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Development of biocomposite films incorporated with different amounts of shellac, emulsifier, and surfactant

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Abstract

This study examined the effects of different ratios of shellac (20–60%), stearic acid (SA) (0–2%), and Tween-20 (0.1–0.5 ml) on the water vapor permeability (WVP) and mechanical properties of the pea starch-guar gum (PSGG) films which were evaluated by using response surface methodology (RSM). The incorporation of shellac into the PSGG film structure led to a slightly increased of film thickness. However the addition of higher concentrations of shellac did not improve the moisture barrier of PSGG film owing to the poor distribution of shellac in the film structure. Film formulated with 40% shellac, 1% SA, and 0.3% Tween-20 exhibited optimal functional properties. Moreover, the influence of the incorporation of different emulsifiers into the optimized film matrix was investigated by studying the physical, mechanical, and optical properties of the films. Films containing oleic acid (OA) showed not only lower thickness, WVP, moisture content, and water solubility, but also higher percentage of elongation (E), tensile strength (TS), and transparency compared with other fatty acids tested. Biocomposite pea starch-guar gum-shellac (PSGG-Sh) films containing OA can be considered to be sufficient for most of food packaging applications.

Keywords: Pea starch; Guar gum; Shellac; Fatty acids, Response surface methodology

1. Introduction

Safety and quality properties of food products which incorporate chemical changes (enzymatic browning and oxidation), microbial stability, sensorial (texture) and physical characteristics, determine the product quality and shelf life (Phan The, Debeaufort, Luu, & Voilley, 2008). The application of edible films and coatings to reduce deteriorative processes, as well as increasing shelf-life and appearance of food, has attracted significant research and industry interest (Bosquez-Molina, Guerrero-Legarreta, & Vernon-Carter, 2003). Edible films made from starch are suitable for food preservation because their resultant films are odorless, tasteless, and transparent with good oxygen barrier properties. Although starch based films have some limitations due to their affinity to water adsorption and retrogradation phenomena which affect their mechanical and barrier properties of films (Cano, Jimenez, Chafer, Gonzalez, & Chiralt, 2014; Jiménez, Fabra, Talens, & Chiralt, 2013). One approach to overcome these shortcomings is development of composites with other polymers or reinforcement substances (Ortega-Toro, Jiménez, Talens, & Chiralt, 2014).

Guar gum (GG) derived from a legume plant *Cyamopsis tetragonoloba* is an appropriate biopolymer for formation of biodegradable films owing to its high molecular weight and wide availability (Saurabh, Gupta, Variyar, & Sharma, 2016). GG is a galactomannan with a backbone of 1, 4-linked β -D-mannose residues and galactose as a side group linked by (1–6) α -d-galactopyranose at every second mannose which establishes short side-branches (Fernandes, Gonçalves, & Doublier, 1993).

Our previous studies demonstrated that GG in combination with pea starch (PS) improved physical, barrier and mechanical properties of films (Sabeti et al., 2017; Sabeti et al., 2016a). The water vapor permeability (WVP) value of pea starch-guar gum (PSGG) biocomposite films exhibited better moisture barrier characteristics than pure PS films (Sabeti et al., 2016b), but these were higher than those of low density polyethylene (LDPE) (Phan The et al., 2008). GG improved

the functional characteristics of PS edible film as both biopolymers are hydrophilic; however, the incorporation of hydrophobic substances for decreasing the water sensitivity of biocomposite films is required.

Lipid components including natural waxes, fatty acids, essential oils, surfactants and resins are commonly applied to reduce water vapor transmission rate in the hydrocolloid matrix (Villalobos, Hernández-Muñoz, & Chiralt, 2006). Shellac is currently used as a moisture barrier in the food industry to extend the shelf-life of products (Phan The et al., 2008), and in the pharmaceutical industry for the moisture protection of drugs, controlled drug delivery system and as an enteric coating for drugs and probiotics (Pearnchob, Dashevsky, & Bodmeier, 2004; Soradech, Limatvampirat, & Luangtana-anan, 2013; Stummer et al., 2010). Shellac is a purified resinous secretion of lac insects, *Kerria lacca*, a parasitic insect found on trees in Southeast Asia (Phan The et al., 2008). However issues related to the application of shellac include its insolubility in an aqueous system, lack of mechanical strength and lower stability, which lead to the reduction in its use (Limmatvampirat et al., 2007; Luangtana-anan et al., 2007). The production of composite films by combination of two natural polymers and the incorporation of some plasticizers, is a novel approach to counter these issues (Soradech et al., 2013).

In addition, the incorporation of fatty acids has been used to decrease water transmission through edible films. Fatty acids are polar lipids and their chain length and unsaturation degree has a significant effect on film properties (Fernández, de Apodaca, Cebrián, Villarán, & Maté, 2007). Other hydrophobic compounds with the potential to improve film characteristics are surfactants. These compounds, such as Tween-20, are amphiphilic substances, which are necessary for preparation emulsion films with suitable properties (Tongnuanchan, Benjakul, & Prodpran, 2014).

The widespread availability and low cost of PS and GG make the use of these compounds ideal for film formation and in combination with shellac as a hydrophobic substance make the combination of these compounds ideal in the development of packaging films. However it is critical to understand the interaction and relationships between these compounds to optimize film properties. In this study, the influence of different amounts of shellac, surfactant and emulsifier based on dry film matter on mechanical (tensile strength and percent of elongation at break) and barrier properties (WVP) of PSGG based films was investigated using response surface methodology (RSM). In addition, different emulsifiers were added to PSGG-Sh composite film based on optimized amount to determine the appropriate emulsifier to make films with improved mechanical, water vapor barrier and optical properties.

2. Materials and methods

2.1. Materials

Canadian non-GMO yellow pea starch with 13.2% moisture, 0.2% protein, 0.5% fat, 0.3% ash, and 36.25±0.32% amylose 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. Food grade shellac was purchased from Castle Chemicals (castlechem.com.au), NSW, Australia. Stearic acid (SA), lauric acid (LA), oleic acid (OA), butyric acid (BA), palmitic acid (PA) and Tween-20 were obtained from Sigma Aldrich, Australia. Glycerol was from Ajax Finechem Pty. Ltd, Australia and used as a plasticizer. All other chemicals were purchased from Merck Millipore, Pty., VIC, Melbourne, Australia.

2.2. Emulsion preparation

Optimized amounts of pea starch (2.5 g), guar gum (0.3 g) and 25% w/w glycerol based on the dry film matter were dissolved in 100 ml degassed deionized water with gentle heating (about 40 °C) and magnetic stirring. In another study, we determined the optimized amount of film components by using Box–Behnken response surface design (BBD) (Saber et al., 2016a). The PSGG-Sh composite mixtures without emulsifier did not form a film, therefore, SA, was used to stabilize the PSGG-Sh composite films. Melted SA (0-2% db) and Tween-20 (0.1–0.5% wb) were added to the PSGG solution and the aqueous suspension was gelatinized at 90 °C for 20 min on a hot plate with continuous stirring. According to preliminary moisture barrier and mechanical tests (data are not shown), shellac was added to the PSGG-SA-Tween 20-glycerol mixture at three different levels (20%, 40% and 60% db). Once the lipids were melted, samples were homogenized for 4 min at 22000 rpm using a T25 Ultra-Turrax (Ika, Staufen, Germany). After homogenization, the film solution was cooled to room temperature with mild magnetic stirring for 1 h to decrease air bubbles. Filmogenic suspensions (20 g) were cast onto Petri dishes (10 cm in diameter) and dried at 40 °C in an oven until reaching constant weight (about 24 h). Films were carefully peeled-off from Petri dishes and conditioned at 25 °C, 65% relative humidity (RH) for 72 h prior to further testing (Saber et al., 2017).

In a second experiment, six film formulations were prepared with and without incorporation of emulsifiers (LA, OA, BA, and PA) with the same amount of the optimized level of SA. All the films were prepared with the same procedure described before. Concentration of shellac and Tween-20 were kept constant throughout the study.

2.3. Experimental design

The effect of process parameters (shellac (X1): 20–60%, SA (X2): 0-2%, and Tween-20 (X3): 0.1–0.5 ml)) on film mechanical and barrier properties was studied by applying a three-level-three-factor, Box–Behnken response surface design (BBD) with three central point replicates. All experimental runs are listed in Table 1. A second-order polynomial model was used to fit the experimental data obtained from the seventeen experimental runs:

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

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 (Saber et al., 2017).

Table 1. Box–Behnken experimental design with process variables (un-coded) and observed responses.*

*THI: Thickness; WVP: Water vapor permeability; TS: Tensile strength; and E: Elongation at break.

Run	Shellac (%w/w)	SA (%w/w)	Tween-20 (ml/100ml)	THI (mm)	WVP $\times 10^{-12}$ (gPa ⁻¹ s ⁻¹ m ⁻¹)	TS (MPa)	E (%)
1	20	1	0.1	0.136	62.633	32.822	15.422
2	20	0	0.3	0.125	47.631	38.576	13.581
3	20	2	0.3	0.15	55.516	22.711	18.714
4	20	1	0.5	0.141	31.232	26.043	16.622
5	40	0	0.1	0.138	28.733	24.386	17.151
6	40	2	0.1	0.152	37.047	11.709	19.577
7	40	1	0.3	0.143	21.412	15.740	20.822
8	40	1	0.3	0.147	20.414	15.842	20.556
9	40	1	0.3	0.146	21.588	15.661	21.101
10	40	0	0.5	0.14	10.46	19.522	19.370
11	40	2	0.5	0.156	15.317	9.665	23.066
12	60	1	0.1	0.149	73.366	13.393	11.133
13	60	0	0.3	0.144	55.315	16.475	10.555
14	60	2	0.3	0.161	64.572	7.441	12.344
15	60	1	0.5	0.152	43.71	11.211	13.636

2.4. Film characterization

2.4.1. Thickness

A digital micro-meter (Mitutoyo, Co., Code No. 543-551-1, Model ID-F125, 139 Japan; sensitivity = 0.001 mm) was used to measure film thickness. The mean value from 10 different points for each film samples was measured (Fakhouri, Fontes, Innocentini-Mei, & Collares-Queiroz, 2009).

2.4.2. Water vapor permeability

Water vapor permeability (WVP) of films was examined gravimetrically using the method explained by Sun, Wang, Kadouh, and Zhou (2014) with some modifications. The films were sealed onto test cups half-filled with anhydrous calcium chloride (CaCl₂) (0% RH) that was dried in a hot air oven at 120 °C for 1 day initially. These cups were then placed in a desiccator containing

saturated NaCl solution (75% RH) and kept at 25 °C. The test cups were weighed as a function of time until changes in the weight were recorded to the nearest 0.001 g. Water vapor transmission rate (WVTR) was calculated by dividing the slope of straight line (g/m) calculated from the weight gain as a function of time data, with film surface area, and WVP was measured as follows:

$$WVP = WVTR \times \frac{\text{Film thickness}}{\Delta P} \quad (2)$$

where ΔP is the water vapor pressure difference between the two sides of the film (Pa). WVP was measured for three replicated samples for each type of films.

2.4.3. Mechanical properties

Tensile strength (TS) and elongation at break (E) of the film were measured using a Texture Analyzer (LLOYD Instrument LTD, Fareham, UK) according to the standard method (ASTM, 2001). Test samples, 40 mm × 15 mm, were cut from each film and fixed with an initial grip separation of 40 mm. Five replicates of each film were then pulled apart at crosshead speed of 1 mm/s. TS (MPa) was calculated by the Equation (1):

$$TS = F_{\max}/A \quad (3)$$

where F_{\max} is the maximum force (N) loaded on the specimen before pulling apart; A is the cross-sectional area (m²) of the specimen. E is defined as the Equation (2):

$$E = \Delta L/L_0 \times 100\% \quad (4)$$

where ΔL is the film elongation at the moment of rupture (mm) and L_0 is the initial length between the grips (Chen, Zhang, & Zhao, 2015).

2.4.5. Calculation of moisture content

The moisture content was measured by reduction of weight after drying at 105 °C from initial weight of casted film forming emulsions until constant weight was reached.

2.4.6. *Water solubility of films*

Film solubility was determined according to the procedure reported by Chiumarelli and Hubinger (2014). Three discs (40 mm × 15 mm) of each film were cut, weighed, immersed in 50 mL of distilled water. Then, they were slowly and periodically agitated during 24 h at 25 °C. The dry mass content of initial and final samples was calculated by drying the samples at 105 °C for 24 h. The measurements were made in triplicate.

2.4.7. *Optical properties*

Films transparency was determined using a UV Vis Spectrophotometer (Varian Australia Pty. Ltd., Melbourne, VIC Australia) as described by (Saber et al., 2016a).

A Minolta colorimeter (CR-300 series, Radiometric instruments Operations, Osaka, Japan) was used to determine the film color. Measurements were performed by placing the films over the standard and three repetitions were conducted. The Hunter laboratory scale was used, and total color difference (ΔE), whiteness index (WI), and yellowness index (YI) were calculated using the following equations:

$$\Delta E = \sqrt{(L^* - L)^2 + (a^* - a)^2 + (b^* - b)^2} \quad (5)$$

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

$$YI = \frac{142.86b}{L} \quad (7)$$

where L^* , a^* , and b^* are the standard color parameter values and ' L ', ' a ', and ' b ' are the color parameter values of the sample (Saber et al., 2016b). The measurements were repeated six times for each film.

2.4.8. Scanning electron microscopy (SEM)

Scanning electron micrographs were taken by a scanning electron microscope (ZEISS, NSW, AU) at an accelerating voltage of 5 kV. Films were mounted on a bronze stub using double-sided adhesive tape, and the films were sputter coated with a layer of gold allowing surface visualization.

2.5. Statistical analysis

The model equations, the 3D contour plots of variable responses and the optimum values for the three independent variables were developed by JMP software (Version 13, SAS, Cary, NC, USA). The sufficiency of the response surface methodology (RSM) second-order polynomial model was determined by the lack of fit and the coefficient of determination (R^2). The results were statistically assessed by analysis of variance (ANOVA) and Multiple Ranges Duncan's test at 5% significance level, using the software SPSS (version 23, SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Preliminary studies

Initial studies showed that using a shellac concentration lower than 20% was not adequate to form a compact film structure. Conversely, the films with shellac concentrations greater than 60% were very soft to peel off; therefore concentrations between 20% and 60% shellac based on dry film matter, were selected for further examination. Similarly different amounts of Tween-20 as the surfactant were also exposed to preliminary assessment to examine their effects on the flexibility

of PSGG films. The levels of Tween-20 between 0.1-0.5 ml/100 ml of filmogenic solution were found to be the most effective concentration. Furthermore, Tween-20 showed the most efficient plasticizing influence in presence of glycerol causing higher flexibility and the resultants films were peeled off easily from petri dishes. In terms of SA, high concentrations of SA made the films brittle and they broke during peeling. Thus, the range of 0–2% (w/w) SA concentrations was more suitable for PSGG films. The incorporation of the same range of SA effectively improved physical and mechanical properties of sodium caseinate edible films .

3.2. Experimental design analysis

The experimental data was analyzed and fitted to the second-order polynomial models to obtain regression equations. The lack of fit, model summary statistics and Pareto analysis of variance (ANOVA) were conducted to evaluate the adequacy of model which represents the effect of process variables over the mechanical and barrier attribute of PSGG based edible films and the results are presented in Table 2. The results show that the coefficient of determination (R^2) of the model for all responses was higher than 0.97, indicating a close correlation between the predicted values and experimental values. The insignificant lack of fit ($p > 0.05$) suggested that the model adequately predicted the responses affected by process variables (Arismendi et al., 2013). The higher PRESS (predicted residual sum of square) and F values and significant difference of p -values of the model also verified the reliability of the model in predicting thickness, WVP and mechanical properties of films.

The adequacy of developed models was estimated using diagnostic plots such as predicted versus actual plots (Fig. 1). The diagnostic plots such as predicted versus actual values determine the model suitability and demonstrate the correlation between predicted and experimental values

(Maran, Sivakumar, Thirugnanasambandham, & Sridhar, 2013) and the results show that the data points on this plot were close to the straight line (Fig. 1). This signifies that the predicted values obtained were very close to the experimental values, suggesting the developed models were reliable and accurate in determining the relationship between the real and predicted data.

By conducting multiple regression analysis on the experimental data, four second order polynomial mathematical models in terms of coded factors (THI, WVP, TS and E) were obtained;

$$\text{THI} = 0.15 + 0.008x_1 + 0.009x_2 + 0.002x_3 - 0.002x_1x_2 - 0.0005x_1x_3 + 0.0005x_2x_3 - 0.0011x_1^2 + 0.0008x_2^2 + 0.0003x_3^2$$

$$\text{WVP} = 21.14 + 4.99x_1 - 12.63x_2 + 3.79x_3 + 0.43x_1x_2 + 0.34x_1x_3 - 0.86x_2x_3 + 32.23x_1^2 - 0.64x_2^2 + 2.39x_3^2$$

$$\text{TS} = 15.75 - 8.95x_1 - 5.93x_2 - 1.98x_3 + 1.71x_1x_2 + 1.15x_1x_3 + 0.71x_2x_3 + 5.05x_1^2 + 0.50x_2^2 + 0.07x_3^2$$

$$\text{E} = 20.83 - 2.08x_1 + 1.63x_2 + 1.18x_3 - 0.83x_1x_2 + 0.33x_1x_3 + 0.32x_2x_3 - 6.31x_1^2 - 0.72x_2^2 - 0.32x_3^2$$

Table 2. Analysis of variance for determination of model fitting.*

Sources of variation	THI (mm)	WVP ×10 ⁻¹² (gPa ⁻¹ s ⁻¹ m ⁻¹)	TS (MPa)	E (%)
Lack of fit	0.381	0.061	0.080	0.167
R ²	0.971	0.990	0.997	0.994
Adjusted R ²	0.919	0.978	0.993	0.983
PRESS	67.689	1167.32	38.997	18.651
F ratio of model	1.769	52.064	98.571	5.138
p of model > F	0.0025*	0.0003*	< 0.0001*	< 0.0001*

* Significant difference with p < 0.05.

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262 3.3. Influence of process variables

263 The linear regression coefficients and their statistical significance for each response are presented
264 in Table 3. The results clearly show that the nature and composition of the films influenced film
265 thickness. The data showed that the film thickness varied between 0.125 to 0.161 mm by increasing
266 shellac, SA and Tween-20 content in the film formulation. The thickness of films had a positive
267 correlation to the three experimental variables. Shellac and SA had a significant linear influence
268 ($p < 0.05$) on the film thickness (Table 3). The “R-Squared” value of 0.971 is in satisfactory
269 correlation with the “Adjusted R-Squared” of 0.919, which is an indicator of the suitability of fit
270 of the model, verifying that 97.1% of the total variation was described by the model (Table 3).
271 While, adjusted R^2 value (0.919) exhibited the significance of the model (Singh, Chatli, & Sahoo,
272 2015). Furthermore, higher precision and reliability of the model was indicated by insignificant
273 value of lack of fit (0.381). With the increase in shellac and SA levels, there was an increase in the
274 thickness of films, but this was not affected by Tween-20 level (Fig. 3A-C). The influence of
275 Tween-20 on the thickness is determined by the level of combination, which was less than 1.0%.
276 A possible reason for the increase in the film thickness after incorporation of lipid compounds
277 could be that there is an increase in total mass, once the solvent is evaporated during the drying
278 process (Fakhouri et al., 2009). A similar effect was observed when fatty acids were added to
279 maize starch and gelatin composite films (Fakhouri et al., 2009), adding oleic acid to tara gum film
280 (Ma, Hu, Wang, & Wang, 2016), and adding different fatty acids to a basil seed gum edible film
281 (Mohammad Amini, Razavi, & Zahedi, 2015). The addition of Tween-20 increases the probability
282 of interaction between the lipid compounds and film polymers thus decreasing the flexibility of
283 polymer chains during film development and resulting in thicker films (Maran et al., 2013).

Analysis of WVP of the films showed that this parameter was dependent on all three independent variables ($p < 0.05$, Table 3). Shellac (linearly and quadratically), SA (linearly) and Tween-20 (linearly) all significantly ($p < 0.05$) affected WVP of films (Table 3). There was no interaction between variables shellac \times SA, shellac \times Tween-20 and SA \times Tween-20. The high R^2 value (0.988) indicated that 98.85% of the total variation fitted to the model (Table 3). The results described in Fig. 2D-F show that the addition of shellac and SA decreased WVP significantly due to the increasing of hydrophobic groups in formulations. In terms of shellac, WVP initially decreased with the addition of shellac, but later WVP increased after reaching an optimal level. Increasing shellac content over the critical value caused a poor dispersion of shellac in the film matrix (Chen et al., 2015). An increase in candelilla wax concentration above 0.5 %, also resulted in increased WVP of oxidized potato starch and carboxymethyl cellulose films (Kowalczyk & Baraniak, 2014). It has been proposed that the addition of hydrophobic compounds over the critical level might induce more disorder in the film network, producing spaces at the polymer-lipid interface and promoting the transmission of water vapor molecules inside the film (Chick & Hernandez, 2002). However, in this study the WVP of the films was significantly ($p < 0.05$) enhanced with the incorporation of Tween-20 in the film matrix. This can be attributed to the hydrophilic-lipophilic balance (HLB) ratio of Tween-20 (HLB of 16.7), which is the most hydrophilic surfactant and presents higher permeability values (Rodríguez, Osés, Ziani, & Mate, 2006).

The results presented in Table 3 shows that TS values were negatively affected by shellac, SA and Tween-20, and positively affected by the interaction of shellac-SA and shellac-Tween-20 and the quadratic term of shellac. Figure 3A-C showed that the amount of TS reduced with increasing the levels of shellac, SA and Tween-20 where the TS values varied from 38.6 to 7.4 MPa. The decrease

in TS with the incorporation of lipid components to the biopolymer films can be associated with their movement to the starch-guar gum interface, disturbing the interactions between them and resulting in a reduction in film resistance (Fakhouri et al., 2009). Lower TS values due to incorporation of increasing amounts of lipids and/or surfactants have been also reported for other biodegradable films (Chen & Lai, 2008; Ziani, Oses, Coma, & Maté, 2008).

The results in Table 3 show that shellac had significant negative linear effect on E, whereas, SA and Tween-20 had significant positive effect. The interaction between shellac \times SA and the quadratic term of shellac and SA were shown to negatively influence the E ($p < 0.05$). The E value of the films decreased by increasing of shellac content due to poor miscibility of shellac in biopolymer matrix at high concentration (Byun, Ward, & Whiteside, 2012). The addition of SA and Tween-20 increased E value, which is probably related to fine dispersion of SA and Tween-20 in the polymer matrix, and the development of interactions causing a plasticizing effect (Jiménez, Fabra, Talens, & Chiralt, 2010). At high concentrations of the surfactant, there is an apparent synergistic effect between glycerol and Tween-20 (Rodríguez et al., 2006). This interaction brings about lower TS and higher E in films. Tween-20 is a small molecule and its hydrophilic component can interact with glycerol or water, promoting its replacement between polymer chains, enhancing even more the chain mobility and increasing the initial plastic effect (Ghanbarzadeh & Almasi, 2011).

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P	DF	THI (mm)		WVP $\times 10^{-12}$ (gPa ⁻¹ s ⁻¹ m ⁻¹)		TS (MPa)		E (%)	
		Estimate	Prob> t	Estimate	Prob> t	Estimate	Prob> t	Estimate	Prob> t
β_0	1	0.145	<.0001*	21.138	<.0001*	15.747	<.0001*	20.826	<.0001*
β_1	1	0.006	0.0006*	4.993	0.0107*	-8.954	<.0001*	-2.083	<.0001*
β_2	1	0.009	0.0002*	-12.632	0.0002*	-5.929	<.0001*	1.630	0.0003*
β_3	1	0.002	0.1064	3.789	0.0298*	-1.983	0.0005*	1.176	0.0012*
β_{12}	1	-0.002	0.1728	0.436	0.8162	1.707	0.0046*	-0.836	0.0218*
β_{13}	1	-0.0005	0.7075	0.343	0.8548	1.149	0.0219*	0.326	0.2564
β_{23}	1	0.0005	0.7075	-0.864	0.6479	0.705	0.1002	0.318	0.2671
β_{11}	1	-0.001	0.4138	32.233	<.0001*	5.049	<.0001*	-6.308	<.0001*
β_{22}	1	0.0008	0.5526	-0.634	0.7454	0.503	0.2259	-0.720	0.0418*
β_{33}	1	0.0003	0.8092	2.387	0.2541	0.069	0.8559	-0.315	0.2870

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336 **Table 3.** 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 shellac, SA and Tween-20; β_{12} , β_{13} , and β_{23} : regression coefficients for interaction between shellac \times SA, shellac \times Tween-20 and SA \times Tween-20; β_{11} , β_{22} , and β_{33} : quadratic regression coefficients for shellac \times shellac, SA \times SA, Tween-20 \times Tween-20.

3.4. Optimization and validation of the models

The desirability function was applied for simultaneous optimization of the multiple responses (Sabeti et al., 2017). The aim was to maximize mechanical properties and to minimize thickness and WVP. The optimum level of different variables by applying the methodology of desired function was achieved with 40 %w/w shellac, 1 %w/w SA and 0.3 ml Tween-20/100 ml with an overall desirability of 0.82. The adequacy of the models was experimentally validated by the above mentioned optimal conditions. Table 4 shows the results of triplicate experiments compared with the predicted values of the responses. The suitability of the methodology developed for the optimization of the process conditions was confirmed by the absolute residual error for the dependent variables.

Table 4. Results of experimental validation of the optimal conditions for development PSGG-Sh fim.*

Responses	Predicted value	Experimental value (n = 3) ^a	Absolute residual error (%) ^b
Thickness (mm)	0.145 \pm 0.006 ^a	0.146 \pm 0.004 ^a	0.694
WVP $\times 10^{-12}$ (gPa ⁻¹ s ⁻¹ m ⁻¹)	21.412 \pm 0.834 ^a	20.047 \pm 1.361 ^a	6.816
Tensile strength (MPa)	15.841 \pm 1.072 ^a	16.291 \pm 1.705 ^a	2.762

Elongation at break (%)	20.561±1.156 ^a	21.636±0.874 ^a	5.078
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* 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 (shellac 40 (% w/w); SA 1 (% w/w); and Tween-20 0.3 (ml/100 ml)).

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

3.5. Effect of the different emulsifiers on PSGG-Sh film physical properties

The first aim of this study showed that the incorporation of 40 %w/w shellac, 1 %w/w SA and 0.3 ml Tween-20 into pea starch (2.5 g), guar gum (0.3 g) and 25 %w/w glycerol in 100 mL of distilled water; made a composite film with satisfactory mechanical and barrier properties. In general, barrier and mechanical characteristics of lipid-containing films are affected by the structure and stability of an emulsion (Kowalczyk et al., 2014). Fatty acids can act as emulsifier and plasticizer in biopolymer films and their action is dependent on the hydrophobic/hydrophilic ratio presented by film components, the polarity, unsaturation number and ramification degree provided by the polymeric chains of the matrix used (Fakhouri et al., 2009; Gontard, Duchez, Cuq, & Guilbert, 1994). Therefore, a variety of fatty acids were studied to determine their efficiency in preparing packaging films.

In the second component of this experiment examined the effect of adding different fatty acids into PSGG-Sh films. Fatty acids selected for the study and their properties are shown in Table 5.

Table 5. Fatty acids selected for the study and their properties

Fatty acid	Formula	Lipid numbers	Molecular weight (g/mol)	Saturation/unsaturation	concentration
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					g/100 g dry matter	mol/100 g dry matter
BA	C ₃ H ₇ COOH	C4:0	88.11	Saturated	1	0.011
LA	C ₁₂ H ₂₄ O ₂	C12:0	200.32	Saturated	1	0.005
PA	C ₁₆ H ₃₂ O ₂	C16:0	256.43	Saturated	1	0.004
SA	C ₁₈ H ₃₆ O ₂	C18:0	284.48	Saturated	1	0.004
OA	C ₁₈ H ₃₄ O ₂	18:1 cis-9	282.47	Unsaturated	1	0.004

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376 All PSGG-Sh films formulated with different fatty acids were peelable, flexible, smooth,
377 homogeneous and transparent. The presence of glycerol, fatty acids and Tween-20 functioned as
378 diluents of shellac improving its miscibility, and modifying hydrophobic phase dispersion during
379 emulsification, affecting homogeneity of the final distribution of hydrophobic constituents in the
380 PSGG matrix. All emulsions produced in this study were considered “stable”, because they did
381 not show creaming during drying. An unstable emulsion creates a gradient of lipid concentrations
382 across the thickness of the film and produces films with a “bilayer-like” structure due to separation
383 of lipid during drying (Shellhammer & Krochta, 1997). Creaming is the main mechanism in which
384 separation of the disperse phase from an emulsion occurs and is typically considered the precursor
385 to coalescence (Kowalczyk et al., 2014). Therefore in this study, all fatty acid molecules
386 successfully incorporated into the PSGG-Sh film without phase separation. Thus the optimized
387 emulsifier obtained by the Box–Behnken experimental design was satisfactory for PSGG-Sh film
388 formation.

389 The WVP, mechanical and transparency of the film are influenced by film thickness, thus this
390 parameter should be precisely and correctly measured. The results showed that the thickness of
391 films varied between 0.129 and 0.146 mm with the addition of various fatty acids (Table 6).
392 Increasing film thickness was related to the presence of fatty acid molecules between the

393 macromolecular chains, thus accordingly extending the structure of film through increasing
394 molecular volume of network (Thakhiew, Devahastin, & Soponronnarit, 2010). In this study, the
395 film formulated with SA produced a film of maximum thickness.

396 The WVP of films was found to be in the range of 36.9×10^{-12} to 16.2×10^{-12} ($\text{gm}^{-1} \text{s}^{-1} \text{Pa}^{-1}$) and
397 was dependent on the type of fatty acid in the following order: **BA > LA > PA > SA > OA** (Table
398 6). This observation maybe due to the hydrophilic-hydrophobic proportion in the film structure,
399 whereby the existence of fatty acids in films could reduce WVP according to the hydrophilic-
400 hydrophobic ratio of the film (Thakur et al., 2016). The different behavior of fatty acids on the
401 reduction of WVP may be related to the different structural arrangement of fatty acids in the film
402 matrix (Kowalczyk et al., 2014). The smallest value for WVP was observed after BA was added
403 to the PSGG-Sh matrix. This maybe because BA has the shortest carbon chain of all fatty acids
404 applied, and therefore facilitated its inclusion into the biopolymer film matrix (Fakhouri et al.,
405 2009). Chain length and the degree of unsaturated fatty acids are important in determining the
406 resultant film properties (Rezvani et al., 2013). Fatty acids with longer hydrophobic tail have low
407 chain mobility and are consequently capable to decrease WVP (Ayranci & Tunc, 2001). The
408 addition of 1% OA was the most effective concentration to improve the water barrier
409 characteristics due to its highly hydrophobic nature (Ghanbarzadeh et al., 2011). This can be also
410 ascribed to the fact that OA dispersed in the film diminished the practical interfacial area exposed
411 to water vapor (Chen et al., 2015). Moreover, it has been proposed that some voids and cracks in
412 film structure are caused by saturated fatty acids because of their crystallisation at ambient and
413 drying temperatures facilitating mass transfer through interior discontinuities and channels of film
414 (**Mohammad Amini** et al., 2015). Moisture transmits through the matrix of continuous phase in
415 edible films prepared as oil-in-water (O/W) emulsion. The moisture permeability of continuous

phase can be affected by interactions between hydrocolloid chains and lipid phase (The, Debeaufort, Voilley, & Luu, 2009). In addition, the polymeric chains may partly immobilize at the interface with the emulsified lipid particles (McHugh & Krochta, 1994), therefore it becomes less mobile and decreases WVP by reducing film free volume and inhibiting the penetration of water via the polymer chains interface (Ghanbarzadeh et al., 2011). The existence of a hydrophobic and disperse phase has been shown to produce a tortuous path for water vapor molecular transmission, thus reducing the WVP in film matrix (Ma et al., 2016).

The mechanical properties of the PSGG-Sh films with different fatty acids including the tensile strength (TS) and elongation at break (E) are summarized in Table 6. The tensile mechanical properties showed a dependency to fatty acid type. The influence of fatty acids on mechanical properties of hydrocolloids film may be determined by the basic matrix characteristics of materials, the interaction of the polymer molecules, the film components and the size distribution of the fatty acid molecules (Chen et al., 2015). In general the results show that TS was reduced by the addition of fatty acids, while E exhibited an increase. Films containing BA showed highest TS and E values because it is lower in size, thus, it can more efficiently interact with polymers than the other fatty acids tested. The small size of BA produced more direct interaction between polymer molecules leading to higher intermolecular cohesive force and higher TS. It is suggested that the interactions between non-polar fatty acid molecules and polar polymer molecules are much lower than those between polar glycerol molecules and polar polymer molecules (Vargas, Albors, Chiralt, & González-Martínez, 2009). The decrease in TS and increase in E with the increase of hydrophobic compounds on mechanical characteristics have been widely reported (Bertan, Tanada-Palmu, Siani, & Grosso, 2005; Colla, do Amaral Sobral, & Menegalli, 2006; Ghasemlou, Khodaiyan, Oromiehie, & Yarmand, 2011; Péroval, Debeaufort, Despré, & Voilley, 2002; Srinivasa, Ramesh,

& Tharanathan, 2007; Yang & Paulson, 2000). In this study, films comprising OA were more flexible and extensible compare to those made by SA. This can be attributed to differences of the solid state of SA, which forms a more rigid dispersed phase in the film and reduces its capability to expanse under the testing conditions, and to the specific plasticizing impact of unsaturated OA in the PSGG-Sh matrix (Rezvani et al., 2013).

As expected, the moisture content of PSGG-Sh films decreased with the incorporation of fatty acids because of the increase in the films' hydrophobicity. With the addition of 1% fatty acid, the moisture content of PSGG-Sh films containing BA, LA, PA, SA, and OA was reduced by 7.1, 17.8, 40.0, 47.4, and 59.5%, respectively. The dispersion of hydrophobic fatty acids in the solution has been shown to decrease the accessibility of hydroxyl groups, which interact with water, thus leading to the immobilization of biopolymer chains and reduction of moisture content by the film (Ma et al., 2016).

Solubility values were significantly ($p < 0.05$) affected with addition of fatty acids (Table 6). The incorporation of fatty acids decreased the water solubility of the films because the total solid levels remained constant in the formulation (Kowalczyk et al., 2014). Through polar–polar interactions between hydrophilic groups of fatty acids and polymer molecules, the less hydrophilic groups in emulsified films were available to interact with water molecules, so a hydrophobic nature developed in film matrix by addition of fatty acids (Cerqueira, Souza, Teixeira, & Vicente, 2012; Ghasemlou et al., 2011).

458 **Table 6.** Effect of different fatty acids on physical properties of PSGG-Sh films.*

Films	Thickness (mm)	WVP $\times 10^{-12}$ (gs ⁻¹ m ⁻¹ Pa ⁻¹)	TS (MPa)	E (%)	Moisture content (%)	Solubility in water (%)
PSGG	0.129±0.016 ^a	1.387E3±100.550	27.786±2.352 ^a	16.281±2.125 ^c	20.136±1.476 ^a	27.768±2.027 ^a
PSGG-Sh	0.139±0.007 ^a	33.209±8.015 ^{ab}	22.086±3.114 ^b	18.453±0.536 ^{de}	15.469±1.861 ^b	24.941±0.730 ^b
PSGG-Sh-BA	0.140±0.006 ^a	36.876±3.611 ^a	21.419±1.985 ^{bc}	27.419±2.093 ^a	14.376±2.642 ^b	22.808±1.128 ^b
PSGG-Sh-LA	0.142±0.009 ^a	29.309±3.666 ^{ab}	19.753±1.898 ^{bcd}	23.086±2.034 ^{bc}	12.709±0.758 ^b	18.124±2.061 ^c
PSGG-Sh-PA	0.144±0.011 ^a	26.976±5.730 ^{bc}	17.786±0.970 ^{cd}	22.453±1.566 ^c	9.289±0.872 ^c	16.458±1.444 ^c
PSGG-Sh-SA	0.146±0.004 ^a	20.372±1.605 ^{cd}	16.553±1.885 ^d	21.348±0.993 ^{cd}	8.129±1.025 ^{cd}	13.474±1.579 ^d
PSGG-Sh-OA	0.142±0.010 ^a	16.166±2.158 ^d	16.953±1.417 ^d	25.953±2.071 ^{ab}	6.263±1.066 ^d	10.242±1.195 ^e

459 * Values are the means of triplicates ± standard deviations. Means at same column with different lower case letters are significantly
460 different (p < 0.05).

3.6. Effect of the different emulsifiers on PSGG-Sh film optical properties

The results presented in Table 7 show the effect of the different emulsifiers on PSGG-Sh film optical properties (total color difference (ΔE), whiteness index (WI), yellowness index (YI), transparency degree). The results show that the PSGG based films were colorless, while the addition of lipid compounds made films more ‘yellowish’ (high positive b), darker (low L) and more ‘greenish’ (high negative a). Films formulated with SA showed the highest values of ΔE , WI, and YI compared with other films. The addition of shellac decreased the transparency of PSGG films; however in the case of BA, LA, and OA there was no significant difference in transparency between the films. Reduction in film transparency after incorporation of lipids has been observed in different studies (Fabra, Pérez-Masiá, Talens, & Chiralt, 2011; Fabra et al., 2009; Kowalczyk et al., 2014; Wang et al., 2014). The size of lipid particles has been shown to cause morphological heterogeneities in emulsion films which consequently scatters visible light through the film, thus affecting its transparency (Kowalczyk et al., 2014). In this study, the lowest transparency values were observed for samples emulsified with SA.

Table 7. Effect of different fatty acids on optical properties of PSGG-Sh films.*

Films	ΔE	WI	YI	Transparency (%)
PSGG	6.538±0.704 ^c	90.267±0.923 ^a	9.548±1.054 ^c	82.275±4.060 ^a
PSGG-Sh	12.976±1.452 ^d	83.735±1.473 ^b	14.713±0.910 ^d	77.275±1.351 ^b
PSGG-Sh-BA	14.992±0.950 ^d	81.662±0.935 ^b	15.931±0.849 ^{cd}	75.608±1.247 ^b
PSGG-Sh-LA	18.470±0.619 ^c	78.248±0.638 ^c	17.675±1.378 ^c	73.942±2.055 ^b
PSGG-Sh-PA	22.148±1.267 ^b	74.521±1.346 ^d	20.636±1.510 ^b	70.112±1.599 ^c
PSGG-Sh-SA	26.492±2.714 ^a	70.205±2.726 ^e	24.792±2.296 ^a	67.778±1.357 ^c
PSGG-Sh-OA	15.753±2.896 ^{cd}	80.897±2.919 ^{bc}	16.366±1.645 ^{cd}	74.942±1.576 ^b

* Values are the means of triplicates ± standard deviations. Means at same column with different lower case letters are significantly different ($p \leq 0.05$).

3.7. Scanning electron microscopy (SEM)

SEM images of the surfaces of PSGG-Sh films with and without fatty acids are presented in Fig. 4 and show surface morphology differences between the films. In general, the surface structure of PSGG-Sh films was nearly smooth and homogenous. Whilst a less smooth, but quite homogeneous surface was observed for films containing saturated fatty acids (Fig. 4A). The presence of long-chain fatty acids produced films with rougher and bumpier surface (Fig. 4). The differences in the morphological structure of films are associated with different internal structure of fatty acid tested (Villalobos, Chanona, Hernández, Gutiérrez, & Chiralt, 2005). The structure of lipid-containing films is widely dependent on the volume fraction of the dispersed lipids, size of lipid aggregations, and their development during the drying process (Fabra et al., 2009). The surface of the PSGG-Sh-OA film showed a more regular, and cohesive structure, with homogenous lipids distribution, which contributed to the good barrier and mechanical properties.

4. Conclusion

Three-levels Box–Behnken response surface design was applied to evaluate main and interaction influence of different amounts of shellac, SA, and Tween 20 on thickness, moisture barrier, and mechanical characteristics of PSGG films. A film formulation composed of 40% shellac, 1% SA and 0.3 ml Tween 20 provided the film with minimum thickness and WVP, as well as improved mechanical properties. After selecting the best concentration of film components, different emulsifiers, BA, LA, PA, SA and OA were tested to determine the appropriate emulsifier for the PSGG-Sh film. According to the physical, optical, WVP and mechanical properties of these films, OA was selected as the most effective emulsifier. The result of this study showed that PSGG-Sh-OA film has potential to be used in food industry.

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Conflict of Interest

The authors declare no conflict of interest.

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686 **Figure captions**

687 **Fig. 1.** Correlations between predicted and experimental thickness (A), water vapor permeability
688 (B), tensile strength (C), and elongation at break (D).

689 **Fig. 2.** Response surface plots showing the interaction effects of process variables on thickness
690 (A-C) and WVP (D-F).

Fig. 3. Response surface plots showing the interaction effects of process variables on tensile strength (A-C) and elongation at break (D-F).

Fig. 4. Micrographs of the surface of PSGG-Sh films with different fatty acids (1000 ×). A: PSGG-Sh; B: PSGG-Sh-BA; C: PSGG-Sh-LA; D: PSGG-Sh-PA; E: PSGG-Sh-SA; F: PSGG-Sh-OA.

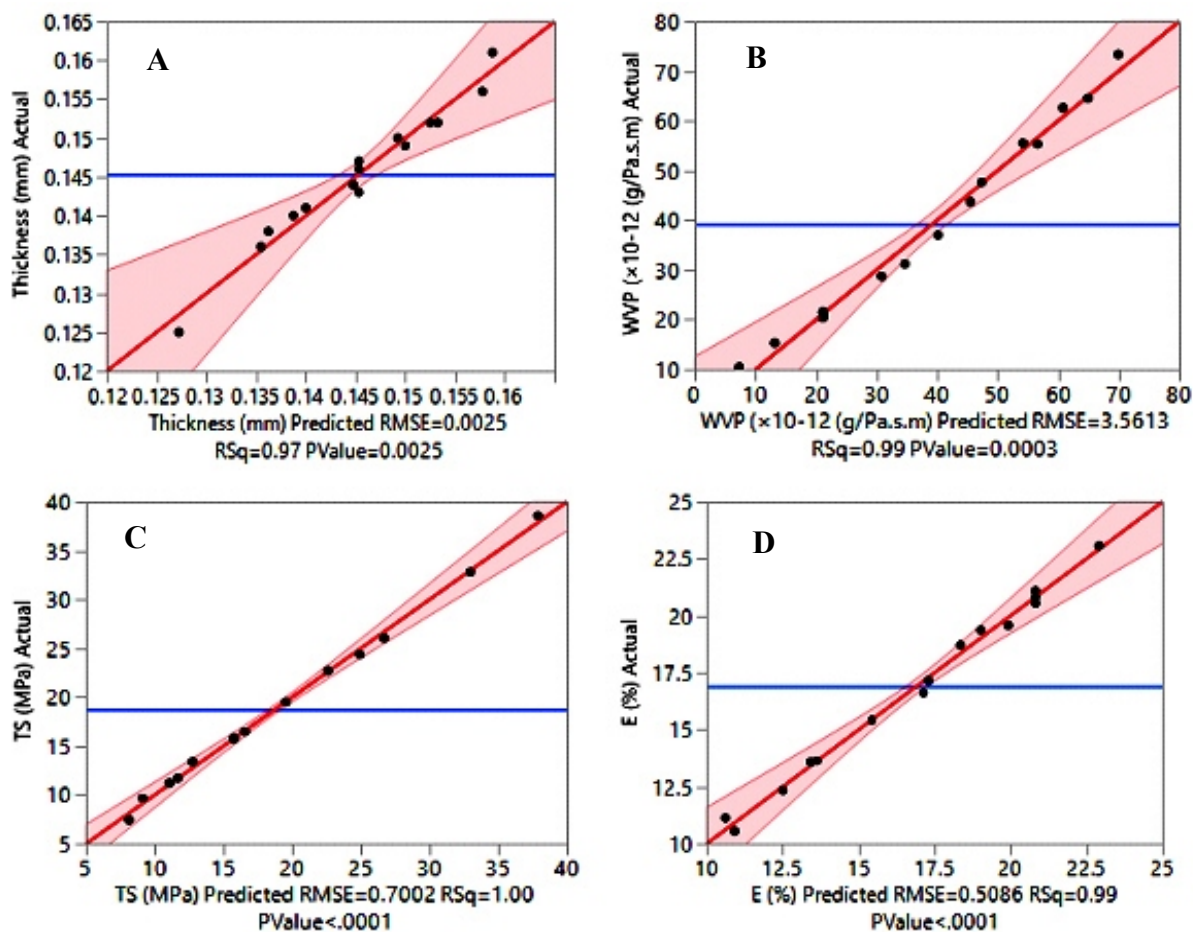
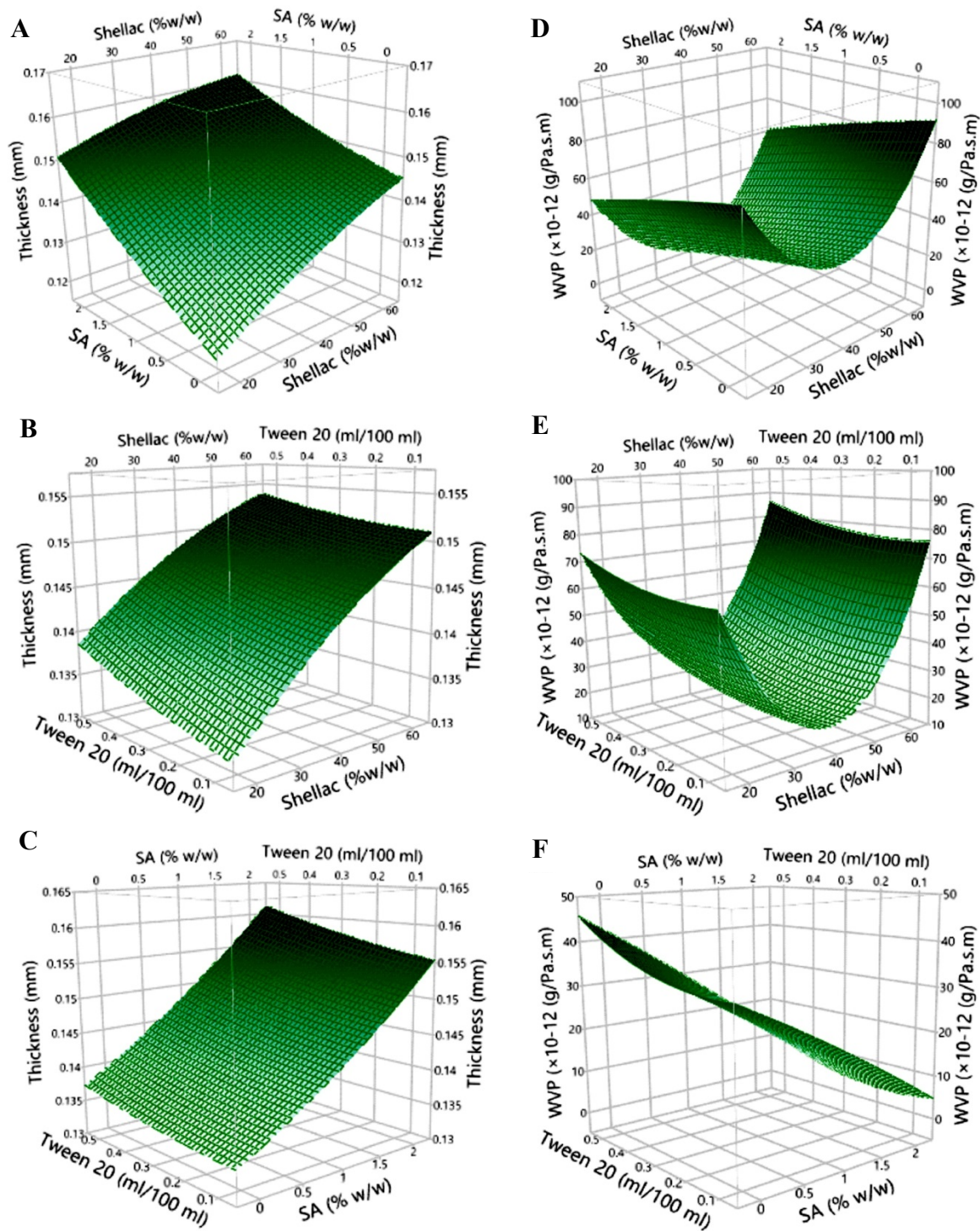


Fig. 1. Correlations between predicted and experimental thickness (A), water vapor permeability (B), tensile strength (C), and elongation at break (D).



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712 **Fig. 2.** Response surface plots showing the interaction effects of process variables on thickness

713 (A-C) and WVP (D-F).

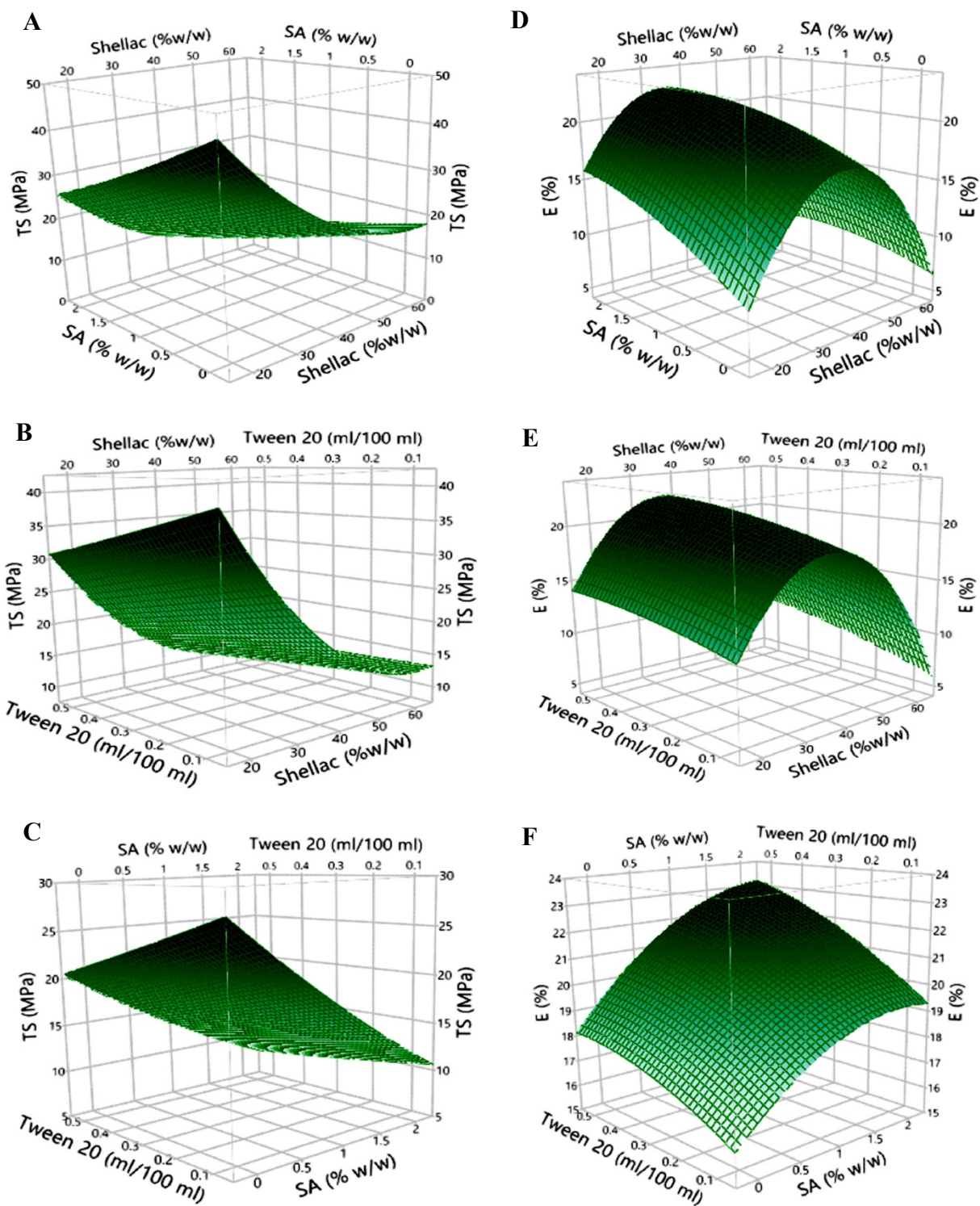
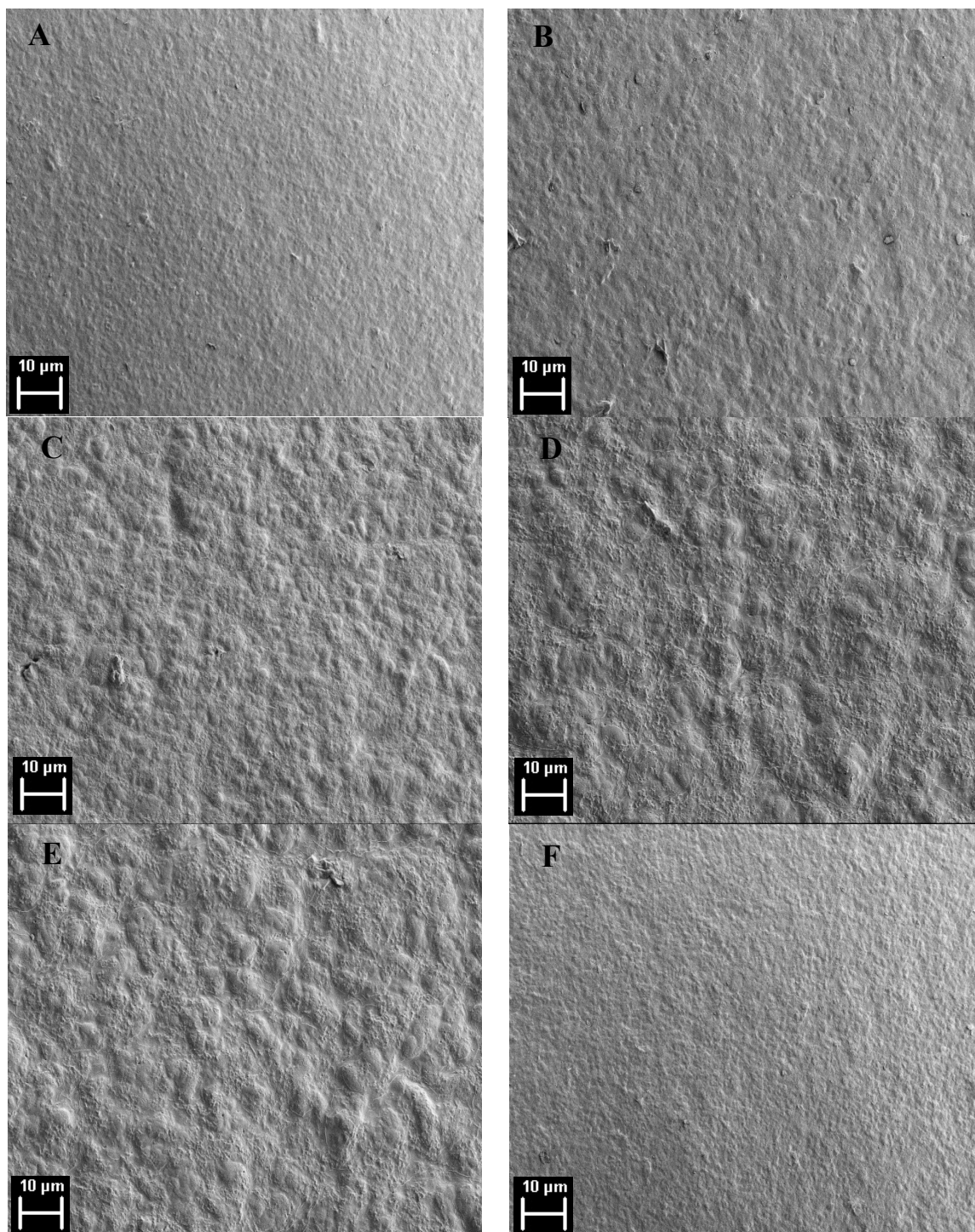


Fig. 3. Response surface plots showing the interaction effects of process variables on tensile

716 strength (A-C) and elongation at break (D-F).



717 **Fig. 4.** Micrographs of the surface of PSGG-Sh films with different fatty acids (1000 ×). A: PSGG-
718 Sh; B: PSGG-Sh-BA; C: PSGG-Sh-LA; D: PSGG-Sh-PA; E: PSGG-Sh-SA; F: PSGG-Sh-OA.