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Erwan Le Goué, Christian Gardrat, Mélanie Romain, Manuela Rollini, Christine Moresoli, et al..
Effect of oleic acid on the release of tetrahydrocurcumin in chitosan-based films. Food Hydrocolloids,
2022, 124, pp.107202. 10.1016/j.foodhyd.2021.107202 . hal-03510940

HAL Id: hal-03510940

<https://hal.science/hal-03510940>

Submitted on 16 Oct 2023

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1 Effect of oleic acid on the release of tetrahydrocurcumin in chitosan- 2 based films 3

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21 22 **Abstract**

23 The first objective of this study was to develop chitosan (CH)-carboxymethyl cellulose
24 (CMC) film forming dispersions (FFDs) containing oleic acid for the encapsulation of a
25 hydrophobic active curcumin derivative (tetrahydrocurcumin-THC). The second objective
26 examined the impact of oleic acid on the release of THC and the associated antioxidant and
27 antimicrobial action of CH-CMC-THC films. Oleic acid had a strong negative impact on THC
28 release potentially due to the stability of the THC encapsulation in the oleic acid droplet, as
29 suggested by ATR-FTIR and SEM analyses. The *in vitro* antifungal activity against *Botrytis*
30 *cinerea*, indicated significant antifungal **activity** of THC and CH-CMC FFD with or without
31 oleic acid. In contrast, *in vivo* experiments consisting of the CH-CMC-THC FFD coating of

32 blackberries had no effect on the microbial charge irrespective of the oleic acid presence. In
33 addition, the visual appearance of the CH-CMC-THC FFDs coated-blackberries seemed
34 better in the absence of oleic acid.

35

36 *Keywords:* chitosan, carboxymethyl cellulose, oleic acid, tetrahydrocurcumin, films,
37 blackberries

38

39

40 **1. Introduction**

41 Biodegradable polymers represent a promising option to replace polymers produced from
42 petroleum. Among them, chitosan (CH) and carboxymethyl cellulose (CMC) obtained in
43 large amounts from chitin and cellulose respectively, are the most abundant natural
44 biopolymers on earth (Gandini & Belkacem, 2008; Peniche, Argülles-Monal, & Goycoolea,
45 2008).

46 CH is a polyaminosaccharide (Fig. S1) consisting of two randomly distributed sub-units,
47 glucosamine and N-acetyl glucosamine, linked by a β -(1,4) bond. The interest of CH in the
48 preparation of food packaging materials, is notably due to its good film forming properties,
49 oxygen barrier properties at low relative humidity, non-toxicity, antimicrobial activity and
50 biodegradability (Kong, Chen, Xing, & Park, 2010; Ansorena, Marcovich, & Pereda, 2018;
51 Mujtaba et al., 2019). Nevertheless, weak mechanical properties and high water vapor
52 permeability of chitosan-based films do not meet the requirements for food packaging (Dutta,
53 Tripathi, Mehrotra, & Dutta, 2009; Bonilla, Talon, Atares, Vargas, & Chiralt, 2013).

54 Blending CH with other biopolymers can improve its packaging material properties (van
55 den Broek, Knoop, Kappen, & Boeriu, 2015). For example, mixing CH and cellulosic
56 derivatives decreased the hydrophilicity of CH films (Moura, Lorevice, Mattoso, & Zucolloto,
57 2011; Vargas, Albors, Chiralt, & Gonzalez-Martinez, 2011). CMC (Fig. S1) is one of the
58 most important cellulose derivatives, is relatively cheap, biocompatible, biodegradable and
59 non-toxic (Paunonen, 2013; Malhotra, Keshawi, & Kharkwal, 2015). CMC is considered to
60 have adequate film forming properties (Ghanbarzadeh & Almasi, 2011; Ebrahimzadeh,
61 Ghanbarzadeh, & Hamishekhar, 2016), but CMC films are very sensitive to moisture (Moura

62 et al., 2011). Blending CH with CMC produces low-cost transparent films with improved
63 barrier and mechanical properties (Tongdeesoontorn, Mauer, Wongruong, Sriburi, &
64 Rachtanapun, 2011). Hydrophobic compounds such as oleic acid or vegetable oils can be
65 added in formulation to improve water vapor permeability of hydrocolloid-based films
66 (Vargas, Albors, Chiralt, & Gonzalez-Martinez, 2009; Ghanbarzadeh & Almasi, 2011;
67 Noshirvani, et al., 2017a; Perdones, Vargas, Atares, & Chiralt, 2014; Valenzuela, Abugoch, &
68 Tapia, 2013; Wang et al., 2014).

69 Generally, CH alone has limited impact on the common microbial strains involved in food
70 spoilage (Dutta et al., 2009). The addition of substances isolated from plants, such as essential
71 oils or natural extracts, can improve the antimicrobial activity of the packaging and the shelf-
72 life of the food products (Ashrafi, Jokar, & Nafchi, 2018; Dutta et al., 2009; Grande-Tovar,
73 Chaves-Lopez, Serio, Rossi, & Paparella, 2018; Li et al., 2019; Ponce, Roura, del Valle,
74 Moreira, 2008; Xiao, Liu, Zhu, Zhou, & Niu, 2014; Zanetti et al., 2018).

75 To the best of our knowledge, few studies have reported on the release properties and
76 antimicrobial properties of chitosan-carboxymethyl cellulose (CH-CMC) films with oleic acid
77 as a matrix for the encapsulation of bioactive hydrophobic molecules. Previous studies
78 reported on films containing ginger or cinnamon essential oils (Noshirvani et al., 2017a),
79 cinnamon essential oil with cross-linked polysaccharide matrices (Valizadeh, Naseri, Babaei,
80 Hosseini, & Imani, 2019) and ZnO particles (Noshirvani, Ghanbarzadeh, Mokarram, &
81 Hashemi, 2017b; Noshirvani, Ghanbarzadeh, Mokarram, Hashemi, & Coma, 2017c). All of
82 these materials exhibited antimicrobial properties **against *Aspergillus niger*** (Noshirvani et al.,
83 2017a, 2017b, 2017c), *Pseudomonas aeruginosa* and *Listeria monocytogenes* (Valizadeh et
84 al., 2019). **The role of oleic acid in the film formulation remains poorly understood. This**
85 **could notably be due to differences in the film preparation but also to the volatility and**
86 **hydrophobicity of the essential oils, creating a dispersion of the active components in the**
87 **hydrocolloid matrix.** These limitations may be addressed by (i) selecting a non-volatile well-
88 identified hydrophobic antimicrobial agent (ii) defining a protocol to obtain films with and
89 without oleic acid and (iii) investigating the release of the antimicrobial agent in the
90 presence/absence of oleic acid.

91 In this study, tetrahydrocurcumin (THC, Fig. S2) was selected as the non-volatile
92 hydrophobic active antimicrobial agent. THC is a metabolite of curcumin exhibiting high
93 antioxidant and antifungal activities (Coma, Portes, Gardrat, Richard-Forget, & Castellan,
94 2011; Portes, Gardrat, & Castellan, 2007; Portes, Gardrat, Castellan, & Coma, 2009; Loron et

95 al., 2021). The initial phase consisted in the development of a protocol to create CH-CMC
96 oleic acid films with uniform properties. The second phase focused on the release of THC
97 from the films with and without oleic acid. *The in vitro antifungal activity of the FFDs was*
98 *determined against *Botrytis cinerea*, selected as target strain being one of the most frequently*
99 *studied fungal pathogen for more than 500 plant species (Williamson, Tudzynski, Tudzynski,*
100 *& van Kan (2007). This microorganism has a disastrous economic impact on major crops*
101 *including grape, strawberry and tomato (Dean et al., 2012), can be present inside fruits, seeds*
102 *and other parts of the plant (Hua et al., 2018) and can occur before maturity or postharvest*
103 *(Pitt & Hocking, 2009; Tournas & Katsoudas, 2005). The developed FFD and the*
104 *corresponding films were then assayed on the shelf-life of blackberries. These fruits, grown*
105 *and harvested inside the garden of one of the authors (M. Rollini, North Italy), were selected*
106 *to ensure their organic conditions, and as an example of wild-harvested specialty fruits with*
107 *health attributes and of commercial value but with a limited shelf life due to fungal*
108 *contamination (mainly *Botrytis* spp.) (Skrovankova et al., 2015).*
109

110 **2. Materials and methods**

111 *2.1. Materials*

112
113 Chitosan (CH, low molecular weight, 20-300 cP, 1 wt. % in 1% (v/w) acetic acid, 75-85%
114 deacetylated), sodium carboxymethylcellulose (CMC, high viscosity, art. C5013),
115 diphenylpicrylhydrazyl radical (DPPH), Nile red (9-(diethylamino)-5H-benzo[a]phenoxazin-
116 5-one) and acetone were purchased from Sigma Aldrich (USA and Germany). Analytical
117 grade glycerol, oleic acid ($\geq 90\%$), acetic acid and Tween 80 were purchased from Merck
118 (Germany) and used as received. Tetrahydrocurcumin (THC, Sabiwhite 95%) was obtained
119 from Sabinsa Corporation (20 Lake Drive, East Windsor, New Jersey).

120 *B. cinerea* was obtained from the culture collection of the LCPO Laboratory at the
121 University of Bordeaux (France). *B. cinerea* was precultured on PDA at 25°C in the dark
122 before use. Sabouraud agar was obtained from Biokar Diagnostics (France) and blackberries
123 were hand picked in a private garden near Milano, Italy.

124

125 *2.2. Elaboration of CH-CMC oleic acid film forming dispersions (FFDs) and films*

126 The following protocol was established by conducting a preliminary study to identify
127 suitable conditions for the preparation of CH-CMC homogenous films. A CH solution: chitosan
128 (0.35 g) was dissolved in distilled water containing acetic acid (50 mL, 0.875% v/v) under
129 magnetic stirring. Tween 80 (0.35 mL) was added as an emulsifier and the solution was stirred
130 overnight. Oleic acid (0.525 mL in 3 mL of ethanol) was added as a hydrophobic carrier under
131 magnetic stirring for 15 min; the pH of the solution was increased to 8.0 using NaOH solution
132 (3M). A CMC solution was prepared separately by dissolving sodium carboxymethylcellulose
133 (CMC, 0.7 g) in hot distilled water (50 mL, 80°C).

134 FFDs were prepared by mixing the CH solution and the CMC solution followed by
135 homogenization with an Ultra-Turrax (Ika; 5 min) and ultrasound (Bandelin Sonopuls,
136 Germany; 15 min, 31 kJ). After degassing under vacuum, glycerol (0.525 mL; glycerol/CH-
137 CMC 63%, w/w) was introduced as a plasticizer under magnetic stirring (10 min). The FFD
138 was casted (4 x 25 mL) in Petri dishes (diameter 9 cm), which were dried at 40°C for two days
139 and then conditioned for additional three days at 25 °C, 50% RH. The dried films were peeled
140 off and weighed before any characterization.

141 Different films were prepared with/without oleic acid, with/without THC. In the case of
142 films containing THC (10.5 mg; THC/CH-CMC 10% w/w), THC was incorporated at the same
143 time as oleic acid in the chitosan solution before pH adjustment. The films without oleic acid
144 were produced with the same protocol, omitting oleic acid addition and THC was solubilized in
145 ethanol.

146 The CH film used for ATR-FTIR analysis was prepared from a chitosan film-forming
147 solution (100 mg) in 100 mL acetic acid solution (0.875% v/v) while the CMC film was
148 prepared from a dispersion of 0.7 g in hot water. The CH-CMC film was prepared following
149 the previous protocol.

150 2.3. Characterization of FFDs

151 2.3.1. Optical microscopy

152 Optical microscope Leica DME (Bioblock Scientific, Illkirch, France) was used. A drop of
153 the FFD was deposited on a slide; a x10 magnification was sufficient to generate suitable
154 images. All images were obtained using the software Motic Images plus 2.0 with an image
155 capture format of 1280 x 1024.

156

157 2.3.2 Particle size distribution

158 Analyzes were performed with a Mastersizer 2000 equipment (Malvern Instruments, Orsay,
159 France). Few drops of the FFD were dispersed in an aqueous medium in the measuring cell.

160

161 *2.4. Characterization of film*

162 *2.4.1 Microscopy*

163 *2.4.1.1 Nile Red staining.* Nile red was dissolved in acetone (0.1 mg/mL) before film staining
164 according to Kimura, Yamaoka, & Kamisaka (2004). After staining, film was analyzed by
165 confocal microscopy (Leica SP5-AOBS) with a thermostated plate (Linkman).

166

167 *2.4.1.2 Scanning electron microscopy (SEM).* Surface microstructure analysis of the film was
168 carried out by SEM with a tungsten source in a Quanta 200 device (FEI, USA). Film samples
169 were observed under low vacuum 50 Pa, large field detector, 3 kV and working distance 7-8
170 mm.

171

172 *2.4.2. Dynamic penetration of water (PDA)*

173 Measurements were recorded on a PDA.2 C 02 Module HVL (high viscous liquid) from
174 Emtec Electronic GmbH (Leipzig, Germany). The film was fixed by using a double-sided
175 adhesive tape on the measuring head, which contains the ultrasound receiver. Water (3.5 mL)
176 was deposited on the horizontal area of the measuring cell, which contained the ultrasound
177 transmitter (frequency 1 MHz). The measuring head of the basic device was lowered together
178 with the film sample until in contact with water.

179

180 *2.4.3. Water contact angle*

181 Water contact angle on the film surfaces was measured by a goniometer Kruss DSA 100
182 (Germany). A drop of distilled water ($\approx 2 \mu\text{L}$) was placed on the film surface (10 mm x 60
183 mm). The initial water contact angle (after 5 seconds) was measured. For each film, three
184 replicates were obtained.

185

186 *2.5. THC release from film and antioxidant activity*

187 THC release was measured for intact film and film after grinding (powder), to evaluate the
188 impact of the physical condition of the film.

189 *2.5.1. Intact film*

190 The films were totally immersed in methanol (100 mL) under magnetic stirring (250 rpm).
191 The absorption spectra of the solutions were recorded at different time intervals (2, 5.30, 10,
192 20, 30, 45 and 60 min) and at 280 nm using a spectrophotometer Cary-100 with quartz cells
193 (length 1 cm). These experiments were repeated three times with different films.

194 The antioxidant activity was evaluated by measuring the DPPH radical scavenging
195 activity. Briefly, a film was immersed in a methanol solution for 5 days (100 mL) in the dark
196 and at room temperature. A 2 mL volume of the methanol solution was mixed with a DPPH
197 solution (150 μ M, 2 mL), vigorously shaken and allowed to rest at room temperature for 30
198 minutes. The absorbance of the methanol solution was measured at 517 nm and the DPPH
199 inhibition (1) was calculated as follows:

$$200 \quad I(\%) = (A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}} * 100 \quad (1)$$

201 where A_{control} is the absorbance of the fresh methanol solution and A_{sample} is the absorbance of
202 the methanol solution after 5 days of film immersion.

203 The presence of THC in the methanol solution was examined by ^1H NMR (Bruker
204 AVANCE III HD 400 MHz spectrometer). The methanol solution was collected after the
205 immersion of a film for 5 days and evaporated. The solid residues were collected with 0.5 mL
206 of deuterated methanol and analysed by NMR.

207 *2.5.2. Grinded film*

208 The film was grinded in liquid nitrogen in order to destroy its structure. The resulting
209 powder was immersed in methanol for 5 days in the dark at room temperature and under
210 magnetic stirring (200 rpm). The antioxidant activity was determined as before, and DPPH
211 inhibition calculated according to equation (1). The presence of THC in the solution was
212 examined by NMR, as previously described.

213 *2.6. ATR- Fourier Transformed Infrared spectroscopy (ATR-FTIR)*

214 ATR-FTIR spectra of the films were obtained using a FTIR spectrometer (PIKE
215 technologies/Gladi Atrvertex 70, Bruker, France). Spectra were collected from 4000 to 400
216 cm^{-1} (32 scans; resolution 4 cm^{-1}).

217

218 *2.7. Antifungal properties of FFDs against B. cinerea*

219

220 2.7.1. *In vitro* antifungal activity

221 The *in vitro* antifungal activity was obtained from growth inhibition tests performed on
222 Sabouraud medium in Petri dishes (9 cm diameter), inoculated in the centre with 10 µL of *B.*
223 *cinerea* spore suspension (10⁴ spores/mL). First, the antifungal activity of THC, oleic acid and
224 Tween 80 was evaluated. For that, the medium was supplemented with THC (0.105 g/L), or
225 oleic acid (0.525 g/L), or Tween 80 (0.35 g/L) after dissolution in 1 wt. % of DMSO. The
226 concentration of THC, oleic acid and Tween was similar as for the FFDs. The effect of the
227 solvent DMSO was obtained with the addition of 1 wt. % DMSO in the culture medium.

228 To perform the *in vitro* assays for FFDs, 1 mL of FFD was added to the Sabouraud medium
229 and Petri dishes were then inoculated in the centre with 10 µl of *B. cinerea* spore suspension
230 (10⁴ spores/mL) as previously described.

231 Growth controls, without any potential inhibitory agents, were conducted in parallel (1 mL
232 H₂O pH 7.5 into Sabouraud medium).

233 All cultures were incubated at 25°C for 5 days and the radial growth of *B. cinerea* was
234 measured every day. Colony diameters were determined as the average of two perpendicular
235 diameter length measurements (expressed in cm). The tests were performed in triplicate.

236 All the analyses were performed in triplicate for the FFDs with and without oleic acid.

237

238 2.7.2. *In vivo* antifungal activity of FFD coated blackberries.

239 Blackberry samples, **harvested at the same day of the trials**, were dipped for 10 s in the FFD
240 (with and without oleic acid) and subsequently left drying on a paper in sterile conditions
241 (under a biohazard type II hood) for 2 h, avoiding direct contact with each other. Uncoated
242 blackberries were used as control and visually inspected after 0, 3, 4 and 5 days of storage at 4
243 °C and 20 °C. The coated blackberries were kept in a Falcon tube with open caps at 4 °C and 20
244 °C and visually inspected after 0, 3, 4 and 5 days. In addition, total bacterial count and yeasts
245 and moulds count were determined according to storage time. For that, blackberry samples (10
246 g) were transferred aseptically into a Stomacher bag (400 mL PE, Barloworld, France)
247 containing 90 mL of sterile peptoned water (10 g/L bacteriological peptone, Costantino, Italy)
248 and blended in a Stomacher (Star Blender LB 400, Biosystem, Belgium) at high speed for 3
249 min. Ten-fold dilution series of the suspension were made in the same solution as for plating.
250 The following culture media were used: TSA (Merck, Germany) for Total Bacterial Count
251 (TBC), and MEA (Malt Extract Agar, Oxoid) for yeasts and fungi. Colonies were counted after
252 incubation at 30 °C for 24 h for mesophile bacteria and 30 °C for 5 d for yeasts and fungi.

253 Microbial counts were performed in triplicate and reported as logarithms of the number of
254 colony forming units (log cfu/g fruit), and means and standard deviation (SD) were calculated.

255
256

257 **3. Results and discussion**

258 *3.1. CH-CMC film preparation*

259 The creation of stable CH-CMC films containing oleic acid required the development and
260 fine tuning of a film forming dispersion methodology as illustrated in Figure 1. The first
261 factor examined was the pH of the CH solution. When CMC solutions (pH 7.40) were added
262 directly to the CH solutions (pH 3.95), physically tight compact hydrogels were obtained,
263 likely due to electrostatic interactions between oppositely charged biopolymers, knowing the
264 anionic character of CMC and cationic character of CH in acidic solutions (Farris, Schaich,
265 Liu, Piergiovanni, & Yam, 2009). Increasing the pH of the CH solution before mixing with
266 the CMC solution can alter the properties of the films (Noshirvani et al., 2017a). In the
267 present study, the pH of the CH solution was adjusted to 8 before mixing with the CMC
268 solution in order to avoid precipitation. In these conditions, CH was not in its protonated
269 form. To avoid aggregation, which occurred during the increase of the CH solution pH at
270 approximately 5.7, an emulsifier was added, Tween 80.

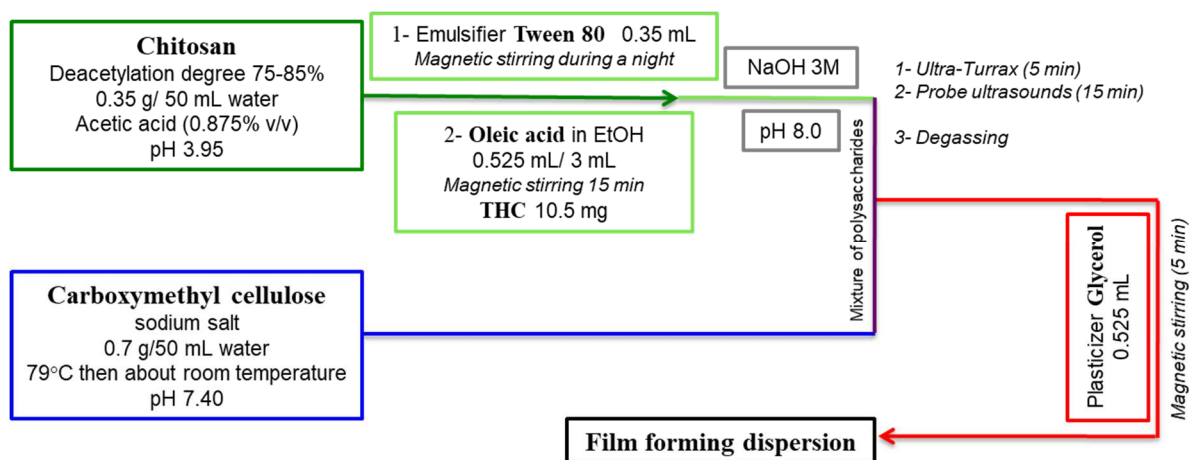
271

272 The addition of oleic acid to the chitosan solution required Ultra-Turrax and ultrasound
273 treatment to obtain a better homogeneous FFD as can be seen in the microscopy images (Fig
274 S3). Large aggregates were observed when using Ultra-Turrax alone (Fig S3a) but
275 disappeared when ultrasounds and Ultra-Turrax were used (Fig S3b). These aggregates are
276 due to the presence of oleic acid (Fig S3c and d).

277

278 The dispersion of oleic acid in the CH solution was improved when Tween 80 was added
279 prior to the addition of oleic acid. In addition, the emulsion stability was improved if Tween 80
280 and oleic acid were added in the solution before pH adjustment to 8 (Fig. S4 and S5).
281 Furthermore, if the oleic acid was pre-solubilized in ethanol before its addition to the CH
282 solution, its particle size decreased from 3.5 μm (Fig. S6a) to 0.5 μm (Fig. S6b), leading to a
283 better emulsion stability. The oleic acid partitioning was examined by using Nile red staining
284 according to Kimura, Yamaoka, & Kamisaka (2004). As can be observed from the confocal

285 microscopy images (Fig. S7), oleic acid was randomly distributed validating the procedure to
 286 elaborate films.
 287



288
 289 **Fig. 1.** Experimental protocol to obtain homogeneous film forming dispersion (FFD).

290
 291 *3.2. Characterization of CH-CMC film*

292
 293 *3.2.1. Visual observation*

294 As illustrated in Figure 2, there was a retraction of the film surface without oleic acid,
 295 potentially due to direct interactions between CH and CMC, leading to a lower film density. As
 296 expected, the addition of oleic acid altered slightly the film transparency but visual
 297 transparency remains sufficient for food coatings applications. The CH-CMC films were
 298 flexible even when no oleic acid was added (well-known plasticizer for polysaccharide-based
 299 materials), which is not generally the case with pure CH films.

300
 301



302
303

304 **Fig. 2.** Visual observations of CH-CMC films with (left side, A) and without oleic acid (right side, B)

305
306

307 3.2.2. Penetration Dynamic Analysis of water

308 The impact of oleic acid on the water penetration in films is presented in Fig. S8. The
309 presence of oleic acid leads to a small decrease of ultrasound intensity after the maximum (at
310 1 s). At the end of the analysis, the increase of the ultrasound intensity is due to the film
311 swelling knowing that the more the water penetration rate, the more the attenuation of the
312 signal.

313 On the contrary, the initial slope of the curve for film without oleic acid decreased quickly
314 in few milliseconds, followed by an increase of ultrasound intensity, which indicates quick
315 water penetration rate and a swelling of the film.

316 The presence of oleic acid improved liquid water barrier properties, especially due of its
317 hydrophobic character. Similar observation was reported previously by Noshirvani et al.
318 (2017a). These authors showed that oleic acid addition to CH-CMC films increased the water
319 contact angle, and even more after adding hydrophobic essential oils. In the current study, the
320 water contact angle of CH-oleic acid and CMC-oleic acid films was 44° and 38°, respectively.
321 For the CH-CMC-oleic acid film, the water contact angle was 48°, indicating that the
322 combination of CH and CMC improved the hydrophobicity of the films. Unexpectedly, the
323 introduction of THC, a hydrophobic compound, reduced the hydrophobicity of the CH-CMC-
324 oleic acid-THC film with a water contact angle of 33°. It seems that THC led to modifications
325 in the CH-CMC network.

326

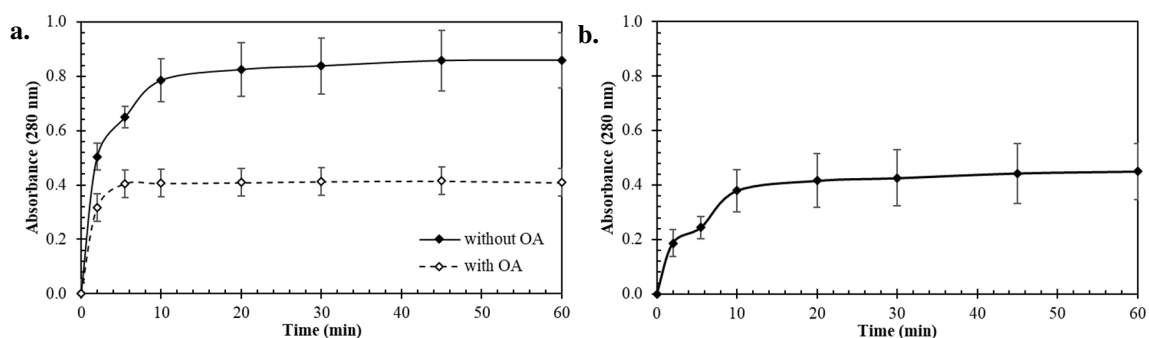
327 3.2.3. THC release from films and antioxidant activity

328

329 In this part, the films containing the following components CH, CMC, Tween 80 and THC
330 with and without oleic acid are called “THC-loaded films”. The release of THC from THC-
331 loaded films with and without oleic acid was studied over time by measuring the absorbance
332 at 280 nm of the methanol solution (Fig. 3a), as THC has a UV-vis spectrum with a λ_{\max} at
333 280 nm (Fig S9). Oleic acid is soluble in methanol, but its absorbance at 280 nm is too low to
334 have a significant influence. Unfortunately, the commercial Tween 80 is also soluble in
335 methanol and displayed an absorbance between 250 and 290 nm (Fig. S9) (Arudi, Sutherland,
336 & Bielski, 1983).

337

338



339

340 **Fig. 3.** Absorbance at 280 nm of the methanol solution *versus* time (a) Absorbance after dipping the THC-
341 loaded CH-CMC films with or without oleic acid (b) Absorbance difference corresponding to THC effectively
342 released from the films. Error bars represent the standard deviation of three measurements on three different
343 films.
344

345 Higher absorbances were observed for THC-loaded films without oleic acid with the
346 maximum absorbance obtained after approximately 1 h. But, because of the unknown detailed
347 composition of Tween 80 and the observed overlap between the absorbance of Tween 80 and
348 THC at 280 nm, the THC released from the films could not be quantified.

349 An alternative approach to distinguish the release of THC from the potential interferences
350 associated with Tween 80 was adopted and was based on the strong antioxidant activity of
351 THC (Portes, Gardrat, & Castellán, 2007), whereas Tween 80 has minimal antioxidant
352 activity (Pérez-Rosés, Rico, Vila, Peñalver, & Cañigüeral, 2014). The absence of DPPH
353 antioxidant activity of Tween 80 was confirmed in the current study (0% DPPH inhibition).
354 The DPPH inhibition of the methanol solution obtained from THC-loaded films are presented
355 Table 1. In order to know if THC remained entrapped in the film matrix, films were reduced
356 to powder and then immersed in a methanol solution for 5 days. The relative inhibition of the
357 film powder with and without oleic acid is presented in Table 1. Higher THC release was
358 obtained for the film powder regardless of the oleic acid presence, indicating that THC could
359 be maintained in the matrix notably by steric hindrance. In addition, higher THC release could
360 also be due to the increase of exchange surface between the film powder and the methanol
361 solution. Without oleic acid present, the THC recovered was double. Similar results were
362 reported previously for THC release from CH-based films (Portes, Gardrat, Castellán, &
363 Coma, 2009) with a plateau reached after 4 hours immersion in methanol and 30% of THC
364 remained in the film, which was fully recovered after film grinding. Another study of CH
365 films containing THC in chitosan particles (Bijoux, 2014) showed that a plateau was reached
366 after two hours and 23% of the THC released in ethanol; and an additional 47% THC was
367 recovered after grinding the film to a powder.

368

369 **Table 1**

370 Impact of the oleic acid content of THC-loaded films on DPPH inhibition of methanol solution after film
371 incubation during 5 days at ambient temperature. Inhibition percentages are presented as means \pm the standard
372 deviations of three replicates.

Sample	DPPH inhibition (%)
--------	---------------------

Film with oleic acid	0
Film without oleic acid	32 ± 6
Film powder with oleic acid	26 ± 6
Film powder without oleic acid	66 ± 7

373 The addition of oleic acid on the THC release in methanol solution for intact and powder
374 THC-loaded films reduced the THC release. These results are in agreement with the ¹H-NMR
375 spectra of the methanol solution (Fig. S10). This could be due to the encapsulation of THC in
376 oleic acid droplets leading to partial release of THC. As THC is soluble in ethanol but poorly
377 soluble in water, it could be easily incorporated into oleic acid micelles because of its higher
378 affinity for the oleic acid matrix. During the film preparation, the addition of the THC and
379 oleic acid ethanol solution to the acidic CH solution pre-emulsified with Tween 80 may have
380 led to the adsorption of CH on the oleic acid or Tween 80-oleic acid droplets (Vargas et al.,
381 2009). Interactions between oleic acid and CH matrices have been proposed previously
382 (Souza, Monte, & Pinto, 2011). As a result, oleic acid could favor the absorption of CH at the
383 droplet interface because of interactions between the amino groups of CH (pK_a ≈ 6.5) and the
384 carboxyl groups of oleic acid (pK_a ≈ 4.8) (Ham-Pichavant, Sebe, Pardon, & Coma, 2005;
385 Vargas et al., 2009) or by the formation of bonds between the carboxyl group of oleic acid
386 and the polar groups of CH, as suggested recently for basil and thyme essential oils in CH-
387 oleic acid matrix (Perdones, Chiralt, & Vargas, 2016). The pH adjustment of the CH-oleic
388 acid and THC solution before mixing with the CMC solution did not alter the organization of
389 the CH-oleic acid and THC system. THC seems to be embedded in the CH-CMC matrix to
390 give stable FFDs. During the drying of the FFD, the presence of oleic acid in the CH-CMC
391 matrix promoted THC retention as observed previously for CH-based films with cinnamon
392 essential oil (Perdones et al., 2014; Valizadeh et al., 2019).

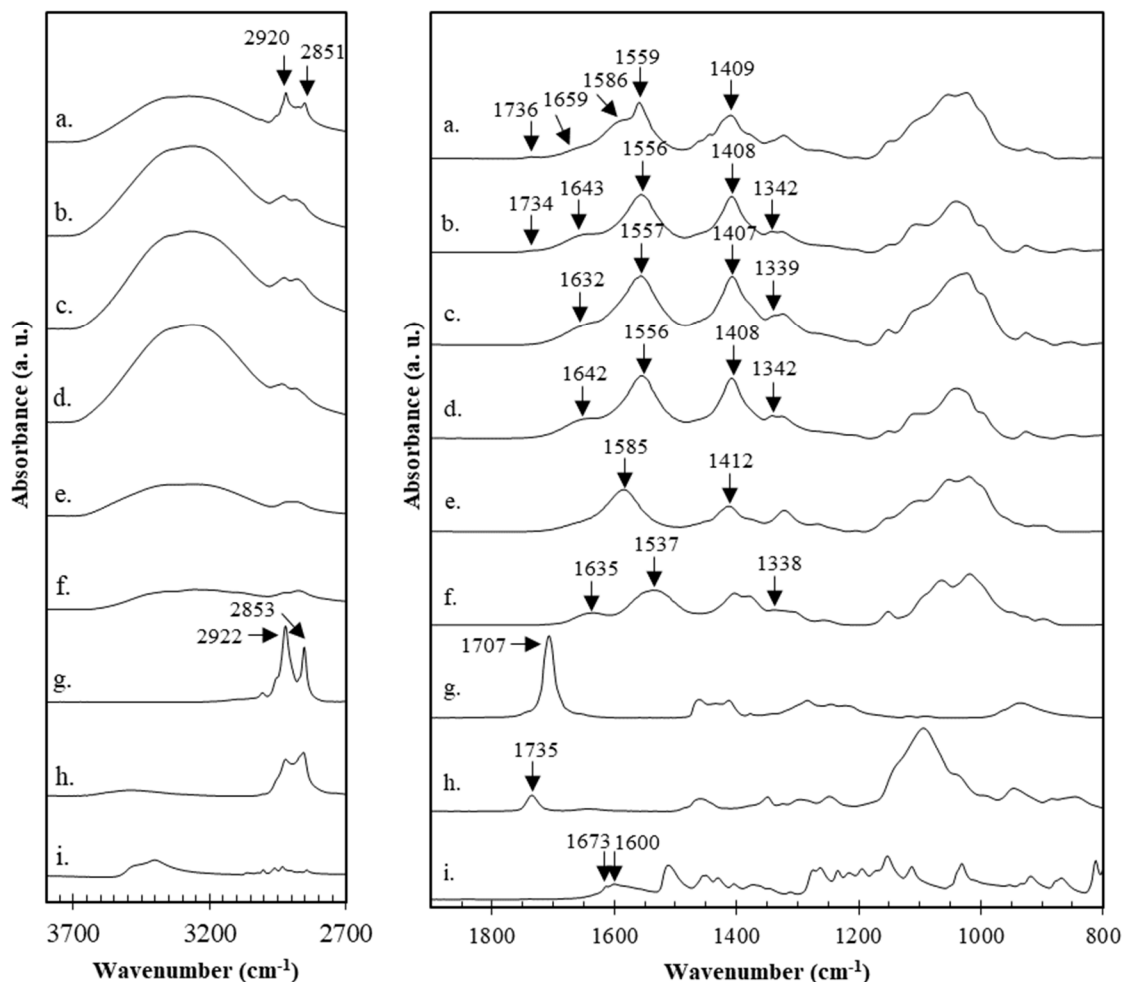
393 Another important observation is the partial release of THC from the THC-loaded film,
394 even without oleic acid. Since no degradation of Tween 80 and THC was observed in
395 methanol for similar duration (UV spectra not shown) and knowing that Tween 80 had no
396 effect on DPPH activity -absence of DPPH activity from intact THC-loaded films with oleic
397 acid- the THC release from fatty acid free films can be evaluated.

398 By assuming that the release of Tween 80 remained the same with and without oleic acid, the
399 difference of the absorbance at 280 nm for THC-loaded films with and without oleic acid
400 (Figure 3a) could be attributed to the release of THC in methanol (presented in Figure 3b).
401 The maximum THC release was about 25 % of THC (calibration curve not shown).

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3.2.4. ATR-FTIR analysis of films

Potential interactions between the different components of the films on the THC release were examined by ATR-FTIR spectroscopy (Fig. 4).



407
408 **Fig. 4.** ATR-FTIR spectra of films (a. CH-CMC-Tween 80-Oleic acid-THC; b. CH-CMC-Tween 80-THC; c.
409 CH-CMC-THC; d. CH-CMC; e. CMC; f. CH) and pure components (g. Oleic acid; h. Tween 80; i. THC).

410
411 CH films (pH \approx 4) displayed broad bands between 3100 and 3400 cm⁻¹ due to overlapped
412 O-H and N-H stretching vibrations. Bands at 1635 and 1537 cm⁻¹ were respectively attributed
413 to $\nu_{C=O}$ (amide I) and $\delta_{NH_3^+}$. The bands between 900 and 1200 cm⁻¹ were characteristic of the
414 carbohydrate structure. CMC films (pH \approx 7.5) displayed a band between 3100 and 3600 cm⁻¹
415 corresponding to O-H stretching vibrations. Bands at 1412 and 1585 cm⁻¹ were due to
416 symmetrical and asymmetrical stretching vibrations of the carboxylate groups, respectively.

417 The bands observed between 900 and 1200 cm^{-1} may be attributed essentially to the
418 polysaccharide skeleton.

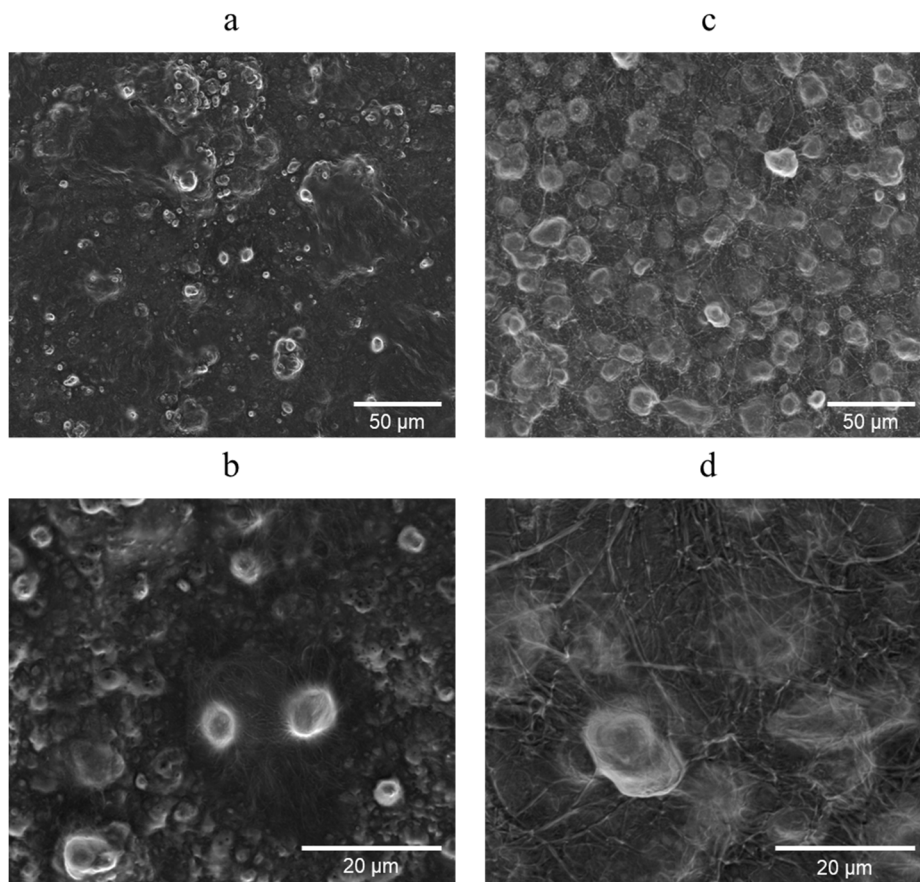
419 CMC-CH films ($\text{pH} \approx 8$), showed shift in both bands belonging to the stretching vibrations
420 of the carboxylate groups of CMC, from 1412 cm^{-1} to 1408 cm^{-1} and from 1585 cm^{-1} to 1556
421 cm^{-1} with a higher intensity of the former band and a higher width of the latter one. The band
422 corresponding to $\delta_{\text{NH}_3^+}$ in chitosan disappeared in agreement with the increase of the pH. The
423 broadening of the band at around 1550 cm^{-1} could be attributed to an overlap with the bending
424 vibration of the amino groups. In the same time, the amide I band of CH was broader. These
425 modifications indicate strong interactions between CMC and CH in films due to their
426 chemical compatibility. The addition of THC in CH-CMC films ($\text{pH} \approx 8$) did not alter
427 strongly the overall shape of the infrared spectrum except a broadening of the band centered
428 on 1557 cm^{-1} , likely due to the overlap with the stretching bands of the aromatic groups of
429 THC. As a consequence, the amide I band of CH gave a smooth shoulder at around 1632 cm^{-1} .
430 After addition of Tween 80, a new band with a small intensity appeared at 1734 cm^{-1} ; as
431 this band has the same wavenumber in the ATR-IR spectrum of Tween 80, it could be
432 concluded that Tween 80 did not strongly interact with the film matrix.

433 The addition of oleic acid to the CH-CMC films led to some modifications: (i) $\nu_{\text{C-H}}$
434 stretchings were clearly apparent at 2920 and 2851 cm^{-1} confirming the presence of oleic acid
435 in the film; (ii) the band at 1736 cm^{-1} (belonging to Tween 80) and the bands at 1559 and
436 1409 cm^{-1} (belonging to the CH-CMC matrix) seemed to have no significant shifts; (iii) the
437 shoulder at $\approx 1659 \text{ cm}^{-1}$ (chitosan amide I vibration) shifted; (iv) a new shoulder appeared at
438 1586 cm^{-1} , due to the transformation of the carboxylic groups of oleic acid into carboxylate
439 ions; (v) two significant modifications appeared in the spectrum of the CH-CMC-Tween 80-
440 Oleic acid-THC film: first, the band at 1559 cm^{-1} was sharper; secondly, the intensity of the
441 weak shoulder at 1342 cm^{-1} decreased strongly. This shoulder corresponds likely to complex
442 vibrations belonging partly to the acetamido groups in CH ($\nu_{\text{C-N}}$, amide III), as suggested from
443 the FTIR spectrum of pure *N*-acetyl- α -D-glucosamine (Kovacs, Nyerges, & Izvekov, 2008).
444 An evolution of this shoulder was observed since it appeared at 1338 cm^{-1} in the ATR-FTIR
445 spectrum of CH films as described previously (Qin, Liu, Yuan, Yong, & Liu, 2019) and at
446 1342 cm^{-1} in CH-CMC-THC-Tween 80 films. These observations suggest interactions,
447 respectively, between oleic acid and THC on the one hand, and between CH and oleic acid on
448 the other hand, in agreement with THC entrapment in oleic acid.

449

450 3.2.5. *Film morphology*

451 SEM images of films with and without THC are presented in Fig. 5 at two magnifications.
452 The addition of THC led to CH-CMC-oleic acid film with irregular surface whereas the
453 absence of THC led to many small structure similar to shells. These shell-like structures were
454 homogeneously distributed at the CH-CMC-oleic acid film surface and were identified as
455 oleic acid droplets by confocal imaging (Fig. S7). The differences observed in the CH-CMC-
456 oleic acid film morphology support the alteration of interactions between oleic acid and the
457 CH-CMC matrix due to the presence of THC. During preparation of FFD, the pH of the CH
458 solution increased, lowering the electrostatic interactions between oleic acid and CH. As
459 consequence, without THC, oleic acid droplets rose at the film surface. The presence of THC
460 modified these interactions and fewer oleic acid droplets were observed at the film surface.
461 The presence of oleic acid droplets at the film surface altered the hydrophobicity of the film
462 and may explain the differences in water contact angle of the CH-CMC-oleic acid films with
463 (33°) and without THC (48°).
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467 **Fig. 5.** SEM image of film surfaces: CH-CMC-oleic acid-THC (a, x1000, b, x4000) and CH-CMC-oleic acid
 468 (c, x1000, d, x4000).

469 **3.2.6. *In vitro* antifungal activity**

470 *In vitro* antifungal activities of the main substances of FFD and the FFD with and without
 471 oleic acid, were evaluated against *B. cinerea*. To examine the putative influence of the FFDs
 472 with and without oleic acid on a food product, *in vivo* experiments were conducted with
 473 blackberries.

474

475 **3.2.6.1. *In vitro* antifungal activity of FFD components and FFD with and without oleic acid**

476 *In vitro* results of the growth of *B. cinerea* in presence of pure DMSO as the solvent, and
 477 THC, oleic acid and Tween 80 in DMSO are reported in Table 2. Plates containing DMSO
 478 showed a reduced fungal development comparatively to the control plate (-16% after 4 days
 479 of incubation), confirming the low antifungal effect of DMSO as previously observed (Elad,
 480 1992). THC in DMSO reduced the fungal growth up to 36% after 4 days incubation, while the
 481 other FFD substances did not show any strong effect compared to the DMSO plate.

482

483 **Table 2**

484 *In vitro* antifungal activity of DMSO, THC, Oleic acid and Tween 80 on the radial growth of *B. cinerea* during
 485 incubation time (expressed in cm), presented as means \pm the standard deviations of three replicates.

Time (day)	Control	DMSO	THC	Oleic Acid	Tween 80
Radial growth (cm)					
0	0	0	0	0	0
2	1.85 \pm 0.20	1.80 \pm 0.14	1.15 \pm 0.08	1.90 \pm 0.06	1.88 \pm 0.10
3	4.12 \pm 0.20	3.75 \pm 0.18	2.37 \pm 0.15	3.70 \pm 0.06	3.43 \pm 0.14
4	7.18 \pm 0.27	6.00 \pm 0.00	4.60 \pm 0.05	6.02 \pm 0.03	5.81 \pm 0.13
		-16%*	-36%*	-16%*	-19%*

486 * Percentage of reduction respect to the growth control at day 4.

487 The anti-fungal activity of the FFD, with and without oleic acid is reported in Table 3.
 488 Lower fungal growth was observed during the first 4 days for the FFDs. No noticeable
 489 difference between the FFD with and without oleic acid was observed. FFDs reduced the
 490 growth up to 20-25%. The lower anti-fungal activity of the FFD without oleic acid may be

491 related to limitation for the diffusion of THC inside the solid culture medium due to the
492 lipophilic nature of oleic acid.
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Table 3

In vitro antifungal activity of film forming dispersion (FFD) with and without oleic acid on the radial growth of *B. cinerea* during incubation time (expressed in cm) presented as means \pm the standard deviations of three replicates.

Time (day)	Control	FFD with oleic acid	FFD without oleic acid
	Radial growth (cm)		
0	0	0	0
1	0.80 \pm 0.00	0	0.25 \pm 0.42
2	2.80 \pm 0.14	2.17 \pm 0.14	1.92 \pm 0.42
3	4.70 \pm 0.14	3.65 \pm 0.16	3.70 \pm 0.28
4	7.05 \pm 0.07	5.27 \pm 0.27	5.62 \pm 0.31
		-25%*	-20%*

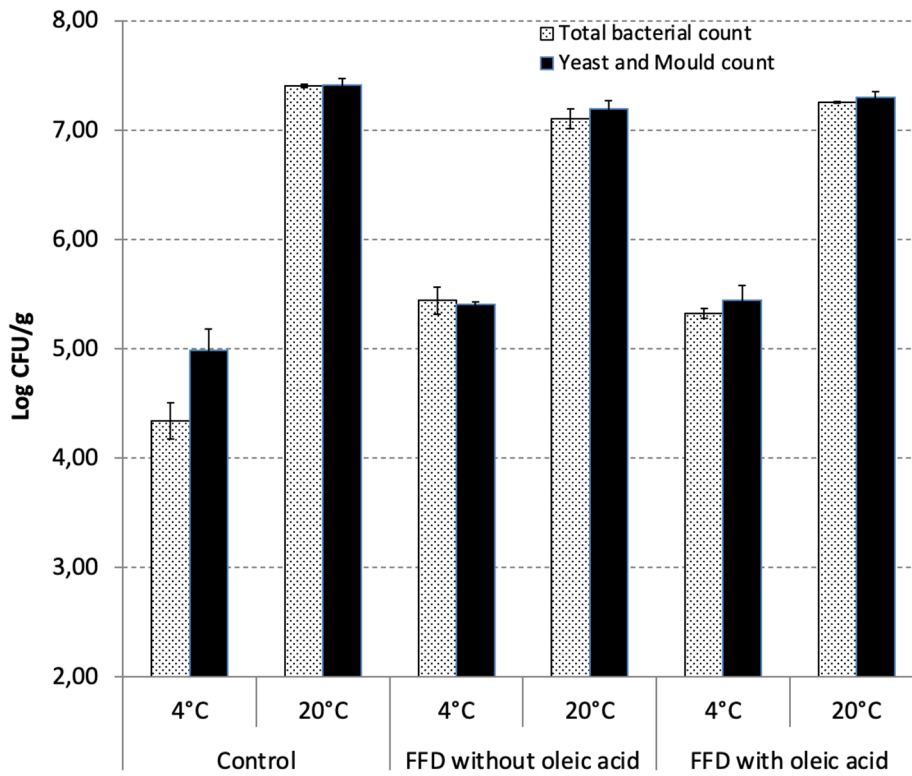
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* Percentage of reduction respect to the physiological growth at day 4.

502 **3.2.6.2. *In vivo* antimicrobial activity of CH-CMC-THC FFD coating on blackberries**

503 The *in vivo* antimicrobial activity, measured as total bacterial count and total yeast and
504 mould count, of non-coated (control) and CH-CMC-THC FFD coated blackberries, during
505 cold storage (4°C) and room temperature storage (20°C) is presented Fig. 6. As expected, the
506 microbial count was higher for storage at room temperature for coated and uncoated
507 blackberries. The CH-CMC-THC FFD coating did not reduce the microbial count for these
508 storage conditions. This result was unexpected due to the partial inhibition of the FFD on *B.*
509 *cinerea* for *in vitro* conditions, but may be explained by the lack of THC activity for the
510 strains present in the blackberries. FFD may also act as a carbon source, explaining the higher
511 counts observed at 4°C. Oleic acid addition had no significant effect on the antimicrobial and
512 antifungal activity of the CH-CMC-THC FFD coatings (Fig. 6).

513 Visual observations of blackberries revealed a loss of consistency due to natural decay and
514 water loss, which was more important when stored at room temperature and for the control
515 and the CH-CMC-THC FFD-coated blackberries with oleic acid (Table 4). off-flavour
516 generation was observed for all blackberries likely due to the presence of oleic acid as
517 reported previously for strawberries (Vargas, Albors, Chiralt, & Gonzalez-Martinez, 2006). In
518 summary, the CH-CMC-THC free of oleic acid offered **the best visual observation.**



520








521 **Fig. 6.** Total bacteria count (TBC) and Yeast and moulds count (log CFU/g) of CH-CMC-THC coated
 522 blackberries after 5 days of incubation at 4°C and 20°C. Errors bars represent the standard deviations of three
 523 replicates.

524

525

526 **Table 4.**

527 Non-coated (control) and CH-CMC-THC FFD coated blackberries after 4 days of storage at different
 528 temperatures.

	Day 0	Storage at 4°C	Storage at 20°C
Control			
FFD with oleic acid	/		
FFD without oleic acid	/		

529

530

531 **4. Conclusion**

532 In the present study, films made with chitosan (CH)-carboxymethyl cellulose (CMC)-
533 Tween 80 incorporating THC (tetrahydrocurcumin) with and without oleic acid were
534 prepared. The addition of oleic acid prevented the THC release from the film matrix. In the
535 absence of the fatty acid, partial release of THC was observed. Microscopic analysis showed
536 the formation of spherical globules attributed to oleic acid in which THC was incorporated as
537 suggested by ATR-FTIR analysis. The presence of these globules may explain the limitation
538 of THC release from CH-CMC-Tween 80 films with oleic acid.

539 In order to create bioactive coatings, *in vitro* experiments showed that THC can exert a
540 slight antifungal activity against the growth of *B. cinerea*. This antifungal activity was
541 observed for CH-CMC-Tween 80-THC films with and without oleic acid. *In vivo* experiments
542 on blackberries, showed that the CH-CMC-THC coating did not reduce the microbial counts,
543 irrespective of oleic acid content. Moreover, the best visual observations were obtained for
544 oleic acid free CH-CMC-THC FFD coated blackberries.

545

546 **Declaration of competing interest**

547 The authors declare that there are no conflicts of interest.

548 **Acknowledgments**

549 The authors thank Chiara Sari for some experiment work.

550 Imaging was performed on the Bordeaux Imaging Center, member of the

551 France BioImaging national infrastructure (ANR-10-INBS-04).

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554 **CRedit authorship contribution statement**

555 **Conceptualization:** ELG, C.G and V.C. **Investigation** ELG, MR and MR. **Methodology**
556 ELG, CG. **Data curation** ELG, MR and MR, CM. **Writing original draft and review**
557 **editing** ELG, CM, VC. **Supervision** VC

558 All authors have read and agreed to the published version of the manuscript.

559 **Conflicts of Interest:** The authors declare no conflict of interest.

560 **References**

- 561 Ansorena, M. V., Marcovich, N. E., & Pereda, M. (2018). Food packaging based on chitosan.
562 In, L. M. T. Martinez, O. V. Kharissova, & B. I. Kharisov (Eds), Handbook of
563 Ecomaterials, Springer International Publishing AG 2018. Doi: 10.1007/978-3-319-68255-
564 6_68.
- 565 Arudi, R. L., Sutherland, M. W., & Bielski, B. H. J (1983). Purification of oleic acid and
566 linoleic acid. *Journal of Lipid Research*, 24, 485-488.
- 567 Ashrafi, A., Jokar, M., & Nafchi, A. M. (2018). Preparation and characterization of
568 biocomposite film based on chitosan and kombucha tea as active food packaging,
569 *International Journal of Biological Macromolecules*, 108, 444-454.
- 570 Bijoux, C. (2014). Films bioactifs à base de chitosane et dérivés de curcumine encapsulés.
571 Libération contrôlée d'agent actif. Master's thesis 2, University of La Réunion.
- 572 Bonilla, J., Talon, E., Atares, L., Vargas, M., & Chiralt, A. (2013). Effect of the incorporation
573 of antioxidants on physicochemical and antioxidant properties of wheat starch-chitosan
574 films. *Journal of Food Engineering*, 118, 271-278.
- 575 Brugnerotto, J., Lizardi, J., Goycoolea, F. M., Argüelles-Monal, W., Desbrières, J., &
576 Rinaudo, M. (2001). An infrared investigation in relation with chitin and chitosan
577 characterization. *Polymer*, 42, 3569-3580.
- 578 Coma, V., Portes, E., Gardrat, C., Richard-Forget, F., & Castellán, A. (2011). *In vitro*
579 inhibitory effect of tetrahydrocurcuminoids on *Fusarium proliferatum* growth and
580 fumonisin B1 biosynthesis. *Food Additives and Contaminants – Part A*, 28, 218-225.
- 581 Dean, R., Van Kan, J. A., Pretorius, Z. A., Hammond-Kosack, K. E., di Pietro, A., Spanu, P.
582 D., Rudd, J. J., Dickman, M., Kahmann, R., Ellis, J., & Foster, G. D. (2012). The top 10
583 fungal pathogens in molecular plant pathology. *Molecular Plant Pathology*, 13, 414–430.
- 584 Dutta, P. K., Tripathi, S., Mehrotra, G. K., & Dutta, J. (2009). Perspectives for chitosan based
585 antimicrobial films in food applications. *Food Chemistry*, 114, 1173–1182.
- 586 Ebrahimzadeh, S., Ghanbarzadeh, B., & Hamishekhar, H. (2016). Physical properties of
587 carboxymethyl cellulose based nano-biocomposites with graphene nanoplatelets.
588 *International Journal of Biological Macromolecules*, 84, 16-23.
- 589 Elad, Y. (1992). The use of antioxidants (free radical scavengers) to control grey mould
590 (*Botrytis cinerea*) and white mould (*Sclerotinia sclerotiorum*) in various crops. *Plant*
591 *Pathology*, 41, 417-426.

592 Farris, S., Schaich, K. M., Liu, L. S., Piergiovanni, L. & Yam, K. L. (2009). Development of
593 polyion-complex hydrogels as an alternative approach for the production of bio-based
594 polymers for food packaging applications: a review. *Trends in Food Science &*
595 *Technology*, 20, 316-332.

596 Gandini, A., & Belkacem, M. N. (2008). The state of the art. In A. Gandini, M.N. Belkacem
597 (Eds), *Monomers, polymers and composites from renewable resources* (pp. 1-16). Elsevier,
598 Amsterdam.

599 Ghanbarzadeh, B., & Almasi, H. (2011). Physical properties of edible emulsified films based
600 on carboxymethyl cellulose and oleic acid. *International Journal of Biological*
601 *Macromolecules*, 48, 44-49.

602 Grande-Tovar, C. D., Chaves-Lopez, C., Serio, A., Rossi, C., & Paparella, A. (2018).
603 Chitosan coatings enriched with essential oils : effects on fungi involved in fruit decay and
604 mechanisms of action. *Trends in Food Science & Technology*, 78, 61-71.

605 Ham-Pichavant, F., Sèbe, G., Pardon, P., & Coma, V. (2005). Fat resistance properties of
606 chitosan-based paper packaging for food applications. *Carbohydrate Polymers*, 61, 259-
607 265.

608 Hua, L., Yong, C., Zhanquan, Z., Boqiang, L., Guozheng, Q., & Shiping, T. (2018).
609 Pathogenic mechanisms and control strategies of *Botrytis cinerea* causing post-harvest
610 decay in fruits and vegetables. *Food quality and Safety*, 3, 111-119.

611 Kimura, K., Yamaoka, M., & Kamisaka, Y. (2004). Rapid estimation of lipids in oleaginous
612 fungi and yeasts using Nile red fluorescence. *Journal of Microbiological Methods*, 56,
613 331-338.

614 Kong, M., Chen, X. G., Xing, K., & Park, H. J. (2010). Antimicrobial properties of chitosan
615 and mode of action: a state of the art review. *International Journal of Food Microbiology*,
616 144, 51-53.

617 Kovacs, A., Nyerges, B., & Izvekov, V. (2008). Vibrational analysis of N-acetyl- α -D-
618 glucosamine and β -D-glucuronic acid. *Journal of Physical Chemistry*, 112, 5728-5735.

619 Li, Z., Lin, S., An, S., Liu, L., Hu, Y., & Wan, L. (2019). Preparation, characterization and
620 anti-aflatoxigenic activity of chitosan packaging films incorporated with turmeric essential
621 oil. *International Journal of Biological Macromolecules*, 131, 420-434.

622 Loron, A., Navikaitė-Šnipaitienė, V., Rosliuk, D., Rutkaite, R., Gardrat, C. & Coma, V.
623 (2021). Polysaccharide matrices for the encapsulation of tetrahydro-curcumin – Potential
624 application as biopesticide against *Fusarium graminearum*. *Molecules*, 26, 3873.

625 Malhotra, B., Keshwani, A., & Kharkwal, H. (2015). Natural polymer based cling films for
626 food packaging. *International Journal of Pharmacy and Pharmaceutical Sciences*, 7, 10-
627 18.

628 Moura, M. R., Lorevice, M. V., Mattoso, L. H. C., & Zucolloto, V. (2011). Highly stable,
629 edible cellulose films incorporating chitosan nanoparticles. *Journal of Food Science*, 76,
630 N25-N29.

631 Mujtaba, M., Morsi, R. E., Kerch, G., Elsabee, M. Z., Kaya, M., Labidi, J., & Khawar, K. M.
632 (2019). Current advancements in chitosan-based production for food technology; a review.
633 *International Journal of Biological Macromolecules*, 121, 889–904.

634 Noshirvani, N., Ghanbarzadeh, B., Gardrat, C., Rezaei, M. R., Le Coz, C., & Coma, V.
635 (2017a). Cinnamon and ginger essential oils to improve antifungal, physical and
636 mechanical properties of chitosan-carboxymethyl cellulose films. *Food Hydrocolloids*, 70,
637 36-44.

638 Noshirvani, N., Ghanbarzadeh, B., Mokarram, R. R., & Hashemi, M. (2017b). Novel active
639 packaging based on carboxymethyl cellulose-chitosan-ZnO NPs nanocomposite for
640 increasing the shelf life of bread. *Food Packaging and Shelf Life*, 11, 106-114.

641 Noshirvani, N., Ghanbarzadeh, B., Mokarram, R. R., Hashemi, M., & Coma, V. (2017c).
642 Preparation and characterization of active emulsified films based on chitosan-
643 carboxymethyl cellulose containing zinc oxide nanoparticles. *International Journal of*
644 *Biological Macromolecules*, 99, 530-538.

645 Paunonen, S. (2013). Strength and barrier enhancements of cellophane and cellulose
646 derivative films: A review. *BioResources*, 8, 3098-3121.

647 Peniche, C., Argülles-Monal, W. M., & Goycoolea, F. M. (2008). Chitin and chitosan: major
648 sources, properties and applications. In A. Gandini, M.N. Belkacem (Eds), *Monomers,*
649 *polymers and composites from renewable resources* (pp. 517-542). Elsevier, Amsterdam.

650 Perdones, A., Vargas, M., Atares, L., & Chiralt, A. (2014). Physical, antioxidant and
651 antimicrobial properties of chitosan-cinnamon leaf oil films as affected by oleic acid. *Food*
652 *Hydrocolloids*, 36, 256-254.

653 Perdones, A., Chiralt, A., & Vargas, M. (2016). Properties of film-forming dispersions and
654 films based on chitosan containing basil or thyme essential oil. *Food Hydrocolloids*, 57,
655 271-279.

656 Pérez-Rosés, R., Rico, E., Vila, R., Peñalver, P., & Cañigüeral, S. (2014). Antioxidant activity
657 of Tween 20 and Tween 80 evaluated through different in vitro tests. *Journal of Pharmacy
658 and Pharmacology*, *67*, 666-672.

659 Pitt, J. I., & Hocking, A. D. (2009). Primary keys and miscellaneous fungi. In *Fungi and Food
660 Spoilage*, Springer, Boston, MA. ISBN: 978-0-387-92207-2.

661 Ponce, A. G., Roura, S. I., del Valle C. E., & Moreira, M. R. (2008). Antimicrobial and
662 antioxidant activities of edible coatings enriched with natural plant extracts: in vitro and in
663 vivo studies. *Postharvest Biology and Technology*, *49*, 294-300.

664 Portes, E., Gardrat, C., & Castellan, A. (2007). A comparative study on the antioxidant
665 properties of tetrahydrocurcuminoids and curcuminoids. *Tetrahedron*, *63*, 9092-9099.

666 Portes, E., Gardrat, C., Castellan, A., & Coma, V. (2009). Environmentally friendly films
667 based on chitosan and tetrahydrocurcuminoid derivatives exhibiting antibacterial and
668 antioxidative properties. *Carbohydrate Polymers*, *76*, 578-584.

669 Qin, Y., Liu, Y., Yuan, L., Yong, H., & Liu, J. (2019). Preparation and characterization of
670 antioxidant, antimicrobial and pH-sensitive films based on chitosan, silver nanoparticles
671 and purple corn extract. *Food Hydrocolloids*, *96*, 102-111.

672 Silva-Weiss, A., Bifani, V., Ihl, M., Sobral, P. J. A., & Gomez-Guillen, M. C. (2014).
673 Polyphenol-rich extract from murta leaves on rheological properties of film-forming
674 solutions based on different hydrocolloids blends. *Journal of Food Engineering*, *140*, 28-
675 38.

676 Skrovankova, S., Sumczynski, D., Mlcek, J., Jurikova, T., & Sochor, J. (2015). Bioactive
677 compounds and antioxidant activity in different types of berries. *International Journal of
678 Molecular Sciences*, *16* (10), 24673-24706.

679 Souza, V. C., Monte, M. L., & Pinto, L. A. A. (2011). Preparation of biopolymer film from
680 chitosan modified with lipid fraction. *International Journal of Food Science and
681 Technology*, *46*, 1856-1862.

682 Tongdeesontorn, W., Mauer, L. J., Wongruong, S., Sriburi, P., & Rachtanapun, P. (2011).
683 Effect of carboxymethyl cellulose concentration on physical properties of biodegradable
684 cassava starch-based films. *Chemistry Central Journal*, *5*, 1-8.

685 Tournas V. H., & Katsoudas, E. (2005). Mould and yeast flora in fresh berries, grappes and
686 citrus fruits. *International Journal of Food Microbiology*, *105*, 11-17.

687 Valenzuela, C., Abugoch, I., Tapia, C. (2013). Quinoa-protein-chitosan-sunflower oil edible
688 film: Mechanical, barrier and structural properties. *LWT-Food Science and Technology*,
689 *50*, 531-537.

690 Valizadeh, S., Naseri, M., Babaei, S., Hosseini, S. M .H., & Imani, A. (2019). Development
691 of bioactive composite films from chitosan and carboxymethyl cellulose using
692 glutaraldehyde, cinnamon essential oil and oleic acid. *International Journal of Biological*
693 *Macromolecules*, *134*, 604-612.

694 van den Broek, L. A. M., Knoop, R. J. I., Kappen, F. H. J., & Boeriu, C. G. (2015). Chitosan
695 films and blends for packaging material. *Carbohydrate Polymers*, *116*, 237–242.

696 Vargas, M., Albors, A., Chiralt, A., & Gonzalez-Martinez, C. (2006). Quality of cold-stored
697 strawberries as affected by chitosan-oleic acid edible coatings. *Postharvest Biology and*
698 *Technology*, *41*, 164-171.

699 Vargas, M., Albors, A., Chiralt, A., & Gonzalez-Martinez, C. (2009). Characterization of
700 chitosan-oleic acid composite films. *Food Hydrocolloids*, *23*, 536-547.

701 Vargas, M., Albors, A., Chiralt, A., & Gonzalez-Martinez, C. (2011). Water interactions and
702 microstructure of chitosan-methylcellulose composite films as affected by water
703 concentration. *LWT-Food Science and Technology*, *44*, 2290-2295.

704 Wang, Z., Zhou, J., Wang, X. X., Zhang, N., Sun, X. X., & Ma, Z.S. (2014). The effect of
705 ultrasonic/microwave assisted treatment on the water barrier properties of soybean protein
706 isolate-based oleic acid/stearic acid blend edible films. *Food Hydrocolloids*, *35*, 51-58.

707 Williamson, B., Tudzynski, B., Tudzynski, P., & van Kan, J. A. (2007). Botrytis cinerea: the
708 cause of gray mould disease. *Molecular Plant Pathology*, *8*, 561–580.

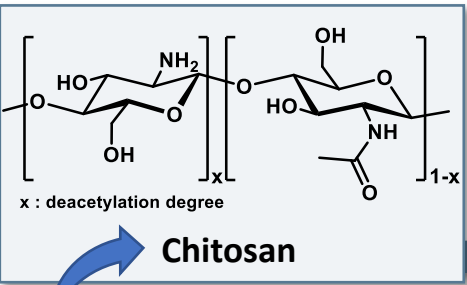
709 Xiao, Z., Liu, W., Zhu, G., Zhou, R., & Niu, Y. (2014). A review of the preparation and
710 application of flavor and essential oils microcapsules based on complex coacervation
711 technology. *Journal of the Science of Food and Agriculture*, *94*, 1482-1494.

712 Zanetti, M., Carniel, T. K., Dalcaton, F., dos Anjos, R. S., Riella, H. G., de Araujo, P. H. H,
713 de Oliveira, D., & Fiori, M.A. (2018). Use of encapsulated natural compounds as
714 antimicrobial additives in food packaging : a brief review. *Trends in Food Science &*
715 *Technology*, *81*, 51-60.

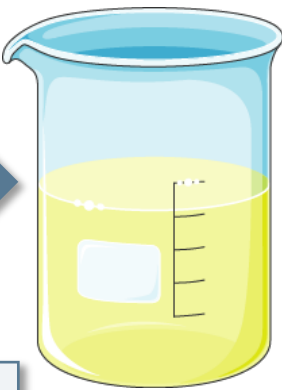
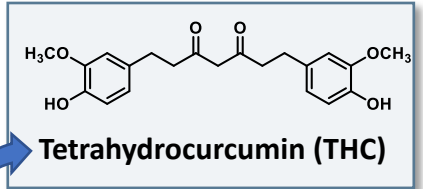
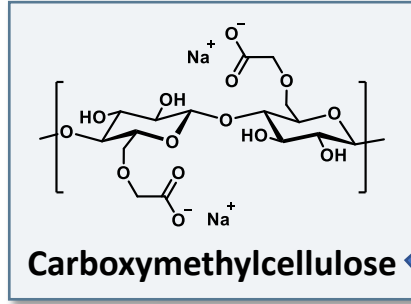
716 Zhang, Q., Wang, A., Meng, Y., Ning, T., Yang, H., Ding, L., Xiao, X., & Li, X. (2015).
717 NMR method for accurate quantification of polysorbate 80 copolymer composition.
718 *Analytical Chemistry*, *87*, 9810-9816.

719

Film-forming dispersions



With or without oleic acid

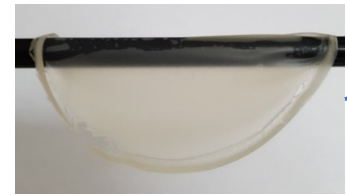


+ Tween 80

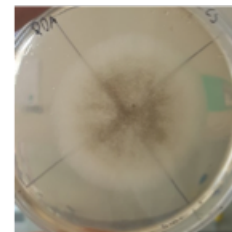
Films



Without oleic acid



With oleic acid



Antifungal activity of solutions and films (*Botrytis cinerea*)

Antioxidant activity (THC released in methanol)

