Cold-pressed milk thistle seed oil: physico-chemical properties, composition and sensory analysis

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Submitted: 25 August 2021; Accepted: 17 November 2021; Published online: 15 December 2022

SUMMARY: Cold pressed oil was produced from milk thistle seeds, and its composition and sensorial properties were determined. The seeds were found to contain 14.98% oil, 17.31% protein and 4.14% ash. The peroxide value of the oil (11.39 meqO₂/kg oil) was within acceptable limits according to codex, but the free fatty acidity value (3.45%) exceeded the limit. The oil melted at -20.18 °C and crystallized at -3.71 °C. Linoleic acid (51.97%), β -sitosterol (67.56 mg/100 g oil) and γ -tocopherol (53.60 mg/kg oil) were determined as the main components, respectively. Six sensory descriptive terms (sweet, spicy, raw vegetable, straw, roasted and throat-catching) were described for the oil. Consumer tests proved that cold-pressed milk thistle seeds oil had intermediate acceptance scores and consumer satisfaction was moderate. In conclusion, it is thought that milk thistle seeds could be used for the production of edible gourmet oil. Further studies regarding the composition of the bio-active molecules in the oil are anticipated.

KEYWORDS: Cold press; Milk thistle seed; Oil; Quality; Sensory.

RESUMEN: Aceite de semilla de cardo mariano prensado en frío: propiedades físico-químicas, composición y análisis sensorial. Se obtuvo aceite prensado en frío a partir de semillas de cardo mariano y se determinó su composición y propiedades sensoriales. Se encontró que las semillas contenían 14,98% de aceite, 17,31% de proteína y 4,14% de ceniza. El índice de peróxido del aceite (11,39 meqO₂/ kg de aceite) se encontraba dentro del límite aceptable según el Codex, pero el índice de acidez libre (3,45%) excedía el límite. El aceite fundió a -20,18°C y cristalizó a -3,71°C. Se determinaron como componentes principales el ácido linoleico (51,97%), β-sitosterol (67,56 mg/100 g de aceite) y γ-tocoferol (53,60 mg/kg de aceite), respectivamente. Se describieron en el aceite seis términos descriptivos sensoriales: dulce, picante, vegetal crudo, pajizo, asado y pegajoso. Las pruebas de consumo demostraron que el aceite de semilla de cardo mariano prensado en frío tenía puntuaciones de aceptación intermedias y la satisfacción del consumidor era moderada. En conclusión, se cree que las semillas de cardo mariano podrían utilizarse para la producción de aceite gourmet comestible. Se requieren más estudios sobre la composición de moléculas bioactivas del aceite.

PALABRAS CLAVE: Aceite; Calidad; Prensado en frío; Semilla de cardo mariano; Sensorial.

Citation/Cómo citar este artículo: Ayduğan A, Ok S, Yilmaz E. 2022. Cold-pressed milk thistle seed oil: physico-chemical properties, composition and sensory analysis. *Grasas Aceites* **73** (4), e481. https://doi.org/10.3989/gya.0894211

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1. INTRODUCTION

Milk thistle (*Silybum marianum* L. Gaert.) is an erect, stout, annual or biennial plant and it is a member of the *Asteraceae* family. It can grow up to 1.5-3 m in height. It has spiny leaves and stems, and purple flower heads. When the leaves and stems are broken, milky sap is released. Each stem ends with a red-purple flower head which is about 5 cm in diameter and each flower head produces approximately 190 seeds (about 6350 seeds per plant). The seeds are 5-8 mm in length, black to brown in color, and have a long white pappus (Bhattacharya, 2011; Karkanis *et al.*, 2011).

Milk thistle is native to some parts of Europe, Africa and Asia and it is now widespread all over the world. It grows as a weed on roadsides or in empty fields; it is also grown specifically as a medicinal plant. For medicinal use, the seeds of milk thistle are mainly utilized. Silymarin is a biologically active compound of milk thistle. It is a mixture of flavonolignans, consisting of silybin, silydianin, and silychristine. Some pharmacological activities of this lipophilic compound are as follows: antioxidant activity, hepatoprotective activity, anti-inflammatory effects, antiviral activities, antidiabetic activities, cardio-protection, hypocholesterolaemic activity, neuroprotective activity, and anticancer activity (Bhattacharya, 2011; Karkanis et al., 2011; Porwal et al., 2019; Murray, 2021). These beneficial health effects were also observed in milk thistle seed oil. The anticancer and anti-inflammatory effects of the oil were studied by Ali et al. (2021). The effects of the oil on the hepatic steatosis and oxidative stress were reported by Zhu et al. (2018). The antioxidant and neuroprotective properties of the oil were studied by Badreddine et al. (2020). The effects of the oil on the cardiovascular and metabolic complications of obesity were reported by Shen et al. (2020).

It was reported that milk thistle seed contains 21.09% oil, 15.46% protein, 26.72% fiber, 4.72% ash, 7.64% moisture, and 24.38% total carbohydrates (Zhang *et al.*, 2020). In addition, the silymarin contents in the seeds of several milk thistle populations were reported as ranging from 23.06 to 77.12 mg/g dry weight (Arampatzis *et al.*, 2018). Hence, milk thistle seeds are nutritionally rich materials and when considering their health

effects and nutritional value, the valorization of these seeds becomes quite important.

Cold pressing is an alternative technique for virgin oil production. It is applied under moderate conditions to keep nutritional compounds and quality safe, in the expense of yield. Clean, safe, sensorially acceptable, and high-quality oils could be produced through the application of the cold pressing technique. In this technique, after pressing, only filtration or centrifugation processes were applied to the oil, and no refining was carried out (Aydeniz *et al.*, 2014; Aydeniz *et al.*, 2017).

In this study, the aim was to produce oil from milk thistle seeds grown in Turkey, by the cold press technique. The physicochemical properties, thermal properties, main components and sensory properties of the oil were determined. Thus, possible uses for this oil were evaluated.

2. MATERIALS AND METHODS

2.1. Materials

Milk thistle seeds (*Silybum marianum* L. Gaert.) were purchased from Aktar Diyarı Co. (İzmir, Turkey). It was acknowledged that the seeds were harvested from cultivation plants located in the İzmir province of Turkey in the 2019 harvest season, and then cleaned and packed before marketing. The seeds were stored in deep-freeze (-18 °C) until cold pressing in our laboratory for two months. All chemicals used in the analyses were purchased from Sigma (St. Louis, MO, USA) or Merck Co. (Darmstadt, Germany).

2.2. Analyses of the milk thistle seeds

The length, width and thickness values of the seeds were determined with a digital caliper (Leo, Nikko Ltd., China). The 1000-seed weight was determined gravimetrically by weighing (Sartorius ED224S, Sartorius, Göttingen, Germany) 25 randomly selected seeds several times, and then multiplying the weight by 40. Color values of the seeds were determined with a Minolta colorimeter CR 400 (Minolta Camera Co., Osaka, Japan). The moisture content of the seeds was measured with a OHAUS MB45 moisture analyzer (Ohaus, Pine Brook, NJ, USA). The total oil, protein, and ash contents were analyzed according to AOAC 920.39 (AOAC, 2002), AOCS Aa 5-38, and AOCS Ba 5a-49 (AOCS, 1998), respectively.

2.3. Cold pressing of the milk thistle seeds

As a result of the pre-experiments, the optimum seed moisture level, which provides maximum oil yield and ease of processing, was determined as 12%. The moisture content in the seeds was adjusted through incubation with added tap water in a closed vessel for 24 h. The amount of added water was calculated by total seed weight and initial seed moisture content. A laboratory scale cold press machine (Koçmaksan, ESM 3710, lzmir, Turkey), with a single head, 1.5 kW power and capacity for 12 kg seed/hour was used for cold-pressed oil production. The cold pressing process was carried out with a screw rotation speed of 18 rpm and a 12 mm exit die. The oil's exit temperature did not exceed 40 °C. After pressing, the oil was centrifuged (Sigma 2-16K, Postfach, Germany) (6797 xg, 10 min) and then filtered through Whatman no. 1 filter paper to remove suspended solid seed materials and moisture. Finally, the oil was placed in amber-colored glasses, flushed with nitrogen gas, and stored at 4 °C during the analyses.

2.4. Physico-chemical analyses of the oil

An oil pycnometer was used to measure the specific gravity of the oil and the analysis was applied according to the AOCS Cc 10c-95 method (AOCS, 1984). Specific extinction coefficient values were measured with a spectrophotometer (Shimadzu UV-1800, Shimadzu Co., Kyoto, Japan) according to the AOCS Ch 5-91 method (AOCS, 2017). An Abbe refractometer (Bellingham and Stanley, Tunbridge Wells, UK) was used to measure refractive index values of the oil. Color values were assessed with a Minolta colorimeter CR-400 (Minolta Camera Co., Osaka, Japan). Apparent viscosity was measured with a Brookfield DV II + Pro Viscometer (Brookfield Eng. Lab., Inc., Middleborough, MA, USA). This analysis was carried out at room temperature with a LV-SC4-18 spindle and rotation speed of 50 rpm.

Free fatty acidity, peroxide value, *p*-anisidine value, iodine number, saponification number, and unsaponifiable matter in the oil samples were determined according to AOCS Ca 5a-40, AOCS Cd 8-53, AOCS Cd 18-90, AOCS Cd 1-25 (AOCS, 1998), AOCS Cd 3-25 (AOCS, 2017), and TSE 894 (TSE, 1970) methods, respectively. The total phenolic content in the oil was determined according to

the Folin-Ciocalteu technique as described by Yilmaz *et al.* (2015).

2.5. Thermal analyses of the oil

Melting and crystallization parameters were determined with a Differential Scanning Calorimeter (Perkin-Elmer DSC 4000, USA) according to Dassanayake *et al.* (2009). About 10 mg oil were placed into an aluminum pan and then sealed hermetically. The analysis was carried out against an empty aluminum pan. The thermal program cycle was as follows: 20 °C to 110 (10 °C/min), 110 °C to -70 °C (10 °C/ min), held at -70 °C for 3 min and -70 °C to 50 °C (5 °C/min). Curves were calculated with Pyris 1 Manager Software.

2.6. Determination of the fatty acid, sterol, and tocopherol compositions of the oil

Fatty acid composition was determined according to AOCS Ce 2-66 (AOCS, 1998). First, 100 mg oil were weighed into a test tube and then 10 mL hexane were added and the oil was dissolved. Then, 100 µL 2 N methanolic KOH were added and mixed for 30 s. Finally, this mixture was centrifuged (Sigma 2-16K, Sartorius, Germany) (6461 xg, 10 min) and the clear phase was taken into a vial. Fatty acid composition was analyzed by GC-MS (GCMS-QP2010, Shimadzu Corporation, Nishinokyo, Japan) equipped with a Rxi-5MS column (30m x 0.25mm ID x 0.25µm film thickness, Restek Co.). The working conditions of GC were as follows: 2 µL injection volume, splitless, 0.83 mL/min flow rate, helium as carrier gas, 250 °C injection temperature. The oven temperature program was as follows: held at 100 °C for 1 min, 100 °C to 160 °C (20 °C/min), held at 160 °C for 1 min, 160 °C to 180 °C (4 °C/min), 180 °C to 330 °C (30 °C/min), held at 330 °C for 4.70 min. The working conditions of the MS detector were as follows: 200 °C ion source temperature, 280 °C interface temperature, 5 min solvent-cutoff time. The library installed in the device was used for the evaluation of the peaks.

The sterol composition of the oil was determined according to the ISO 12228 method (ISO, 1999). First, unsaponifiable matters were extracted. Then, sterol fractions were obtained using Thin Layer Chromatography (TLC). Sterol composition was determined by using a Gas Chromatograph-FID (Agilent Technologies 7890B) equipped with a DB5 capillary column (30 m × 0.25 mm ID × 0.1 µm film thickness, J&W Scientific Co). 1 µL injection volume, 1:100 split ratio, 0.7 mL/min flow rate, 290 °C inlet temperature, and 300 °C detector temperature were used during the analysis. The carrier gas was hydrogen and detector gases were hydrogen (30 mL/min) and dry air (400 mL/min). The thermal program was as follows: held at 60 °C for 2 min, heating to 220 °C (40 °C/min) and held at 220 °C for 1 min, heating to 310 °C (5 °C/min) and held at that temperature for 30 min. Commercial standards were used for the identification of sterols. The amount of sterols was determined from the peak area of α -cholestanol as internal standard.

The tocopherol composition of the oil was analyzed according to Grilo et al. (2014) with minor modifications. 200 µL oil were dissolved in 4.8 mL dichloromethane. The mixture was mixed and placed into a vial. Tocopherol composition was determined by using a HPLC (Shimadzu Corporation, Japan) equipped with Inertsil ODS-3 column (250 mm× 4.6mm× 5 µm, GL Sciences Inc., Japan) and a RF-20A fluorescent detector. The analysis was carried out with 20 µL injection volume, isocratic elution with a flow rate of 1.6 mL/min and 30 °C oven temperature. Methanol: water (97:3, v/v) mixture was used as the mobile phase. Detector wavelengths were set to 290 and 330 nm for excitation and emission, respectively. Commercial standards were used for identification and quantification.

2.7. Sensory descriptive analysis of the oil

Sensorial properties were determined through Quantitative Descriptive Analysis (QDA) (Meilgaard *et al.*, 1991; Altug and Elmacı, 2005). Five female and five male volunteer panelists, between 21 and 47 years of age, were trained for at least 15 hours. Six descriptive terms were developed under the leadership of the panel leader. The descriptive terms, their definitions and references used in the analysis are presented in Table 1. The sample was served in a glass covered with a metal lid to the panelists together with water, unsalted crackers and expectoration cups. The analyses were carried out in daylight at room temperature on different days and sessions. A 10-cm line scale (1 = minimum intensity, 10 = maximum intensity) was used. The analyses were replicated in a randomized order.

2.8. Consumer test

Appearance, smell/aroma, taste/flavor and general acceptance of the oil were evaluated by 50 volunteer consumers. A 5-point hedonic scale was used (1 = Dislike extremely, 5 = Like extremely). A sample was served in a glass covered with metal lid to consumers together with water, unsalted cracker and expectoration cups.

2.9. Statistical analysis

The same batches of seeds harvested in the 2019 season were divided into two parts for two replications of cold-press oil production. After each production, the oils obtained were designed as the replicates. For each replicate oil sample, analyses were performed in triplicate. The data presented were the mean \pm SEM values.

3. RESULTS AND DISCUSSION

3.1. Physico-chemical properties of the seeds

The physico-chemical properties of the milk thistle seeds are shown in Table 2. In one study (El-Haak *et al.*, 2015), length, thickness and width of wild and

TABLE 1. Descriptive terms, their definitions and references used in the sensory descriptive analysis

Descriptive term	Definition	Reference	
Sweet	Flavor associated with sugar solution	Sugar solution (2 g sugar/100 g water)	
Spicy	Flavor associated with spice mixture Red chili pepper, black pepper and thyme		
Raw vegetable	Flavor associated with uncooked/raw vegetable	Green beans/green peppers	
Straw	Flavor associated with dry straw Dry straw		
Roasted	Flavor associated with toasted bread	Toasted bread	
Throat-catching	Burning in the throat 30 seconds after swallowing	Olive oil	

Grasas y Aceites 73 (4), October-December 2022, e481. ISSN-L: 0017-3495. https://doi.org/10.3989/gya.0894211

TABLE 2. Physicochemical properties of the seeds

	Mean ± SEM
Seed dimensions (mm)	
Length	7.08 ± 0.28
Width	3.36 ± 0.16
Thickness	2.15 ± 0.15
1000-seed weight (g)	91.82 ± 0.18
Color	
L*	43.87 ± 2.18
a*	4.70 ± 0.09
b*	16.73 ± 0.51
Moisture (g/100 g seeds)	6.78 ± 0.23
Oil (g/100 g seeds)	14.98 ± 3.68
Protein (g/100 g seeds)	17.31 ± 0.06
Ash (g/100 g seeds)	4.14 ± 0.05

* Results are expressed as mean \pm SEM values of six separate measurements.

TABLE 3. Physiochemical properties of the oil

	Mean ± SEM
Specific gravity (20 °C)	0.82 ± 0.00
Specific extinction	
E232	1.79 ± 0.07
E270	0.20 ± 0.05
Refractive index (40 °C)	1.47 ± 0.00
Viscosity (25 °C, cP)	51.20 ± 0.50
Color	
L*	28.4 ± 0.04
a*	0.5 ± 0.16
b*	3.55 ± 0.14
Free fatty acids (g linoleic acid/100 g oil)	3.45 ± 0.23
Peroxide value (meqO ₂ /kg oil)	11.39 ± 2.26
<i>p</i> -Anisidine value	4.17 ± 0.02
Iodine number (g $I_2/100$ g oil)	85.86 ± 0.04
Saponification value (mg KOH/g oil)	198.30 ± 5.72
Unsaponifiable matter (g/100 g oil)	1.90 ± 0.04
Total phenol content (mg GAE/100 g oil)	11.01 ± 0.1

*Milk thistle seed oil was produced twice, and each analysis for each production was done at least in triplicate. Results are expressed as mean \pm SEM values of six separate measurements.

cultivated milk thistle seeds were found to be about 7 mm, 2 mm and 3 mm, respectively. Our results concur with the literature. 1000-seed weight value is an important parameter to determine seed content. In the study of El-Haak *et al.* (2015), the 1000-seed weights of wild and cultivated milk thistle seeds

were found as 25.23 and 27.40 g, respectively. In another study (Arampatzis et al., 2018), 1000-seed weight values for the seeds in different milk thistle populations were found between 14.91 and 25.90 g. Our results are quite different from the literature, probably because of the differences in milk thistle species and cultivation conditions. L*, a* and b* values of the seeds were measured as 43.87, 4.70 and 16.73, respectively. In the study of Arampatzis et al. (2018), L*, C*, h values were measured as 28.12-42.79, 5.50-14.09 and 72.04-80.71, respectively. Also, in the same study, the seed color was identified as brown. Although the color spaces used in the studies were different, we can say that the colors of the seeds are similar. Moisture, oil, protein and ash contents of the seeds were found as 6.78, 14.98, 17.31 and 4.14%, respectively. In one study (El-Haak et al., 2015), milk thistle seeds were found to contain 28.53-29.68% oil, 22.50-27.54% protein and 3.25-4.50% ash. In another study (Zhang et al., 2020), the proximate composition of milk thistle seeds was determined as 7.64% moisture, 21.09% oil, 15.46% protein and 4.72% ash. There are some minor differences, but the results are generally in line with the literature.

3.2. Physico-chemical properties of the oil

The Physio-chemical properties of the oil are shown in Table 3. Specific gravity and refractive index values mainly depend on the fatty acid composition and therefore these parameters vary according to the type of oil. In one study (Meddeb *et al.*, 2017), the specific gravity and refractive index values of cold-pressed milk thistle seed oils originating from different geographical areas in Tunisia were found as 0.91 and 1.46-1.47, respectively. In the study of Bahl *et al.* (2015), specific gravity of solvent-extracted milk thistle seed oil was determined as 0.885. In the study of Fathi-Achachlouei *et al.* (2019), the refractive index values of milk thistle seed oils were measured as 1.345-1.351. Our results are generally in line with the literature.

Specific extinction values are the indicators of oxidation products. In the codex, it was stated that E232 and E270 values should be 2.50 and 0.22 at the most for extra virgin olive oil, respectively (Codex, 2017). Oil types are different, but production techniques are similar (cold pressing). Therefore, these limit values could be used for comparison. As seen in Table 3, specific extinction values in the oil were lower than the limit values given in the codex. Consequently, it can be said that the oil was in accordance with the codex in terms of oxidation parameters.

Color is an important appearance property for cold-pressed oils in terms of consumer preference. In the study of Meddeb *et al.* (2017), the L*, a* and b* values of milk thistle seed oils originating from different regions were determined as 41.94-66.07, -0.85-1.53 and 5.78-14.95, respectively. The results do not match exactly with this study, but they are similar.

According to codex (Codex, 2017), free fatty acidity and peroxide value should be max. 0.8 % and 20 meqO₂/kg oil for extra virgin olive oil produced by a similar production technique (cold pressing). As seen in Table 3, the peroxide value of the oil was low and within acceptable limits according to codex, but the free fatty acidity value exceeded the limit. The results indicate that during storage of the seeds and the oil, oil hydrolysis might have occurred. Therefore, it is recommended that seeds should be processed without long storage times, and seeds and oil should be stored at low temperature and low humidity. The *p*-Anisidine value is an indicator of secondary oxidation products. In one study (Rokosik et al., 2020), the *p*-anisidine value of cold-pressed milk thistle seed oil was determined as 0.091. In another study (Grajzer et al., 2020), p-anisidine values for cold-pressed milk thistle seed oils were found between 0.13 and 2.19. The *p*-Anisidine value in our study is much higher than these values. Therefore, the storage conditions of the seeds and the oil should be improved.

In our study, the iodine number and the saponification value were found to be 85.86 g $I_2/100$ g oil and 198.30 mg KOH/g oil, respectively. In one study (Meddeb *et al.*, 2017), the iodine number and saponification value for cold-pressed milk thistle seed oils originating from different geographical areas in Tunisia were found between 112.41-118.32 g $I_2/100$ g oil and 128.08-205.16 mg KOH/g oil, respectively. In the study of Bahl *et al.* (2015), the iodine number and saponification value for solvent-extracted milk thistle seed oil were reported as 97 g $I_2/100$ g oil and 199 mg KOH/g oil, respectively. There are some minor differences, but the results are generally in line with the literature.

The unsaponifiable matter content was measured as 1.90%. In the study of Meddeb *et al.* (2017), the unsaponifiable matter content of cold-pressed milk thistle seed oils were found between 1.57 and 5.84%. In another study (Harrabi *et al.*, 2016), milk thistle seeds were collected at different maturity stages and oils were obtained from the seeds following the solvent extraction technique. The unsaponifiable matter contents in these oils were reported as 1.9-3.8%. Our results are in agreement with the literature.

It is known that phenolic compounds in edible oils have various health benefits (Gioxari *et al.*, 2016). In this study, the total phenol content was measured as 11.01 mg GAE/100 g oil. In the study of Grajzer *et al.* (2020), the phenolic contents of cold-pressed milk thistle seed oils were found between 71.7 mg CAE/kg oil and 124.7 mg CAE/kg oil (CAE: Caffeic acid equivalent). In another study (Meddeb *et al.*, 2017), the phenolic contents in cold-pressed milk thistle seed oils were determined as 3.59-8.12 mg GAE/g. It is obvious that there is a great variation between milk thistle seed oils in terms of total phenolic content, probably because of the differences in milk thistle species and cultivation conditions.

3.3. Thermal properties of the oil

The melting and crystallization parameters are presented in Table 4. Just a single peak was observed during melting and crystallization. Melting and crystallization temperatures of the oil were determined as -20.18 °C and -3.71 °C, respectively. It is stated that higher amounts of saturated fatty acids cause higher melting temperatures (Mayfield *et al.*, 2015). The melting point of the oil was lower, as expected, because unsaturated fatty acids were dominant in milk thistle seed oil. In one study (Zhang *et al.*, 2020), it was reported that milk thistle seed oil melts between -44 °C and 10 °C and

TABLE 4. Thermal properties of the oil

	Mean ± SEM
Melting	
Onset _m (°C)	-22.82 ± 0.56
$T_m (°C)$	-20.18 ± 0.17
$\Delta H_{m}(J/g)$	1.12 ± 0.24
Crystallization	
Onset _c (°C)	$\textbf{-0.57} \pm 0.04$
$T_{c}(^{\circ}C)$	-3.71 ± 0.75
$\Delta H_{c} (J/g)$	-7.20 ± 0.61

*Milk thistle seed oil was produced twice, and each analysis for each production was done at least in triplicate. Results are expressed as mean \pm SEM values of six separate measurements.

two major transitions were at -24 °C and -15 °C. In the same study, two transitions, a small peak at -2 °C and a sharp narrow peak at -55 °C, were observed during cooling. In another study (Meddeb *et al.*, 2017), a major peak was observed at around -30 °C during the melting of the oils. Beyond this major peak, they also observed several small peaks from -22.92 °C to 11.62 °C. Although there are some differences, we can say that the results in our study are similar.

3.4. Fatty acid, sterol, and tocopherol compositions of the oil

The physical properties, stabilities, and usage areas of the edible oils mostly depend on their fatty acid compositions. Hence, the determination of fatty acid compositions of oils is very important. As seen in Table 5, milk thistle seed oil contained higher amounts of unsaturated fatty acids. Linoleic acid and oleic acid were the major fatty acids with 51.97 and 27.06%, respectively. The total unsaturated fatty acid content was determined as 79.48%. In one study (Zhang *et al.*, 2020),

TABLE 5. Fatty acid, sterol, and tocopherol compositions of the oil

	Mean ± SEM	
Fatty acids (%)		
Palmitic	10.39 ± 1.35	
Linoleic	51.97 ± 1.1	
Oleic	27.06 ± 0.1	
(E)-octadec-10-enoic	0.45 ± 0.02	
Stearic	5.33 ± 0.2	
Cyclopropaneoctanoic	0.59 ± 0.05	
Arachidic	2.65 ± 0.2	
Docosanoic	1.7 ± 0.1	
Sterols (mg/100 g oil)		
β-Sitosterol	67.56 ± 4.89	
Stigmasterol	9.31 ± 0.89	
Campesterol	6.60 ± 0.72	
∆7-Stigmastenol	20.14 ± 3.92	
Δ 7-Avenasterol	4.93 ± 0.50	
Tocopherols (mg/kg oil)		
δ-Tocopherol	14.91 ± 1.13	
β-Tocopherol	48.87 ± 0.83	
γ-Tocopherol	53.60 ± 1.74	

*Milk thistle seed oil was produced twice, and each analysis for each production was done at least in triplicate. Results are expressed as mean ± SEM values of six separate measurements. linoleic acid (46.19%) and oleic acid (30.59%) were determined as major fatty acids, and total unsaturated fatty acid content was determined as 77.94% for cold-pressed milk thistle seed oil. In another study (Med-deb *et al.*, 2017), the major fatty acids of milk thistle seed oils were found as linoleic acid and oleic acid with 57.00-60.30% and 15.50-22.40%, respectively. In the same study, the total unsaturated fatty acid content was determined as 82.05-83.64%. Generally, the results in this study concur with the literature.

It is known that sterols have important health benefits (Berger et al., 2004). The sterol composition is shown in Table 5. Total sterol content was measured as 108.57 mg/100 g oil. β -Sitosterol was determined as the major sterol with 67.56 mg/100 g oil and it was followed by Δ 7-stigmastenol and stigmasterol. In the study of Zhang et al. (2020), the total sterol content of cold-pressed milk thistle seed oil was found as 291.43 mg/100 g oil and Δ 7-stigmastenol was determined as major sterol, followed by sitosterol. In another study, β-sitosterol was determined as the major sterol for cold-pressed milk thistle seed oil with a total sterol content of 1815.18 mg/kg oil (Rokosik et al., 2020). There are some differences in terms of total sterol content and sterol composition between different studies probably due to differences in milk thistle species and cultivation conditions.

Tocopherols are oil-soluble vitamins which have antioxidant activity. As seen in Table 5, the total tocopherol content was measured as 117.38 mg/kg oil and γ -tocopherol was determined as the major tocopherol with 53.60 mg/kg oil. In a study of Zhang *et al.* (2020), the total tocopherol content was 645.47 mg/kg oil and γ -tocopherol was determined as the major tocopherol for cold-pressed milk thistle seed oil. In another study (Meddeb *et al.*, 2017), α -tocopherol was determined as the major tocopherol for cold-pressed milk thistle seed oils with a total tocopherol contents of 49.57-318.29 mg/kg oil. The results in our study do not exactly match with these previous studies probably due to differences in milk thistle species and cultivation conditions.

3.5. Sensory properties and consumer preferences of the oil

The results from the sensory descriptive analysis are shown in Table 6. Six descriptors were selected to describe the cold-pressed milk thistle seed oil, which were sweet, spicy, raw vegetable, straw, roasted and

TABLE 6. S	Sensory d	lescriptive	properties	of the oil
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Mean \pm SEM		
2.3 ± 1.41		
7.2 ± 2.48		
1.25 ± 0.97		
2.85 ± 2.80		
3.6 ± 2.31		
4.5 ± 2.46		

*Milk thistle seed oil was produced twice, and analysis for each production was done in triplicate. Results are expressed as mean ± SEM values of six separate measurements.

throat-catching. It was observed that spicy flavor was quite dominant compared to other terms and it was followed by throat-catching. To the best of our knowledge, there is no data in the literature about the sensory descriptive terms of milk thistle seed oil. Therefore, this study presents important information to the literature for the first time. In one study (Dhouibi et al., 2020), volatile compounds in cold-pressed milk thistle seed oil were determined. 1,8-Cineole (minty, herbal flavor), methylpyrazine (roasted flavor), 2,5-dimethylpyrazine (roasted flavor) and hexanal (vegetative, herbal flavor) were found as the major volatile compounds in milk thistle seed oil. *p*-Cymene (spicy flavor), isocaryophyllene (woody, spicy flavor) and β-caryophyllene (woody, spicy flavor) were also detected in the oil. The descriptive terms determined for milk thistle seed oil in our study generally match the volatile compound descriptions detected in the study of Dhouibi et al. (2020).

The results from the consumer test are presented in Figure 1. Hedonic scores are generally higher than

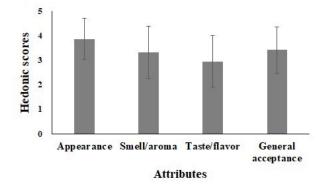


FIGURE 1. The consumer test results of the cold-pressed Milk Thistle seed oil (50 volunteer consumers, n = 6).

3.00 points, which is the neutrality point. Therefore, it could be said that consumer satisfaction was moderate. To the best of our knowledge, there is no data in the literature regarding the consumer preferences of milk thistle seed oil. Therefore, this study presents important information to the literature for the first time.

4. CONCLUSIONS

In this study, milk thistle seed oil was produced by using the cold press technique and the seeds and the oil were characterized. It was observed that cold-pressed milk thistle seed oil was rich in unsaturated fatty acids, sterols and tocopherols. Spicy flavor and throat-catching feeling were determined as dominant sensory properties of the oil. According to the consumer test, coldpressed milk thistle seed oil had intermediate scores and consumer satisfaction was moderate. Therefore, it can be said that this oil could be preferred by consumers. In conclusion, milk thistle seeds can be used to produce high quality cold-pressed oil. Further studies regarding the composition of bio-active molecules and food applications for the oil are foreseen.

ACKNOWLEDGMENTS

This study was prepared from the data contained in the M.Sc. thesis of Ayça Ayduğan completed in the Graduate School of Çanakkale Onsekiz Mart University.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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