

# Accepted Manuscript

Vanillin as a natural cross-linking agent in chitosan-based films: Optimizing formulation by response surface methodology

B. Tomadoni, A. Ponce, M. Pereda, M.R. Ansorena

PII: S0142-9418(19)30294-6

DOI: <https://doi.org/10.1016/j.polymeresting.2019.105935>

Article Number: 105935

Reference: POTE 105935

To appear in: *Polymer Testing*

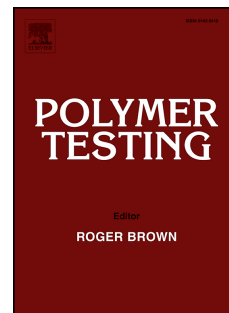
Received Date: 14 February 2019

Revised Date: 26 May 2019

Accepted Date: 15 June 2019

Please cite this article as: B. Tomadoni, A. Ponce, M. Pereda, M.R. Ansorena, Vanillin as a natural cross-linking agent in chitosan-based films: Optimizing formulation by response surface methodology, *Polymer Testing* (2019), doi: <https://doi.org/10.1016/j.polymeresting.2019.105935>.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.



1           **Vanillin as a natural cross-linking agent in chitosan-based films:**  
2           **optimizing formulation by response surface methodology**

3  
4                           Tomadoni B.<sup>1\*</sup>, Ponce A.<sup>2</sup>, Pereda M.<sup>1</sup>, Ansorena M.R.<sup>2</sup>

5  
6           <sup>1</sup>Instituto de Investigación en Ciencia y Tecnología de Materiales (Universidad Nacional  
7           de Mar del Plata - CONICET) Av. Colón 10850 - 7600 Mar del Plata -Argentina

8           <sup>2</sup>Grupo de Investigación en Ingeniería de Alimentos, (Universidad Nacional de Mar del  
9           Plata - CONICET) Av. Juan B. Justo 4302 - 7600 Mar del Plata -Argentina.

10  
11  
12           \*Corresponding author: Tel.: +54 223 626 0627; fax: +54 223 4810046.

13           E-mail: [bmtomadoni@fi.mdp.edu.ar](mailto:bmtomadoni@fi.mdp.edu.ar); [bmtomadoni@hotmail.com](mailto:bmtomadoni@hotmail.com)

14  
15           **Abstract**

16  
17           One way of improving mechanical and water barrier properties of chitosan films is through  
18           cross-linking reactions. Cross-linking agents usually used for this purpose include  
19           glutaraldehyde, glyoxal, and formaldehyde, which may cause some undesirable side  
20           effects and are consider health hazards. Hence, the objective of this work was to optimize  
21           the formulation of chitosan (CH) films with vanillin as a natural antioxidant cross-linking  
22           agent, to simultaneously improve their physical, barrier, antioxidant and mechanical  
23           properties. Three parameters were studied at three levels: vanillin content (0, 25 and 50%  
24           w/w chitosan), glycerol content (30, 45 and 60% w/w chitosan) and drying temperature

25 (35, 50 and 65°C). Response surface methodology (RSM) and desirability function (D)  
26 were used to find the levels of each factor that simultaneously optimize different properties  
27 of the films: opacity value, total soluble matter (TSM), Young modulus (YM),  $T_0$  and  $T_{max}$   
28 parameters (obtained from thermogravimetric analysis), water vapor permeability (WVP),  
29 total phenolic content (TPC) and antioxidant capacity by DPPH method. Fifteen  
30 formulations combining the studied factors were prepared according to a Box-Behnken  
31 design. An optimal chitosan film formulation was found, with vanillin content of 37.5%  
32 (w/w of CH), glycerol content of 45% (w/w of CH) and drying temperature of 57.5°C.  
33 RSM approach using the desirability function was found to be an effective tool to  
34 investigate changes in films properties and to search for the optimal active chitosan film  
35 formulation with a natural cross-linking agent, such as vanillin.

36  
37 **Keywords** *Chitosan films; Response surface methodology; Vanillin; antioxidant film.*

## 38 39 **1. INTRODUCTION**

40  
41 Recently, with the increasing concern on environmental issues, different authors have  
42 focused their attention on the development of natural innovative and active materials for  
43 food packaging applications [1], such as biopolymer formulations based on agricultural  
44 wastes [2] and active packaging as carrier of antimicrobial and antioxidant compounds [3–  
45 7]. Among polysaccharides, chitosan (CH) has been widely studied due to its excellent  
46 film-forming nature [8], antimicrobial properties, biocompatibility and biodegradability  
47 [6,9]. However, comparing biodegradable materials properties (such as CH) with non-

48 biodegradable petrochemical-based plastics, biopolymers have relatively poor mechanical  
49 and barrier properties, which limits their use in the food packaging industry [10,11].

50 One promising way to overcome the poor mechanical and water barrier properties of  
51 hydrocolloid films is to modify their physical properties through chemical, enzymatic, or  
52 physical treatments that induce both inter- and intra-molecular chemical bonding [12].  
53 These treatments basically modify the polymer network through cross-linking of the  
54 polymer chains, i.e. improving the film functionality. Different cross-linking methods and  
55 agents are used depending on the biopolymer and on the extent of improvement in desired  
56 properties. Particularly, chemical cross-linking involves the use of chemical agents (such  
57 as glutaraldehyde [13], glyoxal [14], formaldehyde [15], and polyaspartic acid and its  
58 sodium salt [16]). Nevertheless, these agents may show some undesirable side effects and  
59 could be considered as health hazards. Thus, replacing them with natural non-toxic cross-  
60 linking agents is being encouraged.

61 Therefore, in recent years more attention has been paid on more green and natural cross-  
62 linking agents, such as protein, starch and plant extracts (polyphenols and aldehyde  
63 compounds) [17]. Among these, vanillin, which exists in sugar beet and in vanilla pods, is  
64 one of the most popular flavoring agents and it is widely used in several fields such as  
65 food, beverages, perfumery and pharmaceutical industries [18,19]. Due to its aldehyde  
66 group, vanillin has been identified as a bio-based cross-linking agent. It is an attractive  
67 bio-based monomer, and it has been successfully incorporated into novel polymers with a  
68 wide variety of practical applications [20]. Until now, few studies have been found where  
69 vanillin is applied for enhancement of chitosan film properties [21].

70 On the other hand, incorporation of antioxidant compounds in the biomaterial is an  
71 interesting approach to improve the antioxidant properties of the polymers [22]. Natural

72 antioxidants (mainly vitamins and polyphenols extracted from plants and fruits) such as  
73 vanillin, are being preferred over synthetic ones due to the possible harmful health effects  
74 of the latter ones [23]. For example, rosemary antioxidant extracts have been incorporated  
75 into cassava starch films [24], propolis incorporated into chitosan films [25], different  
76 essential oils incorporated into polysaccharide and protein films [26,27], and some fruit  
77 extracts [28,29] have also been studied to develop antioxidant active food packaging.  
78 Together with microbiological growth, the oxidative processes are one of the main causes  
79 of food quality deterioration [30,31]; i.e. oxidative processes are responsible for texture  
80 modifications, loss of nutritional value, development of undesirable compounds such as  
81 off-flavors, colored and even toxic substances to humans, among others [32]. Therefore,  
82 ~~antioxidant~~ active packaging [33] as carrier of natural antioxidant compounds, such as  
83 vanillin, is being highlighted as a new mechanism to prevent food oxidation [22,34–36].  
84 Most polysaccharide-based films (e.g. chitosan) are naturally brittle, and usually a  
85 plasticizer needs to be incorporated to solve this problem [37]. Plasticizers are compounds  
86 with low molecular weight that are incorporated into the film forming solution to increase  
87 the flexibility and extensibility of the material. However, its permeability also increases  
88 with higher plasticizer content. Therefore, the concentration of plasticizer in the film  
89 forming solution is a parameter that needs to be optimized. The most commonly used  
90 plasticizer for improving edible films properties is glycerol [38].  
91 Besides its formulation, some process parameters can also significantly affect the  
92 properties of the films. For example, drying temperature can affect the physical and  
93 mechanical properties [38]. When preparing films with higher drying temperature, the  
94 resistance and stiffness of the material can be enhanced [39]. However, especially for the  
95 development of antioxidant films, higher drying temperature can reduce the antioxidant

96 properties of the final material. Mayachiew and Devahastin [40] when studying chitosan-  
97 based edible films incorporated with Indian gooseberry extract stated that high drying  
98 temperatures induced degradation of phenolic compounds leading to a lower residual  
99 phenolic content on the films.

100 Hence, this work aimed at optimizing the formulation of edible chitosan-based films  
101 obtained by casting containing vanillin as a natural antioxidant cross-linking agent. The  
102 studied factors were: vanillin content, plasticizer content (glycerol) and drying  
103 temperature. Physical, antioxidant, barrier and mechanical properties were simultaneously  
104 improved by response surface methodology (RSM) and desirability function.

105

## 106 **2. MATERIALS AND METHODS**

107

### 108 **2.1. Materials**

109 Chitosan (CH) (deacetylation degree 90%,  $M_v = 1.61 \times 10^5$  g/mol) was supplied by  
110 PARAFARM, Mar del Plata, Argentina. Glycerol (Gly) was used as plasticizer and  
111 purchased from Biopack (Argentina). Vanillin (V) was incorporated as a natural  
112 antioxidant cross-linking agent purchased from Sigma Aldrich (> 97%; St. Louis, MO,  
113 USA).

114

### 115 **2.2. Experimental design**

116 Three parameters or explanatory factors were chosen based on previous screening  
117 experiments: vanillin content ( $x_1$ , % w/w of CH), glycerol content ( $x_2$ , % w/w of CH) and  
118 drying temperature ( $x_3$ , °C). In order to analyze the effects of these factors on the physical,  
119 barrier, mechanical and antioxidant properties of the chitosan films, Response Surface

120 Methodology (RSM) with a Box–Behnken (BB) design [41] was used. RSM is a statistical  
 121 tool that allows to evaluate the effects of many factors and their interactions on response  
 122 variables. This method has the advantage of reducing the number of experimental runs  
 123 needed to evaluate multiple variables and the ability to identify interactions between them  
 124 [42].

125 For a 3-level–3-factor BB experimental design with three replicates at the central point, a  
 126 total of 15 experimental runs were needed [43], in which each variable was tested at three  
 127 different coded levels: low (−1), middle (0) and high (+1). Table 1 shows the experimental  
 128 design in the coded ( $X_i$ ) and actual ( $x_i$ ) levels of the independent variables.

129

130 **Table 1.** Box-Behnken experimental design matrix.

Exp. Run	Independent variables			Coded variables		
	$x_1$ (V) (% w/w CH)	$x_2$ (Gly) (% w/w CH)	$x_3$ ( $T_d$ ) (°C)	$X_1$	$X_2$	$X_3$
1	0	30	50	-1	-1	0
2	0	60	50	-1	1	0
3	50	30	50	1	-1	0
4	50	60	50	1	1	0
5	25	30	35	0	-1	-1
6	25	30	65	0	-1	1
7	25	60	35	0	1	-1
8	25	60	65	0	1	1
9	0	45	35	-1	0	-1
10	50	45	35	1	0	-1
11	0	45	65	-1	0	1
12	50	45	65	1	0	1
13	25	45	50	0	0	0
14	25	45	50	0	0	0
15	25	45	50	0	0	0

V: vanillin content; Gly: glycerol content;  $T_d$ : drying temperature.

131

132

133 The chosen response variables were opacity, total soluble matter (TSM), Young modulus  
 134 (YM),  $T_0$  and  $T_{\max}$  parameters (obtained from thermogravimetric analysis), water vapor  
 135 permeability (WVP), total polyphenol content (TPC) and antioxidant capacity of the films  
 136 evaluated by DPPH method. A second-degree polynomial (eq. 1) was used to fit the  
 137 measured responses to the coded variables.

$$138 \quad Y_n = \beta_0 + \sum_{i=1}^3 \beta_i X_i + \sum_{i=1}^2 \sum_{j=2, j>i}^3 \beta_{ij} X_i X_j + \sum_{i=1}^3 \beta_{ii} X_i^2 \quad (1)$$

139 where,

140  $Y_n$  is the predicted response,

141  $\beta_0$  is the model constant,

142  $\beta_i$  is the linear coefficient,

143  $\beta_{ii}$  is the quadratic coefficient,

144  $\beta_{ij}$  is the coefficient for the interaction effect, and

145  $X_i$  is a dimensionless coded value of the independent variable  $x_i$ .

146

### 147 2.3. Film preparation

148 Chitosan film-forming solutions (2%, w/v) were prepared by dissolving chitosan powder  
 149 in acetic acid solution (1% v/v) at room temperature ( $23 \pm 2^\circ\text{C}$ ), according to Pereda et al.  
 150 [44]. Appropriate amounts of glycerol (Gly) were added to each sample according to the  
 151 BB design. Once the solutions were homogeneous, vanillin (V) was incorporated as a  
 152 natural antioxidant cross-linking agent in the corresponding proportions (Table 1) and  
 153 dissolved using a magnetic stirrer. Films were prepared by the casting technique, that is, 15  
 154 g of the film-forming solutions were poured into each Teflon Petri dish (diameter = 9 cm)  
 155 and dried at the different drying temperatures according to the BB design, for



156 approximately 24 h in a convection oven (30% RH average). After the excess of water was  
157 evaporated, the obtained films were peeled off from the plates and kept in a closed  
158 reservoir at constant relative humidity (50% RH) and temperature ( $23 \pm 2^\circ\text{C}$ ) for 3 days.  
159 The films were further characterized and tested.

160

#### 161 **2.4. Fourier transform infrared spectroscopy (FTIR)**

162 A Thermo Scientific Nicolet 6700 spectrometer (Wisconsin, EEUU) was used to record  
163 Spectra. 32 scans with resolution of  $4\text{ cm}^{-1}$  were performed between  $400$  and  $4000\text{ cm}^{-1}$  on  
164 every run, using an attenuated total reflection (ATR) accessory with a diamond ATR  
165 crystal.

166

#### 167 **2.5. Film thickness**

168 Film thickness was measured with a 3400-25 Insize 0–25 mm manual micrometer  
169 (Germany) with an accuracy of  $\pm 0.01$  mm. Four random locations were measured for each  
170 film.

171

#### 172 **2.6. Response variables**

173 The effects of vanillin content, glycerol content and drying temperature on chitosan-based  
174 films were simultaneously evaluated through several parameters associated with physical,  
175 barrier, mechanical and antioxidant properties.

176

##### 177 **2.6.1. Film opacity**

178 Film opacity was determined according to the method described by Irissin-Mangata et al.  
179 [45] on rectangular strips directly placed in a UV-Visible spectrophotometer test cell. A

180 UV-Visible spectrophotometer Shimadzu 1601 PC (Tokyo, Japan) was used to obtain the  
181 absorption spectrum of the sample from 400 to 800 nm. Film opacity was defined as the  
182 area under the curve divided by film thickness and expressed as Absorbance Units  $\times$   
183 nm/mm (AU nm/mm). Three measures were performed for each sample.

184

### 185 **2.6.2. Total soluble matter (TSM)**

186 Total soluble matter (TSM) was expressed as the film dry mass percentage dissolved after  
187 24 h immersion in distilled water. TSM measurements were carried out according to the  
188 “wet” method [46], using 30 mL of distilled water with 0.02% of sodium azide to prevent  
189 any possible microbial growth.

190 Three specimens of each film were weighed and then directly immersed in distilled water  
191 under the previously described conditions. After 24 h immersion, samples were oven dried  
192 (105°C, 24 h), to determine the dried remnant insoluble mass ( $m_f$ ). Initial dry mass values  
193 ( $m_0$ ) needed for the TSM calculations were obtained from different specimens cut out from  
194 the same film and oven dried at 105°C for 24 h. TSM was then calculated with eq. 2:

$$195 \quad TSM = \frac{m_0 - m_f}{m_0} 100\% \quad (2)$$

196

### 197 **2.6.3. Mechanical properties: Young modulus (YM)**

198 Prior to running mechanical tests, films were conditioned for 48 h at constant humidity and  
199 temperature ( $50 \pm 5\%$  RH, 25°C). An Instron Universal Testing Machine model 8501  
200 (USA) was used to perform tensile tests at room temperature ( $23 \pm 2^\circ\text{C}$ ). Specimens were  
201 cut according to the ASTM D1708-13 [47]. Five specimens from each film were tested  
202 from a minimum of three films per sample. Crosshead speed was set at 10 mm per min.  
203 Young modulus (YM) was calculated as thoroughly described in ASTM D638-14 [48].

204

205 **2.6.4. Thermal properties: thermogravimetric analysis (TGA)**

206 TGA measurements were carried out on a Shimadzu TGA-50 thermogravimetric analyzer.  
207 Thermal degradation was performed under a nitrogen atmosphere up to 600°C with a  
208 heating ramp of 10°C per min. Samples of 4–10 mg were used. For the determination of  
209 weight loss of the films, initial weight values were taken after moisture evaporation (i.e.  
210 the sample weight at 105°C); hence, comparing only thermal degradation of the films,  
211 independently of their moisture contents.

212

213 **2.6.5. Barrier properties: Water vapor permeability (WVP)**

214 Water vapor transfer rate (WVTR,  $\text{g}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ ) through films was determined  
215 gravimetrically using the ASTM Method E96M-16 [49]. Before testing, films were placed  
216 for 3 days in a chamber at room temperature ( $23 \pm 2$  °C) and humidity of  $63 \pm 2\%$  RH, to  
217 guarantee equilibrium conditions. Then, film specimens were sealed on acrylic permeation  
218 cups (diameter = 5 cm) containing distilled water (100% RH). The cups were weighed  
219 every hour over a 6 h period. Inside the chamber, a fan was used to move the internal air,  
220 hence, ensuring uniform conditions at the entire test location. Linear regression was used  
221 to fit the data, weight vs. time, and to calculate the slope of the resulting straight line in  
222  $\text{g}\cdot\text{s}^{-1}$ . WVP of the films, expressed as  $\text{g}\cdot\text{m}/(\text{Pa}\cdot\text{s}\cdot\text{m}^2)$ , was calculated according to equations  
223 3 and 4. Four specimens were tested for each film type.

224

225 
$$WVP = WVTR \cdot (p_2 - p_1)^{-1} \quad (3)$$

226 
$$WVTR = \Delta W (A \Delta t)^{-1} \quad (4)$$

227

228 where,  
229  $WVTR$  is the water vapor transmission rate,  
230  $y$  is the film thickness,  
231  $(p_2 - p_1)$  is the vapor pressure difference across the film (calculated based on the  
232 chamber temperature and RH inside and outside the cup),  
233  $\Delta W$  is the weight of water absorbed in the cup,  
234  $A$  is the exposed area of the film, and  
235  $\Delta t$  is the time for weight change.

236

### 237 **2.6.6. Antioxidant properties**

238 Polyphenol and antioxidant extraction was performed by a shaker holding 0.5 g of each  
239 film in flasks filled with 20 mL of methanol at room temperature for 24 h in dark  
240 conditions. Afterwards, these flasks were sonicated in an ultrasound chamber (PS-30A,  
241 RoHs, China) during 20 min at room temperature [50]. The final extract was stored at -20  
242 °C to be used in the determination of total phenolic content (TPC) and antioxidant activity  
243 by DPPH method.

244

#### 245 **2.6.6.1. Total phenolic content (TPC)**

246 Total phenolic content (TPC) was determined using the Folin-Ciocalteu reagent (FCR)  
247 according to the methodology proposed by Viacava et al. [51] with modifications. Extract  
248 samples (0.2 mL) were added to 1 mL of 1:10 FCR. After 3 min of incubation at room  
249 temperature, 0.8 mL of sodium carbonate solution ( $\text{Na}_2\text{CO}_3$ , 7.5% w/v) was added and the  
250 reaction mixture was stirred and incubated for 2 h at the same temperature. The absorbance  
251 was measured at 765 nm using a UV 1601 PC UV-visible spectrophotometer (Shimadzu

252 Corporation, Japan). TPC was calculated using a standard curve of gallic acid and  
253 expressed as mg gallic acid equivalents (GAE) per gram of film.

254

#### 255 2.6.6.2. Antioxidant capacity: DPPH radical scavenging method

256 Antioxidant activity was measured using the DPPH (2,2-Diphenyl-1-picrylhydrazyl)  
257 radical, with the methodology previously described by Viacava et al. [51]. Briefly, 0.25  
258 mL of sample extract or ethanol (blank) was mixed with 1 mL of an ethanolic DPPH  
259 solution (100  $\mu\text{mol L}^{-1}$ ). The mixtures were immediately shaken and allowed to stand for  
260 60 min at refrigeration temperature (2 °C) in the dark. After that, the decrease in  
261 absorbance at 517 nm was measured in a spectrophotometer (FLUOstar Omega). DPPH  
262 radical scavenging activity was expressed as percentage of radical scavenging capacity  
263 (%In) calculated according to the following formula:

$$264 \quad \%In = \frac{A_0 - A_s}{A_0} \times 100 \quad (5)$$

265 where,

266 %In is the percentage of DPPH radical inhibition;

267  $A_0$  is the absorbance of the blank sample; and

268  $A_s$  is the absorbance of the sample.

269 Measurements were performed in triplicate for each treatment.

270

### 271 2.7. Simultaneous optimization

272 During optimization, several response variables describing the quality characteristics are  
273 usually to be optimized. Some of these variables were to be maximized while some were

274 to be minimized. In many cases, these responses were competing (i.e. improving one  
275 response may have an opposite effect on another one, which further complicates the  
276 situation). In this work, the responses predicted by the models were optimized by means of  
277 the “desirability optimization methodology” [52]. The desirability function (D) approach is  
278 one of the most widely used methods for the optimization of several responses  
279 simultaneously.

280 The general approach is to first convert each response ( $y_n$ ) into an individual desirability  
281 function ( $d_n$ ). The desirability scale ranges from 0 to 1, where  $d = 0$  for an unacceptable  
282 response value, and  $d = 1$  for a completely desirable one. The individual desirability  
283 functions from the considered responses are then combined to obtain the overall  
284 desirability (D), defined as the geometric average of the individual desirability functions,  
285 also ranging from 0 to 1. An algorithm is then applied to this function to determine the set  
286 of values that maximizes D [53].

287

## 288 **2.8. Validation**

289 In order to test the reliability of the simultaneous optimization, a new set of experiments  
290 using optimal operating conditions obtained with the Desirability function was performed.  
291 The experimental and predicted values of the response variables were compared to  
292 determine the validity of the model.

293

## 294 **2.9. Statistical analysis**

295 SAS software (version 9.0, North Carolina, USA) was use for data analysis. Fit quality of  
296 the model was evaluated by  $R^2$  parameter and analysis of variance (ANOVA) using the  
297 Response Surface Regression (RSREG) procedure. Statistical testing of the model was

298 done by Fisher's statistical test. The robustness of the model was assessed by determination  
 299 coefficient ( $R^2$ ), correlation coefficient ( $R$ ), or F-test.

300 Statistica software (version 7.0, Stat Soft Inc., Tulsa, USA) was used to perform  
 301 simultaneous optimization, desirability functional analysis and 3D plots of the responses.

302

### 303 **3. RESULTS AND DISCUSSION**

304

#### 305 **3.1. FT-IR analysis**

306 Chemical structure of the films often has a close relationship with their behavior at a  
 307 macro scale. Hence, FT-IR analysis could explain the effects of the studied parameters on  
 308 the physicochemical and mechanical properties of the composite films. Table 2 portrays  
 309 the FTIR spectral details. The FTIR spectra of all 15 samples are shown in supplementary  
 310 material (Fig. S1).

311

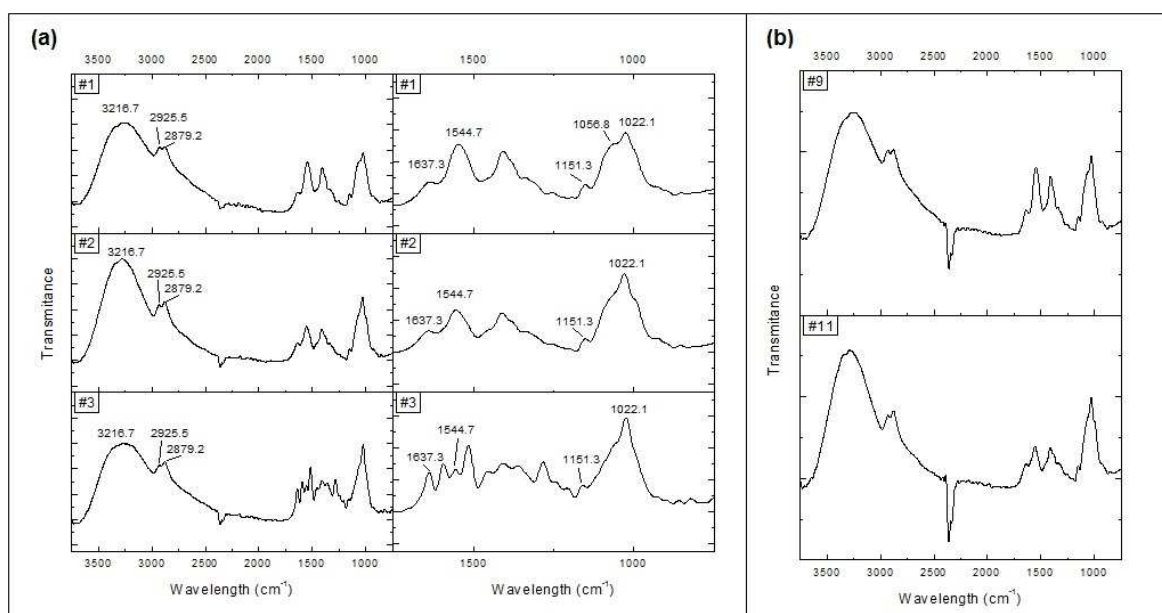
312 *Table 2. FT-IR spectral details.*

Wavelength ( $\text{cm}^{-1}$ )	Responsible functional group
3216.7	Intermolecular hydrogen bonded OH stretching, NH stretching in secondary amides
2925.5	Asymmetrical C-H stretching in $\text{CH}_2$ (aliphatic)
2879.2	symmetrical C-H stretching
1637.3	C=O stretching in secondary amides, and vibration of imine band (Schiff base)
1544.7	-NH bending in secondary amides
1151.3	Symmetrical CH bending in $\text{CH}_3$ (wagging)
1056.8, 1022.1	C-O stretching

313

314 In order to further discuss the interactions between chitosan and glycerol and between  
 315 chitosan and vanillin, FT-IR spectra of samples #1, #2 and #3 were compared (Fig. 1.a).

316



317

318 **Fig. 1:** FT-IR spectra: (a) samples #1, #2 and #3; (b) samples #9 and #11.

319

320 Samples #1 (0% V, 50°C and 30% Gly) and #2 (0% V, 50°C and 60% Gly) were  
 321 compared in order to analyze the effect of glycerol content on the chemical structure of the  
 322 films. The absorption peaks at  $1022.1\text{ cm}^{-1}$  and  $1056.8\text{ cm}^{-1}$  are associated with C-O  
 323 stretching. When glycerol content is increased from sample #1 to sample #2, these peaks  
 324 are joined to become a single peak, suggesting interactions between hydroxyl groups of  
 325 chitosan and glycerol by hydrogen bonding, as was previously described by Leceta et al.  
 326 [54].

327 With regards to chitosan-vanillin interaction, it can be seen when comparing samples #1  
 328 and #2 (CH films without vanillin) with sample #3 (CH film with 50% of vanillin) that the  
 329 band at  $1544.7\text{ cm}^{-1}$  (corresponding to deformation vibration of amino in pure chitosan  
 330 films) decreases its intensity in the composite film. Moreover, the vibration of imine band  
 331 has appeared at  $1637.3\text{ cm}^{-1}$  in the composite film (sample #3), in accordance with results



332 presented by Zhang et al. [21]. These findings support that there were interactions of  
333 vanillin with amino groups in chitosan matrix.

334 Regarding the effect of the drying temperature on the chemical structure of chitosan films,  
335 analysis was performed by comparing the spectra of samples #9 and #11 (Fig. 1.b), which  
336 formulations corresponded to 0% V, and 45% Gly, and drying temperatures of 35°C and  
337 65°C, respectively. Each peak was noted in the same wavelength position for both drying  
338 conditions, but with different intensities. The same result ~~result~~ was found by Jahit et al.  
339 [55] when studying the properties of gelatin/CMC/chitosan films as affected by drying  
340 temperature. It was found that the peaks at  $3216.7\text{ cm}^{-1}$  (which correspond to the  
341 intermolecular hydrogen bonded) were slightly sharper in samples dried at 65 °C  
342 compared to those dried at 35 °C (Fig. 1.b). Hence, higher drying temperature may induce  
343 higher intermolecular interactions. These results were in accordance with those found by  
344 Mayachiew & Devahastin [40], who studied different drying methods, and different drying  
345 conditions on the characteristics of chitosan films enriched with Indian Gooseberry extract.

346

### 347 **3.2. Model fitting**

348 Table 3 shows the mean values of the response variables found for each of the fifteen  
349 formulations of chitosan films. Second order polynomial equations coefficients were  
350 calculated with experimental data to obtain the significance of the model. Table 4 shows  
351 the regression coefficients for the second order polynomial equations together with the  
352 linear, quadratic and interaction terms, and also the correlation coefficients ( $R^2$ ).

353

*Table 3. Mean values of all responses of chitosan edible films with different formulations*

Exp. Run	Experimental responses							
	Opacity (AU nm/mm)	TSM (%)	YM (MPa)	T <sub>o</sub> (°C)	T <sub>max</sub> (°C)	WVP (g.m/Pa.s.m <sup>2</sup> 10 <sup>10</sup> )	TPC (mg GAE/g)	DPPH (inhibition %)
1	1215.2	27.41	355.6	128.9	153.8	4.01	1.65	7.12
2	1113.6	11.39	86.6	127.35	197.1	4.33	1.13	5.22
3	4618.3	27.25	1925.4	139.4	163.2	3.87	7.03	38.1
4	4449.7	30.91	1453.2	132.3	155.2	3.59	7.67	45.02
5	2962.6	26.41	413.0	126.0	161.1	4.07	3.25	25.09
6	3554.7	20.51	1945.7	142.1	171.6	2.96	2.94	10.23
7	2444.1	19.57	77.2	124.9	174.8	3.74	2.29	15.52
8	2671.0	20.98	1030.5	143.6	180.7	3.26	2.59	10.02
9	902.0	24.52	67.0	119.6	182.7	3.82	1.44	6.12
10	2970.9	27.56	1265.2	131.5	176.0	3.61	4.66	30.45
11	1357.1	23.56	361.1	134.7	198.8	3.00	1.45	1.03
12	3353.7	36.37	2188.7	145.7	178.3	3.44	5.95	20.55
13	2739.3	25.83	510.4	128.8	175.0	2.77	3.25	25.88
14	2460.8	21.86	855.8	134.4	197.0	2.92	3.32	22.10
15	2870.0	19.65	651.6	133.8	184.6	2.86	2.53	17.52

354

355

356

357

358

359

360

**Table 4.** Regression coefficients (from coded data) and  $R^2$  of the response surface models

Coefficients	Response variables							
	Opacity (UA nm/mm)	TSM (%)	YM (MPa)	T <sub>o</sub> (°C)	T <sub>max</sub> (°C)	WVP (g.m/Pa.s.m <sup>2</sup> 10 <sup>10</sup> )	TPC (mg GAE/g)	DPPH (inhibition %)
$\beta_0$ (intercept)	2690.1***	22.44***	672.6***	132.3***	185.5***	2.84***	3.03**	21.33**
$\beta_1$ (V)	1350.6***	4.40**	745.3***	4.8***	-7.5**	-0.082	2.45***	14.25***
$\beta_2$ (Gly)	-209.1	-2.34*	-249.1*	-1.1	7.3**	0.000	-0.15	-0.62
$\beta_3$ (T <sub>d</sub> )	207.1	0.41	462.9***	8.1***	4.3	-0.322**	0.16	-4.37
$\beta_{12}$ (V x Gly)	-16.8	4.92*	-50.8	-1.4	-12.8**	-0.150	0.29	2.25
$\beta_{13}$ (V x T <sub>d</sub> )	-18.1	2.44	157.4	-0.2	-3.4	0.160	0.32	-1.25
$\beta_{23}$ (Gly x T <sub>d</sub> )	-91.3	1.83	-144.8	0.6	-1.1	0.157	0.15	2.50
$\beta_{11}$ (V x V)	-301.5	3.96**	193.2	-0.8	-3.1	0.530**	0.97*	0.83
$\beta_{22}$ (Gly x Gly)	460.7	-2.17	89.3	0.5	-15.1**	0.567**	0.36	1.58
$\beta_{33}$ (T <sub>d</sub> x T <sub>d</sub> )	-242.6	1.59	104.6	1.5	1.6	0.087	-0.63	-7.92*
$R^2$	0.936	0.9045	0.959	0.948	0.907	0.939	0.948	0.928

V: vanillin content; Gly: glycerol content; T<sub>d</sub>: drying temperature.

Level of significance:  $p < ^* 0.05$ ;  $^{**} 0.01$ ;  $^{***} 0.001$

361

362

363

364 Predicted models were found to be significant ( $p < 0.01$ ) with high regression coefficients  
 365 ( $0.90 < R^2 < 0.96$ ) and not significant lack of fit ( $p > 0.1$ ). ANOVA results (Table 5)  
 366 indicated that all the independent variables exhibited a significant effect on the different  
 367 film properties. Vanillin concentration was a significant factor ( $p < 0.05$ ) on every  
 368 response, either by the linear term (opacity, YM,  $T_0$ ,  $T_{max}$ , DPPH), the quadratic term  
 369 (TSM, WVP) or both (TPC). Glycerol linear term significantly affected YM and  $T_{max}$ , and  
 370 had a quadratic effect on TSM,  $T_{max}$  and WVP. Drying temperature also significantly ( $p <$   
 371  $0.05$ ) affected TSM, E,  $T_0$  and WVP in linear terms, and in quadratic terms only DPPH.  
 372 The only significant interaction effect found ( $p < 0.05$ ) was between glycerol and vanillin,  
 373 which affected both TSM and  $T_{max}$  values.

374

375 **Table 5.** Results of the ANOVA for regression equation for opacity, TSM, E,  $T_0$ ,  $T_{max}$ , WVP, TPC  
 376 and DPPH.

Responses	Source	DF	F value	Pr > F
Opacity	Linear	3	22.33	0.0025
	Quadratic	3	2.11	0.2173
	Cross-product	3	0.05	0.9825
	Total model	9	8.16	0.0162
	Lack of fit	3	1.36	0.4505
TSM	Linear	3	7.47	0.0270
	Quadratic	3	3.31	0.1152
	Cross-product	3	7.23	0.0356
	Total model	9	8.26	0.0344
	Lack of fit	3	0.85	0.5792
YM	Linear	3	37.60	0.0007
	Quadratic	3	1.05	0.4486
	Cross-product	3	1.09	0.4330
	Total model	9	13.25	0.0055
	Lack of fit	3	2.59	0.2906
$T_0$	Linear	3	29.52	0.0013
	Quadratic	3	0.44	0.7343
	Cross-product	3	0.40	0.7588
	Total model	9	10.12	0.0101
	Lack of fit	3	0.74	0.6188
$T_{max}$	Linear	3	6.36	0.0369
	Quadratic	3	5.51	0.0483
	Cross-product	3	4.44	0.0711
	Total model	9	5.44	0.0284

	Lack of fit	3	0.06	0.9738
WVP	Linear	3	6.98	0.0309
	Quadratic	3	16.28	0.0052
	Cross-product	3	2.30	0.1946
	Total model	9	8.52	0.0148
	Lack of fit	3	7.74	0.2364
TPC	Linear	3	26.50	0.0017
	Quadratic	3	3.15	0.1243
	Cross-product	3	0.46	0.7218
	Total model	9	10.04	0.0103
	Lack of fit	3	4.70	0.1803
DPPH	Linear	3	18.33	0.0040
	Quadratic	3	2.63	0.1621
	Cross-product	3	0.53	0.6810
	Total model	9	7.16	0.0215
	Lack of fit	3	2.64	0.2868

DF: degrees of freedom

377

378

379

### 380 3.2.1. Influence of formulation on physical properties (opacity, TSM)

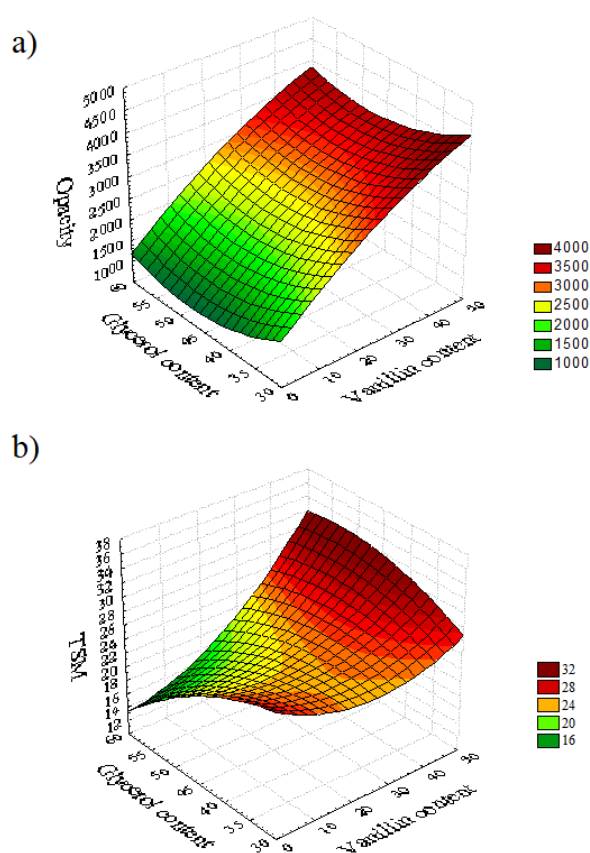
381 Optical properties such as the opacity and transparency of the material are essential to  
 382 define its potential as films and coatings to be applied over a food surface, since these  
 383 affect the appearance of the coated product, which is an important quality factor. In  
 384 general, transparent films are preferred, therefore, the opacity of films should be quite low  
 385 [56,57].

386 Results show that opacity of the film was only significantly affected ( $p < 0.001$ ) by  
 387 vanillin concentration. The linear and positive term of vanillin concentration resulted  
 388 significant in the model. The resulting response surface equation described the opacity of  
 389 the film perfectly ( $R^2 = 0.936$ ). Lack of fit was not significant ( $p = 0.4505$ ) showing that  
 390 the response surface equation adequately described the data (Table 5).

391 Canonical analysis shows that stationary point was a saddle point, suggesting that  
 392 movement away from this point would cause an increased or decreased response,  
 393 depending upon movement direction. Fig. 2.a presents the response surface showing the

394 combined effects of vanillin and glycerol concentrations on the opacity values of the  
395 chitosan films (with  $T_d$  kept constant at middle level). As it can be seen, the opacity of the  
396 films increased with increasing vanillin content, which could be explained by the cross-  
397 linking reaction. The aldehyde group of vanillin molecule with chitosan amine group can  
398 lead to a secondary aldimine, better known as a Schiff base, which is a well-described  
399 reaction [58], as previously seen in the FT-IR analysis.

400



401

402 **Fig. 2:** Response surface curves for physical properties of chitosan-based films. Variation of (a)  
403 opacity and (b) TSM with vanillin and glycerol concentrations. Drying temperature was kept  
404 constant at middle level (50°C).

405

406 In accordance with our findings, Zhang et al. [21] investigated chitosan films properties  
407 modified with vanillin. These authors found that vanillin concentration had a significant ( $p$   
408  $< 0.05$ ) effect on film color and transmittance. This phenomenon is due to the cross-linking  
409 reaction of vanillin with chitosan to form a network structure [21]. Higuera et al. [59]  
410 studied chitosan films with cinnamaldehyde and found a significant change in the optical  
411 properties of the films due to the presence of a conjugated double bond after Schiff base  
412 formation.

413 A simple way to confirm the development of interactions between the main constituents of  
414 our films is to determine their water-soluble fraction. The effect of both vanillin and  
415 glycerol concentrations on the total soluble matter (TSM) of the films is shown on Fig. 2.b.  
416 Both, linear and quadratic terms of vanillin were significant ( $p < 0.05$ ) in the model,  
417 showing an increment in TSM of the films with higher vanillin concentration. Glycerol  
418 linear term was also significant; however, the behavior of TSM with Gly was more  
419 complex with a significant interaction between vanillin and glycerol. Canonical analysis  
420 show that stationary point was a saddle point. Fig. 2.b represents TSM as a function of  
421 glycerol and vanillin content, holding drying temperature at a middle level ( $50^{\circ}\text{C}$ ), since  
422 this variable presented the least influence on this response. At lower vanillin concentration,  
423 an increment in glycerol content resulted in a decrease on the film solubility, as it can be  
424 seen in Fig. 2.b. This result could be related to the observations previously made at the  
425 FTIR section. In the absence of cross-linker, there might be interactions between the  
426 hydroxyl groups of chitosan and glycerol by hydrogen bonding, hence, reducing the  
427 solubility of the film in water [60,61]. On the contrary, as vanillin concentration increases,  
428 the plasticizer has an opposite effect on the film solubility: with higher glycerol content,

429 the TSM increases as well. With vanillin cross-linking chitosan, glycerol-water  
430 interactions may occur, increasing the solubility of the film.

431 Souza et al. [36] studied physical properties of chitosan films incorporated with natural  
432 antioxidants. These authors also reported higher water solubility and moisture in films  
433 incorporated with natural extracts in comparison with pure chitosan films due to the  
434 interaction of water, chitosan and polyphenols present on the studied extracts [36]. Even  
435 though higher water solubility may have positive effects, such as enhancing the film  
436 biodegradability, it would also limit the use of films in food matrices. The bioplastic tends  
437 to solubilize in the food (especially in products with high water content) making it difficult  
438 to separate the plastic from the food being packed [36].

439

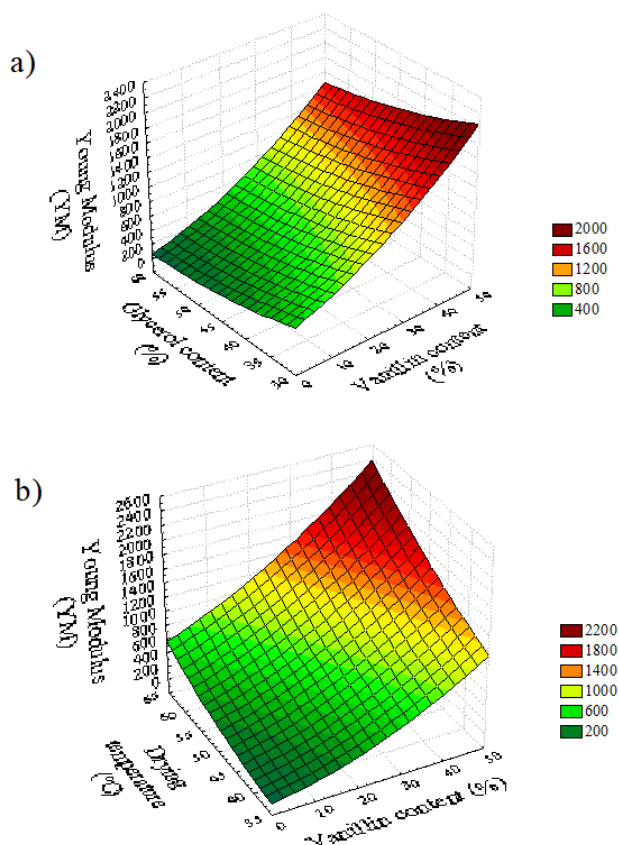
### 440 **3.2.2. Influence of formulation on mechanical properties (YM)**

441 Young modulus (YM) is a measure of the stiffness of the film. The regression analysis of  
442 the data showed that YM was significantly affected by the drying temperature, and vanillin  
443 and glycerol concentration ~~the concentration of vanillin and glycerol~~. The three studied  
444 variables significantly affected the mechanical properties in a linear way.

445 Canonical and stationary point analysis indicated that the stationary point was a point of  
446 minimum response predicting that the estimated conditions were inside the experimental  
447 design region. The relationship between YM and independent variables is depicted in Fig.  
448 3 (a and b).

449





450

451

452 **Fig. 3:** Response surface curves for mechanical properties of chitosan-based films. Variation of  
 453 Young modulus with (a) vanillin and glycerol concentrations, and (b) vanillin concentration and  
 454 drying temperature. The remaining variables were kept constant at middle level.

455

456 Mechanical properties of films are mainly associated with distribution and density of inter-  
 457 and intra-molecular interactions in the chitosan matrix [54].

458 Results showed that the addition of vanillin caused an increment in the young modulus of  
 459 the chitosan films (Fig. 3.a). Zhang et al. [21] also found similar results when studying  
 460 mechanical properties of chitosan films with vanillin. These results indicate that adding  
 461 vanillin, which acts as a cross-linking agent, enhances the structure of chitosan films by  
 462 forming a relatively dense network.

463 With regards to the plasticizer, YM decreased with glycerol content (Fig. 3.a), which could  
464 be induced by the interactions between chitosan and the plasticizer, as it was shown by the  
465 FT-IR study. Mechanical properties of the films can be mainly linked to the physico-  
466 chemical characteristic of the chitosan/glycerol interaction. These results were also found  
467 by Lavorgna et al. [62], who showed a decrease in the elastic modulus when glycerol was  
468 incorporated into chitosan-based films.

469 With regards to  $T_d$ , higher YM were achieved with higher drying temperatures (Fig. 3.b).  
470 In accordance with our findings, Jomlapeeratikul et al. [38] also studied the effect of  
471 drying temperature and glycerol content in Konjac flour films, and found that higher  
472 elastic modulus values (stiffer films) were obtained at higher drying temperatures.

473

### 474 **3.2.3. Influence of formulation on thermal properties (TGA: $T_0$ , $T_{max}$ )**

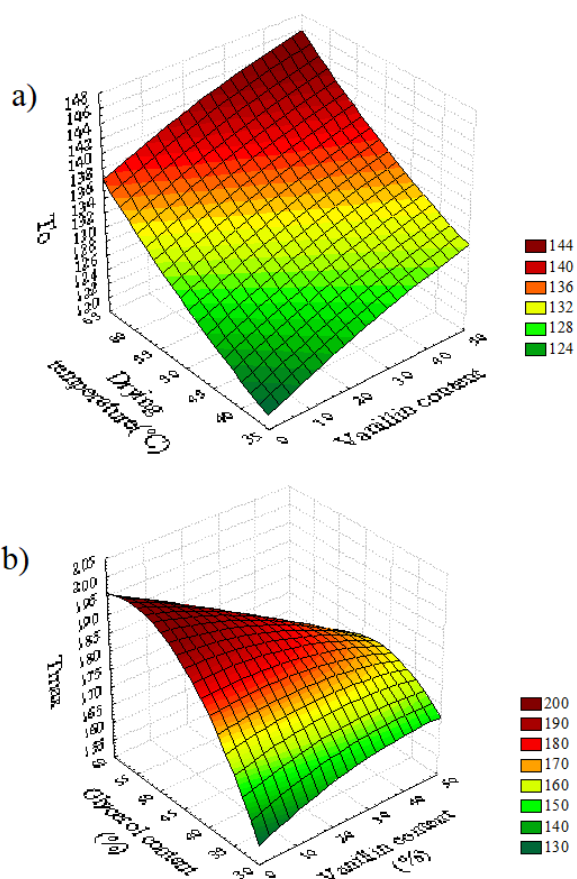
475 The thermal gravimetric analysis (TGA) was used to measure the thermal stability of the  
476 chitosan films. From the curves, two parameters ( $T_0$  and  $T_{max}$ ) were determined.  $T_0$  is  
477 defined as the initial decomposition temperature (IDT, also called onset decomposition  
478 temperature).  $T_0$  differed among samples and ranged between 119 and 146°C (Table 3).

479 TGA showed that the different chitosan films had two well-defined degradation stages (see  
480 Fig S.2 as an example in supplementary data). Region I is related to the loss of water  
481 bounded and absorbed on the polymer structure. The peak temperature (temperature of  
482 maximum rate of weight loss) in this region ( $T_{max}$ ) was detected for each sample from the  
483 first derivative of the weight loss curves (DTG), and ranged between 153 and 199°C  
484 (Table 3). Region II is related to the polymer decomposition. The decomposition peak  
485 temperatures in this region showed little fluctuations among the different samples (271-  
486 282°C).

487 The estimated values of the model coefficients describing the parameters effects on  $T_0$  and  
488  $T_{max}$ , together with their  $p$ -values are presented in Table 4.

489 The variance analysis revealed that  $T_0$  was significantly ( $p < 0.001$ ) affected by vanillin  
490 concentration and drying temperature. For both independent variables, linear and positive  
491 effects were observed, while glycerol exerted no significant effect. Therefore, Fig. 4.a  
492 represents  $T_0$  as a function of vanillin content and drying temperature, holding glycerol  
493 content at middle level (45% w/w of CH). It can be seen that with increasing vanillin  
494 content,  $T_0$  increases. This result shows that the incorporation of vanillin significantly  
495 increases thermal stability of chitosan matrixes, which could be explained by the reaction  
496 between vanillin and chitosan. In accordance with our findings, Kavianinia et al. [63]  
497 studied the effects of different cross-linking agents on thermal properties of chitosan-based  
498 films. These authors also found that the cross-linking of the chitosan matrix increases its  
499 thermal stability.

500



501

502 **Fig. 4:** Response surface curves for thermal properties of chitosan-based films. (a): Variation of  $T_0$   
 503 with vanillin concentration and drying temperature. (b): Variation of  $T_{max}$  with vanillin and  
 504 glycerol concentrations. The remaining variables were kept constant at middle level.

505

506 With regards to  $T_{max}$ , the variance analysis in Table 5 revealed that it was significantly  
 507 affected ( $p < 0.01$ ) by both vanillin and glycerol concentrations. No significant effect of  
 508 drying temperature was observed. Fig. 4.b. shows the effects of both vanillin and glycerol  
 509 content on  $T_{max}$  of the chitosan films. Both linear and quadratic effects of glycerol were  
 510 observed on  $T_{max}$ , and only linear effect was observed for vanillin concentration. The  
 511 interaction between vanillin and glycerol significantly affected ( $p < 0.01$ )  $T_{max}$  in a  
 512 negative manner.

513 As it can be seen in Fig. 4.b, at low glycerol concentrations the peak temperature slightly  
514 increases with increasing vanillin concentration. On the contrary, at higher glycerol  
515 content, the effect of vanillin on  $T_{max}$  was the opposite, with a drastic negative slope. This  
516 result indicates that the vanillin-chitosan matrix tends to retain less water, which in turn  
517 volatilizes more easily than it does in chitosan membranes, suggesting that the cross-linked  
518 chitosan films have lower affinity to water. This indicates the higher hydrophobic  
519 character of chitosan after crosslinking agent could increase the binding of water in the  
520 chitosan matrix. These results are in accordance with those found by Beppu et al. [13] who  
521 studied the effect of glutaraldehyde as a cross-linking agent in chitosan membranes.

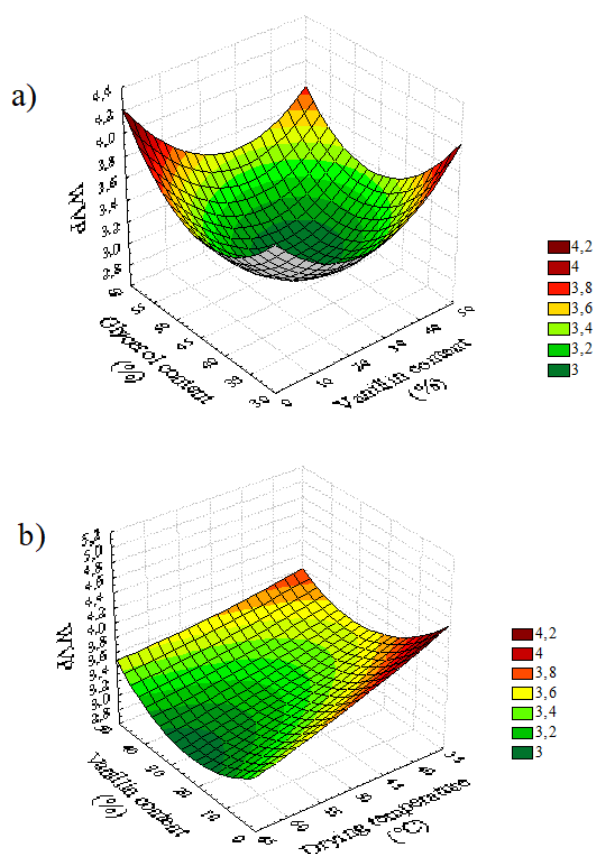
522

#### 523 ***3.2.4. Influence of formulation on barrier properties (WVP)***

524 WVP is considered a crucial property for films potentially used as food films and coatings,  
525 because most natural biopolymers are very prone to absorbing water [64]. One of the main  
526 functions of films is to separate the product from vapor in the surrounding atmosphere to  
527 prevent or retard food deterioration. Hence, to keep foods fresh, the WVP value should be  
528 maintained as low as possible [64].

529 The regression analysis of the data showed that WVP was significantly affected by all  
530 three tested parameters. None of the interactions were significant in the model. The  
531 relationship between WVP and independent variables is shown in Fig. 5 (a and b). A  
532 strong curvature of the surface is observed due to high significance of pure quadratic terms  
533 as shown through the variance analysis.

534



535

536 **Fig. 5:** Response surface curves for barrier properties of chitosan-based films. Variation of water  
 537 vapor permeability with (a) vanillin and glycerol concentrations, and (b) vanillin concentration  
 538 and drying temperature. The remaining variables were kept constant at middle level.

539

540 Vanillin concentration significantly ( $p < 0.01$ ) affected WVP in a quadratic manner. WVP  
 541 tends to decrease with increasing vanillin concentration presenting a minimum at 30%  
 542 vanillin, from which point WVP started to increase with increasing vanillin content.  
 543 Polyphenols such as vanillin, may establish the interactions with chitosan molecules  
 544 through the potential hydrogen bonding, which may limit the interactions between  
 545 hydrophilic groups of chitosan and water molecules due to the competitive binding effect  
 546 [65]; therefore, resulting in the lower WVP.

547 Glycerol concentration also affected WVP in a quadratic manner. At glycerol content less  
548 than 45%, WVP decreased with increasing glycerol concentration, while an increase in this  
549 index was observed with further increase in glycerol content. The results found in previous  
550 studies with respect to the effect of plasticizers in the WVP of films have also been  
551 opposed. The inclusion of glycerol molecules in the chitosan polymer network has  
552 demonstrated to increase the interchain spacing by reducing intermolecular interactions.  
553 The added plasticizer disrupts hydrogen bonding between the polymer chains, reducing the  
554 interchain bonds and thus facilitating the diffusion of water molecules through the film. In  
555 addition, because of the hydrophilic nature of glycerol, this compound acts as a humectant,  
556 enhancing the water-holding capacity of the polymer matrix and also contributing to  
557 higher WVP values of the films [44]. On the other hand, some authors have observed a  
558 reduction in WVP with increasing glycerol concentration, like Xu et al. [66] while working  
559 with corn starch and chitosan blend films. This decrease could be attributed to the  
560 formation of intermolecular hydrogen bonding between  $\text{NH}_3^+$  groups of the chitosan and  
561 hydroxyl groups of the glycerol [67].  
562 Finally, drying temperature significantly affected WVP in a linear way: higher drying  
563 temperatures resulted in lower WVP of chitosan films. In accordance with these results,  
564 Jahit et al. [55] stated that higher temperatures led to increase cohesive strength between  
565 polymer chains, thus, creating a more compact structure with less free volume, which  
566 consequently achieved lower permeability.

567

### 568 ***3.2.5. Influence of formulation on antioxidant properties (DPPH, TPC)***

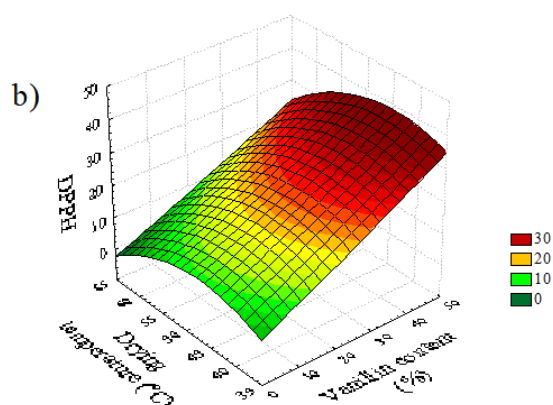
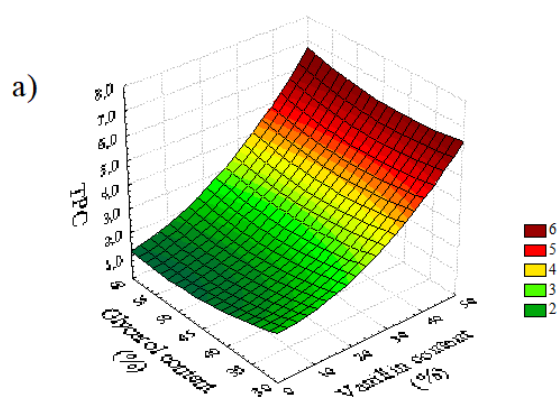
569 Chitosan antioxidant properties have been thoroughly explained in the past and most food  
570 products could benefit from them when chitosan is used to form films [68]. Furthermore,

571 to enhance these inherent characteristics, a possible strategy is to add antioxidant  
572 compounds such as vanillin into the chitosan films.

573 The effects of the independent variables on total phenolic content (TPC) and antioxidant  
574 capacity (DPPH) are shown in Fig. 6 (a and b, respectively). For both responses, the  
575 canonical and stationary point analysis indicated that the stationary point was a saddle  
576 point.

577 Total phenolic content was only significantly affected by vanillin concentration, in both  
578 linear ( $p < 0.01$ ) and quadratic ( $p < 0.05$ ) terms. Results confirmed that increasing vanillin  
579 content significantly increased TPC of the films, as it can be portrayed in Fig. 6.a.

580



581



582 **Fig. 6:** Response surface curves for antioxidant properties of chitosan-based films. (a): Variation  
583 of TPC with vanillin and glycerol concentrations. (b): Variation of DPPH with vanillin  
584 concentration and drying temperature. The remaining variables were kept constant at middle level.

585

586 With regards to DPPH, the variance analysis revealed that the antioxidant capacity was  
587 significantly affected by vanillin content ( $p < 0.001$ ) and drying temperature ( $p < 0.05$ ). A  
588 curvature of the surface is observed due to the significance of the pure quadratic term for  
589 the drying temperature of the films as shown through de variance analysis. However, the  
590 most significant parameter on the antioxidant capacity of the films was vanillin  
591 concentration. A linear and positive effect was observed for vanillin content. As it can be  
592 seen in Fig. 6.b, a similar trend to that found for TPC was obtained for DPPH: the  
593 incorporation of vanillin in the formulation significantly increased the antioxidant capacity  
594 of the films. Therefore, vanillin as a natural cross-linking agent could significantly  
595 enhance antioxidant properties of chitosan films, which could implicate important benefits  
596 for the food industry.

597

### 598 **3.3. Simultaneous optimization and validation**

599 Simultaneous optimization by desirability approach was carried out on the independent  
600 variables to get the optimum levels within the experimental conditions, using the  
601 significant coefficients of the predicted models. In this work, opacity, TSM and water  
602 vapor permeability (WVP) of the films were minimized, while YM,  $T_0$  and  $T_{max}$   
603 parameters, TPC and inhibition of DPPH radical were maximized. In these conditions, it  
604 was found an optimal chitosan film formulation with vanillin content of 37.5% w/w of CH  
605 ( $X_1 = 0.5$ ), Gly content of 45% w/w of CH ( $X_2 = 0$ ) and drying temperature of 57.5°C ( $X_3$   
606 = 0.5).

607 In order to test the reliability of the models in predicting optimal responses, validation  
 608 experiments were carried out at the optimal levels predicted by the simultaneous  
 609 optimization. ~~Predicted values for opacity, TSM, YM, T<sub>0</sub>, T<sub>max</sub>, WVP, TPC and DPPH~~  
 610 ~~were 3328.3 AU nm/mm, 26.85%, 1390.5 MPa, 138.8°C, 182.7°C, 2.84.10<sup>-10</sup> g.m/Pa.s.m<sup>2</sup>;~~  
 611 ~~4.51 mg GA/g and 24.19% of inhibition, respectively. These predicted responses were~~  
 612 ~~verified experimentally, with the following experimental values (mean value ± standard~~  
 613 ~~deviation) for opacity, TSM, YM, T<sub>0</sub>, T<sub>max</sub>, WVP, TPC and DPPH: 3136.9 ± 10.2 AU~~  
 614 ~~nm/mm, 20.6 ± 3.9, 1225.0 ± 134.7 MPa, 147.0 ± 1.0°C, 196.4 ± 1.0°C, 2.95 ± 0.12.10<sup>-10</sup>~~  
 615 ~~g.m/Pa.s.m<sup>2</sup>, 4.95±0.43 mg GA/g, 25±1.2 % inhibition, respectively; confirming the~~  
 616 ~~accuracy of the model generated from the analysis.~~

617 Comparison of predicted and experimental response values at the optimal level are  
 618 depicted in Table 6, confirming the accuracy of the model generated from the analysis.

619

620 *Table 6. Predicted and experimental response values for the optimal chitosan film formulation.*

<i>Response variables</i>	<i>Predicted</i>	<i>Experimental</i>
Opacity (AU nm/mm)	3328.3	3136.9 ± 10.2
TSM (%)	26.85	20.6 ± 3.9
YM (Mpa)	1390.5	1225.0 ± 134.7
T <sub>0</sub> (°C)	138.8	147.0 ± 1.0
T <sub>max</sub> (°C)	182.7	196.4 ± 1.0
WVP (g.m/Pa.s.m <sup>2</sup> 10 <sup>10</sup> )	2.84	2.95 ± 0.12
TPC (mg GA/g)	4.51	4.95 ± 0.43
DPPH (%In)	24.19	25 ± 1.2

621

#### 622 4. CONCLUSIONS

623

624 RSM approach using Box-Behnken design and the desirability function was found to be an  
 625 effective tool to investigate changes in films properties and to search for the optimal active  
 626 chitosan film formulation with a natural antioxidant cross-linking agent, such as vanillin.

627 An optimal chitosan film formulation was found with vanillin content of 37.5% w/w of  
628 CH, glycerol content of 45% w/w of CH, and a drying temperature of 57.5°C. Results  
629 concluded that chitosan-based films with vanillin can be successfully developed with  
630 desirable mechanical, antioxidant and barrier properties to be potentially used in the food  
631 packaging industry.

632

### 633 **Acknowledgments**

634 This work was supported by Consejo Nacional de Investigaciones Científicas y Técnicas  
635 (CONICET), Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT) and  
636 Universidad Nacional de Mar del Plata (UNMDP).

637

### 638 **References**

639

- 640 [1] M.C. Cruz-Romero, T. Murphy, M. Morris, E. Cummins, J.P. Kerry, Antimicrobial  
641 activity of chitosan, organic acids and nano-sized solubilisates for potential use in  
642 smart antimicrobially-active packaging for potential food applications, *Food*  
643 *Control*. 34 (2013) 393–397. doi:10.1016/J.FOODCONT.2013.04.042.
- 644 [2] A. Valdés, A.C. Mellinas, M. Ramos, M.C. Garrigós, A. Jiménez, Natural additives  
645 and agricultural wastes in biopolymer formulations for food packaging, *Front.*  
646 *Chem.* 2 (2014) 6. <https://www.frontiersin.org/article/10.3389/fchem.2014.00006>.
- 647 [3] C. López-de-Dicastillo, D. Pezo, C. Nerín, G. López-Carballo, R. Catalá, R. Gavara,  
648 P. Hernández-Muñoz, Reducing Oxidation of Foods Through Antioxidant Active  
649 Packaging Based on Ethyl Vinyl Alcohol and Natural Flavonoids, *Packag. Technol.*  
650 *Sci.* 25 (2012) 457–466. doi:10.1002/pts.992.

- 651 [4] M. Mujtaba, B. Koc, A.M. Salaberria, S. Ilk, D. Cansaran-Duman, L. Akyuz, Y.S.  
652 Cakmak, M. Kaya, K.M. Khawar, J. Labidi, S. Boufi, Production of novel chia-  
653 mucilage nanocomposite films with starch nanocrystals; An inclusive biological and  
654 physicochemical perspective, *Int. J. Biol. Macromol.* 133 (2019) 663–673.  
655 doi:10.1016/j.ijbiomac.2019.04.146.
- 656 [5] L. Akyuz, M. Kaya, B. Koc, M. Mujtaba, S. Ilk, J. Labidi, A.M. Salaberria, Y.S.  
657 Cakmak, A. Yildiz, Diatomite as a novel composite ingredient for chitosan film  
658 with enhanced physicochemical properties, *Int. J. Biol. Macromol.* 105 (2017)  
659 1401–1411. doi:10.1016/j.ijbiomac.2017.08.161.
- 660 [6] M. Mujtaba, R.E. Morsi, G. Kerch, M.Z. Elsabee, M. Kaya, J. Labidi, K.M.  
661 Khawar, Current advancements in chitosan-based film production for food  
662 technology; A review, *Int. J. Biol. Macromol.* 121 (2019) 889–904.  
663 doi:10.1016/j.ijbiomac.2018.10.109.
- 664 [7] M. Kaya, L. Akyuz, I. Sargin, M. Mujtaba, A.M. Salaberria, J. Labidi, Y.S.  
665 Cakmak, B. Koc, T. Baran, T. Ceter, Incorporation of sporopollenin enhances acid–  
666 base durability, hydrophobicity, and mechanical, antifungal and antioxidant  
667 properties of chitosan films, *J. Ind. Eng. Chem.* 47 (2017) 236–245.  
668 doi:https://doi.org/10.1016/j.jiec.2016.11.038.
- 669 [8] L. Balau, G. Lisa, M.I. Popa, V. Tura, V. Melnig, Physico-chemical properties of  
670 Chitosan films, *Cent. Eur. J. Chem.* 2 (2004) 638–647. doi:10.2478/BF02482727.
- 671 [9] M.A. Khokhlova, M.O. Gallyamov, A.R. Khokhlov, Chitosan nanostructures  
672 deposited from solutions in carbonic acid on a model substrate as resolved by AFM,  
673 *Colloid Polym. Sci.* 290 (2012) 1471–1480. doi:10.1007/s00396-012-2673-3.
- 674 [10] J.-W. Rhim, P.K.W. Ng, Natural Biopolymer-Based Nanocomposite Films for

- 675 Packaging Applications, *Crit. Rev. Food Sci. Nutr.* 47 (2007) 411–433.  
676 doi:10.1080/10408390600846366.
- 677 [11] X.Z. Tang, P. Kumar, S. Alavi, K.P. Sandeep, Recent Advances in Biopolymers and  
678 Biopolymer-Based Nanocomposites for Food Packaging Materials, *Crit. Rev. Food*  
679 *Sci. Nutr.* 52 (2012) 426–442. doi:10.1080/10408398.2010.500508.
- 680 [12] S.F. Sabato, N. Nakamurakare, P.J.A. Sobral, Mechanical and thermal properties of  
681 irradiated films based on Tilapia (*Oreochromis niloticus*) proteins, *Radiat. Phys.*  
682 *Chem.* 76 (2007) 1862–1865. doi:10.1016/J.RADPHYSHEM.2007.02.096.
- 683 [13] M.M. Beppu, R.S. Vieira, C.G. Aimoli, C.C. Santana, Crosslinking of chitosan  
684 membranes using glutaraldehyde: Effect on ion permeability and water absorption,  
685 *J. Memb. Sci.* 301 (2007) 126–130. doi:10.1016/j.memsci.2007.06.015.
- 686 [14] Q. Yang, F. Dou, B. Liang, Q. Shen, Investigations of the effects of glyoxal cross-  
687 linking on the structure and properties of chitosan fiber, *Carbohydr. Polym.* 61  
688 (2005) 393–398. doi:10.1016/j.carbpol.2005.04.017.
- 689 [15] J.Q. Xue, J.X. Li, M. Wu, W. Wang, D.N. Ma, Preparation and Characterization of  
690 Formaldehyde Crosslinked Chitosan, *Adv. Mater. Res.* 239–242 (2011) 279–282.  
691 doi:10.4028/www.scientific.net/AMR.239-242.279.
- 692 [16] Y. Zheng, W. Yang, C. Wang, J. Hu, S. Fu, L. Dong, L. Wu, X. Shen, Nanoparticles  
693 based on the complex of chitosan and polyaspartic acid sodium salt: preparation,  
694 characterization and the use for 5-fluorouracil delivery., *Eur. J. Pharm. Biopharm.*  
695 67 (2007) 621–631. doi:10.1016/j.ejpb.2007.04.007.
- 696 [17] D. Kowalczyk, M. Kordowska-Wiater, J. Nowak, B. Baraniak, Characterization of  
697 films based on chitosan lactate and its blends with oxidized starch and gelatin, *Int. J.*  
698 *Biol. Macromol.* 77 (2015) 350–359. doi:10.1016/J.IJBIOMAC.2015.03.032.

- 699 [18] G.M. Duran, A.M. Contento, A. Rios, beta-Cyclodextrin coated CdSe/ZnS quantum  
700 dots for vanillin sensing in food samples., *Talanta*. 131 (2015) 286–291.  
701 doi:10.1016/j.talanta.2014.07.100.
- 702 [19] H.-B. Zhu, Y.-C. Fan, Y.-L. Qian, H.-F. Tang, Z. Ruan, D.-H. Liu, H. Wang,  
703 Determination of spices in food samples by ionic liquid aqueous solution extraction  
704 and ion chromatography, *Chinese Chem. Lett.* 25 (2014) 465–468.  
705 doi:10.1016/J.CCLET.2013.12.001.
- 706 [20] J.F. Stanzione III, J.M. Sadler, J.J. La Scala, K.H. Reno, R.P. Wool, Vanillin-based  
707 resin for use in composite applications, *Green Chem.* 14 (2012) 2346–2352.  
708 doi:10.1039/C2GC35672D.
- 709 [21] Z.H. Zhang, Z. Han, X.A. Zeng, X.Y. Xiong, Y.J. Liu, Enhancing mechanical  
710 properties of chitosan films via modification with vanillin, *Int. J. Biol. Macromol.*  
711 81 (2015) 638–643. doi:10.1016/j.ijbiomac.2015.08.042.
- 712 [22] F. Tian, E.A. Decker, J.M. Goddard, Controlling lipid oxidation of food by active  
713 packaging technologies, *Food Funct.* 4 (2013) 669–680. doi:10.1039/C3FO30360H.
- 714 [23] M.C.C.B. Santana, B.A.S. Machado, T.N. Silva, I.L. Nunes, J.I. Druzian,  
715 Incorporação de urucum como aditivo antioxidante em embalagens biodegradáveis  
716 a base de quitosana The incorporation of annatto as antioxidant additive based  
717 biodegradable packaging chitosan, *Ciência Rural*. 43 (2013) 544–550.
- 718 [24] D. Piñeros-Hernandez, C. Medina-Jaramillo, A. López-Córdoba, S. Goyanes, Edible  
719 cassava starch films carrying rosemary antioxidant extracts for potential use as  
720 active food packaging, *Food Hydrocoll.* 63 (2017) 488–495.  
721 doi:10.1016/J.FOODHYD.2016.09.034.
- 722 [25] U. Siripatrawan, W. Vitchayakitti, Improving functional properties of chitosan films

- 723 as active food packaging by incorporating with propolis, *Food Hydrocoll.* 61 (2016)  
724 695–702. doi:10.1016/J.FOODHYD.2016.06.001.
- 725 [26] A. Acevedo-Fani, L. Salvia-Trujillo, M.A. Rojas-Graü, O. Martín-Belloso, Edible  
726 films from essential-oil-loaded nanoemulsions: Physicochemical characterization  
727 and antimicrobial properties, *Food Hydrocoll.* 47 (2015) 168–177.  
728 doi:10.1016/J.FOODHYD.2015.01.032.
- 729 [27] Z. Shen, D.P. Kamdem, Antimicrobial activity of sugar beet lignocellulose films  
730 containing tung oil and cedarwood essential oil, *Cellulose.* 22 (2015) 2703–2715.  
731 doi:10.1007/s10570-015-0679-y.
- 732 [28] Z.A. Maryam Adilah, B. Jamilah, Z.A. Nur Hanani, Functional and antioxidant  
733 properties of protein-based films incorporated with mango kernel extract for active  
734 packaging, *Food Hydrocoll.* 74 (2018) 207–218.  
735 doi:10.1016/J.FOODHYD.2017.08.017.
- 736 [29] C. López de Dicastillo, F. Bustos, A. Guarda, M.J. Galotto, Cross-linked methyl  
737 cellulose films with murta fruit extract for antioxidant and antimicrobial active food  
738 packaging, *Food Hydrocoll.* 60 (2016) 335–344.  
739 doi:10.1016/J.FOODHYD.2016.03.020.
- 740 [30] M. Cushen, J. Kerry, M. Morris, M. Cruz-romero, E. Cummins, Nanotechnologies  
741 in the food industry e Recent developments , risks and regulation, *Trends Food Sci.*  
742 *Technol.* 24 (2012) 30–46. doi:10.1016/j.tifs.2011.10.006.
- 743 [31] A. Kołakowska, G. Bartosz, Oxidation of Food Components: An Introduction, in:  
744 G. Bartosz (Ed.), *Food Oxid. Antioxidants Chem. Biol. Funct. Prop.*, CRC Press,  
745 2016: p. 568.
- 746 [32] M. Pazos, I. Medina, Oxidants Occurring in Food Systems, in: G. Bartosz (Ed.),

- 747 Food Oxid. Antioxidants Chem. Biol. Funct. Prop., CRC Press, 2016: p. 568.
- 748 [33] C.E. Realini, B. Marcos, Active and intelligent packaging systems for a modern  
749 society, *Meat Sci.* 98 (2014) 404–419. doi:10.1016/J.MEATSCI.2014.06.031.
- 750 [34] L. Barbosa-Pereira, I. Angulo, J.M. Lagarón, P. Paseiro-Losada, J.M. Cruz,  
751 Development of new active packaging films containing bioactive nanocomposites,  
752 *Innov. Food Sci. Emerg. Technol.* 26 (2014) 310–318.  
753 doi:10.1016/J.IFSET.2014.06.002.
- 754 [35] C. Contini, R. Álvarez, M. O’Sullivan, D.P. Dowling, S.Ó. Gargan, F.J. Monahan,  
755 Effect of an active packaging with citrus extract on lipid oxidation and sensory  
756 quality of cooked turkey meat, *Meat Sci.* 96 (2014) 1171–1176.  
757 doi:10.1016/J.MEATSCI.2013.11.007.
- 758 [36] V.G.L. Souza, A.L. Fernando, J.R.A. Pires, P.F. Rodrigues, A.A.S. Lopes, F.M.B.  
759 Fernandes, Physical properties of chitosan films incorporated with natural  
760 antioxidants, *Ind. Crops Prod.* 107 (2017) 565–572.  
761 doi:10.1016/j.indcrop.2017.04.056.
- 762 [37] R. Sothornvit, J.M. Krochta, Plasticizers in edible films and coatings, *Innov. Food*  
763 *Packag.* (2005) 403–433. doi:10.1016/B978-012311632-1/50055-3.
- 764 [38] P. Jomlapeeratikul, N. Poomsa-Ad, L. Wiset, Effect of Drying Temperatures and  
765 Plasticizers on the Properties of Konjac Flour Film, *J. Food Process Eng.* 40 (2017).  
766 doi:10.1111/jfpe.12443.
- 767 [39] M.M. Andrade-Mahecha, D.R. Tapia-Blácido, F.C. Menegalli, Development and  
768 optimization of biodegradable films based on achira flour, *Carbohydr. Polym.* 88  
769 (2012) 449–458. doi:10.1016/J.CARBPOL.2011.12.024.
- 770 [40] P. Mayachiew, S. Devahastin, Effects of drying methods and conditions on release



- 771 characteristics of edible chitosan films enriched with Indian gooseberry extract,  
772 Food Chem. 118 (2010) 594–601. doi:10.1016/J.FOODCHEM.2009.05.027.
- 773 [41] G.E.P. Box, D.W. Behnken, Some New Three Level Designs for the Study of  
774 Quantitative Variables, Technometrics. 2 (1960) 455–475.  
775 <http://www.jstor.org/stable/1266454>.
- 776 [42] L. Vera Candioti, M.M. De Zan, M.S. Cámara, H.C. Goicoechea, Experimental  
777 design and multiple response optimization. Using the desirability function in  
778 analytical methods development, Talanta. 124 (2014) 123–138.  
779 doi:10.1016/j.talanta.2014.01.034.
- 780 [43] N. Aslan, Y. Cebeci, Application of Box–Behnken design and response surface  
781 methodology for modeling of some Turkish coals, Fuel. 86 (2007) 90–97.  
782 doi:10.1016/j.fuel.2006.06.010.
- 783 [44] M. Pereda, M.I. Aranguren, N.E. Marcovich, Water vapor absorption and  
784 permeability of films based on chitosan and sodium caseinate, J. Appl. Polym. Sci.  
785 111 (2009) 2777–2784. doi:10.1002/app.29347.
- 786 [45] J. Irissin-Mangata, G. Bauduin, B. Boutevin, N. Gontard, New plasticizers for wheat  
787 gluten films, Eur. Polym. J. 37 (2001) 1533–1541. doi:10.1016/S0014-  
788 3057(01)00039-8.
- 789 [46] J.-W. Rhim, J.H. Lee, P.K.W. Ng, Mechanical and barrier properties of  
790 biodegradable soy protein isolate-based films coated with polylactic acid, LWT -  
791 Food Sci. Technol. 40 (2007) 232–238. doi:10.1016/J.LWT.2005.10.002.
- 792 [47] ASTM, Standard Test Method for Tensile Properties of Plastics by Use of  
793 Microtensile Specimens, (2013) ASTM D1708-13. doi:10.1520/D1708.
- 794 [48] ASTM, Standard Test Method for Tensile Properties of Plastics, (2014) ASTM

- 795 D638-14. doi:10.1520/D0638-14.
- 796 [49] ASTM, Standard Test Methods for Water Vapor Transmission of Materials, (2016)
- 797 ASTM E96 / E96M-16. doi:10.1520/E0096\_E0096M-16.
- 798 [50] M.R. Ansorena, F. Zubeldía, N.E. Marcovich, Active wheat gluten films obtained
- 799 by thermoplastic processing, *LWT - Food Sci. Technol.* 69 (2016) 47–54.
- 800 doi:10.1016/J.LWT.2016.01.020.
- 801 [51] G.E. Viacava, S.I. Roura, M.V. Agüero, Optimization of critical parameters during
- 802 antioxidants extraction from butterhead lettuce to simultaneously enhance
- 803 polyphenols and antioxidant activity, *Chemom. Intell. Lab. Syst.* 146 (2015) 47–54.
- 804 doi:10.1016/j.chemolab.2015.05.002.
- 805 [52] G.C. Derringer, A Balancing Act : Optimizing a Product's Properties, *Qual. Prog.*
- 806 27 (1994) 51–58.
- 807 [53] M.R. Ansorena, M.R. Moreira, S.I. Roura, Combined effect of ultrasound, mild heat
- 808 shock and citric acid to retain greenness, nutritional and microbiological quality of
- 809 minimally processed broccoli (*Brassica oleracea* L.): An optimization study,
- 810 *Postharvest Biol. Technol.* 94 (2014) 1–13.
- 811 doi:10.1016/J.POSTHARVBIO.2014.02.017.
- 812 [54] I. Leceta, P. Guerrero, K. de la Caba, Functional properties of chitosan-based films,
- 813 *Carbohydr. Polym.* 93 (2013) 339–346. doi:10.1016/J.CARBPOL.2012.04.031.
- 814 [55] I.S. Jahit, N.N.M. Nazmi, M.I.N. Isa, N.M. Sarbon, Preparation and physical
- 815 properties of gelatin / CMC / chitosan composite films as affected by drying
- 816 temperature, *Int. Food Res. J.* 23 (2016) 1068–1074.
- 817 [56] Y.-H. Yun, C.-M. Lee, Y.-S. Kim, S.-D. Yoon, Preparation of chitosan/polyvinyl
- 818 alcohol blended films containing sulfosuccinic acid as the crosslinking agent using

- 819 UV curing process, *Food Res. Int.* 100 (2017) 377–386.  
820 doi:10.1016/J.FOODRES.2017.07.030.
- 821 [57] S. Mali, M.V.E. Grossmann, M.A. García, M.N. Martino, N.E. Zaritzky, Barrier,  
822 mechanical and optical properties of plasticized yam starch films, *Carbohydr.*  
823 *Polym.* 56 (2004) 129–135. doi:10.1016/J.CARBPOL.2004.01.004.
- 824 [58] M. Fache, B. Boutevin, S. Caillol, Vanillin, a key-intermediate of biobased  
825 polymers, *Eur. Polym. J.* 68 (2015) 488–502. doi:10.1016/j.eurpolymj.2015.03.050.
- 826 [59] L. Higuera, G. López-carballo, R. Gavara, P. Hernández-muñoz, Reversible  
827 Covalent Immobilization of Cinnamaldehyde on Chitosan Films via Schiff Base  
828 Formation and Their Application in Active Food Packaging, (2014).  
829 doi:10.1007/s11947-014-1421-8.
- 830 [60] A.S. Shekarabi, A.R. Oromiehie, A. Vaziri<sup>1</sup>, M. Ardjmand, Ali Akbar Safekordi,  
831 Effect of Glycerol Concentration on Physical Properties of Composite Edible Films  
832 Prepared From, *Indian J. Fundam. Appl. Life Sci.* 4 (2014) 1241–1248.  
833 www.cibtech.org/sp.ed/jls/2014/04/jls.htm 2014 Vol. 4 (S4), pp. 1241-  
834 1248/Shekarabi et al.
- 835 [61] S. Tunç, O. Duman, Preparation and characterization of biodegradable methyl  
836 cellulose/montmorillonite nanocomposite films, *Appl. Clay Sci.* 48 (2010) 414–424.  
837 doi:10.1016/j.clay.2010.01.016.
- 838 [62] M. Lavorgna, F. Piscitelli, P. Mangiacapra, G.G. Buonocore, Study of the combined  
839 effect of both clay and glycerol plasticizer on the properties of chitosan films,  
840 *Carbohydr. Polym.* 82 (2010) 291–298. doi:10.1016/J.CARBPOL.2010.04.054.
- 841 [63] I. Kaviani, P.G. Plieger, N.G. Kandile, D.R. Harding, Preparation and  
842 characterization of chitosan films, crosslinked with symmetric aromatic

- 843 dianhydrides to achieve enhanced thermal properties, *Polym. Int.* 64 (2015) 556–  
844 562. doi:10.1002/pi.4835.
- 845 [64] L. Sun, J. Sun, L. Chen, P. Niu, X. Yang, Y. Guo, Preparation and characterization  
846 of chitosan film incorporated with thinned young apple polyphenols as an active  
847 packaging material, *Carbohydr. Polym.* 163 (2017) 81–91.  
848 doi:10.1016/j.carbpol.2017.01.016.
- 849 [65] U. Siripatrawan, W. Vitchayakitti, Improving functional properties of chitosan films  
850 as active food packaging by incorporating with propolis, *Food Hydrocoll.* 61 (2016)  
851 695–702. doi:10.1016/j.foodhyd.2016.06.001.
- 852 [66] Y.X. Xu, K.M. Kim, M.A. Hanna, D. Nag, Chitosan–starch composite film:  
853 preparation and characterization, *Ind. Crops Prod.* 21 (2005) 185–192.  
854 doi:10.1016/J.INDCROP.2004.03.002.
- 855 [67] T.P. Singh, M.K. Chatli, J. Sahoo, Development of chitosan based edible films:  
856 process optimization using response surface methodology, *J. Food Sci. Technol.* 52  
857 (2015) 2530–2543. doi:10.1007/s13197-014-1318-6.
- 858 [68] M. Moradi, H. Tajik, S.M. Razavi Rohani, A.R. Oromiehie, H. Malekinejad, J.  
859 Aliakbarlu, M. Hadian, Characterization of antioxidant chitosan film incorporated  
860 with *Zataria multiflora* Boiss essential oil and grape seed extract, *LWT - Food Sci.*  
861 *Technol.* 46 (2012) 477–484. doi:10.1016/j.lwt.2011.11.020.  
862

## Highlights

- Vanillin was used as a natural crosslinker in chitosan films
- ~~Vanillin, glycerol content and drying temperature were optimized through RSM~~
- Different properties of the films were simultaneously optimized with Desirability function
- RSM and Desirability function are effective tools in the optimization of the films formulation