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The Effect of Ultrasound-Assisted Ethanolic Extract of Milk Thistle (*Silybum marianum*) Seeds on the Oxidative Stability of Soybean Oil

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ABSTRACT

Due to health concerns related to synthetic antioxidants, extensive research has been conducted on using natural antioxidants as preservatives in food. The present study aimed to evaluate the impact of milk thistle seed extract on the oxidative stability of soybean oil. The extract of milk thistle seeds was prepared using ethanol and ultrasound, its antioxidant properties were assessed and then different concentrations (100, 200, and 400 ppm) of the extract were added to refined soybean oil without any antioxidants. The peroxide value and Thiobarbituric acid index of the oil samples were then monitored during storage under accelerated oxidation conditions (70°C, 12 days). The results indicated that the milk thistle extract contained 34.4 mg of total phenols (as gallic acid equivalents) and 26.2 mg of total flavonoids (as quercetin equivalents) per gram of dry matter. The antioxidant properties of the extract evaluated using three methods (2,2-diphenyl-1-picrylhydrazyl radical scavenging activity, total antioxidant capacity and ferric reducing power) showed increase in the antioxidant activity with increasing the extract concentrations (30 to 150 micrograms). Comparing the antioxidant capacity of different concentrations of milk thistle extract with synthetic antioxidant, butylated hydroxytoluene and the control sample (soybean oil without any antioxidant) revealed that the peroxide and thiobarbituric acid values rose in all samples during storage period. However, increasing the concentration of milk thistle extract in soybean oil mitigated this increase, with the most significant reduction observed at 400 ppm. Ultimately, this study demonstrated that incorporating 400 ppm of milk thistle seed extract was more effective in reducing the oxidation rate of soybean oil compared to the synthetic antioxidant.

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1-Introduction

Edible oils contain essential nutrients and play a crucial role in the diet by maintaining the body's normal physiological functions. With the increasing awareness of consumers, the quality and health aspects of edible oils have gained significant attention. The key quality factors influencing edible oils include fatty acid composition, nutritional content, and oxidative stability. However, oils derived from plant sources often lack a balanced functional profile, optimal nutritional properties, and sufficient oxidative stability.

Polyunsaturated fatty acids (PUFAs) are essential for human health, but unfortunately, they are highly susceptible to oxidation. The oxidation of fatty acids can degrade these compounds, making them unavailable as nutrients while also producing undesirable flavors and toxic compounds. The oxidation process reduces the nutritional quality of oils and may lead to the formation of harmful substances that pose risks to human health [1].

Soybean oil is rich in polyunsaturated fatty acids such as linoleic and linolenic acids, which, due to the low bond dissociation energy of their double bonds, are highly prone to oxidation [2]. Several methods have been developed to prevent oil oxidation, one of which is the addition of antioxidants. Antioxidants are compounds that delay the development of rancidity and off-flavors through various mechanisms, such as controlling oxidation substrates, inhibiting prooxidants, and neutralizing free radicals [3].

Due to health concerns associated with synthetic antioxidants, extensive research has been conducted on the use of natural antioxidants as preservatives in food products. Natural antioxidants include vitamins, tocopherols, carotenoids, and phenolic compounds, all of which can enhance the shelf life of food products. Among these, phenolic compounds are more stable than vitamin-based antioxidants in food systems during processing and storage at high temperatures. In addition to their antioxidant properties, these compounds also exhibit antimicrobial activity [4].

Traditional extraction methods for bioactive compounds, such as maceration and Soxhlet extraction, are often time-consuming, inefficient, and associated with high production costs. Additionally, residual organic solvents (e.g., methanol, acetone, hexane) used in these methods may not only have negative environmental impacts but also cause various health issues in consumers if present in the final product. Consequently, modern extraction techniques have garnered increasing attention from industries and researchers [5].

Ultrasound refers to sound waves with frequencies above 18–20 kHz. Ultrasound-assisted extraction (UAE) is emerging as an efficient alternative to conventional extraction methods due to its higher efficiency, reduced energy and water consumption, and improved recovery of low-molecular-weight bioactive compounds. The enhanced extraction rate achieved by ultrasound is attributed to its ability to disrupt plant cell structures, facilitating the release of intracellular compounds into the extraction medium [6].

Several studies have investigated the use of plant extracts to improve the oxidative stability of edible oils. For instance, Hosseini et al. (2021), Mohammadi Moghaddam et al. (2024), Abdo et al. (2023), and Mohammadi & Arabshahi (2016) used corn meal extract,

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plum peel extract, agricultural waste extract, and frankincense resin extract, respectively, to enhance the oxidative stability of edible oils. These researchers reported that the incorporation of natural antioxidants significantly improved oxidative stability without causing considerable changes in the fatty acid profile of the oils [7, 8, 9, 10].

Milk thistle (*Silybum marianum*), a plant from the *Asteraceae* family, is capable of growing as an annual or biennial herb. This medicinal plant has been used for centuries to treat various ailments, including liver disorders, hypertension, obesity, atherosclerosis, and diabetes. Additionally, milk thistle is rich in flavonoids, tannins, oils, vitamins, and minerals [11]. The primary bioactive component of milk thistle is a flavonolignan complex known as silymarin, which is primarily composed of silibinin, silychristin, silydianin, and isosilybin. Silymarin is present in all parts of the milk thistle plant, including the fruits, seeds, roots, stems, and leaves, with the highest concentration found in the seeds [12].

The objective of this study was to evaluate the effect of ultrasound-assisted ethanolic extract of milk thistle seeds as a natural antioxidant on the oxidative stability of soybean oil, an aspect that has not been previously reported.

2- Materials and Methods

2-1- Materials

Milk thistle seeds were obtained from farms in Galikesh County, Golestan Province, and deodorized soybean oil without antioxidants was purchased from the Ghaneh Company. Gallic acid, quercetin, ferric chloride, ferrozine, ferric chloride, trichloroacetic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), butylated hydroxytoluene (BHT), ferricyanide sodium, and potassium ferricyanide were procured from Sigma. Sodium hydroxide, hydrochloric acid, 96% ethanol, sulfuric acid, hexane, potassium acetate, aluminum chloride, Folin–Ciocalteu reagent, sodium carbonate, sodium phosphate, ammonium molybdate, and methyl red indicator were obtained from Merck.

2-2- Sample Preparation and Extraction of Milk Thistle Seed Extract

Initially, the milk thistle seeds were placed in an electric oven (Memmert, Germany) at 40°C for 48 hours until their moisture content reached approximately 5%. The dried seeds were ground into powder using a laboratory mill, and the obtained powder was defatted using hexane at a 1:5 ratio for 48 hours. The resulting defatted powder was first stored at room temperature and then in an oven at 40°C to completely remove hexane. The powder was then sieved through a 45-mesh sieve. The defatted powder was subjected to extraction using ethanol (1:5 ratio) in an ultrasonic bath (Agilent, USA) made of stainless steel with a frequency of 50 Hz at 20°C for 30 minutes. After the extraction process, the solution was filtered using Whatman No. 1 filter paper. The filtered solution was then concentrated and dried using a rotary evaporator (Heidolph Laborota 400, South Korea) at 60°C with a rotation speed of 70 rpm. The obtained extract powder was stored in a sealed container in a refrigerator at 4°C until further testing [5,6].

2-3- Evaluation of the Antioxidant Properties of Milk Thistle Extract

2-3-1- Total Phenolic Content

The total phenolic content was determined using the Singleton and Rossi (1977) method. Briefly, 20 µL of the extract solution (before drying) was mixed with 1.16 mL of distilled

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water and 100 µL of Folin–Ciocalteu reagent. After 1 to 8 minutes, 300 µL of sodium carbonate solution (20%) was added. The test tubes were shaken and incubated in a water bath (Memmert, Germany) at 40°C for 30 minutes. Absorbance was read at 760 nm using a spectrophotometer (PG Instrument, UK). A standard curve was generated using gallic acid, and the total phenolic content was expressed as milligrams of gallic acid equivalent per gram of extract [13].

2-3-2- Total Flavonoid Content

Total flavonoid content was measured using the aluminum chloride colorimetric method as described by Chang et al. (2002). A mixture of 0.5 mL extract solution (before drying), 1.5 mL of 95% ethanol, 100 µL of 10% aluminum chloride, 100 µL of 1M potassium acetate, and 2.8 mL of distilled water was prepared. The samples were incubated at room temperature for 30 minutes, and absorbance was measured at 415 nm using a spectrophotometer. Quercetin was used as a standard, and flavonoid content was expressed as milligrams of quercetin equivalent per gram of extract [14].

2-3-3- DPPH Radical Scavenging Activity

DPPH radical scavenging activity was determined using the method of Wu et al. (2003). Extract solutions with varying concentrations (30–150 µg/mL) and synthetic antioxidant BHT were prepared in ethanol. A 1.5 mL aliquot of DPPH ethanol solution (0.15 mM) was mixed with 1.5 mL of extract solution, vigorously shaken, and centrifuged at 2500 rpm for 10 minutes (Hanil, South Korea). The samples were kept in the dark for 20 minutes, and absorbance was read at 517 nm. The percentage inhibition of DPPH radicals was calculated using Equation (1):

Equation (1)

DPPH Radical Scavenging Activity **(%)** = $(A_C - A_S)/A_C \times 100$

where A_C is the absorbance of the control and A_S is the absorbance of the sample.

The EC_{50} value, representing the concentration required to scavenge 50% of DPPH radicals, was calculated and compared [15].

2-3-4- Total Antioxidant Capacity

The phosphomolybdenum method was used to evaluate total antioxidant capacity, based on the reduction of Mo(VI) to Mo(V) in an acidic environment, forming a green phosphomolybdenum complex with maximum absorption at 695 nm. Extract solutions (30–150 µg/mL) and BHT were prepared in ethanol. A mixture of 100 µL extract solution and 1 mL reagent (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) was placed in an Eppendorf tube and incubated in a water bath at 95°C for 1.5 hours. After cooling, absorbance was measured at 695 nm. The EC_{50} value was determined as the concentration yielding 0.5 absorbance [16].

2-3-5- Iron (III) Reducing Power

The iron (III) reducing power of each sample was assessed based on the reduction of potassium ferricyanide, which induces a color change from yellow to green or blue. For this purpose, solutions of the extracts at varying concentrations (30–150 µg/mL), as well as the synthetic antioxidant BHT, were prepared in the respective extraction solvents and ethanol. Then, 1 milliliter of the extract solution was combined with 2.5 mL of sodium phosphate buffer (pH = 6.6) and 2.5 mL of 1% potassium ferricyanide solution. The mixture was then incubated in a water bath at 20°C for 50 minutes. Following

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incubation, 2.5 mL of 10% trichloroacetic acid was added to the tubes, and the samples were centrifuged at 1350×g (Centorion, Canada) for 50 minutes at room temperature. After centrifugation, 2.5 mL of the supernatant was mixed with an equal volume of distilled water and 0.2 mL of 0.1% ferric chloride solution. The absorbance of the resulting solution was then measured at 700 nm using a spectrophotometer. A higher absorbance at this wavelength indicates greater reducing power of the sample. Furthermore, the EC50 value, defined as the extract concentration required to achieve an absorbance of 0.5 at 700 nm, was determined and compared among the samples. A lower EC50 value is indicative of stronger antioxidant activity of the extract [17].

2-4- Antioxidant Activity of Milk Thistle Extract in Soybean Oil

To evaluate the antioxidant activity of milk thistle seed extract, refined, deodorized, and bleached soybean oil without additives (e.g., citric acid, synthetic antioxidants) was used. The extract was added at concentrations of 100, 200 and 400 ppm, while BHT was added at 100 and 200 ppm. An oil sample without any antioxidant/extract was used as control. The samples were stored in clear containers and placed in an electric oven at 70°C for 12 days. Oxidation progress was monitored by measuring peroxide and thiobarbituric acid values at 2-day intervals [3,8].

2-4-1- Determination of Peroxide Value

The peroxide value was determined based on the AOCS Cd 8-53 (1994) method. For this assay, 3 g of the oil sample was mixed with 30 mL of an acetic acid-chloroform solution (at a ratio of 2:3). Subsequently, 0.5 mL of saturated potassium iodide solution was added to the mixture, followed by vigorous stirring. Then, 30 mL of distilled water was introduced, and the solution was stirred again. The resulting mixture was then titrated with 0.01 N sodium thiosulfate solution until a light yellow color appeared. To enhance the endpoint detection, 0.5 mL of 1% starch solution was added as an indicator, which imparted a blue color to the solution. The titration was continued until the blue color disappeared, indicating the completion of the reaction. The peroxide value was calculated using Equation (2):

Equation (2)

$$\text{Peroxide Value}(\text{meq}O_2/\text{Kg}) = (S \times M \times 1000)/m$$

Where, S is volume of sodium thiosulfate used (mL), M: Molarity of sodium thiosulfate and m: Mass of the sample (g) [18].

The induction period (IP) defined as the "time required for the peroxide value to reach 20 meqO_2/kg oil at 70°C was also determined. Moreover, the protection factor (PF) was calculated to assess the antioxidant efficacy of the samples [19].

2-4-2- Determination of Thiobarbituric Acid (TBA) Value

The thiobarbituric acid (TBA) value, an indicator of secondary oxidation products, was determined using the AOCS Cd 19-90 method. To prepare the TBA reagent solution, 200 mg of thiobarbituric acid powder was dissolved in 60 mL of butanol by continuous stirring on a magnetic stirrer for 3 hours. The solution was then transferred to a 100 mL volumetric flask and diluted to volume with butanol. For the assay, 200 mg of the oil sample was weighed into a 25 mL volumetric flask and diluted to volume with butanol, followed by thorough mixing to ensure complete dissolution. A 5 mL aliquot of the sample solution was then mixed with 5 mL of the TBA reagent and stirred well. The mixture was incubated in a water bath at

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95°C for 2 hours. After incubation, the samples were cooled to room temperature, and the absorbance of the solution was measured at 530 nm using a spectrophotometer against a blank solution (containing only solvent and reagent). The TBA value was calculated using Equation (3):

Equation (3) $TBA = 50 \times M(A-B)$

Where, TBA is thiobarbituric acid value (as mg malondialdehyde per kg of oil), A: Absorbance of the sample, B: Absorbance of the blank and M: Sample weight (mg) [18].

2-5- Data Analysis

Statistical analysis was performed using SPSS software, and mean comparisons were conducted using Duncan's test at a 5% significance level. Graphs were generated using appropriate software.

3- Results and Discussion

3-1- Antioxidant Properties of Milk Thistle Extract

3-1-1- Total Phenolic and Flavonoid Content of Milk Thistle Extract

The results of total phenolic and flavonoid content measurements in the extract obtained from the defatted milk thistle seed flour are presented in Table (1). The phenolic and flavonoid compounds in milk thistle seeds depend on the plant variety, type of extract (aqueous or other solvents), plant part (root, stem, leaf, or seed), as well as the concentration and purification methods used. Javid et al. (2022) investigated the phenolic and flavonoid content of different parts of the milk thistle plant using a colorimetric method based on the Folin–Ciocalteu reagent and aluminum chloride. Their study reported that the total phenolic content of extracts from leaves, stems, and seeds were (21.79), (17.29), and (1.70) mg gallic acid/g, respectively, while their flavonoid content was (129.66), (114.29), and (24.79) mg quercetin/g, respectively [20]. Similarly, Mohammadi et al. (2016) reported the phenolic content of milk thistle as (29.00) mg gallic acid/g and the flavonoid content as (3.39) mg quercetin/g. The differences between these findings and the results of the present study may be attributed to factors such as plant variety, cultivation conditions, and extraction methods employed [21].

Table 1- The total phenol and flavonoid contents of the whole extract obtained from the defatted flour of milk thistle seeds

Compound	Amount
Total phenols (mg gallic acid/g)	34.40±1.85
Total Flavonoids (mg of quercetin/g)	26.20±1.30

Data are mean of three replicates ± SD

3-1-2- DPPH Free Radical Scavenging Activity

The DPPH free radical scavenging ability is one of the most well-known mechanisms by which antioxidant compounds can inhibit lipid oxidation. In this method, the results are expressed as the percentage reduction in absorbance of DPPH solutions in the presence of the extract compared to the DPPH solution without the extract [22]. The antioxidant capacity of different concentrations of milk thistle seed extract based on its ability to scavenge DPPH free radicals is shown in Figure 1. According to the results, increasing the concentration of milk thistle seed extract enhanced its DPPH free radical scavenging ability. However, at all concentrations, except for the sample containing 150 µg/mL extract, the DPPH radical scavenging ability was lower than that of the synthetic antioxidant BHT ($p < 0.05$).

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Overall, an increase in the concentration of phenolic compounds directly enhanced the extract's ability to scavenge free radicals ($p < 0.05$). At higher concentrations, due to the increased number of hydroxyl groups in the reaction environment, the probability of hydrogen donation to free radicals increased, thereby improving the extract's scavenging capacity [23].

Ahmadi et al. (2007) measured the radical scavenging activity of methanolic extract of wild celery at different concentrations and compared it with BHT, α-tocopherol, and ascorbic acid. Their results showed that increasing the extract concentration enhanced its DPPH radical scavenging ability, which aligns with the findings of the present study [24]. Similarly, Sarseh et al. (2016) examined the DPPH radical scavenging ability of milk thistle seed extract in comparison with the synthetic antioxidants BHA and BHT. Their findings demonstrated that as the concentration increased, the inhibitory power of the extract also increased [25]. The EC_{50} value of the milk thistle seed extract was 62.7 µg/mL, whereas that of BHT was 51.5 µg/mL, indicating that the milk thistle extract had a lower free radical scavenging ability compared to the synthetic antioxidant.

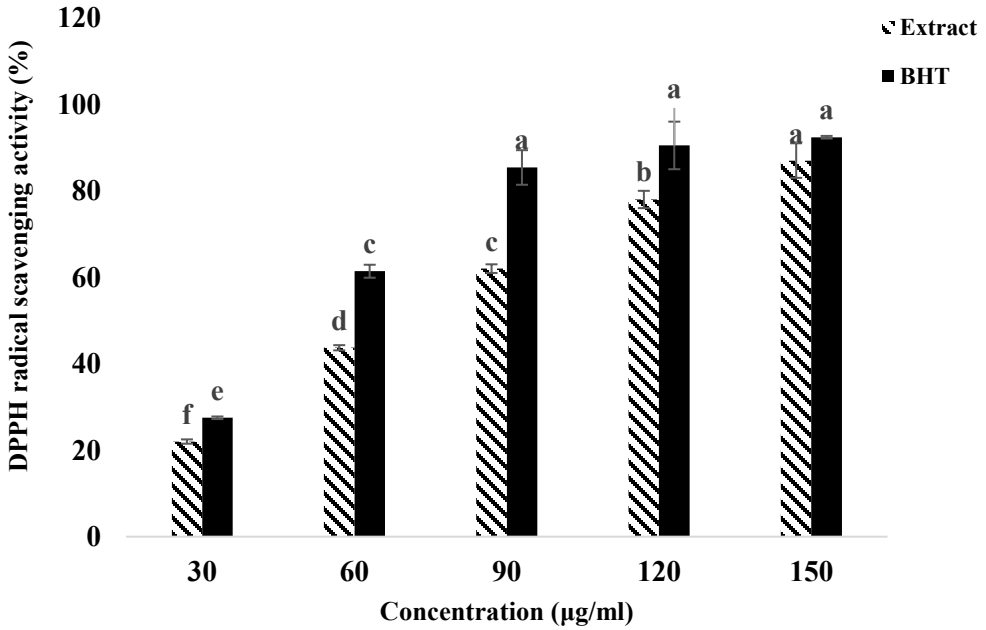

Figure 1- DPPH free radical scavenging activity (%) of different concentrations of milk thistle seed extract compared to BHT

3-1-3- Total Antioxidant Capacity

This method is based on the reduction of molybdenum (VI) to molybdenum (V) in an acidic environment and at high temperatures. This reaction results in the formation of green phosphomolybdenum complexes, which exhibit maximum absorbance at 695 nm. These complexes are highly stable and are not affected by the solvent used for extracting phenolic compounds. Extracts with higher absorbance at this wavelength exhibit greater antioxidant capacity [16]. The antioxidant capacity of different concentrations of milk

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thistle seed extract, based on total antioxidant capacity (absorbance at 695 nm), is shown in Figure 2. According to the results, increasing the concentration of milk thistle seed extract led to an increase in total antioxidant capacity. However, at all tested concentrations, the total antioxidant capacity of the extract was lower than that of the synthetic antioxidant BHT ($p < 0.05$). The upward trend in antioxidant capacity with increasing extract concentration can be attributed to the higher levels of phenolic compounds in the extract. .Kumaran and Karunakaran (2007) reported a positive correlation between the total phenolic content in different varieties of Phyllanthus and their antioxidant capacity [26]. Similarly, Arabshahi and Urooj (2006) demonstrated a direct relationship between the phenolic content of various mulberry leaf extracts and their antioxidant capacity, which aligns with the findings of this study [27]. The EC_{50} value of milk thistle seed extract was 132.8 µg/mL, while that of BHT was 112.5 µg/mL, indicating that the milk thistle extract had a lower antioxidant capacity compared to the synthetic antioxidant.

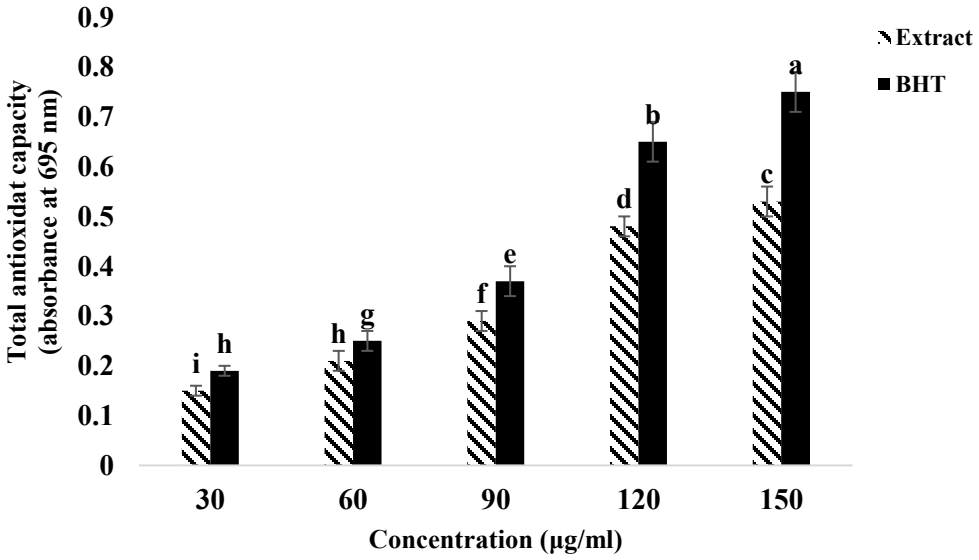

Figure 2- Total antioxidant capacity of different concentrations of milk thistle seed extract compared to BHT

3-1-4- Iron (III) Reducing Power

This method evaluates the ability of extracts to reduce ferric iron (Fe^{3+}) and convert it to ferrous iron (Fe^{2+}). The presence of reducing agents (antioxidants) leads to the reduction of ferricyanide complexes and their conversion into the ferrous form, which is accompanied by a color change in the solution from yellow to various shades of green and blue, depending on the reducing capacity of the extracts being tested [28]. The antioxidant capacity of different concentrations of milk thistle seed extract, based on the reductive power of iron ions (absorption at 700 nm), is shown in Figure 3. According to the results, as the concentration of milk thistle seed extract increased, the reductive power of iron ions also increased. In all concentrations, the reductive power of iron ions in the extract was lower than that of the synthetic antioxidant BHT. The findings from other researchers confirm a correlation between the phenolic compounds in the extract and its reducing power. Nghi et al. (2005), in their study of the reducing power of methanolic, chloroformic, ethyl acetate, and acetone

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extracts of *Ziziphus jujuba* at concentrations ranging from 0.48 to 4.8 mg/mL, reported that the absorbance of the extracts increased with concentration [29]. Oliveira et al. (2008) examined the phenolic compounds and reductive power of iron ions in aqueous extracts from the green husks of five walnut varieties and found that extracts containing higher amounts of phenolic compounds had lower EC50 values [30]. In another study, a high correlation was observed between the phenolic content of cereals and their reductive power of iron ions, which was consistent with the results obtained in this study [31]. The EC50 of milk thistle seed extract was 82.3 µg/mL, while for BHT, it was 70.8 µg/mL, indicating that the milk thistle extract was less effective than the synthetic antioxidant in reducing iron ions. Aziz et al. (2021) reported the reductive power of iron ions in different varieties of milk thistle available in Pakistan, ranging from 9.73 to 17.69 mg/g dry weight [32].

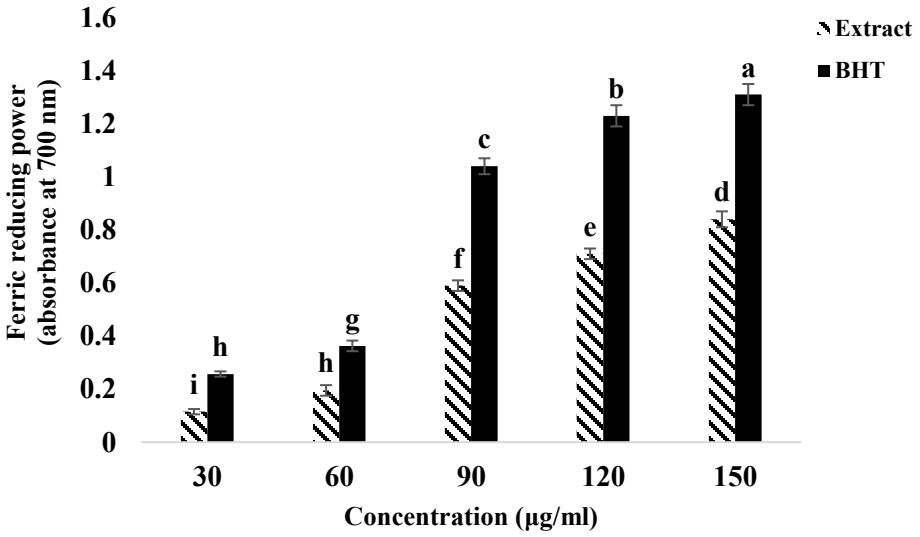

Figure 3- Ferric reducing power of different concentrations of milk thistle seed extract compared to BHT

3-2- Antioxidant Activity of Milk Thistle Extract in Soybean Oil

3-2-1- Peroxide Value

The peroxide value is recognized as an indicator of primary oxidation products, namely hydroperoxides, and serves as an appropriate marker for oxidative changes during the early stages of oil storage. The results of assessing the antioxidant activity of milk thistle extract at various concentrations, compared to the synthetic antioxidant, BHT and the control sample based on peroxide value are presented in Figure 4. Soybean oil, due to its high content of unsaturated fatty acids such as linolenic acid and linoleic acid, is prone to oxidation. Like other oils, it oxidizes more rapidly when exposed to high temperatures, light, oxygen, and metal ions. In this study, the peroxide value of all samples gradually increased with prolonged storage at 70°C ($p<0.05$). The increase in peroxide value is due to the formation of primary oxidation products, namely hydroperoxides. In the early days of the experiment, the rate of formation of these products was low, but after the sixth day, the rate increased more rapidly ($p<0.05$). Thus, it was observed that at the later stages of the experiment, the rate of formation of primary oxidation products slowed down. It is likely that some of the hydroperoxides formed

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during the propagation stage began to decompose and convert into secondary oxidation products such as aldehydes and ketones. The significant increase in the thiobarbituric acid value in the later days of the experiment confirms the observed reduction in the rate of hydroperoxide formation. Hydroperoxides decompose at high temperatures, generating more free radicals, and continue chain reactions. In some cases, stable non-radical products such as aldehydes, ketones, alcohols, and acids are produced from the decomposition of these compounds [33]. Antioxidants, at this stage, break the chain reactions of oxidation by donating electrons to free radicals and prevent the decomposition of peroxides into stable and harmful products through reactions with alkoxy radicals (RO°) [34]. The increase in the antioxidant power of phenolic compounds as a result of higher concentrations can be attributed to an increase in the number of active sites of these compounds to react with free radicals. The results obtained for oil oxidation inhibition are consistent with the results of free radical scavenging tests, reduction, and total antioxidant capacity, as in other methods of antioxidant activity evaluation, the increasing concentration of extracts led to enhanced reduction power and their ability to donate electrons to free radicals [3, 35]. The results of this section align with the findings of Ahmadi et al. (2025) and Arabshahi et al. (2011) [36, 19].

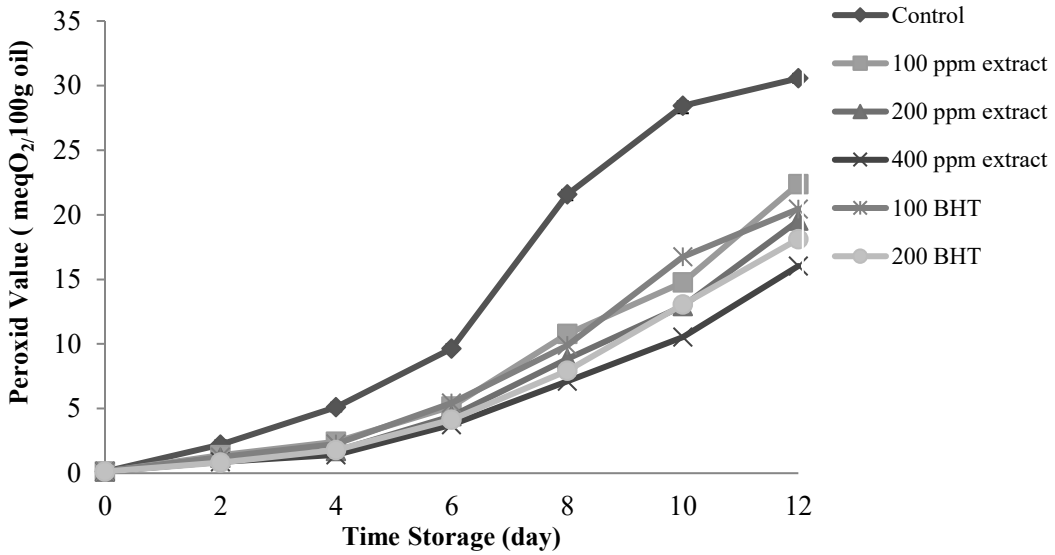

Figure 4- Effect of different concentrations of milk thistle seed extract on the peroxide value of soybean oil during accelerated storage (70°C, 12 days) compared to BHT

Table 2 shows the induction time (the time required for the peroxide value of the sample to reach meqO2/Kg oil 20) as well as the protective factor (PF) for various oil samples. As observed, the addition of milk thistle seed extract and synthetic antioxidants led to an increase in the induction time, indicating the ability of these compounds to delay the onset of oil deterioration and protect it against oxidation. Based on the protective factor, the highest antioxidant activity in the oil was observed at a concentration of 400 ppm of milk thistle extract, while the lowest was at a concentration of 100 ppm of the extract and BHT.

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Table 2- Effect of milk thistle extract and BHT on the stability of soybean oil expressed as induction period (IP) and protection factor (PF) determined by peroxide value

Additive	IP	PF(IP_{sample}/IP_{oil})
None	1.80	0.00
Milk Thistle extract (100 ppm)	2.60	1.45
Milk Thistle extract (200 ppm)	2.87	1.59
Milk Thistle extract (400 ppm)	3.15	1.75
BHT (100 ppm)	2.60	1.45
BHT (200 ppm)	2.97	1.65

3-2-2- Thiobarbituric Acid Value

The results of assessing the antioxidant capability of milk thistle extract at various concentrations, compared to the synthetic antioxidant, BHT and the control sample based on the thiobarbituric acid value are shown in Figure 5. The results indicated that with increased storage time, the thiobarbituric acid index increased in all samples ($p<0.05$), but this trend was less pronounced with higher concentrations of extract in soybean oil ($p<0.05$). The lowest thiobarbituric acid value at the end of the storage period was found in the sample containing 400 ppm of extract, which was even lower than the sample containing 200 ppm of BHT ($p<0.05$). Malondialdehyde is formed as a result of the autoxidation of polyunsaturated fatty acids with two or more double bonds, and this compound is used in very low concentrations as an indicator of fat oxidation. The oxidation products of unsaturated fats form a red complex with thiobarbituric acid, which has a strong absorbance at a wavelength of 532-535 nm. This index reflects the secondary stages of fat oxidation and the presence of secondary oxidation compounds in the sample. Therefore, a higher thiobarbituric acid index in the oil indicates more oxidation and, consequently, lower stability of the oil [3, 35]. The further reduction in the thiobarbituric acid index in the oil sample containing milk thistle extract compared to the control (without antioxidant) can be attributed to the phenolic compounds present in the extract and, consequently, the antioxidant power of these compounds in preventing the decomposition of hydroperoxides. In line with the results of this section, Razghandi et al. (2024) and Ahmadi et al. (2025) also reported that with increasing extract concentration, the intensity of the increase in this index decreases [35, 36].

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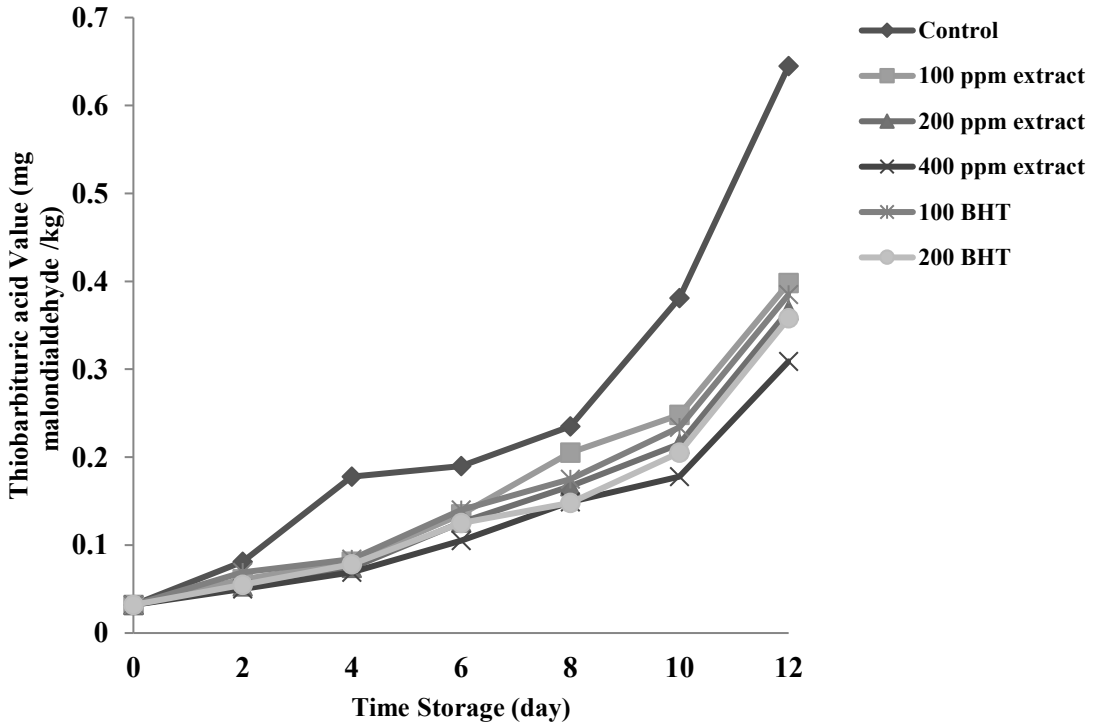

Figure 5- Effect of different concentrations of milk thistle seed extract on the thiobarbituric acid value of soybean oil during accelerated storage (70°C, 12 days) compared to BHT

4- Conclusion

The preservation of food against oxidation under various thermal conditions, such as regular cooking, frying, etc., requires antioxidants with high thermal stability. The extract obtained from milk thistle seeds contains significant amounts of phenolic and flavonoid compounds and exhibits desirable antioxidant properties. Adding this extract to soybean oil results in a delay in oil oxidation, with a concentration of 400 ppm of the extract showing greater effectiveness than BHT in preventing soybean oil oxidation. The notable antioxidant activity of milk thistle seed extract in soybean oil also demonstrates the stability of its antioxidant compounds under heat. Therefore, it can be concluded that milk thistle seed extract has high potential for use as a natural antioxidant in oils and oil-containing foods.

5- References

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تاثیر عصاره اتانولی دانه خار مریم (*Silybum marianum*) استخراج شده با کمک فراصوت بر پایداری اکسایشی روغن سویا

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چکیده

با توجه به نگرانی‌های بهداشتی اخیر مرتبط با آنتی اکسیدان‌های سنتزی، تحقیقات زیادی در راستای استفاده از آنتی اکسیدان‌های طبیعی به‌عنوان نگهدارنده در مواد غذایی انجام شده است. در همین راستا، تحقیق حاضر با هدف ارزیابی تاثیر عصاره استخراج شده از دانه گیاه خار مریم بر پایداری اکسایشی روغن سویا صورت گرفت. به این منظور، پس از عصاره‌گیری دانه خارمریم با حلال اتانول و به کمک فراصوت و بررسی برخی ویژگی‌های آنتی اکسیدانی آن، عصاره حاصله در غلظت‌های مختلف (۱۰۰، ۲۰۰ و ۴۰۰ پی‌پی‌ام) به روغن سویای پالایش شده فاقد آنتی‌اکسیدان افزوده شد و سپس عدد پراکسید و شاخص تیوباربیتوریک اسید نمونه‌های روغن در طول نگهداری در شرایط اکسیداسیون تسریع شده (دمای ۷۰ درجه سانتی‌گراد، ۱۲ روز) اندازه گیری شد. نتایج نشان داد که میزان ترکیبات فنولی و فلاونوئیدی کل در عصاره خار مریم به‌ترتیب برابر با ۳۴/۴ میلی‌گرم (معادل گالیک اسید) و ۲۶/۲ میلی‌گرم (معادل کوئرستین) در گرم ماده خشک بود. در بررسی ویژگی‌های آنتی‌اکسیدانی عصاره با سه روش توانایی مهار رادیکال آزاد ۲،۲-دی فنیل،۱-پیکریل هیدرازیل، ظرفیت آنتی‌اکسیدانی کل و قدرت احیاکنندگی یون آهن مشخص گردید که با افزایش غلظت عصاره از ۳۰ تا ۱۵۰ میکروگرم قابلیت آنتی‌اکسیدانی عصاره افزایش یافت. نتایج ارزیابی قابلیت آنتی اکسیدانی عصاره خار مریم در غلظت‌های مختلف در مقایسه با آنتی‌اکسیدان سنتزی بوتیلیتد هیدروکسی تولوئن و نمونه شاهد در روغن سویا نشان داد که با افزایش زمان نگهداری، میزان عدد پراکسید و تیوباربیتوریک اسید در تمامی نمونه‌ها افزایش یافت ولی با افزایش غلظت عصاره خارمریم در روغن سویا از شدت این افزایش کاسته شد. در نهایت این مطالعه نشان داد که افزودن ۴۰۰ پی‌پی‌ام عصاره آنتی‌اکسیدانی دانه خارمریم منجر به کاهش بیشتری در سرعت اکسیداسیون روغن سویا در مقایسه با آنتی اکسیدان سنتزی می‌شود.