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Development of chitosan/cycloolefin copolymer and chitosan/polycaprolactone active bilayer films incorporated with grape seed extract and carvacrol

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Ece Sogut*[1] (https://orcid.org/0000-0003-4052-993X), Atif Can Seydim[1] (ORCID ID 0000-0003-3808-509X), Amparo Chiralt[2] (ORCID ID 0000-0003-1134-8144)

[1]Department of Food Engineering, Faculty of Engineering, Suleyman Demirel University, Isparta, Turkey

[2]Universitat Politècnica de València, School of Agricultural Engineering and Environment, Department of Food Technology, Institute of Food Engineering for Development, Valencia, Spain

Corresponding author *ececagdas@sdu.edu.tr

ABSTRACT

Chitosan (CH) bilayer films were obtained by casting polycaprolactone (PCL) or cycloolefin copolymer (COC) onto the CH film surface. Active components, such as grape seed extract (GSE, 5%, w/w) and carvacrol (CV, 10%, w/w), were incorporated into the bilayers. Film samples were characterized as the release behavior of CV in ethanol, antioxidant activity, water vapor permeability (WVP), tensile properties, and optical characteristics. The contact angles of COC and PCL chloroform solutions on the CH-based film surface were also analyzed to evaluate the potential wettability of CH films with PCL or COC solutions. A lower release rate was observed in COC-based bilayer films when compared to PCL-based bilayers. Film samples including GSE and CV showed radical scavenging activity, reducing the radical between 10 - 15 %. The PCL or COC layers on CH improved the WVP, while GSE also negatively affected WVP. The combination of GSE and CV in the bilayer film formulations enhanced the films' tensile properties. All film samples showed an internal transmittance value of around 80%.

Keywords: Chitosan, Cycloolefin copolymer, Polycaprolactone, Grape seed extract, Carvacrol, Bilayer film

Declarations

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Conflicts of interest/Competing interests

The authors declare that there is no conflict of interest.

Availability of data and material

The authors confirm that the data supporting the findings of this study are available within the article.

Code availability

Not applicable.

Authors' contributions

Ece Sogut: Formal analysis, Methodology, Writing - original draft, Writing - review & editing. Atif Can Seydim: Conceptualization, Methodology, Writing- Reviewing and Editing. Amparo Chiralt: Conceptualization, Investigation, Methodology, Resources, Writing- Reviewing and Editing.

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Not applicable.

1. INTRODUCTION

In recent years, the demand for biodegradable packaging materials obtained from renewable sources instead of synthetic plastics has increased while requiring additional potential to improve food quality and safety. Combining bio-based plastics with commonly used synthetic plastics has been accepted as the first step for the solutions to the environmental problems arising from the overuse of synthetic plastics [1]. Thus, internal layers, contact with food surfaces, and bio-based coatings have been proposed to enhance the barrier and thermomechanical properties of bio-based materials [2]. Chitin is the raw material used to produce chitosan (CH), a linear cationic (1,4)-2-amino-2-deoxy-β-D-glucan [3]. It is found abundantly in nature and presents intrinsic antimicrobial activity against various microorganisms [4]. Although CH has good gas barrier properties, biocompatible nature, and convenience for food packaging applications, it has limited water vapor barrier capacity due to its hydrophilic nature [5].

Cycloolefin copolymer (COC), a synthetic engineering polymer, is produced by the copolymerization of ethylene and cyclic olefins. COC is highly resistant to chemicals and has low birefringence, low affinity to water, and low specific gravity while showing high transparency [6]. Polycaprolactone (PCL) is another synthetic polymer chemically synthesized from crude oil by the ring-opening polymerization of caprolactone monomer while showing biodegradable properties [7]. By blending or lamination of these synthetic polymers with other polymers have been studied by many researchers to improve the physicomechanical properties, rheological behavior, and thermal properties and to increase the suitability of bio-based plastics intended to be used in food packaging applications [8–12]. Although blending PCL or COC with CH can be a promising method to enhance CH films' properties, a simple solution blending technique is unsuitable for these polymers because of differences in polarities and lack of chemical affinity.

Limited adhesion and low affinity between polymer interfaces may produce phase-separated polymers leading to poor functional properties [13]. The compatibility between CH and other non-polar polymers has been improved by using different techniques. Few studies have been focused on increasing the affinity between polymers by using different compatibilizers such as methylene diphenyl diisocyanate and dioctyl maleate. In contrast, others analyzed the chemical interactions at the interface, providing improvement in blend properties without notable phase separation [4, 14–16].

Furthermore, the combination of PCL and COC with CH by coating the surface of CH can be another choice to enhance the functional properties of CH films. Particularly, barrier properties of layered materials can be improved by combining sheets of polymers with a complementary barrier capacity to water vapor and gases. In this sense, bilayer films of PCL coatings on the CH film surface and COC coatings on the CH film surface were obtained in this study.

Incorporating active agents, with antioxidant or antimicrobial activity, into the polymer layers may improve the potential functionality of the newly developed food packaging materials. In this study, grape seed extract (GSE) and/or carvacrol (CV) have been incorporated in one or both layers of the bilayer assembly to obtain active films. GSE includes monomeric phenolic constituents and anthocyanins, showing various biological effects such as antimicrobial activity and antioxidant activity [17, 18]. Several plant essential oils have also been reported as natural antimicrobial or antioxidant compounds. CV, which is the main component of thyme and oregano essential oils, exhibits strong antimicrobial activity against a wide range of microorganisms [19, 20]. CV has a hydrophobic nature, while GSE is highly hydrophilic. The combinations of natural active agents and their possible synergistic interactions may increase the biological activity of those natural active agents [21].

On the other hand, the strong charges, chain flexibility, surface energy, functional groups such as -OH, -COOH, giving a positive charge on NH_3^+ groups in acidic solutions, and forming attractive forces make CH suitable to adhere to other polymer surfaces, thus this may provide positive interactions at the interface of bilayer films [14]. Besides, the active properties of CH, such as being antimicrobial and antioxidant, may provide a potential to be used as active internal layers [5]. CH also provides the bilayers with high oxygen barrier capacity while having a high barrier to water vapor with the second hydrophobic layer made of PCL and COC films. Researches about bilayer films, including both bio-based polymers, have gained attention in recent years. However, the studies on the combination of bio-based polymers with synthetic polymers, aiming to reduce synthetic polymer use, are limited. Thus, in this study, active films were developed by including GSE and CV into PCL or COC-coated CH bilayer films. Water vapor permeability, tensile and optical properties of bilayer films, and the final content of carvacrol in the films and their antioxidant activity were analyzed.

2. MATERIALS AND METHODS

2.1. Materials

Chitosan (CH) (M_w of 850000 D) and Polycaprolactone (PCL) (M_w of 80,000 D) were provided from Sigma-Aldrich (Sigma–Aldrich Co., LLC, Madrid, Spain). Cycloolefin copolymer (COC) (8007F-04 grade) was kindly supplied from TOPAS Advanced Polymers (Frankfurt-Höchst, Germany). Carvacrol (CV), magnesium nitrate 6-hydrate, phosphorus pentoxide, ethanol, and 2,2-diphenyl-1-picrylhydrazyl (DDPH) were purchased from Panreac Química, S.A. (Castellar del Vallés, Barcelona, Spain). Grape seed extract (GSE) was obtained by solid-liquid extraction (10%, w/w) from grape seeds, supplied from a local winery in Denizli/Turkey, for six h at 25°C. The extract was then freeze-dried (BW-100F, Bluewave Industry Co., Ltd. Shanghai, China) using

125 microcrystalline cellulose as a carrier (4.5%, w/w) after filtration under vacuum to collect the
126 phenolics in powder form. The total phenolic content of the water-soluble GSE was found as
127 4.5±0.5 mg GAE/kg, mainly including gallic acid (4.02±0.15 ppm), catechin (1.64±0.12 ppm),
128 and epicatechin (0.05±0.001 ppm).

129 **2.2. Film preparation**

130 CH films containing grape seed extract (CH-GSE) or without (CH) were obtained using the casting
131 method. CH (1.5%, w/w) was dissolved in 3% acetic acid solution to prepare CH film-forming
132 solution, and then glycerol at 20% (w/w, based on CH) was added to the fully dissolved film-
133 forming solution. Part of the CH film solution was incorporated with GSE (5 g/100 g CH) to obtain
134 bilayers with GSE. Subsequently, the air bubbles were removed from the CH film solution by
135 degassing under a vacuum. Film solutions were poured on a Teflon© petri dish (D=15 cm, 40
136 g/petri) and dried (~72 h) at ambient conditions. PCL and COC were dissolved in chloroform to
137 prepare 7.5% (w/w) film solutions with and without CV (10% w/w, based on solid content). The
138 surface of dried CH films was coated with PCL or COC film solutions, with and without CV (4.5
139 g), using a coating rod (bar thickness 200 μm) to produce bilayer films. In this sense, 4.5 g of PCL
140 or CCOC solution gave a CH/PCL or CH/COC ratio of 2:1 (g/g). Bilayer films were also obtained
141 by using the same procedure with the CH-GSE matrix. Then, eight different bilayers were obtained
142 (Table 1) with or without the active components (GSE and/or CV).

143 The characterization analyses were carried out after conditioning film samples at 25°C and 53%
144 relative humidity (RH) for one week. The thickness of six randomly selected points was measured
145 by a digital micrometer (Palmer-Comecta, Spain, ±0.001 mm).

146 **2.3. Contact angle measurement**

147 The potential wettability of PCL and COC chloroform solutions on the CH film surface was
148 evaluated through the contact angle test [14]. A glass slide was used to mount the CH and CH-
149 GSE films, and then the coating film solutions (PCL and COC) were dropped on the air contact
150 side of CH films. The contact time of film solutions was adjusted at 20 s. The sessile drop fitting
151 method was used to study the shape of drop (0.015 mL) using a video-based contact angle meter
152 (OCA 20, Data Physics Instruments GmbH, Filderstadt, Germany) SCA20 software for image
153 analyses.

154 **2.4. Estimation of carvacrol content in films**

155 The CV amount released from the film matrix into ethanol was determined spectrophotometrically
156 [22]. Before the CV extraction process, films were stored inside a desiccator with P_2O_5 for 24 h to
157 achieve a moisture balance. Afterward, dried films (4 mg) were immersed in ethanol (15 mL), and
158 the extract was further analyzed in the CV content at different contact times (15 min – 120 h). The
159 CV content of films was determined by a CV standard calibration curve, and a film sample without
160 CV was used as a control. All calculations were performed by measuring the absorbance at 275
161 nm using a UV/IR spectrophotometer (Evolution 201, Thermo Scientific).

162 Peleg's equation (Eq. 1) [23] was also used to model the release kinetics of CV in ethanol.

163 $$M_t = t / [(k_1) + (k_2 t)] \quad (1)$$

164 where M_t is the amount of CV released at each time, and the kinetic constants, k_1 and k_2, are the
165 inverse of the initial release rate and the inverse of the asymptotic release value, respectively.

166 **2.5. Antioxidant activity**

167 The potential antioxidant activity of film samples was measured by using the radical 2,2-diphenyl-
168 1-picrylhydrazyl (DPPH) reducing activity [24]. Briefly, film samples (0.1 g) were dissolved in 10

ml of acetic acid solution (3%, w/w) before the treatment with 0.07 mM DPPH solutions and then incubated at 25°C in the dark for 40 min. The absorbance of samples was read at 517 nm against ethanol as the blank, and the DPPH scavenging activity was expressed as the percentage of DPPH reduced.

2.6. Water Vapor Permeability (WVP) and mechanical properties of film samples

ASTM E96-95 gravimetric method [25] was used to measure films' water vapor permeability (WVP). Designated permeability cups (Payne, Elcometer SPRL, Belgium) stored at 53% RH, and 25°C were weighted periodically by exposing the CH layer to 100% RH for every treatment.

The mechanical properties were determined according to the test conditions explained in ASTM standard method D882 [26]. A universal test Machine (TA.XTplus model, Stable Micro Systems, Haslemere, England) was used to measure the elastic modulus (EM), tensile strength (TS), and percent elongation (%ε) at break values of film samples. The stress-strain curves were obtained from force-distance data of film samples (2.5 cm x 2.5 cm) that broke while stretching at 50 mm/min.

2.7. Optical properties of film samples

A Minolta spectrophotometer (CM-3600d model, Minolta Co., Tokyo, Japan) was used to determine the internal transmittance (T_i) of the film samples from the surface reflectance spectra (400–700 nm) on both white and black backgrounds [27].

ASTM D523-14 standard method [28] was followed to measure the surface gloss values of film samples at 60° incidence angle using a surface gloss meter (Multi Gloss 268, Minolta, Germany). Results were expressed as gloss units (GU) relative to a highly polished surface of black glass standard with a value near 100 GU.

2.8. Statistical analysis

The statistical analysis was performed using Minitab 17 software (Minitab Inc., Brandon, UK). Each experiment was replicated twice, with at least five observations for each sample. The differences between samples were determined with an analysis of variance (ANOVA). Data were analyzed using Tukey's multiple comparison tests at a 95 % confidence level, considering the type of formulation as a factor.

3. RESULTS AND DISCUSSION

3.1. Coating extensibility on the chitosan layer

The contact angle is the angle between the solid surface and the droplet, determined by a tangent passing through the triple point of the air, liquid, and solid phases. Geometrically, it is defined as a smooth, chemically homogeneous, rigid, nonreactive, and insoluble surface at equilibrium. Therefore, it was aimed to determine the surface wetting properties of CH and CH-GSE films with the PCL and COC film-forming solutions with or without CV. The obtained contact angle values are shown in Figure 1.

The contact angle will depend on the hydrophobic character of the tested surface of the solid biomaterials: entirely hydrophobic polymer has higher contact angle values (≥90°), whereas polymers displaying hydrophilic nature have lower contact angle values (<90°) [29]. All solutions had values lower than 90° indicating the film-forming solutions' wetting ability on CH film surface. Therefore, it offers a possibility to obtain multilayer films made of CH and PCL or CH and COC. The lower contact angle values were obtained for the PCL film-forming solutions, which might be due to better adsorption of PCL at the CH interface during the coating process [30]. The presence of CV in the PCL solution resulted in higher contact angle values, thus suggesting that CV did not favor the extensibility of the solution on the CH surface. Likewise, the incorporation

214 of GSE into the CH matrix also lowered the extensibility of the PCL film-forming solution on the
215 CH surface. The highest contact angle value was found in COC film-forming solution on the
216 surface of CH-GSE films.

217 Moreover, CH films containing GSE exhibited higher contact angles than pure CH films, while
218 CV incorporation did not favor the wettability of PCL or COC solutions on the CH surface. The
219 non-polar acetyl amide fragments of CH and the incompatibility of chloroform-containing film-
220 forming solutions with CH film might cause a decrease in the values of contact angle. Optimization
221 of wettability means optimizing cohesion and adhesion forces, promoting the spreading ability of
222 a liquid on the surface. Many studies have shown that spreading, swelling, absorption, and
223 evaporation were the mechanisms occurring on the surface of bio-based polymers [31–33]. The
224 contact angle is mainly influenced by the surface interactions of components at the solid-liquid
225 contact area when the evaporation is negligible.

226 **3.2. Carvacrol content in the films deduced from its release in ethanol**

227 CV as a strong antioxidant/antimicrobial has been accepted as a valuable compound for food safety
228 issues. Thus, incorporating CV into multilayer films is one of the easiest ways to control solubility
229 and release of CV into the food substrate. The released amount of CV was determined
230 spectrophotometrically through ethanol as the release medium. The release behavior of CV
231 included in PCL and COC monolayer films and bilayer film samples is presented in Figure 2.
232 Experimental data were well fitted to the Peleg's model (R^2>0.98) to obtain the initial release rate
233 ($1/k_1$) and the equilibrium/asymptotic value ($1/k_2$). These parameters are shown in Table 2 for PCL
234 and COC monolayer and bilayer films.

235 According to Zhu et al. [34], an agent is released from a polymeric matrix in three stages: the
236 penetration of solvent into the polymer, the swelling of the polymer, and the diffusion of the agent
237 from the swollen matrix until it reaches the equilibrium. Slower release rates ($1/k_1$) were obtained
238 for COC-based bilayer films within 6 h compared to PCL-based bilayers, which might be due to
239 the limited diffusion of ethanol into the COC matrix. However, lower release rates were found in
240 PCL-based bilayer films after 24 h due to the reduction in the driving force for mass transfer.
241 Generally, for the first 24 h, diffusion of CV was faster due to the increased CV mobility via a
242 more open structure associated with solvent diffusion and polymer relaxation. Afterward, the
243 release of CV reached a plateau level when equilibrium was achieved.

244 Similarly, Scaffaro et al. [35] reported a faster release followed by a plateau reaching an
245 equilibrium for CV release from polylactic acid and Bio-flex-based bilayer films. The release rate
246 for active compounds from developed films is significant for its potential use in active films or
247 coatings. The equilibrium values of CV release were very close to the incorporated amount in the
248 corresponding monolayers (10%), suggesting that no significant losses of the compound occurred
249 during the film drying step, and a complete release of the compound occurred in the ethanol media.
250 Likewise, the equilibrium values obtained for the CV amount of the bilayer films agree with the
251 added amount of CV to the PCL or COC monolayers regarding the mass ratio of CH/PCL and
252 CH/COC bilayers. Therefore, all film samples reached the practically total release of CV according
253 to their respective incorporated amounts, suggesting that CV might entirely be depleted from
254 swollen bilayer films because of structural rearrangements in the polymer matrix associated with
255 the polymer relaxation in contact with the ethanol.

256 The CH layer did not significantly change the CV release from bilayer films, which can be
257 attributed to the ethanol contact with the hydrophobic layer (COC o PCL) containing CV and the

CV diffusion mainly through these layers to the solvent medium. Similarly, Luis et al. [36] had found high release rates for isopropyl palmitate from zein and pullulan-based bilayer films at 25°C when the surrounding medium was %50 ethanol.

3.3. Antioxidant Activity

Antioxidant packaging can extend the food shelf life by scavenging free radicals due to the reactivity of the phenolic compounds. Thus, film samples including antioxidants provide enhanced nutritional quality for food products while maintaining the sensorial integrity of food products. Figure 3 shows the antioxidant activities of all film samples determined by DPPH assaying the capacity of scavenging free radicals.

The antioxidant activity of film samples was determined whether CV and GSE retained their antioxidant capacity inside the film matrix. The antioxidant activity of pure GSE and CV at the same concentration incorporated into films was also determined and found as 27.8±0.5 % and 22.75±0.04 %, respectively. Even though these values were higher than those obtained for related film samples, incorporating GSE and CV into the film samples has positively affected their antioxidant properties by enhancing their free radical scavenging capacity. As expected, bilayer films containing both CV and GSE showed higher values ($p<0.05$). However, the antioxidant activity of film samples including GSE was higher than film samples, including only CV ($p<0.05$), which can be attributed to the different releases of actives in the reaction media used to determine the DPPH scavenging activity. The incorporation of both GSE and CV in the bilayer films resulted in the highest antioxidant activity, which might be due to the synergistic effect of both compounds. CH/COC-CV and CH/PCL-CV bilayer films had the lowest values, which could be due to the second layer delaying the release of CV owing to the lower available surface. Besides, CH layers not including GSE might behave as a physical barrier, leading to reduced CV release from the

281 layers. These results were similar to those reported by Bharathi et al. [37] for CH-based bilayer
282 films coated with zein nanofibers. On the other hand, Luis et al. [36] did not find any significant
283 increase in the DPPH scavenging activity (%) of zein/pullulan bilayer films when incorporated
284 with isopropyl palmitate.

285 **3.4. Water vapor permeability (WVP) and mechanical properties of film samples**

286 The water vapor permeability (WVP) values of film samples are shown in Table 3. The WVP
287 values of COC-based bilayer films were significantly lower than PCL-based bilayer films
288 ($p<0.05$). Likewise, bilayer films containing CV presented higher WVP values than bilayer films
289 without CV, which might be explained by the plasticizing effect of CV leading to an increase in
290 the chain mobility by diluting and softening the structure [38]. Consequently, CV inclusion slightly
291 promoted water molecule transfer across the film, thus increasing the WVP values. It was also
292 shown that water barrier properties did not change by increasing the hydrophobic character of film
293 structure due to channels for transferring water molecules [39]. Similar behavior was reported by
294 Zhou et al. [40] and Ding et al. [41] for bilayer films composed of polylactic acid/pea starch, and
295 polyvinyl alcohol/chitosan, respectively.

296 Table 3 presents the elastic modulus (EM), tensile strength (TS), and elongation at break (ε, %)
297 values of film samples. Stress-strain curves obtained for film samples are presented in Figure 4.
298 Generally, PCL-based bilayer films had higher EM and TS values, while the highest elongation at
299 break values was found in CH/COC bilayer film ($p<0.05$). The rigidity of a polymeric matrix is
300 related to its elastic modulus. The coating of CH with PCL and COC film solutions, including CV,
301 slightly increase the EM values while a higher increase was observed for GSE-included bilayer
302 films. The addition of GSE might contribute to the attractive forces between water molecules and
303 polymer chains, which are polarized by chain bonds of CH with charged amino groups. Coating

304 the CH surface with film solutions with and without CV affected the mechanical response of the
305 film due to the coupling of the mechanical response of each layer and the contributions of the
306 interfacial adhesion forces of the sheets [42]. The lowest TS value was found in CH/COC bilayer
307 film while having the highest ε (%) value ($p<0.05$). The lowest ε values were obtained for PCL-
308 based bilayer films, with higher EM and TS values ($p<0.05$). In general, higher EM and TS values
309 and a slight decrease in extensibility were observed compared to control films (CH). Similar
310 effects were also reported on chitosan and gelatin bilayer films incorporated with ethyl lauroyl
311 arginate [43].

312 **3.5. Optical properties of film samples**

313 The optical properties of bio-based films and coatings are important parameters in food packaging
314 applications due to both quality preservation and hedonistic point of view. Besides, there is an
315 increasing interest to see through the package leading industries to search for new solutions.
316 Likewise, gloss is associated with surface roughness, influenced by film processing and
317 compatibility of the components. The internal transmittance is related to the film transparency and
318 the microstructure of the polymeric matrices, and the distribution of the compounds. The different
319 degree of structural homogeneity leads to changes in light scattering and optical properties of the
320 material. Thus, the optical properties may provide information about the suitability of the
321 packaging material for different uses and their microstructural homogeneity. The thickness,
322 internal transmittance values, and gloss values (60°) of bilayer faces of the film samples are
323 presented in Table 4.

324 All CH film solutions were cast at the same amount per plate and then coated with the same PCL-
325 and COC-based film solutions. However, film thickness tended to increase when GSE was added
326 to CH films, which might be due to the hydrophilic nature of GSE, leading to retaining more water

327 molecules, thus providing thicker film samples. Similarly, Adilah et al. [44] reported higher
328 thickness values for polyethylene and gelatin-based bilayer films, including mango peel extract,
329 compared to control bilayer films.

330 The gloss of the bilayer films, including GSE, presented lower values, whereas the inclusion of
331 CV resulted in higher gloss values ($p<0.05$), which might be due to the differences in light
332 scattering properties of active compounds [45]. Transparency of bilayer films, including CV was
333 higher than that of films including GSE. CH/PCL film had the highest internal transmittance value
334 (Ti, %), while CH-GSE/PCL-CV film had the lowest transmittance value ($p<0.05$). These lower
335 values might be related to a non-homogeneous surface and morphological irregularities arising
336 from the uneven coating process. Transparency of a bio-based film is related to its morphology
337 even than its chemical structure. CV droplets inside the PCL and COC film structures might
338 increase the light scattering through the film, thus lowering the transmittance. Generally, film
339 samples had high transmittance, around 80% in the visible region, indicating transparent films.
340 The internal transmittance values of film samples were close to each other demonstrating
341 suitability for samples that required visible packaging materials. However, optical properties must
342 be improved by optimizing process parameters such as homogenization thickness, amount, and
343 type of active compounds. Similarly, Haghighi et al. [43] reported that gelatin and CH-based
344 bilayer films showed lower light transmission values after the addition of lauroyl arginate ethyl.
345 Conversely, Velickova et al. [46] found that the coating of chitosan films with beeswax to obtain
346 multilayered film structure caused an increase in the lightness of films.

347 **4. CONCLUSION**

348 The thin coating of CH films with commonly used non-polar synthetic polymers, such as PCL or
349 COC, can enhance the functional properties of the bio-based polymer. The contact angle of the

chloroform solutions of the non-polar polymers was lower than 90° indicating a good wettability of the CH surface with the polymer solutions. CV incorporation into the coating forming solutions and GSE extract into the CH layer provided the antioxidant capacity to the bilayer films. A lower release rate of CV was observed in COC-based films when compared to PCL-based bilayers. The formation of the second layer on CH also improved the WVP and the mechanical properties, while the addition of GSE and CV lowered the transparency of film samples. These results showed that the obtained bilayer films can be used as active layers for food products and could reduce synthetic plastic films use. Bilayer films can also be used in applications requiring a controlled release along with active food packaging applications. However, further analysis and detailed information about the structure of the final bilayers must be carried out. Likewise, the active release and antioxidant performance of obtained bilayer films should be evaluated when in contact with a specific food.

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483

484 **Table 1.** Film description/codes

Code	Explanation
CH/PCL	Chitosan films (CH) coated with polycaprolactone (PCL)
CH/COC	Chitosan films coated with cycloolefin copolymer (COC)
CH-GSE/PCL	Chitosan films including grape seed extract (GSE) and coated with polycaprolactone
CH-GSE/COC	Chitosan films including grape seed extract and coated with cycloolefin copolymer
CH/PCL-CV	Chitosan films coated with polycaprolactone including carvacrol (CV)
CH/COC-CV	Chitosan films coated with cycloolefin copolymer including carvacrol
CH-GSE/PCL-CV	Chitosan films including grape seed extract and coated with polycaprolactone including carvacrol
C-GSE/COC-CV	Chitosan films including grape seed extract and coated with cycloolefin copolymer including carvacrol

485

486

Table 2. Peleg's model kinetics

Sample	Theoretical CV content (g/g film)*	Peleg's model**		
		1/1/k_1 (wt.% /min)	**1/k_2 (g/g film)**	**R^2**
PCL-CV* **	10	0.049±0.001[c]	9.31±0.06[b]	0.980
COC-CV* **	10	0.017±0.001[d]	10.4±0.07[a]	0.984
CH/PCL-CV	3.1	0.100±0.006[a]	3.21±0.02[d]	0.999
CH/COC-CV	3.1	0.017±0.001[d]	3.74±0.01[c]	0.996
CH-GSE/PCL-CV	3.0	0.084±0.004[b]	3.71±0.01[c]	0.999
CH-GSE/COC-CV	3.0	0.018±0.001[d]	3.71±0.02[c]	0.997

Any two means in the same column followed by the same letter were not significantly different ($p > 0.05$) by Tukey's multiple range test.

*Determined from the incorporated amount in the monolayer formulation and mass ratio of each layer in the bilayer.

** k_1 is related to the release rate at the beginning of the process and k_2 is related to the asymptotic value, which is obtained from the inverse of M∞, the amount of active agents released at equilibrium.

*** PCL-CV and COC-CV are monolayers that are PCL and COC based monolayers including CV

Table 3. Tensile properties and water vapor permeability values of film samples.

Film samples	WVP ($g\ mm\ kPa^{-1}h^{-1}m^{-2}$)	EM (GPa)	TS (MPa)	ε (%)
CH/PCL	0.67 ± 0.10^{ab}	0.56 ± 0.11^{bc}	32 ± 2^{ab}	21 ± 6^{b}
CH/COC	0.15 ± 0.08^{c}	0.39 ± 0.06^{c}	29 ± 5^{b}	28 ± 3^{a}
CH-GSE/PCL	0.61 ± 0.07^{b}	1.01 ± 0.14^{a}	32 ± 3^{ab}	8.5 ± 1.2^{c}
CH-GSE/COC	0.03 ± 0.01^{c}	0.67 ± 0.19^{b}	35 ± 4^{a}	15 ± 3^{bc}
CH/PCL-CV	0.83 ± 0.05^{ab}	1.03 ± 0.25^{a}	36 ± 5^{a}	13 ± 7^{bc}
CH/COC-CV	0.13 ± 0.06^{c}	0.40 ± 0.06^{c}	30 ± 4^{ab}	14.9 ± 0.7^{bc}
CH-GSE/PCL-CV	0.87 ± 0.24^{a}	1.08 ± 0.13^{a}	31 ± 3^{ab}	8.2 ± 1.7^{c}
CH-GSE/COC-CV	0.28 ± 0.04^{c}	1.03 ± 0.17^{a}	32 ± 2^{ab}	6.3 ± 1.4^{c}

Any two means in the same column followed by the same letter were not significantly different ($p > 0.05$) by Tukey's multiple range test.

Table 4. Thickness and Optical properties (internal transmittance and gloss) of film samples

Film samples	Thickness (μm)	T_i (% 450 nm)	Gloss (60º)
CH/PCL	69±3^{b}	79.6±0.2^{a}	7.0±1.3^{c}
CH/COC	74±2^{b}	76.6±0.4^{bc}	13.8±1.5^{a}
CH-GSE/PCL	72±2^{b}	76.1±0.8^{bc}	6.7±1.5^{c}
CH-GSE/COC	86±5^{a}	77.0±2.0^{ab}	7.5±2.2^{c}
CH/PCL-CV	73±4^{b}	77.7±0.7^{ab}	9.4±2.0^{bc}
CH/COC-CV	75±2^{b}	78.2±1.8^{ab}	14.0±3.0^{a}
CH-GSE/PCL-CV	73±3^{b}	75.2±1.1^{c}	12.5±1.2^{ab}
CH-GSE/COC-CV	74±2^{b}	75.7±0.8^{bc}	13.1±2.8^{ab}

Any two means in the same column followed by the same letter were not significantly different ($p > 0.05$) by Tukey's multiple range test.

507 **FIGURE CAPTIONS**

508 **Fig. 1** Contact angle values of PCL and COC film solutions with and without CV on the surface
509 of CH and CH-GSE films.

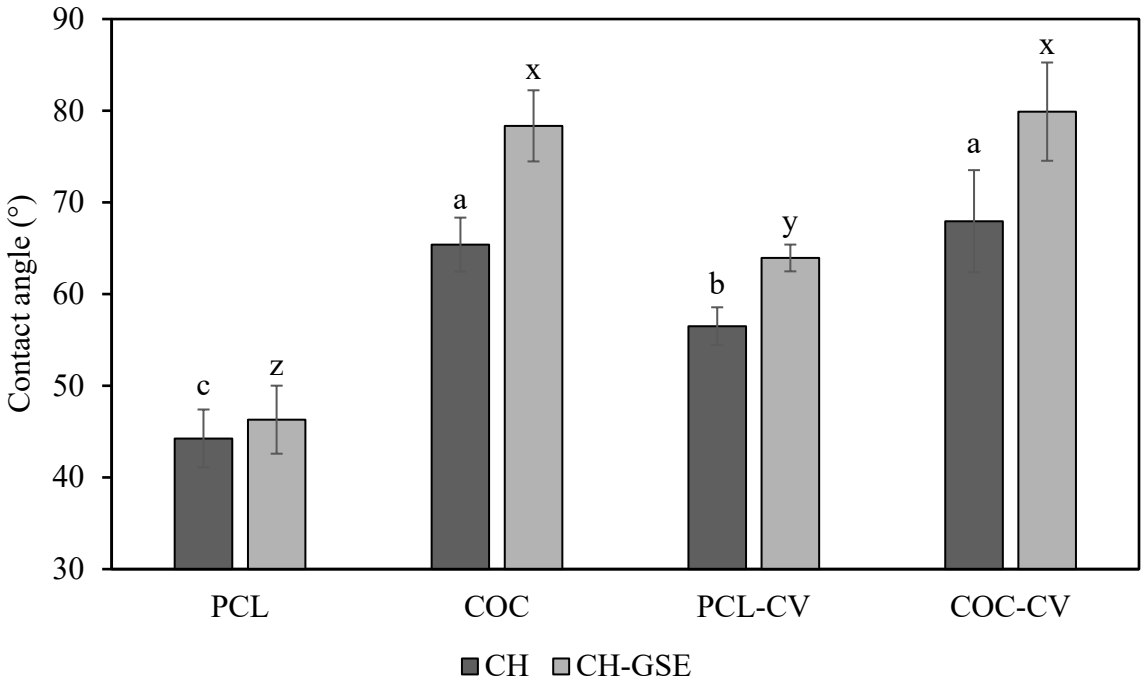
510 **Fig. 2** CV content (Mt: g/100 g film) released from the films into ethanol as a function of time
511 (experimental data points with SD and Peleg fitted model with lines) ("A" corresponds to
512 monolayers of PCL-CV (—●—) and COC-CV (- -○- -); "B" corresponds to bilayers CH/PCL-CV
513 (—■—), CH/COC-CV (- -□- -), CH-GSE/PCL-CV (—▲—), and CH-GSE/COC-CV (- -△- -)).

514 **Fig. 3** Antioxidant activity of film samples.

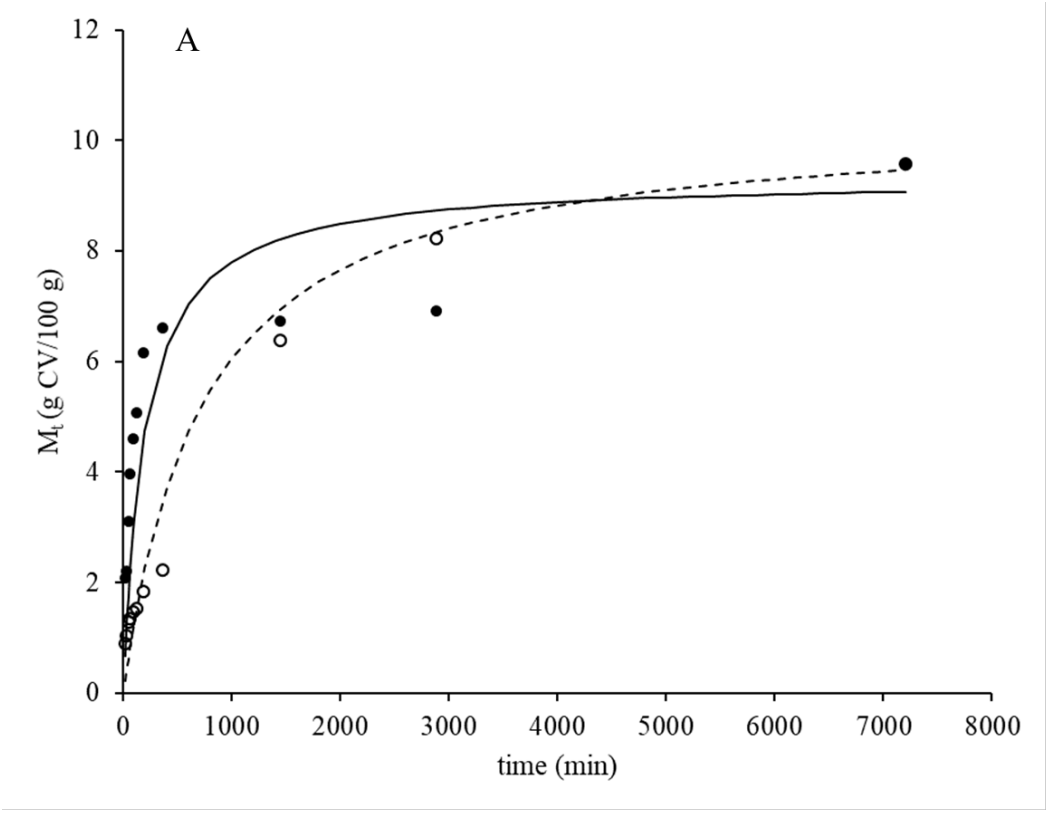
515 Any two means followed by the same letter were not significantly different ($p > 0.05$) by Tukey's
516 multiple range test.

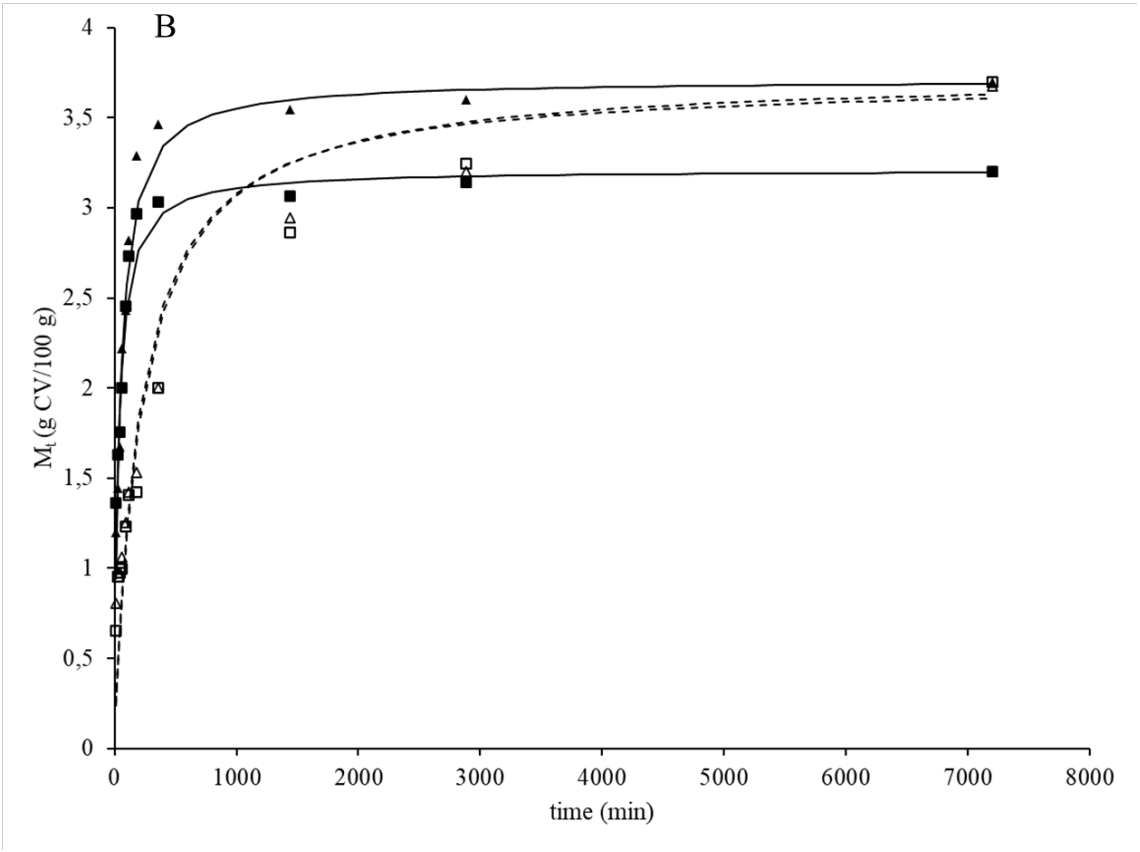
517 **Fig. 4** Stress strain curves of film samples (——CH/COC; – – – CH/PCL; ——CH-
518 GSE/COC; – – – CH-GSE/PCL; ——CH/COC-CV; – – – CH/PCL-CV; ——CH-
519 GSE/COC-CV; – – – CH-GSE/PCL-CV).

520

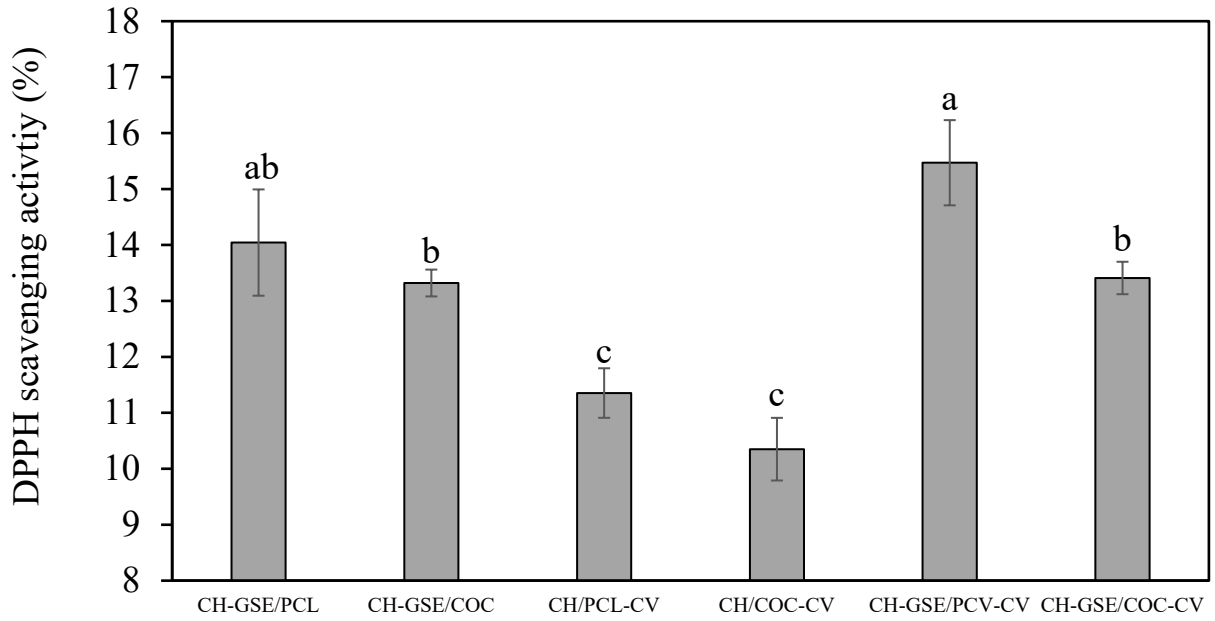

90
80
70
60
50
40
30
Contact angle (°)
c
z
a
x
b
y
a
x
PCL
COC
PCL-CV
COC-CV
CH
CH-GSE

521

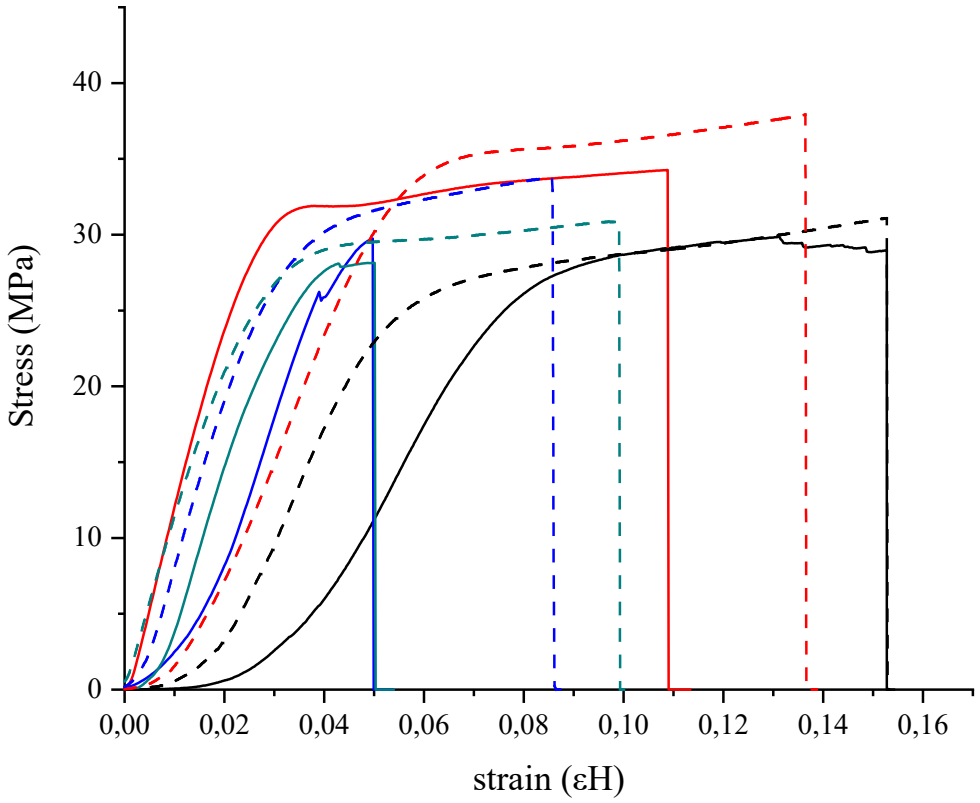

A
12
10
8
6
4
2
0
M_t (g CV/100 g)
0
1000
2000
3000
4000
5000
6000
7000
8000
time (min)


B
4
3,5
3
2,5
2
1,5
1
0,5
0
M_t (g CV/100 g)
0
1000
2000
3000
4000
5000
6000
7000
8000
time (min)

522


DPPH scavenging activtiy (%)
18
17
16
15
14
13
12
11
10
9
8
ab
b
c
c
a
b
CH-GSE/PCL
CH-GSE/COC
CH/PCL-CV
CH/COC-CV
CH-GSE/PCV-CV
CH-GSE/COC-CV

523


40
30
20
10
0
Stress (MPa)
0,00
0,02
0,04
0,06
0,08
0,10
0,12
0,14
0,16
strain (εH)

524