

Viability of Starter Bacteria and Anti-Oxidative Activity of a Functional Yogurt Containing *Silybum marianum* Seed Extract

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Abstract

Background and objective: *Silybum marianum* seed extract (silymarin) is rich in phenolic compounds with anti-oxidative activity that add beneficial and healthful properties to silymarin-enriched products. The present study investigated effects of silymarin on characteristics of a functional yogurt.

Material and methods: In this study, yogurt was enriched with *Silybum marianum* seed extract at concentrations of 0, 25, 50 and 100 mg l⁻¹ milk and the samples were analyzed for physicochemical and sensory properties and viability of starter bacteria during 21 days at 4°C at 7-day intervals.

Results and conclusion: Results showed that increasing silymarin proportion in yogurt samples increased anti-oxidative activity, total phenolic content, total viability of *Lactobacillus delbrueckii* and decreased pH value, viscosity and sensory attributes (p<0.05). Furthermore, pH, viscosity, anti-oxidative activity, phenolic compounds and sensory attributes decreased during storage (P<0.05). In conclusion, 25 mg *Silybum marianum* seed extract per one liter of milk can be used for the preparation of yogurts with healthy properties.

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1. Introduction

Milk thistle (*Silybum (S.) marianum*) is an herbal plant with important medicinal and nutritional uses. Plant is spread throughout the Mediterranean, Middle East, United States and Europe [1]. In Iran, milk thistle is found in northwestern and eastern regions, Kermanshah, Shoosh, Hamidieh, Ramhormoz, Kazeroun, Bushehr and Borazjan [2]. According to Qavami et al. [3], Goli et al. [4], Hadolin et al. [5] and Khan et al. [6], the plant seeds contain high oil levels (nearly 23%) with linoleic acid as a nutritionally important essential fatty acid and high quantities of unsaturated fatty acids. The milk thistle extract, known as silymarin, forms 60% of the plant dry extract [7]. The medicinal and biological properties of the plant seeds have been attributed to their high contents of flavonoids as active ingredients in the plant seeds [3].

Health advantages of silymarin are largely related to flavanolignan constituents including silybinin, isosilybinin, silydianin, silychristin and dihydroflavonol of taxifolin. Seeds and leaves of milk thistle have been used in treatment of liver diseases for over 2000 years [8]. Several medicinal and therapeutic properties of silymarin have been reported, including liver protection [9-12], cholesterol lowering [13], possible laminitis prevention [14], anti-oxidative properties [11,15-19] and of diseases prevention of free radicals. Study results by Juodeikiene et al. [20] suggested that fermented seeds of milk thistle can be used as a good additive to naturally flavor cooked products. Several clinical trials have reported safe and effective oral daily doses of silymarin, ranging from 280 to 800 mg [21].

The concept of functional foods refers to the role of foods in maintaining health and preventing diseases. This has led to increased attentions to healthy foods in scientific and commercial fields [22]. These foods can be prepared by adding herbal ingredients to foods. However, highly consumed fermented products such as yogurt can be enriched with silymarin to enjoy health benefits of this plant. No data have been published on antibacterial effects of silymarin on starter cultures in fermented dairy products.

So, effects of the *S. marianum* seed extract at concentrations of 0, 25, 50 and 100 mg l⁻¹ milk on selective physicochemical properties and viability of yogurt starter were investigated in this study within 21 days of storage at 4°C.

2. Materials and methods

2-1-Material

Raw milk was purchased from Tehran Pegah Dairy, Iran, yogurt starter culture (CH1) from Chr. Hansen (Denmark), Skim milk powder from Aria Rama, Iran and Folin-Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent and monohydrate gallic acid from Sigma-Aldrich, Germany. Other chemicals were purchased from Merck, Germany.

2-2- Preparation of the milk thistle seed extract

Milk thistle seeds provided by the Institute of Medicinal Plants, Karaj, Iran, were frozen at 0°C for 24 h. Seeds (200 g) were powdered and defatted using petroleum ether and Soxhlet apparatus. Then, defatted seed powder was added to hydroalcoholic solvent (80%) and soaked for 24 h. Solution was filtrated and the procedure was repeated for two times. Filtrate was mixed well and the solvent was evaporated using rotary evaporator machine. The residue was collected and dried by incubation at 37°C and then was powdered and preserved in dark containers for further use [23].

2-3- Yogurt production

Cow milk samples enriched with silymarin (0, 25, 50 and 100 mg l⁻¹) were homogenized, pasteurized, cooled and inoculated with starter cultures. During the fermentation, pH was monitored until reached 4.5 ± 0.02. Then, samples were transferred into 250-g containers and stored at 4°C for 21 days.

2-4- Analytical methods

The pH value of yogurts was measured using pH meter (Jenway, UK) according to according to Tomovska et al.

Method [24]. Viscosity was measured using viscometer (DV-II, Brookfield, USA) and Cinbas and Yazici method [25]. Total phenolic content was estimated using Folin-Ciocalteu reagent [26] and gallic acid as a standard. Calculation of radical scavenging activity was carried out using DPPH method [27]. Viabilities of *Lactobacillus* (*L.*) *delbrueckii* and *Streptococcus* (*S.*) *thermophilus* were assessed By Dave and Shah method [28]. Five trained panelists were asked to score yogurt samples using 5-point hedonic scale for flavor, mouthfeel, appearance (color and syneresis) and non-oral texture and standard questionnaires. Yogurt samples were analyzed immediately after production and after 7, 14 and 21 days of storage at 4°C.

2-5-Statistical analysis

Experiments were carried out in triplicate and set up using completely randomized factorial design. Means of the experimental treatments were compared to each other using the least squares test. Data were analyzed using SAS Software v.9.2. The level of significance was set at P≤0.05.

3. Results and discussion

3-1- pH

Results of the sample pH revealed significant differences between the treatments (P<0.05) (Table 1). Sample pH decreased by increasing silymarin. A reason for decreasing pH of the samples with increased silymarin on Day 1 can be attributed to increased *L. bulgaricus* and *S. thermophilus* viabilities and hence to increased production of acids. It is noteworthy that pH of silymarin is normally 6.8. However, results showed no significant differences between the pH values of samples on Day 21 (P>0.05). In all treatments, pH decreased by increasing the storage time. The decreasing trend of pH in various days can be attributed to the fact that starter bacteria in yogurt decrease pH by fermentation of lactose and production of lactic acid. A major problem with fermented products, such as yogurt, is that the quantity of acid increases during storage time due to activation of β-galactosidase enzyme at 0-5°C. Therefore, pH may reach to less than 4.2, resulting in yogurt serum separation and hence affecting viability of lactic acid bacteria due to increase hydrogen ions rather than lactate ions [29]. Acid produced by the bacteria increases acidity in products. The current results of decreases in yogurt pH during storage are similar to previous results [22,30-33].

Table 1. The pH changes in yogurt samples containing silymarin during cold storage*

Treatment	Day 1	Day 7	Day 14	Day 21
T0	4.64 ^a ± 0.05	4.53 ^b ± 0.05	4.15 ^{ef} ± 0.05	4.09 ^{hi} ± 0.05
T1	4.52 ^b ± 0.06	4.33 ^c ± 0.06	4.13 ^{fg} ± 0.06	4.08 ^{hi} ± 0.06
T2	4.23 ^d ± 0.04	4.23 ^d ± 0.07	4.11 ^{gh} ± 0.04	4.07 ⁱ ± 0.06
T3	4.21 ^d ± 0.07	4.23 ^e ± 0.07	4.11 ^{gh} ± 0.04	4.07 ⁱ ± 0.06

*Means with different superscripts differ significantly (p<0.05).

T0, 0 mg *Silybum marianum* seed extract l⁻¹ milk; T1, 25 mg. *Silybum marianum* seed extract l⁻¹ milk; T2, 50 mg *Silybum marianum* seed extract l⁻¹ milk; T3, 100 mg *Silybum marianum* seed extract l⁻¹ milk

T0, 0 mg *S. marianum* seed extract l⁻¹ milk; T1, 25 mg *S. marianum* seed extract l⁻¹ milk; T2, 50 mg *S. marianum* seed extract l⁻¹ milk; T3, 100 mg *S. marianum* seed extract l⁻¹ milk

3-2- Viscosity

In addition to milk composition and dry matter quantity, temperature, heating time, starter type and storage conditions are factors that affect rheological properties of the final products [34]. In the present study, the lowest and the highest viscosity values were reported in T3 and T0 treatments, respectively (Table 2).

Decreases in viscosity with increases in silymarin can be associated to decreases in the product pH, resulting in ultimate watery yogurts. Decreases in pH cause contraction of the casein network [35]. These results did not correlate with those by Ayar and Gurlin [36], who showed that the viscosity of flavored yogurts increased after increasing the proportion of fruit and plant extracts. This possibly occurred because solid and water absorbing compounds were high in fruit and the plant extracts.

Results showed that viscosity decreased by increasing the storage time and the highest viscosity belonged to samples from Day 1. Decline in viscosity over time is due to decreases in water holding capacity of the products. Comparison of the sample viscosity means of Days 1 and 7 demonstrated no significant differences between the samples. This time was exactly the time, when water holding capacity in samples increased and the syneresis effect was at its lowest rate (unreported data). With losses in water and water holding capacity, viscosity decreased more rapidly and thus statistically significant differences were observed in all samples. No significant differences were seen between T2 and T3 samples within all days. Similarly, Lee and Lucey [37] observed that viscosity of yogurts decreased during storage.

3-3- Total phenolic content and anti-oxidative activity

Phenolic compounds include a group of plant aromatic metabolites widely spread throughout plants and include beneficial effects such as anti-oxidative and anti-bacterial activities [38]. Silymarin flavonoids are known as potent antioxidants and free radical scavengers [15-19]. Since

silymarin contains high quantities of phenolic compounds, anti-oxidative activity of these compounds increased in the samples by increasing the extract proportion (Tables 3 and 4), resulting in a significantly higher anti-oxidative activity in silymarin-containing yogurt samples, compared to that in plain yogurt samples. In plain yogurts, catalase, super-oxidase, casein and serum proteins include anti-oxidative activities [39-40].

During storage, phenolic compounds decrease in the samples, resulting in decreases in their anti-oxidative activities. Therefore, Total phenolic content and antioxidant activity of cheese samples decreased with increased storage time. This could contribute to degradation of polymeric phenolic compounds in presence of lactic acid bacteria [41-43] and increase of interactions between the milk proteins and polyphenols, lowering free hydroxyls during storage [41, 44-50] as a part of the total antioxidant capacity was masked by the interactions. The lowest anti-oxidative activity was observed on Day 21 (Table 3).

Factors such as increased storage time and temperature decrease anti-oxidative activity [51-52]. Klimczak et al. [51] showed that anti-oxidative activity decreased due to decreases in polyphenols and vitamin C over time. Changes in phenolic compounds in control samples were not statistically significant and were almost constant. The lowest level of phenolic compounds was seen on Days 14 and 21 in Sample T1 with no significant differences with controls at these two times (Table 3).

According to Gad et al. [53], quantity of the phenolic compounds in yogurt with 10% palm extract was more than that in plain yoghurt, with significant decreases during storage. Furthermore, Zainoldin and Baba [54] reported that addition of 10% of *Hylocereus polyrhizus* extract containing phenolic compounds to yogurt significantly improved phenolic properties of the yogurt, compared to plain yogurt. Similarly, functional yogurt containing 10% of acai extract showed a higher anti-oxidative activity compared to that plain yogurt did [55].

Table 2. Viscosity (Pa.S) changes in yogurt samples containing silymarin during cold storage*

Treatment	Day 1	Day 7	Day 14	Day 21
T0	11.36 ^a ± 0.63	10.47 ^b ± 1.00	9.83 ^{cd} ± 1.96	9.48 ^{de} ± 0.16
T1	10.96 ^a ± 0.42	10.29 ^{bc} ± 2.31	9.57 ^{de} ± 1.40	9.22 ^{ef} ± 0.16
T2	9.68 ^{de} ± 0.57	8.99 ^{fg} ± 2.06	8.66 ^g ± 1.15	6.99 ^h ± 0.9
T3	7.06 ^h ± 0.79	6.72 ^{hi} ± 1.31	6.65 ^{hi} ± 1.91	6.27 ⁱ ± 0.13

*Means with different superscripts differ significantly ($p < 0.05$).

T0, 0 mg *Silybum marianum* seed extract l⁻¹ milk; T1, 25 mg. *Silybum marianum* seed extract l⁻¹ milk; T2, 50 mg *Silybum marianum* seed extract l⁻¹ milk; T3, 100 mg *Silybum marianum* seed extract l⁻¹ milk

Table 3. Total phenolic content (mg gallic acid g⁻¹ sample) and antioxidant activity (% of DPPH radical scavenging activity) changes in yogurt samples containing silymarin during cold storage*

Characteristics	Treatment	Day 1	Day 7	Day 14	Day 21
Total phenolic content	T0	32.69 ⁱ ± 0.21	32 ⁱ ± 0.05	32.11 ⁱ ± 0.18	3107 ⁱ ± 0.07
	T1	49.5 ^d ± 0.14	38.96 ^g ± 0.13	32.2 ⁱ ± 0.13	31.13 ⁱ ± 0.07
	T2	54.96 ^b ± 0.19	47.78 ^e ± 0.11	39.05 ^g ± 0.11	35.09 ^h ± 0.04
	T3	58.26 ^a ± 0.27	51.96 ^c ± 0.07	41.33 ^f ± 0.18	37.45 ^g ± 0.06
Antioxidant activity	T0	7.413 ⁱ ± 0.10	7.397 ⁱ ± 0.19	6.968 ⁱ ± 0.55	6.16 ⁱ ± 0.75
	T1	12.61 ^d ± 0.07	12.065 ^g ± 0.47	11.076 ⁱ ± 0.39	8.26 ⁱ ± 0.35
	T2	14.342 ^b ± 0.09	13.884 ± 0.39	12.487 ^g ± 0.32	8.723 ^h ± 0.40
	T3	17.449 ^a ± 0.13	16.956 ^c ± 0.25	14.71 ^f ± 0.54	9.189 ^g ± 0.6

*Means with different superscripts differ significantly ($p < 0.05$).

T0, 0 mg *Silybum marianum* seed extract l⁻¹ milk; T1, 25 mg. *Silybum marianum* seed extract l⁻¹ milk; T2, 50 mg *Silybum marianum* seed extract l⁻¹ milk; T3, 100 mg *Silybum marianum* seed extract l⁻¹ milk

3-4- Viability of starter bacteria

Results of *L. bulgaricus* viability in yogurt samples (Table 4) showed no significant differences ($P > 0.05$). Despite antibacterial properties of the phenolic compounds in silymarin in present study, silymarin did not show antibacterial activities in growth of *L. delbrueckii*. However, bacterial starter was more viable in samples with higher levels of silymarin. Moreover, differences between viabilities of *L. delbrueckii* in samples were significantly lower after 21 days of storage, compared to those after 7 days of storage ($P < 0.05$). This effect might be due to lactose fermentation and pH reduction leading to an unfavorable environment for the bacterial growth. Other studies have shown that decreased nutrients, increased acidity and oxygen content, competing microorganisms, bacteriocin compounds, antibiotics and fermentation are the most important reasons for the decrease of probiotics [56-57].

According to Donkor et al. [58], *Lactobacillus* species include good cellular stabilities to preserve their concentration through the storage. Zahedi et al. [59] studied viability of starter bacteria in yogurts enriched with orange peel oils and flavonoid extracts and reported a partial role of flavonoids in growth of *L. bulgaricus*. In the present study, viability of *S. thermophilus* in yogurt samples included no significant differences ($P > 0.05$) (Table 4). These results

revealed that silymarin did not directly affect viability of *S. thermophilus*. Regarding mechanisms of action, *Lactobacillus* species include proteolytic activity, degrading proteins such as casein to amino acids such as valine, which act as activators of *S. thermophilus*. Since *S. thermophilus* activates after *L. delbrueckii* acid production and lactose reduction, anti-oxidative activity of silymarin seemed to greatly decrease at this time with no particular effects on *S. thermophilus*.

A reason for increasing *S. thermophilus* on Day 21 could be linked to decreasing activity of *S. thermophilus*. Zahedi et al. [59] studied viability of starter bacteria in yogurt enriched with orange peel oil and its flavonoid extract. They reported that *S. thermophilus* included the highest viability rate in oil-free samples containing 7% of flavonoids during a 15-day study period, while the lowest rate belonged to samples containing 1.5% of oil and 9% of flavonoids. Various results are reported in literatures, regarding viability of the yogurt starter bacteria. For example, Dave and Shah [60] reported that the number of starter bacteria within 28 days of cold storage increased and then decreased with the number reached the lowest on the last day. Similarly, Li et al. [61] reported a gradual decrease in number of starter bacteria in vegetable oil-enriched yogurts at the end of Day 15.

Table 4. *Lactobacillus bulgaricus* and *Streptococcus thermophilus* viabilities (log CFU g⁻¹) of yogurt samples containing silymarin during cold storage*

Characteristics	Treatment	Day 1	Day 7	Day 14	Day 21
<i>Lactobacillus bulgaricus</i>	T0	6.22 ^{ab} ± 0.08	6.19 ^b ± 0.03	6.02 ^{cd} ± 0.12	5.67 ^f ± 0.31
	T1	6.31 ^{ab} ± 0.06	6.25 ^b ± 0.06	6.025 ^{cd} ± 0.08	5.91 ^e ± 0.28
	T2	6.32 ^{ab} ± 0.08	6.24 ^b ± 0.05	6.13 ^c ± 0.07	5.97 ^e ± 0.16
	T3	6.35 ^a ± 0.11	6.24 ^b ± 0.03	6.08 ^c ± 0.11	5.97 ^e ± 0.24
<i>Streptococcus thermophilus</i>	T0	8.58 ^{ab} ± 0.08	8.65 ^{ab} ± 0.03	8.7 ^a ± 0.08	8.77 ^a ± 0.10
	T1	8.64 ^{ab} ± 0.05	8.68 ^{ab} ± 0.08	8.72 ^a ± 0.08	8.81 ^a ± 0.07
	T2	8.67 ^{ab} ± 0.07	8.71 ^{ab} ± 0.07	8.75 ^a ± 0.04	8.83 ^a ± 0.06
	T3	8.7 ^a ± 0.10	8.73 ^a ± 0.04	8.79 ^b ± 0.06	8.88 ^a ± 0.10

*Means with different superscripts differ significantly ($P < 0.05$).

T0, 0 mg *Silybum marianum* seed extract l⁻¹ milk; T1, 25 mg. *Silybum marianum* seed extract l⁻¹ milk; T2, 50 mg *Silybum marianum* seed extract l⁻¹ milk; T3, 100 mg *Silybum marianum* seed extract l⁻¹ milk

3-5- Sensory evaluation

Of yogurt samples containing *S. marianum* seed extract, T1 received the highest scores for all sensory attributes (Table 5). Sensory scores in all samples decreased over time. The control sample (T0) received higher sensory scores than that other treatments did. This suggested that the *S. marianum* seed extract negatively affected sensory attributes of yogurts because the sample pH decreased by increasing the proportion of extract powder. Therefore, the yogurt flavor and syneresis were sourer and higher than those of the controls, respectively. Consuming yogurts containing the extract, panelists felt astringent flavors.

Moreover, primary color of the extract powder was yellow that undesirably affected color yogurts containing *S. marianum* seed extracts.

Overall, it can be concluded that the *S. marianum* seed extract includes negative effects on sensory attributes of yogurts. However, 25 mg *S. marianum* seed extract l⁻¹ milk can be used for the preparation of yogurts with healthy characteristics by masking the extract undesirable sensory attributes using other ingredients.

Table 5. Flavor changes in yogurt samples containing silymarin during cold storage*

Characteristics	Treatment	Day 1	Day 7	Day 14	Day 21
Flavor	T0	2.5 ^a ± 0.35	2.45 ^{ab} ± 0.27	2.17 ^{abc} ± 0.28	2.17 ^{abc} ± 0.18
	T1	2.39 ^{abc} ± 0.33	2.22 ^{abc} ± 0.45	2.11 ^{bc} ± 0.27	2.06 ^c ± 0.39
	T2	1.5 ^{de} ± 0.19	1.56 ^d ± 0.27	1.56 ^d ± 0.18	1.45 ^{de} ± 0.27
	T3	1.06 ^f ± 0.49	1.17 ^{ef} ± 0.55	1.06 ^f ± 0.25	1 ^f ± 0.21
Mouthfeel	T0	2.33 ^a ± 0.30	2.28 ^a ± 0.33 ⁱ	1.89 ^{bc} ± 0.27	1.78 ^c ± 0.34
	T1	2.28 ^a ± 0.33	2.17 ^{ab} ± 0.28	1.72 ^c ± 0.25	1.78 ^c ± 0.34
	T2	1.78 ^c ± 0.40	1.72 ^c ± 0.25	1.61 ^c ± 0.39	1.61 ^c ± 0.14
	T3	1.11 ^d ± 0.34	1.06 ^d ± 0.39	1.06 ^d ± 0.32	1.22 ^d ± 0.34
Appearance	T0	2.61 ^a ± 0.33	2.56 ^a ± 0.40	2.28 ^{abc} ± 0.349	1.78 ^{def} ± 0.17
	T1	2.33 ^{ab} ± 0.59	2.56 ^a ± 0.35	2.06 ^{bcd} ± 0.49	1.84 ^{cde} ± 0.18
	T2	1.67 ^{efg} ± 0.76	1.78 ^{def} ± 0.40	1.72 ^{defg} ± 0.61	1.5 ^{efgh} ± 0.19
	T3	1.33 ^{gh} ± 0.30	1.28 ^h ± 0.49	1.06 ^h ± 0.53	1.06 ^h ± 0.13
Non-oral texture	T0	2.56 ^a ± 0.40	2.67 ^a ± 0.37	2.33 ^{ab} ± 0.60	2 ^{bcd} ± 0.30
	T1	2.44 ^{ab} ± 0.35	2.56 ^a ± 0.27	2.22 ^{abc} ± 0.35	2 ^{bcd} ± 0.30
	T2	2.33 ^{ab} ± 0.42	2.45 ^{ab} ± 0.40	2 ^{bcd} ± 0.56	1.67 ^d ± 0.42
	T3	2.56 ^a ± 0.345	2.34 ^{ab} ± 0.37	1.7 ^{cd} ± 0.35	1.28 ^e ± 0.44

*Means with different superscripts differ significantly (P < 0.05).

T0, 0 mg *Silybum marianum* seed extract l⁻¹ milk; T1, 25 mg. *Silybum marianum* seed extract l⁻¹ milk; T2, 50 mg *Silybum marianum* seed extract l⁻¹ milk; T3, 100 mg *Silybummarianum* seed extract l⁻¹ milk

4. Conclusion

The current study investigated effects of the *S. marianum* seed extract on characteristics of a functional yogurt during storage at 4°C. This study showed that increasing proportion of the *S. marianum* seed extract increased antioxidant activity and total phenolic content, decreased viscosity and pH and improved viability of the yogurt starter bacteria. In general, the yogurt sample containing 25 mg *S. marianum* seed extract l⁻¹ milk can be used for the production of functional yogurts. The product can be scaled up using other ingredients that favor in masking unacceptable sensory attributes.

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6. Conflict of interest

The authors declare no conflict of interest.

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زنده‌مانی باکتری‌های آغازگر و فعالیت ضداکسایشی ماست فراسودمند حاوی عصاره دانه سیلیبوم ماریانوم

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چکیده

سابقه و هدف: عصاره دانه سیلیبوم ماریانوم (سیلیمارین) غنی از ترکیبات فنولی با فعالیت ضداکسایشی است که به فرآورده‌های غنی از آن خواص مفید و سالمی می‌بخشد. مطالعه حاضر اثرات سیلیمارین بر ویژگی‌های ماست فراسودمند را بررسی می‌کند.

مواد و روش‌ها: در این مطالعه، ماست با شیر حاوی عصاره دانه سیلیبوم ماریانوم در غلظت‌های ۰، ۲۵، ۵۰ و ۱۰۰ میلی گرم در لیتر تولید و غنی شد و نمونه‌ها از نظر خواص فیزیکوشیمیایی و حسی و زنده‌مانی باکتری‌های آغازگر در مدت ۲۱ روز نگهداری در دمای ۴ درجه سلسیوس در فواصل زمانی ۷ روز مورد بررسی قرار گرفتند.

یافته‌ها و نتیجه‌گیری: نتایج نشان داد با افزایش میزان سیلیمارین در نمونه‌های ماست، فعالیت ضداکسایشی، میزان ترکیبات فنولی کل، زنده‌مانی کلی لاکتوباسیلوس دلبروکی افزایش یافت، pH، گرانیروی و خواص حسی کاهش یافت ($p < 0/05$). علاوه بر این، pH، گرانیروی، فعالیت ضداکسایشی، ترکیبات فنولی و خواص حسی در مدت نگهداری کاهش یافتند ($p < 0/05$). نتیجه، شیر حاوی ۲۵ میلی گرم عصاره سیلیبوم ماریانوم می‌تواند برای تهیه ماست‌های دارای خواص سلامتی‌بخش مورد استفاده قرار گیرد.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافی مرتبط با انتشار این مقاله ندارند.

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