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### **ORIGINAL PAPER**

# NUTRITIONAL QUALITY AND SAFETY OF MORINGA (MORINGA OLEIFERA LAM., 1785) LEAVES AS AN ALTERNATIVE SOURCE OF PROTEIN AND MINERALS

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#### Abstract

Dry matter, crude protein, crude fat, crude ash, crude fibre and elements (phosphorus, potassium, sodium, calcium, magnesium, zinc, iron, manganese, lead, chromium, molybdenum, cadmium, cobalt, nickel and copper) were determined in extracted and unextracted Moringa oleifera leaves. The leaves were ground in a laboratory grinder (unextracted leaves) and a portion was subjected to continuous comprehensive water extraction at 95-100°C (extracted leaves). Next, densification was carried out in a rotary evaporator, where the material was dried natively in vacuum. The chemical content of the samples was analysed according to standard methods. The concentration of P was determined by the colorimetric method. An Atomic Absorption Spectrometer was used to determine K, Na and Ca by emission flame spectroscopy, and Mg, Zn, Fe, Mn, Pb, Cr, Mo, Cd, Co, Ni and Cu by absorption flame spectroscopy. The nutritional value of herbs depends on the chemical composition of dry mass, especially the content of crude protein, mineral components and fibre fractions. The crude protein content of the extracted and unextracted leaves was 13.6 and 31.6% dry matter (d. m.), respectively. No crude fibre was found in the moringa leaf extract. Dried leaves contained 8% d. m. of crude fibre. Concentrations of crude fat in unextracted leaves implicated that they had five-fold more lipids than extracted from leaves. Total carbohydrates appeared to be the main component of dry mass. More total carbohydrates were found in the extract than in dried leaves (71 and 50% d.m., respectively). The research showed significant differences in the content of macronutrients between extracted and unextracted leaves of moringa. The extracted leaves contained higher quantities of all the studied macroelements. Moringa oleifera leaves may be considered to be a good and safe source of Fe, Zn and Cu. No Ni or Cd was found in the leaves of moringa.

Keywords: chemical composition, Moringa oleifera Lam., macroelements, microelements.

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Supplying an organism with appropriate amounts and ratios of nutrients is necessary for maintaining its correct functions and health. Minerals are very important in this regard, although research indicates that their dietary content in Poland is often inadequately balanced (STEFAŃSKA et al. 2009). Several studies have shown obesity-related abnormalities in the mineral status. The association between being overweight or obese, on the one hand, and the deficiency of iron, calcium and zinc, on the other hand, has been observed in clinical and epidemiological studies (TZANETAKOU et al. 2012). Several investigations suggest that Ca can play a role in the regulation of abdominal fat mass in obese people (ZHU et al. 2013). Alternative leafy vegetables are now recognized as an ally in the fight against deficiencies of macro- and micronutrients, although they have long been overshadowed by other green leafy vegetables of the European origin, such as cabbage and lettuce, which can have a lower nutritional vaue due to conventional intensive production methods (AKTAR et al. 2009). One such alternative leafy vegetable is Moringa oleifera, Lam. a member of the family Moringaceae, widely available in the tropical and subtropical regions. It is a fast growing plant of great economic importance for the food and medical industries (ANWAR et al. 2007). Moringa oleifera is known as the 'horseradish' tree, 'drumstick' tree, 'ben-oil tree', 'benzoil tree', 'cabbage tree', 'mother's best friend' and 'miracle tree'. M. oleifera is native to India, Pakistan, Bangladesh and Afganistan. The plant is becoming increasingly more appreciated around the world. It is considered one of the world's most useful trees, since almost every part of moringa tree can be used for food, medication or industrial purposes. Edible parts (leaves and fresh pods) of moringa tree are used for human food, animal fodder, medicinal products, but when still on trees they provide shade or serve as a windbreak. The tree is valued mainly for the tender pods, which are considered to be a vegetable. In addition, oil is extracted from seeds, which can also be used to purify water (GHEBREMICHAELA et al. 2005). Leaves are eaten as a nutritious vegetable. Moringa leaves can be eaten fresh, cooked or stored as dried powder for several months. Pods, when young, can be cooked and eaten like beans (ANJORIN et al. 2010). The plant contains considerable amounts of various nutrients, and has been suggested as a good supplement for such nutrients as protein, fibre and elements. Inclusion of morenga in a diet to supplement daily nutrient needs could help to prevent many diseases (SHARMA et al. 2012). Leaf extracts show hypocholesterolaemic, hypotensive, bradycardic and anti-ulcerative activity (ANWAR et al. 2007, MENGISTU et al. 2012, GELETA et al. 2016). Extracts from all parts of the plant have pharmacological properties, are widely used and acknowledged by the scientific community (MAKKAR, BECKER 1996). Moringa is rich in health-promoting phytochemicals such as carotenoids, phenols (chlorogenic acids), flavonoids (quercitin and kaempherol glycosides), various vitamins and minerals. One gram of leaf powder has 25-fold more iron than spinach, 17-fold more calcium than milk, 15-fold more potassium than bananas and 9-fold more protein than yoghurt (KOUL, CHASE 2015). Leaves of *Moringa oleifera* are promoted in areas of chronic malnutrition as a nutritional supplement for weaning infants and nursing mothers (DOERR et al. 2009). Most people, however, are unaware of the potential benefits of moringa.

*Moringa oleifera* is important in defeating undernutrition in some parts of Africa. In Europe however, moringa (*Moringa oleifera* Lam.) products are still little known. Therefore, the present research aimed at determining the basic and mineral content of unextracted and extracted moringa leaves.

### MATERIAL AND METHODS

The material consisted of moringa leaves (Moringa oleifera Lam.) brought from India. The material was cleaned and dried in a laboratory convection chamber oven and subsequently shipped to Poland. Until the examination, the leaves had been stored in a dry, shaded, airy place. Then they were ground in a KNIFETEC 1095 (Foss Tecator) laboratory grinder (unextracted leaves) and a portion was subjected to continuous comprehensive water extraction at 95-100°C (extracted leaves). Next, densification was carried out in a rotary evaporator where the material was dried natively in vacuum (without medium). Both samples of moringa leaves were ground in a KNIFETEC 1095 (Foss Tecator, Sweden) laboratory grinder. Before conducting analyses by the weight-dryer method, the dry matter content was determined and afterwards components in dry mass were analysed. The chemical content of the samples was analysed according to standard AOAC methods (2012). Dry matter was assessed by drying at 105°C to constant weight; crude ash was determined by incineration in a muffle furnace at 580°C for 8 h; crude protein (N  $\times$  6.25) was assayed by the Kjeldahl method in a Büchi Distillation Unit B - 324 (Büchi Labortechnik AG, Switzerland); crude fibre was tested in a fibre analyser ANKOM 220 (ANKOM Technology, USA); ether extract was analyed by Soxhlet extraction with diethyl ether. Total carbohydrates were calculated from the difference: 100 - % (moisture + crude protein + crude fat + crude ash + crude fibre).

The concentration of phosphorus was determined by the Egner-Riehm colorimetric method, with ammonium molybdate, at the wavelength 660 nm, on a Specol 221 apparatus. An Atomic Absorption Spectrometer apparatus (iCE 3000 Series, Thermo Fisher Scientific) was used to determine calcium, potassium and sodium by emission flame spectroscopy, and magnesium, iron, zinc, copper, manganese, molybdenum, chromium, cobalt, nickel, cadmium and lead by absorption flame spectroscopy. The material for determinations of macronutrients was subjected to mineralisation in concentrated sulphuric acid ( $H_2SO_4$ ) and perchloric acid (HClO<sub>4</sub>). The material for analyses of the

concentrations of micronutrients was mineralised in a mixture of nitric acid  $(HNO_3)$  and perchloric acid  $(HClO_4)$ . Prior to assays, the content of calcium, potassium and magnesium trials was diluted appropriately. The other mineral compounds were determined in concentrated samples.

Statistical analysis was aided by Statistica 12 software. In order to determine the significance of differences in the chemical composition of *Moringa oleifera* samples, one-way analysis of variance (ANOVA) was conducted. For identification of homogenous subsets of means, the Duncan test was carried out at  $P \leq 0.05$ . The admissible error for determinations of chemical components was 5%.

# **RESULTS AND DISCUSSION**

The nutritional value of herbs depends on the chemical composition in dry mass, especially on concentrations of crude protein, mineral components and fibre fractions. The analyses have suggested that moringa leaves are an interesting source of basic nutrients (Table 1). Most of herbs wilt soon after Table 1

Item	Dry matter (g kg <sup>-1</sup> )	Crude protein	Crude ash	Ether extract	Crude fibre	NFE
Unextracted	$912.2\pm1.3^{b}$	$315.5\pm3.4^a$	$110.9\pm0.6^{b}$	$89.8\pm2.5^a$	$79.9\pm0.3^a$	$499.9\pm2.2^{b}$
Extracted	$959.9 \pm 3.4^{a}$	$136.1\pm0.6^{b}$	$175.4\pm1.3^a$	$17.5\pm0.2^{b}$	$0.0 \pm 0.0^b$	$712.7\pm1.9^a$
P-value	0.003	0.000	0.000	0.000	0.000	0.000

Basic nutrients (g kg<sup>-1</sup> d.m.) in leaves of Moringa oleifera

 $\pm$ SD – Standard Deviation, NFE – Nitrogen-free extract, <sup>*a.b*</sup> Mean values with the same letter in each columns are not significantly different at  $P \leq 0.05$ .

harvest and they have to be preserved in order to retain their quality. The oldest preservation method is drying, which includes draining plants from water and inactivating enzymes. Drying should be done as soon as possible after the harvest. If done properly, it not only prevents rotting and mould growth, but also allows the chemical content to remain unchanged for a long time (GRZESZCZUK, JADCZAK 2006). Extracted moringa leaves contained almost 5% more dry mass than traditionally dried leaves. The high dry mass content in both moringa products confirms their long shelf-life. Moisture (87.8 and 40.1 g kg<sup>-1</sup> d.m.) in food determines the rate of food absorption and preservation of the food quality. The lower the moisture content in food, the higher its nutrient density (DREWNOWSKI 2005).

Moringa leaves are a reservoir of mineral compounds, referred to as ash, which is very important for both dietary and technological reasons. Unextracted moringa leaves contained less crude ash than extracted leaves (by 64.5 g kg<sup>-1</sup> d.m.). However, 11% content of crude ash indicates that the leaves are a good source of minerals, which was confirmed in research by EL SOHAIMY et al. (2015). The ash content in moringa leaves is high, which means that inorganic elements are substantial in the plant (McCLEMENTS et al. 2009).

Protein is one of the elementary nutrients. Moringa leaves contained twice as much crude protein (CP) as the extracted leaves. Similar values were obtained by ATTA ELMNAN and EL AMIN (2015). Other research has reported a variable protein content, ranging between 16 and 40% (LEONE et al. 2015). The CP content in moringa is higher than the values of leaf meals obtained from grasses and vegetable shrubs, which seldom exceed 150 g kg<sup>-1</sup> d.m. (AYSSIWEDE et al. 2010). This level of crude protein is of particular nutritional significance, as it can meet animals' protein and energy requirements and boost the immune system against diseases. It is, however, important to stress that leaf protein concentrate does not constitute food on its own but it possesses some nutritional potential that could be used as a food ingredient, infant formula, food supplement and food formulation.

Crude fibre was not found in the extracted leaves of moringa. Dried leaves, however, contained 8% of crude fibre. Similar values were obtained by AYE and ADEGUN (2013), who compared moringa leaves with leucaena (*Leucaena leucocephala*) and gliricidia (*Gliricidia sepium*) leaf meals. They showed 68, 95 and 86 g kg<sup>-1</sup> d.m. of lipid content, respectively. The tree species, especially *Gliricidia sepium*, are cultivated in homestead gardens or on farms, but can also be found on vacant lots or fallow land of former yam farms and are cultivated in fields in Nigeria. A fraction of the cell walls, that is crude fibre, is composed of cellulose, hemicellulose and lignin, which reduces the digestibility of fibre. However, the low level of fibre in dry *Moringa oleifera* leaves indicates that it is suitable for infants and young children with lower dietary fibre requirements.

Fat and carbohydrates are valuable components, which are the main source of energy for the human body. The extracted leaves contained five-fold less lipids than dried leaves (17.5 and 89.8 g kg<sup>-1</sup> d.m., respectively). Moyo et al. (2011) reported 65 g of fat in moringa plants in Limpopo Province in South Africa. The low level of fat shows that the vegetable is not a source of lipids that could cause arteriosclerosis; hence, a leaf extract would be suitable for individuals suffering from or prone to cardiovascular diseases.

The main component of dry mass appeared to be total carbohydrates, which are the principal source of energy. More total carbohydrates were found in extracted than in unextracted leaves (712.7 and 499.9 g kg<sup>-1</sup> d.m., respectively).

The chemical composition values confirmed that *M. oleifera* leaves are an excellent food source, justifying its direct use in human nutrition or for the development of balanced diets for animal nutrition.

SODAMADE et al. (2013) revealed that Moringa oleifera leaves are nutri-

tionally adequate and constitute a promising source of dietary minerals. The leaf mineral concentration might make the plant an important source of essential elements for the human body. The conducted research showed significant differences in the content of macronutrients between extracted and unextracted leaves of moringa (Table 2). The extracted sample contained Table 2

Item	Р	Ca	Mg	К	Na
Unextracted	$4.82\pm0.0^{b}$	$12.6\pm1.2^a$	$5.65\pm0.0^{b}$	$20.8\pm0.1^{b}$	$2.55\pm0.1^b$
Extracted	$6.51 \pm 0.0^a$	$13.1 \pm 0.0^a$	$13.5 \pm 0.1^a$	$53.9 \pm 0.3^a$	$6.0 \pm 0.4^a$
P-value	0.000	0.054	0.000	0.000	0.000

Content of macroelements (g kg<sup>-1</sup> d.m.) in leaves of Moringa oleifera

Explanations under Table 1

higher quantities of all of the studied macronutrients. The samples contained 5.7 g kg<sup>-1</sup> d.m. of phosphorus, on average. The extracted leaves contained significantly more phosphorus than raw leaves (by 1.69 g kg<sup>-1</sup> d.m., i.e. 35%). According to AJA et al. (2013), Moringa oleifera leaves have  $0.04 \text{ g kg}^{-1}$  of phosphorus. SHARMA et al. (2012) reported a calcium level in moringa leaves at 20.1 g kg<sup>-1</sup>. The present research found 12.8 g kg<sup>-1</sup> d.m. of calcium in leaves. No significant differences were verified in the calcium content between extracted and unextracted leaves. The mean content of magnesium in moringa was 9.6 g kg<sup>-1</sup> d.m. Twice as much of the element was found in extracted than in unextracted leaves (13.5 g kg<sup>-1</sup> d.m.). In other herbs, the magnesium content ranges from 532.7 mg kg<sup>-1</sup> d.m. in Mentha spicata to 4247.5 mg kg<sup>-1</sup> in Rosmarinus officinalis (RAOUF et al. 2014). The mean potassium level in moringa leaves was  $37.3 \text{ g kg}^{-1}$  d.m., which is supported by the research of AMABYE, GEBREHIWOT (2015). Over twice as much potassium was found in extracted than in unextracted leaves. Moringa leaves contained on average 4.3 g kg<sup>-1</sup> d.m. of sodium. According to the literature (SHARMA et al. 2012, SODAMADE et al. 2013), the sodium content in Moringa oleifera ranges from 1.29 to 2.40 g kg<sup>-1</sup>, which agrees with our results. Significantly more sodium (twice as much) was found in extracted than in unextracted leaves. The content of macroelements in the moringa leaf extract decreased in the order: K > Mg > Ca > P > Na, while in the dried leaves it was as follows: K > Ca > Mg > P > Na.

Heavy metals can have various effects on living organisms, depending on their concentrations and forms in the environment. They can stimulate a living organism, e.g. elements essential for metabolic processes (Fe, Mn, Cu, Zn, Mo), or act as poisons, e.g. elements which are harmful for humans and animals even at very low concentrations (As, Hg, Pb, Cd) (OCIEPA-KUBICKA, OCIEPA 2012). Moringa leaves had a significantly higher content of trace metals than the extract (Table 3). In medicinal herbs, the iron content ranges from 2.9 to 6.07 ppm, zinc from 0.74 to 1.83 ppm and copper from 0.31 to

Item		Mean	SD	P-value	
Fe	unextracted	$97.6^{a}$	±2.3	0.000	
	extracted	$11.9^{b}$	±1.1	0.000	
Zn	unextracted	$17.8^{a}$	±0.1	- 0.007	
	extracted	$17.1^{b}$	±0.0		
Cu	unextracted	$3.63^{a}$	±0.2	0.002	
	extracted	$0.0^b$	±0.0		
Mn	unextracted	$27.9^{a}$	±0.1	0.000	
	extracted	$23.2^{b}$	±0.0	0.000	
Mo	unextracted	$37.8^{b}$	±0.4	0.008	
	extracted	$41.7^{a}$	±0.3	0.008	
Cr	unextracted	$6.67^a$	±0.1	0.776	
	extracted	$6.62^{a}$	±0.2		
Со	unextracted	$0.38^a$	±0.0	0.000	
	extracted	$0.01^{b}$	±0.0		
Ni	unextracted	LOQ	-		
	extracted	LOQ	-		
Cd	unextracted	LOQ	-		
	extracted	LOQ	-	-	
Dh	unextracted	$3.14^{a}$	±0.8	0.461	
Pb	extracted	$2.09^{a}$	±1.4		

Explanations under Table 1, LOQ – Limit of Quantitation

1.44 ppm (MAOBE et al. 2012). The content of these elements in moringa plants was 54.7 mg kg<sup>-1</sup> d.m. of iron and 17.4 mg kg<sup>-1</sup> d.m. of zinc. The extracted leaves did not contain copper, whereas unextracted leaves had 3.63 mg kg<sup>-1</sup> d.m. of this metal. The three elements occurred in levels below the WHO limits (300 mg kg<sup>-1</sup> for iron, 50 mg kg<sup>-1</sup> for copper and zinc) (NKANSAH, AMOAKO 2010). Therefore, *Moringa oleifera* may be regarded as a good and safe source of iron, zinc and copper. All of the examined samples contained the amounts of manganese below the WHO limits (200 ppm) for medicinal plants (RAOUF et al. 2014). The plants contained on average 25.5 mg kg<sup>-1</sup> d.m. of manganese. Significantly more of the element was found in leaves (by 20%). FAKANKUN et al. (2013) recorded between 63 and 86 mg kg<sup>-1</sup> in *Moringa oleifera*. Significantly more molybdenum was found in the extracted samples (by 3.9 mg kg<sup>-1</sup> d.m., i.e. 10%). On average, moringa contained 39.7 mg kg<sup>-1</sup> d.m. of molybdenum.

RAOUF et al. (2014) reported that *Tanacetum parthenium*, *Rosmarinus* officinalis and *Matricaria chamomilla* contained 6.7, 12.0 and 6.0 ppm of

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chromium, respectively. *Moringa oleifera* had 6.6 mg·kg<sup>-1</sup> d. m., on average. There was no significant difference found in the chromium concentration between the extracted and unextracted leaves.

There are no threshold limits set by the WHO/FAO for cobalt in herbal plants and preparations (MAOBE et al. 2012). The average cobalt concentration in the morenga plants examined reached 0.19 mg kg<sup>-1</sup> d.m., and significantly more cobalt was found in leaves (by 0.37 mg kg<sup>-1</sup> d.m.). FAKANKUN et al. (2013) did not find any cobalt in *Moringa oleifera*.

All nickel compounds, except for metallic nickel, have been classified as human carcinogens by the International Agency for Research on Cancer (DAs et al. 2008). Cadmium is considered to be a highly toxic metal, able to cause many severe diseases (BATOOL, KHAN 2014). No nickel or cadmium was found in the plant material in the present research, which coincides with the research by FAKANKUN et al. (2013).

There was no significant difference found in the lead content between extracted and unextracted leaves. The mean lead concentration (2.6 mg kg<sup>-1</sup> d.m.) was lower than the WHO limit (RAOUF et al. 2014).

In a balanced diet, it is not only important to supply appropriate quantities of nutrients, but also to consider their ratios (Table 4). The correct calcium

Table 4

Item	Ca:Mg	Ca:P	K:Mg	Na:K
Unextracted	2.27	2.62	3.67	0.12
Extracted	0.97	2.02	3.99	0.11

Selected nutrient ratios in leaves and extract of Moringa oleifera

to magnesium ratio should not be higher than three (MAJKOWSKA-GADOMSKA 2006). Higher than optimal Ca:Mg ratios could indicate magnesium deficiency (FRANCKE 2010). None of the analysed samples had the ratio higher than the threshold value, although unextracted Moringa oleifera leaves had a higher ratio than extracted ones. The Ca:P ratio lower than 0.5 indicates poor quality food source, while a ratio higher than one indicates good quality food source (IHEDIOHA, OKOYE 2011). The Ca:P ratio was higher than one in both extracted and unextracted leaves of moringa, which means that it is a good source of mineral compounds essential for bone synthesis. The K:Mg ratio in a mammalian diet should not exceed six (MAJKOWSKA-GADOMSKA 2006). In the examined samples, the ratio was 3.67 in unextracted leaves and 3.99 in extracted leaves. Too much sodium in relation to potassium causes hypertension. The Na:K ratio in the body is important because it helps control blood pressure (YUSUF et al. 2007). The Na:K ratio should be less than one. Both extracted and unextracted leaves of moringa fulfil this requirement.

# CONCLUSIONS

1. Unextracted moringa leaves contained more crude protein and fat than the extracted samples.

2. Extracted moringa leaves contained significantly more mineral compounds in the form of crude ash.

3. Significantly more of the examined macronutrients were found in extracted leaves from *Moringa oleifera* than in unextracted leaves. The latter contained more heavy metals.

4. Moringa oleifera contained significant quantities of nutrients essential for appropriate functioning of an organism (phosphorus, potassium, calcium, magnesium, iron, zinc, copper, manganese, molybdenum). Adequate Ca:Mg, Ca, K:Mg and Na:K ratios in the plant indicate that it can be a good and safe source of macro- and micronutrients in human and animal diet.

5. Lead concentration in both extracted and unextracted leaves was lower than the WHO limit. No nickel or cadmium were found in the studied samples.

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