Original Research Paper

Preparation of Rosemary Collagen Chitosan Blend Membrane Using Response Surface Methodology (RSM)

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Abstract: The present research provided a formula for the optimization of rosemary/collagen/chitosan blend membrane. Rosemary Extract (RE) was used as additive and membrane was prepared using tensile strength, elongation at break and elongation length as the indexes. The results of single factor experiment showed that the addition ratio of collagen to chitosan, additive content, glycerol content and drying temperature all affected the mechanical properties and thickness of the blend membrane, among which the addition ratio of collagen to chitosan and glycerol content had a great influence. Box-Behnken Design (BBD) of Response Surface Methodology (RSM) was used to optimize the process and the parameters were obtained as follows: The ratio of collagen to chitosan (39:61, v/v), the addition of RE (0.6 mg/mL) and the glycerol content (30.2%). The mechanical properties and thickness of the optimized formula were verified, which proved the feasibility of the scheme and the broad application prospect of rosemary/collagen/chitosan blend membrane in meat packaging industry. The optimal data of tensile strength, elongation at break, elongation length and thickness were 2789.65 g, 49.92%, 117.55 and 0.143 mm, respectively. In the future, the research of blend membrane in food industry will more meet the diversified and high-performance market demand.

Keywords: Blend Membrane, Mechanical Properties, Response Surface Methodology

Introduction

With the improvement of people's requirements on food quality and shelf life as well as the enhancement of people's awareness of environmental protection, the edible packaging made of natural biological materials has become a research hotspot in the field of food packaging (Petkoska *et al*., 2021). Compared with the traditional polymer packaging materials, the edible film is more environmentally safe and easier to degrade. In some aspects, it has the potential to replace the traditional polymer packaging materials (Dou *et al*., 2018).

Collagen is a triple-helical protein capable of self-assembly into the fibrillar structure, which exhibits tensile strength and excellent ability to support cell adhesion (Irawan *et al*., 2021). The manufacturing cost of artificial collagen casing is low (only half of the cost of natural casing) and the preparation process is simple. Previous study has found that the physical properties of pure collagen membrane are far from meeting the requirements of casing (Chu *et al*., 2017). After extraction and in vitro

recombination of collagen in natural materials, the hierarchical structure of collagen is changed and its mechanical properties are significantly reduced (Xiao *et al*., 2021). Unoptimized collagen membrane is prone to poor mechanical strength, adhesion, caking and other problems. Blending with another natural polymer is an effective method to optimize the mechanical properties of collagen membrane (Li *et al*., 2019). To overcome these limitations, biological materials like chitosan can be incorporated with collagen (Gurumurthy *et al* 2018). Chitosan, the most abundant biopolymer second to cellulose, consists of β-(1-4)-2- acetamido-ᴅ-glucose and β-(1-4)-2- amino-ᴅ-glucose units (Sun *et al*., 2017). Chitosan has been found to be nontoxic, biode gradable, biofunctional, biocompatible in addition to possess antimicrobial characteristics (Vedula *et al*., 2021). In addition, chitosan is an excellent component of edible film due to its film-forming capacity and valuable mechanical properties for forming transparent films, which can fulfill various packaging

needs (Singh *et al.*, 2015). Untreated collagen presents a fast biodegradation rate and low mechanical strength. However, addition of chitosan interacting with collagen nanofiber enhances fiber network and increases the pore size of scaffold (in the freeze-dried form), resulting in a significant improvement in mechanical strength (Oliveira *et al.*, 2019).

In addition, natrual active ingredients can be added in edible membrane to improve its antioxidant and antibacterial capacities. For instance, prepared chitosan/β-cyclodextrin/tannic acid biocomposite membrane, the oxidation resistance of the membrane was obtained successfully (Ulu *et al.*, 2021). Li *et al.* (2021) found that bletilla striata polysaccharide can improve antibacterial activity from fabricating bletilla striata polysaccharide/chitosan membranes. Rosemary Extract (RE) are widely used as additives in the food (e.g., flavorings, antioxidant and antimicrobial agent) (Moczkowska *et al.*, 2020). RE from the Lamiaceae family are a source of bioactive ingredients including phenolic acids, flavonoids, diterpenoids and triterpenes (De Paiva *et al.*, 2021). The main polyphenolic compound found in rosemary is rosmarinic acid, which exhibits high antioxidant and antimicrobial properties. The addition of RE to the chitosan films had positive effect on lipid oxidation and microbial contamination of the fresh pork sausages (Jancikova *et al.*, 2019). Natural active ingredients slow down the automatic oxidation of meat and can also affect the physical and mechanical properties of the film. Elongation at break of all films supplemented with RE was reduced by higher 60% compared with the control group (Piñeros *et al.*, 2017). Yan *et al.* (2013) also found that tensile strength and elongation at break of starch-alginate composite membrane decreased after adding RE. Natural phenolic compounds have been successfully used in active packaging. Through increasing the content of phenolic compounds and activities of anti-free radicals, the interaction between macromolecules and active groups have been increased and the physical properties such as the barrier property of the film have been greatly improved (Lei *et al.*, 2019).

Base on the above, chitosan and RE were used in collagen blend membrane in this study. Single factor experimental design and Response Surface Methodology (RSM) was employed to optimize the preparation process of the blend membrane. The objective of this study was to evaluate the effect of ratio of collagen and chitosan, addition of RE and glycerol content on the mechanical properties and thickness of blend membrane, with the aim of optimizing the formulation for food packaging. Our results in this study will provide a basis for optimizing the formulation of blend film for food packaging industry.

Materials

Collagen was purchased from Shandong Zhongtian Biotechnology Co., Ltd (Zibo, China). Water soluble RE was obtained from Zhengzhou Wanbo Chemical Products Co., Ltd (Zhengzhou, China). Chitosan, glycerol and acetic acid were purchased from Sinopac Chemical Reagent Co., Ltd (Beijing, China). All of the reagents used in the experiment were analytically pure.

Methods

Preparation of Blend Membrane

Chitosan was dissolved in 1% (v/v) acetic acid firstly, then collagen and chitosan solutions (6 g: 200 mL m/v) were both prepared using distilled water. RE was added to cowhide collagen solution for mixing. Then the RE-collagen mixture was blended with chitosan solution using digital magnetic stirrers. Meanwhile glycerol was added to the mixture as plasticizer quantitatively. Film forming solution (30 mL) was accurately measured and cast in 10×10 cm polystyrene culture dishes. After removing bubbles on the surface, the film was dried for 16 h.

Single Factor Experimental Design of Blend Membrane

Single factor design was used to analyze the influences of various variables on mechanical properties and thickness of the blend membrane. Ratios of collagen to chitosan were set as 20:80, 30:70, 40:60, 50:50 and 60:40 (v/v), respectively. The count of water-soluble RE addition was set as 0.2-1.0 mg/mL. Glycerol content (percentage of total collagen and chitosan content) was set at 20%-40% and drying temperature was set at 30-50°C.

Determination of Mechanical Properties

Each prepared blend film was cut into six 6 cm×1 cm strip. Tensile strength, elongation at break and elongation length were measured by texture analyze (lotunscienceco Co., Ltd), respectively. The thin film strip was clamped between the gripping clamp probe A/MTG, then stretched at 1 mm/s crosshead capacity, with the initial distance of 40 mm and the tensile speed of 0.5 mm/s.

Determination of the Thickness

Film thickness was measured using the electronic digital display helical thickness gauge (Aipu Metrology Instrument Co., Ltd) at three random sites 20 mm from the edge.
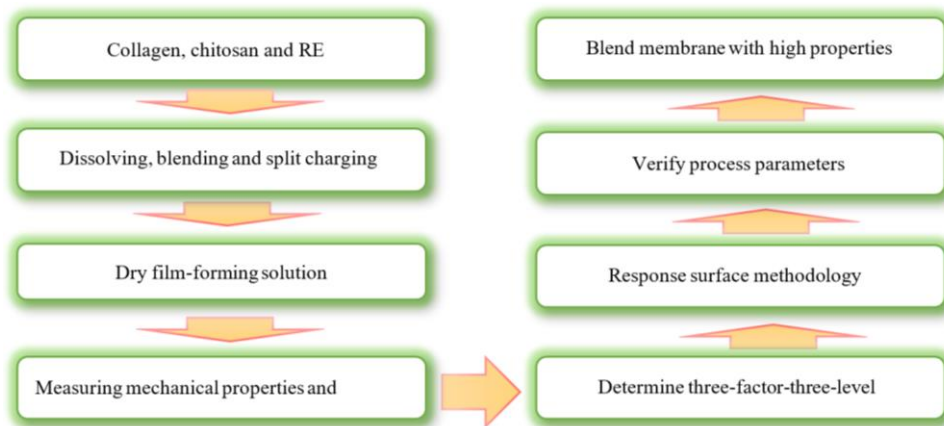
Fig. 1: The process flow diagram of rosemary/collagen/chitosan blend membrane with high properties

Box-Behnken Design (Bbd) for the Blend Membrane

Base on the single-factor test results, the factors and levels affecting the mechanical properties and thickness of RE/chitosan/collagen blend membrane were determined. A three-level-three-factor, BBD was adopted to further optimize the membrane preparation process. Ratios of collagen to chitosan (X_1) (30:70-50:50, v/v), addition of RE (X_2) (0.4-0.8 mg/mL), glycerol content (X_3) (25-35%) on the dependent variables of the films were used in the current study. The stated variables included tensile strength (Y_1/g), elongation at break (Y_2/%), elongation length (Y_3/mm) and thickness (Y_4/mm).

The design matrix and corresponding results of blend membrane process optimization were listed in Table 1. The process flow diagram of blend membrane was shown in Fig. 1. Multiple regression analysis methods were used to process the experimental data (Saberi *et al*., 2016). The relationship between response of film forming performance and test variables can be expressed by the following second-order polynomial equation:

$$Y = \beta_0 + \sum_{i=1}^{i \le 3} \beta_i X_i + \sum_{i=1}^{i \le 3} \beta_{ii} X_i^2 + \sum_{i<j}^{j=3} \sum_{i=1} \beta_{ij} X_i X_j$$

where, Y is the predicted response; β_0, β_i, β_{ii} and β_{ij} are the regression coefficients for intercept, linear, quadratic and interaction terms, respectively. X_i and X_j are the independent variables.

Statistical Analysis

Origin was selected to process and plot the test data of single factor test. Design-expert 8.0.6 was used for data analysis and variance analysis of RSM. p values less than 0.05 were considered to be statistically significant. All data were measured three times and averaged.

Results and Discussion

Effect of Mass Ratio of Collagen to Chitosan on Mechanical Properties and Thickness of Blend Membrane

We investigated the effect of ratio of collagen to chitosaan on mechanical properties of blend membrane. It can be seen from Fig. 2, as the proportion of collagen increasing, tensile strength, elongation at break, elongation length and thickness of blend membrane tended to increase at first and then decrease and peaked at 40:60 (v/v). The peak values of tensile strength, elongation at break, elongation length and thickness of the membrane were 2674.119 g, 18.746%, 41.658 mm and 0.134 mm, respectively. Results in Fig. 2 showed that the addition of chitosan to the blend film led to an increase in tensile strength by approximately 50%. Chitosan contains more polar groups. Under the same humidity, with the increase of the amount of chitosan, moisture absorption rate of blend membrane increases (Glampedaki *et al*. 2011). The plasticizing effect of water improves motility of the molecular chain segment of blend membrane and then enhances elongation at break of composite membrane (Lewandowska *et al*., 2016). As the content of chitosan increases, the degree of collagen cross-linking increases substantially, which reduces fluidity of molecular chain macroscopically showing that elongation at break decreases (Chen *et al*., 2014). As a consequence, with the increasing of the chitosan content, the elongation at break of the membrane firstly increased and then decreased, which was consistent with our research results. In addition, compared with previous studies on fibrin/chitosan based composite membrane added with rosemary extract, the RE/chitosan/collagen blend membrane has better elongation at break (Du *et al*., 2021). Tensile strength of blend membrane was inversely proportional to the proportion of chitosan, possibly because augment of the chitosan content cannot form

sufficient intermolecular force between collagen and chitosan (Yi *et al.*, 2019). The thickness of the blend membrane depends on its composition and processing parameters. Where higher concentrations of biopolymer in the formulation leads to an increase in solids in the polymer matrix, thus the blend membrane becomes thicker evidently (Batista *et al.*, 2019).

Effect of Re on Mechanical Properties and Thickness of Blend Membrane

Adding RE to food products could slow down or prevent the oxidation reactions of processed meat and fishery products (EFSA, 2008). As can be seen in Fig. 3, with the increase of RE addition, the elongation at break and elongation length of blend membrane decreased significantly. With the addition of RE within the set concentration range, the tensile strength and thickness first increased and then decreased and reached the maximum value at 0.6 mg/mL. The possible reason for this phenomenon is that the polyphenols in RE cross-linking with chitosan and then reduce the tensile properties of blend membrane. Previous study found that the chitosan-based film, became stronger and less stretchable due to the enhancement of cross-linking agent after added with thyme extract. (Talón *et al.*, 2017).

Effect of Glycerol on Mechanical Properties and Thickness of Blend Membrane

Glycerol can be used as plasticizer to improve the processing performance of edible collagen membrane. Previous study reported that due to the addition of plasticizer, the elongation at break of composite film could be improved significantly, thus optimized the problem for easy to break (Chen *et al.*, 2014). In the present study, glycerol was employed in preparing blend membrane. As illustrated in Fig. 4, tensile strength, elongation at break, elongation length and thickness of blend membrane all increased with the increase of glycerol content. When the content of glycerol exceeded 30%, thickness of blend membrane had no obvious changes and elongation length of blend membrane began to decrease. The probable explanation might be that glycerol could soft the rigid structure of blend membrane, increase the free volume of system and fluidity of molecular chain by weaking the macromolecular or intramolecular interaction (Ghasemlou *et al.*, 2011).

Effect of Drying Temperature on Mechanical Properties and Thickness of Blend Membrane

Higher heat treatment temperature is conducive to improve strength on the interaction force of collagen and chitosan molecules gradually, sequentially the formed membrane structure tends to be compact (Su *et al.*, 2021). The softness and elongation at break of the composite film

reach ideal condition but viscosity of the film increased when drying temperature is close to 50°C (Chen *et al.*, 2014). Therefore, it was necessary to choose appropriate drying temperature in this research. As can be seen in Fig. 5, tensile strength, elongation at break and elongation length of blend membrane were relatively high, when drying temperature was 30°C, which was consistent with the research results of on the influence of drying temperature on mechanical properties of collagen-chitosan blend membrane (Wang *et al.*, 2020). Therefore, drying temperature was set as 30°C in this study.

Optimization the Preparation of Blend Membrane by RSM

Models Fitting and Statistical Analysis

The influences of the variables (ratio of collagen to chitosan, addition of RE and glycerol content) on tensile strength, elongation at break, elongation length and thickness of blend membrane were investigated through 3^3 factorial experimental design of RSM. The experimental design and observation of BBD dependent variable data were summarized in Table 2. ANOVA was used to judge whether the quadratic model was significant or not. BBD for model fitting was shown in Table 3. Through multiple regression analysis of experimental data, four second-order polynomial mathematical models were obtained. These mathematical models were used to demonstrate the relationship between independent variables and the mechanical properties and thickness of RE/chitosan/collagen blend membrane. The final equations obtained according to the coding factor were as follows:

$$Y_2 = 49.54 - 4.33X_1 + 0.13X_2 + 3.42X_3$$
$$+ 1.77X_1X_2 + 4.91X_1X_3 - 2.4X_2X_3$$
$$- 15.42X_1^2 - 10.38X_2^2 - 6.46X_3^2$$
$$Y_3 = 117.19 - 5.79X_1 + 0.99X_2 + 6.28X_3$$
$$- 3.29X_1X_2 - 4.20X_1X_3 - 0.61X_2X_3$$
$$- 44.41X_1^2 - 31.12X_2^2 - 25.89X_3^2$$
$$Y_4 = 0.15 - 4.75 \times 10^{-3}X_1 - 1.5 \times 10^{-3}X_2$$
$$+ 5 \times 10^{-4}X_3 - 2.75 \times 10^{-3}X_1X_2 - 3.25 \times 10^{-3}X_1X_3$$
$$+ 5.75 \times 10^{-3}X_2X_3 - 0.042X_1^2 - 0.013X_2^2 - 0.013X_3^2$$

The lack of fit test and analysis of variances were used to evaluate the fitted model and suitability of true response surface. The coefficient of determination (R^2) of the model was 1.00, signified that 100% of experimental data can be predictably fitted with model data for tensile strength. The predicted residual sum of square (PRESS) for the model is an indication of how well the predictive model matches each point in the design (Saberi *et al.*, 2016). According to analysis of the model, the high-level F value (15.66) and low-grade p value (<0.01) implied that the model was a sky-high statistically significance.

According to the ANOVA in Table 3, p value of the model was lower than 0.0001, indicated that the regression model was at an extremely significant level, the regression equation could accurately reflect the relationship between tensile strength and various factors in the test conditions. The lack of fit item was not significant as its p value was 0.1967, indicated that the actual test and regression model were well fitted. In this model, R_{Adj}^2 (0.9542) indicated that the variation of tensile strength mainly was distributed among three design factors. The multivariate correlation coefficient R^2 (0.9799) fully indicated that there was an admirable fitting degree between the actual and predicted results.

The determination coefficient (R^2) of elongation at

break model (Table 3) was estimated to be 0.9575, which further indicated that there was a commendable correlation between the predicted value and experimental value. Lack of fit value and p value of the model were calculated to be 0.2587 and 0.0005, respectively, which in turn revealed that the mathematical model was satisfactory for prediction of elongation at break of blend membrane.

The RSM mathematical model of elongation length was calculated and the results showed that elongation length p value was lower than 0.001. The determination coefficient (R^2) of elongation length model (Table 3) was estimated to be 0.97, which in turn revealed that the mathematical model was satisfactory for prediction of elongation length of blend membrane.
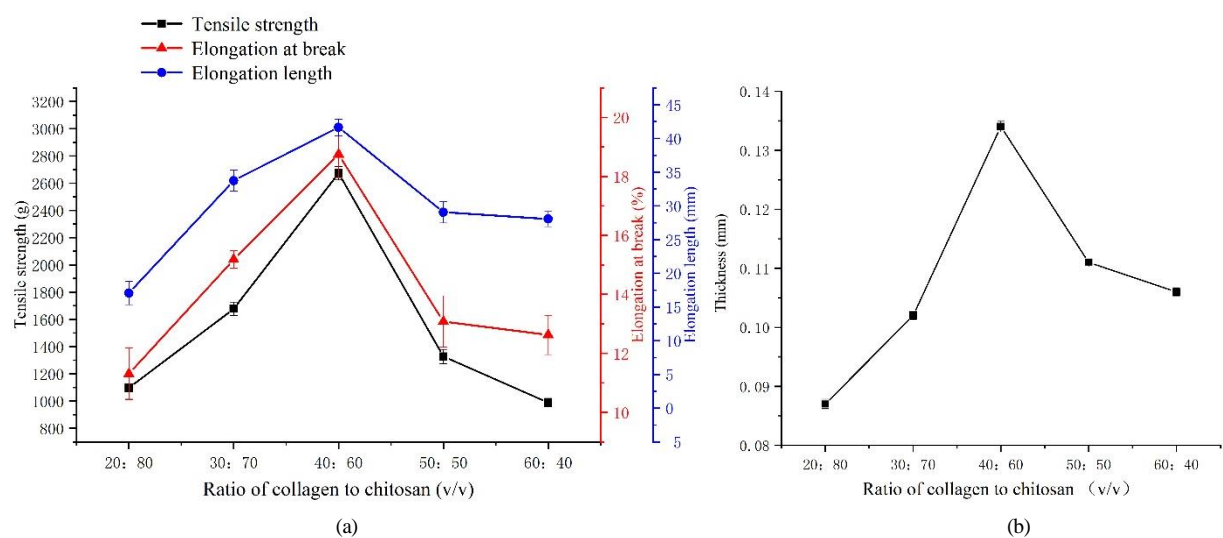

Fig. 2: (a) Effect of ratio of collagen to chitosan on mechanical properties of blend membrane; (b) Effect of ratio of collagen to chitosan on thickness of blend membrane
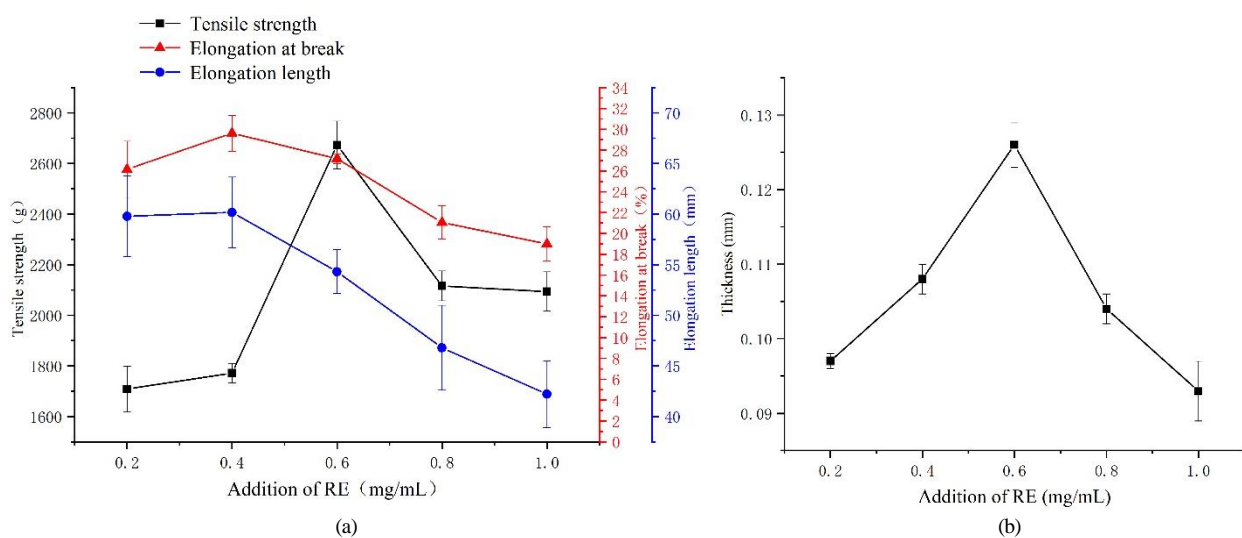

Fig. 3: (a) Effect of RE content on mechanical properties of blend membrane; (b) Effect of RE supplemental addition on thickness of blend membrane
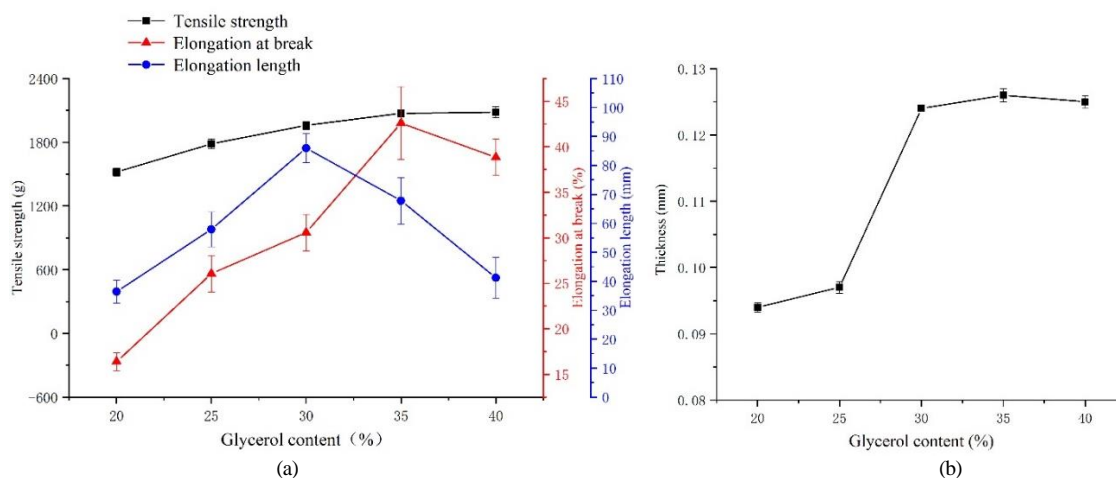
Fig. 4: (a) Effect of glycerol content on mechanical properties of blend membrane; (b) Effect of glycerol content on the thickness of blend membrane
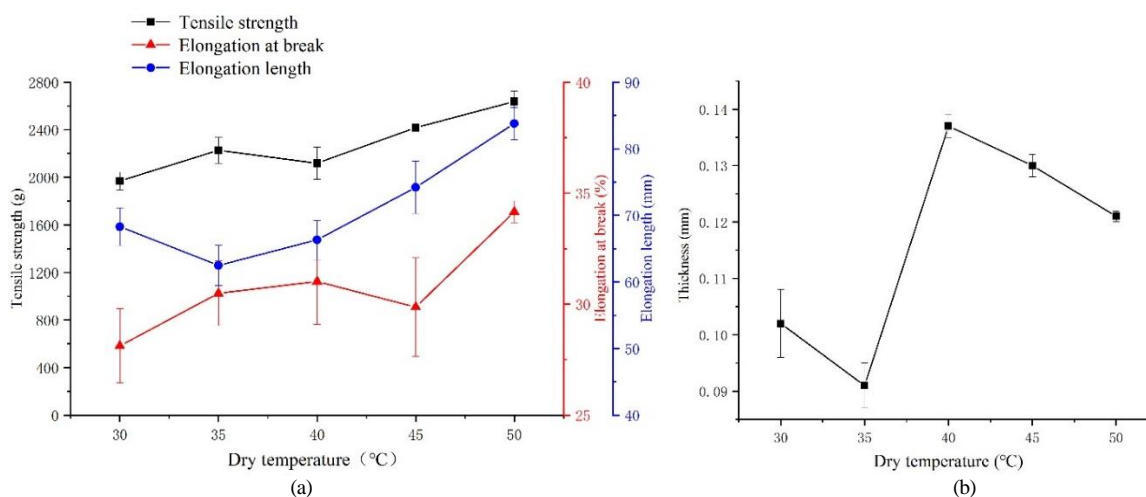

Fig. 5: (a) Effect of dry temperature on mechanical properties of blends membrane; (b) Effect of dry temperature on the thickness of blend membrane

Fitting the model for thickness showed in Table 3 that R^2 value of the models was 0.94. The p value for lack of fit, F value and p value of the model for thickness were 0.28, 12.6 and less than 0.01, respectively. Experimental results verified the reliability of the model in predicting thickness of blend membrane.

Analysis of Response Surface Plot and Contour Plot

Response surface analysis and contour variation represented the interaction between various factors and the degree of influence of different factors on tensile strength, elongation at break, elongation length and thickness. The contour shape showed the strength of interaction between various factors. The circle meant that two factors were not significant, while the ellipse meant that they were relatively significant. The steepness of the 3D graph on the response surface indicated the influence

of various factors on the experimental indicators.

The Effect of Independent Variables on Mechanical Properties

Tensile strength of blend membrane in response surface test ranged from 1174.14 to 2868.83 g. As shown in Table 4, X_1, $X_1 X_3$, X_1^2. X_2^2 and X_3^2 significantly affected the tensile strength of the blend membrane, indicated that the ratio of collagen to chitosan (X_1) was a more significant variable than addition of RE (X_2) and glycerol content (X_3). As can be seen from Fig. 6, when glycerol content was fixed at 0 level, tensile strength increased first and then decreased with ratio of collagen to chitosan (X_1). Moreover, the highest tensile strength values were obtained at 38.8:61.2 (v/v) of ratio of collagen to chitosan and 29.8% of glycerol content, respectively. In Fig. 6B, the more

obvious the ellipse shape of the contour line was, indicated that the interaction between X_1 and X_3 was obvious.

In RSM, elongation at break of blend membrane ranged from 15.55 to 52.68%. As shown in Table 4, coefficients of X_1, X_3 and $X_1 X_3$ were significant based on a 95% confidence level in affect elongation at break of blend membrane, showed that two variables had a significant influence on elongation at break and X_1 and X_3 interacted significantly. Moreover, there was no significant interaction between $X_1 X_2$ and $X_2 X_3$. In order to investigate the effects of the independent variables and interactions between them on elongation at break, response surface plots and contour plots displayed in Fig. 7 were used. The results showed that the ratio of collagen to chitosan and glycerol content had a significant influence on elongation at break of the blend membrane, meanwhile there was a significant interaction between them.

Elongation length of blend membrane varied from 33.44-123.36 mm in RSM experiments (Table 2). The quadratic effects of ratio of collagen to chitosan (X_1^2), addition of RE (X_2^2) and glycerol content (X_3^2) on elongation length were significant ($p<0.01$), however the interaction effects were non-significant. Fig. 8C showed the influence of addition of RE and glycerol content on elongation length. When the ratio was fixed at 0 level, elongation length of both increased continuously and then decreased. According to plasticizer impacted elongation length due to glycerol to reduce the interactions among polymer chains, thus decreased film resistance and increased flexibility (Teixeira *et al.*, 2021).

The Effect of Independent Variables on Thickness

In RSM, the maximum and minimum thickness of blend membrane were 0.079 and 0.152 mm respectively (Table 2). The contour plots of Fig. 9A and 9B were similar, indicated that X_2, X_3, $X_1 X_2$ and $X_1 X_3$ had no significant elongation or interaction on the blend film. According to previous correlated report, the thickness of optimized film did not differ ($p \geq 0.05$) compared to control, which was consistent with the results of our study (Batista *et al.*, 2019).

Verification of Predictive Model

The desirability function was used for simultaneous optimization of the multiple responses (Saberi *et al.*, 2016). The ratio of collagen to chitosan, the addition of RE and glycerol content were 38.97:61.03 (v/v), 0.6 mg/mL and 30.21%, respectively. Tensile strength, elongation at break, elongation length and thickness were 2789.65 g, 49.92%, 117.55 and 0.143 mm, respectively. In order to verify the feasibility of the response surface optimization results, verification tests were carried out. The optimal conditions were determined as follows: Ratio of collagen to chitosan was 39:61 (v/v), addition of RE was 0.6 mg/mL and glycerol mass fraction was 30.2%. It can see from Table 5, there was slightly different between the predicted values and experimental values, indicated that it was feasible and of practical significance to optimize the process of the blend membrane by RSM.

Table 1: Factors and coding value of response surface experimental designment

	Levels		
	-1	0	1
Ratio of collagen to chitosan (X_1) /v/v	30：70	40：60	50：50
Addition of RE (X_2) /mg/mL	0.4	0.6	0.8
Glycerol content (X_3) /%	25	30	35

Table 2: Box-behnken experimental design and results for mechanical properties and thickness

Runs	X_1	X_2	X_3	Y_1 /g	Y_2 /%	Y_3 /mm	Y_4 /mm
1	-1	0	1	1174.14	29.93	68.72	0.093
2	0	0	0	2868.83	50.18	110.96	0.144
3	0	1	-1	1557.72	34.79	59.54	0.104
4	0	0	0	2594.91	52.68	112.08	0.147
5	1	0	-1	1242.15	15.55	33.44	0.096
6	-1	0	-1	1882.82	31.31	34.02	0.097
7	0	0	0	2858.09	49.34	122.32	0.135
8	0	0	0	2776.01	44.43	117.18	0.152
9	1	0	1	1317.73	33.82	51.35	0.079
10	0	1	1	1307.16	35.21	57.13	0.128
11	-1	-1	0	2042.03	33.23	41.63	0.093
12	-1	1	0	2113.44	25.62	55.84	0.102
13	1	-1	0	1372.85	18.32	34.04	0.087
14	0	-1	-1	1189.02	25.39	61.99	0.125
15	0	0	0	2792.71	51.06	123.36	0.135
16	1	1	0	1589.53	17.79	35.09	0.085
17	0	-1	1	1295.2	35.40	62.01	0.126

X_1 = Ratio of collagen to chitosan (v/v), X_2 = Addition of RE (mg/mL), X_3 = Glycerol content (%), Y_1 = Tensile strength (g), Y_2 = Elongation at break (%), Y_3 = Elongation length (mm), Y_4 = Thickness (mm)

**Table 3:** Analysis of variance for determination of model fitting

Sources of variation	Y_1	Y_2	Y_3	Y_4
F ratio of model	38.01	17.51	23.37	12.640
p of model F	<0.0001[a]	0.0005[a]	0.0002[a]	0.0015[a]
PRESS	6837000	2169.24	17571.47	0.01
Lack of fit	0.1967	0.2587	0.0846	0.2803
R^2	0.9799	0.9575	0.9678	0.9420
Adjusted R^2	0.9542	0.9028	0.9264	0.8675

[a]Significant difference with $p<0.05$

Table 4: Analysis of variance for the experimental results

Indicators	df	Y_1 F value	p value	Y_2 F value	p value	Y_3 F value	p value	Y_4 F value	p value
X_1	1	17.87	0.0039[a]	10.88	0.0132[a]	3.21	0.1165	2.05	0.1951
X_2	1	2.80	0.1383	0.01	0.9216	0.09	0.7679	0.20	0.6647
X_3	1	3	0.0929	6.78	0.0352[a]	3.77	0.0931	0.02	0.8844
X_1X_2	1	0.26	0.6232	0.91	0.3718	0.52	0.4948	0.34	0.576
X_1X_3	1	7.69	0.0275[a]	7.01	0.033[a]	0.84	0.3889	0.48	0.5106
X_2X_3	1	1.59	0.2474	1.67	0.2372	0.02	0.8982	1.50	0.2598
X_1^2	1	45.72	0.0003[a]	72.75	<0.0001[a]	99.38	<0.0001[a]	85.54	<0.0001[a]
X_2^2	1	59.81	0.0001[a]	32.94	0.0007[a]	48.81	0.0002[a]	7.81	0.0267[a]
X_3^2	1	173.70	<0.0001[a]	12.78	0.009[a]	33.78	0.0007[a]	8.43	0.0228[a]

[a] Significant difference with $p < 0.05$, df = Degree of freedom
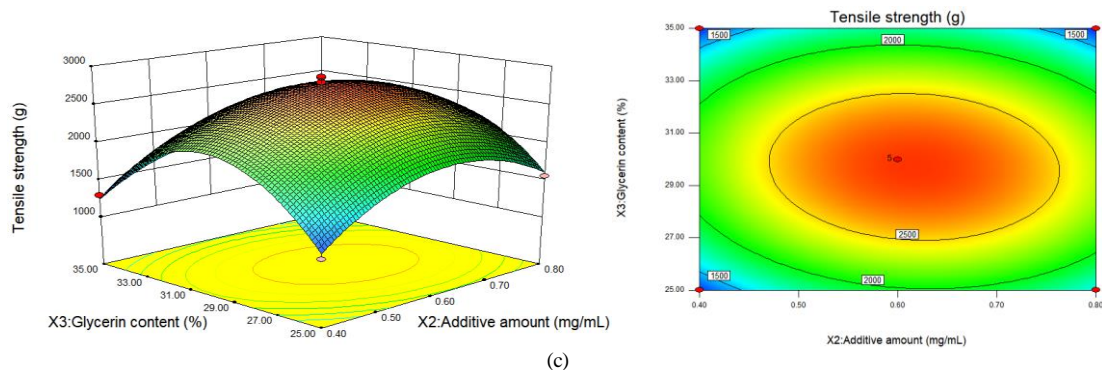

(a)

(b)

(c)
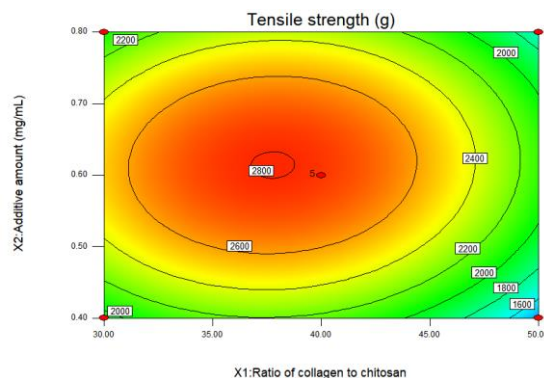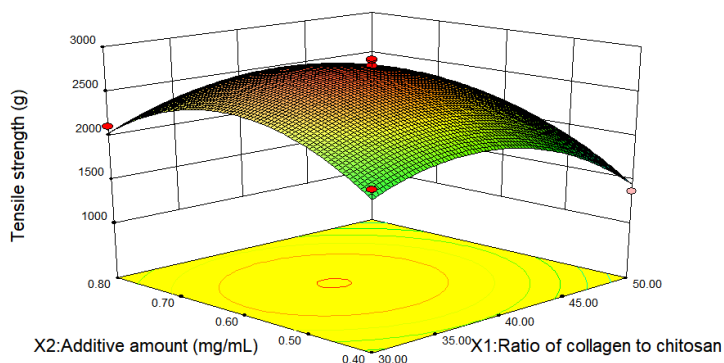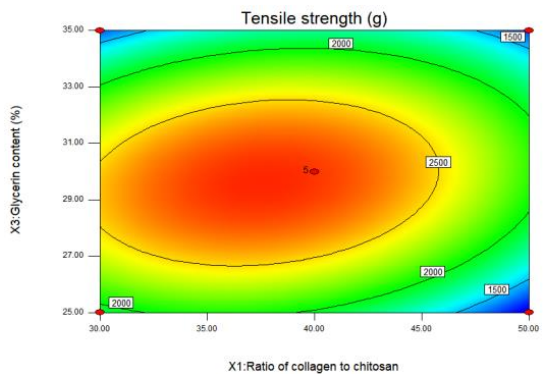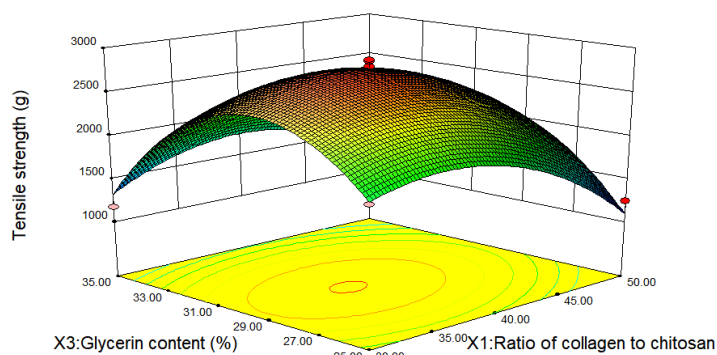
Fig. 6: Response surface plots (3D) and contour plots of tensile strength as a function of significant interaction between factors. (A) Ratio of collagen to chitosan and addition of RE; (B) Ratio of collagen to chitosan and glycerol Content; (C) Addition of RE and glycerol cement
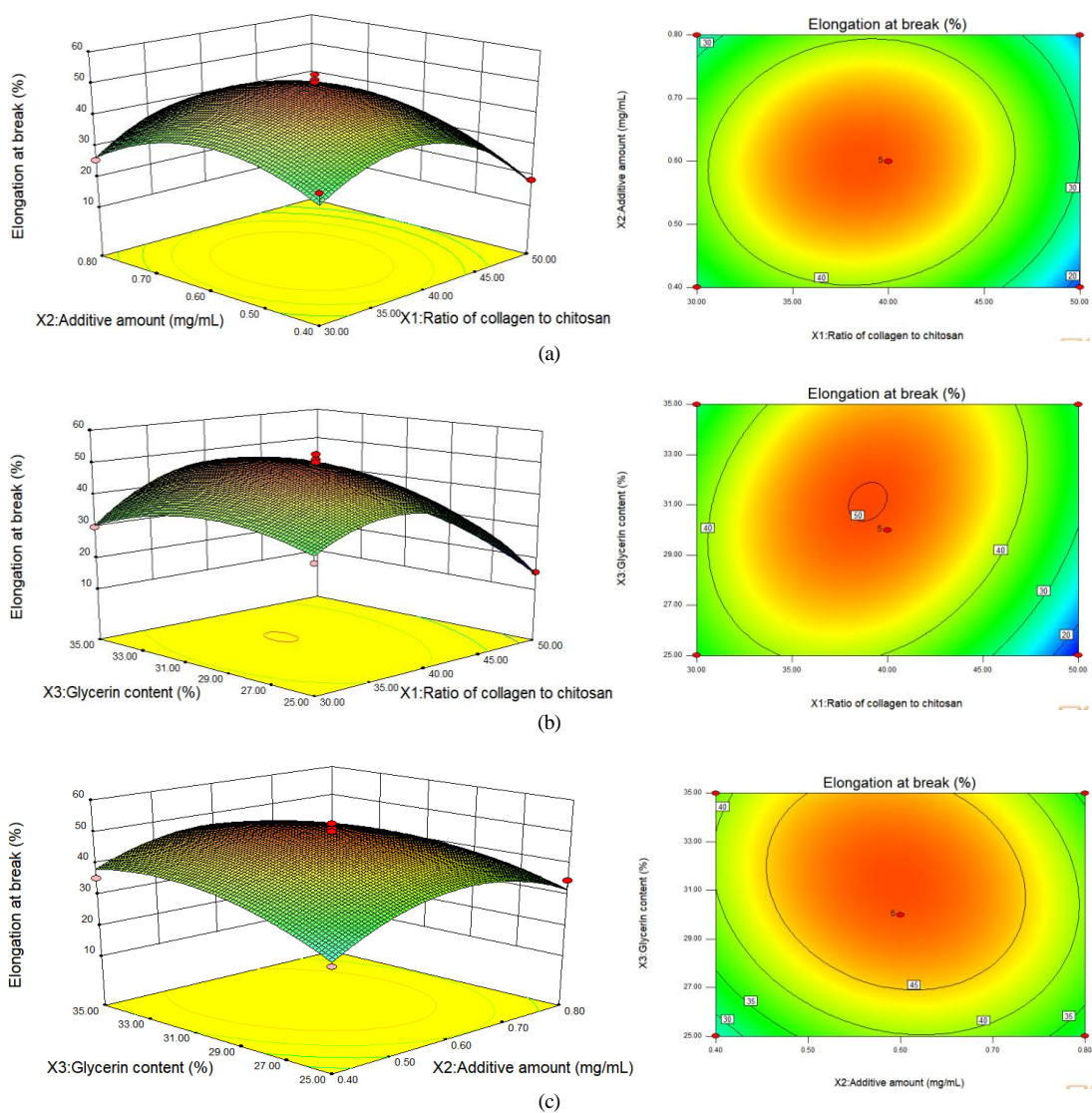

(a)

(b)

(c)

Fig. 7: Response surface plots (3D) and contour plots of elongation at break as a function of significant interaction between factors. (A) Ratio of collagen to chitosan and addition of RE; (B) Ratio of collagen to chitosan and glycerol cement; (C) Addition of RE and glycerol cement
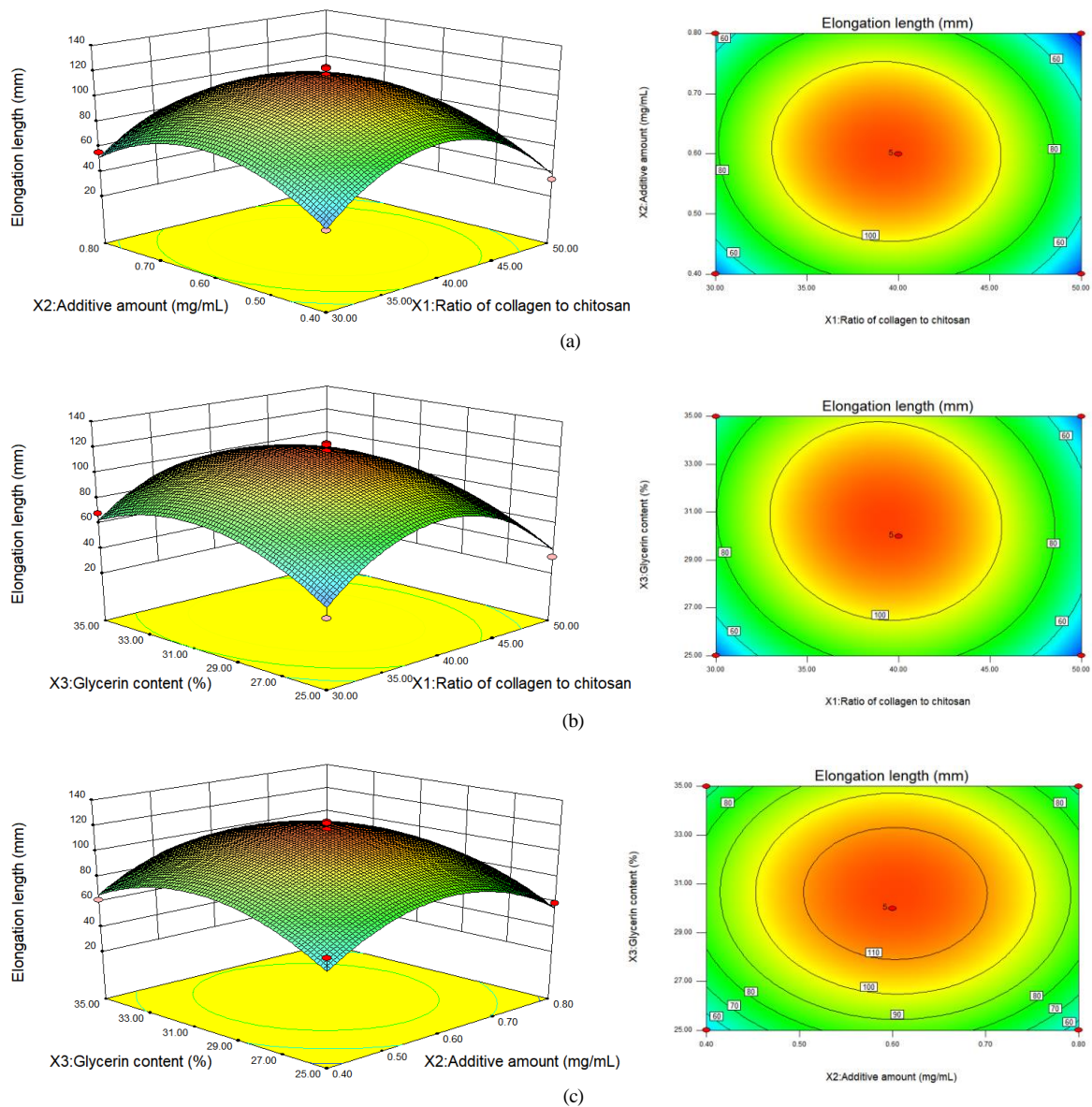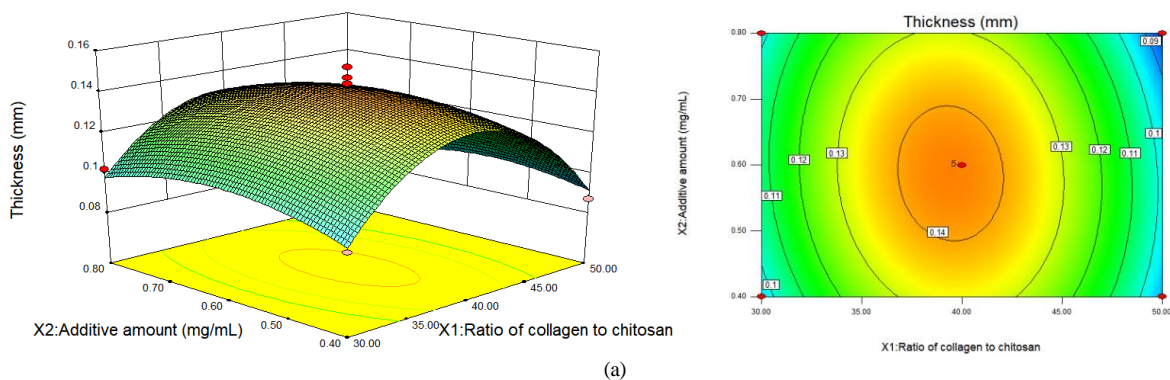
(a)

(b)

(c)

Fig. 8: Response surface plots (3D) and contour plots of elongation length as a function of significant interaction between factors. (A) Ratio of collagen to chitosan and addition of RE; (B) Ratio of collagen to chitosan and glycerol centent; (C) Addition of RE and glycerol centent

(a)

Fig. 9: Response surface plots (3D) and contour plots of thickness as a function of significant interaction between factors. (A) Ratio of collagen to chitosan and addition of RE; (B) Ratio of collagen to chitosan and glycerol Content; (C) Addition of RE and glycerol centent
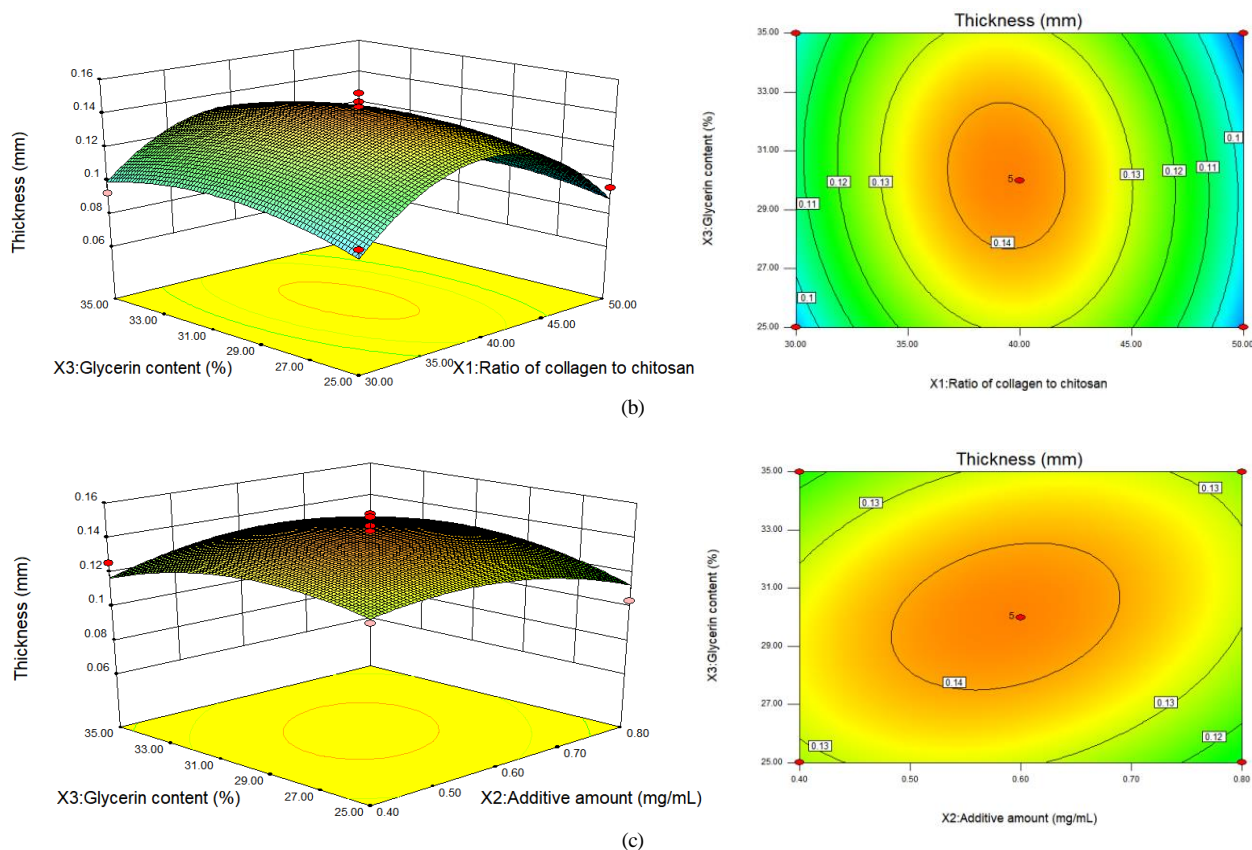
Table 5: Predicted and experimental response values at optimum

Response	Predicted value	Experimental value
Y_1 (g)	2789.65	2716.59±3.8706
Y_2 (%)	49.92	50.42±0.0854
Y_3 (mm)	117.55	119.24±0.7280
Y_4 (mm)	0.143	0.134±0.0004

Conclusion

The optimal formulation of chitosan-based blend membrane was successfully developed from cowhide collagen and RE. The findings showed that ratio of collagen to chitosan and glycerol content significantly affected tensile strength, elongation at break, elongation length of the blend membrane. The optimum formulation of RE/chitosan/collagen blend membrane was found to be ratio of collagen to chitosan of 38.97:61.03 (v/v), addition of RE of 0.6 mg/mL and glycerol content of 30.21%, respectively. Measurement under optimal conditions of tensile strength, elongation at break, elongation length and thickness were 2789.65 g, 49.92%, 117.55 mm and 0.143 mm, respectively. This study manifested that cowhide collagen, RE and chitosan could be used to make the blend membrane having choiceness packaging properties. The resulting blend membrane possessed a potential application value in the meat industry.

Author's Contributions

Ming He: Participated in the whole experiment process and also contributed to the interpretation of the results and manuscript preparation.

Haifang Xiao: Contributed to the study design, the interpretation of the results and manuscript preparation.

Yuhan Zhai, Yuqing zhang and Shuo Xu: Participated in part of the experimental design.

Shaoxuan Yu: Ameliorated the manuscript.

Yuanda Song: Contributed to the guidance of experimental design and ameliorated the manuscript.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

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