Original Research Article

Effects of single and double treatment methods on the micronutrients in *Mucuna pruriens* (velvet bean) seed flour.

ABSTRACT

Mucuna pruriens seed is an underutilized legume with good nutritional value with a possibility of changes in its micronutrients' composition during processing whose data is scanty. *Mucuna pruriens* seed flour was therefore evaluated for the effect of soaking, cooking, roasting, germination and fermentation as well as some double treatments on its vitaminand mineralcontents.Mucuna pruriensseeds were cleaned, washed, soaked, cooked, roasted, germinated and fermented. Vitamins and minerals composition were determined.Soaking, cooking and roasting significantly reduced ($p<0.05$) all the vitamins. Vitamin B_9 was significantly ($p<0.05$) reduced the most with a range of 0.28 – 21.88 mg/100g. Vitamins B_1, B_2, B_3 and B_{12} were significantly increased by fermentation with vitamin B_2 increased the most (0.26 to 1.50 mg/100g; represents 577% increase). Vitamin B_9 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 *Mucuna pruriens* seed to 234.36 mg/100g. Fermentation generally reduced all the minerals significantly ($p<0.05$) except calcium and zinc.Germination and fermentation have proved to be suitable methods for the enhancement of vitamin B_2, B_3, B_{12} and zinc in *Mucuna pruriens* seed flour. Combined process treatments reduce most vitamins and minerals in *M. pruriens* seed flour and are therefore not suitable for their improvement.

Keywords: Mucuna pruriens, vitamin, mineral, germination, fermentation

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 15m in length, almost completely 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].

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] but its contents of some antinutrients limit its food utilizations.*Mucuna pruriens* seed contains 73.5 µg/mL of vitamin A, 147.4 µg/ml of vitamin B_1, 44.5 µg/ml of vitamin B_2, 14.7 µg/ml of vitamin B_3 and 4.9 mg/100g of vitamin C [6], 0.85mg/100g of Vitamin B_6 and 0.37mg/100g of vitamin B_{12}[7]. Raw seed of *Mucuna pruriens* 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 *Mucuna pruriens* 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 *Mucuna pruriens,* 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 *Mucuna pruriens* seed wholesome and effectively utilizable, there are possibilities of reduction or improvement in the vitamins and minerals that it contains of which thedetails are largely lackingin 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 *Mucuna pruriens* (velvet bean) seed flour.

2. MATERIAL AND METHODS

2.1 Materials

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.

2.2Methods

Production of *Mucuna pruriens* seed flour

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

2.2.1 Soaking of *Mucuna pruriens* seeds

Whole *Mucuna pruriens* seeds (1.2kg) were cleaned of 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 during the process. At the end of soaking for each batch, the samples were drained and dried in ahot air 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 a Blender (Binatone BL-1500PRO, China) and stored in low-density polyethylene bags in readiness for analyses.

2.2.2 Cooking of *Mucuna pruriens* seeds

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 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, groundand stored in low-density polyethylene bags in readiness for analysis.

2.2.3 Roasting of *Mucuna pruriens* seeds

Whole *Mucuna pruriens* seeds were sorted and cleaned of 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 15min and batch 3 for 20 min. The samples were then ground and stored in low-density polyethylene bags in readiness for analyses.

2.2.4 Germination of *Mucuna pruriens* seed

The procedure of [5] was used for the germination process. One hundred grams of *Mucuna pruriens* 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 *Mucuna pruriens* seeds

The procedure described by [11] was adopted for the fermentation process. *Mucuna pruriens* 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 inlow-density polyethylene perforated bags (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 *Mucuna pruriens* 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. *Mucuna pruriens* seedflour (2 g) was placed in 25 mL of H_2SO_4 (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 for 8 h. The mixture then wasfiltered 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; 250×4.6 mm i.d., 5μm) through the isocratic delivery mobile phase (A/B 33/67; A:MeOH, B: 0.023 M H_3PO_4, pH = 3.54) at a flow rate of 0.5 mL/min. Ultraviolet (UV) absorbance was recorded at 270 nm at room temperature.

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 *Mucuna pruriens* seed flour.

The minerals in the *Mucuna pruriens* seedflours 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 Vitamincomposition of soaked, cooked, roasted, germinated and fermented *Mucuna pruriens* seed flour.

The vitamincomposition of *Mucuna pruriens* 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 *Mucuna pruriens* seedflours. Soaking, cooking and roasting significantly reduced ($p<0.05$) all the vitamins.Germination significantly ($p<0.05$) increased vitamins B_2, B_3 and B_{12} while 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 *Mucuna pruriens* seed recorded 0.59and 0.74mg/100g for vitamins B_1 and B_3 respectively and were comparable to that of cowpea as documented by [16] with a vitamin B_1 value of 0.524mg/100gand vitamin B_3 value of 0.797mg/100g. The results have shown that *Mucuna pruriens* seed is a good source of vitamins B_1, B_9 and B_{12} 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 B_9 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 B_1, B_2 and B_9 and C as also reported by [19].

For germination, there was a significant increase ($p<0.05$) in some of the vitamins. Vitamin B_2 was significantly increased from 0.26 to 0.31 mg/100g, vitamin B_3 from 0.74 to 1.45 mg/100g and vitamin B_{12} from 0.34 to 0.89 mg/100g. The increasing trend of the three vitamins (B_1, B_3 and B_{12}) during germination was regular. The rest of the vitamins (B_1, B_9, C and A) were significantly reduced during germination in a regular fashion also. Germination had the highest significant reduction effect on vitamin B_9,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 B_1, B_3 and B_{12}.On that same note of increase of some water-soluble vitamins during germination,[21] also observed a significant increasein vitamin B_2 content of germinated Chickpea from 173.3 to 201.33 mg/100g while vitamins B_1, and B_3 were documented to have been reduced by germination.

Table 1: Vitamin compositionof *Mucuna pruriens* seed that received single treatments.

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^{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^{l}	0.23^{hij}	2.66^{e}	1.68^{bc}
3.	S48h	48h soaked	0.23^{gh}	0.17^{h}	0.64^{fgh}	10.25^{j}	0.29^{g}	2.77^{d}	1.60^{ef}
4.	S72h	72h soaked	0.20^{h}	0.23^{ef}	0.66^{fg}	9.78^{k}	0.37^{ef}	2.68^{e}	1.59^{f}
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^{i}	17.65^{f}	0.19^{ijk}	2.79^{cd}	1.51^{h}
9.	R10m	10min roasted	0.60^{c}	0.19^{gh}	0.59^{i}	19.68^{b}	0.18^{ik}	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^{l}	0.55^{c}	2.92^{a}	1.59^{f}

13.	G48h	48h germinated	0.34^{e}	0.30^{d}	1.45^{a}	1.48^{l}	0.71^{b}	2.80^{cd}	1.56^{g}
14.	G72h	72hgerminated	0.33^{de}	0.31^{d}	1.45^{a}	1.46^{l}	0.89^{a}	2.40^{g}	1.47^{l}
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^{l}	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 B_1 (thiamine), B_2 (riboflavin), B_3 (niacin) and B_{12} (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 B_1 was from 0.59 to 0.80 mg/100g, vitamin B_2 from 0.26 to 1.50 mg/100g, vitamin B_3 from 0.74 to 1.10 mg/100g and vitamin B_{12} from 0.34 to 0.55 mg/100g. Vitamin B_2 was the most improved (0.26 to 1.50 mg/100g) representing a 577% increase. These results indicate that vitamin B_2 (riboflavin) can be highly improved in legumes like *Mucuna pruriens* 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 B_9 (folate), vitamin C and vitamin A were significantly reduced ($p<0.05$) by fermentation.Vitamin B_9 was the most significantly reduced ($p<0.05$) from 21.88 mg/100g in raw *Mucuna pruriens* seed to 0.28 mg/100g in 72 hours fermented sample (F72h). The vitamin B_9 reduction may be attributed to the vitamin B_9 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.

3.2 Mineralcomposition of soaked, cooked, roasted, germinated and fermented *Mucuna pruriens* seed flour.

Mineralcomposition (mg/100g) of *Mucuna pruriens* seed flourthat received single treatments of soaking, cooking, roasting, germination and fermentation are presented in Table 2.

Table 2: Mineral composition (mg/100g) of *Mucuna pruriens* seedflours that received single treatments.

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^{l}	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^{i}	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^{de}f	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^{j}	328.00^{c}	220.41^{cd}	1150.0^{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 *Mucuna pruriens* 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 *Mucuna pruriens* seed (CON) were higher than the values reported by [23] for five accessions of *Mucuna pruriens* seed India. These variations in the mineral's composition of *Mucuna pruriens* 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].

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 mg/100g in the raw seed (CON) to 5.10mg/100g. This decrease in minerals during cooking conforms with the details documented by [25].

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 *Mucuna macrocarpa* 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 *Mucuna pruriens* 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 *Mucuna pruriens* 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 Vitamincomposition of *Mucuna pruriens* seed flours that received double treatments.

The vitamins contained in *Mucuna pruriens* 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:Vitamin compositionof *Mucuna pruriens* seed flours that received double treatments.

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	48hgerminated +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	48h 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	72h 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 B_2 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 min roasted sample (FR4) had a value of 0.10 mg/100g. Apart from Vitamin B_2 which was significantly($p<0.05$) increased by some of the double treatments, the rest of the vitamins (B_1, B_3, B_9, B_{12}, 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 min cooking (SC1) significantly increased vitamin B_2 from 0.26 to 0.67 mg/100g while 48 h germinated + 60 min cooked sample (GC3) significantly ($p<0.05$)increased vitamin B_2 from 0.26 to 0.69 mg/100g.

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 B_2 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 B_2 using 72 h soaking + 60 min cooking or 48 h germination + 60 min cooking.

3.4 Mineral composition of *Mucuna pruriens* seed flours that received double treatments

The record of the minerals' composition of *Mucuna pruriens* seed flours that received double treatmentsare presented 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 min

Table 4: Minerals composition (mg/100g) of *Mucuna pruriens* 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.	SC 1	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.	GR 2	48h germinated+ 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.	GC 3	48h 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 *Mucuna pruriens* seed is a good source of vitamins B_1, B_9 and B_{12}. Soaking, cooking and roasting reduced the vitamins in *Mucuna pruriens* seed flour. Vitamin A was least influenced by the treatments and is therefore considered to be the most stable in *Mucuna pruriens* seed. Vitamin B_9 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 *Mucuna pruriens* seed flour. Germination increased vitamin B_2, B_3 and B_{12} in *Mucuna pruriens* seed flour. Vitamins B_1 (thiamine), B_2 (riboflavin), B_3 (niacin) and B_{12} (cyanocobalamin) were all increased by fermentation with vitamin B_2 being the most improved (577% increase) and therefore is suitable for boosting vitamin B_2 in *Mucuna pruriens* seed flour. Fermentationreduces vitamins B_9 (folate), vitamin C and vitamin A contents of *Mucuna pruriens* seed flour.

Soaking reduced the minerals in *Mucuna pruriens* seed flourexcept phosphorus and zinc. Cooking of *Mucuna pruriens* seed reduced the minerals except potassium. Magnesium, iron and calcium did not differ during the roasting of *Mucuna pruriens* 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 *Mucuna pruriens* 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 *Mucuna pruriens* seed flour. Processing of *Mucuna pruriens* seed using double treatments (soaking+cooking, germination+roasting, germination+cooking and fermentation+roasting) reduced many of the water soluble vitamins (B_1, B_3, B9, B_{12} and C), vitamin A as well as minerals (potassium, sodium, magnesium, zinc, manganese and iron) and therefore may not be suitable for their enhancement.

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