Characterization and Optimization of Persian Gum/Whey Protein Bionanocomposite Films Containing Betanin Nanoliposomes for Food Packaging Utilization

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Characterization and Optimization of Persian Gum/Whey Protein Bionanocomposite Films Containing Betanin Nanoliposomes for Food Packaging Utilization

Running title: Biocomposite edible film containing betanin nanoliposomes

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Abstract

In this study, composite packaging films were produced from relatively inexpensive materials including whey protein isolate (WPI) and Persian gum (PG), supplemented with betanin nanoliposomes (NLPs). Using response surface methodology (central composite design), we investigated the effects of two variables (PG [0-2% w/v] and betanin NLPs' [0-10% w/v] content) on the physico-mechanical and antioxidant properties of the film treatments. Afterward, the optimal treatment was evaluated for structural and antimicrobial characteristics. The film samples' permeability to water vapor decreased with the addition of NLP (from 7.38 to 5.46 g/Pa.s.m) but increased with PG incorporation; decreased solubility was observed when either substance was added. Mechanical properties like Young's modulus and tensile strength were weakened by PG addition, but the incorporation of NLPs led to pronounced tensile strength. XRD analysis revealed improved crystallinity through NLPs' addition. The presence of NLPs in the nanocomposite film resulted in an elevated level of antibacterial activity against *Staphylococcus aureus*, while the addition of both PG and betanin NLPs led to improved antioxidative activity (63.45%). Considering the results, PG/WPI films loaded with betanin NLPs could be introduced in active packaging applications for the shelf life extension of perishable food products.

Keywords: Biopolymers; Biodegradable materials; Packaging film; Functional properties; Nanoliposomes

1. Introduction

Environmental pollution represents an inevitable consequence of the utilization of non-biodegradable plastic materials in food packaging systems. However, growing demands among consumers for safe, high-quality foods have led to pronounced scientific interest in the enhancement of bio-based polymer packaging materials [1]. Biopolymer films are preferred because of their edibility, biocompatibility, rapid biodegradation, and the potential to act as vehicles for bioactive substances; drawbacks like water susceptibility and poor mechanical characteristics must also be noted [2]. Owing to such drawbacks, the industrial-scale fabrication of natural, polymer-based packaging films has been limited [3].

Most protein-based edible films act as impressive gas barriers while possessing more desirable mechanical properties relative to films based on fats and polysaccharides. Whey proteins are byproducts of the cheese-making process, purified via ultrafiltration and diafiltration. Whey-based edible films are appreciated for their stable mechanical characteristics and demonstrate good gas barrier resistance while acting as noticeable barriers to edible oils and aromatic compounds. Nevertheless, films based on hydrophilic proteins like whey show a moderate level of moisture resistance [4]. Several methods exist to develop the properties of edible protein films, including physical techniques, enzymatic methods, and strategies involving their combination with hydrophobic substances such as gums.

Persian gum (PG), also named Zedo and Angum, is a natural and low-cost biopolymer that is extracted from *Amygdalus scoparia* Spach (the mountain almond tree), which grows naturally in Iran's forests. Considering its dry weight, PG is constituted by polysaccharides (mainly galactopyranosyl and arabinofuranosyl polysaccharides) and a scarce amount of proteins, thereby having a high water absorption capacity [5, 6]. The adverse ratio of Ara:Gal, the low protein content and the existence of xylose and mannose were highlighted as a distinguishing feature compared to gum Arabic. PG contains 82–90% w/w carbohydrate, around 0.19% w/w

lipid, 0.20–1.02% w/w protein, and 0.60% w/w tannins. The ash content (1.66–3.63% w/w) and the elements (Na, K, Zn, Fe, Mg, Ca) vary widely. Abbasi [7] described the medicinal and health-promoting actions of PG, including mucus reduction, healing of swollen joints, and acting as a remedy for tooth pain. This anionic hydrocolloid possesses emulsifying properties that are similar to those of gum Arabic; it forms brittle films that can be developed by applying a plasticizer [8]. Recently, several investigations have elucidated the capacity of PG in the preparation of edible films or coatings [8-12]. Likewise, the interaction of PG with whey proteins and β-lactoglobulin has been investigated, where the potentiality of these interactions for film-forming solutions was remarked [13, 14].

Packaging fulfills a critical role in food preservation, and the incorporation of antimicrobial/antioxidant agents into film materials is a suitable approach to promote active packaging. It is reported that the addition of these bioactive substances to packaging materials confers more benefits than their direct application to food surfaces.

Betalains are water-soluble and nitrogen-containing pigments found in high concentrations in red beet and they comprise two sub-classes: betaxanthins and betacyanins. As the predominant type of betacyanins, betanin (betanidin 5-O-D-glucoside [schematic 1]; code: E 162) is known as an approved colorant in cosmetic, pharmaceutical, and food products. Betanin is approved as a Generally Recognized as Safe (GRAS) food additive, which can be added to foods as an antioxidant and colorant agents [15, 16]. Some biological functions such as the inhibition of lipid oxidation and the provision of cardioprotective, hepatoprotective, anti-inflammatory, anti-proliferative, and antimicrobial effects are mentioned for this bioactive pigment. Betanin is an unstable substance, and its stability during storage can be affected by factors such as exposure to light, extreme pH, enzymes, high temperature, and oxygen. During the process of betanin decomposition, cyclo-dopa-5-O-glycoside and betalamic acid are formed; these products lack the bioactivity of pure betanin [17, 18].

Bio-nanocomposites are a new generation of biodegradable packagings made from the combination of inorganic/organic materials and biopolymers with the presence of at least one active nano-based compound. Among the advantages of producing bio-nanocomposites is the application of materials that improve the production efficiency, barrier properties, UV-shielding ability, and overall physico-chemical characteristics of the final product [9].

Lipid-based nano-carriers, among them nanoliposomes are desired, because of capability to encapsulate both hydrophobic and hydrophilic ingredients and their amphiphilicity, biocompatibility, non-immunogenicity, and non-toxicity considering the endogenic nature of their constituents. In this study, given our positive experience with the use of liposomes as wall materials for betanin stabilization [19, 20], we aimed to develop PG/whey protein-based biocomposite films embedded with betanin nanoliposomes (NLPs). Furthermore, we characterized the effects of betanin NLPs and PG on the water vapor permeability, thickness, antioxidant activity, mechanical properties, color parameters, morphology, and antimicrobial activity of the fabricated bio-nanocomposites.

2. Experimental

2.1. Materials

Whey protein isolate (WPI; protein 921.6 g/kg as determined by manufacturer) was procured from German Prot, Sachsenmilch Lepperrsdorf GmbH (Saxony, Germany). Dried PG granules were obtained from a reputable herbal market (Shiraz, Iran). The gum kernels were categorized into red, yellow, and white according to their quality and color. Then, the qualified white and yellow gum samples were powdered by a mechanical blender and sieved carefully to obtain a uniform particle size of less than 500 µm; the average molecular weights were 8.4×10^2 and 4.7×10^3 kDa, respectively. Lecithin was purchased from Lipoid Co. (Ludwigshafen, Germany). Betanin, glycerol, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and other analytical grade solvents

and reagents were procured from Sigma-Aldrich (St. Louis, USA); deionized water was used during the experiments.

2.3. Preparation of biocomposite films

Betanin NLPs were prepared by employing the thin film hydration-sonication method described in our previous study [20]. Edible films containing different ratios of PG and NLPs were prepared according to the experimental design. Biocomposite films were made by gently dissolving PG (0–2 %) in distilled water for 1 h at 90 ˚C. PG dispersions were hydrated overnight at room temperature before WPI was applied in a ratio of 5% w/v and the mixture was mixed for 30 min at 80 ˚C. In this way, the ratios of WPI to PG were set in the ranges from 5:0 to 5:2. Subsequently, the NLPs were added at levels of 0-10%, and the mixture was subjected to 15 min of vigorous shaking at 40 ℃. Then, glycerol was added at a constant volume of 40% w/v of dry matter (WPI) for all samples. The pH of the solutions was set at 9 using NaOH before 20 mL dispersions were cast into glass plates. Ultimately, the films were peeled off and re-conditioned at 53 ± 1% relative humidity (RH) and 25 °C for 48 h.

2.4. Characterization of films

2.4.1. Water vapor permeability (WVP)

In line with the procedure described by Khezerlou, Ehsani [9], the WVP of the samples was obtained gravimetrically. Firstly, constant amounts of anhydrous $CaCl_2$ were weighed in cells and then sealed with film samples using liquified paraffin. Next, the RH within the desiccators was adjusted to 75% using saturated NaCl solution, and alterations in the cell weights were recorded every 6 h till reaching a stable weight. Also, the system's weight gain was measured to determine water transfer over the film samples. Ultimately, the mass changes of the permeation cells were plotted against time; the slope of the function was fitted with linear regression (R^2>0.999), and the water vapor transmission rate (WVTR) was estimated according to the following equation:

$$WVTR = \text{slope}/(\text{exposed film area}): \Delta m/\Delta t \times \frac{1}{A} \quad (1)$$

It should be noted that the thickness of the film samples was measured beforehand. Finally, the WVP ($gPa^{-1}s^{-1}\,m^{-1}$) was calculated using Equation 2:

$$WVP = \Delta m/(\Delta t \times A) \times \frac{x}{\Delta p} \quad (2)$$

where Δm/Δt represents the moisture uptake over time (g/s), X is the thickness average (mm), A is the area of the exposed film samples (m^2), and Δp is the difference of water vapor pressure between inside and outside of the cell.

2.4.2. Film solubility in water

The film samples were kept overnight in a desiccator in the presence of calcium sulfate, before the films (Wi~ 400 mg) were mixed with a constant amount of distilled water for 24 h at room temperature. Finally, the remained samples were transferred to the oven 105 ℃ [21]. The dried films were then weighed to obtain the final weight (W_f), and water solubility was calculated according to Equation 3:

$$Water\ solubility\ (\%) = \frac{W_i - W_f}{W_i} \times 100 \quad (3)$$

2.4.3. Determination of antioxidant activity

For the assessment of antioxidant activity, 25 mg of films were submerged in 4 mL of distilled water under constant stirring; then, 2 mL of the solution was incubated with 2 mL of 0.1 mM DPPH methanolic solution. The resultant mixture was kept in dark at room temperature for one hour. Subsequently, the sample absorbance was measured at 517 nm using a UV-Vis spectrophotometer (Model T60 UV, USA). The free radical scavenging activity was approximated using Equation 4:

$$DPPH\ scavenging\ activity\ (\%) = \frac{Abs_c - Abs_s}{Abs_c} \times 100 \quad (4)$$

where Abs_c is the absorbance of the blank DPPH and Abs_s is the absorbance of the samples [22].

144 **2.4.4. Mechanical properties**

145 For each film sample, the ultimate tensile strength (UTS) and Young's modulus (YM) were
146 evaluated at room temperature with a universal testing machine (Model H100K-S, England)
147 according to the standard ASTM E96 method. To this end, the test samples were each cut into
148 a specific shape featuring width and length of 10 and 80 mm, respectively. The gauge size and
149 crosshead speed were 50 mm and 10 mm/min, respectively. Three replicates were done for
150 each process [23].

151 **2.4.5. Instrumental color analysis**

152 The primary color factors were evaluated using a colorimeter (Color Flex, M/s Hunter
153 Colorlab, USA). The parameters measured included L*and a*. The film samples were
154 positioned on a standard white plate.

155 **2.4.6. Scanning electron microscopy (SEM)**

156 The morphological properties of the films' surface and fracture structure were examined with
157 an SEM apparatus (Hitachi 4300S, Japan) at room temperature. The used voltage was 5.0 kV,
158 and the surface was coated by gold with a thickness of a few nanometers. For cross-sectional
159 analysis, the samples were visualized perpendicular to the fracture surface.

160 **2.4.7. X-ray diffraction (XRD) analysis**

161 The films' XRD measurement was completed using a Bruker D8 ADVANCE X-ray
162 diffractometer (Karlsruhe, Germany) working at a Cu Kα wavelength of 0.154 nm. The
163 samples were contacted to the X-ray beam with the generator operating at 40 kV and 40 mA.
164 The distributed radiation was identified at room temperature over the range of diffraction angle
165 $2\theta = 2^{\circ}$- 40° at a rate of 1°/min with a step size of 0.02° [24].

166 **2.4.8. Antimicrobial activity**

167 The inhibition zone method was implemented for the examination of the films' antimicrobial
168 assay against *Staphylococcus aureus* (ATCC 43300) and *Escherichia coli* O157: H7 (ATCC

35218) as typical Gram-positive and Gram-negative pathogenic microorganisms, respectively. To this end, 10 mL of molten Brain Heart Infusion (BHI) agar was poured into plates to which 200 µL of the bacterial cultures, featuring colony counts of 10^8 CFU/mL, were added. The film discs were prepared and situated on the bacterial lawn which were incubated at 37 ˚C for 24 h in an incubator. On the following day, the plates were evaluated for the inhibition zone of the film discs. The diameter of the whole zone was determined and then subtracted from the film disc's diameter.

2.5.Statistical design, analysis, and optimization

In this study, we applied central composite design with two independent variables, namely the levels of betanin NLPs (0-10% w/v) and PG (0-2% w/v), with no blocking of the experiments, resulting in 13 film treatments. Betanin NLPs and PG were considered as continuous numeric variables. Statistically, p-values of less than 0.05 were regarded as significant. Second-order polynomial modeling and data analysis were accomplished using Design Expert 10 (USA) statistical software.

In the next step, the optimum point was obtained considering the following goals: maximizing the,antioxidant capacity, UTS, a* (redness), and antimicrobial activity of the film, while minimizing its WVP, water solubility, and lightness. The optimum treatment was investigated for structural, morphological, and antimicrobial properties.

3. Results and Discussion

3.1. Water vapor permeability (WVP)

In packaging materials, low WVP values are desired for the extension of food shelf life as highly permeable films facilitate rapid product spoilage. Another key parameter is the WVTR, which reflects the hydrophilicity or hydrophobicity of the edible film, where hydrophilic films have higher WVTR values. The contour plots of WVP as a function of NLP and PG concentrations are shown in Figure 1. Our results indicated that the interaction effect of PG

and NLP addition on WVP was significant ($P<0.05$). The WVP capacity of edible films increased with higher PG concentrations but declined with the loading of NLPs to PG-containing films. Polysaccharides promote water permeation despite their suitable barrier properties against gases [11]. However, the incorporation of lecithin NLPs decreases the WVP, water absorption capacity, and moisture content of film samples [25]. In fact, the addition of NLPs to protein films can exert a plasticizing effect and decrease the film's solubility, thereby lowering its WVP. The NLPs, which are located in the links between the polymer matrixes, inhibit gas and water diffusion across the film [26]. Previously, similar trends were reported by Zhang, Liu [27], who showed that WVP declined by about 189% with the loading of only 5% lignin NLPs into a polyvinyl alcohol nanocomposite. However, as reported by Cejudo-Bastante, Cejudo-Bastante [28], red beet extract addition, without wall material, into film material caused an increment in WVP.

3.2. Water solubility

According to the data analysis, the interaction effect of NLPs and PG on solubility was significant ($P<0.05$). Through the simultaneous incorporation of NLPs and PG, the film solubility declined. The lowest solubility was obtained in the treatment with 10% NLPs and 2% PG. Solubility is related to both the hydrophilic/hydrophobic ratio of liposomes and the number of free hydroxyl groups in the polymer matrix available for hydrogen bonding with the polymers [29]. In the case of PG, the reduction in solubility can be attributed to the polymer constituents (soluble and insoluble fraction) and the reaction between the anionic PG and the positively charged protein in the film structure [5, 6]. Moreover, NLPs can bond with macromolecules, restricting molecular movement and thereby reducing the film's water solubility [30]. The notable point is that due to the significance of water solubility for the transportation of bioactive substrates, WPI/PG-based films can be proper instruments for the entrapment of bioactive compounds such as betanin and other anthocyanins. In line with our

observations, Alizadeh-Sani, Khezerlou [31] found that the incorporation of rosemary essential oil into a WPI-based film resulted in a meaningful decrease in the film's water solubility (from 46.66 to 25.33%) and water content.

3.3. Antioxidant activity

As a stable synthetic radical, DPPH binds to hydrogen atoms in a process that can be monitored through the amount of pure violet color loss or the decrease in absorbance. The obtained results confirmed that both NLPs and PG significantly improved the film's antioxidant capacity ($P<0.05$); a high adjusted R^2 value (0.99) was attained for the related predictive model (Table 1). According to the contour plot shown in Figure 2, by increasing the amounts of both PG and NLPs, the antioxidant capacity of the film improved from 30 to 75%. While such antioxidative activity is due to the presence of both PG and NLPs, the latter provides the major effect. Betanin (betanidin 5-O-D-glucoside) is recognized as a powerful antioxidant that exhibits excellent radical-scavenging activity. Persian gum-based polymer structures contain numerous arabinogalactans and probably possess antioxidative activity [32]. Our results are in agreement with those of Amjadi, Nazari [20], who demonstrated that film samples containing uncoated betanin or betanin NLPs offered greater antioxidant activity than control samples and that encapsulation with NLPs protected the betanin and augmented its antioxidative activity from 6.79 to 53.02%.

3.4. Mechanical properties

Films that maintain high UTS are considered suitable for packaging applications. The effects of NLPs and PG on the UTS of the samples were significant ($P<0.05$) and are depicted in Figure 3A. Notably, the maximum increment in UTS (from 1.95 to 4.96 Mpa) was achieved by the addition of 5% NLPs and 1% PG. In a study by Pak, Ghaghelestani [8], it was reported that the UTS of edible films containing PG was related to the gum/plasticizer ratio, such that increased gum content caused a reduction in tensile strength. The increment in UTS with higher

betanin NLP concentrations may be ascribed to the existence of lecithin (as a component of the NLPs) in the film's polymer structure. The incorporation of NLPs into protein films can exert a plasticizing effect and improve film extensibility. The NLPs can expand the film's tensile strength secondary to an intensified interaction between the NLPs and the protein matrix [26]. In this regard, Aziz and Almasi [24] found that by the addition of 15% *Thymus vulgaris* L. extract NLPs to WPI films, the UTS increased from 2.09 to 8.67 Mpa, which is consistent with our data.

Young's modulus (YM) or elasticity is a mechanical property that reflects the stiffness of a packaging film, defined as the association between stress (force per area unit) and strain (relational deformation) in the linear elasticity of a uniaxial deformation. Our results indicated that in the films containing both PG and betanin NLPs, an increase in PG concentration caused a significant decrement ($P<0.05$) in YM from 84.12 to 42.43 MPa. Figure 3B demonstrates the effect of betanin NLPs and PG on the YM of the film samples. These findings are in line with the results of Ghadetaj, Almasi [33], who incorporated the NLPs of *Grammosciadium ptrocarpum* Bioss. essential oil into WPI-based films and found that by the addition of 0 to 1.5% NLPs, the YM decreased significantly from 23.229 to 17.022 MPa ($P<0.05$). These researchers noted that this was because the essential oil molecules lowered the molecular strength of the film by diminishing the intramolecular relations present within the protein matrix. The remarkable effects of PG in the reduction of the UTS and YM of edible films containing gelatin and tragacanth gum were also reported by Khodaei, Oltrogge [10].

3.5. Color parameters

Optical parameters such as color and transparency represent significant aspects pertaining to consumer acceptance. The measured color parameters were L* and a*, which indicate the amount of lightness and redness/greenness, respectively. The results implied a significant effect of PG content on L* ($P<0.05$), while the effect of incorporating betanin NLPs was insignificant

(P>0.05). With the addition of 2% PG, the L* value decreased from 75 to 55. Pak, Ghaghelestani [8] explained that changes in the L* parameter in the presence of PG may be directly related to the homogeneity of the matrix, such that higher homogeneity results in greater transparency.

The a* (red/green) index significantly increased with the incorporation of betanin NLPs but decreased with PG addition. In this view and in agreement with our results, Amjadi, Nazari [20] manufactured a nanocomposite film containing 10% betanin NLPs and found that the L* value increased from 36.70 to 37.56 and the a* value increased from 15.52 to 18.24. It seems obvious that the fall in a* with PG loading is due to the turbidity induced by the presence of this gum in the film.

3.6. Optimization

The main objective of this study was to evaluate the effect of betanin NLP and PG incorporation on the structural and functional features of an edible film. As presented in Table 1, the polynomial second-order models were fitted to the data for each dependent variable through multiple regression analyses. Regression analysis was applied to model different responses as a function of the concentrations of betanin NLPs and PG. The regression coefficients are closely related to the factor effects; the higher the coefficient the higher the effect of that term in the model.

The response surface methodology (RSM) was used for the optimization, and the desirability function for each response was assessed by numerical methods to obtain the overall desirability. The responses of the optimal film are shown in Table 2. The optimal formulation for the whey protein-based film was obtained with a betanin NLP content of 6.90% and a PG concentration of 1.5%. Also, the overall utility of the predicted area for the available variables was 0.718. For reproducibility, two optimal samples were produced, and then tests were

repeated three times to confirm the accuracy. The optimum treatment was investigated in terms of its structural (SEM and XRD) and antimicrobial characteristics.

3.6.1. Scanning electron microscopy (SEM)

Scanning electron microscopy is a useful instrument for the evaluation of the surface and morphological aspects of nanostructures. According to our results, the surface of the control film was soft, continuous, and free of pores (Figure 4A). In contrast, the surface of the optimum treatment had greater roughness (Figure 4B). It must be noted that WPI-based films maintain a fully compact structure, with the spatial position of the polymers remaining consistent within the matrix and thereby preventing surface roughness. This could be related to the hydrophilicity of the proteins during phase separation. Corresponding with our results, Acquah, Zhang [34] reported that WPI films were observed to be homocomposites that formed continuous structures.

3.6.2. X-ray diffraction (XRD) study

The XRD spectra of the pure WPI, the PG, and the betanin NLPs are displayed in Figure 5 along with that of the optimal film sample. Crystal planes originate from crystal lattices and are employed to determine the structure and shape of the unit cell and crystal lattice [20]. Images of XRD provide structural information, including that of crystalline and amorphous polymeric structures like edible films. In XRD spectra, sharp diffraction peaks represent crystalline diffraction. The XRD results elucidated a crystalline structure for pure WPI films; the peak at $2\theta = 25^{\circ}$ indicates that the WPI crystallinity was relatively high. Therefore, the application of the WPI component contributes to the semi-crystalline nature of the biopolymer. Persian gum showed a vast peak at $2\theta = 25^{\circ}$, and the crystallographic index for WPI and PG were 26.45 and 23.11%, respectively. The XRD results for the pure betanin sample featured a broad peak at $2\theta = 22^{\circ}$. In the NLP-containing films, the mentioned peak intensity did not vary considerably from that of the control sample. This implies that the amphiphilic lecithin

molecules in the NLPs interacted with the matrix proteins. Hence, the addition of NLPs not only had no adverse effects on the film structure but also increased the film's density, thereby protecting its crystalline nature. These results indicated a high level of affinity between the WPI and the added betanin NLPs. The observed phenomenon is similar to that reported by Amjadi, Nazari [20], who showed that NLPs fulfill a valuable role in the protection of the film's crystalline nature according to XRD analysis.

3.6.3. Antimicrobial activity

The test for antimicrobial properties was based on the determination of the inhibition zone diameter around the film discs containing the antimicrobial substance. The inhibition zone values for *E. coli* O157:H7 and *S. aureus* were 1.6 and 2.23 mm, respectively. The larger the diameter of the inhibition zone, the greater the desirability of the film antimicrobial properties. The optimum film exerted a stronger effect against *S. aureus* (Gram-positive) than *E. coli* (Gram-negative). This is due to the outer membrane structure of *E. coli*; Gram-negative bacteria have a multiplex cell wall comprised of a peptidoglycan layer and an extra outer membrane. Notably, the outer membrane of a Gram-negative strain reduces the effect of betanin's phenolic groups and minimizes the permeability of radicals like reactive oxygen species [35]. In this light, Wu, Liu [36] reported that the enhanced antibacterial activity of films containing cinnamon essential oil NLPs probably is due to the protection of the compound loaded within the NLPs against the environmental conditions and the subcellular size of the NLPs, which increases their cellular absorption and leads to enhanced antimicrobial activity and decreased mass transfer resistance. In agreement with our findings, Amjadi, Nazari [20] noted that the antibacterial activity of NLP-containing film samples was higher against *S. aureus* than *E. coli*.

4. Conclusion

In this study, betanin NLPs were introduced to the WPI/PG matrix to prepare a novel bionanocomposite film with prominent mechanical and functional properties. The incorporation of the NLPs led to decreased WVP and solubility together with enhanced mechanical, antimicrobial, and antioxidant characteristics in the bionanocomposite films. The films were opaque due to the presence of PG. Furthermore, the interaction of PG with betanin WPI resulted in decreased solubility. However, the addition of PG caused a decrease in mechanical strength. The optimum packaging film was obtained at 6.9% betanin NLPs and 1.5% PG. The results of the current research showed that bio-nanocomposites based on WPI/PG/NLPs may have potential applications as a primary coating or packaging material for fresh and/or perishable products. Investigating the PG and WPI interaction at different pHs as well as the effect of the glycerol to PG/WPI ratio is recommended for further studies.

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Authors' contributions:

Zahra Ghasempour: Conceptualization; Project administration; Writing – original draft

Sepideh khodaeivandi: Investigation; Methodology; Validation; Writing - original draft

Hossein Ahangari: Investigation; Methodology; Writing - original draft

Hamed Hamishehkar: Resources; Supervision; Writing – review & editing

Sajed Amjadi: Investigation; Methodology; Writing – review & editing

Ehsan Moghaddas Kia: Conceptualization; Formal analysis; Writing - original draft

Ali Ehsani: Funding acquisition; Resources; Writing – review & editing

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501 **Table 1.** Regression coefficients of the second-order polynomial model for the response
502 variables.

Response	Regression equation	Brief model
Solubility	Solubility =1.68 - 0.08×A[a] - 0.14×B[b] + 0.05×AB - 0.08×A^2	R-sq = 0.92 R-sq(adj) = 0.88
Thickness	Thickness = 0.24 + 0.01×A + 0.02×B - 0.01×AB	R-sq = 0.96 R-sq(adj) = 0.95
AC[c]	Antioxidant activity = 54.5+ 19.47×A + 2.98×B - 3.94×A^2 + 2.01×B^2	R-sq = 0.99 R-sq(adj) = 0.99
WVP[d]	WVP = 5.99E-07 + 2.14E-09×A + 2.03E - 08×B - 6.28E - 08×AB + 6.26E -08×A^2	R-sq = 0.89 R-sq(adj) = 0.82
UTS[e]	UTS = 4.79 + 0.32×A - 0.31×B - 0.78×A^2	R-sq = 0.89 R-sq(adj) = 0.88
EB[f]	EB = 99.4 - 2570.73A - 1.11B + 33.21A× B + 104852.35A^2 + 0.18B^2	R-sq = 0.99 R-sq(adj) = 0.98
YM[g]	YM = 77.52 + 3.03×A - 9.4×B - 22.23×A^2	R-sq = 0.80 R-sq(adj) = 0.74

503 [a] Betanin nanoliposomes % (W/V), [b] Persian gum % (W/V), [c] antioxidant capacity, [d] water vapor
504 permeability, [e] ultimate tensile strength, [f] elongation at break, [g] Young's modulus

505 **Table 2**. Parameters response of optimal film using response surface methodology (RSM)

NLP[a] (%)	PG[b] (%)	WVP[c] (g/Pa.s.m)	Solubility (%)	AC[d] (%)	UTS[e] (Mpa)	YM[f] (Mpa)	L[g]	a[h]	b[i]	Desirability
6.90%	1.5%	5.46	1.57%	63.45%	4.64	70.75	56.74	-5	29.88	0.71

506 [a] Nanoliposomes, [b] Persian gum, [c] water vapor permeability, [d] antioxidant capacity, [e] ultimate tensile
507 strength, [f] Young's modulus, [g] lightness, [h] red/green, [i] yellow/blue
508

Figure captions:

Schematic 1. Chemical structure of betanin

Figure 1. The interaction effect of betanin nanoliposomes and Persian gum amount on the water vapor permeability in the whey protein isolate-based edible film (toward red indicating higher values of response and toward blue indicating lower values of response).

Figure 2. The effect of betanin nanoliposomes and Persian gum (minimum [0%] and maximum level [2%]) on the antioxidant capacity of the whey protein isolate-based edible film.

Figure 3. The betanin nanoliposomes and Persian gum effect on (A) ultimate tensile strength and (B) Young's modulus of the whey protein isolate-based edible film (toward red indicating higher values of response and toward blue indicating lower values of response).

Figure 4. SEM images: (A) control sample; (B) optimal film containing 6.9% betanin nanoliposomes and 1.5% Persian gum.

Figure 5. XRD spectra: (A) pure whey protein isolate; (B) pure Persian gum; (C) betanin nanoliposomes; (D) optimal film sample (6.9% betanin nanoliposomes and 1.5% Persian gum).

Figures

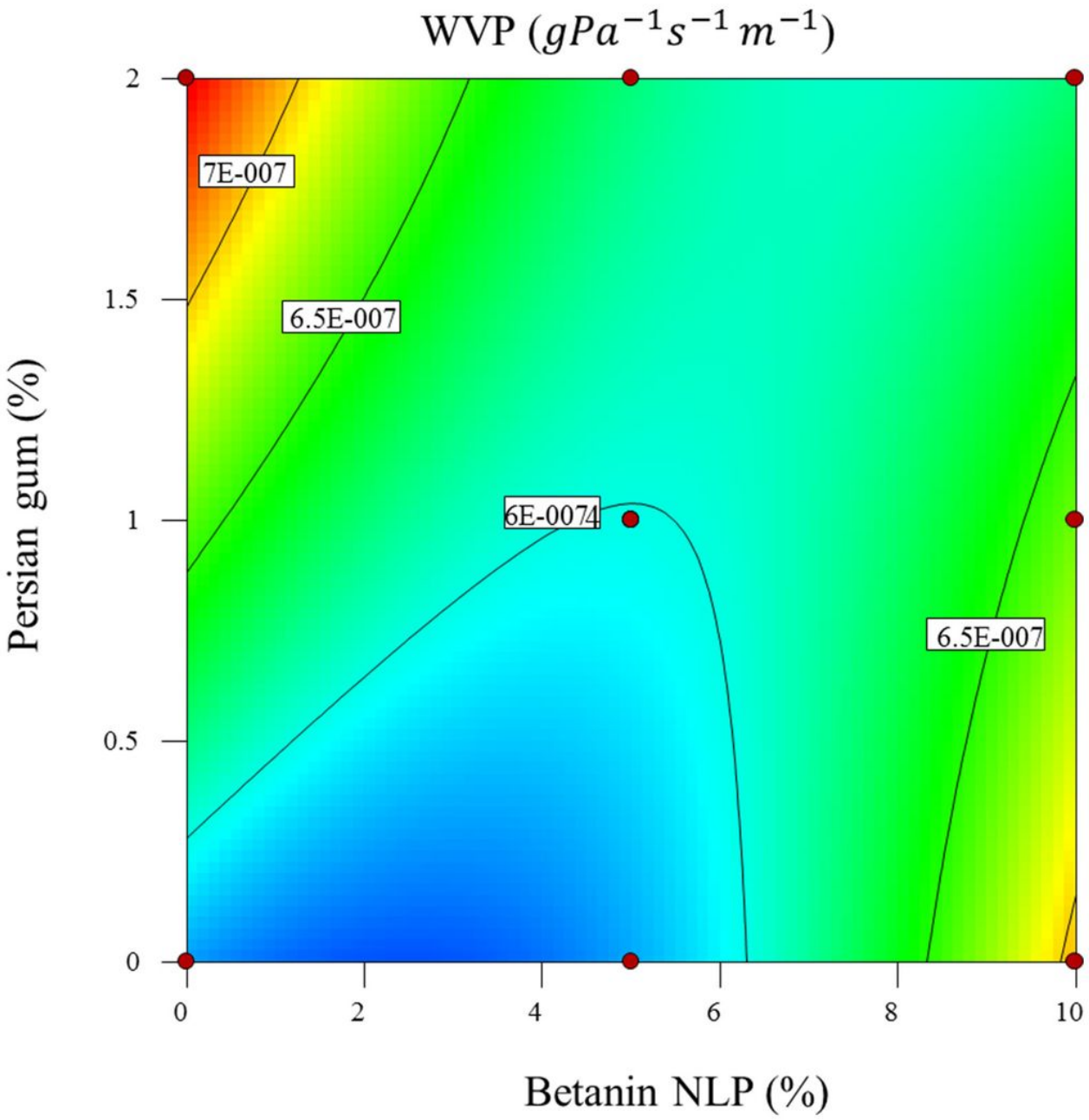

Figure 1

The interaction effect of betanin nanoliposomes and Persian gum amount on the water vapor permeability in the whey protein isolate-based edible film (toward red indicating higher values of response and toward blue indicating lower values of response).

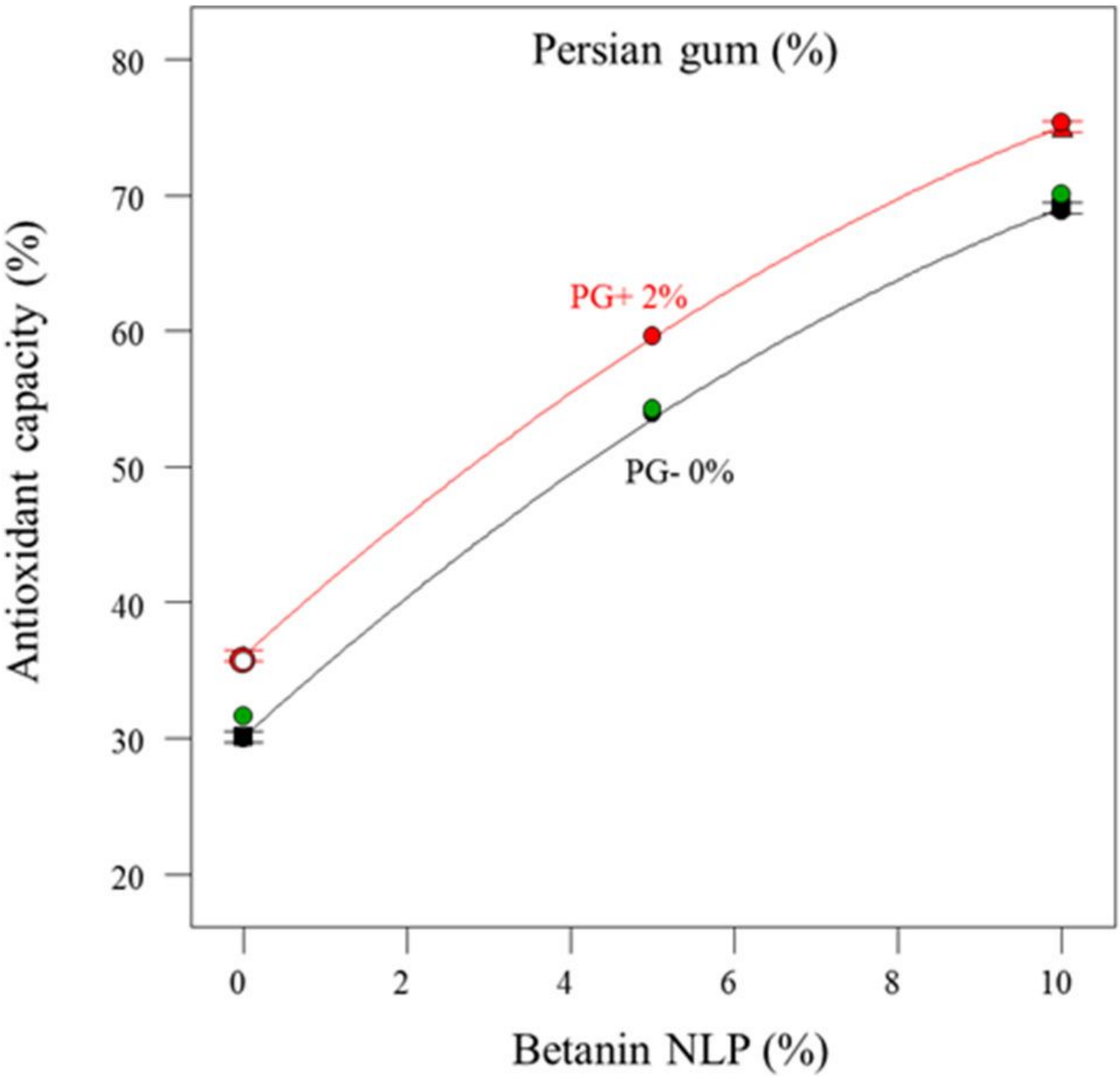

Figure 2

The effect of betanin nanoliposomes and Persian gum (minimum [0%] and maximum level [2%]) on the antioxidant capacity of the whey protein isolate-based edible film.

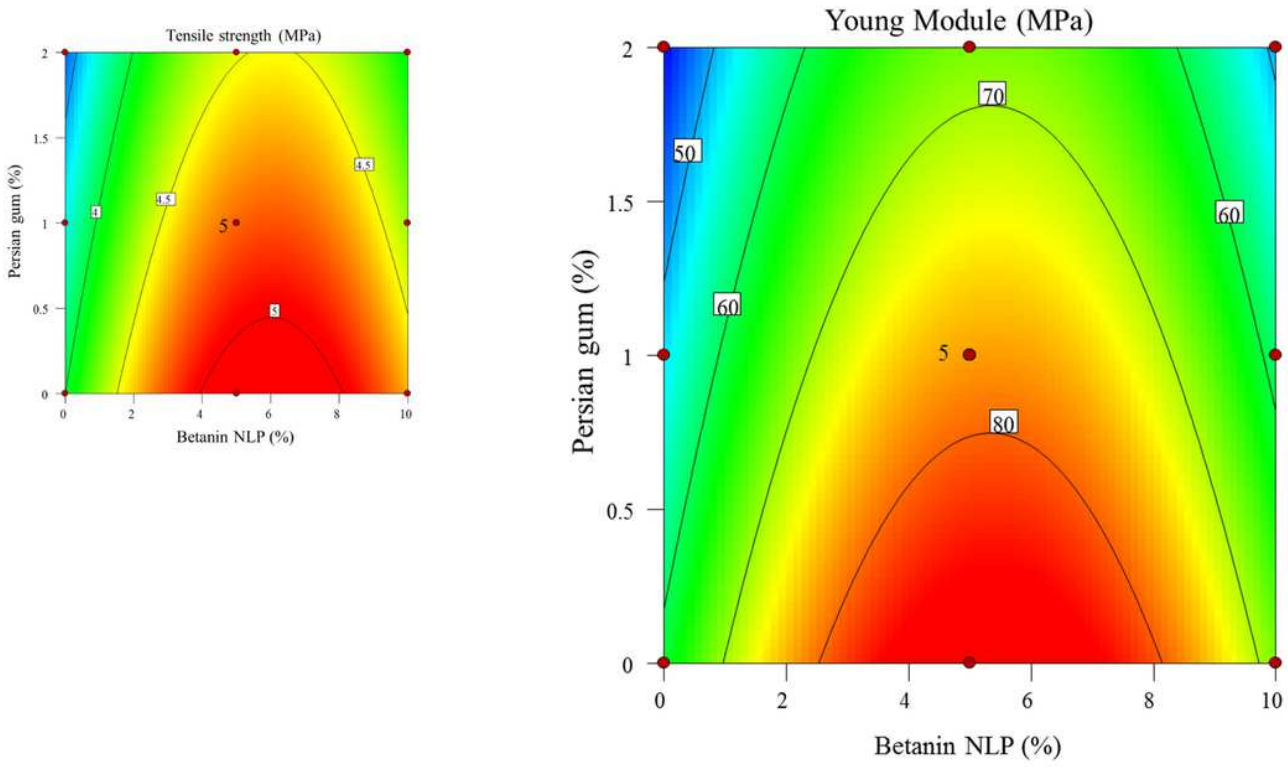

Figure 3

The betanin nanoliposomes and Persian gum effect on (A) ultimate tensile strength and (B) Young's modulus of the whey protein isolate-based edible film (toward red indicating higher values of response and toward blue indicating lower values of response).

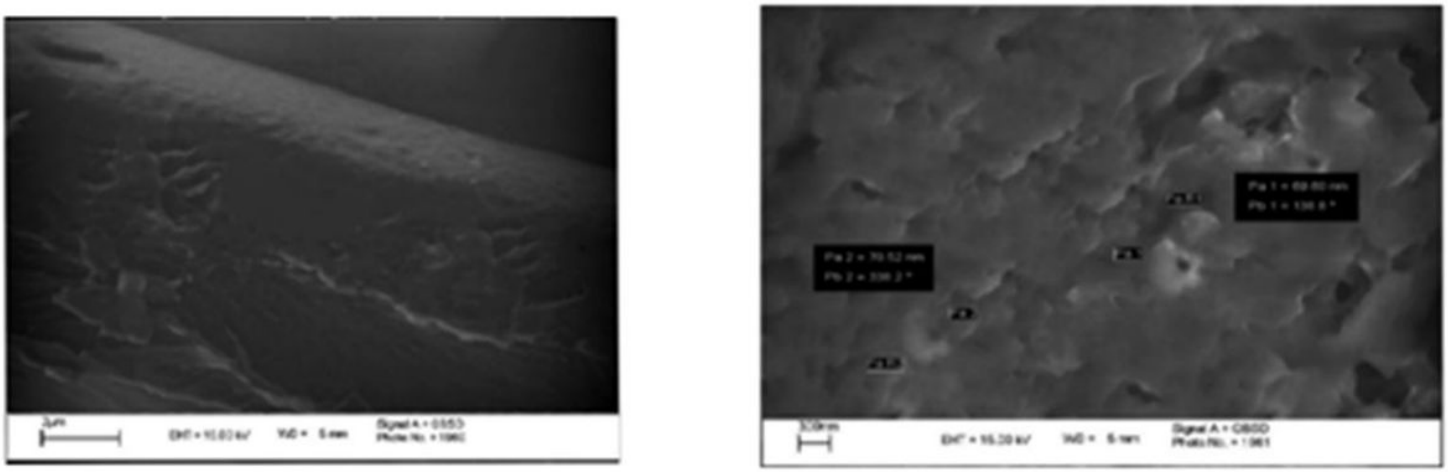

Figure 4

SEM images: (A) control sample; (B) optimal film containing 6.9% betanin nanoliposomes and 1.5% Persian gum.

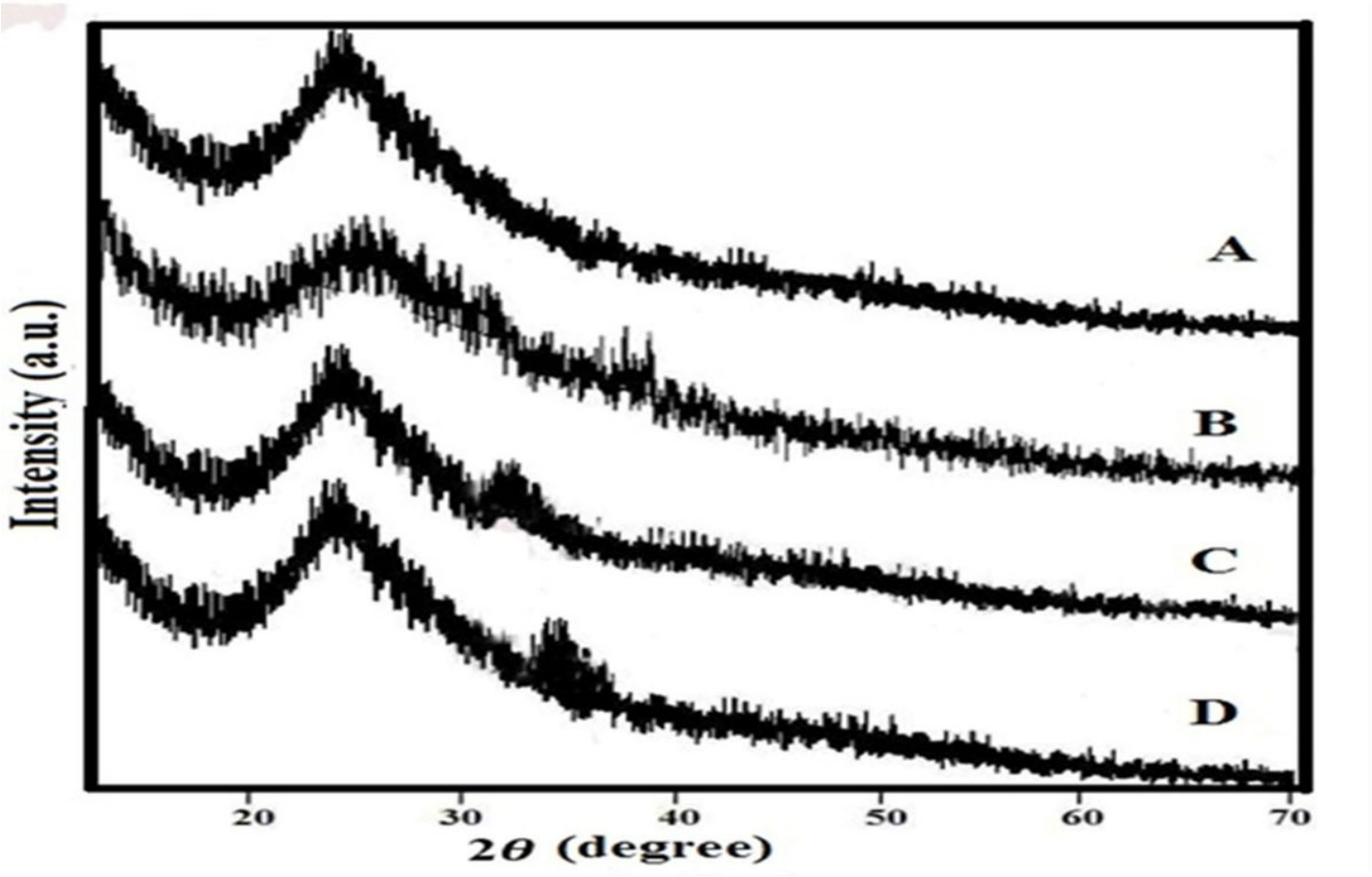

Figure 5

XRD spectra: (A) pure whey protein isolate; (B) pure Persian gum; (C) betanin nanoliposomes; (D) optimal film sample (6.9% betanin nanoliposomes and 1.5% Persian gum).

Image not available with this version

Figure 6

Schematic 1. Chemical structure of betanin