Nutritional compositions of Indian Moringa oleifera seed and antioxidant activity of its polypeptides

Lili Liang | Cong Wang | Shaoguang Li | Xuemei Chu | Kunlai Sun

Abstract
To study the nutritional composition of Indian Moringa oleifera seed and the antioxidant activity of M. oleifera seed polypeptide, Indian M. oleifera seed was used as raw material for composition analysis and content determination. After extraction of the seed protein, enzymatic hydrolysis with flavourzyme, dispase, papain, pepsin, and alcalase was conducted for different time, and the optimal enzymatic hydrolysis conditions was determined with DPPH scavenging capacity as an indicator. The seed polypeptides obtained by enzymatic hydrolysis were ultrafiltered, and the active peptide fragments were tracked with DPPH, HO (•OH), ABTS and superoxide anion (O2•−) free radical scavenging ability and lipid oxidation inhibition rate as indicators. The results showed that the protein content in Indian M. oleifera seed was high to 40.34%, containing seven essential amino acids. The content of macroelements such as potassium, sodium, and magnesium is high, with the potassium content as high as 2,357.71 mg/kg, among the microelements, the iron content as high as 36.2 mg/kg. The optimum enzymatic hydrolysis conditions were as follows: enzymatic hydrolysis with flavourzyme (50°C, pH 6.7) for 300 min, and DPPH scavenging capacity was 84.76%. Activity tracing found that the polypeptide fragment with molecular weight <3.5 kDa had the strongest antioxidant capacity, and the EC50 values of DPPH, •OH, ABTS, and O2•− free radical scavenging rates were 4.0, 4.2, 5.3, and 4.3 mg/ml, respectively. The above results show that Indian M. oleifera seed not only has high nutritional value, but its protease enzymatic hydrolyzate also has significant antioxidant activity, which can be further developed into nutrition products, healthcare products, functional foods, beauty and skin care products, liver protection drugs, etc.

Keywords
antioxidant activity, enzymatic hydrolysis, free radical scavenging activity, Indian Moringa oleifera seed, nutritional composition analysis

1 INTRODUCTION

Moringa oleifera lam is a perennial woody plant of Moringa family, originating in arid or semiarid regions of tropical and southern subtropics (Yiftach & Adina, 2017). At present, more than 30 countries in the world have introduced and cultivated Moringa. Guangdong, Guangxi, Hainan, Sichuan, and Yunnan provinces of China have introduced M. oleifera seeds or cultivation techniques from countries such...
as India and Myanmar. M. oleifera seed is rich in oils, proteins, and minerals. It can be used in food, medicine, cosmetics, and water purification, and has good research prospects. It has significant effects in reducing blood lipids, blood pressure, slimming, regulating the stomach, protecting the liver from alcohol, and enhancing the body's immunity (Du, Sun, Yan, Luo, & Dai, 2017; González et al., 2017; Pereira, Oliverira, & Oliverira, 2011). In addition, studies have confirmed that M. oleifera seed polypeptide is soluble in aqueous solution and can be completely digested (Fan, Shao, Ye, & Yang, 2016). Lin found that M. oleifera seed polypeptide has protective effect on the erythrocytes which suffered oxidative damage (Lin, Zhu, & Zhao, 2018), and Zhao found the extract of M. oleifera leaves can strongly scavenge DPPH and superoxide anion free radical and absorb oxidative free radical (Zhao, Li, Lin, & Yang, 2017). M. oleifera root protein has anti-inflammatory and analgesic functions. At present, there are few studies on M. oleifera seed protein. The only articles are the research provided by Aderinola and his coworkers. Their study showed that M. oleifera seed protein has antioxidant properties (Aderinola, Fagbemi, Enujiugha, Alashi, & Aluko, 2018), especially after hydrolysis by trypsin or alcalase, the hydrolyzate shows in vitro anti-inflammatory and antioxidative properties (Aderinola, Fagbemi, Enujiugha, Alashi, & Aluko, 2019a, 2019b). In this article, we analyze the Indian M. oleifera seed nutrient composition and the antioxidant activity of its peptides hydrolyzed by flavourzyme, dispase, papain, pepsin, and alcalase, expected to provide data support for the in-depth research and development of M. oleifera seed polypeptide.

2 | MATERIALS AND METHODS

2.1 | Materials and reagents

*Moringa oleifera* seeds were collected in the southern Himalayas of Assam, North India (94.1°E, 26.7°N).

DPPH: Shanghai Macklin Biochemical Co., Ltd; FeSO₄ (AR); Wuxi Jingke Chemical Co., Ltd.; ABTS: Beijing Biotopped Science & Technology Co., Ltd.; NBT, NADH: Thermo Fisher Scientific; PMS: Wuxi Jingke Chemical Co., Ltd.; pepsin, flavourzyme, alcalase: Beijing Solarbio Science & Technology Co., Ltd.; NBT, NADH: Thermo Fisher Scientific; PMS: Wuxi Jingke Chemical Co., Ltd.; dispase, papain: Novozymes Biotec Technology Co., Ltd.; anhydrous ethanol, phenanthroline, sodium dihydrogen phosphate, disodium hydrogen phosphate, potassium persulfate, trichloroethanol, thiobarbituric acid, ascorbic acid, etc. were all analytical reagents provided by Sinopharm Chemical Reagent Co., Ltd.

2.2 | Instrument

BSA224 Analytical Balance: Beijing Aoduolisi Scientific Instrument Co., Ltd.; LGJ-10C ordinary vacuum freeze dryer: Four-ring Science Instrument Plant Beijing Co., Ltd.; HH-8 homoeothermic water bath: Jintan Sita Xinbao Instrument Factory; UV-1100 UV spectrophotometer: Shanghai Mapada Instruments Co., Ltd.; WTM-1812G membrane separation equipment: Hangzhou Woteng Membrane Engineering Co., Ltd.; MK3 microplate reader: Thermo Fisher Scientific Co., Ltd.; N-1100 Rotary evaporator: Shanghai Alang Instrument Co., Ltd.; STARTER3100 Precision pH Meter: Shanghai Ohaus Instrument Co., Ltd.; SH8210HP ultrasonic cleaning agent: Shanghai Kudos Ultrasonic instrument Co., Ltd.; JJ-02 high speed pulverizer: Jiangsu Changzhou Huaguo Electric Appliance Co., Ltd.; TGL-16M high speed centrifuge: Shanghai Lu Xiangyi Centrifuge Instrument Co., Ltd.; L-8900 amino acid analysis: Techcomp (China) Scientific Instrument Co., Ltd.; ATN-300 Automatic Kjeldahl Nitrogen Analyzer: Shanghai Hongji Instrument Co., Ltd.; KSW-4D-11-S resistance furnace temperature controller: Shanghai Xian Jian Instrument Co., Ltd.

2.3 | Experimental methods

2.3.1 | Determination of nutrients

### Determination of general nutrients

Determination of crude protein content refers to GB/T5009.5-2016 “Determination of protein in food,” using Kjeldahl method; determination of ash content refers to GB 5009.4-2016 “Determination of ash in food,” using muffle furnace burning method; determination of fat refers to GB 5009.6-2016 “Determination of fat in food,” using Soxhlet extraction method; determination of mineral elements refers to GB5009.268-2016 “Determination of multi-elements in food”; determination of moisture refers to GB5009.3-2016 “Determination of moisture in food,” using direct drying method; determination of crude fat refers to GB 5009.6-2016 “Determination of fat in food,” using Soxhlet extraction method; determination of mineral elements refers to GB5009.268-2016 “Determination of multi-elements in food”; determination of moisture refers to GB5009.3-2016 “Determination of moisture in food,” using direct drying method; determination of nucleic acid refers to GB5009.87-2016 “Determination of phosphorus in food,” using Fiske-Subbarow method; and determination of carbohydrate acid refers to NY/T 2332-2013.

### Determination of amino acid composition and content

After the *M. oleifera* seeds were dried and pulverized, 0.5 g was weighed and was digested with 6 mol/L hydrochloric acid at a constant temperature of 110°C for 22 hr, and then the amino acid content was determined by an L-8900 amino acid analyzer (Hwee & Chee, 2013; Zhang, 2016).

2.3.2 | Method for extracting crude protein from *M. oleifera* seed

Smash, pass 80-mesh sieve → ethyl acetate degreasing (ratio of liquid to material 1:6 mg/ml, 48 hr)→ air-drying (37°C, 48 hr)→ addition of Tris-HCL protein extraction with 1.5 mol/L concentration and 8.8 pH (ratio of liquid to material 1:38 mg/ml, 42°C, 100 min)→ obtain supernatant after centrifugation (2,810 g, 15 min)→ ammonium sulfate precipitation (4.25 mol/L, 25°C)→ dialysis desalting (10 kDa, 25°C, 48 hr)→ freeze-drying (−60°C, 36 hr).

2.3.3 | Determination of the antioxidant activity of *M. oleifera* seed protein, Vc as positive control

Determination of the scavenging ability of *M. oleifera* seed protein to DPPH free radicals

Two milliliters of samples consisting of distilled water and different concentrations of the analytes were placed in cuvettes, and 500 μl
of an ethanolic solution of DPPH (0.02%) and 1.0 ml of ethanol were added. A control sample containing the DPPH solution without the sample was also prepared. In the blank, the DPPH solution was substituted with ethanol. The antioxidant activity of the sample was evaluated using the inhibition percentage of the DPPH radical with the following equation:

$$\text{DPPH free radical scavenging rate} = \frac{A_s - A_c}{A_c} \times 100\%.$$  

where $A_s$ is the absorbance value of the sample group, $A_c$ is the absorbance value of the control group.

Superoxide anion scavenging rate was calculated using the following equation:

$$\text{Superoxide anion scavenging rate} = \frac{A_s - A_c}{A_c} \times 100\%.$$  

where $A_s$ is the absorbance value of the sample group, $A_c$ is the absorbance value of the control group.

### Determination of antilipid peroxidation capacity of *M. oleifera* seed protein

Briefly, a sample (5.0 mg) was dissolved in 10 ml of 50 mM PBS (pH 7.0) and added to 0.13 ml of a solution of linoleic acid and 10 ml of 99.5% ethanol. Then, the total volume was adjusted to 25 ml with deionized water. The mixture was incubated in a conical flask with a screw cap at 40°C in a dark room, and the degree of oxidation was evaluated by measuring ferric thiocyanate values. The reaction solution (100 µl) incubated in the linoleic acid model system was mixed with 4.7 ml of 75% ethanol, 0.1 ml of 30% ammonium thiocyanate, and 0.1 ml of 20 mM ferrous chloride solution in 3.5% HCl. After 3 min, the thiocyanate value was measured at 500 nm following color development with FeCl$_2$ and thiocyanate at different intervals during the incubation period at 40°C (Sudhakar & Nazeer, 2015).

### 2.3.4 Polypeptide separation and purification

#### Enzymatic hydrolysis

The protein solution with concentration of 0.01 g/ml and the enzyme with concentration of 0.005 g/ml were extracted from *M. oleifera* seed, and the enzymatic hydrolysis time was set to 8 time gradients of 30, 60, 120, 180, 300, 360, and 420 min. Enzymatic hydrolysis with flavourzyme (50°C, pH 6.7; Ji, 2017), dispase (50°C, pH 7; Zhong, Chen, & Wen, 2009), papain (50°C, pH 7; Jiang, Wu, Wang, & Zhang, 2014), pepsin (32°C, pH 2.0; Feng, Ruan, Jin, Xu, & Wang, 2018), and alcalase (55°C, pH 10; Yang, Fan, Shao, & Wang, 2015) was conducted, respectively, and DPPH scavenging capacity was used as an indicator to select the optimal enzymatic hydrolysis conditions. The DPPH scavenging capacity is determined as above.

#### Ultrafiltration

Peptide fragments with different molecular sizes of >5 kDa, 3.5–5 kDa, and <3.5 kDa were obtained after ultrafiltration using a 5 kDa, 3.5 kDa ultrafiltration membrane. The three sections of the solution were separately collected for lyophilization, and the dried peptide was stored at ~20°C.

#### Screening for active components

The ability of the peptide fragments with three different molecular sizes to scavenge DPPH, •OH, ABTS, O$_2$• free radicals and the EC$_{50}$
values of lipid antioxidants were studied to screen for more active components. The DPPH•, •OH, ABTS•, O$_2$•$^-$ scavenging power and lipid antioxidants were determined as above.

3 | EXPERIMENTAL RESULTS

3.1 | Proximate composition of *M. oleifera* seed

As can be seen from Table 1, the content of crude protein and crude lipid in Indian *M. oleifera* seed is relatively rich, which are 40.34% and 39.12%, respectively. These are very close to those reported in the literature; however, protein is much more than that in *M. oleifera* leaves (28.73%) (Juhami, Ghafoor, Ahmed, Babiker, & Ozcan, 2017). Compared the content of crude protein and lipid with the well-recognized peanuts (32.65% and 48.06%) and soybean (41.05% and 21.06%) (Liu & Pan, 2016; Zheng, Jin, Geng, & Yu, 2015), the crude protein content of Indian *M. oleifera* seed is equivalent to soybean, higher than peanut; its crude lipid content is much higher than soybeans, slightly lower than peanuts. It is reported that *M. oleifera* seed protein has the effect of water purification and anticoagulant (Aline et al., 2017; Luciana et al., 2013; Marichamy & Ramasamy, 2011). It shows that the Indian *M. oleifera* seed is not only rich in crude protein and lipid, but also has various effects. We can deeply develop its plant protein and vegetable oil, such as functional foods, feed, water purification, cosmetic raw materials, plant growth promoters, and fungicides.

3.2 | Content of mineral elements in *M. oleifera* seed

As can be seen from Table 2, *M. oleifera* seed contains potassium, sodium, magnesium, calcium, and other elements, of which the highest potassium content is 2,357.71 mg/kg and the lowest calcium content is 121.14 mg/kg. The contents of the four macroelements are K > Na > Mg > Ca in order, which is different in order and much less than those contains in leaves (K, Mg, Ca: 3,562.19, 3,813.44, 17,638.41 mg/kg, respectively). At the same time, microelements such as iron, copper, and zinc were detected. The highest iron content is 36.2 mg/kg. The contents of three microelements are Fe > Zn > Cu in order, which is also different in order and much less than those contains in leaves (Fe, Zn, Cu: 1,274.12, 29.51, 8.41 mg/kg, respectively) (Juhami, Ghafoor, Babiker, Matthaus, & Ozcan, 2017). *M. oleifera* seeds also contain four heavy metal elements of arsenic, cadmium, lead, and tin, which are 0.01, 0.018, 0.015, and 0.007 mg/kg, respectively. These are low in content and are in line with GB2762-2017 “The national food safety standard, food contaminants limit” (arsenic is limited to 0.5 mg/kg in grains and their products; cadmium and lead are limited to 0.5 and 0.2 mg/kg, respectively; and in nuts and seeds, tin is limited to 250 mg/kg in food).

3.3 | Amino acid composition and content in *M. oleifera* seeds

As can be seen from Table 3, *M. oleifera* seed contains seven essential amino acids, namely threonine, valine, methionine, isoleucine, leucine, phenylalanine, and lysine, with the total content of 0.824 g/100g, accounting for 25.65% of the total amino acid content. This result is close to the 28.8% of essential amino acids in Liaoning peanuts reported by Shi (Shi, Yu, & Han, 2017). The *M. oleifera* seeds contain seven kinds of hydrophobic amino acids: alanine, valine, methionine, isoleucine, leucine, tyrosine, and phenylalanine, and the total content is 0.834 g/100g. The antioxidant activity of the protein is closely related to its amino acid composition. Peptides containing hydrophobic amino acids can increase their solubility at the water–lipid interface and better interact with free radicals (Zheng, Si, Baseer, Li, & Zhang, 2018).

3.4 | Protein antioxidant activity

As shown in Figure 1, when the *M. oleifera* seed protein was 10 mg/ml, the DPPH•, ABTS•, •OH, and O$_2$•$^-$ scavenging rates were 63.25%, 52.45%, 55.25%, and 59.32%, respectively. And the lipid oxidation inhibition rate was 43.25%. It shows that *M. oleifera* seed protein extraction has good antioxidant activity against different antioxidant models.

3.5 | Optimal enzymatic hydrolysis conditions

Flavourzyme (50°C, pH 6.7), dispase (55°C, pH 7.0), papain (50°C, pH 7.0), pepsin (32°C, pH 2.0), and alcalase (55°C, pH 10.0) were used to conduct enzymatic hydrolysis of *M. oleifera* seed protein for 30, 60, 120, 180, 240, 300, 360, and 420 min, and then the DPPH scavenging rate was determined. The results are shown in Figure 2.

As shown in Figure 2, the DPPH scavenging rate of the five enzymatic hydrolyzates increased gradually with the increase in

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**TABLE 1** General nutrient content of Indian *Moringa oleifera* seed (%), $p \leq 0.05$

| Mineral elements | Crude protein (mg/kg DW) | Ash (mg/kg DW) | Water (%) | Crude lipid (mg/kg DW) | Carbohydrate (mg/kg DW) | Nucleic acid (mg/kg DW) |
|------------------|--------------------------|----------------|-----------|------------------------|-------------------------|------------------------|
| Crude protein    | 40.34 ± 0.45             | 3.5 ± 0.31     | 6.78 ± 0.61| 39.12 ± 0.42           | 8.94 ± 0.28             | 4.26 ± 0.37             |

**TABLE 2** Content of mineral elements in *Moringa oleifera* seed (mg/kg DW, $p \leq 0.05$)

| Mineral elements | Contents (mg/kg DW) | Mineral elements | Contents (mg/kg DW) |
|------------------|---------------------|------------------|---------------------|
| K                | 2,357.71 ± 1.87     | Cu               | 3.29 ± 0.21         |
| Na               | 1,074.09 ± 1.56     | As               | 0.01 ± 0.001        |
| Mg               | 972.06 ± 1.23       | Cd               | 0.018 ± 0.002       |
| Ca               | 121.14 ± 0.87       | Pb               | 0.015 ± 0.001       |
| Fe               | 36.20 ± 0.79        | Sn               | 0.007 ± 0.001       |
| Zn               | 8.37 ± 0.12         |                  |                     |
enzymatic time. After 300 min, the scavenging rate tended to be stable, and the enzymatic hydrolysis was completed at this time. When the flavourzyme was used for the enzymatic hydrolysis of the *M. oleifera* seed protein for 300 min at 50°C and pH 6.7, the DPPH scavenging rate was up to 85%. Therefore, this condition was selected as the optimum enzymatic hydrolysis process for *M. oleifera* seed protein for subsequent studies.

### 3.6 Antioxidant activity of different peptide fragments

After ultrafiltration, the enzymatic peptide was divided into three parts, the molecular weight of the peptide was <3.5 kDa, 3.5–5 kDa, >5 kDa, and the EC_{50} values of DPPH, •OH, ABTS, O_2•^− free radical scavenging rate and the lipid antioxidant inhibition rate are shown in Figure 3.

### 4 CONCLUSIONS

Indian *M. oleifera* seeds are rich in crude protein and crude lipids, with a protein content up to 40.34% and a crude lipids content 39.12%. Its content of macroelements such as sodium, potassium,
and magnesium is much higher than other metals, and the K content is 2.537.71 mg/kg. Indian M. oleifera seeds contain seven essential amino acids and seven hydrophobic amino acids, and the hydrophobic amino acids contribute to the antioxidant activity of M. oleifera seeds. The optimum enzymatic conditions were flavouredzyme hydrolysis of M. oleifera seed protein for 300 min at 50°C and pH 6.7. <3.5 kDa polypeptide fragment has higher EC_{50} values for DPPH•, •OH, ABTS•, and O_2• scavenging rates than fragments 3.5–5 kDa and >5 kDa, and only the lipid oxidation inhibition rate of <3.5 kDa polypeptide fragment is lower than >5 kDa. Therefore, the <3.5 kDa polypeptide fragment is the strongest antioxidant. It has the potential to be further developed into nutritional products, health products, functional foods, beauty and skin care products, and liver protection drugs.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

ETHICS STATEMENT

This study has nothing to do with human and animal testing.

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