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## FOOD SCIENCE & TECHNOLOGY | RESEARCH ARTICLE

# A comparison on the nutritional quality of proteins from *Moringa oleifera* leaves and seeds

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**Abstract:** The aim of this study was to evaluate the nutritional quality of protein from *Moringa oleifera* seeds and leaves. The defatted flours were rich in protein (33.53 and 18.63% for seeds and leaves, respectively) and carbohydrates. Amino acid analysis revealed the presence of all essential amino acids in both leaf and seed flour, with high content of leucine and valine and low content of methionine and cysteine. The total essential amino acids content of leaf flour (42.76 g/16 g N) was higher than that of seed flour (35.07 g/16 g N). Limiting amino acids were lysine and sulfur amino acids. The available lysine content of leaf flour (3.78 g/16 g N) was significantly higher than that of seed flour (1.30 g/16 g N). *In vitro* digestibility studies revealed that leaf proteins were more easily digested by pepsin than seed proteins. Moreover, after a pepsin-pancreatin hydrolysis, digestibility of seed flour (61.12%) was significantly higher than that of leaf flour (57.22%). In addition, the leaf flour showed higher chemical score (72.40%), protein efficiency ratio (3.47–3.71) and protein digestibility corrected amino acid score (41.42%) and available lysine (3.78 g/16 g N) than the seed flour. Therefore, *M. oleifera* seeds and leaves have good potential as nutritional supplements or ingredients in food.

**Subjects:** Food Chemistry; Food Science & Technology; Nutrition

**Keywords:** *Moringa oleifera*; seeds; leaves; proteins quality

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Martin Alain Mune Mune is Senior Lecturer at the University of Maroua, Cameroon, since 2010. He got a PhD in Biochemistry in 2009 at the University of Yaoundé I, and Post Doc in India and Germany. He is presently working on protein and peptide Biochemistry, functional properties and bioactivities, with the major aim of designing new functional products. This study is a contribution on the potential utilization of underutilized plant proteins as food ingredients.

### PUBLIC INTEREST STATEMENT

Protein and calorie malnutrition is one of the most widespread problems in developing countries, with disastrous consequences occurring in children in the forms of two serious diseases namely marasmus and kwashiorkor. Since animal proteins are expensive for people in developing countries, there is a constant search for unconventional legumes as new protein sources. In this connection, *Moringa oleifera* a promising underutilized legume used in the preparation of traditional dishes merited the attention. This study showed that *M. oleifera* seeds and leaves are rich in protein, with higher total essential amino acids content than the FAO/WHO reference pattern. These proteins exhibited high digestibility and good nutritional properties. Therefore, *M. oleifera* seeds and leaves exhibited good potential as nutritional supplements or ingredients in food.

## 1. Introduction

Protein and calorie malnutrition is one of the most widespread problems in developing countries. The most disastrous consequences occur in children where protein energy malnutrition manifests itself in forms of two serious diseases: marasmus and kwashiorkor. Plant proteins are therefore important in the diet of children because animal proteins are unavailable due to high price. Although conventional legumes have been playing a key role as a food and feedstuff in most of these countries, their production is not enough to meet the requirements of the increasing population and animal feed industries (Siddhuraju & Becker, 2003). Therefore, there is a constant search for unconventional legumes as new protein sources. In this connection, *Moringa oleifera* a promising underutilized legume used in the preparation of traditional dishes merited the attention.

Several legumes have been studied and proposed as protein alternatives for human consumption, particularly in developing countries. Generally, legumes are rich in proteins (18–43%) and good sources of slow release carbohydrates (Table 1). They are also good sources of minerals and vitamins. Several reports claim that inclusion of legumes in the daily diet has many beneficial physiological effects in controlling and preventing various metabolic diseases such as diabetes mellitus, coronary heart disease and colon cancer. Legumes also contain antinutritional factors. However, legumes are normally consumed after processing, which not only improves palatability of foods but also increases the bioavailability of nutrients, by inactivating trypsin and growth inhibitors and haemagglutinins (Tharanathan & Mahadevamma, 2003). Extensive research has been conducted on the traditional legumes, e.g. peas, beans and lentils (Pastor-Cavada, Juan, Pastor, Alaiz, & Vioque, 2011; Rebello, Greenway, & Finley, 2014). However, relatively little work has been directed at the seeds of tree legumes. Tree legumes grow extensively in tropical and subtropical regions of the world. Their ability to (a) grow in poor soils because of their nitrogen fixing capability and to (b) withstand long periods of drought makes them ideal low input, high-yielding trees (Marangoni, Alli, & Kermasha, 1988).

*M. oleifera* Lamarck (fam. Moringaceae), is a perennial foliated tree, widely cultivated due to its high adaptability to climatic conditions and dry soils (Okuda, Baes, Nishijima, & Okada, 2001). It is considered as one of the most useful plant in the world because almost all its parts can be used as food, in traditional medicines and for industrial purposes (Fahey, 2005; Khalafalla & Abdellatef, 2010). In addition, seed and leaf flour have been used in the formulation of infant food to increase protein content (Anwar, Latif, Ashraf, & Gilani, 2007). Although *M. oleifera* leaves and seeds represent important source of protein, nutritional quality depends on the essential amino acids content and bioavailability. In fact, it has been shown that vegetable proteins are less susceptible to *in vivo* digestion than animal proteins because of their low sulfur amino acids content, compact structure,

**Table 1. Energy, macronutrient and fiber content of common legumes. Values are per cup of mature dry seeds, cooked (boiled without salt) (Rebello et al., 2014)**

Legume type	Energy (kcal)	Carbohydrate (g)	Protein (g)	Fat (g)	Fiber <sup>†</sup> (g)
Pinto beans	245	44.84	15.41	1.11	15.40
Great Northern beans	209	37.33	14.74	0.80	12.40
Navy beans	255	47.41	14.98	1.13	19.10
Black beans	227	40.78	15.24	0.93	15.00
Black-eyed peas (cowpeas)	198	35.50	13.22	0.91	11.10
Kidney beans	225	40.36	15.35	0.88	11.30
Chickpeas (garbanzo beans)	269	44.97	14.53	4.25	12.50
Split peas	231	41.36	16.35	0.76	16.30
Lentils	230	39.86	17.86	0.75	15.60
Lupin	198	16.40	25.85	4.85	4.60
Soy bean	298	17.08	28.62	15.43	10.30

<sup>†</sup>Does not include all of the resistant starch fraction.

presence of non-protein components (dietary fiber, tannins, phytic acid) and antiphysiological proteins (protease inhibitors, lectins) (Neves, Silva, & Lourenço, 2004). Literature review showed that studies on *M. oleifera* are focused on the isolation of bioactive compounds especially with antioxidant and hypotensive activities. However, there is little information on the protein quality of *M. oleifera*. Hence, the present study was carried out to compare protein quality of *M. oleifera* leaves and seeds as well as *in vitro* protein digestibility (IVPD).

## 2. Materials and methods

### 2.1. Materials

*M. oleifera* seeds and leaves were purchased from Mokolo market (Yaoundé, Cameroon). Dried seeds and leaves were hand-picked and stored in polyethylene bags in the refrigerator (~4°C) until used.

### 2.2. Methods

#### 2.2.1. Preparation of *M. oleifera* seed and leaf flour

*M. oleifera* seeds were dehulled manually, then seeds and leaves were ground into flour and passed through a 150 µm mesh sieve. The flours were extracted twice with the hexane/ethanol (1:1, v/v) solvent system in a 1/3 (w/v) ratio as described by Lu et al. (2009).

#### 2.2.2. Proximate composition

Moisture, protein, ash, total lipids, crude protein ( $N \times 6.25$ ) contents were determined according to AOAC (1990). Carbohydrate content was determined by difference.

#### 2.2.3. Amino acids

Amino acids were determined using a BECKMAN 6300 amino acid analyzer according to the method of Spackman, Stein, and Moore (1958). Hydrolysis of samples was performed in the presence of 6 M HCl, trifluoroacetic acid (TFA, 2:1, v/v) and 5% thioglycolic acid, for 24 h at 100°C. Prior to amino acid analysis, proteins were extracted from seed flour as described by Mune Mune et al. (2010), and from leaf flour as described by Ghaly and Alkoaik (2010).

#### 2.2.4. *In vitro* protein digestibility

IVPD was determined using pepsin-pancreatin enzymatic system as described by Genovese and Lajolo (1998). The nitrogen content of the TCA-soluble matter was determined by the Kjeldahl method (AOAC, 1990). Protein digestibility was expressed as the percentage of the soluble TCA 10% nitrogen, with respect to the total nitrogen content of the undigested sample.

#### 2.2.5. Available lysine

Available lysine (g/16 g N) was determined by dye binding procedure using 1-phenylazo-2-naphthol-6-sulfonic acid (Orange 12), as described by Hurrell, Lerman, and Carpenter (1979). A sample aliquot containing 15 mg of "Arg + His + Lys" was mixed with 4 mL of half saturated sodium Acetate and 40 mL of Orange 12 reagent were added directly for "Arg + His + Lys" determination; or after propionylation of lysine with propionic anhydride for "Arg + His" determination. Difference in absorbance between the two at 475 nm after 2 h reaction in the dark at ambient temperature was used for calculating reactive lysine. Absorbance measurements were performed using a Spectronic Model 601 spectrophotometer (Milton Roy Company, Rochester, NY, 14625, USA).

#### 2.2.6. Determination of nutritional parameters

Nutritional parameters were determined on the basis of the amino acid profiles:

Amino acid score (chemical score) was calculated as: % sample essential amino acids contents/% recommended essential amino acids. The chemical scoring of amino acids was calculated using the FAO/WHO (1991) reference pattern.

Protein Efficiency Ratio (PER) was estimated according to the regression equations developed by Alsmeyer, Cunningham, and Happich (1974), as given below:

$$PER_1 = -0.684 + 0.456 (\text{LEU}) - 0.047 (\text{PRO}) \quad (1)$$

$$PER_2 = -0.468 + 0.454 (\text{LEU}) - 0.105 (\text{TYR}) \quad (2)$$

Protein digestibility corrected amino acid score (PDCAAS) (FAO/WHO 1991) was calculated as: PDCAAS = Lowest uncorrected amino acid score × IVPD

### 2.2.7. Statistical analysis

Results are expressed as mean value ± standard deviation of three different determinations, except for amino acid contents. The data were analysed by the Student–Newman–Keuls test. The computer software used in this study was SPSS (version 20.0, 2011, SPSS Inc., USA).

## 3. Results and discussion

### 3.1. Proximate composition

The proximate composition of *M. oleifera* leaf and seed flour is presented in Table 2. Protein was the major macromolecule in seed and leaf flour (33.53 and 18.63%, respectively) after carbohydrates (49.15 and 52.39%, respectively). Higher protein content values were reported by Bridgemohan, Bridgemohan and Mohamed (2014) and Estelamar, Maria, Valdir, Maraíza and Lucas (2014) for *M. oleifera* seeds and leaves, respectively. In addition, leaf flour reported higher ash content (11%) than seed flour (3.16%). Protein content was significantly ( $p < 0.05$ ) higher in *M. oleifera* seeds compared to leaves. Seed flour was found to have higher protein content than those of several legumes such as cowpea (22%), Bambara bean (24.78%), Chickpea (23.7%), Horse gram (22.5%) (Mune Mune & Sogi, 2015; Sreerama, Sashikala, Pratapa, & Singh, 2012).

### 3.2. Amino acid composition

The amino acid composition of *M. oleifera* seed and leaf flour is presented in Table 3. Regarding essential amino acids content, both seed and leaf flour were found to be rich in leucine (7.17 and 9.70%, respectively) and valine (7.08 and 6.65%, respectively), and total aromatic amino acids (6.68 and 6.78%, respectively). Leaf flour showed higher isoleucine, leucine, lysine and threonine contents than seed flour. It was also observed that seed flour was poor in lysine (1.64%), and seed and leaf flour reported low total sulfur amino acids content (2.11 and 1.81%, respectively). The major non-essential amino acids were observed to be glutamic acid (21.64 and 11.35%) and glycine (12.95 and 10.57%), respectively for seed and leaf flour. The quality of proteins as source of amino acids can usually be adequately assessed by comparison with the FAO/WHO (1991) recommended pattern of essential amino acids. Compared to the seed flour, *M. oleifera* leaf flour reported higher total essential amino acids content, and both seed and leaf flour had higher total essential amino acids than the FAO/WHO (1991) reference pattern. Moreover, histidine, isoleucine, leucine, threonine and valine contents met the FAO/WHO (1991) requirements for infants, while lysine and total sulfur amino

**Table 2. Proximate composition (g/100 g) of *M. oleifera* seed and leaf flour**

Nutrients	Seed flour	Leaf flour
Moisture	7.06 ± 0.12 <sup>b1</sup>	14.79 ± 0.25 <sup>c2</sup>
Protein	33.53 ± 0.38 <sup>e2</sup>	18.63 ± 0.33 <sup>d1</sup>
Ash	3.16 ± 0.00 <sup>a1</sup>	10.99 ± 0.43 <sup>b2</sup>
Lipids	7.10 ± 0.09 <sup>b2</sup>	2.77 ± 0.14 <sup>a1</sup>
Total carbohydrates	49.15 ± 0.19 <sup>d1</sup>	52.39 ± 0.18 <sup>e2</sup>

Notes: Means in the same column with different letters (a–e) are significantly ( $p < 0.05$ ) different. Means in the same line with different numbers (1–2) are significantly ( $p < 0.05$ ) different.

**Table 3. Amino acid composition of *M. oleifera* seed and leaf flour (g/16 g N)**

	Seeds	Leaves	FAO/WHO (1991)
Histidine	2.66	1.98	1.9
Isoleucine	3.54	5.03	2.8
Leucine	7.17	9.70	6.6
Lysine	1.64	5.53	5.8
Threonine	4.09	5.28	3.4
Tryptophan	ND	ND	1.1
Valine	7.08	6.65	3.5
Methionine	1.44	1.40	
Cysteine	0.67	0.41	
Total sulfur amino acids	<b>2.11</b>	<b>1.81</b>	<b>2.3</b>
Tyrosine	1.71	2.20	
Phenylalanine	4.97	4.58	
Total aromatic amino acids	6.68	6.78	6.3
Total essential amino acids	<b>35.07</b>	<b>42.76</b>	<b>33.9</b>
Aspartic acid + asparagine	7.99	9.76	
Serine	3.30	5.75	
Glutamic acid + glutamine	21.64	11.35	
Proline	6.13	5.70	
Glycine	12.95	10.57	
Alanine	7.07	9.79	
Arginine	5.85	4.31	
Total non-essential amino acids	<b>64.93</b>	<b>57.23</b>	
Leucine/isoleucine ratio	<b>2.03</b>	<b>1.93</b>	<b>2.36</b>
First limiting amino acid	<b>Lysine</b>	<b>Cys + Met</b>	
Second limiting amino acid	<b>Cys + Met</b>	<b>Lysine</b>	

Note: ND: non determined.

acids were in non-adequate levels. These results showed that *M. oleifera* seed and leaf flour could be used to complement cereal proteins, which contain high amount of total sulfur amino acids and legume proteins which have low total aromatic amino acids content (Mune Mune, Minka, Mbome, & Etoa, 2011). The leucine/isoleucine ratio in *M. oleifera* seed flour (2.03) was in ideal range suggested by FAO/WHO (1991). Deosthale, Mohan, and Rao (1970) showed that excess leucine in foods interfered with the utilization of isoleucine and lysine.

### 3.3. In vitro protein digestibility

IVPD of *M. oleifera* seed and leaf flour is presented in Table 4. It was observed that *M. oleifera* leaf flour is more susceptible to pepsin digestion than the seed flour, and pancreatin digestion greatly affected seed flour compared to leaf flour. IVPD of *M. oleifera* seed flour (24.34%) was significantly ( $p < 0.05$ ) lower than that of the leaf flour (41.11%) after the action of pepsin. However, due to the

**Table 4. IVPD of *M. oleifera* seed and leaf flour**

Material	Pepsin digestibility (%)	Pancreatin digestibility (%)
Seed flour	24.34 ± 1.69 <sup>a1</sup>	61.12 ± 5.56 <sup>b2</sup>
Leaf flour	41.11 ± 3.33 <sup>b1</sup>	57.22 ± 3.81 <sup>a1</sup>

Notes: Means in the same column with different letters (a-b) are significantly ( $p < 0.05$ ) different. Means in the same line with different numbers (1-2) are significantly ( $p < 0.05$ ) different.

**Table 5. Nutritional parameters and available lysine of *M. oleifera* leaf and seed flour\***

	CS (%)	Limiting amino acids		PER		PDCAAS (%)	Available lysine (g/16 g N)
		First	Second	PER1	PER2		
		Lys	Cys + Met				
Seed flour	28.27	(28.27)	(28.27)	2.30	2.61	17.28	1.30 ± 0.02 <sup>a</sup>
		Cys + Met	Lys				
Leaf flour	72.40	(72.40)	(95.34)	3.47	3.71	41.42	3.78 ± 0.46 <sup>b</sup>

\*CS, Chemical score; PDCAAS, protein digestibility corrected amino acid score; PER, protein efficiency ratio calculated according to Alsmeyer et al. (1974) equations; Means in the same column with different letters (a–b) are significantly ( $p < 0.05$ ) different.

action of pancreatin following that of pepsin, digestibility of seed flour was significantly ( $p < 0.05$ ) higher (61.12%) than that of leaf flour (57.22%). Estelamar et al. (2014) reported lower value for *in vitro* digestibility of *M. oleifera* leaf flour. IVPD of *M. oleifera* seed and leaf flour were in the same range than that of cowpea flour (60%), and lower than that of Bambara bean flour (74.53%) (Mune Mune et al., 2011; Mune Mune, Minka, & Mbome, 2013).

*M. oleifera* seed and leaf proteins probably had different structure in addition to amino acid composition. Moreover, pepsin and pancreatin are endopeptidases with different specificity. Pepsin hydrolyzed preferentially peptide bond where the amino group of aromatic amino acid is committed. Also, pancreatin hydrolyzed peptide bond where the carboxylic group of aromatic amino acids is committed (chymotrypsin), peptide bond where the carboxylic group of basic amino acids is committed (trypsin), and peptide bond where the amino group of aromatic amino acid is committed (chymosin) (Khantaphant & Benjakul, 2010).

### 3.4. Nutritional parameters and available lysine

The nature and quantity of amino acids contained in a dietary protein, determined the efficiency by which an organism could use the protein. Nutritional parameters and available lysine of *M. oleifera* seed and leaf flour is presented in Table 5. The chemical score and protein digestibility corrected amino acid score (PDCAAS) of seed flour (28.27 and 17.28%, respectively) were lower than those of leaf flour (72.40 and 41.42%, respectively). Based on the chemical score, the first and second limiting amino acids of the seed flour were lysine and total sulfur amino acids, respectively, while those of leaf flour were total sulfur amino acids and lysine, respectively. PER of *M. oleifera* leaf flour (3.47–3.71) was in the same range than that reported for tobacco leaves (3.68) *in vivo* by Kung et al. (1980).

Available lysine was significantly ( $p < 0.05$ ) higher in leaf flour (3.78 g/16 g N) compared to seed flour (1.30 g/16 g N). A portion of total lysine in *M. oleifera* seed and leaf flour is probably engaged in chemical reactions. These values were lower than those obtained by the amino acid analysis (5.53 g/16 g N for leaf flour and 1.64 g/16 g N for seed flour). Nevertheless, Waller and Feather (1983) showed that a fraction of non-available lysine could be recovered *in vivo* after acid hydrolysis.

## 4. Conclusion

Results obtained in this study showed that *M. oleifera* leaves and seeds might be used as sources of low-cost protein in nutritional applications, for the benefit of low-income population in developing countries. Seed flour had higher protein content than those of several legumes. *M. oleifera* leaf and seed flour had higher total essential amino acids content than the FAO/WHO (1991) reference pattern, with lysine and total sulfur amino acids being limiting. *M. oleifera* seed flour showed higher protein digestibility than leaf flour. Moreover, leaf flour showed higher chemical score, PER and protein digestibility corrected amino acid score, and available lysine than seed flour.

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### Competing Interests

The authors declare no competing interest.

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