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Effects of Extraction Methods on the Physicochemical Characteristics, Fatty Acid Composition, Total Phenol Content, and Alpha-Tocopherol Levels of *Moringa Peregrine* Seed Oil

Saeed A. Asiri

Food Science & Nutrition Department, Faculty of Agricultural and Food Sciences, King Faisal University, Al-Ahsa, Al-Ahsa 31982; Saudi Arabia

Abstract

This study investigated the impact of various extraction methods on the physical and chemical properties, fatty acid profiles, total phenol content (TPC), and alpha-tocopherol levels in Saudi Arabian Moringa Peregrine seed oil. The seeds underwent extraction through solvent methods using hexane (SEH) or petroleum ether (SEP), as well as cold extraction (CE). Results revealed that oils obtained through solvent extraction exhibited superior physical and chemical characteristics compared to those from cold extraction. Acid values were 0.25 and 0.26% in SEH and SEP oils, respectively, whereas in CE oil, the value was 0.61%. Peroxide values were 6.12 and 6.05 mEq/kg in SEH and SEP oils, respectively, but increased to 8.04 mEq/kg in CE oil. Iodine values were 125.65 g I2/100 g in CE oil, whereas in SEH and SEP oils, they were 115.04 and 110.18 g I2/100 g, respectively. Solvent-extracted oil exhibited darker, greener, and yellower hues compared to the darker, redder, and yellower tones of CE oil. The fatty acid composition of the SEH oil sample notably differed from the other two samples, showing lower levels of oleic, palmitoleic, stearic, arachidic, behenic, and monounsaturated fatty acids. In contrast, the SEH oil contained higher concentrations of palmitic, linoleic, erucic, saturated, and polyunsaturated fatty acids compared to the other samples. Interestingly, the CE oil sample demonstrated slightly higher TPC and alpha-tocopherol content than the solvent-extracted samples. The physical and chemical characteristics of Moringa seed oil suggest potential nutritional advantages, possibly linked to its rich content of unsaturated fatty acids.

Keywords: *Moringa Peregrine*, Solvent extraction, Cold extraction, Physicochemical properties, Fatty acid profiles.

1. Introduction

Moringa Peregrine, commonly known as Arabic Moringa, is the second and most significant species in the Moringaceae family [32]. It is a tree that grows extremely quickly, reaching heights of 5 to 15 meters, with a leg diameter of 20 to 40 cm and a gray-green color. Arabic Moringa is grown in a number of countries, including the Kingdom of Saudi Arabia, Yemen, and Oman [12]. It is grown in the South Hijaz, Jabal FIFA, and Jeddah districts in the Kingdom of Saudi Arabia.

Moringa roots, flowers, bark, and trunk, as well as seeds, have been found to have antimicrobial properties in previous studies [17]. Moringa leaves also have diuretic, anti-inflammatory, antioxidant, anti-hepatitis, blood pressure, cholesterol, and blood

*__Corresponding author email:__ sasir@kfu.edu.sa.;Orcid ID/0000-0001-6515-2281

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sugar-lowering properties [39]. For this reason, *Moringa Peregrine* leaves can be consumed like vegetables and salads due to their high vitamin and mineral, low-fat, and high protein content. In traditional Chinese medicine, leaf extracts are applied topically to the skin to treat rashes, paralysis, and rheumatism and activate heart and blood circulation. According to[12; 46], seed oil is efficient against some harmful bacteria that contaminate food. Moringa seeds are consumed either raw, like peas, or toasted, like peanuts, or as a component of desserts. The oil extracted from them is utilized in cookery, salads, soap, and hair grooming, as a laxative or lubricant, and as a flavor stabilizer in the perfume industry [30] .

Moringa Peregrine seeds are of nutritional relevance since they have an oil production of 48.43%. Moringa oil has a bright yellow color and a pleasant flavor. It has a high concentration of oleic acid (> 73%) and 4.2% linoleic acid, a small quantity of unsaturated fatty acids, and consequently is particularly oxidation-resistant [52]. The significant antioxidant activity of moringa oil can be attributed to the oil's high concentration of vitamin *E* and phytosterols [21]. Given its fatty acid composition and bioactive component, moringa oil shows promising results as a functional food. Both mechanical and solvent extraction methods may be used to get oil from *Moringa Peregrine* seeds. In oil extraction, the goal is to maximize production without compromising oil quality. The oil yield rises as a result of the use of solvent extraction [43].

According to its physicochemical characteristics, the oil from moringa seeds has a promising future in human nutrition [7; 36]. According to [22], the oil derived from moringa is known as Ben oil and has physical and chemical qualities comparable to olive oil. It also includes many tocopherols and contains 70% oleic acid, a MUFA with an 18-carbon chain. Oleic acid has been employed in the food industry because it has superior oxidative stability compared to polyunsaturated fatty acids (PUFAs), allowing for extended storage and high-temperature frying processes [31]. The larger amount of unsaturated fatty acids in moringa seed oil may positively affect human nutrition [20].

Because *M. oleifera* is a vegetative species with high oil content (30–45%), studying its seeds is crucial. This oil has a number of well-known global applications, some of which include biofuel production more precisely, the production of biodiesel for use as lubricant and in cosmetics. These applications show out their enormous potential for cost savings. Monounsaturated and polyunsaturated fatty acids, which are crucial for the body's metabolism of lipids, are abundant in oil. *M. oleifera* oil, commonly referred to as oil of Behen, has a significant amount of oleic acid (about 70%). Oleic acid is a monounsaturated fatty acid that gives this oil a lot of durability against oxidation. Moringa oil is more stable than canola oil for this reason [55]. There is a need to investigate alternative sources of edible oil due to the growing demand and diminishing availability of oil extraction resources. Among the 13 species in the genus Moringaceae, Moringa Oleifera is the most well-known. In the advanced civilization, it held great significance. Edible oil was produced from the seeds by the Romans, Greeks, and Egyptians, who used it for skin lotion and perfume. Moringa plantations in the West Indies sold its oil to Europe in the 19th century for use in machinery lubricants and perfumes [28]. There is also the possibility of using moringa seed oil as a biofuel [47].

One plant species that has the potential to be significant in underdeveloped nations where hunger and malnutrition are big issues is *moringa peregrina*. The mature seeds of the plant are roasted or fried in Malawi, whereas the younger ones are consumed in India. The plant has the potential to be a significant crop in arid and semi-arid areas in the future because of its high nutrient content and ability to withstand severe droughts. Saudi Arabia is one of the natural distribution zones of the tree in the Middle East, along with Yemen and Oman [3; 23].The amount of research conducted on M. peregrina in Saudi Arabia over the past few decades has increased, resulting in a better understanding of the plant's ecology, distribution, and nutrient content in different plant parts, as well as its medicinal properties, threats to the plant, state of conservation, and necessary conservation action for long-term preservation and use [48].

This study was interested in discovering more about the physical and chemical properties of extracted moringa oil by different solvents, as well as its content of antioxidants, given the importance of moringa oil in terms of food and health.

2 Materials and Methods

2.1 Materials

Moringa Peregrine seeds were purchased from a Tabuk farm (2022). All the seeds examined and tested were known to farmers on the farm and were known in advance by the Ministry of Agriculture, Department of Agricultural Extension. Impurities have been removed from all seeds.

2.2 Chemical composition analysis of seeds

Moisture, ash, protein, oil, and fibers were determined by following the International Official Methods of Analysis [6; 40]. Carbohydrates were calculated by substrate all other major contents from 100, as follows:

Carbohydrate= [100 - (Moisture%+ Oil% + protein% + Fiber% + Ash%)].

2.3 Oil extraction

Two different techniques were applied to extract the oil from seeds, including cold extraction (CE) and solvent extractions by either hexane (SEH) or petroleum ether (SEP) [8; 14; 43].

2.3.1 Cold extraction

Seeds were cleaned and sorted to remove impurities before being washed in an air and water cleaning system and allowed to dry completely. In a specialized grinding mill, the seeds were combined, partially cracked, or crushed for hulling and then peeled before being transferred into the funnel of an automated oil press machine to begin the pressing process [13; 28]. Allow the produced oil to go out of the machine and collect it in a clean container to collect the oil and residue separately. Following extraction, the oil was filtered to remove any impurities. It was then collected in its containers and stored at room temperature (25 °C) until use.

2.3.2 Solvent extraction

Using hexane or petroleum ether solvents (40/60 (40/60 °C), the oil was extracted from seeds using the technique outlined by [14; 55]. Briefly, the seeds were thoroughly cleaned with distilled water and let to dry, and then 1000 g of the seeds were crushed and passed through a sieve to produce a soft powder. The powder was then dried in an oven at 100 °C for an hour. 60 g of the soft powder was weighed and placed in cellulose extraction thimbles and then placed in the Soxhlet (Foss SoxtecTM 2043 Based on Tecator TM Technology, Britain). The solvent was used to extract oil from seeds for 8–9 h. The solvent was then evaporated for an hour at 60 °C in the oven. The oil was then maintained at 25 °C in sealed vials for further examination. Each experiment included three replicates that were taken.

2.4 Analysis of physical and chemical properties

The peroxide number, iodine value, saponification value, unsaponifiable matter, refractive index, viscosity value, and acidity (free fatty acid contents) of the oil extract samples were analyzed using the International Official Methods of Analysis [9; 49].

2.5 Color determination

The device (Hunterlab, Hunter Associates Laboratory, Inc. Reston, VA. USA) measured the extracted oil's color. All determinations were completed following the manufacturer-supplied device catalog's recommended procedures. For the purpose of quantifying the color values, the L^* value (representing whiteness), a^* value (representing green), and b^* value (representing yellow) was determined [25].

2.6 Fatty acids composition analysis

The fatty acid composition of the moringa oils was determined using gas chromatography-mass spectrometry (CG-MS) analysis. The Moringa oil was hydrolyzed and then derivatized for the analysis in accordance with the method described elsewhere [15; 51]. The GC-MS (QP2010 plus; Shimadzu, Tokyo, Japan) system used to analyze the samples was equipped with an AOC20i autoinjector (split/splitless mode). The chromatographic separation was carried out using a

DB-5 column (60 m x 0.25 mm x 0.25 m). The oven's starting temperature was 50 °C for 2 min before gradually rising to 300 °C at a rate of 10 °C per min. The detector was maintained at 300 °C, while the injector was held at 280 °C. Helium was the carrier gas and was injected in a split (1:20) mode at a rate of 1.4 mL per min. For the data analysis, Shimadzu's GC/MS 2.6 software was employed. Utilizing the NIST spectrum library database and comparing the retention time and mass spectra of unknown peaks with those of the mix of fatty acid methyl esters (FAME) standard, the molecule was identified.

2.7 Analysis of total phenol content (TPC)

Extracted oil samples were analyzed for total phenol content (TPC) using a modified version of the method reported by Barakat and Ghazal [53]. In short, 10 grams of the extracted oil were combined with 50 mL of 70% (v/v) methanol. The mixtures were shaken for 100 min at 100 revolutions per minute in a dark bottle. The supernatant was collected after centrifugation at 3225 *g* for 10 min, and the residue was re-extracted twice with 25 mL 70 % methanol. All extracts were kept in the dark at -20 °C and analyzed as soon as possible (within 48 h) to prevent oxidation. The Folin-Ciocalteu spectrophotometry method was used to calculate the TPC of the samples in accordance with the method described elsewhere [34]. The total phenolic content was reported as the gallic acid equivalents per gram of sample (mg of GAE/g), which was calculated by comparing the measured values to a standard curve of produced gallic acid (GA) solution.

2.8 Analysis of *alpha*-tocopherols by High-performance liquid Chromatography (HPLC)

High-performance liquid chromatography (HPLC) apparatus (Shimadzu, Kyoto, Japan) equipped with a RF-10AXL fluorescence detector and two binary gradient pumps (Shimadzu LC-10AT), autosampler (SIL 20AC), column oven (CTO-20AC), and a communication bus module (CBM-20A) with valve unit FCV-11AL was used. One gram of oil was weighed, and 10 mL of heptane was added to the volumetric flask. The flask was then covered with aluminium foil to prevent oxidation [18; 24]. Then, 20 µL of the sample was injected into the HPLC instrument, and the elution peak was read using a UV-VIS detector set to 295 nm. The flow rate was set to 1.2 mL per min. Using a known concentration of alpha-tocopherol in a standard solution (Tocopherol, SIGMA-ALDRICH, USA) and the manufacturer-provided software, the estimated alpha-tocopherol concentration was calculated in the samples under investigation, assuming that the baseline is stable.

2.9 Statistical analysis

There were three replicates of each result. Tukey's test was used to do an analysis of variance (ANOVA) and determine the statistical significance of the effects on the results with a 95% level of confidence [16].

3 Results and Discussion

3.1 Chemical composition of moringa seeds

The analysis began immediately after obtaining the necessary samples and similarly drying and pulverizing. The chemical composition of *Moringa peregrine* seeds is presented in Table 1. The data shows that the Moringa seed had a moisture content of 5.06%, an ash content of 3.37%, a protein content of 18.83%, an oil content of 32.25%, a fiber content of 38.13%, a carbohydrate content of 2.34%. The seeds of Arabian Moringa plants growing in Jordan showed positive for 24.1% protein, 53.5% oil, 2.6% ash, and 2.4% moisture [38]. According to the results, the protein has a high concentration of arginine (15.3%), lysine (9%), glycine (8.4%), and proline (8.2%). Due to its high oil, protein, amino acid, fatty acid, and unsaturated sterol content, researchers have suggested using Arabian Moringa seeds in nutrition or feeding. The composition of Saudi-grown *Peregrine* seeds is similar to that reported by [45]. *Moring Peregrine* seeds have roughly 5% less protein and ash than *Moringa oleifera* seeds [44]. *Baraka*t and Ghazal [9] investigated the chemical composition of moringa seeds cultivated in different regions in Egypt, and they reported that protein was in the range of 34.51% - 36.5%, lipid (28.62% - 30.06%), fiber (10.92% - 12.16%), ash (4.22% - 5.06%), and total carbohydrate contents (19.00% - 20.29%).

On the other hand, there is a disagreement between this study and another on the same species of *Peregrine* on the oil content of the seeds. Reasons for these variations include but are not limited to, variations in extraction techniques,

harvest times, farming practices, and even genetic and environmental factors. Oil content in the seeds varied between 30% and 38% in one investigation of many *Moringa oleifera* genotypes in Pakistan [46]. The percentage of oil extracted from *Peregrine* seeds was reported to be between 49.8 and 57.25% by several sources [14; 55], 42.23 % from Egyptian-grown seeds by Abd El Baky and El-Baroty [18], and 25.1% by the cold extraction [28]. When *n*-hexane was used to extract the oil, the percentage rose to 38.3%, and when chloroform was used, the percentage rose to 41.4%. This is due to the difference between the solvents' ability to dissolve oil and the oil's dissolved substances [14].

Table 1: The chemical composition of *Moringa peregrine* seeds

Components (%)	***Moringa peregrine* seeds**
Moisture	5.06 ± 0.55
Protein	18.83 ± 1.25
Oil	32.25 ± 0.89
Ash	3.37 ± 0.15
Fiber	38.13 ± 2.19
Carbohydrate	2.34 ± 0.11

Results were presented as mean ± S.D (n = 3).

3.2 Physical and chemical properties of *Moringa peregrine* oil

Moringa peregrine seeds were extracted by two different methods: the first one was done by cold extraction (CE), and the second one was conducted by solvent extraction, either by hexane (SEH) or by petroleum ether (SEP). The percentage of oil extractions was 29, 40, and 44% in CE, SEH, and SEP oil samples, respectively.

When oil glycerides are decomposed or hydrolyzed as a result of exposure to moisture, heat, and/or the lipolytic enzyme lipase, free fatty acids are produced as a byproduct [26]. The acidity (% estimated as oleic acid) in CE was higher than in both the SEH and SEP samples, as shown in Table 2, which shows that there are significant differences (p<0.05) between the acidity numbers of CE oil extracted by pressure (0.61%) and SEH and SEP oil extracted by solvents (0.25 and 0.26%, respectively). Table 2 shows that there was little difference between the SEH and SEP oil samples. As the fraction of unsaturated fatty acids rises, the acid value rises along with it because of oxidation and hydrolysis processes [26]. In their physical analysis of Moringa oil, Saini et al [49] found that it had an acidity number of 0.7 mg potassium hydroxide per gram of oil. The acid number of peregrine oil is reported to be 0.01 mg potassium hydroxide per gram of oil [50].

The peroxide value of the oil, measured just after it was extracted, ranged from 6.05 to 8.04 mEq/kg oil, indicating that it had experienced some chemical degradation process. The rancidity process is represented by the peroxide value, with a greater peroxide value indicating a more advanced state of oxidation and lipid degradation [2; 21]. Oil with a high peroxide value is theoretically more susceptible to rancidity, which lowers the oil's overall quality [35]. The oil is more suited for long-term storage since oxidative and lipolytic activities are reduced at low peroxide values. Table 2 shows that when comparing the peroxide numbers of cold extraction oil and solvent extraction oil by either hexane or petroleum ether, there are significant differences (p<0.05) between the peroxide numbers of CE oil extracted by pressure (8.04 mEq/kg oil) and SEH and SEP oil extracted by solvents (6.12 and 6.05 mEq/kg oil, respectively). On the other hand, there was little difference between the SEH and SEP oil samples in terms of peroxide number (Table 2). According to research [20], *peregrine* oil has a peroxide number of 15.96 mEq/kg. Egypt-grown *Moringa peregrine* seed oil was reported to have a peroxide number of 0.01 mEq/kg [13].

The drying quality of the oil may be regarded as one of the parameters for oil classification; via the study of the iodine number, it can be classified as non-drying, semi-drying, or drying oil [29]. Table 2 shows that when comparing the iodine numbers of cold extraction oil and solvent extraction oil by either hexane or petroleum ether, there are significant differences (p<0.05) between the iodine numbers of CE oil extracted by pressure and oil extracted by solvents. The CE oil sample showed the highest iodine number (125.65 g I_2/100 g oil), followed by the SEH oil (115.04 g I_2/100 g oil), and the lowest iodine number was obtained for the SEP oil (110.18 g I_2/100 g oil), as listed in Table 2. According to its physical state, which includes being liquid at 25 °C and minimal aeration, the

iodine number for the current research ranged from 110 to 125 g I_2/100 g oil, suggesting that it is a semi-drying or drying oil [26]. The medium or high iodine number represents more unsaturated bonds; thus, the oil tends to go through oxidative rancidity. In contrast, when the oil has fewer iodine numbers (less than 100 g I_2/100 g oil), the low iodine number represents fewer amounts of unsaturated bonds, and thus, the oil has fewer tendencies to go through oxidative rancidity [26; 33].

Table 2: Physical and chemical properties of *Moringa peregrine* oil

Properties	CE	SEH	SEP
Oil moisture %	0.57±0.10	0.63±0.12	0.55±0.11
Acidity % (as oleic acid)	0.61±0.15	0.25±0.10	0.26±0.13
Peroxide value (mEq/Kg)	8.04±0.52	6.12±0.32	6.05±0.44
Iodine number (g I_2/100 g)	125.65±2.13	115.04±1.59	110.18±1.22
Saponification number (mg KOH/g oil)	79.09±1.11	72.04±1.89	81.10±1.55
Unsaponifiable matter (%)	4.92±0.59	3.94±0.43	3.73±0.26
Refractive index at 40 ˚C	1.476±0.10	1.469±0.11	1.471±0.10
Viscosity (CP)	108.32±2.31	106.65±3.11	105.07±2.85

Results were presented as mean ± S.D (n = 3).

According to Salah eldeen et al. [41], the iodine content of *Moringa oleifera* seed oil was between 67.2 and 71.1 g iodine/100 g, whereas that of *Moringa peregrine* seed oil was 67.73. *Moringa oleifera* oil's iodine number was reported to be between 65.46 and 66.83 [42], while commercial olive oil's iodine value was 80.01. *Moringa oleifera* and *Moringa peregrine* had iodine numbers of 74.7 and 71.0, respectively, whereas sunflower oil and olive oil had iodine numbers of 135.4 and 132.8, respectively [5]. Oil extracted from Egyptian-grown *Moringa peregrine* seeds was discovered to have a high iodine content (67.9 g iodine per 100 g [2]. High oxidative stability of seed fats of different Moringaceae species was also reported in the literature [33; 38].

By calculating the amount of alkali that has to be added to the fat in order to turn it neutral, the amount of free fatty acid contained in the oil may be approximated using the saponification value [26]. The tested oil's saponification value ranged from 72 to 81 mg KOH/g oil Table 2. The SEP oil sample showed the highest saponification number (81.10 mg KOH/g oil), followed by the CE oil (79.09 mg KOH/g oil), and the lowest saponification number was obtained for the SEH oil (72.04 mg KOH/g oil), as listed in Table 2. It can be noted that the saponification number of oil samples in this study was significantly lower than those reported in previous studies. For example, *peregrine* seed oil has a saponification number of 187.53 mg of potassium hydroxide per gram of oil, according to a study by Salah eldeen et al. [41]. Although the saponification number of *Moringa peregrine* oil was 182.9 [37], it was reportedly 185 [18] . A saponification number (179 mg potassium hydroxide / g oil) was reported for Egyptian-grown *Moringa peregrine* seed oil [18]. On the other hand, the percentage of oils and fats that are soluble in common fat solvents but are rendered unsaponifiable by caustic alkali is known as the unsaponifiable substance. Hydrocarbon, pigments, waxes, higher molecular weight alcohols, and sterols are examples of unsaponifiable materials that do not react with bases during the synthesis of soap [26]. The unsaponifiable matter was in agreement with saponification results. As listed in Table 2, the SEP oil showed the highest saponification number and the lowest unsaponifiable matter (3.73%), followed by SEH oil with 3.94% unsaponifiable matter, and the CE oil showed the largest percentage of unsaponifiable matter (4.92%).

Table 2 shows that the extracted oil samples all have a similar refractive index ranging from 1.469 to 1.476, with no statistically significant differences (p>0.05). These values are acceptable and can be attributable to the degree of unsaturation, the molecular weight, the length of the hydrocarbon chain, and the conjugation of the fatty acids involved [31]. However, there are statistically

significant (p<0.05) variations in oil sample viscosities. According to Table 2, the CE oil sample had the highest viscosity (108.32 cp), the SEH oil sample had the second-highest (106.65 cp), and the SEP oil sample had the lowest (105.07 cp). According to the literature [19], *peregrine* oil has a refractive index of 1.47 at 25 °C. The refractive index of oil extracted from Egyptian-grown *Moringa peregrine* seeds was reported to be 1.43 at 40 °C [1; 18].

Idris et al. [26] found that Soxhlet's *n*-hexane extraction of oil from *Moringa oleifera* seeds in Sudan yielded a bright yellow oil with a distinctive odor. Also, they reported the physicochemical properties of extracted oil as follows: refractive index at 25 °C: 1.447; iodine value: 96.6 g/100 g of oil; peroxide value: 7.6 mEq/kg of oil; free fatty acids: 0.07%; acid value: 1.4 mg of potassium hydroxide per gram of oil; saponification value: 185.2 mg of potassium hydroxide per gram of oil; unsaponifiable matter: 3.2%. According [25], all physical and chemical parameters of Moringa seed oil exhibited a significant difference (p<0.05) in the two-way interaction impact of location and extraction solvent, except for the refractive index.

3.3 Color values (*L**, *a**, *b**) of *Moringa peregrine* oil

There are statistically significant differences (p<0.05) between the *L** and *a** values of oil extracted by cold pressure and oil extracted by solvents, as shown in Table 3, which displays the degree of the color of *Moringa peregrine* oil using the device (Hunterlab, Hunter Associates Laboratory, Inc. Reston, VA. USA). According to Table 3, the *L** value was 8.32 for CE oil, 6.32 for SEP oil, and 5.45 for SEH oil. While the SEP and SEH oils exhibited negative *a** values, the CE oil showed a high value (6.32). Table 3 shows that SEH oil had the highest *b** value among the oils tested, followed by CE and SEP. The color measurement findings show that the oil samples studied exhibit a dark color when the *L** value is high, a color that is green when the *a** value is low, and a yellow color when the *b** value is high. Anwar et al. [5] found that the color of oil extracted from *Moringa oleifera* seeds cultivated in Pakistan using the solvent technique (hexane) was superior to oil produced from *Moringa peregrine* seeds grown in Saudi Arabia. The color of *Moringa peregrine* seed oil was shown to be 0.9 (red) and 50.0 (yellow) [26; 29]. Three different *Moringa oleifera* cultivars showed a range of 21–23 on the color scale from yellow to red [5]. According to Abdulkarim et al.[1], both solvent-extracted and enzyme-extracted *Moringa oleifera* oil had a red color index of (0.7), but the solvent-extracted oil had a significantly higher yellow color index (5.9) than the enzyme-extracted oil (3). The study found that organic solvents could dissolve some of the pigments in the oilseeds, which is why the oil extracted using solvents was more yellow than the oil extracted using enzymes. There was a statistically significant difference in all three Lab color indicators between moringa oils extracted with and without microwave pretreatment, as reported by [9; 26].

Table 3: Color values (*L**, *a**, *b**) of *Moringa peregrine* oil

Color values	CE	SEH	SEP
*L**	8.32±0.22	5.45±0.11	6.32±0.25
*a**	6.32±0.28	-1.02±0.18	-1.06±0.15
*b**	4.94±0.11	5.19±0.21	2.86±0.16

Results were presented as mean ± S.D (n = 3). *L** scale: Light vs. dark, where a low number (0-50) indicates dark and a high number (51-100) indicates light; *a** scale: Red vs. green, where a positive number indicates red and a negative number indicates green; *b** scale: Yellow vs. blue, where a positive number indicates yellow and a negative number indicates blue.

3.4. Fatty acids composition of *Moringa peregrine* oil

Long-chain hydrocarbon acids are the primary component of seed oils and are recognized as a key factor in differentiating the physicochemical characteristics of the seed oils. In this investigation, 18 different fatty acids—both saturated and unsaturated—were identified. Oleic, stearic, behenic, arachidic, *cis*-11-eicosenoic, palmitic, and palmitoleic acids are in order of increasing proportion (>1%) of fatty acids (Table 4).

There are clear variations between HE oil and the other two oil samples in most fatty acids, as shown in Table 4, which displays the proportion of fatty acids in *Moringa peregrine* oil. However, most fatty acids showed no discernible variation between CE and PEE oils. The table shows that oleic acid is the most abundant unsaturated fatty acid in *Moringa peregrine* seed oil, making up 77.51, 77.46, and 61.25% of CE, PEE, and HE oils, respectively. Other unsaturated fatty acids, including palmitoleic acid C16:1 with ratios (2.15, 2.16, and 1.49%), and *Cis*-11-eicosenoic C20:1 with ratios (1.41, 1.45, and 0.76%) for each of CE, PEE, and HE, respectively. Linolenic acid C18:3 was also discovered in CE and PEE oil samples but not in HE oil, as shown in Table 4. Overall, the percentage of unsaturated fatty acids in CE, PEE, and HE was 81.84, 81.76, and 80.01%, respectively. Based on the value of the oil's unsaturated fatty acids, it appears that the oil has been subjected to slight oxidation and hydrolysis in the current investigation. Storage conditions, in which air in the bottle comes into contact with the oil's surface, may play a role in this oxidation process, leading to oxidized triglycerides into peroxides and hydroperoxides. Consistent with previous research, Adel et al [2] found that oleic acid constituted the majority of the unsaturated fatty acids in *Maringa Peregrine* seed oil, with traces of other acids like C16:1, C17:1, and C20:1, and oil contained 83.5% unsaturated fatty acids. Anwar et al. [5] discovered that Oleifera seed oil had 73.47% unsaturated fatty acids, whereas [26] reported that *Moringa peregrine* seed oil contained 81.24% unsaturated fatty acids. Moringa oil is low in polyunsaturated fatty acids, notably C18:2 and C18:3, compared to other vegetable oils such as olive oil, palm oil, soybean oil, maize oil, and sesame oil, according to studies published previously [19; 21].

Except for the high concentration of C18:2 in HE oil compared to the other two oil samples, their findings were consistent with the findings of this investigation. Because of the high oleic acid content, these seeds may help decrease blood lipids, blood pressure, and cholesterol. However, The seed oil of M. conciseness grown in a drought hit area of Pakistan had higher content of C16 : 0 (11.04%), C20 : 0 (7.09%) and C22 : 0 (3.44%) than those of the oil of the present study [37].

On the other side, it is clear from Table 4 that saturated fatty palmitic acid (C16:0) is found in 16.5, 9.51, and 9.46% in HE, CE, and PEE oils, respectively, followed by stearic C18:0, where found in 3.9, 3.85, and 2.67%, and behenic acid C22:0, where was found in 2.36, 2.31, and 0.15% in PEE, CE, and HE, respectively. In total, the saturated fatty acid content was 20, 18.25, and 18.16% in HE, PEE, and CE, respectively. The results revealed that the overall concentration of saturated fatty acids in peregrine seed oil was 16.53%; the most abundant acid was saturated palmitic acid, followed by C18:0, C22:0, and C20:0. According to Salah Elden et al. [41], the total concentration of saturated fatty acids in Moringa seed oil was 18.76%. While the overall concentration of saturated fatty acids in oleifera seed oil was 14.12%, as determined by [5]. The content of C16:0 in peregrine oil in this study (16.5, 9.51, and 9.46%) for HE, CE, and PEE oils, respectively, was higher than that of oleifera seed oil, with values ranging from 2.5%, to 6.25%, as reported by [5]. For instant, these results are relatively close to those obtained by [1] and [5], for the Malaysian M. oleifera petroleum ether seed extract and the Pakistani *M.oleifera* hexane seed extract, respectively. The detected oleic acid may have favorable nutritional implications and may substantially contribute to the prevention of both cardiovascu lar disease (CVD) and cancer [21; 22].

Table 4: Fatty acids composition and their relative percentages of *Moringa peregrine* oil by GC-MS analysis

FAs composition	CE (%)	SEH (%)	SEP (%)
C14:0 (Myristic)	0.1	0.03	0.09
C15:0 (Pentadecanoic)	0.02	0.01	0.01
C16:0 (Palmitic)	9.51	16.5	9.46
C16:1 (Palmitoleic)	2.15	1.49	2.16
C17:0 (Heptdecanoic)	0.12	0.06	0.12
C17:1 (CIS-10Heptadecanoic)	0.07	0.08	0.07
C18:0 (Stearic)	3.85	2.67	3.9
C18:1n9t (Elaidic)	0.09	0.13	0.09
C18:1n9c (Oleic)	77.51	61.25	77.46
C18:2n6c (Linoleic)	0.53	15.42	0.45
C20:0 (Arachidic)	1.82	0.47	1.87
C20:1n9 (Cis-11-Eicosenoic)	1.41	1.06	1.45
C18:3n3 (A-Linolenic)	0.02	n.d	0.02
C21:0 (Henecosanoic)	0.03	0.03	0.04
C22:0 (Behenic)	2.31	0.15	2.36
C22:1n9 (Erucic)	0.03	0.56	0.03
C20:3n3 (Cis-11,14,17-Eicosatrienoic)	0.03	0.02	0.03
C24:0 (Lignoceric)	0.4	0.08	0.4
Saturated Fatty Acid	18.16	20	18.25
Unsaturated Fatty Acid	81.84	80.01	81.76
Monounsaturated Fatty Acid	81.17	63.68	81.17
Polyunsaturated Fatty Acid	0.58	16.2	0.5
Trans Fatty Acid	0.09	0.13	0.09

3.5 Total phenol content and alpha tocopherol of *Moringa peregrine* oil

Oil produced from *Moringa peregrine* seeds (EC, HE, and PEE) varies greatly in its total phenols concentration as gallic acid. According to Table 5, the TPC content of the oils varied widely, with the greatest being found in the CE oil (11.40 mg of GAE/g), the next highest being found in the PEE oil (11.20 mg of GAE/g), and the lowest being found in the HE oil (10.83 mg of GAE/g). According to Abd El Baky and El-Baroty [18], the value of total phenols in Peregrine oil was 48.31 mg/100 gm oil. This is because phenols have the ability to inhibit oxidation, making them responsible for the antioxidant activity noticed in many oil vegetable seeds. According to Al-Owaisi et al. [4], the highest concentration of phenols and flavonoids was found in oil extracted from Moringa seeds using a methanol solvent method (94.54 and 20.81 mg gallic acid/100 g), followed by ethyl acetate (81.26 and 8.39 mg gallic/100 g) and chloroform (75.53 and 6.55 mg gallic / 100 g). Barakat and Ghazal [10], reported that *Moringa oleifera* has a total phenol content and antioxidant activity of 16.9-18.5 mg GAE/g. TPC's antioxidant capability was positively correlated with its starting concentration, with a higher TPC eliciting a stronger antioxidant reaction.

Tocopherols (fat-soluble vitamins) stand out among the many antioxidant substances in moringa seed oil that give it its medicinal value. The most prevalent form of vitamin *E* in moringa seed is *alpha*-tocopherol, although the seed also includes *beta*- and *gamma*-tocopherols. In addition to protecting polyunsaturated fatty acids and other lipids in the body from oxidation, *alpha*-tocopherol has been linked to a reduced risk of cardiovascular disease [10] . Many researchers note that Moringa seed oil is a sustainable resource for liposoluble vitamins and a possible source of bioproducts of industrial importance [10; 54]. Table 5 shows that the value of *alpha*-tocopherol, extracted using either HE or PEE oils, was 0.120 mg/g, with no significant differences between the two solvents. However, compared to other extracted oils, CE oil has a much higher *alpha*-tocopherol concentration (0.131 mg/g) (Table 5). The total amount of tocopherols in Peregrine oil was calculated to be 20.35 mg/100 gm oil [18]. Tocopherols are a kind of antioxidant that

may be present in many oily plant seeds. Lalas and Tsaknis [33] observed that the *alpha*-tocopherol content of cold-extracted oil from *Moringa oleifera*, hexane, and chloroform-methanol differed from 5.06 to 15.38 to 2.42 mg/kg. The recorded total phenolic content was in close agreement with that reported by [11; 21] for the Indian Moringa hexane seed oil extract. Due to their diverse chemical structures, phenolic phytochemicals have the capacity to protect cellular components against oxidation [17]. But above all, polyphenols have been reported to prevent neurodegenerative disorders, cardio-vascular diseases and cancer [27]. However, the latter reported a higher content of Is it α-, β-, γ-, δ--tocopherol (75.67 mg/kg) and a lower amount of g-tocopherol (39.54 mg/kg) in comparison to that found in the Tunisian Moringa seed oil (10.36 mg/kg vs. 86.87 mg/kg, respectively [21]. According to[24] ,the a-isomer is the most biologically active, while the g-Tocopherol is deemed the optimal lipid oxidation inhibitor as it stabil-izes hydroperoxy and other free radicals. Briefly, the cold-pressed Tuni-sian Moringa seed oil is an ideal dietary source of total a-and g-Tocopherols [21].

Table 5: Total phenol content and *alpha*-tocopherol of *Moringa peregrine* oil

Content	CE	SEH	SEP
TPC (mg of GAE/g)	11.40±1.59	10.83±1.46	11.20±2.12
alpha-Tocopherols (mg/g)	0.131±0.09	0.120±0.06	0.120±0.04

Results were presented as mean ± S.D (n = 3).

4. Conclusions

The research findings emphasize how different extraction methods impact the quality characteristics of Saudi Arabian Moringa Peregrine seed oil. Specifically, solvent extraction techniques, notably employing hexane and petroleum ether, produced oils with superior physical and chemical attributes in comparison to cold extraction. These solvent-extracted oils demonstrated lower acid and peroxide values, distinct color profiles, and varied fatty acid compositions when contrasted with cold-extracted oil. Intriguingly, despite these disparities, cold-extracted oil exhibited higher levels of total phenol content and alpha-tocopherol. The study also revealed the presence of various bioactive compounds in the investigated Moringa seed oil, such as oleic acid, total phenol, and alpha-tocopherol, known for their antioxidant properties. This indicates the potential utility of extracting oil from *Moringa Peregrine* seeds in industries like cosmetics, pharmaceuticals, and medicine. The results suggest that the selection of an extraction method can influence the nutritional benefits of Moringa seed oil, emphasizing the health advantages linked to its abundant unsaturated fatty acids.

Abbreviations

PUFAs: polyunsaturated fatty acids
Cp: Centipoise
CE: Cold extraction.
CG-MS: gas chromatography-mass spectrometry
TPC: Total phenol content
HE: Solvent extraction hexane
PEE: Solvent extraction petroleum ether
GA: Galic acid
Iodine values: g I2 /100g
GAE/g: mg gallic acid equivalents per gram
mEq/kg : milligraphic equivalent per kilogram

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Conflict of interest
The authors declare that they have no conflict of interest, and have not submitted to any other journal in parallel or published previously.

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