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# Effects of Fermentation on the Functional Property, Physicochemical and Suitability of Cassava Starch/Flour for Food Production

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## ABSTRACT

### Keywords

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This study aimed at evaluating the influence of fermentation on Cassava starch structure and its effect on functional property. Cassava roots were washed, peeled and cut to sizes separated to batches; A, B, C, and D. Sample A was peeled and dry immediately, Sample B; C and D was peeled fermented for 24 h, 48 h, 78 h before drying respectively. Each of the dried Samples was analyzed for their Pasting property, Vitamin A content, Physicochemical property, Bulk Density, Swelling index, and Scanning Electron Microscopy (SEM). Pasting temperature results ranged from 75.87 – 77.90, with Sample B and D being the lowest and the highest respectively. Pasting time ranged from 4.27- 4.77 with Sample A and D being the lowest and highest respectively. Physicochemical results, which include: pH ranged of 5.12-6.80 with Sample A having the highest pH; Total Titratable Acidity which was 0.28-0.36 with the lowest as Sample D, while Sample A and C had the highest. Bulk Density ranged 0.53-0.72 for the lowest Sample A and B for the highest. Swelling index ranged was 0.09-0.9 for the lowest and the highest samples A and B respectively. SEM analysis, indicated the starch granules are greatly influenced by fermentation, the starch granules showed gradual disintegration as the fermentation time proceeded, thus hours of fermentation has effects on the starch property.

## Introduction

Cassava flour is versatile gluten-free flour made from the root of the cassava plant (Frediansyah *et al.*, 2017). It's a staple in many cuisines around the world, especially in Africa, South America, and Asia. Cassava flour has gained popularity in recent years due to its

versatility and nutritional benefits. It can be used as a substitute for wheat flour in many recipes, including baked goods, pancakes, and even as a thickening agent in soups and sauces (Elisabeth *et al.*, 2022). One of the key advantages of cassava flour is its gluten-free nature, making it suitable for those with celiac disease or gluten sensitivities. Cassava flour is naturally grain-free,

making it a popular choice for those following grain-free diets. Nutritionally, cassava flour is relatively high in carbohydrates, similar to other flours, but it also contains some fiber and resistant starch, which can have positive effects on digestion and blood sugar levels (Damayanti *et al.*, 2020; Lu *et al.*, 2020).

Physicochemical properties of cassava flour are not only important for understanding its behavior but also for optimizing its applications in the food industry (Aristizábal *et al.*, 2017; Akinyele *et al.*, 2020). For instance, the moisture content of cassava flour directly affects its texture and shelf life. Too much moisture can lead to microbial spoilage, while too little can result in a dry and undesirable texture. Achieving the right balance is crucial for ensuring the flour's stability during storage and its performance in recipes (Vuk and Gáthy, 2025).

Physicochemical properties of cassava flour encompass a diverse range of characteristics that collectively determine its suitability for various food applications. By comprehensively understanding and manipulating these properties, food manufacturers can optimize the functionality, nutritional value, and sensory qualities of cassava flour-based products to meet consumer demands and preferences (Senanayake *et al.*, 2024; Oladeji *et al.*, 2019).

The physicochemical properties of starch extracted from cassava flour are critical in understanding its functionality and suitability for various applications (Chisenga *et al.*, 2019; Vuk and Gáthy, 2025). These properties encompass parameters such as pH, swelling index, TSS and TTA. Each of these characteristics provides insights into how starch behaves under different processing conditions and influences its industrial and culinary uses (Li *et al.*, 2023). Morphological features of Cassava Flour refer to the physical characteristics of the flour particles, including shape, size, surface texture, and overall structure, which are important in determining the flour's quality and functionality.

These features can be studied using various techniques like microscopy, which provides insights into how cassava flour will behave in different food applications. The morphological characteristics of cassava flour determine the final texture of products like breads, cakes, or porridges. For instance, finer particle size results in smoother dough and better crumb structure in baked goods (Osunbade *et al.*, 2025; Taiwo *et al.*, 2025).

Gelatinization and Viscosity; Cassava flour's ability to gelatinize and form a paste when heated is highly dependent on the size and structure of its starch granules. Flour with more intact starch granules will generally produce a thicker and more stable gel. Digestibility: The morphology of cassava flour also impacts its digestibility. Larger, less processed particles may take longer to break down, while smaller particles and damaged granules are easier for enzymes to digest. The granules size of cassava starch depends on process of extraction and milling techniques, starch performance as thickening agent and its cohesiveness depend on its concentration and processing method it is subjected to (Chisenga *et al.*, 2019; Uchechukwu-Agua, 2015; Williams *et al.*, 2019).

Fermentation significantly impacts the quality characteristics of cassava flour, with various methods influencing its final properties (Udoro *et al.*, 2021). One common method is spontaneous fermentation, where natural microorganisms present on the cassava initiate fermentation. This process typically results in a longer fermentation period, allowing for a more complex microbial activity, which can enhance flavor development and reduce cyanogenic compounds (Ardiah *et al.*, 2021). Contrastingly, controlled fermentation involves the use of starter cultures or selected microorganisms to initiate fermentation.

This method provides more predictable results and faster fermentation times compared to spontaneous methods. It's particularly advantageous for maintaining consistency in product quality and achieving specific sensory attributes desired in cassava flour (Elizabeth *et al.*, 2022; Njoku *et al.*, 2020).

The duration of fermentation also plays a crucial role, longer fermentation periods generally lead to more extensive breakdown of complex carbohydrates into simpler sugars, improving digestibility and reducing the bitter taste associated with cyanogenic compounds. However, prolonged fermentation can also affect the texture and shelf stability of the final cassava flour product (Chisenga *et al.*, 2019). The choice of fermentation method and its management significantly impacts the sensory, nutritional, and functional properties of cassava flour. Each method offers unique advantages and challenges, influencing factors such as flavor profile, nutritional content, and overall consumer acceptability of the final product (Egbune *et al.*, 2023; Elizabeth *et al.*, 2022; Udoro *et al.*, 2021).

Fermentation of cassava plays a central role in transforming its inherent qualities, primarily by modifying its physical, chemical, and sensory attributes. The method chosen for fermentation, whether spontaneous or controlled directly influences the outcomes, beginning with the microbial activity that drives the process (Sanni *et al.*, 2024). During fermentation, a dynamic interplay of bacteria and yeasts breaks down the complex structures within cassava, specifically affecting its starch composition and cyanogenic content. This biological action is crucial for improving the edibility of cassava, as the raw tuber contains toxic compounds like cyanogenic glycosides, which, if left unprocessed, can be harmful to consumers (Lopez-Ochoa *et al.*, 2022; Ndjang *et al.*, 2023).

A spontaneous fermentation method relies on the environmental and indigenous microorganisms present on the cassava tuber, often resulting in unpredictable but rich microbial diversity (Yuliana *et al.*, 2023). These microbes contribute to the unique flavors and textures observed in traditionally fermented cassava flours. Their presence, however, can also cause variability in product quality due to the environmental conditions that influence their activity (Osunbade *et al.*, 202). Temperature, humidity, and even the geographic location of fermentation contribute to the microbial profiles, which, in turn, influence the flavor, consistency, and nutritional profile of the flour. Alternatively, controlled fermentation utilizes specific bacterial or fungal strains to guide the process. This method allows producers to influence the fermentation kinetics and, therefore, achieve a more predictable end product (Du *et al.*, 2025; Ye *et al.*, 2019; Setiarto *et al.*, 2024).

Controlled conditions enable the optimization of fermentation parameters, such as pH and temperature, which are crucial in achieving uniform flour quality (Elizabeth *et al.*, 2022). In this scenario, the growth of unwanted microorganisms is minimized, ensuring safety and extending the shelf life of the flour. One of the significant advantages of the fermentation process, regardless of method, is its role in reducing the cyanogenic content of cassava. Enzymatic hydrolysis of cyanogenic glycosides, primarily linamarin and lotaustralin, occurs during fermentation (Ojiako *et al.*, 2018; Karine *et al.*, 2016), leading to the release of less toxic compounds. The effectiveness of cyanide removal depends not only on the fermentation time but also on the microbial strains involved. The reduction in cyanide content not only improves food safety but also affects

the bitterness of the cassava flour, making it more palatable and suitable for diverse culinary applications (Ardiah *et al.*, 2021).

## Materials and Methods

### Sample Collection and Processing

Fresh cassava roots were obtained from Ladoke Akintola University Research Farm. Samples were carefully selected to ensure uniformity in size and maturity. Upon collection, the cassava roots were cleaned, peeled, and washed thoroughly to remove any dirt or debris. Processing took place at Owodunni Food Processing Laboratory, Ladoke Akintola University of Technology, Ogbomosh. The following processing methods were considered, including cabinet dryer and fermentation. Each method was executed according to established protocols to ensure consistency and accuracy. The following processing are; (Peel and dry immediately), (Peel, fermented for 24h and dry) (Peel, fermented for 48hrs and dry), (Peel, fermented for 78h) and each sample were dry by using cabinet drier at 60° C for 20h. Sample A: Non fermented cassava; Sample B: Fermented for 24 hours; Sample C: Fermented for 48 hours; Sample D: Fermented for 72 hours.

### Vitamin A Determination

Vitamin A determination was done by the method described by Onyesife *et al.*, (2014). 20ml of petroleum ether was added to 1g of pulverized sample and put on a shaker for about 30mins. The petroleum ether was decanted and evaporated to dryness. 0.2ml of chloroform-acetic anhydride (1:1 v/v) was added to the residue. Later on, 2ml of trichloroacetic acid- chloroform (1:1 v/v) was added. The absorbance of the solution was then measured at 620 nm. The vitamin A standard was also prepared in the same way at varying concentrations (20-100µg/ml) and a standard curve plotted.

### Swelling Index

To 1 g of sample was added 5 mL of distilled water in a measuring cylinder (10 mL), the volume occupied was recorded before adding the water and left to stand for one hour and the new volume was noted. Swelling index was calculated as follows; swelling index equal to volume occupied by sample before swelling divided by volume occupied after swelling.

## pH

A 20% W/V dispersion of the sample would be shaken in water for 5 minutes and the PH would be determined using a PH meter (Elisabeth *et al.*, 2022).

## Total soluble solid

Total soluble solid were determined using the method described by Adeoye *et al.*, (2022). 10g of sample was homogenized and centrifuged for 20 min at 4°C. The supernatant was used for soluble solids measurement using refractometer.

## Total titratable acidity

The total titratable acidity was determine according to AOAC (2005) official method by direct titration of 2 g of the sample with 0.1 M of sodium hydroxide using phenolphthalein as indicator. The total acidity was calculated as Volume of base x mole of base x mill equivalent of acid x100/wt. of sample.

## Fourier Transform Infrared (FTIR) Spectroscopy Analysis (FTIR ANALYSIS)

The Fourier Transform Infrared (FTIR) spectroscopy was employed to analyze the functional groups present in the cassava flour samples after fermentation. To prepare the samples for FTIR analysis, 2g of each cassava flour sample was dried to ensure moisture removal. The dried samples were then finely ground and mixed with potassium bromide (KBr) at a ratio of 1:100 to form a homogeneous powder. The mixture was compressed into a pellet using a hydraulic press. The prepared pellet was placed in the FTIR spectrometer, and the spectra were recorded in the range of 4000–400  $\text{cm}^{-1}$ . The FTIR spectrum provided information on the characteristic peaks corresponding to various functional groups such as hydroxyl (-OH), carbonyl (C=O), and carboxyl (-COOH) groups, which are key indicators of changes in the molecular structure of the cassava flour due to fermentation.

## Evaluation of starch granules using SEM

Scanning Electron Microscopy (SEM) is utilized to examine the morphology and structural integrity of starch granules extracted from cassava flour. SEM provides detailed images at high magnification, allowing

for the characterization of granule size, shape, surface properties, and internal structure. This analysis is crucial for understanding how processing methods impact the physical characteristics of starch granules and subsequently influence their functional properties in food and non-food applications.

## Result and Discussion

### Pasting Properties of Instant Flour Cassava

The pasting properties of the instant flours were presented in Table 4.1. The peak viscosity values for the instant flour samples ranged from 5251.00 RVU to 6983.59 RVU. Sample A recorded the highest peak viscosity at 5251.00 RVU, while sample B had the lowest at 4.33 RVU. Peak viscosity reflects the viscous load or the amount of energy required during mixing and is often associated with product quality. A higher peak viscosity indicates that the flour can form thicker pastes when cooked. According to Awoyale *et al.*, (2016), a high peak viscosity in *Manihot esculenta* (cassava) is essential for producing pounded yam. This is a critical factor for making instant pounded yam dough with desirable textural qualities.

The trough viscosity for the instant flour samples ranged from 2233.50 to 3042.50 RVU. Final viscosities of the instant flour samples reported in this study ranged from 3177.00 to 4088.00 RVU. Sample C exhibited the highest value of 6983.59 RVU, while sample A recorded the lowest value of 4.27 RVU. Final viscosity is considered the most commonly used parameter for assessing the quality of flour-based samples, as it reflects the flour's ability to form a gel or viscous paste after cooking and cooling, as well as the paste's resistance to shear force during stirring (Olusola *et al.*, 2015).

The setback viscosity for the instant flour samples ranged from 857.50 to 1045.50 RVU. Sample D exhibited the highest value of 1045.50 RVU, while sample B had the lowest value of 857.50 RVU. Setback viscosities indicate the extent of retrogradation, which refers to the separation of amylose from water in the gel formed upon cooling of a starch or flour paste.

Low setback viscosities in starches suggest a lower tendency for the products to retrograde, and vice versa (Paixão e Silva, 2021).

**Table.1** Pasting property of fresh and treated cassava starch flour

Samples	A	B	C	D
Peak Visc.	5251.00±33.94 <sup>d</sup>	5828.80±58.69 <sup>c</sup>	6983.59±60.10 <sup>a</sup>	6451.5±191.62 <sup>b</sup>
Trough Visc.	2233.50±21.92 <sup>b</sup>	2319.50±13.43 <sup>b</sup>	2921.50±156.27 <sup>a</sup>	3042.50±190.21 <sup>a</sup>
Beak Down	3017.50±55.86 <sup>c</sup>	3509.00±45.25 <sup>b</sup>	4062.00±96.17 <sup>a</sup>	3417.00±1.41 <sup>b</sup>
Final Value	3238.00±41.01 <sup>b</sup>	3177.00±11.31 <sup>b</sup>	3843.50±210.01 <sup>a</sup>	4088.00±148.49 <sup>a</sup>
Set Back	1004.50±19.09 <sup>ab</sup>	857.50±24.75 <sup>c</sup>	922.00±53.74 <sup>bc</sup>	1045.50±41.72 <sup>a</sup>
Peak Time	4.27±0.09 <sup>b</sup>	4.33±0.09 <sup>b</sup>	4.47±0.00 <sup>b</sup>	4.77±0.05 <sup>a</sup>
Pasting T.	76.30±0.56 <sup>a</sup>	75.87±1.09 <sup>a</sup>	77.10±1.69 <sup>a</sup>	77.90±0.49 <sup>a</sup>

Means within the same column with different superscripts are significantly different (p<0.05).

Sample A: Non fermented cassava; Sample B: Fermented for 24 hours; Sample C: Fermented for 48 hours; Sample D: Fermented for 72 hours

**Table.2** Vitamin A content of starch flour samples

Samples	Vitamin A Content (mg/g)
A	2.02±0.01 <sup>a</sup>
B	1.77±0.01 <sup>b</sup>
C	1.43±0.00 <sup>c</sup>
D	1.32±0.18 <sup>c</sup>

Means within the same column with different superscripts are significantly different (p<0.05).

Sample A: Non fermented cassava; Sample B: Fermented for 24 hours; Sample C: Fermented for 48 hours; Sample D: Fermented for 72 hours

**Table.3** Physicochemical property of flour sample

Samples	pH	TTA	TSS	Bulk density(g/cm <sup>3</sup> )	Swelling Index
A	6.80±0.01 <sup>a</sup>	0.36±0.01 <sup>a</sup>	1.31±0.01 <sup>a</sup>	0.53±0.01 <sup>a</sup>	0.09±0.01 <sup>d</sup>
B	6.57±0.01 <sup>c</sup>	0.28±0.00 <sup>c</sup>	1.17±0.06 <sup>b</sup>	0.72±0.01 <sup>d</sup>	0.90±0.01 <sup>a</sup>
C	5.67±0.01 <sup>c</sup>	0.36±0.01 <sup>a</sup>	0.77±0.06 <sup>c</sup>	0.68±0.01 <sup>c</sup>	0.50±0.01 <sup>b</sup>
D	5.12±0.01 <sup>d</sup>	0.34±0.01 <sup>b</sup>	0.60±0.01 <sup>d</sup>	0.62±0.01 <sup>b</sup>	0.30±0.01 <sup>c</sup>

Means within the same column with different superscripts are significantly different (p<0.05).

Sample A: Non fermented cassava; Sample B: Fermented for 24 hours; Sample C: Fermented for 48 hours; Sample D: Fermented for 72 hours

**Figure.1 A;** Cassava starch flour, Fig 1B: Morphological feature of Cassava Flour



Fig: 1A

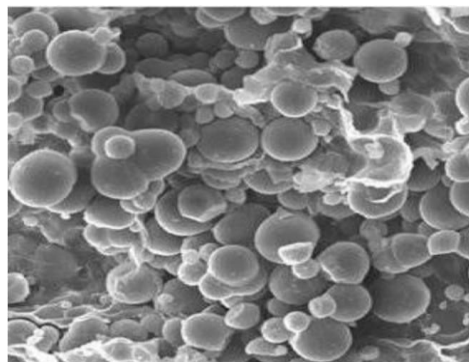


Fig: 1B

Figure.2 MSEM/EDX Micrograph of Sample 1

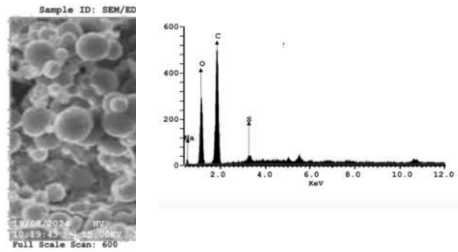


Figure.3 SEM/EDX micrograph Sample-2

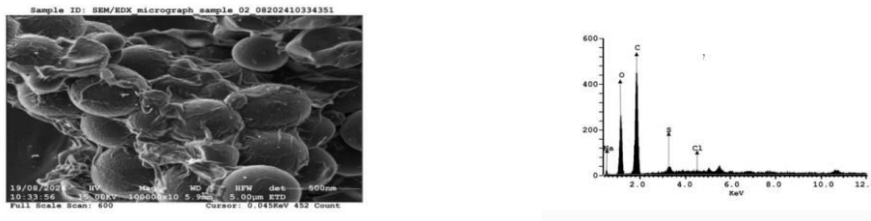
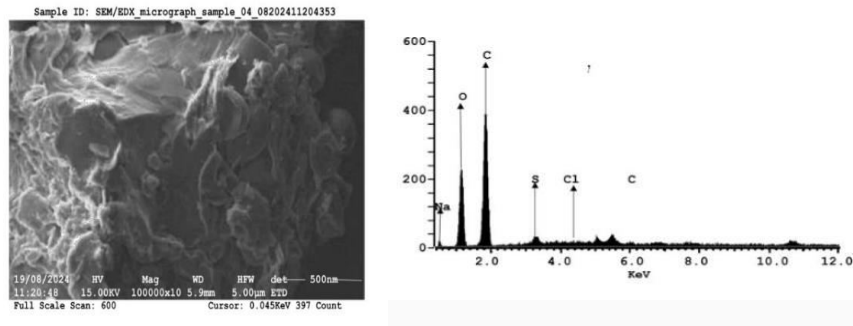


Figure.4 SEM/EDX Micrograph Sample 3



Figure.5 SME/EDX Micrograph Sample 4



The pasting temperature for the instant flours ranged from 75.87 to 77.90 °C. Sample D had the highest value of 77.90 °C, while sample B had the shortest pasting time of 75.87 °C, which is comparable to the 77 °C

which is higher than the result reported by [Aidoo et al., \(2022\)](#). The significant differences in pasting time among the samples can be attributed to the variation in the raw materials used.

Sample A exhibited the highest peak viscosity of 5251.00 RVU, while sample B had the lowest at 4.33 RVU. Peak viscosity provides an indication of the viscous load or the energy required during mixing and has been associated with product quality. A high value implies that the flour sample can form thicker pastes when cooked. As reported by [Aidoo et al., \(2022\)](#), the high peak viscosity of *Manihotesculenta* (cassava) is important for the production of dough. High peak viscosity is a key criterion in producing instant dough formation in composite flour with desirable textural properties.

### **Vitamin A Content of Cassava Flour**

The results of the vitamin content of the instant flours are presented in Table 4.2, with the vitamin A concentration ranging from 1.32 to 2.02 mg/100g. Sample A recorded the highest vitamin A concentration at 2.02 mg/100g, while sample D had the lowest concentration at 1.32 mg/100g. The examination of various quality characteristics of instant cassava flour, as presented in the Table, reveals significant insights into how fermentation methods influence its vitamin A content; increase in fermentation day indicated decrease in the value. Vitamin A is essential for various physiological functions, including maintaining healthy vision, immune system support, and ensuring proper cell function. According to [Adeola et al., \(2021\)](#), the retention of vitamin A in processed flours is highly dependent on the raw material and the processing conditions, where higher temperatures or longer processing times may reduce vitamin content. Thus, instant flours with higher vitamin A concentrations are of greater nutritional value, especially in regions where vitamin A deficiency is prevalent.

The result of physicochemical property of the cassava starch flour was presented in Table 4.3 below. Physical, chemical properties and functional properties are the parameters assessed, the peak viscosity, total titratable acidity (TTA), total soluble solids (TSS), swelling index, and pH levels emerged as critical indicators of flour quality. Each of these characteristics plays a pivotal role in determining the flour's suitability for various culinary applications, nutritional value, and overall consumer acceptability ([Adepoju et al., 2022](#); [Sanni et al., 2024](#)).

Peak viscosity is one of the most critical indicators of the starch's ability to form thick pastes during cooking. In this study, peak viscosity values ranged from 5251.00 to

6983.59 RVU, with sample A demonstrating the highest viscosity. The ability of cassava flour to achieve high peak viscosity is essential for applications as a thickener in food product, where a thick, cohesive texture is desirable. This aligns with the findings of [Awoyale et al., \(2016\)](#), who reported that higher peak viscosity in cassava flour enhances the quality of products requiring substantial thickening agents. The relationship between peak viscosity and cooking performance suggests that fermentation can modify the starch structure, potentially leading to enhanced textural attributes in the final product ([Aidoo et al., 2022](#); [Sanni et al., 2024](#)).

Similarly, the swelling index, which ranged from 0.09 to 0.90, highlights the water absorption capacity of the flour. Sample B's high swelling index indicates its capability to absorb more water, which is vital for achieving desired textures in various food products. A higher swelling index is associated with improved mouth feel and palatability, making fermented cassava flours an excellent choice for products requiring significant moisture retention ([Agua, 2015](#); [Williams et al., 2019](#)).

The analysis of TTA values, which ranged from 0.28 to 0.36, reveals the importance of acidity in enhancing the flavor and preservation of cassava flour. The higher TTA observed in sample A suggests a greater concentration of organic acids resulting from fermentation, which can impart a desirable tangy flavor while inhibiting the growth of spoilage microorganisms. [Adeoye et al., \(2025\)](#) emphasized that the fermentation process can significantly influence the acidity of flours, thus affecting their shelf life and quality attributes.

The pH levels, ranging from 5.12 to 6.80, suggest that the fermentation method not only influences the flavor and safety of the flour but also its functional properties. A balanced pH is critical for the stability and quality of food products, as extreme pH values can lead to undesirable flour for dough production, it reduces the elasticity of the dough, maintaining optimal pH levels during fermentation is essential for producing high-quality flour ([Ayandipe et al., 2022](#); [Olatidoye et al., 2020](#)).

Total soluble solids (TSS), which ranged from 0.60 to 1.31, provide insight into the overall nutritional quality of the flour. A higher TSS indicates a greater concentration of dissolved nutrients, particularly sugars, which are essential for energy. Therefore, optimizing fermentation methods to enhance TSS can significantly

improve the appeal and health benefits of cassava flour. Bulk density ranged from  $0.53 - 0.72 \pm 1 \text{ g/cm}^2$ , the swelling index ranged from 0.09 – 0.30. Both bulk density and swelling index result indicated short fermentation time is favorable for starch food application in area where high viscosity is required. Extensive fermentation tends to cause rupture of starch granules leading to decrease in viscosity as shown in figure 2-5 below.

The findings underscore the importance of fermentation methods in enhancing the quality characteristics of cassava flour. By optimizing fermentation parameters, producers can improve the nutritional profile, functional properties, and consumer acceptance of cassava flour, making it a valuable ingredient in various food applications dependent on the specific application.

### **SEM/EDX Analysis Results**

Scanning Electron Microscope/Energy Dispersive X-ray Spectroscopy (SEM-EDX) analysis as shown in figure 2-5 below displayed the physical morphology of sample surfaces, the starch granules structures and enhanced the analyses of the element compositions, including the visible light elements such as carbon, nitrogen and oxygen. From figure 2-5 the starch granules showed gradual disintegration as the fermentation time proceeded. Sample A showed a homogenous regular spherical shape, which represent the peculiar starch granular matrix of starch structure. There is evidence of gradual swelling in sample 2, with increase fermentation time. From sample 3, the spherical shape became irregular, rough due to absorption of water, at the extreme stage of sample 4, there was complete disruption of starch matrix which resulted to more irregular shape and rupture of starch granules and eventual retrogradation or less viscous mass.

In conclusion, the effect of fermentation method on the quality characteristics of cassava flour is a critical aspect in determining the functionality, nutritional value, and application of cassava-based products. Fermentation, a biochemical process involving microbial activity, plays a pivotal role in the modification of cassava's inherent properties, transforming it into flour with diverse qualities depending on the method employed.

The acidic environment created during fermentation helps reduce cyanogenic glycosides, which are harmful compounds naturally present in cassava. This detoxification process is essential for ensuring the safety

of cassava flour for human consumption, particularly in regions where cassava is a staple. The fermentation also results in the development of sour flavors, which are characteristic of fermented cassava products such as "gari" and "lafun." However, due to the reliance on naturally occurring microbes, the results can vary significantly in terms of the final product's quality, including moisture content, texture, and microbial load. However, prolong fermentation is not favorable for high viscous stable emulsions like salad dressings. There is evidence of reduction in vitamin A with increase fermentation time to maintain good eye health and general wellbeing there is need for innovative process of cassava fermentation to keep up with sustainable development goal number 3 and 12 of United Nation.

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### **Author Contributions**

Adekunle. O. Adeoye: Investigation, formal analysis, writing—original draft. Moses. A. OJO: Validation, methodology, writing—reviewing. Azeezat. O. ADEBIYI-OLABODE:—Formal analysis, writing—review and editing. Grace Mosunmola ADEGBOLA: Investigation, writing—reviewing. Richard. A. AJANI: Resources, investigation writing—reviewing.

### **Data Availability**

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

### **Declarations**

**Ethical Approval** Not applicable.

**Consent to Participate** Not applicable.

**Consent to Publish** Not applicable.

**Conflict of Interest** The authors declare no competing interests.

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