

Effect of Processing on the Nutrient, Anti-nutrient and Functional Properties of *Mucuna flagellipes* (Ox-eyed Bean) Seed Flour; An Underutilized Legume in Nigeria

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Abstract The promotion and consumption of underutilized legumes could help mitigate food insecurity and alleviate malnutrition in developing countries. In this study, the effect of processing on the nutritional, anti-nutritional and functional properties of *Mucuna flagellipes* seed flour was determined. The proximate composition of the samples showed that the flours had a range of 7.37 to 11.46 % moisture, 18.21 to 28.53% crude protein, 2.93 to 3.97% fat, 8.06 to 12.90% crude fibre, 2.74 to 4.69% ash, 38.45 to 59.09% carbohydrate, and 1446.34 to 1285.43KJ/100g energy. Profile of the amino acid showed that the lysine, leucine, tryptophan and aspartic acid in the unprocessed flour were significantly ($P \leq 0.05$) reduced by roasting. The vitamin composition of the samples showed that vitamin A, vitamin C and vitamin E varied between; 0.15 to 3.46 mg/100g, 0.03 to 85.54 mg/100g, 3.41 to 14.24mg/100g respectively. The mineral composition of the flours showed that the calcium, potassium, phosphorus, magnesium, iron and zinc contents of the samples varied between 132.02 to 167.00mg/100g, 85.91 to 145.00mg/100g, 80.95 to 132.87mg/100g; 83.74 to 145.19mg/100g, 19.90 to 33.16mg/100g and 3.96 to 6.26mg/100g, respectively. The following mineral ratios were lower than the reference balance (ideal) and also lower than the minimum in the acceptable ideal range: Ca/Mg, Ca/K, Ca/P and $[K/(Ca+Mg)]$ for all the seed flours except for Ca/P mineral ratio whose value for the roasted seed flour falls within the acceptable ideal range. The mineral safety index showed that Ca, Mg, P and Zn were all lower than the standards for all the seed flours, whereas Fe was higher than the standard in all the seed flour. The anti-nutritional factors of the samples were significantly ($P \leq 0.05$) reduced by autoclaving and roasting than by boiling and soaking methods. The calculated molar ratios of phytate:calcium, phytate:iron, phytate:zinc, oxalate:calcium and $[Phytate][Calcium]/[Zinc]$ were below the critical value and this indicate that the bioavailability of calcium, iron, and zinc in the raw and processed seed flour could be high. The nutrient composition of the flour suggests that it has the potential to be used as both nutritional supplements and functional ingredients in the preparation of a number of baked and complementary food products.

Keywords: *Mucuna flagellipes*, nutrient, anti-nutrient, mineral safety index, bioavailability

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1. Introduction

The formulation of new food in the food industry requires information about which food is healthy and which is not, the functions of food in the body and the use of scientific resources. Man in his existence is solely dependent on nutrients which could be of plant or animal source [1]. The choice of animal as nutrient source for man is affected by some factors which include; cultural

and religious belief, health, cost, food choice and food preference [2]. Plants play significant role in human nutrition, especially as sources of vitamins, minerals, proteins, dietary fibre, carbohydrates and lipids [3]. Legumes and pulses are good sources of protein to man, which makes them protein complements to cereals, vegetables, root and tubers.

Soybean is common protein supplement in feed formulation both for human and animals, due to its excellent protein contents [4]. Its protein is easily digested and rich in essential amino acids such as lysine, threonine,

and tryptophan [5]. Notwithstanding, exploration of unconventional protein supplements is needed to reduce dependency on soybean and other conventional legumes. Unconventional legumes are promising in nutrition, food security, agricultural development and crop rotation [6].

Mucuna is a genus of 100 acknowledged species of climbing vines and shrubs of the family *fabacea*, found worldwide in the woodlands of tropical areas and are in great demand as food, livestock feed and pharmaceutically valued products [7]. Among the species is *Mucuna flagellipes*, a legume that belong to the subfamily *papilionacea* that comprises pods covered with brownish dense whisker-like hairs called trichomes, irritating when in contact with the skin or eyes. Each pod may contain 1 to 3 seeds with a hard coating which is whitish when fresh and immature but turns black when mature and dry [8].

It is known to emanate from Asia, introduced into the hemisphere through Mauritius [9]. It is the most widely cultivated among the numerous varieties of *Mucuna* family, it is known as “Ox-eyed bean” or “Hamburger seed” and popularly called “Ukpo” by the Igbos, “Kararra” by the Hausas and “Yerepe” by the Yorubas.

M. flagellipes seed is an underutilized tropical legume with nutritional qualities comparable to soybean as it contains similar proportions of proteins, lipids, minerals, and other nutrients [10]. Its utilization as human food is limited by the presence of naturally occurring anti-nutritional factors such as protease inhibitors, saponins, tannins, oxalates, phytates, cyanogenic compounds and haemagglutinins in them which may be poisonous to humans or in some cases interfere with the nutrient availability in the body [1]. However, these anti-nutrients can be drastically reduced or eliminated by the use of simple processing techniques such as boiling, roasting, autoclaving, germination, fermentation, microwave, dehulling and soaking in water, acidic or alkaline solutions respectively [11,12].

The flour of *M. flagellipes* seed possesses appreciable good functional properties like water absorption capacity, emulsifying capacity, oil absorption capacity, bulk density, foaming capacity which makes it a useful functional ingredient in the preparation of a good number of food products [13]. Hence, this research work was aimed at assessing the effect of processing on nutrient, anti-nutrient and functional properties of *M. flagellipes* (Ox-eyed bean) seed flour.

2. Materials and Methods

2.1. Sample Collection and Identification

Mature dried *M. flagellipes* seeds were harvested from a local farm in Inyi Oji-River Local Government Area of Enugu State, Nigeria. The seeds were authenticated at herbarium laboratory of Botany department Ahmadu Bello University Zaria, Kaduna State and were given a voucher number 1391842. The seeds were cleaned, sorted, cracked, dehulled and divided into five aliquots of 500g each. Four aliquots were subjected to different processing treatments (soaking, boiling, roasting and autoclaving) while the fifth aliquot was left unprocessed (control).

2.2. Preparation of Raw *M. flagellipes* Seed Flour

The raw *M. flagellipes* seed flour was prepared according to the method of [14]. During preparation, 500g of *M. flagellipes* seeds which were free from extraneous materials were cleaned with 1.5 liters of distilled water and manually dehulled by cracking with stone-like iron followed by winnowing to remove the hulls. The dehulled seeds were spread on trays and dried in a hot air oven (Model DHA 9101 ISA) at 60°C for 6 hours with occasional turning of the seeds at intervals of 30 minutes to ensure uniform drying. The dried seeds were milled in an attrition mill and sieved through a 500 micron mesh sieve. The flour produced were packaged in an airtight plastic container, labelled and stored in a freezer (-18°C) until used.

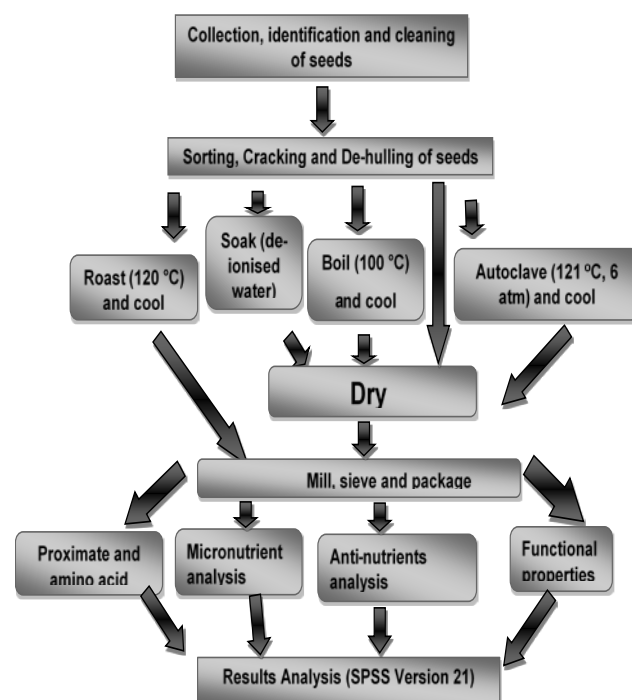


Figure 1. Flowchart of the effect of processing on nutrient, anti-nutrient and functional properties of *Mucuna flagellipes* seed flour

2.3. Preparation of Boiled *M. flagellipes* Seed Flour

The boiled *M. flagellipes* seed flour was prepared according to the method of [15]. During preparation, 500g of *M. flagellipes* seeds which were free from extraneous materials were cleaned with 1.5 liters of distilled water and manually dehulled by cracking with stones-like metal, followed by winnowing to remove the hulls. The dehulled seeds were boiled with 2 liters of distilled water at 100°C for 30 minutes. The boiled seeds were drained, spread on the trays and dried in hot air oven (Model DHA 9101 ISA) at 60°C for 8 hours with constant turning of the seeds at 20 intervals for 30 min to ensure uniform drying. The dried seeds were milled in an attrition mill and sieved through a 500 micron mesh sieve. The flour produced were packaged in an airtight plastic container, labelled and stored in a freezer (-18°C) until used.

2.4. Preparation of Autoclaved *M. flagellipes* Seed Flour

The autoclaved *M. flagellipes* seed flour was prepared according to the method of [14]. During preparation, 500g of the seeds which are free from extraneous materials were cleaned with 1.5liters of distilled water and manually dehulled by cracking with stone-like metal, followed by winnowing to remove the hulls. The dehulled seeds were placed in a beaker and autoclaved at a temperature of 121°C and pressure of 6 atmospheres for 40 minutes in an autoclave (Model 75HG, Britain, UK). The autoclaved seeds were dried in a hot air oven (Model DHG 9101 ISA) at 60°C for 6 hours. The dried seeds were milled in attrition mill and sieved through a 500 micron mesh sieve. The flour produced were packaged in air tight plastic container, labelled and stored in freezer (-18°C) until used.

2.5. Preparation of Roasted *M. flagellipes* Seed Flour

The roasted *M. flagellipes* seed flour was prepared according to the method of [14]. During preparation, 500g of the seeds which were free from extraneous materials were cleaned with 1.5liters of distilled water and manually dehulled by cracking with stone-like metal, followed by winnowing to remove the hulls. The dehulled seeds were placed in a beaker and roasted at a temperature of 120°C for 40 minutes in a hot air oven (Model DHG 9101 ISA). The roasted seeds were milled in an attrition mill and sieved through a 500 micron mesh sieve. The flour produced were packaged in an air tight plastic container, labelled and stored in freezer (-18°C) until used.

2.6. Preparation of Soaked *M. flagellipes* Seed Flour

The soaked *M. flagellipes* seed flour was prepared according to the method of [14]. During preparation, 500g of the seeds which were free of extraneous materials were cleaned with 1.5liters of distilled water and manually dehulled by cracking with stone-like metal, followed by winnowing to remove the hulls. The dehulled seed were soaked in de-ionized water (1:3) in an aluminum bowl for 48 hours at room temperature. Soaking treatment is to allow the seeds to imbibe water. The water was decanted at 6 hour intervals. The soaked seeds were dried in a hot air oven (Model DHG 9101 ISA) at 60°C for 6 hours. The dried seeds were milled in attrition mill and sieved through a 500micron mesh sieve. The flour produced were packaged in an airtight plastic container, labelled and stored in freezer (-18°C) until used.

2.7. Sample Analysis

2.7.1. Determination of Proximate Composition

The moisture content, ash content, crude protein, fat content, and crude fibre content were determined by the methods described by [15] and Carbohydrate was calculated by subtracting the difference of moisture, crude protein, ash and fat from 100 percent [16]. The calorific values or energy content in kilojoule (kJ) of the flours

were calculated by multiplying the crude fat, protein and carbohydrate by Atwater factors of (kJ/kcal) 37, 17 and 17 respectively [12].

2.7.2. Determination of Amino Acid Profile

The method as described by [17] was followed in the extraction of the samples for the amino acid analysis. Ten grams (10g) of each samples was weighed into the 250ml capacity conical flask respectively; the samples were defatted with 30ml of the petroleum spirit three times with soxhlet extractors.

Hydrolysis of samples

About 30mg of the defatted sample was weighed into glass ampoules. 7ml of 6mol/L HCl was added and oxygen expelled by passing nitrogen gas into the samples. The glass ampoules was sealed with a Bunsen flame and put into an oven at 105±5°C for 22h. The ampoule was allowed to cool; the content was filtered to remove humins. The filtrate was evaporated to dryness at 40°C under vacuum in a rotary evaporator. Each residue was dissolved with 5ml of acetate buffer (PH 2.0). The samples were hydrolyzed three times for complete hydrolysis to be achieved before been stored in a plastic specimen bottle and kept in the refrigerator prior to analysis. It should be noted that tryptophan is destroyed by 6N HCL during hydrolysis, hence for tryptophan determination, the sample was hydrolyzed with 4.2 M Sodium hydroxide [18].

Amino acid analysis

Amino acid analysis was done by Amino Acid Analyzer [19] using the model 120a PTH amino acid analyzer. The period of analysis was 76min for each sample. The gas flow rate was 0.50ml/min at 60°C with reproducibility consistent within ±3%. The net height of each peak produced by the chart recorder of the PTH (each representing an amino acid) were measured and calculated. The amino acid values reported were the average of triplicate determinants.

2.7.3. Determination of Vitamins

Pro-vitamin A (beta carotene), Vitamin C and Vitamin E in the samples were determined using the official methods of the Association of Official Analytical Chemists [17]. Exactly 0.2g of each flour sample was weighed into test-tube. For the fat soluble vitamins (vit A and vit E), 5ml of propanol was added to the sample and for the water soluble vitamin (vit C), separately 5ml of methanol was added to the sample. Allow the solution to stand for 2hrs to extract properly and filter. The filtrate was poured into a cuvette and the absorbance taken at 620nm for Vit A, 520nm for Vit C and 520nm for Vit E against the blank using UV-Vis spectrophotometer (Model-Shimadzu 2550).

2.7.4. Determination of Mineral Composition

According to [20], 1g of each sample was weighed into a 125ml Erlenmeyer flask and 20ml of the acid mixture (containing 325ml concentrated nitric acid, 40ml perchloric acid, and 10ml of sulfuric acid) were added. The contents were mixed and heated gently in a digester

(Buchi Digestion unit K-424) at a medium heat under a fume hood and heating continued until dense white fume appeared. Heating continued for 30 seconds and was allowed to cool followed by the addition of 50ml distilled water. The solutions were filtered using filter paper into a 100ml volumetric flask and made up to mark with distilled water. The resultant solutions were read for potassium and iron contents of the samples as determined by the use of a flame photometer (Model 405, Corning, UK) according to the method of [16]. The calcium, magnesium and zinc contents of the flours were determined using atomic absorption spectrophotometer (Perkin-Elmer Model 1033, Norwalk, CT, USA) according to the method of [17]. Phosphorus and iodine were determined using vanadomolybdate colorimetric method of [9].

2.7.5. Estimation of Mineral Ratios

Ratios of Ca/Mg, Na/K, Ca/K, Na/Mg, Zn/Cu, Ca/P, Fe/Cu, Ca/Pb, Fe/Pb, Zn/Cd, Fe/Co, K/Co and [K/ (Ca + Mg)] were all calculated according to the method of [21].

2.7.6. Estimation of Minerals Safety Index

The minerals safety index (MSI) were calculated according to the method of [22], for minerals that have relevant standards for such determination. For the present work, MSI for these minerals were calculated: Fe, Ca, P, Mg and Zn using the formula:

$$\text{Calculated MSI} = \text{MSI} / \text{RAI} \times \text{research data result}$$

Where

MSI = mineral safety index from the Table (standard)

RAI = recommended adult intake.

2.7.7. Determination of Anti-nutritional Factors

The tannin, phytate, oxalate, cyanide and protease inhibitor contents in the seed flour were determined as described by [17].

2.7.8. Estimation of Molar ratios

The molar ratios [Phy]:[Ca], [Phy]:[Fe], [Phy]:[Zn], [Oxalate]:[Ca] and [Phy][Ca]:[Zn] millimolar were calculated by the methods of Igwe *et al.*, (2013), using molecular weights: phytate = 660g/mol, oxalate = 128g/mol, Ca = 40g/mol, Fe = 56g/mol and Zn = 65g/mol.

2.7.9. Determination of Functional Properties

Water absorption capacity, Oil absorption capacity, Gelation capacity, Bulk density were determined by the method described by [16], Swelling index was determined by the method described by [14] and Emulsification capacity (EC) was determined using the method of [23].

2.8. Statistical Analysis

Results are presented as mean \pm SD, all data were analyzed using one way ANOVA. The mean, standard deviation and analysis of variance were calculated using statistical package for social science (SPSS version 21). Difference between means were separated using Turkey's Least Significant Difference (LSD) test. The significant differences were determined at $P \leq 0.05$.

3. Results and Discussion

3.1. Proximate Composition

Among the processed *M. flagellipes* seed flours as shown in Table 1, the boiled sample had the highest mean value of moisture content. This increase could be attributed to the inhibition of large quantity of water by the seeds as a result of boiling during processing. This observation is in line with [24] who reported increased moisture content in boiled flour sample for boiled and roasted *Adenanthera pavonina* L. (Fabaceae) seeds flours. The result is also in agreement with the report of Udensi *et al.* [14] for boiled and soaked *M. flagellipes* seed flours. Moisture is the integral part of the legumes, however, investigations have shown that low moisture content of food samples is a desirable phenomenon, since the microbial activity was reduced [25], this increases the storage periods of the food products while high moisture content in foods encourages microbial growth leading to spoilage of foods [26]. Lower moisture content of flour indicates better gelatinization process [27]. Hence the roasted seed flour with lowest retention of moisture content is acceptable for the storage of foods for a longer period of time.

Table 1. Effect of processing on the proximate composition of *M. flagellipes* seed flour

Parameters (%)	Raw	Processed			
		Soaked	Roasted	Boiled	Autoclaved
Moisture content	11.46 ^b \pm 0.20	10.53 ^{ab} \pm 0.78	7.37 ^a \pm 2.85	10.81 ^{ab} \pm 0.77	10.31 ^{ab} \pm 0.87
Crude protein	28.53 ^c \pm 0.64	19.09 ^{ab} \pm 1.40	23.76 ^{bc} \pm 3.79	18.21 ^a \pm 1.58	27.44 ^c \pm 1.30
Fat content	3.97 ^e \pm 0.03	3.72 ^c \pm 0.03	3.57 ^b \pm 0.03	3.87 ^d \pm 0.03	2.93 ^a \pm 0.03
Ash content	4.69 ^d \pm 0.04	4.59 ^{cd} \pm 0.45	2.74 ^a \pm 0.40	4.28 ^b \pm 0.04	4.51 ^c \pm 0.04
Crude fibre	12.90 ^e \pm 0.10	8.06 ^a \pm 0.04	9.01 ^d \pm 0.03	8.13 ^b \pm 0.01	8.22 ^c \pm 0.02
Carbohydrate	38.45 ^a \pm 0.33	54.01 ^{cd} \pm 0.75	59.09 ^d \pm 3.20	49.14 ^{bc} \pm 3.19	46.60 ^b \pm 0.76
Energy (kJ/100 g)	1285.43 ^a \pm 0.60	1380.39 ^{bc} \pm 0.40	1446.34 ^c \pm 0.80	1382.67 ^{bc} \pm 0.20	1366.85 ^b \pm 0.50
Total fatty acid*	3.17 ^e \pm 0.02	2.97 ^c \pm 0.02	2.86 ^b \pm 0.02	3.10 ^d \pm 0.02	2.34 ^a \pm 0.02
Organic matter	95.31^d \pm 0.04	95.41^{ab} \pm 0.05	97.26^d \pm 0.04	95.72^c \pm 0.04	95.49^b \pm 0.04

Values are Mean \pm SD (n=3).

Values in the same row with different superscripts are significantly different ($P \leq 0.05$).

* Crude fat x 0.80.

The reduction in the protein content of the boiled seed flour could be attributed to progressive solubilisation and leaching out of some soluble nitrogenous substances into boiling water during processing [28]. This observation is in agreement with [13] who reported a reduction in the crude protein content of boiled *M. flagellipes* seed flour. Similar observations were reported by [29] for boiled and roasted *Vigna subterranea* seeds and [30] when boiling Lima beans (*Phaseolus lunatus* L.). The autoclaved seed flour had the highest crude protein content compared to the other processed seed flour, an observation which agrees with the report of [1] for boiled and autoclaved *M. flagellipes* seed flour and thus stated that the high protein content of *M. flagellipes* seed flour makes it a good complement to cereal and a great substitute to soybean in the formulation of complementary meal.

There is a significant ($P \leq 0.05$) reduction in the fat content of the processed seed flour, with the autoclaved seed flour having the lowest fat content and the boiled seed flour having the highest fat content when compared with the other processed seed flour. This observation is in consonance with those earlier reports on certain under-utilized legumes such as *Abrus precatorius*, *Mucuna pruriens* var. *utilis* and *Entada scandens* which showed a reduction in fat content of the processed samples [31] and [32]. The fat content of raw *M. flagellipes* seed flour with 3.97% indicates that it is not an oil seed when compared to soya bean that has up to 23.1% fat content [33]. The high fat content of the boiled and soaked seed samples when compared to other processed seed flour could be due to the fact that fat is insoluble in water as reported by [34] for African yam bean seed.

Ash represents the mineral matter left after food material is burnt in oxygen, it is used as a tool to measure the mineral content in any sample [35]. The result showed that processing has effect on the ash content of the seed flour. This observation is in line with the report of [36] for soaked and boiled *M. flagellipes*, *Colocynthis citrullus* and *Irvingia Gabonensis* respectively.

Processing significantly ($P \leq 0.05$) reduced the crude fibre contents of the seed flour, which could be as a result of the protein-fibre complexes [37] formed after possible chemical modification induced by boiling, autoclaving, roasting and soaking of the dry seed. This agrees with the report of [38] for processed *Lens culinaris*.

Boiling significantly ($P \leq 0.05$) increased the carbohydrate content of the seed flour than autoclaving, which could be as a result of starch gelatinization [13] caused by the breakdown of complex carbohydrates which were otherwise bound in the raw sample by boiling. Carbohydrates provide heat and energy for all forms of body activity. Deficiency can cause the body to divert proteins and body fat to produce needed energy, thus leading to depletion of body tissues [39].

3.2. Amino Acid Profile

The result obtained for amino acid as shown in Table 2 profiles indicates that *M. flagellipes* seed flour on raw basis is adequate in lysine and tryptophan, signifying the seed flour a suitable complement to cereal in the formulation of new food products. Processing significantly ($P \leq 0.05$) reduced the overall amino acid profile of *M. flagellipes* seed flour. Autoclaved seed flour graded the cumulative best amino acid content which indicates that proteins are less denatured by autoclaving. An observation in agreement with the report of [40] for different thermally processed soy bean. However, the result indicated that they are limited in the sulphur containing amino acids particularly methionine.

3.3. Micronutrients

The results obtained from the micronutrient analysis presented in Figure 2 and Table 3 showed that *M. flagellipes* seed was significantly ($P \leq 0.05$) affected by processing as there was reduction in micronutrient of the processed samples.

Table 2. Effect of processing on the amino acid profile of *M. flagellipes* seed flour

Acids (mg/100g)	Raw	Processed			
		Soaked	Roasted	Boiled	Autoclaved
Lysine	5.70 ^f ±0.01	4.91 ^e ±0.02	4.62 ^d ±0.03	5.30 ^d ±0.00	4.79 ^b ±0.02
Histidine	2.91 ^d ±0.02	2.43 ^b ±0.01	2.22 ^a ±0.01	2.60 ^c ±0.01	2.43 ^b ±0.03
Arginine	6.71 ^d ±0.01	4.82 ^a ±0.01	5.01 ^b ±0.01	4.95 ^b ±0.01	6.22 ^c ±0.11
Aspartic Acid	11.67 ^e ±0.06	9.01 ^b ±0.00	8.71 ^a ±0.01	10.58 ^d ±0.02	9.61 ^c ±0.01
Threonine	4.52 ^d ±0.06	2.86 ^{ab} ±0.06	2.77 ^a ±0.11	2.99 ^{bc} ±0.00	3.11 ^c ±0.00
Serine	4.34 ^c ±0.01	2.65 ^c ±0.03	2.01 ^a ±0.02	2.57 ^b ±0.03	3.22 ^d ±0.01
Glutamic Acid	14.04 ^c ±0.05	12.80 ^c ±0.01	12.56 ^b ±0.02	11.27 ^a ±0.01	13.55 ^d ±0.01
Proline	4.35 ^c ±0.02	2.33 ^a ±0.00	3.04 ^b ±0.00	3.46 ^d ±0.01	3.36 ^c ±0.01
Glycine	4.86 ^d ±0.02	3.24 ^b ±0.10	3.02 ^a ±0.02	3.22 ^b ±0.01	3.52 ^c ±0.01
Alanine	4.18 ^d ±0.01	3.14 ^a ±0.00	3.11 ^a ±0.02	3.51 ^b ±0.02	3.70 ^c ±0.00
Cystine	1.87 ^d ±0.04	0.99 ^b ±0.01	1.01 ^b ±0.02	0.64 ^a ±0.00	1.21 ^c ±0.01
Valine	4.51 ^c ±0.02	3.53 ^c ±0.05	2.03 ^a ±0.01	2.65 ^b ±0.03	4.03 ^d ±0.01
Methionine	2.14 ^c ±0.01	1.14 ^b ±0.01	1.43 ^c ±0.03	1.02 ^a ±0.01	1.65 ^d ±0.01
Isoleucine	4.84 ^e ±0.17	2.02 ^a ±0.01	3.22 ^c ±0.02	2.41 ^b ±0.02	4.08 ^d ±0.01
Leucine	8.75 ^d ±0.03	6.83 ^b ±0.02	6.56 ^b ±0.02	6.81 ^b ±0.01	7.29 ^c ±0.05
Tyrosine	4.30 ^e ±0.12	3.28 ^c ±0.01	2.47 ^a ±0.02	2.90 ^b ±0.02	3.44 ^d ±0.05
Phenylalanine	4.43 ^c ±0.01	2.59 ^b ±0.10	2.26 ^a ±0.10	3.62 ^c ±0.02	4.04 ^d ±0.01
Tryptophan	5.24^d±0.05	4.01^b±0.01	3.53^a±0.01	4.05^b±0.05	4.32^c±0.01

Values are Mean ± SD (n=3). Values in the same row with different superscripts are significantly different ($P \leq 0.05$).

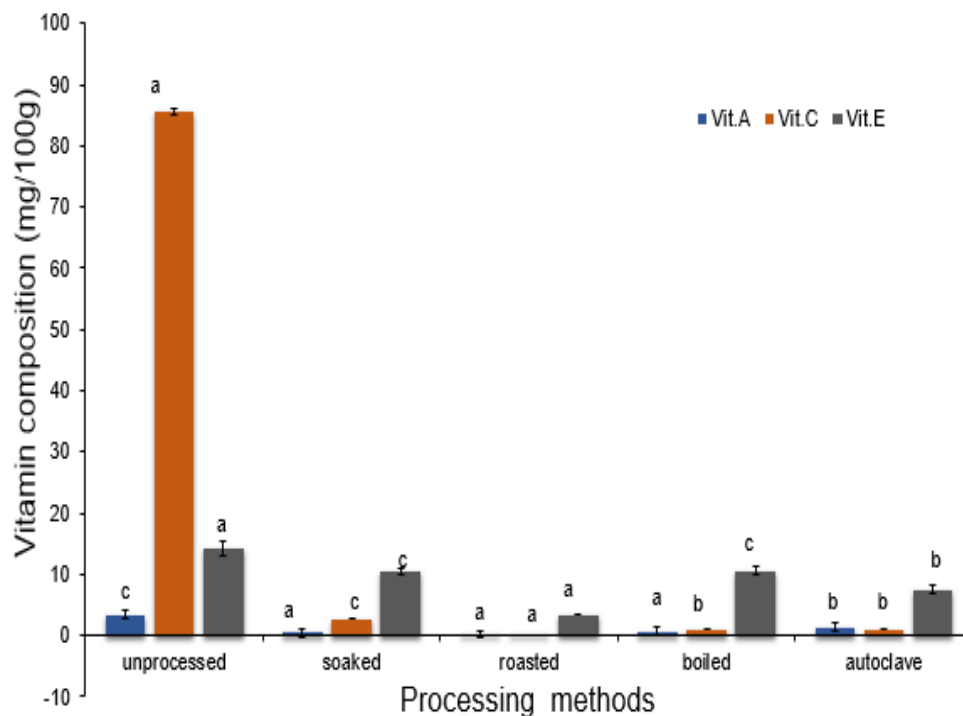


Figure 2. Effect of processing on the Vitamin (A, C and E) content of *M. flagellipes* seed flour. Data are mean \pm SD (n=3) (Different letters within the same type are significantly different ($P \leq 0.05$))

Table 3. Effect of processing on the mineral(mg/100g) contentof *M. flagellipes* seed flour

Elements (mg/100g)	Raw	Processed			
		Soaked	Roasted	Boiled	Autoclaved
Potassium	145.00 ^c \pm 0.84	143.71 ^{bc} \pm 2.86	85.91 ^a \pm 4.61	134.09 ^b \pm 6.20	137.85 ^{bc} \pm 1.03
Phosphorus	139.46 ^c \pm 4.48	133.64 ^{bc} \pm 2.07	80.95 ^a \pm 0.02	132.87 ^b \pm 0.44	135.12 ^{bc} \pm 1.46
Magnesium	145.17 ^c \pm 2.55	139.80 ^b \pm 2.02	83.74 ^a \pm 2.00	138.80 ^b \pm 0.67	139.49 ^b \pm 0.97
Calcium	167.00 ^b \pm 2.24	132.84 ^a \pm 6.88	132.02 ^a \pm 6.93	138.36 ^a \pm 0.72	155.10 ^{ab} \pm 17.43
Iron	33.16 ^c \pm 1.49	27.11 ^b \pm 1.45	19.90 ^a \pm 2.77	28.38 ^b \pm 0.25	29.53 ^{bc} \pm 0.67
Zinc	6.26 ^b \pm 0.25	5.77 ^b \pm 0.67	3.96 ^a \pm 0.31	5.82 ^b \pm 0.15	6.12 ^b \pm 0.21
Iodine	112.22^c \pm 1.25	106^{bc} \pm 1.07	53.41^a \pm 1.02	83.12^b \pm 1.35	107.88^{bc} \pm 1.94

Values are Mean \pm SD (n=3).

Values in the same row with different superscripts are significantly different ($P \leq 0.05$).

The iron content of the samples was significantly ($P \leq 0.05$) higher in autoclaved sample compared to the samples processed by boiling, soaking and roasting seed flours. This observation is in line with the report of [1] on boiled and autoclaved *M. flagellipes* seed flour. Iron is an important component of haemoglobin which is an oxygen carrying pigment in the blood. There was significant ($P \leq 0.05$) difference in the zinc content of processed seed, though a higher significant ($P \leq 0.05$) reduction in the zinc content of boiled, roasted and soaked seed flour than in the autoclaved seed flour. This resulted from zinc leaching into water during processing, a similar observation by [41] on the effect of cooking on the zinc content of cowpea. However, autoclaving, boiling, and soaking processing methods provided a substantial amount of zinc needed in a child's diet, considering the daily recommended intake for children aged 1 \pm 3 years is 3 mg [42].

3.4. Mineral Ratios

The results of the mineral ratios are presented in Table 4. Ca:P ratio came about because of the possibility of proteins and phosphorus rich foods may promote the

loss of calcium in the urine [43]. When Ca:P ratio is low (low calcium, high phosphorus intake), more than the normal amount of calcium will be lost in the urine, which result to decrease in calcium level of the bones. In this study, the Ca:P ratios for the raw, soaked, boiled and autoclaved seed flours were lower than the accepted ideal range of 1.5 to 3.6 well as that of roasted seed flour falls within the range though < 2.6 the reference balance ideal. Roasted seed flour has the Ca:P ratio required for favourable Ca absorption in the intestine for bone formation [44,45]. The levels of Ca:P ratios in the raw, soaked, boiled and autoclaved seed flour will not promote strong bone development to a large extent as expected since absorption under this condition would be low. The Ca:Mg ratio in all the seed flours falls below the accepted ideal range of 3 to 11 and < 7.00 the reference balance ideal. Both Ca and Mg would need adjustment for good health.

The milliequivalent ratios of [K: (Ca + Mg)] of all the seed flours were < 2.2 the reference balance ideal. This indicates that the seed flours may not promote hypomagnesaemia in man [44], contrary to the observations made by [46] in the samples of various parts of bambara groundnut.

Table 4. Mineral ratio of raw and processed *M. flagellipes* seed flour

Parameters	RBI	AIR	Raw	Processed			
				Soaked	Roasted	Boiled	Autoclaved
Ca/P	2.60	1.5 to 3.6	1.22 ^b ±0.02	0.99 ^a ±0.05	1.63 ^c ±0.01	1.04 ^{ab} ±0.01	1.15 ^{ab} ±0.12
Ca/K	4.20	2.2 to 6.2	1.15 ^b ±0.01	0.92 ^a ±0.06	1.54 ^c ±0.03	1.03 ^{ab} ±0.04	1.12 ^b ±0.12
Ca/Mg	7.00	3 to 11	1.15 ^a ±0.01	0.95 ^a ±0.06	1.58 ^b ±0.12	1.00 ^a ±0.00	1.11 ^a ±0.12
[K/(Ca+Mg)]	2.2	Na	0.46^b±0.01	0.53^c±0.01	0.40^a±0.01	0.49^{bc}±0.02	0.47^b±0.03

Values are Mean ± SD (n=3). Values in the same row with different superscripts are significantly different (P≤0.05).

RBI = Reference Balance Ideal

AIR = Accepted Ideal Range

Na = Not available.

3.5. Mineral Safety Index

The mineral safety index (MSI) as calculated for the seed flour samples is in Table 5. The standard MSI for the minerals are Mg (15), P (10), Ca (10), Fe (6.7) and Zn (33). The MSI can easily be explained as thus: the recommended adult intake (RAI) of Ca is 1200mg, its minimum toxic dose (MTD) is 12000 mg or 10times the recommended daily average (RDA) which is equivalent to MSI of Ca, same applied to other minerals whose MSI

were determined. Only the MSI values for Fe in all seed flour samples had their calculated MSI values greater than the standard (table) value thereby giving negative differences whilst others gave positive differences. This is an indication that there might be Fe overload on the consumers when fed with raw, soaked, roasted, boiled or autoclaved seed flour. The calculated MSI lower than the standard MSI meant that such minerals would not constitute mineral overload or become toxic to the seed flour consumers.

Table 5. Mineral safety index (MSI) of some of the minerals of *M. flagellipes* seed flour

Minerals	RAI (mg)	TV of MSI	Raw (CV of MSI)	Processed (CV of MSI)			
				Soaked	Roasted	Boiled	Autoclaved
Ca	1200	10.0	1.39 ^b ±0.02	1.11 ^a ±0.06	1.10 ^a ±0.06	1.16 ^a ±0.01	1.29 ^b ±0.15
P	1200	10.0	1.13 ^b ±0.03	1.11 ^b ±0.02	0.67 ^a ±0.00	1.11 ^b ±0.00	1.21 ^b ±0.15
Mg	400	15.0	5.44 ^c ±0.10	5.24 ^b ±0.07	3.14 ^a ±0.08	5.21 ^b ±0.03	5.23 ^b ±0.04
Fe	15	6.70	14.81 ^c ±0.67	12.11 ^b ±0.65	8.89 ^a ±1.24	12.68 ^b ±0.11	13.18 ^b ±0.32
Zn	15	33.0	13.76^b±0.55	12.70^b±1.48	8.75^a±0.66	12.82^b±0.33	13.46^b±0.46

Values are Mean ± SD (n=3). Values in the same row with different superscripts are significantly different (P≤0.05).

MSI= Mineral Safety index

CV = Calculated value;

TV = Table value;

RAI = Recommended adult intake.

Note: No MSI standard for potassium and Iodine.

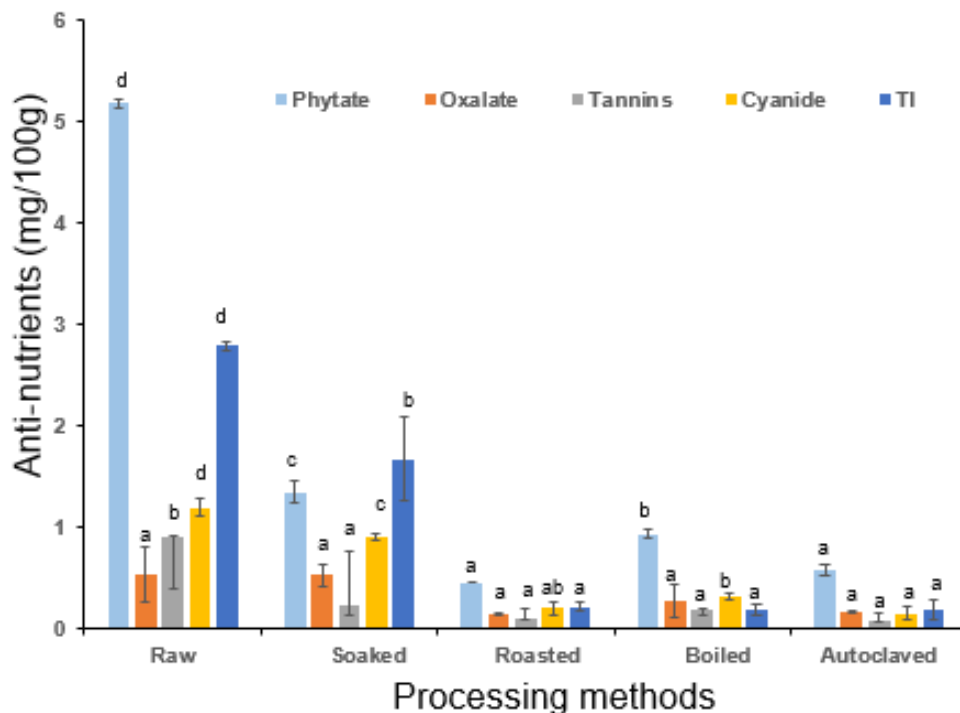


Figure 3. Effect of processing on some anti-nutritional factors of *M. flagellipes* seed flour. Data are mean ± SD (n=3) (Different letters within the same type are significantly different (P≤0.05); TI: Trypsin inhibitor)

3.6. Anti-nutrients

The results obtained for the anti-nutrient is shown in Figure 3. Processing have significant ($P \leq 0.05$) reduction on the anti-nutrients evaluated. Well, as the roasted seed flour is significantly ($P \leq 0.05$) reduced in its phytate and oxalate contents than obtained in the soaked, boiled and autoclaved seed flour, the autoclaved seed flour is significantly ($P \leq 0.05$) reduced in its tannins, cyanide and trypsin inhibitors contents than observed in the soaked, boiled and roasted seed flour. The reductive effectiveness of these anti-nutrients are dependent on the processing methods. Similar decrease in anti-nutrients with different processing methods of *Canavalia plagioperma piper* has been reported by [47]. [29] also reported that processing methods of *Vigna subterranea*. The presence of anti-nutrients and toxic substances in most plant foods including *M. flagellipes* seed flour limits the bioavailability of some essential dietary minerals such as calcium, magnesium, iron and zinc in foods [48]. The lower oxalate and phytate contents in the roasted seed flour distinct from the soaked seed flour could lead to better bioavailability of essential elements like calcium, magnesium and zinc that usually form complexes with these elements. Similarly, the effective reduction in the tannin and trypsin inhibitors contents by autoclaving could lead to better bioavailability of amino acids and iron content of the flour [13].

However, some anti-nutrients such as tannins, phytate, etc in *M. flagellipes* seed flour in variety of ways possess health benefits, anti-oxidant activities (wherein they are 15 – 30 time more efficacious in free radical scavenging activity than Trolox and other simple phenolics), anticarcinogenic activities and hypoglycemic activities [49].

3.7. Molar Ratios

The calcium, magnesium, zinc, oxalate, iron, and phytate molar ratios were calculated for both unprocessed and processed *M. flagellipes* seed flour, the calculation was to evaluate the effects of elevated levels of oxalate and phytate in the bioavailability of dietary minerals. Bioavailability is the ability of the body to digest and absorb the mineral in the food consumed [50]. The calculated values were compared with the reported critical toxicity values for these ratios. The calculated Phy:Ca, Phy: Fe, Phy: Zn, Ox: Ca and [Phy] [Ca]/[Zn] molar ratios of *M. flagellipes* as shown in Table 6, Phytic acids markedly decrease Ca bioavailability and the Phy: Ca molar ratio has been proposed as an indicator of Ca bioavailability. The critical molar ratio of [phy]:[Ca] of

< 0.24 indicating good calcium bioavailability [51]. The values in this study were lower in all groups than the reported critical molar ratio of Phytate to Calcium, indicating that absorption of calcium not adversely affected by phytate in all the groups. Phytate begins to lose its inhibitory effect on iron absorption when phytate:iron molar ratios are < 1.0, although even ratios as low as 0.2 exert some negative effect as stated by [52]. The phytate:iron molar ratios greater than 0.15 regarded as indicative of poor iron bioavailability [53]. This result indicated that, the phytate:iron molar ratios of all the groups are less than the critical value, which implies the absorption of iron in all the groups not inhibited by phytate and as a result the bioavailability of iron is good. The importance of foodstuffs as a source of dietary zinc depends on both the total zinc content and the level of other constituents in the diet that affect zinc bioavailability. Phytate may reduce the bioavailability of dietary zinc by forming insoluble mineral chelates at a physiological pH [54] and the formation of the chelates depends on relative levels of both zinc and phytic acid. Hence, the phytate: Zn molar ratio is considered a better indicator of zinc bioavailability than total dietary phytate levels alone [55]. Therefore, the foods with a molar ratio of Phy:Zn less than 10 showed adequate availability of Zn and problems were encountered when the value was >15. Phytate: zinc molar ratios >15, indicative of poor zinc bioavailability [56]. The values of groups of *M. flagellipes* seed flour were lower than the critical molar ratios of Phy:Zn, which indicates the availability of zinc good. Oxalic acid and its salts can have deleterious effects on human nutrition and health, particularly by decreasing calcium absorption and aiding the formation of kidney stones [54]. The importance of oxalate contents of an individual plant product in limiting total dietary Ca availability is of significance only when the ratio of Oxalate:Ca is >1 [57].

From the result, it was observed that, all groups of *M. flagellipes* seed flour had Oxalate:Ca values are lower than the reported critical value (1.0), which implies that a low level of oxalate could have no adverse effects on bioavailability of dietary calcium in the groups.

The potentiating effect of calcium on zinc absorption in the presence of high phytate intakes has led to the suggestion that the [Phy][Ca]/[Zn] millimolar ratio may be a better index of zinc bioavailability than the [Phy]/[Zn] molar ratio alone [58]. High calcium levels in foods can promote the phytate-induced decrease in zinc bioavailability when the [Ca][phytate]/[Zn] millimolar ratio exceeds 0.5 mol/kg [59]. In this study, the values of [Ca][Phy]/[Zn] millimolar ratios of all the groups were found less than the critical level.

Table 6. Anti-nutrient to mineral molar ratios and mineral bioavailability of *M. flagellipes* seed flour

Molar ratios	Critical limits	Raw	Processed			
			Soaked	Roasted	Boiled	Autoclaved
Phytate:Ca	0.24	$1.9 \times 10^{-3} \pm 0.00^d$	$6.2 \times 10^{-4} \pm 0.00^c$	$2.1 \times 10^{-4} \pm 0.00^a$	$4.1 \times 10^{-4} \pm 0.00^b$	$2.3 \times 10^{-4} \pm 0.00^a$
Phytate:Fe	0.15	$10.0 \times 10^{-3} \pm 0.01^a$	$4.2 \times 10^{-3} \pm 0.00^a$	$2.0 \times 10^{-3} \pm 0.00^a$	$3.1 \times 10^{-3} \pm 0.00$	$1.7 \times 10^{-3} \pm 0.00^a$
Phytate:Zn	10.0	$5.6 \times 10^{-2} \pm 0.04^a$	$2.3 \times 10^{-2} \pm 0.00^a$	$1.1 \times 10^{-2} \pm 0.00^a$	$1.6 \times 10^{-2} \pm 0.00^a$	$9.6 \times 10^{-2} \pm 0.00^a$
Oxalate:Ca	1.00	$1.1 \times 10^{-3} \pm 0.00^a$	$3.6 \times 10^{-3} \pm 0.00^b$	$3.6 \times 10^{-4} \pm 0.00^a$	$6.1 \times 10^{-4} \pm 0.00^a$	$3.4 \times 10^{-4} \pm 0.00^a$
[Phy][Ca]:[Zn] (mol/kg)	0.50	$3.4 \times 10^{-3} \pm 0.00^c$	$7.5 \times 10^{-2} \pm 0.00^b$	$3.7 \times 10^{-2} \pm 0.00^a$	$5.5 \times 10^{-2} \pm 0.00^{ab}$	$3.7 \times 10^{-2} \pm 0.00^a$

Values are Mean \pm SD (n=3). Values in the same row with different superscripts are significantly different ($P \leq 0.05$).

Table 7. Effect of processing on the functional properties (mg/100g) analysis of *M. flagellipes* seed flour

Parameters (mg/100g)	Raw	Processed			
		Soaked	Roasted	Boiled	Autoclaved
WAC	3.34 ^a ±0.42	2.65 ^a ±0.38	4.69 ^a ±0.63	5.22 ^a ±1.11	6.21 ^a ±0.18
OAC	4.16 ^a ±1.62	1.90 ^a ±0.04	4.99 ^a ±0.43	4.05 ^a ±2.42	6.40 ^a ±0.02
S.I	105.85 ^a ±0.29	146.81 ^b ±2.22	156.15 ^b ±1.44	147.02 ^b ±1.42	144.39 ^b ±2.78
Bulk density	0.76 ^a ±0.00	0.61 ^a ±0.02	2.63 ^a ±1.49	1.84 ^a ±0.66	1.96 ^a ±0.74
Emulsification	17.80 ^a ±1.80	31.33 ^b ±2.52	44.46 ^b ±2.80	59.12 ^c ±1.30	66.33 ^c ±1.04
G.C	6.08^a±0.58	12.00^c±0.01	16.11^c±0.86	8.04^a±1.58	16.86^c±1.00

Values are Mean ± SD (n=3). Values in the same row with different superscripts are significantly different ($p \leq 0.05$).

WAC; Water absorption capacity

OAC; Oil absorption capacity

S.I; Swelling index

G.C; Gelation capacity.

3.8. Functional Properties

The results for the functional properties shown in Table 7 revealed that soaking, roasting, boiling and autoclaving had varying effects on the functional properties of the seed flour. All the flours exhibited a significant ($P < 0.05$) increase in the WAC except for soaked seed flour with significant ($P < 0.05$) reduction. The high WAC of the flours could be attributed to loose structure of starch polymers in the flour [60]. The baking quality of flours had been associated with the WAC of the flour [61]. The ability of the flour to absorb water improves its potentials in dough making [62]. The high WAC obtained in this study is a useful indication that *M. flagellipes* seed flour can be incorporated into aqueous formulations especially those involving dough handling.

The OAC is a prominent factor in food formulations as it improves flavor and increases the mouth feel of foods. The OAC was observed to be significantly ($P < 0.05$) increased in the autoclaved seed flour, followed by the roasted seed flour. There was significant ($P < 0.05$) reduction on the boiled and soaked seed flour with the soaked seed flour being the most significant. Oil binding capacity of food component is important for various applications because it relies mainly on this capacity to physically entrap oil by a complex capillary attraction process and this property of flour leads to better flavor retention, a consistency trait and an increase in mouth-feel [63].

Bulk density is generally affected by the particle size and density of the flour. It is very important in determining the packaging requirement, material handling and application in wet processing in food industry [64]. The bulking property of a powder alters according to the preparation methods, different treatments administered and storage. The density of the processed products or the uniqueness of its container determines the amount and strength of packaging material [65]. There was significant ($P < 0.05$) increase in the bulk density of the roasted, boiled and autoclaved seed flour while for the bulk density of the soaked seed flour, there was significant ($P < 0.05$) reduction. In food systems where low bulk density is required like in the formulation of complementary foods, soaking would be the most appropriate way of processing while in others where high bulk density is needed then roasting and autoclaving could be the best option. The gelation capacity of the samples was significantly ($P < 0.05$) higher in autoclaved seed flour than in the soaked, boiled

and roasted seed flour. The variation may be due to differences in carbohydrate content of the flours [66]. Flours with good gelation capacity are desirable for use as soup thickeners.

4. Conclusion

Though underutilized, the results from the study indicated that *Mucuna flagellipes* seeds are good sources of protein, amino acids and a number of micronutrients. They are also rich in fibre and deficient in fat, which may account for some of their proposed health benefits and as used as complement to other food groups especially cereal.

Incorporation of this underutilized inexpensive legume, which meets the requirement of essential amino acids into the diet will no doubt improve the nutritional status and help reduce malnutrition. Its low lipid content and high fibre content adds to its nutritional potential and increases the benefits derived on consumption. Autoclaving is the most efficient processing method in that it drastically reduced the anti-nutrients of the seed flour without much significant reduction in the nutrients.

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