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Investigation of the Physicochemical Properties and Fatty Acid Profile of Moringa Oil

Amin Mirani, Esmaeil Ataye Salehi*

Department of Food Science and Technology, Qu. C., Islamic Azad University, Quchan, Iran

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DOI: 10.48311/fsct.2026.84074.0*Corresponding Author E-
eatayesalehi@iau.ac.ir

ABSTRACT

Moringa oleifera is a native medicinal plant in the Sistan and Baluchestan Province, Iran. In the past, the oil extracted from its seeds was consumed by local residents. Considering the substantial importation of oils, the aim of this research was to evaluate the physicochemical properties and fatty acid profile of *Moringa oleifera* oil as a potential new source of edible oil. The oil was extracted from dehulled seeds using n-hexane. The fatty acid profile, melting point, iodine value, acid value, saponification value, sterols, tocopherols, and total phenolic content of Moringa seed oil were evaluated. Gas chromatography analysis of Moringa oil revealed higher amounts of unsaturated fatty acids compared to saturated fatty acids. Oleic acid (%71) was found to be the dominant fatty acid in *Moringa oleifera* oil. The melting point (°C), iodine value (g/100g), acidity (% w/w), and saponification value (mg KOH/g) of the oil were determined to be 14, 87.7, 1.5, and 194, respectively. The tocopherol content (mg/kg), total phenolic compounds (mg GAE/g) and total sterol content (mg/kg) in the oil were measured as 36, 0.35, and 1977, respectively. Based on the study conducted, Moringa seed oil, with its fatty acid composition and tocopherol content, exhibits similarities to olive oil and can have similar applications.

1- Introduction

Moringa oleifera is a tree adapted to drought conditions and native to tropical and subtropical regions of India and Pakistan (Odee, 1988). In Iran, this species is distributed across Sistan and Baluchestan and Hormozgan provinces over vast areas but at low density and is locally referred to as "Gazorkh" or Moringa oil. On average, Moringa seeds contain about 33% oil, 38% protein, 16% carbohydrates, 5% fiber, and 8% moisture. In addition to its nutritional applications, Moringa seed oil has been consumed in pharmaceutical, cosmetic, and personal care industries (Sadeghi, 2017).

The species grows in arid and semi-arid areas with an annual evapotranspiration exceeding 2000 mm and is usually observed at altitudes of 1000–2000 m above sea level (Gebauer et al., 2007). On a global level, *Moringa peregrina* grows in northeastern Africa and southwestern Asia, while in Iran, its habitat is limited to the moorland and mountains of the southeastern region (from Bashagard to the Pakistan border).

Various *Moringa* species have broad medicinal properties that contribute to their importance. Different parts of this plant, including leaves, roots, and bark, have been used in local medicine to treat various diseases, such as digestive disorders, superficial pimples, cold, skin eruptions, hypertension, seizures, neurological attacks, relapsing fever, dermatological diseases, and rheumatism. Additionally, roots, leaves, flowers, fruits,

and exudates of this species have been used for treating inflammation and cardiovascular diseases (Limaye et al., 1995). In general, Moringa seeds are rich in active compounds, which contribute to antimicrobial, anti-inflammatory, and antitumor activities (Makkar & Becker, 1997). Due to the limited research on Moringa oil, this study aimed to extract oil from Moringa seeds through proximity to n-hexane and evaluate the physicochemical properties of its crude oil.

2- Materials and Methods

Chemicals: All chemicals and solvents used in this research were purchased from Merck (Germany) with the highest purity grade.

Methods

Moringa Seed Preparation: Two kilograms of *Moringa peregrina* seeds were first collected from Fanuj, Sistan and Baluchestan Province. Damaged and low-quality seeds were then removed, and dehulling was performed using a dry process. Finally, the seeds were stored in a cool and dry place to prevent moisture uptake.

Oil Preparation: Oil was obtained through proximity to n-hexane (Organization, 2024) (ISIRI 37:2009). To this end, 20 grams of seed powder were placed in a filter, which was then positioned inside a Soxhlet apparatus. Approximately 200 mL of hexane was poured into the Soxhlet and was heated for

8 to 16 hours. After hexane recovery, the oil content was calculated as 57%.

Melting Point Measurement: The melting point was measured using the Iranian National Standard No. 78 (measuring the melting point by capillary tube method) (Research, 2011) (ISIRI 78:1999).

Iodine Value: Iodine value was measured using the Iranian National Standard No. 4888 (the Iodine value measurement by Wijs method for edible oils and fats) (Research, 2015) (ISIRI 4888:2000).

Acid Value: Acid index was measured according to Iranian National Standard No. 4178 (oil seeds, measurement of oil acidity) (Research, 2011) (ISIRI 4178:1998).

Saponification Value: The saponification value was measured according to the AOCS official method CD 3-25.

Fatty Acid Profile: To determine the fatty acid profile, fatty acid methyl esters (FAME) were first prepared based on Iranian National Standard No. 4090 (Research, 1997) (ISIRI 4090:1997). Thereafter, the fatty acid analysis was performed using gas chromatography, GC MASTER model, equipped with a flame ionization detector and a CP-SIL 88 column according to the AOAC 22/963

method. The column temperature was programmed from 100 to 180°C, with an injection volume of 0.2 µL and an injector temperature of 220°C (AOAC, 2003).

Tocopherols: Tocopherol content was determined according to Iranian National Standard No. 13670 (measuring alpha-beta-gamma, and delta tocopherols, using high-performance liquid chromatography (HPLC)) (Research, 2013) (ISIRI 13670:2013).

Phenolic Compounds: Total phenolic content was measured using the Folin-Ciocalteu assay as described by Velioglu et al. (Velioglu et al., 1998).

Sterols: Sterol content was measured following Iranian National Standard No. 9670 (measuring plant and animal oils, and specific and total sterols using gas chromatography) (Organization, 2013) (ISIRI 9670:2007).

3- Results

Physicochemical Properties of Moringa Oil:

Table 1 compares physicochemical indices between Moringa oil and commonly consumed sunflower, soybean, and olive oils.

Table 1 Comparison of physicochemical indicators of moringa oil with sunflower, soybean and olive oils

Determination	MP oil	Sunflower oil	Soybean oil	Olive oil
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Melting point (°C)	14	-17	-16	-6
IV (g/100g)	85.7	118-141	1249-139	75-94
Acidity (g/100g)	1.5	Max 0.1	Max 0.1	Less or equal 0.3
SV (mg KOH/g oil)	194	188-194	189-195	196-184

MP: Moringa peregrina; IV: Iodine value (g/100g); SV: Saponification value (mg KOH/g oil)

Fatty Acid Profile Table 2 compares the fatty acid profile of Moringa oil with sunflower, soybean, and olive oils.

Table2 The comparison of fatty acid profiles of Moringa oil with refined sunflower oil, soybean oil, and olive oil.

Fatty acids (%wt)	MP oil	Sunflower oil	Soybean oil	Olive oil
(C10:0)	0.043	-	-	-
(C12:0)	0.05	Max 0.1	Max 0.1	-
(C14:0)	0.155	Max 0.2	Max 0.2	Less or equal 0.03
(C16:0)	15.78	5-7.6	8-13.5	7-20
(C16:1)	3.47	Max 0.3	Max 0.2	0.3-3.5
(C17:0)	0.048	-	Max 0.1	Less or equal 0.4
(C18:0)	0.17	2.7-6.5	2.4-5	0.5-5
(C18:1)	71.8	14-39	17-30	55-85
(C18:2)	0.58	48-74	48-59	2.5-21
(C18:3)	2.43	Max 0.5	4.5-11	Less or equal 1
(C20:0)	1.93	0.1-0.5	0.1-0.6	Less or equal 0.6

(C22:0)	3.49	0.3-1.5	Max 0.7	Less or equal 0.2
(C22:1)	0.097	Max 0.3	Max 0.3	-
(C24:0)	0.59	Max 0.5	Max 0.5	Less or equal 0.2

Tocopherol and Phenolic Compounds of Moringa Oil

Table 3 compares the tocopherol and total phenolic compounds (TPC) in Moringa peregrina oil with commonly used oils, including sunflower, soybean, and olive o

Table 3

Comparison of Tocopherol and Total Phenol Content (TPC) In moringa oil with those in sunflower, soybean, and olive oils

Type of MP oil Compound		Sunflower oil	Soybean oil	Olive oil
Alpha-Tocopherol	36	Max 300	500 ¹	141-301
Beta-Tocopherol	ND	ND		<103
Delta-Tocopherol	ND	Max 300		<103
Gamma-Tocopherol	ND	ND		5.4-27.8
Total Tocopherol	36	Max 300		100-300
TPC (mg GAE/g)	0.35	0.447	0.144	0.793

1. means, alone or in combination. *MP*. Moringa peregrina, *ND*. Not detected

Sterols of Moringa Oil

Table 4 compares the sterol profiles in Moringa oil with those of commonly used

oils, including sunflower, soybean, and olive oils.

Table 4 Comparison of Moringa oil sterol compounds with sunflower, soybean, olive oils

Sterol type (%wt)	MP oil	Sunflower oil	Soybean oil	Olive oil
Stigmasterol	31.6	6.5-13	14.9-19.1	Less than campesterol
Campesterol	21.7	6.5-13	15.8-24.2	Less or equal 4
Brassicastrol	ND	ND-0.2	ND-0.3	Less or equal 0.1
β -sitosterol	15.7	50-70	0.6-47	Max or equal 93*
Δ 5-avenasterol	22.4	ND-6.9	1.5-3.7	-
Δ 7-avenasterol	1.21	3-7.5	1.0-4.6	-
Δ 7-stigmastenol	0.91	6.5-24	1.4-5.2	Less or equal 0.5
Total Sterol (mg/kg)	1977	2400-5000	-	Max or equal 1000

1. means, β -sitosterol includes: β -sitosterol + Δ -5-avonaastrol + Δ -5-23 stigmasterol + cholesterol + sitostanol + Δ -5-24 stigmasterol. MP. *moringa peregrina*, ND. Not detected

4- Discussion

Physicochemical Properties of Moringa Oil: The existence of some enzymes in unrefined oils can cause hydrolysis of triglycerides and generate free fatty acids. These enzymes enhance oxygen solubility and accelerate oil oxidation due to pro-oxidant properties (Khushtinat et al., 2005). Moreover, the initial free fatty acids act as catalysts for further fatty acid formation in oils. The low levels of free fatty acids indicate that the Moringa seed oil is free from rancidity and lacks lipase

activity, making it suitable for direct consumption without alkaline refining (Katayon et al., 2006; Ayton et al., 2012). This result is consistent with studies on the effect of the degumming process on the physicochemical properties and oxidative stability of Moringa oil (Sadeghi, 2017).

In a study by Sadeghi et al. (2015), crude Moringa oil was degummed using water and phosphoric acid, and its physicochemical properties and oxidative stability were measured and compared (Sadeghi et al., 2015). Gas chromatography analysis revealed that

oleic acid accounted for approximately 71% of Moringa oil fatty acids, and palmitic, stearic, and behenic acids accounted for about 24%. Degumming reduced peroxide value from 4 to 2.35 meq/kg, free fatty acids from 2.05% to 0.07%, saponification value from 190.11 to 180.55 mg KOH/g, and slightly decreased iodine value. Furthermore, degumming increased the viscosity and density of the resulting oil without affecting the refractive index. Additionally, the flash point of the crude oil increased from 115°C to 205°C due to degumming, but peroxide values were higher in crude oil than in the degummed oil. According to the results, the temperature increased from 3°C to 120°C, enhancing peroxide values in crude and degummed oils. At 80°C and 120°C, Moringa oil lost stability, and its peroxide value considerably increased. Furthermore, when crude and degummed oils were exposed to light, there was a gradual increase in the peroxide value in the first four days, followed by a sharp increase from day four to twelve. Moreover, the oxidative stability measured at 120°C using a Rancimat yielded 7.3 hours for crude oil and 8.9 hours for degummed oil.

Rezvani and Mohammadi (2018) extracted Moringa oil using Soxhlet solvent extraction. To this end, impurities were removed through the degumming of crude oil (Rezvani, 2018). The crude oil epoxidation was also performed using formic acid with concentrated hydrogen

peroxide. Physicochemical and thermal properties were assessed and compared for these three oils. The average crude oil was 45 weight percent (wt%). Results of degumming indicated that this process reduced saponification value and free fatty acids compared to crude oil (190.1 mg KOH/g and 2.05% in crude oil vs. 55.1 mg KOH/g and 0.7% in degummed oil). Comparison of crude, degummed, and epoxidized Moringa oil indicated that removing impurities in the oil degumming and epoxidation process enhanced thermal properties, including melting point, flash point, smoke point, pour point, and cloud point. Furthermore, the stability test results indicated that increasing temperature decreased viscosity for all oils. The stability of Peroxide value increased under light, aeration, and heat over time. At lower temperatures (70-80°C), peroxide stability gradually intensified, while higher temperatures increased production speed and decomposition of peroxides. Oxidative stability at different temperatures indicated that epoxidized oil had the highest stability of about 41 hours at 110°C, but the lowest stability of 6 hours was seen in crude oil at 130°C.

Fatty Acid Profile

The predominance of oleic acid in crude Moringa oil has been reported previously. Due to predominance of oleic acid in Moringa oil and its ability to reduce lipoprotein with a low density, this oil can

be classified as a functional oil (Sadeghi, 2017). Leone et al. (2016) studied the properties and applications of Moringa seeds and oil for human health. Rich in unsaturated fatty acids, sterols, tocopherols, and amino acid-rich proteins, Moringa seeds are important sources for nutritional and non-nutritional programs (Leone et al., 2016). Based on the studies of the Institute of Standards and Industrial Research of Iran (1997), Abdulkarim et al. (2007) compared the quality and stability of Moringa oleifera oil with high oleic acid with canola, soybean, and palm olein under frying (Organization, 2013, Abdulkarim et al., 2007). Results showed that Moringa oleifera oil with high oleic acid displayed superior stability over other oils. Furthermore, its fatty acid composition due to high oleic acid content was greatly similar to olive oil, thereby reducing cholesterol and cardiovascular diseases. These results were consistent with studies on Moringa seed oil properties by Lalas and Tsaknis (2002) and John Tsaknis (1998). (Lalas and Tsaknis, 2002, Tsaknis, 1998)

Tocopherol and Phenolic Compounds of Moringa Oil: The chemical profiles of oils, particularly the level of natural antioxidants, are important indices in forecasting the oil function in the frying process. Tocopherols act as antioxidants by trapping hydroperoxide intermediates and thus stopping the autoxidation reactions. Alpha-tocopherol (α -Tocopherol) has health benefits and

nutritional value for humans, but Gamma-tocopherol (γ -Tocopherol) has strong protective effects in seed components, such as fatty acids. The test results demonstrate that α -tocopherol in Moringa oil has a higher antioxidant activity than γ -tocopherol. Like γ -Tocopherol, Delta-tocopherol (δ -Tocopherol) showed minimal activity, less than that of α -Tocopherol. Therefore, α -Tocopherol shows the highest activity of tocopherol. The unsaponifiable matter is used as a criterion of quality and refinement control for edible fats, ranging from 0.5% to 2.5%. These matters comprise hydrocarbons, triterpene alcohols, carotenoids, phenolics, sterols, and tocopherols. In Moringa seed oil, the content of unsaponifiable matter is within the range of commonly used oils. This remarkable difference arises from higher levels of tocopherols in commonly used oils, which are a key component of the unsaponifiable matter.

Phenolic compounds have both antioxidant and antimicrobial effects. Phenols are, in fact, considered key factors in oxidative resistance in oils. Both tocopherols and phenolic compounds are rapidly depleted during the initial hours of frying. Therefore, they cause resistance to oxidation at early hours of heating (Jafari et al., 2015a; Azad et al., 2015a). The total phenolic content of Moringa oil was 0.35 mg/g, which is lower than that in other vegetable oils, such as olive oil. The presence of tocopherols and phenolic compounds enhances the oxidative

stability. These results were consistent with studies on the characteristics of Moringa seed oil by Lalas and Tsaknis (2002) and John Tsaknis (1998) in addition to studies on stability of olive oil by Mirrezaie and Sahari (2013) (Lalas and Tsaknis, 2002; Tsaknis, 1998; MIRREZAIE and Sahari, 2013) because monounsaturated fatty acids, particularly oleic acid, together with modest amounts of polyunsaturated fatty acids and the presence of natural antioxidants, such as tocopherols, carotenoids, sterols, and phenolic compounds, cause oil stability during storage.

Sterols in Moringa Oil

Sterols are recognized as bioactive anticancer agents. Moringa seed oil contains sufficient amounts of sterol compounds. According to gas chromatography analysis, campesterol, stigmasterol, delta-5-avenasterol, and beta-sitosterol are the most important sterols. Sterols also play a vital role in health due to their antioxidant properties. Beta-sitosterol and campesterol lower blood cholesterol levels. The sterol content of edible oils generally ranges from 0.3% to 2%, which sometimes exceeds 10%. These results are consistent with studies on the properties of Moringa seed oil by Lalas and Tsaknis (2002); Agrik (1999); and John Tsaknis (Lalas and Tsaknis, 2002; Tsaknis et al., 1999; Tsaknis, 1998).

5- Conclusion

The high concentration of essential and non-essential fatty acids in Moringa seed oil is an effective factor that improves its nutritional value. The role of essential fatty acids in human health is well-known, and Moringa oil is rich in minerals and non-essential unsaturated fatty acids, which effectively affect human health. Based on the presented data, Moringa seed oil consumption is scientifically beneficial, and it may serve as a high-quality, flavorful rival for other edible oils, such as olive and sunflower oil. Additionally, the Moringa tree is an economical and accessible source for oil production due to its unique biological characteristics, such as being wild, drought tolerance, special care requirements, long-term productivity under proper use, and the ability to grow in many regions of Iran. Therefore, Moringa seed oil is nutritionally competitive with olive, sunflower, and soybean oils, and also more cost-effective, especially when cultivated in densely planted orchards. With enough content of tocopherols (vitamin E), sterols, antioxidants, essential fatty acids (omega-6 and omega-3), palmitic acid, oleic acid, polyunsaturated fatty acids, and minerals, Moringa oil is both nutritionally and medicinally valuable. Low linolenic acid content (omega-3), oxidizability index, and a suitable iodine value demonstrate

the good stability of this oil. It is hoped that by promoting the use of this oil, which has long been intertwined with the culture, positive steps can be taken towards improving family health and the regional economy.

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مقاله علمی-پژوهشی

بررسی خواص فیزیکی شیمیایی و پروفایل اسیدهای چرب روغن گزروغن (مورینگا)

امین میرانی، اسماعیل عطای صالحی*

گروه علوم و صنایع غذایی، واحد قوچان، دانشگاه آزاد اسلامی، قوچان، ایران

چکیده

اطلاعات مقاله

گزروغن یکی از انواع گیاهان دارویی بومی استان سیستان و بلوچستان است که روغن دانه‌های آن در گذشته توسط روستاییان مورد استفاده خوارکی قرار می‌گرفت. با توجه به وارداتی بودن بخش قابل توجه‌ای از روغن، هدف از این تحقیق ارزیابی ویژگی‌های فیزیکوشیمیایی و پروفایل اسیدهای چرب گزروغن موجود در منطقه فرنوج استان سیستان و بلوچستان به عنوان منبع روغنی جدید بود. بدین منظور ابتدا روغن دانه‌های پوست گیری شده به روش مجاورت با هگزان نرمال استخراج و سپس پروفایل اسیدهای چرب، نقطه ذوب، ان迪س یدی، ان迪س اسیدی، ان迪س صابونی، استرول‌ها، توکوفرول‌ها و فنل تام گزروغن (مورینگا) ارزیابی شد. بررسی روغن مورینگا توسط دستگاه کروماتوگرافی گازی نشان داد که مقدار اسیدهای چرب غیر اشیاع آن از اسیدهای چرب اشیاع بالاتر بود. اسید اولئیک (٪۷۱)، اسید چرب غالب در گزروغن بود. نقطه ذوب این روغن (درجه سلسیوس)، اسیدیته (درصد وزنی)، ان迪س یدی (گرم/۱۰۰ گرم) و ان迪س صابونی (میلی گرم هیدروکسید پتاسیم بر گرم روغن) به ترتیب ۱۴، ۱/۵۴، ۸۵/۷۳ و ۱۹۴/۱۲ و ارزیابی شد. مقدار کل استرول‌ها (میلی گرم/کیلو گرم)، توکوفرول‌ها (میلی گرم/کیلو گرم) و ترکیبات فنلی تام (میلی گرم گالیک اسید/گرم) این روغن به ترتیب (۱۹۷۷، ۳۵/۱۶۵، ۰/۳۵) اندازه گیری شد. بر مبنای نتایج حاصل از این تحقیق از نظر ترکیب اسید چرب و توکوفرول‌ها شباهت زیادی به روغن زیتون دارد و می‌توان کاربردهایی مشابه آن داشته باشد.

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* مسئول مکاتبات:

eatayesalehi@iau.ac.ir