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Biochemical studies on *moringa oleifera* seed oil

Abstract

The purpose of the present study was to examine the physico-chemical properties, fatty acids composition, unsaponifiable matter, tocopherols and phenolic content of *Moringa oleifera* seed oil from Al-Ahsa, Saudi Arabia. In addition, biological evaluation of this oil was determined. *Moringa* oil showed a better overall quality, its, acid, peroxide, iodine, saponification values. Sterol fraction was found rich in β-sitosterol (45.11), stigmasterol (19.20%), campesterol (16.90%) and Δ5-avenasterol (10.00%). The major fatty acids were identified as oleic acid (65.00%). These results strongly suggested in potential use *Moringa* oil as non- conventional seed crop for high quality oil.

Keywords: *moringa oleifera*, physico-chemical properties, fatty acids, biological evaluation

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Introduction

Moringa oleifera lam. (*M. olefera*), a small deciduous tree, is the most widely naturalized species of *Moringaceae* family and is commonly known as the horseradish or drumstick tree.[1] It is native in Asia Minor, Africa, the Indian subcontinent (Bangladesh, India & Pakistan),[2] and is also distributed in the Philippines, Cambodia, Central America, North and South America, and the Caribbean Islands.[1] The tree ranges in height from 5 to 12m and the fruits (pods) are around 50cm long. When mature, the fruit of *M. oleifera* became and has 10-50seeds inside. Fully mature dry seeds are round or triangular in shape and the kernel is surrounded by a light woody shell with three papery wings.[3] All parts of the *Moringa* tree-leaves, flowers fruits, and roots are edible and have long been consumed as vegetables[4] and used to treat many diseases such as abdominal tumors, hysteria, scurvy, paralytic attacks, helmintic bladder, prostate troubles, sores and skin infections.[5] The leaves are highly nutritious, which contain more vitamin A than carrots, more calcium than milk, more iron than spinach, more vitamin C than oranges and more potassium than bananas and more protein than milk and eggs.[6] Moreover, leaves of *Moringa* species are rich in various phytochemicals like carotenoids, amino acids, sterols, glycosides, alkaloids, flavonoids, moringine, moringinine, phytoestrogens, caffeoylquinic acid and phenolic compounds.[7] Fruits and seeds have been reported as a rich source of protein, essential elements (Ca, Mg, K and Fe) and vitamins (A, C, and E). The oil extracted from its seeds (Known as ben or behen oil due to the high behenic acid content) has a 38-40% yield and can be used as a food, a cosmetic, and a lubricant.[8] In African and some parts of Asia, Particularly India, the oil has been used for cooking purposes.[9] In recent years, considering the gap between demand and production of vegetable oils in many developing countries,[9] research focusing on the use of unconventional oilseeds as a source of vegetable oils has become important. There are some reports on the composition and characteristics of *M. oleifera* seed oil varieties from different countries of origin eg: India, Kenya, Malawi, Malaysia, Pakistan,[3,7,10,11] considering its prospect as an alternative vegetable oil source. The refined oil is clear, odorless and rancid-resistant. Seed biomass remaining after the oil extraction can be used as a fertilizer or flocculating agent for water purification.[12] The oil extracted from *M. oleifera* seeds is regarded as having a good commercial interest due to its physical, chemical and pharmacological characteristics.[13]

The objectives of this study were to determine physico-chemical, fatty acid composition, unsaponifiable matter and bioactive components of oil extracted from the seeds of *Moringa oleifera* trees. In addition, biological evaluation of oil was evaluated.

Materials and methods

Plant materials

Moringa oleifera seeds (PKM1, variety) were procured from local market of Al-Ahsa, Saudi Arabia. The seeds were air-dried at room temperature (25°C) for 1week.

Chemicals

All chemicals used were of analytical or HPLC grade from Merck (Darmstadt, Germany) or Sigma Aldrich (St. Louis, Mo, USA). Standards of sterols, tocopherol and phenolic compounds were obtained from FlukaChemie (Buchs, Switzerland).

Oil extraction

The oil from the seeds was extracted with n-hexane using the method described by Tsakins et al.[14]

Proximate analysis

The methods of the AOAC (2012) were used for proximate analysis. *Moringa* flower sample (5grams) was used for determination of moisture content by weighing in crucible and drying in oven at 105°C, until a constant weight was obtained. Determination of ash content was done by ashing at 550°C for 3hr. The kjeldah method was used to determine the protein content. The crude fiber content of the samples was determined by digestion method and the fat was done by Soxhlet extraction method. All determinations were done in triplicate.

Determination of the physico-chemical properties

The extracted *Moringa* oil was analyzed immediately for refractive index, color, acid value, peroxide value, iodine number and saponification number as described in AOAC.[15]

Oxidative stability

The oxidative stability was estimated by measuring the oxidation induction time, on a Rancimat apparatus (Metrohm CH series 679). Air

(20L/h was bubbled through the oil (5.0g) heated at 100°C±2°C, with the volatile compounds being collected in water, and the increasing water conductivity continually measured. The time taken to reach the conductivity inflection was recorded.[16]

Fatty acid composition

Capillary gas chromatograph (HP 6890) was used for the qualitative and quantitative determinations of fatty acids of the oil and reported in relative area percentages. Fatty acids were transesterfied into their corresponding fatty acid methyl esters by shaking a solution of oil (0.1g) in heptane (2ml) with solution methanolic potassium hydroxide (0.2ml, 2N). The fatty acid methyl esters were identified using a gas chromatograph equipped with DB-23 (5%-cyanopropyl-methyl poly siloxane) capillary column (60mx0.32mmX0.25µm film thickness) and flame ionization detector. Nitrogen flow rate was 0.6ml/min, hydrogen and air-flow rates were 45 and 450ml/min, respectively. The oven temperature was isothermally heated 195°C. The injector and the detector temperatures were 230°C and 250°C, respectively. Fatty acid methyl esters were identified by comparing their retention times with known fatty acid standard mixture. Peak areas were automatically computed by an integrator. All GC measurements for each oil sample were made in triplicate and the averages were reported.

Identification of unsaponifiable matter

The unsaponifiable matters of *Moringa* oil was analyzed by an Hp 5890 gas chromatograph equipped with FID detector and DB-5 capillary column (30m, 0.25mm (5% phenyl)-95% methyl polysiloxane, 0.25µm film thickness, 280°C temperature injector and 300°C temperature transfer line. The oven temperature was programmed as follows: initial temperature: 100°C for 2min, increase 10°C/min up to 300°C, and then hold for 20min. The carrier gas was N_2(2ml/min). The identification of the different compounds was performed by comparing of its relative retention times with those of authentic reference compounds.

Determination of total phenolic content

The levels of total polyphenols of fresh crude sidr juice (fruit and leaf) were determined according to the method of Gutfinger.[17] Caffeic acid was served as a standard compound for the preparation of the calibration curve.

Tocopherol analysis

Tocopherol (α, β and δ) analysis was performed using an HPLC system consisting of a L-6000 Merck-Hitachi high pressure ppm connected to an L-4000Merk Hitachi UV detector (Hitachi Instruments Inc., Tokyo, Japan) set at 295nm. Tocopherol contents were identified by comparing the retention times with those of pure standards as described by others.[7,18] AD-2500chromato Integrator (Merck, Darmstadt, Germany) was used for data acquisition and processing.

Animals

Twenty four day old male fisher rats were housed in individual cages and kept under controlled conditions of temperature and humidity with a light/dark cycle of 12-12hr. Diets and water were offered *ad libitum* for 49days food consumption was monitored weekly.

Biological evaluation

For the biological evaluation, three types of diets were prepared: a standard one, containing corn oil (control group), and the test diets containing the *Moringa* oil and olive oil. The animals were divided randomly in the groups of eight rats each: corn oil, *Moringa* oil and olive oil. Diets (Table 1) were prepared according to AOCA[15] and stored under refrigeration (0-4°C) for not more than seven days.

Biochemical determinations

After 49days, the animals were fasted overnight and anesthetized with ether for blood removal through the orbital plexus. Total cholesterol, HDL-cholesterols, triglycerides, albumin, total proteins, hemoglobin, trnasaminases, alkaline phosphatase (ALP), urea and creatinine were determined using the commercial kits (Labtest, Lagoa Santa, MG, Brazil).

Statistical analysis

All experiments and measurements were carried out in triplicate, and the data were suggested to analysis of variance (ANOVA). Analysis of variance and regression analyses were performed according to the MStat C and Excel software. Significant differences between means were determined by Duncan's multiple range tests. P values less than 0.05 were considered statistically significant.

Results and discussion

The results of the proximate composition of *Moringa* seeds are shows in Table 1. The moisture content of the *Moringa* seed was 4.90%. The *Moringa* seed had higher values in the ash, crude fiber and protein and carbohydrate contents. These values were higher than the values observed by Anwar et al.[11] for *Moringa* flour. The high protein content of these flour samples give an indication of their usefulness in human diet and as livestock feed. The *Moringa* seed had higher fat content of 45.00%. The value was higher than the value (42%) reported by Ogunsina et al.[19]

Table 1 Analysis of *moringa oleifera* seeds

Constituents (%)	*M. oleifera* seeds
Moisture	4.90±0.55
Oil	45.00±4.10
Protein	7.10±0.65
Fiber	5.30±0.61
Ash	31.65±2.90
Carbohydrate	10.95±1.15

Values are mean±SD calculated as percentage of dry weight for M. oleifera seeds, analyzed individually in triplicate.

Table 2 shows various physico-chemical characteristics of the extracted *M. oleifera* oil from Saudi Arabia. The values determined for iodine number (69.01gI/100g oil), refractive index at 25°C (1.4570), saponification values (183.20mgKOH/g oil). However, the color at yellow 35.00 (2.00Red) value. Color of the oils is mainly attributed to the presence of natural pigments which are extracted along with the oil during extraction and are effectively removed during the bleaching step of processing of oil. The value of acidity (0.60% as oleic acid) was considerably lower than *M. oleifera* oil from India.[20] Oils with lower values of acidity are more acceptable

for edible applications. The peroxide value (0.83meq.kg^{-1} of oil) which measure hydroperoxides of the oils, this value was lower than those of *M. oleifera* oils from Kenya,[21] and India.[20] The induction period (Rancimat:20L/h, 100°C±2°C) is an important feature which describes the oxidative stability of oil and fats.[4] The induction period of the investigated *M. oleifera* oil (10.50h) was comparable with those *M. oleifera* oils reported from Sindh,[4] but significantly lower than from India[20] and Pakistan.[22]

Table 2 Physico-chemical properties of *Moringa oleifera* oil

Properties	*M. Oleifera* oil
Refractive index (25°C)	1.4570±0.001
Color (Red unit)	2.00±0.33
Acidity (% as oleic acid)	0.60±0.09
Peroxide number (meq. O_2/kg oil)	0.83±0.13
Iodine number (gI/100g oil)	69.01±5.30
Inductions period (hrs)	10.50±1.00

Values are mean±SD for M. oleifera oil, analyzed individually in triplicate.

Phenolic compounds have been proved to be responsible for antioxidant activity on many vegetable seeds oils, it is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides.[23] Total phenolic compounds in the *M. oleifera* seed oil was (160.00µg/g). These values were well comparable to those reported in other *Moringa* species native to Kenya, Pakistan and Egypt.

The data for tocopherol analysis of the *M. oleifera* oil from Saudi Arabia are presented in Table 3. The levels of α, γ and δ-tocopherol in the oil were 150.00, 70.80 and 55.50µg/g, respectively. The content of α-tocopherol in the *M. oleifera* oil was in close agreement with the values reported for soybean, groundnut and palm oils.[24] The contents of γ-tocopherol in the *M. oleifera* oil was slightly lower than those of *M. oleifera* oil from Malawi[25] but considerably higher than those from India.[20] The concentration of δ-tocopherol was slightly lower than the values reported for *M. oleifera* oil from Kenya.[21] Literature revealed that α-isomer of tocopherol has greatest vitamin E potency, whereas, δ-isomer of tocopherol has greater antioxidant efficacy than either γ-or α-tocopherol.[21,24]

Table 3 Phenolic and tocopherol contents (ppm) of *Moringa oleifera* oil

Components	*M. oleifera* Oil
Total phenolic compound	160.00±8.90
α-tocopherol	150.00±7.50
γ-tocopherol	70.80±4.10
δ-tocopherol	55.50±3.15

Values are mean±SD for M. oleifera oil, analyzed individually in triplicate.

The sterols profile of *M. oleifera* oil is shown in Table 4. The sterol fraction of *M. oleifera* oil from Saudi Arabia mainly consisted of β-sitosterol (45.11%), stigmasterol (19.20%), campesterol (16.90%) and Δ^5-avenasterol (10.00%), together with small amounts of clerosterol, 24-methylene cholesterol, Δ^7-campestanol, Δ^7-avenasterol, stigmastanol and 28. isoavenasterol, cholesterol and brassicasterol. The contents of β-sitosterol, stigmasterol, campesterol and Δ^5-avenasterolin the present analysis *of M. oleifera* oil were rather comparable with the values for *M. Oleifera* oil reported from Kenya and India. The sterol composition of the major constituents of the investigated *M. oleifera* oil generally varied to those of most of the conventional edible oils.[24]

Table 4 Sterol composition of Moringa oleifera oil

Sterol (%)	*M. oleifera* Oil
β-sitosterol	45.11±3.11
Stigmasterol	19.20±1.33
Campesterol	16.90±0.91
Δ^5-avensterol	10.00±0.84
Clerosterol	1.20±0.19
24-methylene cholesterol	0.90±0.11
Δ^7-campestanol	0.66±0.09
Δ^7-avenasterol	0.53±0.10
Stigmastanol	0.49±0.07
28-isoavenasterol	0.30±0.01
Cholesterol	0.10±0.01
Brassicasterol	0.07±0.001

Values are mean±SD for M. oleifera oil, analyzed individually in triplicate.

Table 5 shows the fatty acids composition in *M. oleifera* oil. This oil was found to contain a high level of oleic acid (65.00%), palmitic acid (12.31%) and linoleic acid (16.00%), palmitoleic acid (2.10%) and stearic acid (5.10%), respectively. The content of principle fatty acid, i.e. oleic acid was well in line with that reported for *M. oleifera* oil from Kenya[21] and India.[20] High oleic acid in *Moringa* oil makes it desirable in the term of nutrition and high stability cooking and frying.[3] The unsaturated fatty acids are very important for the stability of oils because of the chemical reactions occurring at the double bonds. The rate of those oxidation reactions depend on the number of double bonds in the carbon chain. Therefore, *M. oleifera* oil with high proportion of oleic acid is more stable than the others. In addition, oleic is less susceptible to oxidation than polyunsaturated fatty acid from the linoleic acid. Another interesting fact is that considerable content of linoleic acid as an essential fatty acid in the *M. oleifera* oil may be provide high nutritional remuneration and render beneficial healthy effect on blood lipid, blood pressure and cholesterol contents[26] and it is preferred by industries when oil hydrogenation is required.

Table 5 Fatty acid composition of Moringa oleifera oil

Fatty acids	*M. oleifera* oil
C16:0	12.31±1.88
C16:1	2.10±0.15
C18:0	5.10±0.80
C18:1	65.00±5.13
C18:2	16.00±2.10
C18:3	0.30±0.01

Values are mean±SD for M. oleifera oil, analyzed individually in triplicate.

Table 6 shows sera AST activity of rats fed on *M. oleifera* oil, olive oil and corn oil. They were slight non-significant increase in the activity of AST during the whole experiment for rats fed on *M. oleifera* seed oil, olive oil and corn oil. The data in Table 6 for rat sera activities of ALT and ALP on investigated oils indicate similar results for AST enzyme activity. Table 7 indicates the changes of total lipid contents of rats administered *M. oleifera*, olive and corn oils. The results demonstrate that the administration of oil (*M. oleifera* and olive) used gradual significant decrease in the levels of total lipids. On the contrary, the administration of corn oil caused significant increase in total lipid content of rat sera.[27]

Table 7 shows the sera total cholesterol contents of rats fed on *M. oleifera*, olive and corn oils. The results indicate very little increases (corn oil) and decreases (*M. oleifera* and olive oils). Changes in the levels of low-density lipoprotein cholesterol (LDL-C) of rats administered *M. oleifera*, olive and corn oils are shown in Table 7. The results indicate that there were very little changes in the LDL-C levels of rats fed on diets containing investigated oils.

Table 7 shows the changes in high-density lipoprotein cholesterol (HDL-C) levels of rats fed on diets containing *M. oleifera*, olive and corn oils. The results demonstrate that the above-mentioned oils possessed very little change on the levels of HDL-C.

Table 8 shows the changes of urea and uric acid contents of rats fed on *M. oleifera,* olive and corn oils. The results show that the administration of oils induced very little change on the sera levels of urea and uric acid during the whole experiment.

The results of the present study were demonstrated that most of the characteristics and quality attributes of *M. oleifera* oil from Saudi Arabia are quite identical with those of other *Moringa* oils reported in the literature. Its fatty acids composition revealed that it also fell in the category of high oleic oils just like other *Moringa* oils. Also, contains high considerable amount of tocopherol and phenolic compounds similar to that found in olive oil and could be utilized in diet as a source of vegetable oil human consumption.

Table 6 Influence of M. oleifera oil on the activity of serum AST, ALT, and AP (IU/L) of rats

Blood withdrawal period (week)	Corn oil	Olive oil	*M. oleifera* oil
AST (IU/L)			
0	40.10±2.33	40.10±2.33	40.10±2.33
1	40.23±1.98	40.63±2.54	40.31±1.99
2	40.71±2.46	40.61±2.78	40.45±2.01
3	40.75±2.86	40.60±2.60	40.66±2.75
4	40.33±1.87	40.76±2.98	40.15±1.79
ALT (IU/L)			
0	44.00±3.11	44.00±3.11	44.00±3.11
1	44.15±3.23	44.20±3.25	44.16±3.56
2	44.80±3.81	44.36±3.67	44.40±3.39
3	44.85±3.80	44.76±3.51	44.81±3.78
4	44.46±3.25	44.52±3.49	44.64±3.54
AP (IU/L)			
0	79.00±6.77	79.00±6.77	79.00±6.77
1	79.31±6.54	79.23±6.91	79.19±6.86
2	79.43±6.31	79.35±6.86	79.39±6.97
3	79.67±6.87	79.72±6.76	79.86±6.78
4	79.80±6.90	79.85±6.09	79.81±6.18

Values are mean±SD for M. oleifera oil, analyzed individually in triplicate.

Citation: Basuny AM, Al-Marzouq MA. Biochemical studies on moringa oleifera seed oil. *MOJ Food Process Technol.* 2016;2(2):40–46. DOI: 10.15406/mojfpt.2016.02.00030

Table 7 Influence of M. oleifera oil on sera total lipid, total cholesterol, LDL-cholesterol and HDL-cholesterol (mg/dl) of rats

Blood withdrawal period (week)	Corn oil	Olive oil	*M. oleifera* oil
Total lipids (mg/dl)			
0	280.00±10.67	280.00±10.67	280.00±10.67
1	280.50±10.81	280.33±10.78	280.51±10.88
2	280.70±10.80	280.51±10.85	280.60±10.94
3	280.81±10.66	280.94±10.64	280.85±10.62
4	280.90±10.93	280.96±10.92	280.90±10.23
Total cholesterol (mg/dl)			
0	160.00±7.98	160.00±7.98	160.00±7.98
1	160.81±8.01	160.85±8.16	160.90±8.12
2	160.55±7.99	160.70±8.45	160.85±8.41
3	160.60±8.25	160.65±7.56	160.93±8.43
4	160.66±8.33	160.80±8.09	160.95±8.21
LDL-cholesterol (mg/dl)			
0	59.50±4.11	59.50±4.11	59.50±4.11
1	59.80±4.58	59.71±4.54	59.69±4.25
2	59.85±4.63	59.69±4.32	59.72±4.36
3	59.90±4.28	59.75±4.90	59.81±4.77
4	60.11±4.19	59.90±4.51	59.85±4.58
HDL-cholesterol (mg/dl)			
0	100.10±6.01	100.10±6.01	100.10±6.01
1	100.15±6.12	100.20±6.58	100.33±6.38
2	100.25±6.45	100.35±6.42	100.50±6.78
3	100.30±6.66	100.45±6.23	100.61±6.25
4	100.60±6.39	100.50±6.89	100.81±6.75

Values are mean±SD for M. oleifera oil, analyzed individually in triplicate.

Citation: Basuny AM, Al-Marzouq MA. Biochemical studies on moringa oleifera seed oil. *MOJ Food Process Technol.* 2016;2(2):40–46. DOI: 10.15406/mojfpt.2016.02.00030

Table 8 Influence of M. oleifera oil on the sera urea and uric acid levels (mg/dl) of rats

Blood withdrawal period (week)	Corn oil	Olive oil	*M. oleifera* oil
Urea (mg/dl)			
0	28.20±1.50	28.20±1.50	28.20±1.50
1	28.59±1.45	28.61±1.84	28.55±1.55
2	28.80±1.19	28.66±1.49	28.61±1.84
3	28.97±1.48	28.74±1.78	28.65±1.54
4	29.06±1.76	28.92±1.92	28.77±1.25
Uric acid (mg/dl)			
0	4.10±0.33	4.10±0.33	4.10±0.33
1	4.30±0.39	4.51±0.53	4.20±0.74
2	4.41±0.45	4.55±0.57	4.51±0.54
3	4.50±0.40	4.60±0.65	4.76±0.38
4	4.66±0.56	4.67±0.39	4.81±0.85

Values are mean±SD for M. oleifera oil, analyzed individually in triplicate.

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Conflict of interest

The author declares no conflict of interest.

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