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Optimization of Gelatin-Persian gum-based edible film

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ARTICLE INFO	ABSTRACT
Article History:	In this study, an edible film based on gelatin-Persian gum was prepared and optimization of the edible film was done using Design Expert and
Received 2022/ 04/ 05 Accepted 2022/ 07/ 02	Mixture Design. The resulting films were subjected to various physicochemical tests, including pH, total solid, heat seal ability,
Keywords:	tensile strength, contact angle, calorimetric and moisture absorption. At first, modeling of responses was done using data regression analysis,
Edible film, Gelatin, Heat seal ability, Persian gum, Tensile strength.	and then 3D charts were drawn to show the effects of gelatin (0.5 -1), Persian gum (0.5 -1), and Glycerol (0-0.5) on the film characteristics. Finally, Numerical optimization based on optimization goals was performed and the optimal point with the highest desirability (0.78) was obtained. The ratio of each of the independent variables in the optimal formula was 0.5. Various properties of the optimal film
DOI: 10.22034/FSCT.19.129.1 DOR: 20.1001.1.20088787.1401.19.129.6.3	including pH, total solid, heat seal ability, tensile strength, contact angle, moisture absorption, L*, a* and b* were obtained at 6.66, 95.61%, 84.45 N/M, 64.5 N, 77.34°, 4.5%, 70.98, 0.97 and 0.66 respectively. Generally, the findings proposed that the gelatin-persian
*Corresponding Author E-Mail: Hannan.Lashkari@iau.ac.ir	gum-based edible film can be used as a food packaging material.

1- Introduction

Recently, great attention has been directed to renewable and eco-friendly edible coating and films due to the concerns regarding the quality and safety of various food products as well as the environmental problems associated with the wide application of non-biodegradable plastic wastes [1]. One of the major characteristics of edible films is the substantial water permeability compared with common plastic films [2]. Edible film and coating have been widely used to increase the shelf life of various food products including meat and cheese. In order to prepare such systems, biopolymers and food-including carbohydrates or gums and protein, as well as lipids have been used to prepare film coating agents [3]; [4].

Although synthetic materials have been applied to produce the edible film, gelatinbased materials have attracted substantial attention due to their high strength and translucency [5]. The presence of carboxyl and amino groups in gelatin structure leads to the formation of a positively charged structure at pH values lower than the isoelectric point (pI ~ 8.5 for type A and pI \sim 4.7 for type B) [6]. In the temperatures higher than 60 degrees Celsius gelatin dissolves in water while it forms a gel around 10 degrees Celsius. The physicochemical properties of gelatin could be improved by changing the functional groups in its structure. Considering the formation of positively charge gelatin structures at pH values lower than their pI and the negatively charged structures at pH values higher than its pI, gelatin could be considered a suitable material for the formation of polyelectrolyte complexes with positively charged polymers as well as negatively charged ones [7]; [6]. Gelatin has shown some particular features making it an appropriate material for edible packaging. These properties are great filmforming ability, high abundance and excellent safety record, as well as biodegradability and gas barrier properties. In order to increase the shelf life and safety of meat [8] and fruits [9], during the storage, gelatin coatings have been proposed [8]. Since the application of single gelatin films has resulted in various problems such as weaknesses, brittleness and weak tensile properties [10]. According to this result, single gelatin films can be improved by

adding some kind of polymers such as polysaccharides and lipids.

Persian gum is mainly extracted from almond trees (Amygdalus scoparia Spach) which grow in central regions of Iran. This gum which is also called Shiraz, Farsi, Zudu and Angum gum could be categorized as a natural hydrocolloid material [11]; [12]. Depending on various parameters such as gum color and the origin of the trees, Persian gum could be achieved at different molecular weights. The molecular weight of the gum has shown substantial impact on the interaction with proteins and polysaccharides. In other words, there is an optimal range of molecular weight making the gum ready for complexation and coacervation [13]. The natural Persian gum dissolves in cold water at 30% (w:w) while it is insoluble at 70% (w.w). Soluble fractions are quickly solved in cold water. On the other hand, hot water is able to partially dissolve the other fractions [14]. The water soluble fraction of Persian gum is able to form fragile films while the insoluble fractions have shown no ability to form films even following the addition of metal ions and plasticizers [15]; [16]. These materials are added to improve the physicochemical properties enabling them to form films [17]. Hence, the current study aimed to develop and optimize an oral film containing Persian gum, gelatin and glycerol with good texture and suitable elasticity.

2- Materials and methods

2-1- Materials

Bovine gelatin with bloom 200 was obtained from Gelatin Halal Co. (GHC, Tehran, Iran); Persian gum was purchased from a store in Shiraz (Fars Province, Iran); Glycerol as the plasticizer, was prepared from Merck (Darmstadt, Germany).

2-2- Experimental design

In the current investigation, the effects of gelatin (0.5 -1), Persian gum (0.5 -1), and Glycerol (0-0.5) on the film characteristics were determined by Simplex-Lattice mixture design. This experimental design was carried out in the form of 14 treatments. The levels of raw material for treatments are presented in Table 1.

Table 1	1 The	variables	and	levels	for	experimental 3	k

Run	Gelatin	Persian Gun	Glycerol
1	0.50	0.75	0.25
2	0.50	0.50	0.50
3	0.58	0.83	0.09
4	1.00	0.50	0.00
5	0.50	0.50	0.50
6	0.50	1.00	0.00
7	0.75	0.75	0.00
8	0.67	0.67	0.16
9	0.58	0.58	0.34
10	0.75	0.50	0.25
11	0.75	0.75	0.00
12	0.83	0.58	0.09
13	1.00	0.50	0.00
14	0.50	1.00	0.00

^{* (}ratio of mixture, Gelatin + Persian Gum + Glycerol = 1.5)

2-3-Film preparation

Gelatin (5%, w/v), Persian gum (5%, w/v) and glycerol (3%, w/v) suspension, were made by dispersing them separately in distilled water homogenized with a two-stage homogenizer with 180 Bar. The solutions were mixed according to the volume ratios in the experimental design (Table 1). The mixtures were homogenized at room temperature (25°C) by a stirrer for 1 hour. The films were prepared by casting 30 ml of the mixture on a petri dish. In order to make a thin layer, the solutions were spread on the surface by a glass rod. To dry the suspensions, the mixtures were maintained at (25±2 °C and 50±5% RH for 48 hours and put the petri dish film in-to the sealable desiccator for 24 hr. After this time the film was removed from the petri dishes and stored in ambient conditions.

2-4- pH test

for determining the pH, first, mix the film in 10 cc water and wait for absorbed water then was determined by pH meter from Mettler Toledo company (model: Seven Compact S-220, USA) as ISIRI 2852.

2-5- Total solid

A drying method was used to measure the total solid of the films as described previously. Briefly, the samples were dried in the oven at 103 ± 1 °C in two replications [18]

2-6- Heat seal ability test

In order to assess the heat seal ability, all the samples were cut into similar stripes (5×5 cm) followed by putting one stripe on the top of the previous one. Forty-eight hours prior to the

experiment, all the samples were maintained at 25 ± 2 °C and $50\pm5\%$ RH. Then, drops of distilled water were employed onto the sealing areas followed by inserting the stripes into an automatic heat sealer (AH26 Mod: Go plus 8, IRAN). Sealing temperature, dwell time and pressure were automatically assessed and the resulting welds were analyzed according to ASTM E88-07a (ASTM, 2007) with an Instron universal instrument model no. 5543A (Instron Engineering Corp., Norwood, MA, USA). The seal strength (N/m) was calculated by dividing the maximum peak force (force max) by the film width [19].

2-7- Tensile strength

There are numerous tests for tensile strength measurement and we chose one of them. Our technique that we used, was cut in to 5mm*5mm film using a Horbat slicer and determined the resistance of films from tensile with an Instron universal instrument model no. 5543A (Instron Engineering Corp., Norwood, MA, USA). The seal strength (N/m) was calculated by dividing the maximum peak force (force max) by the film width [20].

2-8- Contact angle

Wetting angles of the edible films were calculated with the images taken by a CCD camera (Canon EOS D30) (standard AT-9995).

2-9- Calorimetric

A Minolta colorimeter (CR-20, Konica Minolta, Inc., Tokyo, Japan) was employed to determine the colorimetric parameters of edible films. The results were reported based

on the CIE L* (luminosity, ranging from 0 - dark- to 100 -bright), a* (color transition from green to red), and b* (color transition from blue to yellow) coordinates. The colorimeter was calibrated with a white reflector plate to prior the experiments.

2.10 Moisture absorption

The method of Angeles and Dufrense was used in order to assess the moisture absorption of the films. The films were cut to 20 mm × 20 mm. a desiccator containing calcium sulfate was used to prevent the effect of humidity. This condition leads to a relative humidity of 0%. Following the initial weighing, the samples were transferred to a desiccator containing potassium sulfate at a relative humidity of 98% and placed at a temperature of 25-25 °C. Then the weight of the samples was measured at 48hr times until reaching a constant weight [21].

2.11 Film microstructure

The surface morphology of films was observed using a scanning electron microscope (TESCAN vega3, Czech Republic). First, film cuts were placed on the specimen holders using aluminum tape and then covered with a gold layer using a sputter coater (Desk Sputter Coater, Nanostructural Company, Iran).

2.12 Statistical analysis

In order to determine the experimental design and analyze the data, Design Expert®9.0 software was employed. In the Simplex-Lattice mixture design, the variables are part of the composition of a mixture, and by changing their ratio, the response will be different. The responses are the same different properties of the mixture that are affected by the change in the ratios of the variables. The relationship between each of the responses with the independent variables was obtained using different models. The models were evaluated with adj-R² coefficients and Lack of Fit test. Finally, based on the mechanical properties of the film, optimization was performed and the optimal formulation for film production was determined [22].

3- Results and discussion

3-1- Models

The properties of the film were modeled in the form of a polynomial equation as a function of independent variables, and using ANOVA, the significance of the linear and interaction effects of regression model coefficients was investigated. The adj-R² coefficients of the models were between 0.77 and 0.99. The Lack of Fit test was no significant for all properties measured at 95% confidence level. Therefore, high adj-R² coefficient and not significant lack of fit of the model confirms the accuracy of the model for predicting features. By substituting different percentages of gelatin, Persian gum and glycerol in the equations, the numerical value of the film properties can be predicted. The Predictive models are presented in Table 2.

Table 2 Predictive models for characteristics of gelatin-Persian gum-based edible film

Responses	Predictive models	Model	adj-R ²
рН	+6.65A+6.25B+6.66C-0.0924AB- 0.0755AC-0.0069BC	Quadratic	0.86
Tensile strength	+4.62A+0.9857B+64.48C+1.05AB- 100.11AC-87.60BC+408.70 A ² BC+460.00 AB ² C- 442.57ABC ²	Special quartic	0.99
Total solid	+96.36A+96.28B+95.61C	Linear	0.77
	+9.40A+1.90B+84.45C+4.47AB-		
Heat seal ability	88.22AC-100.54BC+502.82	Special quartic	0.99
	A ² BC+353.42AB ² C-498.74 ABC ²		
Moisture sorption	+7.64A+7.54B+4.50C-0.3297AB-9.34AC- 9.96BC-42.78ABC	Special cubic	0.91
Contact angle	+72.68A+72.27B+77.36C	Linear	0.97
L*	+70.69A+54.46B+70.98C	Linear	0.95
a*	-0.7034A+1.01B+0.9689C- 0.6873AB+0.9904AC-2.73BC	Quadratic	0.89
b*	+0.7962A+1.02B+0.6571C-3.54AB- 2.49AC-2.54BC	Quadratic	0.86

3-2- pH

The effects of gellatin (A), Persian gum (B) and glycerol (C) on the pH of the film are presented in Fig. 1. The pH showed a considerable variation from 6.63 to 6.72. The value of the calculated coefficient adj-R² of pH (0.86) showed that only about 14% of the total variation for optimization of pH couldn't be described by this model. Increasing the amount of Persian gum in the film caused an increase significantly in pH. Since the pH of Persian gum is 6.9 and is higher than gelatin, it causes and an increase in pH. In this formulation the pH of the glycerol is 6.5 and the pH of the gelatin is 6.6. Dabestani et al. (2018) [14] and Abbasi (2017) [23] reported that the pH of Persian gum solution is between 4.30 to 4.90.

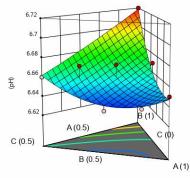


Fig 1 The effects of gelatin (A), Persian gum (B) and glycerol (C) on the pH of the film.

3-3- Tensile strength

The influences of gellatin (A), Persian gum (B) and glycerol (C) on the tensile strength of the film are presented in Fig. 2. The result showed a considerable variation in tensile strength from 0.98 to 65.9 (N). The value of the calculated coefficient adj-R² of the tensile strength (0.99) showed that only about 1% of the total variation for optimization of tensile strength couldn't be described by this model. The tensile strength of the films is a key factor in good film acceptance by users. The highest tensile amount was found in film with 0.5% glycerol and the lower tensile amount showed without glycerol. In fact, with increasing glycerol levels, the tensile index increases and with increasing the amount of gelatin and Persian gum, the tensile decreases. It is well known that plasticizers modify the functional properties of biopolymer films reducing intermolecular forces and increasing the mobility of polymer chains, causing the mechanical strength of the films to be decreased and the flexibility and extensibility enhanced [24]. Jridi et al. (2019) evaluated the mechanical properties of gelatin edible film they reported that the tensile strength of films was between 6.15 to 6.53 MPa [8]. Ghiasi, Golmakani, Eskandari, and Hosseini (2020) studied the mechanical properties of Persian gum. They reported that tensile strength of Persian gum is 4.35 MPa [25]. The electrostatic interaction between WPI and PG can also contribute to strengthening the film network [26].

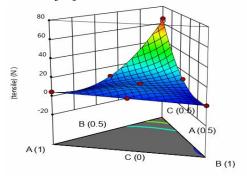


Fig 2 The effects of gelatin (A), Persian gum (B) and glycerol (C) on the tensile amount of the film.

3-4-Total solid:

In fig. 3, the total solid of studied samples are presented. The result showed a considerable variation for total solid from 95.6 to 96.5(%). The value of the calculated coefficient adj-R² of tensile strength (0.77) showed that about 23% of the total variation for optimization of total solid couldn't be describe by this model. According to the fig. 3, with raise in the ratio of gelatin and persian gum significantly increases the total solid of the film. Ghiasi et al. (2020) evaluated the propeties of Persian gum edible film. They reported that the dry matter content of Persian gum edible film with 0.6% glycerol was 92% [25]. Nor, Nazmi, and Sarbon (2017) evaluated the physicochemical propeties of gelatin edible film. They reported that the dry matter content of the film was 92.16%. Also, they reported that gelycerol had significant effects on the moisture content of samples and with increasing glycerol, moisture content increased. Low vapor permeability film that reduces the transfer of moisture between the outer atmosphere and the food environment is a determining factor in the application of film produced in food packaging [27].

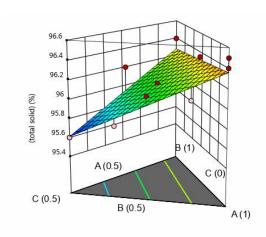


Fig 3 The effects of gelatin (A), Persian gum (B) and glycerol (C) on the total solid of the film.

3-5- Heat seal-ability

The influences of gelatin (A), Persian gum (B) and glycerol (C) on the heat seal-ability of the film are presented in Fig. 4. The heat seal ability of the samples is between 1.9 and 85 (N/M). The value of the calculated coefficient adj-R² of (0.99) showed that about 1% of the total variation for optimization of heat seal ability couldn't be described by this model. The heat seal ability of the films is an important feature because it can ultimately determine the physical properties of packaging materials. If the seal ability of films is weak we cannot use it for packaging because it causes oxygen to penetrate into the film and affects the product [28]. According to the fig 4, whenever the glycerol level increases, the film heat seal ability level increases, it should be noted that with increasing the amount of gelatin and Persian gum, the amount of film heat ability decreases. Nilsuwan, Benjakul, and Prodpran (2017) evaluated the heat seal ability of gelatin film. They reported gelatin film had heat seal ability 38% [29]. Nilsuwan, Benjakul, and Prodpran (2018) determined the heat seal ability of gelatin film. They reported heat seal ability of gelatin film with different concentrations of polylactic acid were between 34.16 to 49.69 % [30].

The remarkable increase in the heat seal strength of the blend films also posted the existence of intermolecular interactions between Konjac glucomannan and gelatin [31].

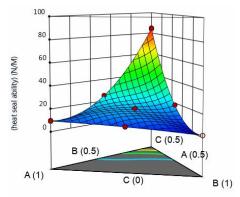


Fig 4 The effects of gelatin (A), Persian gum (B), glycerol (C) on the heat seal-ability of the film.

3-6- Moisture sorption

The influences of gelatin (A), persian gum (B) and glycerol (C) on moisture sorption of the film are presented in Fig. 5. The moisture sorption showed a considerable variation from 2.9 to 7.69 (%). The value of the calculated coefficient adj-R² of (0.91) showed that only about 9% of the total variation for optimization of moisture sorption couldn't be described by this model. At the point with the lowest moisture absorption, the levels of Persian gum, gelatin and glycerol are 0.67, 0.67 and 0.16, respectively. At ratios greater than 0.67 Persian gum and gelatin, the amount of water absorption increases and at ratios less than 0.67 gum and gelatin, the role of glycerol in water absorption increases. Hazaveh, Mohammadi Nafchi, and Abbaspour (2015) studied the water absorption of cold water fish gelatin films as functional properties of They reported the water biopolymers. absorption of the control film was 1 g/g. also, they reported that the contnet of OH group on the structure of polymer is the main factor in the water absorption [32]. Park, Nam, Yun, Jin, and Kwak (2021) evaluated the water absorbtion of fish gelatin film. They reported water absorption capacity of gelatin film was 200%. The absorption occurs because water molecules that diffuse from the surface are entangled in the 3D network structure of the polymer [33]. The monolayer values are important because they represent the ideal moisture content to be obtained both in the drying processes and in the storage of the material, which will guarantee the stability of the product, avoiding chemical and biological reactions [34].

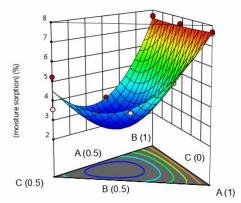


Fig 5 The effects of gelatin (A), Persian gum (B) and glycerol (C) on the moisture sorption of the film.

3-7- Contact angle

The effects of gellatin (A), Persian gum (B), and glycerol (C) on the contact angle of the film are shown in Fig. 6. The contact angle of the sample is between 72 and 77.73°. The value of the calculated coefficient adj-R² of (0.97) showed that only about 3% of the total variation for optimization of Contact angle couldn't be describe by this model. Contact angle monitoring is important in terms of hydrophilicity or hydrophobicity of the production film [35]. The larger the contact angle, the more hydrophilic the film indicates [36]. As can be seen in Figure 6, increasing the ratio of gelatin and Persian gum has reduced the contact angle and in contrast, increasing the glycerol ratio has increased the contact angle. Therefore, the increase of gelatin and Persian gum increases the hydrophilicity of the film. Ghiasi et al. (2020) evaluated the contact angle of persian gum edible films. They reported that the contact angle of persian gum edible film was 43.58 ° [25]. Wang et al. (2018) studied the effect of kafirin and gelatin on the contact angle of edible film. They reported that with the addition of gelatin contact angle significantly increased. The contact angle of kafirin and kafirin/gelatin edible film was 43 and 110°, respectively [37]. Ahammed, Liu, Khin, Yokoyama, and Zhong (2020) evaluated the contact angle of gelatin and zein film. They reported that with the addition of gelatin into zein film contact angle significantly increased from 52.5 to 104.8 ° [38].

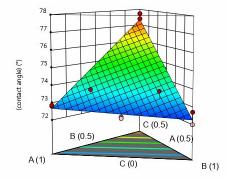


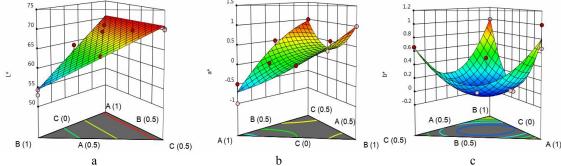
Fig 6 The effects of gelatin (A), Persian gum (B), glycerol (C) on the contact angle of the film.

3-8- Color properties

Color is one of the appearance characteristics of food that affects the quality perception of the consumer of the product and plays an important role in the acceptance of the product by the consumer. In fact, color in food is obtained from natural colors or color compounds produced during the process [39]. The color of films was described with the help of a digital colorimetry method, according to Afshari-Jouybari and Farahnaky (2011) [40]. The L*, a* and b* showed a considerable variation from 53 and 70.33, -1 to +1 and -0.1 to 1, respectively. The value of the calculated coefficient adj- R^2 of L*, a* and b* (0.95, 0.89, and 0.86), respectively showed only about 5, 11 and 14% for optimization of L*, a* and b* of the film of the total variation couldn't be described by the model. The effects of gellatin, Persian gum and glycerol on L*, a* and b* of the film are shown in fig. 7. The L* factor shows the brightness. The value of L=0 is equivalent to darkness and L=100 is equivalent to brightness [41]. The highest brightness is when the amount of gelatin and glycerol is at the maximum amount used and the highest amount of darkness occurred when Persian gum was used in the highest amount used in the films. The a* factor shows the green and red colors in the films. The value of (+a*) is equivalent to red and (-a*) is equivalent to green. As shown in Fig. 7, with increasing Persian gum and glycerol, the amount of a* factor increases, but gelatin decreases a* factor. The b * factor indicated the yellow and blue colors of the films. The value of (+b) is equivalent to the yellow and (b) is equivalent to blue [41]. The yellowing of the film is minimal when the ratios of all 3 variables are in the middle levels. As each of the variables increases, the yellowing of the

film increases. Ghiasi et al. (2020) evaluated the color properties of Persian gum edible films. They reported that the L*, a* and b* value of persian gum edible film was 87.5, 3 and 9.5, respectively [25]. Similar to our results Wang et al. (2018) studied the effect of kafirin and gelatin on the color properties of edible film. They reported that with the addition of gelatin, L value significantly increased a* and b* value significantly

decreased. The L*, a* and b* value of kafirin and kafirin/gelatin edible film was (52.5, 2.2 and 18.5) and (83.9, -1.3 and -0.7), respectively [37]. Also, Ahammed et al. (2020) evaluated the color properties of gelatin and zein film. They reported that with addition gelatin into zein film L* value significantly increased from 90 to 92 and a* value decreased from -2 to -1 and b* value decreased from 12 to 3 [38].

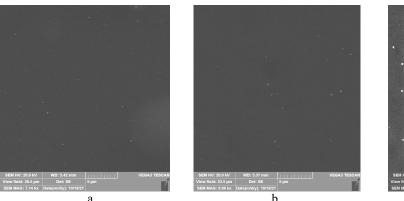


a b c Fig 7 The effects of gelatin (A), Persian gum (B) and glycerol (C) on the L^* (a), a^* (b) and b^* (c) factors of the film.

3-9- Film microstructure

Fig.8 represents the microstructures of the films were obtained by SEM. The surface of the gelatin and Persian gum film was relatively compact, and we can see the homogeneous

and smooth surface microstructure without pores, visible insoluble particles and cracks. In the (fig.8 C) contained a small number of spherical particles which might be attributed to the formation of insoluble complexion.



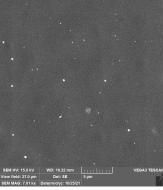


Fig 8 SEM micrographs of gelatin film (a), Persian gum-base film (b) Gelatin-Persian gum-based (1:1) (c)

3-10- Optimization

Numerical optimization is one of the most popular and most frequently used approaches to simultaneous optimization of the desirability function approach. Individual goals (table 3) are combined into a single objective measure to be maximized using a geometric mean function. It is possible to obtain overall desirability from the individual desirabilities [42]. The numerical value of desirability is between zero and one and its

low level indicates that the target is not reachable, while its high level indicates that the target is fully met. When the goal is to optimize multiple responses simultaneously, the desirability of each of the responses is determined and their geometric mean is used as an indicator of the simultaneous fulfillment of the goals. Based on the goals for each property of the film, numerical optimization was performed and the optimal point with the highest desirability (0.78) was obtained with a ratio of independent variables of gelatin,

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Persian gum, and glycerol 0.5. The characteristics of the film at the optimal point were listed in Table 3. Finally, the film was

produced with an optimal formulation, its image can be seen in Fig. 9.

Table 3 optimization Goals and characteristics of the film at the optimal point

Name	Goal	Lower Limit	Upper Limit	Optimal point
Gelatin	is in range	0.5	1	0.5
Persian Gum	is in range	0.5	1	0.5
Glycerol	is in range	0	0.5	0.5
Tensile strength	Maximize	0.98	65.9	64.50
Total solid	is in range	95.6	96.5	95.61
рН	is in range	6.63	6.72	6.66
Heat seal ability	Maximize	1.9	85	84.45
Moisture sorption	Minimize	2.9	7.69	4.50
Contact angle	Maximize	72	77.73	77.36
L^*	Maximize	53	70.33	70.98
a*	is in range	-1	1	0.97
b*	Minimize	-0.1	1	0.66



Fig 9 Optimized edible film based on gelatin-Persian gum

4- Conclusions

The edible film based on gelatin-Persian gum prepared and the effects of gelatin (0.5 -1), Persian gum (0.5 -1), and Glycerol (0-0.5) on the film characteristics were determined by Simplex-Lattice mixture design. Amounts of gelatin, Persian gum and Glycerol had significant effects on the physicochemical film. properties of edible Numerical optimization based on optimization goals was performed and the optimal point with the highest desirability (0.78) was obtained. The ratio of each of the independent variables in the optimal formula was 0.5. This film has a transparent appearance in terms of color and an acceptable amount of tensile strength and heat seal ability, therefore can cover foods and increased their shelf life of them.

5- Data Availability

The data used to support the findings of this study are included within the article, and the raw data are available from the corresponding author upon reasonable request.

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مقاله علمي پژوهشي

شناسایی و بهینه سازی فیلم خوراکی بر پایه صمغ فارسی و ژلاتین محمدصادق عرب ، حنان لشکری * ، مهرداد نیاکو * ر، محمدهادی اسکندری *

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