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COMPARATIVE EVALUATION OF CHEMICAL AND ANTINUTRITIONAL COMPOSITION OF *MORINGA (MORINGA OLEIFERA,* MELON (*CITRULLUS COLOCYNTHIS)* AND SOYBEAN *(GLYCINE MAX*) SEED FLOURS

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ABSTRACT

Moringa (Moringa oleifera) and melon (Citrullus colocynthis) seeds are essential protein sources. However, moringa seed is underutilised, and egusi or melon is limited to soup thickening. Hence, information on the comparative physicochemical properties of the protein-rich seeds is imperative to solving malnutrition problems and promoting health benefits. In this study, the chemical, mineral and antinutritional compositions of defatted moringa (DMOF), melon (DMEF) and the reference protein, soybean (DSBF) seed flours were determined using standard methods. Results obtained showed there was no significant difference in the moisture, fat and carbohydrate contents of DMOF, DMEF and DSBF, which ranged from 6.02-6.28 g/100g, 1.03-1.17 g/100g and 32.67- 43.48 g/100g, respectively. DMOF and DMEF contain zinc (3.25-3.93 mg/100g), magnesium (101.70 -106.19 mg/100g) in higher quantities than DSBF, then calcium (130.01 – 158.40 mg/100 g), vitamins A and B in quantities that meet daily requirements in infants. In comparison, the iron (4.48 mg/100g) and antinutritional factors present in melon were found to be higher than moringa. The results indicate they are suitable alternatives to soybean and other conventional legumes.

Key words: Plant Proteins, *Moringa* Seed, Melon Seed, Chemical Composition, Antinutritional Factors

INTRODUCTION

Plant proteins are considered cost-effective and environmentally sustainable protein sources compared to animal proteins (Sedibe *et al.,* 2023). In addition to their detrimental environmental effects, overconsumption of red and processed meat is associated with chronic illnesses and increased mortality rates. Hence, it is imperative to transit towards high-protein plant-based meals, as they serve as a more nutritious protein source (Zheng *et al.,* 2019; Acquah *et al.,* 2021; Pöri *et al.*, 2023). Also, the issue of climate change, food insecurity, socio-economic challenges, inadequate production of legumes to sustain a growing population like Nigeria, along with the expensive nature of importing soybean used by food companies, lifestyle-related ailments like diabetes, sarcopenia, and malnutrition, have necessitated the exploration of alternative seed-based protein sources (Sonawane and Arya, 2018; Yano and Fu, 2020; FAO/IFAD/UNICEF/WFPD/WHO, 2021; Thapaliya *et al*., 2023).

The use of protein flours from moringa and melon, can meet the nutritional needs of people in Africa and solve problems associated with the use of flours from pulses, such as beany flavour arising from lipoxygenase and alcohol oxidoreductase activities (Acquah *et al*., 2021); flatulence-causing oligosaccharides (Chinma *et al.,* 2023), allergenicity (Singh *et al*., 2023), and abdominal distension (Hlangwani *et al.,* 2023). In addition, they can serve as alternatives to soybean, the number one conventional protein from plant source used in the food industry (Day, 2013), to which leaky intestinal disorder and hormonal imbalance have been associated (Vojdani, 2015, Rizzo *et al.*, 2023).

The Nigerian vegetation is rich in underutilised plant protein sources (Ogundele *et al., 2022)* like moringa and *egusi* melon. Moringa, though widely distributed in Nigeria and contains a high level of protein, is highly underutilised, while *Citrullus colocynthis* is mainly used as a soup thickener (NAS, 2006). *Citrullus colocynthis*, belonging to the family *Cucurbitaceae,* commonly referred to as *egusi*, and more generally referred to as melon, originates from West Africa. It contains 60 % protein by weight of the defatted flour (Olubi *et al.*, 2021). Moringa and melon seed proteins are relatively abundant and contain significant levels of essential amino acids for growth and phytonutrients (Masih *et al*., 2019), which have protective effects

against some chronic degenerative diseases compared to animal proteins (Xu *et al*., 2019, Samtiya *et al*., 2021).

Protein energy malnutrition can be remedied by including protein flours in diets and formulations (Ijarotimi *et al*., 2022). The demand for proteins from inexpensive sources such as moringa and melon with desirable functional, nutritional and health properties that can be incorporated into value-added food products is increasing rapidly due to the costs of proteins from animal origin (Chinma *et al*., 2014). Hence, this work was carried out to compare the chemical, mineral, vitamin and antinutritional compositions of defatted *moringa* and melon seed flours compared to soybean flour.

Chemicals and Reagents: The chemicals and reagents used for this work were obtained from the chemical store of Simbest Chemical Reagent Co., Ltd., Minna, Nigeria. They were of analytical and food-grade quality.

Source of raw materials: Moringa and melon seeds were procured from an Agro dealer in New Bussa market, New Bussa, Niger State, Nigeria in June 2019. At the same time, soybean seeds were purchased from Gusase Soybean Farm, Gusase village, Niger State, Nigeria.

Sample preparation

Preparation of soybean seed flour

Preparing full-fat soybean flour followed the method described by Anuonye (2011). The seeds were dried in the sun and subsequently fragmented into coarse particles using a grinding mill (with Senweimax petrol engine – GX 200, Fuan Shenwei Electro Mechanical Co. Ltd, China). The grits were then winnowed to remove the husks and immersed for 15 hours in distilled water to reduce the antinutritional factors. Subsequently, the grits were meticulously rinsed with fresh water twice before being subjected to boiling at 100 °C for five minutes. The moist grits were finally dehydrated at 40 degrees Celsius and pulverised to acquire full-fat soybean flour**.**

Preparation of defatted flours

Defatted flour samples were prepared from the respective cleaned seeds after 8 hours of solvent extraction of oils at 40°C – 60°C with petroleum ether, using a Soxhlet apparatus (Heating mantles by WHM multi-place, Witeg Labortechnik, Wertheim, Germany). The flours were

subsequently air-dried to evaporate the solvent, labelled, and stored in airtight plastic containers for further analysis. This was done according to the AOAC (2005) method**.**

Proximate analyses of defatted flours

Proximate analyses on the samples were carried out in triplicate according to the methods reported by Onwuka (2018).

Moisture

The moisture content of the samples was determined using the oven drying method. A precise measurement of 2 g of thoroughly blended samples was taken by weighing them in a clean and dried crucible (W1). The crucible was placed inside an oven and subjected to a temperature range of 100-105°C for 6-12 hours until it reached a stable weight. Subsequently, the crucible was transferred to the desiccator and cooled for 30 minutes. After cooling, the object was re-weighed, resulting in a second weight measurement denoted as W2. The % moisture was determined using the following formula:

$$\% \text{ Moisture} = \frac{W_1 - W_2 \times 100}{\text{Weight of sample}}$$

Where

W_1 = Initial weight of crucible + Sample1

W_2 = Final weight of crucible + Sample 2

Ash

Ash content was determined according to this procedure: a clean and empty crucible was placed in a muffle furnace at 550 degrees Celsius for one hour. After cooling in a desiccator, the weight of the empty crucible was recorded as W1. A 2g sample was placed in a crucible (W2) and heated over a hob until it turned into charred residue. Subsequently, the crucible was introduced into a muffle furnace (Boeco MF 8/1100, Germany) to undergo ashing at 550^0C for 4 hours. The presence of grey white ash signifies the complete oxidation of all organic substances in the sample. Once the crucible was ashed, it was allowed to cool down and weighed (W3). The percentage of ash was calculated by using the following formula:

$$\% \text{ Ash} = \frac{\text{Difference in Weight of Ash}}{\text{Weight of Sample}} \times 100$$

W3-W1 = Difference in weight of ash

W2 = Weight of Sample

Crude protein

Protein analysis was performed using the total nitrogen method for the freeze-dried solid residues of moringa, melon, and soybean samples, according to the Kjeldahl method. Percentages of total nitrogen were converted to protein values (%w/v) by multiplying measured percentages by a factor of 6.25 according to the formula:

$$\% \text{ Crude Protein} = 6.25^{*} \times \%N$$

(*Correction factor)

$$N\% = \frac{(S-B) \times N \times 0.014 \times D \times 100}{\text{Weight of Sample} \times V}$$

Where

S = Sample titration reading

B = Blank titration reading

N = Normality of HCI

D = Dilution of sample after digestion

V = Volume taken for distillation

0.014 = Milli equivalent weight of Nitrogen

Crude fat

Crude fat was determined by the ether extract method using a Soxhlet apparatus. Approximately 2g of a moisture-free sample was wrapped in filter paper, placed in a fat-free thimble and then introduced into the extraction tube. A weighed, cleaned and dried receiving

flask was filled with petroleum ether and fitted into the apparatus. The Soxhlet apparatus was assembled and allowed to reflux for 6 hours; the extract was transferred into a clean glass dish with the washing, which was evaporated on a water bath. Then the dish was placed in an oven at 105^{o}C - 110^{o}C for 1hr and cooled in a desiccator. The percentage crude fat was calculated by:

$$\% \text{ Crude Fat} = \frac{\text{Weight of ether extract}}{\text{Weight of sample}} \times 100$$

Crude fibre

2g of the sample was defatted with petroleum ether and boiled under reflux for 30 min with 200 mL of solution containing 1.25g of H_2SO_4 per 100 mL of solution. The solution was filtered through linen cloth placed on a funnel, washed with boiling water until the washings were no longer acidic, then the residue was transferred into a beaker and boiled for 30min with 200 mL of solution containing 1.25 g of carbonate free NaOH per 100 mL. The final residue was filtered through a thin but close pad and washed, then placed in a Gooch crucible then dried in an electric oven and weighed after which it was incinerated, cooled and reweighed. The loss in weight after incineration x 100 is the percentage crude fibre.

Carbohydrate content

The nitrogen-free method described by AOAC (2019) was used. The carbohydrate was calculated as the difference between 100 and the summation of other proximate parameters, as Nitrogen-free Extract (NFE).

Percentage carbohydrate (NFE) = 100- (M+P+F+A+F)

Where: M=moisture, P= protein, F=Fat, A=ash, F=crude fibre

Mineral analysis

Mineral analysis was carried out using inductively coupled plasma optical emission spectrometry (ICP-OES) as described below:

Microwave digestion: A 1 g of each respective sample was weighed into Teflon tubes (MARSXpress – High Throughput Vessels, Matthews, North Carolina) and mixed with 10 mL nitric acid (HNO_3). A blank was also prepared from HNO3, excluding the samples, and all

were digested in a microwave digester (CEM One Touch TM Technology, CEM Technologies, USA). Temperature conditions of the microwave-digester were as follows: the temperature program was ramped to 180 °C for 10 min and kept at 180 °C for another 10 min, followed by immediate ventilation at room temperature for 20 min. The resulting solutions were cooled and made up to mark with Milli-Q water (Millipore, Bedford, MA) in a 50 mL volumetric flask.

ICP-OES Mineral analysis: Stock and working standard solutions were prepared using NIST traceable CRM's of the test minerals. Extracts were analysed on an ICP-OES equipment (Spectro ARCOS, Spectro Analytical Instruments, Kleve, Germany) under the instrumental conditions and parameters of: RF power (emission intensity) – 1400 W; Nebulizer type – Concentric; Nebulizer flow – 1.0 L/min; Sample flow – 1.0 mL/min and Rinse time – 5 min (AOAC, 2019).

Vitamin analysis

Vitamin A

The profiling of vitamin A in samples was carried out according to the method of Liang *et al*. (2021), while external calibration was based on ISO 20633:2015 using a Nexera UHPLC LC-30A system (Shimadzu, Tokyo, Japan). Prepared standard solutions of vitamin A were injected into the Nexera UHPLC LC-30A system, and a standard curve was drawn based on the integrated areas of the chromatographic peaks. Briefly, 1 g of the sample was added to petroleum ether to a volume of 1 mL. Afterwards, 10 µL of the solution was injected into the chromatographic column (250 × 4.6 mm ×3 µm, CNW Athena C30) and isocratically eluted using ultrapure water and methanol (96:4 v/v). The eluted flow rate was 0.8 mL/minute. A UV detector detected vitamin A at wavelengths of 325nm. Vitamin A content was calculated from a standard (Vitamin A) curve.

Vitamin B_{12}

A standard method (Bajaj and Singhal, 2021) was employed to analyse Vitamin B_{12}. Vitamin B_{12} was extracted from the samples by mixing 10g of flour with 20 mL of methanol: deionised water (1:1) in an amber colored conical flask with a stopper on. The flask was kept on a rotary shaker at room temperature (28±1 *o*C) for 30 min, followed by sonication for 15 min in a bath sonicator. This mixture was centrifuged at 5000 g for 15 minutes at 25°C. The supernatant was filtered through Whatman® filter paper 1. This extract was stored in an amber coloured glass bottle at 4°c before analysis. Sample extract was analysed for vitamin B_{12} concentration using high-pressure thin-layer chromatography (HPTLC, CAMAG Linomat 5 autosampler, CAMAG

TLC scanner 3) and connected to a software (Wincats 1.2.2 software, Switzerland). The extraction recovery was determined by adding a known quantity of vitamin B_{12} (100 µg/ g of sample) into the flour, and it was profiled for the concentration of vitamin B_{12} therein.

Vitamin C

Sample Preparation: 50 mg of defatted flour was weighed, and Vitamin C was extracted by homogenising test samples in metaphosphoric acid-acetic acid solution (i.e., 15 g of HPO_3 and 40 mL of HOAc in 500 mL of deionised H_2O). The sample extracts were filtered (and/or centrifuged) and diluted appropriately to a final concentration of 10-100 mg of ascorbic acid/100 mL.

Standard preparation: 50 mg of USP L-ascorbic acid reference standard was weighed and diluted to 50 mL with HPO_3-HOAc extracting solution.

Titration: Triplicates of the standard, test samples, and blank were then titrated with the indophenol reagent (i.e., prepared by dissolving 50 mg of DCIP sodium salt and 42 mg of $NaHCO_3$ in 200 mL of deionised H_2O) to a light but distinctive rose-pink endpoint lasting for about 5 sec to determine the concentration of the indophenol solution as mg ascorbic acid equivalents to 1.0 mL of reagent (Nielsen, 2017).

Determination of antinutritional factors

Phytic acid

The method of Latta and Eskin (1980) was modified to determine the phytate content. Phytate was extracted from 1g of flour with 20 mL of 2.4% (v/v) hydrochloric acid (HCl)by shaking at room temperature for 2h, followed by high-speed centrifugation of the suspension for 15 minutes. The supernatant was decanted and filtered through Whatman No. 1 filter paper. A 3 mL aliquot of filtrate was diluted to 18 mL with distilled water, and the diluted sample was passed through a 200-400 mesh AG1-X8 chloride anion exchange resin (Bio-Rad Laboratories Gmbh, München, Germany). Inorganic phosphorus was eluted with 0.07 M sodium chloride (NaCl), followed by elution of phytate with 0.7 M NaCl. Phytate was determined colorimetrically based on the pink colour of Wade reagent, which is formed upon the reaction of the ferric ion and sulfosalicyclic acid. One millilitre of Wade reagent (0.03% solution of FeCl3×6H2O containing 0.3% (v/v) sulfosalicyclic acid in water) was added to 3 mL of the clear supernatant sample and then centrifuged at 2000 rpm for 15 min. The absorbance was

measured by a spectrophotometer (model Genesys G10S, USA) at 500 nm. The phytate content was calculated using a standard phytic acid curve, and results were expressed as mg/g.

Tannin determination

Tannin content of the defatted flour samples was determined using the method described by Udomkun *et al*. (2019). Tannin content was determined by weighing 0.5 g of the sample and adding 5 mL of 1% (v/v) of HCl in methanol. The sample was allowed to stand at room temperature for 15 min before vortex mixing and centrifugation at 3000 rpm for 10 min. The supernatant of 2.5 mL was transferred to a 10 mL flask containing 7.5 mL of water, then 0.5 mL of Folin-Denis reagent and 1 mL of sodium carbonate were added. The final volume was adjusted to 10 mL with water, and the absorbance was determined after 30 min of incubation at room temperature using a UV/Vis spectrophotometer (model Genesys G10S, USA) at 760 nm. The tannin content was calculated from the standard curve of tannic acid solution.

Trypsin inhibitor

The TIA was assayed following a standard procedure (Liu and Markakis, 1989), using 0.04 g/L of BAPA (Nα-Benzoyl-L-arginine 4 4-nitroanilide hydrochloride) as trypsin substrate. To 1 g of sample, 10 mL of 0.15 mol/L phosphate buffer (pH 8.1 at 4^{o}C) was added and kept overnight. Subsequently, extracts were incubated with trypsin solution (0.004 g/L trypsin in 0.025 mol/L glycine HCl buffer) and diluted to 1 mL with phosphate buffer (pH 8.1). 2.5 mL of 0.001 mol/L BAPA solution in pH 8.1 buffer phosphates was added and warmed to 37^{o}C for 10 min reaction. Trypsin inhibitor was calculated from the absorbance read at 410 nm (Thermo Fisher Scientific) against a reagent blank and the results reported as trypsin inhibitor activity (TIA) unit per mg (dw).

Data analysis

All analyses were conducted in triplicate, and data obtained were subjected to analysis of variance (ANOVA) using the statistical package for social statistics (SPSS, version 20, IBM, Armonk, USA).

RESULTS AND DISCUSSION

Chemical composition of defatted moringa, melon and soybean seed flours

The proximate composition of defatted *moringa* (DMOF), melon (DMEF), and soybean seed (DSBF) flours is presented in Table 1. The results show there was no significant difference in

the moisture content of DMOF, DMEF and DSBF, which ranged from 6.02-6.28 g/100 g and were found to be within the safe threshold of ≤ 10% for storing flour products (Ijah, *et al.,* 2014). This implies that the flour could be stored for long without fear of deterioration or spoilage. A lower moisture content was reported for *Irvingia gabonensis* (4.07%) seed flour (Okagu *et al*., 2021). The fat and carbohydrate contents of the three defatted seed flours ranged from 1.03-1.17 g/100 g and 32.67-43.48 g/100g, respectively, and had no statistical difference. The low-fat content in these flours will be suitable for producing concentrates. The fat content in the flours is consistent with that obtained in green gram (Wani *et al.*, 2020) and other seeds (such as kidney beans and flax seed) that ranged from 1-2% (Lan *et al*., 2020, Wani *et al*., 2020). Low fat content enhances stability, positively impacting flour's quality when stored (Temba *et al.*, 2017; Okagu *et al*., 2021).

The carbohydrate levels of the three defatted seed flours were generally less than 45%, indicating that the seed flours are rich in proteins and thus make them suitable for isolating proteins. The ash content of defatted soybean flour (5.90 g/100g) was significantly higher than moringa (5.77 g/100g) and DMEF (4.86 g/100 g). DMEF had the least crude fibre, while DSBF had the highest quantity of the three defatted seed flours. The ash content of moringa makes it a better source of micronutrients than melon seed flour, thus an indication that the material can be suitable in enhancing the micronutrients of other flours low in micronutrients when used as a composite flour. The fibre content, however, was low and signifies suitability for use as an ingredient in infant formulas.

The crude protein content of the defatted seed flours varied substantially ($p < 0.05$), with the moringa seed flour level being higher than melon. Nevertheless, the protein content of DMOF was significantly lower than DSBF's (49.56 g/100g), the reference. At the same time, DMEF had the lowest protein content ($p<0.05$) among the three defatted seed flours analysed. The DMEF protein level is similar to 40.11% obtained for melon by Imiere *et al.* (2015) in their study. Similarly, the protein content of DMOF obtained in this study corroborates the value (44.00%) reported for defatted moringa flour by Pageo *et al.* (2023). However, the protein levels in defatted moringa, melon, and soybean seed flours exceeded some legumes (chickpea, lentils and green peas) (Tang *et al*., 2021). This implies that the defatted flour samples will be good raw materials for producing functional plant protein ingredients such as protein concentrates, isolates, and hydrolysates.

Mineral and Vitamin Compositions of Defatted Moringa and Melon Seed Flours

The results of the micronutrient compositions (calcium, magnesium, zinc, iron and vitamins A, B, C) are presented in Table 2. Calcium had the highest concentration in the three seed flours. Due to its higher Ca content, defatted moringa seed flour will be more beneficial for bone formulation than C. *colocynthis* seeds. Calcium is the predominant mineral in the human body and is crucial in bone and tooth formation and various physiological processes. Calcium primarily regulates many processes by binding to proteins, such as troponin C, to promote muscle contraction. A lack of calcium is associated with hypertension, osteoporosis and cancer (Walters *et al*., 2018; Okagu *et al*., 2021). The findings are consistent with the study conducted by Okagu *et al*. (2021), which indicates that calcium stands as the predominant mineral present in melon seed. However, *Irvingia wombolu* has a higher calcium concentration than *C. colocynthis* (Okagu *et al.,* 2021).

Table 1: Chemical Composition of Defatted *Moringa*, Melon, and Soybean Seed Flour

!	Moringa seed flour	Melon seed Flour	Soybean seed flour
Moisture (g/100g)	6.28±0.02[a]	6.13±0.01[a]	6.02±0.01[a]
Ash (g/100 g)	5.77±0.01[b]	4.86±0.00[c]	5.90±0.01[a]
Crude fibre (g/100g)	4.35±0.01[b]	3.97±0.02[c]	4.82±0.05[a]
Protein (g/100 g)	44.60±0.14[b]	40.39±0.16[c]	49.56±0.24[a]
Fat (g/100 g)	1.10±0.01[a]	1.17±0.02[a]	1.03±0.01[a]
Carbohydrate (g/100 g)	37.90±0.02[a]	43.48±0.01[a]	32.67±0.01[a]

Mean values and standard deviation of triplicate replications. Means with no common letters within a row significantly differ ($p \leq 0.05$).

However, DSBF had significantly higher amounts of calcium, iron, zinc and ascorbic acid than DMOF and DMEF. The data obtained for DMOF showed higher magnesium (106.19 mg/100g) and lower iron (3.27 mg/100g) concentration than DSBF (reference protein) and defatted melon seed flour. All the minerals analysed were least in melon seed flour except for iron (4.48 mg/100g) and magnesium (101.70 mg/100g). Pageo *et al*. (2023) reported a zinc content of 3.02 mg/kg for *moringa* seed, while another report documented a zinc content of

0.33 mg/100 g in *C. colocynthis* seeds, which are different from the values reported in this study (Okagu *et al*., 2021). Zinc is a cofactor for dehydrogenases, phosphatases, superoxide dismutase, reductases, and polymerases (DNA and RNA polymerases) (Pageo *et al*., 2023, Okagu *et al*., 2021). Hence, consumption of DMOF and DMEF in diets will promote cognitive development, physical growth and boost immunity of children, equally as soybean seed flour.

Vitamin C had the highest concentration among the vitamins analysed in this study and was higher in defatted moringa seed flour (13.60 mAA/ 100 g) than DMEF (10.87 mAA/100 g). Similarly, the concentration of vitamins A and B_{12} were higher in DMOF than DMEF, while the vitamin B_{12} quantity in DSBF was lowest of the three defatted flours (0.05 mg/100g). The micronutrients were above daily recommended allowance except for iron, while vitamin C in all the flours was below the recommended daily requirement (Table 2). Nonetheless, the Vitamin C component of the three flours may contribute to the daily need for physiological functions. A lower value of Vitamin C (1.11 mg/100g) than the flours studied was reported for rice bean (Semwal *et al*., 2023).

The higher magnesium content of moringa than soybean and calcium, zinc, vitamins A and B than recommended dietary allowances (300 – 500 μmg and 0.4 – 0.9 μmg, respectively) (NIH, 2024a and b), could make defatted moringa seed a potential nutraceutical for use in nutritional therapy for the treatment of emerging viral infections and zoonotic diseases as well as in the prevention of micronutrient deficiencies in under five children. On the other hand, the defatted melon seed with higher iron content than DMOF may be used to prevent anaemia in pregnant women.

Antinutrient Composition of Defatted Moringa, Melon and Soybean Seed Flours

Table 3 shows the antinutrient composition of raw *moringa*, melon and soybean seed flours. Soybean contained the significantly ($p < 0.05$) highest concentration of phytic acid (852.41 mg/100g), tannin (3.85 mg/100g) and trypsin inhibitor activity (5.79 TIU/mg) among the three defatted seed protein flours analysed. This was followed by defatted melon seed protein flour, while defatted moringa seed flour had the least trypsin inhibitor activity, phytic acid and tannin concentration.

Table 2: Micronutrient Composition of *Moringa*, Melon, and Soybean Seed Protein Flours

Parameter	*Moringa* seed flour	Melon seed flour	Control (Soybean seed flour)	FAO/WHO 1998-Infants 0-2years
Calcium (mg/100 g)	158.40±0.86b	130.01±0.72c	196.11±0.95a	120-400
Iron (mg/ 100 g)	3.27±0.01c	4.48±0.07b	5.11±0.10a	6.2-18.6
Magnesium (mg/100 g)	106.19±0.23a	101.70±0.48b	96.43±0.61c	26-60
Zinc (mg/100 g)	3.93±0.01b	3.25±0.01c	4.30±0.02a	1.1-2.4
Vitamin A (mg /100 g)	1.25±0.02a	1.07±0.01c	1.31±0.01a	0.3 - 0.5**
Vitamin B_{12} (mg/100 g)	0.17±0.01a	0.13±0.01a	0.05±0.01b	4-9x10^{-4}*
Vitamin C (mgAA/100 g)	13.60±0.03^{b}	10.87±0.09^{c}	14.59±0.07^{a}	25-30

Mean values and standard deviation of triplicate replications. Means with no common letters within a row significantly differ ($p \leq 0.05$). AA = Ascorbic acid equivalent, **mg RAE/ 100g = Retinol activity equivalent, **NIH, 2024a (0-3 years), * NIH, 2024b (0-3 years)

Different authors have reported different values in the defatted flours. The antinutrients (phytic acid, tannin and trypsin inhibitor) differed significantly in the three seed flours, with phytate being of higher concentration than the other ANFs. The results corroborate previous reports that phytic acid is high in the three seeds (Jain *et al.,* 2019; Alam and Judder, 2022; Hymowitz, 2022). The trypsin inhibitor activities of defatted moringa, melon and soybean in this study, were higher than the 1.83 U/mg reported for mung bean, and 2.20 U/mg for pea. Their concentrations were, however, lower than TIA in cowpea (7.52 U/mg) and untreated soybean (94.1 U/mg) (Avilés-Gaxiola *et al.,* 2018).

The amounts of antinutrients (phytate: 1674.9 mg PAE/100g; trypsin inhibitor: 5.7 TIU/mg; tannin: 34.3 mg CE/ 100 g) previously reported for defatted moringa seed flour by León-López *et al.* (2020) were, however, higher than in this work. Various results have been documented on the antinutrient levels of defatted melon, roasted melon, and raw melon flour. Kapoor (2020) gave a higher phytate level of 18.6 mg/g, and a lower tannin (7µg/g) level, while Ogundele (2022) reported a higher level of tannin for defatted melon. Both test defatted flour samples and the reference defatted flour (defatted soybean flour) however, showed lower tannin and phytate levels when compared to sorrel seed flour (phytate level of 32.75 mg/g and tannin level

of 9.03 mg/g) (Ayo-Omogie *et al*., 2023) and kidney beans (Samtiya *et al*., 2020). Phytic acid, tannins and trypsin inhibitor limit protein digestion and mineral availability in vivo (Mahanta *et al.,* 2020), though they possess health benefits. Thus, there is a need to reduce the ANFs in both moringa and melon seeds to acceptable limits.

Table 3: Antinutritional composition of moringa, melon, and soybean seed protein flours

Antinutritional factors	DMOF	DSBF	DSBF
Phytic (mg/100 g)	544.70±1.54^{c}	780.63 ±1.19^{b}	852.41±3.26^{a}
TIA (TIU/ mg)	3.85±0.01^{c}	4.91±0.02^{b}	5.79±0.05^{a}
Tannin (mg/100 g)	2.01±0.00^{c}	2.63±0.02^{b}	3.85±0.01^{a}

Mean values and standard deviation of triplicate replications. Means with no common letters within a row significantly differ ($p \leq 0.05$). TIA= Trypsin inhibitor activity. TIU= Trypsin inhibitor unit

CONCLUSION

The results obtained revealed that defatted moringa and melon seed flours are high-protein flours, equally rich in protein as soybean, with lower antinutritional factors and higher calcium, magnesium, vitamins A and B contents than the reference protein. The flour's rich zinc content may help improve cognitive development and curb stunting, which is prevalent among Nigerian children. Moringa and melon seed proteins are rich sources of macro and macronutrients It is recommended that complementary food formulations, such as blending other iron-rich plant proteins with the seed proteins of this study to reach the daily recommended intake, should be explored. Production of moringa and melon protein concentrates from the flours and comparative characterization for physicochemical, functional thermal, structural and antioxidant properties of the flours to determine their potentials as functional ingredients for incorporation in infant formulas and confectioneries should be carried out.

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