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BIOCHEMICAL COMPOSITION AND PHYSICOCHEMICAL PROPERTIES OF *MORINGA OLEIFERA* SEED OIL

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Moringa oleifera tree has been recognized internationally for its nutritional, therapeutic and medicinal properties. Dry seeds are rich sources of oil with a high potential of commercial exploitation. The present study reports the physicochemical characterization, polyphenol content, DPPH radical scavenging capacity and fatty acid profile of moringa seed oil, and the chemical composition of the seed cultivated in Sonora, Mexico. Moisture, ash, protein and lipid contents in the seed were found to be 4.7, 5.8, 26 and 39%, respectively. The oil showed a refractive index of 1.4642. The saponification number was 183 mg KOH/g oil, iodine value: 75 g I/100 g of oil, acid value: 0.49 (% oleic acid). The polyphenol content was 0.137 mg of gallic acid equivalent/g and DPPH radical scavenging capacity was 87.39%. The moringa seed oil was rich (68%) in the major fatty acid, oleic acid (C18:1n9). Moringa oil extracted by sonication showed a fatty acid profile and physicochemical properties comparable to the oil from seeds grown in different regions of the world. The optimization of the oil extraction process on a large scale shows high potential, as the oil could be marketed as edible vegetable oil, for frying purposes, or as a functional ingredient.

Keywords*: Moringa oleifera* seed oil, fatty acids, polyphenols, DPPH, physicochemical parameters

The *Moringa oleifera* tree is native from northern India, and is widely spread in Pakistan, Afghanistan, Thailand, Philippines and Africa. In recent years, the tree has become naturalized in other regions, such as the United States, Mexico, Peru, Paraguay, Colombia and Brazil (Anwar & Bhanger, 2003). The agronomic benefits of this tree are: its rapid growth, its acclimation to various types of soils, and its adaptation to arid climates (Manzoor et al., 2007). This tree is also known as 'the tree of life' because all parts – leaves, flowers, immature pods, seeds and roots – are edible, and additionally have a high nutritional value (Freiberger et al., 1998). Parts of the tree are popular for use for medicinal and therapeutic purposes (Oliveira et al., 1999). The roasted seeds are used in snacks, like peanuts (Siddhuraju & Becker, 2003; Manzoor et al., 2007).

The seeds of moringa contain 38–42% oil, which is edible vegetable oil. The oil is also used in the production of soap and cosmetics (Tsaknis et al., 1999; Lalas & Tsaknis, 2002). This oil possesses physicochemical properties equivalent to those of olive oil, and contains high level of tocopherols (Tsaknis et al., 1999). In addition, a high proportion of oleic acid is found in the oil (Anwar & Bhanger, 2003). Some studies report that the lipid content, the fatty acid composition and the physicochemical properties of the oil varied widely depending on the species, environmental conditions and extraction method (Lalas & Tsaknis, 2002; Anwar et al., 2005). Different methods are utilized for the extraction of oil from the seeds of moringa, such as extraction by cold pressing followed by solvent extraction (Tsaknis et al., 1999; Lalas & Tsaknis, 2002), Soxhlet extraction (Anwar & Bhanger, 2003; Anwar et al.,

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2005), and extraction by enzymatic methods (Abdulkarim et al., 2005). Another alternative for the extraction of oil from the seeds is the method of sonication using non-polar solvents – hexane, petroleum ether or chloroform (Azuola & Vargas, 2007).

Some non-governmental organizations worldwide have promoted the cultivation of the moringa tree to combat malnutrition, especially among nursing mothers or infants (Fahey, 2005). The Children's Support Foundation (FAI-Sonora) in Sonora, Mexico has considered using the moringa tree as a nutritional alternative for low-income families in rural areas. Intensive campaigns have distributed samples of the moringa leaves, flowers and immature pods to promote consumption of the plant. The encapsulated dried leaves, rich in vitamins, minerals and various medicinal properties are currently being marketed for use as a food supplement.

The cultivation of this tree has been intensified in the arid areas of Northwestern Mexico. Since these plants generate a significant amount of seeds, the present research is focused on the extraction of the oil and the testing of the physicochemical characteristics. The idea is to generate a product with added value which can be marketed by regional producers. The aim of the research was to study the potential benefits of the oil of the moringa seed: the physicochemical parameters, fatty acid profile, phenolic compounds, and the DPPH radical scavenging capacity.

1. Materials and methods

1.1. Materials

The seeds of the moringa were collected from plants in the state of Sonora in Northwest Mexico. Three samples of seeds were harvested and stored in a dry environment without light. After manually removing the seed coat, the seed was coarsely ground, and the oil was immediately extracted. Fatty acid standards FAME MIX C8–C22, gallic acid monohydrate, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), and Folin-Ciocalteu reagent 2N were obtained from Sigma (St. Louis, Missouri, USA). Standard No. 3 PUFA was obtained from Supelco (Bellefonte, Pennsylvania, USA). Methanol, sodium carbonate monohydrate, potassium iodide, sodium thiosulphate, sodium hydroxide, boric acid, sulphuric acid, phenolphthalein, glacial acetic acid, hydrochloric acid, hexane, chloroform, and ethyl acetate were of reagent grade and purchased from Monterrey (Monterrey, Nuevo Leon, Mexico). Anhydrous sodium sulphate was obtained from Fluka (Milwaukee, Wisconsin, USA), while the toluene and potassium carbonate were purchased from Riedel-de Haën (Hannover, Niedersachsen, Germany), and selenium mixture was purchased from Merck (Darmstadt, Hessen, Germany).

1.2. Methods

1.2.1. Oil extraction. During the extractions of seed oil by sonication two alternative solvents, hexane and a mixture of chloroform: methanol (2:1), were tested in independent trials. Samples consisted of 20 g of ground seeds in an Erlenmeyer flask with ground glass stopper. Three extractions with 80 ml of extraction solvent were performed by sonication allowing 20 min for each extraction. The above mixture was elutriated for 1 h, then decanted and filtered to remove any remaining solids. The oil was then recovered using a rotary evaporator (Büchi, Model B-481) with a hexane temperature of 90 °C and 70 °C for the mixture of chloroform:methanol (2:1). Finally, the residual solvent in the oil was removed by oven

drying at 60 °C for 5 h. The solvent-free oil was stored in amber containers under dry and dark conditions until analysed.

1.2.2. Proximate analysis. Moringa seeds and the oilseeds residues (meal) were analysed for moisture content by drying at 110 °C in a convection oven (Fisher Model 281). Crude protein was determined using the micro-Kjeldahl method (Labconco, Model 652), and a conversion factor of 6.25 was applied. Total ash was determined gravimetrically by incineration at 550 °C for 8 h in a muffle (Thermolyne, Model 6000). Total lipids were analysed by the Soxhlet method using hexane as solvent. All procedures were performed according to A.O.A.C. (1984).

1.2.3. Physicochemical analysis of crude oil. Refractive index, peroxide value, saponification value iodine value, and acidity were determined according to A.O.C.S. (1989) Cc 7-25, Cd 8-53, Cd 3-25 Cd 1-25 and F 9a-44, respectively.

1.2.4. Fatty acid composition. Fatty acid methyl esters were prepared by the method of Sánchez-Machado and co-workers (2010) and analysed by gas chromatography. Specifically, 150 mg of oil was weighed in a tube with screw cap and was treated with 2 ml of toluene and 3 ml methanolic HCl of 5%. This mixture was vortexed and placed into a water bath for 2 h, cooled to room temperature, followed by adding 3 ml K_2CO_3 of 6% and 2 ml of toluene, and then agitated in the vortex. The mixture was centrifuged for 5 min at 340 g (Compact II Centrifuge, Clay Adams, USA), the organic phase was separated and dried with Na_2SO_4 anhydride. The organic phase was filtered through a 0.45 µm membrane. The sample was injected into the GC system with flame ionization detector, capillary column (CP-Sil 5 CB), and autoinjector CP-8410, all from Varian Inc. (Palo Alto, CA, USA). The injection volume was 1 µl (at 220 °C), the carrier gas was helium (1 ml min^{-1}), and the detector temperature was maintained at 235 °C. The column temperature was held at 120 °C for 1 min, then was increased to 170 °C at a rate of 3 °C min^{-1} and held for 1 min at this level, and finally was increased to 235 °C at a rate of 6 °C min^{-1}, which was kept for 5 min. Identification of peaks were based on the comparison of retention times to the standards. All samples were analysed in duplicate. The area of the peaks was quantified using the software Galaxie Workstation (Varian Inc., Palo Alto, CA, USA). The relative amount of each fatty acid (% total fatty acids) was quantified by integrating the area under the peak and dividing the result by the total area for all fatty acids.

1.2.5. Total polyphenols and DPPH assay in crude oil and hydrophilic extracts. The hydrophilic extracts were prepared according to the method proposed by Minioti and Georgiou (2010). A 0.5 g of oil was weighed and dissolved in hexane (1:1). The extraction was performed with two portions of 0.5 ml of methanol:water (80:20). The two phases were separated by centrifugation for 5 min at 5000 r.p.m., the hydrophilic fraction was recovered. Finally the two hydrophilic extracts were mixed for analysis.

The amount of total polyphenols was determined by using the Folin-Ciocalteu reagent. Gallic acid was used as calibration standard. All reactions were carried out in darkness. Samples (50 mg oil; 200 µl hydrophilic extracts) were placed in amber glass vials with a screw cap and then 1 ml of 1 N Folin-Ciocalteu reagent was added. This solution was vortexed for 15 s, and left for 5 min at room temperature. Two millilitres of Na_2CO_3 in solution (20%, w/v) was added and the final volume of reaction mixture was adjusted to 5 ml. After 10 min, the absorbance was measured at 760 nm using a spectrophotometer (Thermo Fisher Scientific,

Genesys 10-S Model, Madison, WI, USA). A blank solution was prepared and measured under identical conditions, but without analyte. Results were expressed as mg of gallic acid equivalents/g.

DPPH radical scavenging capacity was determined according to the method proposed by Minioti and Georgiou (2010). This property was analysed in hydrophilic extracts (200 µl) and crude oil in different concentrations (25, 80, 120, 150, 180, 200 and 250 mg). A working solution of 0.1 mM DPPH radical was prepared in ethyl acetate, which shows an absorbance of 1.237 at 515 nm. To each sample 4 ml DPPH solution was added, then was stirred in vortex by 20 s and was left in the dark for 1 h. Subsequently, the absorbances at 515 nm were measured using a spectrophotometer (Thermo Fisher Scientific, Genesys Model 10-S, Madison, WI, USA). As positive control gallic acid (25 mg) was used. Results were expressed as DPPH radical scavenging capacity (%) = [(Ao–A1) / Ao]×100, where Ao=absorbance of control at 60 min, A1=absorbance of the sample at 60 min.

1.2.6. Statistical analysis. Three batches of seeds were utilized for oil extraction and all procedures were performed in triplicate. The SPSS for Windows (SPSS Inc., Chicago, IL, USA) was used for the descriptive statistical analyses, expressing all the data as the mean±standard deviation.

2. Results and discussion

The oil extraction from moringa seeds was performed by sonication with solvents. This extraction method is based on the acoustic waves that increase the interaction of the solvent with the solid surface by a mechanical phenomenon called cavitation. The advantages of sonication are that it reduces the time of oil extraction and the process is carried out at low temperatures. Azuola and Vargas (2007) report that with this procedure the oil extraction from the seeds is more efficient when compared to traditional methods. The extraction yield of oil was calculated by measuring the oil content by the Soxhlet method in moringa seed and in oilseeds residues remaining after oil extraction. The percentage of oil extracted was of 93% with hexane and 91% with the mixture chloroform/methanol (2:1). It was observed that the type of solvent had no significant effect on the extraction yield. However, solvent recovery process was more viable when hexane was used.

Oil extraction by sonication showed high yields and shorter extraction times, and the process is carried out at room temperature. The high efficiency of oil extraction from this study was similar to that obtained by Ogunsina and co-workers (2011), who managed to extract almost 100% of the oil in the moringa seed by cold solvent extraction.

2.1. Proximate composition

The ash, protein and lipid content in moringa seeds and oil seed residues (meal) are shown in Table 1. In the seeds, the major components (approximately 65%) are lipids and proteins. Moringa seeds cultivated in the state of Sonora (Northwest Mexico) had a 39% lipid content, which is similar to that reported for seeds grown in Pakistan (40.39%) (Anwar & Bhanger, 2003). The lipid content in Sonora is greater than the seed grown in Kenya (35.7%) (Tsaknis et al., 1999), Malaysia (30.8%) (Abdulkarim et al., 2005), and in the dry and irrigated regions of Pakistan (30% to 38%) (Anwar et al., 2006). The lipid content in the seeds analysed in the present study is comparable to sunflower seeds (35.7%) (Haro et al., 2007) but greater than soybeans (22.6%) (Minuzzi et al., 2007).

Table 1. Proximate composition of moringa seeds and oilseeds residues (%)[a]

Analysis	Moringa seeds	Oilseed residues (hexane extraction)	Oilseed residues (chloroform/methanol extraction 2:1)
Crude protein	26±0.09	43±0.28	38±1.83
Lipids	39±0.15	2.6±0.04	3.6±0.10
Ash	5.8±0.02	5.5±0.05	5.7±0.04
Total carbohydrate[b]	29	53	53

[a]Data expressed as the mean±standard deviation (n=3, by triplicate); [b]total carbohydrate=100–∑ (protein+lipids+ash)

The variations in lipid content in the seeds of moringa grown in different parts of the world are attributed to genetic and agronomic factors. In addition, Anwar and co-workers (2006) found that the decrease in seeds oil content may be due to the plant growth in areas with deficiency of water. Furthermore, the extraction of oil from the seeds of moringa is of great interest, because the seed oil has physicochemical properties equivalent to that of olive oil. The lipid content of oilseeds residues was less than 4%; which is an indicator of the efficiency of oil extraction by sonication.

The protein content of moringa seeds from the current study (26%) is higher than the protein content reported by Sánchez-Machado and co-workers (2010) in the edible parts of the tree with a content of 22%, 19% and 19% for leaves, immature pods and flowers, respectively.

However, the seed protein content is less than reported by Abdulkarim and co-workers (2005), Anwar and co-workers (2006), and Manzoor and co-workers (2007) with values of 38%, 31% and 30%, respectively. The ash content of the seeds of moringa ranged from 5.4% to 5.7%, values similar to other studies (Anwar & Bhanger, 2003; Abdulkarim et al., 2005).

Oil seed residues (meal) have a high protein content ranging from 37% to 43%. These residues draw the attention of animal feed formulation and have a peptide that has properties useful as a coagulant in water treatment (Liew et al., 2006; Bhatia et al., 2007).

2.2. Physicochemical analysis

Table 2 shows the results of the physicochemical analysis of oil extracted from the seeds of moringa. The saponification number is used to calculate the average molecular weight of the fatty acids present in the oil. The value obtained for the oil of moringa (183 mg KOH/g oil) in this study was comparable with those reported by Anwar and Bhanger (2003) (186 mg KOH/g oil), Anwar and co-workers (2006) (184 mg KOH/g oil), and Manzoor and co-workers (2007) (179 mg KOH/g oil) in the oil of *M. oleifera* cultivated in Pakistan.

The iodine value indicates the degree of unsaturation of the oils; this value is used as a quality parameter (Abdulkarim et al., 2007). Moringa oil showed a iodine value of 75 g of I/100 g of oil, this value is greater than those reported by other authors, ranging from 65 to 71 g of I/100 g of oil in the seed oil of moringa grown in other regions of the world (Anwar & Bhanger, 2003; Abdulkarim et al., 2005, Anwar et al., 2006; Manzoor et al., 2007).

Table 2. Physicochemical properties of moringa seed oil

Parameter	Value[a]
Saponification values (mg of KOH/g of oil)	183±1.90
Acidity (% as oleic acid)	0.49±0.08
Peroxide values (meq kg^{-1} of oil)	ND[b]
Iodine value (g of I/100 g of oil)	75±0.38
Refractive index (35 °C)	1.4642±0.01

[a]Data expressed as the mean±standard deviation (n=3, by triplicate); [b]not detected

The acidity (% oleic acid) evaluates the degree of oil hydrolysis, measured as free fatty acids, the oil analysed presented a value of 0.49%, which is similar to that obtained by Anwar and co-workers (2006), and lower than the value reported by Abdulkarim and co-workers (2005) in the moringa seed oil. The low acidity and the absence of peroxides indicate that the fatty acids in the oil do not exhibit significant oxidation.

The refractive index (RI) of oil is related to the degree of saturation; this value is distinctive for each type of oil and is indicative of its purity. The moringa seed oil analysed presented a RI of 1.4642, its value is comparable with that reported by Manzoor and co-workers (2007) of 1.4648 and differs from the value reported by Anwar and co-workers (2006) of 1.4581. The differences in oxidation stability, physical state, pattern crystallization, iodine value and saponification number of fats and oils are mainly due to its fatty acid composition (Coultate, 2007). The analysis of these indices is useful to know the origin and quality of oil and in establishing the conditions of storage.

2.3. Fatty acid composition

Table 3 shows the fatty acid composition of oil extracted from moringa seed. A total of 12 fatty acids were identified in the oil, of which about 80% are monounsaturated fatty acids. The fatty acid found in greatest proportion was oleic acid (C18:1n9), with percentages of 66% and 68% during the extraction with the mixture of chloroform/methanol (2:1) and hexane, respectively, followed by stearic acid (C18:0) with values of 9.86% and 10.36%, and vaccenic acid (C18:1n7) with 9.81%, 10.47%. The differences found in the fatty acid content with different solvents can be attributed to a variation in the polarity of the solvent used for extraction, which directly influences the extraction capacity of total lipids. According to its fatty acid composition, oils that contain high proportions of oleic fatty acid have been placed in the category of 'high oleic oils'. The high level of oleic acid of moringa seed oil is comparable to olive oil. High oleic oil, labelled as 'high demand' oil, has nutritional benefits, such as reducing the risk of cardiovascular disease. These oils also have high stability during cooking and frying (Abdulkarim et al., 2005). The fatty acid profile obtained in this study for moringa seed oil is similar to the reports by other authors (Lalas & Tsaknis, 2002; Abdulkarim et al., 2005, Anwar et al., 2006; Manzoor et al., 2007; Anwar & Rashid, 2007). Variations in the composition percentage of fatty acids can be attributed to the degree of seed maturity, variety, place of origin, and method of oil extraction. The fatty acid profile of moringa seed oil is comparable to olive oil in its content of oleic and stearic acid (Baccouri et al., 2008).

Table 3. Relative fatty acid contents of moringa seed oil (% total fatty acid content, dry basis)

Fatty acid	Fatty acid (% area)[a]	
	Hexane extraction	Chloroform/methanol (2:1)
Myristic acid (C14:0)	0.23±0.01	0.23±0.06
Palmitic acid (C16:0)	9.86±0.01	10.36±0.39
Palmitoleic acid (C16:1n7)	2.16±0.04	2.73±0.11
Margaric acid (C17:0)	0.08±0.02	0.09±0.03
Stearic acid (C18:0)	5.38±0.23	5.10±0.05
Oleic acid (C18:1n9)	68.01±0.83	66.24±0.84
Vaccenic acid (C18:1n7)	9.81±1.04	10.47±0.30
Linoleic acid (C18:2n6)	0.67±0.01	0.72±0.02
Linolenic acid (C18:3n3)	0.16±0.01	0.27±0.09
Arachidic acid (C20:0)	1.60±0.04	1.58±0.07
Eicosenoic acid (C20:1n9)	0.64±0.04	0.61±0.04
Behenic acid (C22:0)	1.32±0.03	1.88±0.01
Saturated	18.47±0.05	19.24±0.40
Monounsaturated	80.62±1.33	80.05±0.89
Polyunsaturated	0.93±0.01	0.99±0.09

[a]Data expressed as the mean±standard deviation (n=3, by duplicate)

2.4. Total polyphenols and DPPH assay in crude oil and hydrophilic extracts

The total polyphenol content and DPPH radical scavenging capacity in the oil and hydrophilic extracts are shown in Table 4. The oil from the moringa seed showed a higher content of total polyphenols (0.137 mg of gallic acid equivalents/g oil) than the hydrophilic extract (0.063 mg of gallic acid equivalents/g oil). The oil showed a DPPH radical scavenging capacity of 87%, when using a concentration of 250 mg of oil. This value is similar to the one obtained with 25 mg gallic acid standard of 89%. The DPPH radical scavenging capacity was lower in the hydrophilic extract (13.44%) than in the crude oil. Similar results were obtained by Minioti and Georgiou (2010) in hydrophilic extracts and olive oil. The total polyphenol content of moringa seed oil is lower than that reported by Ogbunugafor and co-workers (2011) for moringa seed oil (40.17 mg of gallic acid equivalents/g).

Table 4. Total polyphenols and DPPH assay in crude oil and hydrophilic extracts of moringa seed oil

Sample	Total polyphenols (mg GAE[a]/g of oil)	DPPH radical scavenging capacity (%)
Crude oil	0.137±0.001	87.39±1.11[b]
Hydrophilic extracts	0.063±0.004	13.44±2.65[c]
Gallic acid	NA	89.90±2.04[d]

[a]GAE: gallic acid equivalents; [b]250 mg of oil; [c]200 µl of hydrophilic extracts; [d]25 mg of gallic acid. NA: not applicable

Figure 1 shows the DPPH radical scavenging capacity at different concentrations of the moringa seed oil. Increasing the oil concentration increases the ability of DPPH radical reduction ranging from 31% (25 mg oil) to 87% (250 mg oil). This behaviour is similar to that reported by Minioti and Georgiou (2010) and Ogbunugafor and co-workers (2011).

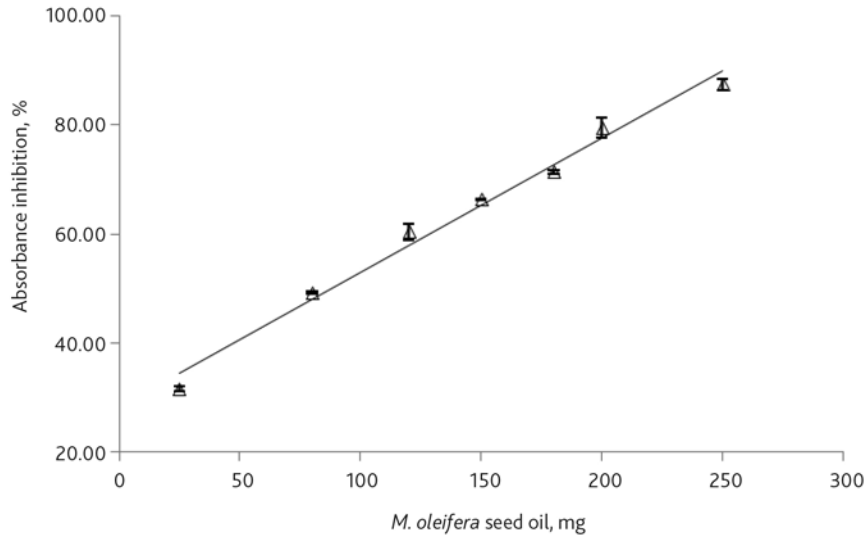

Fig 1. DPPH radical scavenging capacity at different concentrations of moringa seed oil

3. Conclusions

The extraction of oil from the seed of moringa by sonication is a viable alternative in using this natural product. The extraction method used at laboratory level generates high yields, shortens extraction times, and has an acceptable sample/solvent proportion. Moringa seed oil in Northwest Mexico has a high potential for commercialization due to the physicochemical characteristics, fatty acid profile, and total polyphenol content, which are similar to moringa seed oil cultivated in other regions of the world. The moringa seeds are a rich source of oil with significant potential for application in the food industry.

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