

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

Bahareh Saberi, Suwimol Chockchaisawasdee,
John B. Golding, Christopher J. Scarlett
and Costas E. Stathopoulos

This is the accepted manuscript © 2017, Elsevier
Licensed under the Creative Commons Attribution-
NonCommercial-NoDerivatives 4.0 International:
<http://creativecommons.org/licenses/by-nc-nd/4.0/>



The published article is available from doi:
<https://doi.org/10.1016/j.foodhyd.2017.05.042>

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

3 Bahareh Saberi^{a*}, Suwimol Chockchaisawasdee^{a,c}, John B. Golding^b, Christopher J. Scarlett^a,
4 Costas E. Stathopoulos^c

5

6 ^a School of Environmental and Life Sciences, University of Newcastle, Ourimbah, NSW 2258,
7 Australia

8 ^b NSW Department of Primary Industries, Ourimbah, NSW 2258, Australia

9 ^c Division of Food and Drink, School of Science, Engineering and Technology, University of
10 Abertay, Dundee DD1 1HG, UK

11

12 ***Correspondence to:**

13 Bahareh Saberi

14 School of Environmental and Life Sciences, Faculty of Science and Information Technology,
15 University of Newcastle, Brush Road, Ourimbah, NSW 2258, Australia.

16 Tel: +61 449968763; Fax: +61 2 4348 4145; E-mail: bahareh.saberi@uon.edu.au

17

18

19

20

21

22

23

24

25

26

27

28 **Abstract**

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

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

44

45

46

47

48

49 **1. Introduction**

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

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

68 Our previous studies demonstrated that GG in combination with pea starch (PS) improved
69 physical, barrier and mechanical properties of films (Saber et al., 2017; Saber et al., 2016a). The
70 water vapor permeability (WVP) value of pea starch-guar gum (PSGG) biocomposite films

71 exhibited better moisture barrier characteristics than pure PS films (Saber et al., 2016b), but these
72 were higher than those of low density polyethylene (LDPE) (Phan The et al., 2008). GG improved
73 the functional characteristics of PS edible film as both biopolymers are hydrophilic; however, the
74 incorporation of hydrophobic substances for decreasing the water sensitivity of biocomposite films
75 is required.

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

89 In addition, the incorporation of fatty acids has been used to decrease water transmission through
90 edible films. Fatty acids are polar lipids and their chain length and unsaturation degree has a
91 significant effect on film properties (Fernández, de Apodaca, Cebrián, Villarán, & Maté, 2007).
92 Other hydrophobic compounds with the potential to improve film characteristics are surfactants.

93 These compounds, such as Tween-20, are amphiphilic substances, which are necessary for
94 preparation emulsion films with suitable properties (Tongnuanchan, Benjakul, & Prodpran, 2014).

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

105 **2. Materials and methods**

106 *2.1. Materials*

107 Canadian non-GMO yellow pea starch with 13.2% moisture, 0.2% protein, 0.5% fat, 0.3% ash,
108 and $36.25 \pm 0.32\%$ amylose was used in all experiments (supplied by Yantai Shuangta Food Co.,
109 Jinling Town, China). Guar gum (E-412) was purchased from The Melbourne Food Ingredient
110 Depot, Brunswick East, Melbourne, Australia. Food grade shellac was purchased from Castle
111 Chemicals (castlechem.com.au), NSW, Australia. Stearic acid (SA), lauric acid (LA), oleic acid
112 (OA), butyric acid (BA), palmitic acid (PA) and Tween-20 were obtained from Sigma Aldrich,
113 Australia. Glycerol was from Ajax Finechem Pty. Ltd, Australia and used as a plasticizer. All other
114 chemicals were purchased from Merck Millipore, Pty., VIC, Melbourne, Australia.

115 2.2. Emulsion preparation

116 Optimized amounts of pea starch (2.5 g), guar gum (0.3 g) and 25% w/w glycerol based on the dry
117 film matter were dissolved in 100 ml degassed deionized water with gentle heating (about 40 °C)
118 and magnetic stirring. In another study, we determined the optimized amount of film components
119 by using Box–Behnken response surface design (BBD) (Saber et al., 2016a). The PSGG-Sh
120 composite mixtures without emulsifier did not form a film, therefore, SA, was used to stabilize the
121 PSGG-Sh composite films. Melted SA (0-2% db) and Tween-20 (0.1–0.5% wb) were added to the
122 PSGG solution and the aqueous suspension was gelatinized at 90 °C for 20 min on a hot plate with
123 continuous stirring. According to preliminary moisture barrier and mechanical tests (data are not
124 shown), shellac was added the PSGG-SA-Tween 20-glycerol mixture at three different levels
125 (20%, 40% and 60% db). Once the lipids were melted, samples were homogenized for 4 min at
126 22000 rpm using a T25 Ultra-Turrax (Ika, Staufen, Germany). After homogenization, the film
127 solution was cooled to room temperature with mild magnetic stirring for 1 h to decrease air
128 bubbles. Filmogenic suspensions (20 g) were cast onto Petri dishes (10 cm in diameter) and dried
129 at 40 °C in an oven until reaching constant weight (about 24 h). Films were carefully peeled-off
130 from Petri dishes and conditioned at 25 °C, 65% relative humidity (RH) for 72 h prior to further
131 testing (Saber et al., 2017).

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

136 2.3. Experimental design

137 The effect of process parameters (shellac (X1): 20–60%, SA (X2): 0-2%, and Tween-20 (X3): 0.1–
138 0.5 ml)) on film mechanical and barrier properties was studied by applying a three-level-three-
139 factor, Box–Behnken response surface design (BBD) with three central point replicates. All
140 experimental runs are listed in Table 1. A second-order polynomial model was used to fit the
141 experimental data obtained from the seventeen experimental runs:

$$142 \quad Y = \beta_0 + \sum_{i=1}^k \beta_i X_i + \sum_{i=1}^{k-1} \sum_{j=2}^k \beta_{ij} X_i X_j + \sum_{i=1}^k \beta_{ii} X_i^2 + e_i \quad (1)$$

143 where various X_i values are independent variables affecting the responses Y ; β_0 , β_i , β_{ii} , and β_{ij} are
144 the regression coefficients for intercept, interaction coefficients of linear, quadratic and the second-
145 order terms, respectively and k is the number of variables (Saber et al., 2017).

146

147

148

149

150

151

152

153

154

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

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

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

158

159 *2.4. Film characterization*

160 *2.4.1. Thickness*

161 A digital micro-meter (Mitutoyo, Co., Code No. 543-551-1, Model ID-F125, 139 Japan; sensitivity
 162 = 0.001 mm) was used to measure film thickness. The mean value from 10 different points for
 163 each film samples was measured (Fakhouri, Fontes, Innocentini & Mei, & Collares & Queiroz,
 164 2009).

165 *2.4.2. Water vapor permeability*

166 Water vapor permeability (WVP) of films was examined gravimetrically using the method
167 explained by Sun, Wang, Kadouh, and Zhou (2014) with some modifications. The films were
168 sealed onto test cups half-filled with anhydrous calcium chloride (CaCl₂) (0% RH) that was dried
169 in a hot air oven at 120 °C for 1 day initially. These cups were then placed in a desiccator containing
170 saturated NaCl solution (75% RH) and kept at 25 °C. The test cups were weighed as a function of
171 time until changes in the weight were recorded to the nearest 0.001 g. Water vapor transmission
172 rate (WVTR) was calculated by dividing the slope of straight line (g/m) calculated from the weight
173 gain as a function of time data, with film surface area, and WVP was measured as follows:

$$174 \quad \text{WVP} = \text{WVTR} \times \frac{\text{Film thickness}}{\Delta P} \quad (2)$$

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

177 *2.4.3. Mechanical properties*

178 Tensile strength (TS) and elongation at break (E) of the film were measured using a Texture
179 Analyzer (LLOYD Instrument LTD, Fareham, UK) according to the standard method (ASTM,
180 2001). Test samples, 40 mm × 15 mm, were cut from each film and fixed with an initial grip
181 separation of 40 mm. Five replicates of each film were then pulled apart at crosshead speed of 1
182 mm/s. TS (MPa) was calculated by the Equation (1):

$$183 \quad \text{TS} = F_{\max}/A \quad (3)$$

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

186 $E = \Delta L/L_0 \times 100\%$ (4)

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

189 *2.4.5. Calculation of moisture content*

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

192 *2.4.6. Water solubility of films*

193 Film solubility was determined according to the procedure reported by Chiumarelli and Hubinger
194 (2014). Three discs (40 mm × 15 mm) of each film were cut, weighed, immersed in 50 mL of
195 distilled water. Then, they were slowly and periodically agitated during 24 h at 25 °C. The dry
196 mass content of initial and final samples was calculated by drying the samples at 105 °C for 24 h.
197 The measurements were made in triplicate.

198 *2.4.7. Optical properties*

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

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

206
$$\Delta E = \sqrt{(L^* - L)^2 + (a^* - a)^2 + (b^* - b)^2}$$
 (5)

207
$$WI = 100 - \sqrt{(100 - L)^2 + a^2 + b^2}$$
 (6)

208
$$YI = \frac{142.86b}{L}$$
 (7)

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

212 2.4.8. Scanning electron microscopy (SEM)

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

216 2.5. Statistical analysis

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

223 3. Results and discussion

224 3.1. Preliminary studies

225 Initial studies showed that using a shellac concentration lower than 20% was not adequate to form
226 a compact film structure. Conversely, the films with shellac concentrations greater than 60% were
227 very soft to peel off; therefore concentrations between 20% and 60% shellac based on dry film
228 matter, were selected for further examination. Similarly different amounts of Tween-20 as the
229 surfactant were also exposed to preliminary assessment to examine their effects on the flexibility
230 of PSGG films. The levels of Tween-20 between 0.1-0.5 ml/100 ml of filmogenic solution were
231 found to be the most effective concentration. Furthermore, Tween-20 showed the most efficient
232 plasticizing influence in presence of glycerol causing higher flexibility and the resultants films
233 were peeled off easily from petri dishes. In terms of SA, high concentrations of SA made the films
234 brittle and they broke during peeling. Thus, the range of 0–2% (w/w) SA concentrations was more
235 suitable for PSGG films. The incorporation of the same range of SA effectively improved physical
236 and mechanical properties of sodium caseinate edible films .

237 *3.2. Experimental design analysis*

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

247 values of the model also verified the reliability of the model in predicting thickness, WVP and
248 mechanical properties of films.

249 The adequacy of developed models was estimated using diagnostic plots such as predicted versus
250 actual plots (Fig. 1). The diagnostic plots such as predicted versus actual values determine the
251 model suitability and demonstrate the correlation between predicted and experimental values
252 (Maran, Sivakumar, Thirugnanasambandham, & Sridhar, 2013) and the results show that the data
253 points on this plot were close to the straight line (Fig. 1). This signifies that the predicted values
254 obtained were very close to the experimental values, suggesting the developed models were
255 reliable and accurate in determining the relationship between the real and predicted data.

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

$$258 \text{ THI} = 0.15 + 0.008x_1 + 0.009x_2 + 0.002x_3 - 0.002x_1x_2 - 0.0005x_1x_3 + 0.0005x_2x_3 -$$
$$259 0.0011x_1^2 + 0.0008x_2^2 + 0.0003x_3^2$$

$$260 \text{ WVP} = 21.14 + 4.99x_1 - 12.63x_2 + 3.79x_3 + 0.43x_1x_2 + 0.34x_1x_3 - 0.86x_2x_3 + 32.23x_1^2 -$$
$$261 0.64x_2^2 + 2.39x_3^2$$

$$262 \text{ TS} = 15.75 - 8.95x_1 - 5.93x_2 - 1.98x_3 + 1.71x_1x_2 + 1.15x_1x_3 + 0.71x_2x_3 + 5.05x_1^2 +$$
$$263 0.50x_2^2 + 0.07x_3^2$$

$$264 \text{ E} = 20.83 - 2.08x_1 + 1.63x_2 + 1.18x_3 - 0.83x_1x_2 + 0.33x_1x_3 + 0.32x_2x_3 - 6.31x_1^2 -$$
$$265 0.72x_2^2 - 0.32x_3^2$$

266

267

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

Sources of variation	THI (mm)	WVP ×10⁻¹² (gPa⁻¹s⁻¹m⁻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*

269 * Significant difference with p < 0.05.

270

271 3.3. Influence of process variables

272 The linear regression coefficients and their statistical significance for each response are presented
 273 in Table 3. The results clearly show that the nature and composition of the films influenced film
 274 thickness. The data showed that the film thickness varied between 0.125 to 0.161 mm by increasing
 275 shellac, SA and Tween-20 content in the film formulation. The thickness of films had a positive
 276 correlation to the three experimental variables. Shellac and SA had a significant linear influence
 277 (p < 0.05) on the film thickness (Table 3). The “R-Squared” value of 0.971 is in satisfactory
 278 correlation with the “Adjusted R-Squared” of 0.919, which is an indicator of the suitability of fit
 279 of the model, verifying that 97.1% of the total variation was described by the model (Table 3).
 280 While, adjusted R² value (0.919) exhibited the significance of the model (Singh, Chatli, & Sahoo,
 281 2015). Furthermore, higher precision and reliability of the model was indicated by insignificant
 282 value of lack of fit (0.381). With the increase in shellac and SA levels, there was an increase in the
 283 thickness of films, but this was not affected by Tween-20 level (Fig. 3A-C). The influence of
 284 Tween-20 on the thickness is determined by the level of combination, which was less than 1.0%.
 285 A possible reason for the increase in the film thickness after incorporation of lipid compounds
 286 could be that there is an increase in total mass, once the solvent is evaporated during the drying

287 process (Fakhouri et al., 2009). A similar effect was observed when fatty acids were added to
288 maize starch and gelatin composite films (Fakhouri et al., 2009), adding oleic acid to tara gum film
289 (Ma, Hu, Wang, & Wang, 2016), and adding different fatty acids to a basil seed gum edible film
290 (Mohammad Amini, Razavi, & Zahedi, 2015). The addition of Tween-20 increases the probability
291 of interaction between the lipid compounds and film polymers thus decreasing the flexibility of
292 polymer chains during film development and resulting in thicker films (Maran et al., 2013).

293 Analysis of WVP of the films showed that this parameter was dependent on all three independent
294 variables ($p < 0.05$, Table 3). Shellac (linearly and quadratically), SA (linearly) and Tween-20
295 (linearly) all significantly ($p < 0.05$) affected WVP of films (Table 3). There was no interaction
296 between variables shellac \times SA, shellac \times Tween-20 and SA \times Tween-20. The high R^2 value
297 (0.988) indicated that 98.85% of the total variation fitted to the model (Table 3). The results
298 described in Fig. 2D-F show that the addition of shellac and SA decreased WVP significantly due
299 to the increasing of hydrophobic groups in formulations. In terms of shellac, WVP initially
300 decreased with the addition of shellac, but later WVP increased after reaching an optimal level.
301 Increasing shellac content over the critical value caused a poor dispersion of shellac in the film
302 matrix (Chen et al., 2015). An increase in candelilla wax concentration above 0.5 %, also resulted
303 in increased WVP of oxidized potato starch and carboxymethyl cellulose films (Kowalczyk &
304 Baraniak, 2014). It has been proposed that the addition of hydrophobic compounds over the critical
305 level might induce more disorder in the film network, producing spaces at the polymer-lipid
306 interface and promoting the transmission of water vapor molecules inside the film (Chick &
307 Hernandez, 2002). However, in this study the WVP of the films was significantly ($p < 0.05$)
308 enhanced with the incorporation of Tween-20 in the film matrix. This can be attributed to the
309 hydrophilic-lipophilic balance (HLB) ratio of Tween-20 (HLB of 16.7), which is the most

310 hydrophilic surfactant and presents higher permeability values (Rodríguez, Oses, Ziani, & Mate,
311 2006).

312 The results presented in Table 3 shows that TS values were negatively affected by shellac, SA and
313 Tween-20, and positively affected by the interaction of shellac-SA and shellac-Tween-20 and the
314 quadratic term of shellac. Figure 3A-C showed that the amount of TS reduced with increasing the
315 levels of shellac, SA and Tween-20 where the TS values varied from 38.6 to 7.4 MPa. The decrease
316 in TS with the incorporation of lipid components to the biopolymer films can be associated with
317 their movement to the starch-guar gum interface, disturbing the interactions between them and
318 resulting in a reduction in film resistance (Fakhouri et al., 2009). Lower TS values due to
319 incorporation of increasing amounts of lipids and/or surfactants have been also reported for other
320 biodegradable films (Chen & Lai, 2008; Ziani, Oses, Coma, & Maté, 2008).

321 The results in Table 3 show that shellac had significant negative linear effect on E, whereas, SA
322 and Tween-20 had significant positive effect. The interaction between shellac \times SA and the
323 quadratic term of shellac and SA were shown to negatively influence the E ($p < 0.05$). The E value
324 of the films decreased by increasing of shellac content due to poor miscibility of shellac in
325 biopolymer matrix at high concentration (Byun, Ward, & Whiteside, 2012). The addition of SA
326 and Tween-20 increased E value, which is probably related to fine dispersion of SA and Tween-
327 20 in the polymer matrix, and the development of interactions causing a plasticizing effect
328 (Jiménez, Fabra, Talens, & Chiralt, 2010). At high concentrations of the surfactant, there is an
329 apparent synergistic effect between glycerol and Tween-20 (Rodríguez et al., 2006). This
330 interaction brings about lower TS and higher E in films. Tween-20 is a small molecule and its
331 hydrophilic component can interact with glycerol or water, promoting its replacement between

332 polymer chains, enhancing even more the chain mobility and increasing the initial plastic effect
333 (Ghanbarzadeh & Almasi, 2011).

334

335

336

337

338

339

340

341

342

343

344

345

346

347

348

349 **Table 3.** Analysis of variance for the experimental results.*

350

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

351 *Significantly different at $p < 0.05$; P: parameter; β_0 : intercept; β_1 , β_2 , and β_3 : linear regression coefficients for shellac,
352 SA and Tween-20; β_{12} , β_{13} , and β_{23} : regression coefficients for interaction between shellac \times SA, shellac \times Tween-20
353 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
354 Tween-20.

355

356 3.4. Optimization and validation of the models

357 The desirability function was applied for simultaneous optimization of the multiple responses
358 (Saberri et al., 2017). The aim was to maximize mechanical properties and to minimize thickness
359 and WVP. The optimum level of different variables by applying the methodology of desired
360 function was achieved with 40 %w/w shellac, 1 %w/w SA and 0.3 ml Tween-20/100 ml with an
361 overall desirability of 0.82. The adequacy of the models was experimentally validated by the above

362 mentioned optimal conditions. Table 4 shows the results of triplicate experiments compared with
 363 the predicted values of the responses. The suitability of the methodology developed for the
 364 optimization of the process conditions was confirmed by the absolute residual error for the
 365 dependent variables.

366

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

Responses	Predicted value	Experimental value (n = 3) ^a	Absolute residual error (%) ^b
Thickness (mm)	0.145±0.006 ^a	0.146±0.004 ^a	0.694
WVP ×10 ⁻¹² (gPa ⁻¹ s ⁻¹ m ⁻¹)	21.412±0.834 ^a	20.047±1.361 ^a	6.816
Tensile strength (MPa)	15.841±1.072 ^a	16.291±1.705 ^a	2.762
Elongation at break (%)	20.561±1.156 ^a	21.636±0.874 ^a	5.078

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

371 ^a Values obtained at optimum conditions (shellac 40 (% w/w); SA 1 (% w/w); and Tween-20 0.3 (ml/100 ml)).

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

373

374 3.5. Effect of the different emulsifiers on PSGG-Sh film physical properties

375 The first aim of this study showed that the incorporation of 40 %w/w shellac, 1 % w/w SA and 0.3
 376 ml Tween-20 into pea starch (2.5 g), guar gum (0.3 g) and 25 %w/w glycerol in 100 mL of distilled
 377 water; made a composite film with satisfactory mechanical and barrier properties. In general,
 378 barrier and mechanical characteristics of lipid-containing films are affected by the structure and
 379 stability of an emulsion (Kowalczyk et al., 2014). Fatty acids can act as emulsifier and plasticizer

380 in biopolymer films and their action is dependent on the hydrophobic/hydrophilic ratio presented
 381 by film components, the polarity, unsaturation number and ramification degree provided by the
 382 polymeric chains of the matrix used (Fakhouri et al., 2009; Gontard, Duchez, Cuq, & Guilbert,
 383 1994). Therefore, a variety of fatty acids were studied to determine their efficiency in preparing
 384 packaging films.

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

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

Fatty acid	Formula	Lipid numbers	Molecular weight (g/mol)	Saturation/unsaturation	concentration	
					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

388

389 All PSGG-Sh films formulated with different fatty acids were peelable, flexible, smooth,
 390 homogeneous and transparent. The presence of glycerol, fatty acids and Tween-20 functioned as
 391 diluents of shellac improving its miscibility, and modifying hydrophobic phase dispersion during
 392 emulsification, affecting homogeneity of the final distribution of hydrophobic constituents in the
 393 PSGG matrix. All emulsions produced in this study were considered “stable”, because they did
 394 not show creaming during drying. An unstable emulsion creates a gradient of lipid concentrations
 395 across the thickness of the film and produces films with a “bilayer-like” structure due to separation

396 of lipid during drying (Shellhammer & Krochta, 1997). Creaming is the main mechanism in which
397 separation of the disperse phase from an emulsion occurs and is typically considered the precursor
398 to coalescence (Kowalczyk et al., 2014). Therefore in this study, all fatty acid molecules
399 successfully incorporated into the PSGG-Sh film without phase separation. Thus the optimized
400 emulsifier obtained by the Box–Behnken experimental design was satisfactory for PSGG-Sh film
401 formation.

402 The WVP, mechanical and transparency of the film are influenced by film thickness, thus this
403 parameter should be precisely and correctly measured. The results showed that the thickness of
404 films varied between 0.129 and 0.146 mm with the addition of various fatty acids (Table 6).
405 Increasing film thickness was related to the presence of fatty acid molecules between the
406 macromolecular chains, thus accordingly extending the structure of film through increasing
407 molecular volume of network (Thakhiew, Devahastin, & Soponronnarit, 2010). In this study, the
408 film formulated with SA produced a film of maximum thickness.

409 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
410 was dependent on the type of fatty acid in the following order: BA > LA > PA > SA > OA (Table
411 6). This observation maybe due to the hydrophilic-hydrophobic proportion in the film structure,
412 whereby the existence of fatty acids in films could reduce WVP according to the hydrophilic-
413 hydrophobic ratio of the film (Thakur et al., 2016). The different behavior of fatty acids on the
414 reduction of WVP may be related to the different structural arrangement of fatty acids in the film
415 matrix (Kowalczyk et al., 2014). The smallest value for WVP was observed after BA was added
416 to the PSGG-Sh matrix. This maybe because BA has the shortest carbon chain of all fatty acids
417 applied, and therefore facilitated its inclusion into the biopolymer film matrix (Fakhouri et al.,
418 2009). Chain length and the degree of unsaturated fatty acids are important in determining the

419 resultant film properties (Rezvani et al., 2013). Fatty acids with longer hydrophobic tail have low
420 chain mobility and are consequently capable to decrease WVP (Ayranci & Tunc, 2001). The
421 addition of 1% OA was the most effective concentration to improve the water barrier
422 characteristics due to its highly hydrophobic nature (Ghanbarzadeh et al., 2011). This can be also
423 ascribed to the fact that OA dispersed in the film diminished the practical interfacial area exposed
424 to water vapor (Chen et al., 2015). Moreover, it has been proposed that some voids and cracks in
425 film structure are caused by saturated fatty acids because of their crystallisation at ambient and
426 drying temperatures facilitating mass transfer through interior discontinuities and channels of film
427 (Mohammad Amini et al., 2015). Moisture transmits through the matrix of continuous phase in
428 edible films prepared as oil-in-water (O/W) emulsion. The moisture permeability of continuous
429 phase can be affected by interactions between hydrocolloid chains and lipid phase (The,
430 Debeaufort, Voilley, & Luu, 2009). In addition, the polymeric chains may partly immobilize at the
431 interface with the emulsified lipid particles (McHugh & Krochta, 1994), therefore it becomes less
432 mobile and decreases WVP by reducing film free volume and inhibiting the penetration of water
433 via the polymer chains interface (Ghanbarzadeh et al., 2011). The existence of a hydrophobic and
434 disperse phase has been shown to produce a tortuous path for water vapor molecular transmission,
435 thus reducing the WVP in film matrix (Ma et al., 2016).

436 The mechanical properties of the PSGG-Sh films with different fatty acids including the tensile
437 strength (TS) and elongation at break (E) are summarized in Table 6. The tensile mechanical
438 properties showed a dependency to fatty acid type. The influence of fatty acids on mechanical
439 properties of hydrocolloids film may be determined by the basic matrix characteristics of materials,
440 the interaction of the polymer molecules, the film components and the size distribution of the fatty
441 acid molecules (Chen et al., 2015). In general the results show that TS was reduced by the addition

442 of fatty acids, while E exhibited an increase. Films containing BA showed highest TS and E values
443 because it is lower in size, thus, it can more efficiently interact with polymers than the other fatty
444 acids tested. The small size of BA produced more direct interaction between polymer molecules
445 leading to higher intermolecular cohesive force and higher TS. It is suggested that the interactions
446 between non-polar fatty acid molecules and polar polymer molecules are much lower than those
447 between polar glycerol molecules and polar polymer molecules (Vargas, Albors, Chiralt, &
448 González-Martínez, 2009). The decrease in TS and increase in E with the increase of hydrophobic
449 compounds on mechanical characteristics have been widely reported (Bertan, Tanada-Palmu,
450 Siani, & Grosso, 2005; Colla, do Amaral Sobral, & Menegalli, 2006; Ghasemlou, Khodaiyan,
451 Oromiehie, & Yarmand, 2011; Péroval, Debeaufort, Despré, & Voilley, 2002; Srinivasa, Ramesh,
452 & Tharanathan, 2007; Yang & Paulson, 2000). In this study, films comprising OA were more
453 flexible and extensible compare to those made by SA. This can be attributed to differences of the
454 solid state of SA, which forms a more rigid dispersed phase in the film and reduces its capability
455 to expand under the testing conditions, and to the specific plasticizing impact of unsaturated OA
456 in the PSGG-Sh matrix (Rezvani et al., 2013).

457 As expected, the moisture content of PSGG-Sh films decreased with the incorporation of fatty
458 acids because of the increase in the films' hydrophobicity. With the addition of 1% fatty acid, the
459 moisture content of PSGG-Sh films containing BA, LA, PA, SA, and OA was reduced by 7.1,
460 17.8, 40.0, 47.4, and 59.5%, respectively. The dispersion of hydrophobic fatty acids in the solution
461 has been shown to decrease the accessibility of hydroxyl groups, which interact with water, thus
462 leading to the immobilization of biopolymer chains and reduction of moisture content by the film
463 (Ma et al., 2016).

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

471 **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 ^e	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

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

474 3.6. Effect of the different emulsifiers on PSGG-Sh film optical properties

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

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

Films	ΔE	WI	YI	Transparency (%)
PSGG	6.538±0.704 ^e	90.267±0.923 ^a	9.548±1.054 ^e	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

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

491 3.7. Scanning electron microscopy (SEM)

492 SEM images of the surfaces of PSGG-Sh films with and without fatty acids are presented in Fig.
493 4 and show surface morphology differences between the films. In general, the surface structure of
494 PSGG-Sh films was nearly smooth and homogenous. Whilst a less smooth, but quite homogeneous
495 surface was observed for films containing saturated fatty acids (Fig. 4A). The presence of long-
496 chain fatty acids produced films with rougher and bumpier surface (Fig. 4). The differences in the
497 morphological structure of films are associated with different internal structure of fatty acid tested
498 (Villalobos, Chanona, Hernández, Gutiérrez, & Chiralt, 2005). The structure of lipid-containing
499 films is widely dependent on the volume fraction of the dispersed lipids, size of lipid aggregations,
500 and their development during the drying process (Fabra et al., 2009). The surface of the PSGG-
501 Sh-OA film showed a more regular, and cohesive structure, with homogenous lipids distribution,
502 which contributed to the good barrier and mechanical properties.

503 4. Conclusion

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

513 **Acknowledgement**

514 This study was funded by the University of Newcastle, NSW Australia. The authors greatly
515 acknowledge University of Newcastle EMX-ray center for providing access to SEM.

516 **Conflict of Interest**

517 The authors declare no conflict of interest.

518 **References**

- 519 Arismendi, C., Chillo, S., Conte, A., Del Nobile, M. A., Flores, S., & Gerschenson, L. N. (2013).
520 Optimization of physical properties of xanthan gum/tapioca starch edible matrices
521 containing potassium sorbate and evaluation of its antimicrobial effectiveness. *LWT-Food*
522 *Science and Technology*, 53(1), 290-296.
- 523 ASTM. (2001). Standard test method for tensile properties of thin plastic sheeting; Standard D882.
524 In *Annual Book of American Standard Testing Methods*. Philadelphia, PA, USA.
- 525 Ayranci, E., & Tunc, S. (2001). The effect of fatty acid content on water vapour and carbon dioxide
526 transmissions of cellulose-based edible films. *Food Chemistry*, 72(2), 231-236.
- 527 Bertan, L., Tanada-Palmu, P., Siani, A. C., & Grosso, C. (2005). Effect of fatty acids and 'Brazilian
528 elemi' on composite films based on gelatin. *Food Hydrocolloids*, 19(1), 73-82.
- 529 Bosquez-Molina, E., Guerrero-Legarreta, I., & Vernon-Carter, E. J. (2003). Moisture barrier
530 properties and morphology of mesquite gum-candelilla wax based edible emulsion
531 coatings. *Food Research International*, 36(9-10), 885-893.
- 532 Byun, Y., Ward, A., & Whiteside, S. (2012). Formation and characterization of shellac-
533 hydroxypropyl methylcellulose composite films. *Food Hydrocolloids*, 27(2), 364-370.
- 534 Cano, A., Jimenez, A., Chafer, M., Gonzalez, C., & Chiralt, A. (2014). Effect of
535 amylose:amylopectin ratio and rice bran addition on starch films properties. *Carbohydrate*
536 *Polymers*, 111, 543-555.
- 537 Cerqueira, M. A., Souza, B. W., Teixeira, J. A., & Vicente, A. A. (2012). Effect of glycerol and
538 corn oil on physicochemical properties of polysaccharide films—A comparative study.
539 *Food Hydrocolloids*, 27(1), 175-184.
- 540 Chen, C.-H., & Lai, L.-S. (2008). Mechanical and water vapor barrier properties of tapioca
541 starch/decolorized hsian-tsao leaf gum films in the presence of plasticizer. *Food*
542 *Hydrocolloids*, 22(8), 1584-1595.
- 543 Chen, G., Zhang, B., & Zhao, J. (2015). Dispersion process and effect of oleic acid on properties
544 of cellulose sulfate-oleic acid composite film. *Materials*, 8(5), 2346-2360.
- 545 Chick, J., & Hernandez, R. (2002). Physical, Thermal, and Barrier Characterization of Casein
546 Wax-Based Edible Films. *Journal of Food Science*, 67(3), 1073-1079.
- 547 Chiumarelli, M., & Hubinger, M. D. (2014). Evaluation of edible films and coatings formulated
548 with cassava starch, glycerol, carnauba wax and stearic acid. *Food Hydrocolloids*, 38, 20-
549 27.

- 550 Colla, E., do Amaral Sobral, P. J., & Menegalli, F. C. (2006). Amaranthus cruentus flour edible
551 films: influence of stearic acid addition, plasticizer concentration, and emulsion stirring
552 speed on water vapor permeability and mechanical properties. *Journal of Agricultural and*
553 *Food Chemistry*, 54(18), 6645-6653.
- 554 Fabra, M., Pérez-Masiá, R., Talens, P., & Chiralt, A. (2011). Influence of the homogenization
555 conditions and lipid self-association on properties of sodium caseinate based films
556 containing oleic and stearic acids. *Food Hydrocolloids*, 25(5), 1112-1121.
- 557 Fabra, M. J., Talens, P., & Chiralt, A. (2008). Tensile properties and water vapor permeability of
558 sodium caseinate films containing oleic acid–beeswax mixtures. *Journal of Food*
559 *Engineering*, 85(3), 393-400.
- 560 Fabra, M. J., Talens, P., & Chiralt, A. (2009). Microstructure and optical properties of sodium
561 caseinate films containing oleic acid–beeswax mixtures. *Food Hydrocolloids*, 23(3), 676-
562 683.
- 563 Fakhouri, F. M., Fontes, L. C., Innocentini, L. H., & Collares, F. P. (2009). Effect
564 of fatty acid addition on the properties of biopolymer films based on lipophilic maize starch
565 and gelatin. *Starch*, 61(9), 528-536.
- 566 Fernandes, P. B., Gonçalves, M. P., & Doublier, J. L. (1993). Influence of locust bean gum on the
567 rheological properties of kappa-carrageenan systems in the vicinity of the gel point.
568 *Carbohydrate Polymers*, 22(2), 99-106.
- 569 Fernández, L., de Apodaca, E. D., Cebrián, M., Villarán, M. C., & Maté, J. I. (2007). Effect of the
570 unsaturation degree and concentration of fatty acids on the properties of WPI-based edible
571 films. *European Food Research and Technology*, 224(4), 415-420.
- 572 Ghanbarzadeh, B., & Almasi, H. (2011). Physical properties of edible emulsified films based on
573 carboxymethyl cellulose and oleic acid. *International journal of biological*
574 *macromolecules*, 48(1), 44-49.
- 575 Ghasemlou, M., Khodaiyan, F., Oromiehie, A., & Yarmand, M. S. (2011). Characterization of
576 edible emulsified films with low affinity to water based on kefir and oleic acid.
577 *International journal of biological macromolecules*, 49(3), 378-384.
- 578 Gontard, N., Duchez, C., Cuq, J. L., & Guilbert, S. (1994). Edible composite films of wheat gluten
579 and lipids: water vapour permeability and other physical properties. *International Journal*
580 *of Food Science & Technology*, 29(1), 39-50.
- 581 Jiménez, A., Fabra, M., Talens, P., & Chiralt, A. (2010). Effect of lipid self-association on the
582 microstructure and physical properties of hydroxypropyl-methylcellulose edible films
583 containing fatty acids. *Carbohydrate Polymers*, 82(3), 585-593.
- 584 Jiménez, A., Fabra, M. J., Talens, P., & Chiralt, A. (2013). Physical properties and antioxidant
585 capacity of starch–sodium caseinate films containing lipids. *Journal of Food Engineering*,
586 116(3), 695-702.
- 587 Kowalczyk, D., & Baraniak, B. (2014). Effect of candelilla wax on functional properties of
588 biopolymer emulsion films—a comparative study. *Food Hydrocolloids*, 41, 195-209.
- 589 Limmatvapirat, S., Limmatvapirat, C., Puttipatkhachorn, S., Nuntanid, J., & Luangtana-Anan,
590 M. (2007). Enhanced enteric properties and stability of shellac films through composite
591 salts formation. *European Journal of Pharmaceutics and Biopharmaceutics*, 67(3), 690-
592 698.
- 593 Luangtana-anan, M., Limmatvapirat, S., Nuntanid, J., Wanawongthai, C., Chalongsuk, R., &
594 Puttipatkhachorn, S. (2007). Effect of Salts and Plasticizers on Stability of Shellac Film.
595 *Journal of Agricultural and Food Chemistry*, 55(3), 687-692.

- 596 Ma, Q., Hu, D., Wang, H., & Wang, L. (2016). Tara gum edible film incorporated with oleic acid.
597 *Food Hydrocolloids*, 56, 127-133.
- 598 Maran, J. P., Sivakumar, V., Thirugnanasambandham, K., & Sridhar, R. (2013). Response surface
599 modeling and analysis of barrier and optical properties of maize starch edible films.
600 *International journal of biological macromolecules*, 60, 412-421.
- 601 McHugh, T. H., & Krochta, J. M. (1994). Water vapor permeability properties of edible whey
602 protein-lipid emulsion films. *Journal of the American Oil Chemists' Society*, 71(3), 307-
603 312.
- 604 Mohammad Amini, A., Razavi, S. M. A., & Zahedi, Y. (2015). The influence of different
605 plasticisers and fatty acids on functional properties of basil seed gum edible film.
606 *International Journal of Food Science & Technology*, 50(5), 1137-1143.
- 607 Ortega-Toro, R., Jiménez, A., Talens, P., & Chiralt, A. (2014). Properties of starch–hydroxypropyl
608 methylcellulose based films obtained by compression molding. *Carbohydrate Polymers*,
609 109, 155-165.
- 610 Pearnchob, N., Dashevsky, A., & Bodmeier, R. (2004). Improvement in the disintegration of
611 shellac-coated soft gelatin capsules in simulated intestinal fluid. *J Control Release*, 94(2-
612 3), 313-321.
- 613 Péroval, C., Debeaufort, F., Despré, D., & Voilley, A. (2002). Edible arabinoxylan-based films. 1.
614 Effects of lipid type on water vapor permeability, film structure, and other physical
615 characteristics. *Journal of Agricultural and Food Chemistry*, 50(14), 3977-3983.
- 616 Phan The, D., Debeaufort, F., Luu, D., & Voilley, A. (2008). Moisture barrier, wetting and
617 mechanical properties of shellac/agar or shellac/cassava starch bilayer bio-membrane for
618 food applications. *Journal of Membrane Science*, 325(1), 277-283.
- 619 Rezvani, E., Schleining, G., Sümen, G., & Taherian, A. R. (2013). Assessment of physical and
620 mechanical properties of sodium caseinate and stearic acid based film-forming emulsions
621 and edible films. *Journal of Food Engineering*, 116(2), 598-605.
- 622 Rodríguez, M., Osés, J., Ziani, K., & Mate, J. I. (2006). Combined effect of plasticizers and
623 surfactants on the physical properties of starch based edible films. *Food Research*
624 *International*, 39(8), 840-846.
- 625 Saberi, B., Thakur, R., Bhuyan, D. J., Vuong, Q. V., Chockchaisawasdee, S., Golding, J. B.,
626 Scarlett, C. J., & Stathopoulos, C. E. (2017). Development of edible blend films with good
627 mechanical and barrier properties from pea starch and guar gum. *Starch - Stärke*, 69(1-2),
628 1600227-n/a.
- 629 Saberi, B., Thakur, R., Vuong, Q. V., Chockchaisawasdee, S., Golding, J. B., Scarlett, C. J., &
630 Stathopoulos, C. E. (2016a). Optimization of physical and optical properties of
631 biodegradable edible films based on pea starch and guar gum. *Industrial Crops and*
632 *Products*, 86, 342-352.
- 633 Saberi, B., Vuong, Q. V., Chockchaisawasdee, S., Golding, J. B., Scarlett, C. J., & Stathopoulos,
634 C. E. (2016b). Mechanical and Physical Properties of Pea Starch Edible Films in the
635 Presence of Glycerol. *Journal of Food Processing and Preservation*, 40(6), 1339-1351.
- 636 Saurabh, C. K., Gupta, S., Variyar, P. S., & Sharma, A. (2016). Effect of addition of nanoclay,
637 beeswax, tween-80 and glycerol on physicochemical properties of guar gum films.
638 *Industrial Crops and Products*, 89, 109-118.
- 639 Shellhammer, T., & Krochta, J. (1997). Whey protein emulsion film performance as affected by
640 lipid type and amount. *Journal of Food Science*, 62(2), 390-394.

- 641 Singh, T. P., Chatli, M. K., & Sahoo, J. (2015). Development of chitosan based edible films:
642 process optimization using response surface methodology. *Journal of food science and*
643 *technology*, 52(5), 2530-2543.
- 644 Soradech, S., Limatvapirat, S., & Luangtana-anan, M. (2013). Stability enhancement of shellac by
645 formation of composite film: Effect of gelatin and plasticizers. *Journal of Food*
646 *Engineering*, 116(2), 572-580.
- 647 Srinivasa, P., Ramesh, M., & Tharanathan, R. (2007). Effect of plasticizers and fatty acids on
648 mechanical and permeability characteristics of chitosan films. *Food Hydrocolloids*, 21(7),
649 1113-1122.
- 650 Stummer, S., Salar-Behzadi, S., Unger, F. M., Oelzant, S., Penning, M., & Viernstein, H. (2010).
651 Application of shellac for the development of probiotic formulations. *Food Research*
652 *International*, 43(5), 1312-1320.
- 653 Sun, X., Wang, Z., Kadouh, H., & Zhou, K. (2014). The antimicrobial, mechanical, physical and
654 structural properties of chitosan-gallic acid films. *LWT-Food Science and Technology*,
655 57(1), 83-89.
- 656 Thakhiew, W., Devahastin, S., & Soponronnarit, S. (2010). Effects of drying methods and
657 plasticizer concentration on some physical and mechanical properties of edible chitosan
658 films. *Journal of Food Engineering*, 99(2), 216-224.
- 659 Thakur, R., Saberi, B., Pristijono, P., Golding, J., Stathopoulos, C., Scarlett, C., Bowyer, M., &
660 Vuong, Q. (2016). Characterization of rice starch- κ -carrageenan biodegradable edible film.
661 Effect of stearic acid on the film properties. *International journal of biological*
662 *macromolecules*, 93, 952-960.
- 663 The, D. P., Debeaufort, F., Voilley, A., & Luu, D. (2009). Influence of hydrocolloid nature on the
664 structure and functional properties of emulsified edible films. *Food Hydrocolloids*, 23(3),
665 691-699.
- 666 Tongnuanchan, P., Benjakul, S., & Prodpran, T. (2014). Structural, morphological and thermal
667 behaviour characterisations of fish gelatin film incorporated with basil and citronella
668 essential oils as affected by surfactants. *Food Hydrocolloids*, 41, 33-43.
- 669 Vargas, M., Albors, A., Chiralt, A., & González-Martínez, C. (2009). Characterization of
670 chitosan-oleic acid composite films. *Food Hydrocolloids*, 23(2), 536-547.
- 671 Villalobos, R., Chanona, J., Hernández, P., Gutiérrez, G., & Chiralt, A. (2005). Gloss and
672 transparency of hydroxypropyl methylcellulose films containing surfactants as affected by
673 their microstructure. *Food Hydrocolloids*, 19(1), 53-61.
- 674 Villalobos, R., Hernández-Muñoz, P., & Chiralt, A. (2006). Effect of surfactants on water sorption
675 and barrier properties of hydroxypropyl methylcellulose films. *Food Hydrocolloids*, 20(4),
676 502-509.
- 677 Wang, Z., Zhou, J., Wang, X. X., Zhang, N., Sun, X. X., & Ma, Z. S. (2014). The effects of
678 ultrasonic/microwave assisted treatment on the water vapor barrier properties of soybean
679 protein isolate-based oleic acid/stearic acid blend edible films. *Food Hydrocolloids*, 35,
680 51-58.
- 681 Yang, L., & Paulson, A. (2000). Effects of lipids on mechanical and moisture barrier properties of
682 edible gellan film. *Food Research International*, 33(7), 571-578.
- 683 Ziani, K., Oses, J., Coma, V., & Maté, J. I. (2008). Effect of the presence of glycerol and Tween
684 20 on the chemical and physical properties of films based on chitosan with different degree
685 of deacetylation. *LWT-Food Science and Technology*, 41(10), 2159-2165.

686

687 **Figure captions**

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

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

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

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

696

697

698

699

700

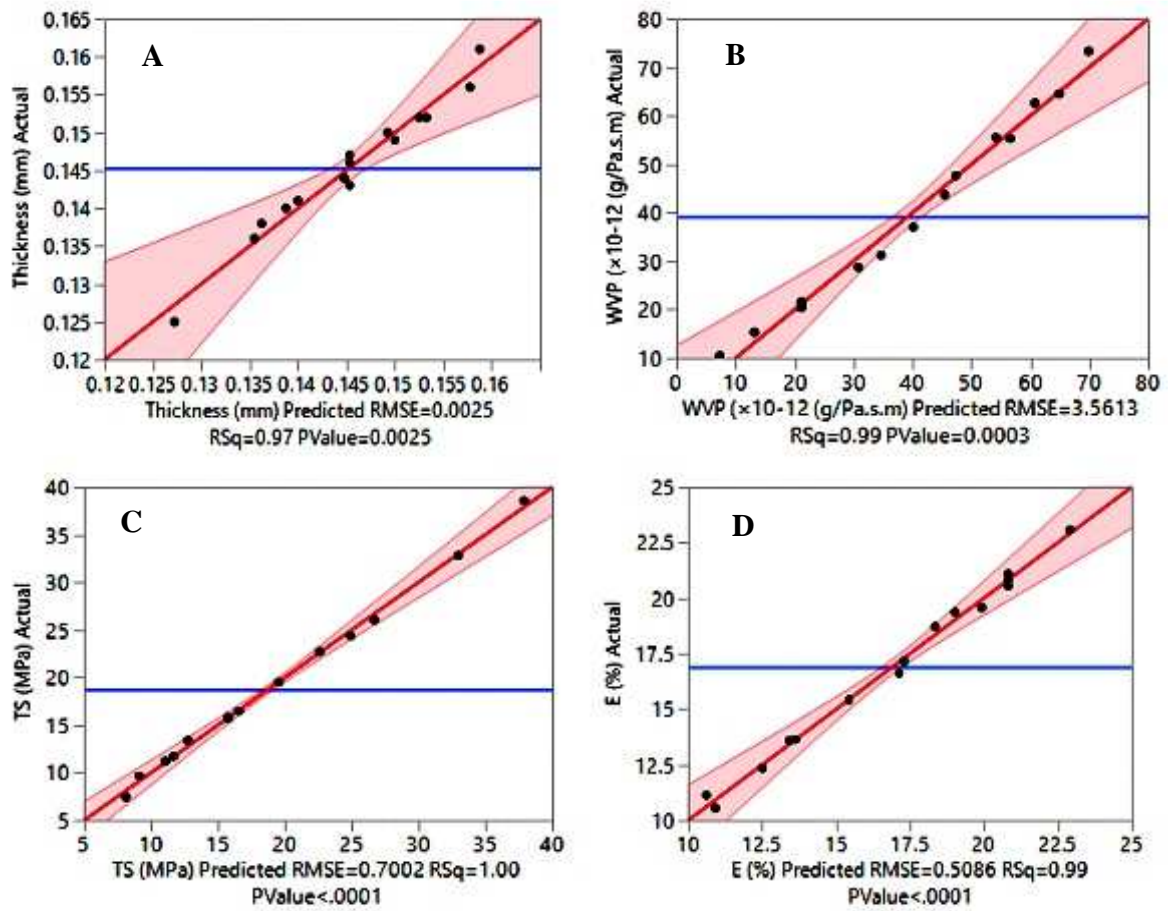
701

702

703

704

705



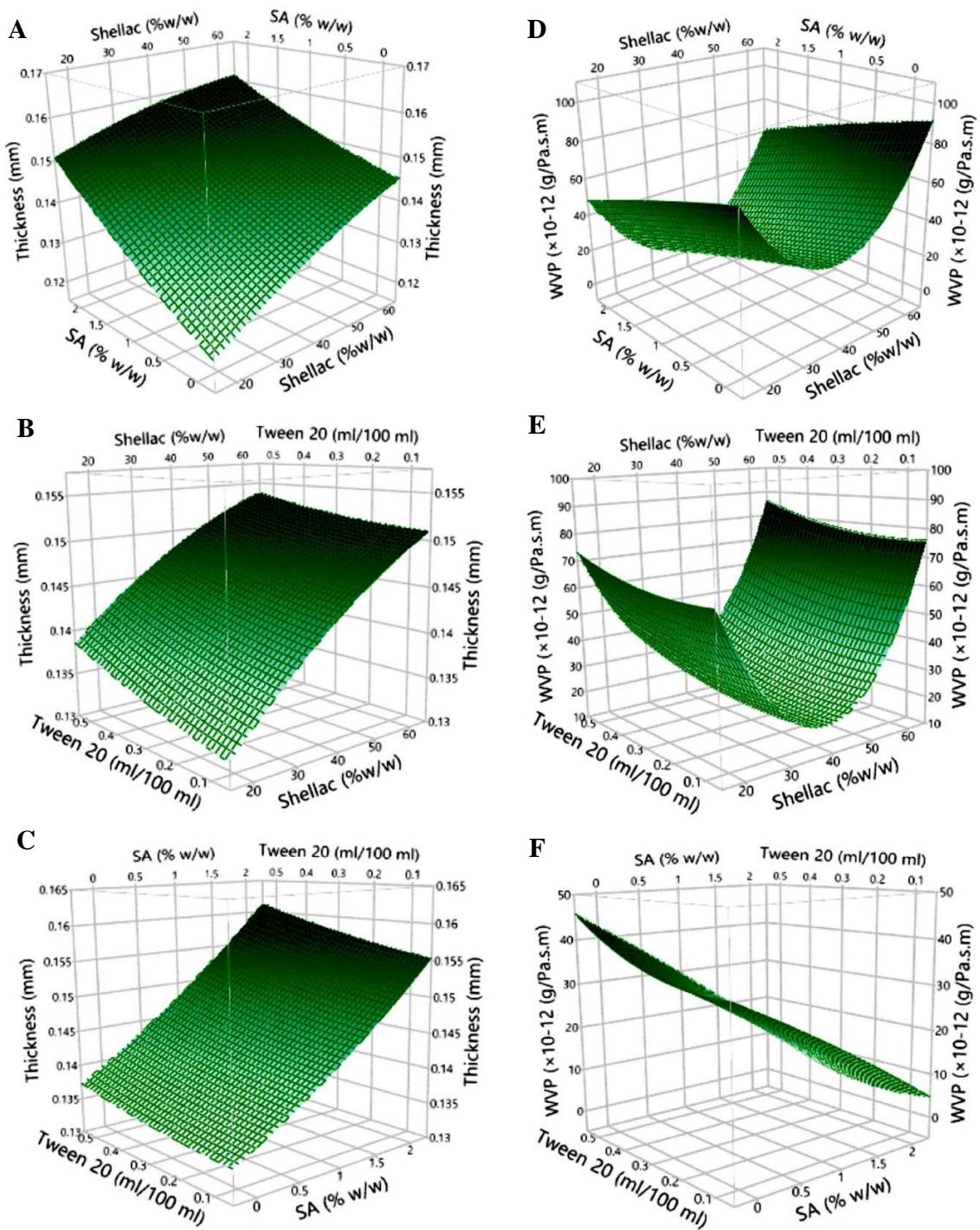
706

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

709

710

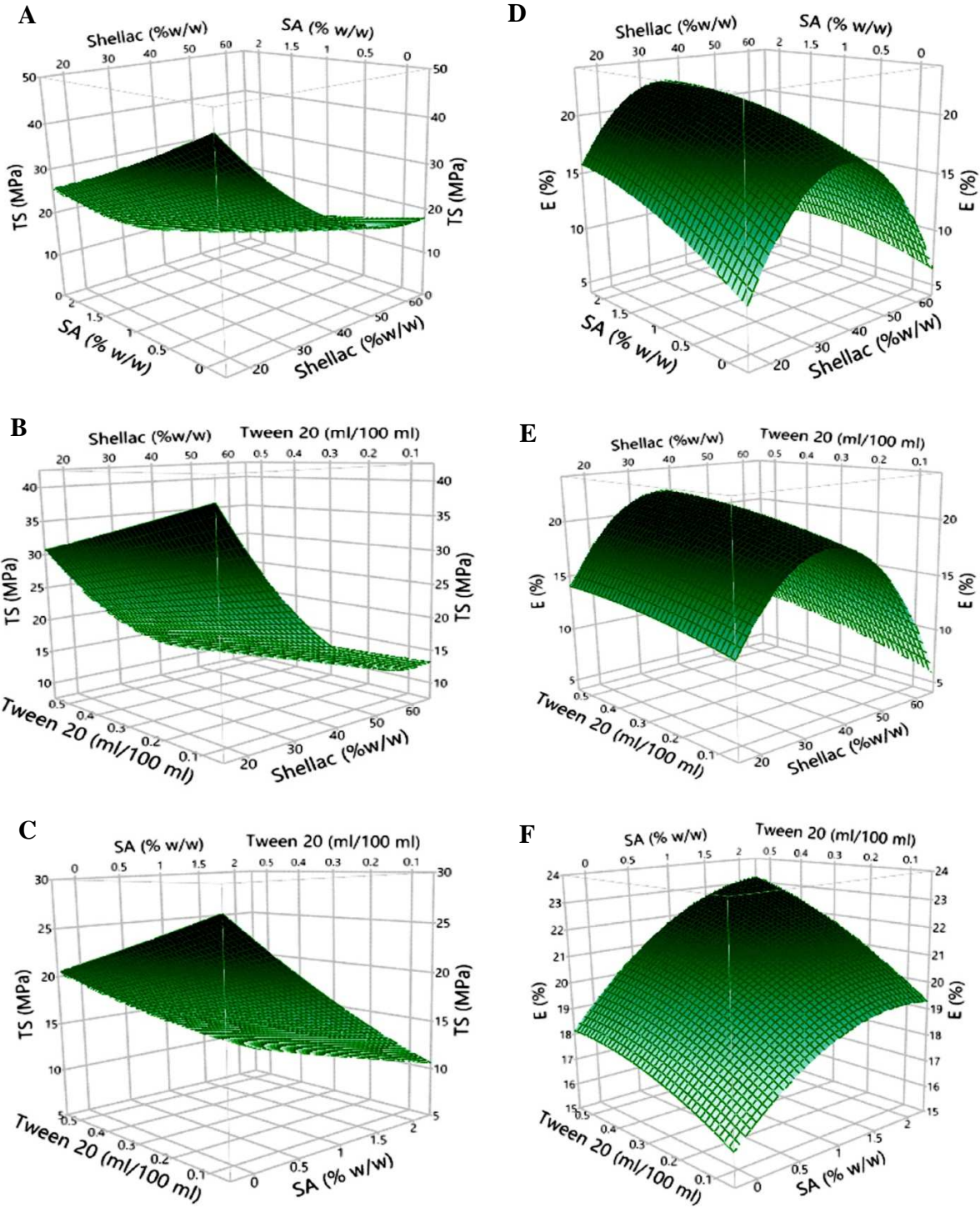
711



712

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

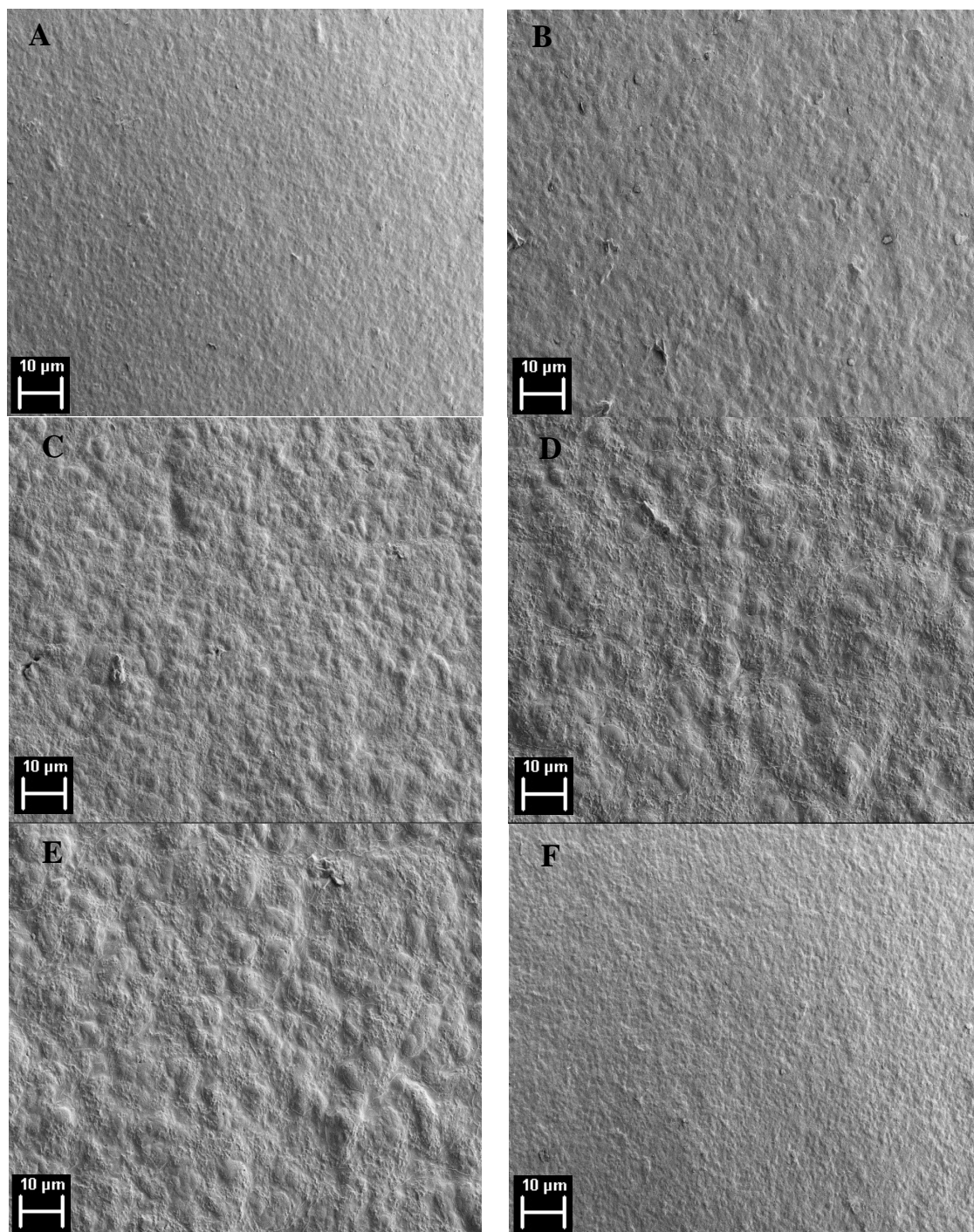
714 (A-C) and WVP (D-F).



715

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

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



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