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Nixtamalization and Fermentation as Treatments for Enhancing the Functional and Nutritional Properties of Foods

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ABSTRACT

This study evaluated the impact of nixtamalization and fermentation on the functional and nutritional properties of selected flours (maize and Cassava). Maize and Cassava were subjected to nixtamalization and fermentation treatments respectively, and their functional properties, proximate composition, mineral content, and antinutrient levels were evaluated. The samples were as follows; Nixtamalized maize (NXM), Non-nixtamalized maize (NNM), Fermented Cassava (FC) and Non-fermented Cassava (NFC). Results revealed that both nixtamalization and fermentation significantly enhanced key functional properties, including water absorption capacity (1.69-2.19 g/g), oil absorption capacity (1.53-1.73 g/g), bulk density (0.57-0.62 g/mL) and swelling index (2.78-3.38 mL/g). The proximate composition showed notable enhancement in protein (3.74-16.27 %), fat (2.26-7.28 %), and fibre content (1.91-3.97 %), while mineral analysis demonstrated elevated levels of essential micronutrients such as calcium (148.49-189.44 mg/100 g), Magnesium (83.44-125.32 mg/100 g), Potassium (149.63-186.32 mg/100 g), Sodium (43.83 49.44 mg/100 g) and Phosphorus (94.79-123.48 mg/100 g). Furthermore, both treatments effectively reduced the antinutrient content, including phytates and tannins, contributing to improved nutrient bioavailability. Overall, nixtamalization and fermentation present promising methods for enhancing the nutritional and functional qualities of flours, making them suitable for use in various food formulations like complementary food, fufu, bread making and many others.

INTRODUCTION

Nutritional deficiencies affect people worldwide, with the heaviest burden falling on low-income populations. This is especially true in developing countries, where many individuals must not only reduce their food intake but also sacrifice nutritional quality due to financial constraints (Erokhin *et al.*, 2021). Inadequate intake of essential nutrients - including proteins, carbohydrates, and vitamins - leads to malnutrition, which has been proven to both stunt cognitive development and diminish work productivity in adults (Erokhin *et al.*, 2021). This is why there is a need for emphasis on pretreatments or treatments, as the case may be, that are applied to raw materials for proper optimization of the quality of foods. The food industry continuously searches for innovative methods to improve both the nutritional value and taste appeal of its products. Among these methods employed, nixtamalization and fermentation stand out as time-honoured practices deeply rooted in the culinary traditions of various cultures worldwide.

Nixtamalization is an ancient method of preparing corn by cooking and soaking it in lime water (a mixture of water and calcium hydroxide) (Matendo *et al.*, 2023). It is a pretreatment given to maize kernel in the processing of several maize-based products, including maize chips, tortillas, etc. (Hassan *et al.*, 2023). This technique was invented and exploited by the Aztec and Mayan civilizations and it is still being used to date. Authors have reported that despite the growing popularity of these maize products, little improvements have been made in the use of this maize processing method (Hassan *et al.*, 2023).

By taking into account people from Africa and Latin America, maize consumption ranges from about 15 % to 56 %, even though it is an ancient practice in Mexico and other Latin American countries (Valderrama-Bravo *et al.*, 2017). This process is not yet ingrained in the culture of the nations of Sub-Saharan Africa that produce and consume maize. Numerous essential foods, such as tortillas, tortilla chips, and snacks, are created using nixtamalization. When it comes to food preparation, grains that have undergone the nixtamalization process have several advantages over untreated grains. Nixtamalized maize is easier to mill, has more nutritional content, has better flavor and aroma, and has a lower likelihood of producing mycotoxins (Ocheme *et al.*, 2010; Santiago-Ramos *et al.*, 2018). Improved protein quality, niacin availability, lysine availability, and higher calcium content are all notable advantages of nixtamalization (Matendo *et al.*, 2023; Santiago-Ramos *et al.*, 2018). According to reports, the nixtamalization procedure has been shown to increase the calcium content in nixtamalized products (Offiah *et al.*, 2016; Rojas *et al.*, 2016; Sefa-Dedeh *et al.*, 2003).

Fermentation is the process by which microorganisms break down complex food materials into simpler forms in their search for energy and carbon. It improves the safety, flavour and digestibility of foods and also the bioavailability of nutrients and shelf life of food

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products under controlled environments (Oladeji *et al.*, 2018). This bioprocess has been known to cultivate desired quality attributes in food products. These qualities encompass their appeal, utility, and functional value post-fermentation. Appeal pertains to the external characteristics, texture, aroma, and flavor of food—critical factors that are discernible by the senses and contribute to consumer satisfaction. Utility aspects entail a range of advantages, including reducing bulk volume, minimizing cooking duration, prolonging shelf life, enhancing nutrient preservation, and capitalizing on the opportunity to transform by-products ("waste") into appetizing value-added food items (Nout, 2005). This process involves chemical changes that occur in an organic substrate through the action of enzymes produced by microorganisms (Erkmen & Bozoglu, 2016). Nixtamalization and fermentation have each played pivotal roles in shaping the culinary heritage of societies for centuries. Their significance, however, extends far beyond cultural heritage as they offer tangible benefits to modern food production, including improved nutritional value, flavor enhancement, and food safety. For context and in this study, maize was employed for the nixtamalization process while cassava was employed for the fermentation process. This piece of work was therefore aimed at exploring the role of nixtamalization and fermentation within the food industry, to enhance the functional and nutritional properties of foods

MATERIALS AND METHODS

Sample Collection

About 10 kg of white Maize (Zea mays) was gotten from the Wurukum market in Makurdi, Benue state, Nigeria. Freshly harvested sweet cultivar of cassava (Manihot esculenta Crantz) was obtained from Wannune market in Benue State Makurdi. All of these raw materials was sorted and cleaned before experiment use. Only analytical-grade reagents were used.

Sample Preparation

Nixtamalized maize flour was prepared by the method of Offiah *et al.* (2016) as in Figure 1. Non-nixtamalized maize flour was prepared as described by Akubor (2019). Maize grains were sorted and cleaned from extraneous materials. These were further milled using a hammer mill and passed through a 40 mm mesh sieve. The flours were packaged in low-density polyethylene bags and stored for further analysis

Cassava flour (nonfermented) was prepared as shown in Figure 2 (Awolu *et al.*, 2022). Matured and freshly harvested cassava tubers was sorted to remove extraneous materials like stones. It was then be peeled using a sharp kitchen knife and washed in clean water. The peeled and washed cassava were size-reduced by cutting it into small pieces. It was oven-dried at 65 oC for 48 h.

Cassava fermentation was done using the Back slopping fermentation method or accelerated natural fermentation as described by Offiah *et al.* (2016). A mixture was prepared by combining 50 g of cassava flour with 150 mL of distilled water in a 500 mL glass beaker. The beaker was covered with aluminum foil and kept at room temperature (30 ± 2°C) for 24 hours. After this period, half of the fermented mixture was used to initiate a new fermentation batch. This back-slopping was repeated every 24 hours throughout the fermentation period. During fermentation, both pH and titratable acidity were measured to track lactic acid bacteria activity. The process was terminated when pH readings stabilized despite continued fermentation cycles. The final fermented product was then dried at 50°C in a hot air fan-driven electric oven for 24 hours, ground in a warring blender, and passed through a 40 mm mesh sieve.

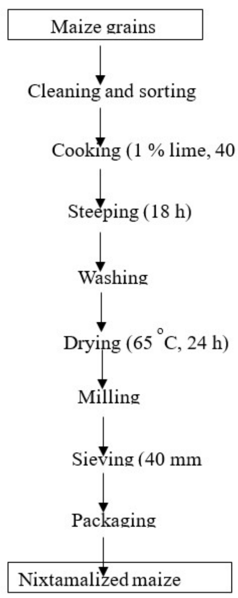

Figure 1: Flow chart for the production of nixtamalized maize flour
Source: (Offiah et al., 2016)

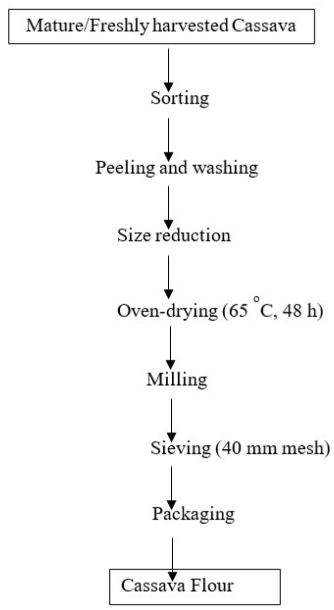

Figure 2: Flow chart for cassava flour production
Source: (Awolu et al., 2022)

Physicochemical Analyses of the Samples

Determination of the Functional Properties

Water absorption capacity (WAC): The method described by the modified method of (Massingue Júnior *et al.*, 2023) was used. The sample was prepared by dispersing 1 g of flour in 10 mL of water in a 15 mL centrifuge tube. The mixture was then agitated on a platform tube rocker for 1 minute at room temperature. After letting it settle for 30 minutes, the sample was centrifuged at 1200 x g for 30 minutes. WAC was then calculated as follows:

WAC=(Amount of water-free water)/(Weight of sample) × density of water × 100 (1)

Oil Absorption Capacity (OAC)

The sample was prepared by dispersing 1 g of flour in 10 mL of vegetable oil in a 15 mL centrifuge tube. The mixture was then agitated on a platform tube rocker for 1 minute at room temperature. After letting it settle for 30 minutes, the sample was centrifuged at 1200 x g for 30 minutes (Onwuka, 2018).

OAC = (Amount of oil-free oil)/(Weight of sample) × density of oil × 100 (2)

Bulk Density (BD)

The bulk density was determined by pouring 50 g of flour into a 100 mL measuring cylinder. The cylinder was tapped repeatedly until the flour volume remained constant. The bulk density (g/mL) was then calculated by dividing the flour weight by its final volume (mL) (Awolu *et al.*, 2022).

BD = (Weight of sample)/(Volume of sample after tapping) × 100 (3)

Swelling Index (SI)

The test was conducted by mixing 1.0 g of flour with 10 mL distilled water in a centrifuge tube. The mixture was heated at 80 °C for 30 minutes with continuous agitation. Following heating, the suspension was centrifuged at 1000 x g for 15 minutes. The supernatant was then removed and the paste weight was measured (Awolu *et al.*, 2022). The swelling power was calculated as follows:

SI(%) = (Weight of sample paste)/(Weight of dry flour) × 100 (4)

Determination of the Proximate Composition of Flours

The proximate parameters namely; moisture, ash, crude protein crude fat, and crude fibre) were determined by standard methods of the Association of Official Analytical Chemists (AOAC, 2012). Carbohydrate was calculated by difference as follows;

% Carbohydrate = 100 - [Protein(%) + Fat(%) + Ash(%) + Fibre(%) + moisture (%)] (5)

Determination of the Mineral Content of the Flours

The Magnesium, Calcium, Potassium, Sodium and Phosphorous contents of samples were determined by the atomic absorption spectrophotometer method (AOAC, 2012). A muffle furnace was used to ignite two grams of the dry samples at 600 °C. Ten milliliters of 5 M HCl were used to dissolve the ash. After the ash was acid-digested on a steam plate, the digested sample was carefully cleaned with distilled water, filtered using Whatman's filter paper, and then diluted to volume in a 50 mL volumetric flask. The Atomic Absorption Spectrophotometer (Perkin-Elmer Analyst 700 spectrophotometer (Norwalk, CT, USA) was then used to evaluate the samples and blanks for the various minerals.

Anti-Nutritional Analysis of Samples

Determination of Tannin Contents

The tannin content was determined using the Burn method (Krishnaiah *et al.*, 2009). Five (5) g of sample was treated with 50 mL methanol and kept for 24 hours before filtration. Five (5 mL) of freshly prepared vanalin hydrochloric acid was added and the solution was allowed to stand for 20 min for colour development. The absorbance was measured at 550 nm using Spectronic 20 and the machine value was used in calculating the tannin content as follows:

$C_1=(C_1C_2)/V_1$

% Tannic acid content=(C1 × 100)/(Weight of sample) (6)

Where;

C_1= Conc. of tannic acid, C_2=Conc. of base, V_1=Volume of tannic acid, V_2= Volume of base

Determination of Phytates Content

The method of Young and Greaves with slight modification was used (Disseka *et al.*, 2018). In a 250 conical flask, precisely 2 g of samples were soaked in 100 mL of 20 % concentrated HCl for 3 hours. The samples were then filtered through filter paper, 50 mL of the filtrate was put in a 250 beaker, and 100 mL of distilled water was added. 10 mL of 0.3% ammonium thiocyanate solution was then added as an indicator, and titration was performed using standard Iron (III) Chloride (0.00915 g/mL). After titrations, the phytate content was calculated as follows;

Phytates Acid = (titre value × 0.00195 × 1.19 × 100)/(sample mass (g)) (7)

Determination of Cyanide

Alkaline picrate reagent was prepared by a modification of the method described by Wasiams and Edwards (1980) as follows: Test tubes with 2mL of 2% KOH and 1 mL of picric acid: Na_2CO_3: H_2O (1:5:200 v/w/v) was prepared (Nwokoro *et al.*, 2010). Standard absorbance curves were created using three Whatman No. 1 filter papers, each measuring 8 × 1 cm. These papers were immersed in an alkaline picrate solution for 15 minutes. After removal, the picrate-treated papers were promptly used for cyanide analysis. Cyanide solutions (ranging from 50 to 200 µg KCN/mL) were prepared in glass containers, acidified with 20% HCl, heated to 80 °C, and immediately sealed with three picrate-treated papers. This setup was left to incubate at room temperature (28 ± 2 °C) for 24 hours.

The resulting red-coloured complex was then extracted with a 50 % ethanol solution for 30 minutes, and the absorbance of the eluate was recorded at 510 nm using a spectrophotometer. Cyanide concentrations in the samples were determined by referencing the standard curve.

Determination of Oxalate Content

This was determined by the standard method of AOAC (2012). One gram (1 g) of the sample was placed in a 250 mL volumetric flask, to which 190 mL of distilled water and 10 mL of 6 M HCl were added. The mixture was then heated in a water bath at 90 °C for 4 hours, followed by centrifugation of the digested sample at 2000 rpm for 5 minutes. The resulting supernatant was diluted to a final volume of 250 mL. Three 50 mL portions of the supernatant were evaporated to 25 mL, after which the brown precipitate was filtered and washed. The combined filtrate and washings were titrated by adding concentrated ammonia dropwise until the salmon-pink color of methyl orange turned faint yellow. The solution was reheated to 90 °C in a water bath, and oxalate was precipitated using 10 mL of a 5 % calcium chloride ($CaCl_2$) solution. The mixture was left to stand overnight and then centrifuged. Each precipitate was then transferred into a beaker using hot 25 % sulfuric acid (H_2SO_4), diluted to 125 mL with distilled water, and after heating to 90 °C, it was titrated with 0.05 M potassium permanganate ($KMnO_4$) until a faint pink color persisted for at least 30 seconds. The oxalate content was calculated by taking;

$$1 \text{ mL of } 0.05M\ KMnO_4 = 2.2 \text{ mg oxalate} \quad (8)$$

Statistical Analysis

Data were collected in triplicate and analyzed using SPSS (Statistical Package for the Social Sciences) Version 27. An Analysis of Variance (ANOVA) test was conducted to assess significant differences between the means, and the Duncan Multiple Range Test was applied for mean comparison and separation. Statistical significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Functional Properties of the Maize and Cassava Flours as Influenced by Nixtamalization and Fermentation

Both nixtamalization and fermentation were seen to affect the functional properties of the different flours, as shown in Table 1.

Nixtamalization significantly enhanced the water absorption capacity from 1.69 % in non-nixtamalized maize flour to 2.14 % in nixtamalized maize flour. Water absorption capacity is the amount of water or moisture that is taken up by a food or flour to achieve the desired consistency for quality food (Chandra *et al.*, 2015). Nixtamalization has been known to increase the water activity of maize flour as was observed in the study by Sefa-Dedeh *et al.* (2004). They observed that nixtamalization increases water absorption capacity in maize, influenced by lime concentration and cooking time, enhancing hydration due to gelatinization and osmotic effects. This variation may be due to lime cooking, which causes gelatinization and thereby exposes the hydrophilic sites in starch. This process reveals a strong inverse relationship between gelatinization and water absorption capacity, meaning that as gelatinization increases in the flour, its water absorption capacity decreases. Several authors have reported that the improved water absorption capacity of the flours is desirable and crucial as water absorption leads to improved texture and handling properties (Ramírez-Miranda *et al.*, 2014; Rodríguez-Martínez *et al.*, 2015). The oil absorption capacity was enhanced by nixtamalization; ranging from 1.61 in NN to 1.53 in NXM. Oil absorption capacity is a measure of the rate at which protein binds to fat in food formulations. It has been attributed to the physical entrapment of oil in a food sample. This has been known to act as a flavor retainer and increases the mouthfeel of foods (Hasmadi *et al.*, 2020). Results in this study agree and follow the same trend as those of (Ocheme *et al.*, 2010; Sefa-Dedeh *et al.*, 2004) who reported a decrease in the oil absorption capacity of the flours with nixtamalization. The bulk density of the flours was observed to be significantly different between the nixtamalized and non-nixtamalized samples. Nixtamalization leads to gelatinization and therefore increase in water absorption or moisture uptake and consequently the bulk of the food sample (Rodríguez-Martínez *et al.*, 2015). Bulk density refers to the mass of numerous particles of flour material divided by the overall volume they occupy. This total volume includes the volume of the particles themselves, the internal pore volume, and the spaces between particles (inter-particle voids) (Awuchi *et al.*, 2019). It is used to determine the packaging requirements of flour as it measures the heaviness of the flour (Hasmadi *et al.*, 2020). The nixtamalized sample had a significantly higher swelling capacity compared to the non-nixtamalized sample. The swelling capacity of a food product is a function and ability of the product to rise while interacting with water. Several factors have been reported to influence the swelling capacity of flour including crop species, size of flour particles, and processing method used (Awolu *et al.*, 2022). Nixtamalization with $Ca(OH)_2$ was seen to enhance the swelling capacity of flour by promoting protein unfolding and increasing the interaction with water. This could be due to the ionizing hydroxyl groups of starch and proteins, which favour the formation of weak non-covalent bonds and electrostatic forces, resulting in strong entanglement and interaction with water (Rincón-Aguirre *et al.*, 2021). This may be attributed to the flour's reduced fat content; research indicates that fats can form complexes with starch, which can restrict swelling. Additionally, the flour's high water absorption capacity also contributes to this effect (Ocheme *et al.*, 2010).

Fermentation on the other hand; as seen in Table 1 significantly affected the functional properties of the flour. It was observed that the water absorption capacity was significantly lower in the fermented sample compared to the non-fermented sample. This is a deviation from the norm as observed by Feyera (2021) who reported that fermentation increases water absorption capacity in complementary food, with values ranging from 163.81 to 180.50 for samples fermented for 24 and 36 hours, respectively, compared to unfermented samples. This could be due to over-degradation of starches and proteins, leading to the loss of molecular structures that are responsible for trapping and holding water. Also, intense fermentation could break down polysaccharides and proteins exposing more hydrophobic ends or reducing the overall hydrophilic sites, thereby leading to a decrease in the water absorption capacity of the flour. Fermentation in sorghum, pearl millet, and maize decreased water absorption capacity, as reported by Alka *et al.* (2012). The oil absorption capacity of the fermented sample was significantly ($p<0.05$) higher than the non-fermented sample. This agrees with this study wherein, African yam bean flour also was used in bread production. They also observed an increase in oil absorption capacity as affected by fermentation (Chinma *et al.*, 2020). The bulk density of the fermented sample (FC, 0.57 %) was significantly lower than the non-fermented sample (NFC, 0.62 %). These results agree with those by Feyera, (2021) who observed that fermentation reduced the bulk density of composite flour from 0.90 to 0.59 g/ml, indicating a substantial decrease in heaviness, which is crucial for improving the acceptability of foods. This reduction could be attributed to the fact that during fermentation, the lactic acid bacteria break down complex polysaccharides and proteins into simpler and less heavy molecules, hence the reduction in the bulk density (Oladeji *et al.*, 2018). The swelling capacity was seen to be significantly lower in the fermented sample (FC, 2.87) compared to the non-fermented sample (NFC, 3.06). While it has been reported that the protein and fat content of flour can inhibit its swelling capacity (Feyera, 2021), it was observed in this study that fermentation led to a decrease in the swelling capacity of the flour. The observed reduction in swelling power due to fermentation in this study aligns with previous findings, which reported a decrease in the swelling capacity of pigeon pea flour after being subjected to various fermentation durations (Feyera, 2021).

Table 1: Functional Properties of Maize and Cassava Flours as influenced by nixtamalization and fermentation

Parameter	Sample			
	NXM	NNM	FC	NFC
WAC (g/g)	$2.14^{b}\pm0.00$	$1.69^{d}\pm0.01$	$1.72^{c}\pm0.00$	$2.19^{a}\pm0.01$
OAC (g/g)	$1.53^{c}\pm0.01$	$1.61^{b}\pm0.01$	$1.73^{a}\pm0.01$	$1.61^{b}\pm0.02$
BD (g/ml)	$0.61^{b}\pm0.00$	$0.59^{c}\pm0.00$	$0.57^{d}\pm0.00$	$0.62^{a}\pm0.00$
SI (mL/g)	$3.38^{a}\pm0.02$	$2.78^{d}\pm0.02$	$2.87^{c}\pm0.03$	$3.06^{b}\pm0.06$

Values are means±standard deviation of triplicate determinations. Means across a row with different superscripts are significantly different at p<0.05

Key:
NXM-Nixtamalized maize,
NNM-Non-Nixtamalized maize,
FC-Fermented cassava,
NFC-Non-Fermented cassava
WAC-Water Absorption Capacity,
OAC-Oil Absorption Capacity,
BD-Bulk Density,
SI-Swelling index

Proximate Composition of Maize and Cassava Flours as Influenced by Nixtamalization and Fermentation

Table 2 presents the proximate composition of the flours as influenced by nixtamalization and fermentation. The moisture content ranged from 10.18 in NXM to 11.51 in NNM while it ranged from 11.75 in NFC to 10.48 in FC. Nixtamalization was seen to significantly reduce ($p<0.05$) the moisture content of the nixtamalized maize flour. This is consistent with previous studies that observed a decrease in the moisture content of nixtamalized products (Matendo *et al.*, 2023). Results in this study however contrast with findings by Sunico *et al.* (2021) who observed an increase in the moisture content of nixtamalized flour. This difference could be owed to difference in lime concentration as well as cooking time. Nixtamalization is known to affect the water absorption capacity and gelatinization (partial) of maize starch. This modifies the starch structure, reducing its ability to retain water and in turn lowers the moisture content. It has also been reported that the calcium salts interfere with the sorptive or hydrophilic sites of starch thereby making them unavailable for moisture absorption (Amador-Rodríguez *et al.*, 2019, 2020). Some researchers have further explained that the use of $Ca(OH)_2$ breaks down the seed coat structure, allowing calcium ions to diffuse into the seed. This process enhances the interaction and absorption of calcium with starch, pectins, and proteins (Santiago-Ramos *et al.*, 2018). The ash content varied from 3.65% in NXM to 3.13% in NNM. Nixtamalization significantly increased the ash content of the nixtamalized maize flour. This was expected as it has been reported that the alkaline treatment releases the bound minerals from antinutrients found mainly in the pericarp which is easily removed by the alkaline nature of the medium (Sunico *et al.*, 2021). The crude protein ranged significantly ($p<0.05$) from 14.02 % (NNM) to 16.27 % (NXM) demonstrating that nixtamalization greatly enhances and increases the protein content of nixtamalized maize and its products. Similar reports have been advanced to enhance the protein content of nixtamalized maize products. For example, Sunico *et al.* (2021) evaluated the effect of nixtamalized maize flour as a substitute in Philippine salt bread. The protein content ranged from 9.78 % in non-nixtamalized maize flour to 10.60 % in nixtamalized bread. Results obtained in this study are higher than those reported by Hassan *et al.* (2023) who observed the protein content to range from 9.71 to 12.88 %, while optimizing the effect of nixtamalization on the nutritional and antinutritional contents of quality protein maize flour. Reporting a protein content ranging from 7.48 to 11.6 %, Hassan *et al.* (2023) demonstrated the effect of nixtamalization in increasing the protein content of nixtamalized products. While assessing the impact of nixtamalization on the proximate, functional, and some anti-nutritional properties of millet flour, ocheme *et al.* (2010) also found that millet flour cooked in lime had a higher protein content than millet flour soaked in water. Santiago-Ramos *et al.* (2018) and Ramírez-Jiménez *et al.* (2019) also observed similar results.

The crude fibre content of the nixtamalized maize flour was 3.97 % higher than observed in the non-nixtamalized counterpart (3.30 %). Other reports have observed similar effects on nixtamalization for example Ocheme *et al.* (2010) observed a crude fibre content ranging from 4.05 to 6.23 % for millet flour. The findings of this research exceeded those found in a comparable investigation by Hassan *et al.* (2023). In their research on how nixtamalization and heat processing affected nutrients in maize-soybean flour, Matendo *et al.* (2023) found lower fiber content between 0.23-1.86%, with nixtamalized maize showing the highest levels. This variation may be attributed to differences in processing techniques. Research has shown that nixtamalization enhances dietary fiber by increasing resistant starch levels in food items (Ekpa *et al.*, 2019; Voss *et al.*, 2017). When studying how nixtamalization affected dietary fiber, starch digestibility and antioxidant properties in blue maize tortilla, Bello-Pérez *et al.* (2015) documented fiber content between 10.13-14.11%. Gutiérrez-Cortez *et al.* (2010) noted that during traditional nixtamalization, the combined effect of heat and alkaline conditions strongly impacts the outer pericarp layers, leading to partial removal of hemicelluloses and some lignin from the fiber matrix of the pericarp. Reports have it that fibre slows down glucsose release into the bloodstream and subsequent absorption (Olosunde *et al.*, 2023), thereby very important in controlling blood glucose levels. The fat content of the maize flours ranged from 6.53 % (NXM) to 7.28 % (NNM). Nixtamalization was seen to significantly reduce the fat content of the maize flour produced. The process alters the fatty acid profile, particularly reducing linoleic acid content due to saponification and the formation of amylose-lipid complexes (Bello-Pérez *et al.*, 2015). This is consistent with numerous literatures that abound. Hassan *et al.* (2023) observed similar but lower results in the range of 3.36 to 6.00 %. (Campechano Carrera *et al.*, 2012) reported 4.4–5.3 % of fat content. The carbohydrate content ranged from 59.60 % in NXM to 60.76 % in NNM. In tortilla which is one of the most popular food product produced by nixtamalization, carbohydrates are the main component. This has been corroborated in other published studies (Bello-Pérez *et al.*, 2015; Matendo *et al.*, 2023).

Researchers also examined how fermentation influenced the basic nutritional composition of cassava flour. The flour's moisture levels varied between 10.48 and 11.75%, where the fermented flour showed the lowest moisture content at 10.48 %. It has been reported that the fermentation process modifies starch granules, thereby making them more porous and susceptible to degradation (Prastiwi *et al.*, 2024), which can lead to moisture loss hence a decrease in moisture content in fermented products as observed in this study The fermentation process modifies starch granules, making them more porous and susceptible to degradation, which can lead to moisture loss. These findings contradict those of Chinma and colleagues (2020), who noted increased moisture levels in fermented African yam bean. The current study's results are also lower than the 10.61-12.69% range reported by Fayemi and Ojokoh (2014) in their investigation of how different fermentation methods affect fufu's nutritional properties. The flour samples' ash content varied from 2.69% in non-fermented cassava (NFC) to 2.83% in fermented cassava (FC). Fermentation was found to significantly boost ash content, suggesting enhanced mineral levels - an observation that aligns with Chinma *et al.* (2020) and Ariahu *et al.* (1999) findings. Protein levels increased significantly through fermentation, ranging from 3.74% (NFC) to 5.19% (FC). While these results exceed Fayemi and Ojokoh's (2014) findings of 1.87-2.32%, they align with Chinma *et al.*'s (2020) observations. This protein increase likely results from reduced carbon ratio in the total mass, as fermenting microorganisms use carbohydrates for energy, producing carbon dioxide and concentrating nitrogen content (Cui *et al.*, 2012). Crude fiber decreased notably from 2.96% (NFC) to 2.26% (FC), possibly due to enzymatic breakdown during fermentation - a trend also noted by Fayemi and Ojokoh (2014). Carbohydrate content showed a slight reduction from 76.95% (NFC) to 76.39% (FC). This decrease likely occurred because microorganisms used carbohydrate compounds for energy, with increased α-amylase activity breaking down polysaccharides into glucose (Olukomaiya *et al.*, 2020).

Table 2: Proximate composition of Maize and Cassava Flours as influenced by nixtamalization and fermentation

Parameter	**Sample**			
	NXM	**NNM**	**NFC**	**FC**
Moisture content	$10.18^{d}\pm0.08$	$11.51^{b}\pm0.03$	$11.75^{a}\pm0.02$	$10.48^{b}\pm0.02$
Ash	$3.65^{a}\pm0.05$	$3.13^{b}\pm0.09$	$2.69^{d}\pm0.10$	$2.83^{c}\pm0.02$
Crude protein	$16.27^{a}\pm0.02$	$14.02^{b}\pm0.05$	$3.74^{d}\pm0.03$	$5.19^{c}\pm0.05$

Crude fibre	3.97[a]±0.04	3.30[b]±0.07	1.91[d]±0.06	2.15[c]±0.05
Crude fat	6.53[b]±0.06	7.28[a]±0.06	2.26[d]±0.06	2.96[c]±0.05
Carbohydrate	59.40[d]±0.09	60.76[c]±0.03	77.60[a]±0.07	75.00[b]±0.07

Values are means±standard deviation of triplicate determinations. Means across a row with different superscripts are significantly different at p<0.05

Key:

NXM-Nixtamalized maize,

NNM-Non-Nixtamalized maize,

FC-Fermented cassava,

NFC-Non-Fermented cassava

Mineral Composition of Maize and Cassava Flours as Influenced by Nixtamalization and Fermentation

The effect of nixtamalization on the mineral content of maize flour is presented in Table 3. The calcium content was significantly enhanced by nixtamalization with NXM having the highest content (189.44 mg/100 g) and NNM having the least (156.32 mg/100 g). Similar findings were reported by Matendo *et al.* (2023) in their study examining how nixtamalization of maize and heat processing of soybean affected nutrients, antinutrients, and mycotoxins in maize-soybean composite flour. The increased calcium levels could be linked to higher alkalinity, as elevated pH helps remove seed pericarp, enabling better calcium interaction with maize endosperm (Rojas-Molina *et al.*, 2007; Sunico *et al.*, 2021). The maize flours' magnesium content varied between 109.50 mg/100 g in non-nixtamalized maize (NNM) and 125.32 mg/100 g in nixtamalized maize (NXM). These values were lower than Santiago-Ramos *et al.*'s (2018) findings of 134.70 to 192.30 mg/100 g. Potassium levels ranged from 154.79 mg/100 g (NNM) to 174.79 mg/100 g (NXM). Similar to other minerals, nixtamalization significantly enhanced potassium content due to calcium's role in improving mineral bioavailability (Santiago-Ramos *et al.*, 2018; Sunico *et al.*, 2021). Sodium content was found to be between 45.63 and 48.78 mg/100 g. This is consistent with findings from (Santiago-Ramos *et al.*, 2018) who observed a range of 13.26 to 24 mg/100 g. The phosphorus content of the flours ranged from 119.84 to 123.48 mg/100 g. This is lower than those reported by (Santiago-Ramos *et al.*, 2018) who observed 301.04 to 370.73 mg/100 g of phosphorus.

The effect of fermentation on the mineral composition of cassava flour is presented in Table 3. Fermentation has been generally known to increase the bioavailability and extractability of minerals in flour by reducing phytate content and other antinutritional factors (Chinma *et al.*, 2020). The calcium content ranged from 148.49 mg/100 g (NFC) to 152.33 mg/100 g (FC). The observance was that fermentation led to an increase in the contents of calcium. The findings from this study exceeded those reported by Fayemi and Ojokoh (2014), while showing agreement with but remaining below values reported by Mudau *et al.* (2022). Calcium, which is crucial for various cellular processes, plays vital structural roles including bone and teeth formation and nerve impulse conduction. Magnesium content varied from 83.44 mg/100 g in non-fermented cassava (NFC) to 98.65 mg/100 g in fermented cassava (FC), with fermentation increasing levels. This aligns with previous research indicating fermentation enhances mineral and macromolecule bioavailability (Chinma *et al.*, 2020; Mudau *et al.*, 2022). Magnesium serves essential bodily functions, including muscle and nerve regulation, blood sugar and pressure control, and protein, bone, and DNA synthesis. Fermentation significantly decreased potassium content, with NFC showing the highest value (186.32 mg/100 g) and FC the lowest (149.63 mg/100 g). These results were lower than Mudau *et al.*'s (2022) findings and contradicted Chinma *et al.*'s (2020) observations of increased potassium in fermented African yam bean. However, they aligned with Oladeji *et al.*'s (2018) findings of reduced potassium in fermented maize. Potassium is essential for fluid balance and organ function, particularly in the brain, nerves, heart, and muscles. Sodium content decreased significantly with fermentation, from 49.44 mg/100 g (NFC) to 43.83 mg/100 g (FC). These values differed from Fayemi and Ojokoh's (2014) higher readings of 0.097-0.555 mg/100 g and contradicted Mudau *et al.*'s (2022) observation of increased sodium post-fermentation, possibly due to different fermentation methods. Sodium maintains blood osmotic pressure and assists in nerve impulse transmission. Phosphorus levels increased significantly with fermentation, reaching 118.85 mg/100 g in fermented samples. These values exceeded Fayemi and Ojokoh's (2014) findings of 0.057-0.152 mg/100 g, similar to observations in fermented African yam bean flour bread.

Table 3: Mineral Composition of Maize and Cassava Flours as influenced by Nixtamalization and Fermentation

Parameter	Sample			
	NXM	**NNM**	**FC**	**NFC**
Calcium	189.44[a]±0.02	156.32[b]±0.02	152.33[c]±0.03	148.49[d]±0.01
Magnesium	125.32[a]±0.02	109.50[b]±0.02	98.65[c]±0.02	83.44[d]±0.02
Potassium	174.79[b]±0.01	154.79[c]±0.01	149.63[d]±0.03	186.32[a]±0.02
Sodium	48.78[b]±0.02	45.63[c]±0.03	43.83[d]±0.03	49.44[a]±0.02
Phosphorus	123.48[a]±0.02	119.84[b]±0.02	118.85[c]±0.05	94.79[d]±0.01

Values are means±standard deviation of triplicate determinations. Values in the same row marked with different superscript letters indicate statistically significant differences at a 95% confidence level (p<0.05).

Key:
NXM-Nixtamalized maize,
NNM-Non-Nixtamalized maize,
FC-Fermented cassava,
NFC-Non-Fermented cassava

Antinutrient Composition of Maize and Cassava Flours as Influenced by Nixtamalization and Fermentation

Table 4.4 presents the antinutrient levels in the produced flours. The analysis revealed that both processing methods - nixtamalization and fermentation - led to a statistically significant decrease (p<0.05) in all measured antinutritional compounds.Nixtamalization significantly reduces antinutrient levels, including phytic acid, in maize and composite flours, thereby improving their nutritional properties (Matendo *et al.*, 2023; Santiago-Ramos *et al.*, 2018; Sunico *et al.*, 2021). Fermentation on the other hand is known to significantly reduce phytic acid content in a number of food products like in lupin flour, maize flour, sorghum flour and Phaseolus vulgaris (Chinma *et al.*, 2020; Ocheme *et al.*, 2010). Fermentation has also been seen to reduce the contents of tannins, oxalates and other antinutritional factors in various flours like maize, sorghum and African breadfruit (Ojha *et al.*, 2018; Ojokoh *et al.*, 2013).

Table 4: Antinutrient Composition of Maize and Cassava Flours as Influenced by Nixtamalization and Fermentation

Antinutrient (mg/100 g)	Sample			
	NXM	NNM	FC	NFC
Tannin	$0.74^b±0.00$	$0.82^a±0.02$	$0.54^c±0.02$	$0.73^b±0.01$
Phytate	$1.18^c±0.00$	$2.16^a±0.00$	$1.08^d±0.00$	$1.73^b±0.03$
Oxalate	$0.78^c±0.00$	$0.94^a±0.00$	$0.61^d±0.01$	$0.83^b±0.03$
Hydrogen cyanide	$1.20^c±0.00$	$1.25^a±0.00$	$1.19^d±0.01$	$1.23^b±0.00$

Values are means±standard deviation of triplicate determinations. Means across a row with different superscripts are significantly different at p<0.05

Key:
NXM-Nixtamalized maize,
NNM-Non-Nixtamalized maize,
FM-Fermented cassava,
NFC-Non-Fermented cassava

CONCLUSION

Flours were successfully produced by the employment of the two treatment methods; nixtamalization and fermentation. The functional properties, which are essential for food product development, were significantly improved by both processing treatments. The basic nutritional composition of the flours showed notable enhancement, particularly with an observed increase in protein content. The mineral contents analysed, especially calcium, magnesium, potassium, sodium and phosphorus were greatly enhanced by both nixtamalization and fermentation. The enhancement in the mineral content was a compliment to the decrease in the antinutrient content of the flours as affected by nixtamalization and fermentation. The integration of nixtamalization and fermentation techniques represents a fascinating intersection of tradition and innovation within the food industry. Through these age-old processes, food manufacturers and artisans alike can enhance not only the nutritional value and flavour profile of their products but also their safety and shelf life. Nixtamalization's ability to improve the digestibility and bioavailability of essential nutrients in maize, coupled with fermentation's capacity to unlock complex flavours and preserve foods, underscores the profound impact these methods have on both culinary practices and public health.

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