AGRO PRODUCTIVIDAD

Colegio de Postgraduados

Chemical composition of twelve accessions of *Moringa oleifera* Lam. grown in Mexico

Ruíz-Hernández, Rafael[1*]; Hernández-Rodríguez, Martha[2]; Pérez-Vázquez, Arturo[3]; Cruz-Monterrosa, Rosy G.[1]; Rayas-Amor, Adolfo A.[1*]

[1] Departamento de Ciencias de la Alimentación. División de Ciencias Biológicas y de la Salud. Universidad Autónoma Metropolitana Unidad Lerma. Av. de las Garzas 10. Col. El Panteón, Lerma de Villada, Estado de México. C. P. 52005.

[2] Postgrado de Recursos Genéticos y Productividad-Genética, Colegio de Postgraduados, Campus Montecillo. Municipio de Texcoco, Estado de México, México. C.P. 56230

[3] Colegio de Posgraduados, Campus Veracruz. Predio Tepetates, Manlio F. Altamirano, Veracruz. Carretera Xalapa-Veracruz km 88.5, CP. 91700.

* Correspondence: r.ruiz@correo.ler.uam.mx; a.rayas@correo.ler.uam.mx

ABSTRACT

Objective: The objective was to evaluate the growth and proximal analysis of twelve accessions of *Moringa oleifera* Lam. grown in Mexico.

Design/Methodology/Approach: The seeds were collected in Veracruz, Oaxaca, Guerrero, Chiapas, and Yucatan. The seeds were sown in a nursery and transplanted in the field in a completely randomized block experimental design. Height, basal diameter, and the number of branches were recorded, and leaves were collected for proximal analysis determination.

Results: Significant statistical differences ($P<0.05$) were identified among the accessions based on tree height, basal diameter, number of branches, moisture content, ash, protein, and fat contents.

Study limitations/implications: Accessions with high growth rates and nutritional characteristics can be selected to establish low-cost food banks.

Result/Finding/Conclusion: The accessions from Chiapas (C1 and C2) were superior to the others in tree height, basal diameter and number of branches, protein, and fat contents.

Keywords: moringa, protein, lipids, genetic improvement.

Citation: Ruíz-Hernández, R., Hernández-Rodríguez, M., Pérez-Vázquez, A., Cruz-Monterrosa, R. G., & Rayas-Amor, A. A. (2022). Chemical composition of twelve accessions of *Moringa oleifera* Lam. grown in Mexico. *Agro Productividad*. https://doi.org/10.32854/agrop.v15i10.2405

Academic Editors: Jorge Cadena Iñiguez and Libia Iris Trejo Téllez

Received: August 15, 2022.
Accepted: October 25, 2022.
Published on-line: November 14, 2022.

Agro Productividad, 15(10). October. 2022. pp: 183-192.

INTRODUCTION

Moringa oleifera Lam. is a fast-growing, drought-resistant tree distributed in tropical, subtropical, arid, and semi-arid zones (Owon *et al.*, 2021). This plant species thrive under medium rainfall conditions, 20 to 30 °C, medium and low fertility soils, and good drainage (Mendieta-Araica *et al.* 2013). Due to its great adaptability, high carbon sequestration capacity, and tolerance to environmental changes, it represents an alternative for food production in the face of climate change scenarios (Gedefaw, 2015). The World Health Organization (WHO) and the Food and Agriculture Organization (FAO) identified and promoted the potential of moringa to address issues related to food and the prevention of various types of diseases (Shil, 2021). The leaves are consumed in Ethiopia, Nigeria,

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India, Malawi, East Africa, and Ghana when combined with traditional foods. Moringa leaves have a higher protein content than other foods and are recommended to address malnutrition problems (Daba, 2016). The range of protein in dried moringa leaves can range from 10.74 to 32.06%, and within this protein are the amino acids valine, threonine, isoleucine, lysine, methionine, phenylalanine, histidine, leucine, arginine, glycine, alanine, serine, aspartic, glutamine, tyrosine, cysteine and proline (Mbailao *et al.*, 2014; Valdez-Solana *et al.*, 2015). They also contain vitamins (A, B1, B2, B6, B12, C, and E) and minerals such as Ca, K, S, P, Mg, K, Na, Zn, Cu, Mn, Fe (Stevens et al., 2021).

In Mexico, moringa is distributed in 14 states and is used for various purposes, mainly food and medicinal, due to its high nutritional content (Olson and Alvaro-Cárdenas, 2016). Studies have been conducted on its nutritional composition by collecting materials in different geographical regions. The results show variations in protein, ash, lipid, and moisture content of the evaluated accessions (Jongrungruangchok *et al.*, 2010; Valdez-Solana *et al.*, 2015; Lamidi *et al.*, 2017). However, the difference in nutritional content may be influenced by soil type, climate, altitude, and type of material evaluated (Bopape-Mabapa *et al.*, 2020). Therefore, the evaluation under homogeneous conditions clarifies the quantitative variation of the Proximal analysis of moringa accessions with greater precision. Identifying materials with higher nutritional content allows the selection of highly nutritious ones and will facilitate the establishment of food banks. Therefore, the objective of this study was to evaluate the Proximal analysis of 12 *Moringa oleifera* Lam. accessions grown in Mexico.

MATERIALS AND METHODS

Biological material

Moringa populations were identified, and seeds were collected in the states of Chiapas, Guerrero, Oaxaca, Veracruz, and Yucatan. Table 1 and Figure 1 show the geographic locations of the accessions evaluated. The research was carried out at the Colegio de Postgraduados, Campus Veracruz, geographically located at 19.27556 N latitude, 96.27556 W longitude at an altitude of 16 masl, with a warm sub-humid climate (AW0) and a mean annual rainfall of 1000 mm.

Sowing and experimental design. Seeds were sown in a substrate composed of soil, manure compost, and sand (5:4:1) under nursery conditions. Two months after sowing, the seedlings were transplanted in a completely randomized block design with five blocks and five replications. The distance between plants and rows was 3 m (Ruiz *et al.*, 2021). Weeds were eliminated when they exceeded 20 cm in height, and regular irrigation was applied. The physicochemical characteristics of the soil of the experimental area were texture, clay loam, sand (37.35%), silt (25.76%), clay (36.89%), pH (6.25), nitrate (0.0 mg L^{-1}), nitrite (0.0 mg L^{-1}), P (2.5 mg L^{-1}), K (21 mg L^{-1}) and 2.58% organic matter (Reyes, 2018).

Data collection: Three-year-old trees were selected for quantitative data collection. Height was measured with a flexometer from the base of the trees to their apex. The basal diameter was measured at ground level with a vernier, and the number of branches was obtained by counting.

Table 1. Geographical location of *M. oleifera* Lam. accessions grown in different states of Mexico.

Accession	State	Municipality	Community	Longitude	Latitude
C1	Chiapas	Tuzantán	Villa Hidalgo	−92.374722	15.108056
C2	Chiapas	Tuxtla Gutiérrez	Colonia La Salle	−93.0868889	16.7429444
G2	Guerrero	Acapulco de Juárez	Parrotillas	−99.6155837	16.8787834
G4	Guerrero	Tecpán de Galeana	Mitla	−99.8934352	16.8789425
O1	Oaxaca	Santa Cruz Xoxocotlán	San Juan Bautista	−96.7280556	16.9791667
O2	Oaxaca	Santa María Huatulco	La Herradura	−96.3658333	15.7772222
O3	Oaxaca	Mariscala de Juárez	Guadalupe la Huertilla	−98.1088889	17.8513889
V1	Veracruz	Soledad de Doblado	El Progreso	−96.4022719	19.0818742
V4	Veracruz	Misantla	Santa Cruz Hidalgo	−96.8628092	19.9555656
V1	Veracruz	Soledad de Doblado	El Progreso	−96.4022719	19.0818742
Y3	Yucatán	Peto	Teshan	−88.62125	20.1486389
Y4	Yucatán	Baca	Felipe Carrillo Puerto	−89.6070099	20.9954688

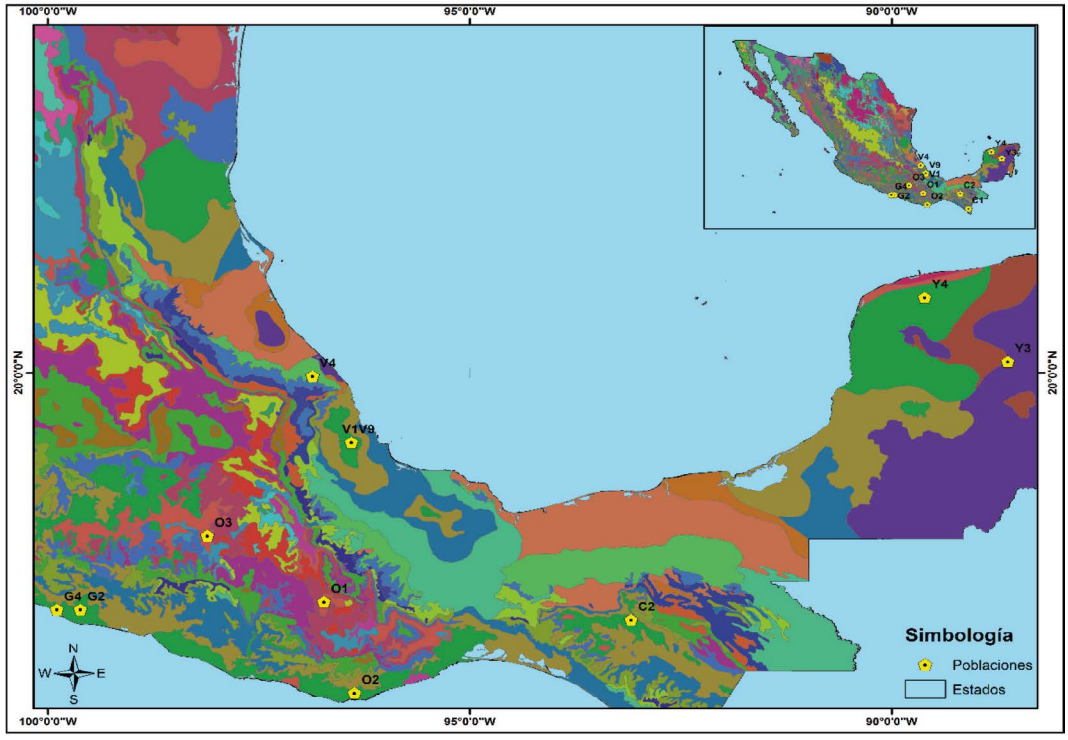

Figure 1. Geographical location of the *M. oleifera* accessions.

Sample collection: 3 kg of mature and healthy leaves were collected from each population. Leaflets were separated from the central rachis, and those with yellowing were removed. Leaves were dehydrated in a forced-air oven at 50 °C for 30 h. Leaves were sprayed with a spray solution. The leaves were pulverized with a plant tissue mill and sieved on a 1 mm mesh. Moringa leaf meal was stored in airtight bags at 20 °C until analysis.

Proximal analysis: moisture, ash, protein, fat, and fiber contents in dried moringa leaves were determined using AOAC (1990) methodology, and samples were analyzed in triplicate.

Moisture determination: Porcelain crucibles were brought to constant weight. One gram of moringa meal was added to the crucible and allowed to dry in an oven at 105 °C until a constant weight was reached. The moisture content was determined under the following equation.

$$Humidity\ (\%) = \frac{Initial\ weight - final\ weight}{sample\ weight} \times 100$$

Ash determination: It was determined through the gravimetric method AOAC 923.03. Porcelain crucibles were brought to constant weight. One gram of moringa meal for each sample was added to the crucible. The samples were burned until carbonization with a Bunsen burner. Subsequently, the crucibles were placed in a flask at 550 °C for five h. The samples were brought to constant weight and room temperature. The crucibles were weighed with the grayish-white ashes. The determination was carried out in triplicate. The total ash content was calculated with the following equation.

$$Ash\ (\%) = \frac{ash\ weight}{sample\ weight} \times 100$$

Protein determination: it was determined by the Kjeldahl method. One gram of moringa meal was added to each Kjeldahl flask, and 0.5 g of $CuSO_4$, 10 g of K_2SO_4, and 25 mL of concentrated H_2SO_4 were added. The tubes were placed in the digesters and heated until a light green, transparent color was reached. They were allowed to cool to room temperature. Sixty mL of 40% (v/v) NaOH was slowly added to each tube and placed in the distiller. The distillate was collected in a 250 mL flask, 50 mL of 0.1 N HCl, and three drops of methyl red indicator. The distillation was concluded when a volume of 100 mL was obtained. Subsequently, the distillate was titrated with NaOH. The protein content was calculated from the total nitrogen content using a factor of 6.25 with the following equation.

$$N(\%) = \frac{(V_B - V_M) \cdot N \cdot Meq}{PM} \times 100$$

$$P(\%) = (N\%)(6.25)$$

Where: V_B=ml of NaOH spent on the blank; V_M=ml of NaOH spent on the problem; N=Normality of NaOH; Meq=Milliequivalent of N; PM=Sample weight; N(%)=Nitrogen; P (%)=Total protein.

Determination of total fat (lipids): 250 ml flasks were brought to constant weight. Three g of moringa flour were placed in each cartridge. The Soxhlet-type extractors were installed, and the cartridges were introduced. 125 ml of hexane (solvent) was added to each sample, and the siphoning effect was performed for five h from the first heating. The total fat content was determined with the following equation:

$$Fat\ (\%) = \frac{Grams\ of\ residue\ in\ flask}{(Weight\ of\ sample\ in\ grams)} \times 100$$

Statistical analysis

The data obtained were recorded in an Excel spreadsheet, version 2016®. Analysis of variance (ANOVA) was performed, and Tukey's test ($p<0.05$) was used to determine the differences between height, basal diameter, number of branches, and Proximal analysis of moringa meal. Correlation analysis of the variables was also performed. Statistical tests were performed with InfoStat software version 2016.

RESULTS AND DISCUSSION

Height, basal diameter, and number of branches

Significant statistical differences ($p<0.05$) were observed in the height of the evaluated accessions. The accessions with the lowest and highest heights were V9 with 3.82 m and C1 with 5.95 m. Ruiz *et al.* (2021) mentioned differences in the height of the evaluated accessions. Accession C1 represents the material with the most significant ($P<0.05$) growth during the three years of development. This genetic material presented more remarkable development under climatic conditions of the sub-humid tropics. Therefore, it can be used for the establishment of protein banks.

The basal diameter of the evaluated accessions showed a progressive development. The statistical test determined significant differences ($P<0.05$) in the basal diameter of the moringa accessions. Accession Y3 presented the most significant ($P<0.05$) diameter growth with a value of 11.30 cm. The lowest basal diameter growth was 7.89 and corresponded to accession O2. De Swaef *et al.* (2015) mentioned that the diameter size of trees is a significant indicator for studies related to drought resistance. Moreover, those accessions with a larger diameter can resist or tolerate this abiotic stress. The number of tree branches indicates the number of leaves and fruits an accession can produce.

In this study, the number of branches fluctuated from 11 to 31 among the accessions. This variation was statistically significant ($p<0.05$), and those materials with a higher number of branches can be selected for fruit and seed production.

Proximal analysis

Table 3 shows the proximate content of 12 moringa accessions. Significant statistical differences ($P<0.05$) were obtained in moisture, ash, protein, and fat content in dried moringa leaves.

Table 2. Height, basal diameter, and the number of branches of the accessions of *M. oleifera* Lam.

Accession	Height±S.D. (m)	Basal Diameter±S.D. (cm)	Number of Branches±S.D.
C1	5.95±0.16^{e}	10.05±0.33^{cde}	19.00±1.29^{bc}
C2	5.18±0.16^{cde}	9.00±0.33^{abc}	24.00±1.29^{c}
G2	4.48±0.16^{abc}	9.67±0.33^{cde}	14.67±1.29^{ab}
G4	4.90±0.16^{bcd}	9.03±0.33^{abc}	18.67±1.29^{bc}
O1	5.40±0.16^{de}	7.70±0.33^{a}	10.50±1.29^{a}
O2	4.30±0.16^{ab}	7.89±0.33^{ab}	13.00±1.29^{ab}
O3	5.41±0.16^{de}	10.13±0.33^{cde}	18.50±1.29^{bc}
V1	4.80±0.16^{bcd}	8.93±0.33^{abc}	16.00±1.29^{ab}
V4	4.94±0.16^{bcd}	10.83±0.33^{de}	31.00±1.29^{d}
V9	3.82±0.16^{a}	7.43±0.33^{a}	11.67±1.29^{a}
Y3	5.42±0.16^{de}	11.30±0.33^{e}	19.00±1.29^{bc}
Y4	4.30±0.16^{ab}	9.40±0.33^{bcd}	15.33±1.29^{ab}

Table 3. Proximal analysis (%) of 100 g of dried leaves of *M. oleifera* accessions from different states of Mexico.

Accession	Hum±S.D.	Ash±S.D.	Protein±S.D.	Fat±S.D.
C1	7.02±0.17 ^{bc}	8.99±0.10 ^{cde}	22.13±0.65^{ef}	5.43±0.14 ^{c}
C2	8.6±0.17 ^{e}	10.86±0.10 ^{h}	20.23±0.65 ^{def}	6.80±0.14 ^{d}
G2	6.9±0.17 ^{bc}	8.59±0.10 ^{bc}	16.60±0.65 ^{abc}	5.69±0.14 ^{c}
G4	5.8±0.17 ^{a}	8.11±0.10 b	20.70±0.65 ^{def}	3.65±0.14 ^{b}
O1	6.54±0.17 ^{ab}	9.33±0.10 ^{efg}	16.73±0.65 ^{abc}	5.15±0.14 ^{c}
O2	6.63±0.17 ^{ab}	8.71±0.10 ^{cd}	18.73±0.65 ^{bcd}	4.00±0.14 ^{b}
O3	7.73±0.17 ^{cde}	9.63±0.10 ^{fg}	14.13±0.65 ^{a}	3.35±0.14 ^{ab}
V1	8.00±0.17 ^{de}	8.93±0.10 ^{cde}	23.50±0.65 ^{f}	5.05±0.14 ^{c}
V4	6.76±0.17 ^{b}	8.55±0.10 ^{bc}	14.57±0.65 ^{a}	3.47±0.14 ^{ab}
V9	6.75±0.17 ^{b}	9.17±0.10 ^{def}	19.50±0.65 ^{cde}	5.01±0.14 ^{c}
Y3	7.31±0.17 ^{bcd}	9.70±0.10 ^{g}	13.67±0.65 ^{a}	3.52±0.14 ^{b}
Y4	7.10±0.17 ^{bc}	7.53±0.10 ^{a}	15.50±0.65 ^{ab}	2.78±0.14 ^{a}
Average	7.09	9.01	18.00	4.49

Humidity

Significant statistical differences ($P<0.05$) were obtained in the moisture content in the dry leaves of the 12 moringa accessions. The minimum and maximum values were 5.8 and 8.6%, corresponding to accessions G4 and C2. The values obtained in this research are like the 6.94 to 8.67% reported by Pérez-Ángel *et al.* (2020). Higher than the 5.7% of Díaz-Fuentes, (2019), 3.34 and 3.06 of Valdez-Solana *et al.* (2015), 4.64% of Chan-Matú *et al.* (2020), and 7.50 of Chelliah *et al.* (2017) and lower than the 22.08% of Mbailao *et al.* (2014). High moisture contents decrease the quality and shelf-life period of dried leaves.

Moisture content in dried leaves depends on dehydration conditions (shade, direct light, and air stoves) and storage materials.

Ash

The range of ashes identified fluctuated from 7.53 to 10.86% among the accessions. These values showed significant statistical differences ($P<0.05$) in ash content in dried moringa leaves under subtropical climate conditions. Pérez-Ángel *et al.* (2020) reported 7.98 to 9.62% when evaluating moringa genotypes in Mexico. The values of 7.22% of Mbailao *et al.* (2014) and 9.3% of Díaz-Fuentes (2019) were also observed. Higher the 3.00 of Chelliah *et al.* (2017), 3.35% of Chan-Matú *et al.* (2020) and lower than 11% of ash Shih *et al.* (2011), 11.18% of Valdez-Solana *et al.* (2015) and 14.60% of ash Sanchez-Machado *et al.* (2010). Accession C2 presented 10.86% ash in dry leaves, and according to Valdez-Solana *et al.* (2015), values similar to 11% ash in dry leaves indicate high mineral content. The ash content represents a valuable source of minerals that can be used in human and animal nutrition and positively favor the organism's functions (Owo *et al.*, 2021). High ash content indicates the plant's ability to absorb minerals from the soil and channel them to the leaves (Guzman *et al.*, 2015). Another factor that can influence ash content in dry leaves is the year's season due to the translocation of minerals by temperature effect (Shih *et al.*, 2011). The macro- and micronutrients contained in moringa leaves could be used to address problems related to malnutrition and obesity in developing countries. Furthermore, in the case of animals, it can serve to minimize mineral deficiency (Miten *et al.*, 2017).

Lipids

Lipid content in leaves represents intense energy in food, and in the evaluated accessions, a range from 2.78 to 6.80% was observed for Y4 and C2 materials, respectively. This variation was statistically significant ($P<0.05$), representing a selection criterion for materials with higher lipid content. The range of values obtained in this research is similar to the 2.94% of Mbailao *et al.* (2014), 4.96% Sánchez-Machado *et al.* (2010), and 5.7 of Díaz-Fuentes, (2019), higher than the 1.90% of Chelliah *et al.* (2017) and lower than the 7.75 of (Shih *et al.*, 2011), 9.17 (Pérez-Ángel *et al.*, 2020) and 10.21 lipids (Valdez-Solana *et al.*, 2015). Lipids in moringa leaves represent an essential source of fatty acids such as myristic, palmitic, palmitoleic, palmitoleic, stearic, oleic, linoleic, linolenic, arachidic, eicosenoic, behenic, lignoceric (Fejér *et al.*, 2019).

Protein

Higher than 10.74 and 11.48% (Valdez-Solana *et al.*, 2015) and similar to 22.05% of Chan-Matú *et al.* (2020), 22.42% of Sánchez-Machado *et al.* (2010) and 22.8% Owon *et al.* (2021). Although some accessions exceeded 20% protein, they were lower than the 24.90-30.36% of Pérez-Ángel *et al.* (2020), 25.29% of Shih *et al.* (2011), 26.9% of Díaz-Fuentes, (2019) and 32.06% of Mbailao *et al.* (2014). Fejér *et al.* (2019) and Owon *et al.* (2021) reported 22.8% protein in dried moringa leaves. They identified amino acids such as tryptophan, valine, threonine, isoleucine, lysine, methionine, phenylalanine, histidine,

leucine, arginine, glycine, alanine, serine, aspartic, glutamine, tyrosine, cysteine and proline which are necessary for the proper functioning of the organism.

This work identified a considerable variation in the protein content of dried moringa leaves under homogeneous edaphoclimatic conditions. Therefore, the variation identified can only be attributed to the genetic constitution of each accession; this is in agreement with Miten *et al.* (2017), who identified differences in Proximal analysis when evaluating the accessions: green, reddish green, red and aromatic green present in Indonesia. The latter authors reported that the aromatic green accession presented higher protein and amino acid content when compared with the other accessions.

Dried moringa leaves represent a source of protein for people in developing countries who do not have access to nutritious and low-cost food (Mbailao *et al.*, 2014). Moringa leaves are an alternative for food fortification and enrichment or food supplements (Lamidi *et al.*, 2017). Materials with higher protein content can produce high-quality forage and facilitate animal meat production in areas where high-quality feed is deficient (Méndez *et al.*, 2018).

Correlation analysis

The Pearson correlation matrix between the evaluated variables is presented in Table 4. A positive correlation was found between basal diameter and the number of branches (r=0.68). A negative correlation was identified between basal diameter and protein content (r=−0.46).

CONCLUSIONS

Moringa accessions present an essential nutritional content that can be used to establish food banks with high nutritional content and little agronomic management. Evaluating the Proximal analysis of moringa accessions allows for taking advantage of the nutritional properties of the cultivars present in Mexico. The identification of elite materials facilitates the development of programs focused on selecting and reproducing this multipurpose species.

Table 4. Correlation matrix of variables recorded for 12 accessions of *M. oleifera* from different states of Mexico.

	Height	Diameter	Branch Number	Humidity	Ash	Protein	Fat
Height	1						
Diameter	0.51	1					
Branch number	0.36	0.68	1				
Humidity	0.23	0.21	0.26	1			
Ash	0.42	0.02	0.17	0.66	1		
Protein	−0.03	−0.46	−0.17	0.09	0.04	1	
Fat	0.13	−0.34	−0.09	0.39	0.58	0.56	1

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