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Nutritional and technological evaluation of milk thistle seeds and their application in functional biscuits

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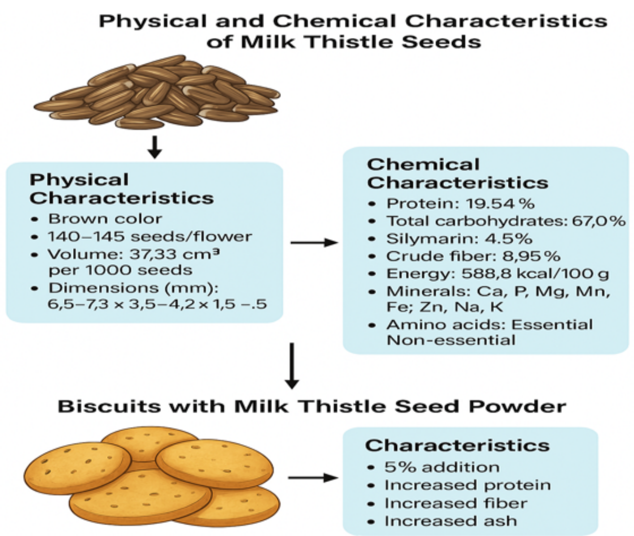
Abstract

Milk thistle (*Silybum marianum* L.) seeds are a rich source of bioactive compounds, particularly phenolic constituents and flavonolignans, which may offer nutritional and functional advantages when incorporated into food products. This study aimed to evaluate the effects of partially substituting wheat flour with milk thistle seed powder (MTSP) on the physicochemical properties, antioxidant activity, and sensory quality of cookies. Cookies were prepared with increasing levels of MTSP substitution (e.g., 5–20%), and their proximate composition, mineral content, total phenolic content, radical scavenging activity, color parameters, texture profile, and sensory acceptance were assessed. The incorporation of MTSP significantly increased protein, dietary fiber, ash, and mineral contents compared with the control formulation, while also enhancing total phenolic content and antioxidant capacity in a concentration-dependent manner. Technological evaluation indicated measurable changes in color attributes and textural properties; however, formulations containing moderate substitution levels maintained acceptable sensory scores comparable to the control cookies. These findings demonstrate that milk thistle seed powder can be successfully utilized as a functional ingredient to improve the nutritional profile and antioxidant potential of bakery products without compromising consumer acceptability. Overall, the results support the feasibility of incorporating milk thistle into cereal-based foods as a natural source of bioactive compounds with potential functional benefits. Further investigations focusing on bioavailability, shelf stability, and in vivo physiological effects are recommended to substantiate health-related claims and expand industrial applications.

Keywords: physical, chemical characteristics, milk thistle, amino acid, fatty acid, biscuits and bioactive components.



Introduction

Milk thistle (*Silybum marianum* (L.) Gaertn., a member of the Asteraceae family and Asteroideae subfamily, is an annual plant native to Southern Europe, North Africa, and Western Asia.[1–3] In Egypt, *S. marianum* commonly grows as a wild plant or weed along roadsides and in cultivated fields, especially in nutrient-poor soils.[4] It is well adapted to drought prone Mediterranean environments, where minimal rainfall is generally sufficient for its growth. However, irrigation during the seed development and filling stages has been shown to enhance the accumulation of silymarin, while both water excess and deficiency can inhibit its biosynthesis.[5,6]

The entire plant has traditional medicinal uses, but the seeds (achenes) are particularly valued for their high content of bioactive compounds, including silymarin, as well as lipids and proteins.[1,7] These seeds are extensively utilized in the pharmaceutical and cosmetic industries and are increasingly being incorporated into functional foods and animal feed due to their nutritional value.

Milk thistle seeds are rich in macronutrients such as proteins, lipids, and carbohydrates, including significant levels of crude fiber. These properties make them a promising source of plant-based protein and oil, as well as a potential functional ingredient for enriching fiber-deficient food products such as processed meats.[4] Notably, the seeds can contain up to 25% (w/w) oil[8] with reported oil content reaching 28–30% in some cultivars.[9,10] Silymarin content in the seeds typically ranges from 1.0% to 3.0% of the seed dry matter, though it may exceed 8% under certain conditions. Breeding efforts are therefore focused on developing high-silymarin varieties.[7]

In addition to silymarin, Milk thistle seeds typically exhibit a mean lipid content of 21.6%, of which 51.6% is linoleic acid and 16.1% total protein. They also contain substantial levels of crude fiber (266.0 g/kg dry matter), neutral detergent fiber (418.0 g/kg), acid detergent fiber (344.0 g/kg), and acid detergent lignin (114.0 g/kg)).[11] Drought stress has been found to improve oil quality by increasing the amount of polyunsaturated fatty acids, which can make up to 77% of the total oil with unsaturated fatty acids comprising as much as 75.1%.[12]

Traditionally, the young fleshy stems have been consumed by local populations, valued for their antioxidant content and medicinal use in treating liver and biliary tract disorders.[2] Among all plant parts, the seeds are considered the most potent in terms of medicinal efficacy. Extracts derived from milk thistle seeds have long been established as herbal remedies for liver protection, detoxification, and regeneration key functions essential for maintaining human health.[2,13,14] Furthermore, silymarin demonstrates promising antioxidant and antimicrobial properties, indicating its potential application as a natural additive in the food industry.[15]

The study aimed to determine the physical properties to estimate the major and minor chemical components of milk thistle seeds as well as to studied chemical and physical properties of milk thistle seed oil grown in Egypt. Additionally, it involved the preparation of biscuits enriched with 2%, 3%, and 5% milk thistle seed powder (MTSP).

Materials and methods

Materials

Milk thistle seeds were collected from Eldawalta village, Bani Suef Governorate, Egypt. The seed was cleaning and saving. The seed of milk thistle was grinded by Brown Multiquick blender at speed 2 to 3 min and sift by 60 mesh sieves. The seeds of milk thistle powder were placed in polyethylene bags and stored at −20 °C in a deep freezer for subsequent analyses. In this study, all raw materials were obtained from local markets in Giza, Egypt, and all chemicals were analytical grade

Methods

Preparation of wheat and milk thistle biscuit: Samples of biscuits (semi-hard sweet biscuits) were manufactured following the standard protocol of BiscoMisr Company, Cairo, Egypt. Biscuit was created by partially substituting soft wheat flour and 2%, 3%, and 5% of milk thistle powder (MTP). Replacement percentages were increased until it was acceptable without causing off flavors. The ratios were (wheat flour: milk thistle powder) 100:0 (control), 98:2, 97:3, and 95:5%.

Physical characteristics of biscuits are made with milk thistle: Hardness (N), Diameter (mm), Thickness, expansion factor (D/T), weight (g) and volume (cm^3) was appreciated by six of biscuit made with milk thistle was measured according to (A.A.C.C).[49]

Major and minor components of milk thistle seeds and biscuit made from milk thistle: The crude protein, either extract, ash, fiber, moisture, minerals, physiochemical characteristics of milk thistle oil, amino acid, fatty acid profiles and quality criteria of protein were evaluated following the methodologies outlined in the A.O.A.C.[49] The amino acid score was utilized to estimate the amino acid requirements of adolescent adults as follows: Amino acid score = (milligrams of amino acid in 1 gram of tested protein /milligrams of amino acid in required pattern) x 100 The Protein Efficiency Ratio was calculated = 0.456 + (Leucine) 0.454 - (Proline) 0.047. whereas the (PER) was calculated = 0.498 + (Leucine) 0.454 - (Tyrosine) 0.105. The Biological Value was evaluated using the equation proposed by,[16] Biological Value% = 39.55 + 8.89 × lysine (g/100gprotein). Additionally, total carbs were calculated by difference, and Silymarin was quantified using the method established by Radjabian et al.[17]

Statistical analysis: The data were subjected to analysis of variance, and Duncan's multiple range tests at the $P \leq 0.05$ significance level was applied to compare the means. The analysis was carried out using the PRO ANOVA procedure of the Statistical Analysis System (SAS).[18]

Results and discussion

Physical properties of milk thistle seed

The physical k thistle seeds, properties of milk thistle seeds, including color, seeds per flower, volume of 1000 seeds (cm^3), weight of 1000 seeds (g/1000 seeds), thickness (mm), length (mm), and seed width (mm), cultivated in Beni Suef Governorate, Egypt, were analyzed and are presented in (Figure 1) and (Table 1) The quality of milk thistle seeds is indication of their physical qualities. The results from the collected seeds indicated that the majority exhibited high quality across all physical attributes, with a brown coloration. The substantial seed count per flower and weight of a 1000 seeds signify the superior quality of mil demonstrating their economic viability for cultivation. The length seeds are 5 to 8 mm in, featuring a long white pappus and exhibiting colors that range from black to brown. The weight of 1000 milk thistle seeds are 28 to 30 g.[6] Each flower head yields approximately 190 seeds, Seeds can remain viable in the soil for up to nine years.[19] The weight of thousand MS seeds ranges from 28 to 30 grams.[6]

Figure 1 Morphological milk thistle.

Table 1 Physical properties of milk thistle seed.

Parameter	Measurements*
Color	Brown
Seed per flower (range)**	140 - 145 ± 3.3
Volume of 1000 seeds (cm^3)	37.33±1.04
Weight of 1000 seeds (g)	26.66±1.20
Seed thickness (mm)	1.5 -2.5 ±0.50
Seed length (mm)	6.5- 7.3 ± 1.02
Seed width (mm)	3.5-4.22± 1.01

*M±SD = means and standard deviation of triplicates. **range in the same plant.

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Major and minor components of milk thistle seeds

The chemical content of milk thistle seed and sunflower meal was analyzed, and results are presented in Table 2. Data revealed that milk thistle seed meal exhibited a higher concentration of chemical constituents compared to sunflower meal, with 19.54 ± 0.14% versus 4.02 ± 0.03% crude protein, 26.96 ± 0.15% versus 19.33 ± 0.33% ether extract, 67.0 ± 1.36% versus 15.99 ± 1.11% total carbohydrate, and 588.8 ± 1.55 versus 254.01 ± 1.36 kcal/100g energy, respectively. The milk thistle seed meal exhibited reduced crude fiber and ash contents compared to sunflower meal, with values of 8.95 ± 0.49% and 3.50 ± 0.10% versus 38.26 ± 0.09% and 14.15 ± 0.16%, respectively, alongside carbohydrate and energy contents of 358.63 ± 0.12% and 328.98 ± 0.05% on a dry weight basis. Silymarin content of milk thistle seeds is 4.5±0.49 g per 100 g of seeds, but sunflower meal shows no detectable silymarin. Reports indicate that milk thistle fruits contain a considerable oil content, ranging from 20% to 31%. Milk thistle seed contains 1.5% crude fiber, 0.70% ash, 22.00% crude protein, 25.00% total lipids, 0.48% total phenols, 24.22% total carbs, and 2.40% flavonoids.[20] The primary biologically active component of milk thistle is silybin and accountable for its pharmacological effects.

Table 2 Chemical composition of milk thistle and Sunflower seed % (as dry weight).

Chemical Composition	Milk thistle	Sunflower
Moisture	3.55*±0.20	8.25± 0.22
Protein	19.54±0.14	4.02±0.03
Ether extract	26.96±0.15	19.33±0.13
Ash	3.50 ±0.10	14.15±0.16
Crude fiber	8.95±0.49	38.26±0.09
Silymarin	4.5±0.49	N.D
Total carbohydrate	67.0 ±1.36	15.99±1.11
Energy (kcal/100g)	588.8±1.55	254.01±1.36

*Means and standard deviations of triplicates are equal to M±SD.

The silybin is utilized for self-treating hepatic problems (Hadolin *et al.,* and Abenavoli *et al.,*).[21–23] That milk thistle seeds comprise 25-30%protein and 20-30% oil. Wichtl and Bisset.[24] Additionally, according to Wallace *et al,*[8] and Abenavoli et al.,[22,23] plant seeds contain roughly 30% protein and 15–30% lipids. According to Beis et al., (2002), safflower seeds contain 20.0% crude fiber, 19.0% protein, 40.0% oil, and 2.2% ash. According to Wallace et al. (2005),[8] milk thistle seeds contained 25% w/w oil. Khalil[20] found exceedingly crude protein (22%), ash (0.7%), and low values for crude fibers (1.5%). Weather circumstances exhibited a more significant impact on alterations in chemical composition than agrotechnical conditions the overall oil content diminished by 4% because of heightened water constraint, although the concentration of polyunsaturated fatty acids escalated under drought conditions. (Malekzadeh *et al.*).[25] The alterations in total protein content were minimal, however the changes in crude fiber and macroelement content, particularly calcium and magnesium, were significant.[6,11] On average, the fruits included 21.6% fat, 51.6% linoleic acid, and 16.1% total protein. On average (g/kg^{-1} of dry matter), the fruits contained the following: 114.0 of acid detergent lignin (ADL), 344.0 of acid detergent fiber (ADF), 418.0 of neutral detergent fiber (NDF), and 266.0 of crude fiber.[6,11] Due to dry weight, the silymarin content of milk thistle seeds ranged from 4% to 6%, with some samples having as much as 8% silymarin Khalil.[20] Shokrpour *et al.,*[26] found that milk thistle seeds grown in Poland had an average silymarin content of 2.18%. Sadowska et al.[6]

The observed increase in protein and mineral content following milk thistle incorporation is consistent with previous reports describing the nutritional richness of oilseed by-products used in cereal formulations. Improvements in ash and fiber content are particularly relevant from a nutritional standpoint, as dietary fiber enrichment in bakery products has been associated with improved glycemic response and digestive health benefits. Moreover, the presence of silymarin and phenolic compounds in milk thistle seeds may provide additional functional value, given their well-documented antioxidant and hepatoprotective properties reported in recent literature.

Mineral content of milk thistle seed

The mineral composition (calcium, phosphorus, magnesium, manganese, iron, zinc, sodium, and potassium) of milk thistle seed, together with its percentage of the recommended daily intake for adults, is shown in mg/100 g on a dry weight basis in Table 3. The mineral composition of milk thistle seed flour reveals elevated levels of calcium, phosphorus, potassium, and magnesium, measuring 910±1.19, 785±1.55, 755±2.11, and 435±1.11 mg/100 g on a dry weight basis, respectively. In contrast, the concentrations of iron, sodium, zinc, and manganese are significantly lower, recorded at 79.89±0.85, 10.5±1.26, 7.40±1.55, and 6.57±1.91 mg/100 g on a dry weight basis, respectively. The results indicate that milk thistle seeds contain iron, potassium, manganese, and magnesium, surpassing the recommended dietary allowances by significant margins (998.63%, 443.28%, 215.71%, 164.25%, and 135.94% respectively). Meanwhile, the composition of phosphors, zinc, and calcium constitutes at least 62.8% for each element (112.14-62.8, 92.5-67.27, and 91-75.83%). Conversely, it comprises a minimal sodium proportion (10.5+1.26), accounting for 2.3-2.1%. Iron deficiency is the primary cause of iron deficiency anemia. Baseline survey data on iron deficiency anemia in Egypt from 2010 indicated that 47% of women aged 20 to 50 years, 40% of children under 5 years, and 35% of children aged 6 to 18 years were anemic (NNI).[27]

Table 3 Mineral content of milk thistle seed (mg/100gm).

Minerals	Minerals Contents mg/100g	RDA (FDA, 2011) 14-70 year
Calcium	910+1.19	1000-1200
Phosphors	785+1.55	700-1250
Magnesium	435+1.11	320-420
Manganese	6.57 ± 1.91	4.0-5.0
Iron	79.89+0.85	8.0-18.0
Zinc	7.40+1.55	8.0-11.0
Sodium	10.5+1.26	1.3 -1.5 gm
Potassium	755+2.11	4.5-4.7

Mineral salts are necessary nutrients. They comprise microminerals (calcium, phosphorus, sodium, potassium, chloride, and magnesium) and trace elements (iron, zinc, selenium). Micronutrient deficiency is a prevalent public health issue, particularly affecting vulnerable populations (infants, children, adolescents, pregnant women, and lactating mothers) in numerous low- and middle-income nations. Anemia, together with deficiencies in vitamin A and zinc, poses significant risks to the development of the group[28–30] assert that bone mass in the elderly is influenced by the rate of mineral loss and the mass accrued during skeletal development, which is contingent upon dietary calcium and vitamin D levels. Minerals are situated in the germ; so, we may anticipate that they are not eliminated during the refining process. The macroelement concentration of milk thistle seeds in g.kg-1 of dry matter was as follows: phosphorus – 6.1, potassium – 4.95, calcium – 7.6, magnesium – 2.6. A significant concentration of iron was detected — 82.3 mg/kg of dry matter. The elevated cellulose-lignin content in whole milk thistle fruits restricts

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its usage to ruminants alone.[6, 11] Magnesium 502 and 560, Potassium 732 and 755, Manganese 4.44 and 4.89, Copper 0.75 and 0.88, Iron 10.5 and 15.56, Phosphorus 411 and 487, Zinc 4.1 and 5.66, Calcium 86.3 and 89.56, and Sodium 2.44 and 1130.55 have been the mineral values (mg/100 grams) for quinoa flour Abdelazim.[29]

Amino acid composition of milk thistle seeds

The amino acid composition of milk thistle seeds was quantified as grams per 100 grams of protein, with the results presented in Table 4. The results indicated that the total essential amino acids (TEAA) and total non-essential amino acids (TNEAA) content of milk thistle seeds was 55.2 g and 79.03 g per 100 g of protein, respectively, compared to 14.69 g and 22.37 g per 100 g of protein in sunflower seeds. The essential amino acid profile reveals that milk thistle seeds contain greater percentages of phenylalanine (6.1%), tyrosine (5.41, lysine, %), leucine (9.84%), and lysine (7.38%). Certain amino acids, including arginine methionine, and glycine, induce a hypocholesterolemia impact, rendering them very important (Mortia et al.,).[31] Evaluations of protein quality for the analyzed formula: The FAO[32] reported that the amino acid score pattern was calculated and presented in Table 5, based on the quantity of the first limiting amino acid. This aimed to propose the amino acid requirement pattern to assess the quality of dietary protein for each age group, according to the FAO/WHO/UNU[32] expert council, based on prior human studies. The calculation of the chemical score was compared based on the scoring pattern of grams per gram of protein needed for adolescent adults.

Table 4 Amino acids composition of milk thistle seeds (as g amino acid/100 g protein).

Amino acids	Milk thistle seed	Amino acid score (AAS)	Sunflower seed*	Amino acid score (AAS)	FAO/WHO Adult
Essential Amino Acids (EAA)					
Tyrosin	5.41	180.33	0.97	32.33	3
Leucine	9.84	166.78	2.42	41.02	5.9
Valine	7.97	204.36	1.92	49.23	3.9
Lysine	7.38	164	1.07	23.78	4.5
Phenylalanine	6.1	203.33	2.32	77.33	3
Isoleucine	5.41	180.33	1.69	56.33	3
Methionine	2.46	111.82	0.59	26.82	2.2
Cysteine	2.16	98.182	1.17	53.18	2.2
Histidine	3.35	223.33	1.09	72.67	1.5
Threonine	5.12	222.61	1.45	63.04	2.3
Total Essential Amino Acids (TEAA)	55.2		14.69		
Non-Essential Amino Acids (NEAA)					
Glutamic	29.62		6.95		
Glycine	8.27		2.55		
Arginine	12.59		3.07		
Serine	7.67		1.73		
Proline	7.28		3.3		
Aspartic	6.91		3.19		
Alanine	6.69		1.58		
Total Non-Essential Amino Acids (NEAA)	79.03		22.37		

The protein quality characteristics are computed and displayed in Table 5. The protein quality of milk thistle seed exhibited elevated values for the protein efficiency ratio (PER). The values and biological value percentage (B.V.) of sunflower seeds were 4.58, 4.39, and 105.16%, compared to 1.39, 1.49, and 49.06%. The analysis indicated that the lowest score for essential amino acids in milk thistle protein, compared to sunflower seed, suggests that the most limiting amino acid provides an initial estimate of its utilization efficiency in adolescents, facilitating an adjustment of protein requirements based on dietary protein quality. Cysteine was identified as the primary limiting amino acid, whereas tyrosine combined with methionine and lysine in comparison to cysteine were the second and third limiting amino acids in milk thistle relative to sunflower seed. Millward[33] emphasizes that leucine and lysine are the most prevalent amino acids in growth requirements. The Essential Amino Acid Index (EAAI) assesses protein quality by comparing the quantity of all essential amino acids to that of the egg reference amino acid profile. This is a quick technique for assessing and optimizing the amino acid composition of food formulations (Suzanne).[34] Milk thistle seed proteins possess a substantial concentration of important amino acids, including leucine, isoleucine, valine, lysine, and threonine, which are absent in wheat flour.[35]

Table 5 Evaluation of protein quality for Milk thistle and Sunflower seeds.

Items	Milk thistle	Sunflower
Protein Efficiency Ratio (PER)a	4.58	1.39
Protein Efficiency Ratio (PER)b	4.39	1.49
Biological value% (B.V)	105.16	49.06
Essential amino acid index (EAAI) EAAI (%)		
First	Cysteine	Methionine
Second	Tyrosine+ methionine	lysine
Third	lysine	Cysteine

Physiochemical characteristics of milk thistle seeds oil

The physical and chemical parameters of crude milk thistle oil were analyzed in comparison to refined sunflower oil, with the results presented in Table 6.The acid values of milk thistle oil (crude oil) were 0.21±0.03 mg KOH/g oil, but sunflower oil (refined oil) exhibited values of 0.65±0.22 mg KOH/g oil, indicating the freshness of the oils.

Data indicated that neither lipolysis nor oxidative rancidity transpired in the milk thistle oil (crude oil). As a result, crude oil from milk thistle can be used as an edible vegetable oil. The refractive index of 1.4681, an acidity of 0.65 ± 0.22% as oleic acid, a peroxide value of 1.0 ± 0.03 equivalent of active oxygen per kg of oil, a saponification value of 178 ± 2.13, and an unsaponifiable matter of no more than 1.5 ± 0.13%, the physical and chemical characteristics of sunflower oil were found to be in line with the values set by the Egyptian Standard Specifications (ESS)[36] for sunflower seed oil. Milk thistle seed oil had a refractive index of 1.4335 (Parry). [37]According to a certain study, milk thistle oil is a good source of vitamin E and an edible oil (El Mallah et al.).[12] Milk thistle seed oil is abundant in oil, which is significant for both nutritional and medicinal purposes; so, the cultivation of this oilseed for edible oil production is warranted.[38]

Table 6 The physiochemical properties of sunflower oil and milk thistle seeds.

Parameter	Milk thistle oil (Crude oil)	Sunflower oil (Refined oil)
Odor	Acceptance	Acceptance
Color	Dark Yellow	Yellow
Refractive index	1.4639	1.4681
Acidity (as % oleic acid)	0.21± 0.03	0.65± 0.22
Acid value (mg KOH/g oil)	3± 0.11	0.106± 0.01
Iodine value*	115.49	118
Peroxide value (meq O/kg oil)	1.56± 0.11	1.0± 0.03
Unsaponifiable matter(g/kg)	1.18±0.22	1.5± 0.13
Saponification value (mg /g oil)	182± 3.33	178± 2.13

IV calculated from fatty acid composition IV = (0.95 × C16:1) 16.99 + (0.86 × C18:1n-9)31.62 + (1.732 × C18:2n-6)57.59 + (2.616 × C18:3n-3) 9.29+ (0.785 × C20:1).

The proportion of fatty acids in milk Sunflower oil and milk thistle seeds

The fatty acid content of milk thistle and sunflower oil is contrasted in Table 7. Milk thistle seed oil has been shown to contain nine different fatty acids: myristic acid, palmitic acid, arachidonic acid, stearic acid, arachidic acid, palmitoleic acid, oleic acid, linoleic acid, and α-linolenic acid. In comparison to sunflower oil, which has values of 55.1% and 2.79%, the results showed that milk thistle has ±-Linolenic acid and Linoleic acid at amounts of 33.25% and 3.55%, respectively. This means that in terms of ∑Polyunsaturated fatty acids (∑PUFA), milk thistle has a total of 36.8%, while sunflower oil has 57.89%. Additionally, the relative percentages of the total monounsaturated fatty acids (∑MUFA), specifically (16:1) and (18:1), were 17.88% and 36.77%, respectively, as opposed to 0.20% and 27.4%. As a result, the cumulative total is 31.86%, whereas the total saturated fatty acids (∑SFA) are 13.67%. The ratios of ∑PUFA/∑SFA, MUFA/∑PUFA, and ∑USFA/∑SFA were 1.15, 1.49, and 2.87, respectively, rather than 4.23, 0.49, and 6.29, which supported the findings. This indicates that milk thistle oil possesses a high TU/TS ratio of 6.25. This suggests that the fatty acid composition of milk thistle oil, which includes the crucial fatty acid C18:2, has a significant degree of unsaturation. Additionally, the five saturated fatty acids found in quinoa seeds are myristic, palmitic, stearic, and arachidonic acids. Their relative percentages are 1.13%, 17.55%, 9.71%, and 0.69%, respectively, whereas those found in flatbread are 0.13%, 8.03%, 4.32%, and 0.23%. Every mammal needs omega-3 fatty acids. It is considered important to consume 2 to 3 grams of α-linolenic acid per day for both primary and secondary prevention of coronary heart disease.[39] The human body requires polyunsaturated fatty acids (PUFAs) in its diet, particularly α-linolenic acid (18:3n-3) and linoleic acid (18:2n-6), (Huang and Brenna).[38] Milk thistle seed oil may be a significant source of polyunsaturated fatty acids, according to this study, making it a notable nutritional choice. The percentage of polyunsaturated fatty acids in oil can rise to 77% during drought stress, which can significantly improve oil quality (El-Mallah et al.,).[12] revealed that S. marianum (L.) seed oil extracted using a chloroform-methanol (2:1 V/V) mixture provided 75.1% of the UFAs. Our results' fatty acid content supported the claims made by Fathi-Achachlouei and Azadmard.[40] (Qavami et al; Hadolin et al.,) [21,41,42,] It includes fatty acids such as palmitic acid, stearic acid, oleic acid, linolenic acid, and linoleic acid. The diet's saturated to unsaturated fatty acid ratio is important because polyunsaturated fatty acids help protect people against a variety of illnesses.[43]

Table 7 Milk thistle and sunflower oils' fatty acid content.

Fatty acids (%)	Milk thistle	Sunflower oil*
Myristic acid (C14:0)	1.13	0.13
Palmitic acid (C16:0)	17.55	8.03
Arachidonic acid C 20:0	0.69	0.23
Stearic acid (C18:0)	9.71	4.32
Arachidic acid (C22:0)	2.78	0.96
∑Saturated fatty acids (SFA)	31.86	13.67
Palmitic (C16:1)	17.88	0.20
Oleic acid (C18:1)	36.77	27.4
∑Monounsaturated fatty acids (MUFA)	54.65	27.6
Linoleic acid (C18:2)	33.25	55.1
α-Linolenic acid (C18:3)	3.55	2.79
∑Polyunsaturated fatty acids (PUFA)	36.8	57.89
Others	0.55	0.36
∑PUFA /∑ SFA	1.15	4.23
∑MUFA / ∑PUFA	1.49	0.49
∑USFA / ∑SFA	2.87	6.29
C18:2 / C18:3	9.37	19.75

From a lipid perspective, the fatty acid composition of milk thistle oil, characterized by a substantial proportion of unsaturated fatty acids, aligns with current recommendations promoting the consumption of plant-based lipids with favorable PUFA/SFA ratios. Incorporation of such lipid profiles into food systems may contribute to improved nutritional quality, although the stability of these compounds during thermal processing requires further evaluation.

The chemical composition of biscuits

Recent interest in functional bakery products has increasingly focused on the incorporation of plant-derived powders rich in bioactive compounds, dietary fiber, and unsaturated lipids to improve nutritional quality while maintaining consumer acceptability. Oilseed derived ingredients such as flaxseed, chia, and defatted seed meals have demonstrated significant potential in bakery fortification due to their balanced amino acid composition and functional properties, including water-binding capacity and lipid contribution to texture development. Similar to these matrices, milk thistle seed powder exhibited the ability to enhance protein and fiber content in biscuits while preserving acceptable sensory characteristics, suggesting technological compatibility with wheat-based systems.

The chemical composition of biscuits at 2%, 3%, and 5% using soft wheat flour and replacement milk thistle powder displayed in (Figure 2). The biscuits made with milk thistle powder had higher levels of protein, ether extract, fiber, ash, and silymarin than the control samples

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made with wheat flour, according to the data. The protein content of biscuits made with four different levels of milk thistle powder replacement varied from 11.43±0.11 at 2% to 11.64±0.12 at 3% and 12.03±0.11 at 5% replacement. This contrasts with the 11.05±0.21% protein content of the control biscuits. The extract gradually increased in biscuits manufactured with alternatives for milk thistle powder. When compared to other biscuits, the ether extract used to replace 5% of the milk thistle powder in biscuits was greater (13.86±0.24). The results showed that biscuits made with different milk thistle powder replacements had higher levels of ash and fiber than the control. The fiber and ash (0.73±0.02, 0.82±0.01and 0.98±0.03 content were (0.73±0.02, 0.82±0.01 and 0.98±0.03) and (1.03±0.02,1.05±0.02 and 1.14±0.01) in biscuits respectively. The amount of silymarin gradually increased in biscuits made with various substitutes for milk thistle powder, (0.08±0.01 at 2 %,0.13±0.01at 3% and 0.25±0.01 at 5%). It was observed that the higher percentage of milk thistle powder, as compared to the control sample, gradually increased the chemical composition (protein, fiber, and ash). Illustrated in Figure 3 how adding milk thistle powder reduced the overall amount of carbohydrates when compared to the control sample. The biscuits made with composite milk thistle powder are more nutrient-dense than those made with soft wheat flour. This demonstrated that the milk thistle powder has a high ash content, fiber, either extract, and protein content. Therefore, it might be used as a nutritional ingredient in the creation of nutritious food items. Our results agree with Hassan et al.,[44] Apostol et al.[35] Abdelazim et. al.[29] Krystyjan et. al,[45]and Dockalova et.al.[46]

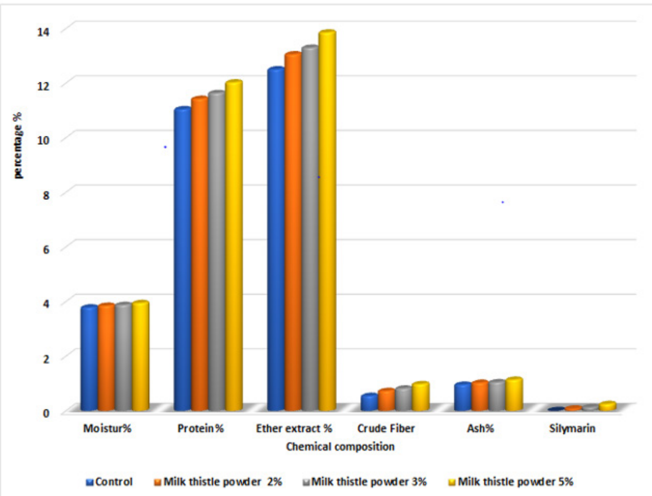

Figure 2 Chemical parameters of biscuits made from wheat flour 72% ex and milk thistle powder biscuits.

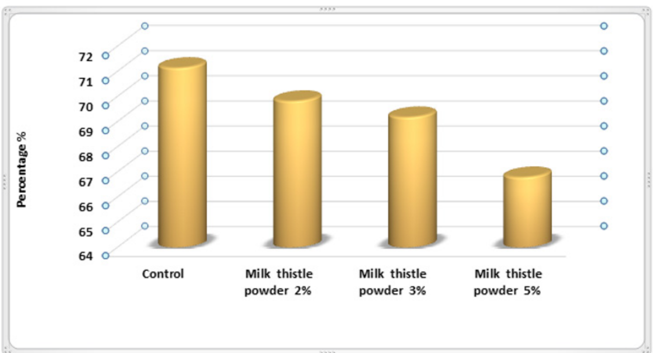

Figure 3 Total carbohydrate of biscuits made from wheat flour 72% ex and milk thistle powder biscuits

The physical characteristics of biscuits

The physical parameters of biscuits manufactured with varying substitutions of milk thistle powder include hardness (N), diameter (mm), weight (g), expansion factor, biscuit volume (cm^3), specific volume (cm^3/g), and thickness (mm), as illustrated in (Figure 4). The mass of biscuits increased with a higher proportion of milk thistle powder. The weight rise of 5% surpassed that of 3%, which was greater than 2%. When compared to the control sample, biscuits with a higher percentage of milk thistle powder expanded, thickened, and increased in volume.

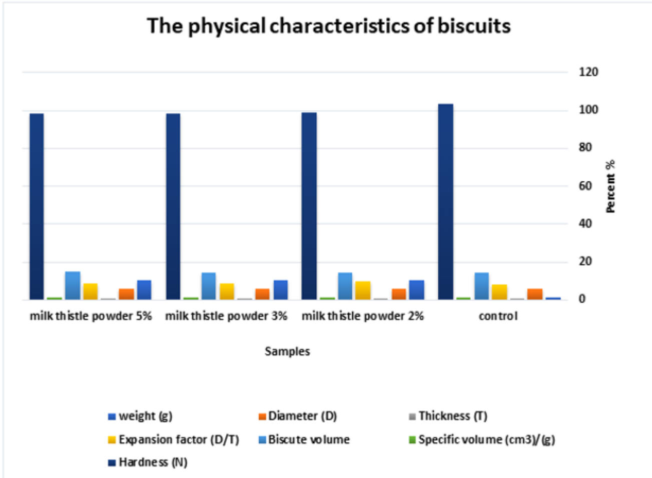

Figure 4 Physical characteristics of biscuits made with various milk thistle powder substitutes.

The specific volume exhibited a marginal increase with the incorporation of milk thistle powder. The specific volume of the 5% biscuit samples was marginally higher than that of the control. The specific volumes of 2% and 3% are comparable. The results are in line with those of Hassan et al. Apostol et al. and Abdelazim et al.[45,35,29]

The sensory qualities of biscuits

The statistically assessed results in Table 8 pertains to biscuits created with varying substitutions of 72% wheat flour and milk thistle powder at three distinct amounts. The milk thistle powder biscuits containing 2%, 3%, and 5% exhibited the highest color values. These results indicate that the organoleptic scores of biscuits significantly improved with the incorporation of milk thistle powder (2%, 3%, and 5%) compared to control biscuits produced with 72% wheat flour. The biscuits with 5% milk thistle powder received the highest evaluation compared to all other treatments and the control biscuits. The sensory approval levels for the flavor of biscuits made from milk thistle were the highest among all sensory attributes evaluated. The higher the percentage of milk thistle powder in the biscuit samples, the better the general acceptance. Overall, 5% milk thistle powder had the highest acceptance rate (94.23±1.33). The sensory evaluation of biscuits indicates that substituting naked barley flour varieties positively influences the biscuits' qualities, hence diminishing their sensory appeal in most attributes Abdelazim et al.[29] These findings are consistent with those published by Hassan et al., Prodhan et al., Apostol et al., and Maray et. al.[35,45,47–52]

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Table 8 Sensory qualities of biscuits made with different substitutions milk thistle powder and wheat flour 72%ex.

Samples	Color (20)	Appearance (20)	Texture (20)	Taste (20)	Odor (20)	Overall acceptability (100)
Control flour 72%ex	18.20^{b}±0.23	18.30^{bc}±0.57	17.66^{c}±022	18.2^{b}±0.11	18.30^{c}±0.09	90.5^{d}±2.00
Milk thistle powder 2%	18.11^{bc}±0.53	18.21^{bc}±1.11	17.64^{cd}±1.2	19.5^{b}±0.35	18.70^{b}±0.67	91.3^{c}±1.50
Milk thistle powder 3%	18.2^{b}±0.89	18.33^{b}±0.88	18.58^{ab}±0.82	19.08^{c}±0.91	18.70^{b}±1.06	93.11^{b}±2.09
Milk thistle powder 5%	19.0^{a}±0.88	18.96^{a}±0.51	18.81^{a}±0.54	19.85^{a}±0.83	19.12^{a} ±1.25	94.23^{a}±1.33

The sensory acceptance observed at moderate substitution levels confirms that milk thistle powder can be integrated into bakery formulations without compromising consumer perception, which is a critical determinant of functional food success. Comparable findings have been reported in recent studies on fortified biscuits containing plant protein sources, pseudocereal flours, and herbal powders, where optimization of substitution levels was necessary to balance nutritional enhancement with desirable texture and flavor characteristics.

Conclusion

This study demonstrated that milk thistle seeds represent a nutrient-dense plant resource characterized by considerable levels of protein, dietary fiber, essential minerals, bioactive compounds (notably silymarin), and unsaturated fatty acids. The physicochemical properties of the extracted oil confirmed its potential suitability for edible applications, while the amino acid profile indicated favorable protein quality compared with conventional plant sources. Incorporation of milk thistle seed powder into biscuit formulations, particularly at a 5% substitution level, significantly improved nutritional composition without negatively affecting technological or sensory attributes, supporting its feasibility as a functional ingredient in bakery products.

The enhancement observed in protein, fiber, mineral content, and bioactive compounds suggests that milk thistle powder could contribute to the development of value-added cereal-based foods with improved nutritional profiles. However, the present work focused primarily on compositional and technological evaluation; therefore, direct physiological or clinical health effects cannot be inferred. Further investigations are warranted to evaluate the stability and bioavailability of silymarin during processing and digestion, antioxidant functionality in finished products, and potential metabolic or health outcomes through in vitro and in vivo studies. From an industrial perspective, the utilization of milk thistle seeds may provide an opportunity for diversification of functional ingredients derived from underutilized crops, supporting sustainable food innovation and nutraceutical development. Milk thistle seeds can serve as a valuable functional ingredient in biscuit production due to their rich nutritional and antioxdant profile. Nevertheless, moderate consumption and proper formulation are essential to avoid undesirable sensory effects or possible health related complications in sensitive populations. Careful evaluation of dosage, consumer health status, and product quality characteristics is recommended when developing functional bakery products containing milk thistle seeds.

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Conflict of interest

No potential conflict of interest was reported by the authors.

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