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Effect of Fermentation on the Nutritional, Anti-Nutritional and Functional Properties of Horse Eye Beans (*Mucuna urens*) Flour

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Authors' contributions

This work was carried out in collaboration between both authors. Author FAB designed and supervised the study. Author VTU managed the analyses, managed the literature search, performed the statistical analysis and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: To evaluate proximate, mineral, anti-nutrient and functional properties of unfermented and fermented horse eye bean (*Mucuna urens*) flour.
Study Design: Single factor (one way).
Place and Duration of Study: Department of Food Science and Technology, Faculty of Agriculture, University of Uyo, Uyo, Akwa Ibom State, Nigeria between August 2016 to October 2016.
Methodology: The seeds were sorted, washed, cracked open, dehulled and divided into four groups. The first portion was milled and sieved to get fine flour which served as control (UMUA). The second, third and fourth portions were fermented for 24, 48 and 72 h and coded as FMUB, FMUC and FMUD, respectively. The fermented beans were drained, dehulled, cubed into smaller sizes oven dried, milled and sieved to get fine flour. The flours obtained were analysed using standard analytical methods.
Results: The result obtained from proximate composition showed that fermentation significantly ($p<0.05$) increased the crude protein, crude fat and ash content of the samples when compared

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with the unfermented sample (control). However, crude fibre and carbohydrate contents were significantly decreased as fermentation period increased. The selected minerals analysed (i.e. iron and phosphorus) exhibited a significant ($p<0.05$) increase in fermented flour samples except magnesium, potassium and sodium content that showed a significant decrease. More so, fermentation showed a significant decrease in all the anti-nutrients analysed especially phytate which usually limits the bioavailability of minerals and essential trace elements like iron which strengthens the immune system. Functional properties of fermented samples showed significant decreased in bulk density, with significant ($p<0.05$) increase in water and oil absorption capacity as well as gelation temperature.

Conclusion: Fermentation improves the nutrient composition, reduces anti-nutritional composition of horse eye beans thereby increasing its safety for human consumption and enhanced its functionalities in food formulations.

Keywords: Legume; mucuna; nutrients; flour; anti-nutrients.

1. INTRODUCTION

Grain legumes are major sources of dietary proteins in the developing countries, as animal proteins are expensive [1]. In addition to their protein contributions, legumes are also rich in other nutrients such as starch, dietary fibre, phytochemicals, vitamins and mineral. Indigenous legumes are an important source of affordable alternative protein to people in many tropical countries especially in Africa where they are predominantly consumed. Horse eye bean (*Mucuna urens*), a specie of *Mucuna* seed is a leguminous plant naturally found in tropical and sub-tropical regions of the world. The wild legume *Mucuna* consists of about 100 varieties which are in great demand as food, pharmaceutically valued products and livestock feed [2]. Horse eye bean is commonly found in the home gardens in the South Eastern parts of Nigeria, where the Efik's, Ibibio's and Igbo's use the seeds as a major soup condiment for thickening. It is called 'ibaba' by the Efik's and Ibibio's, 'ukpor' by the Ibo's, 'yerepe' by the Yorubas and 'karasau' by the Hausas. It is an underutilized legume in Nigeria and studies had shown that the bean is a good source of high protein, high carbohydrates, high fibre, low lipids, and adequate minerals [3,4,5,6].

The utilization of raw horse eye bean as human food is limited by the presence of naturally occurring anti-nutritional factors such as phytic acid (phytate), tannins, cyanogenic glucoside (cyanide), oxalate and gossypol. These factors negatively affect the nutritive value of the beans through direct and indirect reactions by inhibiting proteins and carbohydrate digestibility, induce pathological changes in the intestine and liver tissues thus affecting metabolism [7]. Soaking, dehulling and fermentation are important traditional methods used to reduce phytic acid. Generally, a significant increase in quantity as well as quality of the food proteins is observed while the anti-nutritional factors show a decline during fermentation [8]. Fermentation actually holds promise as a food processing method that can be used to diversify the food uses of some under exploited plant foods like horse eye bean. Currently, no attempt has been made to analyze the effect of fermentation on horse eye bean. Therefore, the objectives of this study are to evaluate proximate, minerals, anti-nutrients and functional properties of horse eye bean.

2. MATERIALS AND METHODS

2.1 Source of Sample and Preparation

Horse eye bean (*Mucuna urens*) was bought from Akpan Andem market in Uyo metropolis. The seeds were treated separately. First portion, which served as the control (UMUA) was sorted, washed with water, cracked open and dehulled. The sample were washed again, drained and cubed into smaller sizes for easy drying in the conventional air oven (model pp 22 US, Genlab, England) at 60°C for 24 h. After oven drying, the sample was milled using local attrition mill and sieved to pass through 0.25 mm mesh sieve to get fine flour. Second, third and fourth portion was sorted, washed with water, cracked opened and fermented by soaking the seeds in distilled water using ratio 1:5 (w/v). The samples were coded as FMUB, FMUC, and FMUD for 24, 48 and 72 h, respectively. After fermenting, the bean was drained, dehulled, cubed into smaller sizes and oven dried in conventional air oven (model pp 22 US, Genlab, England) at 60°C for 24 h, milled using local attrition mill and sieved to pass through a 0.25 mm mesh sieve to get fine flour.

The flour was packaged in plastic containers, labeled and stored at 4°C for analysis.

2.2 Sample Analysis

2.2.1 Determination of proximate composition

The crude protein, crude fat, crude fibre and ash of the unfermented and fermented horse eye bean flour were determined according to the method described by [9]. Carbohydrate content was determined from the percentage difference of the other proximate indexes as follows:

%carbohydrate = 100 – (%crude protein + %crude fat + %crude fibre + %ash).

2.2.2 Determination of mineral content

Selected minerals were determined in unfermented and fermented flour samples using the method described by [9]. Two (2) grams of the samples were ashed in a furnace at 550°C for 4 h. The ash was allowed to cool and leached with 5 ml of 6 M HCl. The volume was made up to 20 cm^3 with distilled water. The blank determination was also carried out in a similar way as described above except for the omission of sample. Potassium in the solution was determined by flame photometry method using an instrument called flame photometer while other selected minerals (sodium, magnesium, phosphorus and iron) were determined from the resulting solution using Atomic Absorption Spectrophotometer (AAS Model SP9).

2.2.3 Determination of anti-nutritional composition

This was determined by Spectrophotometric method as described by [10]. Selected anti-nutrients determined in unfermented and fermented horse eye beans flour were tannins, phytates, cyanide, oxalates and saponin.

2.2.4 Functional properties determination

The bulk density, water absorption capacity, oil absorption capacity and gelation temperature of the samples were determined using the method described by [10].

2.3 Statistical Analysis

All triplicate data were subjected to one-way analysis of variance and the significance differences between the means were analyzed using Duncan's new multiple range test via SPSS software for window version 20.0 statistical packages (SPSS Inc.). All statistical tests were performed at a 5% ($p<0.05$) significance level.

3. RESULTS

The result of proximate composition for unfermented and fermented horse eye beans is presented in Table 1. Fermentation significantly ($p<0.05$) increased the crude protein content of the flour samples from 13.42 to 18.63% for UMUA (control) and FMUD (72 h fermented sample). There was no significant ($p>0.05$) difference between FMUB (24 h fermented samples) and FMUC (48 h fermented samples). Crude fat showed an increase from 5.70% in UMUA to 9.13% in FMUD. Crude fibre was seen to have decreased from 3.27% in UMUA to 2.31% in FMUD. No significant ($p>0.05$) difference was observed in FMUB and FMUC. Ash content also reflected an enormous increase from 1.73% in UMUA to 3.15% in FMUD while carbohydrate content decreased from 75.88% (UMUA) to 66.78% (FMUD).

Table 2 shows the result of mineral composition of unfermented and fermented horse eye beans flour samples. Iron content was significantly ($p<0.05$) higher in fermented flour when compared to unfermented flour (UMUA). It ranged between 6.15 (UMUA) to 8.75 mg/100 g (FMUD). The magnesium content in the flour samples decreased from 83.66 in UMUA to 70.33 mg/100 g in FMUD. The entire samples were significantly ($p<0.05$) different. The most abundant mineral in the horse eye bean flour was potassium. An observable difference was noticed where there was significant ($p<0.05$) decreased from 500.00 mg/100 g in UMUA to 85.00 mg/100 g in FMUD. Phosphorous had an increase from 185 (UMUA) to 340.00 mg/100 g (FMUD). Sodium content decreased from 10.30 to 2.30 mg/100 g in UMUA and FMUD, respectively.

Fermentation significantly ($p<0.05$) reduced the anti-nutrients content of horse eye beans flour samples (Table 3). There was decreased in anti-nutrients content with longer fermentation period. The trend shows that tannin decreased from 1.00 (UMUA) to 0.47 mg/100 g (FMUD). The fermented samples were significantly ($p<0.05$) different except for FMUB and FMUC which were not significantly ($p>0.05$) different. Phytate also reduced from 2.91 (UMUA) to 0.79 mg/100 g

(FMUD). The samples were significantly different except UMUA and FMUC which were not significantly (p>0.05) different. Reduction in hydrogen cyanide content was observed from 1.08 to 0.54 mg/100 g for UMUA and FMUD, respectively. Oxalate content decreased from 0.33 (UMUA) to 0.12 mg/100 g (FMUD). The samples were significantly different except FMUC and FMUD which were not significantly (p>0.05) different. Saponin also decreased from 1.20 to 0.61 mg/100 g for UMUA and FMUD.

The result of functional properties of unfermented and fermented horse eye beans flour samples is presented in Table 4. Bulk density decreased from 0.83 (UMUA) to 0.60 g/ml (FMUD).

Table 1. Effect of fermentation on proximate composition (% dry basis) of *Mucuna urens* flour samples

Sample code	Crude protein	Crude fat	Crude fibre	Ash	Carbohydrate
UMUA	13.42^{c}±0.69	5.70^{d}±0.42	3.27^{a}±0.36	1.73^{c}±0.12	75.88^{a}±0.02
FMUB	14.51^{b}±0.50	7.13^{c}±0.25	2.80^{b}±0.30	2.37^{b}±0. 24	73.19^{b}±0.01
FMUC	14.66^{b}±0.34	8.20^{b}±0.62	2.68^{b}±0.28	3.01^{a}±0.13	71.45^{c}±0.05
FMUD	18.63^{a}±0.43	9.13^{a}±0.23	2.31^{c}±1.03	3.15^{a}±0.09	66.78^{d}±0.02

Values are means ± SD of triplicate determination. Means in the same column with different superscript are significantly different at (p<0.05). UMUA=Control (Unfermented Mucuna urens flour), FMUB=Fermented Mucuna urens flour (24 h), FMUC= Fermented Mucuna urens flour (48 h), FMUD=Fermented Mucuna urens flour (72 h)

Table 2. Effect of fermentation on mineral composition (mg/100 g) of *Mucuna urens* flour samples

Sample code	Iron	Magnesium	Potassium	Phosphorus	Sodium
UMUA	6.15^{b}±0.07	83.66^{a}±0.57	500.00^{a}±10.00	185.00^{d}±1.00	10.30^{a}±0.36
FMUB	6.50^{b}±0.39	80.00^{b}±1.00	350.00^{b}±10.00	236.06^{c}±2.00	7.56^{b}±0.51
FMUC	7.85^{a}±0.39	74.00^{c}±1.00	250.00^{c}±10.00	256.66^{b}±1.00	4.27^{c}±0.25
FMUD	8.75^{a}±0.83	70.33^{d}±1.52	85.00^{d}±5.00	340.00^{a}±2.00	2.30^{d}±0.48

Values are means ± SD of triplicate determination. Means in the same column with different superscript are significantly different at (p<0.05). UMUA= Unfermented Mucuna urens flour (Control), FMUB=Fermented Mucuna urens flour (24 h), FMUC= Fermented Mucuna urens flour (48 h), FMUD=Fermented Mucuna urens flour (72 h)

Table 3. Effect of fermentation on anti-nutritional composition (mg/100 g) of *Mucuna urens* flour samples

Sample code	Tannins	Phytate	Cyanide	Oxalate	Saponin
UMUA	1.00^{a}±0.01	2.91^{a}±0.03	1.08^{a}±0.07	0.33^{a}±0.02	1.20^{a}±0.02
FMUB	0.72^{b}±0.08	1.91^{b}±0.07	0.89^{b}±0.02	0.22^{b}±0.02	0.84^{d}±0.03
FMUC	0.62^{b}±0.11	1.73^{b}±0.09	0.75^{c}±0.07	0.17^{c}±0.02	0.72^{c}±0.03
FMUD	0.47^{c}±0.05	0.79^{c}±0.17	0.54^{d}±0.05	0.12^{c}±0.20	0.61^{d}±0.01

Values are means ± SD of triplicate determination. Means in the same column with different superscript are significantly different at (p<0.05). UMUA= Unfermented Mucuna urens flour (Control), FMUB=Fermented Mucuna urens flour (24 h), FMUC= Fermented Mucuna urens flour (48 h), FMUD=Fermented Mucuna urens flour (72 h)

Table 4. Effect of fermentation on functional properties of *Mucuna urens* flour samples

Sample code	Bulk density (g/ml)	Water absorption capacity (ml/g)	Oil absorption capacity (g/g)	Gelatinization Temp (˚C)
UMUA	0.83^{a}±0.03	4.20^{d}±0.02	1.80^{d}±0.05	58.00^{c}±2.00
FMUB	0.81^{a}±0.01	4.80^{c}±0.01	2.00^{c}±0.04	60.00^{bc}±1.00
FMUC	0.69^{b}±0.01	4.90^{b}±0.02	2.10^{b}±0.03	62.00^{ab}±1.00
FMUD	0.60^{b}±0.05	5.00^{a}±0.05	2.20^{a}±0.03	64.00^{a}±2.00

Values are means ± SD of triplicate determination. Means in the same column with different superscript are significantly different at (p<0.05). UMUA= Unfermented Mucuna urens flour (Control), FMUB=Fermented Mucuna urens flour (24 h), FMUC= Fermented Mucuna urens flour (48 h), FMUD=Fermented Mucuna urens flour (72 h)

There was no significant ($p>0.05$) difference between UMUA and UMUB, and also UMUC and UMUD. Water absorption capacity significantly ($p<0.05$) increased from 4.20 g/ml for the UMUA to 5.00 g/ml for UMUD. Oil absorption capacity reflected an increase from 1.80 to 2.20 g/ml in UMUA and UMUD, respectively. Gelling temperature also showed an increase from 58.00 in UMUA to 64.00 g/ml in UMUD.

4. DISCUSSION

Increase in crude protein contents in fermented horse eye been flour especially when fermented for longer periods could be attributed to increase in microbial mass during fermentation which led to the extensive hydrolysis of the protein molecules to amino acid [11,12]. This observation agrees with the increased values obtained for fermented locust bean and mesquite bean [13] but differs with the reduction in values obtained from horse eye bean subjected to soaking, cooking and toasting at different period [14]. Fermented horse eye bean flour could therefore be used as an alternative source of protein in the diet and protein supplement in food processing. Fermentation periods also caused significant ($p<0.05$) increase in crude fat due to extensive breakdown of large molecules of fat into simple fatty acids [15]. Reduction in crude fibre could be as a result of the fermenting micro floral which hydrolyze and metabolize them as carbon source (substrate). The value obtained was lower than the values reported for *Afzelia Africana* flour [12]. Fermentation significantly ($p<0.05$) increased the level of ash content in all the fermentations periods. This may be attributed to the reduction of the anti-nutritional factors, specifically phytates which are thought to be responsible in mineral bio-availability [16]. Similar finding was reported for *C. lanatus* and *C. vulgaris* [17]. The carbohydrate values for the fermented samples decreased significantly ($p<0.05$) in contrast to unfermented flour samples. The reduction in carbohydrate during fermentation could be due to bioconversion of the substrate which is usually accompanied by the release of heat. Similar finding was reported for fermented whole and ground African breadfruit (*Treculia africana*) seeds [18].

Minerals are important constituents of human diet as they serve as cofactors for many physiological and metabolic processes [19]. Iron is required for a number of biological functions, including proper functioning of the immune system, gene regulation, cell growth as well as binding and transport of oxygen [20]. The increase in iron and phosphorus contents supports the reports of [14] and [21]. The desirable changes in the nutrient contents may be due to the breakdown of complex compounds into simpler forms. Moreover, magnesium, potassium and sodium content in fermented flour reduces drastically, showing that some quantities might have been lost due to leaching in fermenting water. [22] reported similar finding on African oil bean seeds.

Reduction in the amount of tannins in fermented horse eye beans may be due to its solubility in water [23]. Decrease in phytate content as fermentation period increases may be due to the activity of the endogenous phytases from both raw ingredient and inherent microorganisms which hydrolyses phytic acid in many fermented food preparations into inositol and orthophosphate [24]. Cyanide reduction could be as a result of endogenous hydrolytic enzymes that occurred during fermentation leading to the hydrolysis of cyanogen. [25] also noted that prolonged processing like water soaking could reduce cyanide. Oxalate and phytates combines with phosphorous respectively to form complexes [26]. Reduction in saponin level had a great health benefit as it had been reported to alter cell wall permeability and therefore produce some toxic effects when ingested [18]. The current finding is in agreement with the results reported by [6] and [27].

The bulk density was lowered with longer fermentation period and as such the flour is advantageous in complimentary foods [28]. Less bulky flours have higher nutrient density [29]. Proteins has both hydrophilic and hydrophobic properties therefore, can interact with water and oil in foods [30]. Water holding capacity depends on the water bounding capacities of food components. The high water absorption capacity reported in this study indicates that fermented horse eye bean flour may be used as thickening agent in soup preparation and baked products. [12] reported increased value for fermented mahogany bean (*Afzelia africana*) flour. The values obtained from this studies did not agree with the decreased value reported for hamburger seed flour as the fermentation period increased [6]. The observed increase in oil absorption capacity suggests that it would be useful in formulation of foods like doughnuts and pancakes where high oil absorption is required. The presence of protein exposes more non-polar amino acids to the fat and enhances hydrophobicity as a result of which the flour absorbs more oil [31]. Interactions between carbohydrates, proteins and lipids have been reported to be responsible for the gelation capacity of legumes and oil seed proteins [32]. The increase in the fermentation period led to the increase in the gelling temperature. The ability of the sample to form gels and provide a structural matrix for holding water, flavours, sugars and food ingredient is useful in food formulations and in new product development.

5. CONCLUSION

The result obtained from this study showed that the fermentation significantly ($p<0.05$) increased the content of crude protein, crude fat, ash, iron, potassium, water and oil absorption capacity as well as gelation temperature. Reduction in crude fibre, carbohydrate, magnesium, potassium, sodium and bulk density was observed as fermentation period increased. The highest fermentation period (which was 72 h in this research work) resulted in lowering the concentration of the anti-nutritional factors in horse eye bean flour. Fermentative applications can therefore be developed for improving the nutritional properties, reducing the anti- nutrients content and thereby enhancing the functionalities of horse eye beans flour.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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