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Optimization of physical and optical properties of biodegradable edible films based on pea starch and guar gum

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ABSTRACT

The influence of process variables (pea starch, guar gum and glycerol) on the viscosity (V), solubility (SOL), moisture content (MC), transparency (TR), Hunter parameters (L , a , and b), total color difference (ΔE), yellowness index (YI), and whiteness index (WI) of the pea starch based edible films was studied using three factors with three level Box–Behnken response surface design. The individual linear effect of pea starch, guar and glycerol was significant ($p < 0.05$) on all the responses. However, a value was only significantly ($p < 0.05$) affected by pea starch and guar gum in a positive and negative linear term, respectively. The effect of interaction of starch $\times$ glycerol was also significant ($p < 0.05$) on TR of edible films. Interaction between independent variables starch $\times$ guar gum had a significant impact on the b and YI values. The quadratic regression coefficient of pea starch showed a significant effect ($p < 0.05$) on V, MC, L , b , ΔE , YI, and WI; glycerol level on ΔE and WI; and guar gum on ΔE and SOL value. The results were analyzed by Pareto analysis of variance (ANOVA) and the second order polynomial models were developed from the experimental design with reliable and satisfactory fit with the corresponding experimental data and high coefficient of determination (R^2) values (> 0.93). Three-dimensional response surface plots were established to investigate the relationship between process variables and the responses. The optimized conditions with the goal of maximizing TR and minimizing SOL, YI and MC were 2.5 g pea starch, 25 % glycerol and 0.3 g guar gum. Results revealed that pea starch/guar gum edible films with appropriate physical and optical characteristics can be effectively produced and successfully applied in the food packaging industry.

Keywords: Pea starch; Guar gum; Edible films; Response surface methodology

1. Introduction

Edible films and coatings as an emerging packaging technique contribute to increasing food quality, prolonging shelf life and, potentially, developing the economic proficiency of packaging resources (Rojas-Graü et al., 2009). Edible films provide substitute and/or reinforcement of natural layers to avoid moisture losses, while permitting for controlled interchange of important gases involved in respiration processes, such as oxygen, carbon dioxide, and ethylene. A film or coating can also prevent loss of other important components and afford surface sterility (Pavlath and Orts, 2009). Edible coatings are part of the final food product and should provide adequate color, odor, taste, flavor, and texture to the coated product (Valencia-Chamorro et al., 2011). They can also improve mechanical characteristics and perform as active films while carrying food additives (antimicrobials or antioxidants) serving in food quality preservation (Min and Krochta, 2005).

Edible films based on starch have been mainly developed because they display physical properties similar to those of synthetic polymers; they are transparent, odorless, tasteless, semi-permeable to CO₂ and resistant to O₂ diffusion (Nisperos-Carriedo, 1994), while they can be edible, biocompatible, non-toxic, non-polluting and low costing (Vásconez et al., 2009).

Pea starch has been reported to produce films with improved physical and mechanical properties in comparison with films prepared from other starches due to the high amount of amylose (Chen et al., 2009b; Corrales et al., 2009; da Matta et al., 2011; Han et al., 2006; Mehyar and Han, 2004; Saberi et al., 2015a, b; Sun et al., 2013; Wu et al., 2010; Zhang and Han, 2006; Zhang and Han, 2008). Pea starch films demonstrate numerous weaknesses such as their poor mechanical properties and hydrophilic behavior, similar to other hydrocolloids, when compared to plastic polymers. Since the physicochemical characteristics of starch-based films are restricted by their

crystallization degree, recent investigations have scrutinized the influence of other carbohydrates incorporated into starch to modify the physicochemical properties of the films (Jiménez et al., 2012). Composite biopolymer films can bring about improved mechanical and physical properties if components are structurally compatible. Thus, blending a starch with other hydrocolloids can produce films with interesting properties and potential for novel applications.

Guar gum is a galactomannan with a mannose to galactose ratio of 1:6 obtained from the endosperm of an annual legume plant, *Cyamopsis tetragonoloba* (Cunha et al., 2005). The backbone of guar gum is a linear chain of (1–4) β -D-mannopyranose with galactose as a side group linked by (1–6) α -D-galactopyranose (Fernandes et al., 1993). Cui et al. (2006) has reported the synergism of guar gum with other components such as xanthan gum, agar, carageenan, and starch. There are a very few reports on biodegradable packaging film based on guar gum. A water resistant biocide film has been developed by modifying guar gum into guar gum benzamide by Das et al. (2011). Mikkonen et al. (2007) improved the mechanical properties of guar gum based films by enzymatic depolymerization. The simultaneous impacts of gamma irradiation and addition of nanoclays on the mechanical and barrier properties of guar gum based biodegradable films was studied by Saurabh et al. (2015). Rao et al. (2010) developed composite films based on chitosan and guar gum to evaluate optical, mechanical, barrier and antimicrobial properties of these films. The objective of this study was to evaluate via a response surface methodology (RSM), the influence of pea starch, guar gum and glycerol levels on the physical and optical properties of pea starch based edible films, with the aim of optimizing the formulation for food packaging.

2. Materials and Methods

2.1. Materials

Canadian non-GMO yellow pea starch with 13.2% moisture, 0.2% protein, 0.5% fat and 0.3% ash, was used in all experiments (supplied by Yantai Shuangta Food Co., Jinling Town, China). Guar gum (E-412) was purchased from The Melbourne Food Ingredient Depot, Brunswick East, Melbourne, Australia. All other chemicals were purchased from Merck Millipore Pty. Ltd., Victoria, Australia.

2.2. Preparation of film-forming solution

The film-forming solution was prepared by dissolving pea starch (2-3 g) and guar gum (0.1-0.5 g) in 100 ml degassed deionized water with gentle heating (about 40 °C) and magnetic stirring, followed by the addition of 15%, 25%, and 35% glycerol based on the dry film matter. The dispersion was then heated at 90 °C for 20 min with gentle magnet stirring to allow complete gelatinization of the starch. After gelatinization, the film solution was cooled to room temperature with gentle magnetic stirring for 1 h to reduce air bubbles (Sabeti et al., 2015b).

2.3. Viscosity of film forming solutions

A viscometer (Brookfield, DV-II+S04, Brookfield Viscometers Ltd, Harlow, UK) was used to determine the viscosity (V) of each film suspension at 100 rpm and 25 °C by using spindle No. 2. Viscosity was measured for three replicated samples for each type of film (Sabeti et al., 2015a).

2.4. Film preparation

All the films were prepared by casting method where 20 g of filmogenic suspensions were poured onto Petri dishes (10 cm in diameter). Films were formed by drying at 40 °C in an oven until reaching constant weight (about 24 h). The prepared transparent and homogenous films were

peeled-off from Petri dishes and equilibrated at 25 °C, 65 % relative humidity for 72 h prior to further examination.

2.5. Solubility in water

The method described by Farahnaky et al. (2013) was applied to calculate the solubility of films in water. The films were cut into (15 mm × 40 mm) specimens, weighed and immersed in 50 ml of distilled water. The beaker was sealed to prevent the evaporation of water and stored at 25 °C for 24 h with gentle agitation (10 rpm). After that period, the remaining pieces of films were filtered and dried at 110 °C to constant weight (final dry weight). The percentage of the solubility (SOL) of the films was calculated using the following equation:

$$\% \text{ SOL} = \frac{\text{initial dry weight} - \text{final dry weight}}{\text{initial dry weight}} \times 100 \quad (1)$$

2.6. Moisture content

Film moisture content was determined from the weight loss through which the films underwent a 24 h oven drying at 90 °C. The temperature was chosen to avoid loss of plasticizer (Khazaei et al., 2014). Moisture content (MC) was calculated using the following equation:

$$\text{Moisture content (MC)} = \frac{M_i - M_f}{M_i} \times 100 \quad (2)$$

where M_i and M_f are the masses of initial and dried samples, respectively. Three replicates were obtained for each sample.

2.7. Transparency

Films transparency (TR) was determined using a UV Vis Spectrophotometer (Varian Australia Pty. Ltd., Melbourne, VIC Australia) as described by Ozdemir and Floros (2008). The films were

cut into rectangular shapes (15 mm × 40 mm) and placed inside the spectrophotometer cell at 560 nm. Three replicates of each film were tested and tests were conducted at 25 ± 2 °C and 50 ± 5 % RH. The percent transparency was calculated as follows:

$$\% \text{ TR} = \left(\frac{I_r}{I_0} \right) \times 100 \quad (3)$$

where I_r is the light intensity with the specimen in the beam and I_0 is the light intensity with no specimen in the beam (Prakash Maran et al., 2013b).

2.8. Color

The color of each film was determined with a Minolta colorimeter (CR-300 series, Radiometric instruments Operations, Osaka, Japan). A white color plate was used as a standard for calibration and as a background for color measurements of the films. The lightness (L^*) and chromaticity parameters ' a^* ' (red-green) and ' b^* ' (yellow-blue) were measured (Rubilar et al., 2013). The total color difference (ΔE), yellowness (YI) and whiteness (WI) indexes of samples were calculated:

$$\Delta E = \sqrt{(L^* - L)^2 + (a^* - a)^2 + (b^* - b)^2} \quad (4)$$

$$YI = \frac{142.86b}{L} \quad (5)$$

$$WI = 100 - \sqrt{(100 - L)^2 + a^2 + b^2} \quad (6)$$

where L^* , a^* , and b^* are the standard color parameter values and ' L ', ' a ' and ' b ' are the color parameter values of the sample (Khazaei et al., 2014). The measurements were repeated six times for each film.

2.9. Experimental design

A three-level-three-factor, Box–Behnken response surface design (BBD) with five central point replicates was applied for studying the effect of process parameters (pea starch (X_1): 2–3 g, glycerol (X_2): 15–35 %, and guar gum (X_3): 0.1–0.5 g on film properties (Table 1). The optimum levels of the independent variables were selected from preliminary single factor tests. All experimental runs are listed in Table 2. The experimental data obtained for the seventeen experimental runs were fitted to the following second-order polynomial model:

$$Y = \beta_0 + \sum_{i=1}^k \beta_i X_i + \sum_{i=1}^{k-1} \sum_{j=2}^k \beta_{ij} X_i X_j + \sum_{i=1}^k \beta_{ii} X_i^2 + e_i \quad (7)$$

where various X_i values are independent variables affecting the responses Y ; β_0 , β_i , β_{ii} , and β_{ij} are the regression coefficients for intercept, interaction coefficients of linear, quadratic and the second-order terms, respectively and k is the number of variables (Bhuyan et al., 2015).

Table 1. Independent variables and their code variable levels used for the Box–Behnken design.

Coded variable levels	Independent variables		
	Pea starch (g)	Glycerol (% w/w)	Guar gum (g)
+1	2	15	0.1
0	2.5	25	0.3
-1	3	35	0.5

2.10. Statistical analysis

168 JMP software (Version 11, SAS, Cary, NC, USA) was used to establish the model equation to
169 graph the the 3D- and 2D contour plots of variable responses and to predict the optimum values
170 for the three independent variables. The adequacy of the response surface methodology (RSM)
171 second-order polynomial model was determined based on the lack of fit and the coefficient of
172 determination (R^2). SPSS statistical software (version 16.0) was utilized to compare the means
173 analysis by independent samples *t*-test. The differences between the mean values in the performed
174 experiments were taken to be statistically significant at $p < 0.05$.

175 **Table 2.** Box–Behnken experimental design with process variables (un-coded) and observed responses.*

176

Run	Starch (g)	Glycerol (% w/w)	Guar gum (g)	V (mPa.s)	SOL (%)	MC (%)	TR (%)	<i>L</i>	<i>a</i>	<i>b</i>	ΔE	YI	WI
1	2	25	0.1	42.533	21.038	9.741	82.385	92.724	-3.881	6.899	4.822	10.629	89.243
2	2	15	0.3	135.333	24.829	11.686	81.452	92.324	-3.941	7.232	5.311	11.198	88.731
3	2	35	0.3	95.133	27.471	13.205	80.487	91.742	-3.911	7.566	5.986	11.783	88.107
4	2	25	0.5	348.000	32.582	15.552	78.285	91.213	-3.989	7.970	6.663	12.478	87.454
5	2.5	15	0.1	62.333	23.804	16.374	87.575	94.846	-3.776	6.391	2.652	9.627	90.954
6	2.5	35	0.1	50.267	26.904	17.774	86.321	94.302	-3.722	6.566	3.250	9.945	90.506
7	2.5	25	0.3	192.900	28.090	19.488	83.412	93.631	-3.812	6.732	4.113	10.417	89.820
8	2.5	25	0.3	193.100	27.884	19.488	84.653	93.124	-3.827	6.761	4.051	10.405	89.805
9	2.5	25	0.3	190.100	27.582	18.738	83.409	93.612	-3.710	6.815	4.062	10.421	89.812
10	2.5	25	0.3	190.800	31.078	18.572	83.271	92.993	-3.803	6.826	4.151	10.412	89.831
11	2.5	25	0.3	193.400	28.100	19.125	83.409	93.662	-3.816	6.833	4.021	10.426	89.828
12	2.5	15	0.5	659.740	34.299	20.700	82.916	93.390	-3.848	7.167	4.320	10.963	89.486
13	2.5	35	0.5	538.400	37.563	23.374	80.532	92.663	-3.798	7.307	5.064	11.265	88.912
14	3	25	0.1	70.667	28.100	25.840	90.249	95.546	-3.676	6.151	1.914	9.197	91.558
15	3	15	0.3	446.667	32.433	26.684	89.583	94.879	-3.703	6.484	2.701	9.761	90.924
16	3	35	0.3	371.333	36.100	29.376	85.916	94.179	-3.687	6.651	3.409	10.086	90.387
17	3	25	0.5	805.933	42.266	31.402	84.916	93.503	-3.762	6.874	4.101	10.504	89.780

177 * V: Viscosity; SOL: Solubility; MC: Moisture content; TR: Transparency; *L*, *a*, and *b*: Hunter parameters; ΔE: Total color difference; YI: Yellowness index; WI:
178 Whiteness index.

3. Results and Discussions

3.1. The response surface methodology fitting model

The influence of the variables: starch, glycerol and guar gum on the V, SOL, MC, TR, Hunter parameters (L , a , and b), ΔE , WI, and YI was investigated through a 3^3 factorial experimental design with response surface methodology (RSM) and Box–Behnken design. The Box–Behnken experimental design and the observed dependent variables data are summarized in Table 2. It is essential to apply the ANOVA analysis in order to conclude whether or not the quadratic model is significant (Liu et al., 2015). The analysis of variance of the Box–Behnken design for establishing the model fit is demonstrated in Fig. 1 and Table 3.

The lack of fit test and the analysis of variance were used to evaluate the fitted model and the suitability of the true response surface. The coefficient of determination (R^2) of the model was 1.00, signifying that 100 % of the experimental data can be predictably fitted with the model data for viscosity. The predicted residual sum of square (PRESS) for the model, which is an indication of how well the predictive model matches each point in the design (Sahin and Samli, 2013), was 25259.34. The lack of fit value, F value and p -value of the model were calculated to be 0.061, 401.48 and < 0.0001 , respectively, which in turn revealed that the mathematical model was satisfactory for prediction of viscosity of film forming solutions.

The RSM mathematical models for solubility were also calculated. The results (Fig. 1B) revealed p value for solubility of < 0.0001 . Coefficient of determination (R^2) for the solubility model (Table 3) was estimated to be 0.98, further specifying a close correlation between the predicted values and experimental values. PRESS values (54.84) and F values (32.29) showed that the mathematical model was successful predictor of solubility properties of the pea starch edible films.

201 Fitting the model for MC and TR showed that R^2 value of the models was 1.00 and 0.99,
202 respectively. The p -value for lack of fit, PRESS, F value and p -value of the model for MC were
203 0.63, 6.55, 422.61 and < 0.0001 , respectively, and for TR were 0.78, 7.85, 74.21 and < 0.0001 ,
204 respectively. The results verified the reliability of the model in predicting MC and TR of films.

205 The results for Hunter parameters (L , a , and b) and ΔE also confirmed the competency of the
206 model. The R^2 values of L , a , b and ΔE were 0.99, 0.93, 0.99, and 1.00, respectively (Fig. 1E–H).
207 The p -values for lack of fit for all Hunter parameters and ΔE were found insignificant ($p > 0.05$,
208 Table 3). The p -values (< 0.0001 , < 0.0034 , < 0.0001 and < 0.0001 , respectively), F values (35.561,
209 9.68, 94.10 and 307.47, respectively) and PRESS values (1.09, 0.03, 0.30 and 0.78, respectively)
210 of the model also supported the efficiency of these models in correctly evaluating the Hunter
211 parameters and ΔE of pea starch edible films.

212 The coefficient of determination (R^2) of the model was 1.00, signifying that 100 % of the
213 experimental data can be predictably fitted with the model data for YI and WI. The predicted
214 residual sum of square (PRESS) for the model, which is an indication of how well the predictive
215 model matches each point in the design (Sahin and Samli, 2013), was 0.82 and 0.61 for YI and
216 WI, respectively. The lack of fit value, F value and p -value of the model were calculated to be
217 0.102, 155.80 and < 0.0001 for YI, and 0.444, 346.88 and < 0.0001 for WI, which in turn revealed
218 that the mathematical model was satisfactory for prediction of YI and WI of films.

Fig. 1. Correlations between predicted and experimental viscosity (A), solubility (B), moisture content (C), transparency (D), L (E), a (F), b (G), total color difference (H), yellowness index (I), and whiteness index (J).

223

224 **Table 3.** Analysis of variance for determination of model fitting.*

Sources of variation	V (mPa.s)	SOL (%)	MC (%)	TR (%)	<i>L</i>	<i>a</i>	<i>b</i>	ΔE	YI	WI
Lack of fit	0.061	0.744	0.629	0.783	0.961	0.944	0.156	0.060	0.102	0.444
R ²	0.998	0.976	0.998	0.990	0.989	0.926	0.992	0.997	0.995	0.998
Adjusted R ²	0.995	0.946	0.996	0.976	0.951	0.830	0.981	0.994	0.987	0.995
PRESS	25259.34	54.836	6.545	7.853	1.089	0.027	0.301	0.777	0.817	0.606
F ratio of model	401.48	32.29	422.607	74.211	35.561	9.680	94.099	307.473	155.800	346.873
<i>p</i> of model χ^2 F	< 0.0001*	< 0.0001*	< 0.0001*	< 0.0001*	< 0.0001*	< 0.0034*	< 0.0001*	< 0.0001*	< 0.0001*	< 0.0001*

225 * Significant difference with $p < 0.05$.

3.2. Development of second order polynomial mathematical models

By conducting multiple regression analysis on the experimental data, eight second order polynomial mathematical models were obtained. These mathematical models were applied to demonstrate the relationship between independent variables and the responses on the optical and mechanical characteristics of the pea starch based edible films (Prakash Maran et al., 2013a). The final equations developed in terms of coded factors are given below:

$$V = 192.06 + 134.20x_1 - 31.12x_2 + 265.78x_3 - 8.78x_1x_2 + 107.45x_1x_3 - 27.32x_2x_3 + 29.58x_1^2 + 40.48x_2^2 + 95.15x_3^2 \quad (8)$$

$$SOL = 28.55 + 4.12x_1 - 1.58x_2 + 5.86x_3 + 0.26x_1x_2 + 0.66x_1x_3 + 0.041x_2x_3 + 1.01x_1^2 + 0.65x_2^2 + 1.44x_3^2 \quad (9)$$

$$MC = 19.082 + 7.890x_1 + 1.036x_2 + 2.662x_3 + 0.293x_1x_2 - 0.062x_1x_3 + 0.318x_2x_3 + 1.117x_1^2 + 0.038x_2^2 + 0.434x_3^2 \quad (10)$$

$$TR = 83.631 + 3.507x_1 - 1.034x_2 - 2.485x_3 - 0.675x_1x_2 - 0.308x_1x_3 - 0.283x_2x_3 + 0.176x_1^2 + 0.553x_2^2 + 0.152x_3^2 \quad (11)$$

$$L = 93.404 + 1.263x_1 - 0.319x_2 - 0.831x_3 - 0.031x_1x_2 - 0.133x_1x_3 - 0.046x_2x_3 - 0.339x_1^2 + 0.215x_2^2 + 0.181x_3^2 \quad (12)$$

$$a = -3.793 + 0.111x_1 + 0.019x_2 - 0.042x_3 - 0.003x_1x_2 + 0.005x_1x_3 - 0.001x_2x_3 - 0.030x_1^2 + 0.012x_2^2 - 0.005x_3^2 \quad (13)$$

$$b = 6.793 - 0.438x_1 + 0.102x_2 + 0.414x_3 - 0.042x_1x_2 - 0.088x_1x_3 - 0.009x_2x_3 + 0.153x_1^2 + 0.037x_2^2 + 0.027x_3^2 \quad (14)$$

$$\Delta E = 4.080 - 1.332x_1 + 0.341x_2 + 0.939x_3 + 0.008x_1x_2 + 0.087x_1x_3 + 0.037x_2x_3 + 0.413x_1^2 - 0.141x_2^2 - 0.117x_3^2 \quad (15)$$

$$YI = 10.416 - 0.818x_1 + 0.191x_2 + 0.727x_3 - 0.065x_1x_2 - 0.136x_1x_3 - 0.004x_2x_3 + 0.271x_1^2 + 0.019x_2^2 + 0.014x_3^2 \quad (16)$$

$$WI = 89.819 + 1.139x_1 - 0.273x_2 - 0.829x_3 + 0.022x_1x_2 + 0.003x_1x_3 - 0.032x_2x_3 - 0.369x_1^2 + 0.087x_2^2 + 0.058x_3^2 \quad (17)$$

3.3. The effect of independent variables on viscosity

The linear regression coefficients and their statistical significance are presented in Table 4. All the three independent process variables: starch (2–3 g), glycerol (15–35 %) and guar gum (0.1–0.5 g) had significant effect on the viscosity of film forming solutions ($p < 0.05$). The viscosity increased steadily when starch and guar gum increased; however, the levels of viscosity decreased when glycerol exceeded from 15 % to 35 % (Fig. 2 A-C). Similar observations were made by Farahnaky et al. (2013), Bertuzzi et al. (2007), and López et al. (2008) who indicated that a decrease in glycerol content significantly increased viscosity of filmogenic suspensions. Plasticizers restrict polymeric chain interactions reducing the stiffness of the network, creating a less organized film structure, causing lower viscosity (Sothornvit and Krochta, 2005). As concentrations of starch and gum increase, starch-starch and starch-gum associations become dominant and a greater amount of water molecules are immobilized, bringing about additional increase of apparent viscosity (Bertuzzi et al., 2007). Interaction between independent variables starch $\times$ guar gum, and glycerol $\times$ guar gum had a significant impact on the viscosity ($p > 0.05$, Table 4). The values of this

266 parameter were positively influenced by the positive quadratic effect of the starch, glycerol and
267 guar gum.

Table 4. Analysis of variance for the experimental results.*

P	DF	V		SOL		MC		TR		L	
		Estimate	Prob ^{>}	Estimate	Prob ^{>}	Estimate	Prob ^{>} t	Estimate	Prob ^{>}	Estimate	Prob ^{>}
			t		t				t		t
β_0	1	192.06	<.0001*	28.55	<.0001*	19.082	<.0001*	83.631	<.0001*	93.404	<.0001*
β_1	1	134.20	<.0001*	4.12	<.0001*	7.890	<.0001*	3.507	<.0001*	1.263	<.0001*
β_2	1	-31.12	0.0006*	1.58	0.0087*	1.036	<.0001*	-1.034	0.0006*	-0.319	0.0086*
β_3	1	265.78	<.0001*	5.86	<.0001*	2.662	<.0001*	-2.485	<.0001*	-0.831	<.0001*
β_{12}	1	-8.78	0.2815	0.26	0.6924	0.293	0.1736	-0.675	0.0283*	-0.030	0.8200
β_{13}	1	107.45	<.0001*	0.66	0.3266	-0.062	0.7567	-0.308	0.2487	-0.133	0.3222
β_{23}	1	-27.32	0.0084*	0.04	0.9492	0.318	0.1441	-0.283	0.2868	-0.046	0.7249
β_{11}	1	29.58	0.0050*	1.01	0.1401	1.117	0.0006*	0.176	0.4861	-0.339	0.0272*
β_{22}	1	40.48	0.0009*	0.65	0.3162	0.038	0.8443	0.553	0.0538	0.215	0.1204
β_{33}	1	95.15	<.0001*	1.44	0.0488*	0.434	0.0548	0.152	0.5441	0.181	0.1813

*Significantly different at $p < 0.05$; P: parameter; β_0 : intercept; β_1 , β_2 , and β_3 : linear regression coefficients for pea starch, glycerol and guar gum; β_{12} , β_{13} , and β_{23} : regression coefficients for interaction between starch $\times$ glycerol, starch $\times$ guar gum and glycerol $\times$ guar gum; β_{11} , β_{22} , and β_{33} : quadratic regression coefficients for starch $\times$ starch, glycerol $\times$ glycerol, guar gum $\times$ guar gum.

Table 4 (continue). Analysis of variance for the experimental results.

* Significantly different at $p < 0.05$; P: parameter; β_0 : intercept; β_1 , β_2 , and β_3 : linear regression coefficients for pea starch, glycerol and guar

P	DF	<i>a</i>		<i>b</i>		ΔE		YI		WI	
		Estimate	Prob ^{>} t	Estimate	Prob ^{>} t	Estimate	Prob ^{>} t	Estimate	Prob ^{>} t	Estimate	Prob ^{>} t
β_0	1	-3.793	<.0001*	6.793	<.0001*	4.076	<.0001*	10.416	<.0001*	89.819	<.0001*
β_1	1	0.112	<.0001*	-0.438	<.0001*	-1.332	<.0001*	-0.818	<.0001*	1.139	<.0001*
β_2	1	0.019	0.1985	0.102	0.0021*	0.341	<.0001*	0.191	0.0021*	-0.273	<.0001*
β_3	1	-0.043	0.0143*	0.414	<.0001*	0.939	<.0001*	0.727	<.0001*	-0.829	<.0001*
β_{12}	1	-0.004	0.8556	-0.042	0.2127	0.008	0.8616	-0.065	0.2127	0.022	0.5749
β_{13}	1	0.005	0.7792	-0.087	0.0246*	0.087	0.0997	-0.136	0.0246*	0.003	0.9428
β_{23}	1	-0.001	0.9588	-0.009	0.7822	0.037	0.4499	-0.004	0.7822	-0.032	0.4225
β_{11}	1	0.029	0.1539	0.153	0.0013*	0.413	<.0001*	0.217	0.0013*	-0.369	<.0001*
β_{22}	1	0.012	0.5330	0.037	0.2531	-0.141	0.0158*	0.019	0.2531	0.087	0.0467*
β_{33}	1	-0.005	0.8084	0.027	0.3879	-0.117	0.0334*	0.014	0.3879	0.058	0.1492

β_{12} , β_{13} , and β_{23} : regression coefficients for interaction between starch $\times$ glycerol, starch $\times$ guar gum and glycerol $\times$ guar gum;

β_{11} , β_{22} , and β_{33} : quadratic regression coefficients for starch $\times$ starch, glycerol $\times$ glycerol, guar gum $\times$ guar gum.

3.4. *The effect of independent variables on solubility*

The ideal value for the film solubility depends on its application or proposed use. When the water activity is high, biodegradable films can perform as packaging material to enhance product integrity and water resistance in water-rich foods such as peeled fruits (Cerqueira et al., 2012). On the other hand, water solubility of the film for immediate consumption of food and coating might be useful in encapsulation of food or additives (Maizura et al., 2007). Analysis of the solubility assays of the films revealed that this parameter was influenced by all three independent variables ($p < 0.05$, Table 4). The interaction between variables starch $\times$ glycerol, starch $\times$ guar gum and glycerol $\times$ guar gum was shown to have no significant consequences on the solubility assay.

The water solubility changed from 21.04 % to 42.27 % with increasing starch, guar gum and glycerol content in films (Fig. 2). In swollen film structure, crosslinking between biopolymer molecules was most likely decreased due to the high affinity of guar gum to absorb water, which resulted in higher solubility in water (Ahmadi et al., 2012). Increased concentrations of glycerol decreased the polymer network interaction density by disturbing the network with hydrogen bonds, which increased film solubility in water. It has been observed that water solubility values ranged between 17.33-19.89 %, 26.6-38.8 %, and 40–50 % for potato starch film, cassava starch/konjac glucomannan composite film, and konjac glucomannan film, respectively (Li et al., 2006; Nair et al., 2011; Zavareze et al., 2012).

Fig. 2. Response surface plots showing the interaction effects of process variables on V: viscosity (A-C) and SOL: solubility (D-F).

3.5. *The effect of independent variables on moisture content*

One of the most fundamental aspects of edible films for food packaging purpose, particularly for high water activity foods, is high water resistance (Maran et al., 2013). Moisture content of film contributes to understanding the interaction between pea starch and guar gum that affects the water bonding capacity of edible films (Martins et al., 2012). The data showed that the moisture content of films had a positive correlation to the three experimental variables. Starch, glycerol and guar gum were all shown to have a significant influence on the moisture content. However, the interaction between independent variables starch $\times$ glycerol, starch $\times$ guar gum and glycerol $\times$ guar gum had no significant influence on the moisture content of films ($p > 0.05$, Table 4). The values of this parameter were positively influenced by the positive quadratic effect of the starch. It was found that, the moisture content of film was increased from 9.741 to 31.402 %, owing to the increasing of starch, glycerol and guar content in the film formulation. Prakash Maran et al. (2013b) stated that maize starch films with higher agar/glycerol concentration had higher water content.

The presence of guar gum could result in a higher affinity of the films to water, possibly owing to higher molecular entanglement and viscosity leading to higher retaining of water molecules during drying of the films (Singh et al., 2015). Guar gum incorporation delivers a higher hydrophilicity to the films caused by the exposure of its hydroxyl groups (Xu et al., 2003). The possibility of the network structural modifications by addition of guar gum and relatively high hydrophilic characteristics of the guar gum itself could not be excluded (Chen et al., 2009a). The addition of glycerol caused the rising of moisture content of the pea starch films, because its high hygroscopic

character can contribute to the decreasing of the forces between the adjacent macromolecules (Sobral et al., 2001).

3.6. The effect of independent variables on transparency

Transparency of any material is an indication of the degree to which light is permitted to pass through it (Saurabh et al., 2015). With different proportions of glycerol and guar gum, pea starch based film forming solution resulted in transparent and homogeneous films and the transparency determined for each film samples are presented in Table 2. Table 4 shows that starch had significant positive linear effect on transparency, whereas, glycerol and guar gum had significant negative effect. Interaction between factors starch $\times$ glycerol was also shown to negatively influence the TR ($p < 0.05$, Table 4). Figure 3 (D-F) demonstrates that the highest transparency values were mainly obtained at high pea starch concentrations. The transparency of films showed a reduction tendency, when the glycerol and guar gum concentration was increased ($p < 0.05$), probably due to the higher polymeric chain compaction modifying the refractive index and restricting the passage of the light through the film matrix (Ortega-Toro et al., 2014). The loss of transparency at high levels of guar gum maybe due to phase separation because amylose and amylopectin can interact with hydrocolloids forming different network structures. This information is important since film transparency is an essential aspect based on film applications, principally if the film will be applied as a surface food coating or for amending product appearance (Prakash Maran et al., 2013b).

Fig. 3. Response surface plots showing the interaction effects of process variables on MC: moisture content (A-C) and TR: transparency (D-F).

3.7. The effect of independent variables on color

Films color was represented in Table 2 as Hunter system L (lightness), a (redness) and b (yellowness) values. Pea starch-guar gum films, containing different quantities of glycerol, were almost homogeneous, colorless, with a high brilliancy. Table 4 indicates that all the linear coefficients for L value were highly significant. The results showed a positive influence of pea starch and a negative influence of glycerol and guar gum on the L response. The quadratic effect of pea starch level on L value was also negative showing an antagonistic effect. Figure 4 (A-C) represents higher L values at low guar gum and glycerol contents, and an increase of this response as pea starch concentration was higher, reaching a maximum value at 3 g of gum per 100 ml of slurry. Arismendi et al. (2013) reported the highest L values for the lowest hydrocolloids concentration for xanthan gum/tapioca starch edible.

As can be observed in Table 2, a parameter was always negative suggesting the films had a green tone. The analysis of variance showed that the linear coefficients of pea starch and guar gum significantly ($p < 0.05$) affected a value of the films. When guar gum was incorporated in pea starch films there was decrease of a value; however, a value increased when the percentage of pea starch and glycerol increased in the mixture.

Generally, b parameter always took positive values suggesting that these films tend to be slightly yellowish. Table 4 shows that b value was significantly influenced by all linear coefficients, however pea starch linear term had antagonistic effect. The interaction between variables starch $\times$ guar gum and quadratic term of pea starch was shown to have significant consequences on the b value. It could be observed that b showed the highest values for the highest guar gum and glycerol

concentrations, showing an opposite tendency to that observed for L response. Leceta et al. (2013) and Singh et al. (2015) also reported that yellowness value increased with the incorporation of glycerol.

The total color difference (ΔE) explains how far apart two colors are in the color space and is influenced by L , a and b values (Rao et al., 2010). It was seen that ΔE values changed by incorporation of guar gum and glycerol owing to changes in L , a , and b values of film. Increase in b parameter suggests increased yellowness of films, while decrease in L and a values signifies increased darkness and greenness of the films, respectively. The linear and quadratic coefficients of all variables were significant (Table 4). Moreover, ΔE values increased with the increase of the guar gum and glycerol and the decrease of the pea starch (Fig. 5D-F). The increases observed on the total color difference (ΔE) of the material can possibly be associated with the natural color of the individual raw materials used for making the films (Veiga-Santos et al., 2005).

The YI parameter is an indication of the yellowness contributed to film visual evaluation, and needs to be considered regarding the consumer's acceptance (Flores et al., 2010). Overall, it is favorable to have a low YI value to preserve organoleptic acceptability (Espinell Villacres et al., 2014). Table 4 shows that linear term of guar gum and glycerol and quadratic term of pea starch have significant positive effects on YI, whereas, linear coefficient of pea starch and pea starch $\times$ guar gum interaction coefficient have significant negative effects. The YI increased with increasing of glycerol and guar gum concentration, so the maximum YI was obtained with maximum value of glycerol and guar. This is associated with the development of inter-molecular hydrogen bonds between guar and starch and also the cohesive molecular structure of the films resulting in a tighter structure, thus yellowish color of the sample (Singh et al., 2015).

Analysis variance of the WI assays of the films revealed that this parameter was influenced by all three independent variables ($p < 0.05$, Table 4). The interaction between variables starch $\times$ glycerol, starch $\times$ guar gum and glycerol $\times$ guar gum was shown to have no significant consequences on the WI assay. These results highlight that pea starch presence in the film formulation allowed the formation of a whiter matrix. WI value improved with the increase in starch level however, it decreased with the increase in glycerol and guar gum level.

Fig. 4. Response surface plots showing the interaction effects of process variables on *L* value (A-C) and *a* value (D-F).

412

413 **Fig. 5.** Response surface plots showing the interaction effects of process variables on b value (A-
414 C) and ΔE : total color difference value (D-F).

3.8. Optimization and validation of the models

The desirability function was used for simultaneous optimization of the multiple responses. This function enables a combination of independent variables that simultaneously optimizes the requirement for each response in the design (Maran et al., 2013). The aim was to maximize transparency and to minimize SOL, YI and MC. Therefore, these responses were considered to study the possibility of choosing one formulation which optimizes the properties of studied edible films. The maximum, minimum, and average values of these variables experimentally achieved in the Box–Behnken experimental design (Table 2) were applied for calculation of the desirability function. The optimum level of different parameters by applying the methodology of desired function was obtained under condition of pea starch of 2.5 g, glycerol of 25 %, and guar of 0.3 g with an overall desirability of 0.77.

The above mentioned optimal conditions were experimentally validated to confirm the adequacy of the models. Triplicate experiments were performed to compare the experimental results with the predicted values of the responses (Table 5). The absolute residual error for the dependent variables ranged from 0.40 % to 11.62 %, indicating suitability of the methodology developed for the optimization of the process conditions, and validity of the surface responses obtained by the Box–Behnken experimental design.

Table 5. Results of experimental validation of the optimal conditions for the development of pea starch-guar gum edible film.*

Responses	Predicted value	Experimental value (n = 3) ^a	Absolute residual error (%) ^b
Viscosity (mPa.s)	199.356±24.414 ^a	203.631±20.101 ^a	2.099
Solubility (%)	28.771±2.234 ^a	27.673±2.724 ^a	3.968
Moisture content (%)	19.509±1.104 ^a	20.134±1.482 ^a	3.104
Transparency (%)	83.820±7.376 ^a	82.272±4.671 ^a	1.881
<i>L</i>	93.471±11.177 ^a	93.842±2.483 ^a	0.395
<i>a</i>	-3.787±0.942 ^a	-3.542±0.522 ^a	6.917
<i>b</i>	6.770±0.274 ^a	6.238±0.704 ^a	8.528
ΔE	4.009±0.638 ^a	4.536±0.508 ^a	11.618
Yellowness Index	10.373±1.217 ^a	9.496±1.061 ^a	9.235
Whiteness Index	89.879±7.152	90.546±7.472 ^a	0.737

* All the values are means ± standard deviations and those in the same row not sharing the same superscript letter are significantly different from each other ($p < 0.05$).

^a Values obtained at optimum conditions (pea starch 2.5 g; glycerol 25 %; and guar gum 0.1 g).

^b Absolute Residual Error = [(experimental value – predicted value)/experimental value] × 100.

4. Conclusion

The optimal formulation of starch based edible films was successfully developed from pea starch, glycerol, and guar gum using three level Box–Behnken response surface design. The findings showed that pea starch, glycerol, and guar gum significantly affected viscosity, solubility, moisture content, transparency and colour of the films. The optimum formulation of pea starch based films was found to be pea starch of 2.5 g, glycerol of 25 % w/w, and guar gum of 0.3 g, respectively. This study revealed that pea starch and guar gum could be used to make a composite film having

satisfactory packaging properties and this film can be applied to coat fruits and vegetable to extend their self-life. Future study is recommended to determine mechanism and interaction of pea starch and guar gum to further elucidate their roles in the film formation.

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Conflict of Interest

The authors declare no conflict of interest.

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