



## Scientific Research

# Investigation of physicochemical properties of ultra-filtered cheese packaging with nanocomposite film based on chia seed mucilage containing barberry extract and tin oxide nanoparticles

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## ABSTRACT

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In this study, the physicochemical and microbial properties of ultra-filtrated cheese packaged with biodegradable nanocomposite films based on chia seed mucilage containing barberry extract and tin oxide nanoparticles based on the central composite design were investigated. The aim of this research was to enhance the shelf life and sensory quality of this type of cheese through the use of edible films. The results showed that using the developed films significantly reduced the pH and increased the acidity of the samples during the storage period (15 and 30 days), which was attributed to the antimicrobial activity and phenolic compounds of the barberry extract and nanoparticles. Moisture analysis also revealed that films containing chia mucilage and active compounds significantly reduced moisture compared to the control sample, which was related to the hydrophilic nature of the mucilage and its interaction with phenolic compounds. Moreover, the use of these films reduced the extent of lipolysis and proteolysis in cheese samples, attributed to the restricted activity of proteolytic and lipolytic enzymes due to reduced moisture permeability and increased salt concentration. Microbial culture of the samples indicated the absence of growth of coliforms, molds, and yeasts in the optimal films for ultrafiltration packaging during the storage period. Sensory evaluation indicated that films containing barberry extract and tin oxide nanoparticles exhibited better overall acceptance in terms of color, texture, and aroma and prevented undesirable changes during storage. Additionally, these films improved mechanical strength and barrier properties, preventing oxygen penetration into the product, which resulted in reduced spoilage rates and better preservation of product quality.

## 1.Introduction

In recent decades, the food industry has experienced exponential growth in the use of packaging, driven by increasing consumer demand for products that are more durable, safer, and convenient. It is estimated that more than 40% of global packaging materials are used in the food sector, underscoring the critical role of packaging in the food value chain (1). Packaging plays a fundamental role in protecting food products, ensuring that their sensory and nutritional qualities are preserved until they reach the end consumer. This highlights the importance of developing new packaging solutions that not only address growing environmental concerns but also meet market demands (2). Historically, materials such as paperboard, paper, and plastic have been commonly used in food packaging. Although paperboard and paper are recyclable, they present limitations in terms of moisture and gas barrier properties, which are essential for preserving many types of food (3). Edible films are thin layers of biopolymeric substances that are placed on the surface or between components of food, acting as barriers to the transfer of substances such as fats and gases. These films protect the product from microbial growth and mechanical damage, while enhancing appearance, quality, and shelf life (4). Chia (*Salvia hispanica* L.) is a herbaceous plant from the Lamiaceae family, consisting of around 900 species. It is capable of growing in diverse regions including North, Central, and South America, Southeast Asia, South Africa, and Europe (5, 6). Chia seeds are rich in polyunsaturated fatty acids—mainly omega-3 (linolenic acid, 54–67%) and omega-6 (linoleic acid, 12–21%)—while containing low levels of saturated fatty acids. They also contain protein (15–24%), dietary fiber (18–30%), carbohydrates (26–41%), and minerals (4–6%) (7). Approximately 24% of the seed's weight is protein, primarily globulin, which constitutes 52% of the total protein content. Other proteins include albumins (17%), glutelins (14%), and prolamins (12%). Chia's protein content is higher than that of many other crops and has good digestibility (78.9%), comparable to that of casein (88.6%) (8).

Chia seed mucilage exhibits outstanding properties such as high water-holding capacity, good viscosity, gel formation, syneresis control, and emulsion stabilization, making it a promising material for edible film production. The mucilage is composed of a branched matrix of xylose, glucose, and glucuronic acid (9, 10). It contains a high amount of xylans (38%), which contribute to cross-linking ability in films and can absorb 10 to 100 times their weight in water. Additionally, the mucilage has high hemicellulose content, giving it excellent barrier properties such as oil resistance and low oxygen permeability (11). In recent years, plant extracts have gained prominence as functional additives in the food industry due to their antimicrobial and antioxidant properties. They delay the development of off-flavors, improve color stability, reduce microbial load, and enhance antioxidant capacity in food products (12). Barberry (*Berberis vulgaris*), from the Berberidaceae family, comprises nearly 500 species worldwide (13). The best-known species, commonly referred to as European barberry or Épine-Vinette, is native to Central and Southern Europe, parts of Asia (including northern Pakistan and Iran), and the northeastern United States (14). Barberry contains ascorbic acid, triterpenoids, vitamin K, over ten phenolic compounds, and more than thirty types of alkaloids. The primary alkaloid isolated from barberry is berberine, an isoquinoline alkaloid known for its anti-tumor properties, which include promoting apoptosis in tumor cells through modulation of related genes and enzymatic activity (15). Berberine also possesses anti-inflammatory effects and may support the treatment of central and peripheral nervous system disorders (16). The incorporation of nanoparticles into food packaging can regulate gas exchange within packaging layers, thereby prolonging shelf life. These materials also help scavenge unwanted gases that could shorten the product's life span. Enhanced barrier and thermal properties, antimicrobial effects, and altered surface characteristics such as wettability and hydrophobicity are key features of nanoparticle-containing packaging systems (17). Tin oxide (SnO<sub>2</sub>) nanoparticles possess a high surface area-

to-volume ratio, which enhances their ability to act as radical scavengers and antioxidant carriers. They are also used as antibacterial agents due to their small size, which allows them to penetrate microbial cell membranes (18). In the past, SnO<sub>2</sub> nanoparticles were used in the manufacturing of food cans for their antimicrobial effects. Today, they find broader applications in areas such as lithium-ion batteries, transparent conductive films, dye-sensitized solar cells, catalysts, environmental monitoring systems, biochemical sensors, and ultra-sensitive gas sensors, owing to their microstructural and electrical properties (19). Cheese is one of the most widely consumed dairy products globally, with over a thousand varieties produced across countries, each offering unique flavors, textures, and forms (20). One of the most important Iranian white cheeses is ultrafiltration (UF) cheese, which is produced via ultrafiltration, a membrane process that selectively removes macromolecules (molecular weights of 1,000–200,000 Da) from solvents and solutes (21). UF cheese has high consumption rates in Iran and is classified as a fresh cheese with a soft, spreadable texture, as it is marketed immediately after production without undergoing ripening. This product typically contains 34% dry matter, 66% moisture, a maximum of 1.75% acidity, and 12% protein. During storage, various biochemical reactions affect cheese quality. Due to its high moisture content, UF cheese is particularly susceptible to microbial spoilage, necessitating effective packaging solutions to preserve its quality (22). Given the nutritional value and high consumption of cheese, recent years have seen increasing interest in using compounds with antibacterial and antioxidant properties to extend shelf life and improve nutritional quality. Among these, edible films containing plant extracts and nanoparticles have attracted attention for their functional benefits

(23). For instance, Motelica et al. (2021) developed biodegradable antibacterial films based on alginate containing silver nanoparticles and lemongrass essential oil for innovative cheese packaging. The addition of antimicrobial agents like silver nanoparticles significantly enhanced the antibacterial activity of the films (24). Similarly, Hassani et al. (2024) studied the structural and physicochemical properties of potato starch-gum Arabic nanocomposite films containing boron oxide nanoparticles and anise hyssop essential oil. Their results indicated improved antioxidant capacity and increased layer thickness, although elongation at break decreased with higher levels of nanoparticles and essential oil. The films also demonstrated improved antimicrobial activity (25).

The aim of this study was to investigate the physicochemical properties of ultrafiltration cheese packaged with a chia seed mucilage-based nanocomposite film containing barberry extract and tin oxide nanoparticles.

## 2. Materials and Methods

### 2.1. Materials

Chia seeds were purchased from a local market in Urmia, Iran. Barberry fruits were obtained from orchards in Urmia. Tin oxide nanoparticles were supplied by Pishgaman Nano Materials Iranian Company (Iran). Glycerol was purchased from Sigma-Aldrich (USA), and sodium hydroxide, hydrochloric acid, potassium dichromate, and sodium sulfate were sourced from Merck (Germany). The commercial cheese starter culture was obtained from Danisco (Germany), and raw milk with 3.5% fat content was purchased from Pegah Dairy Company in Urmia, Iran.





Fig 1 Examples of different films prepared

## 2.2. Preparation of Biodegradable Films Based on Chia Seed Mucilage Containing Barberry Extract and Tin Oxide Nanoparticles

To prepare the biodegradable film, 3 grams of chia seed mucilage were mixed with 100 mL of distilled water and stirred for 15 minutes at 60°C using a magnetic stirrer. The resulting solution was filtered to remove impurities. Glycerol (30% w/w of dry polymer content) was added as a plasticizer, and the mixture was stirred again for 10 minutes.

Based on the Central Composite Design (CCD) shown in Table 1, barberry extract was added at three levels (0, 3, and 6 cc/g of dry polymer) and homogenized using a D9I12 homogenizer (Heidolph, Germany) at 13,000 rpm for 2 minutes. Tin oxide nanoparticles were added at different levels (0, 2, and 4 mg/g of dry polymer), and the mixture was stirred for 10 minutes to achieve a uniform dispersion. The solution was then subjected to ultrasonic treatment for 20 minutes to evenly distribute the nanoparticles. Subsequently, glycerol was added again at 40% of the total dry weight as a secondary plasticizer, followed by 15 minutes of stirring and 10 minutes of degassing to remove air bubbles. Finally, 25 mL of the prepared solution was poured into petri dishes and dried in an oven at 38°C to form the

films. After drying, the films were carefully removed and evaluated for further analysis [26].

## 2.3. Preparation of Ultrafiltration (UF) Cheese

UF cheese samples were produced at Pegah West Azerbaijan Dairy Company (Urmia, Iran) using the ultrafiltration cheese production process recommended by the manufacturer. Raw milk (3.5% fat) was pasteurized using a high-temperature short-time (HTST) method and then concentrated using a tubular membrane ultrafiltration system at 50°C until the dry matter content reached 34%. The concentrated milk underwent standard cheese-making steps, including homogenization, pasteurization, starter inoculation, rennet and salt addition, packaging, and incubation at 28°C for 24 hours until the pH reached approximately 4.7. The resulting cheese was stored in a cold room at 4°C until analysis [27].

## 2.4. Packaging UF Cheese with Nanocomposite Films

After preparing the UF cheese, the biodegradable films described above were used as antimicrobial packaging to preserve the quality of the cheese samples. Cheese blocks of 50–60 grams with dimensions of 7 × 7 × 1 cm were cut and packaged using the optimized films. The packages were labeled and stored for evaluation. Tests were conducted after 15 and 30 days of storage to assess various quality parameters.



Fig 2 Examples of packed cheeses with different films prepared

### 3. Analysis of UF Cheese Packaged with Biodegradable Films

#### 3.1. Acidity

To measure the acidity of the packaged UF cheese samples, 10 grams of cheese were weighed, mixed thoroughly with 10 mL of distilled water, and diluted to a final volume of 250 mL. The mixture was filtered, and 25 mL of the clear filtrate was titrated with 0.1 N NaOH using 3–4 drops of phenolphthalein as an indicator until a stable pink color appeared. Acidity, expressed as lactic acid percentage, was calculated using the following formula: Acidity (% lactic acid) =  $0.045 \times \text{Volume of NaOH used (mL)} \times 100$ . This test was repeated in triplicate for each sample, and the average value was reported [28].

#### 3.2. pH Measurement

The pH of cheese samples was determined using a calibrated pH meter (Metrohm, Switzerland), which had been calibrated with buffer solutions at pH 4 and 7. For analysis, 10 grams of cheese

were mixed with 50 mL of distilled water and homogenized thoroughly. The pH of the resulting mixture was measured directly. Each test was performed in triplicate, and the mean values were reported [28].

#### 3.3. Salt Content

To measure salt content, 10 grams of cheese were dissolved in 50 mL of boiling water and diluted to a final volume of 100 mL. The solution was filtered using Whatman filter paper. A 25 mL aliquot of the filtrate was titrated with 0.1 N silver nitrate using 0.5–1 mL of potassium dichromate as an indicator until a brick-orange color appeared. Salt content was calculated using the following formula:

$$\text{Salt (\%)} = (\text{Normality of AgNO}_3 \times \text{Volume of AgNO}_3 \text{ used} \times 8.5 \times 100) / \text{Sample weight (g)}$$
 [29]

#### 3.4. Lipolysis

The acid value was used as an indicator of lipolysis in the UF cheese samples. For this, 10 grams of cheese were mixed with 60 mL of diethyl ether and 6 grams of anhydrous sodium



sulfate using a magnetic stirrer. The mixture was filtered, and the residue was washed twice with diethyl ether. The combined filtrate was then titrated with 0.1 N KOH in the presence of phenolphthalein until a stable violet color (lasting for at least 20 seconds) appeared. The ether was evaporated under a hood, and the remaining fat was weighed. The acid value was reported in milliequivalents per 100 grams of fat [30].

### 3.5. Proteolysis

Proteolysis was evaluated by measuring total nitrogen using the Kjeldahl method and determining the non-protein nitrogen content soluble in 12% trichloroacetic acid (TCA). For soluble nitrogen analysis, 30 grams of cheese were mixed with distilled water and adjusted to the isoelectric point (pH 4.6) using 1 N HCl and NaOH. Samples were incubated at 40°C for 30 minutes, centrifuged, and filtered through Whatman paper. The soluble nitrogen was measured using the Kjeldahl method.

To determine non-protein nitrogen, 20 mL of the previous filtrate was mixed with 4 mL of 12% TCA and incubated in the dark at room temperature for 30–45 minutes. Samples were centrifuged for 10–15 minutes, filtered again, and the resulting non-protein nitrogen content was measured via the Kjeldahl method [31].

### 3.6. Sensory Evaluation

Sensory evaluation of the packaged UF cheese samples was conducted using a five-point hedonic scale by 20 trained panelists from the Food and Drug Control Laboratory in Urmia. The cheese samples were coded randomly and left at

room temperature for one hour before testing. Panelists scored organoleptic properties including color, texture, odor, and overall acceptability on a scale of 1 (very poor) to 5 (excellent). The results were analyzed statistically [32].

## 4. Statistical Analysis

To design the experiments, a Central Composite Design (CCD) was employed using Design-Expert software version 11 (Stat-Ease Inc., Minneapolis, USA). In this experimental design, the effects of two independent variables—barberry extract (0, 3, and 6 cc/g of dry polymer) and tin oxide nanoparticles (0, 2, and 4 mg/g of dry polymer)—on various physicochemical and microbiological parameters of UF cheese were investigated.

Each experiment was performed in triplicate, and the data were statistically analyzed using analysis of variance (ANOVA). Differences between mean values were evaluated by Tukey's post hoc test at a significance level of  $p < 0.05$ . Results were reported as mean  $\pm$  standard deviation. Additionally, response surface methodology (RSM) was used to determine the optimal levels of the independent variables for maximizing or minimizing the desired responses.

Table 1-A Statistical analysis table of prepared film samples

Run	Extract%	NPs% SnO <sub>2</sub>
1 (Blank)*	0	0
2 (Max NPs)*	0	4
3	3	0
4	0	2
5	3	2
6	3	2
7	3	2
8	3	2
9	3	2
10	6	2
11 (Max Extract)*	6	0
12 (Optimum) *	6	4
13	3	4

Blank Film: Chia seed mucilage nanocomposite film

Max SnO<sub>2</sub> Film: Chia seed mucilage nanocomposite film containing the highest percentage of tin oxide nanoparticles

Max Extract Film: Chia seed mucilage nanocomposite film containing the highest percentage of barberry extract

Optimum Film: Chia seed mucilage nanocomposite film containing the highest percentage of tin oxide nanoparticles and barberry extract

Table 1-B. Selected films for Cheese samples packaging

Run	A: Extract (%)	B: SnO <sub>2</sub> NPs (%)
R <sub>1</sub>	0	0
R <sub>2</sub>	0	4
R <sub>11</sub>	6	0
R <sub>12</sub>	6	4

## 5. Results and Discussion

### 5.1. pH and Acidity

Figure 1 illustrates the pH and acidity trends of UF cheese samples during storage. According to the analysis of variance (Table 3), both the concentration of barberry extract and the amount of tin oxide nanoparticles had a statistically significant effect ( $p < 0.05$ ) on the pH and acidity of the cheese samples.

During storage, pH decreased, and acidity increased in all treatments. The reduction in pH was more prominent in samples with higher levels of barberry extract, which is attributed to its rich organic acid and phenolic content that

may intensify microbial activity and acid production. This aligns with the findings of Soto et al. (2016), who reported that the use of plant extracts in cheese packaging can lead to lower pH due to lactic acid formation by lactic acid bacteria [33].

Conversely, the increase in acidity was correlated with the breakdown of lactose into lactic acid, facilitated by enzymatic and microbial processes. The samples treated with both barberry extract and tin oxide nanoparticles showed higher acidity levels than the control. These changes reflect both microbial metabolism and the inherent properties of the active compounds used in the films, confirming their influence on fermentation activity and acidification kinetics.

Table 2 The results of pH and acidity of UF packaged cheese

Sample	pH		Acidity (g/100)	
	15th day	30th day	15th day	30th day
Control	4.7±0.6 <sup>Aa</sup>	4.7±0.4 <sup>Aa</sup>	1.081±0.28 <sup>Ad</sup>	1.098±0.11 <sup>Bd</sup>
CSM	4.7±0.5 <sup>Aa</sup>	4.6±0.3 <sup>Ba</sup>	1.221±0.25 <sup>Abc</sup>	1.314±0.12 <sup>Bbc</sup>
CSM/Ex	4.8±0.6 <sup>Aa</sup>	4.6±0.5 <sup>Ba</sup>	1.303±0.17 <sup>Aab</sup>	1.336±0.34 <sup>Bab</sup>
CSM/NP	4.8±0.5 <sup>Aa</sup>	4.6±0.4 <sup>Ba</sup>	1.299±0.21 <sup>Ab</sup>	1.343±0.17 <sup>Bab</sup>
CSM/Ex/NP	4.6±0.4 <sup>Ab</sup>	4.4±0.2 <sup>Bb</sup>	1.351±0.15 <sup>Aa</sup>	1.384±0.22 <sup>Ba</sup>

Capital dissimilar letters in each row indicate significant differences between different days ( $P < 0.05$ )

Small non-identical letters in each column indicate significant differences between different samples ( $P < 0.05$ )

### 5.2. Moisture Content

The moisture content of the UF cheese samples decreased significantly during storage, as shown

in Figure 2. Based on the analysis of variance (Table 3), both barberry extract and tin oxide nanoparticle levels had a significant effect ( $p < 0.05$ ) on moisture retention.

Cheeses packaged with chia seed mucilage-based films exhibited lower moisture loss compared to the control group. This is attributed to the excellent water-holding capacity of chia mucilage, which forms a dense, gel-like network capable of limiting water migration. Additionally, the incorporation of barberry extract and tin oxide nanoparticles improved the structural integrity of the films, further enhancing

their moisture barrier properties. These findings align with previous studies demonstrating that biopolymer-based nanocomposite films can act as effective barriers to moisture transfer. For instance, Moradi et al. (2020) reported that starch-based films reinforced with natural extracts reduced moisture loss in cheese by more than 20% compared to conventional polyethylene packaging [34].

Table 3 The results of moisture of UF packaged cheese

Sample	Moisture (g/100)	
	15th day	30th day
Control	64.243±1.8 <sup>Aa</sup>	61.069±1.9 <sup>Bb</sup>
CSM	62.711±1.4 <sup>Ab</sup>	62.31±1.6 <sup>Ba</sup>
CSM/Ex	62.748±2.5 <sup>Ab</sup>	61.066±1.8 <sup>Bc</sup>
CSM/NP	62.743±1.5 <sup>Ab</sup>	61.068±2.1 <sup>Bc</sup>
CSM/Ex/NP	62.741±2.1 <sup>Ab</sup>	61.06±2.1 <sup>Bc</sup>

Capital dissimilar letters in each row indicate significant differences between different days ( $P < 0.05$ )

Small non-identical letters in each column indicate significant differences between different samples ( $P < 0.05$ )

### 5.3. Salt Content

The salt content of UF cheese samples increased over the storage period, as illustrated in Figure 3. Statistical analysis indicated that both the barberry extract and tin oxide nanoparticle concentrations had significant effects ( $p < 0.05$ ) on salt content. The observed increase in salt content is likely due to a reduction in moisture, which results in the concentration of remaining solids, including salt. Additionally, the packaging films—particularly those containing tin oxide nanoparticles—

exhibited low water vapor permeability, which may have contributed to reduced water loss and relatively higher salt concentrations.

This trend supports the findings of Pirsā et al. (2021), who reported that cheese packaged with active biodegradable films experienced a slower rate of salt diffusion, helping retain flavor and structural properties over time [35]. In this study, the highest salt levels were found in samples with high nanoparticle content, reinforcing the role of film composition in modifying cheese ripening dynamics.

Table 4 The results of salt of UF packaged cheese

Sample	Salt (g/100)	
	15th day	30th day
Control	2.62±0.17 <sup>Aa</sup>	2.58±0.47 <sup>Bcd</sup>
CSM	2.55±0.54 <sup>Ab</sup>	2.60±0.24 <sup>Bbc</sup>
CSM/Ex	2.52±0.32 <sup>Ac</sup>	2.61±0.24 <sup>Bb</sup>
CSM/NP	2.53±0.18 <sup>Ac</sup>	2.62±0.32 <sup>Bb</sup>
CSM/Ex/NP	2.51±0.24 <sup>Acd</sup>	2.64±0.17 <sup>Ba</sup>

Capital dissimilar letters in each row indicate significant differences between different days ( $P < 0.05$ )

Small non-identical letters in each column indicate significant differences between different samples ( $P < 0.05$ )

### 5.4. Proteolysis

Proteolysis is one of the most important biochemical processes during cheese storage and

ripening. It involves the breakdown of proteins into peptides and free amino acids by enzymatic action, contributing to flavor development and



texture

changes.

Figure 4 presents the total nitrogen, soluble nitrogen, and non-protein nitrogen (NPN) content of UF cheese samples packaged with nanocomposite films. According to statistical analysis, both barberry extract and tin oxide nanoparticles had significant effects ( $p < 0.05$ ) on proteolysis indicators.

Cheese samples packaged with the active films showed lower levels of soluble nitrogen and NPN compared to the control group. This suggests that

the nanocomposite films inhibited enzymatic activity, possibly due to reduced moisture content and the antimicrobial properties of the incorporated compounds. These effects are beneficial for controlling over-ripening and off-flavor development during storage.

The results are consistent with the findings of Bahrami et al. (2019), who demonstrated that the use of edible coatings containing essential oils and nanoparticles slowed proteolytic activity in dairy products and extended shelf life [36].

Table 5 The results of proteolysis of UF packaged cheese

Sample	Proteolysis (g/100)	
	15th day	30th day
Control	0.245±0.018 <sup>Aa</sup>	0.115±0.011 <sup>Ba</sup>
CSM	0.158±0.021 <sup>Ab</sup>	0.098±0.011 <sup>Bb</sup>
CSM/Ex	0.108±0.13 <sup>Ad</sup>	0.086±0.009 <sup>Bc</sup>
CSM/NP	0.13±0.17 <sup>Ac</sup>	0.083±0.009 <sup>Bd</sup>
CSM/Ex/NP	0.095±0.025 <sup>Ae</sup>	0.073±0.012 <sup>Be</sup>

Capital dissimilar letters in each row indicate significant differences between different days ( $P < 0.05$ )  
Small non-identical letters in each column indicate significant differences between different samples ( $P < 0.05$ )

### 5.5. Lipolysis

Lipolysis refers to the enzymatic degradation of milk fat into free fatty acids, which play a crucial role in the development of cheese flavor. However, excessive lipolysis may result in off-flavors and spoilage.

Figure 5 shows the acid value of UF cheese samples during storage. The data revealed that samples packaged with nanocomposite films containing barberry extract and tin oxide nanoparticles had significantly lower acid values ( $p < 0.05$ ) compared to the control group. This reduction in lipolytic activity can be attributed to several factors:

- The reduced moisture permeability of the active

films limited water availability for enzymatic reactions.

- The antioxidant and antimicrobial properties of barberry extract and SnO<sub>2</sub> nanoparticles inhibited lipase-producing microorganisms.

- The dense film matrix may have acted as a barrier to oxygen, which is necessary for some lipid degradation pathways.

These results are supported by Pirsā and Asadzadeh (2020), who demonstrated that incorporating nanoparticles into biodegradable films slowed lipolysis and helped maintain the sensory quality of cheese over time [37].

Table 6 The results of lipolysis of UF packaged cheese

Sample	Lipolysis (meq/g)	
	15th day	30th day
Control	0.265±0.04 <sup>Aa</sup>	0.92±0.6 <sup>Ba</sup>
CSM	0.202±0.12 <sup>Ab</sup>	0.851±0.07 <sup>Bb</sup>
CSM/Ex	0.148±0.09 <sup>Ad</sup>	0.789±0.07 <sup>Bd</sup>

CSM/NP	0.164±0.07 <sup>Ac</sup>	0.814±0.05 <sup>Bc</sup>
CSM/Ex/NP	0.141±0.011 <sup>Ae</sup>	0.78±0.09 <sup>Bd</sup>

Capital dissimilar letters in each row indicate significant differences between different days ( $P < 0.05$ )  
Small non-identical letters in each column indicate significant differences between different samples ( $P < 0.05$ )

### 5.6. Microbial Analysis

Figure 6 presents the microbial counts in UF cheese samples stored under different packaging conditions. According to the analysis of variance, barberry extract and tin oxide nanoparticles had a statistically significant effect ( $p < 0.05$ ) on the microbial load of the samples.

In the control samples (cheese without active packaging), coliforms, yeasts, and molds were detected after 15 and 30 days of storage. However, in samples packaged with films containing barberry extract and nanoparticles—especially at higher concentrations—no microbial growth was observed during the same periods.

This antimicrobial effect can be attributed to:

- The presence of phenolic compounds and alkaloids (especially berberine) in barberry extract, which have known antimicrobial properties.
- The tin oxide nanoparticles, which can disrupt microbial cell membranes and interfere with vital cellular processes.
- The barrier properties of the nanocomposite films, which limited moisture and oxygen exchange, thus inhibiting microbial proliferation.

These results are in agreement with Noshirvani et al. (2017), who reported that incorporating herbal extracts and nanoparticles into edible films

significantly reduced microbial growth in dairy products [38].

### 5.7. Sensory Evaluation

The sensory attributes of the UF cheese samples were evaluated using a five-point hedonic scale (1= very poor, 5= excellent). The parameters assessed included color, texture, odor, and overall acceptability.

As shown in Figure 7, cheeses packaged with films containing barberry extract and tin oxide nanoparticles—particularly at higher concentrations—received significantly higher scores ( $p < 0.05$ ) compared to the control group. These films helped maintain the natural white color, firm texture, and pleasant odor of the cheese throughout storage. The improvements in sensory quality can be attributed to the following:

- The antioxidant properties of the barberry extract, which prevented oxidative discoloration and off-odors.

- The antimicrobial activity of both barberry and tin oxide nanoparticles, which preserved freshness.
- The mechanical integrity of the films, which reduced physical damage and moisture loss. These findings are in agreement with the study by Ehsani et al. (2021), who reported that active films with plant-based additives improved both microbial safety and consumer acceptance of dairy products [39].

Table 7 The results of sensory evaluation of UF packaged cheese

Sample	15th day			
	Color	Texture	Odor	Total acceptance
Control	5±0.01 <sup>A</sup>	4±0.01 <sup>A</sup>	4.6±0.01 <sup>A</sup>	4.2±0.001 <sup>D</sup>
CSM	5±0.001 <sup>A</sup>	4±0.002 <sup>A</sup>	4.2±0.02 <sup>D</sup>	4.4±0.002 <sup>C</sup>
CSM/Ex	4.75±0.02 <sup>B</sup>	4±0.01 <sup>A</sup>	4.55±0.001 <sup>B</sup>	4.6±0.01 <sup>A</sup>
CSM/NP	5±0.01 <sup>A</sup>	4±0.02 <sup>A</sup>	4.5±0.001 <sup>C</sup>	4.5±0.02 <sup>B</sup>
CSM/Ex/NP	5±0.001 <sup>A</sup>	4±0.01 <sup>A</sup>	4.5±0.01 <sup>C</sup>	4.6±0.01 <sup>A</sup>

Sample	30th day			
	Color	Texture	Odor	Total acceptance
Control	5±0.01 <sup>A</sup>	4±0.02 <sup>A</sup>	4.6±0.02 <sup>A</sup>	3.8±0.01 <sup>E</sup>
CSM	5±0.01 <sup>A</sup>	4±0.01 <sup>A</sup>	4.15±0.03 <sup>E</sup>	3.95±0.003 <sup>C</sup>
CSM/Ex	4.7±0.01 <sup>B</sup>	4±0.01 <sup>A</sup>	4.50±0.01 <sup>B</sup>	4.1±0.02 <sup>B</sup>
CSM/NP	4.9±0.002 <sup>A</sup>	4±0.001 <sup>A</sup>	4.45±0.01 <sup>C</sup>	3.9±0.01 <sup>D</sup>
CSM/Ex/NP	4.9±0.01 <sup>A</sup>	4±0.01 <sup>A</sup>	4.4±0.001 <sup>D</sup>	4.25±0.01 <sup>A</sup>

Non-identical letters in each column indicate significant differences between different samples (P<0.05)

## 6. Conclusion

The results of this study demonstrate the effectiveness of biodegradable nanocomposite films based on chia seed mucilage enriched with barberry extract and tin oxide nanoparticles in improving the quality and shelf life of UF cheese. These films significantly influenced physicochemical properties such as pH, acidity, moisture content, salt concentration, proteolysis, and lipolysis. They also effectively inhibited microbial growth, with no detectable coliforms, molds, or yeasts in samples treated with higher levels of barberry extract and nanoparticles. Additionally, sensory evaluations showed that active films improved color, odor, and texture, enhancing overall consumer acceptance.

The improvements can be attributed to the films' moisture and oxygen barrier properties, as well as the antimicrobial and antioxidant activities of the natural additives. These findings suggest that such films can serve as an eco-friendly and health-promoting alternative to conventional cheese packaging.

In conclusion, chia seed mucilage-based nanocomposite films containing barberry extract and tin oxide nanoparticles represent a promising approach for the active packaging of dairy products, offering both quality preservation and environmental sustainability.

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## بسته‌بندی پنیر فراپالایش با فیلم نانوکامپوزیت بر پایه موسیلاژ دانه چیا حاوی عصاره زرشک و نانوذرات اکسید قلع و بررسی ویژگی‌های فیزیکوشیمیایی

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اطلاعات مقاله	چکیده
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<sup>1</sup> - Central Composed Design