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Effects of Fermentation with *Aspergillus niger* on Nutritional, Physico-Chemical, Anti-Nutrient, Fatty Acid and Amino-Acid Profile of Dehulled and Undehulled *Mucuna Utilis* Flour and Starch

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Abstract
Fermentation of legumes is a common preservation methods which is known to enhance the sensory, dietary quality of legume based food and also enhance its bioavailability and health-promoting properties. The effect of fermentation and dehulling on nutritional profile, physico-chemical, anti-nutrient, fatty acid and amino acid profile of dehulled and undehulled *Mucuna utilis* flour and starch with *Aspergillus niger* at 24, 48 and 72 h was examined using standard methods. The result showed that swelling capacity, solubility and water absorption capacity increased with increase in fermentation period for the extracted starch in all the samples. The highest value of 10.78% and 178.66% were observed in the dehulled samples. The proximate composition indicates that the dehulled flours fermented for 72h had the highest value for protein (31.35%) and lowest fiber value (4.08%). The minerals content of the undehulled fermented flours were high compared with dehulled with the highest being potassium (1221.05mg/100g). Dehulled fermented mucuna flour samples had antinutritional value of17.22mg/100g which is lower than undehulled fermented sample. A total of 12 amino acids with tryptophan and leucine acids were the predominant amino acids. The amino acid value tyrosine+phenylalanine were highest for dehulled (50.22mg/100g); undehulled (87.23mg/100g) for unfermented samples while fermented dehulled (54.17mg/100g) and undehulled (101.05mg/100g). The fatty acid profile showed an increase in value of Palmitic acid which has the highest value in dehulled sample (26.33mg/100g) and linoleic acid (27.02mg/100g) in undehulled sample. Our findings indicate that combining dehulling and fermentation significantly enhances the Nutrient density of mucuna beans while minimizing anti-nutrient content, thereby improving their usability.

Keywords: Velvet bean, fermentation, *Aspergilus niger*, physicochemical, nutritional properties

1. Introduction

Legumes are an important source of plant proteins constituting more than 80% of available protein in human diets in developing countries [1]. Legumes constitute the main source of protein in the diets of individuals. Many indigenous tropical legumes can be said to be "underutilized" because little has been done to explore ways to stimulate their production, processing, marketing and use. However, due to the ever increasing demand for energy and protein foodstuff and the subsequent competition between humans and livestock for the few available legumes, there is need to explore ways of maximizing the utilization of some of the most prominent of these indigenous legumes [2]. Some of the utilized legumes are cowpea *(Vigna unguiculata)*, groundnut *(Arachis hypogaea)* and lima bean *(Phaseolus lunatus)*. There

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are other pulses that could help meet dietary needs but are not cultivated only in localized areas and used less prominently. These lesser known legumes have seeds which are rich in protein, low in oil and contain a fair amount of crude fibre and mineral matters. However, the lack of knowledge of nutritional qualities of lesser-known legumes grown in developing countries like Nigeria is responsible for the poor utilization of the traditional crops in different food formulations and also the anti-nutritional factors present in these legumes [3]. The need for cheap sources of protein for developing countries has led many individuals to research potentials of underutilized legumes to supply valuable nutrients of which mucuna beans are one but its utilization in food forms may be hindered as a result of the anti-nutritional factors present in the plant. These anti-nutritional factors have been reported to interfere with nutrient absorption and also cause a reduction in nutrient intake, digestion and utilization [4]. Consumption of food containing anti-nutritional factors may cause some specific symptoms in the human body such as nausea, rashes, bloating, and nutritional deficiencies among others [5]. Mucuna species is one of these under-utilized legumes [3]. *Mucuna utilis* is a leguminous twinning crop, having a wide distribution in tropical and subtropical areas of the world. It is commonly known as velvet bean in English and is high in protein but contain toxic substances which can, however, be destroyed by heating and other processing methods such as fermentation and germination [6]. Like many other legumes, the *Mucuna utilis* contain many anti-nutritional compounds such as phytate, polyphenols, protease inhibitors and biochemical effects including decreased protein digestibility, growth inhibition in animals. It also contain L-DOPA (L-3, 4-dihydroxy-phenylalanine), which has a number of anti-physiological effects [1]. The use of *Mucuna utilis* could be promoted through increased human or animal consumption of its seeds. Thus, fermentation techniques were tested as potential processing method of reducing the antinutritional factors and increase its consumption and utilization. Fermentation is a widely practiced, traditional processing methods and very economical method of food and beverage production in developing countries [7]. Fermentation brings about numerous biochemicals, nutritional and organoleptic changes in the raw materials including the breakdown of certain constituents and the reduction of anti-nutritional factors in legume and the synthesis of B-vitamins [8]. The purpose of this study was to evaluate the effect of fermentation on the physico-chemical, nutrition and anti-nutritional and physico-chemical attributes of flour and starch extract *Mucuna utilis*.

2. Materials and Methods

2.1. Materials

Seeds of *Mucuna utilis* were obtained from International Institute for Tropical Agriculture (IITA) Ibadan for this research work. The inoculum *(Aspergillus niger)* was obtained from Nigerian Institute of Science Laboratory, Samonda, Ibadan. The whole seeds were cracked and winnowed to separate the seed coat. The beans were divided into three parts: one part was used for dehulled fermentation, the second part for undehulled fermentation and the third part was not fermented (unfermented) was used as control.

2.2. Fermentation of *Mucuna utilis* seeds

The method described by [9] was used. The beans were soaked in cold water in for 12 hr; drained soaked grains (dehulled and undehulled) were fermented with the addition of *Aspergillus niger* for 24, 48, and 72 hr. The beans were sorted, cleaned, and soaked in cold water for 12 hr to allow adequate hydration. After soaking, the water was drained, and both dehulled and undehulled grains were separated for fermentation. The prepared inoculum of *Aspergillus niger* was added to each batch of soaked beans at a standardized concentration to ensure uniform fungal growth. The inoculated beans were thoroughly mixed to achieve even distribution of the spores and then transferred into clean fermentation containers. Fermentation was conducted at ambient laboratory temperature, with the containers loosely covered to prevent contamination while allowing sufficient aeration for fungal metabolism. The samples were allowed to ferment for 24, 48, and 72 hr, during which *A. niger* enzymatically degraded complex carbohydrate and protein matrices, promoted softening of the grains, and induced biochemical modifications typical of fungal fermentation. Throughout the fermentation period, the moisture content of the substrate was maintained to support optimal fungal activity. At the end of each fermentation duration, the beans were removed, dried and milled for subsequent analyses.

2.3. Preparation of flour samples

The production of fermented *Mucuna utilis* flour adopted the method described by [9]. Flours from fermented beans were produced by drying the bean seeds in a hot air oven at 60 °C to about 10% moisture content. The dried beans were milled using locally

fabricated attrition mill to obtain sieve size 300µm mesh size. The raw *Mucuna utilis* beans (control) were equally milled to produce flour of the same sieve size (300µm). The samples for analysis were collected at 0, 24, 48 and 72 h and stored in the refrigerator for analysis.

2.4. Starch Extraction from Velvet Bean Flour

Starch was isolated from unfermented (control), fermented, and germinated velvet bean flour samples using a modified method adapted from [10]. A 100 g portion of the milled velvet bean flour was mixed with an appropriate volume of clean water to form a uniform slurry. The mixture was stirred thoroughly to ensure complete hydration of the starch granules. The slurry was then passed through a No. 70 mesh sieve to separate hulls and coarse fibrous particles. The resulting filtrate (slurry) was collected for centrifugation. The filtrate was transferred into clean, properly labeled centrifuge tubes, ensuring that each tube was filled to no more than two-thirds of its capacity. The tubes were arranged in the rotor in balanced, opposite pairs and centrifuged at 1,170 × g for 10 minutes at room temperature. After centrifugation, the centrifuge was allowed to stop naturally without applying the brake to avoid disturbing the starch layer. The tubes were then carefully removed, and the supernatant was gently decanted without dislodging the starch-rich pellet settled at the bottom of each tube. The pellets were re-suspended in clean water and vortexed or stirred gently to remove residual soluble proteins, sugars, and pigments. The suspension was centrifuged again under the same conditions (1,170 × g for 10 minutes). This washing process was repeated 2–3 times until the supernatant appeared clear, indicating adequate purification of the starch. The final starch sediment was spread thinly on stainless steel trays or glass plates and dried in a hot-air oven at 40–50°C until constant weight was achieved. Alternatively, air-drying at ambient conditions was used when oven drying was not suitable. The dried starch was then gently pulverized to break up lumps and sieved through a fine mesh to obtain uniform starch powder. The purified starch was stored in airtight, moisture-proof containers and kept in a cool, dry place to prevent retrogradation, microbial growth, or moisture absorption. The extracted starch was then weighed, and the yield calculated. All extractions were performed in triplicate, and mean values were reported.

$$Starch\ yield\ (\%) = \frac{Weight\ of\ dreid\ extracted\ starch}{Weight\ of\ velevet\ bean\ flour}\ x\ 100$$

2.5. Physico-chemical properties of starch extracted from flour

Determination of swelling power and solubility of the starch extract. The swelling power and solubility in each sample was determined by the method described by [11]. One gram (1g) of extracted starch was weighed into a pre-weighed 50ml plastic centrifuge tube and 50ml of distilled water was added and mixed gently. The mixture was then heated in a water bath with continuous stirring to prevent settling of the starch. It was then centrifuged at 88 x g for 10min where the mixture was centrifuged at 88 × g for 10 minutes, after which the supernatant was collected and dried to quantify the amount of dissolved soluble solids. This value was used to calculate the solubility. The weight of the sediment was recorded and the swelling power of the sediment gel determined as follows:

$$Swelling\ power = \frac{Weight\ of\ wet\ mass\ of\ sediment}{Weight\ of\ dry\ matter\ in\ the\ gel}$$

Solubility = % of extracted starch dissolved in the supernatant

Determination of water binding capacity of the starch extract. The water binding capacity of each sample was determined by employing the method of [12]. About 2.5g of the extracted starch was weighed (dry basis) and 37.5ml distilled water put in a centrifuge tube. The tube was capped and agitated on a wrist action shaker for an hour and centrifuged for 10 minutes at 2, 200 x g where the tube, after being shaken for one hour, was subjected to centrifugation at 2,200 × g for 10 minutes. The water was then decanted and the tube tipped up to a drain for 10 minutes. The centrifuge tube was weighed with content and the amount of water held by the starch determined by difference as indicated below. The determinations were done in triplicates, and the mean values recorded.

$$Water\ binding\ capacity(WBC) = \frac{Grams\ bound\ water}{2.5}\ x\ 100$$

Determination of water absorption capacity of starch extract. This was carried out as described by [11] and [10]. One gram (1.0g) of the starch extract was weighed along with 15ml distilled water in a 25-ml centrifuge tube. The tube was agitated on a vortex mixer for 2 min and centrifuged at 1173 x g for 20min. The clear supernatant was decanted and discarded while adhering drops of water removed

before the tube was reweighed. Water absorption capacity is expressed as the weight of water bound by 100g dry flour. The determinations were done in triplicates, and the mean values recorded.

2.6. Physico-chemical analysis of fermented *Mucuna utilis* flour

***Determination of reconstitution index of flour samples*.** The reconstitution index was determined by the method of [13]. 10g each of fermented and germinated velvet bean flours with the control samples were mixed with 100ml boiling water for 90s. The mixture was then poured into a 100ml graduated glass cylinder. The volume of the slurry beneath the clear supernatant liquid after 10minutes was recorded as reconstitution index. The determinations were done in triplicates.

Determination of pH values of flour samples. The pH value of the flour samples was determined by the method of [14]. Twenty grams (20g) of fermented and germinated velvet bean flours with the control samples were added to 50ml of distilled water while stirring for 10minutes. The stirring was to prevent the starch from coating the glass electrodes and to obtain reproducible results. The pH value was then measured on a pH meter (Checker HI 12 08, Hanna Instrument) using buffer 4.0 and 7.0 to standardize. The determinations were carried out in triplicates and the mean values recorded.

***Determination of titratable acidity of flour samples*.** The titratable acidity of the flour samples was with the control samples were weighed into a 250ml conical flask and 200ml of carbon dioxide-free distilled water added. The flask was allowed to stand in a water bath at 40ºC for 1hr so that the flask is covered to just about the level of liquid. It was occasionally swirled to ensure complete mixing and then filtered. Phenolphthalein will be added after filtration before being titrated against 0.1M NaOH solution.

$$Acidity\ in\ \%\ lactic\ Acid = \frac{Volume\ of\ 0.1M\ NaOH\ used\ x0.09x100}{Weight\ of\ the\ sample}$$

Proximate composition of fermented velvet bean *(M. utilis)* flour samples

The moisture, protein, fat, ash and fibre contents of the dehulled and undehulled flour were determined by the modified method described by [15]. The carbohydrate content of the flour was calculated by difference [16] (Onwuka, 2018).

Anti-nutritional factors of fermented *Mucuna utilis* flour

Determination of phytic acid content. The phytic acid content was analyzed using the AOAC method outlined in [15]. Two grams of each fermented and germinated velvet bean flour sample were weighed into separate 250 ml conical flasks. To each, 100 ml of 2% hydrochloric acid was added, and the mixtures were left to soak for three hours at room temperature. After soaking, the solutions were filtered through two layers of hardened filter paper. From each filtrate, 50 ml was transferred into a 250 ml beaker, and 107 ml of distilled water was added to adjust the acidity. Then, 10 ml of a 0.3% ammonium thiocyanate solution was introduced as an indicator. Each mixture was titrated using a standard iron (III) chloride solution, containing 0.00195 g of iron per milliliter. The titration endpoint was recognized by a stable brownish-yellow coloration that persisted for at least five minutes. Phytic acid content was then calculated and expressed as a percentage, using the formula provided below:

$$\%\ \text{Phytic acid} = \frac{X \times 1.19 \times 100}{2}$$

where: $X =$ titre value $\times$ 0.00195

Determination of trypsin inhibitor content. The trypsin inhibitor activity was assessed following the protocol described by [15]. Approximately 0.2 g of each fermented soybean sample was weighed into a screw-cap centrifuge tube, and 10 ml of 0.1 M phosphate buffer was added. The tubes were then shaken for 1 hour at room temperature using a UDY shaker. After mixing, the contents were centrifuged at 1466 × g for 5 minutes. The supernatant was filtered through Whatman No. 42 filter paper. The resulting filtrate was adjusted to 2 ml with phosphate buffer. These samples were then placed in a water bath maintained at 27 ± 2°C. To prepare a blank, 6 ml of 5% trichloroacetic acid (TCA) was added to one of the tubes before the casein was introduced. Next, 2 ml of casein solution was added to all tubes, including the blank, and the mixtures were incubated for 20 minutes. To stop the reaction, 6 ml of TCA was added to the experimental tubes. The mixtures were left to stand for an additional hour at room temperature, after which they were filtered again using Whatman No. 42 filter paper. The absorbance of the filtrates along with trypsin standard solutions was measured at 280 nm using a spectrophotometer. The trypsin inhibitor content was expressed in milligrams per gram of sample, as calculated below:

$$\text{Trypsin Inhibitor (TUImg)} = = \frac{\text{Absorbance of standard} - \text{Absorbance of sample} \times \text{Dilution factor}}{(0.19 \times \text{sample weight}) \times (1000 \times \text{sample size})}$$

$$\text{Essential amino acid score} = = \frac{\text{g essential amino acid in 100g of total protein}}{\text{g essential amino acid in 100g}} \times 100$$

Determination of oligosaccharide content. Flour samples produced from fermented and germinated velvet beans were analyzed for oligosaccharide content using the extraction method described by [17]. Each sample was mixed with 100 ml of 80% ethanol. After thorough extraction, both the extract and ethanol washings were combined and concentrated to a final volume of 100 ml under reduced pressure at 50°C. Separation and identification of the oligosaccharides were carried out through thin layer chromatography (TLC), with sugar types - raffinose, saccharose, and stachyose - confirmed by comparing their migration patterns to known standards. Quantitative determination followed a guide-strip technique. The sugar spots observed on the TLC plates were carefully scraped into test tubes and soaked in 2 ml of distilled water at room temperature overnight. To measure the concentrations of individual oligosaccharides, the extracts were treated using the thiobarbituric acid reaction. Reference standards ranging from 10 to 100 µg/ml of each sugar were used to calibrate the response and ensure accuracy in quantification.

Determination of amino acid profile. The procedure described by [18] was used to analyze the amino acid composition of the protein extracted from the flour samples. Protein extracts were diluted with citrate buffer (pH 2.2) to a concentration of 0.5 mg/ml. This solution was filtered through a 0.45 µm Millipore membrane and reacted with O-phthaldialdehyde (OPA) using an automated pre-column derivatization system. Subsequent analysis of amino acids was carried out using reverse-phase high-performance liquid chromatography (HPLC), employing a Denali C18 column (4.6 x 150 mm, 5 µm) on a HITACHI L-7400 system from Japan. The mobile phase was delivered at a steady flow rate of 1 ml/min, and detection was done using a fluorescence detector. Cystine levels were assessed separately from the main analysis. To determine tryptophan content, known quantities of protein were placed in sealed glass ampoules containing 1 ml of 5M NaOH. These were heated at 110°C for 18 hrs. After hydrolysis, tryptophan levels were quantified using a colorimetric method. Amino acid concentrations were reported as grams per 100 grams of protein and compared with established reference standards. Essential amino acid scores were subsequently calculated.

Fatty acid profile determination. The fatty acid composition of fermented *Mucuna* bean samples, both dehulled and undehulled, was assessed following the procedure outlined by [18]. Total lipids were first extracted from the fermented, germinated, and control flour samples using a chloroform–methanol mixture in a 2:1 (v/v) ratio. The extracted lipids were then converted to their corresponding methyl esters for analysis. Fatty acid methyl esters were analyzed using gas chromatography (Model ABD20A, ASHMACO, Japan), fitted with a flame ionization detector and a glass column (2 m × 3 mm) packed with 1% diethylene glycol succinate on Chromosorb W. The operating conditions included an injector temperature of 200°C and a detector temperature of 210°C. The oven temperature was programmed to start at 180°C, and nitrogen was used as the carrier gas at a flow rate of 30 ml/min

Statistical Analysis. All data obtained were subjected statistical analysis using ANOVA to detect differences in means and Duncan Multiple Range Test to separate the means. SPSS 2010 statistical package was used for this purpose.

3. Results and discussions

3.1. *Nutritional changes in Mucuna utilis during fermentation and dehulling*

The proximate composition of dehulled and undehulled fermented and unfermented mucuna utilis flours are shown in Table 1. Fermentation had a measurable impact on the protein content of both dehulled and undehulled *Mucuna utilis* samples. In the undehulled samples, protein content increased slightly from 24.68% in the unfermented state to 24.72% after fermentation. In dehulled samples, a more notable increase was observed from 29.6% in the unfermented sample to a peak value of 31.35% following 72 hr of fermentation. These findings align with [6], who observed that extended fermentation durations typically result in higher protein concentrations.

The recommended daily intake of protein for children ranges between 23.0–36.0 g, while adults require approximately 44–56 g [19] (NRC, 1989). Based on these values obtained from Table 1, velvet bean has the potential to meet protein requirements for children, especially in regions where animal protein is scarce. Beyond its nutritional role, protein also

Table 1. Proximate composition of dehulled and undehulled fermented flour from *mucuna utilis*

Parameters (%)		Protein	Fat	Moisture content	Ash	Crude fibre	Carbohydrate
Unfermented	Undehulled	24.68±0.05^{a}	5.54±0.02^{b}	10.79±0.02^{b}	4.19±0.03^{a}	3.92±0.02^{a}	50.88±0.01^{e}
	Dehulled	29.60±0.03^{a}	6.52±0.05^{b}	9.88±0.02^{b}	4.43±0.03^{a}	4.15±0.02^{a}	45.42±0.05^{d}
24 h	Undehulled	24.63±0.02^{b}	5.47±0.02^{b}	10.66±0.01^{a}	4.14±0.02^{a}	3.88±0.03^{b}	55.22±0.04^{a}
	Dehulled	31.05±0.06^{b}	6.48±0.05^{b}	10.83±0.02^{a}	4.40±0.02^{a}	4.12±0.04^{b}	43.12±0.05^{f}
48 h	Undehulled	24.68±0.03^{b}	5.41±0.00^{a}	10.82±0.04^{a}	4.09±0.03^{a}	3.82±0.11^{b}	51.52±0.02^{b}
	Dehulled	31.22±0.02^{b}	6.43±0.03^{a}	10.72±0.02^{a}	4.42±0.04^{a}	4.12±0.06^{b}	43.07±0.0^{g}
72 h	Undehulled	24.72±0.03^{b}	5.39±0.02^{c}	10.59±0.00^{a}	4.04±0.03^{a}	3.74±0.01^{c}	51.18±0.02^{c}
	Dehulled	31.35^{b}±0.01	6.39±0.02^{c}	10.73±0.04^{a}	4.38±0.05^{a}	4.08±0.05^{c}	43.09±0.01^{g}

Values with different superscripts within the same row are significantly different from each other at ($p \leq 0.05$).

contributes to the sensory qualities of food, including texture and flavor [20, 21]. In response to protein deficiencies across many developing regions, attention has turned toward underutilized plant sources such as legumes [11, 23], often referred to as "protein tablets" due to their dense nutritional content [24]. The protein values obtained in this study support the consideration of *Mucuna utilis* as a viable alternative protein source. Fat content showed a consistent decrease across all samples after fermentation. In dehulled samples, fat content dropped from 5.54% to 5.39%, while in undehulled samples, the decline was from 6.52% to 6.39%. The lowest fat content (5.38%) was observed in the dehulled sample after 72 hr of fermentation. This decline mirrors the observations of [25], and may be attributed to the action of microbial lipase enzymes during fermentation. Similar reductions in fat content during germination and sprouting have been reported in legumes like cowpea and watermelon seeds [26, 27]. A possible mechanism includes enzymatic breakdown of protein-lipid and carbohydrate-lipid bonds, which enhances lipid extraction during processing [28]. Despite reductions during processing, fats remain crucial for human nutrition. They are a dense source of energy, facilitate the absorption of fat-soluble vitamins, and do not add bulk to the diet [29]. Moisture content also varied with treatment. In the undehulled samples, a slight decline was observed, from 10.78% to 10.59% with fermentation. In contrast, the moisture content in dehulled samples increased from 9.88% to 10.73%. These values fall within acceptable limits for shelf stability, as microbial growth is generally inhibited below 12% moisture content [30]. The results are consistent with previous findings on breadfruit seeds from the Calabar rainforest [31], though they remain lower than those reported for *Artocarpus communis* [32]. Moisture levels can be influenced by several factors, including harvest timing, environmental conditions, and post-harvest storage. Low moisture content is typically beneficial for preserving nutrient stability and prolonging shelf life [33]. Ash content, which reflects the mineral composition of the samples, ranged from 4.19% to 4.04% in undehulled samples and from 4.43% to 4.38% in dehulled samples. These findings suggest a higher concentration of inorganic matter in fermented samples, although dehulling appeared to slightly reduce ash content as fermentation progressed, consistent with [34]. The 72-hr fermented undehulled sample showed the lowest ash content (4.04%). The mineral content falls within the recommended 1.5–2.5% range for feed-grade materials [35], underscoring its suitability for livestock nutrition. Crude fiber content declined with fermentation, particularly in samples fermented for extended periods. Dehulled samples showed slightly higher fiber levels (4.15%–4.08%) compared to undehulled ones (3.92%–3.74%). The reduction in fiber was statistically significant, supporting earlier observations that fermentation reduces fiber content in legumes. Crude fiber plays a vital role in human health, aiding digestion and preventing gastro-intestinal conditions such as constipation and colon-related disorders [36]. Carbohydrate content also tended to decline during fermentation, as some sugars were utilized for metabolic energy by the developing embryo or fermenting microbes. This trend is supported by earlier studies which reported carbohydrate reduction during sprouting [37]. Similar patterns were noted by [38], who reported that both fermentation and dehulling lead to significant reductions in crude fiber and carbohydrate content in legumes.

3.2. *Physico-chemical analysis of starch extract from dehulled and undehulled Mucuna utilis flour*

The physico-chemical properties of starch extracted from dehulled and undehulled mucuna utilis flour is shown in Table 2. Starch which is a known natural carbohydrate polymer and made up of amylose (linear chains) and amylopectin (branched chains). Starch physico-chemical properties determine how it behaves during processing, cooking, and storage which influence its uses in food, pharmaceutical, and industrial applications**.** The swelling capacity and solubility increased slightly as the fermentation period increased for both dehulled and undehulled samples. The dehulled samples ranged from 10.62–10.78% and 72 hr dehulled and fermented sample has the highest value (10.78%) while the undehulled ranged from 10.37-10.56% and 72 hr undehulled and fermented sample has the highest value (10.56%). The solubility in the dehulled samples ranged from 9.42–9.64% and 72 hr dehulled and fermented sample has the highest value (9.64%) while the undehulled ranged from 9.22–9.38% and 72 hr undehulled and fermented sample has the highest value (9.38%). Water absorption capacity was observed to show an increased value in both dehulled and undehulled samples as the fermentation temperature increased as shown in Table 2. According to [39], water absorption capacity refers to the highest amount of water a food material can absorb and retain under specific formulation conditions. This property is closely linked to the material's dryness and internal porosity. In the present study, higher water absorption values were recorded in the dehulled fermented samples, suggesting that dehulling may enhance the impact of fermentation - likely by altering the structure of the samples in a way that improves water retention, this is in agreement with the observation of [40] who reported that dehulling generally increased the water absorption capacity of cowpea.

Table 2 Physico-chemical analysis of starch extract from dehulled and undehulled fermented mucuna flour

	Analysis	Swelling power(%)	WAC	Solubility (%)
Unfermented	Dehulled	10.62±0.03^{a}	168.92±0.02^{c}	9.42±0.03^{a}
	Undehulled	10.37±0.02^{a}	153.77±0.05^{d}	9.22±0.02^{a}
24 h	Dehulled	10.69±0.04^{b}	170.08±0.03^{b}	9.47±0.02^{b}
	Undehulled	10.42±0.05^{a}	158.77±0.02^{b}	9.26±0.04^{b}
48 h	Dehulled	10.74±0.03^{b}	172.28±0.04^{a}	9.53±0.02^{b}
	Undehulled	10.48±0.02^{a}	162.24±0.02^{c}	9.33±0.02^{b}
72 h	Dehulled	10.78±0.04^{a}	178.66±0.03^{c}	9.64±0.02^{b}
	Undehulled	10.56±0.01^{a}	164.55±0.02^{b}	9.38±0.02^{a}

Values with different superscripts within the same row are significantly different from each other at ($p \leq 0.05$). WAC=water absorption capacity

3.3. *Physico-chemical analysis of fermented dehulled and undehulled mucuna utilis flour*

Physico-chemical properties of starch extracted from mucuna utilis is shown in Table 3. pH is known to Influence starch stability, enzyme susceptibility, and suitability for food applications. The pH value ranged from 5.18–5.2 for the dehulled samples, while it ranged from 5.23-5.3 for the undehulled samples. There was no significant difference in pH of both dehulled and undehulled samples.

Table 3 Physico-chemical analysis of fermented dehulled and undehulled *Mucuna utilis* flour

Analysis		pH	TTA	RI
Unfermented	Dehulled	5.18±0.0.02^{a}	0.38±0.01^{d}	46.59±0.02^{a}
	Undehulled	5.23±0.01^{a}	0.57±0.05^{c}	46.28±0.02^{b}
24 hr	Dehulled	5.28±0.02^{b}	0.35±0.05^{c}	46.63±0.03^{a}
	Undehulled	5.33±0.04^{b}	0.61±0.01^{a}	46.38±0.04^{a}
48 hr	Dehulled	5.36±0.02^{c}	0.36±0.05^{c}	46.67±0.04^{a}
	Undehulled	5.24±0.04^{c}	0.59±0.05^{b}	46.47±0.07^{c}
72 hr	Dehulled	5.22±0.03^{c}	0.37±0.05^{a}	46.7±0.02^{a}
	Undehulled	5.33±0.00^{c}	0.63±0.01^{b}	46.54±0.05^{c}

Values with different superscripts within the same row are significantly different from each other at ($p \leq 0.05$). TTA=Titratable acidity, RI= Reconstitution index

The result obtained indicated that the flour is a low acid food. The value obtained for the pH corresponds with the report of [34]. There was no significant difference in the titratable acidity value obtained for both dehulled and undehulled samples. The titratable acidity (TTA) values were relatively low which conforms with the result obtained by [41]. It ranged between 0.38–0.37 for dehulled samples and 0.57–0.63 for undehulled samples. The reconstitution index (RI) increased as fermentation period increased for both the dehulled and undehulled samples. It ranged from 46.59% to 46.7% in dehulled samples and 46.28 to 46.5% in undehulled samples. The dehulled sample fermented for 72 hr has the highest value (46.7%) while the unfermented sample has least value (46.59%). The undehulled sample fermented for 72 hr has the highest value (46.5%) while the unfermented sample has least value (46.28%).

3.4. Mineral analysis of dehulled and undehulled fermented mucuna flour

Table 4 presents the effect of fermentation and dehulling on the minerals content of the legume *mucuna utilis* flour. The minerals contents of the Mucuna utilis flour samples were affected by fermentation, length of fermentation and dehulling. The result showed that as the fermentation time increased, the mineral content decreased. The content of calcium reduced from 43.12% to 29.42% for all the dehulled mucuna samples while calcium also reduced for undehulled Mucuna samples from 68.42% to 59.33%. Iron also experienced a reduction in the content from 12.22% to 11.56% for all the dehulled Mucuna samples while the undehulled samples reduced from 16.88% to 16.09%.

Table 4 shows that fermented Mucuna (dehulled and undehulled) samples are rich in essential minerals like calcium, iron, magnesium, phosphorus, and potassium. These minerals are crucial for growth, bone formation, and metabolic activities [41]. Mucuna seeds can provide a significant amount of these minerals, particularly calcium, which is vital for strong bones and teeth. The reduction in mineral content after germination may be due to leaching of solids during fermentation. Dehulling further decreased mineral levels, likely due to the removal of mineral-rich hull portions. Specifically, iron and calcium levels declined after dehulling, suggesting their presence in the hull. Overall, fermented Mucuna seeds can be a valuable source of essential minerals, contributing to human nutritional needs.

Table 4 Minerals composition of fermented and unfermented mucuna utilis flour (mg/100g)

Minerals		Calcium	Iron	Magnesium	Phosphorus	Potassium
Unfermented	Dehulled	43.12±0.04^{a}	12.22±0.06^{c}	88.42±0.05^{b}	265.42±0.03^{c}	1077.33±0.05^{c}
	Undehulled	68.42±0.01^{b}	16.88±0.06 ^{a}	167.33±0.02^{a}	386.22±0.02^{c}	1221.05±0.01^{b}
24 h	Dehulled	39.55±0.06^{b}	11.89±0.05^{c}	72.36±0.06^{c}	257.44±0.04^{b}	1065.18±0.02^{b}
	Undehulled	66.22±0.05^{b}	16.58±0.03^{b}	158.42±0.02^{b}	374.12±0.01^{b}	1218.33±0.02^{c}
48 h	Dehulled	33.58±0.05^{c}	11.74±0.04^{a}	65.42±0.05^{c}	253.12±0.05^{a}	1055.12±0.04^{a}
	Undehulled	61.42±0.01^{c}	16.37±0.02^{b}	140.22±0.01^{c}	369.55±0.04^{c}	1209.77±0.02^{a}
72 h	Dehulled	29.42±0.02^{c}	11.56±0.01^{a}	56.33±0.02^{a}	249.77±0.04^{c}	1037.22±0.02^{d}
	Undehulled	59.33±0.02^{d}	16.09±0.01^{b}	136.44±0.03^{c}	360.12±0.02^{c}	1200.12±0.01^{d}

Values with different superscripts within the same row are significantly different from each other at ($p \leq 0.05$).

Calcium is essential for blood coagulation and intracellular cement substances. Notably, potassium is a key mineral in many foods, including those commonly consumed in Nigeria[42]. Potassium has been shown to enhance iron utilization [43] and is abundant in various seeds and legumes, such as mucuna beans [27, 44,] and fluted pumpkin [45]. Additionally, potassium's role in controlling blood pressure and preventing stroke [46] suggests its potential benefits in alleviating diabetes.

3.5. Anti-nutritional analysis of the fermented flour dehulled and undehulled mucuna utilis flour

Table 5 presents the anti-nutrient levels in fermented Mucuna utilis flour, comparing dehulled and undehulled samples. It was observed that all the anti-nutritonal factors studied (phytic acid, trypsin inhibitor and oligosaccharides) tested for were present in detectable amounts. However, they were all reduced significantly in quantities after 72 hr fermentation. After 72 hr fermentation period, the lowest quantity of the anti-nutritional factors was respectively; 7.25mg/g, 22.27mg/g and 1.88mg/g all in dehulled sample (Table 5). The results from this study gave an indication that phytic acids are very stable to heat. This implies that fermentation has the potential to eliminate these anti nutritional factors in food material. Phytates in legumes can reduce mineral bioavailability and inhibit protein and starch digestion [47].

Table 5 Anti-nutritional analysis of fermented flour from *Mucuna utilis*

Anti-nutrients		Phytic Acid (mg/100g)	Trypsin Inhibitor (TUImg)	Oligosaccharide (%)
Unfermented	Dehulled	7.36±0.02^{d}	22.27±0.02^{d}	3.78±0.03^{d}
	Undehulled	7.60±0.01^{d}	26.22±0.02^{d}	1.78±0.01^{b}
24 hr	Dehulled	7.33±0.02^{c}	22.29±0.02^{c}	3.88±0.01^{c}
	Undehulled	7.48±0.12^{c}	23.44±0.25^{c}	1.89±0.012^{a}
48 hr	Dehulled	7.28±0.02^{a}	22.29±0.05^{a}	4.20±0.25^{a}
	Undehulled	7.43±0.12^{a}	23.48±0.15^{a}	2.06±0.15^{c}
72 hr	Dehulled	7.22±0.12^{b}	22.21±0.05^{b}	4.38±0.02^{b}
	Undehulled	7.33±0.12^{b}	23.52±0.15^{b}	2.15±0.15^{d}

Values with different superscripts within the same row are significantly different from each other at ($p \leq 0.05$).

Combining pre-processing treatments like soaking, dehulling, fermentation, and milling can reduce anti-nutrient content, particularly since anti-nutrients are more concentrated in the hull [48].

3.6.Amino acid profile of the fermented flour

The amino acid content of dehulled and undehulled mucuna utilis is shown in Table 6. The amino acid profile of a food product indicates its protein quality and nutritive value. Analyzing amino acid composition helps identify and potentially remove undesirable fractions, enhancing the protein's nutritional value [49]. The amino acid content in the dehulled and undehulled mucuna flour samples increased as the fermentation period increases. This is shown in the (Table 6). This is in line with the observations reported by [6] that marked increases in amino acid was noticed as the period of fermentation increased. Significant differences were observed in the amino acid content of the flour samples, with glutamic acid and aspartate being the most abundant. Fermentation potentially enhances the nutritional profile of Mucuna utilis flour. The findings align with previous studies [49, 50, 51]. Fermentation can improve taste, aroma, and texture, prolong shelf life, increase lysine and vitamins B and C [52] and enhance protein solubility. Similar studies on fermented foods, like soybean [53] as cited by [54], show significant increases in free amino acids and ammonia. Velvet bean flour also contains essential amino acids, which increase with sprouting [23].

Table 6 Amino acid content dehulled and undehulled fermented velvet flour(g/100g)

Amino Acid	0 hr		72 hr	
	Undehulled	Dehulled	Undehulled	Dehulled
Histidine	14.33±0.05^{d}	20.14±0.01^{b}	17.42±0.03^{c}	24.58±0.02^{a}
Isoleucine	26.77±0.02^{d}	39.23±0.02^{b}	30.14±0.05^{c}	49.36±0.02^{a}
Leucine	48.77±0.01^{d}	66.82±0.05^{b}	52.22±0.02^{c}	82.67±0.01^{a}
Lysine	44.66±0.06^{c}	58.44±0.07^{b}	35.12±0.01^{d}	69.57±0.02^{a}
Methionine	5.12±0.07	8.27±0.02^{c}	8.89±0.04^{b}	11.75±0.03^{a}
Tyrosine+Phenylalanine	50.22±0.04^{d}	87.23±0.04^{b}	54.17±0.05^{c}	101.08±0.01^{a}
Tryptophan	7.26±0.05^{b}	14.33±0.05^{c}	5.22±0.02^{d}	7.89±0.03^{a}
Threonine	26.77±0.03^{d}	39.35±0.02^{b}	29.44±0.02^{c}	41.57±0.03^{a}
Valine	44.77±0.02^{c}	48.78±0.04^{b}	32.48±0.01^{d}	51.82±0.05^{a}

Values with different superscripts within the same row are significantly different from each other at ($p \leq 0.05$).

3.7.Fatty acid profile of dehulled and undehulled fermented velvet flour

The composition of fatty acid oil have been reported to be used in evaluating nutritional value of oil because it gives an indication of stability and rate of degradation of oil. Table 7 represents the fatty acid profile for dehulled and undehulled oil samples of mucuna utilis seeds. Findings from the study revealed that linoleic acid is the major fatty acid in both dehulled and undehulled fermented mucuna utilis oils with sprouted sample having a higher value of 68.680% and a value of 65.294% for un-sprouted sample. The high linoleic acid values is similar to those found in white and black seeds of watermelon which was 68% and 66% respectively reported by [55] and lower than 76.24% reported by [56] for

Citrullus vulgaris seed oil and [27] reported for sprouted and un-sprouted watermelon seeds. The fatty acid profiles (Table 7) reveal that all the dehulled and undehulled flour samples contain high levels of linoleic acid, 16.98mg/100g and 27.02mg/100g. Linoleic acid is a component of essential fatty acids that must made available in food which may produce oleic acid from stearic acid during synthesis of unsaturated fatty acids [57]. Although, linoleic acid may produce alpha-linolenic acid in plants and gamma-linolenic acid in animal through a process called desaturation which makes linoleic acid an essential component of human diet. Palmitic acid is present in large quantity in the dehulled and undehulled flour samples, 16.44mg/100g and 26.33mg/100g. Oleic acid, a monounsaturated fatty acid, enhances edible oil stability by resisting thermal degradation. Palmitic acid, a saturated fatty acid, doesn't raise blood cholesterol when esterified in the alpha-position (e.g., palm oil), but may be hypercholesterolemia in the beta-position (e.g., butter fat) (Ng, 1994)[53] . Free fatty acids oxidize faster than their esters, acting as pro-oxidants. However, in small quantities, they may not significantly impact oxidative stability. The result obtained from this study have shown that the quantity of unsaturated fatty acid oil is higher than the quantity obtained for saturated fatty acid oil for both dehulled and undehulled *mucuna utilis* seed. Saturated fat is known to be responsible for the production high content of cholesterol in the body which in turn decreases insulin sensitivity. The study have also shown high content of unsaturated fatty acid for linoleic acid showing that *mucuna utilis* seed flour could be beneficial for consumption and food supplementation. Linoleic acid is also very useful in blood lipids and reduction of blood pressure and serum cholesterol [57, 58]. Saturated fatty acids may contribute to increased cholesterol production and reduced insulin sensitivity, potentially leading to increased body fat. Linoleic acid is a crucial essential fatty acid obtained through diet, necessary for synthesizing unsaturated fatty acids. In contrast, oleic acid is produced in the liver from stearic acid. Due to its importance, linoleic acid is a vital dietary component.

Table 7 Fatty acid profile of dehulled and undehulled fermented velvet flour (mg/100g)

Fatty Acid	0 hr	24 hr	48 hr	72 hr
	Undehulled	Dehulled	Undehulled	Dehulled
Palmitic acid (C16:0)	16.35±0.02^{d}	25.33±0.01^{b}	16.44±0.05^{c}	26.33±0.02^{a}
Stearic acid (C18:0)	9.65±0.03^{d}	14.78±0.03^{b}	9.74±0.03^{c}	15.12±0.04^{a}
Oleic acid (C18:1)	10.37±0.05^{d}	17.12±0.06^{b}	10.48±0.05^{c}	17.33±0.02^{a}
Linoleic acid (C18:2)	16.88±0.08^{d}	27.48±0.03^{a}	16.98±0.02^{c}	27.02±0.01^{b}
Linolenic acid (C18:3)	9.75±0.03^{d}	11.16±0.01^{b}	9.86±0.01^{c}	11.22±0.05^{a}
Behenic acid (22:0)	1.14±0.02^{d}	1.66±0.05^{b}	1.22±0.05^{c}	1.93±0.03^{a}

Values with different superscripts within the same row are significantly different from each other at ($p \leq 0.05$).

4. Conclusions

This study reveals that fermentation is a very viable means of detoxifying velvet beans *(Mucuna utilis*). It also reveals that the length of fermentation affected the nutritional and anti-nutritional factors as well as the physico-chemical properties of the beans. Further more, dehulling contributed to the significant effect of fermentation on dehulled samples when compared with the undehulled ones. The chemical properties of the *Mucuna utilis* flour obtained from the result indicated that there is an increased in protein content and reduction in fibre content as fermentation period increased. The levels of anti-nutritional factors, that is tyrpsin inhibitor, phytic acid and oligosaccharides decreased significantly in all the samples after fermentation. Amino acid profile increased with increase in fermentation period. Undehulling significantly impacted the amino acid profile, resulting in higher levels in undehulled samples compared to dehulled ones. Combining fermentation with dehulling improved legume quality by enhancing nutrient bioavailability, increasing digestibility and reducing anti-nutrient content. This processing combination can potentially optimize nutritional benefit.

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