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Investigate the components and thermal behavior of milk thistle seed oil

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Abstract

Milk thistle seed oil has recently been approved as a novel food resource. Despite its growing popularity, there is limited information on the constituents and properties of this oil. This study aims to compare the lipid composition, nutraceutical content, antioxidant activity, and thermal properties of milk thistle seed oils extracted through different methods (using hexane/ethanol or by cold press) from Iranian milk thistle seeds. The findings reveal that the extraction method does not significantly affect the fatty acid and triacylglycerol profiles. The main fatty acids identified are linoleic acid (45.83–46.41%) and oleic acid (30.12–30.59%), with oleic-linoleic-linoleic (OLL, ~20–21%) being the most prevalent triacylglycerol, followed by linoleic-linoleic-linoleic (LLL, ~18%), palmitic-oleic-linoleic (POL, ~15%), and palmitic-linoleic-linoleic (PLL, ~11%). Conversely, the extraction method significantly influences the minor component content and antioxidant activity of the oil. Ethanol-extracted oil contains less total vitamin E and sterols but more tocotrienols and exhibits stronger free radical scavenging capacity. Additionally, cold-pressed oil displays a more complex melting

profile compared to solvent-extracted oil. These results are valuable for the quality assessment and industrial production of milk thistle seed oil.

Keywords: Milk thistle seed oil , Thermal analysis , Components, Vitamin E

1. Introduction

Milk thistle (Silybum marianum L. Gaertn.) is an annual or biennial herbaceous plant belonging to the Asteraceae family (Fathi-Achachlouei & Azadmard-Damirchi, 2009). Native to southern Europe, northern Africa, the Americas, and parts of Asia, milk thistle is now widespread globally (Soleimani, Delghandi, Moallem, & Karimi, 2019). The plant's leaves, flowers, young stems, and sprouts are consumed as vegetables in soups and salads (Andrzejewska, Martinelli, &Sadowska, 2015; Ben Rahal, Barba, Barth, & Chevalot, 2015). Its fruits and seeds have been used as herbal remedy for liver and biliary disorders for over 2000 years (Post-White, Ladas, & Kelly, 2007).

Recent studies have highlighted milk thistle's antioxidant, antiatherosclerotic, antihypertensive, anti-obesity, anti-diabetic, anti-inflammatory, and anticarcinogenic properties (Fanoudi, Alavi, Karimi, & Hosseinzadeh, 2018; Tajmohammadi, Razavi, & Hosseinzadeh, 2018). In China, milk thistle is recognized as an important herb in the Chinese Pharmacopoeia (Tan et al., 2014). It grows natively throughout the country and is commercially cultivated in at least six provinces, covering an area of more than 65,000 hectares (Li et al., 2012).

The primary pharmaceutical compound in milk thistle is silymarin, a mixture of flavonolignans including silibinin, silychristin, silidianin, and isosilybin (Tajmohammadi et al., 2018). Silymarin is present throughout the plant—fruit, seed, root, stem, and leaves—but is most concentrated in the seeds (Andrzejewska et al., 2015). China, as the largest consumer and exporter of silymarin products, has developed a comprehensive industrial chain from cultivation to the processing of drug products (Tan et al., 2014).

Apart from silymarin, milk thistle seeds are rich in oil, containing between 20% and 30% by weight. The oil must be removed from the seeds before extracting silymarin, making milk thistle seed oil an important byproduct of silymarin production. This oil is particularly notable for its high concentration of unsaturated fatty acids, especially linoleic and oleic acids, which

have health benefits including the prevention of arteriosclerosis, diabetes, and cancer (Kazazis, Evangelopoulos, Kollas, & Vallianou, 2014).

Some investigations have suggested that milk thistle seed oil might be suitable for use as an edible oil (Hassanein, Elshami, & Elmallah, 2003). Additionally, it has been recommended as a potential source of natural antioxidants due to its high vitamin E content (Hadolin, Skerget, Knez, & Bauman, 2001). In China, the Ministry of Health authorized milk thistle seed oil as a new edible oil resource in 2014, allowing its use in various food products, except for infant food.

The composition of milk thistle seed oil can also vary depending on the extraction methods used. This study comprehensively evaluates the fatty acid profile, triacylglycerol distribution, bioactive components (sterols and vitamin E), thermal properties, and antioxidant activity of milk thistle seed oils extracted using solvents (hexane and ethanol) and cold press methods. The results will enhance the understanding of the applications of milk thistle seed oil in the food and pharmaceutical industries.

2. Materials and Methods

2.1. Materials

Milk thistle seeds used in this study were purchased from the Iran herb medicine market in Tehran. The raw seeds were dried at 40°C for 24 hours, then ground into a powder with a particle size of less than 380 μm using an FW-100 grinder (Beijing Ever Light Medical Equipment Co., LTD, Beijing, China). The powder was packed in high-density polyethylene bags and stored in a refrigerator at -20°C until use.

Standards of fatty acid methyl esters (FAME) and sterols were obtained from Sigma-Aldrich (Shanghai, China). Standards for each vitamin E isomer, including four tocopherols and four tocotrienols, were procured from Beijing Sunky Biological Technology Co., Ltd. (Beijing, China). All reagents used in this study were of analytical or chromatographic grade.

2.2. Composition Analysis of Milk Thistle Seeds

The moisture, oil, protein, crude fiber, and ash contents of milk thistle seeds were measured according to the AOCS official methods Ba 2a-38, Ba 3-38, Ba 4a-38, Ba 6-84, and Ba 5a-49, respectively (AOCS, 1997). The crude protein content was determined from the nitrogen content, measured using a Foss 8400 Kjeltec analyzer equipped with a Foss DT 208 digester

(Foss Analytical Co., Ltd., Höganäs, Sweden), by applying a multiplication factor of 6.25. The crude fiber content was analyzed using an FT-350 fiber analyzer (Foss Scino Co., Ltd., Suzhou, China). Total carbohydrates were determined by the difference method (Nyam, Tan, Lai, Long, & Che Man, 2009). All results were expressed as grams per 100 grams of seed on a wet basis (g/100 g, w.b.).

2.3. Extraction of Milk Thistle Seed Oil by Different Methods

2.3.1. Cold Pressing

Whole seeds were pressed under a constant pressure of 40 MPa using a hydraulic press (Zhengzhou Bafang Machinery Equipment Co., Ltd, Zhengzhou, China) for 30 minutes at ambient temperature. This process was repeated four times. The collected oil was then centrifuged at 5180×g for 10 minutes to obtain clear oil free from suspended substances.

2.3.2. Extraction with Hexane

Two hundred grams of milk thistle seed powder were mixed with 1200 mL of n-hexane in a beaker. The mixture was agitated at room temperature (~25°C) for 6 hours using a mechanical stirrer and then centrifuged at 1860×g for 15 minutes. The supernatant was filtered under vacuum, and the filtered liquid was collected and evaporated at 45°C in a rotary evaporator (RE-2000A, Yarong Biochemical Instrument Factory, Shanghai, China) to remove the hexane. The oil was then flushed with nitrogen to remove any residual hexane.

2.3.3. Extraction with Ethanol

Milk thistle seed oil was extracted with ethanol using a Soxhlet extractor with thermal cycles at 90°C for 12 hours. The majority of the solvent was evaporated under vacuum at 45°C. Subsequently, the concentrated substances were centrifuged to separate the oil phase after settling at 4°C for 24 hours. Any residual solvent was further removed by flushing with 99.9% nitrogen.

All extracted oils were stored separately in brown glass bottles at -20°C until further analysis.

2.4. Analysis of Fatty Acid, Triacylglycerol, Sterol, and Vitamin E Composition

The oil samples for fatty acid composition determination were methylated using the BF3-methanol method as described in the ISO standard 5509 (2000). The triacylglycerol distribution and phytochemical composition (sterols and vitamin E) of the milk thistle seed oil were analyzed as described in our recently published papers (Zhang et al., 2018, 2019). Detailed information is provided in the supplementary material of this manuscript.

2.5. Antioxidant Activity Assays of Milk Thistle Seed Oil

The antioxidant activity of milk thistle seed oil was evaluated using methods described in the literature (Gao, Liu, Jin, & Wang, 2019). Two grams of milk thistle seed oil were placed in a centrifuge tube and dissolved in 4 mL of methanol. The mixture was vortexed vigorously for 10 minutes and then centrifuged at 4770×g for 15 minutes. The supernatant was collected for further analysis. These procedures were repeated three times.

2.5.1. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Assay

A 200 µL sample of the oil extract was diluted with 1.8 mL of methanol, followed by the addition of 2 mL of DPPH methanolic solution (0.1 mmol/L). The mixture was vortexed for 2 minutes and stored in the dark for 2 hours. The absorbance of the mixture was then recorded at 517 nm using a UV–Vis spectrophotometer (Model T6, Beijing Purkinje General Instrument Co., Ltd., Beijing, China) against a blank solution without DPPH. The DPPH antioxidant activity was calculated as percentage inhibition using the following formula (Samaram et al., 2015):

$$\text{DPPH \%} = (\text{blank} - \text{sample})\ \text{blank} \times 100 \quad \text{DPPH \%} = A\text{blank}\ (A\text{blank} - A\text{sample}\ ) \times 100$$

Where:

- A is the absorbance at 517 nm.

2.5.2. Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay was conducted following the method described by Benzie and Strain (1999) with slight modifications. Initially, 100 µL of the oil extract was mixed with 2 mL of freshly prepared FRAP reagent (a mixture of 300 mmol/L acetate buffer solution, 10 mmol/L 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) solution, and 20 mmol/L FeCl3 solution in a ratio of 10:1:1) in a glass tube. The mixture was diluted to 10 mL with distilled water and incubated at 37°C for 10 minutes. The absorbance of the mixture was then recorded at 593 nm against a blank reagent. Results were calculated using a calibration curve (absorbance plotted against known Trolox concentration) and expressed as µmol Trolox equivalents per kg of oil sample (µmol TE/kg).

2.6. Thermal Analysis of Milk Thistle Seed Oil

Temperature and heat flow calibration were conducted using indium (melting point = 156.6°C, enthalpy = 28.45 J/g) and mercury (melting point = -38.8°C, enthalpy = 11.47 J/g) before thermal analysis. For the thermal analysis of the oil sample, 10 mg of the sample was weighed

and hermetically sealed in an aluminum pan, with an empty pan used as a reference. The sample was then subjected to the following temperature program: heating to 60°C at a rate of 5 K/min and holding at 60°C for 1 minute, followed by cooling to -80°C at a rate of 2 K/min and holding at -80°C for 1 minute. Finally, the sample was heated again to 60°C at a rate of 2 K/min. The thermal characteristics were analyzed using TA Universal Analysis 2000 software (Version 4.5A, TA Instruments), with the peak temperature considered as the phase transition temperature.

2.7. Statistical Analysis

All determinations were conducted in triplicate, and the results were expressed as mean ± standard deviation (SD, n = 3). Analysis of variance (ANOVA) and Duncan's test were employed to determine the differences among the mean values. Significance was considered at the level of $p < 0.05$. All statistical analyses were performed using SPSS (version 24.0) statistical software (IBM Corp, Armonk, NY, USA).

3. Results and Discussion

3.1. Chemical Composition of Milk Thistle Seed

The proximate composition of milk thistle seeds is presented in Table 1. Milk thistle seeds contained approximately 21 g of oil per 100 g of seed, a comparable content to soybeans (18–22 g/100g), the most prevalent oilseed globally. Considering that oil is a byproduct during milk thistle seed processing, cultivating milk thistle can be highly profitable. However, compared with data from the literature, the milk thistle seeds used in this study contained less oil and protein but more crude fiber. For instance, Dabbour et al. (2014) reported an oil content of 26.90 g/100g in milk thistle seeds grown in Jordan, while Fathi-Achachlouei and Azadmard-Damirchi (2009) found oil contents ranging from 26 to 31 g/100g in four varieties of milk thistle planted in Iran.

Table 1
Proximate composition of milk thistle seed (g/100 g, w.b.).

Component	This work	Literature values [a]
oil	21.09 ± 0.33	26.90 ± 1.10
Protein	15.46 ± 0.61	17.64 ± 1.12
Fiber	26.72 ± 0.69	25.32 ± 1.12
Ash	4.72 ± 0.41	5.10 ± 1.15
Moisture	7.64 ± 0.21	4.61 ± 0.96
Total carbohydrate [b]	24.38 ± 0.86	20.43 ± 1.10

Note: Results are the mean ± standard deviation ($n = 3$).
[a] The results reported by Dabbour et al. (2014).
[b] Total carbohydrate obtained by difference as described by Nyam et al. (2009).

3.2. Fatty Acid Composition of Milk Thistle Seed Oil

The fatty acid composition is a crucial characteristic of edible oil, significantly influencing its physicochemical and nutritional properties. Table 2 shows the fatty acid composition of milk thistle seed oils extracted using different methods. Ten types of fatty acids were identified in all oil samples studied. Linoleic (C18:2), oleic (C18:1), palmitic (C16:0), and stearic (C18:0) acids were the most predominant, constituting over 95% of the total fatty acids. Interestingly, the extraction method had no significant effect on the fatty acid composition. Ethanol emerged as a promising green substitute for hexane in milk thistle seed oil extraction, aligning with findings by Baümler, Carrín, and Carelli (2016) on sunflower oil extraction kinetics.

Table 2
Fatty acid composition of milk thistle seed oil (%).

Fatty acids	Cold press	Hexane extraction	Ethanol extraction
C14:0	0.08 ± 0.01^{a}	0.10 ± 0.02^{a}	0.12 ± 0.01^{a}
C16:0	7.87 ± 0.04^{a}	8.19 ± 0.01^{b}	8.15 ± 0.06^{b}
C16:1	0.07 ± 0.01^{a}	0.09 ± 0.03^{a}	0.08 ± 0.03^{a}
C18:0	6.69 ± 0.04^{a}	6.65 ± 0.04^{a}	6.59 ± 0.12^{a}
C18:1	30.59 ± 0.17^{a}	30.47 ± 0.15^{a}	30.12 ± 0.22^{a}
C18:2	46.19 ± 0.25^{a}	45.83 ± 0.11^{a}	46.41 ± 0.85^{a}
C20:0	4.26 ± 0.08^{a}	4.23 ± 0.03^{a}	4.18 ± 0.13^{a}
C18:3	1.09 ± 0.02^{a}	1.16 ± 0.02^{ab}	1.14 ± 0.03^{b}
C22:0	2.65 ± 0.06^{a}	2.69 ± 0.04^{a}	2.68 ± 0.15^{a}
C24:0	0.50 ± 0.03^{a}	0.60 ± 0.06^{a}	0.53 ± 0.10^{a}
SFA	22.06 ± 0.05^{a}	22.46 ± 0.04^{a}	22.11 ± 0.37^{a}
MUFA	30.66 ± 0.18^{a}	30.55 ± 0.13^{a}	30.17 ± 0.22^{a}
PUFA	47.28 ± 0.24^{a}	46.99 ± 0.09^{a}	47.62 ± 0.73^{a}
U/S	3.53 ± 0.01^{a}	3.45 ± 0.01^{a}	3.49 ± 0.12^{a}

Note: Results are the mean ± standard deviation ($n = 3$). Values in the same row with different letters are significantly different ($p < 0.05$).
SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; U/S, unsaturated fatty acid/saturated fatty acid.

The fatty acid composition observed in this study differs slightly from published results. For instance, Hassanein et al. (2003) reported different proportions in milk thistle seed oil native to Egypt, while Harrabi et al. (2015) reported variations in Tunisia milk thistle seed oil. Additionally, Fathi-Achachlouei and Azadmard-Damirchi (2009) observed different compositions in Iranian milk thistle seed oil. These differences suggest that fatty acid composition depends on geographical location and genotype.

3.3. Triacylglycerol Composition of Milk Thistle Seed Oil

Triacylglycerol (TAG) constitutes the most abundant component of vegetable oil, representing over 98% of its composition (Tan, Chong, Hamzah, & Ghazali, 2018). Analyzing TAG species is crucial for understanding the physical and chemical properties of vegetable oil. The TAG composition of milk thistle seed oil was determined using the HPLC-APCI-MS method. Table 3 presents the equivalent carbon number (ECN), diacylglycerol fragment, and relative percentage content of identified TAGs, while a typical HPLC chromatogram of TAG composition is illustrated in Fig. 1.

Table 3
Mass spectral and identification of triacylglycerol composition of milk thistle seed oils by HPLC-APCI-MS (%).

TAG	ECN	$[M+NH_4]+$	Diacylglycerol fragments (m/z)			Content (%)		
			1	2	3	Cold press	Hexane	Ethanol
LLLn	40	894.5	597.3			1.03	1.83	2.05
LLL	42	896.5	599.3			17.89	18.68	18.24
OLL	44	898.5	601.3	599.3		21.01	20.39	20.09
PLL	44	872.5	575.3	599.3		10.51	10.78	11.01
GLL	46	926.5	599.3	629.3		trace	trace	trace
OOL	46	900.5	601.3	603.4		9.08	9.05	8.64
SLL	46	900.5	599.3			trace	trace	trace
POL	46	874.5	577.3	601.3		15.70	14.96	14.58
PPL	46	848.5	575.3	551.3		0.58	1.05	0.98
GOL	48	928.5	601.3	629.4		trace	trace	trace
OOO	48	902.5	603.3			0.25	0.55	0.53
POO	48	876.5	577.3	603.4				
SOL	48	902.5	601.3			11.98	11.37	11.59
ALL	48	928.5	599.3	631.3				

PPO	48	850.5	551.3	577.3		1.30	1.22	2.19
PSL	48	876.5	575.3	603.3	579.3	0.23	0.16	0.18
BLL	50	956.5	599.3	659.4		trace	trace	trace
AOL	48	930.5	601.3	633.3	631.5	trace	trace	trace
OOS	50	904.5	605.3			6.53	6.30	6.07
POS	50	878.5	577.3	605.4		0.99	0.97	0.93
PAL	50	904.5	575.3	607.4	631.3			
LgLL	52	984.5	599.3	687.4		trace	trace	trace
AOO	52	932.5	603.4	633.4		2.46	2.20	2.42
PLB	52	932.5	575.2	635.4	659.4			
BOL	52	958.5	601.3	659.4	661.4	trace	trace	trace
AOP	52	906.5	577.3	633.3		0.46	0.48	0.49
OSS	52	906.5	605.4	607.4				

Note: P, Palmitic (C16:0); S, Stearic (C18:0); O, Oleic (C18:1); L, linoleic (C18:2); Ln, linolenic (C18:3); A, Arachidic (C20:0); G, Gadoleic (C20:1); B, Behenic (C22:0); Lg, Lignoceric (C24:0).

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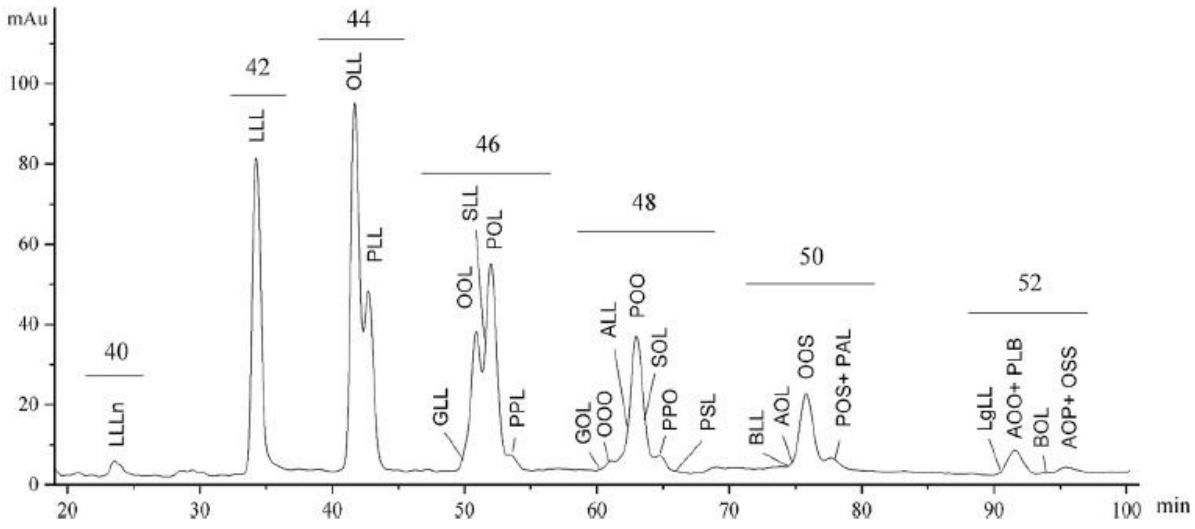

Fig. 1. HPLC chromatogram of the triacylglycerol composition of milk thistle seed oil extracted with *n*-hexane extraction.

As TAGs are eluted in order of increasing ECN, they are generally separated, except for positional isomers (Holčapek, Jandera, Zderadička, & Hrubá, 2003). Table 3 reveals that milk thistle seed oils extracted using different methods exhibit similar TAG compositions, comprising 7 types of ECN. The predominant ECNs are ECN 44 (~39%), ECN 46 (~35%), and ECN 48 (~14%), collectively accounting for approximately 88% of total TAGs. Irrespective of extraction methods, at least 27 TAG types were identified, with OLL (~20–21%) being the most abundant, followed by LLL (~18%), POL (~15%), and PLL (~11%). Additionally, the contents of OOL, POO, and OOS exceeded 5%.

These findings contradict previous reports, suggesting variations in milk thistle seed oil composition depending on the extraction method and other factors. Further research is warranted to elucidate these discrepancies and understand the factors influencing TAG composition in milk thistle seed oil.

3.4. Sterol Composition of Milk Thistle Seed Oil

Sterol, a major component of unsaponifiables in vegetable oil, is advocated for inclusion in edible oil due to its ability to reduce serum low-density lipoprotein (LDL) cholesterol and its beneficial effects in anti-inflammatory and anti-tumor activities (Fathi-Achachlouei, Azadmard-Damirchi, Zahedi, & Shaddel, 2019). Table 4 reveals the identification of eight types of sterols in milk thistle seed oil, with Δ7-stigmastenol and sitosterol being the predominant components, followed by stigmasterol, Δ7-avenasterol, and Δ7-campesterol. These findings align with previous reports in the literature (Harrabi, Curtis, Hayet, & Mayer, 2016; Hassanein et al., 2003).

Table 4
Sterol composition of milk thistle seed oil (%).

Sterol type	Cold press	Hexane extraction	Ethanol extraction
Campesterol	4.97 ± 0.17^{a}	4.79 ± 0.03^{a}	5.71 ± 0.13^{b}
Stigmasterol	6.20 ± 0.07^{a}	6.31 ± 0.04^{a}	6.71 ± 0.15^{b}
Δ7-Campesterol	7.40 ± 0.18^{c}	4.42 ± 0.14^{a}	6.02 ± 0.26^{b}
Sitosterol	28.97 ± 0.60^{a}	37.36 ± 0.78^{b}	37.95 ± 0.67^{b}
Δ5-Avenasterol	4.07 ± 0.24^{a}	8.92 ± 0.24^{b}	3.49 ± 0.03^{a}
Δ5,24-Stigmastadienol	2.98 ± 0.15^{c}	1.84 ± 0.12^{a}	2.56 ± 0.10^{b}
Δ7-Stigmastenol	36.57 ± 0.32^{c}	25.62 ± 0.42^{a}	30.15 ± 0.18^{b}
Δ7-Avenasterol	7.74 ± 0.02^{c}	4.76 ± 0.15^{a}	6.66 ± 0.24^{b}
Unknown1	1.10 ± 0.10^{a}	0.94 ± 0.07^{a}	0.73 ± 0.20^{a}
Unknown2	–	3.33 ± 0.10	–
Unknown3	–	1.71 ± 0.04	–
Total content (mg/100 g)	291.43 ± 8.57^{a}	447.32 ± 15.41^{b}	302.8 ± 6.00^{a}

Note: Results are the mean ± standard deviation (n = 3).Values in the same row with different letters are significantly different ($p < 0.05$).

Notably, the total sterol content and relative content of each sterol in milk thistle seed oil are influenced by the extraction methods. Delta 7-stigmastenol was the most predominant sterol compound in cold-pressed milk thistle seed oil, whereas sitosterol, followed by Δ7-stigmastenol, was predominant in hexane-extracted and ethanol-extracted oils. The higher

relative content of Δ7-stigmastenol and Δ7-campesterol in ethanol-extracted oil compared to hexane-extracted oil may be attributed to their greater solubility in ethanol (Lim & Nyam, 2016). Furthermore, the total sterol content in hexane-extracted oil (447.32 ± 15.4 mg/100g) and ethanol-extracted oil (302.8 ± 6.00 mg/100g) was higher than that in cold-pressed oil (291.43 ± 8.57 mg/100g), indicating the greater efficiency of solvent extraction in extracting sterols from oilseeds.

These results corroborate findings by Azadmard-Damirchi, Habibi-Nodeh, Hesari, Nemati, and Achachlouei (2010), who studied the oxidative stability and nutraceutical content of rapeseed oils extracted using solvents or cold press methods, respectively. However, the appearance of a few unknown peaks in the HPLC chromatogram of hexane-extracted oil necessitates further investigation for identification.

3.5. Vitamin E Composition of Milk Thistle Seed Oil

Vitamin E, an essential lipid-soluble antioxidant present in vegetable oil, primarily comprises tocopherols and tocotrienols. It plays a crucial role in inhibiting lipid peroxidation and converting lipid radicals into more stable products, thereby enhancing the oxidative stability of vegetable oils (Gai et al., 2013). In this study, six vitamin E isomers, including α-, β-, γ-, and δ-tocopherol, as well as α- and γ-tocotrienol, were identified in milk thistle seed oils (Fig. 2). The quantification of these compounds is detailed in Table 5.

Table 5
Vitamin E composition and antioxidant activity of milk thistle seed oil (mg/kg).

Isomers	Cold press	Hexane extraction	Ethanol extraction
α-TP	527.89 ± 1.53^{c}	504.22 ± 1.73^{b}	452.44 ± 2.76^{a}
α-TT	21.33 ± 0.18^{a}	27.94 ± 0.39^{b}	30.80 ± 0.57^{c}
β-TP	43.24 ± 0.36^{b}	56.42 ± 0.32^{c}	40.33 ± 0.99^{a}
γ-TP	30.86 ± 0.56^{c}	24.62 ± 0.17^{a}	26.83 ± 0.70^{b}
γ-TT	10.03 ± 0.50^{b}	5.93 ± 0.01^{a}	10.01 ± 0.29^{b}
δ-TP	12.13 ± 0.10^{b}	11.72 ± 0.14^{b}	10.05 ± 0.49^{a}
ΣTP	614.10 ± 2.54^{c}	596.97 ± 2.36^{b}	529.64 ± 3.53^{a}
ΣTT	31.36 ± 0.32^{a}	33.87 ± 0.40^{b}	40.80 ± 0.86^{c}
Total Vitamin E (mg/kg)	645.47 ± 2.23^{c}	630.84 ± 2.12^{b}	570.45 ± 4.39^{a}
DPPH (%)	34.81 ± 0.75^{a}	43.71 ± 1.20^{b}	77.82 ± 1.97^{c}
FRAP (μmol/kg)	221.02 ± 2.98^{a}	319.48 ± 12.3^{b}	607.94 ± 7.79^{c}

Note: Results are the mean ± standard deviation (n = 3).Values in the same row with different letters are significantly different ($p < 0.05$).

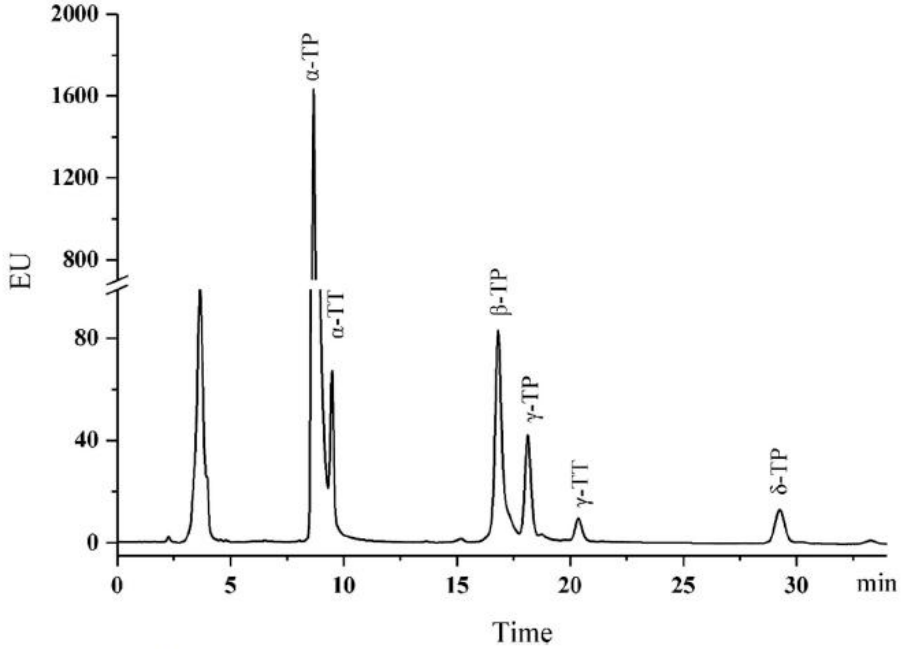

Fig. 2. Typical HPLC chromatogram of vitamin E of milk thistle seed oil extracted with *n*-hexane extraction.

As observed in Table 5, α-tocopherol is the most abundant vitamin E isomer (452.44–527.89 mg/kg), followed by β-tocopherol (40.33–56.42 mg/kg), γ-tocopherol (24.62–30.86 mg/kg), α-tocotrienol (21.33–30.80 mg/kg), and small amounts of δ-tocopherol and γ-tocotrienol. The total vitamin E content ranges from 570.45 to 645.47 mg/kg. These results align with values reported in the literature, where the vitamin E content varied from 224 to 1015 mg/kg, with α-tocopherol being the dominant form (Fathi-Achachlouei et al., 2019; Hassanein et al., 2003). However, differences exist in the minor vitamin E isomers, likely due to variations in planting locations and milk thistle varieties.

Notably, the extraction method significantly affects ($p < 0.05$) the content and composition of vitamin E. The α-tocopherol content in ethanol-extracted oil was the lowest among all samples studied, resulting in a lower total vitamin E content in ethanol-extracted oil. However, the tocotrienol content in ethanol-extracted oil exceeded that in cold-pressed and hexane-extracted oil. Tocotrienol is recognized as a superior antioxidant to α-tocopherol, exhibiting remarkable anti-cancer, hypocholesterolaemic, and neuroprotective effects (Zhang et al., 2019). Consequently, ethanol-extracted oil may possess excellent antioxidant capability owing to its tocotrienol content. Additionally, the vitamin E content in cold-pressed oil was significantly higher ($p < 0.05$) than that in solvent-extracted oils, as indicated by the results in Table 5.

3.6. Antioxidant Activity of Milk Thistle Seed Oil

The antioxidant activity of milk thistle seed oil was evaluated using two well-established methods: DPPH and FRAP. Both methods yielded similar results regarding the antioxidant activity of the oil.

Extraction method significantly influenced ($p < 0.05$) the antioxidant activity of milk thistle seed oil. Specifically, ethanol-extracted oil exhibited much higher antioxidant activity compared to cold-pressed and hexane-extracted oils. Similar findings have been reported for other oils such as oat oil and walnut kernel oil.

The high antioxidant activity of vegetable oils is often attributed to their rich content of endogenous antioxidant compounds, including tocopherols, tocotrienols, phytosterols, polyphenols, carotenoids, and flavonoids, which can scavenge free radicals and active oxygen. Interestingly, in this study, there was no correlation or even a negative correlation between antioxidant activity and the total sterol content, total vitamin E content, or tocopherol content. However, a significant ($p < 0.05$) positive correlation was observed between antioxidant activity and tocotrienol content. This suggests that tocotrienols may play a crucial role in preventing milk thistle seed oil from oxidation. Moreover, it indicates that other antioxidant compounds besides sterols and tocopherols may be involved in inhibiting the oxidation of milk thistle seed oil.

3.7. Thermal Property of Milk Thistle Seed Oil

The differential scanning calorimetry (DSC) curves of milk thistle seed oils are illustrated in Figure 3. These curves reveal that the melting of milk thistle seed oil occurs within the temperature range of -44 to 10 °C. Specifically, there are five endothermic transitions observed in the heating curves, with two major transitions occurring at around -24 and -15 °C, respectively. Additionally, three minor transitions are observed at -32, -6, and 9 °C. Furthermore, an additional endothermic transition is noted at 5 °C for cold-pressed and ethanol-extracted oils.

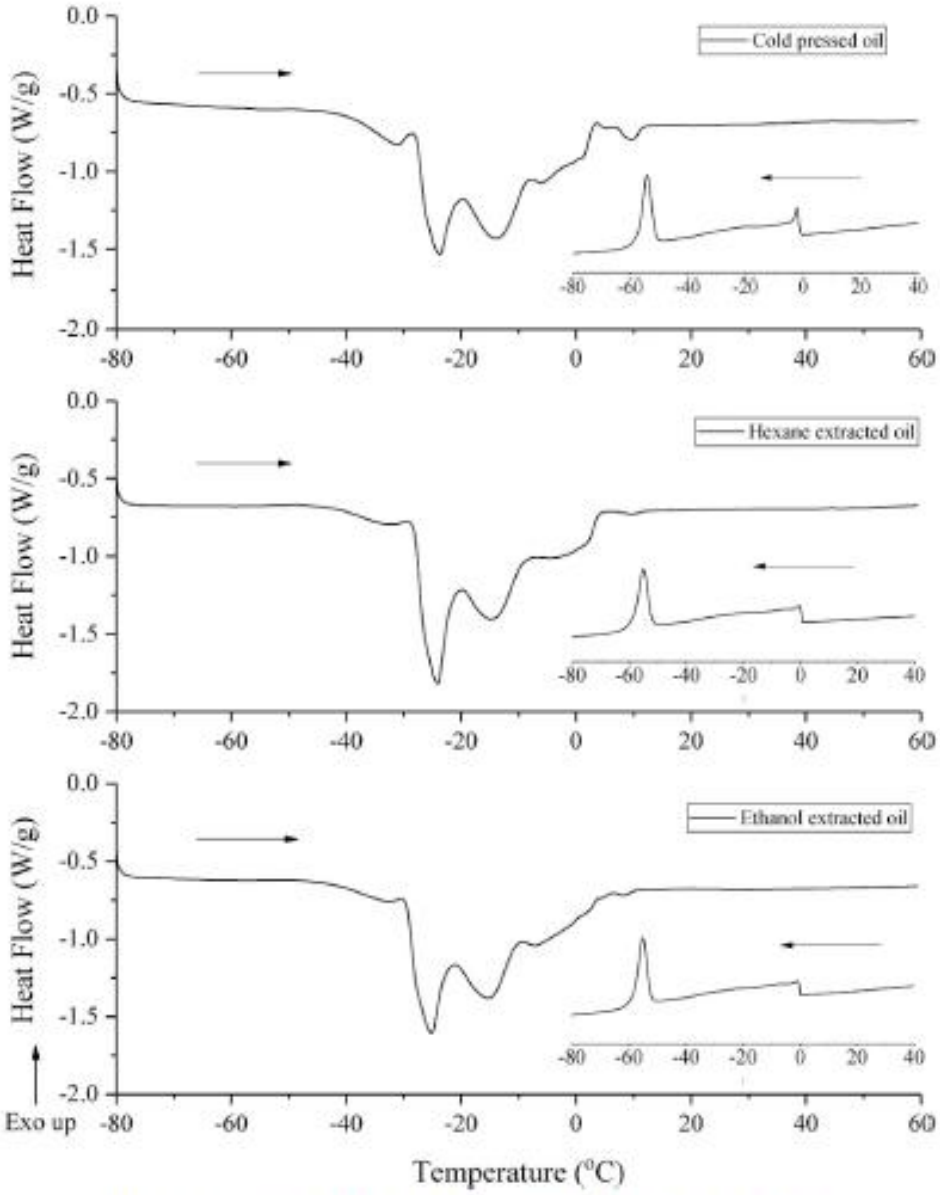

Fig. 3. DSC curves of milk thistle seed oils obtained with three different methods.

These findings differ from those reported by Meddeb et al. (2017), who studied the thermal behavior of Tunisian milk thistle seed oil. Their study revealed a major peak occurring at -30 °C and several secondary endothermic transitions ranging from -49 to 12 °C. This discrepancy may be attributed to variations in the fatty acid composition of the oils.

Typically, endothermic peaks at lower temperatures result from the melting of unsaturated fatty acids and triacylglycerols (TAGs), while those at higher temperatures are associated with the melting of saturated fatty acids and TAGs.

The cooling curves of milk thistle seed oils display two exothermic transitions: a small peak at approximately -2 °C and a sharp, narrow peak at approximately -55 °C. The peak at lower

temperatures is attributed to the melting of tri- and di-unsaturated TAGs, while the one at higher temperatures is associated with the melting of monounsaturated TAGs.

In conclusion, milk thistle seed oils obtained through different methods exhibit similar melting and crystallization behaviors. These thermal data are valuable for controlling oil fractionation and crystallization during production and can aid in the identification of unknown seed oil samples.

4. Conclusion

This study highlights the distinct chemical composition of Iranian milk thistle seeds and their extracted oil compared to previous reports. While the extraction methods (hexane extraction, ethanol extraction, and cold pressing) did not significantly impact the fatty acid and triacylglycerol profiles of the oil, they did affect the content and composition of vitamin E and sterols.

Solvent extraction proved to be more efficient in extracting sterols from milk thistle seeds, while cold-pressed oil showed higher vitamin E content. Antioxidant activity, as assessed by DPPH and FRAP assays, was found to be influenced by the tocotrienol content rather than the total sterol and vitamin E contents.

Additionally, milk thistle seed oil displayed unique melting characteristics, providing a potential method for its identification. These findings contribute to a better understanding of milk thistle seed oil and its potential applications in various industries.

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