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Milk Thistle (*Silybum marianum* (L.) Gaertner) Endosperm as an Alternative Protein Source for a Sustainable Food System (SFS)—Pilot Studies

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Abstract: Milk thistle is a plant that perfectly meets the needs of sustainable agriculture. Despite their high protein content, the seeds and endosperm of *S. marianum* have not been considered as raw food material so far. Therefore, the work aimed to characterize milk thistle endosperm in terms of its possible use in producing novel food. The nutritional and energy value of the raw material, profile of amino acids), fatty acids, and health quality indices of lipids were characterized. The main components of milk thistle endosperm (MTE) were protein (>20% dry matter (DM)), fat (>39% DM), and fiber (>31% DM). MTE protein is characterized by a high content of sulfur, aromatic amino acids, and tryptophan, comparing the FAO/WHO patterns. The PDCAA S (Protein Digestibility Corrected Amino Acid Score) value for lysine is low but can be higher in combination with other proteins. Milk thistle fat is dominated by unsaturated fatty acids, constituting about 80% of total fatty acids, of which over 56% are polyunsaturated fatty acids. Low values of atherogenicity and thrombogenicity indices of MTE fat testify to its potentially beneficial properties towards the cardiovascular system.

Keywords: milk thistle; plant proteins; amino acids; PDCAAS; fatty acids; nutritional value

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1. Introduction

With the growth of the population, the demand for protein as a nutrient increases dynamically. The protein crop of the greatest importance in the economy is invariably soybean. The allergenicity of this raw material, the spread of genetically modified varieties [1,2], and the fact that this plant cannot be grown effectively in all climatic zones have become the cause of many controversies and consumer doubts about soy-based products. The review of literature data shows that research on the development of methods for extracting proteins and obtaining their concentrates and isolates from sources other than soybean was carried out in many research centers around the world. Lo et al. [3] described the current state of knowledge on lupine proteins, focusing on the characteristics of isolation methods and technical and functional properties. Research on pea protein isolates (structure, extraction, functionality) was presented in detail in their review work by Lam et al. [4] and Daba and Morris [5]. There is also an increase in scientists' interest in potato protein [6], rapeseed protein [7], rice protein [8], and hemp protein [9]. According to the European Vegetable Protein Association (EUVEPRO), the prospective raw materials for the food protein industry are peas, fava beans, chickpeas, lentils, lupins, rice, potatoes, and wheat [10]. Plant proteins and their role in creating SFS are given more and more space in economic, environmental, or political debates and numerous scientific studies.

The production of vegetable proteins is one of the most important and fastest-growing branches of sustainable industry. The Plant-Based Protein Global Market Report 2022: By

Type, Source, Form, and Application [11] shows that the global market for these products was valued at USD 14.58 billion in 2022. It is projected that by 2026 it will reach a CAGR (Compound Annual Growth Rate) of 9.9%. Data from Farm Animal Investment Risk and Return (FAIRR) [12] indicate that as many as 28% of major food producers have adopted formal goals related to expanding the vegetable protein market. The scale of this phenomenon is mainly due to the need to implement the objectives of the global environmental policy resulting from the real risk of the climate crisis. Issues related to the prospects for producing and acquiring plant proteins for the agri-food sector have been discussed many times at the EU level. The report from the Commission to the Council and the European Parliament on the development of plant protein production in the European Union [13] clearly states that there is a significant shortage of vegetable proteins in the Community, which forces the needs of the EU agricultural sector to be met by import. The further development of plant protein production in Europe has several economic benefits for farmers and food and feed producers while positively impacting the environment and climate. The potential of this sector is based on strong links with current social problems, including in the field of health policy (reduction in the risk of civilization diseases, incidence of food allergies to animal products), ethics (care for animal welfare), current consumer trends (increase in the popularity of elimination diets) or the implementation of the global concept of Sustainable Development. Replacing milk and meat with alternative proteins can improve the sustainability of Europe's food supply.

Although a significant part of the production of vegetable proteins in Europe is still directed to the feed market, the interest in this product within the sustainable food sector has developed to such an extent that this direction of protein management is currently considered the most promising. The EUVEPRO Association, representing producers of vegetable proteins intended for human consumption in the EU, now brings together ten entities, among which there are leading players on the food market, e.g., Belgian corporation Cargill NV, Irish KERRY ingredients and Flavors Ltd. or BUNGE Netherlands B.V. Milk thistle (*Silybum marianum*), despite the centuries-old tradition of safe use by humans, remains unnoticed in the forecasts regarding the global development of the plant protein production sector. Its seeds contain about 25–30% of protein [14]—several times more than oats (10.9%), wheat (11.6%), corn (9.4%), rice (8.1%), potatoes (8.4%), but comparable to lentils (25.6%), chickpeas (21.3%), peas (22.1%) or broad beans (26.1%) [EUVEPRO, 2019]. Culturing milk thistle is possible on various soil types (pH approx. 5.8–6), also in monoculture conditions, lasting up to 20 years. Sown two years in a row in the same place, it can grow without sowing in the following years. An additional advantage of milk thistle is the low cost of its cultivation, as low nutritional requirements characterize this plant. Plantation care is not expensive. It is limited to the fight against weeds, which can be carried out mechanically, e.g., using a tractor weeder [15]. An undoubted advantage of milk thistle is its resistance to pests and diseases. In addition, due to its drought resistance, *S. marianum* is considered a typical non-irrigated crop, and in most cases, the average rainfall is sufficient for good seed production [16,17]. From the point of view of environmental protection and the implementation of the principles of Sustainable Development in agriculture, these are essential features because they seriously reduce the consumption of water, fertilizers, and plant protection products in the cultivation process. Regarding processing, milk thistle seeds and waste products obtained after producing silymarin (endosperm) or oil (pomace) are valuable raw materials for further use.

Over the last 20 years, there has been a significant, successive increase in interest in plant proteins in the context of scientific research in many fields. The data from the *Web of Science* database [18] covering the years 2003–2022 (search term: "plant protein"; search criterion: "topic") show that over 207,500 publications in this field were published in the indicated period. In 2003, 5270 papers on plant proteins were published; in 2022, their number exceeded 18,700. Global remodeling of economic, environmental, and social policies toward implementing the principles of Sustainable Development results in many initiatives, including those related to creating a Sustainable Food System (SFS). Limiting the production and processing of meat, investments in the development of the alternative protein sector, and technologies for processing plant raw materials to obtain substitutes for dairy and meat products are important elements of the SFS.

Several environmental impacts are associated with food production, including greenhouse gas emissions, water footprint, and land use. Discussions on this subject have been going on for a long time. However, in light of the need for the Community countries to implement the concept of Sustainable Development, the impact of various agricultural production systems on the environment has become particularly important. In the total pool of emitted greenhouse gases (GHG), as much as 25% of methane and 60% of nitrogen oxides come from agricultural production. The primary source of methane and ammonia emissions is animal production [19]. According to the European Environment Agency, livestock supply chains are responsible for 7.1 gigatonnes of CO_2 equivalent yearly, 14.5% of all anthropogenic greenhouse gas emissions. Cattle (raised for meat and milk) are responsible for about 65% of these emissions, and about 44% of livestock emissions are methane [20]. The average water footprint for meat increases depending on the species and ranges from poultry (4325 m^3/t), pork (5988), and mutton (10,412) to beef (15,415). Milk production, on the other hand, consumes 1020 m^3 of water/t of milk [21]. About 26% of the planet's ice-free land is used for grazing livestock, and 33% of all arable land is used to grow animal feed [20]. These and many other environmental issues have drawn the world's attention to the need to reduce the negative impact of animal production on the environment and to a broader interest in, among others, animal production, plant production, which can provide substitute product solutions, such as alternative sources of protein. Reducing the consumption of animal products reduces greenhouse gas emissions. About 10 kg of feed is needed for every 1 kg of animal protein. In addition, a diet that includes animal products requires 4.5 times more crops than the production of plant products. Cow milk production is associated with about three times more GHG emissions than plant-based drinks. Moreover, milk typically requires nine times more land use than plant-based "milk" and more than 20 times more water than soy drink production [22].

Plant-based milk substitute drinks can be essential to people's diets with confirmed cow's milk protein allergy [23]. Despite the importance of this problem among the general population, especially in children, at the moment, only soy milk has a protein content similar to cow's milk (2.5–3.16 mg/100 mL) [24] and is treated as an excellent alternative to it. However, it should be emphasized that soy is a strong allergen, and the hydrolysis of soy protein isolate does not solve this problem [25]. Other plant-based drinks, such as rice, almond, and oat, usually contain well below 1 g of protein/100 mL. The lower protein content of plant-based milk alternatives and the variety of these products can potentially reduce the protein intake of their consumers, whose numbers are steadily increasing. Many adverse health effects can also arise from consuming inadequate amounts of essential amino acids [26]. Using milk thistle seeds to produce milk substitutes and alternative protein preparations is highly prospective in this context. So far, no allergic reactions to the protein of this raw material have been reported [27]. Other studies [28] indicate that fermented milk thistle drink contains over 2 g of protein/100 mL. At the same time, it has all the essential amino acids. In defatted milk thistle flour, the highest concentrations include arginine (12.59%) > leucine (9.84%) > valine (7.97%) > lysine (7.38%) [29].

Not only milk thistle seeds can be a potential source of alternative protein and raw material for the production of milk substitutes and milk products. During the processing of this raw material into silymarin (currently the main direction of using *S. marianum* for humans), significant amounts of a by-product, endosperm, are generated [30]. It is a precious raw material containing substantial amounts of protein (approx. 20%) but used only as fodder. This fact is crucial considering the SFS's assumptions and the intensification of global activities to strengthen the circular economy. Using resources more sustainably is a fundamental element of this model.

However, a review of the literature data indicates the need to answer several research questions:

RQ1 What is the nutritional value of milk thistle endosperm?
RQ2 Is the amino acid profile of milk thistle endosperm proteins and the fatty acid profile valuable in the context of applicable standards in human nutrition?
RQ3 What directions of *S. marianum* endosperm processing can be considered prospective for SFS?

Therefore, this work aimed to characterize milk thistle endosperm as a new, plant material for the sustainable production of novel foods. Studies were conducted to determine the nutritional and energy value, the amino acid profile (high-performance liquid chromatography; HPLC), the fatty acid profile (gas chromatography; GC), and milk thistle lipid quality indices.

2. Materials and Methods

2.1. Analytical Standards and Reagents

The amino acids standards and supplemental amino acids were purchased from Agilent Technologies (Santa Clara, CA, USA). For milk thistle endosperm fatty acid identification (GC-FID analysis), 37 Component FAME Mix of Supelco, (Bellefonte, PA, USA) was used.

The derivatization reagents for amino acids HPLC analysis, i.e., 9-fluorenylmethyl chloroformate in acetonitrile (FMOC) and *ortho*-phthaldialdehyde and 3-mercaptopropionic acid in borate buffer (OPA), as well 0.4 M borate buffer were from Agilent Technologies (Santa Clara, CA, USA). Acetonitrile, water, methanol, chloroform (HPLC grade), 10% solution of BF_3, and NaH_2PO_4 monohydrate were purchased from Sigma-Aldrich (Merck, Darmstadt, Germany).

2.2. Plant Material

Milk thistle endosperm (*Sylibum marianum;* MTE) was obtained from the Poznań Herbal Company 'Herbapol' S.A. (Poznań, Poland) in 2022. MTE weighing 30 kg was tightly packed in a PP bag and delivered for testing within 24 h. Plant material intended for analysis was assessed macroscopically, then portioned, vacuum-packed (Profi Line 410, Hendi, The Netherlands) in PA/PE bags (single sample weight m = 1.00 kg), and stored at a temperature of approx. −20 °C without access to light.

2.3. Nutrition and Energy Value of Milk Thistle Endosperm

The parameters of the nutrition/energy value of MTE were selected on the basis of the European Parliament requirements [31]. According to the methods previously described by [32] the content of dry matter, crude protein, fat, sugars, digestible carbohydrates, dietary fiber, ash, and salt in MTE were determined.

2.4. Fatty Acid (FA) Profile Determination (GC-FID Analysis)

For lipid extraction, milk thistle endosperm (±5.00 g) was homogenized with the mixture of chloroform and methanol (2:1; v/v) containing 0.001% of BHT as antioxidant. The solvent was evaporated in a nitrogen stream. Next, the crude MTE lipid extract was mixed with 0.5 M methanolic solution of KOH. According to the official method [33], for fatty acids transesterification the BF_3 (boron trifluoride) solution in methanol was used.

The saponified chromatographic separation conditions were in accordance with those proposed in Wołoszyn et al. publication [34]. The fatty acids in the form of methyl esters (FAME) were quantified by GC (Agilent 7890 A series, Agilent Tech. Inc., St. Clara, CA, USA) coupled with FID (flame-ionization detector), and fused silica capillary column J&W Scientific HP-88 series (length: 100 m, diameter: 0.25 mm, film: 0.20 μm; Agilent Tech. Inc., St. Clara, CA, USA).

2.5. Lipid Health Quality Indices

Taking into account the results of the FA analysis, selected indices characterizing the potentially health-promoting quality of milk thistle endosperm lipids have been determined.

The n-6/n-3 ratio, and atherogenicity index (AI) have been calculated according to [35]. The thrombogenicity index (TI) calculation formula was previously described by [36], and hypocholesterolemic/hypercholesterolemic index (h/H) by [37].

2.6. Amino Acids Content Determination (HPLC-DAD)

The analysis of amino acids content in milk thistle endosperm was determined using Agilent 1100 Series high-performance liquid chromatograph coupled with the UV DAD detector. The parameters of the chromatographic column (AAA Eclipse Zorbax; Agilent Technology Inc., St. Clara, CA, USA) were as follows: length: 150 mm; diameter: 3.0 mm; porosity: 3.5 µm. Milk thistle endosperm preparation was in accordance with analytical procedure described by [38].

Detailed conditions for the chromatographic separation of amino acids were described in publications [39] [40]. Tryptophan content in MTE was determined after alkaline hydrolysis of the sample by the method proposed by Çevikkalp et al. [41]. Cysteine and methionine were determined as cysteic acid and methionine sulfone by performic acid oxidation before its digestion using 6M HCl, according to Untea et al. [42]. The cysteic acid and methionine sulfone formed after the samples' oxidation were converted to cysteine and methionine, respectively.

2.7. Calculation of Protein Digestibility-Corrected Amino Acid Score (PDCAAS)

The PDCAAS was calculated according to the following equation:

PDCAAS = AAS (Amino Acid Score) * true fecal digestibility (TD), where:

AAS = [(g of amino acid in 100 g of a test protein/g of amino acid in 100 g of requirement pattern)] $\times$ 100%

The amino acid with the lowest percentage is called the limiting amino acid.

The assumed TD value was 86.92% [43].

2.8. Statistical Analysis

Nutrition value parameters of milk thistle endosperm and fatty acids analysis data were presented as the mean ± SD (*n* = 3).

3. Results and Discussion

3.1. Nutritional and Energy Value of Milk Thistle Endosperm

The main distinguishing feature of the nutritional value of milk thistle endosperm is the high protein content exceeding 20% in the raw material's dry matter (DM) (Table 1). The protein's amino acid profile is discussed in detail later in this paper (Section 3.2).

Table 1. General nutritional characteristics of milk thistle endosperm (±SD).

Parameter	Content in Fresh Matter (FM)	Content in Dry Matter (DM)
Energy value [kJ/100 g]	1966.50 ± 10.61	2120.53 ± 9.95
Energy value [kcal/100 g]	477.00 ± 2.83	514.56 ± 2.69
Crude protein [%]	19.63 ± 0.01	21.10 ± 0.00
Fat (total) [%]	36.32 ± 0.47	39.40 ± 0.47
Saturated fatty acid (SFA) [g/100 g]	7.23 ± 0.06	7.77 ± 0.06
Total sugars [%]	1.82 ± 0.03	1.96 ± 0.03
Digestible carbohydrates [%]	2.40 ± 0.52	2.57 ± 0.56
Total ash [%]	5.00 ± 0.21	5.21 ± 0.22
Fiber [%]	29.41 ± 0.24	31.78 ± 0.24

Research by Li et al. [44] indicates that the dominant fraction of *Silybum marianum* proteins are albumins and, to a lesser extent, globulins. The amount of prolamins and glutelins is small. These fractions consist of polypeptides in the molecular weight range of 16–112 kDa. The comparison of the protein content in the tested material with the content of this component in other plants, considered promising for alternative proteins of plant origin, looks very favorable. Milk thistle endosperm is a richer source of protein than potato tubers (PC range 7.82–10.10 g/100 g DM) [45,46], rice (6.0–11.4 g/100 g DM) [47], corn (6–12 g/100 g DM) [48], oats (8.13–12.69 g /100 g) [49], as well as raw materials of animal origin, including cow's milk (3.1–3.8 g/100 g), mare's milk (1.5–2.8 g/100 g) [50], turkey (breast with skin; 18.7 g/100 g), pork neck (18.8 g/100 g), and, e.g., mutton (shoulder; 15.6 g/100 g) [51]. Importantly, milk thistle is considered a non-allergenic raw material [27] and safe [52]. This fact makes it competitive with other plants that produce alternative proteins, especially soy. The European Center for Allergy Research Foundation (ECARF) reports that about 0.3% of Europeans are allergic to soy [53]. Another important food allergen is gluten—a storage protein found, among others, in wheat, which must be avoided by patients with celiac disease (1% of the global population), Dühring's disease, people diagnosed with wheat allergy (10–25% of people with food allergy) and non-celiac gluten sensitivity [54]. There are no data on the allergenicity of milk thistle protein in the literature. *S. marianum* is also not included in the allergen database created by the University of Nebrasca-Lincoln, which includes 430 species and 913 taxa protein groups [55]. However, the anti-allergic potential of this raw material should be finally verified to approve *S. marianum* as a new source of protein in human nutrition. Taking into account the EFSA requirements in this regard, it is necessary not only to identify potential allergens but also to assess the impact of the digestion process on the allergenicity of this protein, to estimate the dose of exposure to the protein, its physicochemical properties, and cross-reactivity, and to study the individual immune response in animals [27,43] proved that the milk thistle protein (SMP) formula has an excellent balance of all essential amino acids and is of good nutritional quality. Using differential scanning calorimetry, they found that SMP has higher thermal stability than soy protein isolate (SPI). In vitro sequential pepsin digestibility measurement by SDS-PAGE showed that SMP was more readily digested than SPI.

Fat accounted for over 39% of the dry matter of milk thistle endosperm, and saturated acid (SFA) content was 7.77 g/100 g DM of the tested raw material. The fatty acid (FA) profile of *S. marianum* endosperm is described in detail in Section 3.3. According to Aziz et al. [56], milk thistle seeds contain between 19.74 and 23.19% fat. Keshavarz Afshar et al. [57], who studied the effect of irrigation and organic fertilizers on the oil content and FA composition in milk thistle seeds, indicated higher values, i.e., 25.3–27.9%. In general, milk thistle oil can be considered a good source of PUFA for human consumption [58] and used in food, similar to cottonseed, sunflower, and soybean oil [59]. Among the non-food applications of this raw material, there are, e.g., the production of biodiesel [60] and use in cosmetics (e.g., improvement of elasticity, density, and color of the skin) [61].

As shown in our research, milk thistle endosperm is distinguished by a high content of fiber (>29% in FM and 31% in DM) and ash ($\geq$5% in FM and DM; Table 1). These values are close to those obtained by Bedrníček et al. [62], who determined the content of macronutrients in milk thistle oilseed cake flour fractions (OCFF). Depending on the particle size in the tested OCFF samples (coarse >710 µm; medium 315–710 µm; and fine <315 µm), the fiber content was 49.44–46.81–30.41 g/100 g FW, and the ash content was 5.52–5.43–8.78, respectively g/100g FW. The main components of insoluble fiber OCFF were cellulose > lignin > hemicellulose [62].

3.2. Amino Acid Profile of Milk Thistle Endosperm

Searching for new protein sources is the current trend in food technology. The biological value of this vital food component is determined by a balanced composition of essential amino acids [63]. Proteins consist of twenty amino acids, eight are essential for humans and should be provided with a diet [64]. The Report of an FAO Expert Consultation [63] gives values for the requirements of indispensable dietary amino acids. According to the report, protein quality can be determined, among others, by comparing the contents of individual, essential amino acids in this protein to the human requirements for the amino acids and correcting the received value by the true fecal digestibility of the protein determined by the rat balance method (PDCAAS—Protein Digestibility Corrected Amino Acid Score). Thistle endosperm contains all essential amino acids (Table 2). However, the amino acid limiting the nutritional value of this protein, as in the case of cereal proteins, is lysine (PDCAAS from 57.77 to 68.61%). Leucine and valine are the second and third most deficient amino acids in comparison to the FAO/WHO patterns.

Table 2. Profile and contents of amino acids (AA) and PDCAAS value of milk thistle endosperm (± SD).

Amino Acid	Amino Acid Contents			PDCAAS (Children 6 Months to 3 Years) * [%]	PDCAAS (Older Children, Adolescents, Adults) * [%]
	g/100 g of DM	g/100 g of FM	g/100 g of all Amino Acids in FM		
Asp (aspartic acid)	1.18 ± 0.04	1.10 ± 0.04	9.55		
Glu (glutamic acid)	2.47 ± 0.08	2.30 ± 0.07	19.96		
Ser (serine)	0.65 ± 0.02	0.60 ± 0.02	5.24		
His (histidine)	0.35 ± 0.02	0.33 ± 0.01	2.84	123.61	154.51
Gly (glycine)	0.70 ± 0.01	0.65 ± 0.01	5.64		
Thr (threonine)	0.47 ± 0.01	0.43 ± 0.01	3.78	105.96	131.40
Arg (arginine)	1.25 ± 0.04	1.16 ± 0.04	10.07		
Ala (alanine)	0.52 ± 0.02	0.49 ± 0.02	4.24		
Tyr (tyrosine)	0.47 ± 0.02	0.44 ± 0.02	3.78		
Val (valine)	0.53 ± 0.01	0.49 ± 0.01	4.25	85.85	92.29
Phe (phenylalanine)	0.54 ± 0.03	0.50 ± 0.03	4.35		
Ile (isoleucine)	0.48 ± 0.02	0.45 ± 0.02	3.90	105.80	112.86
Leu (leucine)	0.77 ± 0.03	0.72 ± 0.03	6.24	82.23	88.97
Hyp (hydroxyproline)	0.06 ± 0.01	0.06 ± 0.01	0.49		
Pro (proline)	0.49 ± 0.07	0.46 ± 0.06	3.99		
Lys (lysine)	0.47 ± 0.04	0.44 ± 0.04	3.79	57.77	68.61
Cys (cysteine)	0.39 ± 0.01	0.37 ± 0.01	3.19		
Met (methionine)	0.22 ± 0.06	0.21 ± 0.07	1.79		
Trp (tryptophan)	0.36 ± 0.01	0.34 ± 0.02	2.91	298.05	383.85
TOTALS	12.37	11.51	100.0		
Sulfur AA (Met+Cys)	0.61	0.58	4.98	160.38	188.27
Aromatic AA (Phe+Tyr)	1.01	0.94	8.13	135.97	172.45

* Values for PDCAAS (Protein Digestibility Corrected Amino Acid Score) were calculated using the scoring patterns recommended by FAO/WHO [63]. The reference scoring pattern (g/100 g protein) for children: His 2.0; Ile 3.2; Leu 6.6; Lys 5.7; sulfur AA 2.7; aromatic AA 5.2; Thr 3.1; Trp 0.85; Val 4.3; The reference scoring pattern (g/100g protein) for older children, adolescents, and adults: His 1.6; Ile 3.0; Leu 6.1; Lys 4.8; sulfur AA 2.3; aromatic AA 4.1; Thr 2.5; Trp 0.66; Val 4.0; for PDCAAS calculations a digestibility value of 86.92% was used [43].

The sum of essential amino acids in the thistle endosperm protein (40.85 g/100 g protein) is similar to that of milk protein—casein (45.8 g/100 g protein) [65]. The high sulfur AA and Trp content are noteworthy. The content of sulfur AA in the endosperm (4.98 g/100 g protein) is higher than in total milk protein (3.3 g/100 g protein), as well as in the protein of sunflower seeds [66], rapeseed, soybeans, peas, and beans (3.7; 3.3; 2.7, 2.1, 2.2 g/100 g protein, respectively [67]. Trp content, on the other hand, is comparable to its content in milk protein beta-lactoglobulin (2.91 v/s 2.3 g/100 g protein, respectively) and is higher than in legume proteins such as beans, broad beans, peas, and soybeans (0.0; 0.8; 0.9 and 1.3 g/100 g protein), as well as oilseeds such as rapeseed and sunflower (1.1–1.3 g/100 g protein) [65,67,68]. The second distinguishing feature of thistle endosperm protein is its high content of the aromatic AA tyrosine and phenylalanine (8.13 g/100 g protein). The aromatic AA content of endosperm protein is slightly higher than that of pork and beef protein (7.5 and 7.2 g/100 g protein, respectively) and milk protein beta-lactoglobulin (7.8 g/100 g protein) [65], but lower than that of soybean and bean protein (8.7 and 9.5 g/100 g protein, respectively) [68].

In summary, thistle's endosperm protein is characterized by a high content of sulfur, aromatic amino acids, and tryptophan, comparing the FAO/WHO patterns. Regarding sulfur amino acids (methionine and cysteine), milk thistle proteins are similar to animal proteins. This makes products containing milk thistle proteins an excellent addition to a vegan diet. The presence of sulfur amino acids in the diet is significant because it is a prerequisite for synthesizing glutathione and taurine. Taurine has a broad spectrum of biological effects—it stimulates brain function, plays an essential role in neurotransmission and neuromodulation in the central nervous system, helps digest fats, and regulates osmotic pressure. Conversely, glutathione is a key endogenous antioxidant, a free radical scavenging enzyme system component, and a significant factor in brain detoxification. It is also a compound on which the proper work of the immune system depends, as it affects the replication of lymphocytes [69]. Tryptophan participates in the formation of neurotransmitters (serotonin, dopamine, norepinephrine), positively affects mood, and improves sleep self-efficacy. Low levels of tryptophan can contribute to impaired memory, lowered mood, and increased depressive symptoms in people prone to depression and exacerbate aggressive behavior in non-some individuals. Elevated tryptophan content is found in foods high in protein (meat, fish, eggs, cheese, nuts, and oilseeds), including milk thistle, which indicates the high nutritional quality of its protein [70]. The PDCAAS value of milk thistle (for lysine) is low but can be higher in combination with other proteins. Combining milk thistle products with, e.g., soy, peas, or milk (of which limiting amino acids are Met + Cys) can increase the PDCAAS value of the diet and provide a complete source of amino acids, increasing its nutritional quality [71,72]. According to Guidi et al. [73], a strategy to improve the nutritional value of a product is to mix proteins from different sources to balance out deficiencies. For example, it is reported to be beneficial to combine peas and lentils (rich in lysine) with cereal grains, which have a complementary essential amino acid profile (i.e., poor in lysine, rich in sulfur amino acid) [4]. Moreover, mixing plant proteins can also influence their sensory properties (including color), gel strength, and water-holding capacity [73].

The above results indicate the wide possibilities of using milk thistle protein to produce various categories of novel food. The market for plant-based milk and yogurt alternatives, vegan meat substitutes, snack products (e.g., protein bars), drinks, sports nutrition, bread, or pasta seems particularly promising. *S. marianum* proteins were tested for functional properties (solubility-pH profile, foaming, emulsifying properties, water holding, and fat absorption capacities) [44]. The authors suggested their universal suitability for applications in the food industry.

The problem is the lack of data on the sensory profile of milk thistle protein, which is essential from the point of view of consumer acceptance of both milk thistle products (e.g., plant-based substitutes for yogurt, milk, and ice cream) and products to which MTE protein can be added (protein mixtures, confectionery, and bakery products, etc.).

3.3. Fatty Acid Profile of Milk Thistle Endosperm

Table 3 presents the profile of fatty acids contained in the endosperm of milk thistle and the values of selected health quality indices of lipids (LHI) of the tested raw material.

Milk thistle fat is dominated by unsaturated fatty acids (UFA), constituting about 80% of TFA (total fatty acids), of which over 56% are polyunsaturated fatty acids (PUFA). The share of saturated fatty acids (SFA) did not exceed 20% of TFA. The main SFA of the tested raw material was palmitic acid > stearic acid > arachidic acid > behenic acid, with a share in the range of 8.27–2.61% TFA, respectively (Table 3). The percentage of other SFAs was small and did not exceed 0.11% in the case of myristic acid (C14:0). All SFAs identified in milk thistle endosperm can be classified as long-chain fatty acids. According to Hewlings [74], this group includes FA containing 14 to 24 carbon atoms in the chain.

Among monoenoic acids (MUFA), accounting for approx. 24% of TFA in milk thistle endosperm, the highest share was found in C18:1 oleic acid. It is the main MUFA in the human circulatory system. According to Martinez and Mougan [75], in the brain, it is an important component of membrane phospholipids and is present in high concentrations in myelin. A significant decrease in oleic acid is observed in the brains of patients suffering from Alzheimer's and severe depressive disorders [76]. Considering the polyene FA (PUFA) profile, milk thistle fat is distinguished by a high content of LA (Table 3) with a low content of ALA. Moreover, a trace amount of eicosadienoic acid (C20:2 n-9) was also found. The increased consumption of vegetable oil in the last century has significantly increased the intake of LA while reducing the intake of *n*-3 FA, including ALA, EPA, and DHA [77]. Generally, *n*-6 PUFAs are thought to exhibit pro-inflammatory activity, while n-3 PUFAs are anti-inflammatory. According to Hajeyah et al. [78], enzymatically oxygenated PUFAs (oxylipins) include a broad range of derivatives, for example, specialized pro-resolving mediators. Oxylipins made from n-3 PUFAs have less potent proliferative and pro-inflammatory activity compared to oxylipins created from n-3 PUFAs [79]. It is recommended that the consumption of n-6 should be balanced with the consumption of *n*-3 PUFA with a ratio of *n*-6 to *n*-3 between 1:1 and 2:1, a pattern of consumption humans have followed throughout evolution [80]. Despite the imbalance between these FAs in milk thistle oil, the calculated AI (Atherogenicity Index) and TI (Thrombogenicity Index) values are favorable. From a nutritional point of view, low AI and TI values are required. The recommended value of AI in the human diet is below 1.0, and TI is below 0.5 [34,37]. In milk thistle oil, they were 0.11 and 0.33, respectively, which is consistent with the results of Rokosik et al. [81]. This suggests that the fatty acids in milk thistle oil may prevent the development of cardiovascular disease. This hypothesis seems to be confirmed by Shen et al. [82]. These authors used milk thistle oil (MTO) to treat mice fed a high-fat diet for 20 weeks. The experiment investigated whether MTO affects the metabolic and cardiovascular complications associated with obesity. A multidirectional, beneficial effect of MTO was found. It was manifested by the reduction in obesity, hypertension, hyperglycemia, and the reduction in markers typical of inflammation (liver, adipose tissue) in mice. It suggests that milk thistle oil could be used in humans to treat metabolic syndrome in the future [82]. Moreover, according to Gaber et al. [83], milk thistle oil (MTSO) has effects to improve the function of the injured liver. The authors found that MTSO had antioxidant, antimicrobial and anticancer effects in vitro and hepatotherapeutic impact on the tested markers of liver function. It should be emphasized that edible oils' nutritional and potentially health-promoting properties are influenced not only by the fatty acid profile but also by the presence of other biologically active compounds. Milk thistle oil is a valuable source of tocopherols and phytosterols [81], the content of which significantly exceeds that of olive oil [84].

Table 3. Characteristics of milk thistle endosperm lipids (± SD).

Fatty Acid (FA)		[%]	g of FA/100 g FM	g of FA/100 g DM
myristic	C14:0	0.11 ± 0.01	0.04 ± 0.00	0.04 ± 0.00
palmitic	C16:0	8.27 ± 0.23	3.00 ± 0.06	3.23 ± 0.07
palmitoleic	C16:1 *n*-7	0.09 ± 0.01	0.03 ± 0.01	0.04 ± 0.01
margaric	C17:0	0.06 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
stearic	C18:0	5.32 ± 0.18	1.93 ± 0.05	2.07 ± 0.06
oleic	C18:1 *n*-9	22.47 ± 0.76	8.16 ± 0.33	8.77 ± 0.35
cis-vaccenic	C18:1 *n*-7	0.57 ± 0.06	0.21 ± 0.02	0.22 ± 0.03
linoleic (LA)	C18:2 *n*-6	55.64 ± 0.86	20.25 ± 0.24	21.76 ± 0.28
α-linolenic (ALA)	C18:3 *n*-3	0.42 ± 0.04	0.15 ± 0.02	0.16 ± 0.02
arachidic	C20:0	2.93 ± 0.04	1.06 ± 0.01	1.14 ± 0.01
cis-11-eicosenoic	C20:1	0.80 ± 0.08	0.29 ± 0.03	0.31 ± 0.03
eicosadienoic	C20:2 *n*-9	0.05 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
behenic	C22:0	2.61 ± 0.49	0.95 ± 0.19	1.02 ± 0.20
lignoceric	C24:0	0.60 ± 0.00	0.22 ± 0.00	0.23 ± 0.00
nervonic	C24:1	0.09 ± 0.00	0.03 ± 0.00	0.04 ± 0.00
SFA [%]		19.89 ± 0.06	7.23 ± 0.03	7.77 ± 0.06
UFA [%]		80.11 ± 0.06	29.14 ± 0.08	31.31 ± 0.09
MUFA [%]		24.01 ± 0.76	8.72 ± 0.16	9.37 ± 0.35
PUFA [%]		56.11 ± 0.81	20.42 ± 0.09	21.95 ± 0.26
n-6/n-3 ratio		132.46		
AI		0.11		
TI		0.33		
h/H		9.44		

4. Conclusions

Milk thistle is a plant with promising utility potential. The directions of its processing do not have to be limited to obtaining drugs and dietary supplements containing silymarin, as is the case today. The high protein content with a favorable amino acid profile and fat in milk thistle seeds and endosperm is an important feature of this raw material. It is a starting point for the search for new applications of *S. marianum*, e.g., the production of alternative proteins, plant milk substitutes, or fermented beverages. According to own research [28] and other authors' experiences [85], milk thistle protein extract is a good nutrient for lactic acid bacteria, allowing it to produce innovative milk yogurt substitutes. In the future, however, research on the multidirectional assessment of the allergenicity of milk thistle proteins, their cytotoxicity, and their digestibility, e.g., after the lactic fermentation process should be intensified, which would allow supplementing the existing knowledge about milk thistle and the possibility of its use for a sustainable food system. Another essential element of future research on using MTE for food applications should be the sensory evaluation of products containing this raw material and the protein extracted from it. This topic has not been presented in the literature, but it is crucial for determining consumer acceptance of milk thistle food.

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