Iraqi Journal of Market Research and Consumer Protection
(2026) 18(1): 24-36.
DOI: http://dx.doi.org/10.28936/jmracpc18.1.2026.(3)
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المجلة العراقية لبحوث السوق
وحماية المستهلك

# STUDY OF THE PHYSICOCHEMICAL AND STORAGE PROPERTIES OF LOCALLY CULTIVATED *MORINGA OLEIFERA* LAM

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**Received 25/ 3/ 2024, Accepted 14/ 5/ 2024, Published 31/ 3/ 2026**



## ABSTRACT

**The study aimed to characterize the properties of *Moringa oleifera* Lam.The seed oil of Moringa was grown in the fields of the College of Agricultural Engineering Sciences – University of Baghdad in summer season 2022-2023. The total composition of the seeds was estimated, and it was noted that the oil content in the seeds was high at 38.11%. The total fatty acid content of the oil extracted by the cold pressing method was estimated, the oleic acid was characterized as a high content of fatty acid, at 72.38%, followed by stearic acid, at 6.27%. Palmitic acid (6.17%). The antioxidants activity of Moringa were estimated as the total phenolic content (119 ppm), such as sterols, tocopherols and carotenoids at (1700, 88 and 17) ppm, respectively. Whereas the proportions of glycosides, tannins and saponins were (4.22, 5.88, 5.89) % respectively. Some physical properties of the oil were estimated at a temperature of 25°C. The refractive index value was 1.499, the specific gravity was 0.9238, and the melting point of the oil was 12.9°C. In addition the chemical properties of the extracted oil, the iodine value free fatty acids peroxide value and thiobarbituric acid were (120.8, 0.16,0.24 and 0.051) meq/kg oil, respectively, of TBARS (milligrams of malonaldehyde per kilogram of sample). The results of the antioxidant test showed that *Moringa oleifera* seed oil was higher thanascorbic acid in scavenging free radicals (Diphenyl-2-picrylhdrazyl DPPH). Finally,the oil was high stability through the Rancimat test was more than 5 hours at a temperature of 200 degrees Celsius., respectively. Thus, *Moringa oleifera* seed oil was the most important strategic oils that can be used to provide a healthy source of oil is was included in sustainable development programs after the success of its cultivation in many parts of the world, including Iraq, to ensure food and health security.**

**Keywords:** Physicochemical properties, *Moringa oleifera* Lam. Oil, Oleic acid, DPPH, Rancimat.

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# دراسة الصفات الفيزيوكيميائية والخزنية لزيت بذور المورينجا اوليفيرا (*Moringa oleifera Lam*) المزروعة محليا

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**الخلاصة**

**هدفت الدراسة الى توصيف خصائص زيت بذور المورينجا اوليفر(*Moringa oleifera* Lam.) المنزرعة في حقول كلية علوم الهندسة الزراعية– جامعة بغداد للموسم الصيفي 2022-2023 وقدرت نسب المكونات الكلية للبذورولوحظ ارتفاع نسبة الزيت في البذور بلغت 38.11%، قدرت نسب الأحماض الدهنية الكلية للزيت المستخلص بطريقة العصر البارد وتميز بارتفاع نسبة حامض الأوليك وبلغت 72.38 % يليه حامض الستياريك الذي بلغت نسبته 6.27 % وكانت نسبة حامض البالمتيك 6.17%, وتميز الزيت المستخلص باحتوائه على العديد من مضادات الأكسدة الطبيعية حيث بلغت اجمالي الفينولات الكلية (119 ppm) والستيرولات 1700 ppmوبلغ كل من اجمالي التوكوفيرولات والكاروتينويدات 88 و17 ppmعلى التتالي , ونسبة كل من الكلايكوسيدات والتانينات والصابونين (4.22, 5.88, 5.89) %على التتالي.قدرت بعض الخواص الفيزيائية للزيت عند درجة حرارة 25° م وبلغت قيمة معامل الأنكسار 1.499 والكثافة النوعية 0.9238 وكانت نقطة انصهار الزيت 12.9 °م .اما الخواص الكيميائية للزيت المستخلص فقد بلغ الرقم اليودي 120.8 و نسبة الاحماض الدهنية الحرة 0.16 %وبلغت قيمة رقم البيروكسيد (0.24meq\kg oil ) وكانت قيمة حامض الثيوباربيتوريك (0.051 mg/kg of TBARS) . milligrams of malonaldehyde per kilogram of sample. واشارت نتائج فحص مضادات الأكسدة تفوق زيت بذور المورينجا اوليفيرا على حامض الأسكوربيك في كبح الجذور الحرة (Diphenyl-2-picrylhydrazyl DPPH) كما امتازالزيت بثباتية عالية من خلال فحص(Rancimat) لمدة تزيد على5 ساعات على درجة حرارة 200 درجة مئوية وبذلك يعد زيت بذور المورنجا اوليفيرا احد اهم الزيوت الاستراتيجية التي يمكن استعمالها في توفير مصدر زيت صحي يدخل ضمن برامج التنمية المستدامة بعد نجاح زراعته في مناطق عديدة من العالم ومنها العراق لضمان تحقيق الأمن الغذائي والصحي.**

**الكلمات المفتاحية :** الخصائص الفيزيائية والكيميائية، زيت المورينجا أوليفيرا لام، حامض الأوليك، Rancimat.,DPPH .

## INTRODUCTION

Moringa oleifera trees are perennial and fast-growing in most regions and climatic conditions. They have a unique ability to tolerate drought. Almost all parts of the tree are used to obtain essential nutrients. Moringa oleifera trees flourish globally in all tropical and subtropical regions **(Ibrahim & Ameen, 2017)**. Studies have shown that the leaves and pods of *M. oleifera* trees are the cheapest and reliable alternatives for good nutrition **(Ismet *et al.*, 2018).** M oringa Oleifera trees was agood source of nutrients which important for human health. In addition to their nutritional uses, they are also used in plant production, fertilizer manufacturing, water purification, and etc **(Sidney & Michael, 2015) (Abd, *et al.* (2023).** They have been used to produce natural food supplements and have been studied by researchers in many fields, including medicine and nutrition. The different parts of the tree (such as stems, leaves, seeds, bark, and flowers) have been used**. (Araújo *et al*, 2013)** It was used in the past to treat inflammation, infectious diseases, and disorders of the heart, liver, and kidneys. The use of plants for medicinal purposes has been linked to their possession of antioxidants, as they work to quench and suppress the formation of reactive oxygen species and

free radicals or inhibit oxidation processes inside the living body **(Govardhan Singh *et al*, 2013).** Moringa oleifera seeds have been used to treat eye diseases, headache, fever, snake and scorpion bites, warts, ulcers, gastritis, skin inflammations, bladder infections, scurvy, abdominal tumors, schistosomiasis, and to inhibit the growth of harmful bacteria. **(Hussin, *et al.*, 2022).** It is also used to treat ulcers, gastric inflammation, skin inflammations, bladder infections, arthritis, abdominal tumors, schistosomiasis, and to inhibit the growth of harmful bacteria **(Anwar *et al*., 2007)**.

The seeds of Moringa oleifera were of nutritional importance such as rich in proteins (30-35%) and oils (**Stohs, & Hartman, 2015**)., with oil content reaching (38-45)%. They are also rich in fiber and essential minerals for the human body, in addition to containing a reasonable amount of carbohydrates **(Elsorady, 2023).** The seeds of *Moringa oleifera* trees are considered a good source of edible oil, and this plant has been identified as one of the sources of vegetable oils (**Al-Taweel & Al-Anbari, 2019).** Furthermore, Moringa seed oil is rich in vitamins (Vit A, Vit D) and natural antioxidants such as flavonoids and glucosides **(Lalas & Tsaknis, 2002) (Hussin, et al., (2022).** It is used as a laxative and in the treatment of leprosy and ulcers. It also treats rheumatism, gout, causes of skin diseases, lupus, and bladder disorders **(Ariani *et al*., 2023).**

The seed oil of *Moringa Oleifera* is characterized by containing more than 75% of unsaturated essential fatty acids, while oleic acid constituting over 70% of its content**.** **(Asgari-Kafrani, *et al.* 2020**). Its oil is distinguished by its ability to counteract oxidative stress and oxidative degradation, making it ideal for nutrition, medicinal, and commercial uses **(Rong Liu *et al*, 2022).**

## MATERIALS AND METHODS:

Seeds of *Moringa Oleifera* were obtained from the fields of the College of Agricultural Engineering Sciences, University of Baghdad, for the seasons 2022-2023 when they reached full maturity. The seeds were separated from the dry pods and manually dehulled.

**Estimation of Total Components:** The total components of *Moringa Oleifera* seeds were estimated as follows:

**Moisture Content Estimation:** The percentage of moisture was estimated using the method outlined in (**A.O.A.C. 2016**), employing oven drying until a constant weight was achieved.

**Protein Content Estimation:** The Micro Kjeldahl method was used to estimate the protein content in the seeds, following the procedure described in, (**AOAC., 2017**).

**Fat Content Estimation:** The fat content in *Moringa Oleifera* seeds was determined using the Soxhlet fat extraction method, as per (**AOAC 2016)),** utilizing a Soxhlet fat extraction apparatus provided by Electrotherma**.( Al-Anbary, 2012).**

**Ash Content Estimation:** The ash percentage in the samples was determined by ashing the seed powder using a Muffle furnace according to the method outlined in (**AOAC 2016)**.

**Estimation of Fiber Content:** The percentage of fiber in the samples was determined according to the method outlined in (**AOAC., 2017)**.

**Estimation of Total Carbohydrate Percentage:** Total carbohydrates were estimated mathematically by subtracting the sum of the total components from 100, as mentioned in **AOAC (2017). (Zouboulis, *et al.*, (2023).**

**Extraction of Moringa Seed Oil:** The oil was extracted from dried Moringa seeds using the cold mechanical pressing method, as described by (**Al-Anbari & Ali 2022),** using a press machine provided by Oimaster, China.

**Estimation of Total Fatty Acid Percentages:** The total fatty acid percentages for *Moringa Oleifera* seed oil were determined using a Gas Chromatography-Mass Spectrometry (GC-MS) apparatus provided by Agilent Technology (Model: 7820-A).

Column Specifications:

- Length: 30 meters
- Inner diameter: 250 microns
- Column thickness: 0.25 microns
- Pressure: 11.933 psi
- Temperature: 250 degrees Celsius
- Helium gas was used as the carrier gas.

The spectra of the components were compared with the spectra of updated components stored in the library of standards and technology at the National Institute **(Uduman, *et al*., 2017; Al-Hayali, *et al.*, 2023).**

**Physical and Chemical Properties of Moringa Oleifera Seed Oil:** Refractive index (RI), Free Fatty Acids (FFA), Peroxide Value (PV), Iodine Value, Thiobarbituric Acid (TBA) value, Saponification value, and melting point were estimated according to (**AOAC 2016**).

**Quantitative Estimation of Some Active Compounds**: Total Phenolic Determination: Total phenolic compounds were estimated as described by (**Ali Khoddami *et al.* 2013).**

**Total Flavonoids Determination**: Total flavonoids were estimated according to (**Zangana & Al-Bahadly 2017) (Jahan, *et al.* 2018).**

**Total Tannins Content Estimation:** Tannins were estimated using the method mentioned in (**El-Geddawy *et al.*, 2019).**

**Total Saponin Content Determination:** Saponins were estimated as per (**Valentina *et al.* 2023).** Percentage of saponins = (W2 – W1 / Wt. of sample) X 100

**Total Glycosides Estimation:** Glycosides were estimated as described by (**Akinlabu, 2019).**

**Estimation of Free Radical Scavenging Activity (Diphenyl-2-picrylhydrazyl DPPH):** The free radical scavenging activity was estimated as described by (**Shamrad & Shaki 2019).** The percentage inhibition of DPPH was calculated using the following equation:

**DPPH scavenging effect (%) or Percent inhibition = (A0 - A1) / A0 × 100**

Where:

- A0 is the absorbance of the blank.
- A1 is the absorbance in the presence of the test sample.

**Examination of Antioxidant Resistance (Rancimat Test):** The stability of *Moringa Oleife*ra seed oil was examined according to (**Pedro *et al.*, 2013).** This test was conducted using a Rancimat 743 apparatus provided by Metrohm, which measures the stability and resistance

of oil to oxidation. Five milliliters of the sample placed in the apparatus tubes were exposed to a stream of air at a constant temperature (200 degrees Celsius) and air pressure (20 liters/hour). Secondary oxidation products that vaporize are transferred from the reaction vessel to the measuring vessel with an air stream, where they are absorbed in distilled water (measurement liquid). The electrical conductivity is observed; an increase in conductivity indicates an increase in vaporized compounds. The time required from the start of the test until the appearance of these compounds is noted and referred to as the Induction time, which indicates the stability of the oil before model failure **(Lucía Félix & Irwin, 2021).**

**Storage Stability Tests:** Periodic tests were conducted every 10 days over a duration of 90 days for Moringa Oleifera seed oil (*Moringa Oleivera*) to assess the Free Fatty Acids (FFA), Peroxide Value (PV), and Thiobarbituric Acid (TBA) value, as outlined by (**Geisa Pazzoti1 *et al.*, 2018).**

## RESULTS AND DISCUSSION

The results showed that the percentages of moisture, total ash, protein, total fat, fiber, and carbohydrates were (4.08, 3.25, 32.11, 38.11, 7.55, 14.1) % respectively. The results were consistent with those found by ((**Leone *et al.*, 2016)** and **Pareek *et al.* (2023).** The protein percentages were (31.4 and 31.7) %, fat (36.7 and 38.4) %, fiber (7.3 and 6.75) %, and carbohydrates (18.4) % for each of the researchers respectively. There was a relative difference in the percentage composition compared to what (**Barakat & Ghazal 2016**) found, as they reported moisture (7.5) %, ash (4.73) %, protein (35.54) %, fat (29.61) %, fiber (10.92) %, and carbohydrates (20) %. The results also contrasted with those reported by (Ijarotimi OS *et al*, (2013). ((Ashutosh Pareek et al 2023) where moisture, protein, fat, and carbohydrates were (10.6, 18.86, 13.35, 53.36) % respectively. This could be due to the high moisture content in the samples or differences in environmental factors.

**Table (1):** chemical Composition of *Moringa Oleifera* Seed.

| Component | | Percentage (%) |
|---|---|---|
| Moisture | | 4.08 |
| Total Ash | | 3.25 |
| Protein | | 32.11 |
| Total Fat | | 38.11 |
| Fiber | | 7.55 |
| Carbohydrates | | 14.1 |

Table (2) illustrates the percentages of fatty acids composing *Moringa Oleifera* seed oil. The results indicated that oleic, palmitic, and stearic acids could be consider the major components, which is consistent with findings of **Christos *et al.* (2023).** The percentage of oleic acid was

72.38%, palmitic acid was 6.17%, and stearic acid was 6.27%. This aligns with previous studies by **(Lalas** &**Tsaknis 2002)** and (**Obikili, 2010).**

Some biological activities of different fatty acids in Moringa seed oil have been observed, such as antioxidant activity attributed to palmitic and oleic acids **(Mohammed, *et al.*, 2003; Al-Anbari, 2013; Annamaria *et al.*, (2015) (Deusdelia, 2018)** mentioned a liver protective effect due to palmitoleic acid. Oleic acid has been found to have anticancer activity. The biological results obtained by researchers confirm that Moringa seed oil protects the liver from fibrosis (**Asgari-Kafrani *et al.*, 2020).**

**Table (2):** Fatty Acid Composition in Moringa Seed Oil.

| Fatty acid | Chemical Formula | Percentage% | Common Name | Commented |
|---|---|---|---|---|
| Palmitic acid | $C_{16}H_{32}O_2$ | 6.17 | hexadecanoic acid | Saturated |
| Stearic acid | C18H36O2 | 6.27 | Octadecanoic acid | Saturated |
| Arachidic acid | C20H40O2 | 3.85 | Eicosanoic acid | Saturated |
| Behenic acid | C22H44O2 | 5.91 | docosanoic acid | Saturated |
| Lignoceric acid | $C_{24}H_{48}O_2$ | 1.01 | Tetracosanoic acid | Saturated |
| Palmitolic acid | C16H28O2 | 1.13 | 9-hexadecynoic acid | Mono-saturated |
| Oleic acid | $C_{18}H_{34}O_2$ | 72.38 | cis-9-Octadecenoic acid | Mono-saturated |
| Linoleic acid | C18H32O2 | 0.47 | -9,12-Octadecadienoic acid | Poly-saturated |
| Linolenic acid | $C_{18}H_{30}O_2$ | 0.17 | 9,12,15-Octadecatrienoic acid | Poly-saturated |
| Gadoleic acid | C20H38O2 | 2.23 | cis-9-eicosenoic acid | Poly-saturated |
| Total Saturated Fatty Acids | | 23.21 | | |
| Total Monounsaturated Fatty Acids | | 73.51 | | |
| Total Polyunsaturated Fatty Acids | | 2.87 | | |

Table (3) illustrates the physicochemical properties of *Moringa Olei*fera oil. The results indicate that the physical properties of the oil (density and melting point) fall within the normal range for liquid oils **(Félix, & Donis 2021**). As for the chemical properties, the decrease in both the peroxide value and free fatty acids, along with the value of thiobarbituric acid, is evidence of the oil's resistance to auto-oxidation **(Al-Anbari, *et al.*, 2013).** All values are within the acceptable range for edible vegetable oil **(Laveena, & Chandra, 2018).**

**Table (3):** illustrates the physicochemical properties of Moringa Oleifera seed oil.

| physicochemical properties | Value |
|---|---|
| Saponification Number (Sapo) (g/mg KOH) | 192.6 |
| (Peroxide Value) P.V (Meq.100gm) | 0.24 |
| Acidity No. | 0.9541 |
| Free Fatty Acid (FFA)% | 0.16 |
| Thiobarbituric acid (TBA) (mg MDA/gm) | 0.051 |

| | |
|---|---|
| Refractive index (RI) (25◦C) | 1.499 |
| Density (25◦C) | 0.9238 |
| Iodine No. | 120.8 |
| Melting point | 12.9 |

Table (4) highlights that Moringa seed oil contains numerous active compounds (**Ahmed, & Saeed, 2013)**. Phenolic are a broad group of chemical compounds containing a phenol group (-OH) attached to the benzene ring**(.Bouzenna, *et al.* (2019).** These compounds encompass various classes of molecules such as flavonoids, simple phenols, tannins, stilbenes, and others (**Jahan *et al.*, 2018**). Sterols play a crucial role in cellular structure and body functions, with cholesterol being the most important sterol, vital for cell membrane structure and hormone production. Plant sterols, such as phytosterols found in vegetable oils, play a significant role in reducing cholesterol levels in the blood **(Rong Liu, *et al*., (2022) (Hafidh & Al-Anbari, 2023).**

Tocopherol (Vitamin E) is an antioxidant that plays a crucial role in protecting cells from damage caused by free radicals **Hussin, *et al* (2022).** Carotenoids are also considered antioxidants and have health benefits as they contribute to protecting cells from damage caused by free radicals. Some carotenoids are converted into vitamin A in the body, which is essential for vision, skin health, and the immune system **(Awad & Hind, 2016).**

**Table (4):** Active Compounds in Moringa Seed Oil.

| No | content | Ppm |
|---|---|---|
| 1 | Total phenolic | 105.8 |
| 2 | Total flavonoid | 72.58 |
| 3 | Total alkaloid | 14.89 |
| 4 | Total glycoside | 5.89 |
| 5 | Total saponins | 4.22 |
| 6 | Total Tannin | 5.88 |

Table (5) illustrates the assessment of antioxidant activity using DPPH to determine the oil's ability to donate hydrogen atoms to inhibit the resulting free radicals, commonly used in evaluating antioxidant activity **(Jahan, *et al*. 2022).** The comparison was made with vitamin C at various concentrations (30, 60, 120, 250, 500 ppm). The inhibition percentage for 30 minutes was recorded as follows: for Moringa seed oil, it was 22.56%, 48.97%, 71.25%, 84.56%, and 92.66%, respectively, compared to vitamin C, which exhibited percentages of 12.49%, 27.89%, 40.25%, 65.98%, and 80.55% in the same order. Moringa seed oil demonstrated higher inhibition capacity than vitamin C. These results suggest the potential use of Moringa seed oil as an effective natural antioxidant compound in dietary systems due to its richness in phenolic compounds, tocopherols, and carotenoids, which aligns with previous findings **(Ogbunugafor *et al.*, 2011) (Khan, *et al* 2015).**

**Table (5):** Comparing the antioxidant activity of different concentrations of Moringa seed oil with the same concentrations of vitamin C using the DPPH assay.

| Concentration (ppm) | Vitamin C | Moringa Seed Oil |
|---|---|---|
| 30 | 12.49 | 22.56 |
| 60 | 27.89 | 48.97 |
| 120 | 40.25 | 71.25 |
| 250 | 65.98 | 84.56 |
| 500 | 80.55 | 92.66 |

Table (6) indicating the high storage stability of Moringa seed oil through monitoring of free fatty acids, peroxide value, and thiobarbituric acid value. After 90 days of storage at room temperature (25±2°C), the values were (1, 4.4, 0.8) ml/Kg respectively (**Ijarotimi *et al.*, 2013**). This stability can be attributed to the presence of a high proportion of oleic fatty acid, which acts as a natural antioxidant, along with a good percentage of active compounds such as tocopherols and carotenoids. These findings are consistent with those reported by **Safa Bejaoui *et al.*, (2021)** and **Akintola *et al.*, (2022),** The increase in peroxide value during the storage period may be due to the oxidation of unsaturated fatty acids, yet the increase is slight and considered acceptable within the limits specified by the Iraqi standard for oils (1582), which sets the permissible increase in peroxide value at 10 milliequivalents per kilogram of fat.( **Jahan, *et al.*, 2022**). The researchers noted that the increase is natural throughout the storage period, although the results may vary depending on the type of oil and its storage stability, as mentioned by **(Leone, *et al.*, 2016)**. **(Pérez-Pérez, *et al.* 2020).**

**Table (6):** The changes in free fatty acids (FFA), peroxide value (PV), and thiobarbituric acid (TBA) over a 90-day storage period at room temperature (25±2°C).

| Chemical analysis | Duration (day) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 |
| FFA% | 0.16 | 0.2 | 0.32 | 0.38 | 0.4 | 0.48 | 0.53 | 0.62 | 0.7 | 0.8 |
| PV meq/Kg oil | 0.24 | 0.7 | 1 | 1.3 | 1.9 | 2.4 | 2.9 | 3.2 | 3.8 | 4.4 |
| TBA ml/Kg | 0.05 | 0.09 | 0.16 | 0.28 | 0.45 | 0.56 | 0.6 | 0.74 | 0.9 | 1 |

(Figure, 1) illustrates the resistance curve of *Moringa Ole*ifera seed oil to oxidation through the Rancimat test. It can be observed that the oil resisted oxidation after five hours of exposure to air at a temperature of 200°C. This resistance is attributed to the high proportion of natural antioxidants in Moringa seed oil, which is consistent with what was mentioned by (**Lalas & Tsaknis 2002) (García-Moreno, *et al* 2013).,** stating that Moringa seed oil exhibits high stability due to its high content of oleic acid, tocopherols, and carotenoids**. ( Zouboulis, *et al.* 2023)**

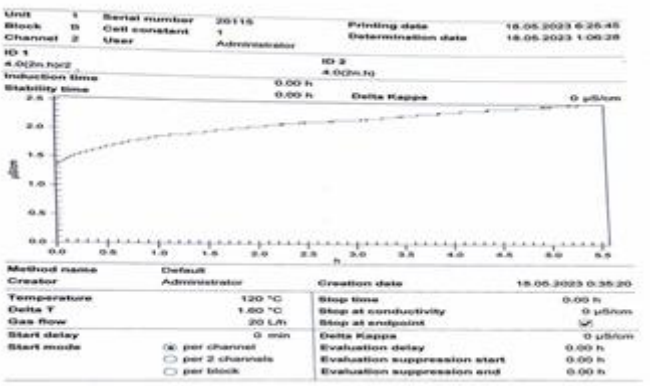


**Figure (1):** Shows the resistance of Moringa oleifera seed oil to oxidative rancidity.

## CONCLUSIONS

The study results emphasize the nutritional importance of *Moringa Oleifera* seeds due to their high protein and oil content. Its oil is considered one of the most valuable nutritional and therapeutic oils due to its high content of oleic acid and natural antioxidants, which give it high storage stability compared to other vegetable oils. It can be used as a food product that meets all the standards of the standard specifications for vegetable oils and can be considered among oils with high economic value. Additionally, it can be used in the pharmaceutical industries due to its content of fat-soluble vitamins, natural antioxidants, and essential fatty acids.

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