A Study of the Physical, Chemical, Phytochemical and Nutritional Properties of Wild *Silybum marianum* L. Seeds Oil to Investigate Its Potential Use to Boost Edible Oil Self-Sufficiency in Egypt

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ABSTRACT

Food security is now a threat to Egypt's economy. Egypt relies on large quantities of imports of edible oil. Securing those supplies led Egypt to become one of the top 10 importers of edible oils in the world. This work aimed to investigate the *Silybum Marianum* seeds (SMS) as a potential novel source of edible oil. Fatty acids composition, physicochemical characteristics, selected bioactive components and nutritional quality of SMS cold-pressed oil (SMSO) were examined. The results showed that SMS is a promising source of crude fat (28.04%). USFAs quantitatively accounted for 82.89% of total fatty acids, with linoleic (C18:2) being the most abundant (50.31%), followed by oleic (C18:1) at 31.76%. Unfortunately, only 0.28% represented linolenic (C18:3). The physicochemical characteristics of SMSO were in accordance with Egyptian Standards for vegetable edible oils. SMSO contained a wide range of bioactive components including silymarin 2530.79 mg/100g, α-tocopherol 307.84 mg/100g, β-carotene 30.52 mg/100g, total phenolic content 2165.83 mg GAE/100g and flavonoids 837.86 mg QE/100g. SMSO exhibited valuable nutritional quality indicators represented in the low (atherogenic and thrombogenic) indices and the high (PUFAs/SFAs, USFAs/SFAs, and hypocholesterolemic/ hypercholesterolemic) rations, while ω6/ω3 ratio was far from the recommended values. From the nutritional standpoint, SMSO is a strong candidate to serve as a functional ingredient of the human diet, whether used alone or blended with oils that are rich in ω-3 PUFAs to achieve a wide spectrum of nutritional and health benefits. Based on the obtained results, milk thistle should be cultivated extensively as an innovative resource of oil to boost edible oil self-sufficiency in Egypt.

Keywords: *Silybum marianum*, Cold-pressed oil, physicochemical characteristics; Fatty acids, bioactive compounds, nutritional quality.

INTRODUCTION

Edible oils are a substantial element in human diets. The main sources of edible oil in Egypt are cotton seeds, soybeans, sunflower seeds, maize embryos, and flaxseeds. In terms of imports, the edible oils group ranks second behind wheat (Hassan and Sahfique, 2010). There is currently a wide gap between edible oil consumption and edible oil production from local oilseeds in Egypt (El-Hamidi *et al*., 2020). According to the Egyptian Ministry of Agriculture and Land Reclamation, the current production-consumption gap in edible oil is estimated to be 97% as the population is growing by around 1.5 million people annually (CAPMAS, 2014; El-Hamidi and Zaher, 2018). The amount of edible oils consumed in Egypt per year is around 2.5 million tons, about 48.5 thousand tons of which are produced by oilseed extraction in local extraction plants. Egypt produces only 2% of its edible oil consumption needs through the extraction of local and imported oilseeds; the remaining 98% is imported (El-Hamidi *et al*., 2020). Egypt's sunflower oil prices increased by 32% as a result of the Russian-Ukraine war, causing Egypt's edible oil prices to reach unsustainable levels. Because 73% of sunflower oil is supplied by Russia and Ukraine, the war has already disrupted Egypt's sunflower seed supply. Egypt already needs to find alternatives (Riyad, 2022).

Milk thistle (*Silybum marianum*) is an annual herb that grows wild in Egypt, mostly abundant along streams in the Nile (Delta) and Faiyum regions (El-Mallah *et al*., 2003). Milk thistle belongs to the sunflower family, and is native to northern Africa, some parts of Asia, southern Europe, and both Americas (Soleimani *et al*., 2019). Numerous studies have demonstrated that milk thistle possesses antioxidant, anti-atherosclerotic, hepatoprotective, antihypertensive, anti-obesity, anti-diabetic, anti-inflammatory and anticarcinogenic activities (Abenavoli *et al*., 2010; Meddeb *et al*., 2017; Fanoudi *et al*., 2018; Tajmohammadi *et al*., 2018; Shen *et al*., 2020 and El Hassanen *et al*., 2021). The seed of milk thistle has been shown to contain a high amount of oil ranging from 20 to 31%, and to be rich in unsaturated fatty acids, particularly linoleic and oleic acids, which are beneficial to human health (Fathi-Achachlouei & Azadmard-Damirchi, 2009; Dabbour *et al.*, 2014; Kazazis *et al*., 2014 and Harrabi *et al*., 2015). The Chinese Ministry of

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Health has authorized milk thistle seed oil as a novel source of edible oil in China (Zhang *et al*., 2020).

Unfortunately, data available on the physicochemical characteristics, phytochemicals, and nutritional quality of wild milk thistle seed oil in Egypt are scarce. The present study aims at identifying the physicochemical characteristics, fatty acids profile, nutritional value and selected bioactive components of *Silybum Marianum* seed cold-pressed oil (SMSO). The findings may indicate the potential use of SMSO in human diets and food industries.

MATERIALS AND METHODS

1. Materials

1.1. Plant material

Wild Milk thistle (*Silybum Marianu*m) growing beside public irrigation canals was collected from Mit Ghorab Village, Sinbillawain Center, Dakahlia Governorate, Egypt. The plant material was identified and authenticated by the plant taxonomy experts at the Faculty of Agriculture, Menoufia University, Shebin El-Kom, Egypt. The seeds were obtained from the dried mature flowers, dried under direct sunlight, cleaned from foreign bodies and stored in airtight glass containers until oil extraction.

2. Methods

2.1. Proximate chemical composition of *Silybum Marianum* seeds (SMS)

The moisture, crude protein, crude fat, crude fiber and ash of SMS were determined following AOAC (2000). Nitrogen free extract (NFE) was estimated as follows: %NFE= 100 - (%moisture + %protein + %fat + %fiber + %Ash).

2.2. Cold-pressing of SMS

Intact SMS seeds were pressed three times at room temperature using a laboratory Manual hydraulic press (Xiamen Tmax Battery Equipments Limited, Xiamen, Fujian, China) at a constant pressure of 40 MPa for 30 minutes. The yield oil was centrifuged at 10000 rpm for 10 minutes to obtain clear oil free of suspended particles.

2.3. Physical properties of SMS cold-pressed oil (SMSO)

The physical properties of SMSO included measurements of the color, physical state (4 ºC), density (g/ml, 20 ºC), refractive index (20 ºC) and specific gravity (kg/m^3) were performed in triplicate by the methods of Siger *et al*. (2017). The water content of the oil was determined using the Karl Fischer method (ISO8534: 2017).

2.4. Chemical properties of SMSO

The chemical properties of SMSO involved acid value (mg KOH/g), peroxide value (meq O_2/kg), saponification value (mg KOH/g), free fatty acid (%), ester value (mg KOH/g) and iodine value (g/100g) were determined in triplicate as described by AOAC (2000). The value of *p*-Anisidine was estimated by ISO 6885 (2007) standard methods. A pH meter (Kent EIL 7020) was used to measure the pH. The oleic/linoleic (O/L) acid ratio was calculated according to Kaya (2020). The ratio obtained is expressed as "%".

2.5. Fatty acids composition of SMSO

The oil sample (200 mg) was dissolved in hexane (4 mL) in a conical tube and 200 µL of 2 M methanolic potassium hydroxide solution was added. The samples were neutralised with potassium hydrogen phosphate after vigorous shaking in a vortex for 1 minute. The FAME-containing organic layer was filtered, and 1 µL was injected into the gas chromatograph (GC). A Varian CP-3800 (GC) equipped with a flame ionisation detector (FID) was used to undertake qualitative and quantitative analysis of fatty acid content. For separation, a SUPELCO fused-silica SP-2560poly (biscyanopropyl siloxane) capillary column (100 m x 0.25 mm i.d.; film thickness 0.20 m) was utilized and operated according to the Danish and Nizami technique (2019).

2.6. Bioactive components of SMSO

Total phenolics in SMSO were estimated using the Folin-Ciocalteu reagent following Singleton & Rossi (1965) and Wolfe *et al*. (2003). The results were presented as gallic acid equivalents (GAE). The total flavonoid content was determined by the colorimetric assay according to Zhishen *et al*. (1999) and expressed as quercetin equivalents, QE. Total chlorophyll content was determined by using a portable chlorophyll meter, hand-held chlorophyll analyzer, Model, Chlorophyll meter SPAD-502Plus, Sasha, China. Results expressed as mg 100g^{-1}. The α-tocopherol was determined by the colorimetric method mentioned by Emmerie and Engle (1938). The β-carotene content in SMSO was extracted and analyzed according to Dóka *et al*. (2013). The content of β-carotene was assessed from the absorbance measured at 450 nm (UV–visible spectrophotometer, Shimadzu Corporation, Tokyo, Japan). The Silymarin content in SMSO was determined following the methods of Sathish *et al*. (2005).

2.7. Nutritional quality indices of SMSO

Based on the composition of fatty acids in SMSO. The nutritional quality of the oil was assessed using six distinct criteria. The atherogenic index (AI) and the thrombogenic index (TI) were estimated in accordance with Ulbricht and Southgate (1991). The ratio of hypocholesterolemic/hypercholesterolemic (HH) was estimated following the method described by Guimarães *et al*. (2013). USFAs/SFAs were computed by dividing the total amount of unsaturated fatty acids by the total amount of saturated fatty acids according to Kaya and Eryiğit (2020). The PUFAs/SFAs and ω6/ω3 ratio were also calculated according to Ahmad *et al*. (2020).

$$AI = \frac{C12:0 + 4 * C14:0 + C16:0}{\sum MUFA + \sum \omega6 + \sum \omega3}$$

$$TI = \frac{C14:0 + C16:0 + C18:0}{0.5 * \sum MUFA + 0.5 * \sum \omega6 + 3 * \sum \omega3}$$

$$HH = \frac{C18:1\omega9 + C18:2\omega6 + C20:4\omega6 + C18:3\omega3 + C20:5\omega3 + C22:5\omega3 + C22:6\omega3}{C14:0 + C16:0}$$

3. Statistical Analysis

All measurements were performed in triplicate and data were presented as mean ± standard deviation (SD, n=3).

RESULTS AND DISCUSSION

1. Proximate chemical composition of *Silybum Marianum* seed (SMS)

The proximate chemical composition of SMS was presented in Table (1). It could be noticed that SMS contained a high percentage of crude fat (28.04%), while crude protein, crude fiber, ash and nitrogen free extract contents were (23.85, 6.50, 2.47 and 31.58 %), respectively. SMS used in this work contained a relatively similar percentage of oil compared with data found in the previous studies. Fathi-ashashloy and Azadmard-Demirchi (2009) revealed that the seed oil content of four different genotypes of milk thistle cultivated in Iran ranged from (26 to 31 g/100 g). While, the oil content of Jordanian milk thistle seed was 26.90 g/100g (Dabbour *et al*., 2014), also Harrabi *et al*. (2015) reported the level of total lipid was 30.5% in the milk thistle seed growing wild in Tunisia. While milk thistle seed cultivated in China contained approximately 21 g of oil/100 g (Zhang *et al*., 2020). The variations of oil levels in milk thistle seed owing to the differences in genotype and environmental conditions. In addition, drought stress has been shown to reduce the total oil content of milk thistle seeds (Malekzadeh *et al*., 2011). The oil content in the SMS is relatively commensurate to that of the world's most predominant oilseed crops such as cotton seeds (15–24%), soybean (18–22%) and olives (20–25%) (Matthäus and Ozcan, 2006), and sunflower seed (35–42%) (Premnath *et al*., 2016). These data suggested that SMSO may serve as a natural promise of an innovative source of edible oil.

Table 1. Proximate chemical composition of *Silybum marianum* seeds

Component (g/100g)	Values
Moisture	7.56 ± 0.14
Crude fat	28.04 ± 0.53
Crude protein	23.85 ± 0.44
Crude fiber	6.50 ± 0.12
Ash	2.47 ± 0.05
Nitrogen free extract (NFE)	31.58 ± 0.28

Data expressed as the mean value of three replicates ± SD.

2. Physical properties of *Silybum marianum* seed cold-pressed oil (SMSO)

The physicochemical properties of SMSO are an important criterion for the purity of the oil. The cold-pressed oil SMSO was subjected to some physical analyses as shown in Table (2). SMSO was yellow to deep yellow colour and was liquid at 4°C. The colour may be affected by the existing chlorophyll and carotenoid pigments in the oil. The mean values of the density (g/ml, 20 ºC), water content (ppm), specific gravity (kg/m^3) and refractive index (20 ºC) were (0.915 ± 0.004, 814.33 ± 17.90, 0.827 ± 0.021 and 1.471 ± 0.002), respectively. These findings are compatible with those found by Rokosik *et al*. (2020) except for the water content which was (779 ± 22) ppm. Also, Ciocarlan *et al*. (2018) reported that *S. marianum* oil had a specific gravity of 0.860-0.890 g/cm^3 (20°C) and a refractive index of 1.4705-1.4760. On the other hand, the specific gravity value of SMSO was lower than those stated by Meddeb *et al*. (2017) who stated that the specific gravity values in milk thistle seed oils originating from Bizerte, Zaghouan, and Sousse, in Tunisia were 0.91, 0.91and 0.91 respectively. These variations may be due to the differences in environmental factors and climatic conditions from one country to the other. According to the Egyptian Standards ES 49-1, 3, 4, 6 & 7 (2005) for vegetable edible oils, the refractive index value of SMSO was comparatively similar to those of sesame seed, maize, linseed, soybean and sunflower seed oils (1.465-1.469, 1.465-1.468, 1.474-1.482, 1.466-1.470 and 1.461-1.468), respectively. The refractive index indicates the relation between the speed of light in air and oil, and it rises with chain length and the unsaturation of fatty acids. Since SMSO contains more long-chain fatty acids (>16 carbons) and unsaturation (Table 4), its refractive index is somewhat high. Also, the SMSO density (g/ml, 20 ºC) was compatible with those of ES for edible oils which ranged from 0.912 to 0.936 according to ES 49-1, 3, 4, 5, 6, 7 & 8 (2005).

Table 2. Physical properties of *Silybum marianum* seed cold-pressed oil

Physical properties	Values
Colour	Yellow to Deep Yellow
Physical state (4 ºC)	Liquid
Density (g/ml, 20 ºC)	0.915 ± 0.004
Water content (ppm)	814.33 ± 17.90
Refractive Index (20 ºC)	1.471 ± 0.002
Specific gravity (kg/m^3)	0.827 ± 0.021

Data expressed as the mean value of three replicates ± SD.

3. Chemical properties of *Silybum marianum* seed cold-pressed oil (SMSO)

The chemical characteristics of SMSO are a crucial criterion to determine the quality of the oil (Table 3). The fresh oil samples of SMS cold-pressed oil showed the following characteristics: acid value (2.16±0.07 mg KOH/g), peroxide value (1.17±0.05 meq O2/kg), *p*-Anisidine value (0.11±0.02), saponification value (127.36±3.97 mg KOH/g), free fatty acid (2.58±0.05%), ester value (125.2±0.82 mg KOH/g), iodine value (120.46±3.64 g/100g) and pH value (8.05±0.07). Meddeb *et al*. (2017) found differences in the physicochemical properties indices of SMS oils that are collected from three different regions (Bizerte, Zaghouan and Sousse) in Tunisia, such as saponification values that recorded (205.16, 194.72 and 128.08 mg KOH/g of oil), respectively. The peroxide and the anisidine values are essential parameters for assessing the oil's oxidation quality. In our study, the peroxide value in SMSO was extremely low, even lower than the level recommended for cold-pressed oils by Codex Alimentarius (CODEX-STAN: 210-1999), (maximum 10 meqO2/kg oil). Also, the Egyptian Standards ES 49-1, 3, 4, 5, 6, 7 & 8 (2005) specified the maximum peroxide values of virgin and refined vegetable oils by (15 and 10 meqO_2/kg oil), respectively. The anisidine value in SMSO was slightly higher than that detected by Rokosik *et al*. (2020) for cold-pressed milk thistle (0.091). The acid value, which depends on the amount of free fatty acids, was lower than the value accepted for virgin edible oils by ES 49-1, 3, 4, 5, 6, 7 & 8 (2005) being 4 mg KOH/g. The iodine value, which reveals the content of unsaturated bonds in one gram of oil, is another property that influences the nutritional value of oil. SMSO's iodine value was greater than that observed by Meddeb *et al*. (2017), however consistent with values of sesame seed 104-120, maize 103-135, sunflower 118-141, and cotton seed 100-123 (g/100g) oils according to the ES 49-1, 3, 7 & 8 (2005) for vegetable edible oils. Additionally, SMSO has low acidity and free fatty acid values, indicating that it is appropriate for edible and might be shelf-stable. In general, the variation of physicochemical properties is likely attributable to differing levels of saturated and unsaturated fatty acids in the SMS oil which depend on the region where the seeds were grown, the climatic conditions, and the type of soil

Besides the aforementioned properties, the quality factor, oleic acid/linoleic acid (O/L) ratio was calculated according to the data explained in Table (4) which indicated that the O/L ratio in SMSO was (0.63%). The stability and bitterness of the oils may be assessed using the quality index of O/L (León *et al*., 2008). Under the effect of organic fertilizer types, the O/L acid ratio for cowpea (*Vigna unguiculata L*.) seed oil ranged from 0.402 to 1.043% (Yürürdurmaz, 2022). In fact, oils with a high O/L ratio are more stable and have a lower bitterness (León *et al*., 2008). These results fairly demonstrated the physiochemical characteristics of SMSO are very similar to that found in the most common edible oils in Egypt.

Table 3. Chemical properties of *Silybum marianum* seed cold-pressed oil

Chemical properties	Values
Acid value (mg KOH/g)	2.16 ± 0.07
Peroxide value (meq O_2/kg)	1.17 ± 0.05
p-Anisidine value	0.11 ± 0.02
Saponification value (mg KOH/g)	127.36 ± 3.97
Free fatty acid (%)	2.58 ± 0.05
Ester value (mg KOH/g)	125.2 ± 0.82
Iodine value (g/100g)	120.46 ± 3.64
pH value	8.05 ± 0.07
oleic acid/linoleic acid (O/L)	0.63 ± 0.004

Data expressed as the mean value of three replicates ± SD.

4. Fatty acid composition of *Silybum marianum* seed cold-pressed oil (SMSO)

The fatty acids content of SMSO was determined by GC and presented in Table (4). From such data, it was clear that 9 fatty acids were identified, palmitic (16:0), palmitoleic (16:1), stearic (18:0), oleic (18:1), linoleic (18:2), linolenic (18:3), arachidic (20:0), eicosenoic (20:1) and lignoceric (24:0) acids. Unsaturated fatty acids USFAs quantitatively represented the absolute majority 82.89% of total fatty acids. The main constituents in SMSO were polyunsaturated linoleic acid (18:2, ω-6) was the most plentiful at 50.31%, followed by the monounsaturated oleic acid (18: 1, ω-9) at 31.76%, while linolenic acid (18:3, ω-3) unfortunately represented only 0.28%.

Table 4. Fatty acids composition of *Silybum marianum* seed cold-pressed oil

Fatty acids (g/100g)		Values
Saturated fatty acids (SFA %)		
Palmitic acid C16:0		7.66 ± 0.48
Stearic acid C18:0		6.34 ± 0.22
Arachidic acid C20:0		2.66 ± 0.52
Lignoceric acid C24:0		0.45 ± 0.03
Unsaturated fatty acids (USFA %)		
Palmitoleic acid C16:1		0.24 ± 0.06
Oleic acid C18:1 (ω-9)		31.76 ± 0.31
Linoleic acid C18:2 (ω-6)		50.31 ± 0.78
Linolenic acid C18:3 (ω-3)		0.28 ± 0.03
Eicosenoic acid C20:1		0.30 ± 0.03
∑	SFAs	17.11 ± 1.01
	MUFAs	32.3 ± 0.25
	PUFAs	50.59 ± 0.76

Data expressed as the mean value of three replicates ± SD. SFAs: saturated fatty acids, MUFAs: monounsaturated fatty acids, PUFAs: Polyunsaturated fatty acids

On the other hand, saturated fatty acids SFAs account for about 17.11% of the total fatty acids (the primary SFAs being palmitic 7.66% and stearic 6.34%). The fatty acid composition in SMSO was partially different from the published results. Zhang *et al*. (2020) reported that linoleic (45.83–46.41%), oleic (30.12–30.59%), palmitic (7.87-8.19%), and stearic 6.59–6.69% were the most abundant fatty acids in milk thistle seed cultivated in China. Whereas Harrabi *et al*. (2015) revealed the oil seed content of wild milk thistle growing in Tunisia for linoleic, oleic, palmitic and stearic acids were 59.98, 21.26, 12.74, and 3.24%, respectively. Ciocarlan *et al.* (2018) demonstrated that linoleic acid is the most prevalent unsaturated fatty acid, accounting for 48.88%, followed by monounsaturated fatty acid at 31.94%, palmitic acid at 7.61% and stearic at 4.31%. These findings are somewhat inconsistent with the results of El-mallah *et al*. (2003) who clarified that unsaturated fatty acids constitute 75.1 % of SMS oil. The variations in fatty acids composition are due to the differences in genetic type, geographical location and may be the extraction methods.

The total of MUFAs and PUFAs was high in SMSO. This reflects that SMSO is a rich source of fatty acids with cardio-protective effects. Fatty acids are essential in human nutrition. USFAs, in particular, have been linked to lower the risk of certain diseases like cardiovascular disease; autoimmune disorders such as asthma; certain malignancies such as colon, breast, and prostate cancer; inflammation, and arthritis (Simopoulos, 2002). Moreover, PUFAs are important in a variety of biological systems, including the prevention of cardiac and degenerative diseases, as well as bio-membrane components such as mitochondrial and plasma membranes (Ramadan *et al*., 2009; Kaithwas and Mujumdar, 2010).In this context, Saoussem *et al*. (2009) reported that milk thistle oil has fatty acid compositions compatible with maize kernel oil, with linoleic acid being the most prevalent fatty acid (49.7-62.7%), followed by oleic (23.5-34.9%), and palmitic (9.5-11.5%). Also, milk thistle oil has a very similar fatty acids composition to sunflower and pumpkin oils (Orsavova *et al*., 2015). The seed oil of milk thistle could be consumed as edible oil (El-mallah *et al*., 2003). Furthermore, in 2014, the Chinese Ministry of Health authorized milk thistle seed oil as a novel source of edible oil (Zhang *et al*., 2020).

Our findings confirmed that SMSO is rich in unsaturated fatty acids, including linoleic and oleic acids, which are essential in the human diet and exert a wide range of nutritional and health benefits. The SMSO is therefore recommended as an innovative source of edible oil in Egypt.

5. Bioactive components of *Silybum marianum* seed cold-pressed oil (SMSO)

SMS cold-pressed oil contained a wide range of bioactive compounds. Total phenolics and flavonoids contents, which have a substantial role in seed oil antioxidant capacity were determined in SMSO, the mean values were 2165.83 mg GAE/100g and 837.86 mg QE/100g respectively, as shown in Table (5). Several studies have reported that flavonoids and many other phenolic compounds act as antioxidants, anticancer, antibacterial, cardio-protective, anti-inflammatory, and immune-stimulating agents (Tungmunnithum, 2018; Abd Elalal *et al*., 2022 and Gharib *et al.*, 2022). SMSO is rich in α-tocopherol (307.84 mg/100g) and β-carotene (30.52 mg/100g) which are essential antioxidant compounds in vegetable oils. The α-tocopherol, a naturally occurring phenolic antioxidant component present in varying levels in vegetable oils (Yoshida, 2003), is the most biologically active member of the vitamin E family. It is a significant component of vegetable oils and a crucial lipid oxidation inhibitor in biological systems and foods (Badawy, 2009; Aly *et al*., 2017 and Elhassaneen *et a*l., 2017). The α-tocopherol content in SMSO was lower that determined by Zaborowska and Przygoński (2016) and Rokosik *et al*. (2020) in their studies on milk thistle (530.2 mg/kg, 590 mg/kg), respectively. In turn, cold-pressed oils of rapeseed and sunflower contained about 110-178 mg/kg of α-tocopherol content (Krygier *et al*., 1998). As SMSO is not refined, it has a high chlorophyll content (51.20 mg/100g). The presence of chlorophyll and carotenoid pigments are important, not only for their effect on the oil's color but also because of their nutritional and technological properties, especially their anti- and pro-oxidant properties (Wroniak *et al*., 2006). Furthermore, SMSO had a high silymarin content (2530.79 mg/100g). The silymarin found in milk thistle seeds can preserve liver cells or cells that have not yet been permanently damaged by reducing oxidative stress and consequent cytotoxicity (Gillessen and Schmidt, 2020).

Table 5. Bioactive components of *Silybum marianum* seed cold-pressed oil

Components	Values
Total phenolics (mg	2165.83 ± 94.48
Flavonoids (mg QE/100g)	837.86 ± 39.89
Silymarin (mg/100g)	2530.79 ± 125.43
α-tocopherol (mg/100g)	307.84 ± 14.13
β-carotene (mg/100g)	30.52 ± 1.30
Chlorophyll (mg/100g)	51.20 ± 1.92

Data expressed as the mean value of three replicates ± SD. QE, Quercetin equivalents; GAE, Gallic acid equivalents

As a result of its antioxidant and anti-inflammatory properties, silymarin exerts potential hepatoprotective effects (Feher & Lengyel, 2012; Badawy, 2021 and Elhassaneen *et al*., 2021), protecting the liver from chemically induced liver damage, dietary steatosis and oxidative stress. (Clichici *et al*., 2015; Ni and Wang, 2016), as well as immunomodulatory effects (Katiyar, 2005) and anticancer (Vue *et al*., 2016). Based on the obtained results, we recommend using SMSO in food preparation as a functional ingredient.

6. Nutritional quality indices of *Silybum Marianum* seed cold-pressed oil SMSO

SMSO nutritional quality was evaluated using several combinations and ratios of fatty acids as illustrated in Table (6). It was obvious that the PUFAs/SFAs ratio of 2.96 indicated that SMS oil has an interesting fatty acid balance. It is worth mentioning that diets with a PUFAs/SFAs ratio less than 0.45 are considered insufficient owing to their potential to elevate blood cholesterol levels (Wood *et al*., 2004). In addition, USFAs/SFAs ratio in SMSO was 4.84 this high value is nutritionally desirable (Gonçalves *et al*., 2016). USFAs are precious compounds in the human diet, but their qualitative index of ω6/ω3 ratio is vital for the human health. Excessive omega-6 consumption is associated with decreased ingestion of omega-3 fatty acids is a risk factor for cardiovascular disorders. The estimated ω6/ω3 ratio in SMSO was (179.68: 1) which was far from the recommended level (5:1 to 10:1) according to WHO (1995), also Simopoulos (2008) suggested that the ratio of (ω6: ω3) fatty acids in the human diet should not exceed 4 to prevent arteriosclerosis and coronary heart disease. Although linolenic *ω*-3 acid represented only 0.28% of total fatty acids in SMSO, however, the ω-6/ω-3 ratio was identical to sunflower oil (Orsavova *et al*., 2015).

The fatty acids present in SMSO were assessed for their biological activity against cardiovascular disease by measuring their atherogenic index (AI), thrombogenic index (TI), and hypocholesterolemic/ hypercholesterolemic ratio (HH). The AI and TI indices are considered risk factors for cardiovascular disease. AI indicates the suppression of plaque accumulation while TI indicates the tendency to cause thrombosis in blood vessels. In our present study, SMSO recorded AI and TI ratios of 0.09 and 0.33, respectively which were less than (one), this may indicate that the fatty acids in SMSO may prevent the development of cardiovascular diseases. From the nutritional perspective, oils that have a relatively high HH indicator are the most preferable in the human diet, since they take into consideration the specific effects of fatty acids on cholesterol metabolism.

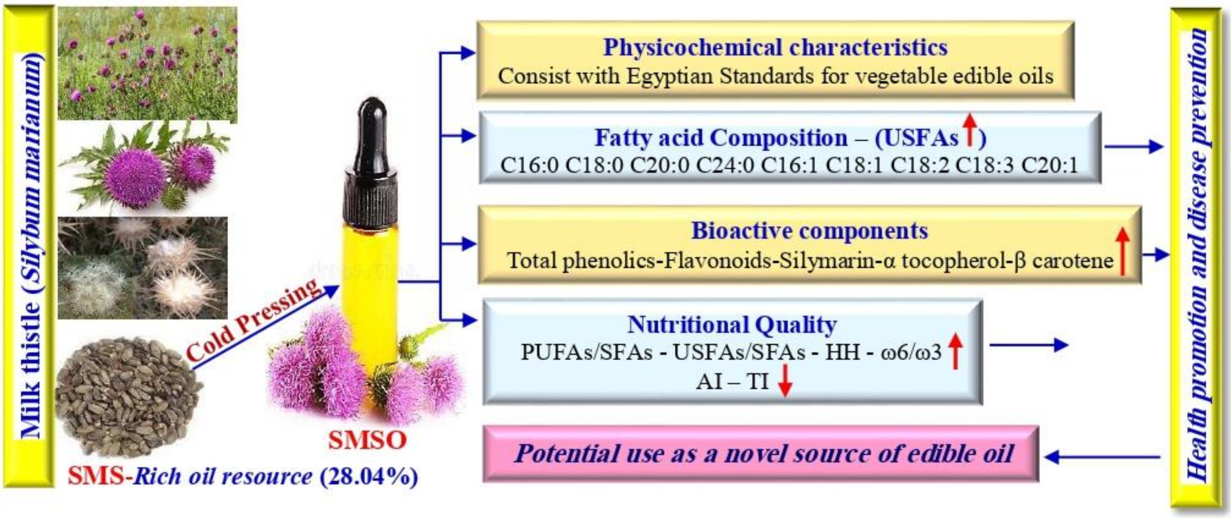
Table 6. Nutritional quality indices of *Silybum Marianum* seed cold-pressed oil

Index	Values
PUFAs/SFAs ratio	2.96 ± 0.23
USFAs/SFAs ratio	4.84 ± 0.36
ω6/ω3 ratio	179.68 ± 20.63
Atherogenic index (AI)	0.09 ± 0.01
Thrombogenic index (TI)	0.33 ± 0.02
Hypocholesterolemic/ hypercholesterolemic ratio (HH)	10.83 ± 0.84

Data expressed as the mean value of three replicates ± SD.

The HH ratio in SMSO was 10.83, which was consistent with the results published by Rokosik *et al*. (2020), and greater than that discovered by Guimares *et al*. (2013) in sesame oil (4.82) but lower than that of flaxseed oil (14.85). Additionally, Shen *et al*. (2020) demonstrated that milk thistle cold pressed oil (MTO) is effective at attenuating dietary obesity-induced weight gain, hypertension, hyperglycemia, and inflammation in mice fed a high-fat diet and suggested that MTO supplementation may helpful for patients exhibiting metabolic syndrome symptoms.

According to the evaluation of the nutritional quality, SMSO is a strong candidate to serve as a functional ingredient as a part of the human diet, as a cooking and salad dressing oil, whether used alone or blended with oils that are rich in ω-3 PUFAs to achieve a broader spectrum of nutritional and health benefits.



Graphical Abstract

CONCLUSION

This study indicated that wild SMS is a promising natural source of oil and the physiochemical characteristics of SMS cold-pressed oil were within the range of Egyptian Standards of vegetable edible oils. SMSO is a rich source of the USFAs and PUFAs; and has a low level of SFAs. Furthermore, SMSO is distinguished by containing a wide range of bioactive compounds such as silymarin, α-tocopherol, β-carotene, and high total phenolics and flavonoids content which exert potential beneficial therapeutic activities. Additionally, the nutritional quality of SMSO using different indices and ratios between fatty acids (PUFAs/SFAs, USFAs/SFAs, ω6/ω3, AI, TI, and HH) indicated that SMSO characterized by high nutritional value mainly thanks to its fatty acid composition that seems to be highly beneficial to human health. In summary, SMSO has the potential to be used as a functional component in human nutrition and could be exploited as a novel resource of edible oil.

ETHICAL APPROVAL

All experiments of the study were approved by the Scientific Research Ethics Committee, Faculty of Home Economics, Menoufia University, Shebin El-Kom, Egypt (Approval # 24- SREC- 01 -2022).

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this paper.

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الملخص العربي

دراسة الخصائص الفيزيائية والكيميائية والكيميائية النباتية والتغذوية لزيت بذور شوك الحليب البري للتحقق من امكانية استخدامه لتعزيز الاكتفاء الذاتي من زيت الطعام في مصر

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يشكل الأمن الغذائي الآن هاجسا للإقتصاد المصري، حيث تعتمد مصر على كميات كبيرة من واردات زيت الطعام، ولقد أدى تأمين هذه الإمدادات إلى أن تصبح مصر واحدة من أكبر ١٠ مستوردين لزيوت الطعام في العالم. لذلك كان الهدف من هذاه الدراسة هو التحقق من امكانية استخدام بذور شوك الحليب *Silybum marianum* (SMS) كمصدر جديد محتمل لزيت الطعام. تم فحص مكونات الزيت من الأحماض الدهنية، الخصائص الفيزيائية والكيميائية، المركبات النشطة بيولوجيا، والجودة الغذائية للزيت المستخلص بطريقة الضغط على البارد (SMSO). ولقد أظهرت النتائج أن SMS تعتبر بمثابة مصدرا واعدا للزيت الخام (٢٨,٠٤%). تمثل الأحماض الدهنية غير المشبعة من الناحية الكمية ٨٢,٨٩% من إجمالي الأحماض الدهنية بالزيت، حيث كان اللينوليك (C18: 2) هو الأكثر وفرة (٥٠,٣١ %)، يليه الأوليك (C18: 1) بنسبة ٣١,٧٦%، في حين لم يمثل اللينولييك (C18: 3) سوى ٠,٢٨% فقط. كانت الخصائص الطبيعية والكيميائية لـ SMSOمطابقة للمواصفات القياسية المصرية لزيوت الطعام النباتية. كما أظهر التحليل الكيميائى أن SMSO يحتوي على مجموعة كبيرة من المكونات النشطة بيولوجيًا بما في ذلك سيليمارين ٢٥٣٠,٧٩ مجم/١٠٠ جم، ألفا توكوفيرول ٣٠٧,٨٤ مجم/١٠٠ جم، بيتا كاروتين ٣٠,٥٢ مجم/١٠٠ جم، محتوى الفينولات الكلية ٢١٦٥,٨٣ مجم GAE/١٠٠ جم، والفلافونويدات ٨٣٧,٨٦ مجم QE/١٠٠ جم. أظهر الـ SMSOمؤشرات جودة غذائية قيمة تمثلت في انخفاض مؤشرات تصلب الشرايين وتجلط الدم، وارتفاع نسبة الأحماض الدهنية عديدة عدم التشبع للأحماض الدهنية المشبعة، ونسبة الأحماض الدهنية غير المشبعة للأحماض الدهنية المشبعة، بالاضافة الى نسبة (خفض كوليسترول الدم الى رفع كوليسترول الدم)، بينما كانت نسبة الأوميجا ٦ الى الأوميجا ٣ بعيدة عن القيم الموصى بها. من وجهة النظر التغذوية، يعد SMSO مرشحًا قويًا ليكون بمثابة مكون وظيفي في النظام الغذائي للانسان، سواء تم استخدامه بمفرده أو ممزوجًا بالزيوت الأخرى الغنية بالأوميجا ٣ لتحقيق مجموعة كبيرة من الفوائد الغذائية والصحية. وبناءًا على النتائج التي تم الحصول عليها، يجب زراعة شوك الحليب على نطاق واسع لتكون بذورها موردا مبتكرا للزيت لتعزيز الاكتفاء الذاتي من زيت الطعام فى مصر.

الكلمات المفتاحية: شوكة الحليب، زيت مستخلص بالضغط على البارد، الخصائص الطبيعية والكيميائية، الأحماض الدهنية، المركبات النشطة بيولوجيا، الجودة الغذائية.