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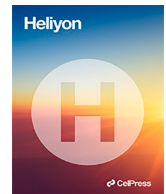

Proximate composition and anti-nutritional factors in *Mucuna pruriens* (velvet bean) seed flour as affected by several processing methods

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ABSTRACT

Mucuna pruriens seed being an underutilized legume with antinutrients was processed to reduce antinutrients and improve nutritional quality. The seeds were cleaned, washed and subjected to single treatments of soaking (24 h, 48 h, 72 h), cooking (20 min, 40 min, 60 min, 80 min), roasting (10 min, 15 min, 20 min), germination (24 h, 48 h, 72 h) and fermentation (24 h, 48 h, 72 h). Combined treatments: soaking (72 h) + cooking (60 min); germination (48 h) + roasting (15 min); germination (48 h) + cooking (60 min); fermentation (72 h) + roasting (15 min) were also separately carried out. Proximate composition and antinutrients were evaluated. Crude protein ranged from 25.34 to 29.50%, ash 3.02–3.82% and crude fibre 0.70–4.69. Crude protein was increased by single and double treatments while ash content was increased by only single treatments. All the treatments reduced the crude fibre. For single and double treatments, phenol, L-3,4-dihydroxyphenylalanine and trypsin inhibitor were in the range of 0.69–3.49%, 0.01–6.83% and 0.00–12.58 TIU/mg protein respectively. This research indicated that the use of up to 72 h soaking, 80 min cooking, 20 min roasting, 72 h germination or 72 h fermentation (*Rhizopus oligosporus*) is not adequate to reduce phenol and L-3,4-dihydroxyphenylalanine in *Mucuna pruriens* seed flour to below the recommended safe limits of 0.003% and 0.1% respectively. The double treatment of 72 h fermentation +15 m roasting was the only treatment that reduced L-3,4-dihydroxyphenylalanine to safe limit of 0.01%.

1. Introduction

Mucuna pruriens commonly known as velvet bean, cowitch or cowhage is of the family leguminosae, genus; Mucuna and Specie; *Mucuna pruriens* [1]. Traditionally in Enugu, the south eastern part of Nigeria, *Mucuna pruriens* is known as “Egbara” or “Agbara”. It consists of about 100 species of climbing vines and shrubs and it is found in tropical Africa, India and the Caribbean [2]. It is a twining annual crop that can reach 15 m in length, almost completely covered with fuzzy hair when young, but almost free of hairs when older

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[3]. The fruits have curved longitudinal pods which usually contain 4 to 6 seeds and cause irritating blisters or itching if they come in contact with human skin. The seeds are shiny black or brown, ovoid and of about 12 mm long [3].

Mucuna pruriens is a good source of crude protein (24–31.44%), crude carbohydrate (42.79–64.88%), crude lipid (4.1–14.39%), crude fibre (5.3–11.5%), ash (2.9–5.5%) [4] and its digestibility is comparable to that of other pulses like soybean, rice bean and lima bean [1,5].

Despite its nutritional value and potential, *Mucuna pruriens* seed has remained an underutilized minor food crop largely as a result of the presence of anti-nutritional factors in it. These anti-nutritional factors include L-3,4-dihydroxyphenylalanine (L-DOPA), trypsin inhibitor, phytate, tannins, glycosides, alkaloids, phenols, raffinose and stachyose [5,6]. The longer time and greater resources/energy input required for its processing has also contributed to its under-utilization [5].

To reduce or eliminate the anti-nutritional factors, improve nutritional quality and also increase the utilization of legumes, many food processing methods can be adopted [7]. These processing methods include soaking, dehulling, cooking, fermentation, germination, toasting/roasting and autoclaving [5,7,8].

Although single process treatments reduce antinutrients in *Mucuna pruriens* seed [5,7,8], the levels/magnitudes of single treatments for reduction of antinutrients have not been extensively researched on. Double treatments of *Mucuna pruriens* seed to achieve the reduction of antinutritional factors have also been scantly documented. During the application of these process treatments (single and double treatments) geared towards the reduction of the anti-nutritional factors in *Mucuna pruriens* seed, there are also possibilities of loss or improvement in nutrients (proximate parameters) whose details are also largely lacking. The aforementioned challenges and gap in knowledge present the need for further research in improving the utilization of *Mucuna pruriens* seed as food. Hence this study was carried out to evaluate the effects of soaking, cooking, roasting, germination and fermentation as well as their combined treatments on the proximate and anti-nutritional factors in *Mucuna pruriens* (velvet bean) seed flour.

2. Materials and methods

2.1. Source of raw materials and equipment

Raw *Mucuna pruriens* seeds was bought from New Market in Enugu State, Nigeria. Equipment and analytical grade reagents in the Department of Food Science and Technology, Federal University of Technology, Owerri (FUTO) and National Arbovirus and Vectors Research Centre (NAVRC), Enugu State were used.

2.2. Processing of raw materials

Soaking, boiling, roasting, germination and fermentation were the methods adopted in processing *Mucuna pruriens* seed.

2.3. Soaking of Mucuna pruriens seed

Whole *Mucuna pruriens* seeds (1.2 kg) were cleaned of any extraneous materials while in dry form, sorted and washed with distilled water. The seeds were divided into three batches which were coded S24h, S48h and S72h (400 g each) and were soaked in distilled water (1:5 w/v) for 24 h, 48 h and 72 h, respectively. A 6 h interval change of distilled water was maintained during the process. At the end of soaking for each batch, the samples were drained and dried in an oven (Laboratory Oven, England Labscience, DHG-9053A) at 70 °C (for 18 h with constant turning after every 4 h) to constant weight. They were finally ground (Binatone BL-1500PRO, China) and stored in a low-density-polyethylene bags in readiness for analyses.

2.4Cooking of Mucuna pruriens seed

Whole Mucuna pruriens seeds were cleaned, sorted and washed with distilled water. The seeds were divided into four batches of 400 g each. The four batches coded C20 m, C40 m, C60 m and C80 m were boiled in distilled water (1:5 w/v) for 20, 40, 60 and 80 min, respectively. The samples were drained, dried in an oven (Laboratory Oven, England Labscience, DHG-9053A) at 70 °C (for 18 h with constant turning after every 4 h) to constant weight, grinded and stored in a low-density polyethylene bag in readiness for analysis.

2.5Roasting of Mucuna pruriens seed

Whole Mucuna pruriens seeds were sorted and cleaned of unwanted and extraneous materials. The seeds were divided into three batches (R10 m, R15 m and R20 m) of 400 g per batch. The batches were roasted in an Oven (Electric hot Oven, Saisho Model: S-936R) at 150 °C; Batch 1 (R10 m) was roasted for 10 min, batch 2 (R15 m) for 15min and batch 3 for 20 min, respectively. The samples were then ground and stored in a low-density polyethylene bag in readiness for analyses.

2.6. Germination of Mucuna pruriens seed

The procedure described by Mugendi et al. [5] was used for the germination process. 100 g of *Mucuna pruriens* seed was soaked in ethanol (1:2 w/v) for 1 min to aid decontamination. Seeds were soaked in distilled water (1:10, w/v) for 12 h at room temperature (27 ± 2 °C). The water was drained, the seeds were divided into three (3) groups (G24h, G48h and G72h) and spread on jute bags that were

placed on top of a wool-cloth, covered with a black-coloured low density polyethylene bag and allowed to germinate in the dark. The seeds in group 1 (G24h) were removed after 24 h, group 2 (G48h) removed after 48 h and group 3 (G72h) removed after 72 h. The raw, 24 h, 48 h and 72 h germinated *Mucuna pruriens* seeds were presented in Fig. 1a, b, 1c and 1d respectively. Seeds were afterwards dried in an oven (Laboratory hot air Oven, England Labscience, DHG-9053A) at 70 °C (for 18 h with constant turning after every 4 h) to constant weight. Dried germinated seeds were ground into powder and stored in low density polyethylene bags for analyses.

3. Double treatments

3.1. Fermentation of Mucuna pruriens seed

The procedure described by Egounlety [9] was adopted for the fermentation process. *Mucuna pruriens* seeds were boiled in distilled water for 45 min (1 kg/6l), hand-dehulled, chopped into 2–3 pieces per grain, soaked twice (1 kg/3l) for 12 h with removal of soak water after each soaking period, re-cooked for 45 min (1 kg/6l), drained and cooled. To prevent the growth of the other

Fig. 1. aRaw *Mucuna pruriens* seed (control), b: 24 h germinated *Mucuna pruriens* seed, c: 48 h germinated *Mucuna pruriens* seed c: 48 h germinated *Mucuna pruriens* seed, d:72 h germinated *Mucuna pruriens* seed, e: Unfermented *Mucuna pruriens* seed f: 24 h fermented *Mucuna pruriens* seed, g: 48 h fermented *Mucuna pruriens* seed h: 72 h fermented *Mucuna pruriens* seed, i: Soaked (72 h) and cooked (60 min) j: Germinated (48 h) and roasted (15 min) *Mucuna pruriens* seed. *Mucuna pruriens* seed, k: Germinated (48 h) and cooked (60 min) l: Fermented (72 h) and roasted (15 min).

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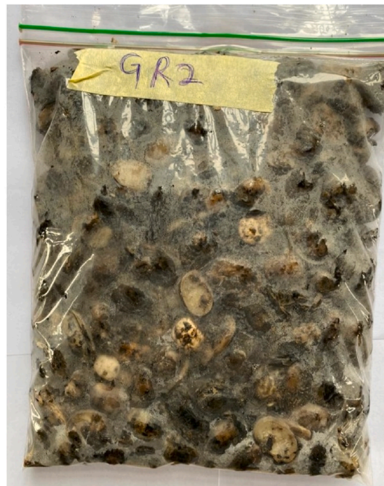

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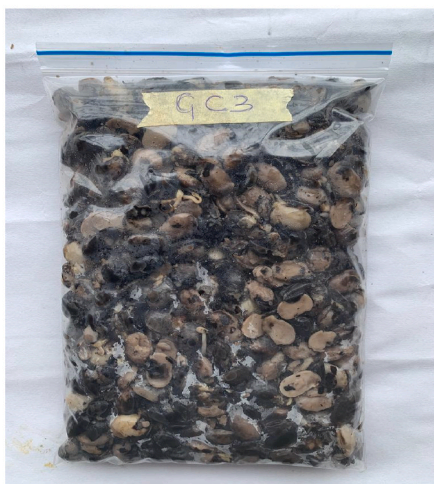

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Fig. 1. (*continued*).

microorganisms and to maintain the pH for the convenient growth of *R. oligosporus*, pH of the substrate was adjusted using vinegar of grapes at 2.85 ml per 100 g of substrate [10]. The grains obtained were divided into three portions, inoculated with *Rhizopus oligosporus* (0.4 g/kg drained grain), packed in polyethylene perforated bags (50 μm) and fermented (29 °C) for 24 h, 48 h and 72 h to obtain samples F24h, F48h and F72h, respectively. The samples were presented in Fig. 1e, f, 1g and 1h respectively for unfermented sample, 24 h, 48 h and 72 h fermented samples respectively.

Single treatments with optimal reduction of antinutrients and better nutrients retention were combined to form the double treatments. Lower treatment intensities or durations which had reduction in antinutrients and nutrients retention effect were selected instead of the selection of higher intensities or durations. Other chosen treatment combinations include.

i. 72 h soaking +60 min cooking, and the processed seeds were presented in Fig. 1i.
ii. 48 h germination +15 min roasting and the processed seeds were presented in Fig. 1j.
iii. 48 h germination +60 min cooking and the processed seeds were presented in Fig. 1k.
iv. 72 h fermentation +15 min roasting and the processed seeds were presented in Fig. 1l.

3.2. *Determination of the proximate composition of Mucuna pruriens seed flour*

Mucuna pruriens seed flours were assessed for % moisture, % ash, % fat, % crude fibre, % crude protein and % carbohydrate as described by AOAC [11].

3.3. *Determination of anti-nutritional factors in Mucuna pruriens seed flour*

The anti-nutritional factors in *Mucuna pruriens* seed flour were determined as follows.

3.4. *Determination of tannin*

Tannin content of the sample was determined by Folin Denis Colorimetric method as reported by Nwosu et al. [12]. 1 g of the processed sample was mixed with distilled water in the ratio of 1:10 (W/V). The mixture was agitated for 30 min at room temperature and filtered to obtain the extract. A standard tannic acid solution was prepared 2 ml of the standard solution and equal volume of distilled water were dispersed into a separate 50 ml volumetric flasks to serve as standard and reagent blank respectively. Then 2 ml of each of the sample extracts was put in their respective labelled flasks. The content of each flask mixed with 35 ml distilled water and 1 ml of the Folin Denis reagent was added to each. This was followed by 2.5 ml of saturated Na_2CO_3 solution. Thereafter each flask was diluted to the 50 ml mark with distilled water and incubated for 90 min at room temperature. Their absorbance was measured at 710 nm in a colorimeter (Jenway 6051) with the reagent blank at zero. The tannin content was calculated as shown below:

$$\%\ Tannin = 100/W \times a_u/a_s \times C \times V_t/V_a \times D \tag{1}$$

where W = weight of sample, a_u = absorbance of test sample, a_s = absorbance of standard tannin solution, C = concentration of standard tannin solution, V_t = total volume of extract, V_a = volume of extract analyzed, D = dilution factor.

3.5. *Determination of saponins*

This assay was done by the double solvent extraction gravimetric method [11]. 2 g of the processed sample was mixed with 100 ml of 20% aqueous ethanol solution and incubated for 12 h at a temperature of 55 °C with constant agitation. After that, the mixture was filtered through whatman No 42 grades of filter paper. The residue was re-extracted with 50 ml of the ethanol solution for 30 min and the extracts weighed together. The combined extract was reduced to about 40 ml by evaporation and then transferred to a separating funnel and equal volume (40 ml) of diethyl ether was added to it. After mixing well, there was a partition and the upper layer was discarded while the lower aqueous layer was re-extracted with the ether after which its pH was reduced to 4.5 with dropwise addition of NaOH solution. Saponin in the extract was taken up in successive extraction with 60 ml and 30 ml portion of n-butanol. The combine extract (ppt) was washed with 5% of NaCl solution and evaporated with a water bath in a previously weighed evaporation dish. The saponin will then dried in an oven (Gallenkamp Hot box Oven) at 60 °C (to remove any residual solvent), cooled in a desiccator and re-weighed. The saponin content was calculated as shown below:

$$\%\ Saponin = \frac{W_2 - W_1}{W} \tag{2}$$

where W = weight of sample used, W_1 = weight of empty evaporation dish, W_2 = weight of dish + saponin extract.

3.6. *Determination of total phenols*

Total phenols were determined by the folin-ciocalteau spectrophotometric method [11]. The total phenol was extracted from 0.2 g of the sample with 10 ml concentrated methanol. The mixture was shaken for 30 min at room temperature. The mixture centrifuged at 500 rpm for 15 min and the supernatant (extract) used for the analysis. One ml portion of the extract from each sample was treated

with equal volume of folin-ciocalteau reagent followed by the addition of 2 ml of 2% Na_2CO_3 solution. Standard phenol solution was prepared and diluted to a desired concentration. One ml of the standard solution was also treated with the Folin-ciocalteau reagent and Na_2CO_3 solution. The intensity of the resulting blue colouration was measured (absorbance) in a colorimeter (Jenway 6051) at 540 nm wavelength. Measurement was made with a reagent blank at zero. The phenol content was calculated using the formula below:

$$\% \text{ Phenol} = \frac{100}{W} \times \frac{a_u}{a_s} \times C \times \frac{V_t}{V_a} \times D \quad (3)$$

where: W = weight of sample, a_u = absorbance of test sample, a_s = absorbance of standard phenol solution, C = concentration of standard phenol solution, V_t = total volume of extract, V_a = volume of extract analyzed, D = dilution factor.

3.7. Determination of hydrogen cyanide

This was determined by the method described by Nwosu et al. [12]. 1 g of the sample was dispersed in 50 ml of distilled water in a 50 ml conical flask. An alkaline pikrate paper hung over the sample mixture and the blank in their respective flasks. The set up was incubated overnight at room temperature and each pikrate paper eluted (or dipped) into 60 ml distilled water. A standard cyanide solution was prepared and diluted to a required concentration. The absorbance of the eluted sample solution and that of the standard was measured at 540 nm wavelength with the reagent blank at zero. The cyanide content was determined by the formula shown below

$$\text{Hydrogen cyanide} \left(\frac{mg}{kg}\right) = 1000 \times \frac{a_u}{W} \times \frac{C}{a_s} \times D \quad (4)$$

Where W = weight of sample analyzed, a_u = absorbance of test sample, a_s = absorbance of standard hydrogen cyanide solution, C = concentration of standard in mg/dl, D = dilution factor where applicable.

3.8. Determination of phytate

The method described by Nwosu [13] was used for the determination of phytate. The phytic acid in the samples was precipitated with excess $FeCl_3$ after extraction of 1 g of each sample with 100 ml 0.5 N HCl. The precipitate was converted to sodium phytate using 2 ml of 2% NaOH before digestion with an acid mixture containing equal portions (1 ml) of conc. H_2SO_4 and 65% $HClO_4$. The solution was filtered with filter paper and 5 ml of 5% molybdate solution was added. It was allowed to stand for 30 min for colour development through the formation of phosphomolybdate complex. The absorbance of the solution was measured colorimetrically (Jenway 6051 Colorimeter) at 520 nm after colour development with molybdate solution. The percentage phytate was thus calculated:

$$\% \text{ Phytate} = \frac{100}{W_t} \times \frac{a_u}{a_s} \times C \times \frac{V_t}{V_a} \quad (5)$$

where W_t = weight of sample used, a_u = absorbance of test sample, a_s = absorbance of standard phytate solution, C = concentration of standard phytate solution, V_t = total volume of extract, V_a = volume of extract analyzed.

3.9. Determination of trypsin inhibitor

This was done using the spectrophotometric method as described by Nwosu et al. [12]. 10 g of the test sample was dispersed in 50 ml of 0.5 M NaCl solution and stirred for 30 min at room temperature. It was centrifuged and the supernatant filtered through whatman No.42 filter paper. The filtrate was used for the assay. Standard trypsin was prepared and used to treat the substrate solution (N – benzoyl-DI-arginine-P-anilide; BAPA). The extent of inhibition was used as a standard for measuring the trypsin inhibitory activity of the test sample extract. Into a test tube containing 2 ml of extract and 10 ml of the substrate (BAPA), 2 ml of the standard trypsin solution was added. Also, 2 ml of the standard trypsin solution was added in another test tube containing only 10 ml of the substrate. The latter served as the blank. The content of the tubes was allowed to stand for 30 min and then absorbance of the solution measured at 430 nm wavelength with a colorimeter (Jenway 6051). One trypsin activity unit inhibited is given by an increase of 0.01 absorbance unit at 430 nm. Trypsin unit inhibited was thus calculated:

$$\text{Trypsin inhibitor} = a_u \times \frac{0.01}{a_s} \times F \quad (6)$$

where a_u = absorbance of test sample, a_s = absorbance of standard (uninhibited) sample.

F = experimental factor given as:

$$\frac{V_f}{V_a} \times \frac{1}{W} \quad (7)$$

Where V_f = total volume of extract, V_a = volume of extract analyzed, W = weight of sample analyzed.

3.10. Determination of oxalate

This was carried out by the procedures described by Nwosu et al. [12]. 1 g of the sample was weighed into a 100 ml beaker, 20 ml of 0.30 N HCl was added and warmed to (40–50 °C) using magnetic hot plate and stirred for 1 h. It was extracted three times with 20 ml flask. The combined extract diluted to 100 ml mark of the volumetric flask. The oxalate was estimated by pipetting 5 ml of the extract into a conical flask and made alkaline with 1.0 ml of 5 N ammonium hydroxide. A little indicator paper was placed in the conical flask to enable know the alkaline regions. It will also be made acid to phenolphthalein (3 drops of this indicator added, excess acid decolorizes solution) by dropwise addition of glacial acetic acid. 1.0 ml of 5% $CaCl_2$ was then added and the mixture allowed to stand for 3 h after which it was then centrifuged at 300 rpm for 15 min. The supernatant was discarded. 2 ml of 3 N H_2SO_4 was added to each tube and the precipitate dissolved by warming in a water bath (70–80 °C). The content of all the tubes was carefully poured into a clean conical flask and titrated with freshly prepared 0.01 N $KMnO_4$ at room temperature until the first pink colour appeared throughout the solution. It was allowed to stand until the solution became colourless. The solution will then be warmed to 70–80 °C and titrated until a permanent pink colour that persisted for at least 30 s was attained. The percentage (%) oxalate content will thus be calculated:

$$\% \text{ Oxalate} = \frac{100}{\text{W}} \times 0.00225 \times \text{Total titre volume} \tag{8}$$

where W = weight of sample used.

3.11. Determination of L-3, 4 dihydroxyphenylalanine (L-DOPA)

L-DOPA content of the samples was determined using the procedure described by Rahmani-Nezhad et al. [14]. *Mucucna pruriens* seed *was* purchased from New Market in Enugu State, Nigeria. To prepare the extract, 1 g of each plant sample (dried seed) was grinded to powder and soaked in solution (10 ml) containing 0.1 N HCl- Ethanol (1:1) in darkness. After 5 days, each solution was filtered using whatman filter paper (No. 1) and centrifuged at 3500 rpm for 10 min. The resulting solution was used for the determination of L-DOPA content in each sample using spectrophotometric method. A stock solution of L-DOPA (USA, Cat. No. 92630) was prepared by dissolving 10 mg of L-DOPA in a 100 ml volumetric flask. A series of nine 25-ml volumetric flasks respectively containing 1, 2, 3, 4, 5, 6, 7, 8, and 9 ml of L-DOPA stock solution were prepared. Then, sodium nitrite (3%, 2 ml) and hydrochloric acid (1 mol l^{-1}, 1 ml) was added to those volumetric flasks, left to stand 5 min (turned yellow), rendered alkaline with sodium hydroxide (1 mol l^{-1}, 3 ml), left to stand 5 min (turned red), and finally diluted to the corresponding volume with distilled water. Next, λ_{max} was determined by scanning one of the solutions in the range of 400–800 nm using a blank (containing the same components without analyte) and λ_{max} = 470 nm was obtained. Finally, the absorbance of all the solutions was measured at 470 nm and the related calibration curve was plotted to determine the linearity in the concentration range of 4–36 μg/ml and regression value of 0.9978. The L-DOPA content of each sample was estimated using the above validated spectrophotometric method.

3.12. Statistical analysis

All the data generated from the various analyses were subjected to Analysis of Variance (ANOVA) using Statistical Package for Social Science - SPSS, version 25.0. Mean separation was done using the Duncan's Multiple Range test at 5% probability level.

4. Results and discussion

4.1. Observations after the processing of Mucuna pruriens seeds

For the germinated seeds, the appearance of the raw (ungerminated) seeds was presented in Fig. 1a while that of the germinated seeds were presented in Fig. 1b, c and 1d for 24 h, 48 h and 72 h germination respectively. Sprouts were observed after 24 h (Fig. 1b) which increased as the germination duration increased to 48 h (Figs. 1c) and 72 h (Fig. 1d). The observed sprouts were not unexpected as correct germination procedure is expected to generate sprouts and is a confirmation that germination has been successfully achieved.

For the fermented seeds, the appearance of the unfermented seeds was presented in Fig. 1e while that of the fermented seeds were presented in Fig. 1f, g and 1h for 24 h, 48 h and 72 h fermentation respectively. White-coloured mycelial mass was observed after 48 h (Fig. 1g) which increased as the fermentation duration increased to 72 h (Fig. 1h). The white mycelial mass is one of the characteristics associated with Fungi like *Rhizopus oligosporus*.

The appearances of the *Mucuna pruriens* seeds that received double treatments were shown in Fig. 1i, j, 1k and 1l. The appearance of 72 h soaked and 60 min cooked *Mucuna pruriens* seed were shown in Fig. 1i and there were signs of water imbibition and dark colouration of some seed with soft seed texture. Fig. 1j shows the appearance of 48 h germinated and 15 min roasted *Mucuna pruriens* seed and a dark colouration of the seeds was also observed. The appearance of 48 h germinated and 60 min cooked *Mucuna pruriens* seed was presented in Fig. 1k which indicated a dark colouration of the seeds with soft seed texture. Fig. 1l shows the appearance of 72 h fermented and 15 min roasted *Mucuna pruriens* seed with an observed dark seed colour seed as well as dark coloured mycelial mass. The observed dark colour of the seeds after various treatments could have been a result of the reaction and conversion of Phenolic compounds to O- Quinones and *O*-diphenolics leading to the formation of characteristic dark polymeric compounds [15].

4.2. Proximate composition of soaked, cooked, roasted, germinated and fermented Mucuna pruriens seed flour

The moisture, ash, crude protein, crude fibre, fat and carbohydrate content of *Mucuna pruriens* seed that received single treatments (soaking, cooking, roasting, germination and fermentation) are shown in Table 1. The different treatments effected significant ($p < 0.05$) changes in many of the proximate parameters.

The raw *M. pruriens* seed contained 10.99% moisture, 3.82% ash, 25.34% crude protein, 4.69% crude fibre, 5.94% fat and 49.22% carbohydrate. The crude protein (25.34%) of raw *Mucuna pruriens* seed recorded in this study is comparable to the 27.87% recorded by Mugendi et al. [5] and also for that of lentil (26.9%), fenugreek (26.30%) but higher than that of lima bean (21.5%) and Mung bean (23.6%) [7].

Soaking significantly reduced ($p < 0.05$) the ash, crude protein, crude fibre and fat content of *Mucuna pruriens* seed while moisture and carbohydrate content increased significantly. Both the decrease and also the increase observed in some of the nutrients during the treatments were continuous as the duration of the treatment increased. There is the possibility of leaching of nutrients into the soak water [5] which could lead to the reduction of many of these nutrients. Moisture absorption during soaking would have contributed to the increase in moisture content during soaking.

Cooking also significantly ($p < 0.05$) reduced all the proximate parameters except moisture content and carbohydrate the same way soaking did. The reduction in the nutrients during cooking was greater than that observed during soaking. The reduction trend was continuous and regular with increase in cooking time. Greatest nutrients reduction was witnessed during the 80 min

cooking. The aggregate effect of heating, diffusion and leaching may have led to the reductions observed for ash, crude protein, crude fibre and fat. A similar report of significant reductions in.

Roasting generally reduced significantly ($p < 0.05$) all the proximate parameters with the exception of carbohydrate. The reductions though significant but were not very great. Increase in roasting time caused an inverse effect on the proximate parameters (except carbohydrate). Roasting for 20 min had the greatest reduction impact on all proximate parameters except carbohydrate which was significantly increased ($p < 0.05$) the most. Roasting burn off organic matter and volatile compounds (lipids and vitamins) with a tendency of disintegrating the compounds [5] which could have contributed to the slight significant reduction trends observed. Seena et al. [17] recorded similar significant ($p < 0.05$) reduction trend in moisture, crude protein, crude fibre and ash during the roasting of *Canavalia cathartica* but with an increase in crude lipid.

Germination caused significant differences ($p < 0.05$) in the proximate parameters of *Mucuna pruriens* seed. Ash, crude protein and carbohydrate significantly increased ($p < 0.05$) while moisture, crude fibre and fat significantly decreased. Germination decreased crude fibre the most from 4.69% in the raw *Mucuna pruriens* seed to 0.74%, 0.71% and 0.70% in the 24h, 48h and 72h germinated samples respectively. The effect of 24h, 48h and 72h germination on crude fibre did not differ ($p > 0.05$) from each other. Germination activates endogenous enzymes [18], destroys antinutrients [19] and synthesizes structural proteins [5]. These effects would most likely have resulted in the significant increase in ash, crude protein and carbohydrate while decrease in moisture, crude fibre and fat may have been as a result of the nutrients' need during germination. Similar to the finding of this research, Anaemene and Fadupin [19] recorded a significant ($p < 0.05$) increase in the crude protein and a significant decrease in crude fibre of pigeon pea. A significant

Table 1
Proximate composition of soaked, cooked, roasted, germinated and fermented Mucuna pruriens seed flour.

			Parameters					
S/No	Sample code	Treatment	Moisture Content	Ash	Crude protein	Crude fibre	Fat	Carbohydrate
1.	CON	Raw (control)	10.99abcd	3.82de	25.34g	4.69a	5.94a	49.22l
2.	S24h	24h soaked	11.14abcd	3.70ef	25.27gh	4.31c	5.18de	50.40k
3.	S48h	48h soaked	11.27abcd	3.58g	25.11hi	2.99j	5.04f	52.01j
4.	S72h	72h soaked	11.40abc	3.48g	25.03i	2.83k	4.83gh	52.43i
5.	C20m	20min cooked	11.09abcd	3.71ef	24.25k	4.16d	3.14i	53.65g
6.	C40m	40min cooked	11.24abcd	3.63 fg	24.14k	3.67f	3.06ij	54.26ef
7.	C60m	60min cooked	12.20 ab	3.54g	22.35l	3.40g	3.08ij	55.43b
8.	C80m	80min cooked	12.44a	3.14hi	22.14m	3.10i	2.97j	56.21a
9.	R10m	10min roasted	9.97bcd	3.28h	25.17ghi	3.88e	5.28d	52.42i
10.	R15m	15min roasted	9.71cd	3.15hi	24.78j	3.84e	5.48c	53.04h
11.	R20m	20min roasted	9.62cd	3.02i	24.75j	3.26h	4.87g	54.48de
12.	G24h	24h germinated	9.91cd	3.88cd	25.90f	0.74l	5.44c	54.13f
13.	G48h	48h germinated	9.08d	3.95cd	26.30e	0.71l	5.00f	54.96c
14.	G72h	72h germinated	9.05e	4.01c	26.90c	0.70l	4.72h	54.62d
15.	F24h	24h fermented	11.40abc	4.00c	26.70d	4.51b	5.74b	47.65m
16.	F48h	48h fermented	11.80abc	5.00b	27.91b	4.20d	5.60c	45.49n
17.	F72h	72h fermented	11.72abc	5.80a	29.50a	3.30h	5.12ef	45.56n
	SEM		0.24	0.09	0.23	0.18	0.14	0.46

Values are means of three replicates. Means in the same column with different superscripts are significantly different ($p < 0.05$); SEM - standard error of the mean.

ash, crude protein, crude fibre and fat content of *Mucuna flagellipes* seed during cooking was reported by Obi and Okoye [16]. A reduction in these proximate parameters and increase in carbohydrate content of cooked goat pea (*Securigera securidaca* L.) was also observed by Ref. [7]. They observed that the reduction in crude protein was significant while the reduction in others were not significant.

decrease in crude fibre was also observed for kidney bean, mung bean, soybean and peanut during germination [20].

There were significant differences ($p < 0.05$) in all the proximate parameters during fermentation. Moisture, ash and crude protein were significantly increased while crude fibre, fat and carbohydrate were significantly decreased by fermentation process. Fermentation had the highest increasing effect on ash and crude protein. Fermentation increased crude protein content from 25.34% (raw seed) to 26.70%, 27.91% and 29.50% in 24h, 48h and 72h germinated samples respectively. Fermentation activates α-amylase and maltase which reduce starch to simple sugars that serve as substrates to fermenting organisms and fermenting organisms may also utilize fat for energy generation [18]. These two reasons may have contributed to the decrease in fat and carbohydrate contents of the fermented *Mucuna pruriens* seeds. The multiplication of the *Rhizopus oligosporus* used for the fermentation, loss of dry matter and the synthesis of proteins may have led to the increase in the crude protein content. Fermentation increases the crude protein content of legumes like pigeon pea [19] and *Mucuna cochinchinesis* [21].

4.3. Anti-nutritional factors in soaked, cooked, roasted, germinated and fermented Mucuna pruriens seed flour

The anti-nutritional factors in *Mucuna pruriens* seed are shown in Table 2. Generally, there were significant reductions ($p < 0.05$) in many of the anti-nutritional factors when subjected to the various treatments. The reduction in many of the anti-nutritional factors generally got greater with increase in time/duration of each process treatment.

The results of this study show that raw *Mucuna pruriens* seed contains phenol (1.82%), phytate (1.50%), tannins (0.38%), oxalate (0.09%), saponin (0.38%), hydrogen cyanide (9.86%), trypsin inhibitor activity (12.58 TIU/mg protein) and L-DOPA (6.83%). The phytate (1.50%), trypsin inhibitor activity (12.58 TIU/mg protein) and L-DOPA (6.83%) of the raw seed recorded in this study are comparable to those reported by Ref. [5] for phytate (1.35%), trypsin inhibitor activity (9.32 TIU/mg protein) and L-DOPA (5.71%) while phenol (5.20%) and tannin (0.00%) varied largely. Some of these variations in the anti-nutritional factors in legumes could result from the climate and ecological conditions and the stage of maturity before harvest [22].

Soaking significantly reduced ($p < 0.05$) all the anti-nutritional factors evaluated. Anti-nutritional factors decreased with increase in soaking time and the trend was regular. Leaching of antinutrients would have contributed largely to this reduction trend [5]. The highest reduction in anti-nutritional factors during soaking was observed for phytate which was reduced from 1.50% in the raw *Mucuna pruriens* seed to 0.42% in 72 h soaked sample (S72h). This decrease represents a 72% reduction. This implies that soaking can be used to reduce to a great extent the phytate content of *Mucuna pruriens* seed and possibly that of other pulses. Conversely, the lowest reduction was observed in hydrogen cyanide which was reduced from 9.86 mg/kg to 8.35 mg/kg (15% reduction) after 72 h of soaking. The low reduction value for hydrogen cyanide could be attributed to the fact that the *Mucuna pruriens* seed has a tough nature and was in whole seed form (not broken) which led to lower moisture absorption rate with consequent lower rate of hydrogen cyanide reduction. Soaking is one of the simple and inexpensive techniques of legumes processing used to soften pulses, shorten cooking time and/or reduce anti-nutrients [23,24]. Similar report which correlates with that of this present research as regards the reduction of

Table 2
Anti-nutritional factors in soaked, cooked, roasted, germinated and fermented *Mucuna pruriens* seed flour.

S/No	Sample code	Treatment	Phenol (%)	Phytate (%)	Tannins (%)	Oxalate (%)	Saponin (%)	HCN (mg/kg)	TIA (TIU/mg protein)	L-DOPA (%)
1.	CON	Raw (control)	1.82d	1.50d	0.38c	0.09a	0.38a	9.86a	12.58a	6.83a
2.	S24h	24h soaked	0.92e	0.82e	0.35d	0.09a	0.35b	9.73b	8.04b	6.01c
3.	S48h	48h soaked	0.80fg	0.58f	0.33e	0.08ab	0.30c	9.11c	7.21d	5.83e
4.	S72h	72h soaked	0.73h	0.42gh	0.30gh	0.07bc	0.27de	8.35d	6.08g	4.26h
5.	C20m	20min cooked	0.85f	0.56f	0.24i	0.05de	0.37a	8.11e	5.94h	4.96g
6.	C40m	40min cooked	0.82fg	0.46g	0.20j	0.05de	0.34b	7.51g	1.12m	3.63i
7.	C60m	60min cooked	0.70h	0.41gh	0.15k	0.04e	0.24g	4.94j	0.78n	2.93j
8.	C80m	80min cooked	0.69h	0.40h	0.12l	0.04e	0.18i	4.34k	0.00p	1.27o
9.	R10m	10min roasted	0.78g	0.58f	0.32ef	0.06cd	0.35b	9.84a	6.04g	6.22b
10.	R15m	15min roasted	0.71h	0.46g	0.31fg	0.06cd	0.28d	7.21h	5.21i	5.92d
11.	R20m	20min roasted	0.69h	0.35i	0.29h	0.05de	0.26ef	4.97j	3.48j	5.19f
12.	G24h	24h germinated	1.82d	1.32	0.20j	0.09a	0.38a	9.71b	7.54c	1.56m
13.	G48h	48h germinated	1.82d	1.08	0.20j	0.08ab	0.37a	8.01f	7.11e	1.34n
14.	G72h	72h germinated	1.81d	0.90	0.20j	0.06cd	0.35b	7.07i	6.82f	1.30o
15.	F24h	24h fermented	2.02c	4.06a	0.50a	0.07bc	0.30c	2.56l	0.46o	2.80k
16.	F48h	48h fermented	2.82b	3.50b	0.49a	0.06cd	0.25fg	2.34m	1.31l	2.50l
17.	F72h	72h fermented	3.02a	3.09c	0.41b	0.06cd	0.20h	2.01n	2.29k	1.30o
	SEM		0.11	0.16	0.02	0.00	0.01	0.38	0.47	0.28

Values are means of three replicates. Means in the same column with different superscripts are significantly different ($p < 0.05$); SEM - standard error of the mean. HCN- hydrogen cyanide, TIA -trypsin inhibitor activity, L-DOPA – L-3,4-dihydroxyphenylalanine.

phytic acids, tannins, saponin, phenolics, flavonoids and (L-DOPA) by soaking was documented by Ref. [25]. A similar observation in the reduction of antinutrients (flavonoids, saponins, tannins, phenol and Hydrogen cyanide) was made by Nwaoguikpe et al. [8] as a result of 48 h soaking of *Mucuna pruriens* seed. Soaking has also been reported to reduce anti-nutritional factors (polyphenols and tannins) in other legumes like red gram, bengal gram, green gram and lentil [26], as well as tannin, phytic acid and trypsin inhibitor in cowpea after 16 h soaking [27].

Cooking also significantly reduced ($p < 0.05$) in a regular sequence all the anti-nutritional factors evaluated. Cooking exhibited a better anti-nutritional factors' reduction effect than the other treatments that were used. This is in agreement with Shi et al. [28] that reported a better antinutrients reduction by cooking as against soaking. The anti-nutritional factors were observed to have decreased with increase in cooking time; the concentration of anti-nutritional factors showed an inverse relationship with cooking time. The reduction in the anti-nutritional factors during cooking in this research is in agreement with that of Shi et al. [28] for phytic acid and oxalate in pea, lentil, faba bean, chick pea, red kidney bean, and soybean after 1 h cooking. Hefnawy [29] also reported similar results during cooking as regards the reduction of trypsin inhibitor activity, tannin and phytic acid in lentil seed as well as for saponin in chickpeas and lentils [30]. Cooking for 80 min destroyed the entire trypsin inhibitor activity. Trypsin inhibitors antagonize the activities of trypsin and chymotrypsin through the synthesis of indigestible complexes resulting to the impedance of digestion of proteins [30]. Similarly, a total elimination of trypsin inhibitor activity was documented by Avilés-Gaxiola et al. [31] for lentil and faba bean after 1 h cooking as well as for soybean after 1 h of cooking [28].

Roasting also significantly reduced ($p < 0.05$) the anti-nutritional factors to varying concentrations. Roasting was generally less effective than cooking in reducing the anti-nutritional factors. This indicates that cooking which is a wet heat treatment is better than roasting which is a dry heat treatment in reducing antinutrients in whole *Mucuna pruriens* seed. L-DOPA was the least reduced by roasting from 6.83% in raw seed to 5.19% in 20 min roasted sample (R20 m) representing a 24% reduction. This little reduction effect of roasting on L-DOPA could be attributed to the relatively higher time that may be needed to reach the core of the whole seed and higher stability to dry heat than wet heat. This indicates that roasting may not be a suitable treatment for *Mucuna pruriens* seed in its whole (uncracked) form. L-DOPA is a toxic non-protein amino acid and a precursor of dopamine which can cause vomiting, nausea, anorexia and diarrhea when consumed in high amounts [32]. Roasting for 15 min reduced phytate the most from 1.50% to 0.35% which is a 77% reduction. Phytate, an inositol hexaphosphate derivative is the cardinal form for the storage of phosphorus in plants. Phytate forms complexes with minerals and amino acids thereby reducing minerals and amino acids bioavailability [33]. Attempts have already been made by Refs. [5,34] in investigating the effect of roasting on the antinutrients in *Mucuna pruriens* seed at 100 °C for 1 h and 2 h. They discovered that roasting reduced most of the antinutrients except phenol which increased significantly ($p < 0.05$) [34] and also L-DOPA [5].

Anti-nutritional factors in *Mucuna pruriens* seed as affected by germination is contained in Table 2. There were significant differences ($p < 0.05$) in the anti-nutritional factors during germination. Germination is a catabolic process which has the ability to alter the chemical components (nutrients or antinutrients) in seeds [35]. During the germination of *Mucuna pruriens* seed, all the anti-nutritional factors (phytate, tannins, oxalate, saponin hydrogen cyanide, trypsin inhibitor activity and L-DOPA were significantly reduced ($p < 0.0$) except phenol which did not differ significantly. The reduction in the antinutrients was continuous and regular as the hours of germination increased except for tannin which was reduced from 0.38% in the raw seed (CON) to 0.20% after 24 h germination but remained constant at 0.20% after 48 h and 72 h germination periods. These reductions in most of the anti-nutritional factors in *Mucuna pruriens* seed during germination in this research are similar to the report of Mugendi et al. [5] on L-DOPA, trypsin inhibitor activity and phytate in *Mucuna pruriens* seed; Sangronis and Machado [36] on trypsin inhibitor activity, phytic acid and tannin in black beans (*Phaseolus vulgaris* L.), white beans (*Phaseolus vulgaris* L.) and Pigeon pea (*Cajanus cajan*); Anaemene and Fadupin [19] on tannin, phytate, oxalate, saponin and polyphenol in pigeon pea.

Unlike other ant-nutrients, phenol was not significantly reduced. The phenolic content of many legumes (African bread fruit, Bambara nut, red bean, pigeon pea, African yam bean, oil bean and groundnut) have been found not to decrease but rather increased during germination [37]. Increase in polyphenols during germination has also been reported by Duenas et al. [38] for lupin and Khang et al. [39] for black bean, mung bean, peanuts, Adzuki beans, soybean and cowpea. The non-significant ($p > 0.05$) reduction of phenol in *Mucuna pruriens* seed as corroborated by the reports of other scholars as noted earlier indicates that germination may not be a suitable technique for the reduction or elimination of phenol in *Mucuna pruriens* seed and possibly for other pulses. Germination can lead to the solubilization of phenols in germinated seeds thereby increasing its content [37]. Phenols are known to inhibit the activities of digestive enzymes and are therefore detrimental to the process of digestion [38]. Among all the anti-nutritional factors, L-DOPA recorded the highest reduction during germination from 6.83% in the raw seed (CON) to 1.30% in 72 h germinated sample (G72h) which represents 81% reduction. The reduction in L-DOPA content was also regular as the germination time was increased from 24 h to 48 h and finally to 72 h.

The effect of fermentation using *Rhizopus oligosporus* on the anti-nutritional factors in *Mucuna pruriens* seed is shown in Table 2. There were significant differences ($p < 0.05$) in all the anti-nutritional factors during fermentation. Phenol, phytate, and tannin were all increased significantly ($p < 0.05$) by 24, 48 and 72 h fermentation while oxalate, saponin, hydrogen cyanide, trypsin inhibitor activity and L-DOPA were all decreased significantly by 24, 48 and 72 h fermentation. The decrease in oxalate, saponin, hydrogen cyanide and L-DOPA was regular in pattern while that of trypsin inhibitor was irregular. During fermentation treatment, 72 h fermentation reduced the anti-nutritional factors the most except phenol and trypsin inhibitor activity. Trypsin inhibitor activity was decreased from 12.58 TIU/mg protein in raw seed to as low as 0.46 TIU/mg protein after 24 h fermentation and got increased to 1.31 TIU/mg protein and 2.29 TIU/mg protein after 48 h and 72 h fermentation respectively. In the same vein, the use of fermentation in reducing anti-nutrients in *Mucuna pruriens* seed have been reported by Ogudoro et al. [34]. Ogudoro et al. [34] reported a reduction in saponin, hydrogen cyanide, oxalate, alkaloids and flavonoids. They also observed a reduction in tannin, phytate and phenol which is

contrary to the findings of this present research that indicated an increase in the three anti-nutrients. These variations in the report on tannin, phytate and phenols is attributed to the use of a different fermenting organism (*R oligosporus*) and use of dehulling in this research which could cause a variation in the food matrix and chemical composition. A research was carried out by Ojokoh et al. [40] wherein they recorded a reduction in the phytate, oxalate and tannin content of *Mucuna pruriens* after 72 h of fermentation with *lactobacillus plantarum* as against the increase in phytate and tannin in this present research. The use of *Rhizopus oligosporus* in this present research as against the *Lactobacillus plantarum* used byOjokoh et al. [40] would have contributed to this variation. For the significantly increased anti-nutritional factors (phenol, phytate and tannin), the increase in phenol had a regular pattern while that wasn't the case for phytate and tannins. These results suggest that fermentation using *Rhizopus oligosporus* may not be a suitable technique for the reduction or elimination of phenol, phytate and tannin in *Mucuna pruriens* seed and possibly other species of Mucuna as well as pulses at large. The increase in phytate and tannin during fermentation witnessed in this research is in line with the report of Mugendi et al. [5] who equally reported an increase in both phytate and tannin but a decrease in total phenolics. For L-DOPA, being the anti-nutritional factor with very high concentration and of greatest concern in *Mucuna pruriens* seed, it was notably reduced from 6.83% in the raw seed (CON) to 2.80%, 2.50% and 1.30% after 24, 48 and 72 h fermentation periods respectively. The disparity in the report on phenol may be attributed to the use of different micro-organisms for fermentation as *Rhizopus oligosporus* was used in this research while Mugendi et al. [5] used *Bacillus subtilis*. Furthermore, many phenolic compounds can be synthesized during fermentation [18,41]. Fermentation involves microbial/enzymatic processing of food which is capable of altering the biochemical composition of foods [40].

Though the single treatments (soaking, cooking, roasting, germination and fermentation) generally significantly ($p < 0.05$) reduced many of the antinutrients but phenol, saponin and L-DOPA were not reduced by any of the treatments below the recommended safe limits of 0.003%, 0.02% and 0.1% respectively.

4.4. Proximate composition of Mucuna pruriens seed flours that received double treatments

Mucuna pruriens seed flours that were subjected to double treatments were evaluated for their proximate composition and their values recorded in Table 3. Single treatments were merged to achieve double treatments based on optimal reduction of antinutrients and better nutrients retention. These double treatments include: 72 h soaking +60 min cooking; 48 h germination +15 min roasting; 48 h germination +60 min cooking; 72 h fermentation +15 min roasting. There were significant differences ($p < 0.05$) in the proximate parameters (moisture, ash, crude protein, crude fibre, fat and carbohydrate) as a result of the double treatments. The moisture content, ash, crude fibre and fat in *M. pruriens* seed were significantly decreased during the double treatments except for the crude fibre content of 48 h germinated +15 m roasted sample (GR2). The range for the crude protein of the sample was 24.10–30.40% while crude fibre had a range of 4.13–5.49%. The carbohydrate and crude protein content of the various sample significantly increased ($p < 0.05$) except the crude protein content of 72 h soaked + 60 m cooked sample (SC1). The sample that received the double treatment of 72 h fermentation +15 m roasting (FR4) had its crude protein content significantly increased the most from 25.34% (raw seed) to 30.40%.

The proximate parameters of double treated samples when compared with those of that received single treatments show that the double treatments had comparable effect with single treatments (Table 1) on the moisture and carbohydrate content. The ash and fat content appear to have been decreased the more by the double treatments (Table 3) than the single treatments (Table 1) while the crude protein and crude fibre were significantly increased the more by the double treatments than the single treatments as shown in Table 1. The observed increasing effect of many of the double treatments may have been as a result of the overlapping increasing effect of germination and fermentation with the other treatments they were combined with during the double treatment, as well as the unique nature of the make-up and matrix of the samples that received double treatments. This shows that 48 h germination +15 m roasting, 48 h germination +60 m cooking and 72 h fermentation +15 m roasting can be adopted to increase the crude protein content of *M. pruriens* seed.

Table 3
Proximate composition (%) of *Mucuna pruriens* seed flours that received double treatments.

			Parameters					
S/No	Sample code	Treatment	Moisture Content	Ash	Crude protein	Crude fibre	Fat	Carbohydrate
1.	CON	Raw (control)	10.99^a	3.82^a	25.34^d	4.69^b	5.94^a	49.22^e
2.	SC1	72 h soaked + 60 m cooked	11.15^a	3.40^b	24.10^e	4.36^c	4.94^b	52.05^a
3.	GR2	48 h germinated + 15 m roasted	9.95^b	1.39^e	28.78^b	5.49^a	4.30^c	50.09^d
4.	GC3	48 h germinated +60 m cooked	11.04^a	1.93^c	27.04^c	4.60^b	4.21^c	51.18^b
5.	FR4	72 h fermented +15 m roasted	10.11^b	1.71^d	30.40^a	4.13^d	2.80^d	50.85^c
	SEM		0.14	0.26	0.61	0.13	0.28	0.26

Values are means of three replicates. Means in the same column with different superscripts are significantly different ($p < 0.05$); SEM - standard error of the mean.

4.5. Anti-nutritional factors in Mucuna pruriens seed flours that received double treatments

The anti-nutritional factors in *Mucuna pruriens* seed flours that received double treatments are shown in Table 4. Single treatments were merged to achieve double treatments based on optimal reduction of antinutrients and better nutrients retention. The selected double treatments that were adopted include: 72 h soaking +60 min cooking; 48 h germination +15 min roasting; 48 h germination +60 min cooking; 72 h fermentation +15 min roasting. There were significant differences ($p < 0.05$) in the anti-nutritional factors in *M. pruriens* seed that was given double treatments. All the anti-nutritional factors were significantly reduced ($p < 0.05$) except phenol whose values got significantly increased by the double treatments. L-DOPA which is an anti-nutritional factor of great concern in *M. pruriens* seed had a range of 0.01%–6.83% and was reduced the most (99.85% reduction) by 72 h fermentation +15 m roasting (FR4) from 6.83% to 0.01% which is below the recommended safe limit of 0.1% for L-DOPA. This same treatment of 72 h fermentation +15 m roasting also reduced the most all the anti-nutritional factors evaluated.

In comparison with the effect of the single treatments on the anti-nutritional factors in *M. pruriens* seed (Table 2), the double treatments on the overall had better reduction effect. The general reduction effect of the double treatments on antinutrients (Table 4) when compared with that of single treatments (Table 2) was more on tannins, oxalate, saponin, hydrogen cyanide, trypsin inhibitor activity and L-DOPA and less on phenol and phytate. This is an indication that the

combined treatments may not be suitable for the reduction of phenol and phytate in *M. pruriens* seed. The combined effect of the treatments that were merged during double treatment in processing *M. pruriens* seed would have probably summed up to yield a greater magnitude of effect which resulted to the observed greater reduction effect on the anti-nutrients in *M. pruriens* seed flour as against that of single treatments.

5. Limitations

Tannin and phytate were determined using a Colorimeter because of the unavailability of a Spectrophotometer at the time of their determination. Spectrophotometers generally give results with higher accuracy and precision than Colorimeters.

6. Conclusion

In conclusion, the findings from this research proved that germination and fermentation with *Rhizopus oligosporus* have a positive impact on the crude protein and ash content of *Mucuna pruriens* seed flour, while soaking, cooking, and roasting lead to a reduction in these nutrients. The study reveals that soaking, cooking, roasting, germination, and fermentation are effective in reducing various anti-nutritional factors, with the exception of phenol, phytate, and tannin, which are not significantly reduced through fermentation with *Rhizopus oligosporus*. Remarkably, 80 min cooking proved to be highly effective in completely eliminating trypsin inhibitor in *Mucuna pruriens* seed flour. However, the highest level of single treatments (72 h soaking, 80 min cooking, 20 min roasting, 72 h germination and 72 h fermentation with *Rhizopus oligosporus*) are not suitable for the reduction of phenol, saponin and L-DOPA in *Mucuna pruriens* seed flour below the recommended safe limits of 0.003%, 0.02% and 0.1% respectively.

Among the combination of treatments evaluated, 72 h of fermentation with 15 min of roasting was the most suitable processing combination that reduced L-DOPA to 0.01% which is below the recommended safe limit of 0.1%. Hence, *Mucuna pruriens* seed should be fermented (72 h) and roasted (15 min) before usage in food products. There is need for future research on the Protein Efficiency Ratio and Biological Value of the processed *M. pruriens* seed flour to evaluate its protein quality and the level of protein bioavailability.

Author contribution statement

Smith Nkhata: Analyzed and interpreted the data; Wrote the paper.

Clement Chinedum Ezegbe: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Justina Nne Nwosu: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Clifford Ifeanyi Owuamanam: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Tope Adeyemisi Victor-Aduloju: Performed the experiments; Analyzed and interpreted the data.

Data availability statement

Data included in article/supplementary material/referenced in article.

Additional information

No additional information is available for this paper.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to

Table 4
Anti-nutritional factors in *Mucuna pruriens* seed flours that received double treatments.

S/No	Sample code	Treatment	Parameters							
			Phenol (%)	Phytate (%)	Tannins (%)	Oxalate (%)	Saponin (%)	HCN mg/kg	TIA (TIU/mg protein)	L-DOPA (%)
1.	CON	Raw (control)	1.82^{d}	1.50^{a}	0.38^{a}	0.09	0.38^{a}	9.86^{a}	12.58^{a}	6.83^{a}
2.	SC1	72 h soaked + 60 m cooked	2.89^{b}	0.87^{b}	0.14^{b}	0.02	0.09^{b}	8.38^{b}	2.52^{b}	2.10^{b}
3.	GR2	48 h germinated + 15 m roasted	3.49^{a}	0.68^{b}	0.12^{b}	0.01	0.05^{b}	6.90^{d}	1.38^{c}	1.23^{c}
4.	GC3	48 h germinated +60 m cooked	2.68^{c}	0.80^{b}	0.16^{b}	0.01	0.06^{b}	7.69^{c}	2.64^{b}	1.31^{c}
5.	FR4	72 h fermented +15 m roasted	1.71^{d}	0.09^{c}	0.11^{b}	0.00	0.03^{b}	2.17^{e}	0.50^{d}	0.01^{d}
	SEM		0.18	0.12	0.03	0.02	0.04	0.70	1.18	0.63

Values are means of three replicates. Means in the same column with different superscripts are significantly different ($p < 0.05$); SEM - standard error of the mean. HCN- hydrogen cyanide, TIA-trypsin inhibitor activity, L-DOPA – L 3,4-dihydroxyphenylalanine.

influence the work reported in this paper.

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