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Food Chemistry

Influence of fermentation period on the chemical and functional properties, antinutritional factors, and in vitro digestibility of white lima beans flour

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Abstract: This study evaluated the variation in chemical and functional properties, antinutritional factors, and in vitro digestibility during the natural fermentation of white lima bean (*Phaseolus lunatus*) at different fermentation periods of 0, 24, 48, 72, and 96 h using standard methods. The results showed that an increase in the fermentation period resulted in a significant ($p < 0.05$) increase in protein and ash content, while fiber and fat content decreased with the length of fermentation. Also, there was an optimum increase by 92%, 56.39%, and 58.16% in β-carotene, vitamin B2, and vitamin B3 at 24 h fermentation. Results showed that the fermentation period increased the mineral composition except for sodium which had a slight reduction though no significant ($p < 0.05$) difference was observed in the fermented samples. The antinutritional factors decreased linearly as fermentation progresses from 19.05–13.26 mg/100 g, 35.29–19.05 mg/100 g, 18.00–7.15 mg/100 g, and 3.09–1.35 mg/100 g for phytate, tannins, alkaloids, and oxalate, respectively. Fermentation significantly decreased the bulk densities, and swelling index, while water and oil absorption capacity, foaming properties, and emulsion capacities increased as fermentation progresses. Furthermore, protein digestibility improved from 50.33% to 58.50% and the glycemic index (GI) increased significantly ($p < 0.05$) with GI values of 57.18, 62.36, 62.67, and 62.82 for 24, 48, 72, and 96 h, respectively. This implies that these are all intermediate GI foods. This study showed that fermentation periods influence the quality of lima beans and this can be used to improve nutrition especially in the rural communities and find applications in food product development.

KEYWORDS
antinutrients, chemical properties, digestibility, fermentation period, lima beans

Practical Application: Lima beans are underutilized crops in comparison with other legumes. This is attributed to problems associated with digestion on consumption and its long hours in cooking described as "hard to cook" phenomenon which is reported to be attributed to the presence of significant amount of

antinutrients such as tannins and phytates. The nutritional value of lima beans will be increased, along with their acceptance and consumption as food, by the reduction or inactivation of these antinutritional factors.

1 | INTRODUCTION

Lima beans *(Phaseolus lunatus)* are a tropical and subtropical minor legume cultivated for their edible seeds. Lima beans are a member of the *Fabaceae* (Ezeagu & Ibegbu, 2010). Butter bean is another name for lima bean; it is also called the following: Madagascar beans, Chad beans, and by the Yoruba tribe of Nigeria as "pakala" (Adebo, 2023). Its seeds are mostly brown, a few varieties are black, creamy white, red, green which could be kidney shaped, spherical, or curved making up the lima bean pod and a notable starchy flavor (Adebo, 2023; Farinde et al., 2017). Lima beans are an affordable alternative to the high-priced soybeans and groundnut that make up the majority of traditional protein sources (Adebayo & Okoli, 2017).

The classification of lima beans as an underutilized crop in comparison with other legumes is attributed to problems associated with digestion on consumption and its long hours in cooking described as "hard to cook" phenomenon. This has been attributed to the presence of significant amount of antinutrients such as tannins and phytates (Adebo, 2023; Adegbehingbe & Daramola, 2019). The nutritional value of lima beans will be increased, along with their acceptance and consumption as food, by the reduction or inactivation of these antinutritional elements.

Fermentation is one of the traditional techniques applied in food processing and fermented food products are desired all around the globe. Fermentation produces a variety of foods that are sometimes categorized as "functional foods" containing bioactive ingredients attributed to their positive health impacts (Adebo et al., 2022). The fermentation process is of less energy demand and has been used to improve some legumes' nutritional composition (Adebo et al., 2022).

Legumes generally have nutritional compounds that are nonbioavailable and several antinutrients that may decrease the digestibility of other nutrients or cause physiological discomfort (Verni et al., 2022). Fermentation has been reported to reduce antinutritional factors (ANFs) generally in legumes, and this was substantiated by some researchers (Adegbehingbe & Daramola, 2019; Nzi' et al., 2021) on black lima beans and some other varieties. The in vitro estimation of carbohydrates digestibility is an important factor in nutrition as it predicts the glycemic of complex food ingredients which can be used in assessing its utilization by some vulnerable groups.

According to Adebo (2023), the exploitation and utilization of crops mostly depend on existing knowledge. Nevertheless, extended further research is required to investigate the effect of fermentation duration specifically on white lima beans concerning the chemical properties, digestibility, functional, and physical property.

Therefore, findings from this study can stimulate its use for varying food applications and thus increasing its demand and more income for the locals, establishing the most suitable optimal fermentation period for advantageous nutritional constituents with minimal or removal of undesirable components and overall improvement in consumption of fermented lima beans as part of a healthy diet with particular nutritional and health benefits.

2 | MATERIALS AND METHODS

Five kilograms of white lima bean seeds *(P. lunatus)* were purchased from the local Keffi market, Nassarawa State, Nigeria. Chemicals and reagents used for the analyses were obtained from Department of Food Science and Technology, Federal University of Technology, Minna, and were of analytical grade.Supporting Information

2.1 | Material handling and sample preparation

Fermented lima bean flour was prepared according to Anteneh and Omprakash (2017) with slight modifications (Figure 1). Raw lima bean seeds (Plate 1) were wet cleaned to ensure nonadherence of particles and other foreign materials and then solar dried for 72 h. The cleaned and dried samples (Plate 2) were grounded into flour using a fabricated attrition mill to obtain lima beans flour (LBF0). The lima bean flour was divided into five portions out of which four were used for the fermentation process. Four portions of 120 g each of lima bean flour were weighed and 80 mL of distilled water was added. Each portion was allowed to naturally ferment for 24, 48, 72, and 96 h, respectively. To produce fermented lima beans flour FLB24 (24 h fermentation), FLB48 (48 h fermentation), FLB72 (72 h fermentation), and FLB96 (96 h fermentation), each soaked sample was oven dried for 72 h, cooled and grounded into flour using a blender and allowed to pass through 250 μm sieve to produce fermented lima beans flour samples.

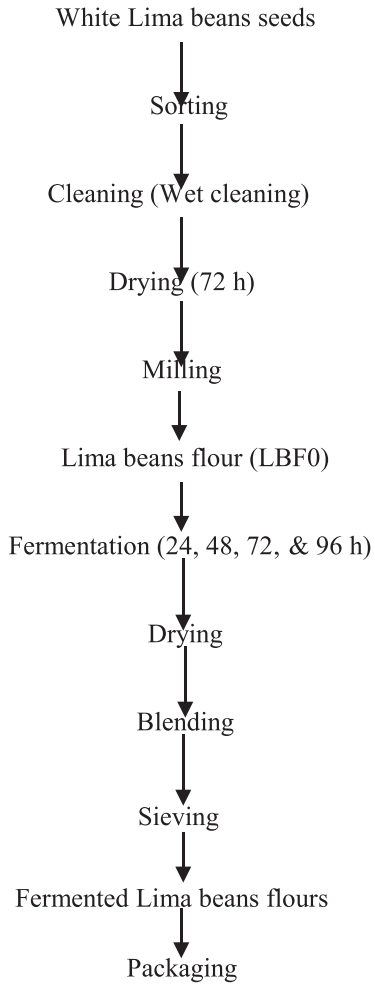

FIGURE 1 Production of fermented lima beans flour. *Source*: Anteneh and Omprakash (2017)

PLATE 1 Raw white lima beans.

The unfermented samples (LBF0) served as the control. These flour samples were then packed separately into zip-lock bag for subsequent analysis. In all, 25 samples were produced. Five samples from each portion, and all determinations were done in triplicates.

PLATE 2 Cleaned and dried white lima beans.

2.2 | Chemical analyses of lima beans flour samples

White lima beans flour samples were analyzed to determine moisture content using AOAC (2000) method No. 925.10, ash (method 923.03), protein by Kjeldahl method of 920.87, fat content (Soxhlet extraction method 945.16), and total dietary fiber method 985.29. The carbohydrate content of the various white lima beans flours samples was calculated by difference using the formula;

$$\text{Carbohydrate (g/100 g)} = 100 - (\text{Protein} + \text{Fat} + \text{Ash} + \text{Moisture} + \text{Fiber}) \quad (1)$$

2.2.1 | Determination of β-carotene, vitamin B2, and vitamin B3

β-Carotene was determined according to the method described by Kumar et al. (2011). Essentially, 100 mg of each sample was mixed with 10 mL of acetone–hexane (67-64-1; 99%; Supelco) and allowed to stand for a minute and then filtered. The absorbance was read at three different wavelengths (453, 505, and 663 nm). The β-carotene contents were calculated using the formula: β-carotene (mg/100 mg) = $0.216 \times A_{663} - 0.304 \times A_{505} + 0.452 \times A_{453}$.

Vitamin B2 was determined using the procedure described by Okwu (2005). Samples (5 g) were extracted using 50% ethanol (100 mL; 64-17-5; 99% purity; Merck) and shaken for 1 h. The mixture was filtered into 100 mL of 30% hydrogen peroxide (3317-61-1; 98% purity; Supelco) and allowed to stand over a hot water bath for 30 min. Thereafter, 2 mL of 40% sodium sulfate (7757-82-6; 99% purity; Sigma-Aldrich) was added to make up to the 50 mL mark and the absorbance was read at 470 nm in a

spectrophotometer.

$$\text{Vitamin B2} = \left(\frac{\text{Abs of sample}}{\text{Abs of Standard}}\right) \times \text{concentration of standard} \times \left(\frac{\text{Volume of solvent}}{\text{weight of sample}}\right) \quad (2)$$

For vitamin B3 determination, the various white lima beans flour samples (0.1 g) were weighed and 9.87 mL of sulfuric acid (7664-93-9; 97% purity; Supelco) was added and mixed thoroughly. The mixture was allowed to stand for 20 min after which two drops of ammonia were added. This was followed by centrifugation for 5 min at 2000 rpm. Two hundred and fifty microliter of 0.1 M potassium cyanide (151-50-8; 98% purity; Supelco), 500 μL of the supernatant (liquid lying above the solid residue after centrifugation) and 100 μL of 0.02 M sulfuric acid (7664-93-9; 97% purity; Supelco) were added to the residue. The blank used as standard was prepared with niacin at different concentrations to simulate the acidic nature of the reagents. The absorbance was measured at 470 nm and the concentration of the vitamin B3 in the samples were calculated from the prepared standard curves.

2.2.2 | Mineral content determination

The atomic absorption spectrophotometer (Reagent Kit – TECO Diagnostic) method described by AOAC (2005) was employed in the determination of macrominerals: potassium (K), sodium (Na), calcium (Ca), magnesium (Mg), iron (Fe), and zinc (Zn). Nitric acid and hyperchloric acid were used in the digestion of the mixtures.

2.2.3 | Antinutrient determination

The spectrophotometric procedure described by Bainbridge et al. (1996) was adopted in the estimation of the tannin content. This includes the introduction of 1 mL of methanolic extract into the test tube and 5 mL vanillin reagent was added and placed in the dark for 20 min. The absorbance was read with a spectrophotometer at 500 nm against a blank. Distilled water (5 mL) was used to replace vanillin in each test for blank preparation. The tannin content in the various white lima beans samples was determined using a standard range established from a tannic acid solution (2 mg/mL; 1401-55-4; Merck) under the same conditions as the test. Phytate was determined using the method described by Latta and Eskin (1980). One gram (1 g) of each of the flour samples was homogenized in 20 mL of 2% HCl (7647-01-0; 37% purity; Sigma-Aldrich) and the mixture was stirred for 12 h at room temperature followed by centrifuging at 3000 rpm for 40 min. The supernatant was collected and 0.5 mL of the supernatant was added to 3 mL of Wade's reagent (64-17-5; Sigma-Aldrich).

Blanks were also prepared for each sample using 0.5 mL of distilled water in the test tubes to replace Wade's reagent. The tubes were left to stand for 20 min in the dark and the optical density was read with a spectrophotometer at 490 nm against a blank. The amount of phytate was determined and expressed as mg/100 g using a standard range established from a sodium phytate (10 mg/mL; 14306-25-3; 95% purity; Sigma-Aldrich) under the same conditions as the test. The alkaloid content was gravimetrically determined as reported by Zubair et al. (2023). Essentially, 5 g of the various white lima bean samples were weighed and dispersed in 50 mL of 10% acetic acid (64-19-7; 99% purity; Sigma-Aldrich) in ethanol. The mixture was shaken for thorough mixing and allowed to stand for 4 h before filtration. Thereafter, evaporation of the filtrate to one-quarter of its initial volume was carried out using a hot plate and concentrated ammonium hydroxide was added in drops in order to precipitate the alkaloids. A filter paper of known weight was used to filter the precipitate off and washed with ammonium hydroxide solution (1%; 1336-21-6; 99% purity; Sigma-Aldrich). The filter paper containing the precipitate was dried in an oven at 60°C for 30 min, transferred into a desiccator to cool and reweighed until a constant weight was obtained. The differences in weight were expressed in percentages of the weight of the samples analyzed in order to calculate the content of alkaloid.

The saponin content was determined using the procedure outlined by Obadoni and Ochuko (2001). Briefly, 100 mL of 20% aqueous ethanol (64-17-5; 99% purity; Merck) was added to 5 g of the flour sample in a 250 cm^3 conical flask. The mixture was heated over a hot water bath at 55°C for 4 h with continuous stirring. Filtration of the mixture was done after cooling and re-extraction of the residue with 100 mL of 20% ethanol followed thereafter. Evaporation of the combined extract was done at 40 cm^3 over a water bath at 90°C and diethyl ether (60-29-7; 99.7% purity; Sigma-Aldrich) was added to the concentrate in a separator funnel of 250 cm^3 and then agitated vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated and 60 mL of n-butanol was added and washed twice with 10 mL of 5% aqueous NaCl (7647-14-5; 99% purity; Thermo Fisher Scientific). The sodium chloride layer was then discarded and the remaining solution was heated in a water bath, followed by oven drying at 100°C into a constant

weight. The saponin content was calculated as:

$$\text{Saponins} = \frac{\text{weight of saponins}}{\text{weight of sample}} \times 100 \quad (3)$$

2.2.4 | Determination of functional properties of lima beans flour samples

Packed and loose bulk density

The packed and loosed bulk density of the samples was determined using the method adopted by Chinyere et al. (2015) with minor modifications. The method described by Elkhalifa et al. (2005) with slight modifications was adopted in the determination of the water and oil absorption capacities (OACs). The method described by Njoku and Banigo (2006) was employed in the determination of the swelling index. The least gelation capacities of the samples were determined using the method described by Ojinnaka et al. (2016), while foaming capacity (FC), foaming stability (FS), and emulsion capacity were determined using the method described by Onwuka (2005).

2.2.5 | Determination of color

The color of the samples was measured with a CHN Spec CS-10 (Hang Zhou CHN Spec Co. Ltd). The colorimeter operates on the Commission International de I' Edairage (CIE) L^*, a^*, b^* color scheme. The colorimeter was calibrated against a standard white reference tile standardized. About 5 g of samples were put on clean paper and the colorimeter and the sensor were allowed to touch the sample. Triplicate reading was taken.

Reading for L^* was taken directly. The mechanism of the instrument is a display of three-dimensional differences in color in uniform color space coordinates. Uniform color space indicates three dimensions, a light-to-dark direction called L^*, a red-to-green direction called a^*, and a blue-to-yellow direction called b^*. Triplicate readings were taken.

2.2.6 | In vitro starch and protein digestibility analysis of lima beans flour samples

In vitro starch digestibility was determined using the method of Englyst et al. (1992) with modification by Chung et al. (2008). The supernatant of 2.70 mL was collected by dispersing 0.45 g porcine pancreatic alpha-amylase in 4 mL sterile distilled water and centrifuging at 1500 × *g* for 12 min followed by mixing with 0.3 mL amyloglucosidase (9032-08-0; Sigma-Aldrich) and 0.20 mL invertase (9001-57-4; Sigma-Aldrich). For each evaluation of starch digestibility, the mixed enzyme was freshly prepared. Thereafter, 100 g of flour was weighed and 5 mL of 7 M KOH (1310-58-3; 90% purity; Sigma-Aldrich) was added followed by preheating in a shaking water bath for 10 min at 37°C and incubating for 30 min. The hydrolyzed solutions were removed at various times (0, 20, 30, 60, 90, and 120 min) and mixed with 4 mL of 95% ethanol to inactivate the enzymes. The glucose content was assessed using the glucose assay following 5-min centrifugation at 1500 rpm. These formulas were used to determine the fractions of rapidly digestible starch (RDS), slowly digestible starch (SDS), resistant starch (RS), total starch (TS), and glycemic index (GI.)

$$\text{RDS} = G_{20} \times 0.9 \quad (4)$$

$$\text{SDS} = (G_{120} - G_{20}) \times 0.9 \quad (5)$$

$$\text{RS} = \text{TS} - (\text{SDS} + \text{RDS}) \quad (6)$$

$$\text{Glycaemic index} = \text{GI} = \frac{\text{iAUCtest food}}{\text{iAUCglucose}} \times 100 \quad (7)$$

where iAUC is the incremental area under the glucose curve; G20 is the glucose released within 20 min; TS is the total starch content; G120 is the glucose released within 120 min; RS is the resistant starch; SDS is the slowly digestible starch; and RDS is the rapidly digestible starch.

2.2.7 | Determination of in vitro protein digestibility

In vitro protein digestibility (IVPD) was carried out according to the method described by Elkhalil et al. (2001) with slight modification. To determine the protein digestibility, 20 mg of the samples in triplicates were digested in 10 mL of trypsin (0.2 mg/mL in 100 mm Tris–HCl buffer, pH 7.6). Incubation of the suspension was done at 37°C for 2 h, and 5 mL of 50% trichloroacetic acid (TCA; 76-03-9; 99% purity; Sigma-Aldrich) was added to end hydrolysis. The mixture was allowed to stand for 30 min using a centrifuge (Ostrode am Harz model 4515). The resultant precipitate was dissolved in 5 mL of NaOH (1310-73-2; 98% purity; Thermo Fisher Scientific) and protein concentration was determined using the micro-Kjeldahl method of AOAC (2000). Digestibility was calculated as:

$$\text{Protein Digestibility (\%)} = (\text{Total Protein} - \text{Digested Protein}) \times 100 \quad (8)$$

TABLE 1 Chemical properties and antinutritional factors of unfermented and fermented lima beans flours.

Parameters	LBF0	FLB24	FLB48	FLB72	FLB96
Moisture content (%)	5.00 ± 0.10^{b}	6.95 ± 0.35^{a}	6.35 ± 0.21^{a}	6.60 ± 0.28^{a}	6.45 ± 0.77^{a}
Crude protein (%)	16.46 ± 0.50^{d}	17.52 ± 0.04^{c}	18.83 ± 0.07^{b}	20.20 ± 0.07^{a}	20.21 ± 0.06^{a}
Crude fat (%)	3.67 ± 0.14^{e}	3.12 ± 0.04^{c}	3.36 ± 0.02^{b}	3.58 ± 0.06^{a}	3.03 ± 0.06^{d}
Ash (%)	2.65 ± 0.21^{c}	3.50 ± 0.20^{a}	3.25 ± 0.35^{b}	3.25 ± 0.35^{b}	3.12 ± 0.17^{b}
Crude fiber (%)	4.62 ± 0.01^{a}	2.49 ± 0.04^{c}	2.43 ± 0.00^{b}	2.43 ± 0.00^{d}	2.40 ± 0.01^{c}
Carbohydrate (%)	67.60 ± 0.46^{a}	66.43 ± 0.23^{b}	65.78 ± 0.38^{b}	63.94 ± 0.89^{c}	64.79 ± 0.61^{d}
Vitamin and mineral composition					
β-Carotene (mg/100 g)	8.63 ± 0.02^{d}	16.55 ± 0.06^{a}	14.23 ± 0.03^{b}	12.93 ± 0.07^{c}	12.90 ± 0.03^{c}
Vitamin B2 (μg/100 g)	16.03 ± 0.06^{e}	25.07 ± 0.06^{a}	22.43 ± 0.38^{b}	22.50 ± 0.10^{b}	20.56 ± 0.06^{d}
Vitamin B3 (μg/100 g)	32.67 ± 0.06^{e}	51.67 ± 0.06^{a}	50.67 ± 0.10^{b}	47.53 ± 0.06^{c}	34.20 ± 0.10^{d}
Potassium (mg/100 g)	989.03 ± 0.50^{c}	1052 ± 0.10^{b}	1110 ± 1.05^{a}	1100 ± 0.40^{ab}	1095 ± 0.20^{ab}
Sodium (mg/100 g)	137.63 ± 0.13^{a}	136.26 ± 0.32^{b}	136.38 ± 0.12^{b}	135.54 ± 0.04^{c}	135.45 ± 0.10^{c}
Calcium (mg/100 g)	10.26 ± 0.01^{d}	10.93 ± 0.01^{c}	10.94 ± 0.05^{c}	11.05 ± 0.02^{b}	11.12 ± 0.03^{a}
Magnesium (mg/100 g)	9.88 ± 0.00^{e}	10.11 ± 0.02^{d}	10.93 ± 0.02^{c}	12.93 ± 0.02^{b}	13.06 ± 0.03^{a}
Iron (mg/100 g)	4.68 ± 0.00^{e}	4.96 ± 0.06^{d}	7.08 ± 0.02^{c}	7.90 ± 0.00^{b}	8.28 ± 0.00^{a}
Zinc (mg/100 g)	4.73 ± 0.08^{e}	4.85 ± 0.05^{d}	5.99 ± 0.09^{c}	6.17 ± 0.06^{a}	6.22 ± 0.08^{a}
Antinutritional factors					
Phytate (mg/100 g)	19.05 ± 0.10^{a}	17.44 ± 0.60^{b}	13.73 ± 0.26^{c}	13.24 ± 0.09^{d}	13.20 ± 0.23^{d}
Tannins(mg/100 g)	35.29 ± 0.09^{a}	30.04 ± 0.96^{b}	24.31 ± 0.46^{c}	19.82 ± 0.24^{d}	19.05 ± 0.43^{e}
Alkaloids (mg/100 g)	18.00 ± 0.08^{a}	10.29 ± 0.62^{b}	8.22 ± 0.55^{c}	7.73 ± 0.05^{d}	7.15 ± 0.32^{e}
Oxalate (mg/100 g)	3.09 ± 0.00^{a}	2.37 ± 0.00^{b}	2.07 ± 0.00^{c}	1.55 ± 0.10^{d}	1.35 ± 0.00^{e}

Values are expressed as mean ± SD ($n = 3$). This means in the same row with different superscripts is significantly different from each other at $p < 0.05$.
Abbreviations: FLB24, 24 h fermented lima beans flour; FLB48, 48 h fermented lima beans flour; FLB72, 72 h fermented lima beans flour; FLB96, 96 h fermented lima beans flour; LBF0, unfermented lima beans flour.

2.2.8 | Statistical analysis

Data collected were subjected to statistical analysis using analysis of variance (ANOVA). The means were separated using the Duncan Multiple range test at a 95% confidence level (SPSS version 23 computer software was used).

3 | RESULTS AND DISCUSSION

3.1 | Chemical properties

The fermented white Table 1 shows the chemical properties of the white lima beans fermented flours and control. The moisture content ranged from 5.00% to 6.95% with higher moisture content observed in all the fermented lima beans flour compared to the unfermented sample (control). However, these moisture contents are within the range ≤10% recommended as a safe limit for extended preservation of flours. The slight increase in value for moisture content of fermented lima beans flour compared to unfermented lima bean flour (control) could be a result of the addition of water to mix the substrate before fermentation and subsequent absorption by the beans flour during the fermentation period (Ogodo et al., 2018). The observation is in agreement with the report of Agblemanyo and Abrokwah (2019) and Ogodo et al. (2018) for African locust beans and bambara nuts flours fermented at different hours, respectively. On the other hand, Adegbehingbe and Daramola (2019) reported moisture decrease as the fermentation period increases in lima beans. There was also a significant difference ($p < 0.05$) between the control and the fermented samples at each 24 h interval in the protein content. Noticeable gradual increases in protein content at 6.44%, 14.40%, 22.72%, and 22.78% as fermentation progresses were observed. Fermentation has been reported to be one of the best food processing techniques that can improve the protein levels of legumes (Adhikari et al., 2013; Kumitch, 2019). This might be due to the release of protein initially bound to ANFs, an increase in the microbial population, which results in the release and modification of structural proteins that are integral parts of the microbial cells into the substrates, synthesis of amino acids and breakdown of the protein molecules to amino acids, and other simple peptides (Adebiyi et al., 2019). This also implies that the fermenting

microorganisms could serve as probiotics in the flour. The observation is in agreement with the report of Adebiyi et al. (2019) who reported in the protein content of unhulled Dawa Dawa (a fermented condiment) by approximately 18%. On the contrary, N'zi et al. (2021) reported a decrease in protein content in black lima beans as spontaneous fermentation progresses.

The crude fat content ranged between 3.03% and 3.67%. The fermented white lima bean flours had lower fat content with a 17.44% decrease at FLB96. This is supported by N'zi et al. (2021), they reported a decrease from 1.76% to 1.25% while Adegbehingbe and Daramola (2019) reported an increase (3.68%–4.74%) both within 0–72 h fermentation of black lima beans. The decrease in fat content could be attributed to the metabolism of lipids by the organisms responsible for fermentation as well as the leaching of soluble organic salts. Generally, studies on the fermentation of legumes, such as African yam beans, cowpea and lima beans, reported reduced fat content between 0.63% and 58.70% (Chinma et al., 2020; Difo et al., 2014; Farinde et al., 2017) during fermentation. The reduction was attributed to the breakdown of lipids by lipase, the use of lipids as the food source by fermenting organisms and lipolytic enzymes, fat denaturation and leaching of fat-related components into the water during processing, and loss of total solids during soaking (García et al., 2019; Onwurafor et al., 2014).

Unfermented lima beans flour (LBF0) contained 2.65% ash and there was a 32.08% increase after 24 h of fermentation, followed by a gradual decrease by 22.64% for samples FLB48 and FLB72 and 17.74% at 96 h fermentation period (FLB96). The reduction in ash content could be a result of increased enzyme activities and the resultant loss of dry matter fermentation period progresses (Chinma et al., 2020). The results demonstrated that the ash content of fermented lima beans flour at different fermentation times was higher than the control with the optimal increase at 24 h fermentation period. N'zi et al. (2021) reported a gradual decrease in ash content for black lima beans (4.11%–1.88%) as fermentation progresses and this ash content reported falls within the range of this study.

There is a slight decrease in crude fiber content within the fermented products and this is largely influenced by the fermentation's progression. The crude fiber content of 4.62% decreased by 46.10% (FLB24), 47.40% (FLB48 and FLB72), and 47.52% (FLB96). The reduction in crude fiber content could probably be attributed to the microbial activities whereby fiber-related components are utilized by the microorganisms, hydrolysis, and leaching of fiber into the fermentation medium. This could be advantageous as reduced fiber content could significantly increase the bioavailability of micronutrients and proteins, thereby enhancing the nutritional quality of the food (Nzi et al., 2021). The results of this study are in partial agreement with Adegbehingbe and Daramola (2019) who also reported a decrease in crude fiber content but at 4.28% and 6.70% for 48 and 72 h fermentation periods of lima beans. On the other hand, Agblemanyo and Abrokwah (2019) reported increased crude fiber content on all hours of fermentation on African locust beans compared with the control.

The carbohydrate content of the lima beans flour (LBF0) at 67.60 g/100 decreased by 1.73%, 2.69%, 5.41%, and 4.16% for FLB24, FLB48, FLB72, and FLB96, respectively. The highest reduction was observed in sample FLB72. These results agree with the findings of Asensio-Grau et al. (2020) who reported a decrease in the carbohydrate content of kidney beans by 17% when compared with the unfermented sample (control) and also a 3% decrease in the carbohydrate content reported by Xiao et al. (2018) during the fermentation of red bean (*Phaseolus angularis*). Adebo et al. (2022) stated that fermentation microorganisms utilize carbohydrates-related compounds as source of energy as well as the conversion of oligosaccharides to monosaccharides, this can be responsible for the varying decrease in the carbohydrate contents in the fermented samples of this study.

The fermented white lima bean flour samples had a higher content of β-carotene, vitamin B2, and vitamin B3 content compared with the control. A sharp increase of 92%, 56.39%, and 58.16% was observed at 24 h fermentation for β-carotene, vitamin B2, and vitamin B3, respectively. The decrease was linear with an increase in the fermented period with the least values at 96 h fermentation period. This has been attributed to enzyme interactions with macromolecules such as starch, protein, and other important biosynthetic precursors, which stimulate the synthesis of vitamins in the bound form. The decrease in higher fermentation period could be a result of the utilization of these constituents for metabolism by microorganisms, as well as losses due to discarding the supernatant (Ejuigi et al., 2005). The percentage increase in this study agrees with the 5%–106% increase reported for β-carotene, vitamin B2, and vitamin B3 in cowpea and mung beans (Doblado et al., 2003; Onwuruafor et al., 2014).

Potassium had the highest value ranging between 989.00 and 1110.00 mg/100 g and the highest increase was by 12.00% at 48 h fermentation period. The least change in all was observed in sodium content of 137.63 mg/100 g, having a slight decrease of 1.00%–1.58% within the fermented samples. The level of potassium which is high and low level of sodium is beneficial to people with high blood pressure who require high potassium but low sodium necessary for the electrolytic balance of the body (Levings & Gunn, 2014). The magnesium content

of 9.88 mg/100 g of the white lima beans flour increased by 2.33%, 10.63%, 30.87%, and 32.19% for FLB24, FLB48, FLB72, and FLB96 respectively while the calcium content of 10.26% increased steadily by 6.53%–8.38% within the fermented samples. Iron and zinc content of 4.68% and 4.73% of unfermented white beans sample (FLB0) increased by 5.98%–76.92% and 2.50%–31.50% respectively within the fermented samples. Study by Adebiyi et al. (2019) reported that fermentation enhance the extraction and bioavailability of minerals due to the breakdown of chelated mineral elements/antinutrients complexes, this can be responsible for the increase in the mineral element. These results are similar to the increase in mineral elements reported by Onwurafor et al. (2014) for Ca, Mg, P, Zn, Cu, Mn, and Fe as fermentation progresses in mung beans. In addition, the collection of these mineral elements in the fermented products indicates that these can provide the body with the necessary food component.

The ANFs in various samples are shown in Table 1. Food fermentation has been reported to effectively decrease the levels of antinutrients in foods. Reduction observed in the antinutritional contents of lima bean seeds largely depends on the ingredients used in the preparation. Phytate is widely distributed in legumes (Wcislo and Szarlej-Wcislo, 2014), the phytate and tannin contents at 35.29 mg/100 g and 19.05 mg/100 g decreased by 8.45%–30.71% and 14.88%–46.01%, respectively. The highest reduction in ANFs was in the range of 42.83%–60.28%, for the alkaloids content. While the oxalate content of 3.09 mg/100 g decreased by 23.30%–56.31% and saponins with 22.47 mg/100 g decreased by 19.05%–32.80%. The fermented samples had lower contents of ANFs with fermentation at 96 h (FLB96) the lowest. The reduction in the ANFs could probably be attributed to leaching out during soaking, and degradation by fermenting microbial enzymes such as phytase which degrades phytic acids. Similar reported reductions by 27% in oxalate concentrations after fermentation was reported by Adebiyi et al. (2019) in *Dawa Dawa* and for legumes, a decrease of 24%–79% in ANFs of fermented African oil bean was reported by Onwuliri et al. (2004).

3.2 | Functional properties and color characteristics of fermented and unfermented lima beans flour

The utilization of flour as raw material is strongly dependent on its functional properties which is influenced by its composition and indirectly reveals the chemical interactions between the components of the flour (Caruso et al., 2020). The results of the effects of the fermentation period on lima bean flour are presented in Table 2. The packed bulk density (PBD) and loose bulk density (LBD) represent the important quality of a flour product and play a significant role in package design, material treatment, and application in food products (Malomo et al., 2012). The results of the PBD indicated a significant decrease ($p < 0.05$) by 8.51% at 24 h (FLB24) and 48 h fermentation (FLB48), followed by 12.77% and 14.89% at FLB 72 and FLB 96. Likewise, for the LBD which is defined as the lowest density that is attainable without density without compression (Olubunmi et al., 2017), the unfermented lima beans flour (LBF0) had the highest value of 0.74 g/mL which decreased by 5.41% and 8.11% at 24 h (FLB24) and 48 h fermentation (FLB48) respectively with the optimum decrease by 27.03% at FLB72 and FLB96 fermentation period. Fermentation significantly ($p < 0.05$) reduced the PLB and LBD of the lima bean flour samples.

The reduction of bulk densities in the fermented samples could be due to the breakdown of starch during fermentation. High-bulk densities are undesirable in packaging as this could incur higher costs because of more packaging materials requirements. However, high-bulk density is important for mixing purposes. The lower value of bulk density has the advantage of a higher number of flour particles staying together hence, a better packaging advantage. Low-bulk density flours have wider food applications especially in bakery products and in low-bulk weaning foods (Olubunmi et al., 2017).

Water absorption capacity (WAC) influences food preparations' functional and sensory properties. Using flours with a higher WAC may aid in the preservation of a soft texture (Du et al., 2014), on the other hand, thinner gruels are made from flour of lower absorption capacity. There is a significant increase ($p < 0.05$) in WAC as the fermentation period increases. The results reveal a notable twofold increase at 96 h fermentation. The OAC of food is attributed to the physical entrapment of oil which is considered important as a flavor retainer and enhancer of foods mouth feel. These results demonstrated that the OAC of fermented lima bean flour at different fermentation times was higher than the control (LFB0). OAC is influenced by the large number of available nonpolar side chains in its protein molecules (Du et al., 2014). The OACs were generally lower than those of the WAC. This implies that protein molecules have a higher proportion of hydrophilic than hydrophobic groups on their surfaces (Caruso et al., 2020). Overall, FLB96 exhibits good water and OAC and they will be more desirable for use in the preparation of complementary or baby foods.

The EC results exhibited a gradual increase of 2.94%–40.06% with an increase in fermentation time. EC implied the maximum amount of oil that could be emulsified by protein dispersion (Oloyede et al., 2016). FC and FS are the foaming properties used in determining the whipping characteristics of food flour. The FC and FS increased

TABLE 2 Functional properties and color characteristics of unfermented and fermented lima beans flours.

Parameter	LBF0	FLB24	FLB48	FLB72	FLB96
PBD (g/mL)	0.94 ± 0.01^{a}	0.86 ± 0.02^{b}	0.86 ± 0.01^{b}	0.82 ± 0.00^{c}	0.80 ± 0.00^{d}
LBD (g/mL)	0.74 ± 0.3^{a}	0.70 ± 0.00^{b}	0.68 ± 0.20^{c}	0.54 ± 0.00^{d}	0.54 ± 0.00^{d}
WAC (%)	2.00 ± 0.00^{a}	2.95 ± 0.07^{b}	3.95 ± 0.07^{b}	3.80 ± 0.00^{c}	4.17 ± 0.03^{d}
OAC (%)	1.48 ± 0.02^{b}	1.54 ± 0.80^{b}	1.60 ± 0.10^{b}	1.75 ± 0.07^{b}	2.00 ± 0.00^{a}
EC (%)	18.72 ± 0.03^{c}	18.82 ± 0.10^{c}	24.85 ± 0.21^{b}	25.26 ± 0.36^{b}	26.22 ± 0.03^{a}
FC (%)	37.25 ± 0.00^{b}	39.95 ± 0.33^{d}	40.33 ± 0.96^{a}	43.04 ± 0.14^{c}	50.88 ± 0.14^{b}
FS (%)	82.19 ± 0.12^{e}	96.42 ± 0.24^{d}	96.63 ± 0.00^{c}	98.63 ± 0.00^{b}	99.08 ± 0.29^{a}
SI	2.16 ± 0.05^{a}	1.66 ± 0.00^{b}	1.59 ± 0.01^{c}	1.59 ± 0.01^{c}	1.35 ± 0.01^{d}
LGC (%)	0.40 ± 0.00^{b}	0.60 ± 0.01^{a}	0.60 ± 0.00^{a}	0.60 ± 0.02^{a}	0.60 ± 0.01^{a}
Color					
L*	87.33 ± 0.11^{a}	70.77 ± 0.20^{b}	66.52 ± 0.42^{c}	64.46 ± 0.20^{d}	62.49 ± 0.04^{e}
a*	2.14 ± 0.02^{e}	8.30 ± 0.05^{d}	9.75 ± 0.04^{c}	10.01 ± 0.05^{b}	10.55 ± 0.20^{a}
b*	5.53 ± 0.01^{e}	27.47 ± 0.12^{d}	28.08 ± 0.08^{c}	28.48 ± 0.07^{b}	28.60 ± 0.05^{a}

Abbreviations: EC, emulsion capacity; FC, foaming capacity; FLB24, 24 h fermented lima beans flour; FLB48, 48 h fermented lima beans flour; FLB72, 72 h fermented lima beans flour; FLB96, 96 h fermented lima beans flour; FS, foam stability; LBD, loose bulk density; LBF0, unfermented lima beans flour; LGC, least gelation capacity; OAC, oil absorption capacity; PBD, packed bulk density; SI, swelling index; WAC, water absorption capacity.

significantly during fermentation by 7.25%–36.59% and 16.51%–20.55%, respectively. FC and FS of flours depend generally on some factors such as the surface tension formed by proteins which keeps air bubbles in suspension and slows down the rate of coalescence, methods of processing and protein type. This increase could be a result of an increase in solubilized protein during fermentation which invariably increased the foaming properties. These foaming properties of the lima bean flour especially the fermented lima beans could be exceptionally useful as an aerating agent in food systems like beans pudding (*moi-moi*) and fried beans (*akara*), and so forth as well as other product which require FS during production.

The swelling index is a measure of hydration capacity and it is determined by measuring the weight of the swollen starch granules and their occluded water. Food eating quality is frequently linked with the retention of water in the swollen starch granules (Vengaiah et al., 2013). The swelling index in the unfermented samples is higher than that of the fermented sample. A decrease from 2.16 by 23.15%–37.50% was observed. This decrease might be due to a decrease in the hydrophilic groups and inhibition of granular swelling during fermentation and drying (Ukom et al., 2018). High SI is important in bakery products (Kusumayanti et al., 2015). This implies that these samples can be applied to a wide range of foods.

The Hunter color values (L*, a*, b*) of the various lima bean flour samples as affected by the fermentation period are presented in Table 2. Among these samples, unfermented white lima beans flour (LBF0) was significantly lighter with a high L* of 87.33%, while the highest values of highest greenness (a*) of 10.55 and yellowness (b*) value of 28.60 were observed for the FLB96 sample. The decrease in lightness (L*), high a* and b* observed in all the fermented flours could be due to the Maillard browning reaction which results from the chemical reaction between macromolecules such as simple sugars and protein amino acids facilitated as a result of fermentation and subsequent drying of the flours. Also, the metabolism of sugar which leads to the production of organic acid by fermenting microorganisms may have brought about the darkening of the fermented white lima beans flour samples. The changes in the color of the fermented white lima bean flours conform to the review of Oyarekua (2011) and Yakubu et al. (2022) on legumes. This suggests the chances of favorable color change which could be a factor determining acceptability and subsequent use in foods such as pastry.

The effect of fermentation on the in vitro starch digestibility is shown in Table 3. The postprandial plasma glucose is rapidly elevated by RDS, which is a starch fraction that is readily and completely digested in the small intestine. The control (LFB0) had the lowest RDS of 9.06% and an increase in the RDS contents of the fermented samples of lima beans flour by 11.15%–25.05% was observed with the highest at FLB96. The SDS decreased by 8.33% at 24 h fermentation (FLB24) followed by an increase of 9.41%–14.68% for FLB48 and FLB72 and followed by a slight decrease of 1.48% at 96 h fermentation (FLB96). These changes could be due to the chemical nature of the starch and a decrease in enzyme action which may be a result of fluctuating acidic environment (Elkhalifa, 2005). SDS and RDS have potential health effects linked to diabetes management. However, products with low RDS may be of preference to diabetic patients (Giuberti & Gallo, 2018).

TABLE 3 Effect of fermentation time on lima beans in vitro starch and protein digestibility and glycemic index.

Parameters	LBF0	FLB24	FLB48	FLB72	FLB96
RDS	9.06 ± 0.01^{e}	14.22 ± 0.01^{a}	10.07 ± 0.01^{d}	10.21 ± 0.01^{c}	11.33 ± 0.01^{b}
SDS	31.68 ± 0.00^{c}	29.04 ± 0.00^{e}	34.97 ± 0.00^{b}	36.33 ± 0.00^{a}	31.21 ± 0.01^{d}
TG	52.05 ± 0.01^{e}	52.62 ± 0.01^{d}	55.25 ± 0.01^{b}	58.03 ± 0.00^{a}	54.21 ± 0.01^{c}
TS	46.85 ± 0.01^{e}	47.34 ± 0.03^{d}	49.73 ± 0.01^{b}	52.23 ± 0.00^{a}	48.79 ± 0.00^{c}
RS	6.12 ± 0.01^{a}	4.10 ± 0.00^{e}	4.69 ± 0.01^{d}	5.68 ± 0.01^{c}	5.92 ± 0.02^{a}
GI	57.11 ± 0.21^{b}	57.18 ± 0.01^{b}	62.38 ± 0.72^{a}	62.67 ± 0.58^{a}	62.82 ± 0.89^{a}
IVPD	50.33 ± 0.06^{e}	53.17 ± 0.14^{d}	55.07 ± 0.00^{c}	57.84 ± 0.01^{b}	58.50 ± 0.01^{a}

Abbreviations: FLB24, 24 h fermented lima beans flour; FLB48, 48 h fermented lima beans flour; FLB72, 72 h fermented lima beans flour; FLB96, 96 h fermented lima beans flour; GI, glycemic index; IVPD, in vitro protein digestibility; LBF0, unfermented lima beans flour; RDS, rapidly digested starch; RS, resistance starch; SDS, slowly digested starch; TG, total glucose; TS, total starch.

The control (LFB0) had the lowest TG and TS of 52.05% and 46.85%, respectively. This could be attributed to the restriction in the accessibility of starch caused by endosperm protein fractions (Elkhalifa, 2005). RS is a natural component of several foods and it is the sum of starch and the products of starch degradation that are not absorbed in the small intestine but are fermented in the large intestine of healthy individuals. There was a significant ($p < 0.05$) difference in the RS of the various samples with the control having the highest RS of 6.12%. A decrease by 33.11%, 23.37%, 7.19%, and 3.27% for FLB24, FLB48, FLB72, and FLB96 was observed. The higher RS in the control (LBF0) could be attributed to starch contained within the granules which are unaffected by hydrolytic enzymes and consequently most indigestible (Kuar et al., 2015). Siddhuaraju and Becker (2005) also attributed the decrease to the partial loss of soluble components, such as oligosaccharides and phenolic substances.

The variation of GI and IVPD was dependent on fermentation time. Though a slight increase was observed in the GI as fermentation time increased, there was no significant ($p < 0.05$) difference at FLB48–FLB96, whereas, for the IVPD the increase was in the order FLB96 > FLB72 > FLB 48 > FLB24 > LBF0. The increase was likely due to the ease of digestion and absorption of glucose as a result of the degradation of fiber by microorganisms during fermentation (Nkhata et al., 2018). The GI of a food is the rate at which sugar is released from that food into the blood and the lower the number, the slower the process, therefore LBF0 has a better GI compared with the fermented lima beans flours. These samples fall within the range of intermediate (medium) glycemic foods which range between 56 and 69 on the GI scale (Eleazu, 2016).

The IVPD assay reflects the dietary performance of protein concerning its amino acid and bioavailability (Yakubu et al., 2022). The IVPD increased by 5.64%, 9.42%, 14.92%, and 16.23% which could be attributed to increased availability of amino acids, proteolysis which resulted in proteins release that were more vulnerable to enzyme action, inducing structural modifications the proteins becomes more accessible coupled with the reduced ANFs (Chinma et al., 2020; Ogodo et al., 2017). This increase is higher than the 10% and 4.40% increase in IVPD in African yam bean and fermented chickpea reported by Chinma et al. (2020) and Xiao et al. (2014) but within the range of 16.00%–32.50% reported for fermented lupin flours by Olukomaiya et al. (2020).

3.3 | Mechanism and effects of fermentation on white lima beans flour

Fermentation of white lima beans flour was due to activities of lactic acid bacteria (LAB) which release amino acids, bioactive compounds and breakdown ANFs in white lima beans flour through activation of microbial enzymes and endogenous enzymes in the beans thereby improving in vitro protein. This improvement in amino acid profile of fermented white lima beans flour occurred due to proteolytic activity of the fermenting microorganisms (Jalili et al., 2023; Senanayake et al., 2023). Fermentation also improves quality of protein of white lima beans flour due to increase in the amounts of essential amino acids like lysine and methionine and thereby enhanced the production of bioactive peptides which exhibit antioxidant, anti-inflammatory, and antimicrobial properties (Senanayake et al., 2023).

Fermented foods also have major metabolites of fermenting microorganisms such as lactate (Marco et al., 2017; Stiemsma et al., 2020). LAB in fermented foods are known to produce short-chain fatty acids (SCFAs) via fermentation of the prebiotic nondigestible carbohydrates which serve as a source of energy by bacteria in the colon thereby preventing the growth of intestinal pathogens and regulate various metabolic pathways such as cholesterol synthesis and the secretion of appetite hormones (Masood et al., 2011; Stiemsma et al., 2020).

Fermentation also increases the phenolic content, antioxidant activity, formation of new bioactive compounds, and bioavailability of nutrients and

phytochemicals as well as reduces the concentration of ANFs like tannins and phytate which bind minerals in white lima beans (Marco et al., 2017; Senanayake et al., 2023; Stiemsma et al., 2020).

4 | CONCLUSIONS

This present study indicated that the different fermentation period of 0, 24, 48, 72, and 96 h of lima beans flour samples influenced the chemical, antinutritional, and in vitro digestibility characteristics. A significant increase was observed in the protein and micronutrients while the ANFs decreased noticeably. This could be attributed to influence of fermenting microorganisms that remove protease inhibitors that reduce the digestion and use of protein; as well as well as phytates that bind minerals and affect mineral utilization. All the samples were grouped as moderate glycemic food and the fermented samples were digested more rapidly than the unfermented lima bean flour sample. This showed that fermentation period if controlled can improve and/or promote desirable health properties. The fermentation process could be beneficial and improve the potential use of while lima beans for industrial-scale applications which could add practical relevance and enhances community nutrition and helps in food security particularly in communities where white lima beans are stable food.

AUTHOR CONTRIBUTIONS

Mofoluwaso O. Ojo: Conceptualization; investigation; writing—original draft; methodology; validation; writing—review and editing; software; formal analysis; data curation; supervision. **Oyekunle K. Oni**: Conceptualization; investigation; writing—original draft; methodology; validation; writing—review and editing; software; formal analysis; data curation. **Adeiza B. Zubair**: Conceptualization; investigation; writing—original draft; methodology; validation; software; formal analysis; data curation. **Fortune A. Femi**: Conceptualization; investigation; writing—original draft; methodology; validation; software; formal analysis; data curation; writing—review and editing. **Yohanna Audu**: Conceptualization; investigation; writing—original draft; methodology; validation; software; formal analysis; data curation. **Blessing Etim**: Conceptualization; investigation; writing—original draft; methodology; validation; writing—review and editing; software; formal analysis; data curation. **Samuel A. O. Adeyeye**: Conceptualization; investigation; writing—original draft; writing—review and editing; methodology; validation; software; formal analysis.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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