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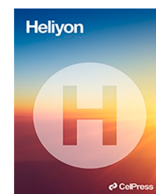

Characterizations and effects of pectin-coated nanoliposome loaded with Gijavash (*Froriepia subpinnata*) extract on the physicochemical properties of cheese

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ABSTRACT

In this study, pectin-coated nanoliposomes containing *Gijavash* extract were used to formulate cheese and evaluate its shelf life, physicochemical, and sensory aspects. The study used a central composite design with three independent variables to prepare the cheese. The results showed that the optimal particle size, zeta potential, encapsulation efficiency, and DPPH radical antioxidant activity were 201.22 nm, −29.33 mV, 61.87%, and 57.54%, respectively. Adding nanoliposomes with varying extract amounts improved pH and lowered acidity in fortified cheeses. Moisture and lipolysis indices also improved after applying nanoliposomes. Sensory evaluation revealed that sensory acceptance was highest in the cheese with 15% extract. The study suggests that adding pectin-coated nanoliposomes containing *Gijavash* extract to cheese formulations may create novel products and improve their physicochemical properties.

1. Introduction

Cheese is a dairy product with high nutritional value, resulting from the enzymatic coagulation of milk. Cheese is a fermented food that can increase healthy gut bacteria, affecting blood cholesterol levels [1]. It contains significant amounts of vitamins and protein and minerals, such as sodium, calcium, zinc, phosphorus, potassium, and iron. Due to its nutritional value and high consumption, there is an increasing trend to use natural preservatives with antibacterial features and antioxidants to promote nutritional value and shelf life [2–4]. The application of natural preservatives in the human diet can help reduce the risk of age-related diseases [5,6].

Gijavash (*Froriepia subpinnata*) is a plant from the family Apiaceae, native to Europe and West Asia, and naturally distributed in the northern regions of Iran [7]. It has stone-breaking, anti-inflammatory, and anticancer effects, as well as preventing chronic diseases. The plant contains bioactive compounds with antioxidant characteristics, including polyphenols and flavonoids, some of which are tannins and hydroxyl citronella [7]. In the Gijavash, bioactive compounds (polyphenolics and flavonoids) with antioxidant characteristics can be found, some of which are tannins (a α-bisabolol) and hydroxycitronellal, making it practical. Furthermore, this plant contains a large content of citronella, a sedative utilized to treat depression and mental illness [7]. Nanotechnology is widely used in the food industry for product development, formulation, and packaging. It involves the use of nanoparticles, nanoemulsions, nanocomposites, nanoliposomes, and nanostructured materials, which can improve taste, texture, and consistency of food products [8]. Nanotechnology can also be used to develop new functions and tools for food safety, such as nanosensors. Nanoparticles can be used as

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carriers for foodstuffs, drugs, and bioactive substances, and the preparation methods of colloidal nanocarriers is selected based on various factors. Nanotechnology can improve the texture, appearance, taste, and nutritional values of food, and it can also be used in food packaging to increase shelf life [9]. Lipid-based nanocarriers, including nano- and microemulsions, multiple emulsions, nanoliposomes, lipid nanoparticles, polymer-based hydrogel particles, and nanocarriers, are used in the food industry for product development, delivery, formulation, and packagin [10]. Nanoliposomes are spherical particles composed of polar lipids [11,12]that can be used to deliver substances with different solubility, pragmatic compounds, and food additives, as well as improving their bioavailability [13]. They can be used in the pharmaceutical, cosmetic, health, and food industries due to compatibility, biodegradability, and non-toxic constituents. Nanoliposomes can also be used as models for biological membranes [12].

Today, nanoparticles are used in many natural and processed foods [14]. The transfer of toxins from nanoparticles to food is a complex process that some studies show [15,16]. The toxicity of nanoparticles depends on several molecular and physicochemical mechanisms, and their physicochemical properties also depend on how they interact with food [17]. When nanoparticles are mixed with other materials, they may become toxic, even if they are not toxic when used alone. The effects of nanoparticles depend on a wide range of factors including size, structure, coating, dose, and consumption [18]. Therefore, to minimize the risk of toxicity, it is important identify the sources of nanoparticles and their effects, to determine how nanoparticles interact with living organisms in the food chain and environmental systems.

Pectin is a polysaccharide with a non-toxic linear anionic structure, which is rich in galacturonic acid. It is applied as a gelatinizing, thickening, and stabilizing agent in the food and pharmaceutical industries. Pectins are classified into high- and low-methoxyl categories based on the degree of methoxylation [19]. The main sources of pectin are plant cell walls such as the white part of the inner layer of citrus peel, apple pulp, sugar beetroot, sunflower waste, and carrots [20]. Pectin is a natural fiber that leads to the excretion of more waste products from the intestine, consequently lowering the risk of gastrointestinal tract-related diseases due to the connection with fats and their excretion, less cardiovascular diseases, and help with losing weight [21]. Pectin is suitable for micro-coating processes such as liposomes, causing stability and promoted transmission in systems. Various pectin-liposome systems have been fabricated, including pectin-liposome nanocomplexes and coatings, and pectin-loaded with liposomes. The properties of pectin affect the final characteristics of the coating carrier [22].

The present study examined the effect of pectin-coated nanoliposome incorporating Gijavash extract with antioxidant activity in the cheese formulation, as well as evaluating its physicochemical, and sensory features, and shelf life.

2. Materials and methods

Gijavash was collected from northwest Iran, and fresh cow's milk was purchased from a local market. Additionally, DPPH powder and selective medium Violet Red Bile Agar were obtained from Merck Chemical Reagent Co. in Germany. The LM apple pectin with the DM of 5.1% (Classic AU 910) was supplied by Herbstreith & Fox KG in Germany, while soybean lecithin and cholesterol were provided by Sinopharm Chemical Reagent Co. Ltd. in Shanghai, China. The other reagents used in the tests were not of analytical grade.

2.1. Preparation of Gijavash extract

To prepare Gijavash, it was dried under shade at room temperature away from wind flow for a week, ground, and packaged in thick polyethylene bags in a refrigerator. The percolation method was employed to extract the plant, in which a percolator device, often utilized as a cylinder or funnel, was used. Briefly, Gijavash was soaked with an adequate amount of ethanol (96%) and placed in closed containers for 4 h. Afterward, it was packaged and entered into a percolator, and the outlets were closed. After 24 h of soaking the plant in ethanol solvent (96%), the percolator output was returned to remove the extract gently. Finally, the extract was filtered using a rotary apparatus [23].

2.2. Formulation of the nanoliposomes enriched with extracts

Liposomes were prepared using the thin layer hydration technique. To do this, lecithin and cholesterol were first weighed according to the statistical design and dissolved in 15 ml of ethanol in a round bottom balloon. The ethanol was then evaporated on a rotary evaporator to form a thin film on the wall. In the next step, the fat film was hydrated using 5 ml of distilled water containing the extract based on the statistical design. The liposomes were then suspended with distilled water up to 10 ml and homogenized for 20 min at 50 °C at 20,000 rpm. Finally, the liposome solution was sonicated by turning the sonicator on and off, 8 times for 1 min at 25 °C and 20 kHz [24].

2.3. Preparation of pectin solution

To obtain the apple pectin solution, 1 g of pectin was dissolved in 100 ml of distilled water and stirred at 50 °C for 30 min. The solution was then stored overnight at room temperature. The pH of the solution was adjusted at 6 using phosphate buffer, and the product was kept overnight in the refrigerator. Additionally, 0.004% sodium azide was added to the buffer solution to prevent microbial growth [25].

2.4. Preparation of pectin-coated nanoliposomes

To fabricate pectin-coated nanoliposomes, the primary nanoliposomes, and pectin solution (70:30) were stirred with a magnetic stirrer at room temperature for 30 min at 600 rpm. The sample was then stored for 4 h at 4 °C before characterization [26].

2.5. Nanoliposome tests

2.5.1. Particle size and dispersion index

The particle size and polydispersion index (PDI) of nanoparticles were determined using photon correlation spectroscopy based on the dynamic light scattering (DLS, Malvern, UK). To prevent multiple scattering, the samples were prepared with a ratio of 1:50 by diluting with distilled water. Then, the measurement was performed at 25 °C, 657 nm, and 90° angle [27].

2.5.2. Zeta potential

To determine the zeta potential of nanocomplexes, the zeta-sizer from Malvern, England was used. The zeta-sizer was rinsed twice with deionized water, and the sample was injected directly into the cell using a syringe. The cell was then placed in an appropriate chamber [27].

2.5.3. Encapsulation efficiency

In this step, 1 ml of each sample was centrifuged for 30 min at 13,000 rpm to separate the capsules and filtered through a sterile syringe filter (0.22 μm) to remove the undissolved particles entirely. Then, 40 μl of each sample was diluted to 2 ml with acetone, and the absorbance of which was read on a visible-ultraviolet spectrophotometer at 290 nm. The different concentrations of the extract were applied to draw calibration curves. A linear equation was obtained from the curve, which was used to calculate the total extract content. Ultimately, encapsulation efficiency was calculated using the following equation [27]:

$$EE\% = (TUE\text{-}FE/TUE) \times 100$$

where TUE represents the total amount of utilized extract, and FE reflects the free extract level.

2.5.4. Antioxidant capacity

The DPPH radical antioxidant activity method was employed to measure the antioxidant capacity of the nanoliposomes. To this end, 250 μl of samples was added to a 3 ml solution of 60 μM 1, 1-diphenyl-2 picrylhydrazyl (in ethanol) and kept in the dark for 30 min. The absorbance of the samples was recorded at 517 nm, and the inhibition percentage of DPPH radical antioxidant activity was calculated the following equation [28]:

$$\text{Percentage of free radical quenching} = (A_{blank} - A_{sample})/A_{blank} \times 100$$

where A indicates adsorption, blank and sample denote the control and original samples, respectively.

2.5.5. FTIR

To obtain the spectrum of the grind samples, they were pressed with the powder of an alkaline halide (KBr) to form pellet. The pellet was then placed in a condenser beam in the sample cell of an FTIR spectrometer (Bomem MB-series). Finally, the spectrum of the samples was obtained in the range of 400–4000 cm^{-1} [29].

2.5.6. Morphological examination

The morphology of the nanoliposomes was assessed on a scanning electron microscope (SEM) (Leo 1430 vp, Oberkochen, Germany) [30].

2.5.7. Preparation of the cheese treated with the pectin-coated nanoliposomes containing Gijavash

To prepare cheese samples, the pasteurized cow's milk of Pegah (1.2% fat) was heated to 35 °C. Pectin-coated nanoliposomes with Gijavash (5%, 15%, and 25%) were then added to 500 ml of the milk and mixed for 15 min based on the statistical design shown in Table 1. Further, 0.004 g of starter was added to the milk, which was stored at 35 °C for 55 min until it reached a pH of 6.2. The, 0.25 g of rennet was added, and the mixture was given 50 min to form a clot. The clot was added into special molds, pressed, and positioned in a saline solution for 16 h. The cheese samples were then placed in air-impermeable plastic containers, and their surface was covered

Table 1
Organoleptic quality.

Score	Flavour and Color	Overall acceptability
1	Very unfavorable	Dislike
2	Unfavorable	Neither like
3	Normal	Like Slightly
4	Well	Like moderately
5	Very well	Like very much

with a 10% saline solution. Finally, the samples were kept in the refrigerator at 4 °C. After two months of ripening, the cheese samples were tested [24].

2.5.8. *Cheese sample analyses*

2.5.8.1. PH. The pH of the samples was determined by using a digital pH meter according to the standard [24].

2.5.8.2. Acidity. Regarding the acidity, 10 g of the sample was dissolved in 10 ml of distilled water, stirred until complete homogenization, poured into a 250 ml volumetric flask, and strained. Then, 25 ml of the filtrated solution was transferred to a beaker and titrated with 0.1 M sodium hydroxide until the appearance of pink color after adding 0.5 ml of phenolphthalein (AACC, 2000).

$$\text{Acidity (in terms of lactic acid)} = \text{Volume of consumed sodium hydroxide (ml)} \times 0.45 \times 100$$

2.5.8.3. Lipolysis index. Lipolysis intensity was measured by evaluating the acid index, total free fatty acids in cheese (meq/100 gr fat), as a lipolysis index. Briefly, 10 g of cheese sample was thoroughly mixed with 6 g of anhydrous sodium sulfate, on which 60 ml of diethyl ether solution was poured while stirring with the magnetic stirrer. The solution was strained through a filter paper, titrated with 0.1 N KOH solution in the presence of phenolphthalein, and placed under the laboratory hood. Finally, the remaining was weighed and the total fatty acid amount of cheese was expressed in meq/100 g fat [31].

2.5.8.4. Sensory evaluation. The samples were numbered and examined for sensory characteristics such as color, texture, smell, and tasted by 10 panelists based on the five-point hedonic scale test (Table 1). In this scale, scores five and one demonstrate the maximum and minimum quality of sensory properties in the samples, respectively [32].

2.5.8.5. Statistical analysis. The extract-incorporating nanoliposomes were prepared through using the central composite design in three levels and three replications at the central point. Furthermore, the three independent variables of Gijavash extract (5, 15, and 25%), lecithin (25, 37.5, and 50%), and cholesterol (50, 62.5, and 75%) were considered (Table 2). The optimal nanoliposomes were utilized in cheese.

Design Expert 7.0.0 software was applied to analyze the data and draw curves. At this step, the test results were analyzed by using SPSS 21 software in a completely randomized design including the cheese containing the pectin-coated nanoliposome enriched with Gijavash extract at three concentrations of 5, 15, and 25%, as well as a control sample (cheese without nanoliposomes) with 3 replications. Duncan's multiple ranges were applied at a significance level of 95% to compare the mean of treatments.

3. Results and discussion

3.1. *Results of nanoliposomes*

3.1.1. *Particle size*

The results of ANOVA revealed that the single factors of lecithin and Gijavash extract significantly influenced the particle size of nanoliposomes ($P \leq 0.05$). In the lecithin-cholesterol interaction, particle size increased and diminished by elevating lecithin and cholesterol percentage when the extract content was 15%, respectively (Fig. 2a–c) (see Fig. 1). Regarding the lecithin-extract

Table 2
Experimental design of the response surface method in the form of a central composite design.

Run	Lecithin (W/V %)	Cholesterol (W/V %)	Gijavash extract (W/V %)
1	25	75	5
2	50	5/62	15
3	50	75	15
4	5/37	5/62	5
5	5/37	75	15
6	25	50	25
7	25	5/62	15
8	5/37	5/62	25
9	5/37	5/62	15
10	25	50	5
11	5/37	50	15
12	25	75	25
13	50	50	25
14	5/37	5/62	15
15	50	75	5
16	5/37	5/62	15
17	50	50	5

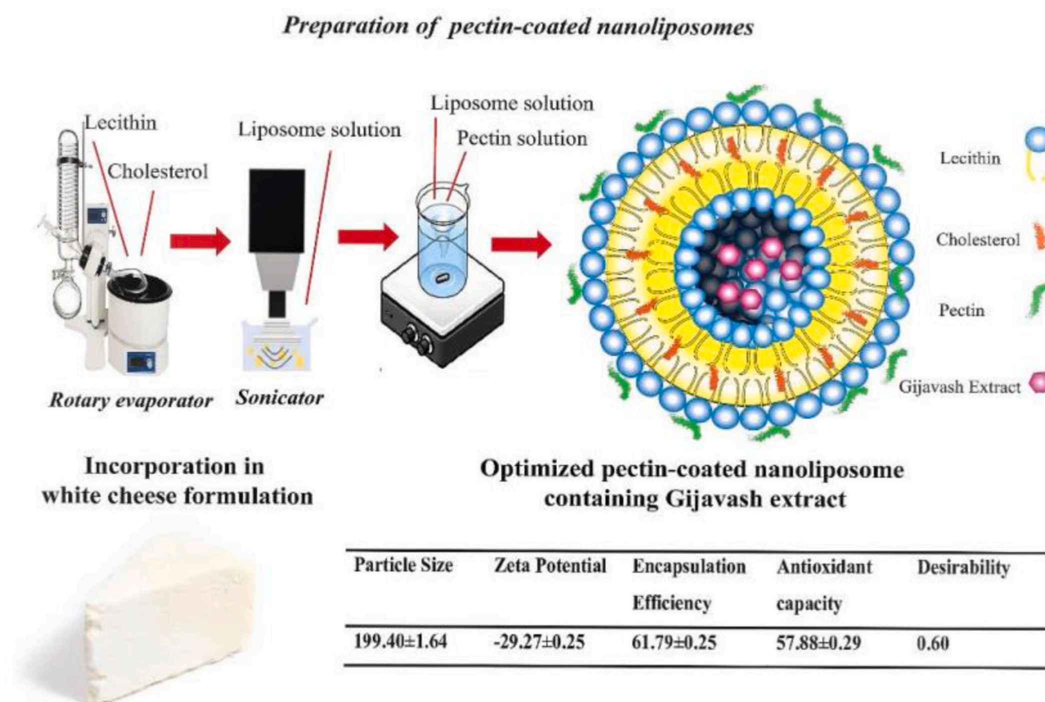


Fig. 1. Cheese treated with the pectin-coated nanoliposomes containing Gijavash.

interaction with the constant amount of cholesterol (62.5%), a rise in the lecithin level enhanced the particle size, while the size slightly improved in the higher extract concentration. In addition, lower and greater particle sizes were respectively detected following an increase in cholesterol and extract content in the cholesterol-extract interaction with a fixed lecithin amount (37.5%). The least particle size was related to the cholesterol-extract interaction. Lecithin, as the main skeleton of nanoliposomes and cholesterol, was used in the formulation to promote the stability and stabilization of the formed nanoliposomes. Thus, the liposome membranes rich in lecithin, as well as the arrangement of acyl chains in one direction reduce the spaces created by the bulky polar groups in the lipid head, leading to more contact and interaction between chains [33]. Further, an enhancement in lecithin percentage decreases the movement of oil droplets (cholesterol) and prevents their movement to the top according to Stoke's law [34]. Furthermore, the higher cholesterol level may bind hydrophobic molecules and decline bilayers in membranes through one of the following mechanisms, which ultimately diminishes particle size. The mechanisms involve competing fatty acid molecules (e.g., phospholipids) with cholesterol ones to capture lipophilic space in lipid membranes or causing lipophilic molecules to join the lipid membrane by cholesterol though reducing the flexibility of bilayer membrane [33,35]. Additionally, the greater diameter of the extract-loaded nanoliposomes indicated that phenolic compounds were not encapsulated only inside the nanoliposomes and some of them may be trapped in the bilayer membrane or adsorbed on the nanoliposome surface [22]. The results of the present study are consistent with those of surface [22,36] in the presence of more lecithin and saffron extract, as well as Savaghebi, Barzegar [37] following an improvement in the percentage of *Sargassum boveanum* algae extract. However, Ghanbarzadeh, Works [38] examined the effect of the various concentrations of lecithin-cholesterol on the particle size characteristics of the nanoliposomes incorporating vitamin A palmitate and reported no significant change in the particle size after adding cholesterol. Hamidi, Pirozifard [39] found that the particle size of the nanoliposomes containing black grape seed extract decreases in all concentrations by elevating cholesterol level in the particle formulation. According to Viriyaroj, Ngawhirunpat [40], the use of cholesterol and sterol compounds reduces liposome size. The model predicted for this feature was significant, the R^2 and R^2-Adj of which were 0.92 and 0.82 which is not significant ($P > 0.05$), respectively. The low coefficient of variation reflects the efficiency of the proposed model: $DLS = 281.27 + 30.79 \times A - 7.46 \times B + 15.40 \times C - 4.04 \times AB + 9.84 \times AC + 12.43 \times BC - 45.08 \times A^2 - 0.93 \times B^2 - 5.30 \times C^2$

3.1.2. Polydispersity index (PDI)

The results represented the significant effect of the single factors of cholesterol, Gijavash extract, and lecithin-extract and cholesterol-extract interactions on the PDI of nanoliposomes ($P \leq 0.05$). In the case of the lecithin-cholesterol interaction when the extract equaled 15%, PDI did not change significantly by increasing lecithin percentage, while the higher cholesterol level resulted in declining the index slightly (Fig. 3a–c). The lecithin-extract interaction with a constant cholesterol percentage (62.5%) led to no significant change, as well as enhanced PDI in the presence of more extract. Regarding the cholesterol-extract interaction with a fixed lecithin concentration (37.5%), an insignificant change and a promotion were respectively observed in the PDI of the samples after improving cholesterol content and adding extract. The index was minimized in lecithin-cholesterol interaction. Based on the statistical

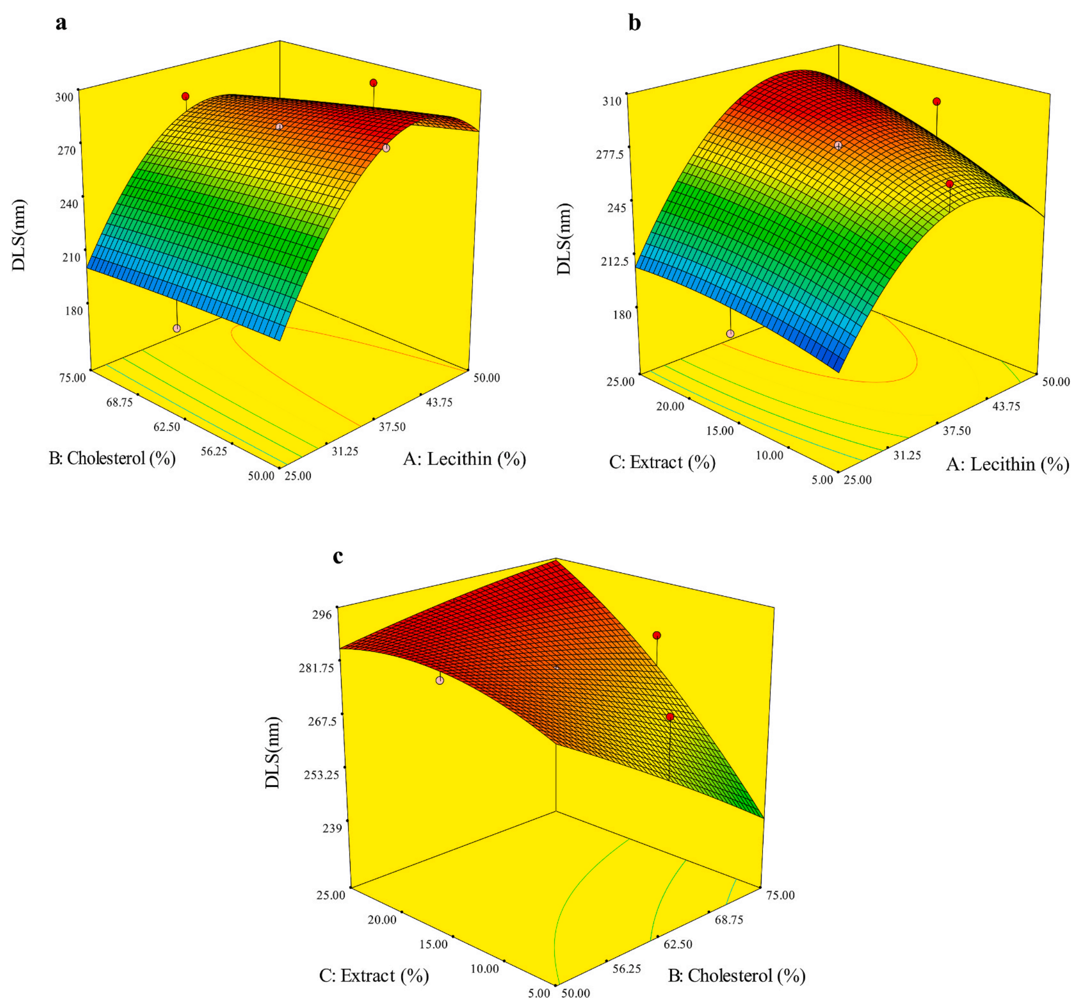


Fig. 2. (a–c) Particle size of Pectin-coated Nanoliposome loaded with Gijavash Extract; (a) interaction between different concentration of Cholesterol and Lecithin. (b) Interaction between different concentration of Extract and Lecithin. (c) Interaction between different concentration of Extract and Cholesterol.

results and shapes, the particles were seemingly in a good position in terms of PDI. Given the different amounts reported for the index in the various studies (less than 0.1 to more than 0.7), the results of the present study (0.2–0.3) reflected a favorable condition for nanoliposomes. The lower PDI exhibits more homogeneous colloidal system. Further, samples with a homogeneous PDI are more resistant to instability [41]. Thus, all of the intended samples with the different concentrations of lecithin, cholesterol, and Gijavash extract were thin and homogeneous. The results are in line with those of Takahashi, Inafuku [42] which demonstrated the homogeneous particle size distribution of the nanoliposomes produced by using soy lecithin with various contents. According to Mohammadi, Ghanbarzadeh [43], the greater level of vitamin and cholesterol fails to affect the particle size and size distribution of vitamin D3 nanoliposomes significantly. Hamadou, Huang [44] compared the marine phospholipid (MPL) nanoliposomes enriched with β-carotene with chicken egg phosphatidylcholine (EPC) nanoliposomes. They found the appropriate size distribution and homogeneous dispersion of the MPL nanoliposomes compared to the EPC ones. Regarding the property, the proposed model was significant due to the R^2 of 0.98 and R^2-Adj of 0.96 with non-significant mismatch ($P > 0.05$). In this model, a low coefficient of variation indicates that the model is efficient.

$$PDI = 0.28 + 0.0003 \times A - 0.011 \times B + 0.05 \times C + 0.001 \times AB - 0.009 \times AC - 0.012 \times BC + 0.017 \times A^2 - 0.002 \times B^2 + 0.001 \times C^2$$

3.1.3. Zeta potential

Based on the results, the zeta potential of the samples was significantly influenced by the single factors of lecithin and cholesterol ($P \leq 0.05$). As shown in Fig. 4(a–c), the zeta potential diminishes following an increase in lecithin percentage in the lecithin-cholesterol interaction, as well as the extract level of 15%. In this regard, an elevation in cholesterol amount leads to no significant change.

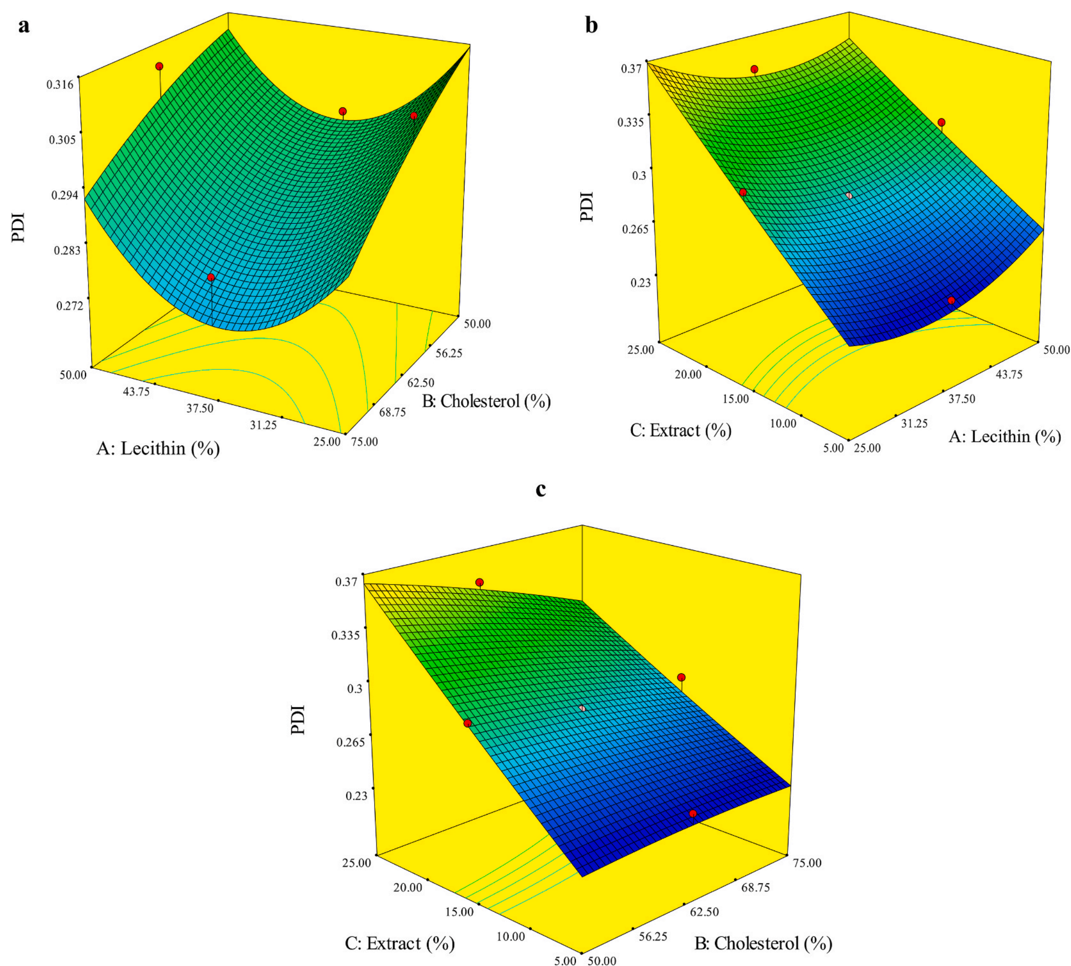


Fig. 3. (a–c) Polydispersity index (PDI) of Pectin-coated Nanoliposome loaded with Gijavash Extract; (a) Interaction between different concentration of Cholesterol and Lecithin. (b) Interaction between different concentration of Extract and Lecithin. (c) Interaction between different concentration of Extract and Cholesterol.

Considering the lecithin-extract interaction with the constant cholesterol concentration (62.5%) and cholesterol-extract one with a fixed lecithin content (37.5%), the zeta potential of particles reduces in the presence of more lecithin and cholesterol, respectively. However, it does not significantly change when the extract level enhances. All nanoliposomes possess a zeta potential above −25 mV, the highest negative one of which belongs to the cholesterol-extract interaction. Basically, the zeta potentials about 25 mv (positive or negative) are considered as a measure for the separation of electrically-charged surface particles (up and down). The colloidal systems containing particles with low zeta potential (positive or negative) are highly prone to gathering [45]. The negative charge of the particles can be attributed to the formation of a hydrogen bond between the choline group in lecithin (phosphatidylcholine) and hydroxyl one in cholesterol head. Furthermore, a rise in the negative zeta potential following the use of more cholesterol is ascribed to the fact that cholesterol is a neutral molecule. The formation of this hydrogen bond results in pulling the positively-charged choline and negatively-charged phosphatidyl groups into the membrane and its surface, respectively. Accordingly, the negative charge and electrostatic repulsion of the particles improve, and more negativity of zeta potential reveals stronger repulsion, and consequently more stability in nanoliposomes [46]. Some researchers have obtained larger zeta potential by adding cholesterol to the niacin-incorporating liposomes and barrage essential oil-loaded nanoliposomes [47]. Along with being located inside liposomes, phenolic compounds can be absorbed to or join the membrane surface [48]. In addition, more than 80% of the polyphenols in grape seed extract are attached to the surface of liposome membrane. Therefore, it seems that the negative charge of the system can be reduced by a further increase in the extract amount because of covering the surface of nanoliposomes as much as possible, and subsequently diminishing the surface charge. Machado, Pinheiro [49] introduced the reaction between the phenolic compounds of algae extract with the negatively-charged groups of liposome membrane as a reason for altering zeta potential. In this respect, the suggested model was significant with the R^2 and R^2-Adj of 0.98 and 0.97, respectively, which had nonsensical mismatch ($P > 0.05$), as well as a low coefficient of variation implying its efficiency.

$$\text{Zeta} = -36.14 - 9.16 \times A - 1.01 \times B - 0.18 \times C - 0.11 \times AB - 0.11 \times AC - 0.88 \times BC - 4.87 \times A^2 + 2.66 \times B^2 - 0.96 \times C^2 a$$

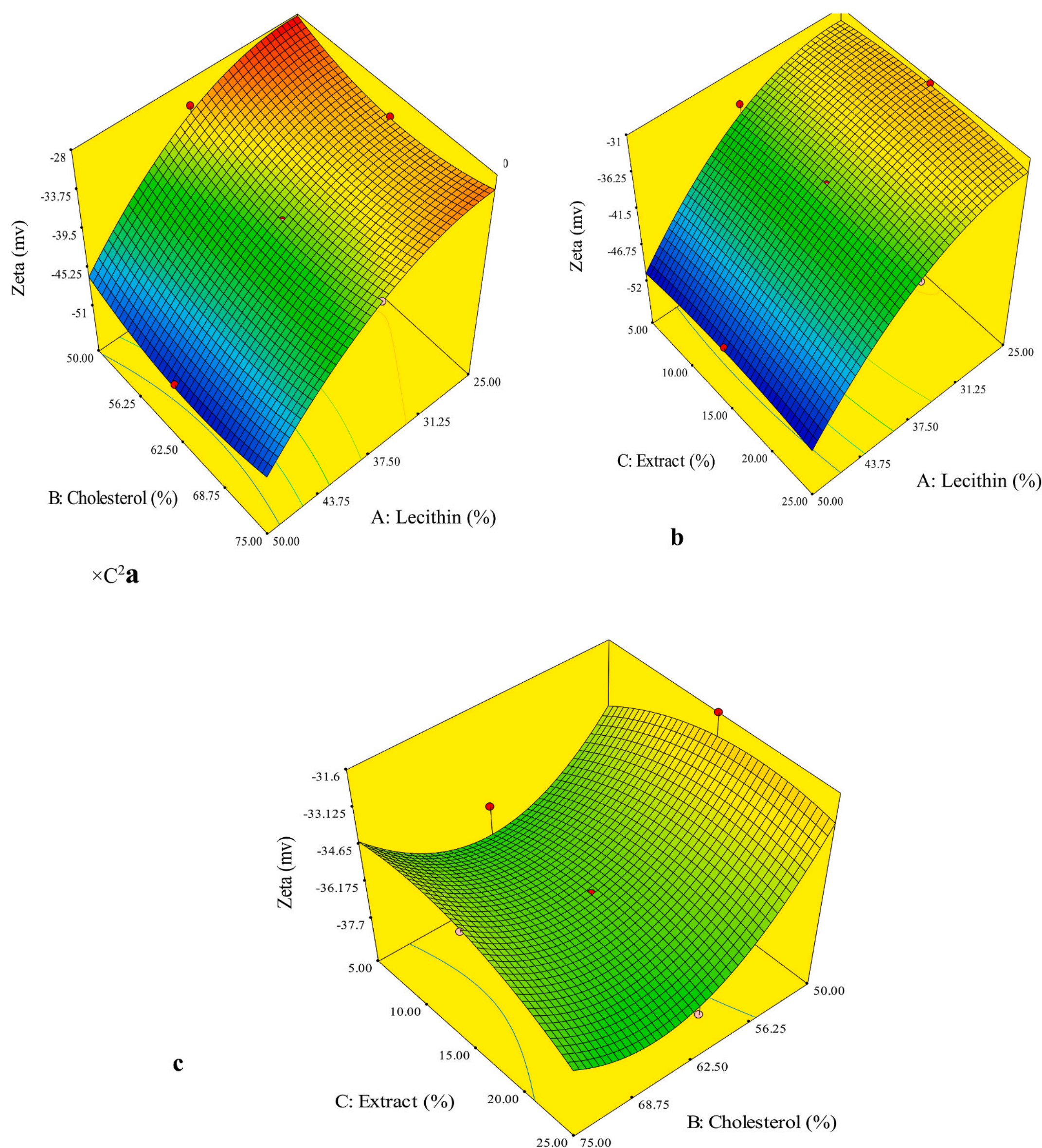


Fig. 4. (a–c) Zeta potential of Pectin-coated Nanoliposome loaded with Gijavash Extract; (a) Interaction between different concentration of Cholesterol and Lecithin. (b) Interaction between different concentration of Extract and Lecithin. (c) Interaction between different concentration of Extract and Cholesterol.

3.1.4. Encapsulation efficiency (EE)

The results indicate that the effect of lecithin, cholesterol, and lecithin-cholesterol interaction as single factors had a significant impact on the EE of the samples ($P \leq 0.05$). When the extract level of 15% was used, an increase in lecithin and a decrease in cholesterol led to an enhancement and a reduction in EE, respectively, in the lecithin-cholesterol interaction (Fig. 5a–c). No significant change was detected in the greater percentage of extract in the lecithin-extract interaction with a cholesterol concentration of 62.5% and in the cholesterol-extract interaction with a constant lecithin amount of 37.5%. However, EE was promoted by elevating the level of lecithin and cholesterol, respectively. Furthermore, the lecithin-extract interaction maximized the EE, while an increase in cholesterol concentration reduced the EE. Additionally, an increase in cholesterol concentration of the liposome formula promoted extract retention and enhanced stability. However, a greater rise resulted in filling the space between two layers with cholesterol and declining the extract loading in the liposome layers, leading to competition between cholesterol and extracts to fill the interstitial space. As phospholipids are the main building blocks of liposomes, their higher amount led to the encapsulation of more active compounds. The membrane hardening by cholesterol prevents the opening of liposome membranes, causing less hydrophobic active

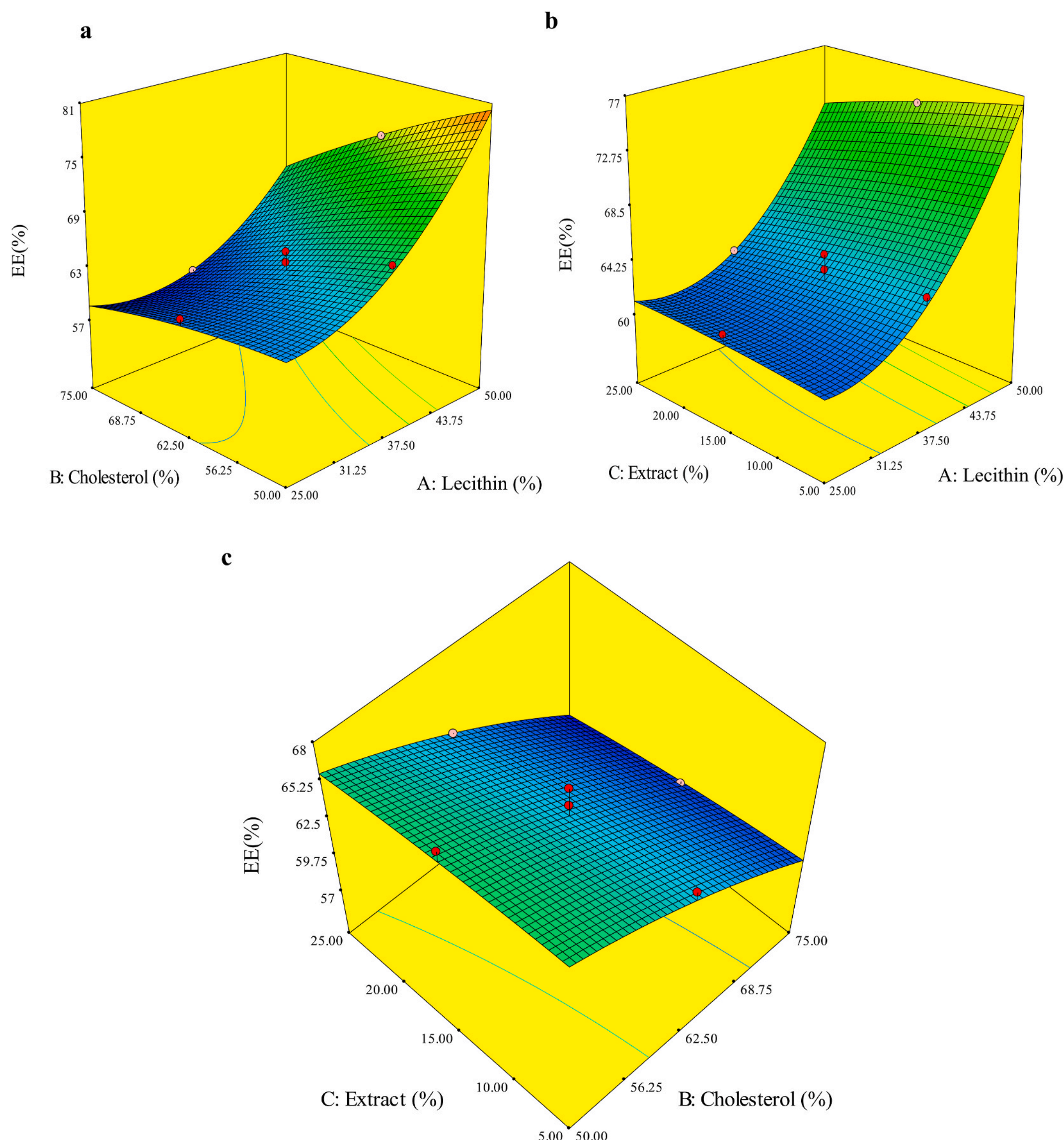


Fig. 5(a–c). Encapsulation efficiency of Pectin-coated Nanoliposome loaded with Gijavash Extract; (a) Interaction between different concentration of Cholesterol and Lecithin. (b) Interaction between different concentration of Extract and Lecithin. (c) Interaction between different concentration of Extract and Cholesterol.

substances [50]. According to Ref. [51], the EE improves when the stiffness of nanoliposomes increases, while it diminishes by adding lubricating compounds such as cholesterol. Similarly, Ref. [50,52] reported a smaller EE following cholesterol addition to the niacin-containing liposomes. In the present study, the high EE percentage in lecithin-extract interaction can be attributed to the low solubility of Gijavash extract in water, and consequently mixing of the extract in the hydrophobic part of liposome membrane. Additionally, the suitable ratio of Gijavash extract and lecithin led to large EE due to the presence of sufficient space in lecithin structure to bond the active groups of the extract [53]. The results are with those of [53] on the nanoliposomes of turmeric extract as a potential antioxidant and antimicrobial nanocarrier for loading food applications. Hosseinnia, Khaledabad [54] evaluated the microencapsulation of cockatiel essential oil and found a higher EE by adding the essential oil to biopolymer. In the case of this feature. The predicted model was a significant model with an R^2 of 0.97 and R^2-Adj of 0.93, and a non-significant mismatch ($P > 0.05$). The low coefficient of variation of the model indicates its performance. $EE = 63.33 + 6.70 \times A - 4.09 \times B - 0.88 \times C - 2.37 \times AB - 1.17 \times AC - 0.22 \times BC + 4.43 \times A^2 - 0.65 \times B^2 - 0.23 \times C^2$

3.1.5. Antioxidant capacity

The study found that the single factors of lecithin-cholesterol and cholesterol-extract interaction had a significant impact on the antioxidant capacity of the samples ($P \leq 0.05$). A greater antioxidant capacity is observed in the higher level of lecithin and cholesterol

when considering the lecithin-cholesterol interaction with the extract concentration of 15% (Fig. 6a–c). In the interaction of lecithin-extract with a constant cholesterol content of 62.5%, the use of more lecithin and extract leads to a slight enhancement and an elevation in the antioxidant capacity, respectively. Further, the larger percentage of cholesterol fails to change the parameter in the cholesterol-extract interaction with a fixed lecithin content of 37.5%. In this regard, the capacity promotes following an improvement in extract percentage. The highest antioxidant capacity is related to the cholesterol-extract interaction, which can be ascribed to the synergistic effect (synergy) of cholesterol and Gijavash extract. Furthermore, the presence of a phenolic group in the extract can also be addressed as a reason for its high antioxidant capacity since the hydroxyl group of phenol can trap free radicals [55]. The results are consistent with those of Zorzi et al. (2016) on preparing a nanoemulsion from the extract of a plant (*Achyrocline satureioides*) incorporating quercetin. Reference [55] encapsulated the essential oil of pistachio shell with nanoliposomes to increase its antioxidant and antibacterial activities, and phenolic compound stability. They suggested the usability of the product as carriers to transfer bioactive substances (phenolic compounds) with high antimicrobial properties in the food industry. According to Ref. [55], the antioxidant effects of the burger samples refrigerated for 12 days improve by using the nanoliposomes enriched with quinoa peptides. The model predicted for this feature was significant, the R^2 and R^2-Adj of which were respectively equal to 0.97 and 0.94 with a non-significant mismatch ($P > 0.05$). The low coefficient of variation represents the efficiency of the proposed model.

$$\text{DPPH radical antioxidant activity} = 50.60 + 4.51 \times A + 2.08 \times B + 10.85 \times C - 2.24 \times AB + 0.43 \times AC + 2.19 \times BC + 0.95 \times A^2 + 2.09 \times B^2 + 5.49 \times C^2$$

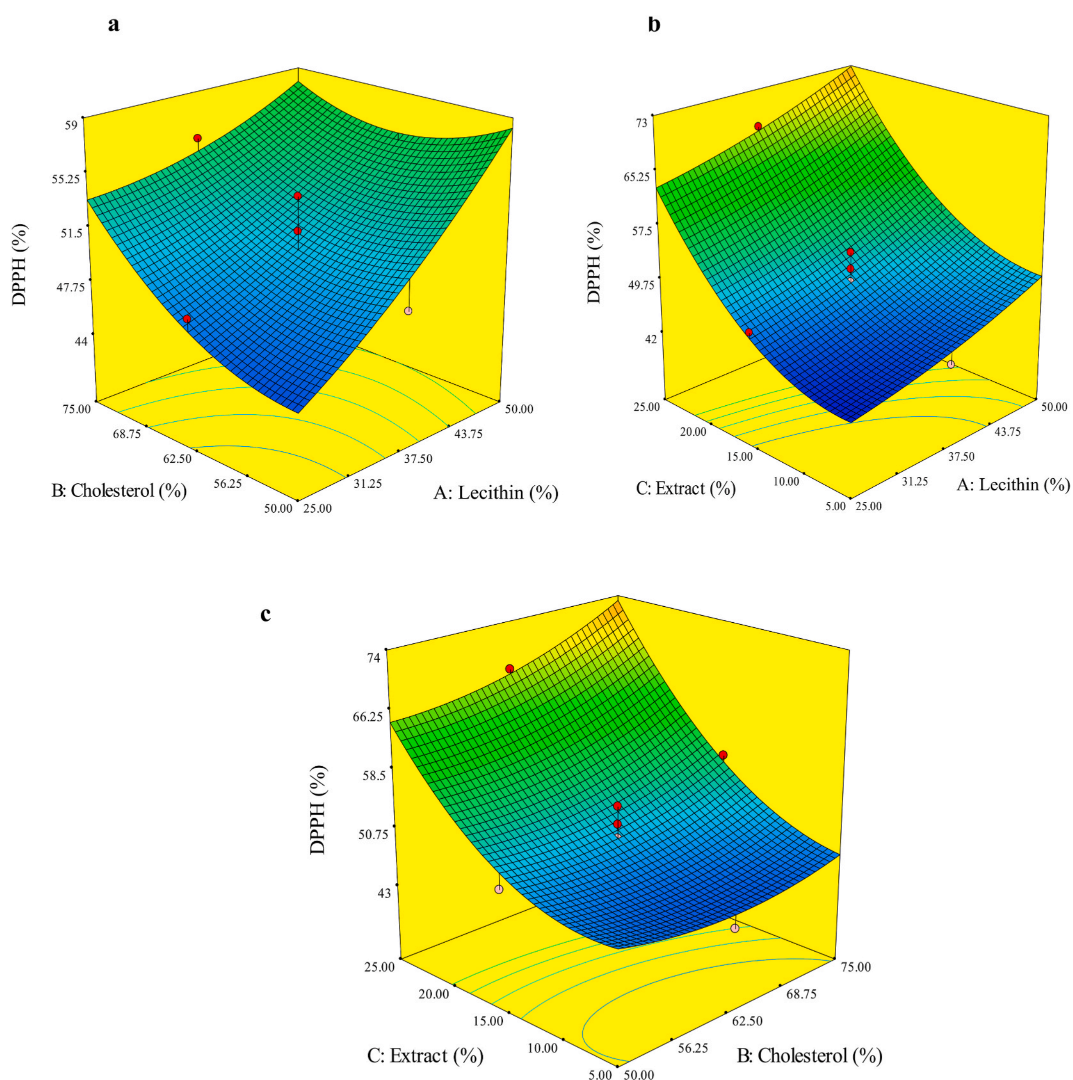


Fig. 6. (a–c) Antioxidant activity of Pectin-coated Nanoliposome loaded with Gijavash Extract; (a) Interaction between different concentration of Cholesterol and Lecithin. (b) Interaction between different concentration of Extract and Lecithin. (c) Interaction between different concentration of Extract and Cholesterol.

3.1.6. Optimization results

After performing the Design Expert analysis, the regression coefficients for the properties of nanoliposomes were displayed in (Table 3). The optimization was performed to achieve minimum particle size and size distribution, as well as maximum encapsulation efficiency and antioxidant capacity. The results of the statistical analysis are summarized in (Table 4). Based on the optimization results, the best quality was detected in the nanoliposomes replaced with 25% of lecithin, 50% of cholesterol, and 25% of Gijavash extract. In addition, the desirability, optimum particle size, zeta potential, EE, and antioxidant capacity of the optimized nanoliposomes were 0.60, 201.22 nm, −29.33 mV, 61.87%, and 57.54%, respectively. In the next step, the optimized pectin-coated nanoliposome containing Gijavash extract were utilized in cheese, followed by implementing the desired tests.

3.1.7. FTIR

The FTIR spectrum of the optimal sample is shown in Fig. 7(a). The spectrum reveals stretching vibrations of phenolic O–H group in Gijavash extract and aliphatic C–H bonds appear at 3400 [56] and 2938 cm^{-1} [57], respectively. Poor adsorption of C–C bonds was observed at 1636 cm^{-1} [58]. Further, the band at 1750 and 1300-1000 cm^{-1} corresponded to C]O bond [20] and C–O bonds in the optimal nanoliposome [59]. These results indicate the electrostatic interactions between the extract constituents and carriers during a simple mixing process. The position of pectin-coated nanoliposome loaded with Gijavash extract in the FTIR spectrum and the lipophilic nature of the extract, Gijavash extract was combined in the structure of lipophilic liposome, and subsequently entered the inner layer of phospholipids [27]. The emergence of the peak related to polyphenols in the FTIR spectrum of pectin-coated nanoliposome incorporating with Gijavash extract confirms the presence of the extract phenolics in the structure of nanoliposomes [60].

3.1.8. Morphology

The nanoparticle morphology and particle size of the optimal sample were evaluated using SEM.

The SEM image in Fig. 7b shows spherical vesicles with a size of approximately 200 nm. The particle size was also confirmed using device measurement. Sarabandi, Jafari [61] and Karimi, Ghanbarzadeh [62] reported similar results regarding the match of SEM image with nanoliposome particle size.

4. Results of cheese analysis

4.1. Lipolysis index

The lipolysis index of cheese treated with pectin-coated nanoliposome incorporating different levels of Gijavash extract during storage is shown in Fig. 8a, along with the control. Statistical analysis revealed a significant increase in the lipolysis rate in all samples during storage ($P < 0.05$). The lipolysis index of the control sample was the highest from day 1 to day 60 compared to other liposome samples. The index decreased until the 20th day of storage with a higher percentage of Gijavash compared to the control sample. After day 20, the index significantly increased ($P < 0.05$), indicating the continuous hydrolysis of the lipid portion during cheese storage. The high lipolysis rate in the sample containing 25% of the extract may be related to the extract's antimicrobial effects and growth retardant properties related to lactic acid bacteria [63]. Similar studies have reported increased lipolysis in soft cheese enriched with ginger extract [64] and cheese enriched with tomato powder extract during storage [63].

4.2. pH

Based on the results related to the pH of the cheese containing pectin-coated nanoliposome loaded with the various amounts of Gijavash extract, as well as the control during storage (Fig. 8b), the pH of all samples during storage significantly declined during the

Table 3
Regression coefficients for the characteristics of nanoliposomes.

Factors	Particle Size (nm)	Polydispersity index (PDI)	Zeta Potential (mv)	Encapsulation efficiency (%)	Antioxidant capacity (%)
Model	**9.23	**48.51	**67.87	**27.94	**31.30
Intercept	281.27	0.28	36.14-	63.33	50.60
A	**30.79	0.0003	**9.16-	**6.70	**4.51
B	7.46-	**0.01-	*1.01-	**4.09-	*2.08
C	*15.40	**0.05	0.18-	0.88-	**10.85
AB	4.04-	0.001	0.11-	**2.37-	*2.24-
AC	9.84	*0.009-	0.11-	1.17-	0.43
BC	12.43	**0.01-	0.88-	0.22-	*2.19
A^2	*45.08-	*0.01	**4.78-	**4.40	0.95
B^2	0.93-	0.002-	**2.66	0.65-	2.09
C^2	5.30-	0.001	0.96-	0.23-	**5.49
R^2	0.92	0.98	0.98	0.97	0.97
Adj-R^2	0.82	0.96	0.97	0.93	0.94
Model mismatch	0.7^{ns}	4.79^{ns}	3.55^{ns}	0.43^{ns}	1.45^{ns}
Coefficient of variation%	2.50	1.34	3.26	2.63	1.85

*:$p \leq 0.05$, **:$p < 0.01$, ns(non-significant):$p \geq 0.05$, A: Lecithin, B: Cholesterol, C: Gijavash Extract.

Table 4
Optimization amounts for a variable and answered parameters based on the response surface method.

Particle Size(nm)	Zeta Potential (mv)	Encapsulation Efficiency (%)	Antioxidant capacity (%)	Desirability
199.40 ± 1.64	−29.27 ± 0.25	61.79 ± 0.25	57.88 ± 0.29	0.60

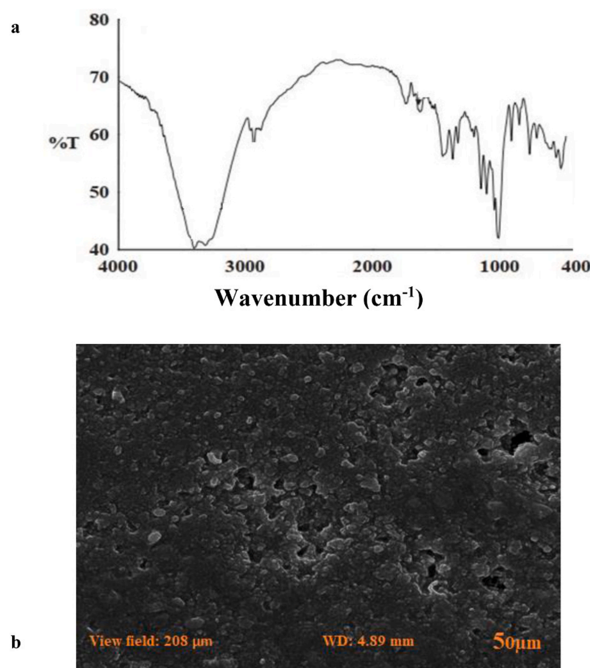


Fig. 7. (a–b) FTIR and SEM of the optimal sample; (a) Fourier transform infrared (FTIR) spectra of Pectin-coated Nanoliposome loaded with Gijavash extract. (b) Morphology of Pectin-coated Nanoliposome loaded with Gijavash extract confirmation by SEM.

storage compared to the control. An enhancement in the extract content led to a significant decrease in the pH on the first day in compared to the control, as well as an increase pH since the day 20 ($P < 0.05$). The pH of the control sample on the first day was the highest compared to other liposome samples. The greater pH by elevating the extract percentage in cheese can be attributed to a diminution in lactate-protein ratio, and consequently the lower buffering features of curd cheese. Thus, the removal of lactic acid increases the solubility of calcium and phosphorus, the main factors causing the buffering properties in cheese, which eventually raises the pH [65]. According to Jamdar, Mortazavi [66], the pH of refined white cheese enriched with fine-grained wheat germ extract promotes during shelf life. They introduced the type and concentration of the wheat germ extract as a reason for the enhancement. This extract influences the amount of free amino acids in cheese because of the presence of enzymes and proteins, and improves pH because of forming alkaline materials such as ammonia, creating non-acidic decomposition products, and decomposing lactic acid at the end of ripening. However, Arce and Ustunol [67] obtained to a smaller pH in the cheese samples enriched with microencapsulated iron during storage. Furthermore, Fernandes, Guimarães [68].

4.3. Acidity

The acidity of the cheese treated with pectin-coated nanoliposomes incorporating the different percentages of Gijavash extract, as well as the control during storage time is outlined in Fig. 8(c). The results indicate a significant effect of storage time and extract amount on the acidity of the enriched samples ($P < 0.05$). With the increase of shelf life, the acidity of the samples increased and also the control sample showed the highest amount of acidity. The sample loaded with 15% of the extract had the minimum acidity on the 60th day because of increasing the acidity of all samples and control one during the 60-day period so that that of the control reached 0.98% in terms of lactic acid in the 60th day. The control sample and 25% liposome had the highest value of acidity compared to other liposome samples. The higher acidity of the samples stored for 60 days is consistent with the results of pH, which reflected a significant decline in their pH ($P < 0.05$). The continued growth of lactic bacteria in cheese, and subsequently lactose fermentation, and its conversion to lactic acid and other metabolites can be addressed as a reason for enhancing the acidity of the samples over time [69]. An improved extract concentration in the nanoliposome slightly diminished acidity, which may be ascribed to the placement of cholesterol particles in lecithin coating, as well as the interaction between cholesterol and oxidation-accelerating agents, which was minimized, and further acidity increase was prevented [70]. Acidity elevation over time is in line with the results of some studiesFernandes, Guimarães [68,70].

4.4. Sensory evaluation

The results of the sensory evaluation of cheese containing pectin-coated nanoliposome enriched with various percentages of Gijavash extract, as well as the control during storage, are shown in Fig. 8(d–e). Based on the results, nanoliposomes loaded with different levels of extract significantly affected the general acceptance, smell, and taste of the samples ($P < 0.05$). The sensory evaluation revealed a reduction in the smell and taste index, and general acceptance of the sample containing 5% of extract and control until the 40th day of storage, as well as a rise in this regard on the day 60 ($P < 0.05$). The control sample has the highest amount of taste and smell and overall acceptance compared to other liposome samples. Further, lower smell and taste indices were detected in the samples with 15 and 25% of the extract until the 20th day, followed by an increase ($P < 0.05$). Thus, the sample incorporating 15% of extract exhibited higher smell and taste indices than the others. In terms of taste characteristics and general acceptance, the best smell and general acceptance were related to the cheese loaded with 15% of Gijavash extract, so that further increase of extract amount to a decrease in the acceptance of the indices. The special smell of cheese with aromatic compounds in Gijavash can be considered as a reason for its low smell scores following the addition of more extract. Solhi, Azadmard-Damirchi [63] reported a diminution in the sensory features of the cheeses enriched with tomato powder extract. They referred to the sample containing 2% of the extract as the best treatment in terms of sensory evaluation. Reference [71] used walnut leaf extract to enrich yogurt and found significantly worse smell and taste in the yogurt samples after enhancing the extract content compared to the control. Finally, the smell and taste scores declined over time, the maximum and minimum of which were respectively observed on the first day and in front of end of the storage period.

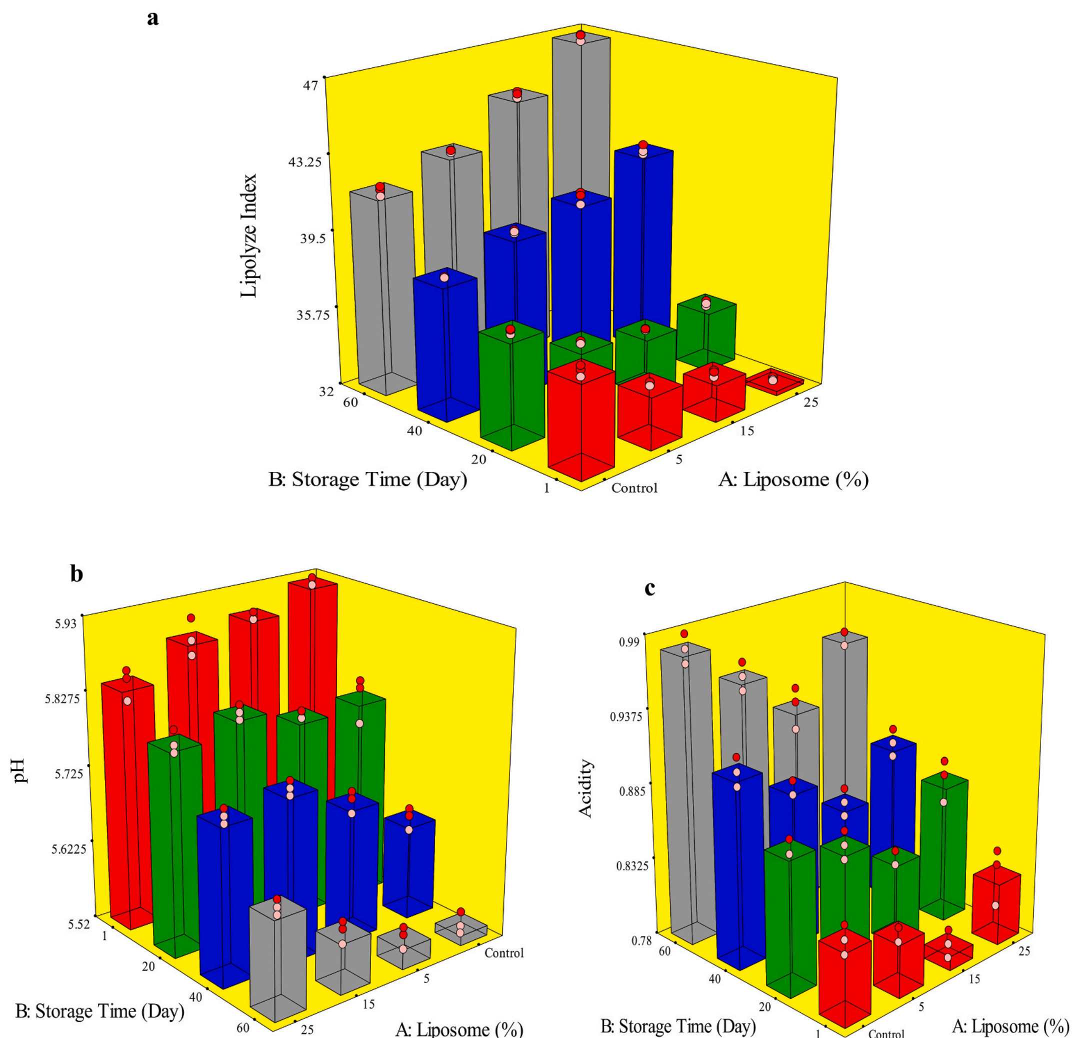
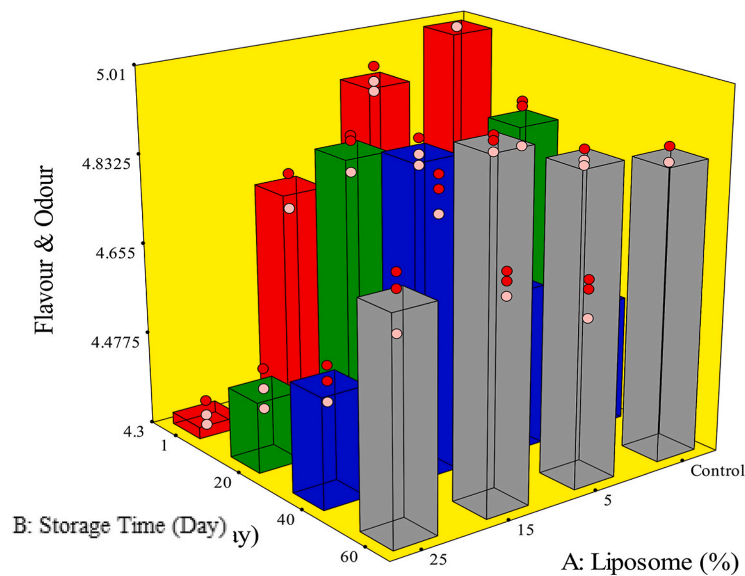


Fig. 8. (a–e) Result of cheese analysis; (a) lipolysis index, (b) PH, (c) acidity, (d) Sensory evaluation, and (e) overall acceptability.



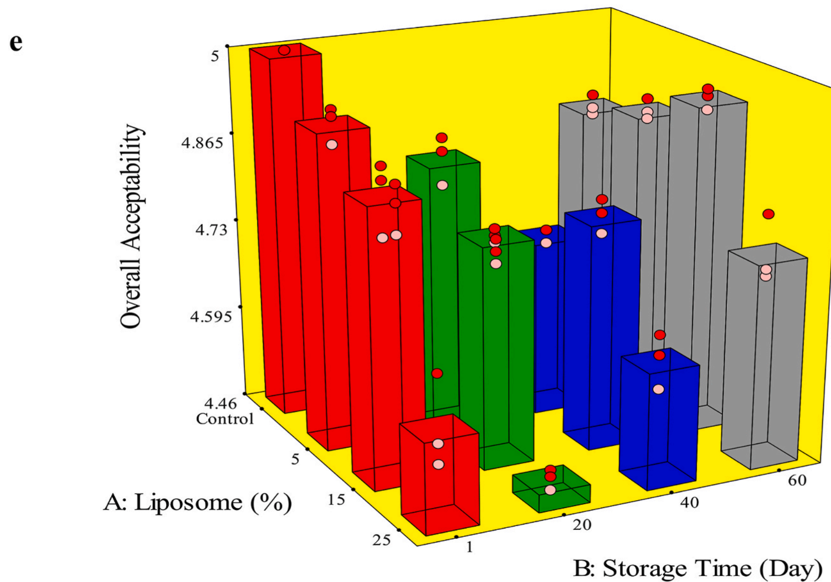


Fig. 8. (*continued*).

5. Conclusion

The results of the study indicated that pectin-coated nanoliposome enriched with Gijavash extract can be effectively used to optimize the stability of the physicochemical properties of cheese. The proposed models were significant with respect to R^2 and R^2-Adj, and their coefficient of variation was low, indicating the efficiency of the models in predicting the features of the subject under study. The following observations were made. The least particle size and highest negative zeta potential belonged to the cholesterol-extract interaction, while the greatest particle size distribution was found in the lecithin-extract interaction. The EE (74.5% lecithin-extract and 62.25% cholesterol-extract) and antioxidant capacity (55.5% lecithin-extract and 66% cholesterol-extract) were maximized in the lecithin-extract and cholesterol-extract interactions. FTIR spectra exhibit the electrostatic interactions between nanoliposomes, which confirm the results of the morphology of particles by using a particle size measuring device. Furthermore, the optimization results suggested 201.22 nm, −29.33 mV, 61.87%, and 57.54% as the optimal amounts for particle size, zeta potential, EE, and antioxidant capacity, respectively. Regarding the enriched cheeses, the addition of nanoliposomes with various percentages of the extract significantly increased pH and decreased acidity ($P < 0.05$). The samples had a higher moisture content and lipolysis in the presence of more extract. Finally, the greatest sensory acceptance was detected in the cheese containing 15% of Gijavash extract.

Ethics statement

Sensory evaluators have participated in the sensory evaluation test knowingly and with full satisfaction.

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Availability of data and material

Data will be made available on request from the authors.

Data availability statement

Data included in article/supplementary material/referenced in article.

Author agreement/declaration

All authors reviewed and approved the final form of the submitted paper was viewed and accepted by all authors. The authors declare that this work is original and that all information, including all tables and figures, was created by the authors and has not been published or is currently under consideration elsewhere.

Additional information

No additional information is available for this paper.

CRediT authorship contribution statement

Farzad Mohammadi: Writing – review & editing, Writing – original draft, Methodology. **Mahsa Yousefi:** Data curation, Methodology, Software, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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