Available online at http://www.ifgdg.org

Int. J. Biol. Chem. Sci. 18(3): 779-786, June 2024

ISSN 1997-342X (Online), ISSN 1991-8631 (Print)

International Journal of Biological and Chemical Sciences

***Original Paper*** http://ajol.info/index.php/ijbcs http://indexmedicus.afro.who.int

# ICP-MS determination of iron, zinc and others minerals bioaccessibility and their potential contribution in *Moringa oleifera Lam* leaf powder from Senegal

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***ACKNOWLEDGMENTS***

*The authors thank the FPL and CIWA Projects for funding the work.*

Received: 02-09-2023 Accepted: 14-10-2023 Published: 30-06-2024

## ABSTRACT

Moringa oleifera is an edible tree whose leaf powder is increasingly used as the main source of iron and other minerals such as zinc, magnesium, phosphorus and calcium. The objective of this present work was to determine the bioaccessibility of these minerals and their potentials contribution in *Moringa oleifera Lam* leaf powder (Mlp) from Senegal by Inductively Coupled Plasma Spectrometry (ICP-AES). Minerals experiments were done in duplicate and analyzed twice. Bioaccessibility experiments were done in duplicate with three times for intestinal stage (each stage was performed two times). Mlp sample had the following mineral contents: iron (42.8 ± 2.97 mg/100g), zinc (1.88 ± 0.024 mg/100g), magnesium (506.28 ± 3.45 mg/100g), phosphorus (317.3 ± 2.04 mg/100g) and calcium (2273.39 ± 17.72 mg/100g). Mineral bioaccessibility (percentage) were respectively 9.04 ± 0.038, 66.32 ± 2.065, 87.64 ± 0.346, 69.44 ± 0.200 and 55.44 ± 0.316% for Fe, Zn, Mg, P and Ca. The total amount of bioaccessible minerals were respectively 3.87 ± 0.016, 1.25 ± 0.039, 443.71 ± 1.752, 220.34 ± 0.635, 1260.46 ± 7.176 mg/100g for Fe, Zn, Mg, P and Ca. These results show that Mlp is an excellent source of minerals except iron whose bioaccessibility is very low; they can well be used by target cells. A daily consumption of moringa leaf powder divided into number of intakes during the day can provide the required amount of mineral.


**Keywords**: *Moringa oleifera*, minerals, bioaccessibility, ICP-AES.

## INTRODUCTION

*Moringa oleifera* Lam. or *Moringa pterygosperma* Gaertner (Haman, 2010) is a medicinal plant native to the Himalayan sub-regions of North West India, Pakistan, Bangladesh and Afghanistan. It is also found almost everywhere in the world, namely in South America, Arabia, South-East Asia, the Pacific, the Caribbean and in several African countries with great economic importance (Price, 2007; Hêdji et al., 2014).

It is considered one of the most useful trees in the world. Almost all parts of *Moringa oleifera* Lam. can be used in food, industry and pharmacy (Abdellatef et al., 2010). Recently, there has been a lot of interest in the nutritional properties of moringa in most countries where it is not native. Studies in several countries

 
DOI : https://dx.doi.org/10.4314/ijbcs.v18i3.4

have shown that the leaves are rich in amino acids, minerals and vitamins. This is how they have been used to fight malnutrition in infants and women (Moyo et al., 2011). According to Brisibe et al. (2009) and reported by Moyo et al. (2011), there are considerable differences between the nutritional values of Moringa oleifera Lam., which vary according to the genetic context, the environment, and the culture methods.

In Senegal, the areas of intensive moringa cultivation are Kolda and Ziguinchor, but we find it everywhere in the other regions of the country. The leaves are either eaten cooked as a sauce called "Mbuum" or dried and reduced to powder. In general, it is the powder that is most used by households. Hence, our objective was to assess the bioaccessibility of the minerals and their potential contribution contained in the Moringa oleifera Lam leaf powder (Mlp) from Senegal by the method of induced plasma mass spectrometry (ICP-MS).

## MATERIALS AND METHODS

Plant material of *M. oleifera Lam* was purchased from local retail supermarket. The sample was analyzed by the method of ICP-MS at Central Analytical Facilities (CAF), STELLENBOSCH University.

### Minerals

***Microwave assisted acid digestion***

Acid digestion of Mlp was performed using concentred nitric acid plus hydrogen peroxide according to EPA method 3051A (U.S. EPA, 2007). The sample and acid are placed in a quartz microwave vessel. The vessel is sealed and heated in the microwave unit for a specified period of time. After cooling, the vessel contents are filtered, centrifuged, or allowed to settle and then diluted to volume.

***Minerals contents***

Minerals contents (Fe, Zn, Mg, P, and Ca) of digested Mlp were analysed by EPA method 200.7 (U.S. EPA, 1994) using inductively coupled plasma atomic emission spectrometry (ICPOES-AES) (iCAP 6000 series, Thermo Fisher Scientific, Waltham, USA). Elements were analysed using wavelengths 239.5nm for Fe, 206.7nm for Zn, 285.2nm for Mg, 214.9nm for P and 315.8nm for Ca. To ensure accuracy, samples were analysed against National Institute for Standards, Technology (NIST) traceable standards, and independent quality control solutions. A calibration acceptance criterion of R2 > 0.9995 was used and an internal standard technique was also used to ascertain the accuracy of the result. Two independent samples were analysed in duplicate.

### *In vitro* dialysability mineral bioaccessibility

The Mlp were subject to *in vitro* digestion to simulate human gastric and intestinal digestion. The *in vitro* dialysability method of Miller et al. (1981) was used. The gastric stage was done in duplicate with 1.8 g of pepsin solution (16 g de pepsine+100 ml de HCL 0.1 M) at pH 2. The intestinal stage was repeated 3 times using dialysis tubing Spectra/Por 7 (Ø = 20.4 mm) with a molecular mass cut-off of 10000 Da (G.I.C. Scientific, Johannesburg, South Africa) and 3 g of pancreatin-bile extract mixture solution consisting of 4 g of pancreatin (Sigma. P-1750, from porcine pancreas), 25 g of bile extract (Sigma, B-8631, porcine) and 1 litre of 0.1 M $NaHCO_3$ at pH 7,5. Mineral contents of the dialysates were determined by ICP-AES as described above, but without the digestion step. Two independent dialysability assay experiments were performed, with the intestinal step being each time performed in triplicate. Mineral bioaccessibility (%) was calculated as the percentage of the mineral in the dialysate as compared to the total mineral content in the digest:

$$\% \, Mineral \, Bioaccesibility = \frac{concentration \, of \, mineral \, in \, the \, dialysate \times 100}{concentration \, of \, mineral \, in \, pepsin \, digest}$$

### Potential contribution

The Amount of Bioaccessible mineral in 100 g food sample was determined according to Burgos et al. (2018):

$$Amount \, of \, Bioaccessible \, mineral \, in \, 100 \, g \, food = \frac{\% \text{ bioaccessible mineral } \times \text{Fe content of food sample (mg/100 g)}}{100}$$

### Data analyses

Minerals experiments were done in duplicate and analyzed twice (n=4). Bioaccessibility experiments were done in duplicate with three times for intestinal stage (n=6). The mean ± standard deviation calculations for minerals, bioaccessibility and amount of bioaccessible mineral were performed by using descriptive statistics with XLSTAT 6.1.9 software. LSD Fisher test was applied to determine significant differences between specific means at a confidence level of 95% ($p < 0.05$).

## RESULTS

### Minerals contents and their potential contribution

In the present study, Mlp sample had the following mineral contents: iron (42.8 ± 2.97 mg/100g), zinc (1.88 ± 0.024 mg/100g), magnesium (506.28 ± 3.45 mg/100g), phosphorus (317.3 ± 2.04 mg/100g) and calcium (2273.39 ± 17.72 mg/100g) (Figure 1). These results show that the moringa leaf powder from Senegal is an excellent source of minerals.

### *In vitro* dialysability mineral bioaccessibility

The percentage of minerals bioaccessibility of Mlp from Senegal were 9.04 ± 0.038, 66.32 ± 2.065, 87.64 ± 0.346, 69.44 ± 0.200 and 55.44 ± 0.316% respectively for Fe, Zn, Mg, P and Ca (Figure 2). These percentages of bioaccessibility represent the amount of mineral to come in contact with a living organism, which may then absorb it. This means that only 3.87 ± 0.016 mg/100g for iron, 1.25 ± 0.039 mg/100g for zinc, 443.71 ± 1.752 mg/100g for magnesium, 220.34 ± 0.635 mg/100g for phosphorus and 1260.46 ± 7.176 mg / 100g for calcium reached the target cells likely to absorb them (Figure 1).

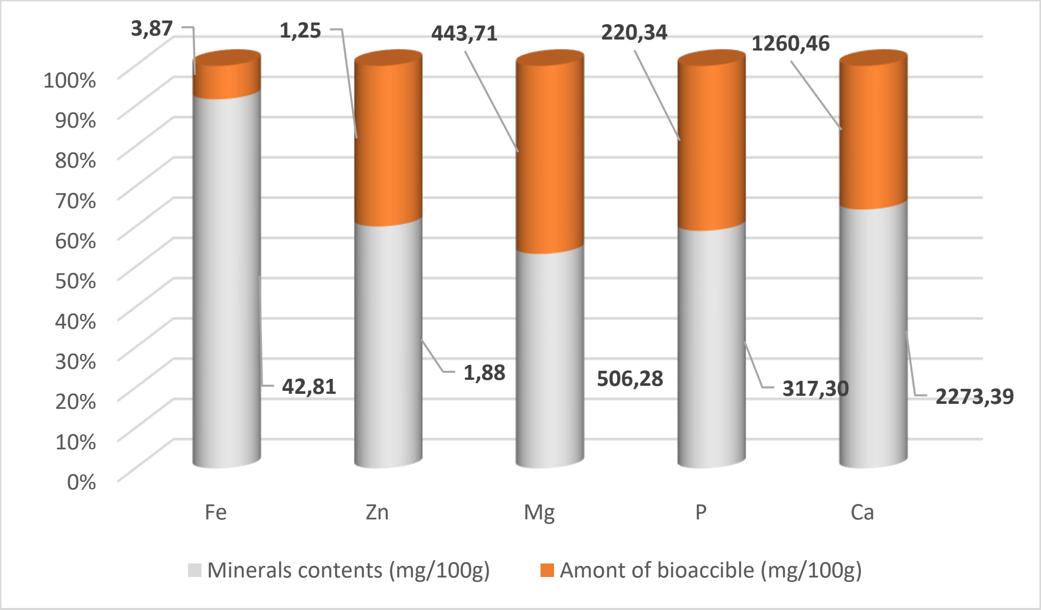


**Figure 1:** Minerals contents of Mlp and their potential contribution.

Values are the mean ± 1 SD of two independent samples of Mlp analysed in duplicate (n=4).

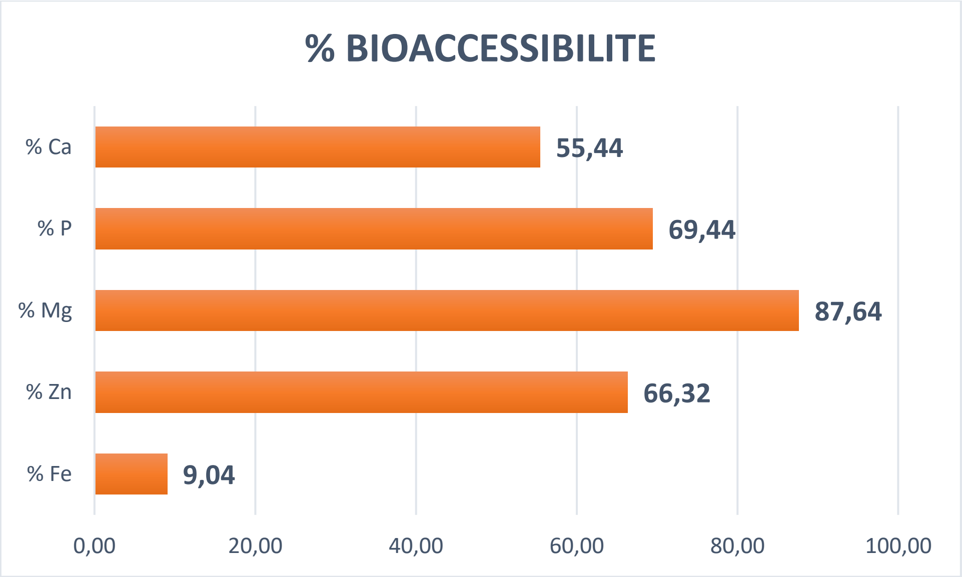


**Figure 2**: Percent of mineral bioaccessibility in 100g of moringa leaf powder (mg/100g).
Values are the mean ± 1 SD of two independent dialysability assay experiments with the intestinal step being each time performed in triplicate (n=6).

**DISCUSSION**

In the present study, the moringa leaf powder contained 42, 8 mg / 100g of Fe. This value is higher compared to that found by Adetola et al. (2019) (19.1mg / 100g). It is also higher than values found by Leone et al. (2015) who studied the nutritional composition of dry moringa leaves from 3 countries: Chad, Sahwari Camp and Haiti. The values obtained were respectively 17.03, 41.68 and 11.91 mg / 100g. Olusanya et al. (2019) found a higher iron value in their moringa powder which is 109.15 mg/100g. The same is true for Van der Merwe et al. (2019) who found that their moringa powder contained 58.4 mg/100g of iron and for Adetola et al (2021) who also found that their “SupaNutri” moringa powder contained 114.4 mg/100g of iron. Zinc content was 1.88 mg/100g, a value much lower than those found by Leone et al. (2015) which were 2.48, 3.09 and 2.18 mg / 100g respectively for moringa powders from Chad, Sahwari Camp and Haiti. Olusanuya et al. (2019) obtained 2.75 mg/100g; Moyo et al. (2011) found 3.10 mg/100g and 2.28 mg/100g by Van der Merwe et al. (2019). Practically, Adetola et al. (2019) obtained the same value of 2 mg/100g found the same value. Mg content in moringa powder was 506.28 mg/100g, a value much lower than those found by Adetola et al. (2019) (896 mg/100g). In their moringa powders, Leone et al. (2015) found 562.49 mg/100g (from Chad), 489.94 mg/100g (from Sahwari Camp) and 533.51 mg/100g (from Haiti). Phosphorus content in moringa powder is 317.3 mg/100g. This value is slightly lower than those found by Adotela et al. (2019) (330 mg/100g of phosphorus) and higher than that found by Van der Merwe et al. (2019) (284 mg/100g). Calcium content in moringa powder is 2273 4 mg/100g, a value higher than that found by Van der Merwe et al. (2019) (2115 mg/100g) and lower than that found by Adotela et al. (2019) (4252 mg/100g). For calcium, Leone et al, 2015 found 1,839.10; 2,743.38 and 2,150.26 mg/100 g respectively in moringa powders from Chad, Sahwari camp and Haiti.

According to Apostoli (2006), “substances must be bioaccessible before they can become bioavailable to human beings. A substance is defined as bioaccessible if it is possible for it to come in contact with a living organism, which may then absorb it. Bioaccessibility is a major consideration in relation to particulates, where species internal to the particles may never become bioaccessible. Elemental species that are accessible on the surface of particles or in solution may be bioavailable if mechanisms exist for their uptake by living cells". According to Adotela et al. (2019), the direction of the effect of mineral bioaccessibility essay is a more reliable guide. So, assuming that this positive effect would be to improve mineral bioavailability.

There are two types of dietary iron: nonheme (plant foods and animal tissues) and heme iron (hemoglobin and myoglobin). Nonheme iron is usually much less well absorbed than heme iron. In plant-based diets such as Moringa oleifera, the main inhibitor of iron absorption is myo-inositol hexaphospate (phytic acid). We have also calcium, proteins and polyphenols. The negative effect of phytate on iron absorption has been shown to be dose dependent and start at very low concentrations of 2-10 mg/meal. Calcium negative effect is too dose dependent and starts at 75 mg. The presence of these inhibitors of iron absorption may explain its low rate of bioaccessibility in Mlp, which is 9.04% (out of 42.8 mg / 100g, only 3.87 mg / 100g reach the cell). However, ascorbic acid is the only main absorption enhancer in vegetarian diets. Many studies have shown ascorbic acid increases the absorption of both native and fortification iron. The enhancing effect is largely due to its ability to reduce ferric ($Fe^{3+}$) to ferrous ($Fe^{2+}$) iron. (Hurell et al, 2010). The RDA of iron (Table 1) is much higher than the amount of bioaccessible iron in moringa leaf powder (3.87 mg / 100g) for healthy individuals in a group. However, a daily consumption of Mlp divided into number of intakes during the day can provide the required amount of iron (Iron, National Institutes of Health).

Zinc is an essential mineral present in some foods. The major function of zinc in human metabolism is DNA synthesis, cell division, wound healing, immune function and it has required for the catalytic activity of approximately 100 enzymes. In dietary supplement, zinc is in several forms: zinc gluconate ($C_{12}H_{22}O_{14}Zn$), sulfate ($ZnSO_4$) and acetate ($ZnC_4H_6O_4$). Research has not determined whether differences exist among forms of zinc in absorption, bioavailability or tolerability. The Mlp has a higher zinc bioaccessibility, which is 66.32 % (out of 1.88 mg/100g, 1.25 mg/100g reach to the cell), but this value is lower than the RDA for zinc (Table 1) for healthy individuals in a group. A daily consumption of Mlp can reach the adequate intake of zinc that is recommended (the body has no specialized zinc storage system) (Zinc, National Institutes of Health).

Magnesium is an abundant mineral in the body and is naturally present in many foods, or added to other food product. It is an important element for health and disease. It has been identified as a cofactor in over 300 enzymatic reactions involving energy metabolism, proteins and nucleic acid synthesis. The majority of information about magnesium comes from of his determination in serum (Elin, 2010). Magnesium supplement exists in several forms: magnesium oxide ($MgO$), citrate ($C_6H_6MgO_7$) and chloride ($MgCl_2$). Small studies reveal that Mg in the spartate, citrate, lactate and chloride form is absorbed more completely and is more bioavailable than magnesium oxide and sulfate (Magnesium, National Institutes of Health). In Mlp, Mg had a very higher bioaccessibility, which is 87.64% (out of 506.28 mg/100g, 443.71 mg/100g reach to the cell). Moringa oleifera leaf powder from Senegal is thus a principal source of magnesium. This value is also higher than the RDA for Mg (Table 1) for healthy individuals in a group.

Phosphorus is an essential mineral and a component of RNA, DNA, teeth and bones. It is also a component of cell membrane structure and of the body's key energy source ATP. As dietary supplement, phosphorus is usually found in the form of phosphate salts or phospholipids. Phosphate salt are approximatively bioavailable at 70%, but others form of phosphate have not been determined in human (Phosphorus, National Institutes of Health). In Mlp, phosphorus was bioaccessible at 69.44% or 220.34 of 317.3 mg

/ 100g arrive at the cell. The quantity of phosphorus bioaccessible was higher than the infants RDA for phosphorus (Table 1) but very than the adolescents and adults RDA for phosphorus. A daily consumption of moringa can reach the adequate intake of phosphorus.

Calcium is present in some medicine such as antacids, in some foods or added to others. Calcium carbonate ($CaCl_3$) and citrate [$Ca_3(C_6H_5O_7)_2$] are two main form of calcium. The percentage of calcium absorbed depends on the total amount of elemental calcium consumed at on time. Calcium carbonate is absorbed most efficiently when taken with food due to its dependence on stomach acid (Calcium, National Institutes of Health). In Mlp, calcium was bioaccessible at 55.44% or 1260.46 of 2273.39 mg/100g arrive at the cell. Thus, value is practically the same of RDA for calcium (Table 1) for healthy individuals in a group.

**Table 1:** Recommended Dietary Allowances and Adequate Intakes for Fe, Zn, Mg, P and Ca.

| Life stage group | Iron (mg/d) | Zinc (mg/d) | Mg (mg/d) | P (mg/d) | Ca (mg/d) |
|---|---|---|---|---|---|
| **Infants** | | | | | |
| 0-6 mo | 0.27* | 2* | 30* | 100* | 200* |
| 7-12 mo | 11 | 3 | 75* | 275* | 260* |
| **Children** | | | | | |
| 1-3 y. | 7 | 3 | 80 | 460 | 700 |
| 4-8 y. | 10 | 5 | 130 | 500 | 1000 |
| **Males** | | | | | |
| 9-13 y. | 8 | 8 | 240 | 1,250 | 1300 |
| 14-18 y. | 11 | 11 | 410 | 1,250 | 1300 |
| 19-30 y. | 8 | 11 | 400 | 700 | 1,000 |
| 31-50 y. | 8 | 11 | 420 | 700 | 1,000 |
| 51- 70 y. | 8 | 11 | 420 | 700 | 1,000 |
| > 70 y. | 8 | 11 | 420 | 700 | 1,200 |
| **Females** | | | | | |
| 9-13 y. | 8 | 8 | 240 | 1,250 | 1,300 |
| 14-18 y. | 15 | 9 | 360 | 1,250 | 1,300 |
| 19-30 y. | 18 | 8 | 310 | 700 | 1,000 |
| 31-50 y. | 18 | 8 | 320 | 700 | 1,000 |
| 51- 70 y. | 8 | 8 | 320 | 700 | 1,200 |
| > 70 y. | 8 | 8 | 320 | 700 | 1,200 |
| **Pregnancy** | | | | | |
| 14-18 y. | 27 | 12 | 400 | 1,250 | 1,300 |
| 19-30 y. | 27 | 11 | 350 | 700 | 1,000 |
| 31-50 y. | 27 | 11 | 360 | 700 | 1,000 |
| **Lactation** | | | | | |
| 14-18 y. | 10 | 13 | 360 | 1,250 | 1,300 |
| 19-30 y. | 9 | 12 | 310 | 700 | 1,000 |
| 31-50 y. | 9 | 12 | 320 | 700 | 1,000 |

**Source:** Food and Nutrition Board, Institute of Medicine, National Academies, National Institute of Health. *Adequate Intakes

## Conclusion

This study allowed us to assess the bioaccessibility of iron, zinc, magnesium, phosphorus and calcium contain in *Moringa oleifera Lam* leaf powder from Senegal. The results showed significant bioaccessibility of Mg, followed by Zn, P and Calcium and a very low bioaccessibility of iron. This low rate of iron bioaccessibility can be explained by the presence of phytate, calcium and polyphenols in moringa leaf powder. Currently, a research team from the Food Technology Institute (ITA) is carrying out studies on the increase of iron's bioaccessibility and bioavailability in moringa leaf powder by cooking-extrusion. In addition, shortly the results will be published.

## COMPETING INTERESTS

The authors state that there is no competing interest.

## AUTHORS' CONTRIBUTIONS

MD is the principal author of the manuscript. AS, AD and ED participated in the sampling and the manipulations of products. CN is the coordinator of the FPL and SMIL projects (Senegal). DT and MLG are the works supervisors.

## ACKNOWLEDGMENTS

The authors thank the ITA for product development, and the Stellenbosch University (ICP-MS Laboratory, Central Analytical Facilities) for bioaccessibility analysis.

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