Calcium and Phosphorus Contents of Non-Bioprocessed and Bio-Processed *Mucuna pruriens* (Egbara) Seed Flour

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KEYWORDS

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ABSTRACT

This study evaluated the effect of non-bioprocessing and bioprocessing on the calcium and phosphorus content of Mucuna pruriens seed flour. The seeds were cleaned, washed, soaked in distilled water (24 h, 48 h and 72 h), cooked (20 min, 40 min, 60 min and 80 min), roasted (10 min, 15 min and 20 min), germinated (24 h, 48 h and 72 h) and fermented with Rhizopus oligosporus (24 h, 48 h and 72 h). Calcium and phosphorus contents of the samples were determined. Calcium ranged from 187.10 – 425.68 mg/100 g while phosphorus ranged from 778.00 – 1790 mg/100 g. Germination (24 h) and fermentation (24, 48 and 72 h) significantly ($p < 0.05$) increased calcium while other treatments decreased it. Roasting for 10 min significantly increased phosphorus content while others decreased it. Fermentation with Rhizopus oligosporus and roasting for 10 min are therefore recommended for the improvement in the calcium and phosphorus contents of Mucuna pruriens seed flour respectively.

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INTRODUCTION

Mucuna pruriens commonly known as velvet bean, cowitch or cowhage is of the family leguminosae, genus; Mucuna and Specie; *Mucuna pruriens* (Pathania *et al*., 2020). In Nigeria, *Mucuna pruriens* is known as "Egbara" or "Agbara" in south Eastern part of Nigeria or "Werepe" in the Western part of Nigeria. *Mucuna pruriens* is a good source of crude protein (24 - 31.44 %), crude fibre and ash (2.9 - 5.5 %) and its digestibility is comparable to that of other pulses like soybean, rice bean and lima bean (Pathania *et al*., 2020). Raw seed of *Mucuna pruriens* contains minerals in the following range; Potassium: 806 - 2,790 mg/100 g, Sodium: 4 – 70 mg/100 g, Calcium: 104 – 900 mg/100 g, Phosphorus: 98 - 498 mg/100 g, Magnesium: 85 – 477 mg/100g, Iron: 1.3 – 15 mg/100 g, Copper: 0.33 - 4.34 mg/100 g, Zinc: 1 – 15 mg/100 g and Manganese: 0.56 - 9.26 mg/100 g (Pathania *et al*., 2020).

In the body, Calcium (Ca) is needed for maintenance of good health and the integrity of several systems like the musculoskeletal system, nervous system, and the heart (Buturi *et al*., 2021). Maintaining good bone, tooth, and mineral homeostasis is essential (Buturi *et al*., 2021). Calcium also acts as a cofactor in many enzymatic reactions and contributes to parathyroid function (Beto, 2015). Phosphorus is a macro-mineral required by the body for the first steps in carbohydrate metabolism and B-group Vitamins utilization (Okaka *et al*., 2002). More than 80% of phosphorus in the human body is bound to calcium as hydroxyapatite in teeth and bones (Okaka *et al*., 2002).

To reduce/eliminate the anti-nutritional factors and improve the nutritional quality and effectively utilize grain legumes like *Mucuna pruriens*, soaking, dehulling, cooking, fermentation, germination,

toasting/roasting and autoclaving can be adopted. During these processes geared towards making *Mucuna pruriens* seed wholesome, there are possibilities of reduction or improvement in the calcium and phosphorus content whose details are largely lacking. Hence this study was embarked on to investigate the effects of soaking, cooking, roasting, germination and fermentation on the calcium and phosphorus content of *Mucuna pruriens* seed flour.

MATERIALS AND METHODS

Mucuna pruriens seeds were purchased from New Market in Enugu State, Nigeria. Soaking, boiling, roasting, germination and fermentation were used in processing *Mucuna pruriens* seed. A completely randomized design was adopted in this experiment.

Soaking of *Mucuna pruriens* seeds

As outlined by Mugendi *et al*. (2010), 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 (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-hour 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 a hot 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 using a blender (Binatone BL-1500PRO, China) and stored in a high-density polyethylene bag in readiness for analysis.

Boiling of *Mucuna pruriens* seeds

As described by Mugendi *et al*. (2010), 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 a hot air oven (Laboratory Oven, England Labscience, DHG-9053A) at 70°C (for 18 h with constant turning after every 4 h) to constant weight, ground and stored in high-density polyethylene bags in readiness for analysis.

Roasting of *Mucuna pruriens* seeds

As described by Mugendi *et al*. (2010), whole *Mucuna pruriens* 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, China) 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 allowed to cool, ground and stored in high-density polyethylene bags in readiness for analysis.

Germination of *Mucuna pruriens* seeds

The procedure of Mugendi *et al.* (2010) was used for the germination process. One hundred grams (100 g) of *Mucuna pruriens* seeds were soaked in ethanol (1:2 w/v) for 1 min to aid decontamination. The ethanol was drained afterwards. 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 (27±2°C) 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 with a blender (Binatone BL-1500PRO, China) into flour and stored in high-density polyethylene bags for analysis.

Fermentation of *Mucuna pruriens* seeds

The procedure described by Egounlety (2003) was adopted for the fermentation process. *Mucuna pruriens* seeds were boiled in distilled water for 45 min (1kg/6 L), hand-dehulled, chopped into 2-3 pieces per grain, soaked twice (1 kg/3 l) for 12 h with removal of soak water after each soaking period, re-cooked for 45 min (1kg/6 L), 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. The grains obtained were divided into three portions, inoculated with *Rhizopus oligosporus* (Ragi Tempe, Raprima Inokulum Tempe, PT.Aneka Fermentasi Industri, Sandung 40553 -Indonesia) (0.4 g/kg drained grain), packed in low-density polyethylene perforated bags (50 μm) and allowed to ferment (29°C) in an incubator (LAB-TECH 150421, India) for 24 h, 48 h and 72 h to obtain samples F24h, F48h and F72h, respectively.

Determination of calcium and phosphorus

The minerals in the *Mucuna pruriens* seed flours were analysed using the method described by Asaolu *et al*. (2012). An aliquot of 2.0 g 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 100 mL standard flask and made to mark with distilled. The appropriate lamps for the minerals were used. After digestion and appropriate dilution, the digested samples were aspirated into an air–acetylene flame to burn the elements into atomic components, which were then detected in a spectrophotometer (Buck scientific model 211A, USA) at 422.7 nm for calcium. Phosphorus content in the digested extract was determined colorimetrically by the addition of 1mL molybdate reagent with shaking gently to mix thoroughly and it was allowed to stand for 15 min. Afterwards, 1mL of 1% ascorbic acid was added and also allowed to stand for 15 min. The absorbance of the solution was then measured at 660 nm. The concentrations of the minerals were determined using standard curves generated with standard solutions (0.5, 1.0, 2.0, 5.0 mg/L) of the respective minerals (Sigma Chemical Co., USA). The same procedure was used for blank solutions but devoid of the respective mineral solutions and the values deducted accordingly.

RESULTS AND DISCUSSION

Calcium content of non-bioprocessed and bioprocessed *Mucuna pruriens* seed flour

Calcium content was in the range of 187.10 – 425.68 mg/100 g. The various treatments effected significant ($p < 0.05$) differences in the calcium content of the samples. The treatments significantly reduced the calcium content except 24 h germination and fermentation (24 h, 48 h and 72 h) which significantly increased Calcium from 218.17 mg/100 g in the raw *Mucuna pruriens* seed flour (CON) to 234.36 mg/100 g and 425.68 mg/100 g respectively. The microbial biomass of *R. oligosporus* formed during fermentation contribute to the overall increase in minerals like calcium of the fermented legume seeds (Toor *et al*., 2021). Germination also has the ability to increase the calcium content of legumes as corroborated by Luo and Xie (2013) who reported an increase in calcium after germination of Faba Bean and Soybean. Fermentation for 24, 48 and 72 h significantly ($p < 0.05$) increased calcium in an irregular pattern. Calcium, along with vitamin D, may improve bone health and is associated with a reduced risk of various types of cancer (Castiglione *et al*., 2018). The Recommended Daily Allowance (RDA) for Calcium is between 1,000 - 1,300 mg per day (Buturi *et al*., 2021). Calcium (187.10 – 425.68 mg/100 g) content of the samples were lower than the RDAs of 1,000 mg/d for calcium as documented by Benayad and Aboussaleh (2021).

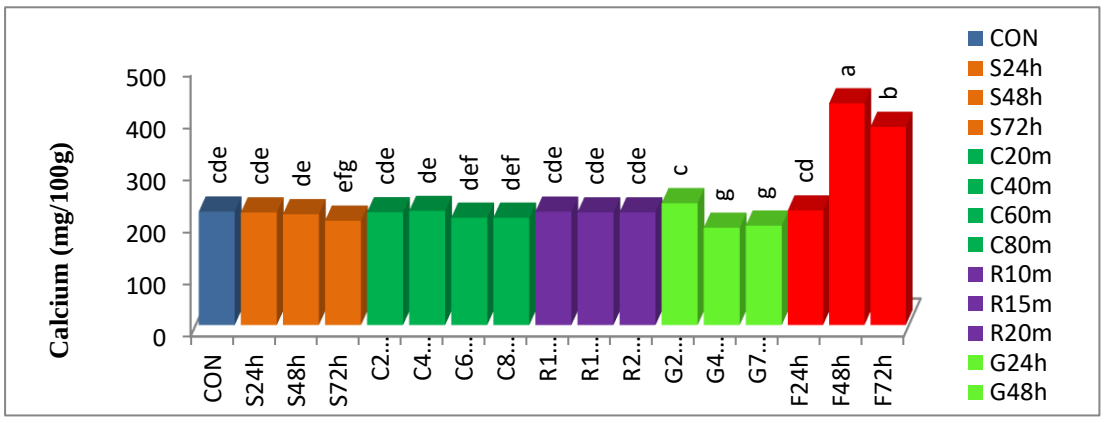


Figure 1: Calcium content of *Mucuna pruriens* seed flour that received single treatments

CON = Control; S24h = 24 h soaked; S48h = 48 h soaked; S72h = 72 h soaked; C20m = 20 min cooked; C40m = 40 min cooked; C60m = 60 min cooked; C80m = 80 min cooked; R10m = 10 min roasted; R15m = 15 min roasted; R20m = 20 min roasted; G24h = 24 h germinated; G48h = 48 h germinated; G72h = 72 h germinated; F24h = 24 h fermented; F48h = 48 h fermented; F72h = 72 h fermented.

Phosphorus content of non-bioprocessed and bioprocessed *Mucuna pruriens* seed flour

Phosphorus content ranged from 778.00 – 1790 mg/100 g. The phosphorus content was significantly ($p < 0.05$) reduced by many of the treatments except cooking (20 min) and roasting (10 and 15 min). These findings regarding the increase in phosphorus during roasting agree with the findings of Aware *et al*. (2019) which indicated an increase in phosphorus after the roasting of *Mucuna macrocarpa* seeds. Phosphorus functions as part of the high energy storage molecules adenosine tri-phosphate and adenosine di-phosphate, which are important for the phosphorylation of several components required for optimal

body function (Okaka *et al*., 2002). Suboptimal phosphorus intake can cause rickets, osteomalacia, and fragile bones in adults (Okaka *et al*., 2002). The recommended daily intake of phosphorus is 1,200 mg/day by Benayad and Aboussaleh (2021) and many of the values recorded for phosphorus in this research were higher than the 1,200 mg/day Recommended Dietary Allowance (RDA) for Phosphorus.

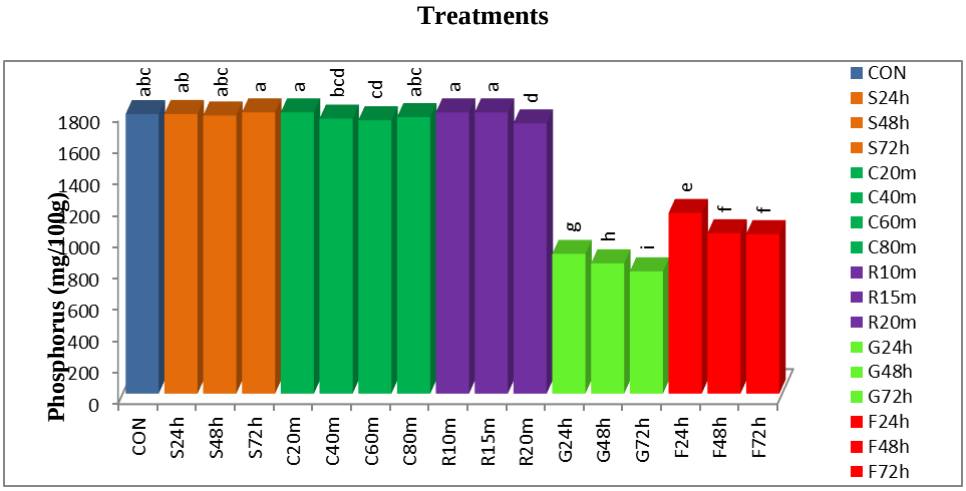


Figure 2: Phosphorus content of *Mucuna pruriens* seed flour that received single treatments

CON = Control; S24h = 24 h soaked; S48h = 48 h soaked; S72h = 72 h soaked; C20m = 20 min cooked; C40m = 40 min cooked; C60m = 60 min cooked; C80m = 80 min cooked; R10m = 10 min roasted; R15m = 15 min roasted; R20m = 20 min roasted; G24h = 24 h germinated; G48h = 48 h germinated; G72h = 72 h germinated; F24h = 24 h fermented; F48h = 48 h fermented; F72h = 72 h fermented.

CONCLUSION

Germination for 24 h and fermentation for 24 h, 48 h and 72 h are suitable for the improvement of calcium in Mucuna pruriens seed flour. Soaking, cooking, roasting, germination and fermentation (*Rhizopus oligosporus*) of *Mucuna pruriens* seed are not very suitable for the enhancement of phosphorus in Mucuna pruriens seed flour.

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