

# **Physical, Barrier and Antioxidant Properties of Pea Starch-Guar Gum Biocomposite Edible Films by Incorporation of Natural Plant Extracts**

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1 **Physical, Barrier and Antioxidant Properties of Pea Starch-Guar Gum Biocomposite**  
2 **Edible Films by Incorporation of Natural Plant Extracts**

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30 **Abstract**

31 Active food packaging based on pea starch and guar gum (PSGG) films containing natural  
32 antioxidants (NAs) was developed. Four kinds of NAs (epigallocatechin gallate (EGCG),  
33 blueberry ash (BBA) fruit extract, macadamia (MAC) peel extract, and banana (BAN) peel  
34 extract) were added into PSGG-based films as antioxidant additive. The effects of these  
35 compounds at different amounts on physical and antioxidant characteristics of PSGG film were  
36 investigated. The antioxidant activity was calculated with three analytical assays: DPPH radical  
37 scavenging ability assay, cupric reducing antioxidant capacity (CUPRAC) and ferric reducing  
38 activity power (FRAP). EGCG-PSGG films showed higher antioxidant activity, followed by  
39 BBA-PSGG, MAC-PSGG and BAN-PSGG films, at all concentrations (0.75-3 mg/mL) and  
40 with all procedures tested. Additionally, the antioxidant activity of films showed a  
41 concentration dependency. The results revealed that addition of NAs made the PSGG film  
42 darker and less transparent. However, the moisture barrier was significantly improved when  
43 NAs were incorporated into the film. The FTIR spectra were examined to determine the  
44 interactions between polymers and NAs. The results suggested that incorporation of EGCG,  
45 BBA, MAC, and BAN into PSGG film have great potential for use as active food packaging  
46 for food preservation.

47 **Keywords** Pea starch . Guar gum . Active edible film . Natural extracts . Antioxidant activity

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## 52 **Introduction**

53 Oxidation is the major cause of food degradation which can reduce the shelf life of food (Miller  
54 and Krochta 1997), decrease nutritional quality, increase toxicity, develop off-odor, and alter  
55 texture and color (Perazzo et al. 2014). The direct incorporation of antioxidants in food  
56 products is limited due to the high probability for rapid depletion of the antioxidants as well  
57 as the very high initial concentrations required to prevent this oxidation (Finley and Given  
58 1986). Edible films and coatings can be developed as oxygen barrier layer and carrier for  
59 antioxidant delivery to prevent oxidative damage (Moreno et al. 2015). The increased attention  
60 on food safety and consumer health has prompted researchers to examine and develop  
61 functional ingredients from natural resources such as antimicrobial enzymes, essential oils,  
62 bacteriocins and phenolic compounds, rather than synthetically manufactured ingredients  
63 (Ramos et al. 2012; Vodnar 2012). Active packaging aims to combine active ingredients  
64 including nutrient supplementation, antimicrobial, and antioxidant agents into packaging  
65 materials to preserve food quality, safety and shelf life (Coma 2008; Gutiérrez et al. 2009;  
66 Vermeiren et al. 1999; Wang et al. 2015b). The addition of phenolic compounds and extracts  
67 in active packaging not only allows the phenolics to prevent oxidation in the food, but it can  
68 also increase their direct human consumption to improve human health (Komes et al. 2010;  
69 Sun et al. 2014).

70 Many polyphenols including flavonoids and proanthocyanidins are derived from vegetables  
71 and fruits and are considered sources of bioactive compounds (Apak et al. 2007). These  
72 compounds are widely consumed in the human diet where their effective antioxidant  
73 characteristics have positive health advantages including the inhibition of cancer,  
74 cardiovascular diseases, obesity and diabetes (Vuong et al. 2014).

75 Catechins are the main tea polyphenols in green tea extract mostly such as epicatechin (EC),  
76 epigallocatechin (EGC), epicatechin gallate (ECG), epigallocatechin gallate (EGCG) (Yu et al.  
77 2015). EGCG is an active polyphenolic catechin and comprises around 59% of the total  
78 catechins from the leaves of the green tea (Steinmann et al. 2013). To the best of our  
79 knowledge, the effect of EGCG on antioxidant and physical properties of PSGG edible film  
80 has not yet been investigated.

81 Blueberry ash (*Elaeocarpus reticulatus* Sm.) is a plant that belongs to *Elaeocarpaceae* family  
82 grown in rainforest and coastal scrub along the east coast of Australia (Rickard 2011). There  
83 is limited information on phytochemical and antioxidant characteristics of blueberry ash fruits.  
84 In this study, the potential application of blueberry ash fruit extract as an antioxidant compound  
85 to PSGG edible film was investigated.

86 The macadamia is recognized as an evergreen, native Australian tree with two more common  
87 species, the *Macadamia integrifolia* (smooth shelled) and the *Macadamia tetraphylla* (rough  
88 shelled) (Munro and Garg 2008). The skin/husk of the macadamia has been suggested to have  
89 plenty of phenolic compounds (Alasalvar and Shahidi 2009; Dailey and Vuong 2016).  
90 Therefore, active biodegradable packaging can be developed by incorporation of phenolic  
91 compounds derived from macadamia skin.

92 Banana peel accounts for approximately 40% of total weight of the fresh fruit (Anhwange  
93 2008). The peel of banana as a natural source of antioxidants and phytochemical content  
94 specially catecholamines (Kanazawa and Sakakibara 2000), galliccatechin (Someya et al.  
95 2002), phenolic (Baskar et al. 2011; del Mar Verde Méndez et al. 2003; Fatemeh et al. 2012;  
96 Nguyen et al. 2003), dopamine (Kanazawa and Sakakibara 2000), lutein (Davey et al. 2006),  
97 as well as carotenoid compounds (Davey et al. 2006; van den Berg et al. 2000) has been taken

98 into account. So far, there is no report on the impact of the incorporation of banana peel extract  
99 to the pea starch-guar gum edible films.

100 Therefore, this study was conducted to analyse the effect of various natural plant extracts on  
101 antioxidant properties of pea starch-guar gum films. In addition, the effect of these extracts was  
102 examined on the barrier, physical, and optical characteristics of pea starch-guar gum edible  
103 film.

## 104 **Materials and Methods**

### 105 **Materials**

106 In all experiments Canadian non-GMO yellow pea starch (supplied by Yantai Shuangta Food  
107 Co., Jinling Town, China) with 13.2% moisture, 0.2% protein, 0.5% fat, 0.3% ash, and 36.25%  
108 amylose was used. Guar gum (E-412) was provided by The Melbourne Food Ingredient Depot,  
109 Brunswick East, Melbourne, Australia. All other chemicals were purchased from Sigma-  
110 Aldrich Pty Ltd, Castle Hill, NSW, Australia. Commercial **epigallocatechin gallate** Teavigo™  
111 EGCG was obtained from RejuvaCare, Sydney, NSW, Australia. It was in the form of dry  
112 powder stored at 5-8 °C until needed.

### 113 **Preparation of Extracts**

114 The method described by Dailey and Vuong (2015) was used for extraction from macadamia  
115 skin (*Macadamia tetraphylla*). In short, the extraction process was performed on the dried and  
116 ground skin of macadamia harvested in the Central Coast region, New South Wales, Australia  
117 (latitude of 33.4° S, longitude of 151.4° E). The extraction process was performed in an  
118 ultrasonic bath (Soniclean, 220 V, 50 Hz and 250 W, Soniclean Pty Ltd., The Barton, Australia)  
119 with pre-set conditions for temperature of 40 °C, time of 35 min, power of 200 W, sample to  
120 solvent ratio of 5:100 g/mL and a mixture of acetone: water (1:1 v/v).

121 The extraction of phenols from blueberry fruits was carried out as described by Saberi et al.  
122 (2017). Blueberry ash (*Elaeocarpus reticulatus* Sm.) fruits were collected in August 2015 from  
123 the Central Coast region of New South Wales (NSW), Australia. The extraction solvent (50%  
124 acetone) at a solvent-to-sample ratio of 100:1 mL/g of dried sample was applied to extract  
125 bioactive compounds from blueberry ash fruits using ultrasound assisted extraction (UAE).  
126 The extraction process was performed in an ultrasonic bath with pre-set conditions for  
127 temperature of 35 °C, time of 30 min and power of 150 W, followed by agitation for 3 s once  
128 every 5 min using a Vortex. The extracts were immediately cooled on ice to room temperature,  
129 after the ultrasonic extraction was completed. The extract was then filtered using a 5 mL  
130 syringe fitted with a 0.45 µm cellulose syringe filter (Phenomenex Australia Pty. Ltd., Lane  
131 Cove, Australia). The filtered extract was kept at 4 °C before further analysis.

132 The extraction of phenols from banana peel was carried out according to Vu et al. (2016).  
133 Briefly, the ripe bananas (*Musa acuminata cavendish*) were purchased from a local market,  
134 Central Coast, NSW, Australia. Peels from ripe mature banana fruit were manually separated  
135 and cut into pieces (1 × 2 cm). The extraction process was conducted at UAE temperature of  
136 30 °C, UAE time of 5 min, UAE power of 60% (150 W), sample to solvent ratio of 8:100 g/mL  
137 and acetone concentration of 60%.

#### 138 Film Preparation

139 The film-forming solution was made by dissolving optimized amounts of pea starch (2.5 g),  
140 guar gum (0.3 g) and 25% w/w glycerol based on the dry film matter in 100 mL degassed  
141 deionized water with gentle heating (about 40 °C) and magnetic stirring for about 1 h. In  
142 another study, we optimized the film ingredients by using Box–Behnken response surface  
143 design (BBD) (Saberi et al. 2016b). After gelatinization at 90 °C for 20 min, the film solution  
144 was cooled to room temperature with mild magnetic stirring for 1 h to decrease air bubbles.

145 Plant extracts at defined concentrations (0.75 mg/mL, 1.5 mg/L, 2.25 mg/mL, and 3 mg/mL)  
146 were added. According to preliminary experiments, PSGG film with active compounds lower  
147 than 0.75 mg/mL possessed a weak antibacterial activity and therefore higher levels of extracts  
148 were tested in this study (Saber et al. 2017). Filmogenic suspensions (20 g) were cast onto  
149 Petri dishes (10 cm in diameter) and dried at 40 °C in an oven until reaching constant weight  
150 (about 24 h). Films were peeled-off carefully from Petri dishes and conditioned at 25 °C, 65%  
151 relative humidity (RH) for 72 h prior to further tests.

## 152 Moisture Content

153 Moisture content (MC) of the films was calculated gravimetrically by using a ventilated oven  
154 at  $105 \pm 1$  °C for 24 h until constant weight was reached. All the tests were performed in  
155 triplicate, and the means were reported. Moisture content was determined by the following  
156 equation:

$$157 \quad MC (\%) = \frac{M_w - M_d}{M_w} \times 100 \quad (1)$$

158 where  $M_w$  is the weight of the films conditioned in 65% RH to moisture equilibrium and  $M_d$  is  
159 the dry weight of the films (Wang et al. 2015b).

## 160 Water Solubility (WS), Gel Fraction (GF) and Swelling Degree (SD)

161 Film samples in 40 mm × 15 mm pieces conditioned in 65% RH to moisture equilibrium was  
162 weighed to the nearest 0.1 mg, and the amount was referred as  $M_w$ . The film specimens were  
163 submerged into 50 mL of distilled water in a 50 mL-beaker with gentle agitation at room  
164 temperature for 24 h. The film was filtered under vacuum through MN-640 m filter papers  
165 (Macherey-Nagel, Germany) and weighed in an analytical balance with a precision of 0.1 mg  
166 (the amount was referred to as  $W_w$ ), then dried at 110 °C in a vacuum oven to constant weight



167 (the amount was referred to as  $W_d$ ). The following equations were applied to measure water  
168 solubility, gel fraction and swelling degree of films (Delville et al. 2002; Abdollahi et al. 2012):

$$169 \quad WS (\%) = \frac{M_w (1-MC) - W_d}{M_w (1-MC)} \times 100 \quad (2)$$

$$170 \quad GF (\%) = \frac{W_d}{M_w (1-MC)} \times 100 \quad (3)$$

$$171 \quad SD = \frac{W_w}{W_d} \quad (4)$$

172 where MC is the moisture content of the film specimens conditioned in 65% RH. Three  
173 replicates were performed and averaged for each sample.

#### 174 Water Vapor Permeability

175 Water vapor permeability (WVP) of films was examined using the method explained by Sun  
176 et al. (2014) with some modifications. The films were sealed onto test cups half-filled with  
177 anhydrous calcium chloride ( $\text{CaCl}_2$ ) (0% RH) and then placed in a desiccator containing  
178 saturated NaCl solution (75% RH) and kept at 25 °C. The test cups were weighed as a function  
179 of time until changes in the weight were recorded to the nearest 0.001 g. Water vapor  
180 transmission rate (WVTR) was calculated by dividing the slope of straight line (g/m) obtained  
181 from the weight gain as a function of time data, with film surface area, and WVP was measured  
182 as follows:

$$183 \quad WVP = WVTR \frac{\text{Film thickness}}{\Delta P} \quad (5)$$

184 where  $\Delta P$  is the water vapor pressure difference between the two sides of the film (Pa). WVP  
185 was measured for three replicated samples for each type of films.

#### 186 Optical Properties

187 A UV Vis Spectrophotometer (Varian Australia Pty. Ltd., Melbourne, VIC Australia) was used  
188 to determine films transparency (Saber et al. 2016c). The films were cut into rectangular  
189 shapes (5 mm × 40 mm) and placed inside the spectrophotometer cell and the film transparency  
190 was taken at 560 nm.

191 The color of each film was measured with a Minolta colorimeter (CR-300 series, Radiometric  
192 instruments Operations, Osaka, Japan). The lightness ('L') and chromaticity parameters 'a'  
193 (red-green) and 'b' (yellow-blue) were analysed, as well as the total color difference ( $\Delta E$ ) of  
194 samples were calculated (Saber et al. 2016c):

$$195 \Delta E = \sqrt{(L^* - L)^2 + (a^* - a)^2 + (b^* - b)^2} \quad (6)$$

196 The  $L^*$ ,  $a^*$  and  $b^*$  values were the color of a white color plate used as a standard for calibration  
197 and as a background for color measurements ( $L^* = 97.27$ ,  $a^* = -3.52$ , and  $b^* = 5.36$ ) and 'L',  
198 'a', and 'b' are the color parameter values of the sample. The measurements were repeated six  
199 times for each film.

#### 200 Fourier-Transform Infrared (FTIR) Spectroscopy

201 The method described by Thakur et al. (2016) was applied to study IR spectra of the films using  
202 an infrared spectrometer (FTIR) (Thermo Fisher Scientific Inc., Nicolet iS10, USA). The  
203 spectrums were obtained at the range of between 450 and 4000  $\text{cm}^{-1}$ , using 40 scans at a  
204 resolution of 4  $\text{cm}^{-1}$ .

#### 205 Total Phenolic Content (TPC)

206 Each film sample (25 mg) was dissolved in 5 mL of distilled water for 24 h. The total phenolic  
207 content (TPC) was determined using Folin-Ciocalteu reagent, as described by Dailey and  
208 Vuong (2015). One mL of film extract was added to 5 mL of 10% (v/v) Folin-Ciocalteu reagent

209 and then 4 mL of 7.5% (w/v) Na<sub>2</sub>CO<sub>3</sub> was added. The final solution was incubated in the dark  
210 at room temperature for 1 h and the absorbance was measured at 760 nm. Gallic acid was used  
211 for plotting standard curve and the results were then reported in milligrams of gallic acid  
212 equivalents per gram of sample (mg GAE/g).

### 213 Total Flavonoids

214 Total flavonoid **content** (TFC) was measured by mixing 0.5 mL of film extract solution with 2  
215 mL of distilled water and 0.15 mL of 5% (w/v) NaNO<sub>2</sub> and leaving at room temperature for 6  
216 min. Afterwards, 0.15 mL of 10% (w/v) AlCl<sub>3</sub> was mixed and kept at room temperature (25  
217 °C) for another 6 min. Finally, the final solution was prepared with addition of 2 mL 4% (w/v)  
218 NaOH and 0.7 mL of distilled water and incubated at room temperature for 15 min and the  
219 absorbance was read at 510 nm. The results were then expressed in milligrams of rutin  
220 equivalents per gram of sample (mg RUE/g) (Zhishen et al. 1999).

### 221 DPPH Radical Scavenging Activity

222 The antioxidant properties of the film samples was calculated using a DPPH (2, 2-diphenyl-1-  
223 picrylhydrazyl) free radical scavenging assay following the technique of Papoutsis et al.  
224 (2016), and the results were specified as mg of trolox equivalents per gram of sample (mg  
225 TE/g).

### 226 Cupric Reducing Antioxidant Capacity (CUPRAC)

227 The procedure defined by Apak et al. (2004) was used to measure CUPRAC with some  
228 adjustments. Film extract solution (1.1 mL) was added to working CUPRAC solution (1 mL  
229 of CuCl<sub>2</sub>, 1 mL of neocuproine and 1 mL of NH<sub>4</sub>Ac) and after mixing well, the mixture was  
230 incubated at room temperature for 1.5 h before reading the absorbance at 450 nm. The results  
231 were determined as milligram of trolox equivalents per gram of sample (mg TE/g).

232 FRAP Assay

233 FRAP (Ferric reducing antioxidant power) was determined as explained by Vu et al. (2016). A  
234 standard curve was plotted using trolox and the results were calculated as milligram of trolox  
235 equivalents per gram of sample (mg TE/g).

236 Statistical Analysis

237 Analysis of variance was performed and the results were separated using the Multiple Ranges  
238 Duncan's test ( $P < 0.05$ ) using statistical software of Statistical Package for Social Science,  
239 SPSS (version 23, SPSS Inc., Chicago, IL, USA). All tests were carried out at least in triplicate.

## 240 **Results and Discussion**

241 Moisture Content

242 Moisture contents of the PSGG films with different ratios of active compounds are given in  
243 Table 1. Among samples, films with EGCG performed the highest moisture content value from  
244 20.3% to 17.5%, which could be due to the more hydrophilic nature of the EGCG and the  
245 availability of its hydroxyl groups to bind water molecules (Kanmani and Rhim 2014). There  
246 was no significant difference between films with 0.75 mg/mL of EGCG and BBA. Increasing  
247 the ratio of EGCG and BBA to 1.5 mg/mL increased the moisture content value to 25.6% and  
248 22.7%, respectively, which is related to plasticization effect and disintegration of film matrix.  
249 This phenomenon increased the absorption of water molecules in polymer chains by hydrogen  
250 bonding (Jouki et al. 2014). Incorporation of EGCG and BBA at higher amounts into the PSGG  
251 films caused a significant decrease in the MC. Addition of MAC and BAN considerably ( $P <$   
252  $0.05$ ) decreased moisture content of PSGG film. Lower moisture content of PSGG-MAC and  
253 PSGG-BAN films may be because of their lower hydrophilicity which can influence the  
254 capacity of the film to absorb water. Another possible reason for the reduction of MC could be

255 owing to interactions between hydroxyl groups of polymers and hydroxyl groups of phenolic  
256 compounds, which result in the lack of interactions sites for water in glucosemonomers of  
257 polymers during drying (Talja et al. 2007). The difference of films in MC may have an  
258 association with the dissimilarity in chemical structure of constituent contained in extracts (Li  
259 et al. 2014). Reduction in moisture content was also noticed in the chitosan films with *Lycium*  
260 *barbarum* fruit extract (Wang et al. 2015b), green tea extract (Siripatrawan and Harte 2010),  
261 and tea polyphenols (Wang et al. 2013).

262 Water Solubility (WS), Gel Fraction (GF) and Swelling Dgree (SD)

263 Water solubility of biodegradable films is an essential aspect since it can contribute to the water  
264 resistance of films, particularly in humid environment (Ashwar et al. 2015), and their  
265 biodegradability (Rotta et al. 2009). Water insolubility of films is also influential in specific  
266 circumstances in which it is necessary to improve the product integrity, moisture barrier  
267 characteristics and product shelf life (Tongdeesoontorn et al. 2011). The WS, GF, and SD of  
268 the PSGG films formulated with antioxidant compounds are summarized in Table 1. The  
269 results indicated that the reduction in WS of the PSGG films was significant ( $P < 0.05$ ) when  
270 weight ratio of active agents was increased from 0.75 mg/mL to 3 mg/mL. This might be a  
271 consequence of crosslinking of antioxidant compounds which can stabilize polymers structure  
272 and reduce its solubility in aqueous medium (Ashwar et al. 2015). The results were consistent  
273 with the film made from chitosan and *Lycium barbarum* fruit extract (Wang et al. 2015b),  
274 gelatin with green tea extract (Wu et al. 2013), and myofibrillar protein-based film formulated  
275 with grape seed and green tea polyphenols (Nie et al. 2015). Moreover, water solubility in  
276 EGCG- and BBA-incorporated films was moderately higher than those in MAC- and BAN-  
277 incorporated films. It might be due to the higher level of hydroxyl groups in EGCG and BBA  
278 molecules. All ingredients used in this study were completely soluble in water, but the water

279 solubility of the obtained films was not 100%, signifying gel formation. The interactions  
280 between polymers and antioxidant compounds are reasons for the gel formation. Consequently,  
281 the gel fraction increased as the concentration of natural extracts to PSGG film increased  
282 indicating the higher quantity of macromolecules engaged to produce gel (Wang et al. 2015b).  
283 Additionally, the data revealed that with the increasing amount of natural antioxidant agents,  
284 swelling degree (SD) of the films declined noticeably. The accessibility of the hydrophilic  
285 groups in the macromolecule networks to water reduced suggesting the more interaction  
286 between polymer chains and active compounds (Wang et al. 2015a). Cross-linking of  
287 compounds with PSGG diminished polymer relaxation and distribution of water into polymer,  
288 thereby decreased the SD of films (Yu et al. 2015). The degree of swelling of film is determined  
289 by drying temperature and the extent and the nature of intermolecular chain interactions  
290 (Mayachiew and Devahastin 2010; Di Pierro et al. 2006). It should be noted that the molecular  
291 characteristics of phenolic compounds significantly contributed to the strength of film matrix  
292 (Moradi et al. 2012).

### 293 Water Vapor Permeability

294 Table 1 shows WVP of the PSGG films with different contents of antioxidant compounds. The  
295 data presented that the reduction in WVP of the PSGG films containing phenolic compounds  
296 was significant ( $P < 0.05$ ) when their weight ratio to PSGG film was enhanced from 0.75 to 3  
297 mg/mL ( $P < 0.05$ ). With regard to the influence of phenolic compounds on water vapor  
298 transmission, incorporation of EGCG or BBA made the films more penetrable, which might be  
299 explained by more hydrophilic property of their phenolic compounds (Siripatrawan and Harte  
300 2010), higher WVP for EGCG-PSGG and BBA-PSGG films at 1.5 mg/mL can be accounted  
301 by their higher water absorption ability. The high tendency of EGCG-PSGG and BBA-PSGG  
302 films for water may solubilize and break the interaction with polymer chains, leading to higher

303 plasticization and consequent increase in WVP (Ashwar et al. 2015). Another reason may be  
304 due to the existence of EGCG or BBA bringing about less crystalline films and providing more  
305 free hydroxyl-hydrophilic position to water molecules and inducing high WVP (Rubilar et al.  
306 2013). The increase in WVP of these films can be described by the similar hypothesis  
307 established for moisture content. The diminished WVP of PSGG-based films with higher  
308 amounts of active compounds probably originated from the interactions between PS and GG  
309 with phenolic compounds, which enhanced intermolecular interactions and resulted in  
310 decreased interchain space of the polymer, as can be demonstrated by reduction of the  
311 transmission of water vapor molecules in the film matrix (Wang et al. 2012). Reduction in  
312 WVP has been observed by addition of natural plant extracts to edible films (Cheng et al. 2015;  
313 Wang et al. 2015b; Wang et al. 2015a; Wang et al. 2012; Li et al. 2014).

#### 314 Optical Properties

315 Color characteristics are imperative for film appearance concerning consumer acceptance and  
316 general appearance for the packed products (Wang et al. 2015a; Wang et al. 2015b). The color  
317 properties of PSGG films formulated with different natural antioxidants can be seen in Table  
318 2. The incorporation of all natural phenolic compounds influenced the appearance of edible  
319 PSGG films in both color and transparency. Edible PSGG films with filled EGCG or BBA  
320 became darker red-blueish as observed by the decreased  $L$  and  $b$ , and increased  $a$  values when  
321 the weight ratio of these compounds in the film enhanced (Table 2). The PSGG films  
322 formulated with MAC or BAN demonstrated a light yellowish tint, which is an indication of  
323 increased  $b$  value. The native color of the edible PSGG films changed because the incorporation  
324 of different combinations could structurally attached to the film matrix (Moradi et al. 2012).  
325 The color variation was closely associated with the quantity of phenolic acids and flavonoids  
326 contained in the various compounds (Corrales et al. 2009).  $\Delta E$ , as a parameter of the total color

327 changes of films, enhanced with increasing the amount of natural compounds, resulting in more  
328 colored films. Similar trends in film color have been also evidenced in chitosan (Moradi et al.  
329 2012; Wang et al. 2015b), hydroxypropyl methylcellulose (HPMC) (Chana-Thaworn et al.  
330 2011), apple puree (Du et al. 2011), soy protein (Sivarooban et al. 2008) and pea starch  
331 (Corrales et al. 2009) edible films.

332 Incorporating all active compounds into the PSGG edible film led to a reduction in its  
333 transparency. The decline in transparency could probably be owing to the light scattering from  
334 the hindering of light transmission of the edible PSGG films and phenolic compounds added  
335 into the edible PSGG films (Chana-Thaworn et al. 2011).

#### 336 Fourier-Transform Infrared (FTIR) Spectroscopy

337 Since changes were observed in the physical properties of the films, complementary study at  
338 the molecular level was performed to scrutinize interaction between functional groups in the  
339 films. FTIR spectrums of PSGG films with different active compounds at weight ratio of 3  
340 mg/ml are shown in Fig. 1. The chemical associations among different compounds can be  
341 revealed by variations in the characteristic spectra peaks (Xu et al. 2005). As it can be seen, the  
342 major appearances of the FTIR spectra of PSGG film did not alter by incorporation of active  
343 compounds, so representing no main changes of the polymers backbone, **no phase separation**  
344 **and thus** the miscibility and compatibility of employed compounds with PSGG films (Wang et  
345 al. 2012). The peak linked to the stretching vibration of free, inter- and intramolecular bound  
346 hydroxyl groups between  $3000\text{ cm}^{-1}$  to  $3600\text{ cm}^{-1}$  (Zhang and Han 2006), turned into wider and  
347 sharper when PSGG film formulated with natural extracts, which revealed that polyphenols in  
348 these ingredients comprised a number of O-H and C=O bands to create the intramolecular and  
349 intermolecular hydrogen bond (**cross-links**) (Li et al. 2014). Moreover, the intensity of C-O  
350 and C-C bands at  $1000\text{-}1300\text{ cm}^{-1}$  was found to increase by addition of these extracts.



351 Simultaneously, the sharp peak at 2700-3000  $\text{cm}^{-1}$  associated with C–H stretching (Park et al.  
352 2000), became more obvious with extracts added to film. Furthermore, the peaks between 1500  
353  $\text{cm}^{-1}$  and 1675  $\text{cm}^{-1}$ , corresponding to the stretching vibration of C=O bands and bending  
354 vibration of C-O-H bands were more recognized in films with extracts, because these bands  
355 can simply make the intermolecular hydrogen bond with O-H bands in polyphenol compounds  
356 (Siripatrawan and Harte 2010). The results of FTIR showed that addition of EGCG, BBA,  
357 MAC and BAN into PSGG film brought about interactions happening between polymers and  
358 active compounds. These intramolecular and intermolecular hydrogen bonds decrease the free  
359 hydrogen, which can constitute hydrophilic bonding with water leading to improved water  
360 barrier characteristics (Gómez-Guillén et al. 2007; Curcio et al. 2009).

#### 361 Total Phenolic Content (TPC) and Total Flavonoids (TF)

362 The most antioxidant active metabolites from plants are considered phenolic and polyphenolic  
363 compounds (Bors et al. 2001). There is a significant association between the content of  
364 phenolic compounds and antioxidant capacity (Pan et al. 2008), because these combinations  
365 have the efficiency to make available hydrogen or electrons beyond their capability to scavenge  
366 free radicals and protect against oxidative process (Genskowsky et al. 2015). Total phenol  
367 (TPC) and total flavonoid (TFC) content of PSGG edible films containing different extracts  
368 was shown in Fig. 2. These factors can be applied as influential signs of the antioxidant capacity  
369 for any produce utilized as a natural source of antioxidants in functional foods (Viuda-Martos  
370 et al. 2011). Pure PSGG films did not show the existence of phenolic and flavonoid compounds  
371 (Fig. 2). The results exhibited that TPC and TFC in the PSGG films considerably was improved  
372 ( $P < 0.05$ ) with increasing concentration of compounds (Fig. 2). The EGCG-incorporated  
373 PSGG film had the highest TPC and TFC compared with other films, while the lowest values  
374 were observed in films incorporated with 0.75 mg/mL of banana peel extract.

## 375 Antioxidant Properties

376 The antioxidant capacity of the films has been determined as a percentage of free radical-  
377 scavenging capacity (DPPH), cupric reducing antioxidant capacity (CUPRAC) and ferric  
378 reducing antioxidant power (FRAP) in Table 3. More than one method is essential to calculate  
379 the antioxidant capacity of plant material extracts in vitro (Pérez-Jiménez et al. 2008), because  
380 of the differences in their ability to produce free radicals, the mechanism to determine the end  
381 point of the prevention reaction, and the affinity towards the various reducing molecules in the  
382 sample (Roginsky and Lissi 2005). Film without any compound did not show a free radical-  
383 scavenging, cupric and ferric reducing antioxidant activity. The presence of natural extracts  
384 into PSGG films increased their antioxidant activities in comparison to the PSGG films and  
385 this increase was determined by the concentration applied. Additionally, the results displayed  
386 that PSGG-EGCG, followed by PSGG-BBA, PSGG-MAC and PSGG-BAN film, comprises  
387 more phenolic to reduce free radicals and to cause more stable products. Phenolic compounds  
388 contain one or more aromatic rings bearing hydroxyl groups and are consequently capable to  
389 quench free radicals by developing resonance-stabilized phenoxyl radicals (Dudonné et al.  
390 2009). Though, it should be taken into account that the antioxidant properties of natural extracts  
391 is not only due to phenolic compounds. Other components including ascorbates, reducing  
392 carbohydrates, tocopherols, carotenoids, terpenes, and pigments might give rise to antioxidant  
393 capacity (Babbar et al. 2011). In this study close relationship between TPC or TFC and  
394 antioxidant capacity (DPPH, CUPRAC and FRAP values) of PSGG films incorporated with  
395 various extracts was achieved and the results are illustrated in Table 4. This table shows the  
396 correlation of TPC and TFC with antioxidant properties of films formulated with natural  
397 compounds measured by DPPH, FRAP and CUPRAC. The higher value shows that the  
398 antioxidant activity of film is as a result of phenolic and flavonoid compounds in the extract.

399 **Conclusion**

400 Active packaging films based on pea starch and guar gum formulated with antioxidants  
401 compounds were effectively developed. The physical, optical and barrier characteristics of the  
402 PSGG films were mostly dependant on incorporated phenolic compounds. After incorporating  
403 active compounds into PSGG film, MC, WS, SD, WVP, and transparency of the films were  
404 significantly reduced. Results obtained from FTIR analysis exhibited that the modifications in  
405 the physical properties of films were nearly related to the interactions of polymers with  
406 antioxidant substances. The antioxidant activity of the PSGG film was noticeably enhanced  
407 after addition of natural compounds, which indicated the great potential of these films as active  
408 food packaging. Further studies should be taken into account regarding the use of these active  
409 packaging materials in vitro to determine the migration of phenolic compounds from the films  
410 and their effects on extending shelf-life during storage time.

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414 and macadamia skin extracts.

415 **Conflict of Interest**

416 The authors declare no conflict of interest.

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## 648 **Figure captions**

649 **Fig. 1** FTIR spectra of PSGG films containing different natural antioxidant compounds at 3  
650 mg/mL in the region 400-4000  $\text{cm}^{-1}$ .

651 **Fig. 2** Total phenol contents (A) and total flavonoid (B) of PSGG films formulated with  
652 different natural compounds at different concentrations.

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- 1 **Table 1** Moisture content (MC), water solubility (WS), gel fraction (GF), swelling degree (SD), and water vapour permeability (WVP) of edible
- 2 PSGG films as a function of natural compound concentrations.

Natural compounds concentration (mg/mL)	MC (%)	WS (%)	GF (%)	SD	WVP ( $\times 10^{-10}$ gs <sup>-1</sup> m <sup>-1</sup> Pa <sup>-1</sup> )
PSGG**	20.13 ± 1.48 <sup>cd</sup>	27.67 ± 2.72 <sup>a</sup>	15.87±2.75 <sup>m</sup>	56.85±5.50 <sup>a</sup>	13.87±1.11 <sup>bc</sup>
0.75	20.29±2.02 <sup>c</sup>	25.71±2.02 <sup>ab</sup>	38.96±8.33 <sup>ghi</sup>	45.63±3.03 <sup>bc</sup>	13.16±0.75 <sup>cd</sup>
1.5	25.63±1.59 <sup>a</sup>	23.56±1.59 <sup>bcd</sup>	55.63±9.39 <sup>def</sup>	52.29±6.05 <sup>ab</sup>	16.49±1.02 <sup>a</sup>
2.25	18.83±0.52 <sup>cdef</sup>	22.18±0.52 <sup>cde</sup>	67.29±4.33 <sup>ab</sup>	32.83±3.77 <sup>de</sup>	12.59±1.25 <sup>cde</sup>
3	17.49±0.76 <sup>efg</sup>	20.31±0.76 <sup>efg</sup>	76.71±5.74 <sup>a</sup>	25.83±3.28 <sup>efg</sup>	10.20±0.99 <sup>fg</sup>
0.75	19.33±0.95 <sup>cde</sup>	24.58±0.95 <sup>bc</sup>	29.96±1.53 <sup>ijk</sup>	40.83±4.87 <sup>c</sup>	12.83±0.85 <sup>cd</sup>
1.5	22.75±2.71 <sup>b</sup>	21.23±2.71 <sup>def</sup>	42.29±3.95 <sup>fgh</sup>	45.96±4.57 <sup>bc</sup>	14.99±2.08 <sup>ab</sup>
2.25	17.78±1.89 <sup>defg</sup>	19.18±1.89 <sup>fgh</sup>	58.29±8.07 <sup>bcd</sup>	27.74±4.76 <sup>def</sup>	11.41±0.66 <sup>def</sup>
3	16.49±1.05 <sup>fghi</sup>	17.64±1.05 <sup>hi</sup>	64.38±8.51 <sup>bc</sup>	21.16±1.59 <sup>fghi</sup>	9.32±0.57 <sup>gh</sup>
0.75	15.62±0.74 <sup>ghij</sup>	21.72±0.74 <sup>def</sup>	21.16±2.49 <sup>klm</sup>	33.23±6.23 <sup>d</sup>	10.89±0.33 <sup>efg</sup>
1.5	14.90±1.26 <sup>hij</sup>	20.23±1.26 <sup>efgh</sup>	33.02±5.39 <sup>hij</sup>	26.56±2.60 <sup>defg</sup>	9.71±0.64 <sup>fgh</sup>
2.25	13.49±0.90 <sup>jk</sup>	17.64±0.90 <sup>hi</sup>	45.06±7.62 <sup>efg</sup>	19.56±3.74 <sup>ghi</sup>	7.53±0.51 <sup>i</sup>
3	11.16±0.75 <sup>k</sup>	15.11±0.75 <sup>i</sup>	51.05±7.65 <sup>def</sup>	14.56±1.29 <sup>ij</sup>	7.05±0.80 <sup>i</sup>
0.75	17.29±0.95 <sup>efgh</sup>	22.25±0.95 <sup>cde</sup>	17.83±4.65 <sup>lm</sup>	29.83±2.71 <sup>de</sup>	11.49±0.72 <sup>def</sup>
1.5	15.90±0.43 <sup>ghij</sup>	20.89±0.43 <sup>efg</sup>	27.69±2.89 <sup>jkl</sup>	22.19±1.86 <sup>fgh</sup>	10.16±1.04 <sup>fg</sup>
2.25	14.57±0.80 <sup>ij</sup>	18.52±0.80 <sup>gh</sup>	39.63±3.03 <sup>ghi</sup>	17.89±3.05 <sup>hij</sup>	8.19±0.92 <sup>hi</sup>
3	13.49±1.90 <sup>jk</sup>	15.97±1.90 <sup>i</sup>	46.88±5.38 <sup>efg</sup>	12.56±0.76 <sup>j</sup>	7.39±1.27 <sup>i</sup>

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

4 \*\* Please refer to Saberi et al. (2016b) and Saberi et al. (2016a).

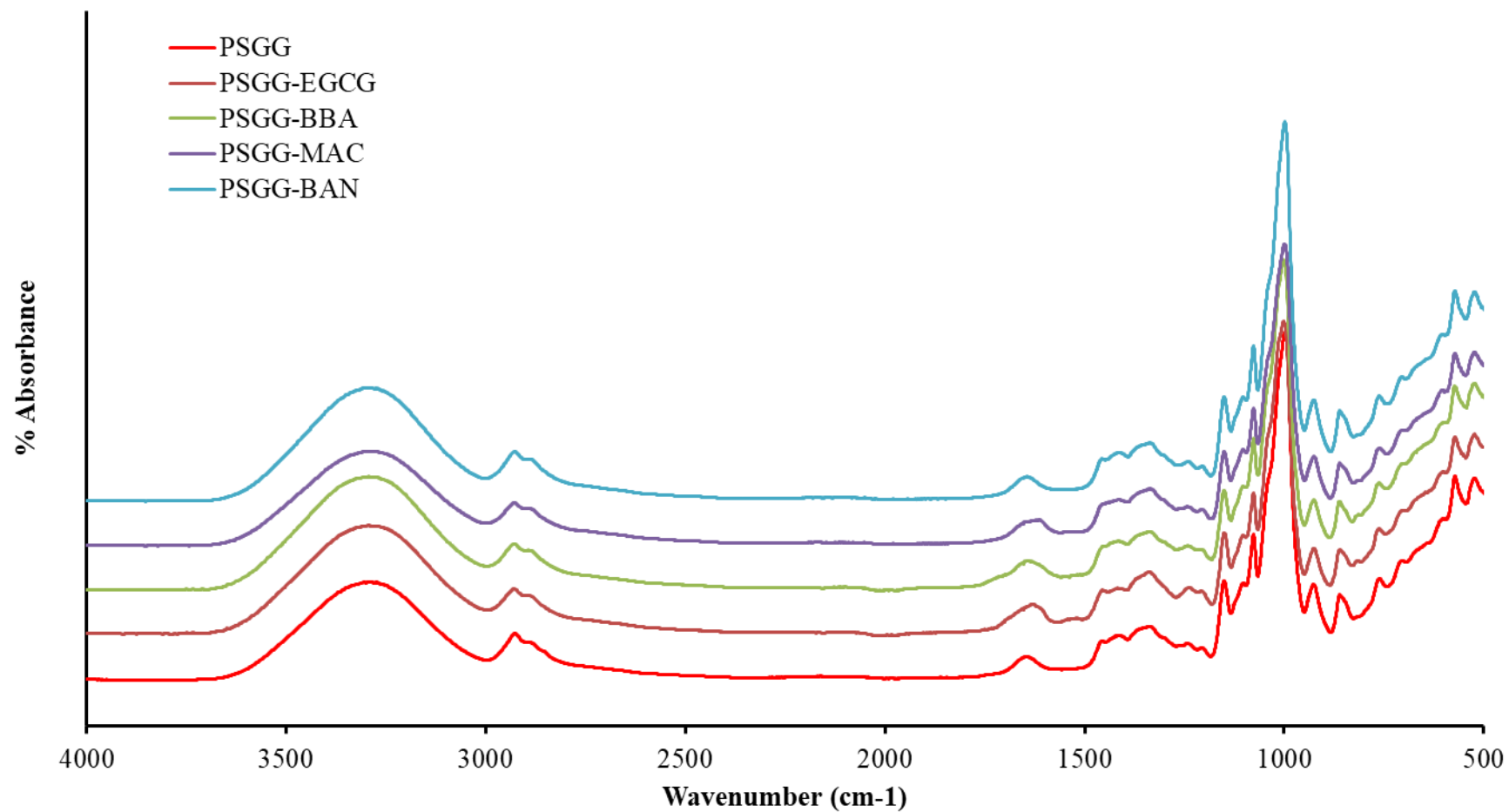
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1 **Table 2** Optical properties of edible PSGG films as a function of natural compound concentrations.

Natural compounds concentration (mg/mL)	<i>L</i>	<i>a</i>	<i>b</i>	$\Delta E$	Transparency (%)
PSGG**	93.84±2.48 <sup>a</sup>	-3.54±0.52 <sup>e</sup>	6.24±0.70 <sup>efg</sup>	4.54±0.51 <sup>i</sup>	82.27±4.67 <sup>a</sup>
0.75	91.71±0.64 <sup>bc</sup>	-2.04±0.27 <sup>d</sup>	6.38±0.37 <sup>ef</sup>	5.53±0.38 <sup>hi</sup>	82.11±0.78 <sup>a</sup>
1.5	90.85±0.80 <sup>bcd</sup>	-1.01±0.95 <sup>c</sup>	6.04±0.73 <sup>efg</sup>	6.70±0.69 <sup>fgh</sup>	81.54±0.77 <sup>ab</sup>
2.25	89.63±0.76 <sup>cdef</sup>	1.04±0.89 <sup>b</sup>	5.58±0.70 <sup>fgh</sup>	8.07±0.78 <sup>def</sup>	79.54±0.91 <sup>abcd</sup>
3	87.65±0.77 <sup>fghi</sup>	2.52±0.23 <sup>a</sup>	4.91±0.49 <sup>h</sup>	10.41±1.13 <sup>bc</sup>	78.21±1.75 <sup>cde</sup>
0.75	92.04±0.27 <sup>ab</sup>	-2.38±0.37 <sup>d</sup>	6.51±0.23 <sup>def</sup>	5.80±0.65 <sup>ghi</sup>	82.04±0.89 <sup>a</sup>
1.5	91.18±0.77 <sup>bc</sup>	-1.71±0.64 <sup>cd</sup>	6.18±0.67 <sup>efg</sup>	6.77±0.72 <sup>efgh</sup>	81.11±0.38 <sup>ab</sup>
2.25	90.67±0.46 <sup>bcd</sup>	0.98±0.21 <sup>b</sup>	5.84±0.44 <sup>efgh</sup>	8.89±0.72 <sup>d</sup>	80.21±1.92 <sup>abc</sup>
3	88.83±1.23 <sup>defg</sup>	1.44±0.54 <sup>b</sup>	5.18±1.00 <sup>gh</sup>	10.88±0.58 <sup>abc</sup>	79.21±0.78 <sup>bcd</sup>
0.75	90.51±0.59 <sup>bcd</sup>	-3.76±0.39 <sup>e</sup>	7.51±0.61 <sup>cd</sup>	7.12±0.56 <sup>efgh</sup>	81.38±0.37 <sup>ab</sup>
1.5	89.62±0.64 <sup>cdef</sup>	-4.00±0.66 <sup>ef</sup>	8.49±0.47 <sup>bc</sup>	8.31±0.58 <sup>de</sup>	80.05±0.12 <sup>abc</sup>
2.25	87.03±1.51 <sup>ghi</sup>	-4.33±0.29 <sup>ef</sup>	9.50±0.56 <sup>ab</sup>	11.10±1.31 <sup>ab</sup>	76.40±1.00 <sup>e</sup>
3	86.05±0.45 <sup>i</sup>	-4.82±0.50 <sup>f</sup>	10.17±0.68 <sup>a</sup>	12.29±0.67 <sup>a</sup>	73.83±1.49 <sup>f</sup>
0.75	91.04±0.73 <sup>bc</sup>	-3.70±0.33 <sup>e</sup>	6.94±0.13 <sup>de</sup>	6.44±0.67 <sup>gh</sup>	81.58±0.23 <sup>ab</sup>
1.5	90.28±0.80 <sup>bcd</sup>	-3.90±0.04 <sup>ef</sup>	7.52±0.48 <sup>cd</sup>	7.33±0.83 <sup>efg</sup>	80.57±0.70 <sup>abc</sup>
2.25	88.30±1.94 <sup>efgh</sup>	-4.10±0.31 <sup>ef</sup>	8.06±0.39 <sup>c</sup>	9.41±1.79 <sup>cd</sup>	77.06±1.62 <sup>de</sup>
3	86.72±1.02 <sup>hi</sup>	-4.32±0.35 <sup>ef</sup>	9.32±0.75 <sup>ab</sup>	11.34±0.72 <sup>ab</sup>	75.72±0.53 <sup>ef</sup>

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

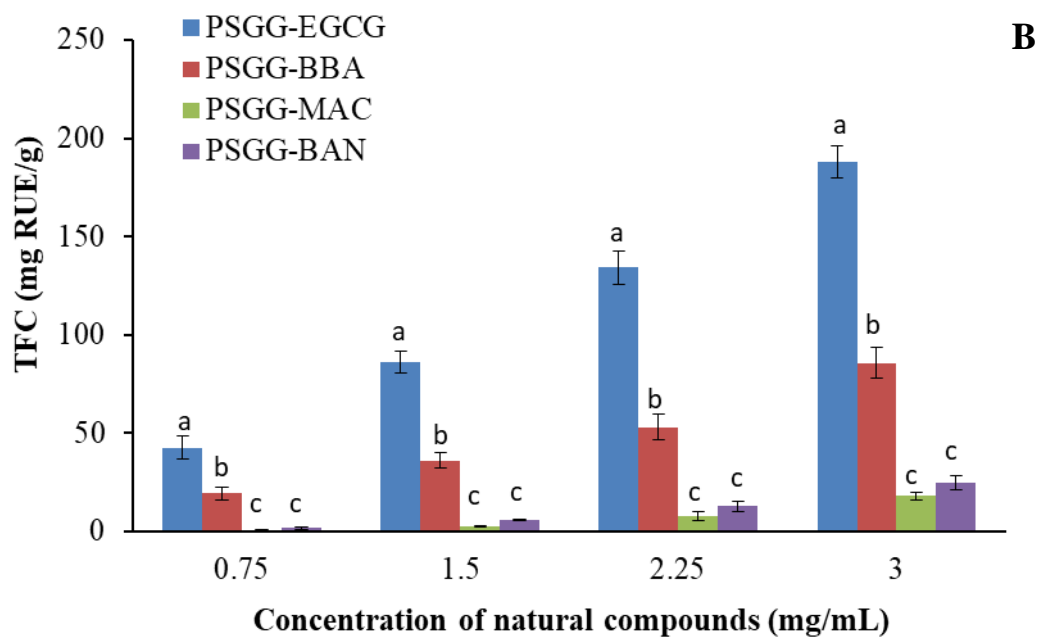
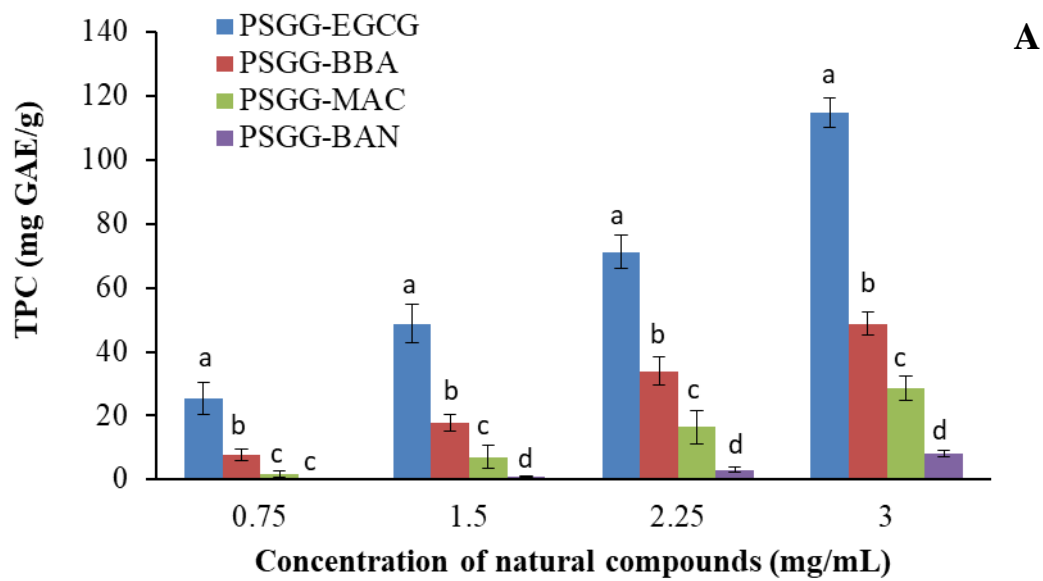
3 \*\* Please refer to Saberi et al. (2016b) and Saberi et al. (2016a).



1

2 **Fig. 1** FTIR spectra of PSGG films containing different natural antioxidant compounds at 3 mg/mL in the region 400-4000 cm<sup>-1</sup>.

3



1 **Fig. 2** Total phenol content (A) and total flavonoid content (B) of PSGG films formulated with  
 2 different natural compounds at different concentrations.

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4

1 **Table 3** Antioxidant effect of PSGG edible films incorporated with natural compounds at  
 2 different concentrations by means of three different antioxidant tests such as DPPH, CUPRAC,  
 3 and FRAPS assays.

Natural compounds concentration (mg/mL)	DPPH (mg TE/g)	CUPRAC (mg TE/g)	FRAP (mg TE/g)
0.75	4.34±0.70 <sup>efgh</sup>	2.62±0.55 <sup>def</sup>	9.08±0.83 <sup>de</sup>
1.5	8.95±2.46 <sup>cd</sup>	8.74±1.52 <sup>c</sup>	16.23±2.19 <sup>c</sup>
2.25	19.83±3.18 <sup>b</sup>	17.95±2.08 <sup>b</sup>	27.11±5.86 <sup>b</sup>
3	39.78±3.16 <sup>a</sup>	29.78±3.16 <sup>a</sup>	59.78±7.02 <sup>a</sup>
0.75	1.23±0.48 <sup>hi</sup>	0.95±0.27 <sup>efg</sup>	0.88±0.16 <sup>gh</sup>
1.5	5.36±1.35 <sup>ef</sup>	3.35±1.10 <sup>d</sup>	3.78±0.47 <sup>fgh</sup>
2.25	12.03±2.44 <sup>c</sup>	7.68±1.63 <sup>c</sup>	10.45±1.06 <sup>d</sup>
3	19.75±2.80 <sup>b</sup>	17.11±1.08 <sup>b</sup>	16.64±1.48 <sup>c</sup>
0.75	0.47±0.09 <sup>i</sup>	0.13±0.10 <sup>g</sup>	0.13±0.10 <sup>h</sup>
1.5	1.51±0.73 <sup>ghi</sup>	0.85±0.34 <sup>efg</sup>	0.60±0.34 <sup>gh</sup>
2.25	4.54±0.73 <sup>efg</sup>	3.35±0.87 <sup>d</sup>	2.83±0.54 <sup>fgh</sup>
3	10.21±2.24 <sup>c</sup>	8.68±1.55 <sup>c</sup>	6.74±1.35 <sup>def</sup>
0.75	0.17±0.09 <sup>i</sup>	0.06±0.032 <sup>g</sup>	0.02±0.01 <sup>h</sup>
1.5	0.69±0.13 <sup>i</sup>	0.48±0.06 <sup>fg</sup>	0.37±0.26 <sup>gh</sup>
2.25	2.90±0.82 <sup>fghi</sup>	3.02±0.68 <sup>de</sup>	1.50±0.17 <sup>gh</sup>
3	6.23±1.37 <sup>de</sup>	7.35±1.09 <sup>c</sup>	4.84±0.71 <sup>efg</sup>

4 \* Values are the means of triplicates ± standard deviations. Means at same column with different lower case are  
 5 significantly different ( $P < 0.05$ ).

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**Table 4** Correlation between phytochemicals and antioxidant properties of films containing natural compounds.

Films with natural compounds	DPPH	CUPRAC	FRAP
TPC	0.981	0.993	0.967
TFC	0.945	0.991	0.909
TPC	0.997	0.944	0.996
TFC	0.993	0.940	0.986
TPC	0.979	0.965	0.972
TFC	0.950	0.998	0.997
TPC	0.993	0.997	0.996
TFC	0.992	0.985	0.965