislike and 7 meant extremely like.

2.2. Statistical Analysis

All analyses were performed in triplicate and presented as mean ± standard deviation. Statistical Analysis of Variance (ANOVA) was applied for evaluating the means, using SPSS version 22 (SPSS, Inc., USA). Furthermore, the Duncan Multiple Range Test (DMRT) at an acceptable level of $p<0.05$ was utilized for the separation of means [17]

3. Results

Table 1 Mean values of pH and microbial load of the steep water during akamu processing

Duration (h)	pH value	*Lactobacillus* spp. (CFU/mL)	Coliform (CFU/mL)	Fungi (CFU/mL)
0	6.17±0.002^{c}	0.93x10^{3}±0.223^{a}	3.27x10^{3}±0.177^{b}	3.63x10^{3}±0.021^{a}
24	5.30±0.051^{b}	4.14x10^{3}±0.108^{b}	6.61x10^{3}±0.211^{c}	4.81x10^{3}±0.033^{b}
48	4.95±0.027^{ab}	6.07x10^{3}±0.201^{b}	3.92x10^{3}±0.183^{b}	5.96x10^{3}±0.018^{c}
72	4.03±0.010^{a}	8.42x10^{3}±0.191^{c}	2.38x10^{3}±0.074^{a}	6.62x10^{3}±0.013^{d}

Means in the same column with same superscript letters are not significantly different at p≤0.05.

Table 2 Microbial isolates from the steep water obtained during akamu processing

Microorganisms		Duration (Hours)			
		0	24	48	72
Bacteria	*Bacillus subtillis*	+	+	-	-
	Escherichia coli	+	+	+	-
	Lactobacillus fermentum	+	+	+	+
	Lactobacillus plantarum	+	+	+	+
	Pseudomonas chlororaphis	-	+	+	-
	Staphylococcus aureus	+	+	+	-
Fungi	*Aspergillus niger*	+	+	+	+
	Penicillium spp	+	+	+	-
	Saccharomyces cerevisiae	_	+	+	+

Present (+); Absent (-)

Table 3 Proximate composition of the enriched akamu flour samples

Samples	Crude Protein content (%)	Crude Fibre content (%)	Ash content (%)	Moisture content (%)	Fat content (%)	Carbohydrate content (%)	Energy content (kcal/100 g)
A	8.64±0.041^{a}	1.70±0.070^{a}	0.86±0.051^{a}	10.44±0.112^{bc}	3.59±0.157^{a}	74.77±0.360^{e}	365.96±0.613^{d}
B	10.95±0.035^{b}	2.28±0.372^{b}	0.91±0.04^{a}	10.87±0.119^{c}	3.54±0.042^{a}	71.44±0.420^{d}	361.48±0.848^{c}
C	13.53±0.032^{c}	2.54±0.089^{bc}	1.14±0.030^{b}	10.68±0.048^{c}	3.49±0.090^{a}	68.35±0.151^{c}	358.91±0.402^{bc}
D	15.54±0.04^{d}	2.81±0.047^{c}	1.86±0.076^{c}	10.15±0.153^{ab}	3.79±0.076^{b}	65.84±0.344^{b}	359.64±1.925^{b}
E	19.24±0.026^{e}	3.72±0.031^{d}	2.83±0.051^{d}	9.74±0.025^{a}	4.27±0.046^{c}	60.20±0.071^{a}	356.20±0.422^{a}

Means in the same column with same superscript letters are not significantly different at p≤0.05. A: 100% Maize (control); B: 90% Maize, 10% Bambara; C: 80% Maize, 20% Bambara; D: 70% Maize, 30% Bambara, E: 100% Bambara

Table 4 Sensory Evaluation of the Akamu samples

	Appearance	Taste	Consistency	Overall Acceptability
A	6.59±0.178^{d}	6.77±0.068^{d}	6.56±0.131^{d}	6.58±0.064^{d}
B	6.09±0.076^{c}	5.78±0.058^{b}	6.05±0.064^{c}	6.21±0.100^{c}
C	5.64±0.066^{b}	6.07±0.056^{c}	5.66±0.120^{b}	5.80±0.058^{b}

D	3.66±0.111[a]	4.54±0.030[a]	2.51±0.105[a]	3.38±0.071[a]

Means in the same column with same superscript letters are not significantly different at p≤0.05. A: 100% Maize (control); B: 90% Maize, 10% Bambara; C: 80% Maize, 20% Bambara; D: 70% Maize, 30% Bambara.

4. Discussion

4.1. Microbial profile

Table 1 presents results for the pH and microbial load of the steep water from 0 to 72 h (at intervals of 24 h). It was observed that the pH and coliform count values of the steep water decreased progressively as time increased. This is because of the increase in the lactic acid content of the medium as fermentation continued [17]. Also, a lower pH value makes it unfavourable for most bacteria to survive. On the contrary, the *Lactobacillus* spp and yeast increased from $0.93x10^3$ and $3.63x10^3$ to $8.42x10^3$ and $6.62x10^3$ CFU/mL, respectively.

Table 2 shows the microorganism isolated during the steeping stage of maize fermentation for akamu production. The presence of bacterial such as *Bacillus subtillis, Escherichia coli, Lactobacillus fermentum, Lactobacillus plantarum, Pseudomonas chlororaphis, Staphylococcus aureus;* as well as fungi such as *Aspergillus niger, Penicillium* spp *and Saccharomyces cerevisiae* were observed at different stages of fermentation.

The distribution of these microbes during different fermentation stages in akamu processing was similar to the older observations [7] [191].They reported that microbes like *Corynebacteria, Lactobacillus, and Saccharomyces cerevisiae* were found in the fermentation medium from the second day onward. Furthermore, Afolabi et al. (2018) reported that organisms such as *Lactobacillus fermentum, Pseudomonas cepacia, Bacillus alvei, Flavobacterium rigense, and Lactobacillus plantarum* etc., were present at different stages of fermentation including fungi *Candida tropicalis, Aspergillus niger, Penicillium claviforme, Rhizopus stolonifer, and Saccharomyces cerevisiae*. Similar organisms were also reported by [19].

In this study, *Bacillus subtillis, Escherichia coli, Lactobacillus fermentum, Lactobacillus plantarum, Staphylococcus aureus* as well as fungi such as *Aspergillus niger* and *Penicillium sp*, were found in the fermentation medium on the first day but *Bacillus subtillis* disappeared by 48 h. *Pseudomonas chlororaphis* and *Saccharomyces cerevisiae* were present from 24 h. However, *the Lactobacillus fermentum, Lactobacillus plantarum, Aspergillus niger* and *Saccharomyces cerevisiae* were the only organisms present by 72 h. Other microbes disappeared. The appearance of some microbes by 48 h of fermentation could be attributed to changes in the acidity of the fermentation medium. For example, a decrease in pH increases fermentation activity as Lactic Acid Bacteria (LAB) strive more at acidic pH.

The presence of some other bacteria up to 48 h and 72 h differed from Mbata et al. (2009), who recorded a complete disappearance of all bacteria after 24 h. [14] did not change their steep water until after 72 h. However, in this study, the steep water was changed every 24 h as that is the regular akamu processing practice in south-eastern Nigeria because it reduced the level of sourness and spoilage of the final product. Therefore, introducing new, steep water gave room for other bacteria to appear aside from *Lactobacillus spp* and *Escherichia coli*, which are indicator organisms. Their presence could result from fecal contamination of maize, most likely from materials used for storage or even handling. Also, microbes such as *Penicillium* sp and *Aspergillus niger*, could be because of unsanitary handling or contamination.

The microorganisms isolated during the process were mainly composed of a succession of gram-negative enteric bacteria and a mixed fungal population containing lactic acid bacteria and yeasts, respectively. Research [19] that looked into the symbiotic relationship between yeast and lactic acid bacteria in traditional African fermented foods. This has been linked to the development of the distinctive flavor of these fermented foods. Some of the yeasts have also been shown to have amylolytic, proteolytic, and phytate-breaking properties. This enzymatic ability may aid in the breakdown of cereals such as sorghum, maize and millet starch, as well as improved access to nutritionally essential minerals.

4.2. Proximate composition

The proximate composition of the flour samples is presented in Table 3. Crude protein, crude fibre fat and ash contents showed a significant increasing pattern from sample A to E. The trend was similar to previous research [6], while moisture, carbohydrate and energy values varied among the samples.

The use of Bambara groundnut in this study resulted in a significant increase in the protein contents of the supplemented akamu when compared to the control (sample A). Results indicated that the increase in protein content was proportional to the amount of Bambara groundnut that was included in the blend. Sample D with the highest protein content of 15.54% is recommended as a nutritious food. The percentage increase in protein when the control sample A was compared to samples B, C and D was 26.7%, 56.6% and 79.9%, respectively. However, increasing the Bambara groundnut content beyond 20% reduced the overall acceptability of akamu [19]. Therefore, care should be taken to ensure a balance between the nutritional improvement of a food product and its acceptability.

The fibre composition of the samples increased significantly as the quantity of Bambara groundnut increased, except for sample C (2.54%), which was significantly similar to samples B and D. Sample E had the highest crude fibre content of 3.72%. This could be so because akamu is made of maize starch while, Bambara groundnut flour is made from the whole nut, which contains other food components aside from starch. Also, variations in the species of Bambara groundnuts utilized can be a factor [6].

Similarly, the ash content of the supplemented samples significantly increased from samples A to E, although samples A and B were not significantly different. While sample E had the highest ash content of 2.83%, sample A had the lowest value of 0.86%. This could be because akamu, with mainly starch components, had lost most of its minerals/ ash content during the processing stage. This increasing pattern with the addition of Bambara ground agrees with previous research [14].

Moisture content showed a variation in values ranging from 9.74 % to 10.87 % for samples E and B, respectively. Significant differences occurred amongst some samples whereas sample D was similar to samples A and E. In all, the moisture content range is within the acceptable limits for dry flour shelf stability of ≤ 12 % [18].

The fat content of the supplemented samples was not significantly different from the control (3.59%) until the addition of up to 30% Bambara groundnuts in sample D (3.79 %). This implies that if no significant improvement is required for the fat content, there would be no need to add Bambara groundnut beyond 20% in the supplemented akamu flour blend. Also, higher fat content could lead to oxidative rancidity if not adequately preserved [20].

The carbohydrate content decreased significantly from samples A to E (74.77 % to 60.20 %), indicating that as Bambara groundnut content increased, carbohydrate decreased. This carbohydrate content decrease trend in the supplemented akamu with increasing protein agreed with the findings of [10]. The authors stated that including legumes resulted in carbohydrate reductions in supplemented maize gruel (Akamu). This reduction in carbohydrate values was determined by difference. Therefore, an increase in other components like crude protein, fat, ash and crude fibre would cause a decrease in carbohydrates.

4.3. Total Energy Value

Adding Bambara with the lowest energy value (356.20 kcal/100 g), reduced the energy content of the flour blends significantly. However, sample C was similar to samples B and D, while other samples were significantly different from each other. The control sample has the highest energy value of 365.96 kcal/100g. These energy values are similar to earlier findings [22]. This is expected as the energy values directly depend on the fat, protein and carbohydrate content, going by its method of determination.

4.4. Sensory Evaluation

Sensory evaluation values are presented in Table 4. Selected attributes of appearance, taste, consistency and overall acceptability were analyzed to suit the observable features of most consumers when taking akamu. The control sample (A) had the highest significant values of all the sensory attributes investigated when compared to the flour blends, with its taste having the highest value of 6.77. It was observed that increasing the Bambara groundnut content of the flour blend resulted in a significant decrease across all the sensory attributes. Especially the consistency of sample D showed the lowest score of 2.51, meaning that the panelist strongly disliked the consistency of that sample. It was found that increasing bamabara ground in cereal gruels directly enhanced its nutritional composition but, reduced the sensory acceptability of the food [20]. This is because their poorer acceptance scores may have been the result of a comparison with the control sample. Furthermore, this study was carried out in the southeastern part of Nigeria, where maize constitutes most of the cereal consumed [6] and many people regularly consume ‘akamu made from maize. Their familiarity with the consistency, taste, appearance and overall acceptance of the samples may have affected their preference. If sampled in another part of the world where Bambara groundnut gruel consumption is common or a variety of other legumes, this sensory study may produce a different sensory assessment result. Therefore, if sensory

attributes are the main criteria for selecting these sample blends, then sample B with the 10% Bambara addition will be more acceptable due to its higher overall acceptability of 6.58

5. Conclusion

Therefore, adding bamabara groundnut flour to akamu flour improved the nutritional composition by enhancing its protein content. Although, enrichment of akamu with Bambara ground beyond 20 % did not show favourable sensory acceptability. Hence, it is essential to balance nutritional improvement with overall acceptability. The microbial profile of the steep water exhibited diverse compositions of microorganisms at different stages of fermentation for akamu production. Hence, this study helped to establish a nutritional improvement of akamu, cereal starch gruel, with Bambara groundnut, aimed at addressing Protein- Energy Malnutrition.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

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