

Original Research Article

Effect of single and double treatment methods on the micronutrients in *Mucunapruriens* (velvet bean) seed flour.

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ABSTRACT

Aims: *Mucunapruriens* seed is an underutilized legume with good nutritional value with a possibility of changes in its micronutrients' composition during processing whose data is scanty. *Mucunapruriens* seed flour was therefore evaluated for the effect of soaking, cooking, roasting, germination and fermentation as well as some double treatments on its vitamins and minerals contents.

Place and Duration of Study: Department of Food Science and Technology, Federal university of technology, Owerri, Imo State, Nigeria.

Methodology: *Mucunapruriens* seeds were cleaned, washed, soaked, cooked, roasted, germinated and fermented. Vitamins and minerals composition were determined.

Results: Soaking, cooking and roasting significantly reduced ($p < 0.05$) all the vitamins. Vitamin B₉ was significantly ($p < 0.05$) reduced the most with a range of 0.28 – 21.88 mg/100g. Vitamins B₁, B₂, B₃ and B₁₂ were significantly increased by fermentation with vitamin B₂ increased the most (0.26 to 1.50 mg/100g; represents 577% increase). Vitamin B₉ was the most significantly reduced ($p < 0.05$) from 21.88 to 0.28 mg/100g by 72 h fermentation. Cooking reduced all the minerals except potassium and sodium. Potassium was increased from 690.50 to 930.75, 760.50 and 730.00 mg/100 in 10, 15 and 20 minutes roasted samples respectively. Germination significantly ($p < 0.05$) reduced all the minerals except 24 h germination which increased calcium from 218.17 mg/100g in the raw *Mucunapruriens* seed to 234.36 mg/100g. Fermentation generally reduced all the minerals significantly ($p < 0.05$) except calcium and zinc.

Conclusion: Germination and fermentation have proved to be suitable methods for the enhancement of vitamin B₂, B₃, B₁₂ and zinc in *Mucunapruriens* seed flour. Combined process treatments reduce most vitamins and minerals in *M. pruriens* seed flour and are therefore not suitable for their improvement.

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Keywords: *Mucunapruriens*, vitamin, mineral, germination, fermentation

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1. INTRODUCTION

Mucunapruriens commonly known as velvet bean, cowitch or cowhage is of the family leguminosae, genus; *Mucuna* and Specie; *Mucunapruriens*[1]. Traditionally in Enugu, the south eastern part of Nigeria, *Mucunapruriens* 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 15m in length, almost completely

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covered with fuzzy hair when young, but almost free of hairs when older [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].

Mucunapuriens 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 %) [4] and its digestibility is comparable to that of other pulses like soybean, rice bean and lima bean [1, 5] but its contents of some antinutrients limit its food utilizations. *Mucunapuriens* seed contains 73.5 µg/ml of vitamin A, 147.4 µg/ml of vitamin B₁, 44.5 µg/ml of vitamin B₂, 14.7 µg/ml of vitamin B₃ and 4.9 mg/100g of vitamin C [6], 0.85mg/100g of Vitamin B₆ and 0.37mg/100g of vitamin B₁₂ [7]. Raw seed of *Mucunapuriens* contains 4.69mg/100g Sodium, 164.5mg/100g Potassium, 77.4mg/100g calcium, 244.59mg/100g Phosphorus, 29.29mg/100g magnesium, 14.61mg/100g iron, 3.27mg/100g copper, 5.03mg/100g Zinc, 3.26mg/100g manganese and 19.00mg/100g selenium [8]. The antinutritional factors found in *mucunapuriens* include phenolics, tannins, 3,4-dihydroxyphenylalanine, phytic acid, hydrogen cyanide, trypsin inhibitor (Kala *et al.*, 2010).

In order to improve the nutritional quality and effectively increase utilization of grain legumes like *Mucunapuriens*, many food processing methods can be adopted to reduce/eliminate the anti-nutritional factors [9]. These processing methods include soaking, dehulling, cooking, fermentation, germination, toasting/roasting and autoclaving [5, 9, 10].

During these process treatments geared towards making *Mucunapuriens* seed wholesome and effectively utilize it, there are possibilities of reduction or improvement in the vitamins and minerals that it contains whose details are largely lacking in the scientific literature. Hence this study was embarked on to investigate the effects of soaking, cooking, roasting, germination and fermentation as well as double (combined) treatments on vitamins and minerals in *Mucunapuriens* (velvet bean) seed flour.

Provide a factual background, clearly defined problem, proposed solution, a brief literature survey and the scope and justification of the work done.]

2. MATERIAL AND METHODS

2.1 Source of raw materials and equipment

Raw *Mucunapuriens* 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 Methods

Processing of raw materials

Soaking, boiling, roasting, germination and fermentation were the treatment methods adopted in processing *Mucunapuriens* seed into flour.

2.2.1 Soaking of *Mucunapuriens* seed

Whole *Mucunapuriens* seeds (1.2kg) were cleaned of any extraneous materials while in dry form, sorted and washed with distilled water. The seeds were divided into three batches (400 g each) which were coded S24h, S48h and S72h 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

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Comment [U14]: Delete with 'utilizable'

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Comment [U17]: Replace with 'Materials'

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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 with (Binatone BL-1500PRO, China) and stored in a low-density polyethylene bags in readiness for analyses.

Comment [U22]: Mention type of oven used

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2.2.2 Cooking of *Mucunapuriens* seeds

Whole *Mucunapuriens* seeds were cleaned, sorted and washed with distilled water. The seeds were divided into four batches of 400 g each. The four batches coded C20m, C40m, C60m and C80m 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 bags in readiness for analysis.

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2.2.3 Roasting of *Mucunapuriens* seeds

Whole *Mucunapuriens* seeds were sorted and cleaned of unwanted and extraneous materials. The seeds were divided into three batches (R10m, R15m and R20m) of 400 g per batch. The batches were roasted in an Oven (Electric hot Oven, Saisho Model: S-936R) at 150 °C; Batch 1 (R10m) was roasted for 10 min, batch 2 (R15m) for 15 min and batch 3 for 20 min, respectively. The samples were then ground and stored in a low-density polyethylene bags in readiness for analyses.

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2.2.4 Germination of *Mucunapuriens* seed

The procedure of [5] was used for the germination process. One hundred grams of *Mucunapuriens* seeds were soaked in ethanol (1:2 w/v) for 1 minute 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. 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.

2.2.5 Fermentation of *Mucunapuriens* seeds

The procedure described by [11] was adopted for the fermentation process. *Mucunapuriens* seeds were boiled in distilled water for 45 min (1kg/6L), hand-dehulled, chopped into 2-3 pieces per grain, soaked twice (1kg/3L) for 12 h with removal of soak water after each soaking period, re-cooked for 45 min (1kg/6L), drained and cooled. To prevent the growth of the other 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 [12]. The grains obtained were divided into three portions, inoculated with *Rhizopus oligosporus* (0.4 g/kg drained grain), packed in polyethylene perforated bags

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(50µm) and fermented (29°C) for 24 h, 48 h and 72 h to obtain samples F24h, F48h and F72h, respectively.

2.3 Determination of Vitamins in soaked, cooked, roasted, germinated and fermented *Mucunapuriens* seed flour.

2.3.1 Determination of B-Group Vitamins

The method as described by [13] was adopted in the determination of vitamins. HPLC-grade solvents were used for the analysis. *Mucunapuriens* seed powder (2 g) was placed in 25 mL of H₂SO₄ (0.1 N) solution and incubated for 30 min at 121°C. Then, the content was cooled and adjusted to pH 4.5 with 2.5 M sodium acetate and 50 mg Takadiastase enzyme was added. The preparation was stored at 35°C overnight. The mixture then was filtered through a Whatman No. 4 filter, and the filtrate diluted with 50mL of potable water and filtered again through a micropore filter (0.45µm). Twenty microlitres of the filtrate was injected into the HPLC system. Quantification of B group vitamins was accomplished by comparison to vitamin B group standards. Standard stock solutions for thiamine, riboflavin, niacin, pyridoxine, and cobalamin was prepared and Chromatographic separation was achieved on a reversed phase- (RP-) HPLC column (Agilent ZORBAX Eclipse Plus C18; 250x4.6 mm i.d., 5µm) through the isocratic delivery mobile phase (A/B 33/67; A:MeOH, B: 0.023 M H₃PO₄, pH = 3.54) at a flow rate of 0.5 mL/min. Ultraviolet (UV) absorbance was recorded at 270 nm at room temperature.

Comment [U31]: Delete with 'flour'

Comment [U32]: Specify the period of storage

2.3.2 Determination of Vitamin C

Vitamin C was extracted according to the method described by [13]. Ten (10g) of the sample was blended and homogenized with an extracting solution containing metaphosphoric acid (0.3 M) and acetic acid (1.4 M). The mixture was placed in a conical flask and agitated at 10,000 rpm for 15 min. The mixture was then filtered through a Whatman No. 4 filter and samples extracted in triplicate. The ascorbic acid standard was prepared by dissolving 100 mg of l-ascorbic acid in a metaphosphoric acid (0.3 M)/acetic acid (1.4 M) solution at a final concentration of 0.1 mg/mL. The calibration line was converted to a line arrange based on four measured concentration levels. Quantification of ascorbic acid content was performed on an Agilent HPLC system. Chromatographic separation was achieved on an RP-HPLC column through isocratic delivery of a mobile phase (A/B 33/67; A: 0.1 M potassium acetate, pH = 4.9, B: acetonitrile:water [50:50]) at a flow rate of 1 mL/min. UV absorbance was recorded at 254 nm at room temperature.

2.4 Determination of minerals in soaked, cooked, roasted, germinated and fermented *Mucunapuriens* seed flour.

The minerals in the *Mucunapuriens* seed flours were analysed using the method described by [14]. An aliquot of 2.0g of the samples was digested with concentrated nitric acid and concentrated perchloric acid in ratios 5:3(v/v), the mixture was placed on a water bath for 3 hours at 80°C. The resultant solution was cooled and filtered into 100ml standard flask and made to mark with distilled. The minerals were then determined using atomic absorption Spectrophotometer (Buck scientific model 210/211).

3. RESULTS AND DISCUSSION

3.1 Vitamins composition of soaked, cooked, roasted, germinated and fermented *Mucunapruriens* seed flour.

The vitamins composition of *Mucunapruriens* seed that was soaked, cooked, roasted, germinated and fermented are shown in Table 1. The treatments induced significant differences ($p < 0.05$) in the vitamin contents of *Mucunapruriens* seed flours. Soaking, cooking and roasting significantly reduced ($p < 0.05$) all the vitamins while germination and fermentation significantly ($p < 0.05$) increased many of the vitamins. Vitamins are very important micro-nutrients in the body without which some disease conditions may develop [15]. The stability and/or retention of vitamins depend on their structure and processing conditions [15].

Raw *Mucunapruriens* seed recorded 0.59, 0.26 and 0.74 mg/100g for vitamins B₁, B₂ and B₃ respectively and were comparable to that of cowpea as documented by [16] with a vitamin B₁ value of 0.524 mg/100g, vitamin B₂ value of 0.072 mg/100g and vitamin B₃ value of 0.0797 mg/100g. The results have shown that *Mucunapruriens* seed is a good source of vitamins B₁, B₉ and B₁₂ when compared with other pulses [16].

Soaking, cooking and roasting significantly reduced ($p < 0.05$) all the vitamins evaluated. The trend of reduction was regular during cooking but was irregular during soaking and roasting. Vitamin A had a range of 1.47 – 1.70 mg/100g and was least influenced by the treatments followed by vitamin C. Vitamin A which was the least influenced during soaking and cooking may be as a result of the fact that vitamin A is not water soluble and relatively more stable than the B-grouping of vitamins.

Vitamin B₉ was significantly reduced the most by the treatments adopted in this research and had a range of 0.28 – 21.88 mg/100g. Soaking and boiling have the ability to hydrate and soften pulses leading to the loss of nutrients like water soluble vitamins through leaching [17, 18]. The heat generated during cooking and roasting may have also led to the destruction of some of the heat labile vitamins like vitamin B₁, B₂ and B₉ and C as also reported by [19].

For germination, there was a significant increase ($p < 0.05$) in some of the vitamins. Vitamin B₂ was significantly increased from 0.26 to 0.031 mg/100g, vitamin B₃ from 0.74 to 1.45 mg/100g and vitamin B₁₂ from 0.34 to 0.89 mg/100g. The increasing trend of the three vitamins (B₁, B₃ and B₁₂) during germination was regular. The rest of the vitamins (B₁, B₉, C and A) were significantly reduced during germination in a regular fashion also. Germination had the highest significant reduction effect on vitamin B₉, reducing it from 21.88 mg/100 in the raw seed to 1.46 mg/100g in 72 hours germinated sample (G 72h.). Germination has the ability to improve B-group of vitamins due to their synthesis by the new sprouts [20], just as observed in this study for B₁, B₃ and B₁₂. On that same note of increase of some water-soluble vitamins during germination, El- Adaway (2002) also observed a significant increase in vitamin B₂ content of germinated Chickpea from 173.3 to 201.33 mg/100g while vitamins B₁, and B₃ were documented to have been reduced by germination.

Table 1: Vitamins composition of *Mucunapruriens* seed that received single treatments.

S/No	Sample code	Treatment	Vitamin B ₁ (mg/100g)	B ₂ Vitamin (mg/100g)	B ₃ Vitamin (mg/100g)	B ₉ Vitamin (µg/100g)	B ₁₂ Vitamin (µg/100g)	C Vitamin (mg/100g)	A Vitamin (µg/100g)
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Comment [U35]: From the results (Table 1), germination treatment, improved vitamins B₂, B₃ and B₁₂. What is the justification for this claim since out of seven vitamins analysed, only three vitamins got improved significantly ($p < 0.05$) by this germination treatment.

Comment [U36]: In your statement here, use of 'comparable' should not be applied to vitamins B₂ and B₃ since your results and those of the cited author varied much. I.e., for vitamin B₂ ($0.26 - 0.072 = 0.188$ mg/100g); and for vitamin B₃ ($0.74 - 0.0797 = 0.6603$ mg/100g)

Comment [U37]: Please recheck from Table 1; it should be 0.31 and not 0.031

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1.	CON	Raw (control)	0.59 ^c	0.26 ^e	0.74 ^e	21.88 ^a	0.34 ^f	2.92 ^a	1.70 ^a
2.	S24h	24h soaked	0.26 ^{fg}	0.22 ^{fg}	0.55 ^j	13.78 ⁱ	0.23 ^{hij}	2.66 ^e	1.68 ^{bc}
3.	S48h	48h soaked	0.23 ^{gh}	0.17 ^h	0.64 ^{gh}	10.25 ^j	0.29 ^g	2.77 ^d	1.60 ^{ef}
4.	S72h	72h soaked	0.20 ^h	0.23 ^{et}	0.66 ^{fg}	9.78 ^k	0.37 ^{et}	2.68 ^e	1.59 ⁱ
5.	C20m	20min cooked	0.38 ^d	0.19 ^{gh}	0.67 ^f	19.22 ^c	0.21 ^{hijk}	2.88 ^b	1.66 ^d
6.	C40m	40min cooked	0.26 ^{fg}	0.17 ^h	0.66 ^{fg}	18.88 ^d	0.16 ^k	2.83 ^c	1.61 ^e
7.	C60m	60min cooked	0.25 ^{fg}	0.17 ^h	0.63 ^{gh}	18.57 ^e	0.17 ^k	2.81 ^{cd}	1.60 ^{ef}
8.	C80m	80min cooked	0.23 ^{gh}	0.17 ^h	0.59 ^j	17.65 ^t	0.19 ^{jk}	2.79 ^{cd}	1.51 ^h
9.	R10m	10min roasted	0.60 ^c	0.19 ^{gh}	0.59 ^j	19.68 ^b	0.18 ^{jk}	2.88 ^b	1.70 ^a
10.	R15m	15min roasted	0.27 ^f	0.25 ^{ef}	0.61 ^{hi}	16.84 ^g	0.24 ^{ghi}	2.68 ^e	1.70 ^a
11.	R20m	20min roasted	0.25 ^{fg}	0.22 ^{fg}	0.76 ^e	15.30 ^h	0.25 ^{gh}	2.58 ^f	1.67 ^{cd}
12.	G24h	24h germinated	0.36 ^{de}	0.26 ^e	1.42 ^a	1.49 ^j	0.55 ^c	2.92 ^a	1.59 ^f
13.	G48h	48h germinated	0.34 ^e	0.30 ^d	1.45 ^a	1.48 ^j	0.71 ^b	2.80 ^{cd}	1.56 ^g
14.	G72h	72h germinated	0.33 ^{de}	0.31 ^d	1.45 ^a	1.46 ^j	0.89 ^a	2.40 ^g	1.47 ⁱ
15.	F24h	24h fermented	0.61 ^c	1.50 ^a	1.10 ^b	0.38 ^m	0.47 ^d	2.60 ^f	1.70 ^a
16.	F48h	48h fermented	0.71 ^b	0.95 ^b	1.00 ^c	0.30 ⁿ	0.55 ^c	2.30 ^h	1.69 ^{ab}
17.	F72h	72h fermented	0.80 ^a	0.90 ^c	0.93 ^d	0.28 ⁿ	0.40 ^e	2.00 ⁱ	1.68 ^{bc}
	SEM		0.03	0.05	0.04	1.14	0.03	0.04	0.01

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.

Fermentation was also notable in significantly increasing ($p < 0.05$) many of the vitamins. Vitamins B₁ (thiamine), B₂ (riboflavin), B₃ (niacin) and B₁₂ (cyanocobalamin) were all significantly ($p < 0.05$) increased by fermentation, although the increasing trend of the vitamins were not regular for all. The maximum increase during fermentation for vitamin B₁ was from 0.59 to 0.80 mg/100g, vitamin B₂ from 0.26 to 1.50 mg/100g, vitamin B₃ from 0.74 to 1.10 mg/100g and vitamin B₁₂ from 0.34 to 0.55 mg/100g. Vitamin B₂ was the most improved (0.26 to 1.50 mg/100g) representing a 577% increase. These results indicate that vitamin B₂ (riboflavin) can be highly improved in legumes like *Mucunapuriens* seed through fermentation. Riboflavin promotes the absorption of Zinc and Iron in the body and therefore plays an essential indirect role in supporting growth and immunity [22]. Fungal and bacterial fermentation has the ability to produce very high amounts of riboflavin [22] as observed in this research. Fermentation has also been reported to improve B-group of vitamins [11, 20]. On the contrary, vitamins B₉ (folate), vitamin C and vitamin A were significantly reduced ($p < 0.05$) by fermentation. Vitamin B₉ was the most significantly reduced ($p < 0.05$) from 21.88 mg/100g in raw *Mucunapuriens* seed to 0.28 mg/100g in 72 hours fermented sample (F72h). The vitamin B₉ reduction may be attributed to the vitamin B₉ need of the fermenting fungi (*Rhizopus oligosporus*). Second to the most reduced is vitamin C, which was significantly reduced from 2.92 to 2.00 mg/100g, followed by vitamin A which was reduced from 1.70 to 1.68 mg/100g.

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3.2 Minerals composition of soaked, cooked, roasted, germinated and fermented *Mucunapuriens* seed flour.

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Minerals composition (mg/100g) of *Mucunapuriens* seed flour that received single treatments of soaking, cooking, roasting, germination and fermentation are presented in Table 2.

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Table 2: Minerals composition (mg/100g) of *Mucunapruriens* seed flours that received single treatments.

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S/No	Sample code	Treatment	Potassium	Sodium	Calcium	Phosphorus	Magnesium	Zinc	Manganese	Iron
1.	CON	Raw (control)	690.50 ^g	343.94 ^{bc}	218.17 ^{cde}	1779.00 ^{abc}	42.78 ^a	5.00 ^h	4.00 ^b	10.15 ^b
2.	S24h	24h soaked	340.90 ^m	309.61 ^d	216.56 ^{cde}	1780.00 ^{ab}	42.46 ^a	5.20 ^g	0.80 ^g	9.19 ^c
3.	S48h	48h soaked	380.75 ^j	305.17 ^{de}	213.11 ^{de}	1770.00 ^{abc}	42.65 ^a	5.10 ^{gh}	0.80 ^g	9.02 ^c
4.	S72h	72h soaked	580.85 ⁱ	291.35 ^e	200.67 ^{efg}	1790.00 ^a	42.52 ^a	4.50 ⁱ	0.25 ^j	8.01 ^d
5.	C20m	20min cooked	890.90 ^b	360.35 ^b	217.00 ^{cde}	1790.00 ^a	42.58 ^a	5.10 ^{gh}	0.60 ^h	9.26 ^c
6.	C40m	40min cooked	860.25 ^c	310.75 ^d	219.40 ^{de}	1750.00 ^{bcd}	42.41 ^a	5.00 ^h	0.45 ^h	9.11 ^c
7.	C60m	60min cooked	900.05 ^b	196.40 ^f	206.45 ^{def}	1740.00 ^{cd}	42.41 ^a	4.47 ⁱ	0.55 ^h	8.66 ^{cd}
8.	C80m	80min cooked	830.15 ^d	82.55 ^g	206.45 ^{def}	1760.00 ^{abc}	42.38 ^a	4.40 ⁱ	0.20 ⁱ	8.11 ^d
9.	R10m	10min roasted	930.75 ^a	344.90 ^{bc}	218.09 ^{cde}	1790.00 ^a	42.43 ^a	3.00 ^k	0.80 ^g	8.22 ^d
10.	R15m	15min roasted	760.50 ^e	383.50 ^a	216.77 ^{cde}	1790.00 ^a	42.43 ^a	3.50 ^j	0.60 ^h	8.15 ^d
11.	R20m	20min roasted	730.00 ^f	309.40 ^d	216.77 ^{cde}	1720.00 ^d	40.24 ^a	4.50 ⁱ	0.55 ^h	8.06 ^d
12.	G24h	24h germinated	120.92 ⁿ	89.23 ^g	234.36 ^c	889.30 ^g	28.45 ^b	7.20 ^d	3.80 ^c	0.50 ^f
13.	G48h	48h germinated	110.14 ^{no}	78.48 ^g	187.10 ^g	828.90 ^h	23.70 ^b	6.70 ^e	1.16 ^f	0.50 ^f
14.	G72h	72h germinated	98.00 ^o	80.56 ^g	191.21 ^{fg}	778.00 ⁱ	20.20 ^b	6.00 ^f	1.02 ^f	0.50 ^f
15.	F24h	24h fermented	502.72 ^l	328.00 ^c	220.41 ^{cd}	1150.00 ^{eo}	4.72 ^c	12.50 ^c	2.30 ^e	4.00 ^e
16.	F48h	48h fermented	401.84 ^k	299.02 ^{de}	425.68 ^a	1020.40 ^f	6.90 ^c	13.00 ^b	3.56 ^d	12.40 ^a
17.	F72h	72h fermented	601.51 ^h	368.79 ^a	380.52 ^b	1014.68 ^f	5.30 ^c	14.00 ^a	4.32 ^a	3.50 ^e
	SEM		39.55	18.14	0.09	56.63	2.10	0.46	0.06	0.50

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.

There were significant differences (p<0.05) in the mineral compositions of many of the samples. The process treatments (soaking, cooking, roasting, germination and fermentation) adopted in this study generally reduced significantly (p<0.05) many of the minerals although some of the minerals were also significantly increased. Potassium, calcium and zinc were relatively more stable than other minerals during the treatments; zinc was the most stable. Germination had the most significant decreasing effect (p<0.05) on the minerals while fermentation induced the most significant increasing effect.

Raw *Mucunapruriens* seed (CON) contained high amounts of potassium, sodium and phosphorus as their values were above the 100mg per 100g which is above 100mg benchmark for macro-minerals for a day. The sodium, phosphorus, zinc and iron contents of the raw *Mucunapruriens*seed (CON) were higher than the values reported by [23] for five accessions of *Mucunapruriens* seed India. These variations in the mineral's composition of *Mucunapruriens* seed in comparison with those from other regions/countries are influenced by differences in climate, composition of soil, temperature, volume of rainfall and surrounding vegetation [8, 23,24].

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Soaking caused significant decrease (p<0.05) in almost all the minerals except for phosphorus and zinc. This reduction trend in many of the minerals during soaking is not unexpected as nutrients like minerals can diffuse and get leached into soak-water. Soaking for 24 and 72 h caused a significant increase (p<0.05) in phosphorus from 1779.00 mg/100g to 1780.00 mg/100g and 1790.00 mg/100g respectively. Similarly, 24 and 48 h soaking

caused a significant increase in Zinc from 5.00mg/100 to 5.20 and 5.10mg/100g respectively.

Cooking significantly reduced ($p < 0.05$) all the micro-minerals (Zinc, manganese and Iron) except 20 minutes cooking which increased zinc content from 5.00 gm/100g in the raw seed (CON) to 5.10mg/100g. This decrease in minerals during cooking conforms with the details documented by [25].

Comment [U46]: Delete with 'mg'

Macro-minerals were also significantly reduced by cooking except potassium which recorded significant increase when compared with the value for the raw sample (CON). Potassium content in cooked samples was the most significantly increased, and ranged from 690.50 to 900.05 mg/100g for cooked samples.

Roasting significantly increased ($p < 0.05$) some of the minerals like potassium, sodium and phosphorus. Potassium was significantly ($p < 0.05$) increased from 690.50 mg/100g in the raw seed (CON) to 930.75, 760.50 and 730.00 mg/100 for 10, 15 and 20 min roasted samples respectively. Sodium was also significantly increased during 15 minutes roasting from 343.94 mg/100g in the raw seed to 383.50 mg/100g while phosphorus was significantly increased from 1779.00 to 1790.00 mg/100g for both 10 and 15 min roasting respectively. These findings regarding the increase in potassium, sodium and phosphorus during roasting agree with the findings of [25] which indicated an increase in phosphorus, potassium and sodium during the roasting of *Mucunamacrocarpa* seeds. Magnesium, iron and calcium did not differ significantly ($p > 0.05$) during roasting for 10, 15 and 20 minutes which implies greater stability during exposure to the dry heat of roasting. Minerals show relative higher stability on exposure to high temperatures [26].

Germination had the most reduction effect on many of the minerals when compared with other treatments. Amongst all the minerals, iron was reduced the most during germination from 10.15mg/100g in the raw seed (CON) to 0.05 mg/100g in the three germinated samples (G24h, G48h and G72h) respectively. Germination significantly reduced ($P < 0.05$) all the minerals except 24 hours germination which increased calcium from 218.17 mg/100g in the raw *Mucunapruriens* seed (CON) to 234.36 mg/100g and also Zinc from 5.00 mg/100g raw seed) to 7.20mg/100g.

Fermentation generally reduced many of the minerals significantly ($p < 0.05$) except for calcium, zinc and iron. This implies that calcium, zinc and iron content of *Mucunapruriens* seed can be improved through fermentation. Fermentation for 24, 48 and 72 h significantly increased ($p < 0.05$) calcium in an irregular pattern and zinc in a regular pattern. Potassium, phosphorus and magnesium were significantly reduced at the different levels of fermentation with their reduction trend being irregular. It is worthy to note that iron was significantly reduced from 10.15mg/100g in the raw seed (CON) to 4.00mg/100g after 24 h but significantly rose to a high value of 12.40 mg/100g after 48hours fermentation and got significantly reduced back again to a very low value of 3.50 mg/100g after 72 h germination.

Minerals are micro-nutrients which are vital for many metabolic processes and normal functioning of the body. There are two classes of minerals: macro-minerals (potassium, sodium, calcium, magnesium, phosphorus and Sulphur) are minerals needed in concentrations above 100mg each day in the body while micro-minerals (copper, zinc, iron, manganese, iodine, cobalt and fluorine) are needed in amounts that are lower than 100mg each day in the body [27].

Potassium is vital in blood pressure regulation, calcium is needed for good bone mass and phosphorus is necessary for ATP synthesis [24]. Zinc and manganese are associated with antioxidation activities as well as strengthening of immune system [8].

3.3 Vitamins composition of *Mucunapruriens* seed flours that received double treatments.

Comment [U47]: Delete

The vitamins contained in *Mucunapruriens* seed flours after the various double treatments are shown in Table 3. There were significant differences ($p < 0.05$) in the vitamins evaluated.

Table 3: Vitamins composition of *Mucunapruriens* seed flours that received double treatments.

Comment [U48]: Delete

S/No	Sample code	Treatment	Vitamin B ₁ (mg/100g)	Vitamin B ₂ (mg/100g)	Vitamin B ₃ (mg/100g)	Vitamin B ₉ (µg/100g)	Vitamin B ₁₂ (µg/100g)	Vitamin C (mg/100g)	Vitamin A (µg/100g)
1.	CON	Raw (control)	0.59 ^a	0.26 ^b	0.74 ^a	21.88 ^a	0.34 ^a	2.92 ^a	1.70 ^a
2.	SC1	72h soaked + 60 m cooked	0.31 ^b	0.67 ^a	0.16 ^b	9.19 ^b	0.20 ^{ab}	1.93 ^b	1.26 ^b
3.	GR2	48h germinated + 15m roasted	0.17 ^b	0.23 ^b	0.11 ^b	2.41 ^d	0.09 ^b	0.99 ^d	0.44 ^c
4.	GC3	48 h germinated + 60 m cooked	0.21 ^b	0.69 ^a	0.12 ^b	4.59 ^c	0.06 ^b	1.26 ^c	1.43 ^b
5.	FR4	72 h fermented + 15 m roasted	0.30 ^b	0.10 ^b	0.01 ^b	1.53 ^e	0.03 ^b	0.67 ^e	0.48 ^c
	SEM		0.04	0.07	0.07	2.00	0.04	0.21	0.14

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.

Vitamins B₂ ranged from 0.10 - 0.69 mg/100g with the 48 h germinated + 60 m cooked sample (GC3) having the highest value of 0.69 mg/100g while 72 h fermented + 15 m roasted sample (FR4) had a value of 0.10 mg/100g. Apart from Vitamin B₂ which was significantly ($p < 0.05$) increased by some of the double treatments, the rest of the vitamins (B₁, B₃, B₉, B₁₂, C and A) were all significantly reduced ($p < 0.05$) by all the double treatments. The sample that was treated using 72 h soaking + 60 m cooking (SC1) significantly increased vitamin B₂ from 0.26 to 0.67 mg/100g while 48 h germinated + 60 m cooked sample (GC3) significantly ($p < 0.05$) increased vitamin B₂ from 0.26 to 0.69 mg/100g.

Comment [U49]: What does 'm' stand for?

Comment [U50]: Use the correct symbol for minute as used in the previous sections

Comment [U51]: ??

When compared with vitamins values obtained during single treatments (Table 1), the double treatments generally had more reduction effect on the vitamins except for vitamin B₂ which had values similar to those of single treatments. The generally lower vitamins values of double treated samples would have most probably emanated from the effect of the combination of the treatments. This also indicates that the double treatments are not ideal for the enhancement of vitamins in *M. pruriens* seed except for vitamins B₂ using 72 h soaking + 60 m cooking or 48 h germination + 60 m cooking.

Comment [U52]: Use the correct symbol

Comment [U53]: Please, correct as pointed out earlier

3.4 Minerals composition of *Mucunapruriens* seed flours that received double treatments

Comment [U54]: Delete

The record of the minerals' composition of *Mucunapuriens* seed flours that received double treatments are represented in Table 4. The double treatments caused significant reductions ($p < 0.05$) in almost all the minerals evaluated except for calcium and phosphorus. Calcium was significantly increased from 218.17 to 259.62 mg/100g by 48 h germination + 15 m

Comment [U55]: Delete

Comment [U56]: From the Table 4., Calcium had significant ($p < 0.05$) increase for all the double treatments except for 'GR2'. Please, correct the statement.

Comment [U57]: Use 'min.'

Table 4: Minerals composition (mg/100g) of *Mucunapuriens* seed flours that received double treatments.

S/No	Sample code	Treatment	Potassium	Sodium	Calcium	Phosphorus	Magnesium	Zinc	Manganese	Iron
1.	CON	Raw (control)	690.50 ^a	343.94 ^a	218.17 ^b	1779.00 ^b	42.78 ^a	5.00 ^a	4.00 ^b	10.15 ^a
2.	SC1	72 h soaked+ 60 m cooked	435.02 ^d	230.44 ^b	161.45 ^e	782.76 ^e	25.24 ^e	2.25 ^d	1.00 ^e	5.89 ^d
3.	GR2	48hgerminated+ 15 m roasted	517.88 ^c	151.33 ^d	259.62 ^a	1334.25 ^c	31.23 ^c	4.50 ^b	4.72 ^a	6.39 ^b
4.	GC3	48 h germinated+ 60 m cooked	517.88 ^c	192.61 ^c	187.63 ^d	1791.53 ^a	42.06 ^b	2.25 ^d	1.92 ^d	4.97 ^e
5.	FR4	72 h fermented + 15 m roasted	607.64 ^b	230.44 ^b	187.84 ^c	1120.77 ^d	26.52 ^d	3.85 ^c	2.80 ^c	6.09 ^c
	SEM		23.38	17.14	8.97	103.71	2.01	0.30	0.36	0.48

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. CONCLUSION

[Raw *Mucunapuriens* seed is a good source of vitamins B₁, B₃ and B₁₂. Soaking, cooking and roasting reduced the vitamins in *Mucunapuriens* seed flour. Vitamin A was least influenced by the treatments and is therefore considered to be the most stable in *Mucunapuriens* seed. Vitamin B₉ was significantly ($p < 0.05$) reduced the most by the treatments adopted in this research, and therefore soaking, cooking, roasting, germination and fermentation are not suitable for its enhancement in *Mucunapuriens* seed flour. Germination increased vitamin B₂, B₃ and B₁₂ in *Mucunapuriens* seed flour. Vitamins B₁ (thiamine), B₂ (riboflavin), B₃ (niacin) and B₁₂ (cyanocobalamin) were all increased by fermentation with vitamin B₂ being the most improved (577% increase) and therefore is suitable for boosting vitamin B₂ in *Mucunapuriens* seed flour. Fermentation has the tendency to reduce vitamins B₉ (folate), vitamin C and vitamin A contents of *Mucunapuriens* seed flour.

Comment [U58]: Why the use of 'tendency' when you have results already?

Soaking reduced the minerals in *Mucunapuriens* seed flour except phosphorus and zinc. Cooking of *Mucunapuriens* seed reduced the minerals except potassium. Magnesium, iron and calcium did not differ during the roasting of *Mucunapuriens* seed for 10, 15 and 20 min which implies greater stability during exposure to the dry heat of roasting. Germination had the most reduction effect on many of the minerals except calcium after 24 h. Germination of *Mucunapuriens* seed is not a good method for the improvement of iron as iron was greatly reduced during germination. Fermentation has proved to be a good method for the improvement of calcium and zinc in *Mucunapuriens* seed flour. Processing of *Mucunapuriens* seed using double treatments (soaking+cooking, germination+roasting,

germination+cooking and fermentation+roasting) reduced many of the water soluble vitamins (B₁, B₃, B₉, B₁₂ and C), vitamin A as well as minerals (potassium, sodium, magnesium, zinc, manganese and iron) and therefore may not be suitable for their enhancement.

REFERENCES

1. Pathania R, Chawla P, Khan H, Kaushik R, Khan MA. An assessment of potential nutritive and medicinal properties of *Mucunapruriens*: a natural food legume. *Biotech.* 2020;10(6): 261-275.
2. Eze PC, Eze CN, Agu RS. Determination of physicochemical properties of velvet bean (*Mucunapruriens*) from south Eastern Nigeria. *Nigerian J of Tech.* 2017;36(2): 628-635.
3. Kavitha C, Thangamani C. Amazing bean "*Mucunapruriens*": A comprehensive review. *J of Medicinal plants Research.* 2014;8(2): 138-143.
4. Natarajan K, Narayanan N, Ravichandran N. Review on "*Mucuna*"- The wonder plant. *Inter J. of Pharm Sciences Review and Research.* 2012;17(1): 86-93.
5. Mugendi JB, Njagi EN, Kuria EN, Mwasaru MA, Mureithi JG, Apostolides Z. Effects of processing technique on the nutritional composition and anti-nutrient content of *Mucuna* bean (*Mucunapruriens* L.) *African J. of Food Sci.* 2010;4(4):156-166.
6. Ikechukwu, EL, Okafor PN, Egba SI. In vitro assessment of the anti-sickling properties of *Buchholziaceae* and *Mucunapruriens* seed extracts. *In Vitro Cellular & Developmental Biology - Animal.* 2020; doi:10.1007/s11626-020-00512-y.
7. Udengwu OS, Umejiaku CM, Umeh SI. *Mucunapruriens* (L.) Dc. (Var. Okeagbaloko) Can bridge the protein gap in the menu of the rural poor in South Eastern Nigeria. *World Applied Sciences Journal* 2018;36(7): 806-818. doi: 10.5829/idosi.wasj.2018.806.818.
8. Bhat R, Sridhar KR, Sahadevan S. Nutritional quality evaluation of Velvet bean seeds (*Mucunapruriens*) exposed to gamma irradiation. *International Journal of Food Sciences and Nutrition.* 2008;59(4): 261-278.
9. El-suhaibani M, Ahmed MA, Osman MA. Study of germination, soaking and cooking effects on the nutritional quality of goat pea (*Securigerasecuridaca* L.). *J. of King Saudi University-Sci.* 2020;32:2029-2033.
10. Nwaoguikpe, RN, Braide W, Ujowundu CO. The effects of processing on the proximate and phytochemical composition of *Mucunapruriens* seeds (velvet beans). *Pakistan Journal of Nutrition.* 2011;10(10): 947-951.
11. Egunlety M. Processing of velvet bean (*Mucunapruriens Varutilis*) by fermentation. *Trop. and Subtropical Agroecosystems.* 2003;1(3): 173-181.
12. Erkan SB, Gürler HN, Bilgin DG, Germec M, Turhan I. Production and characterization of Tempehs from different sources of legume by *Rhizopus oligosporus*. *LWT - Food Science and Technology,* 2020;119: 108880. Doi: <https://doi.org/10.1016/j.lwt.2019.108880>.
13. Sami R, Li Y, Qi B, Wang S, Zhang Q, Han F, Ma Y, Jing J, Lianzhou J. HPLC analysis of water-soluble vitamins (B₂, B₃, B₆, B₁₂ and C) and fat-soluble vitamins (E, K, D, A and β -carotene) of Okro (*Abelmoschus esculentus*). *Journal of Chemistry.* 2014;(1):1-6.
14. Asaolu SS, Adefemi OS, Oyakilome IG, Ajibulu KE, Asaolu MF. Proximate and mineral composition of Nigerian leafy vegetables. *Journal of Food Research.* 2012;1(3):214-218.
15. Gulati P, Brahma S, Rose DJ. Impacts of extrusion processing on nutritional components in cereals and legumes: Carbohydrates, proteins, lipids, vitamins, and minerals. In: *Extrusion Cooking.* Editor: Girish M. Ganjyal (2nd Edition). Woodhead Publishing. 2020; pp. 415–443. doi:10.1016/b978-0-12-815360-4.00013-4.
16. Venkidasamy B, Selvaraj D, Nile AS, Ramalingam S, Kai G, Nile SH. Indian pulses: A review on nutritional, functional and biochemical properties with future perspectives.

- Trends in Food Science & Technology. 2019;88: 228–242. doi:10.1016/j.tifs.2019.03.012.
17. Prinyawiwatkul W, Eitenmiller RR, Beuchat LR, Mcwatters KH, Phillips RD. Cowpea Flour Vitamins and Trypsin Inhibitor Affected by Treatment and Fermentation with *Rhizopusmicrosporus*. Journal of Food Science.1996;61(5): 1039–1042. doi:10.1111/j.1365-2621.1996.tb10928.x.
 18. Huma N, Anjum M, Sehar S, Khan MI, Hussain S. Effect of soaking and cooking on nutritional quality and safety of legumes. Nutr.and Food Sci. 2008;38(6): 570–577.
 19. Hotz C, Gibson RS. Traditional food-processing and preparation practices to enhance the bioavailability of micronutrients in plants-based diets. Journal of Nutrition.2007;137: 1097–1100. Doi: <https://doi.org/10.1093/jn/137.4.1097>.
 20. Nkhata SG, Ayua E, Kamau EH, Shingiro J. Fermentation and germination improve nutritional value of cereals and legumes through activation of endogenous enzymes. Food Sci and Nutr. 2018;6:2446-2458.
 21. El-Adaway T. Nutritional composition and nutritional factors of chickpeas (*Cicerarietinum* L.) undergoing different cooking methods and germination. Plant Foods for Human Nutrition. 2002;57:83-97. Doi: <http://dx.doi.org/10.1023/A:1013189620528>.
 22. Zhang J.-R, Ge Y.-Y, Liu P.-H, Wu D.-T, Liu H.-Y, Li H.-B, Corke H, Gan R.-Y. Biotechnological Strategies of Riboflavin Biosynthesis in Microbes. Engineering, 2022;12(5):116-128. doi:10.1016/j.eng.2021.03.018.
 23. Kalidass C, Mohan VR. Nutritional and antinutritional composition of itching bean (*Mucunapruriens* (L.) DC var. *pruriens*): An underutilized tribal pulse in Western Ghats, Tamil Nadu. Tropical and Subtropical Agroecosystems.2011;14: 279-293.
 24. Tavares RL, Silva AS, Campos ARN, Schuler ARP, Aquino JDS. Nutritional Composition, Phytochemicals and Microbiological quality of the legume, *Mucunapruriens*. African Journal of Biotechnology.2015;14(8): 676-682.
 25. Aware C, Patil R, Vyavahare G, Gurav R, Bapat V, Jadhav J. Processing effect of L-DOPA, in vitro protein and starch digestibility, proximate composition and biological activities of promising Legume: *Mucunamacrocarpa*. J. of the American College of Nutr. 2019;38(5): 447-456.
 26. Singh J, Dean A, Prakash S, Bhandari B, Bansal N. Ultra-high temperature stability of milk protein concentrate: Effect of mineral salts addition. Journal of Food Engineering. 2021;300:110503. Doi:10.1016/j.jfoodeng.2021.110503.
 27. Fitriyah H, Anwar F, Palupi E. Morphological characteristics, chemical and amino acids composition of flours from velvet beans tempe (*Mucunapruriens*), an indigenous legume from Yogyakarta. Journal of Physics: Conference Series.2021;1869: 012012 doi:10.1088/17426596/1869/1/012012.