

Onopordum acanthium (Linnaeus, 1753) seeds from Southern Bulgaria: A promising natural source of lipid-soluble bioactive components

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Abstract. The current study aimed to evaluate the chemical and lipid composition of seeds of *Onopordum acanthium* grown in South Bulgaria. The seeds were characterised with a high amount of carbohydrates (56.76%), and similar contents of proteins (16.05%) and glyceride oil (16.45%). The share of dietary fiber was very high, at 38.4%, and that of the ash content was 3.42%. The chemical composition of the seeds determined their good energy value, which was 439 kcal/100 g. The glyceride oil was rich in lipid-soluble bioactive components – unsaponifiable matter (2.70%), sterols (0.69%), tocopherols (172.50 mg/kg), and phospholipids (0.36%), as well as in various essential fatty acids, linoleic acid being the predominant one (69.3%). The other fatty acids found in high amounts were oleic (21.4%) and palmitic (5.3%) acids. The main sterols identified in the seed oil were β -sitosterol (62.2%), followed by stigmasterol (21.7%) and campesterol (8.6%). Two tocopherols were detected in the fraction: γ -tocopherol (74.7%) and α -tocopherol (25.3%), while the predominant phospholipids were phosphatidylinositol (33.7%), phosphatidylcholine (13.1%), phosphatidylserine (12.9%), and phosphatidylethanolamine (11.9%). Important lipid indices were also calculated for *O. acanthium* seed oil – index of atherogenicity (0.0625), index of thrombogenicity (0.1735), hypocholesterolaemic/hypercholesterolaemic (HH) ratio (16.83), peroxidability index (70.64), and oxidation stability index (0.78). These characteristics depicted the health benefits and oxidative stability of the examined glyceride oil, determining its potential implementation in functional foods, nutraceuticals, and therapeutic formulations.

Key words: *Onopordum acanthium*, chemical composition, glyceride oil, lipid-soluble biologically active components.

Introduction

Bulgaria has a diverse and rich flora, with more than 4,100 plants found within its varied landscapes, 2800 of which are higher species. The flora comprises approximately 275 endemic species, the majority of which are found in mountainous regions such as Rila, Pirin, and the Rhodopes (Stoev et al., 2022). Bulgaria also plays a role as a reservoir of medicinal biodiversity, with significant implications for biomedical research and

sustainable development. The country is also home to a myriad of medicinal plants, with nearly 800 wild taxa being legally controlled by the national Medicinal Plants Act and more than 3,600 species being cultivated in botanical gardens. They find application in numerous areas, including traditional medicine, ethnobotany, veterinary medicine, and aromatherapy. Many species possess pharmacologically active secondary metabolites, such as polyphenols, alkaloids, and essential oils,

with high potential for therapeutic, nutraceutical, and cosmetic applications (Zahariev, 2022; Ivanova et al., 2025).

Most studies on medicinal plants predominantly focus on the analysis of the above-mentioned components, while the lipid composition, particularly that of the seeds, remains largely underexplored and insufficiently characterised. The lipids of the plants also contain valuable biologically active components with very important functions in the human body: tocopherols play a role in protecting cellular membranes from oxidative damage due to their antioxidant properties; sterols contribute to the regulation of blood cholesterol levels and support membrane structure and fluidity; phospholipids are essential for the formation and maintenance of biological membranes and participate in cell signaling pathways; and essential fatty acids, such as linoleic and α -linolenic acid, are precursors of eicosanoids and are involved in inflammatory regulation, cardiovascular health, and neural development (Belitz et al., 2009).

A widespread plant in Bulgaria is *Onopordum acanthium* (common names include Scotch thistle, cotton thistle, and camel thorn), a member of the family Asteraceae, which originates from South and Central Europe (Zhelev et al., 2014). It is a biennial flower plant with a height of up to 200 cm (Garsiya et al., 2019). All parts of this plant are edible, and also take part in folk medicine: it can be used in the treatment of some types of cancer; extracts of its aerial parts have a calming effect on the nervous system and possess diuretic properties (Garsiya et al., 2019; Zhelev et al., 2014). The seeds of *O. acanthium* are characterised by a relatively high content of glyceride oil, ranging from 16 to 28%. Protein content is about 18–22%, and that of the total carbohydrates is about 30–35%. The amount of dietary fibers varies from 12 to 15%, which has a supporting effect on the proper function of the gastrointestinal tract and metabolic regulation. The ash content usually ranges from 3 to 5% (Al-Snafi, 2020; Matthaus et al., 2014). Recent studies found that the seeds of *O. acanthium* contain a complex lipid composition comprising fatty acids, tocopherols, sterols, and phospholipids, which have significant nutritional and pharmacological importance. The predominant fatty acid in the seed oil is oleic (342–530 g/kg), followed by linoleic (176–511 g/kg) and palmitic

acid (99–150 g/kg), indicating high levels of unsaturated fatty acids in the lipids suitable for dietary applications. β -Tocopherol is the major component of the tocopherol fraction, reaching concentrations up to 911 g/kg, which suggests strong antioxidant potential (Zhelev et al., 2014). In the sterol fraction, β -sitosterol (546–632 g/kg) and campesterol (128–156 g/kg) dominate, contributing to cholesterol-lowering and membrane-stabilising effects. Phosphatidylinositol is the most abundant phospholipid in the seeds, with levels about 320 g/kg (Zhelev et al., 2014). In addition, a study that examines the changes occurring in the oil content, fatty acid, and phytosterols profile of *O. acanthium* during seed development has identified nine fatty acids and six phytosterols, with notable shifts in the composition during seed maturation, including the presence of erucic and pentadecanoic acids in later stages (Arfaoui et al., 2014). These findings depict that *O. acanthium* seeds are a promising source of bioactive lipids with potential applications in functional foods, nutraceuticals, and therapeutic formulations. Additionally, the information on the entire chemical and lipid composition of seeds from *O. acanthium* grown in the southern regions of Bulgaria is lacking.

Therefore, the aim of the present study was to evaluate the seeds of *O. acanthium* in terms of their chemical and lipid composition, grown in South Bulgaria, in order to provide information about their nutritional properties and evaluate their possible medicinal potential.

Materials and methods

Material

The plant was identified as *Onopordum acanthium*, and the material (ripe seeds) was collected during the growing season of 2023 from natural habitats (dry fields and ruderal places) of Southern Bulgaria (Plovdiv and Pazardzhik regions). The seeds were separated and precisely ground with a laboratory mill, and then directly subjected to analysis.

Methods

Chemical composition

The glyceride oil was extracted from the seeds by a Soxhlet apparatus with n-hexane (ISO 659, 2014). Total proteins, dietary fibers, ash, and moisture content were determined following standard

procedures (AOAC, 2016). Total carbohydrates were calculated using the formula: **100 - (% protein + % lipids + % water + % ash in the seeds)** (FAO, 2003). The starch content was determined by BS 13488 (1976).

The energy value of the seeds was determined following the formula (FAO, 2003):

$$\text{Energy value} = \% \text{ Carbohydrates} \times 4 + \% \text{ Lipids} \times 9 + \% \text{ Proteins} \times 4 \text{ (kcal/100g)}.$$

Fatty acid composition

Fatty acid composition was determined by gas chromatography (GC) (ISO 12966-1, 2014). The glyceride oil was transesterified with sulfuric acid in methanol when the fatty acid methyl esters (FAMES) were obtained (ISO 12966-2, 2017). A GC instrument, Agilent 8860 (Santa Clara, CA, USA), was used for the analysis. It was equipped with a capillary column DB-Fast FAME (Agilent, USA, 30 m × 0.25 mm × 0.25 μm) with a flame ionisation detector (FID). The conditions of the column were: starting from 70°C (1 min), increasing to 250°C at a rate of 5°C/min (3 min); injector temperature was 270°C, and the detector temperature was 300°C. For identification a standard mixture of FAMES (37 comp. FAME mix, Supelco, Bellefonte, PA, USA) was used.

The iodine value was calculated following the formula (AOCS, 1999):

$$\text{IV (g I}_2\text{/100 g)} = [(\% \text{ Oleic acid} \times 90) + (\% \text{ Linoleic acid} \times 181) + (\% \text{ Linolenic acid} \times 274)]/100$$

in which 90, 181, and 274 are the iodine values of pure oleic, linoleic, and linolenic acids.

Sterols

The unsaponifiables were extracted with n-hexane from the glyceride oil (ISO 18609, 2000). Total sterols were isolated from the unsaponifiable matter by thin-layer chromatography (TLC) and measured spectrophotometrically at 597 nm (Ivanov et al., 1972).

Sterol composition was determined on an Agilent 8860 gas chromatograph (Santa Clara, CA, USA) with a capillary column DB 5 (25 m × 0.25 mm × 0.25 μm) and FID. The starting temperature was 90°C (3 min), then at a rate of 15 °C/min increased up to 290°C, and after that up to 310°C at a rate of 4°C/min (10 min). The injector temperature was 300°C, and the detector temperature was 320°C. A

standard mixture of sterols was used for the identification of the components (ISO 12228-1, 2014).

Tocopherols

Tocopherols were determined on a high-performance liquid chromatograph Merck-Hitachi system (Burladingen, Germany) with column Nucleosil Si 50-5 (250 × 4 mm, particle size: 5 μm), and fluorescent detection at 295 nm excitation and 330 nm emission. The following conditions were used: the mobile phase was hexane: dioxane, 96:4 (v/v), and the flow rate was 1 mL/min (ISO 9936, 2016).

Phospholipids

A mixture of chloroform and methanol (2:1, v/v) was used for the extraction of the polar lipids in the seeds (Folch et al., 1957). Individual phospholipids were isolated by two-dimensional TLC, and the spots were scraped and mineralised with perchloric and sulphuric acid (1:1, v/v) (Schneiter & Daum, 2006). The quantification was performed spectrophotometrically at 700 nm (ISO 10540-1, 2014).

Lipid indices

The indices of atherogenicity and thrombogenicity, as well as the hypocholesterolemic/hypercholesterolemic (HH) ratio, were calculated using the formulas (Ulbricht & Southgate, 1991; Santos-Silva et al., 2002):

$$\text{IA} = (\text{C12:0} + 4 \times \text{C14:0} + \text{C16:0})/(\sum \text{MUFA} + \sum \text{PUFA})$$

$$\text{IT} = (\text{C14:0} + \text{C16:0} + \text{C18:0})/[(0.5 \times \sum \text{MUFA}) + (0.5 \times \sum \text{n-6 PUFA}) + (3 \times \sum \text{n-3 PUFA}) + (\sum \text{n-3 PUFA}/\sum \text{n-6 PUFA})]$$

$$\text{HH ratio} = [\text{C18:1 (n-9)} + \text{C18:2 (n-6)} + \text{C18:3 (n-6)} + \text{C18:3 (n-3)} + \text{C20:2 (n-6)} + \text{C20:5 (n-3)}]/(\text{C12:0} + \text{C14:0} + \text{C16:0})$$

in which C18:1 (n-9) - oleic acid, C18:2 (n-6) - linoleic acid, C18:3 (n-6) - γ-linolenic acid, C18:3 (n-3) - α-linolenic acid, C20:2 (n-6) - eicosadienoic acid, C20:5 (n-3) - eicosapentaenoic acid, C12:0 - lauric acid, C14:0 - myristic acid, and C16:0 - palmitic acid, C18:0 - stearic acid, ∑MUFA is the amount of the monounsaturated fatty acids, ∑PUFA - polyunsaturated fatty acids, ∑n-6 PUFA - polyunsaturated fatty acids (n-6), ∑n-3 PUFA - polyunsaturated fatty acids (n-3).

The peroxidability index (PI) was calculated by the formula described (Yun & Surh, 2012):

$$\text{PI}=(\% \text{ monoenoic FA} \times 0.025) + (\% \text{ dienoic FA} \times 1) + (\% \text{ trienoic FA} \times 2) + (\% \text{ tetraenoic FA} \times 4) + (\% \text{ pentaenoic FA} \times 6) + (\% \text{ hexaenoic FA} \times 8).$$

Oxidation Stability Index (OSI), Allylic Position Equivalent (APE), and Bis-Allylic Position Equivalent (BAPE) were calculated using the equations (Kumar & Sharma, 2014; Pinto et al., 2021):

$$\text{APE}=2 \times (\% \text{ C18:1} + \% \text{ C18:2} + \% \text{ C18:3})$$

$$\text{BAPE}=\% \text{ C18:2} + (2 \times \% \text{ C18:3})$$

$$\text{OSI}=3.91 - (0.045 \times \text{BAPE})$$

in which: C18:1 - the content of the oleic acid (%), C18:2 - linoleic acid (%), C18:3 - linolenic acid (%).

Statistics

All measurements were carried out in triplicate ($n = 3$), and the results were depicted as mean value \pm standard deviation (SD).

Results and Discussion

The chemical composition of the plants gives key information about their nutritional properties as well as their possible medicinal potential and ecological interactions. The main nutrients found in the *Onopordum acanthium* seeds are presented in Table 1.

The seeds were characterised as having a moderate content of glyceride oil and total proteins (16.45% and 16.05%), and a very high percentage of carbohydrates (56.76%). Despite that, it was ob-

vious that the dietary fibers (38.46% in the seeds) occupied the main share of the carbohydrate content, which was more than half of their amount. The ash content in *O. acanthium* seeds was 3.42% and the moisture accounted for 7.32%. The calculated energy value was relatively high (439 kcal/100g), which was probably due to the high carbohydrate content of the seeds.

The examined seeds from Southern Bulgaria had lower glyceride oil content than those from the Northern part of the country (212 g/kg) (Zhelev et al., 2014). On the other hand, Matthaus et al. (2014) established that the lipid content of the seeds from the same plant grown in Turkey was slightly lower (15.71%) than that reported in the current study. The amount of glyceride oil present in the *O. acanthium* was lower than some commonly used edible seeds, such as sunflower seeds (43 - 52%), sesame seeds (up to 58%), and olives (about 50%), but had similar quantities to the grape seeds (up to 20%), soybeans (17-24%) and cotton seeds (20-25%) (Stoyanova et al., 2006). The total protein, fiber, ash content, and energy value of the examined seeds were similar to those of *Centaurea benedicta* ((L.) L., 1763): 16.4%, 32.2%, 4.1% and 439 kcal/100g, while the carbohydrate content of the same plant was higher (68.5%) (Teneva et al., 2024).

A myriad of biologically active components is present in the glyceride oils, some of which are important for cellular signaling, immune modulation, and maintaining skin barrier integrity (Table 2).

Table 1. Chemical composition of the *O. acanthium* seeds.

Chemical composition	Content
Glyceride oil, %	16.45 \pm 0.23
Proteins, %	16.05 \pm 0.05
Carbohydrates, %	56.76 \pm 0.44
Fibers, %	38.46 \pm 0.47
Ash, %	3.42 \pm 0.06
Moisture, %	7.32 \pm 0.10
Energy value, kcal/100g	439

Table 2. Content of biologically active components of *O. acanthium* seed oil.

Biologically active components	Content
Unsaponifiable matter, %	
- in the oil	2.70±0.14
- in the seeds	0.44±0.02
Sterols, %	
- in the unsaponifiable matter	25.54±2.21
- in the oil	0.69±0.06
- in the seeds	0.11±0.00
Tocopherols, mg/kg	
- in the oil	172.50±2.12
- in the seeds	28.38±0.35
Phospholipids, %	
- in the oil	0.36±0.05
- in the seeds	0.06±0.01

O. acanthium seed oil was relatively rich in lipid-soluble bioactive components. The unsaponifiable matter was 2.70% which usually consisted of sterols, tocopherols, hydrocarbons, waxes, etc. Total sterols in the oil were 0.69% and they accounted for 25.54% of the whole unsaponifiable substances. The results for the total sterols found in the examined seed oil were slightly higher than those of the same plant grown in Northern Bulgaria (5 g/kg) and lower than those of *C. benedicta* (0.9%) (Zhelev et al., 2014; Teneva et al., 2024). The amount of tocopherols was rather lower (172.50 mg/kg) than some commonly utilised oils, such as sunflower seed oil (440 – 1520 mg/kg), soybean oil (600 – 3370 mg/kg), and canola oil (430 – 2680 mg/kg) (CXS210, 1999). Zhelev et al. (2014) reported a higher content of total tocopherols in *O. acanthium* seed oil grown in the northern region of Bulgaria (0.246 g/kg), as well as Oueslati et al. (2019), who established a much higher amount in the same species from Tunisia (1809.22 mg/kg). The total amount of phospholipids in the examined glyceride oil was 0.36% and 0.06% in the whole seeds. Phospholipids play a central part in cell membranes, forming their bilayers that act as a semipermeable barrier, which is involved in the transportation of nutrients, ions, and waste products needed for cellular metabolism (Belitz et al.,

2009). The results of this component in *O. acanthium* seed oil grown in South Bulgaria were about five times lower than the total phospholipids in the glyceride oil from the same plant grown in the northern part of the country (19 g/kg) (Zhelev et al., 2014). Generally, it was observed that the content of the lipid-soluble biologically active constituents of the plant from the north was much higher than from the south. This confirmed the importance of latitude and habitat conditions for the yield of glyceride oil from *O. acanthium* seeds and its composition. One of the reasons for that is the different environmental conditions, such as lower temperatures, lesser intensity of sunlight, and greater humidity, which influence the biosynthesis of these compounds in plants (Stoyanova et al., 2006).

The individual composition of sterols, tocopherols, and phospholipids in *O. acanthium* seed oil from Southern Bulgaria is presented in Table 3. It was visible that the main sterol was β -sitosterol, whose content was more than 60% in the whole fraction. The following components with a relatively high quantity were stigmasterol (21.7%) and campesterol (8.6%). With a moderate amount, were also found Δ^7 -campesterol (4.0%) and brassicasterol (1.6%). The latter is a precursor of brassinosteroids, which help with the growth and

development of the seeds, improve the yields, and ensure stem cell integrity (Chakraborty et al., 2025). The other components were found in very small amounts, ranging from 0.2% (Δ^5 -avenasterol and Δ^7 -stigmasterol) to 1.2% (cholesterol). Similar results were obtained for the sterol composition of the same plant grown in the northern regions of Bulgaria. Zhelev et al. (2014) also established that the major sterol was β -sitosterol (632 g/kg), but campesterol (128 g/kg) had a higher content than stigmasterol (33 g/kg). β -Sitosterol,

stigmasterol, and campesterol were also the main components in *C. benedicta* seed oil, whose quantity was as follows: 59.5%, 19.4%, 7.5%, respectively (Teneva et al., 2024). On the other hand, the sterol composition of *O. acanthium* seed oil was quite different from *Silybum marianum* ((L.), Gaertn., 1791) seed oil, in which the major components from this fraction were sitosterol (1511.82 mg/kg), Δ^7 -stigmasterol (1333.32 mg/kg), stigmasterol (345.97 mg/kg) and cholesterol (323.11 mg/kg) (Maaloul et al., 2024).

Table 3. Individual composition of sterols, tocopherols, and phospholipids of *O. acanthium* seed oil.

Components	Content, %
Sterols	
Cholesterol	1.2±0.1
Brassicasterol	1.6±0.1
Campesterol	8.6±0.1
Stigmasterol	21.7±0.3
Δ^7 -Campesterol	4.0±0.2
β -Sitosterol	62.2±0.4
Δ^5 -Avenasterol	0.2±0.0
Δ^7 -Stigmasterol	0.2±0.0
Δ^7 -Avenasterol	0.4±0.0
Tocopherols	
α -Tocopherol	25.3±0.3
γ -Tocopherol	74.7±0.6
Phospholipids	
Phosphatidylinositol	33.7±0.8
Phosphatidylcholine	13.1±0.2
Phosphatidylethanolamine	11.9±0.4
Phosphatidylserine	12.9±0.6
Monophosphatidylglycerol	8.7±0.2
Diphosphatidylglycerol	9.4±0.1
Phosphatidic acids	10.3±0.4

In the glyceride oil extracted from *O. acanthium* seed, two tocopherol isomers were identified: γ -tocopherol, which was present in higher amounts (74.7%), and α -tocopherol (25.3%) (Table 3). Tocopherols possess vitamin E activity and scavenge reactive oxygen species, i.e., they have

antioxidant properties (Belitz et al., 2009). Zhelev et al. (2014), who examined the tocopherol composition of the same plants grown in North Bulgaria, identified different tocopherol isomers in the oil: α -tocopherol (911 g/kg) and α -tocotrienol (89 g/kg). One possible reason for that can also be due

to the environmental conditions in which the plants were developed. Tocotrienols usually accumulate in plants under stress, which can be caused by lower temperatures, humidity, etc. The tocopherol composition of the examined *O. acanthium* seed oil was completely different from the composition of some other plant species from the family Asteraceae. For example, Teneva et al. (2024) established that *C. benedicta* seed oil was abundant in α -tocopherol (472 mg/kg) and a small presence of β -tocopherol (20 mg/kg). Individual tocopherols identified in *S. marianum* seed oil were α -tocopherol (400.833 mg/kg), γ -tocopherol (about 70.0-80.0 mg/kg), and δ -tocopherol (4.58 mg/kg) (Maaloul et al., 2024). According to CXS210 (1999), sunflower oil was rich mainly in α -tocopherol, ranging from 403 to 935 mg/kg, and had negligible amounts of β -tocopherol (0-45 mg/kg), γ -tocopherol (0-34 mg/kg), and δ -tocopherol (0-7 mg/kg).

Seven phospholipids were detected in *O. acanthium* seeds, among them the predominant ones were phosphatidylinositol (33.7%), phosphatidyl-

choline (13.1%), phosphatidylserine (12.9%), and phosphatidylethanolamine (11.9%) (Table 3). The other present components were in relatively low amounts, varying from 8.7% (monophosphatidylglycerol) to 10.3% (phosphatidic acids). *O. acanthium* seeds grown in the northern regions of the country were also characterised by the predominant quantities of phosphatidylinositol (320 g/kg), followed by phosphatidylethanolamine (188 g/kg) and phosphatidylcholine (183 g/kg), but a distinctive difference was found in the absence of phosphatidylserine in the samples (Zhelev et al., 2014). Completely different phospholipid composition was observed by Teneva et al. (2024) for *C. benedicta* seeds, in which the major components were phosphatidylethanolamine (45.4%) and phosphatidylinositol (37.1%), followed by a drastically low amount of phosphatidylcholine (6.1%).

The fatty acid composition of the oils plays a part in evaluating their nutritional value, oxidative stability, functionality, and biological effect. The fatty acid composition of the studied seed oil is depicted in Table 4.

Table 4. Fatty acid composition of *O. acanthium* seed oil.

Fatty acids		Content, %
C _{14:0}	Myristic	0.1±0.0
C _{16:0}	Palmitic	5.3±0.2
C _{17:0}	Margaric	0.1±0.0
C _{17:1}	Heptadecenoic	0.1±0.0
C _{18:0}	Stearic	2.6±0.1
C _{18:1}	Oleic	21.4±0.2
C _{18:2} (n-6)	Linoleic	69.3±0.4
C _{18:3} (n-3)	Linolenic	0.1±0.0
C _{20:0}	Arachidic	0.4±0.0
C _{20:1}	Gadoleic	0.2±0.0
C _{20:5} (n-3)	Eicosapentaenoic	0.1±0.0
C _{22:0}	Behenic	0.3±0.0

The predominant fatty acid in the glyceride oil was linoleic acid (69.3%). The second-highest content was assigned to the oleic acid, whose amount was about 20%. The most abundant saturated fatty acids in the seed oil were palmitic acid, which accounted for 5.3%, and stearic acid (2.6%). The amount of the other fatty acids in the fraction

ranged from 0.1% (myristic, margaric, heptadecenoic, linolenic, and eicosapentaenoic acids) to 0.4% (arachidic acid). A lower content of linoleic acid was reported by Zhelev et al. (2014), at 511 g/kg, while the levels of oleic acid were higher (342 g/kg) than in the present study (21.4%). The same authors also detected some other fatty acids, which

were not present in the seed oil from *O. acanthium* grown in South Bulgaria, such as lauricoleic (11 g/kg), myristoleic (19 g/kg), and palmitoleic (1 g/kg) (Zhelev et al., 2014). Similar results to those in the present study were observed by Mohammadi et al. (2025) for the glyceride oil of the seeds from the same plants grown in Iran: linoleic acid (63.41%), oleic acid (28.21%), palmitic acid (5.86%), and stearic acid (3.73%). Several other authors also confirmed that the main fatty acids of *O. acanthium* seed oil from different world regions were those mentioned above, whose content varied as follows: linoleic (57.65 – 65.9%), oleic (18.8 – 28.79%), palmitic (5.8 – 8.81%), and stearic (2.6 – 4.43%) (Tonguç & Erbaş, 2012; Matthaus et al., 2014). The same pattern of fatty acid composition was observed in *S. marianum* seed oil, in which linoleic acid (54.725%) also predominated, followed by oleic acid (16.979%), palmitic acid (13.090%), and stearic acid (5.299%) (Maaloul et al., 2024). Based on the amount of the oleic, linoleic, and linolenic acids, the iodine value of the examined seed oil was calculated (145 gI₂/100 g). This value indicated a

very high degree of unsaturation and classified it as a strongly drying oil (Stoyanova et al., 2006).

The distribution and the content of the main classes of fatty acids - saturated (SFA), unsaturated (UFA), mono- (MUFA), and polyunsaturated (PUFA) fatty acids found in *O. acanthium* seed oil are presented in Fig. 1. The main ones were UFAs, which accounted for 91.2% of the fraction, while the amount of the SFAs was below 10%. The predominant UFAs were the polyunsaturated ones (69.5%), the reason for which was the highest amount of linoleic acid in the oil. The content of the MUFAs was 21.7%, the component which determined this result was the sum of oleic, heptadecenoic, and gadoleic acids. The levels of the UFAs of the seed oil from the same plant grown in the northern regions of Bulgaria were slightly lower (885 g/kg) than in the current study, and the amount of the saturated ones was higher (115 g/kg) (Zhelev et al., 2014). The same authors reported that the quantity of MUFA and PUFA of this oil was 374 g/kg and 511 g/kg, respectively.

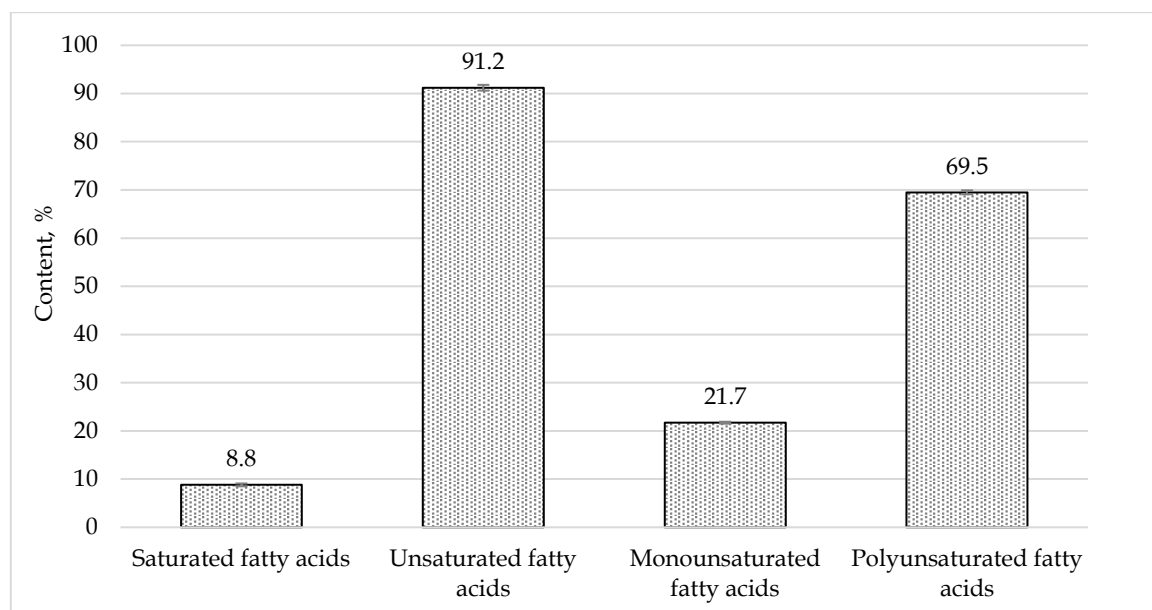


Fig. 1. Content of saturated, unsaturated, mono- and polyunsaturated fatty acids.

O. acanthium seed oil appeared to have a better fatty acid composition than *S. marianum* seed oil, which was characterised with a higher content of saturated fatty acids (23.199%), and lower amounts of mono- (18.517%) and polyunsaturated fatty acids (55.832%) (Maaloul et al., 2024). In this

regard, it can be concluded that *O. acanthium* seed oil may have better values for the lipid indices, respectively, better health-promoting effects.

Based on the determined fatty acid composition, the main lipid indices for *O. acanthium* seed oil were calculated (Table 5).

Table 5. Lipid indices (ratio of polyunsaturated to saturated fatty acids (PUFA/SFA), index of atherogenicity (IA), index of thrombogenicity (IT), and hypocholesterolemic/hypercholesterolemic ratio (HH ratio)), peroxidability index (PI), and oxidation stability index (OSI) of *O. acanthium* seed oil.

Indices	Value
PUFA/SFA	7.90
Index of atherogenicity (IA)	0.0625
Index of thrombogenicity (IT)	0.1735
Hypocholesterolemic/hypercholesterolemic ratio (HH ratio)	16.8333
Peroxidability index (PI)	70.64
APE	181.6
BAPE	69.5
Oxidation Stability Index (OSI)	0.7825

The ratio of PUFA/SFA was extremely high. Generally, a ratio of approximately 0.45 was considered beneficial for cardiovascular health, indicating a high content of polyunsaturated fatty acids that support the proper functioning of the heart (Chen & Liu, 2020). Higher values of this indicator were also observed in the sunflower oils that ranged from 4.75 to 4.94 (Chen & Liu, 2020). The index of atherogenicity of the examined seed oil was very low (0.0625). The favourable value for this index is below 1, which indicates a low potential of *O. acanthium* seed oil to promote atherosclerosis (i.e., to form plaque in the arteries) (Chen & Liu, 2020). The value of the index of thrombogenicity was also very low (below 1), which suggested a reduced risk of clot formation when this oil is consumed regularly (Chen & Liu, 2020). The calculated hypocholesterolemic/hypercholesterolemic ratio (HH ratio) was extremely high (16.833), which showed that the content of cholesterol-lowering fatty acids predominated over those that cause an increase in the cholesterol level in the body. Generally, the values of this indicator above 2.5 depict a beneficial effect on the organism (Chen & Liu, 2020). The value of the peroxidability index of *O. acanthium* seed oil was high (70.64), indicating its susceptibility to reactive oxygen species. What is more, the lower result for the oxidation stability index (0.7825) also depicted weak oxidative stability of this oil, which was caused by the high content of fatty acids with allylic and bisallylic positions in their structure (APE was 181.6 and BAPE was 69.5).

Conclusions

Onopordum acanthium seeds from southern Bulgaria were proven to be a promising natural source of major nutrients and important lipid-soluble bioactive components. It was confirmed that they were rich in macrocomponents (carbohydrates, glyceride oil, and proteins), which determined their good calorific effect. The seed oil was characterised with a relatively high content of sterols and phospholipids, and a bit low amounts of tocopherols. The specific individual sterol, tocopherol, and phospholipid composition of the examined *O. acanthium* seed oil positioned it into the group of high-value functional lipids. The presence of essential fatty acids in the oil (especially linoleic acid) established its favourable values of lipid indices, which indicated the potential of the oil to have health benefits in the human diet. This may suggest that *O. acanthium* seed oil can find application in various functional foods, nutraceuticals, and therapeutic formulations in the future.

Acknowledgements

This study is financed by the European Union-NextGenerationEU, through the National Recovery and Resilience Plan of the Republic of Bulgaria, project No. BG-RRP-2.004-0001-C01.

References

Al-Snafi, A.E. (2020). Constituents and pharmacology of *Onopordum acanthium*. *IOSR Journal of Pharmacy*, 10(3), 7-14.

- AOAC. (2016). *Official methods of analysis* (20th ed.). Association of Official Analytical Chemists. Benjamin Franklin Station, Washington DC.
- AOCS. (1999). Official methods and recommended practices of the American Oil Chemists Society: Calculated iodine value (5th ed., Cd 1c-8). AOCS Press, Champaign, IL.
- Arfaoui, M.O., Renaud, J., Ghazghazi, H., Boukhchina, S., & Mayer, P. (2014). Variation in oil content, fatty acid and phytosterols profile of *Onopordum acanthium* L. during seed development. *Natural Product Research*, 28(24), 2293–2300. doi: [10.1080/14786419.2014.940944](https://doi.org/10.1080/14786419.2014.940944)
- Belitz, H.D., Grosch, W., & Schieberle, P. (2009). *Food chemistry* (4th ed.). Springer Berlin, Heidelberg. doi: [10.1007/978-3-540-69934-7](https://doi.org/10.1007/978-3-540-69934-7)
- BS 13488. (1976). Bulgarian State Standard. Grain. Method for Determining the Content of Starch. [in Bulgarian]
- Chakraborty, N., Ganguly, R., Sarkar, A., Dasgupta, D., Sarkar, J., Acharya, K., Burachevskaya, M., Minkina, T., & Keswani, C. (2025). Multifunctional role of brassinosteroids in plant growth, development, and defense. *Journal of Plant Growth Regulation*, 44, 2627–2640. doi: [10.1007/s00344-024-11593-4](https://doi.org/10.1007/s00344-024-11593-4)
- Chen, J., & Liu, H. (2020). Nutritional indices for assessing fatty acids: A mini-review. *International Journal of Molecular Sciences*, 21(16), 5695. doi: [10.3390/ijms21165695](https://doi.org/10.3390/ijms21165695)
- Codex Alimentarius Commission. (1999). *Standard for named vegetable oils (CXS210-1999)*. Food and Agriculture Organization of the United Nations & World Health Organization.
- Folch, J., Lees, M., & Sloane-Stanley, G.H. (1957). A simple method for isolation and purification of total lipids from animal tissues. *Journal of Biological Chemistry*, 226, 497–509.
- Food and Agriculture Organization of the United Nations (FAO). (2003). *Food energy – Methods of analysis and conversion factors*. FAO Food and Nutrition Paper No. 77, Rome, 93 p.
- Garsiya, E.R., Konovalov, D.A., Shamilov, A.A., Glushko, M.P., & Orynbasarova, K.K. (2019). Traditional medicine plant, *Onopordum acanthium* L. (Asteraceae): Chemical composition and pharmacological research. *Plants*, 8(2), 40. doi: [10.3390/plants8020040](https://doi.org/10.3390/plants8020040)
- ISO 10540-1. (2014). Animal and vegetable fats and oils – Determination of phosphorus content – Part 1: Colorimetric method.
- ISO 12228-1. (2014). Animal and vegetable fats and oils – Determination of individual and total sterols contents – Part 1: Gas chromatographic method.
- ISO 12966-1. (2014). Animal and vegetable fats and oils – Gas chromatography of fatty acid methyl esters – Part 1: Guidelines on modern gas chromatography.
- ISO 12966-2. (2017). Animal and vegetable fats and oils – Gas chromatography of fatty acid methyl esters – Part 2: Preparation of methyl esters of fatty acids.
- ISO 18609. (2000). Animal and vegetable fats and oils – Determination of unsaponifiable matter – Method using hexane extraction.
- ISO 659. (2014). Oilseeds – Determination of oil content (Reference method).
- ISO 9936. (2016). Animal and vegetable fats and oils – Determination of tocopherol and tocotrienol contents by high-performance liquid chromatography.
- Ivanov, S., Bitcheva, P., & Konova, B. (1972). Méthode de détermination chromatographique et colorimétrique des phytosterols dans les huiles végétales et les concentrés stéroliques. *Revue Française des Corps Gras*, 19, 177–180.
- Ivanova, A., Bogdanova, S., Petrov, V., & Gechev, T. (2025). Plants from Bulgarian botanical gardens: Some selected species with potential for health food and medical applications. *Plants*, 14(14), 2176. doi: [10.3390/plants14142176](https://doi.org/10.3390/plants14142176)
- Kumar, M., & Sharma, M.P. (2014). Potential assessment of microalgal oils for biodiesel production: A review. *Journal of Materials and Environmental Science*, 5, 757–766.
- Maaloul, S., Ghzaïel, I., Mahmoudi, M., Mighri, H., Pires, V., Vejux, A., Martine, L., de Barros, J.-P. P., Prost-Camus, E., Boughalleb, F., Lizard, G., & Abdellaoui, R. (2024). Characterization of *Silybum marianum* and *Silybum eburneum* seed oils: Phytochemical profiles and antioxidant properties supporting important nutritional interests. *PLoS ONE*, 19(6), e0304021. doi: [10.1371/journal.pone.0304021](https://doi.org/10.1371/journal.pone.0304021)
- Matthaus, B., Ozcan, M. M., & Al-Juhaimi, F. (2014). Fatty acid, tocopherol, and mineral contents of *Onopordum acanthium* seed and oil. *Chemistry of Natural Compounds*, 50, 1092–1093. doi: [10.1007/s10600-014-1166-7](https://doi.org/10.1007/s10600-014-1166-7)
- Mohammadi, S., Movafeghi, A., Delazar, A., Hamedeyazdan, S., Bahadori, M.B., & Naze-

- miyeh, H. (2025). Isolation and characterization of bioactive compounds from Scotch thistle (*Onopordum acanthium* L.) seeds. *Pharmaceutical Sciences*, 31(3), 288-293. doi: [10.34172/PS.025.40963](https://doi.org/10.34172/PS.025.40963)
- Oueslati, M.A., Gunenc, A., Rigane, G., Ghazghazi, H., Valencia, C., Salem, R.B., Boukhchina, S., Willmore, W.G., & Hosseinian, F. (2019). Chemical composition, antioxidant and cytotoxic activities of *Onopordum acanthium* L. crude oil and defatted meal. *Revue Roumaine de Chimie*, 64(6), 503-510. doi: [10.33224/rrch/2019.64.6.06](https://doi.org/10.33224/rrch/2019.64.6.06)
- Pinto, T.I., Coelho, J.A., Pires, B.I., Neng, N.R., Nogueira, J.M., Bordado, J.C., & Sardinha, J.P. (2021). Supercritical Carbon Dioxide Extraction, Antioxidant Activity, and Fatty Acid Composition of Bran Oil from Rice Varieties Cultivated in Portugal. *Separations*, 8(8), 115. doi: [10.3390/separations8080115](https://doi.org/10.3390/separations8080115)
- Santos-Silva, J., Bessa, R.J.B., & Santos-Silva, F. (2002). Effect of genotype, feeding system and slaughter weight on the quality of light lambs: Fatty and composition of meat. *Livestock Production Science*, 77, 187-194. doi: [10.1016/S0301-6226\(02\)00059-3](https://doi.org/10.1016/S0301-6226(02)00059-3)
- Schneiter, R., & Daum, G. (2006). Analysis of yeast lipids. In: Xiao, W. (Ed.) *Yeast Protocol. Methods in Molecular Biology*, 313, 75-84. Humana Press, Totowa, NJ. doi: [10.1385/1-59259-958-3:075](https://doi.org/10.1385/1-59259-958-3:075)
- Stoev, P., Hubenov, Z., Ganeva, A., Blagoev, G.A., & Barov, B. (2022). Biodiversity of Bulgaria: Characteristics, protection and trends. *Biodiversity Information Science and Standards*, 6, e95683. doi: [10.3897/biss.6.95683](https://doi.org/10.3897/biss.6.95683)
- Stoyanova, A., Perifanova-Nemska, M., & Georgiev, E. (2006). *Raw material science about glyceride and essential oils*. Agency 7D Publishing, Plovdiv.
- Teneva, O., Petkova, Z., Dobрева, A., Dzhurman-ski, A., Stoyanova, L., & Angelova-Romova, M. (2024). *Centaurea benedicta* – A potential source of nutrients and bioactive components. *Plants*, 13(24), 3579. doi: [10.3390/plants13243579](https://doi.org/10.3390/plants13243579)
- Tonguç, M., & Erbaş, S. (2012). Evaluation of fatty acid compositions and some seed characters of common wild plant species of Turkey. *Turkish Journal of Agriculture and Forestry*, 36(6), 673-679. doi: [10.3906/tar-1201-22](https://doi.org/10.3906/tar-1201-22)
- Ulbricht, T., & Southgate, D. (1991). Coronary heart disease: Seven dietary factors. *The Lancet*, 338 (8773), 985-992. doi: [10.1016/0140-6736\(91\)91846-M](https://doi.org/10.1016/0140-6736(91)91846-M)
- Yun, J.M., & Surh, J. (2012). Fatty acid composition as a predictor for the oxidation stability of Korean vegetable oils with or without induced oxidative stress. *Preventive Nutrition and Food Science*, 17(2), 158-165. doi: [10.3746/pnf.2012.17.2.158](https://doi.org/10.3746/pnf.2012.17.2.158)
- Zahariev, D. (2022). The medicinal plants in Bulgaria: List of species, usable parts, fields of application, toxicity and contraindications. *Acta Scientifica Naturalis*, 9(1), 33-46. doi: [10.2478/asn-2022-0004](https://doi.org/10.2478/asn-2022-0004)
- Zhelev, I., Merdzhanov, P., Angelova-Romova, M., Zlatanov, M., Antova, G., Dimitrova-Dyulgerova, I., & Stoyanova, A. (2014). Lipid composition of *Carduus thoermeri* Weinm., *Onopordum acanthium* L., and *Silybum marianum* L., growing in Bulgaria. *Bulgarian Journal of Agricultural Science*, 20, 622-627.

Received: 17.10.2025
Accepted: 03.12.2025