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Review

Chemical characteristics and fractionation of proteins from *Moringa oleifera* Lam. leaves

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ABSTRACT

Moringa oleifera Lam. is a leguminous plant, originally from Asia, which is cultivated in Brazil because of its low production cost. Although some people have used this plant as food, there is little information about its chemical and nutritional characteristics. The objective of this study was to characterise the leaves of *M. oleifera* in terms of their chemical composition, protein fractions obtained by solubility in different systems and also to assess their nutritional quality and presence of bioactive substances. The whole leaf flour contained 28.7% crude protein, 7.1% fat, 10.9% ashes, 44.4% carbohydrate and 3.0 mg 100 g^{-1} calcium and 103.1 mg 100 g^{-1} iron. The protein profile revealed levels of 3.1% albumin, 0.3% globulins, 2.2% prolamin, 3.5% glutelin and 70.1% insoluble proteins. The hydrolysis of the protein from leaf flour employing sodium dodecyl sulfate (SDS) and 2-mercaptoethanol (ME) resulted in 39.5% and 29.5%, respectively. The total protein showed low *in vitro* digestibility (31.8%). The antinutritional substances tested were tannins (20.7 mg g^{-1}), trypsin inhibitor (1.45 TIU mg g^{-1}), nitrate (17 mg g^{-1}) and oxalic acid (10.5 mg g^{-1}), besides the absence of cyanogenic compounds. β-Carotene and lutein stood out as major carotenoids, with concentrations of 161.0 and 47.0 μg g^{-1} leaf, respectively. Although *M. oleifera* leaves contain considerable amount of crude protein, this is mostly insoluble and has low *in vitro* digestibility, even after heat treatment and chemical attack. *In vivo* studies are needed to better assess the use of this leaf as a protein source in human feed.

Contents

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1. Introduction

Nutritional deficiencies are one of the most serious problems worldwide and it represents a challenge for public health in Brazil. The overall strategy here is to try to change eating patterns, focusing on the recovery of regional dietary practices related to the consumption of local food with high nutritional value. *Moringa oleifera* Lamarck (fam. Moringaceae), is a perennial foliaged tree, widely cultivated due to its high adaptability to climatic conditions and dry soils (Okuda, Baes, Nishijima, & Okada, 2001). It is considered one of the most useful trees in the world because almost all parts of this plant can be used as food, in medicines and for industrial purposes (Khalafalla & Abdellatef, 2010). This especies comes from NW India, but the interest in its cultivation has been extended to countries where it is not native (Oduro, Ellis, & Owusu, 2008), considering its nutritional, therapeutic and prophylactic properties (Fahey, 2005), besides claims of increased animal productivity. The leaf flour has been used as an alternative food source to combat malnutrition, especially among children and infants (Anwar, Latif, Ashraf, & Gilani, 2007). In Brazil there is an effort in order to spread the use and cultivation of *M. oleifera*, taking into account the high protein, vitamin and mineral contents and the low toxicity of the seeds and leaves (Ferreira, Farias, Oliveira & Carvalho, 2008).

Although its leaves represent an important source of proteins, the nutritional quality depends on the absolute and relative contents of essential amino acids and its bioavailability after digestion and absorption. Neves, Silva, and Lourenço (2004) demonstrated that vegetable proteins are less susceptible to *in vivo* digestion than animal proteins. The low content of sulfur amino acids, compact structure, presence of non-protein components (dietary fiber, tannins, phytic acid) and antiphysiological proteins (protease inhibitors, lectins) can affect digestion.

Due to its use into the herbal medicine, studies of *M. oleifera* have focused on the isolation of bioactive compounds especially with antioxidant and hypotensive activities. However, there is little information on the effects of *M. oleifera* in the human diet. Considering its advantages, the scientific assessment of its potential as an alternative nutritional source is convenient and necessary. This study was carried in order to characterise the leaves of *M. oleifera* regarding chemical composition, protein fractionation by solubility in several systems and also to assess their nutritional quality by determinig *in vitro* protein digestibility and bioactive substances (mainly carotenoids and antinutritional substances).

2. Materials and methods

2.1. Material

The leaves of *M. oleifera* Lam harvested from trees located in Minas Gerais, SE Brazil, were washed and then dried at 35 °C in an air circulating oven for approximately 24 h. The material was ground in a knife mill and sieved (60 mesh), and the flour obtained was lyophilised and stored in jars with airtight lids. The flour was defatted by double lipid extraction with n-hexane in the ratio 1:5 (w: v).

2.2. Methods

2.2.1. Analysis of the chemical composition

The percentages of moisture, crude protein, lipids and ash were determined in the lyophilised flour by standard methods of the Association of Official Analytical Chemists (AOAC., 2011). Mineral content was determined by the method described by Sarruge and Haag (1974). The non-protein nitrogen was determined in the supernatant obtained after the precipitation of proteins with 5% trichloroacetic acid (TCA) as indicated by Campos, Nussio, and Nussio (2004).

2.2.2. Protein fractionation

The sequential extraction of leaf flour proteins was carried by the Osborne and Voogt (1978) procedure with some modifications. The defatted flour was dispersed in water (pH 7.0, ratio 1:30, w:v), subjected to mechanical stirring (60 min) and centrifuged (15,000 rpm for 40 min). The supernatant, after extensive dialysis against distilled water in appropriate membranes (pore size around 10 KDa) for 24–36 h, was centrifuged again, resulting in albumins (supernatant) and part of the globulins (precipitated), and the residue was submitted to re-extraction under the same conditions. The precipitated residue was extracted with 0.5 M NaCl (pH 7.0, ratio 1:30, w:v) and after centrifugation, the supernatant was dialyzed as described above and centrifuged again, resulting in another portion of precipitated globulins, which was added to the first. The residue from NaCl extraction was successively extracted with 70% ethanol and 0.1N NaOH to separate prolamin and glutelin fractions, respectively, from the final residue. The percentages of protein extracted at each stage were calculated with respect to the total protein content in defatted flour and analyzed by nitrogen determination.

2.2.3. In vitro protein digestibility

The extent of the enzymatic hydrolysis of the protein from the defatted Moringa leaves flour was determined by three different treatments: heating at 121 °C for 15 min, using sodium dodecyl sulfate (SDS), and a last one using 1% 2-mercaptoethanol (ME). The pretreated proteins were hydrolyzed sequentially with the enzymes pepsin and pancreatin as described by Akeson and Stahmann (1964). The degree of hydrolysis (% DH) of each sample was calculated as a percentage of soluble nitrogen relative to the total nitrogen in the sample.

2.2.4. Bioactive substances

The extraction, hydrolysis, separation and identification of the carotenoids were performed as described by Rodriguez-Amaya (2001). The activity of trypsin inhibitors was determined by the procedure described by Kakade, Simons, and Liener (1969), using Nα-benzoyl-DL-arginine-p-nitroanilide (BAPNA) as the substrate for trypsin. The determinations of total tannins were analyzed as described by Joslyn (1970). The levels of nitrate (Mantovani, Cruz, Ferreira & Barbosa, 2005), oxalic acid (Loures & Jokl, 1990) and cyanogenic glycosides (AOAC, 2011).

Table 1
Chemical composition from *Moringa oleifera* leaves flour.

Constituents	Amount (g.100 g^{-1})
Moisture	9.0 ± 0.17
Protein	28.65 ± 0.04
Lipids	7.09 ± 0.43
Ash	10.9 ± 0.8
Carbohydrates[a]	44.36
Minerals	(mg.100 g^{-1})
Calcium	2.97
Magnesium	1.9
Zinc	1.58
Potassium	4.16
Iron	103.12
Copper	3.38

[a] Obtained by difference.