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Determination of Proximate and Mineral Composition of *Moringa oleifera* Leaves and Pods

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ABSTRACT

Background and Objective: *Moringa oleifera* is a multipurpose plant known for its high nutritional and medicinal value. Despite its widespread cultivation, comprehensive data on the nutrient composition of its leaves and pods in Bangladesh are limited. This study aimed to determine the proximate, vitamin, and mineral composition of *M. oleifera* leaves and pods to evaluate their potential for dietary supplementation and food fortification. **Materials and Methods:** Leaves and pods of *M. oleifera* were collected from BIRTAN research fields, Araihazar, Narayangonj, Bangladesh, in triplicate. Samples were cleaned, dried, and analyzed for proximate composition (moisture, protein, fat, ash, carbohydrate, dietary fiber, dry matter, and energy) using standard AOAC (2000, 2005) methods. Vitamin content (Vitamin C, β-carotene, B1, B2, B 3, B6, B9) was quantified using High-Performance Liquid Chromatography (HPLC). Mineral content (Ca, K, Na, Mg, Zn, Mn, Fe, Cu, P) was determined using Atomic Absorption Spectroscopy and Flame Photometry. Data were expressed as Mean±SD of triplicate analyses. p-values≤0.05 were considered statistically significant. **Results:** Leaves were richer in protein (7.60±0.14 g/100 g), fat (1.81±0.04 g/100 g), carbohydrate (15.55±0.31 g/100 g), and energy (108.88±0.38 kcal/100 g) compared to pods, which contained higher moisture (87.04±1.74 g/100 g) and dietary fiber (5.36±0.25 g/100 g). Vitamin analysis showed leaves had significantly higher Vitamin C (123.85±0.77 mg/100 g) and β-carotene (15,649.95 µg/100 g) than pods (Vitamin C: 70.66±1.48 mg/100 g; β-carotene: 0.096±0.02 mg/100 g). B-complex vitamins, including B6 (1.36±0.004 mg/100 g) and folate B9 (356.18±1.25 µg/100 g), were also higher in leaves. Mineral profiling revealed leaves had elevated Ca (393.08±7.86 mg/100 g), Fe (2.82±0.06 mg/100 g), Mg (75.61±1.51 mg/100 g), Na (48.30±0.47 mg/100 g), and P (99.67±2.00 mg/100 g), while pods were richer in K (337.99±2.08 mg/100 g) and Zn (0.744±0.011 mg/100 g). **Conclusion:** *Moringa oleifera* leaves are nutrient-dense, providing high levels of protein, essential vitamins, and minerals, while pods contribute dietary fiber, potassium, and zinc. Both plant parts can be utilized strategically for food fortification and dietary supplementation, highlighting the potential of *M. oleifera* as a sustainable resource to combat malnutrition and enhance nutritional security.

KEYWORDS

Moringa oleifera, nutrient composition, proximate analysis, vitamins, minerals, dietary fiber, HPLC, atomic absorption spectroscopy

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INTRODUCTION

Moringa oleifera, commonly known as the "drumstick tree," is a fast-growing, multipurpose species originally native to South Asia but now cultivated across much of the tropics and subtropics. It exhibits remarkable environmental resilience, thriving in semi-arid and humid regions, tolerating drought, and growing in marginal soils[1]. Because of these traits, *M. oleifera* is increasingly promoted as a sustainable crop for food security and climate-resilient agriculture[2].

The leaves of *Moringa oleifera* are particularly valued for their nutrient richness, containing high levels of protein, essential amino acids, vitamins, minerals, and bioactive compounds[3,4]. In many regions, these leaves are integrated into diets to reduce malnutrition, especially among children and lactating women[5]. Comparisons with conventional foods emphasize Moringa's nutritional edge: Dried leaves may contain 25 times more iron than spinach, 17 times more calcium than milk, 15 times more potassium than bananas, and nine times more protein than yogurt[6,7], although vitamin C content declines upon drying[8]. Recent reviews confirm Moringa is low in fat and carbohydrates but dense in protein, micronutrients, and antioxidants[9]. Advances in mineral profiling using techniques like ICP-OES have revealed that leaves generally contain high levels of calcium (676 mg/100 g), potassium, and manganese, while seeds contribute more zinc and copper[10].

Beyond nutrition, traditional medicine uses *M. oleifera* parts to manage hypertension, diabetes, respiratory and gastrointestinal diseases, inflammatory conditions, infections, and to support wound healing[11]. Modern studies have also documented its antioxidant, anti-inflammatory, cytoprotective, and antimicrobial properties[12]. Seed oil is rich in unsaturated fatty acids and has potential in nutraceutical and cosmetic applications, while pods provide dietary fiber, certain vitamins, minerals, and immunomodulatory effects[13].

Moringa oleifera has gained global recognition due to its exceptional nutritional and medicinal properties[14]. Its leaves and pods are particularly noted for their high content of vitamins, including vitamin C, β-carotene, and several B-vitamins[15]. Accurate quantification of these vitamins is crucial for understanding their nutritional contributions and potential health benefits. High-Performance Liquid Chromatography (HPLC) is an established analytical technique offering high sensitivity and specificity for the determination of water-soluble vitamins in plant matrices[16].

Despite extensive studies on leaves, direct comparative data on different edible parts especially regarding proximate composition, vitamin profiles, and mineral contents remain limited. Understanding these differences is critical for food formulation, nutritional recommendations, and value-added strategies.

Accordingly, this study was designed to:

- Determine the proximate composition of dried Moringa leaves
- Analyze and compare the vitamin content of Moringa leaves and pods
- Evaluate and contrast the mineral composition of Moringa leaves and pods

MATERIALS AND METHODS

Description of the study area: The Moringa research plot was established at the BIRTAN head office field at Araihazar, Narayangonj, Bangladesh (Fig. 1a-c). The study was conducted from April to November, 2024. Leaves and pods were used for this research purpose. Fresh leaves of local cultivars of *M. oleifera* were collected from the BIRTAN research field, Araihazar, Narayangonj, Bangladesh. The sample was washed and cleaned with deionized water, and after removal of excess water, the sample was packed and then transferred to the lab for analysis. This study was carried out at the Waffen Research Laboratory, Mirpur, Dhaka, and the BIRTAN Research Laboratory, Araihazar, Narayangonj.

Fig. 1(a-c): Moringa field at BIRTAN HQ, (b) Leaf of moringa and (c) Pod of moringa

Taxonomy:

- Kingdom: Plantae
- Order: Brassicales
- Family: Moringaceae
- Genus: Moringa
- Species: *M. oleifera* Lam

Moisture: The moisture content of Moringa oleifera samples was determined using the method described by Anwar *et al.*[5]. Samples were collected in pre-weighed, tared porcelain crucibles. The crucibles containing the samples were weighed and then placed in an oven maintained at 105°C for 24 hrs. After drying, the crucibles were transferred to desiccators and allowed to cool to room temperature. The final weights of the crucibles with the dried samples were then recorded. The percent weight loss was calculated and reported as the moisture content using the following formula:

$$\text{Moisture content (\%)} = \frac{\text{Initial weigh (g)} - \text{Final weight (g)}}{\text{Initial weight (g)}} \times 100$$

Where:
Initial weight = Sample weight+crucible weight (before heating)
Final weight = Dry sample weight+crucible weight (after heating)

Protein content: The AOAC Official Method 2001.11 is a standard Kjeldahl-based procedure for determining crude protein in animal feed, forage, grains, and oilseeds[17]. The method comprises three main stages: Digestion, distillation, and titration. In the digestion step, a homogenized sample (typically 0.2-1.0 g) is treated with concentrated sulfuric acid in the presence of a copper catalyst, with or without potassium sulfate to elevate the boiling point. The mixture is heated in a block digester until the organic matter is completely decomposed, leaving ammonium sulfate in solution[18]. During distillation, the cooled digest is diluted with water, rendered strongly alkaline using sodium hydroxide, and subjected to steam distillation. Released ammonia is collected in a boric acid solution containing a mixed indicator[19]. In the titration step, the ammonia trapped in the boric acid is quantified by titration with standardized acid, commonly 0.1 N HCl or H_2SO_4, until the indicator endpoint is reached. Nitrogen content is calculated from the titration data, and crude protein (%) is obtained by multiplying N (%) by the conventional factor, usually 6.25[20]. This method is widely used due to its accuracy, reproducibility, and suitability for routine analysis of feeds and agricultural products, although it requires strict safety precautions because of the use of concentrated acids, alkali, and high temperatures[18].

$$N\ (\%) = \frac{V_{net} \times V_{acid} \times 1.4007}{\text{Sample mass (g)}}$$

Where:

V_{net} = V_{sample}-$V_{blank\ (mL)}$

N_{acid} = Normality of titrant (eq/L)

$$\text{Crude protein (\%)} = \text{Nitrogen (\%)} \times 6.25$$

$$\text{Crude protein (factor 6.25)} = 3.4458 \times 6.25 = 21.54\% \text{ protein}$$

Determination of fat: Fat was determined using the continuous solvent extraction gravimetric method using a Soxhlet apparatus, as described by Hewavitharana *et al.*[21] and de Faria *et al.*[22]. Samples were weighed into an extraction thimble and covered with cotton wool. The recovered aluminum cap was weighed. About 50 mL of organic solvent (diethyl ether) was poured into the cap connected to the thimble and the sample. The extractor was connected to a heating mantle. The extract obtained was dried in a hot air oven and held in desiccators for cooling, after which it was weighed.

$$\text{Fat (\%)} = \frac{\text{Weight of fat}}{\text{Original weight of sample}} \times 100$$

Determination of ash: The ash content was determined gravimetrically[23] method 08-01. About 3 g of the Moringa flour sample was weighed on a pre-ignited and cooled porcelain crucible. Ashing of the sample was done using a muffle furnace model B 180, Germany, adjusted to 550°C for three hours. After cooling in desiccators, the ash (%) was calculated from the mass difference on a dry matter basis.

Calculation:

The percent of ash was calculated using the following formula:

$$\text{Weight of ash} = \text{(Weight of crucible+ash)-weight of crucible}$$

$$\text{Ash (\%)} = \frac{\text{Weight of ash (g)}}{\text{Weight of sample (g)}} \times 100$$

Carbohydrates: The carbohydrate content, expressed as nitrogen-free extract (NFE), was determined by difference. Specifically, the sum of the percentages of moisture, fat, crude protein, ash, and crude fiber was subtracted from 100%, providing the NFE value[24].

$$\text{Carbohydrate (\%)} = \text{100-(moisture+fat+ash+crude fibre+crude protein (\%))}$$

Organic matter: The organic matter content was estimated by subtracting the percentages of moisture and ash content from one hundred[19].

$$\text{Organic matter (\%)} = \text{100-(moisture+ash (\%))}$$

Energy value: The caloric value of *Moringa oleifera* samples was calculated using the Atwater factors, by multiplying the amounts of crude protein, lipid, and carbohydrate by 3.99, 9.1, and 3.99, respectively, and summing the results[17].

Determination of vitamin content

Sample collection and preparation: *Moringa oleifera* leaves and pods were harvested from mature trees. The samples were washed, dried, and ground into fine powders. Extraction of vitamins was performed using a suitable solvent, followed by filtration and concentration of the extract[25].

HPLC analysis: The vitamin content was determined using an HPLC system equipped with a UV-Vis detector. The separation was achieved on a C18 column with a mobile phase optimized for the separation of water-soluble vitamins. Detection wavelengths were set according to the specific absorbance maxima of the vitamins under investigation. Quantification was performed by comparing the peak areas of the sample chromatograms with those of standard solutions[26].

Mineral content: The mineral content of different parts of the Moringa sample was determined[19]. One gram of the sample was used in the determination of the mineral content. The ash in the porcelain crucible was dissolved with a few drops of distilled water, followed by 5 mL of 2N hydrochloric acid and filtered through Whiteman filter paper into a 100 mL volumetric flask. The minerals, such as calcium (Ca), Magnesium (Mg), Zinc (Zn), Manganese (Mn), and Iron (Fe), were determined using an Atomic Absorption Spectrometer Novaa 350, Analytic Jena, Germany. In contrast, Sodium (Na) and Potassium (K) were then determined by using the Flame photometer FP-902PG instrument, while phosphorus (P) content was determined using a UV-spectrophotometer model spectra-5200.

Data analysis: All the data are expressed as the Mean±SD with a minimum of triplicate analysis. Statistical comparisons were made by One-Way Analysis of Variance (ANOVA) with *post hoc* Duncan multiple comparisons[27]. Predetermined p-values$\leq$0.05 were considered statistically significant.

The significance between mean values (mean separation) was expressed by the Least Significant Difference Test (LSD) method.

RESULTS AND DISCUSSION

Proximate composition: The proximate composition of *Moringa oleifera* leaves and pods is presented in Table 1. The leaves were found to be richer in protein (7.60 g/100 g), fat (1.81 g/100 g), carbohydrate (15.55 g/100 g), and total energy (108.89 kcal/100 g), indicating their role as a more nutrient-dense component of the plant. In contrast, the pods contained higher levels of moisture (87.04 g/100 g) and dietary fiber (5.36 g/100 g), but relatively lower protein and caloric values compared to the leaves. Such differences suggest that while leaves may serve as an excellent source of concentrated nutrition and energy, pods may be more beneficial for hydration and digestive health due to their higher water and fiber content. These findings are consistent with previous reports that *M. oleifera* leaves provide substantial amounts of proteins, carbohydrates, and essential micronutrients, making them useful in combating malnutrition, especially among vulnerable groups such as children and nursing mothers[5]. Conversely, the high fiber content in pods has been linked to improved gut motility and cholesterol-lowering effects, thereby promoting gastrointestinal health[8,28].

For the fat, their contents have been found in very weak concentrations compared to the proteins and carbohydrates. Thus, the concentrations of fat were observed to be 1.81 g/100 g and 0.33 g/100 g in moringa leaves and pods, respectively. In general, vegetables are not considered rich sources of fats reported. *Moringa oleifera* leaves are characterized by a relatively low fat content, which is considered nutritionally favorable. In addition, the fat fraction of the leaves contains a higher proportion of polyunsaturated fatty acids (PUFAs) compared to saturated fatty acids (SFAs). This composition is desirable since a diet rich in PUFAs and low in SFAs has been associated with the prevention of chronic diseases and the promotion of overall health[8,11].

Table 1: Proximate composition and energy value of moringa leaves and pods at WB

Parameter	Unit	Moringa leaf	Moringa pod
Energy (calories)	Kcal/100 g	108.88±0.38	49.55±2.14
Protein	g/100 g	7.60±0.14	2.87±0.08
Fat	g/100 g	1.81±0.04	0.33±0.01
Moisture	g/100 g	75.43±1.51	87.04±1.74
Ash	g/100 g	1.66±0.03	0.98±0.02
Carbohydrate	g/100 g	15.55±0.31	8.78±0.18
Total dietary fiber	g/100 g	3.42±0.17	5.36±0.25
Dry matter	g/100 g	26.62±0.12	12.96±0.47

WB: wet basis and values are expressed as the Mean±SD (minimum of triplicate analysis)

The reduced moisture content of the leaf is a critical determinant of its extended shelf stability. Consequently, prolonged storage of the powdered form does not predispose it to microbial deterioration, thereby substantiating the conventional practice of preserving the material in a dehydrated state. Moisture content is also recognized as one of the most fundamental and widely employed parameters in the assessment of food processing, preservation, and storage quality[29].

Ash content, on the other hand, represents the inorganic residue obtained following complete desiccation and subsequent incineration of all organic constituents, including lipids, proteins, carbohydrates, vitamins, and organic acids[30]. Accordingly, the ash fraction of dried leaf powder is regarded as a direct index of its mineral composition. The present findings demonstrate that dried Moringa leaves exhibit substantial mineral deposits, corroborating earlier reports in the literature[31].

The leaf of *Moringa oleifera* is a rich source of carbohydrates, contributing significantly to its caloric value and potential to meet the body's energy requirements. Carbohydrates are an indispensable component of a balanced diet and are recommended to constitute approximately 50% of the daily caloric intake[32].

Dietary fiber, composed predominantly of cellulose and smaller proportions of lignin, is resistant to digestion in the human gastrointestinal system. The relatively low fiber concentration reported in this study is of particular interest, as it deviates from the higher levels typically found in most forage species. This is significant since the fiber fraction exerts a major influence on both the rate and efficiency of feed digestion[33]. While an appropriate level of dietary fiber can enhance digestibility and facilitate the absorption of microelements, glucose, and fat, excessive fiber intake may lead to intestinal irritation, reduced digestibility, and lower nutrient utilization[34]. The dietary fiber content of Moringa leaves in this study (3.42±0.17%) was considered acceptable, suggesting that Moringa leaves are a promising ingredient for both human and animal diets.

Dry matter (DM) represents the portion of a plant or feed that remains after all water has been removed, including protein, fat, carbohydrates, fiber, and minerals. Expressing nutrients on a dry matter basis allows for accurate comparison between samples with differing moisture contents and is essential for proper dietary formulation and evaluation of nutrient availability[33]. In this study, Moringa leaves contained 26.62±0.12% DM, while Moringa pods had 12.96±0.47% DM.

Vitamin content: Vitamin composition of *Moringa oleifera* leaves and pods was determined using the HPLC method. The findings showed that the leaves are significantly richer in vitamins compared to the pods. Specifically, the leaves contained 123.86 mg/100 g of vitamin C and 15,649.95 µg/100 g of β-carotene, confirming their importance as dietary sources of antioxidant nutrients. In addition, substantial levels of B-complex vitamins were observed, particularly vitamin B_6 (1.38 mg/100 g) and folate (vitamin B_9, 356.18 mg/100 g), which are critical for amino acid metabolism, red blood cell synthesis, and fetal neural development (Table 2).

Table 2: Vitamin composition of Moringa leaf and pod

Parameter	Unit	Moringa leaf	Moringa pod
Vitamin C	mg/100 g	123.85±0.77	70.66±1.48
Beta carotene	mg/100 g	15.64±0.38	0.096.19±0.02
Vitamin B_1	mg/100 g	0.05±0.002	0.049±0.002
Vitamin B_2	mg/100 g	0.42±0.01	0.72±0.05
Vitamin B_3	mg/100 g	0.84±0.03	0.70±0.02
Vitamin B_6	mg/100 g	1.36±0.004	0.38±0.053
Vitamin B_9	µg/100 g	356.18±1.25	78.99±2.87

Table 3: Mineral composition of *Moringa oleifera* leaf and pod

Parameter	Unit	Moringa leaf (Mean±SD)	Moringa pod (Mean±SD)
Sodium	mg/100 g	48.30±0.47	35.74±1.87
Potassium	mg/100 g	308.43±1.20	337.99±2.08
Zinc	mg/100 g	0.63±0.01	0.744±0.011
Calcium	mg/100 g	393.08±7.86	26.19±0.25
Iron	mg/100 g	2.82±0.06	0.749±0.009
Phosphorus	mg/100 g	99.67±2.00	97.06±0.41
Magnesium	mg/100 g	75.61±1.51	37.84±0.34
Copper	mg/100 g	0.14±0.003	0.111±0.002

Values are expressed as the Mean±SD (minimum of triplicate analysis)

These results are consistent with earlier reports indicating that *M. oleifera* leaves provide superior amounts of essential vitamins compared to pods[8]. The high vitamin C and β-carotene content supports their role in reducing oxidative stress and strengthening immune function[35], while folate and vitamin B6 highlight their relevance in addressing maternal and child nutrition, particularly in regions prone to micronutrient deficiencies[11]. Collectively, the data reinforce the nutritional potential of Moringa leaves as a functional food capable of contributing to both general health and disease prevention.

The leaves of *Moringa oleifera* are a rich source of vitamins, especially those that are water-soluble. Among these, vitamin C is present in notably high concentrations and is recognized for its role in facilitating the absorption of iron within the human body[31]. Reports indicate that the vitamin C content of Moringa leaves surpasses that of many plant species traditionally regarded as substantial sources of this essential nutrient.

Mineral compositions: The mineral profile of *Moringa oleifera* leaves and pods is presented in Table 3. Sodium content was higher in the leaves (48.30±0.47 mg/100 g) than in the pods (35.74±1.87 mg/100 g). Potassium was abundant in both parts, with slightly higher levels in the pods (337.99±2.08 mg/100 g) compared to the leaves (308.43±1.20 mg/100 g). Zinc concentrations were modest but comparable, with 0.63±0.01 mg/100 g in leaves and 0.744±0.011 mg/100 g in pods.

Calcium showed the greatest disparity, being markedly higher in leaves (393.08±7.86 mg/100 g) than in pods (26.19±0.25 mg/100 g), confirming the importance of leaves as a major dietary source of this mineral. Calcium plays a vital role in the development and preservation of bone and dental structures, thereby contributing to the prevention of osteoporosis. In addition, adequate calcium levels are essential for proper blood coagulation and the normal functioning of the nervous system. Similarly, iron levels were elevated in leaves (2.82±0.06 mg/100 g) relative to pods (0.749±0.009 mg/100 g). Phosphorus concentrations w ere nearly equivalent between leaves (99.67±2.00 mg/100 g) and pods (97.06±0.41 mg/100 g). Magnesium was also more abundant in leaves (75.61±1.51 mg/100 g) than pods (37.84±0.34 mg/100 g). Copper levels were low in both, with slightly higher values in leaves (0.14±0.003 mg/100 g) compared to pods (0.111±0.002 mg/100 g).

Overall, the results highlight that *Moringa oleifera* leaves are superior in calcium, iron, magnesium, sodium, and phosphorus, whereas pods provide slightly higher potassium and zinc. These findings suggest that the leaves serve as an excellent source of micronutrients crucial for bone health, hemoglobin synthesis, and enzymatic functions, while the pods may contribute to electrolyte balance and immune function.

These observations align with earlier reports that *M. oleifera* leaves are particularly rich in calcium and iron, making them a valuable dietary supplement in regions affected by malnutrition and micronutrient deficiencies[5,8]. The relatively high potassium levels in pods are consistent with their potential role in regulating blood pressure and cardiovascular function[11].

Moringa leaves should be promoted as a nutrient-dense food source to address protein and vitamin deficiencies, particularly among vulnerable populations. Similarly, Moringa pods can be utilized to support digestive and cardiovascular health. Incorporating both leaves and pods into functional foods, flours, and dietary supplements can enhance overall nutrient intake. Further research is recommended to investigate the bioavailability, antioxidant activity, and health impacts of nutrients derived from different parts of Moringa. Studies should also examine the presence of antinutritional factors and evaluate how various cooking and processing methods affect nutrient retention. Moreover, integrating Moringa-enriched products into community-based nutrition programs can play a vital role in improving public health outcomes, especially in areas with a high prevalence of micronutrient deficiencies.

CONCLUSION

The findings of this study confirm that *Moringa oleifera* is a highly nutrient-dense plant with distinct nutritional profiles in its leaves and pods. The leaves are particularly rich in protein, energy, vitamins, and minerals such as calcium, sodium, manganese, zinc, and iron, whereas the pods are notable for potassium, zinc, and dietary fiber. These complementary nutrient profiles suggest that both parts of the plant can be strategically utilized to enhance food quality and fortification programs. The HPLC analysis further demonstrated that the leaves are an excellent source of essential vitamins, including Vitamin C, β-carotene, and B-complex vitamins, underscoring their potential as a dietary supplement. Overall, *Moringa oleifera* is a versatile and sustainable plant resource capable of contributing to nutritional security, health promotion, and the development of nutrient-enriched food products.

SIGNIFICANCE STATEMENT

This study discovered the exceptional nutritional and functional potential of *Moringa oleifera*, with leaves rich in protein, vitamins, and minerals, and pods abundant in dietary fiber and essential micronutrients, that can be beneficial for improving dietary quality, addressing micronutrient deficiencies, and supporting public health. This study will help researchers to uncover the critical areas of nutrient utilization and functional food development that many researchers were not able to explore. Thus, a new theory on the role of Moringa in enhancing nutrition and health outcomes may be arrived at.

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