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ORIGINAL PAPER

Some physical properties of Persian lime (*Citrus Latifolia*) seeds and physicochemical properties of the seed oil as affected by solvent extraction and cold pressing methods

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Abstract
In this study, some physical properties of Persian lime seed and some physicochemical properties of its cold-pressed and solvent-extracted oil were investigated. Bulk density, true density, angle of repose, thousand seed mass, and coefficients of static friction were higher in fresh seeds than in dried seeds, but porosity was lower ($p < 0.05$). Cold pressing resulted in a significantly lower oil extraction yield (34.4%), as compared to the solvent extraction (44.2%). Cold-pressed oil showed lower free fatty acid content, peroxide value, color L, a*, b* parameters, total carotenoids, iodine value, and sterol content, and higher phenolics, α-tocopherol contents, and oil stability index ($p < 0.05$). Both oil samples contained similar amounts of chlorophyll and unsaponifiable matter ($p > 0.05$). The major fatty acids of cold-pressed Persian lime seed oil were linoleic (30.72%), palmitic (27.09%), oleic (26.06%) and linolenic acids (6.98%). The oil was mainly composed of PLO (18.15%), PLL (16.12%), PPL (9.87%), POO (8.87%), LLO (6.95%), PPO (5.67%), LLL (3.76%), OOO (5.86%), and PLLn (5.26%) triacylglycerols (TAG). The cold-pressed oil contained slightly lower amounts of palmitic acid, linolenic acid, and PPL and LLL TAGs but slightly higher amounts of oleic acid, linoleic acid, and PLO, PLL and OOO TAGs than the solvent extracted oil ($p < 0.05$). β-sitosterol, campesterol, and Delta-5 avenasterol were the dominant sterols in both oil samples. Based on the differential scanning calorimetry, the oil started melting at around − 24 °C and fully crystallized at around − 8 °C.

Keywords Persian lime seed · Physical properties · Cold pressing · Solvent extraction · Physicochemical properties

Introduction

Persian lime (*Citrus latifolia*), also known as sour lemon, is a triploid cross between key lime (*C. aurantiifolia*) and lemon (*C. limon*). It is a medium-sized, nearly thornless tree in the *Rutaceae* family that produces the lime fruit. Persian lime is mainly used for the production of lime juice [1]. One of the valuable by-products remained after the juice extraction is lime seeds. Citrus seeds comprise 2–7% of the whole fruit [2] and contain 34 to 43% oil (dry basis). The almost high oil content makes citrus seeds valuable and interesting especially for oil extraction particularly in countries with low domestic edible oil production. Citrus seed oils can be used in the formulation of various edible oil and fat products, as well as in the production of cosmetics, soaps, and detergents. Accordingly, some researchers have studied the chemical composition and yield of different citrus seed oils [3–5]. According to Juhaimi et al. [6], palmitic (19.6–26.2%), oleic (21.3–31.4%) and linoleic (32.3–43.7%) acids are the main fatty acids of oils extracted from seeds of some citrus genus, that makes citrus seed oils good candidate for cooking and frying applications. The protein fraction of the citrus seeds, on the other hand, is also of great importance. The protein content of citrus fruit seeds was reported to be in the range of 15.9–19.9% (dry basis) [3, 5]. Citrus seeds protein showed interesting water and fat absorption capacities, and gelation, foaming, and emulsification properties, as well. Generally, the amino acid profile of

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citrus seeds, particularly in terms of sulfur-containing amino acids and total essential amino acid content is excellent [3].

On an industrial scale, vegetable oils are usually extracted using hard/cold press, solvent, or prepress-solvent extraction systems. The choice of extraction process depends on the oil content of the oilseeds and affects the oil yield and physicochemical properties of the extracted oil [7, 8]. Compared to the solvent extraction method, the cold press method yields lower oil but is a more rapid, environmentally–friendly, and cheaper process. Cold pressing does not involve chemical treatments, and the cold-pressed oil has a distinctive flavor and aroma. Cold pressing is also regarded as an edible oil valorization technique for unconventional, less common oil-bearing seeds and kernels, which are currently considered as the source of oils that have relatively high and diverse amounts of minor bioactive components [7, 9–11]. Cold press [5, 12], solvent extraction [3–5], and supercritical carbon dioxide extraction [13, 14] methods were used for the extraction of different citrus seed oil. The effects of enzyme (naringinase, hesperinidase) and roasting pretreatments on the yield and chemical composition of grapefruit and orange seed oil extracted using cold press were also studied [12, 15]. However, the physicochemical properties of cold-pressed Persian lime seed oil have not been reported.

Considering the potential of citrus seed for oil and protein extraction, basic information about the physical characteristics of the seed is needed for further processing. In fact, designing various separation, handling, storing, and drying equipment requires data of important physical properties including density, porosity, angle of repose, coefficient of friction, and so on [16]. At present, no citrus seed oil is produced commercially in Iran and there is no report on the biophysical properties of the Persian lime seed. On the other hand, knowledge about the physicochemical properties of lime seed oil can help us to develop the extraction and refining processes and find potential applications for the obtained oil. Accordingly, in the first step, some physical properties of Persian lime seeds (dried and fresh) were determined. In the next step, lime seed oil was extracted using solvent extraction and cold pressing methods and characterized in terms of physicochemical properties.

Materials and methods

Sample preparation

Persian lime (*C. latifolia*) seeds were obtained from a lime juice extraction plant (Asiashoor Food Industry and Trade Inc., Tabriz, Iran) as a juice extraction by-product composed of peels, seeds, and pulp. The by-product which contained about 9% seeds was first poured in water and stirred using a spatula to help detachment of seed from the other materials. The settled seeds were then separated, rinsed, dried by direct sun drying (for 48 h), packed in black nylon bags, and stored at 4 °C.

Proximate composition of Persian lime seeds

Moisture, protein, ash, and fat content of Persian lime seeds were measured according to the American Oil Chemists' Society (AOCS) methods Ca 2d-25, Ba 4d-90, Ba 5a-49, Am 2-93, respectively [7]. Total carbohydrate content was determined as described elsewhere [12]. Kernel and shell proportion was determined by manual deshelling of 50 g of seeds and was reported as a percent of the whole seed.

Physical properties of seeds

The geometrical dimensions namely length (L), width (W), and thickness (T) of the seeds were measured using a Vernier caliper (Mitutoyo, Japan) with an accuracy of ± 0.01 mm. The arithmetic mean diameter (D_a), geometric mean diameter (D_g), and sphericity (Φ) of seeds were calculated from the geometrical dimensions as described by Mohsenin [16]. The aspect ratio (the ratio of seed width to its length, indicative of tendency toward oblong shape) was calculated according to Pradhan et al. [17]. Surface (S) and volume (V) was calculated using the equation described by Nazmi [18]. To determine the thousand-seeds weight (M1000), various sets of 100 randomly taken seed samples were weighted using an electronic balance with 0.001 g accuracy (AND, EJ303, Japan) and the mean weight was multiplied by 10. Bulk density (ρb) of seeds was determined by filling a cylindrical container of 500 ml volume with the seeds [17], true density (ρs) was determined using the toluene displacement method [10] and porosity (ε) was calculated using the relationship described by Nazmi [18]. The coefficient of static friction (μ_s) of seeds against surfaces of steel, galvanized iron, plywood, glass, and rubber was determined according to Nazmi [18]. A wooden topless and bottomless box of 100 × 100 × 40 mm was filled with the seeds and placed on a changeable inclined plate, faced with the test surface. To prevent contact of the box with the sloping surface it was lifted slightly (5–10 mm). The inclination angle of the test surface was increased gradually with a screw device until the box just started to slide down. The angle of slope (α) was read from a graduated scale and tan(α) was regarded as the coefficient of static friction. For each replication, the sample in the box was evacuated and refilled with a new sample of seeds [18].

To obtain the angle of repose (θ), seeds were poured from 15 cm height on a wooden horizontal surface. The height of seeds stack above the floor (H) and the diameter of the heap (D) were measured and used to determine the angle of repose for filling using the following relationship [18]:

$$\theta = \text{Arctan}\frac{(2\text{H})}{\text{D}} \quad (1)$$

Extraction of Persian lime seed oil

The moisture content of the seeds was first adjusted to 8% by the addition of distilled water. The required amount of water was calculated using the following equation [19]:

$$\text{Q} = \frac{\text{W}_{\text{i}}\left(\text{M}_{\text{f}} - \text{M}_{\text{i}}\right)}{100 - \text{M}_{\text{f}}} \quad (2)$$

in where Q is the mass of water added (kg), W_i = initial mass of sample in kg, M_i = initial moisture content (% dry basis) of the sample, M_f = final moisture content (% dry basis) of the sample. After the addition of the water, seeds were conditioned by packaging in polyethylene bags and storing at 4 °C in a refrigerator for 48 h [19]. The conditioned seeds were then cold-pressed using a laboratory-scale cold press machine (Iran cold pressing, Kerman, Iran; single head, 0.75 kW power, 10 kg seed/h capacity) at 10 rpm screw rotation speed. During the extraction, the temperature of the extracted oil was lower than 40 °C.

For solvent extraction, dried and ground seeds (50 g) were placed into a cellulose paper cone and the oil was extracted using hexane (at 60–70 °C) in a 250 ml Soxhlet extractor for 8 h. The collected micelle was then vacuum-evaporated (Heidolph Rotavapor, Germany, 40 °C, vacuum 0.6 bar and rpm 50) to obtain the extracted oil. The cold-pressed and hexane-extracted oils were immediately centrifuged (Universal, PIT 320, Tehran, Iran) at 9000 rpm for 15 min, placed in amber-colored glasses, flushed with nitrogen, and stored in the fridge until the analyses.

Physicochemical analysis of Persian lime seed oil

Specific gravity was measured using an oil pycnometer according to the AOCS method Cc 10c-95 at 25 °C [20]. Refractive index was determined using an Abbe AR4 (Kruss, Germany) refractometer at 25 °C according to the AOCS Cc 7-25 [20]. Apparent viscosity was measured using a Brookfield Viscometer (DRV model, Brookfield Eng. Lab., Inc., MA, USA) with 6 spindles (RV/HA/HB) at 30 rpm and 25 °C according to the AOCS method Ja 10-87 [20]. The instrumental color values (L, a* and b*) were determined using a Colorimeter (CR-400 Konica Minolta, Inc., Osaka, Japan) [5]. Free fatty acid (FFA) contents, peroxide value (PV), iodine value (IV), saponification value, and unsaponifiable matters were measured according to the AOCS Ca 5a-40, Cd 8-53, Cd 1d-92, Cd 3-25, and Ca 6a-40 methods [20], respectively. Total phenolic content was measured using the Folin–Ciocalteu reagent [21]. Total carotenoid and chlorophyll content were determined according to Jabri-Karoui and Marzouk [22] using a UV–Vis spectrophotometer (Pharmacia Biotech Ltd., Cambridge, UK) at 470 and 670 nm, respectively.

Fatty acid, sterol, triacylglycerol and tocopherol composition of the oil

Fatty acid methyl esters (FAMEs) were prepared according to the AOCS method Ce 2-66 [20] and quantified using a gas chromatograph (Shimadzu, 2030 Nexis, Kyoto, Japan) equipped with a flame ionization detector (FID) (Agilent Technologies, Palo Alto, CA, USA), and a DM-2330 capillary column (60 m × 0.25 mm i.d., 0.2 µm film thickness, Dikma Technologies Inc., USA). The analytical GC conditions were as follows: initial oven temperature of 60 °C for 2 min, increase to 200 °C (10 °C/min) and hold for 10 min, increase to 240 °C (5 °C/min) and hold for 5 min; 1 µL injection volume, injector split ratio of 1:60; 2 mL/min flow rate of carrier gas. Hydrogen was used as the carrier gas, and the injector and detector were set at 250 °C and 260 °C, respectively.

Sterol composition was determined following the method COI/T.20/Doc. No 30 of the International Olive Council [23] using a gas chromatograph (YL6500, YL Instruments Co., Ltd., Anyang, Korea) equipped with FID (Agilent Technologies, Palo Alto, CA, USA) and a Supelco 28097-U Equity-5 capillary column (30 m × 0.25 mm i.d., 0.1 µm film thickness, Supelco, Sigma-Aldrich, USA). The analysis conditions were as follows: start at 240 °C for 2 min, increase to 300 °C (10 °C/min) and hold for 1 min, increase to 305 °C (1 °C/min) and hold for 10 min; injection volume: 1 µL; injector split ratio: 1:100. Hydrogen was used as carrier gas at a flow rate of 1.5 mL/min and injector and the detector temperatures were 300 and 310 °C, respectively.

Triacylglycerol (TAG) composition was determined according to the ISO method ISO/TS 17383 [24] using capillary gas chromatography (Shimadzu, 2030 Nexis, Kyoto, Japan) with FID (Agilent Technologies, Palo Alto, CA, USA), equipped with an Rtx-65TG capillary column (30 m × 0.25 mm i.d., 0.1 µm film thickness, Restek, Bellefonte, PA, USA). The analysis conditions were as follows: initial oven temperature, 100 °C for 2 min, increase to 325 °C (30 °C/min), increase to 345 °C (1 °C/min), increase to 370 °C (5 °C/min) and hold at 370 °C for 5 min; injection volume of 1 µL; injector split ratio of 1:100. Hydrogen was used as carrier gas at a flow rate of 1.5 mL/min. Injection was done via the cold on-column method and the detector temperature was set at 370 °C.

Tocopherol composition was determined using HPLC (Young Lin Acme 9000, YL Instruments Co., Ltd., Anyang, Korea) according to AOCS Ce 8-89 method [20]. Before the HPLC, 2 g of the seed oil was diluted and made up to 25 ml in a 25 ml volumetric flask and then 20 µl of the sample was injected. The sample was analyzed using a normal-phase silica HPLC column (150 mm × 4.6 mm × 0.5 µm, Sil-M51001546, HECTOR-M, RStech, Korea) and detected by a fluorescence detector (Jasco FP-4025, JASCO International Co., Ltd. Japan, λ excitation = 290 nm, and λ emission = 330 nm). Isopropanol: hexane (0.5:99.5 v/v) was used as the mobile phase isocratically at a flow rate of 0.5 ml/min and 30 °C. A mixed tocopherol standard (Sigma Chemical Co., St. Louis, MO, USA) in a hexane solution (2 µg/ml) was used as the external standard.

Thermal analysis of the oil

A differential scanning calorimeter (DSC, DSC 3, Mettler Toledo, Schwerzenbach, Switzerland) was used to assess the thermal parameters of the Persian lime seed oil according to AOCS Cj 1-94 method [20]. Heat flow calibration was achieved using indium (heat of fusion 28.45 J/g). Temperature calibration was carried out using hexane (mp of − 93.58 °C), water (mp of 0.0 °C), and indium (mp of 156.6 °C). Samples were prepared by carefully weighing 10 mg of the oil into the 160 mL aluminum DSC pans and closed without hermetic sealing. An empty pan was used as the reference. The temperature program was as follows: heating from room temperature to 80 °C at a rate of 10 °C/min and holding at the final temperature for 10 min, cooling to − 60 °C at a rate of 10 °C/min and holding at the final temperature for 30 min for full crystal formation and finally heating the sample again to 80 °C at a rate of 5 °C/min. The thermal parameters including melting temperature (T_m), melting enthalpy (DH_m), crystallization temperature (T_c), and crystallization enthalpy (DH_c) were calculated using the STAR^e (ver.9, Mettler Toledo, Switzerland) software.

Oxidative stability index (OSI) of the oil was determined at 110 °C with an airflow rate of 9 L h^{-1} using a Rancimat instrument (Model 743; Metrohm Ltd., Herisau, Switzerland) according to the AOCS Cd 12b-92 method [20].

Statistical analysis

Cold pressing and solvent extraction of Persian lime seed oil and all the listed analyses were performed at least three times. Analysis of variance (ANOVA) and Tukey's mean comparing test at 5% significance level was performed using the Minitab ver. 18.1 (Minitab, Pennsylvania, USA) program.

Results and discussion

Proximate composition of Persian lime seeds

The proximate composition of Persian lime seeds is shown in Table 1. Persian lime seed was composed of 72.39% ± 0.01 kernel and 27.61% ± 0.01 shell (shell to kernel ratio of 0.38). Oil was the main constituent (44.2%) of the seeds. The oil content of the Kütdiken and Interdonato varieties of *Citrus limon* (from Turkey) and *Citrus limon Osbeck* seeds was found to be 45.1, 45.8% and 34.9 (dry basis), respectively [4, 25]. Because of its high oil content, Persian lime seed can be considered as an economically valuable raw material for vegetable oil extraction.

Carbohydrates (28.2%) and protein (20.6%) were the other major constituents of Persian lime seeds. Habib et al. [26] reported that Egyptian lime contained 42.65% lipid, 13.75% protein, 40.56% carbohydrate and 2.19% ash (dry basis). A protein content of 19.41% (dry basis) and an ash content of 1.41% (dry basis) has been reported for *Citrus limon L.* (Kütdiken variety), as well [5]. Different harvest year, variety, and extraction and analysis methods could be the reason for these differences.

Physical properties of Persian lime seed

The average length, width, thickness, geometric mean diameter (Dg), and arithmetic mean diameter (Da) of the seeds are presented in Table 1. It seems that gradual drying (sun drying for 48 h) and the rigid shell structure prevented seeds shrinkage. Accordingly, seed length and width did not change by drying ($p > 0.05$). However, thickness, geometric mean diameter, and arithmetic mean diameter of the seeds decreased by drying (Table 1, $p < 0.05$).

Drying had not any significant effect on the sphericity and aspect ratio of Persian lime seeds (Table 1, $p > 0.05$). Sacilik et al. [27], Garnayak et al. [19], and Dursun and Dursun [28] considered a grain as spherical when the sphericity value was higher than 80, 73%, and 70%, respectively. Accordingly, Persian lime seeds could not be regarded as a sphere (Table 1).

Surface area, volume, thousand-seed mass, bulk density, and true density of dried Persian lime seeds were lower than those of fresh seeds (Table 1, $p < 0.05$); but the porosity was found to increase by drying (Table 1, $p < 0.05$).

The angle of repose and static coefficients of friction (μ_s) of the Persian lime seeds (on five different surfaces including steel, galvanized iron, plywood, glass, and rubber) are presented in Table 1. It was observed that the angle of repose and static coefficient of fresh seeds were higher than those of dried seeds ($p < 0.05$). In fact, the water present in the seed offers a cohesive force on the contact surface resulting in better stability and less flow ability. In terms of the effect of the contact surface type on the static coefficient, the maximum friction was offered by rubber, followed by plywood, galvanized iron, steel, and glass surface. This difference could be due to the different roughness of the various surfaces

Table 1 Proximate composition (% dry basis) and physical properties of dried and fresh Persian lime (*Citrus Latifolia*) seeds

Proximate composition of lime seeds (%)					
Moisture fresh[A] (dried)	Protein	Oil	Carbohydrate	Ash	Shell/kernel ratio
44.6 ± 0.4 (4.4 ± 0.6)	20.6 ± 1.1	44.2 ± 1.6	28.2 ± 0.1	2.15 ± 0.05	0.38 ± 0.01
Physical properties of lime seed*					
Property	Dried	Fresh	Property	Dried	Fresh
L (mm)	8.39 ± 0.19^a	8.50 ± 0.06^a	V (mm^3)	78.24 ± 1.62^b	87.37 ± 2.6^a
W (mm)	3.31 ± 0.26^a	3.39 ± 0.04^a	M_{1000} (g)	41.13 ± 1.99^b	63.90 ± 1.10^a
T (mm)	5.13 ± 0.13^b	5.46 ± 0.09^a	ε (%)	42.14 ± 0.61^a	35.43 ± 0.40^b
D_g (mm)	5.22 ± 0.06^b	5.40 ± 0.05^a	ρ_s (g/cm^3)	0.74 ± 0.026^b	1.21 ± 0.00^a
D_a (mm)	5.61 ± 0.03^b	5.78 ± 0.04^a	ρ_b (g/cm^3)	0.43 ± 0.01^b	0.78 ± 0.00^a
Φ (%)	62.24 ± 2.17^a	63.55 ± 0.81^a	θ	20.47 ± 0.54^b	32.00 ± 0.59^a
S (mm^2)	71.92 ± 1.73^b	77.02 ± 1.45^a			
Aspect ratio (%)	39.5 ± 4.1^a	39.9 ± 0.7^a			
Coefficient of static friction (μ_s) at different Surface material[#]					
Surface	Glass	Steel	Galvanized iron	Plywood	Rubber
Dried	0.247 ± 0.041^{bE}	0.314 ± 0.017^{bD}	0.349 ± 0.032^{bC}	0.367 ± 0.031^{bB}	0.457 ± 0.019^{bA}
Fresh	0.413 ± 0.017^{aE}	0.438 ± 0.019^{aD}	0.533 ± 0.027^{aC}	0.555 ± 0.037^{aB}	0.618 ± 0.025^{aA}

Values given are the means of three replicates ± standard deviation

L Length, *W* width, *T* thickness, D_g geometric mean diameter, D_a arithmetic mean diameter, *Φ* sphericity, *S* surface, *V* volume, M_{1000} thousand seed weight, ρ_s true density, ρ_b bulk density, *ε* porosity, *θ* angle of repose

[A]Reported as wet basis

*Means with different letters within a row are significantly different at $p < 0.05$ (using Tukey's Multiple Range Test)

[#]Means with different capital letters within a row and small letters within a column are significantly different at $p < 0.05$ (using Tukey's Multiple Range Test)

Physicochemical properties of Persian lime seed oil

Oil extraction yield (gram oil extracted per gram seed) and physicochemical properties of the cold-pressed and solvent-extracted oil samples are given in Table 2. The oil extraction yield was significantly higher in the solvent extraction method (P < 0.05). Specific gravity, refractive index, viscosity, saponification value and unsaponifiable matter content of cold-pressed and solvent-extracted oil samples were not significantly different (Table 2, p > 0.05). The specific gravity, refractive index and viscosity of different citrus seed oils was reported to be as 0.884–0.962 g/cm^3, 1.4672–1.4684 (at 25 °C), and 0.05–0.08 Pa s, respectively [3, 29]. The results of this study agree with those reported in the literature. Pigments are responsible for the color of oils. The L-value, a* value and b* value of the solvent-extracted oil were higher than those of the cold-pressed oil. This shows that higher amounts of pigments (such as carotenoids, Table 2) are extracted using the solvent. However, the chlorophyll contents of the oil samples were not significantly different (p < 0.05, Table 2). Similar results have been previously reported for the color of the cold-pressed and solvent-extracted seed oil of *Citrus limon* L. (Kütdiken variety) [5]. A low concentration of chlorophyll is preferable because of its possible sensitizing effect in oil photooxidation.

FFA content of cold-pressed oil was significantly lower than that of the solvent-extracted sample (p < 0.05, Table 2). The FFA content of cold-pressed and solvent-extracted Kütdiken lemon seed oil samples were reported as 0.64 and 1.05%, respectively [5], which were lower than those obtained in this study.

PV of the cold-pressed sample was significantly lower than that of the solvent-extracted sample (p < 0.05, Table 2). This indicates the advantage of cold pressing over the solvent extraction. The PV of solvent-extracted and cold-pressed Kütdiken lemon seed oil has been reported as 9.49 and 39.11 meq O_2/kg oil, respectively [5], which were significantly higher than those obtained in this study. El-Adawy et al. [3] reported the PV of four different citrus seed oils (extracted with n-hexane) as 5.90–6.37 meq O_2/kg oil which was in general agreement with those reported in this study.

Although the IV of the two oil samples were statistically different (p < 0.05, Table 2), the saponification value and unsaponifiable matter contents were quite similar (p > 0.05, Table 2). The IV of sweet lemon seed oil was 110 g iodine/100 g oil [8]. Similarly, the IV of

Table 2 Physicochemical properties of the cold-pressed and solvent-extracted Persian lime seed oils

Property	Persian lime seed oil	
	Solvent extracted	Cold pressed
Oil extraction yield (g oil/100 g seed)	44.2 ± 1.6[a]	30.4 ± 0.6[b]
Specific gravity (g/ml) (25 °C)	0.92 ± 0.01[a]	0.92 ± 0.01[a]
Refractive index (25 °C)	1.46 ± 0.01[a]	1.46 ± 0.01[a]
Viscosity (25 °C, cP)	55.7 ± 0.2[a]	57.2 ± 0.5[a]
Color		
L	32.15 ± 0.97[a]	26.68 ± 0.81[b]
a*	1.83 ± 0.07[a]	0.38 ± 0.01[b]
b*	17.64 ± 0.52[a]	4.40 ± 0.13[b]
Free fatty acid (%, linoleic acid)	1.40 ± 0.08[a]	1.14 ± 0.1[b]
Peroxide value (meq O_2/kg oil)	7.30 ± 0.19[a]	4.92 ± 0.47[b]
Iodine value (g I/100 g oil)	98.8 ± 1.4[a]	90.8 ± 1.4[b]
Saponification value (mg KOH/g oil)	191.0 ± 2.3[a]	190.5 ± 1.8[a]
Unsaponifiable matter (%)	0.79 ± 0.02[a]	0.77 ± 0.01[a]
Total phenolics (mg GA/kg oil)	43.22 ± 0.74[b]	58.34 ± 1.20[a]
Total Chlorophylls (mg/kg oil)	3.15 ± 0.04[a]	3.27 ± 0.04[a]
Total carotenoids (mg/kg oil)	5.30 ± 0.26[a]	4.46 ± 0.11[b]

Means with different letters within a row are significantly different at $p < 0.05$ (using Tukey's Multiple Range Test)

Values are the means of three replicates ± standard deviation

cP centipoise

some citrus seed oils ranged from 91.54 to 102. 57 g iodine/100 g oil [3]. Cold-pressed and solvent-extracted lemon seed oil samples were reported to have an IV of 117.49 and 129.11 g iodine/100 g oil, respectively [5]. The IV determined in this study (Table 2) was lower than those reported in the literature for citrus seed oils.

The saponification values of the cold-pressed and solvent-extracted oil samples were 190.5 and 191.0 mg of KOH/g of oil (Table 2). According to Anwar et al. [8], the saponification values of sweet lemon, grapefruit, orange and mandarin orange seed oils were 180.90, 198.85, 189.50 and 186.00 mg of KOH/g of oil, respectively. In another study [3], the saponification values of four citrus seed oils ranged from 187.2 to 190.2 mg of KOH/g of oil. Overall, the saponification values determined in this study appear to be higher than those reported in the literature. This difference can be attributed to the source and variety of seeds, climate, and other factors such as extraction conditions.

Cold-pressed and hexane-extracted Persian lime seed oil contained 0.77 and 0.79% unsaponifiable matter, respectively ($p > 0.05$, Table 2), which were higher than those reported for hexane-extracted sweet lemon seed oil (0.31%) [8], close to that of cold-pressed grapefruit seed oil (0.79%) [12], and lower than that reported for hexane-extracted orange seed oil (1.57%) [29].

The total phenolics content of cold-pressed Persian lime seed oil was significantly higher than that of the solvent-extracted one ($p < 0.05$, Table 2). According to Al Juhaimi et al. [30] and Gafoor et al. [31] phenolic compounds are sensitive to heat, So the low temperature and relatively fast process of cold pressing, as compared to the solvent extraction, may preserve more phenolics in the oil. Similar to the results of this study cold-pressed lemon seed oil had higher total phenolics than the solvent-extracted one [5].

Fatty acid, sterol, triacylglycerol and tocopherol composition of Persian lime seed oil

Table 3 shows the fatty acid composition of the Persian lime seed oil and some other citrus seed oils. It seems that the extraction type had a significant, but little, effect on the fatty acid composition of oil (Table 3). Cold-pressed oil had higher contents of stearic, oleic, linolenic but lower contents of palmitic and linoleic acid than the solvent-extracted oil ($p < 0.05$). Like the other citrus seed oils (lemon, grapefruit and orange seed oils, Table 3), linoleic, palmitic and oleic acids were the main fatty acids of Persian lime seed oil. Fatty acid composition of Persian lime seed oil was different from that reported for Egyptian lime seed oil (palmitic (19.08%), oleic (13.07%), linoleic (18.62%) and linolenic (42.22%) acid), that contained linolenic acid as its main fatty acid [26]. The palmitic acid content of Persian lime seed oil was in the range of that of grapefruit seed and orange seed [32]. Similar to other citrus oils (Table 3), the rare fatty acid palmitoleic acid was also found in Persian lime seed oil. The oleic acid content of Persian lime seed oil was similar to that of the other citrus seed oils.

The TAG composition (as carbon number (CN) and TAG species) of Persian lime seed oil is shown in Table 4. Persian lime seed oil was mainly composed of CN 52 TAGs. This may be attributed to the fact that the oil was mainly (about 85%) composed of palmitic, oleic and linoleic acids (with similar ratios, Table 3). Nine TAGs, namely PPO and PPL (from CN50 TAGs), PLO, PLL, POO and PLLn (from CN 52 TAGs) and OOO, LLL and LLO (from CN 54 TAGs) were the main TAGs of the oil and constituted about 81% of the total TAGs. Persian lime seed oil contained PLO as the most abundant TAG, followed by PLL, PPL, POO and LLO. The solvent-extracted and cold-pressed oil samples had almost similar TAG compositions. However, as compared to the solvent-extracted oil, the cold-pressed oil had higher contents of PLO, PLL, OOO and LLnLn and lower contents of PPL, PSO and LLL ($p < 0.05$). According to Saloua et al. [29] The major TAGs of Osage orange (*Maclura pomifera)* seed oil were LLL (33.48%) followed by PLL (24.26%), POL + SLL (20.30%), OLL (10.12), OOL (7.50%)

Table 3 Fatty acid composition of solvent-extracted and cold-pressed Persian lime seed oil and some other oils

Fatty acids (%)	Persian lime seed oil		Lemon seed oil [5, 32]	Grape fruit seed oil [32]	Orange seed oil [32]
	Solvent-extracted	Cold-pressed			
C12:0	0.04 ± 0.01	ND	0.04	0.5	–
C14:0	0.30 ± 0.05[a]	0.24 ± 0.01[a]	0.10	1.0	1.0
C16:0	28.36 ± 0.04[a]	27.09 ± 0.03[b]	20.88–21.40	18.0–29.0	21.0–29.0
C16:1	0.66 ± 0.03[b]	0.72 ± 0.01[a]	0.26–0.65	0.0–1.0	1.0
C18:0	4.45 ± 0.05[b]	4.59 ± 0.01[a]	2.3–4.32	2.0–8.0	4.0–8.0
C18:1 n-9	26.01 ± 0.03[b]	26.62 ± 0.04[a]	20.8–36.6	20.0–28.0	20.0–37.0
C18:2 n-6	31.48 ± 0.01[a]	30.72 ± 0.03[b]	31.4–44.3	36.0–51.0	36.0–38.0
C18:3 n-3	6.18 ± 0.03[b]	6.98 ± 0.03[a]	6.9–8.96	5.0–6.0	1.0–7.0
C20:0	0.52 ± 0.02[a]	0.55 ± 0.01[a]	0.31	0.5-2.0	0.2
C22:0	0.11 ± 0.03[b]	0.22 ± 0.02[a]	0.1	–	–
C24:0	0.28 ± 0.01[a]	0.28 ± 0.01[a]	0.17	–	–
SAFA	34.42 ± 0.01[a]	33.35 ± 0.01[b]	–	–	–
MUFA	27.09 ± 0.01[b]	27.80 ± 0.01[a]	–	–	–
PUFA	37.66 ± 0.01[a]	37.70 ± 0.01[a]	–	–	–

Means with different letters within a row are significantly different at $p < 0.05$ (using Tukey's Multiple Range Test)

Values are the means of three replicates ± standard deviation

SAFA saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids

and PPO (4.32%). The oil of Musk lime (*Citrus microcarpa)* seed contained POL (18.9%) as the most prominent TAG, followed by PLL (13.7%) and OLL (11.9%) [33], which as almost similar to the result obtained here.

As shown in Table 5, the solvent-extracted oil had higher total sterol content than the cold-pressed oil ($p < 0.05$). Eight sterols were quantified in the Persian lime seed oil samples (Table 5). The most abundant one was β-sitosterol (approximately 76%), followed by campesterol, Δ-5-avenesterol and stigmasterol. Δ-7-avenasterol and cholesterol were found in contents lower than 1% of total sterols. Similar sterol compositions were reported for lemon and orange seed oils [5, 15]. The Kütdiken lemon seed oil contained 75.6, 10.4, 4.4, 2.7 and 2.5% β-sitosterol, campesterol, stigmasterol, cholesterol and Δ-5-avenasterol, respectively [5] and the main sterols of grapefruit seed oil were β-sitosterol (80.85–81.74%), campesterol (9.0–9.75%) and stigmasterol (2.18–2.71%) [12].

Cold-pressed Persian lime seed oil had significantly higher α-tocopherol content than the solvent-extracted sample. α-tocopherol content of cold-pressed and solvent-extracted lemon seed oils was 155.00 and 110.20 mg/kg, respectively [5], which were similar to the results of this study. In another study, lemon seed oil contained 102.49 mg/kg α-tocopherol, 2.20 mg/kg β-tocopherol, 1.33 mg/kg γ-tocopherol and 18.98 mg/kg δ-tocopherol [4]. However, higher α-tocopherol content has been reported for grapefruit seed oil [12]. The α-tocopherol content of Persian lime seed oil was lower than that reported in the literature for peanut oil (399 mg/kg), flaxseed oil (589 mg/kg), sunflower oil (635 mg/kg) and soybean oil (1798 mg/kg) [34]. Since tocopherols are strong antioxidants and possess vitamin E activity, they are extremely important for oil stability and increase the nutritional value of the oils, as well [11, 25].

Thermal properties of lemon seed oil

The crystallization and melting temperatures and enthalpies of the Persian lime seed oil samples are shown in Fig. 1 and Table 6. The melting of cold-pressed and solvent-extracted oil started (the onset temperature) at − 23.38 and − 24.13 °C, respectively. Three other different fractions having melting temperatures of − 20.68, − 5.20 and − 1.16 °C were observed in the cold-pressed lemon seed oil. In the solvent-extracted sample, two peaks at − 18.20 and − 4.38 °C and a shoulder at around 0–3 °C were visible (Table 6 and Fig. 1).

The onset of crystallization was recorded as 2.13 and − 3.37 °C for solvent-extracted and cold-pressed oil samples, respectively (Table 6). Two exothermic peaks were detected during the cooling of solvent extracted seed oil (Fig. 1a). Since saturated TAGs crystallize at higher temperatures than the unsaturated ones, the smaller exothermic peak (at 1.2 °C) can be reasonably attributed to the phase transition of an oil fraction composed mainly of saturated FAs, such as palmitic and stearic acid. The bigger exothermic peak, which was observed at − 7.79 °C, may be related to the phase transition of a low-melting highly unsaturated oil fraction. However, only one exothermic peak (− 8.38 °C) was detected during the cooling of cold pressed-seed oil (Fig. 1b). This may be explained by the

Table 4 Triacylglycerol (TAG) composition of Persian lime seeds oil extracted by solvent extraction and cold-pressing

TAG (%)	Solvent-extracted	Cold-pressed
CN48		
PPP	0.26 ± 0.05[a]	0.16 ± 0.07[a]
UI	0.34 ± 0.17[a]	0.33 ± 0.18[a]
Subtotal	0.60 ± 0.21[a]	0.49 ± 0.25[a]
CN50		
PPS	0.23 ± 0.07[a]	0.19 ± 0.03[a]
PPO	6.53 ± 0.13[a]	5.67 ± 1.03[a]
PPL	11.90 ± 0.25[a]	9.87 ± 1.32[b]
UI	3.14 ± 0.18[b]	3.79 ± 0.10[a]
Subtotal	21.81 ± 0.49[a]	19.51 ± 2.48[a]
CN52		
PSS	0.01 ± 0.00[a]	0.04 ± 0.03[a]
PSO	2.09 ± 0.34[a]	1.62 ± 0.04[b]
POO	8.27 ± 0.23[a]	8.87 ± 0.64[a]
PLO	16.92 ± 0.26[b]	18.15 ± 0.43[a]
PLL	15.39 ± 0.20[b]	16.12 ± 0.24[a]
PLLn	4.77 ± 0.50[a]	5.25 ± 0.11[a]
UI	3.54 ± 0.14[a]	3.18 ± 0.49[a]
Subtotal	50.98 ± 0.24[b]	53.23 ± 0.81[a]
CN54		
SOO	1.06 ± 0.22[a]	1.03 ± 0.27[a]
OOO	4.87 ± 0.06[b]	5.86 ± 0.47[a]
OOL	1.43 ± 0.37[a]	1.52 ± 0.17[a]
LLO	6.85 ± 0.29[a]	6.95 ± 0.82[a]
LLL	5.23 ± 0.31[a]	3.76 ± 0.57[b]
OLLn	1.63 ± 0.16[a]	1.27 ± 0.47[a]
LLnLn	0.24 ± 0.04[b]	1.02 ± 0.31[a]
UI	5.31 ± 0.55[a]	5.35 ± 0.22[a]
Subtotal	26.61 ± 0.46[a]	26.76 ± 1.142[a]

Means with different letters within a row are significantly different at $p < 0.05$ (using Tukey's Multiple Range Test)

Values are the means of three replicates ± standard deviation

P Palmitic acid, *L* linoleic acid, *O* oleic acid, *S* stearic acid, *Ln* linolenic acid, *CN* carbon number, *UI* un-identified

lower saturated fatty acid (Table 3), tri-saturated and di-saturated TAG content (Table 4) of cold-pressed oil.

According to Yilmaz and Güneşer [5], cold-pressed and solvent-extracted Kütdiken lemon seed oils started crystallization at around − 4 to − 5 °C and fully crystallized at around − 6 to − 8 °C, respectively. The melting started at around − 25 °C in both samples and three other different fractions were observed in the cold-pressed lemon seed oil. But in the solvent-extracted sample, only two additional fractions were observed. Compared to the DSC thermogram of Osage orange seed oils [29], Persian lime seed oil had different crystallization and melting temperatures but similar enthalpy values. The DSC data can be used in the setup of fractionation/winterizing experiments to achieve a cold-stable oil.

Table 5 Sterol and tocopherol compositions of Persian lime seed oil

Property	Persian lime seed oil	
	Solvent-extracted	Cold-pressed
Sterols (%)		
Cholesterol	0.69 ± 0.01[a]	0.54 ± 0.02[b]
Brassicasterol	0.39 ± 0.03[a]	0.29 ± 0.01[b]
Campesterol	8.05 ± 0.02[b]	8.34 ± 0.03[a]
Stigmasterol	3.57 ± 0.05[b]	4.41 ± 0.01[a]
Beta-sitosterol	76.36 ± 0.65[a]	74.21 ± 0.5[b]
Δ-5 avenasterol	4.77 ± 0.04[b]	6.8 ± 0.01[a]
Δ-7 stigmastenol	0.77 ± 0.02[a]	0.76 ± 0.01[a]
Others	4.78 ± 0.03[a]	3.22 ± 0.01[b]
Total sterol (mg/kg oil)	4183.97 ± 1.97[a]	3896.52 ± 1.21[b]
Tocopherols (mg/kg oil)		
α-Tocopherol	102.91 ± 9.27[b]	203.80 ± 6.31[a]
β-Tocopherol	ND	ND
γ-Tocopherol	ND	ND
δ-Tocopherol	ND	ND

Means with different letters within a row are significantly different at $p < 0.05$ (using Tukey's Multiple Range Test)

Values are the means of three replicates ± standard deviation

ND Not detected

The OSI (oil stability index) of Persian lime seed oil samples at 110 °C is presented in Table 6. OSI of cold-pressed oil was higher than that of the solvent-extracted oil ($p < 0.05$). In general, the fatty acid composition and the presence of other minor components, such as waxes, sterols, and phenols, greatly affect the thermal stability of edible oils [15]. In fact, the higher total phenolics and tocopherol content of the cold-pressed oil may explain its higher oxidative stability. According to Yilmaz and Güneşer [5] the of cold-pressed and solvent-extracted lemon seed oil were not significantly different. According to Güneşer and Yilmaz [15] microwave roasting of orange seed oil increased the induction period of oxidation of the cold-pressed oil, significantly.

Conclusion

In this work, some physical properties of Persin lime seed were determined, which can be helpful in the design and development of lime seed handling and processing equipment. Persian lime seed gave an oil yield of 34.4% and 44.2% by cold pressing and solvent extraction, respectively. As compared to the solvent extraction, cold pressing resulted in oil with lower PV, IV, FFA content, color L, a*, b* parameters and carotenoid and sterol content but higher phenolics and α-tocopherol contents (*p < 0.05*). Linoleic, palmitic,

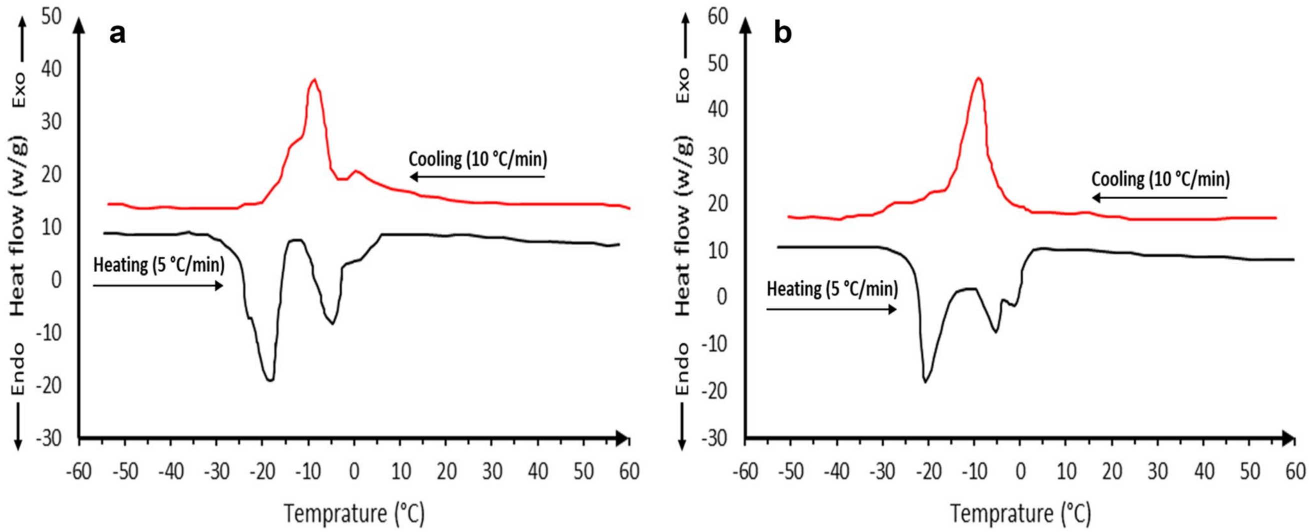

Fig. 1 Differential scanning calorimetery thermograms of melting and crystallization of solvent extracted (**a**) and cold-pressed (**b**) Persian lime seed oil

Table 6 Thermal properties of Persian lime seed oils

Property	Persian lime seed oil	
	Solvent extracted	Cold pressed
Melting		
Onset$_m$ (°C)	− 24.13 ± 0.19^a	− 23.38 ± 0.21^a
Tm1 (°C)	− 18.20 ± 0.21^a	− 20.68 ± 0.19^a
Tm2 (°C)	− 4.38 ± 0.44^a	− 5.20 ± 0.18^a
Tm3 (°C)	ND	− 1.16 ± 0.92
AH$_m$ (J/g)	43.71 ± 0.27^a	45.52 ± 0.38^a
Crystallization		
Onset$_c$ (°C)	2.13 ± 0.17^a	− 3.37 ± 0.78^a
Tc1 (°C)	1.2 ± 0.02^a	− 8.38 ± 0.34^a
Tc2 (°C)	− 7.79 ± 0.53	ND
AH$_c$ (J/g)	− 17.59 ± 0.73^a	− 16.18 ± 0.81^a
OSI (h)	2.35 ± 0.14^b	2.73 ± 0.13^a

Means with different letters within a row are significantly different at $p < 0.05$ (using Tukey's Multiple Range Test)

Onset$_m$ melting start, *Tm* melting temperature, *AH$_m$* melting enthalpy, *Onset$_c$* crystallization start, *Tc* crystallization temperature, *AHc* crystallization enthalpy, *OSI* oil stability index, *ND* not detected

oleic and linolenic acids were the main fatty acids, PLO, PLL, PPL, POO, LLO, PPO, LLL, OOO, and PLLn were the main TAGs, and β-sitosterol, campesterol, and Delta-5 avenasterol were the main sterols of Persian lime seed oil. As determined by differential scanning calorimetry, Persian lime seed oil starts melting at around − 24 °C and crystallizes at around − 8 °C. Cold pressing resulted in a higher oil stability index (2.73 h) than the solvent extracted method (2.35 h). Results obtained here show the potential of Persian lime seed in the production of vegetable oil with good physicochemical properties.

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