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Macronutrients and micronutrients in germinated and non-germinated seed flour and moringa leaves (*Moringa oleifera L.*)

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Abstract

Moringa (*Moringa oleifera L.*) is a plant highly valued for its content of macronutrients and bioactive elements. The objective of the study was to evaluate the composition of macronutrients and micronutrients of leaf flour, germinated or non-germinated seeds of moringa in the district of La Matanza in the department of Piura, Peru. The proximal analysis revealed that the moringa shoots presented a high protein value on days 2 and 3, with values of 39.25±0.01 g/100 g and 39.26±0.01 g/100 g, respectively. The fat content in the moringa leaf was significantly lower compared to the seed ($p \leq 0.05$). The fibre content in the raw seed was higher than the leaf content and the germinated seed (19.95 mg/100 g). The iron and calcium values were higher in the leaves (27.77 mg/100 g) and (1620 mg/100 g), respectively. The results of this study show that both moringa leaves and germinated and non-germinated seeds could significantly provide adequate amounts of protein, fat, fibre, and minerals such as iron and calcium.

1. Introduction

Moringa oleifera plant is a type of soft shrub, 5 to 15 m tall belonging to the genus Moringa (Saa *et al*., 2019). It is indigenous to South Asia, specifically the sub-Himalayan areas of northern India (Leone *et al*., 2016). Some studies indicate that it can grow in conditions of water scarcity and has a great capacity for adaptation and growth in different soil conditions and agronomic practices (Coello *et al*., 2020). This plant is cultivated in all tropical and subtropical regions of the world (Sultana, 2020), as well as on the Peruvian coast. It is known worldwide by different common names such as radish tree, pearl tree, marango, miracle tree, among others (Tshabalala *et al*., 2019), the latter is due to its enormous importance in preventing health problems that could otherwise be considered incurable (Martin *et al*., 2013). The importance of using this plant is due to the fact that it has a wide spectrum of metabolites with possible nutritional and medicinal properties (Ma *et al*., 2020).

Moringa leaves are round or oval (Ijarotimi *et al*., 2013). They are usually used as a vegetable (Anjorin *et al*., 2010) and traditionally have been consumed by Asians and West Africans as a healthy food product (Ijarotimi *et al*., 2013). Scientific evidence has shown that moringa leaves contain high nutritional value (Anwar *et al*., 2007). The leaves have been used to decrease the risk of malnutrition among vulnerable populations such as infants and nursing mothers (Moyo *et al*., 2011). Apart from promoting wound healing, the leaves have been used in the treatment of various diseases, such as nervous weakness, paralysis, asthma, diarrhoea, fever, cough, cholera, spasms, enlarged liver and spleen, infections and ulcers and inflammation (Mishra *et al*., 2011). Some studies have placed a lot of emphasis on its nutritional properties, which include antioxidant, anti-inflammatory, and immunomodulatory capacity (Mehta *et al*., 2003; Jaja-Chimedza *et al*., 2017). These benefits are attributed to its high content of bioactive properties, such as phenolic compounds,

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alkaloids, and terpenes. In addition, they contain vitamins, especially A, B, C and E, minerals and essential amino acids (Guzmán-Albores *et al*., 2019). These nutritional characteristics make the consumption of moringa an attractive approach to preventing the development and treat chronic diseases (Coello *et al*., 2020).

Regarding the moringa seed, several studies have highlighted its nutritional value due to its high content of proteins, polyunsaturated fatty acids, vitamins, and minerals (Saa *et al*., 2019). On the other hand, previous studies carried out on moringa mention that seeds germinated for four days provided 14.62% lipids and 23.69% protein compared to ungerminated seeds (Ijarotimi *et al*., 2013). Another study found that germination of moringa seeds improved fibre, fat, riboflavin content, as well as antioxidant potential (Coello *et al*., 2020). The germination process improves the nutritional composition and phytochemical content of edible seeds (Gan *et al*., 2017). In addition, germination favours a considerable increase in the content of micronutrients such as complex vitamins B and C (Rico *et al*., 2020), macronutrients, such as proteins, bioactive compounds, and γ-aminobutyric acid (GABA) in the seeds (Aparicio-García *et al.*, 2020). Variations in the nutritional composition and physiology of the seeds are influenced by factors such as soaking time and germination conditions (presence and/or absence of light, temperature, and time) (Rico *et al*., 2020). Consequently, the improvement of nutritional value and nutritional properties depends on the careful selection of the germination conditions of each seed (Tomé-Sánchez *et al*., 2020).

Although, various studies describe the nutritional and medicinal properties of moringa leaves (Anwar *et al*., 2007; Mishra *et al*., 2011; Ma *et al*., 2020). However, there are considerable variations between the nutritional composition of the different moringa species that are influenced by various factors, such as genetic background, type of soil, environment, organic contribution and frequency of irrigation (Brisibe *et al*., 2009; Guzmán-Albores *et al*., 2019). Furthermore, as far as is known, the nutritional composition and characterization of the germinated moringa leaf and seed of the Peruvian ecotype have not been previously evaluated, this is the first study to include the nutritional composition profile of the macronutrients, micronutrients of the leaves and germinated seeds. This could help in the formulation of new and healthy diets and food products according to the needs and realities of the Peruvian population.

Therefore, the objective of this study was to determine the composition of macronutrients and micronutrients of the leaf and germinated seeds of *Moringa oleifera* of the Peruvian ecotype. The appropriate conditions for the germination process were taken into account. In addition, the different stages of the germination process were monitored at different times (days one, two, and three).

2. Materials and methods

2.1 Procurement of plant materials

Moringa leaves and seeds were obtained from cultivated trees from the La Matanza district of the department of Piura, located on the north coast of Peru. The materials were provided by the residents in coordination with the Asociación Promoción de la Gestión Rural Económica y Social (PROGRESO), Piura, Peru. Moringa leaves and seeds were harvested during December of the current year and were stored in the Diet Techniques laboratory of the Universidad Peruana Unión.

2.2 Leaf flour processing

Any impurities were removed from the leaves to minimize the presence of any foreign material. Subsequently, the drying process was carried out and the sheets were expanded into trays. They were dried in the shade for 5 days at room temperature. They were then ground using an Oster® laboratory blender (model BLSTVB-G00) until obtaining a fine powder. The screening process was carried out to remove impurities. The flour obtained from the leaves was packed in bags and subsequently sealed to be stored at room temperature (27°C) until use.

2.3 Raw moringa seed flour

The raw seeds were sanitized with tap water and soaked in sterilized water (ratio 1: 6, w/v) for 30 mins at room temperature (23-25°C). They were dehisced and taken to the Excalibur® dehydrator at 50°C (Power: 440 W, Model: 4526T220FB/FW, United States) for 24 hrs. The seeds were pulverized using an Oster® laboratory mixer (model BLSTVB-G00). The flour was stored in a vacuum-sealed plastic container at room temperature (27°C) until further analysis.

2.4 Germination process and obtaining moringa flour

Based on previous studies, the seeds were washed with running water and then the soaking process was carried out for 24 hrs at 27°C (Cáceres *et al*., 2014). The water was drained and the hydrated seeds were divided into three subsamples. Subsequently, they were sieved in 3 numbered trays (1, 2, and 3) with a wet filter paper. The germination process was carried out according to the

International Seed Testing Association (ISTA, 1985) and the temperature used for cultivation in geographical areas similar to the northern region of Peru was taken into account. The seeds germinated on the second day (first day of germination), so the germination process of tray 1 was stopped. The same procedure was repeated with trays 2 and 3 on the second and third day of germination, respectively. During the process, non-germinated seeds were excluded. The germinated seeds were derailed and washed manually with distilled water; subsequently, they were dried in the Excalibur® dehydrator at 50°C (Power: 440 W, Model: 4526T220FB/FW) for 24 hours. They were also ground with an Oster® laboratory blender (model BLSTVB-G00). The flour was packed in a vacuum-sealed plastic container at room temperature (27°C) until later use.

2.5 Proximate analysis

2.5.1 Crude protein

Proteins were determined through the micro-Kjeldahl method, one of the most used techniques for the determination of organic nitrogen (Pearson, 1976). This process consisted of the disintegration of organic matter through the use of acids. Consequently, in the presence of catalysts, ammonium borate was obtained which was titrated in the presence of HCL. The method assumes that the nitrogen in the sample is presented in protein form, the calculation of the amount of nitrogen was according to the following formula:

$$\%N = [(V \times N \times 0.014)/M] \times 100$$

Where V represents the volume of hydrochloric acid used in the titration. N represents the normality of hydrochloric acid and M represents the mass of the sample in grams.

To obtain the crude protein, the percentage of Nitrogen (N) was multiplied by the factor 6.25 (% crude protein =% N × 6.25).

2.5.2 Crude fat

The Soxhlet apparatus was used for the extraction of fat. Approximately 3 g of the sample were placed on filter paper. Subsequently, the package was introduced into the Soxhlet apparatus, in the fat balloon, 200 mL of hexane was placed on the stove. After the hexane volatilization process in an oven, the fat content was determined by the difference in weight of the ball with fat and its tare, using the following formula: %F = ([w1-w2)/M]×100. Where, w1 represents the weight of the ball with fat, w2 is the weight of the empty balloon and M is the mass of the sample in grams.

2.5.3 Crude fibre

Crude fibre determination was obtained by successive digestions with diluted acids and bases, according to the methodology proposed by AOAC (AOAC, 2012). The defatted sample was worked with and placed in a beaker (1 L), then boiled with 250 mL of 1.25% H_2SO_4. Then, it was digested for 30 mins. At the end of the time, it was filtered with a Whatman filter (No. 42) and washed to discard the residual acid with hot water. The acid-free sample was heated with 200 mL of boiling 1.25% NaOH for 30 mins. The mixture was then filtered as described above and washed with water. The remaining sample was placed in a crucible and dried in an oven at 105°C until reaching a constant weight. The sample was then cooled in a desiccator to room temperature and weighed. Finally, the sample was incinerated in a muffle at 600°C for 4 hrs, dried and weighed. The variability of the sample weights after incineration determined the amount of crude fibre (Weight of crude fibre = (Weight of crucible + crude fibre + ash) - (Weight of crucible + ash).

2.5.4 Ashes

The ashes were obtained through the calcination method, according to the recommendations of the AOAC (AOAC, 2012). Three grams of sample (leaves and seeds) were placed in a crucible and placed in a muffle furnace at a temperature of 600°C for 9 hrs. The ash content was determined by the weight loss of the sample (% ash = [(Weight of crucible with sample - Weight of crucible with ash)/Weight of sample] × 100).

2.5.5 Carbohydrates

The percentage of carbohydrates (nitrogen-free extract) was obtained from the difference of the subtraction of 100% minus the sum of the percentages of Moisture, crude protein, fat, fibre and ash (% Carbohydrates = 100% - [%M + %P + %F +%F + %A]).

2.6 Statistical analysis

All data were expressed as the mean±standard deviation (SD), considering a minimum triplicate analysis of the samples. Statistical comparisons were made using one-way analysis of variance (ANOVA) with Duncan's post hoc multiple comparisons. All analyses were performed using the IBM SPSS statistical software package (SPSS Inc., Chicago, IL, USA). Default p values ≤0.05 were considered statistically significant.

3. Results and discussion

The proximal composition of moringa leaf meal and

raw seeds is presented in Table 1. The protein content of moringa seed flour (27.94 g/100 g) was significantly higher compared to raw moringa leaf flour (25.91 g/100 g). The high protein content may be due to the fact that moringa seeds are one of the main dietary sources of protein. This observation was similar to the findings of other studies, in which high levels of protein were reported in raw moringa seed meal with values ranging between 26.7 g/100 g (Eme *et al*., 2012) and 37.2 g/100 g (Bridgemohan *et al*., 2014). However, in a study conducted by Govardhan *et al*. (2011), when evaluating the protein content of the defatted moringa seed, it was reported that its protein composition reached 62.8 g/100 g. Moreover, it is important to mention that the protein value of moringa seeds can cover only the requirements of some essential and semi-essential amino acids for people such as histidine, threonine, tyrosine, leucine, isoleucine and phenylalanine (Saa *et al*., 2019).

The fat content of the raw moringa seed meal was significantly ($p \leq 0.05$) higher (25.40 g/100 g) than the moringa leaf meal sample (4.12 g/100 g) (Table 1). The fat content values of leaf flour agree with the values reported in other studies (4.8 g/100 g) (Devisetti *et al*., 2016). Regarding the fat content of moringa seed flour, other studies reported values between 14% and 46% (Abiodun *et al*., 2012; Ijarotimi *et al*., 2013). While the lipids present in moringa seed flour contain a low content of monounsaturated and saturated fats (Ijarotimi *et al*., 2013). However, the oil is an important source of bioactive elements such as phytosterols and tocopherols (Saa *et al*., 2019). Various studies have emphasized the importance of moringa seed oil in people's nutrition, prevention and treatment of non-communicable diseases due to its physicochemical characteristics and the high biological value of its oil (Coello *et al*., 2020).

Table 1. Nutritional composition (g/100 g) of raw moringa leaves and seed flour.

Macronutrients (g/100 g)	MOLM	MOSM
Protein	25.91±0.02[a]	27.94±0.02[b]
Fat	4.12±0.02[a]	25.40±0.01[b]
Fibre	5.37±0.00[d]	19.95±0.01[e]
Ash	8.71±0.00[c]	3.19±0.00[a]
Carbohydrates	48.18±0.01[e]	18.30±0.00[d]

Values are means±standard deviation of triplicate samples. Values with different superscripts within the same column are significantly different ($p<0.05$). MOLM, *M. oleifera* leaf meal; MOSM, *M. oleifera* seed meal.

The crude fibre in moringa seed meal was significantly high ($p \leq 0.05$) compared to leaf meal (19.95±0.01 g/100 g vs. 5.37±0.001 g/100 g) (Table 1). The fibre content in the current study was quite high compared to the values observed for the moringa leaf in another study that analysed the functional properties of the leaves, in which a high content of dietary fibre was observed (5.03±0.07 g/100 g to 6.17±0.03 g/100 g) (Saa *et al*., 2019). However, in another study, the fibre content ranges between 6.00±0.40 g/100 g and 9.60±0.29 g/100 g (Sultana, 2020). The high fibre content in our study is of interest considering that the fibre fraction present in the food defines the degree and rate of digestibility (Rubanza *et al*., 2005). On the other hand, Jimoh and Oladiji (2005), indicate that a high level of fibre can cause intestinal irritation, lower digestibility, and lower absorption of nutrients. However, crude fibre plays an important role in improving digestibility, it favours the absorption of microelements and chemicals such as glucose and fat. In our study, the fibre content in both leaves and moringa seed flour is at an adequate level, which makes moringa an ideal food for human consumption.

The ash content values of the moringa leaf meal were significantly higher compared to the values of the seed meal (8.71±0.00 g/100 g vs. 3.19±0.001 g/100 g) (Table 1). These values are consistent with those reported in other studies (between 8.05±0.39 g/100 g to 10.38±0.45 g/100 g (Sultana, 2020). Similarly, another study showed that whole moringa leaf flour contained 6.53 g/100 g (Abiodun *et al*., 2012). The results of the current study suggest that the *Moringa oleifera* leaf meal represents an important source of minerals.

Regarding the carbohydrate content, a higher content was observed in moringa leaf flour compared to moringa seed flour (48.18±0.01 g/100 g vs. 18.30±0.001 g/100 g) (Table 1). The carbohydrate content of moringa leaf flour in the current study is comparable to the values reported for moringa leaf flour in a similar study in which the value ranges between 47.25±0.39 g/100 g and 54.12±0.86 g/100 g (Sultana, 2020). Moringa leaf flour is high in carbohydrates, in addition, it is of great biological value, therefore, it is an ideal food to contribute to the body's energy requirements. Carbohydrates from whole foods are an essential part of a balanced and balanced diet and should represent between 50% to 60% of the daily intake of total calories.

The protein content of moringa seeds increased significantly on days 2 and 3 of germination (39.25±0.01 g/100 g) and (39.26±0.01 g/100 g), respectively (Table 2). These values are consistent with those previously reported, in which the protein content increased significantly during germination (Coello *et al*., 2020). Similarly, a similar study reported a significant increase in protein content in germinated moringa seeds after 4 days of germination (23.69±0.11 g/100 g) (Ijarotimi *et al*., 2013). It is worth mentioning that, in general, sprouted foods have high levels of protein (Oshodi *et al.*,

1999). Two possible mechanisms could explain the high protein content in germinated moringa seed flours. First, the loss of carbohydrates caused by respiration during the germination process could favour an increase in other nutrients such as proteins (Coello *et al*., 2020). Second, the higher protein content of the germinated seed meal may be due to the biochemical activities of the seeds during the germination process (Ijarotimi *et al*., 2013). Along the same line of idea, other studies show that during germination, carbohydrates are mobilized for the synthesis of amino acids, which stimulates the growth of seedlings (Abdelrahaman *et al*., 2007; Ocheme and Chinma, 2008; Cáceres *et al*., 2014).

Germinated moringa seeds exhibited significantly lower fat content on the third day of germination ($p \leq 0.05$) (Table 2). In addition, the fat content on the first day of germination was slightly higher than on the second day, but without a significant difference ($p > 0.05$). These results are similar to the findings found in other studies in which higher lipid contents have been reported in moringa seeds in the first, 24 and 60 hours of germination 30.13±0.04 g/100 g and 31.89±0.30 g/100 g, respectively (Coello *et al.,* 2020). Moreover, in another study, the germinated seed showed a higher lipid content (26%) compared to the non-germinated seeds (Ijarotimi *et al*., 2013).

Table 2. Nutritional composition (g/ 100 g) of moringa seeds according to germination day.

Macronutrients	GSM1	GSM2	GSM3
Protein	38.40±0.02^{c}	39.25±0.01^{d}	39.26±0.01^{d}
Fat	37.55±0.01^{e}	37.24±0.01^{e}	30.51±0.00^{c}
Fibre	2.53±0.01^{a}	2.56±0.001^{b}	3.08±0.001^{c}
Ash	3.55±0.001^{c}	3.55±0.001^{c}	3.52±0.01^{b}
Carbohydrates	13.06±0.001^{b}	12.01±0.001^{a}	16.78±0.001^{c}

Values are means±standard deviation of triplicate samples. Values with different superscripts within the same column are significantly different ($p<0.05$). GSM1, germinated seed meal of *M. oleifera* on day 1; GSM2, germinated seed meal of *M. oleifera* on day 2; GSM3, germinated seed meal of *M. oleifera* on day 3.

Regarding the total content of dietary fibre, a significant increase was observed during the third day of the germination process (Table 2). The presence of dietary fibre in food provides many health benefits and can be very effective in the prevention of chronic diseases, such as diabetes mellitus, cardiovascular disease, coronary heart disease, hypertension, gastrointestinal disorders, obesity, gastric cancer, breast cancer and colorectal (Ben *et al*., 2014; Yao *et al*., 2014; Quagliani and Felt-Gunderson, 2017). In addition, fibre intake is associated with digestive benefits by increasing stool volume and decreasing intestinal transit time (Anderson *et al*., 2009). According to the recommendations of the Institute of Medicine of the United States, fibre consumption should range between 19 g and 38 g per day, depending on the sex and age of the person (Food and Nutrition Board, Institue of Medicien of the National Academies, 2005). Taking into account these guidelines, the consumption of 100 g of *Moringa oleifera* sprouts per day can cover the decrease in fibre consumption.

The ash content was similar in moringa seed meal for the three days of germination processes (3.55 g/100 g, 3.55 g/100 g and 3.52 g/100 g, respectively) (Table 2). Other data from the literature reported similar content (Olagbemide and Philip, 2015; Liang *et al*., 2019). The ash content in moringa sprouts is considered a measure of the mineral content in this food.

The carbohydrate content was significantly higher on the third day of germination 16.78±0.005 g/100 g (Table 2), however, this content was below the values observed in the raw seed meal. Other studies reported higher carbohydrate levels in moringa sprouts than the current study (Ijarotimi *et al*., 2013; Coello *et al*., 2020). The low carbohydrate content in the present study could be due to the fact that, during the germination process, the carbohydrate content is decreased, which can be explained by the release of reducing sugars and the hydrolysis of starch that provide energy to the seedling growing (Abdelrahaman *et al*., 2007; Ocheme and Chinma, 2008; Cáceres *et al*., 2014).

Table 3 shows the micronutrient composition (iron and calcium) of the leaf meal, germinated and non-germinated moringa seeds with respect to time. The findings indicate that the *Moringa oleifera* leaf meal and the seed germinated during the first day, which contains a high deposit of minerals such as iron and calcium. Moringa leaf flour exhibited iron levels of 27.77 mg/100 g compared to raw seed flour (21.33 mg/100 g). Regarding the germinated seed, it was observed that the iron content was significantly higher (16.54 mg/100 g) on day one of the germination processes compared to days two (12.25 mg/100 g) and three (9.94 mg/100 g). Similarly, the results indicate that the calcium content in moringa leaf flour was 1620 mg/100 g. These results are in agreement with the findings of previous studies (Moyo *et al*., 2011; Sultana, 2020). Minerals are involved in multiple processes. The inadequate intake of these elements is related to various health conditions such as diabetes, anaemia, cardiovascular and kidney diseases, obesity, accelerated ageing, and risk of fractures (Heffernan *et al*., 2019). In the particular case of iron, considering that the body cannot synthesize it, it is important to acquire it through the diet. Adequate iron

Table 3. Mineral composition (mg/100 g) of raw moringa leaf and seed flour and according to the germination day.

Nutrients (mg/100 g)	MOLM	MOSM	GMS1	GMS2	GMS3
Iron	27.77±0.01^{e}	21.33±0.01^{d}	16.54±0.01^{c}	12.25±0.01^{b}	9.94±0.01^{a}
Calcium	1620±0.00^{e}	280±0.00^{d}	290±0.00^{c}	250±0.00^{b}	230±0.00^{a}

Values are means±standard deviation of triplicate samples. Values with different superscripts within the same column are significantly different ($p<0.05$). MOLM, *M. oleifera* leaf meal; MOSM, *M. oleifera* seed meal. GSM1, germinated seed meal of *M. oleifera* on day 1; GSM2, germinated seed meal of *M. oleifera* on day 2; GSM3, germinated seed meal of *M. oleifera* on day 3.

intake prevents malnutrition, malabsorption disorders, or conditions that can lead to a loss of iron in haemoglobin caused by blood loss (Moustarah and Mohiuddin, 2021). As for calcium, its intake is necessary for the formation and maintenance of healthy bones and teeth; in addition, it prevents osteoporosis. Calcium is also necessary for normal blood clotting and nerve function (Sultana, 2020).

4. Conclusion

In this study, the germination process improved the nutritional quality of protein, fat, and calcium content of the sprouts and moringa seeds, and this nutritional improvement depends on the germination time of the seeds.

The calcium iron content decreased on the third day of germination. Similarly, the fibre, ash, and carbohydrate content decreased with germination. On the other hand, moringa seeds exhibited significant values of protein, ash and carbohydrates. In addition, the content of microelements such as iron and calcium were very high in the current study.

The results of this study shed light on the nutritional qualities of the germinated and non-germinated leaves and seeds of moringa for consumption as such and their possible use in the production of highly beneficial products for health through the preparation of functional foods, instant foods in powder, beverages, bakery products and beauty and skincare products.

Conflict of interests

Authors have no potential conflicts of interest

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