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Abstract The aim of this study was to develop an optimal formulation for preparation of an edible film from chitosan, pea starch and glycerol using response surface methodology (RSM). Three independent variables were assigned comprising chitosan (1-2%), pea starch (0.5-1.5%) and glycerol (0.5-1%) to design an empirical model best fit in physical, mechanical and barrier attributes. Impacts of independent variables on thickness, moisture content (MC), solubility, tensile strength (TS), elastic modulus (EM), elongation at break (EB) and water vapor permeability (WVP) of films were evaluated. All the parameters were found to have significant effects ($p<0.05$) on physical and mechanical properties of film. The optimal formulation for preparation of edible film from chitosan, pea starch and glycerol was 1% chitosan, 1.5% pea starch and 0.5% glycerol. An edible film with good physical and mechanical properties can be prepared with this formulation and thus this formulation can be further applied for testing on coating for fruit and vegetables.

Keywords Pea Starch. Chitosan. Plasticizer. Edible films. Box-Behnken Design

Introduction

Many efforts have been made to develop and test edible films for further utilisation to extend shelf life of fresh produce (Arnon et al. 2014; Dhall 2013; Gómez-Estaca 2009; Valencia-Chamorro et al. 2010). Chitosan has been found to have a great potential for wide range of application in formulation of edible films due to its biodegradability, biocompatibility, antimicrobial activity and non-toxicity (Pelissari et al. 2009; Sánchez-González et al. 2010). Pea starch has small granules size, 2-40µm (Ratnayake et al. 2002) and high amylose content (60-70 %) (Hilbert and Macmasters 1945), thus it can provide a good transparent film with good physical, and mechanical properties with composite materials. Glycerol has been widely used as plasticizer for development of starch based films (Santacruz et al. 2015).

Previous studies indicated that physical and mechanical properties of the films could be significantly affected by ingredients concentration of the formulation (van den Broek et al. 2015; Zhang et al. 2015). RSM was applied for optimisation because it has been useful in finding the relationships between different independent and response variables while minimizing the number of experiments and usage of resources (Dailey and Vuong 2016). The findings of this study can be utilised for further application on coating fruit and vegetables. Therefore the aim of this study was to develop an optimal formulation for preparation of edible film from pea starch, chitosan and glycerol using RSM.

Material and methods

Materials

Chitosan (medium molecular weight Poly (D-glucosamine) deacetylated chitin, ≥ 75% deacetyleated) and acetic acid were purchased from Sigma-Aldrich USA. Pea starch was supplied by Yantai Shuangta Food Co. Ltd China and was used as a film forming material. Glycerol was purchased from Ajax Finechem Pty Ltd. Australia.

Edible Film Preparation

Films were prepared by the casting process and dehydrating the suspension solution in petri plates. Suspension solution was prepared by dissolving 1 g chitosan (1-2 %) in 100 ml of 0.7 % (v/v) of aqueous acetic acid solution (Maciel et al. 2014). Pea starch powder (0.5-1.5 %) was mixed to the above solution under control heating conditions (80^{o}C) with continuous stirring until the gelatinization temperature was reached. The ranges of chitosan (1-2 %), pea starch (0.5-1.5%) and glycerol (0.5-1%) were selected based on previous studies (Chillo et al. 2008; Maran et al. 2013b; Santacruz et al. 2015) and our preliminary studies (results not shown). The film forming dispersion solution of starch-chitosan was cooled to room temperature before glycerol (plasticizer; 0.5-1%) was added. The solution was stirred for further 20 minutes to allow through mixing and removal of air bubbles. Film forming suspension solution (about 20 g) was casted in the petri dishes (10 cm in diameter) and dried at 30 ^{o}C for 24 hrs. Dried films were peeled off and used for further analysis.

Characteristics of pea starch-chitosan film

Physical properties

Thickness

The thickness of film was measured according to previously reported method (Saberi et al. 2015) using a digital micro-meter (Mitutoyo, Co., Model ID-F125, Japan). The sensitivity of the instrument was 0.001 mm. Film sample was placed under the nobe and thickness values in mm was recorded. Random value from at least 10 different points was noted for individual film sample and average was calculated. Results from thickness measurement were also used for further calculation of water vapour permeability (WVP) of the samples.

Moisture content (MC)

Films were cut into 15 x 40 mm strips and placed into the aluminum dishes for drying at 110 ^{o}C for 24 hrs. Films were then cooled for 2 hrs after removal from the oven and the weight was measured using a four decimal balance (HA-180 M, A & D company Ltd, Japan). MC was calculated based on weight difference (Eq. 1). All the measurements were carried out in triplicate and the values are expressed as means ± standard deviations.

$$MC(\%) = \frac{M_i - M_f}{M_i} X\ 100 \quad (1)$$

Solubility

Solubility of film was measured according to the method reported in a previous study (Ojagh et al. 2010). Film specimens (40 × 15 mm) were dried to a constant weight at 110^{o}C for 24 hrs. Each sample was then placed into the glass-jar containing 50 ml of distilled water and subsequently shaken at 25 rpm at room temperature for 24 hrs. Undissolved portion of the film was collected and dried in the oven at 110^{o}C for 24 hrs to reach a constant weight. Solubility was calculated based on weight difference as shown in Eq. 2.

$$S(\%) = \frac{S_{initial} - S_{final}}{S_{initial}} \times\ 100 \quad (2)$$

Barrier properties

Water vapour permeability (WVP)

Gravimetrically method, ASTM E96 procedure (ASTM 1996), with a 75 % RH gradient at 25^{o}C was used to measure the WVP of the film. Permeation cells (0.7065 mm^2 film area) containing anhydrous $CaCl_2$ (0 % RH) were sealed tightly by the sample film using parafilm. Covered permeation cells were placed in a desiccator having saturated NaCl solution (75 % RH). RH inside the permeation cell was always lower than outside, and water vapour transport was determined using the weight gain of the cell at a steady state of transfer. Changes in the

weight of the cell were recorded and plotted as a function of time. The slope of each line was evaluated by linear regression (R^2 > 0.99), and the water vapour transmission rate was calculated through the slope of the straight line (g/s) divided by the test area (m^2). After the permeation tests, the film thickness was measured and WVP (g $Pa^{-1}s^{-1}m^{-1}$) was calculated as:

$$\text{WVP} = \frac{\Delta m}{A\,\Delta t}\,\frac{X}{\Delta P} \quad (3)$$

$\Delta m/\Delta t$ = weight of moisture gain per unit time (gs^{-1}) and can be calculated by the slope of the graph. A= area of the exposed film surface (m^2), T = thickness of the film (mm), ΔP= represents the water vapour pressure difference inside and outside of the film (Pa) (Saberi et al. 2015).

Mechanical properties

The mechanical properties of the films were determined according to the method described by (Saberi et.al 2015) with modification using a Texture Analyzer (LLOYD Instrument LTD, Fareham, UK). Film specimens (15× 40 mm) were used for all mechanical tests. The maximum load (N) and extension (mm) curves were recorded to calculate tensile strength (TS), elongation at break (E) and Elastic Modulus (EM) of the films using a tensile test at crosshead speed of 1 mm/s and initial grip distance 40 mm.

Experimental design and statistical analysis

Response surface methodology (RSM)

The statistical analysis and regression model study was performed with JMP software (Version 22, SAS, Cary, NC, USA). A Box-Behnken design at three levels for each independent variables at three center points replicates was employed for study. Fifteen different edible coating formulations comprising chitosan (1-2 %), pea starch (0.5-1.5%) and glycerol (0.5-1%) were used to get the best optimal combination. Effect of polysaccharide biopolymers blended

with plasticizer (independent variables) on properties of casted film (response functions, Y) comprising thickness, WVP, solubility, moisture content, elongation at the break, elastic modulus, and tensile strength, was observed. In the process of optimization of coating formulation, response variables were related to independent variables by a second order polynomial equation (Eq. 4).

$$Y = \beta_o \sum_{i=1}^{k} \beta_i X_i + \sum_{\substack{i=1 \\ i<j}}^{k-1} \sum_{i=2}^{k} \beta_{ij} X_i X_j + \sum_{i=1}^{k} \beta_{ii} X_i^2 \quad (4)$$

X_i = independent variables

β_0= intercept

β_i, β_{ii}, β_{ij} = regression coefficients for intercept, linear, quadratic, and interaction terms

k = number of variables

The independent variables and their code variable levels are shown in Table 1. The JMP software was also employed to develop the model equations, to graph 3D plots, 2D contour plots of the responses, as well as predicting the optimum conditions of the independent variables. The three independent variables were assigned as: X1 (chitosan concentration %), X2 (pea starch, %) and X3 (glycerol, %). Thus, the function containing these three independent variables is expressed as follow

$$Y = \beta_o + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{23} x_3^2 \quad (5)$$

Statistical analysis

JMP (Version 11, SAS Cary, NC, USA) was used to predict the optimal conditions of independent variables using 3D contour plots. Analysis of variance ANOVA, the coefficient of determination (R^2) and adjusted the coefficient of determination (Adj-R^2) were used to assess the validity of the model. Analysis of variance (one-way ANOVA) was used to compare the mean differences of the samples. SPSS 16.0.0 statistical software for windows (SPSS IBM, USA) was used for data treatment and statistical analysis. Comparison of the mean was considered to be statistically significant at $P<0.05$.

Results and discussion

Fitting of the model

Different analysis sources of variation, such as lack of fit, R^2, Predicted Residual Sum of Square (PRESS) for the models, F ratio and Prob > F were analyzed to identify the fitting of the RSM mathematical models. The results (Table 2 and Fig. 1) showed that the value of the coefficient of determination (R^2) was in the range of 0.79 to 0.97, reflecting that at least 79 % of the predicted values could be matched with the actual values. Values of F ratio for physical parameters (thickness, solubility and moisture content) (2.791, 3.26 and 2.84) and lack of fit (0.5, 0.51 and 0.78) showed that the designed model was efficient in predicting the physical properties of the film.

For barrier properties of the film, statistics showed that values of PRESS, F value and lack of fit were 1377.8, 3.26 and 0.51, indicating that the mathematical model is a good predictor of WVP of film (Table 2). The results also indicated that predicted model for mechanical properties of the film had high PRESS values for EM, EB and TS (957664.1, 38.65 and 89595)

and the coefficient of determination (R^2) ranged from 0.79 to 0.94, indicating that the model is also reliable for predicting the mechanical properties of the chitosan-pea starch edible film.

Empirical model for prediction of film properties

Through applying multiple regression analysis on the experimentally attained data, the empirical model was developed by fitting the experimental data obtained from Box-Behnken design into second order polynomial mathematical equation (Eq. 4). The model could be fitted to the following second order polynomial equations (6) – (12). In order to investigate the relationship between process variables and response variables from the developed mathematical model equations 3D contour plots were constructed between two independent variables while keeping the 3rd variable constant.

$$Y_{Thickness} = 0.11 + 0.01845x_1 + 0.00923x_2 + 0.017x_3 + 0.000725x_1x_2 + 0.00287x_1x_3 + 0.018x_2x_3 - 0.0074x_1^2 - -0.00143x_2^2 - 0.0038x_3^2 \quad (6)$$

$$Y_{WVP} = 10.66 + 7.31x_1 + 4.52x_2 - 1.225x_3 + 4.315x_1x_2 - 1.0425x_1x_3 + 2.062x_2x_3 + 3.36x_1^2 + 0.9316x_2^2 - 0.6808x_3^2 \quad (7)$$

$$Y_{Solubility} = 49.98 - 1.15x_1 + 0.392x_2 + 2.8x_3 + 3.185x_1x_2 - 1.64x_1x_3 - 4.175x_2x_3 - 2.28x_1^2 - 0.919x_2^2 + 0.660x_3^2 \quad (8)$$

$$Y_{Moisture} = 29.47 - 6.31x_1 - 3.90x_2 + 6.67x_3 + 1.95x_1x_2 - 1.23x_1x_3 + 0.445x_2x_3 - 1.1929x_1^2 - 0.262x_2^2 - 2.395x_3^2 \quad (9)$$

$$Y_{Elastic\ modulus} = 354.9 + 213.18x_1 + 591.28x_2 - 926x_3 + 90.39x_1x_2 - 381.9x_1x_3 - 1325x_2x_3 - 399.9x_1^2 + 595.7x_2^2 + 834.85x_3^2 \quad (10)$$

$$Y_{Elongation\ at\ break} = 5.5 - 1.47x_1 - 1.325x_2 + 0.725x_3 + 0.65x_1x_2 - 0.4x_1x_3 + 0.35x_2x_3 + 0.275x_1^2 + 0.525x_2^2 + 0.27x_3^2 \quad (11)$$

$$Y_{Tensile\ strength} = 57.61 + 37.20x_1 + 37.32x_2 - 42.05x_3 + 0.918x_1x_2 - 8.05x_1x_3 - 46.6x_2x_3 + 2.43x_1^2 + 20.42x_2^2 + 26.50x_3^2 \quad (12)$$

Effect of operating parameters on properties of film

Thickness

Thickness affects the structure of film in relations of drying kinetics, WVP and film opacity (Maran et al. 2013a). Results showed that thickness varies from 0.06 mm to 0.16 mm with the change in the amount of dry matter in film suspension solution (Table 1). Analysis results showed that chitosan and glycerol had a significant impact on the thickness of the film (Table 2) whereas, starch did not show any significant impact on thickness of the film (p >0.05). In addition, the results in Fig. 2 (a) showed that higher the content of chitosan resulted in the formation of film with greater thickness. Chitosan is a positively charged molecule and being a positively charged moiety it have wide hydration layers with highly retained water molecules which participates in the film structure thus inhibiting the chain approximation and giving rise to thicker films. Similar explation related to the chitosan concentration and thickness of the films has been reported previously in the literature (Bonilla et al. 2013). The other possible explanation could be the over loading of the suspension solution. Unoptimized formulations in most of the previous studies is subject to increase in the unwanted film thickness as a result of overloading of suspension solution which hinders the permeability control of film eventually (Bof et al. 2015). Correlation between permeability properties and thickness can be explained by fick's law of diffusion, which says that permeability is inversely proportional to the thickness of the film. Hence higher the thickness lesser will be the mass transfer thorugh the film due to more resistance. Similar explation has been provided in the literature on the water vapour permeability and thicknes effect of hydrophilic films by McHugh et al. (1993).

Water vapour permeability

WVP is the main parameter used to explain the possible mass transfer mechanisms through the film surface. For edible coatings it should be low to prevent moisture loss from the fresh produce (Ma et al. 2008). The value of WVP varied significantly ($p<0.05$) with the concentration of polymer and plasticizer. Coating formulation comprising 1% chitosan; 0.5% starch and 0.75% glycerol showed the minimum permeability values (4.1×10^{-10} $gs^{-1}m^{-1}Pa^{-1}$) whereas increased concentration of starch and glycerol (1.5% pea starch and 0.75% glycerol) resulted in to higher WVP response (34.4×10^{-10} $gs^{-1}m^{-1}Pa^{-1}$ (Table 1). Biopolymer-plasticizer chemistry proposed a significant impact on WVP of the casted film. Binding between the NH_2 and OH functional groups forms a crosslinking network in the film structure and slow down the rate of permeability. Fig. 2 (b) shows the behaviour of increasing starch concentration on the film permeability attributes which could be due to the hydrophilic nature of starch that fails to resist the migration of water through the film surface. These results are in line with the previous study (Pelissari et al. 2009) where increased concentration of starch enhanced the WVP rate. Another possible reason for these observations behaviour could be explained on the basis of the oval granular structure of the pea starch, where the arrangement may leave some inter-granular spaces which at lower concentration are filled by other partaking ingredients but at higher concentration are available for free mass transfer. The WVP of the coated film gradually decreased as the concentration of chitosan increased from 1 to 2% (Table 1). The decreasing WVP value may be due to hydrophobic acetyl group of incompletely deacetylated chitosan or due to intense hydrogen bond interactions between NH_2 and OH functional groups. These interaction may be dominated over hydrophilic interactions thus reducing the availability of free hydrophilic groups at lower starch concentration. Glycerol also increases the mobility by reducing the rigidity and destabilisation of chain arrangements (by easily interacting with the starch chain) by minimizing the starch intermolecular and intramolecular hydrogen bonds

with starch-glycerol hydrogen bonds which disrupted the crystailline pattern of starch and facilitates the movement of water (Singh et al. 2009; Xu et al. 2005).

Solubility

Water solubility describes the water resistance and integrity of the edible film. Solubility of the chitosan-starch film was significantly affected by glycerol and chitosan concentrations ($p<0.05$). Results showed that the solubility of casted film was in the range of 40.8 to 55.2% and increases with the increase in glycerol concentration (Table 1; Fig. 2c) and decreases with higher chitosan concenteration. The possible reason may be due to glycerol which can disrupt the crystalline structure of the starch and causes breakage of hydrogen bonds and formation of new hydrogen bonds between exposed OH group of amylose and amylopectin and glycerol (Ratnayake et al. 2002). These findings are also in agreement with the previous reported work (Maran et al. 2013a; Mehyar and Han 2004). These results could be explained from the fact that higher chitosan concentration induces the strong interactions between the two polymers and lowers the resulting soulubility. These observations supports the previous studies where the solubility proportionally decreased as starch was blended with chitosan at higher concentration (Bourtoom and Chinnan 2008; Kanmani and Lim 2013).

Moisture content (MC)

MC describes the available moisture present in the film. Variations in the moisture content are shown in the Fig. 3 (a). Glycerol and starch were found to affect the moisture level in the film significantly ($p<0.05$) and MC was higher at higher concentration of glycerol, 1% (Table 1). This may be due to the hydrophilic nature of the glycerol which assist in the formation of hydrogen bonding with free OH groups (Cerqueira et al. 2012). Similar results were reported in previous studies on the increasing effect of glycerol on MC (Saberi et al. 2016; Sanyang et al. 2015b). Starch also facilitates the retention of MC (due to its hydrophilic nature) in films as

compared to chitosan (Fig. 3a). Lower MC at higher chitosan concentration may be due to higher interactions among the molecules leaving behind no free hydrophilic groups for interaction with the water molecules.

Mechanical properties: Tensile strength (TS), Elongation at break (EB) and Elastic modulus (EM)

TS is an important property of the films as it greatly affects the utility of the film for its application in shelf life extension of fresh produce during storage. The stability of film was measured on the basis of TS, EB, and EM. Fig. 3 (b-e) shows the effect of additives on the mechanical properties of edible film. Pea starch and chitosan blend provided a film with good mechanical properties which illustrate the compatibility of hydrocolloids. TS, EB and EM values of chitosan– pea starch film are presented in Table 1. It was found that strength of the film was significantly affected by varying polymeric concentration ($p<0.05$). TS was maximum with 1.5% chitosan and 1.5% of starch blended with lowest concentration of glycerol (0.5%) and depicts the greatest integrity of film forming components. TS varied between 26.6 to 266.9 N/m (Table 1). This is due to the formation of dominating inter-molecular hydrogen bonding between NH_2 and OH groups of chitosan-starch which increases with the increase in polymer concentration. Interaction of polymers increases the stability because of the participating functional groups in the bonding. Chitosan-starch hydrogen bonding is the intrinsic factor which supports the mechanical and physical properties of the film. These results are in agreement with previous research where tensile properties of starch films were improved significantly when chitosan was incorporated in to the starch solution (Xu et al. 2005; Zhai et al. 2004).

294 It is important to note that plasticizer also has a significant ($p<0.05$) effect on the TS of the film
295 (Fig. 3c). TS increases with the decrease in the plasticizer concentration and was maximum
296 (266.9 N/m) at 0.5% glycerol. This phenomenon can be explained by the role of glycerol on
297 diminishing the strong intra–molecular hydrogen bonding between starch and chitosan
298 molecules as previously reported by Sanyang et al. (2015a). The effectiveness of glycerol for
299 TS reduction is due to hydrophilic nature of the compound which hold more H_2O molecules
300 and resulted in to more intense plasticizing effect. This arrangement increases the spatial
301 difference between the polymer chains and decrease the TS. Moreover, a more vigorous
302 relationship between tensile properties and moisture content was observed portraying the
303 negative effect of moistire which might have caused extra plasticizing effect hence lower the
304 tensile strength of starch film. The results are in line with the presious study reported by
305 Chinma et al. (2015) which showed that tensile properties of starch films decreased with
306 increase in film humidity.

307 The % E and EM varies between 3.4-10.5 mm and 191.4-5815.4 N/m^2 respectively (Table 1).
308 % E was greater when higher chitosan concentration was applied and lower with higher starch
309 concentrations. Both chitosan and starch were found to affect the elongation property of films
310 at a significant ($p<0.05$) rate. Similarly EM value was higher for 1% chitosan, 1.5% starch and
311 0.5% glycerol (5815.4 N/m^2) and minimum with higher concentration of glycerol. High starch
312 concentration results in a lower ability of edible film for stretching where plasticizer influences
313 the flexibility of film by occupying the free space between the polymers.

314 **Optimization and validation of coating formulation**

315 Optimal edible film formulation was achieved by optimizing chitosan, starch and glycerol for
316 physical, mechanical and barrier properties. RSM was used for the optimization of the coating
317 formulation. Based on the effect of independent variables (chitosan, starch and glycerol) on the

response values for physical and mechanical properties of film, the optimal conditions for fomulation of this film were determined to be chitosan 1%, starch 1.5% and glycerol 0.5 %. To validate these predicted conditions, this fomulation was tested in triplcate experiments and the results showed that the actual values for physical and mechanical properties were found to be similar to the predicted values (Table 3). These results shows that this fomulation can be applied to prepare the pea starch film with good physical and mechanical properties for further utilisation.

Conclusion

RSM has been sucessfully applied to optimize the best fomulation for preparation of pea starch film for further utilisation in coating vegetable and fruits. All three tested ingridients (pea starch, chitosan and glycerol) were found to have different effects on physical, and mechanical properties of film. The results showed that optimal fomulation for preparation of pea starch film were chitosan 1% pea starch 1.5% and glycerol 0.5%. which had satisfactory thickness, good WVP, solubility, moisture content and mechanical properies. These findings can be further applied for coating vegetables and fruit.

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438 **List of tables**

439 **Table 1** Box- Behnken design employed for formulation of edible coating composition

Run	Independent variables			Dependent variables						
	Factors			Responses						
	X_1 (%)	X_2 (%)	X_3 (%)	T (mm)	WVP × 10^{-10} ($gs^{-1}m^{-1}Pa^{-1}$)	S (%)	M (%)	EM (N/m^2)	EB (mm)	TS (N/m)
1	1	1	0.5	0.0768	9.62	44.7	22.7	401.62	6.2	54.125
2	1	0.5	0.75	0.0736	4.16	52.7	42.1	681.93	10.5	26.64
3	1	1.5	0.75	0.0911	8.5	44.4	27.9	372.17	5.7	54.545
4	1	1	1	0.0841	5.09	53.1	40.4	372.79	8.2	30.46
5	1.5	0.5	0.5	0.0859	9.56	40.8	24.2	670.85	6.7	70.59
6	1.5	1.5	0.5	0.0677	10.56	52.6	18.0	5815.4	4.2	266.96
7	1.5	1	0.75	0.1246	16.25	54.0	31.0	191.50	5.6	65.64
8	1.5	1	0.75	0.1135	8.63	48.9	29.7	407.86	5.4	57.105
9	1.5	1	0.75	0.0919	7.12	47.1	28.0	465.56	5.5	50.105
10	1.5	0.5	1	0.1055	7.15	55.2	34.8	406.23	7.7	35.47
11	1.5	1.5	1	0.1596	16.4	50.3	30.3	249.92	6.6	45.165
12	2	1	0.5	0.1074	23.7	46.9	13.9	1970.9	4.7	149.015
13	2	0.5	0.75	0.1096	12.8	42.8	24.2	548.57	5.6	78.3
14	2	1.5	0.75	0.13	34.4	47.3	17.8	600.39	3.4	142.935
15	2	1	1	0.1262	15	48.8	26.6	414.15	5.1	93.145

440 Independent variables: X_1, = Chitosan (1-2%), X_2= Starch (0.5-1.5%), X_3= Glycerol (0.5-1%)

441 Responses (Y): T= Thickness (mm); WVP= Water vapour permeability ($gs^{-1}m^{-1}pa^{-1}$); S= Solubility (%); M= Moisture (%): EM= Elastic modulus
442 (N/m^2); EB= Elongation at break (mm), TS= tensile strength (N/m).

Table 2 ANOVA study for the model fitting.

Parameters	T (mm)	WVP× 10^{-10} ($gs^{-1}m^{-1}Pa^{-1}$)	S (%)	MC (%)	EM (N/m^2)	EB (mm)	TS (N/m)
Lack of fit	0.51	0.51	0.78	0.19	0.01	0.01	0.03
R^2	0.83	0.85	0.84	0.97	0.79	0.94	0.90
Adjusted R^2	0.54	0.59	0.54	0.91	0.40	0.84	0.71
F ratio of model	2.791	3.26	2.84	17.67	2.05	9.09	4.83
Prob>F	0.50	0.51	0.74	0.003	0.22	0.01	0.05
Press	0.0158	1377.8	338.5	376.0	957664.1	38.65	89595.6

WVP = Water vapour permeability ($gs^{-1}m^{-1}pa^{-1}$), S= Solubility (%), MC= Moisture content (%), EM= Elastic modulus (N/m^2), EB= Elongation at break (mm), TS= Tensile strength (N/m).

Table 3 Validation of predicted values for physical, mechanical and barrier properties of pea starch: chitosan blended film.

Variables Response	Predicted value	Experimental value (n=3)
Thickness (mm)	0.055 ± 0.01	0.058 ± 0.03
WVP ($gs^{-1}m^{-1}Pa^{-1}$)	5.29 ± 0.07	5.27 ± 0.03
Solubility (%)	45.53± 0.1	48.12±0.06
Moisture Content (%)	17.73±0.03	19.14±0.08
Elastic modulus (N/m^2)	3543.53 ±2.56	3559.25±5.69
Elongation at break (mm)	4.6±0.92	5.0 ±1.34
Tensile Strength (Nm^{-2})	173.7±2.13	181.8± 1.78

459 **Figure captions**

460 **Fig 1:** Correlation between predicted and experimental values for thickness (a), WVP (b), solubility (c), moisture content (d), tensile strength (e),
461 Elongation at break (f) and elastic modulus (g),

462 **Fig 2**: Response surface plots showing the interaction impact of independent variables on the thickness (a) WVP (b) and solubility (c) of the edible
463 film

464 **Fig 3:** 3 D Contour plots for moisture % (a), tensile strength (b-c), elongation at break (d) and Elastic modulus (e) showing the interaction impact
465 of independent variables on the pea starch: chitosan film

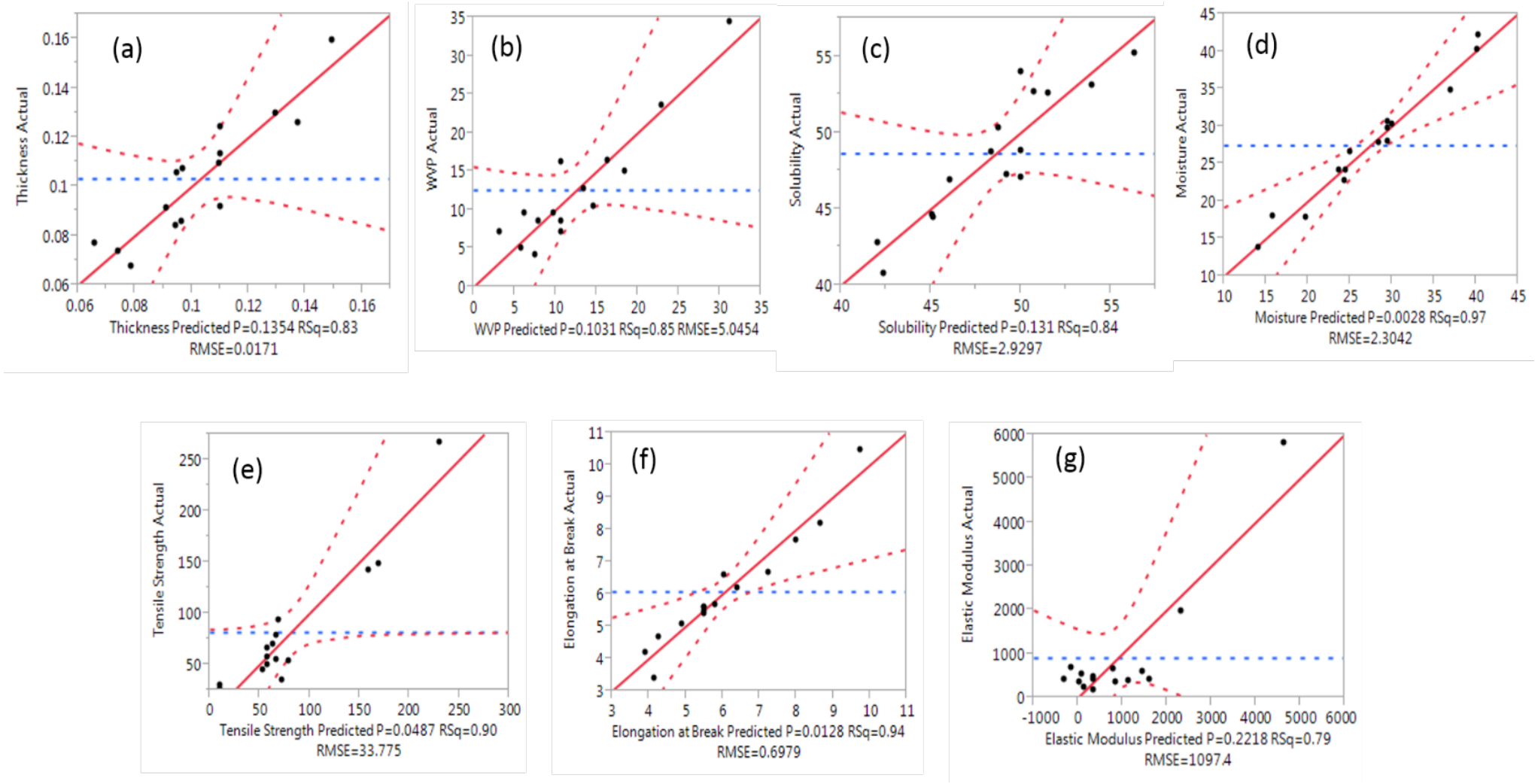
(a)
Thickness Actual
Thickness Predicted P=0.1354 RSq=0.83
RMSE=0.0171
(b)
WVP Actual
WVP Predicted P=0.1031 RSq=0.85 RMSE=5.0454
(c)
Solubility Actual
Solubility Predicted P=0.131 RSq=0.84
RMSE=2.9297
(d)
Moisture Actual
Moisture Predicted P=0.0028 RSq=0.97
RMSE=2.3042
(e)
Tensile Strength Actual
Tensile Strength Predicted P=0.0487 RSq=0.90
RMSE=33.775
(f)
Elongation at Break Actual
Elongation at Break Predicted P=0.0128 RSq=0.94
RMSE=0.6979
(g)
Elastic Modulus Actual
Elastic Modulus Predicted P=0.2218 RSq=0.79
RMSE=1097.4

Fig 1

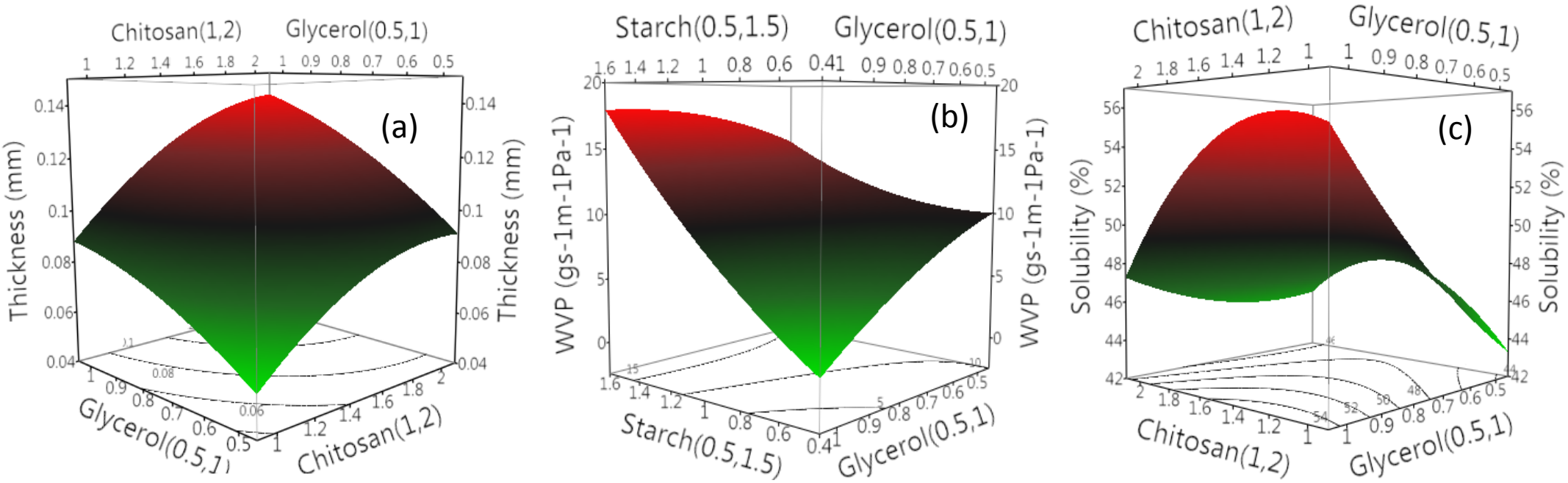
Chitosan(1,2)
Glycerol(0.5,1)
(a)
Thickness (mm)
Glycerol(0.5,1)
Chitosan(1,2)
Starch(0.5,1.5)
Glycerol(0.5,1)
(b)
WVP (gs-1m-1Pa-1)
Starch(0.5,1.5)
Glycerol(0.5,1)
Chitosan(1,2)
Glycerol(0.5,1)
(c)
Solubility (%)
Chitosan(1,2)
Glycerol(0.5,1)

Fig 2

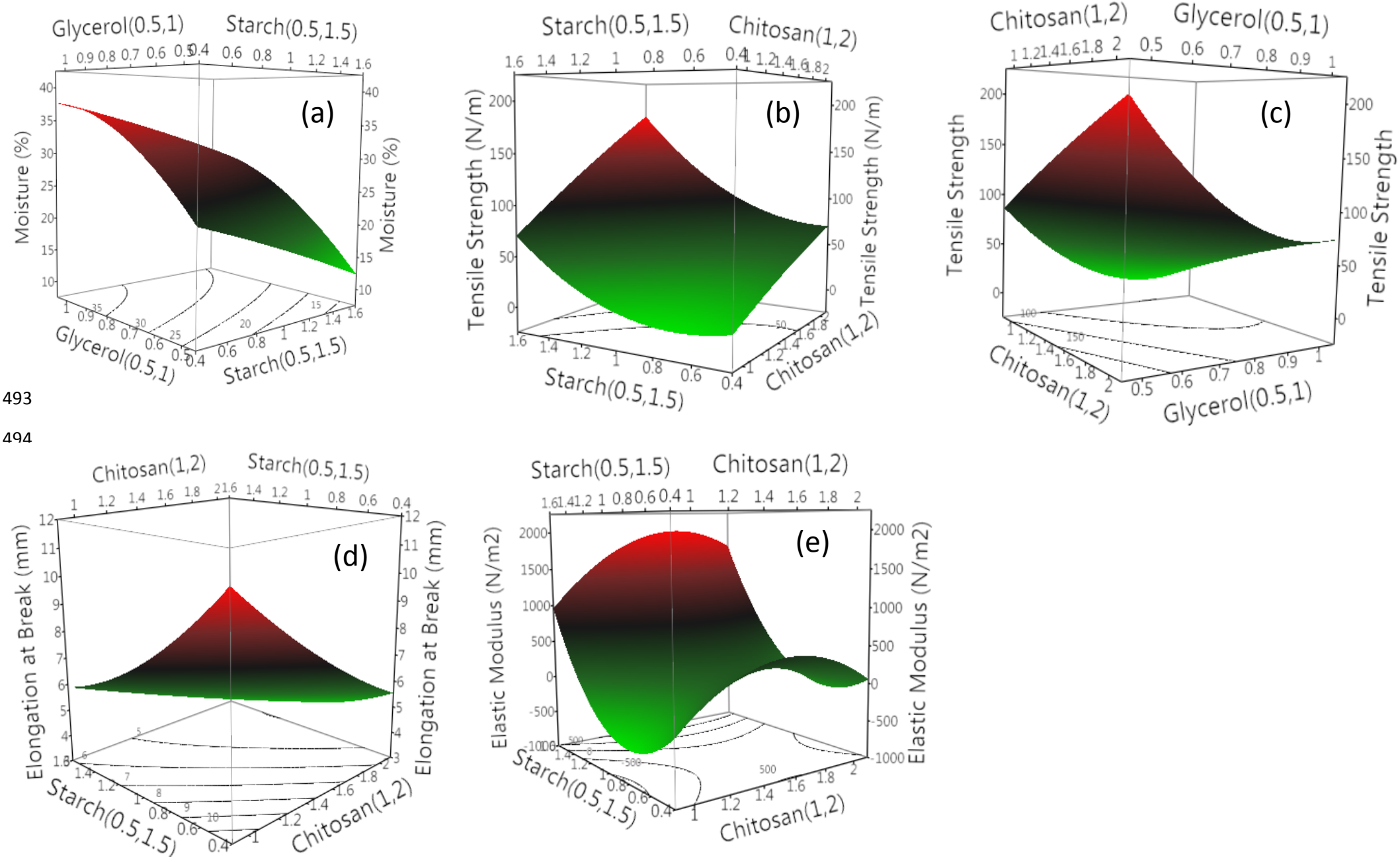
Glycerol(0.5,1)
Starch(0.5,1.5)
Moisture (%)
(a)
Starch(0.5,1.5)
Chitosan(1,2)
Tensile Strength (N/m)
(b)
Chitosan(1,2)
Glycerol(0.5,1)
Tensile Strength
(c)
Chitosan(1,2)
Starch(0.5,1.5)
Elongation at Break (mm)
(d)
Starch(0.5,1.5)
Chitosan(1,2)
Elastic Modulus (N/m2)
(e)

Fig 3