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VALORISING *MUCUNA PRURIENS* BEANS, AN INDIGENOUS LEGUME, THROUGH FERMENTATION FOR NUTRIENT AVAILABILITY AND L-DOPA REDUCTION

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ABSTRACT

Food insecurity remains a pressing issue, particularly in developing countries in sub-Saharan Africa where malnutrition remains a major public health problem. Resources as well as the knowledge to prepare quality over quantity food are limited. Indigenous legumes such as *Mucuna pruriens* are important dietary components; although the nutrients in the beans are low, fermentation would render them available for absorption and utilization in the body. While affordable, consumption is limited by anti-nutritional factors particularly L-DOPA which reduce nutrient utilization and may cause adverse effects if not properly processed. *Mucuna pruriens* is a potential alternative protein source. This study evaluated the impact of fermentation on the *Mucuna pruriens* whole seed form. The primary experimental factor was fermentation, and a Completely Randomized Design (CRD) was used, yielding 27 treatments including unfermented or controls. Factors were variety (Black, White, Mottled), fermentation method (brine at 5% and 10% NaCl, or yeast (*Saccharomyces cerevisiae*)1 g/500 g), temperature (25°C or 37°C), and time (24 h or 72 h). After fermentation, samples were ground and analysed for proximate and mineral composition using AOAC methods; L-DOPA was quantified by UV-spectrophotometry. Data were analysed in R (ANOVA; Tukey's HSD at $p< 0.05$). Carbohydrate content increased significantly ($p< 0.05$), for example from 54.01% to 64.12% p=0.02 in Black beans and from 56.00% to 63.09% p=0.013 in Mottled beans, while crude fat and fibre were significantly reduced ($p < 0.01$). Yeast fermentation at 37°C for 72 hours led to a highly significant reduction of L-DOPA by 40 to 60% ($p< 0.001$) from about 6–8 mg/100g in raw beans to 3–4 mg/100g after fermentation, thereby enhancing the safety of the beans. Mineral content also increased, for Mottled variety, calcium content was (19.57 mg/100g to 23.68 mg/100g, p=0.001), and iron was (0.5mg/100g to 1.17 mg/100g, p=0.001). Brine fermentation at 25°C had minimal effect. Among the tested combinations, yeast fermentation at 37°C for 72 h is recommended as the most effective option for improving the nutritional profile and reducing anti-nutritional factors in *Mucuna pruriens* seeds, making them a safer and more viable food option. Further research should focus on optimizing fermentation conditions, microbial selection and preservation to promote its sustainable use.

Key words: Anti-nutritional factors, Bioavailability, Fermentation, Food security, L-DOPA, *Mucuna pruriens*

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INTRODUCTION

Anti-nutritional factors are natural or synthetic compounds that reduce the nutritional quality of many commonly consumed foods like legumes, limiting people's ability to achieve food security[1]. Legumes such as beans contain phytates that can inhibit iron absorption by up to 50%, contributing to iron-deficiency anaemia which affects 40% of pregnant women and 42% of children under five globally [2]. Additionally, lectins, protease inhibitors, tannins, phytates and oxalates impair protein digestibility and mineral absorption, particularly calcium, thereby affecting bone health and overall contributing to malnutrition [3, 4, 5]. One promising solution lies in utilizing underexploited legumes like *Mucuna pruriens*, also known as velvet bean. It can be an important source of food in the diets of people all over the world. Though the nutrients in the beans are low, fermentation would render them available for absorption and utilization in the body [6]. Further, they are crucial in the conservation of agriculture because they enhance the nitrogen fixation in the soil, and thus reducing the need for application of synthetic fertilizers, case study in Western and Coastal regions Kenya [7, 8]. *Mucuna pruriens*, enhances the soil quality and can also act as weed suppressor when grown as a cover crop thus enhancing the yield of next crops [9]. It is commonly cultivated in Eastern and Southern Africa (Kenya, Uganda and Rwanda) in three main varieties: black, white and mottled (speckled), which are typically distinguished by seed coat colour and agronomic traits. These are taxonomically classified *as Mucuna pruriens* var. *pruriens, Mucuna pruriens* var. *utilis* and *Mucuna pruriens* var. *cochinchinensis* [10]. Its high yields and other agronomic characteristics are considered to be an asset as previous studies have even estimated that, in the best of conditions it is capable of producing 3 to 4 tons per hectare [11].

In food context, this legume is known for having a high protein content roughly 20-30% protein and a host of other health advantages [12]. Due to their availability and affordability, beans such as *Mucuna pruriens* can play a role of providing protein especially to those in the rural areas [13]. *Mucuna pruriens* is endowed with an amino acid derivative called L-3, 4-dihydroxyphenylalanine (L-DOPA) that is a precursor to dopamine, widely used in treating Parkinson's disease by refilling depleted dopamine levels in the brain, improving motor function [14]. *Mucuna pruriens* is traditionally used in the diets and healing practices of indigenous communities and also serves as a potential income source for smallholder farmers [15]. However, if not handled well during preparation, anti-nutrients that are specific to *Mucuna pruriens* can reduce protein digestibility and cause toxicity, which may be fatal if consumed in excess with side effects such as nausea, low blood pressure and long-term complications like dyskinesia (involuntary movements), hence its low acceptability as a pulses [16]. Fermentation is a traditional food processing method

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known to reduce anti-nutritional factors and enhance mineral bioavailability in legumes, while also promoting gut health through beneficial microbes [17, 18]. Other methods like leaching, boiling and germination have also been used, but fermentation has shown greater effectiveness in significantly reducing anti-nutrient levels and improving overall nutritional content in other legumes [19].

In order to address persistent food insecurity in sub-Saharan Africa, this study explored the potential of *Mucuna pruriens*, an underutilized legume with promising nutritional and agronomic attributes. Although other bean varieties are widely available, *Mucuna pruriens* is affordable, climate resilient and traditionally used in parts of Africa in many rural areas, however, its use has largely been limited to animal feed or medicinal purposes, leaving its potential as a human food source underexploited. The renewed interest in this legume stems from its ability to thrive under marginal conditions and its high protein content, despite the presence of anti-nutritional factors, especially L-DOPA, which hinder safe utilization. Fermentation was therefore investigated as a low cost, accessible process to reduce anti-nutrients and improve nutrient bioavailability, thereby enhancing the safety and nutritional value of *Mucuna pruriens* for food insecure communities.

MATERIALS AND METHODS

Study area and Sources of raw *Mucuna pruriens* beans and Sampling

This study was conducted at Food Science Laboratory of Jaramogi Oginga Odinga University of Science and Technology (JOOUST), Siaya County in Kenya**.** Dried *Mucuna pruriens* beans were obtained from Bungoma County, Kenya. The samples were generously donated by Dr. Marystella Wabwoba. A total of 2kg of each of the varieties of *Mucuna pruriens* beans (black, white, and mottled) scientifically named as *Mucuna pruriens* var*. pruriens., Mucuna pruriens* var*. utilis., Mucuna pruriens* var*. cochinchinensis,* respectively were collected. The samples were transported in sealed plastic bags and packaged in paper bags to JOOUST Food science laboratory for further processing and analysis.

Research design

A Completely Randomized Design (CRD) was used to assign 27 treated and untreated combinations derived from different fermentation methods, time and temperatures. For each variety, eight fermentation treatments were applied. This design was chosen due to the manageable number of samples and the homogeneity of experimental conditions (fermentation method: brine at 5% and 10% NaCl, and 1 g yeast, temperature: 25°C and 37°C and duration: 24 hours and 72 hours) [1, 15, 26].

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Each variety had corresponding control and treatment groups: Control for White (COW), Control for Black (COB) and Control for Mottled (COM). This design enabled the evaluation of interactions between treatment factors and their effect on the nutritional, biochemical and physicochemical properties of *Mucuna pruriens*, with a focus on optimizing L-DOPA reduction and nutrient content [20, 21].

Preparation of *Mucuna pruriens* beans Treatments

For brine fermentation, 500 g *Mucuna pruriens* beans were sorted, cleaned, and soaked over 72 hours at room temperature and boiled in water for two hours, then brine solutions were prepared at 5% and 10% salt concentrations into 5L. The brine was prepared by dissolving sodium chloride (NaCl) in distilled water, ensuring complete dissolution before submerging the beans. The fermentation process was done under controlled conditions at either 25°C or 37°C for 24 or 72 hours, allowing natural microbial activity and enzymatic breakdown to occur. Then dried and grinded into a fine powder by the aid of laboratory grinder (YUZHONG, model YZ-15-T-2000A) for further analysis.

For yeast fermentation, 1g *Saccharomyces cerevisiae* per 500 g *Mucuna pruriens* beans (sorted, cleaned and soaked over 72 hours at room temperature and boiled for two hours) was used as the sole fermenting agent [26]. The beans are cleaned and pre-soaked before being inoculated with an active yeast culture. The fermentation medium was cool boiled water adjusted to promote yeast activity and enhance fermentation efficiency. The process was carried out at either 25°C or 37°C, with durations of 24 or 72 hours**,** ensuring sufficient yeast metabolism for biochemical and sensory modifications. The beans were then dried and grinded into a fine powder by the aid of laboratory grinder (YUZHONG, model YZ-15-T-2000A) for further analysis.

The untreated (controls) *Mucuna pruriens* beans were sorted, cleaned, dried and directly milled under the same laboratory conditions.

Determination of nutritional content of *Mucuna pruriens* beans before fermentation (controls: COW, COB, COM) and after fermentation (treated samples)

Nutritional analyses of fermented and non-fermented(controls) *Mucuna pruriens* beans were performed following the standard methods outlined by the Association of Official Analytical Chemists (AOAC) [20]. For all analyses, the procedure involved the preparation of *Mucuna pruriens* samples (fermented and non-fermented); it included grinding of seeds into a fine powder by the aid of laboratory grinder (YUZHONG, model YZ-15-T-2000A). Moisture content was determined by oven-drying method, approximately 4 g of each flour sample in pre dried crucibles at 105°C for 5 hours using a hot air oven (Model: ELSKLO). Ash content was

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determined by incinerating 10g of each sample in a muffle furnace at 600°C for 2 hours and expressed as a percentage of dry weight [20]. Protein content was determined using the Biuret assay. One gram of each powdered sample was homogenised at 100 rpm in 10 mL of distilled water for 12 hours at 4°C to extract soluble proteins. The extracts were centrifuged at 6000 rpm for 10 minutes to remove insoluble material, and the supernatant was collected. Calibration curves were generated using bovine serum albumin (BSA) standards (0.25–4 mg/mL). For the assay, 2 mL of each extract was mixed with 2 mL of Biuret reagent (containing 1.5 g copper sulphate, 45.0 g sodium hydroxide, and 2.5 g potassium sodium tartrate per litre of distilled water), incubated at room temperature for 15 minutes, and absorbance measured at 540 nm using a Thermo Scientific GENESYS FSD UV-Vis spectrophotometer. Protein concentrations were calculated by comparing sample absorbance values with the BSA standard curve [22]. Crude fat content was determined using a modified Bligh and Dyer procedure, where 2 g of each powdered sample was extracted with a chloroform–methanol (2:1 v/v) mixture, and the lipid residue was quantified gravimetrically after solvent evaporation [7]. Crude fibre was analysed using sequential digestion in 200 millilitres of 1. 2g of 25% sulphuric acid and 0. 31 N sodium hydroxide. The mixture was boiled for 30 minutes and then washed twice, first with ethanol and then with petroleum ether. The resulting residues were carefully transferred into clean, dry crucibles and dried 5 minutes at 100°C to complete the hydrolysis process. The dried residues were weighed to calculate crude fibre content [23]. Carbohydrate content was calculated by difference, subtracting the total percentages of protein, fat, fibre, ash and moisture from 100% [24]. Total energy content was estimated using the Atwater conversion factors of 4, 9 and 4 kcal/g for protein, fat and carbohydrate, respectively, as described by Boniface [7]. For mineral analysis, 2 g of each sample was incinerated in a muffle furnace at 550°C for 3 hours to obtain ash, which was digested in a mixture of hydrochloric and nitric acids. The digests were diluted to volume in 100 mL volumetric flasks and analysed for calcium (Ca), iron (Fe) and zinc (Zn) using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) [25, 26].

Determination of L- DOPA content before fermentation (controls: COW, COB, COM) and after fermentation (treated samples) *Mucuna pruriens*

The amount of L-DOPA in *Mucuna pruriens* was determined using UV-spectrophotometry, with slight modifications based on the methods described by Chittasupho and Tesore [27, 28]. The procedure involved the preparation of *Mucuna pruriens* samples; it included grinding of seeds into a fine powder by the aid of laboratory grinder (YUZHONG, model YZ-15-T-2000A). L-DOPA was extracted from the *Mucuna pruriens* flour using 0.1 M hydrochloric acid. The extract was first filtered through Whatman filter paper, followed by filtration through a 0.45 µm membrane

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filter to remove any remaining particles. For standard preparation, a stock solution was made by dissolving 100 mg of pure L-DOPA in 100 mL of 0.1 M hydrochloric acid, yielding a concentration of 1000 mg/L. Working standards were then prepared by serial dilution of the stock solution to obtain concentrations of 0 mg/L, 6 mg/L, 11 mg/L, 16 mg/L, 24 mg/L, 29 mg/L, 30 mg/L, and 40 mg/L. Each standard solution was thoroughly vortexed to ensure it was particle free. The absorbance of each standard solution was measured at 280 nm using Thermo Scientific GENESYS FSD UV-Vis spectrophotometer. A calibration curve was generated by plotting absorbance against concentration. The concentration of L-DOPA in the *Mucuna pruriens* samples was then determined by measuring the absorbance of the sample extracts at 280 nm and comparing the values to the calibration curve. The calibration curve derived from the L-Dopa standards was utilised to ascertain the concentration of L-Dopa in the extract solutions by comparing their absorbance values to the curve. The concentration (mg/L) is calculated using the formula:

$$Concentration\ \left(\frac{mg}{L}\right) = \frac{(Absorbance - \ y\ intercept\ )}{Slope}$$

The concentration was then calculated as the product of the extract volume and the amount of L-Dopa. Lastly, when the original sample weight was provided, the L-Dopa amount was adjusted to mg/g of the sample using a specific formula:

$$L - Dopa\ content\ (mg/g)\ = \frac{Amount\ of\ L{-}Dopa\ (mg).}{Weight\ of\ the\ sample\ (g)}$$

Data analysis

The nutrient composition data of *Mucuna pruriens* beans before fermentation (controls: COW, COB, COM) and after fermentation (treated samples) were analysed using descriptive statistics (Mean ± SE). Normality and homogeneity of variance were assessed using Shapiro–Wilk's and Levine's tests, respectively. One-way ANOVA was applied to detect significant differences among treatments, and mean separation was performed using Tukey's HSD at $p< 0.05$. For the changes in L-Dopa concentration across treatments (fermentation method, time and temperature) were evaluated using ANOVA, with data transformations or non-parametric alternatives considered where assumptions were not met. A factorial ANOVA assessed the main and interaction effects of fermentation method, duration and temperature on nutrient composition and anti-nutritional factors. Post hoc comparisons were performed using Tukey's HSD, and all analyses were conducted in R software version 4.2.1 (R Core Team).

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RESULTS AND DISCUSSION

Nutrient content of three *Mucuna pruriens* beans varieties before fermentation (controls: COW, COB, COM) and after fermentation (treated samples)

The proximate and mineral composition of fermented and non-fermented *Mucuna pruriens* beans showed significantly increasing carbohydrate and mineral content, while reducing crude fat and fibre (Table 1). Compared to brine fermentation, yeast fermentation was superior in affecting nutritional components, thus in range of previous studies as this fermentation had bigger impacts on diminishing anti-nutritional components like phytate, tannins, oligosaccharides and L-Dopa [29]. Dry matter significantly decreased with fermentation in white (90.42 ± 0.83% control to 87.29 ± 0.58% at 5% brine; p= 0.03) and mottled beans (90.50 ± 0.95% control to 86.44 ± 0.57% yeast; p= 0.003), but not black (p= 0.53). This reduction is linked to water uptake during hydrolysis of structural polysaccharides and microbial metabolism. Comparable declines were reported in proximate composition and anti-nutritional factors in *Mucuna pruriens* seed flour as affected by processing methods (1.5 to 2.5%), and in *Mucuna deeringiana* subjected to lactic fermentation (2 to 3%) [20, 35]. In this study slightly larger reductions suggest that yeast fermentation at 37 °C promotes greater hydration and enzymatic softening compared with lactic cultures. Crude Fibre decreased in black (8.10 ± 0.49% to 5.09 ± 0.28%; p= 0.01) and white (5.70 ± 0.42% to 3.92 ± 0.25%; p= 0.002) but was unchanged in mottled (p= 0.23). Total Ash was stable in black and mottled (both p> 0.05) but increased in white (7.61 ± 0.27% to 9.95 ± 0.25%; p= 0.01). The significantly difference in crude fibre indicated that enzymatic degradation of structural polysaccharides by microbial cellulases and the absence of change in mottled beans suggested varietal resistance, possibly due to differences in cell wall architecture. Ash content could be attributed to relative concentration effects and improved mineral solubilisation due to acidification during fermentation where organic matter degradation and acid mediated release of minerals from phytate complexes and fibre losses (6.8% to 4.1%; p < 0.01) reported [6, 26]. In addition, previous studies reported significant changes in crude fibre, which decreased from 7.9 g/100 g to 3.7 g/100 g, and in total ash, which increased by about 3.5 g/100 g following fermentation. These shifts were attributed to microbial breakdown of structural polysaccharides and enhanced mineral release during processing. In this study, findings suggested fermentation enhances mineral density, improving the nutritional profile of white beans [15, 21]. Crude Protein remained statistically unchanged in black (6.59–9.16%; p= 0.11) and white (6.62–7.14%; p= 0.89), but significantly changed in mottled under yeast fermentation (8.80 ± 0.73% to 5.15 ± 0.52%; p= 0.001). This change reflects microbial proteolysis and amino acid catabolism related to the documented reduction from 27.3% to 21.5% crude protein (p < 0.01) in fermented Mucuna, and between

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20 to 30% protein losses in other underutilized legumes[17, 18, 26]. In contrast, another study reported stable protein in lactic-fermented *Mucuna deeringiana* [35]. Thus, this study's results indicated a varietal difference, with mottled beans more prone to protein loss than black or white. Crude fat significantly changed across all varieties (black: 6.67 ± 1.39% to 2.00 ± 0.31%; p= 0.001; white: 4.78 ± 0.49% to 2.44 ± 0.30%; p= 0.008; mottled: 7.55 ± 0.62% to 1.63 ± 0.27%; p= 0.001). This is consistent with lipase mediated triglyceride hydrolysis and microbial metabolism of free fatty acids [31]. Previous studies observed a reduction from 6.4% to 3.1% crude fat ($p < 0.01$) [20], while reported 50% decreases in fermented *Mucuna* beans [33]. This study observed a 60–75% decline in fat content, which was more pronounced under yeast fermentation, suggesting stronger lipid catabolism in those conditions. Carbohydrate content significantly changed with fermentation (black: 54.01 ± 1.43% to 64.12 ± 1.42%; p= 0.02; mottled: 56.00 ± 0.02% to 63.09 ± 1.30%; p= 0.013), this indicates enzymatic degradation of structural polysaccharides by microbial cellulases. These results were in range of those in a previous study on 'the effects of different processing methods on proximate composition of *Mucuna pruriens'* which found carbohydrate content to be 53.47±1.48 [29, 32]. Energy declined where microbial utilisation was greatest, with white beans dropping from 332.29 ± 4.60 to 307.43 ± 2.52 kcal/100 g ($p = 0.008$) and mottled beans from 327.23 ± 8.26 to 286.24 ± 4.61 kcal/100 g ($p = 0.006$). These reductions align with trends in other fermented African legumes, where microbial lipolysis enzymes break down triglycerides into glycerol and free fatty acids, which are subsequently converted into energy and organic acids. Controlled fermentation of *Mucuna pruriens* has also been shown to reduce energy values by 8 - 12% depending on treatment, with differences significant at $p < 0.05$. Similarly, documented energy reductions in *Mucuna pruriens* is linked to degradation of fat and non- digestible carbohydrates, though the magnitude was smaller (around 5%) than observed here [33, 34]. Mineral responses were heterogeneous: calcium fell markedly in white (44.01 ± 2.62 to 22.81 ± 1.18 mg/100 g; p= 0.001) and mottled (46.12 ± 4.13 to 16.47 ± 2.03 mg/100 g; p= 0.001), zinc shifted significantly in black (0.70 ± 0.09 to 0.56± 1.05 mg/100 g; p= 0.009) and white (1.16 ± 0.16 to 0.72± 0.88 mg/100 g; p= 0.003) but not mottled (p= 0.14), and iron decreased most in mottled (2.12 ± 0.07 to 0.50± 1.17 mg/100 g; p= 0.001). These results are similar with the findings of other researchers, reporting that fermentation of *Mucuna pruriens* decreased calcium and zinc by 30 to 40% ($p< 0.05$), though they also observed that mineral bioavailability improved due to phytate hydrolysis [15]. Similarly, to the study demonstrated that indigenous processing methods (soaking, germination, fermentation) reduced total mineral content, with iron losses ranging from 15 to 25% ($p < 0.05$), but enhanced solubility and extractability of these minerals. The main difference of this study observed a much

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sharper decline in iron for mottled beans (58%), patterns consistent with fermentation driven organic acid formation altering mineral extractability and solubility [19, 30].

Effect of time and Temperature during fermentation on the nutritional quality of *Mucuna pruriens*

Time dependent fermentation (0, 24 and 72 h) resulted in significant nutrient shifts across varieties (Table 2). Time effects confirmed that 72 h fermentation maximizes fat hydrolysis, fibre breakdown and mineral shifts, with black beans showing the highest carbohydrate enrichment, white beans experiencing mineral losses, and mottled beans displaying energy depletion. In black beans, crude fat fell sharply from 6.67 ± 1.39% (0 h) to 2.25 ± 0.28% (24 h) and to 2.03 ± 0.31% (72 h) ($p = 0.001$), crude fibre declined (8.10 ± 0.49% to 5.19 ± 0.32% and to 5.92 ± 0.54%, $p = 0.02$), and carbohydrate increased (54.01 ± 1.43% to 62.93 ± 1.33% and to 61.24 ± 1.64%, $p = 0.04$), while protein and ash were unchanged ($p = 0.69, 0.25$). Energy showed a non- significant downward trend (312.61 ± 5.31 kcal/100 g(0h) to 302.97±3.67kcal/100 g(24h), and 294.26 ± 4.50 kcal/100 g (72h), $p = 0.10$). Similar results of fat and fibre reductions were reported that fat decreased from 6.4% to 3.1% ($p < 0.01$) and fibre from 6.8% to 4.1% in *Mucuna pruriens* after 72 h fermentation [20]. Pulikkalpura [25] also demonstrated lipase driven lipid hydrolysis and cellulose mediated fibre degradation in legume fermentations, supporting these mechanisms.

White beans showed significant dry matter (90.42 ± 0.83% to 87.96 ± 0.46% and to 87.71 ± 0.42%, $p = 0.03$), fat reduction (4.78 ± 0.49% to 3.17 ± 0.34% and to 2.44 ± 0.34%, $p = 0.01$), ash increase (7.61 ± 0.27% to 9.51 ± 0.38% and to 9.54 ± 0.27%, $p = 0.03$), and a marked energy drop (332.29 ± 4.60 to 311.83 ± 2.94 and to 306.55 ± 2.69 kcal/100 g, $p = 0.002$); proteins and carbohydrates were stable ($p = 0.99, 0.77$). Minerals in white variety declined with time, Ca 44.01 ± 2.62 to 29.21 ± 2.75 and to 25.44 ± 1.48 mg/100 g ($p = 0.003$) and Zn 1.16 ± 0.16 to 0.75 ± 0.06 and to 0.82 ± 0.03 mg/100 g ($p = 0.003$) while Fe was unchanged ($p = 0.69$). In mottled beans, fat decreased strongly (7.55 ± 0.62% to 2.63 ± 0.39% and to1.97 ± 0.29%, $p = 0.001$), with dry matter reduced (90.50 ± 0.95% to 86.29 ± 0.57% and to 86.75 ± 0.53%, $p = 0.007$) and energy lowered (327.23 ± 8.26 to 293.04 ± 5.14 and to 296.36 ± 8.67 kcal/100 g, $p = 0.002$). Carbohydrate and fibre were statistically unchanged in mottled ($p = 0.35, 0.66$), but Ca fell steeply (46.12 ± 4.13 to 19.42 ± 1.94 and to22.28 ± 1.51 mg/100 g, $p = 0.001$) and Fe dropped (2.12 ± 0.07 to 0.92 ± 0.06 and to 0.89 ± 0.14 mg/100 g, $p = 0.001$). Spontaneously, the consistent fat losses reflect lipase mediated triglyceride hydrolysis with subsequent microbial metabolism; carbohydrate gains (notably in black) indicate hydrolysis of fibre-bound

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polysaccharides; and energy declines in white and mottled track macronutrient utilisation during fermentation [21]. Time linked mineral decreases (Ca, Fe, Zn) likely arise from organic acid complexation and altered extractability, even as overall bioavailability may improve with phytate reduction [20, 35].

Temperature effects were variety specific (Table 3). In the black variety, none of the parameters differed significantly between 25°C and 37°C (dry matter 87.23 ± 0.30 vs 87.64 ± 0.40%; protein 7.71 ± 0.76 vs 8.16 ± 0.58%; fat 3.04 ± 0.57 vs 2.14 ± 0.34%; all $p > 0.05$), indicating minimal temperature sensitivity for this genotype. In white beans, 37°C reduced crude fat from 3.53 ± 0.33% to 2.39 ± 0.33% ($p= 0.02$) and lowered energy from 317.63 ± 2.80 to 304.42 ± 2.73 kcal/100 g ($p= 0.003$); it also decreased Ca and Zn (Ca: 32.17 ± 2.87 to 25.51 ± 1.01 mg/100 g, $p= 0.006$; Zn: 0.89 ± 0.05 to 0.74 ± 0.05 mg/100 g, $p= 0.04$), while dry matter, protein, carbohydrate, fibre, ash and Fe were unchanged ($p > 0.05$). In the mottled variety, 37°C markedly reduced fat (3.73 ± 0.59 to 1.83 ± 0.39%, $p= 0.01$) and energy (300.44 ± 5.24 to 285.31 ± 4.63 kcal/100 g, $p= 0.04$); other traits, including minerals, did not differ ($p > 0.05$). Collectively, 37°C consistently enhanced lipid hydrolysis and microbial utilisation of energy substrates (lower fat and energy in white and mottled), with limited or no impact on protein and carbohydrates. The mineral decreases in white beans (Ca, Zn) suggest variety-dependent complexation/solubility shifts under more active fermentation, a pattern noted for legume fermentations where organic acids modulate extractable mineral fractions [1, 36, 37]. Given prior evidence that 37°C yeast fermentation accelerates anti-nutrient (for example, L-DOPA) degradation while improving digestibility [25]. Comparable findings have been reported, showing that prolonged microbial activity at elevated temperatures promotes carbohydrate utilisation and organic acid generation, ultimately diminishing caloric density [33].

Effects of temperature, time, and fermentation methods on L-DOPA concentration of *Mucuna pruriens* beans varieties

Across varieties, temperature (25 and 37°C) (Figure 1) did not significantly influence L-DOPA concentration in black (p = 0.64), white (p = 0.11), or mottled beans (p = 0.16). This stability suggested enzymatic equilibrium in the shikimate and phenylpropanoid pathways. In the black variety, L-DOPA concentration was 0.61 mg/g at 25°C compared with 0.56 mg/g at 37°C. In the white variety concentration decreased from 0.69 mg/g at 25°C to 0.45 mg/g at 37°C and in the mottled variety , L-DOPA levels declined from 0.52 mg/g at 25°C to 0.39 mg/g at 37°C. within the tested thermal range compare to other study reported that 16.1 mg/g tested in fresh Mucuna seed at room temperature while severe temperatures (>80°C) can cause fast L-Dopa depletion, past investigations have indicated that modest processing temperatures (30-50°C) have no effect on its content [38, 39].

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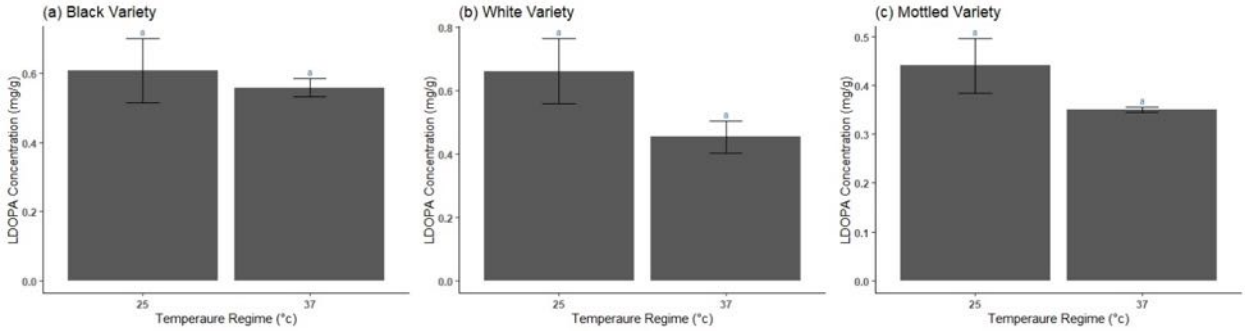

Figure 1: Effects of temperature on L-DOPA concentration of *Mucuna pruriens* beans varieties. Mean ± standard error (SE). Bars with different letter in the same graph differ significantly ($p<0.05$)

In contrast, fermentation time (Figure 2) significantly reduced L-DOPA levels in all varieties (p = 0.001). In the black variety, L-DOPA declined from 1.23 mg/g at 0 h to 0.63 mg/g at 24 h, and further to 0.55 mg/g at 72 h. In the white variety, L-DOPA decreased from 1.22 mg/g at 0 h to 0.58 mg/g at 24 h, and then to 0.45 mg/g at 72 h. In the mottled variety, concentrations fell from 0.82 mg/g at 0 h to 0.35 mg/g at 24 h, stabilizing at 0.34 mg/g at 72 h. This aligns with biochemical evidence that polyphenol oxidase (PPO) and peroxidase (POD) catalyse oxidative degradation of L-DOPA into melanin derivatives [25]. Microbial metabolism further contributes, with some strains degrading while others release bound L-DOPA [35]. L-DOPA is inherently unstable and undergoes auto-oxidation, in addition to being degraded through enzymatic oxidation. L-Dopa can undergo spontaneous oxidation to dopamine quinone, particularly in conditions characterised by varying pH and oxygen levels [1]. Additionally, microbial metabolism over time may influence L-Dopa concentration. Some microorganisms can degrade or enhance L-Dopa levels based on their metabolic pathways. The observed differences with change in time highlight the importance of appropriate storage conditions and time management in preserving the bioavailability of L-Dopa in Mucuna based food and pharmaceutical products [7].

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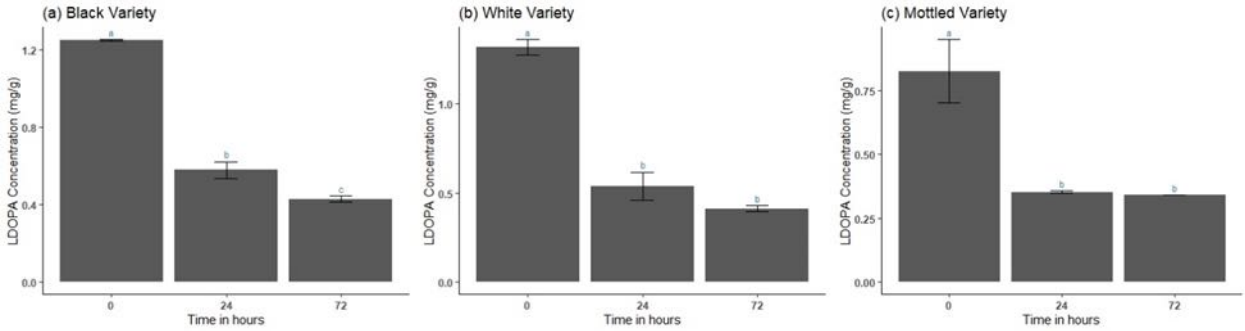

Figure 2: Effect of time on L-DOPA concentration of *Mucuna pruriens* beans varieties. Mean ± standard error (SE). Bars with different letter in the same graph differ significantly (p<0.05)

The different fermentation levels (Figure 3) significantly influenced L-DOPA concentration of all varieties (p=0.001). In the black variety, the control sample recorded the highest L-DOPA concentration (1.23 mg/g). Fermentation progressively reduced these levels, with 5% brine lowering L-DOPA to 0.74 mg/g, yeast fermentation to 0.58 mg/g, and the strongest decline observed with 10% brine (0.41 mg/g). In the white variety, L-DOPA concentration in the control was 1.22 mg/g, while fermentation treatments resulted in marked reductions; yeast yielded 0.62 mg/g, and both 10% brine (0.44 mg/g) and 5% brine (0.45 mg/g) showed comparable effects. For the mottled variety, the control contained 0.82 mg/g, which declined significantly following fermentation, reaching 0.34 mg/g with 10% brine, 0.35 mg/g with yeast, and 0.34 mg/g with 5% brine. This suggests that microbial activity is absolutely vital in changing L-Dopa levels. Microbial enzymes include β-glucosidases and proteases can break down protein bound precursors during fermentation, therefore releasing free L-Dopa. By hydrolysing bound forms into more bio accessible molecules, some *Lactobacillus* species have been demonstrated to increase L-Dopa availability [30, 40]. Though some fermentation settings could increase L-Dopa content, others can cause degradation. Through enzymatic oxidation and microbial metabolism, either prolonged fermenting times or the presence of oxidative bacteria might encourage L-dopa breakdown. The important need of optimising fermentation length, microbial strains and environmental variables such pH and oxygen levels is shown by the notable changes in L-Dopa levels under various fermenting settings [5, 29].

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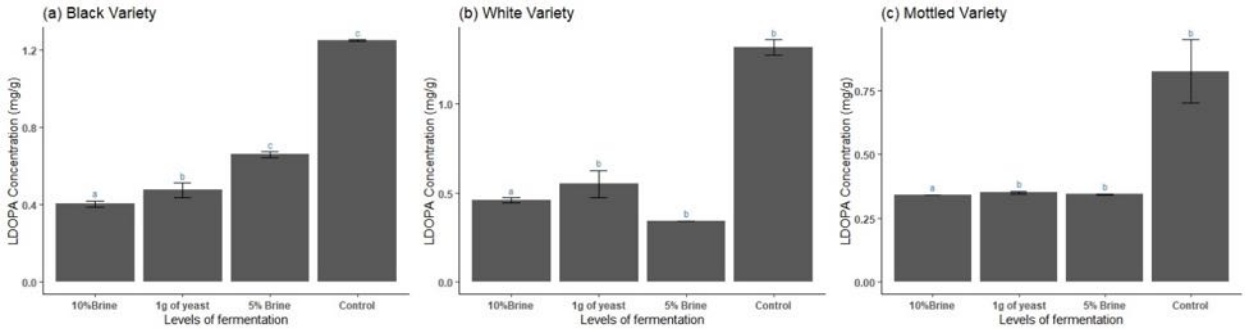

Figure 3: Effect of fermentation on L-DOPA concentration of *Mucuna pruriens* beans varieties. Mean ± standard error (SE). Bars with different letter in the same graph differ significantly ($p<0.05$)

CONCLUSION AND RECOMMENDATIONS FOR DEVELOPMENT

Fermentation enhanced the carbohydrate content in both black and mottled *Mucuna pruriens* varieties. There was marked reduction in crude fat and fibre levels, especially in yeast-fermented samples of the mottled variety, where crude fat dropped significantly and confirm the effectiveness of fermentation in improving nutritional quality by increasing energy yielding components while reducing less desirable constituents. Though the protein was preserved, brine fermentation did not lower L-DOPA. Yeast fermentation at 37°C for 72 hours turned out to be the most efficient in lowering L-DOPA concentration. Higher temperatures and longer fermenting times helped to break down complicated molecules, hence boosting digestibility and mineral bioavailability. To maximise these benefits, further optimisation of fermentation parameters such as microbial strains, pH, and duration is recommended. Developing acceptable and affordable fermented Mucuna-based foods, particularly complementary flours, and fortified products, would support dietary diversification and address food insecurity. Long-term feeding studies are necessary to validate safety for vulnerable populations. Policymakers are also urged to integrate *Mucuna pruriens* into national nutrition and food security strategies, given its potential as a climate resilient legume that enhances both soil fertility and human nutrition.

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Ethics Statement

This study did not involve human or animal subjects.

Conflict of Interest

The authors declare no conflict of interest.

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Table 1: Nutrient content of three *Mucuna pruriens* bean varieties before fermentation (controls: COW, COB, COM) and after fermentation (treated samples)

Variety	*Fermentation*	*Mean ± SE percentage of nutrients contained in Mucuna beans before and after fermentations*									
		Dry matter (%)	**Crude protein (%)**	**Crude fat (%)**	**Total Ash (%)**	**Crude fiber (%)**	**Carbohydrate (%)**	**Energy (kcal/100g)**	**Calcium (mg/100g)**	**Zinc (mg/100g)**	**Iron (mg/100g)**
Black											
	Control	88.33^{a}±0.74	9.14^{a}±0.61	6.67^{a}±1.39	10.41^{a}±0.25	8.10^{a}±0.49	54.01^{a}±1.43	312.61^{a}±5.31	26.09^{a}±2.04	0.70^{a}±0.09	1.22^{a}±0.02
	10% Brine	87.00^{a}±0.41	8.70^{a}±0.45	2.50^{b}±0.44	9.86^{a}±0.55	6.67^{ab}±0.67	59.26^{b}±1.93	294.37^{a}±3.41	23.20^{a}±1.51	0.56^{b}±0.09	1.03^{a}±0.08
	5% Brine	87.25^{a}±0.52	9.16^{a}±1.37	2.06^{b}±0.35	10.11^{a}±0.44	5.09^{b}±0.28	60.85^{ab}±2.03	298.49^{a}±3.92	25.59^{a}±6.99	1.05^{ab}±0.09	1.56^{a}±0.12
	1g Yeast	87.48^{a}±0.40	6.59^{a}±0.71	2.00^{b}±0.31	9.54a±0.82	5.24^{b}±0.48	64.12^{ab}±1.42	300.81^{a}±5.46	34.13^{a}±3.27	0.96^{b}±0.08	1.54^{a}±0.14
	P-value	**0.53**	**0.11**	**0.001**	**0.90**	**0.01**	**0.02**	**0.38**	**0.22**	**0.009**	**0.06**
White											
	Control	90.42^{b}±0.83	6.99^{a}±1.29	4.78^{b}±0.49	7.61^{b}±0.27	5.70^{b}±0.42	65.33^{a}±1.81	332.29^{b}±4.60	44.01^{c}±2.62	1.16^{b}±0.16	1.37^{b}±0.01
	10% Brine	88.67^{a}±0.51	6.84^{a}±0.48	2.54^{a}±0.57	9.29^{a}±0.52	5.01^{a}±0.24	64.98^{a}±1.68	310.12^{a}±3.55	29.07^{a}±0.89	0.82^{a}±0.03	1.35^{a}±0.07
	5% Brine	87.29^{b}±0.58	6.62^{a}±0.49	3.78^{b}±0.37	8.88^{ab}±0.49	5.18^{a}±0.28	62.83^{a}±1.01	311.79^{b}±5.67	34.59^{ab}±4.43	0.88^{b}±0.05	1.48^{ab}±0.07
	1g Yeast	87.69^{ab}±0.46	7.14^{a}±0.36	2.44^{ab}±0.30	9.95^{ab}±0.25	3.92^{a}±0.25	64.22^{a}±0.73	307.43^{b}±2.52	22.81^{bc}±1.18	0.72^{ab}±0.05	1.04^{ab}±0.09
	P-value	**0.03**	**0.89**	**0.008**	**0.01**	**0.002**	**0.56**	**0.008**	**0.001**	**0.003**	**0.006**
Mottled (speckled)											
	Control	90.50^{a}±0.95	8.80^{a}±0.73	7.55^{a}±0.62	12.67^{a}±0.84	5.09^{a}±0.51	56.00^{ab}±0.02	327.23^{a}±8.26	46.12^{b}±4.13	2.37^{a}±0.81	2.12^{c}±0.07
	10% Brine	86.58^{b}±0.67	9.41^{b}±0.23	2.39^{b}±0.35	12.91^{a}±0.90	5.47^{a}±0.42	56.78^{a}±1.55	286.24^{b}±4.61	19.57^{a}±2.11	0.85^{a}±0.39	0.50^{a}±0.06
	5% Brine	86.63^{b}±0.92	7.60^{ab}±1.04	3.56^{bc}±0.47	11.65^{a}±0.74	5.33^{a}±0.65	58.49^{b}±1.94	296.36^{a}±8.67	16.47^{b}±2.03	0.95^{a}±0.36	0.80^{b}±0.02
	1g Yeast	86.44^{b}±0.57	5.15^{b}±0.52	1.63^{c}±0.27	12.25^{a}±0.95	4.29^{a}± 0.29	63.09^{ab}±1.3	287.75^{ab}±4.48	23.68^{b}±1.66	1.16^{a}±0.24	1.17^{c}±0.09
	P-value	**0.003**	**0.001**	**0.001**	**0.86**	**0.23**	**0.013**	**0.006**	**0.001**	**0.14**	**0.001**

Note. Values presented as the mean ± standard error (SE) values of key nutritional parameters (dry matter, crude protein, crude fat, total ash, crude fibre, carbohydrate, energy, calcium, zinc, and iron) in three *Mucuna pruriens* varieties (Black, White, and Mottled (speckled)) different fermentation methods (no fermentation, 10% brine, 5% brine, and yeast fermentation). The significance (p-values) of differences among treatments is also provided. Different superscript letters indicate statistically significant differences at $p < 0.05$

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Table 2: Effect of fermentation time on the nutritional quality of *Mucuna pruriens* beans

Varieties	*Time*	*Mean ± SE percentage of nutrients contained in Mucuna beans before and after fermentation in different time intervals*									
		Dry matter (%)	Crude protein (%)	Crude fat (%)	Crude Ash (%)	Crude fiber (%)	Carbohydrate (%)	Energy (Kcal/100g)	Calcium (mg/100g)	Zinc (mg/100g)	Iron (mg/100g)
Black											
	0 hours	88.33^{a}±0.74	9.14^{a}±0.61	6.67^{a}±1.39	10.41^{a}±0.25	8.10^{a}±0.49	54.01^{a}±1.43	312.61^{a}±5.31	26.09^{a}±2.04	0.70^{a}±0.09	1.22^{a}±0.02
	24 hours	87.23^{a}±0.33	7.75^{a}±0.94	2.25^{a}±0.28	9.09^{a}±0.51	5.19^{ab}±0.32	62.93^{ab}±1.33	302.97^{a}±3.67	31.17^{a}±4.78	1.00^{a}±0.08	1.63^{ab}±0.08
	72 hours	87.38^{a}±0.39	7.76^{a}±0.58	2.03^{b}±0.31	10.42^{a}±0.68	5.92^{b}±0.54	61.24^{b}±1.64	294.26^{a}±4.50	27.36^{a}±1.82	0.76^{a}±0.09	1.21^{b}±0.08
	P-value	**0.41**	**0.69**	**0.001**	**0.25**	**0.02**	**0.04**	**0.10**	**0.68**	**0.07**	**0.04**
White											
	0 hours	90.42^{a}±0.83	6.99^{a}±1.29	4.78^{a}±0.49	7.61^{a}±0.27	5.70^{a}±0.42	65.33^{a}±1.81	332.29^{a}±4.60	44.01^{a}±2.62	1.16^{a}±0.16	1.37^{a}±0.01
	24 hours	87.96^{b}±0.46	6.92^{a}±0.38	3.17^{b}±0.34	9.51^{a}±0.38	4.45^{a}±0.28	63.92^{a}±0.86	311.83^{b}±2.94	29.21^{b}±2.75	0.75^{b}±0.06	1.24^{a}±0.08
	72 hours	87.71^{b}±0.42	6.95^{a}±0.33	2.44^{b}±0.34	9.54^{b}±0.27	4.57^{a}±0.28	64.21^{a}±0.88	306.55^{b}±2.69	25.44^{b}±1.48	0.82^{b}±0.03	1.21^{a}±0.09
	P-value	**0.03**	**0.99**	**0.01**	**0.03**	**0.14**	**0.77**	**0.002**	**0.003**	**0.003**	**0.69**
Mottled (speckled)	0 hours	90.50^{a}±0.95	8.80^{a}±0.73	7.55^{a}±0.62	12.67^{a}±0.84	5.46^{a}±0.42	56.00^{a}±0.02	327.23^{a}±8.26	46.12^{a}±4.13	2.37^{a}±0.81	2.12^{a}±0.07
	24 hours	86.29^{b}±0.57	6.55^{a}±0.74	2.63^{b}±0.39	11.56^{a}±0.75	4.77^{a}±0.43	60.77^{a}±1.35	293.04^{b}±5.14	19.42^{b}±1.94	0.93^{a}±0.20	0.92^{b}±0.06
	72 hours	86.75^{b}±0.53	7.11^{a}±0.74	1.97^{b}±0.29	12.98^{a}±0.78	4.74^{a}±0.31	59.96^{a}±1.64	296.36^{b}±8.67	22.28^{b}±1.51	1.13^{a}±0.28	0.89^{b}±0.14
	P-value	**0.007**	**0.39**	**0.001**	**0.39**	**0.66**	**0.35**	**0.002**	**0.001**	**0.07**	**0.001**

Note: Examination of how fermentation duration (0 hours, 24 hours, and 72 hours) influences the nutrient profile of the beans
Mean (± SE) percentage values with different superscript letters in the same row are significantly different at $p < 0.05$

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Table 3: Effect of fermentation temperatures on the nutritional quality of *Mucuna pruriens* beans

Variety	*Temperature*	*Mean ± SE percentage of nutrients contained in Mucuna beans before and after fermentation under different temperatures*									
		Dry matter (%)	Crude protein (%)	Crude fat (%)	Total Ash (%)	Crude fiber (%)	Carbohydrate (%)	Energy (Kcal/100g)	Calcium (mg/100g)	Zinc (mg/100g)	Iron (mg/100g)
Black											
	25^0c	87.23^a±0.30	7.71^a±0.76	3.04^a±0.57	10.05^a±0.61	6.07^a±0.45	60.37^a±1.34	299.71^a±4.48	27.76^a±2.01	0.78^a±0.09	1.32^a±0.09
	37^0c	87.64^a±0.40	8.16^a±0.58	2.14a±0.34	9.56^a±0.44	5.56^a±0.47	62.22^a±1.72	300.75^a±3.24	30.35^a±4.52	0.97^a±0.09	1.50^a±0.14
	P-value	**0.42**	**0.66**	**0.21**	**0.55**	**0.45**	**0.39**	**0.86**	**0.58**	**0.12**	**0.27**
White											
	25^0c	88.62^a±0.44	7.12^a±0.34	3.53^a±0.33	9.00^a±0.33	4.62^a±0.27	64.35^a±0.67	317.63^a±2.80	32.17^a±2.87	0.89^a±0.05	1.26^a±0.09
	37^0c	87.50^b±0.43	6.72^a±0.36	2.39^a±0.33	9.70^a±0.30	4.68^a±0.28	64.01^a±0.99	304.42^b±2.73	25.51^a±1.01	0.74^a±0.05	1.22^a±0.07
	P-value	**0.09**	**0.43**	**0.02**	**0.14**	**0.14**	**0.77**	**0.003**	**0.006**	**0.04**	**0.75**
Mottled (speckled)											
	25^0c	87.57^a±0.63	7.64^a±0.55	3.73^a±0.59	12.06^a±0.73	5.06^a±0.25	59.07^a±1.06	300.44^a±5.24	24.71^a±3.21	1.25^a±0.29	1.02^a±0.16
	37^0c	86.20^a±0.51	6.30^a±0.79	1.83^b±0.39	12.63^a±0.65	4.55^a±0.43	60.90^a±1.72	285.31^b±4.63	22.34^a±1.85	1.09^a±0.22	1.07^a±0.12
	P-value	**0.12**	**0.17**	**0.01**	**0.58**	**0.29**	**0.35**	**0.04**	**0.55**	**0.68**	**0.83**

Note. This table compares the impact of different fermentation temperatures (25°C and 37°C) on the nutrient composition of *Mucuna pruriens* beans. The results show significant changes in crude fat, energy, and mineral content, indicating the optimal conditions for improving nutritional bioavailability
Mean (± SE) percentage values with different superscript letters in the same row are significantly different at $p < 0.05$

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