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Microbiological and Nutritional Characteristics of Locally Fermented Fufu Commonly Sold in Amai, Delta State, Nigeria

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ABSTRACT: Cassava (*Manihot esculenta*) is the raw material for various local dishes around the world with popular ones such as garri, eba, fufu, tapioca abacha and starch in Nigeria, however, microbial and nutritional quality information are scanty on these dishes. Consequently, the objective of this paper was to evaluate the microbiological and nutritional characteristics of Locally Fermented Fufu commonly sold in Amai, Delta State, Nigeria using appropriate standard methods. Data obtained from the sample analysis revealed the presence of various species of microorganisms, including *Staphylococcus spp*., *Lactobacillus spp*., *Pseudomonas spp*., and *Bacillus spp*. *Aspergillus spp* and *Rhizopus spp* however, *Aspergillus spp* was the predominant fungi. The outcome of the nutritional analysis of the fufu product reveals some parameters such as moisture content, protein content, carbohydrate content. The result revealed high carbohydrate (87.26%) indicating fufu as an energy-giving food, other compositions such as fiber content (3.75%) and ash content (2.22%) suggest that fufu promotes gut health and digestibility. The results of this study shed light on the cultural, macroscopic, microscopic, and nutritional characteristics of the of fufu samples. The proximate analysis of the fufu provided valuable insight to its nutritional content which specifies that fufu is rich in energy giving nutrient (carbohydrate).

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Fermentation is a traditional food processing technique that has been practiced for centuries in various cultures around the world. It involves the conversion of organic substrates by microorganisms, leading to the production of a varied range of fermented foods with unique flavors, textures, and nutritional properties. Fermented foods play an important role in the diets of several communities, particularly in Africa, where they are important sources of essential nutrients and contribute to food security. One such fermented food in West Africa is fufu, a staple made from starchy tubers such as cassava, yam, or plantain.

Fufu is a popular traditional food consumed in numerous West African nations, such as Ghana, Cote d'Ivoire, and Nigeria. It is prepared by fermenting peeled and grated cassava, yam, or plantain, followed by cooking and pounding the fermented mash into a smooth, elastic dough-like consistency. The

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fermentation process is crucial for the production of fufu as it improves the finished product's flavor, texture, and digestibility. During fermentation, microorganisms present in the environment or introduced intentionally, like molds, yeasts, and lactic acid bacteria, participate in breakdown of complex carbohydrates, proteins, and other components of the raw materials, leading to the production of alcohols, organic acids, and aroma compounds. The fermentation of cassava for fufu includes the action of microorganisms, principally lactic acid bacteria (LAB), yeast, and molds. These microorganisms utilize the carbohydrates present in cassava, breaking them down into simpler compounds through enzymatic activities. This process leads to the creation of organic acids and other metabolites, which contribute to the characteristic taste, aroma, and texture of fufu. As a fermented food product, fufu shares similarities with other fermented foods such as yogurt, cheese, sauerkraut, and kimchi, which also rely on the action of microorganisms to transform the starting ingredients. These fermentation processes not only improve the sensory properties of food but also result in food safety and preservation by preventing the growth of spoilage and pathogenic microorganisms. The microbial community involved in the fermentation process, as well as the biochemical changes that occur, are essential factors that influence the value and wellbeing of the final product.

Several studies had reported the isolation of lactic acid bacteria (LAB) from fermented cassava products, including fufu (Ezemba *et al*., 2021, Igyor, 2023). These LAB performance a crucial role in the fermentation process, contributing to the sour taste, texture, and nutritional value of fufu (Abban, 2023). LAB have been revealed to contribute to nutrient content of fermented foods, including fufu (Agyei, 2017). These microorganisms can increase the levels of essential amino acids, vitamins, and bioactive compounds, ultimately improving the total health benefits of fufu (Ballini, 2022). Fermented foods, including fufu, have a lesser risk of contamination with foodborne pathogens due to the production of antimicrobial compounds such as organic acids, hydrogen peroxide, and bacteriocins by LAB (Amodio, 2020). The presence of these protective substances in fermented fufu could help avoid the growth of spoilage and pathogenic microbes, ensuring the safety of product for consumers (Tamang, 2016). Fermentation also contributes to the nutritional quality of fufu. During the process, LAB synthesize vitamins, such as vitamin B_{12} and folate, and enzymes that break down complex carbohydrates, improving digestibility and nutrient availability. Moreover, the fermentation process reduces the levels of toxic compounds like cyanogenic glycosides in cassava, making fufu safer for consumption. Fufu also contains some dietary fiber, which can aid in digestion. However, its nutritional value largely depends on the ingredients used and the accompanying dishes. Hence, the objective of this paper is to evaluate the Microbiological and Nutritional Characteristics of Locally Fermented Fufu commonly sold in Amai, Delta State, Nigeria

MATERIALS AND METHODS

Collection of samples: Fufu were purchased from diverse locations in Amai, Delta State Nigeria. The samples collected were taken in sterile bottles in a polyethylene bag to the laboratory for further analysis.

Enumeration and isolation of microbes: Isolation of microorganism from the samples was done using pour plate method. 10g of the fufu samples was weighed and homogenized and about 90ml of water was added. Serial dilution was done by mixing 1 ml of the sample carefully with 9 ml of sterile distilled water to give 1:10 dilution. The dilution was made up to 10^{-10}. Using sterilized pipette, 1ml of the diluent was plated out using different media (Harrigan and McCance, 1976).

Culture preservation: The pure cultures of the bacteria and fungi isolates were sub-cultured into slants incubated at 37°C till growth becomes noticeable. The slants remained kept as stock cultures under refrigeration (4°C) for up to four weeks.

Characterization and identification of isolated bacteria and fungi: Characterization of bacteria and fungi isolates obtained from fufu samples was carried out by employing morphological and biochemical tests.

M*macroscopic examination:* The cultural features of each of the isolates were observed. They were grouped based on their colonial appearances such as shape, elevation, pigmentation, size, surface, opacity, edge and consistency.

Microscopic examination

Gram staining procedure: This was done to classify the bacteria based on retention of primary or secondary stain according to Prescott *et al.,* (2005).

Biochemical test: The biochemical tests include:

Catalase Test: This test was done to determine the presence or absence of catalase enzyme. (Olutiola *et al.,* 1991). The enzyme catalyzes breakdown of Hydrogen peroxide to release free oxygen gas and water.

$$2H_2O_2 \rightarrow 2H_2O + O_2.$$

A few drops of freshly prepared 3% hydrogen peroxide was added to a smear of 18-24 hrs old culture of the bacteria on a clean glass slide. Evolution of gas as white bubbles indicated a positive test for catalase.

Oxidase Test: Oxidase test was done to determine the presence of oxidase enzymes (Olutiola *et al.*,1991).

Endospore Staining procedure: Endospore staining was used to determine sporulating bacteria (Olutiola *et al*., 1991). Smear of pure culture were prepared on clean grease free glass slides and heat fixed. The slides were flooded with Malachite green reagent and gently heated until boiling and then left for 5 minutes to cool after which the stain was rinsed off and the smear counter stained with safranin for 30-60 seconds, rinsed with distilled water and dried by blotting with filter paper. The slide was then observed under the microscope.

Citrate Utilization Test: This was done to determine the capability of the bacteria to use up citrate (Olutiola *et al*., 1991). Simmons's citrate agar was prepared and dispensed into screw cap tubes and sterilized at 121^{o}C for 15minutes.The tubes were left to solidify and inoculated with the test isolates. It was incubated for 2-3 days. Appearance of blue coloration indicates a positive result while the initial green coloration was retained for negative results.

Proximate analysis of fufu: The percentage crude protein, moisture, ash, crude fibre, fat and carbohydrate contents of fufu were carried out by applying the methods described by Ibitoye (2005). The carbohydrate content was determined by their differences (AOAC, 2012).

Analysis of Data: ata collected was analysed using analysis of variance (ANOVA) and means was separated using duncan multiple range test (DMRT) at 95% level of significance.

RESULTS AND DISCUSSION

The cultural and biochemical characteristics of bacteria isolated from local fermented fufu sold in Amai, Delta State are shown in Table 1. It revealed the presence of various species of organisms, including *Lactobacillus spp*., *Staphylococcus spp*., *Pseudomonas spp*., and *Bacillus spp*.

Table 2: shows the macroscopic and microscopic characteristics of the fungal from local fermented fufu sold in Amai, Delta state displayed different colony morphologies, colors, and textures, indicating the presence of multiple fungal species. Microscopic analysis showed the presence of the fungal hypae, spores, and other structures, allowing for the identification of genera such as *Aspergillus* and *Rhizopus*.

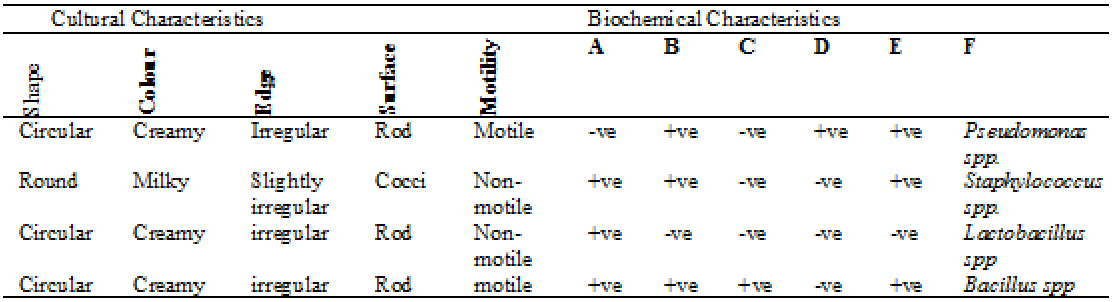
Table 3: shows the mean proximate composition of local fermented fufu. Proximate composition shows the difference in nutritional content of different fufu sold in different locations in Amai, Delta State. The highest carbohydrate content was obtained in Sample A (87.26%) followed by sample B (81.49%) while the least was recorded in sample D (79.31). The highest moisture content of 7.25% was seen in Sample C and D. The highest ash content is obtained from Sample C (2.22%) while the lowest ash content is seen in sample A (1.85%). Sample D has the highest protein content of 4.50% while Sample A has the lowest ash content of (1.68%).

Plate1: shows the image of *Aspergilllus niger* which was a prevailing fungi in most of the samples cultured. The fungal specie *Aspergilllus* was dominant in the isolated samples of fufu sold in Amai, Delta State. The proximate composition analysis of the final fufu product reveals its nutritional content. The moisture content of 6-7% indicates the water content present in the fermented fufu, which is consistent with findings from other studies indicating that adequate moisture content is crucial and it contributes to the product's desirable soft and pliable texture. The protein content, ranging from 1-4%, aligns with the generally low protein profile of fufu, which provides limited but essential protein nutrition. Similarly, the low fat content of 1.20 -1.30% suggests that fufu is a low-fat food. The carbohydrate content was notably high, ranging from of 79.3 - 87.26% reflects the presence of complex carbohydrates derived from the raw materials (cassava) used in the fermentation process, which provides a significant energy source. The ash content, observed at 1.85 – 2.22% represents the inorganic mineral content, while the fiber content of 1-3% indicates the presence of dietary fiber, which is beneficial for digestive health. This is in line with the works of Akinmoladun *et al.,* 2020; Osei *et al.,* 2019; Nielsen *et al.,* 2018;Iwe *et al.,* 2021. The presence of bacterial species such as *Lactobacillus spp*., in the

fufu samples is known to play a crucial role in the fermentation process by converting carbohydrates into organic acids, contributing to the characteristic flavor, texture, and preservation of the product (Gänzle *et al.*, 2016).The presence of these bacteria underscores their importance in ensuring the quality and safety of fermented fufu.

Plate 1: Fungal growth in Potato Dextrose Agar (PDA)

Table 1: Cultural and biochemical characteristics of bacteria isolated from fufu samples



Cultural Characteristics					Biochemical Characteristics					
Shape	Colour	Edge	Surface	Motility	A	B	C	D	E	F
Circular	Creamy	Irregular	Rod	Motile	-ve	+ve	-ve	+ve	+ve	*Pseudomonas spp.*
Round	Milky	Slightly irregular	Cocci	Non-motile	+ve	+ve	-ve	-ve	+ve	*Staphylococcus spp.*
Circular	Creamy	irregular	Rod	Non-motile	+ve	-ve	-ve	-ve	-ve	*Lactobacillus spp*
Circular	Creamy	irregular	Rod	motile	+ve	+ve	+ve	-ve	+ve	*Bacillus spp*

Where: A = Gram Stain; B = Catalase; C = Spore Stain; D = Oxidase; E = Citrate; F = Probable Organisms

Table 2: Macroscopic and microscopic characteristics of the fungal isolated from fufu samples

Isolates	**Colonial/ Structural morphology**	**Microscopic morphology**	**Probable organism**
1. Sample A	Conidial heads are radiate initially, splitting into columns at maturity. Hypae are septate.	Phialids are straightforwardly on the globes vesicle sclerotia and erect conidiopores. Conidia are brown with warts, irregular ridges.	*Aspergillus niger*
2. Sample B	Wooly and Initially white but change to black after a few days producing conidial spore.	Has smooth coloured conidiophores and conidia are brown with warts, irregular ridges.	*Aspergillus niger*
3. Sample C	Whitish colony at the underlying, later become earthy colored to dark.	Branched, Non-septate mycelia, with sporangiousphore, the sporangium is spherical.	*Rhizopus stolonifer*
4. Sample D	Whitish green which turns dark green with time, has cottony surface.	Hyphae is septate, unbranched conidiophores expanded at the tip. The phialids that produce conidia is available.	*Aspergillus flavus*

The isolation of fungal genera such as *Aspergillus*, and *Rhizopus* highlights the diverse microbial population involved in fufu fermentation. These fungi are integral to the fermentation process, contributing to the development of flavor and texture in the final product (Ogbo *et al.*, 2015). The macroscopic and microscopic characteristics of the fungal isolates provide insight into their growth patterns, spore structures, and potential roles in the fermentation process. This fungal contributes to the breakdown of complex carbohydrates, protein hydrolysis and the development of different flavors (Cheng *et al.*, 2022).Particularly; *Aspergillus niger* was identified as the most prevalent fungus in the samples. This organism is noted for its resilience and ability to produce heat-resistant spores, which can survive under harsh conditions (Miller, 2019). Although *A. niger* can produce mycotoxins under unfavorable storage conditions such as when the food is stored improperly or exposed to high moisture levels posing potential health risks (Pitt and Hocking, 2020).The presence of these fungi underscores their importance in the fermentation process but also highlights the need for proper handling and storage to prevent potential contamination.

Table 3: Mean of the proximate composition of fufu samples

S/N	Parameters	Sample (A)	Sample (B)	Sample (C)	Sample (D)
1	Moisture (%)	6.52	7.13	7.25	7.25
2	Protein (%)	1.68	3.42	3.69	4.50
3	Fat (%)	1.32	1.22	1.20	1.22
4	Ash (%)	1.85	2.13	2.22	2.14
5	Crude fiber (%)	1.41	2.21	3.75	2.55
6	Carbohydrate (%)	87.26	83.49	81.45	79.31

It is imperative to note that the proximate composition of fufu can vary depending on factors such as the specific fermentation process, the types of raw materials used, and the geographical location as stated in the work of Nielsen *et al.*, 2018.

These variation in both proximate composition and microbial profile among the fufu samples from Amai, Delta State, highlights the influence of different fermentation practices, raw materials, and environmental factors on the final product. These variations underscore the need for standardized practices to ensure consistent quality and safety in local fermented fufu production. It also emphasizes the need for continuous monitoring and quality control to maintain the nutritional and safety standards of fermented products.

Conclusion: In conclusion, the fermentation process is a critical step in the traditional production of fufu, contributing to the unique texture, flavor, and potential dietary benefits of this staple food. The result of the study provides information on the nutritive value of fufu. The proximate analysis of the fufu provided valuable insight to its nutritional content which specifies that fufu is rich in energy giving nutrient (carbohydrate). Fufu as a staple food promotes food security and enhances livelihoods and as such it should be produced in a clean environment that will prevent contamination of fermented products.

Conflicts of Interest: The authors declare no conflict of interest.

Data Availability: Data are available upon request from the first author/corresponding author.

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