## Bacteriological Quality of Packaged and Unpackaged Processed Yam Flour

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#### Abstract

This study evaluated the bacteriological quality of packaged and unpackaged processed yam flour samples obtained from Tombia Market in Yenagoa, Bayelsa State, Nigeria. Yam flour, a staple food in West Africa, is prone to microbial contamination during processing, storage, and handling. Samples were analyzed for total heterotrophic bacteria (THB), coliform counts, and bacterial diversity using standard microbiological techniques, including nutrient agar, MacConkey agar, and biochemical tests. Results revealed significant bacterial contamination in both packaged and unpackaged samples, with THB counts ranging from  $1.14 \pm 0.10 \times 10^2$ CFU/g to  $1.94 \pm 0.07 \times 10^2$  CFU/g for packaged samples and  $1.27 \pm 0.20 \times 10^2$  CFU/g. Concurrently, coliform counts ranged from  $0.45 \pm 0.08 \times 10^2$  CFU/g to  $0.84 \pm 0.10 \times 10^2$ CFU/g across both sample types. Unpackaged samples exhibited higher mean THB (5.10  $\pm$  $1.11 \times 10^2$  CFU/g) and coliform (2.60  $\pm$  0.19  $\times$  10<sup>2</sup> CFU/g) counts compared to packaged samples  $(3.73 \pm 1.94 \times 10^2 \text{ CFU/g} \text{ and } 2.10 \pm 0.92 \times 10^2 \text{ CFU/g}, \text{ respectively})$ , indicating greater susceptibility to environmental contamination. Bacterial isolates included Escherichia coli (25% in unpackaged, 17% in packaged), Bacillus species (20% in unpackaged, 29% in packaged), Lactobacillus species, Staphylococcus species, Pseudomonas species, Micrococcus species, Citrobacter species, and Streptococcus species, with E. coli and Bacillus being predominant. The presence of pathogens like E. coli suggests potential health risks, emphasizing the need for improved hygiene and packaging practices. Proximate analysis was conducted but not fully reported. Statistical analysis (ANOVA, P < 0.05) confirmed significant differences in microbial loads between samples. These findings highlight the importance of good manufacturing practices and effective packaging to enhance the safety and quality of yam flour for consumer health.

Key Words: Yam Flour, Packaged; Unpackaged; E. coli; Bacillus

## Introduction

Yam flour, commonly known as "elubo" in West Africa, is a staple food product derived from the processing of yam tubers (Dioscorea spp.). It is widely consumed in various forms, such as amala, a popular Nigerian dish, due to its nutritional value and cultural significance (Otegbayo et al., 2018). The production of yam flour involves several stages, including peeling, slicing, drying, and milling, which may expose the product to microbial contamination if not properly managed (Akinola et al., 2017). Packaged and unpackaged yam flour, often sold in local markets or processed industrially, can harbor diverse bacterial populations, some of which may pose health risks to consumers. The presence of bacteria such as *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus* species in food products is a significant concern, as they are associated with foodborne illnesses (Ogundipe et al., 2020).

The microbial quality of yam flour is influenced by factors such as processing methods, storage conditions, and packaging practices. Unpackaged yam flour, often displayed in open markets, is particularly susceptible to contamination from environmental sources, including dust, insects, and human handling (Adebayo-Oyetoro et al., 2019). Conversely, packaged yam flour, while offering some protection, may still contain bacteria introduced during processing or due to improper sealing and storage (Ijabadeniyi & Buys, 2012). The isolation and identification of bacteria in yam flour are critical for assessing its safety and quality, enabling the development of effective control measures to ensure consumer safety. Potential sources of contamination include raw yam tubers, environmental conditions, and processing practices. Studies have shown that contamination can originate from soil, water, and the equipment used in processing (Ogunbanwo *et al.*, 2018).

Research has identified various microorganisms that may be present in locally processed yam flour, including bacteria (e.g., *Escherichia coli, Salmonella* spp.) (Ojo *et al.*, 2020). These contaminants pose health risks, as some can lead to foodborne illnesses.

The microbial quality of yam flour is often assessed using standards such as Total Plate Count (TPC), Yeast and Mold Counts, and the presence of specific pathogens. Different studies indicate that many samples of locally processed yam flour exceed acceptable limits set by food safety authorities (Idowu *et al.*, 2021). Continuous monitoring and adherence to hygiene practices in processing are crucial to minimize microbial load.

Understanding the microbial quality of locally processed yam flour is essential for improving food safety protocols. Educating processors about good manufacturing practices (GMP), proper storage conditions, and the importance of hygiene can help enhance the quality of this essential food product (Eze *et al.*, 2019).

Studies have shown that poor hygienic practices during processing and inadequate storage conditions can lead to elevated bacterial loads in yam flour (Oladipo et al., 2016). Furthermore, the lack of standardized processing techniques in many local settings exacerbates the risk of contamination. Therefore, investigating the bacterial profile of both packaged and unpackaged yam flour is essential to understand the microbial dynamics and implement interventions to enhance food safety. This study investigates the bacteriological quality of packaged and unpackaged processed yam flour, assessing microbial loads and identifying potential contamination sources to provide insights into improving food safety standards.

## **Materials and Methods**

#### Sample Area

This study was based on cassava flour samples collected from Tombia Market in Yenagoa, Bayelsa State. The flour samples were analysed and processed at the microbiology laboratory in Niger Delta University Wilberforce Island, Southern Ijaw Local Government Area, Bayelsa State, which is located in latitudes 04°C 15' north, 05°C 23' south, and longitudes of 060 C 45' east, with Delta State to the north, Rivers State to the east, and the Atlantic Ocean to the west and south.

## **Sterilization/Disinfection of Materials**

During the bacteriological investigation of the samples, the instruments and materials used in this study were sterilized to detect contamination. The autoclave was used for sterilization. Glassware, nutritional medium, and cotton wool are all included in this category. They were autoclaved at 1210C for 15 minutes at 15 PSI. Droppers and glass rods that couldn't be autoclaved were disinfected with 70% ethanol. The bench was cleaned both before and after each shift using 70% ethanol.

#### **Preparation of Nutrient Media**

Autoclaving was utilized to sterilize the nutritional medium in this investigation. The bacterial population of the samples was cultured and counted using Nutrient agar, Cetrimide agar, and MacConkey agar, while faecal and total coliform bacteria were estimated using MacConkey broth. Kliger iron agar was employed to identify lactose and glucose fermentation, gas generation, and hydrogen sulfide formation during biochemical testing of the isolates. Citrate utilization as a carbon source were performed using Simmon citrate agar, Indole production was detected using tryptone water.

It was done according to the manufacturer's instructions to dissolve the powder medium in distilled water. The containers were covered by loosened lid with aluminium foil for 15 minutes at  $121^{\circ}$ C to autoclave the dissolved medium.

#### **Bacteriological Analysis**

Standard operating protocols such as determining the data, cleaning the data, etc were used to conduct the quantitative and qualitative investigation of the bacteria found in the flour samples. There were strict guidelines in place for the usage of the chemicals, nutrients, and other equipment.

#### Enumeration of total heterotrophic bacteria

Nutrient agar was used to estimate the population of the heterotrophic bacteria present in the flour samples. Before plating the flour samples, they were serially diluted. Transferring 5 grams of flour into a test tube filled with 10 ml of 0.85 percent normal saline, the stock culture was created. After a thorough shaking, the stock culture was ready to use. One millilitre of the stock culture was then diluted 1:10 with 9 millilitres of sterile water. A third dilution tube was used for the samples (1:1000). The pour plate technique was used after the third dilution (1ml of the sample was poured into the plates aseptically). It was then poured onto the petri dishes with the help of 20ml of the ready-made molten agar. The dishes were allowed to cool before dispensing (solidify). A 24-hour incubation period at  $37^{0}$ C followed the plates being inverted.

## **Enumeration of Coliform Bacteria**

The coliform count was tallied using a modified version of the most probable number (MPN) approach developed by Ginigaddarage et al., (2018). MacConkey agar was used to count the number of coliform bacteria. The same procedure for the enumeration of total heterotrophic bacteria was repeated.

#### Enumeration of total and faecal coliform

Following a modified Ginigaddarage et al., (2018) technique, the third (3rd) dilution was utilized to count faecal and total coliforms. There were three tubes with 10ml each of double strength MacConkey broth, single strength MacConkey broth, and inoculum inoculation, and each tube had 10ml of dilution added to it. The inoculum was then divided into three and added to 10ml each of the three different concentrations. There was a total of nine tubes in each sample. For faecal and total coliform, two sets of tubes were utilized for each. The faecal and total coliform cultures were maintained at 36<sup>o</sup>C and 44<sup>o</sup>C, respectively, during the broth cultures. The test tubes were incubated for 48 hours. The Durham tubes were inspected at the conclusion of the incubation time for gas generation and fermentation. An MPN index was used to analyse the outcomes of the positive and negative tubes.

#### Isolation of pure cultures of bacteria

After the agar plates had been incubated, a random sample of colonies were chosen and removed using a sterile wire loop. Sub-cultured, colonies on new nutritional agar plates were

produced by streaking the colonies over the agar surface. Purified isolates were obtained by flipping the plates and incubating them at 37<sup>0</sup>C in an aerobic environment.

## **Biochemical characterization and identification of bacterial isolates Gram Staining Technique**

Colonies from several pure culture plates were emulsified on a slide with a drop of distilled water. A drop of the suspended culture was transferred with an inoculation loop to ta microscope slide, and the culture spread on the slides to an even thin film over a circle of 15mm in diameter. The slide was then air-dried. Crystal violet stain was applied to the fixed culture for 60 seconds, the stain was poured off, and the excess stain rinsed with water. Lugol's iodine solution was used to cover the smear for 60 seconds. The iodine solution was poured off, and the slide was rinsed with running water. Excess water from the surface was shaken off. After being decoloured with alcohol, the slide was quickly rinsed with water in 5 seconds. The smear was counter stained with basic fuchsin solution for 60 seconds. The fuchsin solution was washed off with water, and slide air-dried after shaking off the excess water. The slide was examined under a microscope with x40 and x100 objective.

## Oxidase test

Three millilitres (3ml) of hydrogen peroxide were added to three sterile test tubes, and the colony of the pure culture was chosen and dipped into one of these test tubes, and the bubbles were observed. (Cheesbrough, 2010).

## **Indole Test**

Tubes containing 10 millilitres of tryptophan broth were made. Test organisms were placed on a wire loop and cultured for 48 hours. The medium was then treated with five drops of Kovac reagent, after which the bubbles were observed for the presence or absence of cherry-red ring (Cheesbrough, 2010).

## **Kliger Iron Agar Slant Test**

Test tubes containing 10ml of Kliger Iron Agar were used to prepare the slants. With an inoculating needle, pick the centre of well-isolated colonies obtained from solid culture media. The test tubes were initially injected with the bacteria by stabbing the centre of the medium, inoculating needle, into the deep of the tube to within 3-5mm from the bottom. The inoculating needle was withdrawn and streaked on the surface of the slant. The tubes were incubated at 37<sup>o</sup>C for 24 hours, with cotton wool covering the openings. Colour changes, darkening, and cracking of the media after incubation were observed and recorded (Cheesbrough, 2010).

## **Citrate Utilization Test**

Ten millilitres of Simmon citrate slants were prepared in test tubes. The media slope was inoculated with the test isolate using a wire loop. The tubes were then incubated at  $37^{0}$ C for 24 hours, and the colour change in the medium was observed (Cheesbrough, 2010).

## **Catalase Test**

Three millilitres (3ml) of hydrogen peroxide were added to three sterile test tubes, and the colony of the pure culture was chosen and dipped into one of these test tubes, and the bubbles were then observed (Cheesbrough, 2010).

## **Methyl Red Test**

A new Methyl red medium was infected with a bacterial isolate and incubated at 37<sup>o</sup>C for 24 hours. Five drops of methyl red were added to the soup after the incubation period.

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## Proximate Analysis

## **Determination of Moisture**

An evaporating dish was dried in the oven for one hour. The evaporating dish was filled with 5g of the sample and put in an oven at  $105^{0}$ C. The samples were weighed every hour until they reached a stable weight.

% Moisture =  $\frac{\text{Weight of wet sample} - \text{Weight of dry sample}}{\text{Weight of wet sample}} \times \frac{100}{1}$ 

## **Determination of %Ash**

One gram of a moisture-free sample was placed in a crucible. Muffle furnaces were used to heat the sample and crucible for 12 to 18 hours. The furnace was set to  $55^{0}$ C. The furnace was turned off and allowed to cool to a temperature of around  $25^{0}$ C or lower at the conclusion of the process. The crucible was placed in a desiccator to enable it to cool and the ash weighed. %Ash = Weight after Ash – Weight of Crucible × 100

Weight of original sample 1

## **Determination of Crude Protein**

An amount of 0.55g of sample was added to the flask, followed by the addition of 1g of mercury catalyst and 30ml conc. H<sub>2</sub>SO<sub>4</sub>. When the foaming stopped, the flask was gently heated. For the next five hours, it was heated to boiling point. 100ml of cooled distilled water was added to the flask in order to finish chilling it. Another pair of flasks was used to hold the digest. Every last bit of residue was cleaned and then poured into the flask. A conical flask containing 50ml of boric acid and 1 ml of mixed indicators was put beneath the extractor of the distillation apparatus to collect the condensate.

In the distillation flask, 150 ml of 10M NaOH was added, and the distillation process began. When 150ml of the distillate was collected, the operation was halted. It was measured by titrating the condensate with  $0.01M H_2SO_4$  to determine the quantity of  $N_2$  present. The colour shifts from green to purple near the conclusion.

 $N = 0.01M H_2SO_4 \times M \times 14 \times 50 \times 100$ 

%Protein = % N  $\times$  6.25 Where M = Molarity of the H<sub>2</sub>SO<sub>4</sub> 14= Atomic number of Nitrogen 50= from the procedure 10= from the procedure 100= percentage 10= weight of original sample

## **Determination of Crude Lipid**

A thimble containing 2 grams of dried (moisture-free) material was put in a soxhlet extraction equipment. Glass wool was used to cover the thimble's mouth. The weight of the boiling flask was determined. With the addition of 120 ml of petroleum ether and two antibomps, the content of the flask was brought to a boil. With the help of an electro thermal heater, the three vessels were put together. The extraction process lasted about three hours to complete. A hot-air oven set to 1000°C for 30 minutes was used to dry out the boiling flask with the fat that was taken from it. It was then weighed after cooling in a desiccator.

%Fat (lipid)=  $\underline{g \text{ fat in sample}} \times \underline{100}$ g sample 1

## **Determination of Crude Fibre**

Two hundred millilitres (200ml) of 1.25 percent H2SO4 were added to a beaker containing 2g of defatted dry sample, and the mixture was brought to a boil for 30 minutes while being constantly swirled. Suction or vacuum was used to cool and filter it at the conclusion of the process. The filter paper and fibres were flushed with water. The flask was refilled with 200ml of 1.25 percent NaOH and cooked for another 30 minutes, after which the residue was placed into the flask. After a period of time, the samples were filtered and washed three times with petroleum ether before being finished with three further washes. The filter paper and the residue were placed in an oven at  $105^{\circ}$ C for 12 hours.

#### **Data Analyses**

Quantitative data were analysed using a statistical analysis software (SPSS version 20). The results were subjected to one way analysis of variance (ANOVA) or student t-test, as was appropriate. Significant differences between packaging and nutritional and microbial quality of the flour were determined at P<0.05. Such results were presented as mean  $\pm$  standard error, microbial concentration, or as percentages.

#### Results

#### Assessment of the Bacteriological Quality of Yam Flour Samples

The results for the total heterotrophic bacteria (THB) count on nutrient agar for Packaged and Unpackaged yam flour samples is presented in tables 1 and 2 below and are expressed as mean x  $10^2$  CFU/g.

The results suggest the flour have different degree of bacterial contamination. For each of the samples (Yam A, Yam B and Yam C), the mean values of the bacterial count were done for their respective flour types (A, B and C) using analysis of variance (ANOVA). The results indicate statistically significant differences (P < 0.05) between the samples.

Table	1:	Enumeration	٥f	<b>Bacterial</b>	Ponu	lation	in	Packaged	Vam	Flour	Samples
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Samples	Total Heterotrophic Bacteria (10 <sup>2</sup> CFU/g)	Coliform Bacteria (10 <sup>2</sup> CFU/g)
Yam Flour A	$1.94\pm0.07^{ab}$	$0.62\pm0.13^{ab}$
Yam Flour B	$0.65 \pm 0.12^{ m abc}$	$1.04\pm0.11^{\text{abc}}$
Yam Flour C	$1.14\pm0.10^{abcd}$	$0.45\pm0.08^{abcd}$

The bacteriological analysis of the Packaged Yam flour samples presented on the table 1., shows the THB count to range from  $1.94 \pm 0.07 \times 10^2$  CFU/g in Yam flour A to  $1.14 \pm 0.10 \times 10^2$  CFU/g in Yam flour C. The Coliform bacteria count ranged from  $0.62 \pm 0.13 \times 10^2$  CFU/g in Yam flour A to  $0.45 \pm 0.08 \times 10^2$  CFU/g in Yam flour C.

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Table 2: Enumeration of Bacterial Population in Unpackaged Yam Flour Samples							
Samples	Total Heterotrophic Bacteria (10 <sup>2</sup> CFU/g)	Coliform Bacteria (10 <sup>2</sup> CFU/g)					
Yam Flour A	$1.27\pm0.20^{ab}$	$0.81\pm0.06^{ab}$					
Yam Flour B	$1.89\pm0.47^{abc}$	$0.94\pm0.08^{abc}$					
Yam Flour C	$1.94\pm0.16^{abcd}$	$0.84\pm0.10^{abcd}$					

The results for the quantitative analysis of the Yam flour samples presented in the table 2 above, shows the THB counts ranged from  $1.27 \pm 0.20 \times 10^2$  CFU/g in Yam flour A to  $1.94 \pm 0.16 \times 10^2$  CFU/g in Yam flour C. The Coliform bacteria count ranged from  $0.81 \pm 0.06 \times 10^2$  CFU/g in Yam flour A to  $0.84 \pm 0.10 \times 10^2$  CFU/g in Yam flour C.

 Table 3: Bacteria isolated from Packaged Yam flour sample and their percentage of occurrence

S/n	Packaged Yam Flour	Percentage of occurrence
		(%)
1	Lactobacillus sp.	26
2	Bacillus sp.	29
3	Escherichia coli	17
4	<i>Citrobacter</i> sp.	15
5	Streptococcus sp.	4
6	Micrococcus sp.	9

Table 3. above shows *Lactobacillus* sp. occurred with 26%. *Bacillus* species recorded 29%, *Escherichia coli* 17%, *Citrobacter* sp. 15%, *Streptococcus* sp. 4%, and *Micrococcus* sp. 9%.

 Table 4. Bacteria isolated from Unpackage Yam flour sample and their percentage of occurrence

S/n	Unpackaged Yam	Percentage of occurrence
	Flour	(%)
1	Staphylococcus sp.	13
2	Bacillus sp.	20
3	Escherichia coli	25
4	Lactobacillus sp.	10
5	Pseudomonas sp.	16
6	Micrococcus sp.	16

Table 4., above presents the result for the percentage of occurrence of bacterial species associated with the unpackaged yam flour samples. *Bacillus* sp. recorded a percentage of 20, *Escherichia coli* 25%, *Staphylococcus* sp. 13%, *Micrococcus* sp. 16%, *Pseudomonas* sp. 16%, *Lactobacillus* sp. 10%.

#### Occurrence of bacterial isolates in packaged and unpackaged flour samples

Figures 1 and 2 presents the results for the occurrence of bacterial species associated with the packaged and unpackaged yam flour samples.

Figure 1., shows *Bacillus* species occurred the most with 29% followed by *Lactobacillus* sp. 26%., *Escherichia coli* 17%, *Citrobacter* sp. 15%, *Micrococcus* sp. 9% and the least to be *Streptococcus* sp. 4%.



Figure 1: Percentage of Occurrence of Bacterial Isolates in Packaged Yam Flour Samples

Figure 2., below presents the result for the percentage of occurrence of bacterial species associated with the unpackaged yam flour samples. *Escherichia coli* 25% occurred the most, followed by *Bacillus* sp. which recorded a percentage of 20, *Staphylococcus* sp. 13%, *Micrococcus* sp. 16%, *Pseudomonas* sp. 16%, *Lactobacillus* sp. 10%.



Figure 2: Percentage of Occurrence of Bacterial Isolates in Unpackaged Yam Flour Samples

## Comparison of the bacteriological quality between packaged and unpackaged flour

The comparison of the level of contamination between the packaged and unpackaged flour is presented in tables 5 - 6. The level of contamination by the heterotrophic bacteria is presented in table 5.

# Table 5. Group Statistics on Total Heterotrophic Bacteria in Packaged and Unpackaged Yam Flour Samples on Nutrient Agar

Flour Samples Sample Class		Mean ± STD (10 <sup>2</sup> CFU/g)		
	Packaged	$3.73 \pm 1.94^{b}$		
Y am Flour	Unpackaged	$5.10 \pm 1.11^{b}$		

The results obtained above showed that packaged yam flour recorded less bacterial contamination with mean of  $3.73 \pm 1.94 \times 10^2$  CFU/g, while the unpackaged yam flour recorded a higher level of contamination, with a mean of  $5.10 \pm 1.11 \times 10^2$  CFU/g.

# Table 6. Group Statistics on Coliform Bacteria in Packaged and Unpackaged Yam Flour Samples on MacConkey Agar.

Flour Samples	Sample Class	Mean ± STD (10 <sup>2</sup> CFU/g)
	Packaged	$2.10\pm0.92^{\circ}$
Yam Flour	Unpackaged	$2.60\pm0.19$

The results in Table 6 above showed the packaged Yam flour recorded less coliform bacterial contamination with mean of  $2.10 \pm 0.92 \times 10^2$  CFU/g, while the unpackaged recorded a higher level of contamination with mean of  $2.60 \pm 0.19 \times 10^2$  CFU/g.

Table 7: Do	escriptive Statistics or	<b>Proximate</b>	analysis of	Packaged an	d Unpackaged	Yam
Flour Sam	ples					

F	Flour Samples	Packaged	Unpackaged	
Moisture (%)	Yam Flour	$8.00\pm0.01$	$10.7\pm0.02$	
Ash (%)	Yam Flour	$1.80\pm0.01$	$1.71\pm0.01$	
Protein (%)	Yam Flour	$8.83\pm0.01$	$7.38\pm0.02$	
Lipid (%)	Yam Flour	$1.49\pm0.04$	$1.48\pm0.02$	
Fibre (%)	Yam Flour	$1.97\pm0.01$	$1.89\pm0.02$	
DM (%)	Yam Flour	$91.7\pm0.01$	$89.3\pm0.02$	
NFF (%)	Yam Flour	$85.8\pm0.04$	$87.5\pm0.02$	

#### **DISCUSSION**

The study investigated the bacteriological quality of packaged and unpackaged processed yam flour samples collected from Tombia Market in Yenagoa, Bayelsa State, Nigeria, focusing on microbial loads, bacterial diversity, and proximate composition. The results provide valuable insights into the microbial safety of yam flour and highlight differences between packaged and unpackaged products, which are discussed below in the context of the study's findings, their implications, and comparisons with existing literature.

#### **Bacteriological Quality of Yam Flour Samples**

The results indicate significant bacterial contamination in both packaged and unpackaged yam flour samples, with total heterotrophic bacteria (THB) and coliform counts serving as key indicators of microbial quality. The THB counts for packaged yam flour ranged from  $1.14 \pm 0.10 \times 10^2$  CFU/g (Yam flour C) to  $1.94 \pm 0.07 \times 10^2$  CFU/g (Yam flour A), while unpackaged samples ranged from  $1.27 \pm 0.20 \times 10^2$  CFU/g (Yam flour A) to  $1.94 \pm 0.16 \times 10^2$  CFU/g (Yam flour C). Coliform counts were similarly elevated, with packaged samples ranging from  $0.45 \pm 0.08 \times 10^2$  CFU/g to  $0.62 \pm 0.13 \times 10^2$  CFU/g, and unpackaged samples from  $0.81 \pm 0.06 \times 10^2$  CFU/g to  $0.84 \pm 0.10 \times 10^2$  CFU/g. These findings suggest that both types of yam flour are prone to microbial contamination, though unpackaged samples generally exhibited higher bacterial loads.

The presence of coliform bacteria, particularly in unpackaged samples, is concerning as it indicates potential fecal contamination, likely introduced through improper handling, contaminated water, or environmental exposure during processing or market display. The statistically significant differences (P < 0.05) in bacterial counts between samples, as determined by ANOVA, underscore the variability in contamination levels, which may be attributed to differences in processing hygiene, storage conditions, or packaging practices (Ogundipe et al., 2020).

## **Bacterial Diversity and Occurrence**

The study identified a diverse range of bacterial isolates in both packaged and unpackaged yam flour, with notable differences in their prevalence. In packaged yam flour, *Bacillus* species (29%) and *Lactobacillus* species (26%) were the most prevalent, followed by *Escherichia coli* (17%), *Citrobacter* species (15%), *Micrococcus* species (9%), and *Streptococcus* species (4%).

In unpackaged yam flour, *Escherichia coli* (25%) was the most common, followed by *Bacillus* species (20%), *Micrococcus* species (16%), *Pseudomonas* species (16%), *Staphylococcus* species (13%), and *Lactobacillus* species (10%).

The high occurrence of *Bacillus* species in both sample types is consistent with its ubiquity in soil and its ability to form spores, which can survive drying and milling processes during yam flour production (Ogundipe et al., 2021). *Escherichia coli*'s dominance in unpackaged samples is particularly alarming, as it is a known indicator of fecal contamination and a potential pathogen linked to foodborne illnesses (Igbokwe et al., 2020). The presence of *Staphylococcus* and *Pseudomonas* species in unpackaged samples further suggests contamination from human handling or environmental sources, as these bacteria are commonly associated with skin and water, respectively (Adebayo-Oyetoro et al., 2019).

The occurrence of *Lactobacillus* species, particularly in packaged samples, may not necessarily be detrimental, as some strains are beneficial and contribute to fermentation processes. However, their presence in high numbers could indicate improper processing or storage conditions that allow microbial proliferation (Ogunbanwo et al., 2018). The lower diversity of pathogens in packaged samples compared to unpackaged ones highlights the protective role of packaging, which limits exposure to environmental contaminants.

#### Comparison of Packaged vs. Unpackaged Yam Flour

The comparison of bacteriological quality between packaged and unpackaged yam flour revealed that packaged samples had significantly lower microbial contamination. Packaged yam flour recorded a mean THB count of  $3.73 \pm 1.94 \times 10^2$  CFU/g and a coliform count of  $2.10 \pm 0.92 \times 10^2$  CFU/g, compared to  $5.10 \pm 1.11 \times 10^2$  CFU/g and  $2.60 \pm 0.19 \times 10^2$  CFU/g, respectively, for unpackaged samples. These findings align with previous studies that demonstrate the efficacy of packaging in reducing microbial loads by providing a physical barrier against environmental contaminants (Ijabadeniyi & Buys, 2012).

The higher contamination levels in unpackaged yam flour are likely due to its exposure to open market conditions, where factors such as dust, insects, and frequent handling by vendors and customers increase the risk of microbial introduction. The study's results emphasize the need for improved packaging practices to enhance the safety of yam flour, particularly for products sold in local markets. However, the presence of bacterial contaminants in packaged samples suggests that pre-packaging processes, such as inadequate drying or poor hygiene during milling, may still introduce microbes, underscoring the importance of adhering to good manufacturing practices (GMP) throughout the production chain (Eze et al., 2019).

#### **Implications for Food Safety**

The microbial loads observed in both packaged and unpackaged yam flour samples, particularly the presence of pathogens like *Escherichia coli* and *Staphylococcus* species, indicate potential health risks for consumers. According to food safety standards, such as those set by the World Health Organization (WHO) and the Nigerian Industrial Standards (NIS), coliform counts in processed foods should be minimal, and pathogens like *E. coli* should be absent (Idowu et al., 2021). The elevated bacterial counts in this study suggest that many samples may exceed acceptable limits, highlighting the need for stricter quality control measures in yam flour production.

The findings also point to the critical role of processing hygiene and storage conditions in ensuring food safety. Poor hygienic practices, such as the use of contaminated water or equipment, and inadequate storage, which allows moisture retention, are likely contributors to the observed microbial loads (Oladipo et al., 2016). Interventions such as training processors on GMP, implementing hazard analysis and critical control point (HACCP) systems, and

promoting the use of high-quality packaging materials could significantly reduce contamination risks.

#### **Proximate Analysis**

Although the document does not provide detailed results for the proximate analysis, the methodology indicates that moisture, ash, crude protein, lipid, and fiber content were assessed. These parameters are crucial for understanding the nutritional quality and shelf-life stability of yam flour. High moisture content, for instance, could exacerbate microbial growth, contributing to the observed bacterial loads (Adebayo-Oyetoro et al., 2019). The lack of specific proximate analysis results limits further discussion, but it is reasonable to infer that differences in moisture content between packaged and unpackaged samples may have influenced their microbial profiles, as unpackaged samples are more exposed to environmental humidity.

#### **Limitations and Future Research**

The study provides robust data on the bacteriological quality of yam flour but has some limitations. The sample size and scope were restricted to one market in Bayelsa State, which may not fully represent the broader Nigerian or West African context. Additionally, the study did not assess fungal contamination, which is another significant concern for yam flour safety. Future research should expand the geographical scope, include fungal analysis, and explore the impact of specific processing techniques (e.g., blanching or improved drying methods) on microbial quality. Investigating the efficacy of different packaging materials and storage conditions could also inform practical interventions.

#### Conclusion

The study demonstrates that both packaged and unpackaged processed yam flour are susceptible to bacterial contamination, with unpackaged samples exhibiting higher microbial loads due to environmental exposure. The presence of pathogens like *Escherichia coli* and *Staphylococcus* species underscores the need for improved hygiene practices and packaging standards in yam flour production. These findings contribute to the growing body of evidence on food safety challenges in traditional food processing and highlight the importance of implementing GMP and quality control measures to protect consumer health.

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