

ANALYSIS OF THE PHYSICOCHEMICAL PROPERTIES OF MILK THISTLE SEED OIL AND EVALUATION OF ITS BIOACTIVE POTENTIAL

Sina MAKOU EI, Jolanta MAŁAJOWICZ✉, Joanna BRYŚ

Warsaw University of Life Sciences – SGGW, Poland

Abstract: Milk thistle (*Silybum marianum* L. Gaertn.) is the Asteraceae family's extensively distributed herbaceous plant. Its seeds are famous for their medicinal and industrial potential due to their lipid fraction, richness in valuable fatty acids, and bioactive compounds, including silymarin and polyphenolics. The study aimed to evaluate the chemical composition and antioxidant properties of commercial milk thistle seed oil. The fatty acid profile, triacylglycerol distribution, thermal property, and antioxidant activity were analysed. Research on the physicochemical properties of Polish cold-pressed oil from milk thistle seeds has shown that this oil is a valuable source of unsaturated fatty acids, mainly linoleic ($49.83 \pm 0.02\%$) and oleic ($27.36 \pm 0.11\%$). Beneficial nutritional and health values characterise it, but due to the high value acid number (4.43 ± 0.14 mg KOH/g) and low polyphenol content (0.559 ± 0.004 mg GAE/g), it is unstable, quickly turns rancid, and loses its sensory properties.

Key words: milk thistle seed oil, fatty acids profile, antioxidant properties, oxidative stability

INTRODUCTION

In recent years, oils derived from non-conventional seeds have gained increasing significance. These include oils extracted from, among others, flaxseeds, safflower, pumpkin seeds, grape seeds, pomegranate seeds, and milk thistle seeds [1]. The popularity of oils from unconventional oil seeds, especially those rich in health-promoting ingredients [2], results from the increasing awareness of consumers, their care for their health, and disease prevention through the proper selection and balance of dietary ingredients. The principles of healthy eating are associated with limiting the consumption of saturated fatty acids, which is why there is growing interest in alternative plant oils characterised by a high content of unsaturated fatty acids.

Milk thistle (*Silybum marianum* L. Gaertn.), a member of the Asteraceae family, is a herbaceous plant native to the Mediterranean Basin, North Africa, and the Middle East [3–5], however, the plant is presently widespread throughout the world. Poland is a significant European producer of milk thistle seeds. Currently, the domestic variety, Silma, is cultivated on plantations and is characterised by a high yield of achenes (approx. 1.5 t/h) [6].

Sina Makouei – Institute of Food Sciences, Department of Chemistry, 02-787 Warszawa, 159C Nowoursynowska St.;
e-mail: sina_makouei@sggw.edu.pl; <https://orcid.org/0000-0002-3137-9995>

✉ Jolanta Małajowicz – Institute of Food Sciences, Department of Chemistry, 02-787 Warszawa, 159C Nowoursynowska St.;
e-mail: jolanta_malajowicz@sggw.edu.pl; <https://orcid.org/0000-0001-5197-0187>

Joanna Bryś – Institute of Food Sciences, Department of Chemistry, 02-787 Warszawa, 159C Nowoursynowska St.;
e-mail: joanna_brys@sggw.edu.pl; <https://orcid.org/0000-0002-7852-3624>

Milk thistle is commercially cultivated for its remarkable medicinal properties [7]. It is a safe and well-tolerated herbal product with no known health risks or side effects. The leading pharmaceutical compound of milk thistle is a mixture of at least six flavonolignans, popularly called silymarin. It consists mainly of silibinin, isosilibinin, silychristin, isosilychristin, silydianin, and one flavonoid – taxifolin. Silibinin is the predominant compound in silymarin and is considered to be the primary active ingredient [7–9]. According to Zheljazkov et al. [10], the contents of individual active ingredients in milk thistle seeds are at the level of 0.26–0.36% for taxifolin, 0.69–0.99% for silydianin, 0.27–0.39% for isosilibinin and 1.31–1.78% for silibinin, respectively. In addition to the flavonoid complex, milk thistle seeds contain quercetin, histamine, tyramine, phytosterols, tannins, organic acids, and mineral compounds [11,12]. They also contain high concentrations of oil (20–30%), protein (25–30%) and sugars (approx. 37%) [13–15].

Since the oil must be removed from the seeds before the silymarin can be extracted, milk thistle seed oil is an essential by-product worthy of attention. It has a high concentration of unsaturated fatty acids, which are beneficial to human health in preventing arteriosclerosis, diabetes, and cancer [16]. Moreover, milk thistle seed oil is recommended as a potential source of a natural antioxidant – vitamin E [17]. It also contains other valuable ingredients, including micro and macro elements, at a significant level, such as calcium, potassium, and copper (17 mg/g) [1,18]. In China, since 2014, the Chinese Ministry of Health has approved milk thistle seed oil as a new source of edible oil, allowing its use except for infant food [19].

In recent years, many studies have been conducted to better demonstrate the benefits of milk thistle seed oil. In Poland, milk thistle seeds and the oil that can be extracted from them have been becoming increasingly popular. This research was aimed at assessing the physicochemical and health-promoting properties of *Silybum marianum* L. seed oil extracted by cold pressing (at a temperature not exceeding 40°C), unrefined. This work shows the fatty acid profile, triacylglycerol distribution, thermal property, antioxidant activity and health indicators of milk thistle seed oil. These results contribute to a comprehensive understanding of the role of milk thistle seed oil and complement the existing knowledge of its physicochemical properties.

MATERIAL AND METHODS

Materials

The research material was milk thistle seed oil. It came from the commercial offer of Białystok PPHU JS Słodkie Zdrowie company and Szamotuły SemCo Sp. z o.o. (Poland).

It was cold pressed, unrefined oil. The oil was tested in the first month after pressing, within its declared expiration date. The oil was stored in a refrigerator at approximately 10°C during the analysis. All reagents and solvents, including hexane, ethanol, methanol, diethyl ether, potassium hydroxide, sodium carbonate, acetic acid, and chloroform, were purchased from Sigma-Aldrich and were of analytical quality. The solvents used for the spectrophotometric and chromatographic measurements were of HPLC grade

Determination of fatty acid composition

The compositions of fatty acids in the commercial milk thistle oils were determined using gas chromatography (GC), with a capillary column and a flame ionisation detector. The EN ISO 5509:2001 standard [20] was applied to determine the composition of fatty acids present in the oil. To generate volatile derivatives of fatty acids, the studied samples of oil were applied to esterification with methanol, resulting in fatty acid methyl esters (FAME). A gas chromatograph (YL6100 GC), equipped with a BPX-70 capillary column (0.25 µm film thickness, 60 m length, and 0.25 mm internal diameter), was used to analyse the samples. The carrier gas inside the column was nitrogen. The FAME separation conditions were as follows: initial temperature of 70°C was maintained for 0.5 min, the temperature rise increment was 15°C/min within the range from 70°C to 160°C, then the temperature rise increment was 1.1°C/min within the range from 160°C to 200°C, and finally 30°C/min within the range from 200°C to 225°C. The end temperature of 225°C was maintained for 15 min; the temperatures of the detector and injector were 250°C and 225°C, respectively. Fatty acids were identified based on retention time values compared with standards purchased from Sigma Aldrich, Supelco Analytical, Bellefonte, PA, USA [21].

Determination of triacylglycerol structure using enzymatic hydrolysis

The distributions of fatty acids in commercial milk thistle oils were determined with regard to their positions – internal or external of triacylglycerols – in accordance with the methods described in papers by Bryś et al. [21]. The analysed oils were subjected to enzymatic hydrolysis using pancreatic lipase, which breaks the ester bonds

in the external positions of triacylglycerols. The resulting products were extracted with diethyl ether. Following that, the products of enzymatic deacylation of triacylglycerols, dissolved in ether, were separated using preparative thin-layer chromatography. Isolated *sn*-2 monoacylglycerols, together with a gel, were removed from chromatoplates, followed by their elution with diethyl ether. The fatty acid composition of the obtained *sn*-2 monoacylglycerols was determined by gas chromatography. Based on the compositions of isolated *sn*-2 monoacylglycerols and the starting triacylglycerols, the composition of the fatty acid in the *sn*-1,3 positions was determined.

Determination of acid value and free fatty acid content

The degree of hydrolysis of the analysed oils was determined by the acid value according to the ISO 660:2009 method [22]. The acid value was determined using titration of oil samples dissolved in the mixture of diethyl ether:ethanol (1:1, v/v) with 0.1 M ethanolic potassium hydroxide solution. The free fatty acids content was then computed using the value of the molar mass of oleic acid and the acid value for the studied samples.

Determination of peroxide value

The content of the primary oxidation products of the oils was examined by the peroxide value according to the ISO 3960:2007 method [23]. The peroxide value was determined by the iodometric technique.

Determination of oxidation induction time

Pressure Differential Scanning Calorimetry (PDSC) was used to define the oxidative stability of milk thistle oil. To determine the induction time for the oxidation reaction of the oils, experiments were carried out with the help of a DSC Q20 apparatus (TA Instruments) linked to a high-pressure chamber. The weight of the oil used in the test ranged from 3 to 4 mg. Oil samples were placed in small aluminium pans in an oxygen atmosphere under a pressure of 1,400 kPa. Measurements were taken isothermally at 120°C. The oxidation induction time was determined from the PDSC curves.

Health indicators of oil

The composition of fatty acids was used to calculate health indicators. The hypocholesterolemia/hypercholesterolemia (h/H) ratio was obtained using Eq. 1, and the atherogenicity index (AI) and thrombogenicity index (TI) were obtained using Eq. 2 and 3, respectively.

$$h/H = \frac{(\text{cis-C18:1} + \sum \text{PUFA})}{(\text{C12:0} + \text{C14:0} + \text{C16:0})} \quad (1)$$

$$AI = \frac{(\text{C12:0} + (4 \times \text{C14:0}) + \text{C16:0})}{(\sum \text{PUFA})} \quad (2)$$

$$TI = \frac{(\text{C14:0} + \text{C16:0} + \text{C18:0})}{(0.5 \times \sum \text{MUFA} + (0.5 \times \sum \text{n-6PUFA}) + (3 \times \sum \text{n-3PUFA}) + \frac{(n-3)}{(n-6)})} \quad (3)$$

Determination of total phenolic content

The phenolic content in the samples was quantified using Folin-Ciocalteu's reagent. One gram of each *Silybum marianum* L. seed oil was dissolved in 5 ml of *n*-hexane and extracted with 5 ml of methanol by vortexing at ambient temperature. After centrifugation, the methanolic layer was separated from the lipid phase. Subsequently, 0.5 ml of the methanolic layer was diluted in water, and then 0.5 ml of Folin-Ciocalteu's reagent and 1 ml of a sodium carbonate solution (20%) were added. The absorbance was measured at 760 nm after 60 minutes with the samples kept in the dark. The total phenolic content in the sample was determined using a standard curve plotted for gallic acid ($y = 8086x + 0.0237$; $R^2 = 0.9952$). The results were expressed as milligrams of gallic acid equivalent (GAE) per gram of oil.

DPPH scavenging activity

The antioxidant power was estimated by the DPPH (2,2'-diphenyl-1-picrylhydrazyl) test. The DPPH solution was prepared by dissolving 10 mg of DPPH in 100 mL of methanol. 50 μ L of the methanolic solutions of the oil sample was added to 2.95 mL of the methanolic solution of DPPH. The mixture was shaken vigorously and left at room temperature in the dark for 30 minutes. Then the absorbance of the resulting solution was measured

at 517 nm using a spectrophotometer (RAYLLEIGH UV-1601). The inhibition of the free radical DPPH as a percentage [I(%)] was calculated as follows (Eq. 4):

$$Inh (\%) = 100 \times \frac{(A_{\text{blank}} - A_{\text{sample}})}{A_{\text{blank}}}, \quad (4)$$

where:

A_{blank} – the absorbance of the control (containing all reagents except the test compound),

A_{sample} – the absorbance of the test compound.

Data analysis

All the analyses were performed in triplicate and the results were expressed as mean values \pm standard deviations (*SD*). The statistical analysis was performed using the Statistica software, version 13.3 (StatSoft, Krakow, Poland). Analysis of variance (ANOVA) was used. A *p*-value of ≤ 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Fatty acid profile in milk thistle seeds oil

The composition of fatty acids is a crucial characteristic of edible oil, which has a huge influence on its physicochemical and nutritional properties. Their composition in the tested cold-pressed milk thistle seed oils is shown in Table 1. In the oil samples studied, 11 types of fatty acids were identified based on GC analysis. Linoleic (C18:2, 49.83 \pm 0.02%; 48.97 \pm 1.02), oleic (C18:1, 27.36 \pm 0.11%; 27.06 \pm 0.09), palmitic (C16:0, 8.47 \pm 0.02%; 10.39 \pm 1.35) and stearic (C18:0, 6.23 \pm 0.11%; 5.33 \pm 0.02) were the most predominant fatty acids, which accounted for more than 90% of the total fatty acids. Polyunsaturated acids were present in more than twice the concentration of monounsaturated acids, and the ratio of unsaturated to saturated was approx. 4.31 \pm 0.22 and 3.95 \pm 0.02, respectively, for Białystok PPHU JS Słodkie Zdrowie and Szamotuły SemCo Sp. z o.o. company.

Table 1. Fatty acid composition of commercial milk thistle seeds oils

Fatty acid	Fatty acids composition [%]	
	thistle seeds oils from Białystok PPHU JS Słodkie Zdrowie company	thistle seeds oils from Szamotuły SemCo Sp. z o.o. company
C14:1	0.10 \pm 0.01 ^a	–
C16:0	8.47 \pm 0.02 ^b	10.39 \pm 1.35 ^b
C16:1	0.06 \pm 0.01 ^a	0.04 \pm 0.01 ^a
C18:0	6.23 \pm 0.11 ^b	5.33 \pm 0.02 ^b
C18:1 <i>n</i> -9	27.36 \pm 0.11 ^c	27.06 \pm 0.09 ^c
C18:2 <i>n</i> -6	49.83 \pm 0.02 ^d	48.97 \pm 1.02 ^d
C18:3 <i>n</i> -3	0.20 \pm 0.01 ^a	0.11 \pm 0.07 ^a
C20:0	3.76 \pm 0.03 ^a	4.28 \pm 0.12 ^b
C20:1	1.11 \pm 0.02 ^a	2.88 \pm 0.08 ^a
C20:4 <i>n</i> -6	2.51 \pm 0.17 ^a	1.21 \pm 0.12 ^a
C24:0	0.40 \pm 0.04 ^a	0.32 \pm 0.08 ^a
SFA	18.85 \pm 0.05	20.32 \pm 0.03
MUFA	28.62 \pm 0.03	29.98 \pm 0.10
PUFA	52.54 \pm 0.10	50.29 \pm 0.09
U/S	4.31 \pm 0.22	3.95 \pm 0.02

Note: Results presented in the table are the mean \pm standard deviation (*n* = 3). Values in the same row with different letters are statistically 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.

Source: own elaboration.

The results for the percentage of fatty acid composition of milk thistle seed oil in this study differ slightly from published data. Meddeb et al. [4] analysed the fatty acid composition of oil from three varieties of milk thistle seeds from different areas in Tunisia (Bizerte, Zaghouan, and Sousse). Among the fatty acids, linoleic acid was

the most abundant, accounting for 57.0% to 60.3%. The content of oleic acid was 15.5–22.4% and palmitic acid was 5.5–11.4%. Hassanein et al. [24] reported 53.3% for linoleic, 20.8% for oleic, 9.4% for palmitic, and 6.6% for stearic acid in the milk thistle seed oil native to Egypt. In addition, Fathi-Achachlouei and Azadmard-Damirchi [3] observed 49.7–53.6% for linoleic, 22.8–28.9% for oleic, 7.3–8.4% for palmitic, and 4.6–6.8% for stearic acid present in Iranian milk thistle seed oil. The differences indicate that the fatty acid composition of milk thistle seed oil depends on the geographical location and genotype. The presence of more linoleic and less oleic acid in milk thistle seed oil makes it valuable in terms of its nutritional value, but more susceptible to oxidation processes compared to the popular rapeseed or sunflower oil.

Physicochemical characterisation of cold pressed milk thistle seed oil

In order to more fully characterise the milk thistle seed oil, further research determined the acid and peroxide values, oxidation induction times (Table 2) and the composition of fatty acids in the external (*sn*-1,3) and internal (*sn*-2) positions of triacylglycerols (Table 4).

The acid value is an indicator of the degree of fat hydrolysis. It is defined as milligrams of KOH needed to neutralise the free fatty acids present in 1 g of oil. According to the Codex Alimentarius, the acid value in edible oil should not be higher than 4 mg KOH/g of oil. The conducted research shows (Table 2) that Polish commercial milk thistle oil is characterised by a quite high acid value – 4.43 ± 0.14 mg KOH/g and thus exceeds the permissible normative value. An increased acid value is associated with an increased content of free fatty acids. The oil contained approximately 2.23% of free fatty acids, which were formed as a result of enzymatic hydrolysis by lipases, which could have occurred during the storage of seeds and oil. 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 [25]. Additionally, it is worth noting that FFAs are more susceptible to oxidation than the fatty acids that are present in the triacylglycerol molecules. Hence, the higher the level of FFAs in the seed oil, the higher the risk of oxidation. This phenomenon causes the oil to become rancid, which negatively affects its sensory value.

Table 2. Acid value (AV), free fatty acid (FFA) content, peroxide value (PV) and oxidation induction time (OIT) of analysed oils

Specification	AV [mg KOH/g]	FFA [%]	PV [meq O ₂ /kg]	OIT [min]
Thistle seeds oil from Białystok PPHU JS Słodkie Zdrowie	4.43 ± 0.14	2.23 ± 0.07	24.16 ± 4.22	31.54 ± 1.99
Thistle seeds oil from Szamotuły SemCo Sp. z o.o.	6.02 ± 0.11	3.45 ± 0.23	22.11 ± 3.02	28.72 ± 0.13

Source: own elaboration.

An indicator of the content of primary oxidation products is the peroxide value. It is defined as the number of ml of standard sodium thiosulphate solution needed to titrate the iodine separated from a potassium iodide solution as a result of the action of the peroxides contained in 1 g of oil. Peroxide value is expressed in milliequivalents of oxygen per kg of oil. The peroxide value for cold-pressed oils in accordance with the Codex Alimentarius should not exceed 15 meq O₂/kg and, according to the PN-EN ISO 3960:2017-03 [26] standard – 10 meq O₂/kg. Research on the peroxide value in the tested oils (Table 2 – PV over 24 meq O₂/kg oil) showed that the content of primary oxidation products significantly exceeded both the Codex Alimentarius standard and the ISO standard. The peroxide value of the tested oil differs significantly from the value of this parameter determined in milk thistle oils from other parts of the world. The acid and peroxide values of the various milk thistle seed oils characterised by other scientists were very low, even lower than those determined by the Codex Alimentarius [4, 27,28]. Comparing the results obtained in this study with the results of other researchers examining freshly pressed oil, it can be concluded that the oils immediately after pressing were characterised by a lower content of peroxides than commercial oils [4, 27]. The higher acid and peroxide values of the tested milk thistle seed oil indicate that this type of oil, cold pressed, without refining should be consumed in a short time. The acid and peroxide values may also have been exceeded due to poor storage of the oil or poor quality of the seeds intended for pressing, as well as an incorrect pressing process itself.

In order to provide a more complete characterisation of the analysed oil, samples were also tested using Pressure Differential Scanning Calorimetry (PDSC). The PDSC tests conducted at an isothermal temperature of 120°C showed an average 30.13-minute oxidation induction time for the tested oil (Figure 1).

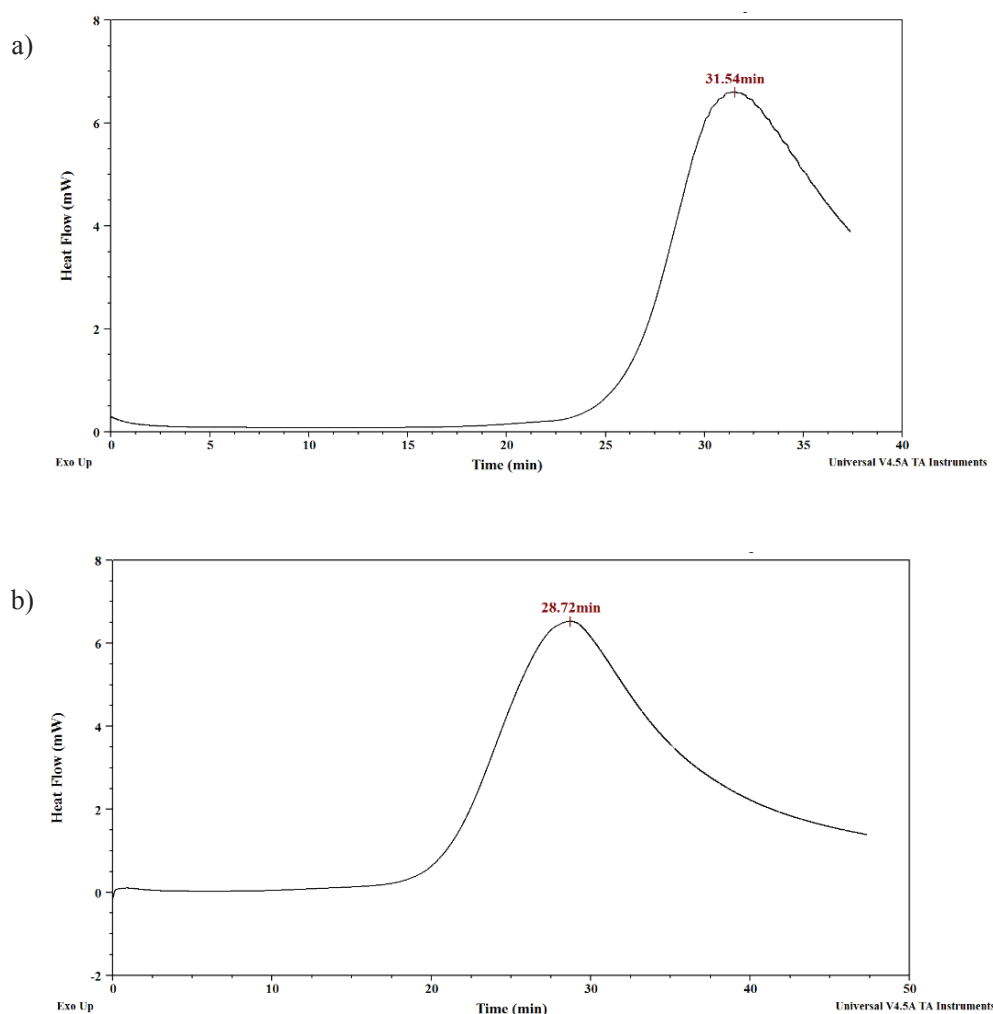


Figure 1. PDSC curves of commercial milk thistle seeds oil where: a) – PDSC curve for thistle seeds oils from Białystok PPHU JS Słodkie Zdrowie; b) – Thistle seeds oils from Szamotuły SemCo Sp. z o.o.

Source: own elaboration.

This value was slightly lower compared to milk thistle oil (about 33 min), which was the subject of research by Bryś et al. [21] on a human milk fat substitute obtained from mixtures of lard and milk thistle oil. Scientists also tested lard using the same method, for which the induction time was over 45 minutes. In general, oils contain a high concentration of unsaturated fatty acids, which are characterised by short induction times and, consequently, worse oxidative stability. Therefore, special attention should be paid to oils such as milk thistle oil during processing and storage, as they are very susceptible to oxidative changes and partial loss of quality.

Analysis of health indices for tested commercial milk thistle seeds oil

Specific health indicators of hypocholesterolemia/hypercholesterolemia, atherogenicity and thrombogenicity provide information on the impact of the fatty acids present in oils on human health in terms of the risk of atherosclerosis and the likelihood of thrombosis. The oils recommended for consumption are characterised by a high hypocholesterolemia/hypercholesterolemia (h/H) index and low atherogenicity ($AI < 1.0$) and thrombogenicity ($TI < 0.5$) indices. Consumption of products with a lower AI is associated with a reduction in total cholesterol levels and low-density lipoprotein (LDL) cholesterol in human plasma, while consuming products with a lower TI and a higher h/H ratio may be beneficial in the prevention of cardiovascular heart disease [29]. The analysed oil was characterised by a low atherogenicity index ($AI = 0.16$) and a bit high thrombogenicity index ($TI = 0.90$); (Table 3). Milk thistle oil, which is the subject of this research, is also characterised by the highest h/H index value (9.44). Summarising the obtained results, it can be stated that the tested oil is characterised by very beneficial nutritional and health values.

Table 3. Health indices for tested commercial milk thistle seeds oil

Health indices		
AI*	TI	h/H
0.161	0.899	9.438

Note: AI – atherogenic index; TI – thrombogenic index; h/H – hypocholesterolaemic/hypercholesterolaemic index.

Source: own elaboration.

Analysis of fatty acid composition in external and internal positions of triacylglycerols

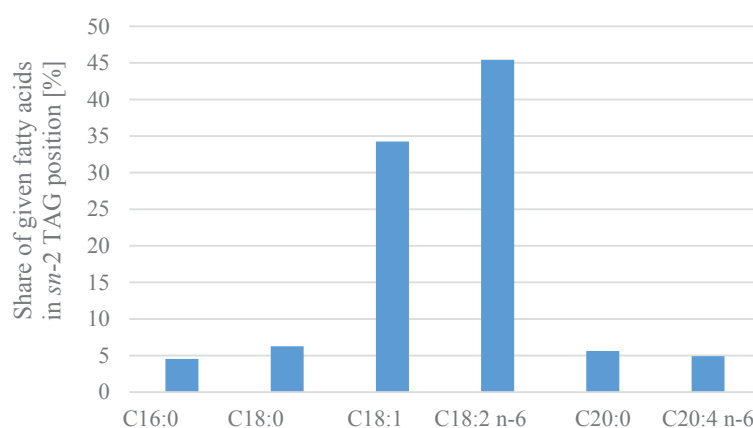
Based on enzymatic hydrolysis and chromatographic analysis of the products, the structure of the triacylglycerols in the tested oil was also examined. Taking into account the obtained results (Table 4, Figure 2), it can be concluded that in the tested oil, saturated fatty acids such as palmitic acid (C16:0) and stearic acid (C18:0) are found mainly in the external positions of triacylglycerols, because their share in the central TAG position is very small. However, unsaturated acids such as oleic (C18:1) and linoleic (C18:2) acids occupy mainly the internal TAG position, as their share in this position is over 80% (Figure 2).

The structure of triacylglycerols is responsible, among other things, for the proper absorption of fats and oil from food. It also prevents the formation of insoluble calcium salts. This is because pancreatic lipase is an enzyme that selectively hydrolyses fatty acids in the *sn*-1,3 positions, producing free fatty acids and 2-monoacylglycerols. Palmitic acid in the form of monoacylglycerol is better absorbed than free palmitic acid, because the latter can bind calcium and magnesium ions, among others creating insoluble salts excreted in the faeces. From this point of view, the TAG structure of milk thistle oil is not suitable because palmitic acid is present mainly in the external positions of the TAG. However, this fatty acid does not occur in significant amounts in this oil [30,31].

Table 4. Distribution of the most important fatty acids in the external (*sn*-1,3s) and internal (*sn*-2) triacylglycerols (TAG) positions

Fatty acids	<i>sn</i> -2 [%]	<i>sn</i> -1,3 [%]
C16:0	1.16	12.12
C18:0	1.17	8.18
C18:1 n-9	28.12	26.98
C18:2 n-6	67.91	40.79
C20:0	0.64	5.32
C20:4 n-6	0.37	3.58

Source: own elaboration.

Figure 2. Percentage of a given fatty acid in *sn*-2 position of TAG of milk thistle seeds oil

Source: own elaboration.

Analysis the Total Phenolic Content (TPC)

The level of total phenolic content in the analysed commercial seed oils from *Silybum marianum* L. was on average 0.559 ± 0.004 mg GAE/g. This value is lower than those reported by Parry et al. [32] and Dabbour et al. [33] for cold-pressed milk thistle seed oil (3.07 mg GAE/g and 1.16 mg GAE/g, respectively). The content of phenolic compounds in the tested oil was also significantly lower than oil pressed from several varieties

of seeds grown in Tunisia, where the TPC ranged from 3.59 to 8.12 mg GAE/g [4]. According to Meddeb et al. [4], differences in TPC may be due to differences in the climate and plant growing conditions, such as soil, irrigation, and temperature. The content of phenolic compounds is correlated with the oxidative stability of the tested oil. A low TPC content (0.559 ± 0.004 mg GAE/g) contributes to its low oxidative stability. This relationship is confirmed by research by Aparicio et al. [34], where the authors indicate a significant contribution of phenolic compounds (51%) to oxidative stability.

Antioxidant activity of milk thistle seed oil

The antioxidant activity of milk thistle seed oil was assessed using the DPPH method. Its value was approx. $19.13 \pm 1.60\%$. In general, the high antioxidant activity of vegetable oil could be attributed to its abundance of endogenous antioxidant ingredients such as tocopherol/tocotrienol, phytosterols, polyphenol, carotenoid, flavonoid, etc., which are able to scavenge free radicals and active oxygen. Zhang et al. [19] suggest that tocotrienol might play an important role in preventing milk thistle seed oil from oxidising.

CONCLUSIONS AND FUTURE PERSPECTIVES

Milk thistle is a plant with promising utility potential. Research on the physicochemical properties of milk thistle seed oil has shown that this oil is a valuable source of essential fatty acids, mainly linoleic and oleic. The ratio of unsaturated to saturated acids was approximately 4.31 ± 0.22 and 3.95 ± 0.02 . The determined indicators of hypocholesterolemia/hypercholesterolemia ($h/H = 9.438$) as well as atherogenicity ($AI = 0.161$) and thrombogenicity ($TI = 0.899$) showed that this oil is characterised by beneficial nutritional and health values and can be an attractive food product. The research carried out complements the existing knowledge of the physicochemical properties of this type of oil and can constitute a basis for further experiments using more advanced techniques.

Acknowledgements

Research equipment was purchased as part of the 'Food and Nutrition Centre – modernisation of the WULS campus to create a Food and Nutrition Research and Development Centre (CŻiŻ)' project co-financed by the European Union from the European Regional Development Fund under the Regional Operational Programme of the Mazowieckie Voivodeship for 2014–2020 (Project No. RPMA.01.01.00-14-8276/17).

REFERENCES

- [1] Sumara A., Stachniuk A., Montowska M., Kotecka-Majchrzak K., Grywalska E., Mitura P., Saftić Martinović L., Kraljević Pavelić S., Fornal E. 2023. Comprehensive review of seven plant seed oils: chemical composition, nutritional properties, and biomedical functions. *Food Reviews International* 39(8): 5402–5422.
- [2] Yang R., Zhang L., Li P., Yu L., Mao J., Wang X., Zhang Q. 2018. A review of chemical composition and nutritional properties of minor vegetable oils in China. *Trends in Food Science & Technology* 74: 26–32.
- [3] Fathi-Achachlouei B., Azadmard-Damirchi S. 2009. Milk thistle seed oil constituents from different varieties grown in Iran. *Journal of the American Oil Chemists' Society* 86: 643–649.
- [4] Meddeb W., Rezig L., Abderrabba M., Lizard G., Mejri M. 2017. Tunisian milk thistle: An investigation of the chemical composition and the characterization of its cold-pressed seed oils. *International Journal of Molecular Sciences* 18(12), 2582: 1–13.
- [5] Tukan S.K., Takruri H.R., Al-Eisawi D.M. 1988. The use of wild edible plants in the Jordanian diet. *International Journal of Food Sciences and Nutrition* 49: 1889–1895.
- [6] Seidler-Łożykowska K. 2010. Hodowla i odmiany roślin zielarskich. *Hodowla i Nasiennictwo* 3: 16–20.
- [7] Hackett E.S., Twedt D.C., Gustafson D.L. 2013. Milk thistle and its derivative compounds. A review of opportunities for treatment of liver disease. *Journal of Veterinary Internal Medicine* 27: 10–16.
- [8] Crocenzi F.A., Roma M.G. 2006. Silymarin as a new hepatoprotective agent in experimental cholestasis: New possibilities for an ancient medication. *Current Medicinal Chemistry* 13: 1055–1074.
- [9] Shaker E., Mahmoud H., Mnaa S. 2010. Silymarin, the antioxidant component and *Silybum marianum* extracts prevent liver damage. *Food and Chemical Toxicology* 48(3): 803–806.
- [10] Zheljazkov V., Zhalnov I., Nedkov N. 2006. Herbicides for weed control in Blessed Thistle (*Silybum marianum*). *Weed Technology* 20:1030–1034.
- [11] Baranowska B., Kurzepa K., Marczak E., Szczucińska A., Lipkowski A. 2003. Utylizacja odpadu nasion ostropestu plamistego II. Biologicznie czynne peptydy z odpadu nasion ostropestu plamistego. *Rośliny Oleiste* 24: 725–732.

- [12] **Malekzadach M., Mirmazloun S., Mortazavi S., Panahi M., Angorani H. 2011.** Physicochemical properties and oil constituents of milk thistle (*Silybum marianum* Gaertn. Cv. Budakalaszi) under drought stress. *Journal of Medicinal Plants Research* 5(13): 2886–2889.
- [13] **Qavami N., Naghdi Badi H., Labbafi M.R., Mehrafarin A.A. 2013.** Review on pharmacological, cultivation and biotechnology aspects of milk thistle (*Silybum Marianum* (L. Gaertn.). *Journal of Medicinal Plants* 12(47): 19–37.
- [14] **Alemardan A., Karkanis A., Salehi R. 2013.** Breeding objectives and selection criteria for milk thistle (*Silybum marianum* L. Gaertn.) improvement. *Not Bot Horti Agrobo* 42(2): 340–347.
- [15] **Aliyas I.M. 2017.** Wild milk thistle unique fatty plant. *International Journal of Science and Research* 6(1): 1227–1229.
- [16] **Kazazis Ch.E., Evangelopoulos A.A., Kollas A., Vallianou N.G. 2014.** The therapeutic potential of milk thistle in diabetes. *The Review of Diabetic Studies* 11(2): 167–174.
- [17] **Hadolin M., Skerget M., Knez Z., Bauman D. 2001.** High pressure extraction of vitamin E-rich oil from *Silybum marianum*. *Food Chemistry* 74(3): 355–364.
- [18] **Marceddu R., Dinolfo L., Carrubba A., Sarno M., Di Miceli G. 2022.** Milk thistle (*Silybum marianum* L.) as a novel multipurpose crop for agriculture in marginal environments: A review. *Agronomy* 12, 729: 1–25.
- [19] **Zhang Z.S., Wang S., Liu H., Li B.Z., Che L. 2020.** Constituents and thermal properties of milk thistle seed oils extracted with three methods. *LWT – Food Science and Technology* 126, 109282: 1–8.
- [20] **PN EN ISO 5509:2001.** Animal and vegetable fats and oils – preparation of methyl esters of fatty acids. Polish Committee for Standardization, Warsaw, Poland.
- [21] **Bryś J., Vaz I.F., Górska A., Wirkowska-Wojdyła M., Ostrowska-Ligęza E., Bryś A. 2017.** Use of GC and PDSC methods to characterize human milk fat substitutes obtained from lard and milk thistle oil mixtures. *Journal of Thermal Analysis and Calorimetry* 130(1): 319–327.
- [22] **ISO 660:2009.** Animal and vegetable fats and oils – determination of acid value and acidity. International Organization for Standardization, Geneva, Switzerland.
- [23] **ISO 3960:2007.** Animal and vegetable fats and oils – determination of peroxide value –iodometric (visual) endpoint determination. International Organization for Standardization, Geneva, Switzerland.
- [24] **Hassanein M., Elshami S.M., Elmallah M.H. 2003.** Detailed studies on some lipids of *Silybum marianum* (L.) seed oil. *Grasas y Aceites* 54: 397–402.
- [25] **Ayduğan A., Ok S., Yılmaz E. 2022.** Cold-pressed milk thistle seed oil: physico-chemical properties, composition and sensory analysis. *Grasas Aceites* 73(4): e481.
- [26] **PN-EN ISO 3960:2017-03.** Vegetable and animal oils and fats. Determination of peroxide number (Reference Method). Polish Committee for Standardization, Warsaw, Poland.
- [27] **Khan I., Khattak H.U., Ullah I., Bangash F.K. 2007.** Study of the physicochemical properties of *Silybum marianum* seed oil. *Journal of The Chemical Society of Pakistan* 29: 545–548.
- [28] **Nyam K.L., Tan C.P., Lai O.M., Long K., Che Man Y.B. 2009.** Physicochemical properties and bioactive compounds of selected seed oils. *Journal of Food Science and Technology* 42: 1396–1403.
- [29] **Siol M., Dudek A., Bryś J., Mańko-Jurkowska D., Gruczyńska-Sękowska E., Makouie S., Palani B.K., Obranović M., Koczoń P. 2024.** Chromatographic and thermal characteristics, and hydrolytic and oxidative stability of commercial pomegranate seed oil. *Foods* 13(9), 1370: 1–16.
- [30] **Bryś J., Wirkowska M., Górska A., Ostrowska-Ligęza E., Bryś A., Koczoń P. 2013.** The use of DSC and FT-IR spectroscopy for evaluation of oxidative stability of interesterified fats. *Journal of Thermal Analysis and Calorimetry* 112: 481–487.
- [31] **López-López A., Castellote-Bargalló A.I., Campoy-Folgoso C., Rivero-Urgel M., Tormo-Carnicé R., Infante-Pina D., López-Sabater M.C. 2001.** The influence of dietary palmitic acid triacylglyceride position on the fatty acid, calcium and magnesium contents of at term newborn faeces. *Early Human Development* 65: S83–S94.
- [32] **Parry J., Hao Z., Luther M., Su L., Zhou K., Yu L.L. 2006.** Characterization of cold-pressed onion, parsley, cardamom, mullein, roasted pumpkin and milk thistle seed oils. *Journal of the American Oil Chemists' Society* 83: 847–854.
- [33] **Dabbour I.R., Al-Ismail K.M., Takruri H.R., Azzeh F.S. 2014.** Chemical characteristics and antioxidant content properties of cold pressed seed oil of wild milk thistle plant grown in Jordan. *Pakistan Journal of Nutrition* 13: 67–78.
- [34] **Aparicio R., Roda L., Albi M.A., Gutiérrez F. 1999.** Effect of various compounds on virgin olive oil stability measured by Rancimat. *Journal of Agricultural and Food Chemistry* 47: 4150–4155.