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1 ACTIVE PACKAGING FILMS CONTAINING ANTIOXIDANT EXTRACTS FROM  
2 GREEN COFFEE OIL BY-PRODUCTS TO PREVENT LIPID OXIDATION

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4

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21 **ABSTRACT**

22           The residual biomass of cold-pressed green coffee oil (GCO), rich in chlorogenic  
23 acids (CGA), was reused by incorporating the press cake (CE) and sediment (SE) extracts  
24 into carboxymethyl cellulose (CMC) films. The effect of these extracts combined with  
25 GCO was investigated on the physicochemical, barrier, and antioxidant properties, and  
26 on the ability of the active films to delay fish oil oxidation. The films with added CE and  
27 GCO (C-CE) or SE and GCO (C-SE) showed high antioxidant activity,  $3.61 \pm 0.01$  and  
28  $2.03 \pm 0.01$  mmol Trolox eq/g dry film, respectively. These findings are in line with the  
29 CGA content in CE and SE (9.8 and 9.0% w/w, respectively), as determined by HPLC.  
30 The addition of SE and GCO slightly affects the oxygen barrier of CMC films, while  
31 providing them with high Ultraviolet-Visible (UV-Vis) absorption. The evolution of  
32 peroxide value (PV) and thiobarbituric acid reactive substances (TBARS) in fish oil  
33 samples covered by C-CE and C-SE films and inert headspace was significantly lower  
34 than those of controls (storage at 40°C for 16 days). The antioxidant release from films  
35 with added CE and SE showed an antagonistic behaviour into the food simulants.  
36 Although both active films are promising for active packaging, the C-SE film appeared  
37 as more advantageous for oil-rich food protection.

38

39 **Keywords:** Bio-based films; carboxymethyl cellulose; chlorogenic acids; cod liver oil;  
40 cold-pressed oil; release of antioxidants.

41

42

## 43 **Highlights**

44 1. Residues extracts from GCO pressing showed an important amount of CGA and  
45 caffeine

46

47 2. Strong antioxidant capacity of CMC films is provided by CGA able to migrate from  
48 matrix and trapping O<sub>2</sub>

49

50 3. Lipid components from GCO and its residues extracts improve UV-Vis absorption of  
51 films but slightly affect oxygen barrier

52

53 4. CMC films with added CGA from GCO residues play a key role in protecting fish oil  
54 against oxidation when used as lid combined with inert headspace

55

## 56 **1. Introduction**

57         The first synthetic plastic produced in 1907, called Bakelita, marked a shift in the  
58 development of new plastics. In 1950, the world saw an outpouring of new plastic  
59 materials, stimulated by their unique properties, such as low cost, strength, durability,  
60 light weight and easy production (Andrady and Neal, 2009). Nowadays, more than 380  
61 million tonnes of plastic are produced per year, which has a dramatic negative impact on  
62 oceans and wildlife health. The packaging sector is responsible for generating almost half  
63 of the world's total plastic pollution owing to the use of primary plastics, and also to their  
64 very short lifetime (Ritchie and Roser, 2018). Thus, the food industry is increasingly  
65 interested in developing more ecofriendly materials. Films based on hydrocolloids are  
66 promising alternatives to petrol-derived plastic films. They may display a better oxygen  
67 barrier than synthetic polymer owing to a great deal of bonds between chains which form  
68 a tortuous pathway for gases throughout the film matrix (Sahraee et al., 2019). Moreover,  
69 they can be combined with natural lipids, such as emulsion-based films, to improve their

70 water barrier resistance, which is otherwise quite low and one of their main drawbacks  
71 (Bourlieu et al., 2009; Tongnuanchan et al., 2015).

72         Among hydrocolloids, cellulose is an insoluble material that is also the most  
73 abundant biomass resource on earth, offering great opportunities as food-packaging  
74 material (Nieto, 2009). Carboxymethyl cellulose (CMC) obtained by reacting sodium  
75 monochloroacetate with cellulose in alkaline medium is one of the most relevant  
76 products, owing to its economic importance as a thickening and emulsifying agent, with  
77 wide use in pharmaceuticals, cosmetics, and food (Yaradoddi et al., 2020). CMC is a  
78 highly water-soluble anionic polysaccharide that generally forms transparent films. These  
79 films, when used in a low water activity ( $a_w$ ) range, in which CMC water sensitivity is  
80 not a drawback, display good mechanical and barrier properties against oxygen and lipids  
81 (Arik Kibar and Us, 2013; Ballesteros et al., 2018).

82         The incorporation of bioactive compounds in biodegradable films plays a crucial  
83 role in active packaging technology, resulting in innovative solutions for extending the  
84 shelf-life, and maintaining or monitoring the quality of foods (Ganiari et al., 2017).  
85 Functional molecules with antioxidant and antimicrobial properties, such as polyphenols,  
86 can be recovered from wine pomaces. Thus, chemical, enzymatic or physical processes  
87 should be investigated and optimized from a sustainable perspective (Gontard et al.,  
88 2018). These molecules can be exploited as green additives in polymeric systems to  
89 impart specific properties to the matrix (Kirschweng et al., 2017). In recent studies, the  
90 addition of agricultural waste extracts into bio-based films has significantly improved  
91 their antioxidant activity, and protective effects have been found against lipid oxidation  
92 in lipid food systems (de Moraes Crizel et al., 2018; Serrano-León et al., 2018; Wu et al.,  
93 2018).

94 Cold pressing of unroasted green coffee beans (*Coffea Arabica* L.) generates  
95 abundant residues for production of green coffee oil (GCO), an attractive ingredient for  
96 cosmetic formulations with UVB absorption, and anti-aging and emollient properties  
97 (Nosari et al., 2015; Oliveira et al., 2018). Two main residues are generated after GCO  
98 processing: a solid residue called the press cake (~90%, w/w total residues) and a  
99 sediment produced after clarification of raw GCO; both of them remain rich in  
100 chlorogenic acids (CGA) and oil (Castro et al., 2018; Lombo Vidal et al., 2020; Oliveira  
101 et al., 2019). CGA are responsible for the strong antioxidant properties of green coffee  
102 extracts against lipid oxidation (Budryn et al., 2014; Dziki et al., 2015; Gawlik-Dziki et  
103 al., 2014). The radical scavenging efficiency of CGA is due to the hydroxyl groups  
104 bonded to the aromatic ring in an *ortho* position with respect to each other (Badhani et  
105 al., 2015).

106 CGA-rich extracts from GCO residues have recently been incorporated into bio-  
107 based films (Lombo Vidal et al., 2020); however, the techno-functional and putative  
108 antioxidant properties found in these films required test applications to confirm their  
109 efficiency as active food packaging material. Fish oil is an interesting food system  
110 because it is very prone to oxidation with a significantly faster rate than that of other oils  
111 owing to its high content in n-3 long chain polyunsaturated fatty acids (PUFAs).  
112 However, mammals are not able to synthesize the main n-3 precursor C18:3, alpha-  
113 linolenic acid, which must be provided by the diet. Since fish oil is very prone to  
114 oxidation, improper processing and/or storage conditions (e.g., temperature abuse,  
115 exposure to light and humidity) can lead to rapid deterioration (Wang W. et al., 2017;  
116 Yeşilsu and Özyurt, 2019).

117 The aim of this study was to evaluate and compare the effect of two residue  
118 extracts (Cake Extract - CE and Sediment Extract - SE) from cold-pressed GCO and GCO

119 on the antioxidant activity, antioxidant release, O<sub>2</sub> barrier properties, Ultraviolet-Visible  
120 (UV-Vis) absorption capacity and physicochemical properties of resulting active CMC-  
121 based films. Phenolic compounds in raw materials (cake, sediment and oil), were  
122 identified and quantified by HPLC. The capacity of active films to retard the oxidation  
123 progress in fish oil during storage, in two configurations (1-indirect contact combined  
124 with inert headspace, 2-direct contact with no headspace), was monitored by measuring  
125 primary (peroxides) and secondary products of oxidation (Thiobarbituric acid reactive  
126 species - TBARS).

## 127 **2. Materials and methods**

### 128 *2.1. Raw materials and chemicals*

129 Green coffee beans (*Coffea arabica* L.) were kindly donated by São Francisco  
130 Farm (São José do Vale do Rio Preto, Brazil). Sodium carboxymethyl cellulose (average  
131 MW 250,000 Da, degree of substitution 0.9), DPPH radical (2,2-diphenyl-1-  
132 picrylhydrazyl), trolox (6-hydroxy-2,5,7,8- tetramethylchroman-2-carboxylic acid), 4-  
133 and 5-caffeoylquinic acid (CQA) (95%), caffeic acid (> 98%), caffeine (>99%), methanol  
134 (MeOH) and water (both HPLC grade, >99.9%) were purchased from Sigma-Aldrich  
135 (Saint-Quentin-Fallavier, France). Glycerol, ferrous chloride and tween 20 were  
136 purchased from Merck Co. (Darmstadt, Germany), and *n*-butanol (BuOH) and ethanol  
137 (EtOH) P.A. from Honeywell (Germany). Hydrogenated Lecithin Phospholipon® 90H  
138 was kindly supplied by Lipoid (Ribeirão Preto, Brazil) and MilliQ pure water was  
139 purchased from Millipore Corporation (USA). All other reagents and solvents were of  
140 analytical grade and commercial fish oil (cod liver oil) was purchased in a local store  
141 (Montpellier, France).

142 *2.2. Screw cold-pressing and quality parameters*

143 The clarified oil, the cake and the sediment were obtained as described in Lombo  
144 Vidal et al. (2020). Briefly, in this study, green coffee beans were immersed in liquid  
145 nitrogen for 30s and then ground (IKA II Basic, USA). GCO was obtained by triple  
146 pressing 500 g of ground coffee after progressively sieving at 2.000 mm and 850 mm  
147 with a continuous expeller press fitted with a gravity feeding system (Oekotec, CA59G,  
148 Germany). To decrease the residual oil in the cake, small nozzle size (4 mm), and low  
149 screw speed ( $\pm 30$  rpm) was used. The temperature of the oil exiting the expeller,  
150 measured by a thermo-par coupled to a digital thermometer, ranged between 40 and 45°C.  
151 The raw oil was centrifuged (Multifuge X3R, Thermo Fisher scientific, Germany) in  
152 falcon tubes (50 mL) for 20 min at 3,100 x g at 25°C. The clarified oil, the press cake and  
153 GCO sediment residue were stored at -18°C, until further use.

154 The following quality parameters were determined, unsaponifiable matter (ISO  
155 18609: 2000), free fatty acid expressed as oleic acid equivalent (method Cd3d-63);  
156 peroxide value (method Cd8-53); Moisture content of ground green coffee, cake and  
157 sediment was determined by gravimetric analysis, placing 1 g of the sample at 105°C to  
158 constant weight. GCO yield is defined as the ratio of clarified oil recovered from pressing  
159 to the amount of ground green coffee originally used.

160 *2.3. Determination of dry matter and residual oil content in the cake and the sediment*

161 The cake and the sediment were defatted with three purposes: 1) to determine the  
162 residual oil content in these by-products after pressing; 2) to prepare the hydroalcoholic  
163 extracts for HPLC characterization of bioactive compounds and 3) to evaluate the  
164 possible contribution of lipid fraction compounds for UV-Vis absorption of films. The  
165 raw materials were placed into an envelope-shaped paper filter, dried at 105°C until  
166 constant weight, and then extracted with hexane for 10 h in a Soxhlet apparatus. Finally,



167 the envelopes were placed at 20°C for 12 h, dried at 105°C and weighed (Laguna et al.,  
168 2018). Fat content (%) was determined gravimetrically based on the weight difference of  
169 the sample (the residue and envelope) before and after delipidation (Laguna et al., 2018).  
170 Dry matter content was determined for both the cake and the sediment by carefully  
171 weighing 1 g of the sample into an aluminium capsule and drying them at 105°C until  
172 constant weight (difference between two weighings <1%).

#### 173 *2.4. Extraction of phenolic compounds from raw materials: GCO, CE and SE*

174 For both the cake and the sediment, the procedure was adapted from Laguna et  
175 al., (2019). Briefly, 50 mg of defatted sample was weighed and 5 mL of ethanol 70% was  
176 added. The mixture was stirred in an orbital shaker (350 rpm) at 75°C for 20 min,  
177 centrifuged for 5 min at 2,147×g, and the final extracts were filtered (0.22 µm). For GCO,  
178 2.5 g was weighed and added with 5 mL of hexane, followed by 6 mL of methanol-water  
179 (60:40, v/v). The mixture was vortexed and then centrifuged at 3,500×g and 4°C for 10  
180 min. The methanol phase was removed and the operation was repeated three times (Wang  
181 X. et al., 2017). The MeOH extracts were evaporated at 35°C under a nitrogen stream,  
182 and the residue was resuspended in 500 µL of ethanol-water (70:30, v/v). The residue and  
183 the GCO extracts were kept at -20°C until HPLC analysis.

#### 184 *2.5. HPLC-DAD analysis of chlorogenic acids and caffeine in GCO and its residues of* 185 *pressing*

186 Chlorogenic acids and caffeine were quantified with an XR UFLC Shimadzu  
187 liquid phase chromatograph fitted with an SPD- M20A diode array detector (Kyoto,  
188 Japan). Separation was carried out with an ACE C18 reversed phase column (5 µm, 250  
189 mm×4.6 mm, Phenomenex, Le Pecq, France). Injection volume, flow rate, and oven  
190 temperature were set at 20 µL, 1 mL/min and 30°C, respectively. The gradient elution  
191 was performed using HPLC grade water with 0.1% (v/v) acetic acid (A) and methanol

192 with 0.1% (v/v) acetic acid (B) in the following conditions: 15% B (0-5 min), 15-80% B  
193 (5-30 min), 80-100% B (30-31 min), 100% B (31-35 min), 100-15% B (35-40 min). The  
194 detection wavelength was set at 328 nm for CGA and at 272 nm for caffeine. The  
195 identification of the CGA isomers was confirmed by comparison with retention time of  
196 the respective standards and literature data. The concentration of each CGA isomer was  
197 calculated by selecting 5-CQA as the suitable reference standard and by using it to  
198 calculate the response factor of other six CGA.

#### 199 *2.6. Preparation of residue extracts (CE and SE) for film elaboration*

200 The residues of GCO pressing were extracted as previously described by Lombo  
201 Vidal et al., (2020). In brief, for both CE and SE, ethanol-water (70:30, v/v) was used in  
202 a 1:10 ratio (w/v). For SE, the sample-solvent mixture was placed in an ultrasound bath  
203 for 6 min and then for 2 h in an orbital shaker at 55°C and 150 rpm. After centrifugation  
204 (20 min, 3,000×g, 25°C), the hydroalcoholic phase was removed and the pellet was re-  
205 extracted as described above. CE was ground for 1 min using a coffee blender and mixed  
206 with solvent heated at 50°C. The mixture was transferred to a shaker-incubator for 90 min  
207 at 55°C and 150 rpm. After extraction, the mixture was centrifuged (5 min, 2900×g,  
208 25°C). Both extracts (SE and CE) were filtered with paper Whatman no. 3, dried in a  
209 SpeedVac device (Savant, Thermo Scientific®), and the solid residue extracts were stored  
210 at -20°C. To determine the total solid contents of SE and CE, 1 mL of each extract  
211 (triplicate) was dried in an oven at 105 °C to constant weight.

#### 212 *2.7. Film elaboration*

213 The film-forming solutions of the active films were prepared by dissolving 2 g of  
214 CMC in 70 mL of ultrapure water and transferred to a shaker incubator for 6 h (35°C, 150  
215 rpm). Separately, 1 g of dried residue extracts (SE or CE) was dissolved in 30 mL of

216 ultrapure water for 5 min at 25°C using an Ultra-turrax T25 (IKA, USA). Afterwards, the  
217 solutions were mixed to obtain a 1:2 extracts/CMC mass ratio, followed by addition of  
218 0.5 mL glycerol as a plasticizer (25wt% based on CMC), 0.08 g of lecithin as an  
219 emulsifier (4wt% based on CMC), and 0.3 mL of GCO (10wt% based on CMC), and  
220 dispersed with an Ultra-turrax homogenizer at 13,500 rpm for 10 min. A control film of  
221 pure CMC was prepared by dissolving 2 g of CMC in 100 mL of ultrapure water and a  
222 second control was added with the same proportions of glycerol and lecithin that are used  
223 to active films. The resulting solution was poured on polystyrene petri dishes (80 mm  
224 diameter) and dried for 24 h at 25°C in a fume hood. Dried films were manually peeled  
225 off the plates and conditioned at 58% relative humidity (RH) in desiccators over a  
226 saturated solution of NaBr prior to all analyses.

227 Finally, four different formulations of films were obtained: (1) CMC control films  
228 produced only with carboxymethyl cellulose film; (2) C-G-L for films containing CMC  
229 (C), glycerol (G) and lecithin (L), also used as control; and films containing GCO added  
230 to residue extracts referred as C-CE (CMC + glycerol + lecithin + GCO + cake extract)  
231 and C-SE (CMC + glycerol + lecithin + GCO + sediment extract).

## 232 *2.8. Film characterization*

### 233 *2.8.1. Film thickness*

234 Thickness was measured using a micrometer (Mitutoyo, Japan; 0.001 mm  
235 precision), based on 10 readings taken at random locations of the films. Total solids of  
236 film-forming solutions were used to calculate the appropriate mass to obtain  
237 homogeneous thickness.

238 2.8.2. *Film moisture content*

239 Film samples were cut into small pieces and 0.2 g ( $w_1$ ) was placed in an  
240 aluminium pan (previously weighed dry) and dried at 105°C to constant weight ( $w_2$ ).

241 Film moisture content (%) was provided by eq. (1):

242 (1) Moisture (%) =  $\left(\frac{w_1 - w_2}{w_1}\right) \times 100$

243 2.8.3. *Light transmission and film transparency*

244 The UV-Vis light barrier property of films was determined by exposing film strips  
245 (10 x 40 mm) at wavelengths ranging from 200 to 800 nm (Bitencourt et al., 2014), using  
246 a UV-vis spectrophotometer UV-2700 (Shimadzu, Japan). An empty test cell was used  
247 as a reference. Three replicates were performed for each film formulation. Prior to the  
248 test, two new films were prepared with previously defatted SE as described in section  
249 (2.3.) to evaluate the possible contribution of lipid fraction compounds on this property.

250 The transparency value of the films was calculated using the following equation (2):

251 (2)  $\tau = \frac{-\log T_{600}}{\Delta x}$

252 Where,  $T_{600}$  is transmittance at 600 nm and  $\Delta x$  is the corresponding film thickness (mm).

253 The higher the transmittance values, the lower the transparency of the films.

254 2.8.4. *Oxygen permeability ( $O_2P$ ) and  $O_2$  permeance of films*

255 The  $O_2P$  value of the films was determined at 23°C and 50% RH by an oxygen  
256 permeability tester (PreSens, Fibox 4, Germany). Briefly, this system consists of two  
257 chambers separated by a grid where the film is placed with an open testing area of 0.00126  
258 m<sup>2</sup>. After a nitrogen flush was applied on both sides of the films to remove all surrounding  
259 O<sub>2</sub>, the lower chamber was exposed to an oxygen flow (50 mL/min), while the upper  
260 chamber was hermetically closed. By permeation, the O<sub>2</sub> molecules could pass through

261 the film where the sensor stood; the system measured oxygen ingress in % for 2 days.  
262 The analysis was performed in triplicate, and the results were averaged.

263 Oxygen permeability (O<sub>2</sub>P) of films was calculated using the following equation

264 (3)  $O_2P = J(\Delta x)/A(\Delta p)$

265 Where, O<sub>2</sub>P is the O<sub>2</sub> permeability (mol·m·m<sup>-2</sup>·s<sup>-1</sup>·Pa<sup>-1</sup>), J is the transmission rate  
266 of O<sub>2</sub> (mol/s), Δx is the film thickness (m), A is the surface area of the film (m<sup>2</sup>), and p  
267 is the differential partial pressure of the permeant gas across the film (Pa).

268 O<sub>2</sub> Permeance of films were calculated as the ratio of oxygen permeability (O<sub>2</sub>P)  
269 and film thickness (Δx).

### 270 *2.9. Antioxidant activity of raw materials and films*

271 The antioxidant capacity of the films and raw materials (CE, SE, GCO and its  
272 unsaponifiable fraction) was determined by using the DPPH method (Brand-Williams,  
273 Cuvelier & Berset, 1995) adapted to the microplate assay. For active films, an aliquot was  
274 prepared according to Lombo et al., (2020). Briefly, 20 μL of the aliquot solution of the  
275 films (20 mg/mL in water) was mixed with 180 μL of 150 μM DPPH methanolic solution  
276 in a microplate well (clear 96-well microplate). Measurements were taken at 515 nm and  
277 37°C every 15 min with a microplate reader (Infinite M1000 2, TECAN, Grödig, Austria)  
278 until the reaction reached a plateau. It is worth mentioning that the DPPH/sample ratio  
279 used in the present work was 1:9 (v/v) in comparison with previous work 1:15 (v/v), and  
280 thus 3-fold more DPPH concentrated. Also, it is important to consider that the time of  
281 analysis was set at 120 min in a kinetic mode, instead of a single measurement at 30 min  
282 in previous work (Lombo Vidal et al., 2020).

283 A standard curve of trolox was established using a range of concentrations from 4  
284 to 26 μmol. The DPPH radical scavenging activity of films and raw materials was

285 expressed as mmol of trolox equivalent (TE)/g dried film or extract on a dry matter basis  
286 (d.b.). All the measurements were performed in triplicate. The DPPH radical scavenging  
287 activity was calculated with the following equation:

$$288 \quad (4) \text{ DPPH} \cdot \text{inhibition (\%)} = 100 \times \frac{(\text{Blank absorbance} - \text{sample absorbance})}{(\text{Blank absorbance})}$$

289 In order to determine the contribution of each raw material to the total antioxidant  
290 activity of the active films, the samples were prepared according to the most suitable form  
291 of every material for the DPPH test. For the cake and the sediment, the solid extract (as  
292 described in section 2.6) was resuspended in ethanol 70% (v/v). For GCO, a phenolic  
293 extraction was prepared according to Lombo et al., (2020); and its unsaponifiable fraction  
294 was resuspended in ethanol 70% (v/v).

#### 295 *2.10. Release of phenolic compounds from films*

296 The release of phenolic compounds from the films was determined as follows: the  
297 samples were cut into small pieces (2 cm<sup>2</sup>) and then immersed in 5 mL water (to mimic  
298 aqueous food) or in 95% (v/v) ethanol to mimic a less polar medium. Then, all samples  
299 were placed in a shaker incubator at 25 °C and 125 rpm for 7 days (Piñeros-Hernandez et  
300 al., 2017). After the exposure period, the migration of phenolic compounds to the simulant  
301 medium was estimated by the Folin-Ciocalteu method (Singleton, Orhofer & Lamulea-  
302 Raventos, 1999); 100 µL of each medium was mixed with 700 µL of milli-Q water, 50  
303 µL of Folin-Ciocalteu reagent and 150 µL of sodium carbonate 20% (v/v). The mixture  
304 was vortexed and incubated in a water bath at 40°C for 30 min. Then, absorbance was  
305 measured at 750 nm using a microplate reader FlexStation III (Molecular Devices, San  
306 Jose, USA). The results were expressed as mg of gallic acid equivalents (GAE)/g of dried  
307 film.

## 308 2.11. Food system using fish oil

309 Commercial cod liver fish oil (25 mL) was poured into glass pots, and the active  
310 films were tested in two ways (**Fig. 1**). Firstly, in indirect mode, the film samples were  
311 cut into circles (3.5 cm diameter) and set into perforated screw-on lids. These covers were  
312 screwed on the glass cell in such a way that the film was in contact with the external  
313 atmosphere (upper side) and in contact with the internal headspace of the glass cell (lower  
314 side). The headspace (33 mL) was flushed with N<sub>2</sub> to get rid of internal residual O<sub>2</sub> at the  
315 beginning of the experiments and after each sampling. In the second configuration, the  
316 direct mode, active films (0.24 ± 0.03 g) and residue extracts of CE or SE (0.07 g) were  
317 immersed into fish oil, while the glass cell was left open in contact with the external  
318 atmosphere. Control fish oil systems covered with an aluminium foil were used as a  
319 reference for the indirect mode, and control without any protection, *i.e.*, directly exposed  
320 to ambient air, were used as a negative control for the direct mode, *i.e.* when active films  
321 and residue extracts were immersed within fish oil (**Fig. 1**). All experiments were run in  
322 triplicate.

323 Two aliquots (20 µL) of fish oil were taken from each pot sample every four days  
324 to determine peroxide value (PV) and TBARS, according to the method outlined by Hu  
325 et al. (2004) and Ghani et al. (2017), respectively. After each sampling, N<sub>2</sub> flush was used  
326 to remove air which had entered the headspace during sampling. All samples were  
327 maintained in an orbital shaker protected from light at 40°C and 110 rpm for 16 days.

### 328 2.11.1. Peroxide value (PV)

329 Fish oil (20 µL) was diluted in a methanol/butanol (30:70, v/v) solution according  
330 to Hu et al. (2004), adapted for the microplate reader. Then, 100 µL from this mixture  
331 was directly added to the microplate containing 160 µL of MeOH/BuOH (3:7, v/v), 2.5  
332 µL of 30% ammonium thiocyanate (w/v) and 2.5 µL of ferrous chloride solution (10 g/L)

333 in 3.5% HCl (w/v). After 10 min, absorbance was read at 500 nm in a TECAN microplate  
334 reader. PV was calculated on the basis of a standard curve using cumene hydroperoxide  
335 (0.7-65  $\mu$ M). The result was then expressed as meq O<sub>2</sub>/kg oil.

#### 336 2.11.2. Thiobarbituric acid-reactive substances (TBARS)

337 TBARS was determined according to Ghani et al. (2017). A fish oil sample (20  
338  $\mu$ L) was weighed and diluted in 1 mL of a methanol: *n*-butanol solution (30:70, v/v); 25  
339  $\mu$ L was taken out and diluted in a 1:4 ratio (v/v) with ultrapure water to react with 200  
340  $\mu$ L of a prepared solution of 15% (w/v) trichloroacetic acid, 0.375% (w/v) of  
341 thiobarbituric acid and 0.25 N of HCl. The mixture was heated in a water bath at 90°C  
342 for 15 min and the reaction was stopped in an ice bath after it turned pink. It was then  
343 centrifuged (3,354 $\times$ g, 10 min and 15 °C), and the absorbance of the supernatant was  
344 recorded at 532 nm in a TECAN microplate reader. The TBARS value was established  
345 using a standard curve of 1,1,3,3-tetramethoxypropane, a precursor for malonaldehyde  
346 (MDA) at 0.01 to 0.2 mM. The results were expressed in meq MDA/kg.

#### 347 2.12. Statistical analysis

348 One-way analysis of variance (ANOVA) and Tukey's multiple tests were  
349 performed using XLSTAT (Addinsoft 2020, NY, USA). For all statistical tests, a 95%  
350 confidence level was used.

### 351 3. Results and discussion

#### 352 3.1. Screw pressing and oil quality parameters

353 The quality parameters determined in cold-pressed GCO and its residues of  
354 pressing are shown in **Table 1**. Based on the PV obtained for GCO (2.5 meq O<sub>2</sub>/kg), there  
355 was no extensive oxidative degradation during the processing of green coffee beans  
356 and/or oil extraction. However, the high free fatty acid value found (6.0%, as oleic acid)



357 could be related to the moisture content and endogenous lipase activity of green coffee  
358 beans. In this study, moisture of the coffee seeds ranged between 9.3 and 11.2%, which  
359 facilitated the pressing process and resulted in an oil yield of 4.2%. Interestingly, low  
360 moisture content increases the force needed to initiate seed structure rupture, while high  
361 moisture content may also decrease oil yield owing to the lack of friction during pressing  
362 (Lazouk et al., 2015).

363 Cold pressing of GCO produced abundant residual biomass corresponding to cake  
364 (91%), as well as residual oil in sediment (58.1%), which raises interest in the valorization  
365 of these residues. The content of unsaponifiable matter reached 13.1%. This fraction, in  
366 green coffee beans (that concentrates bioactive compounds) is extremely high in  
367 comparison with most vegetable oils (0.1-1.0% of unsaponifiable) (Speer and Kölling-  
368 Speer, 2006). Certain unusual properties of GCO, such as its high moisture binding  
369 properties and its antioxidant and UV-Vis absorption properties, could be explained by  
370 the compounds of this unsaponifiable fraction (Chiari et al., 2014; Wagemaker et al.,  
371 2015).

### 372 *3.2. Identification and quantification of phenolic compounds in GCO and its residues*

373 Table 2 shows the compounds identified and quantified by HPLC-DAD in GCO  
374 and the ethanolic residue extracts (70% v/v). A total of seven CGA, including three  
375 caffeoylquinic acids (3-, 4- and 5-CQA), two feruloylquinic acids (4- and 5-FQA), two  
376 dicaffeoylquinic acids (3,5-diCQA and 4,5-diCQA), and caffeine, were identified in CE  
377 and SE. Total CGA content was 9.8% and 9.0% for CE and SE, respectively. These values  
378 of CGA are in line with the ones reported in regular green coffee beans, that usually range  
379 from 4 to 12% for *Coffea arabica* L. (Farah et al., 2005). As expected, the global chemical  
380 profiles in CGA of CE and SE were similar, and heavily dominated by caffeoylquinic

381 acids (CQA) which accounted for 68-74% of total CGA in these two by-products. This  
382 global similarity of CGA profile can be explained by a similar histological origin of these  
383 residues from the bean of the coffee fruit. Perrone et al., (2008) found that these three  
384 isomers (3-, 4- and 5-CQA), account for about 83% of total CGA in green coffee beans  
385 of *C. arabica* cultivars from Brazil.

386 CGA were not detected in GCO, probably due to the low affinity of phenolic  
387 compounds for oil. Caffeine was detected in GCO (0.2%) but it was more concentrated  
388 in CE (3.7%) and even more in SE (4.5%). We can hypothesize that SE concentrates more  
389 caffeine as it is formed by the precipitation of finer and more hygroscopic materials than  
390 CE. Interestingly, caffeine and CGA interact physico-chemically, forming a well-known  
391 complex, easily found and stored intracellularly in raw coffee beans (Waldhauser and  
392 Baumann, 1996). Oliveira et al. (2019) highlighted that the mechanical pressing of ground  
393 coffee bean materials would aid the subsequent extraction of CGA by breaking the cell  
394 wall of the beans.

### 395 *3.3. Film characterization*

#### 396 *3.3.1. Total solid, thickness and moisture of films*

397 **Table 3** shows thickness, total solids and moisture of the films. After addition of  
398 the residue extracts, thickness of active films was higher than that of control CMC-film  
399 ( $p < 0.05$ ) but similar amongst active films ( $p > 0.05$ ). Incorporation of GCO residues  
400 extracts but also glycerol and lecithin decreased film moisture content ( $p < 0.05$ ), probably  
401 owing to the presence of hydrophobic compounds.

#### 402 *3.4. Oxygen permeability ( $O_2P$ ) and permeance*

403 CMC films typically have good oxygen barrier properties, as most hydrocolloids-  
404 based films (Bourlieu et al., 2009). It can be explained by their large number of hydrogen

405 bonds, which helps adjacent chains bind tightly to each other, resulting in a dense polymer  
406 matrix (Sahraee et al., 2019). The O<sub>2</sub>P and permeance values at 50% RH and 23 °C of  
407 CMC-based active films developed in this study (**Table 3**) ranged from 8.3 to 9.9×10<sup>-17</sup>  
408 mol·m·m<sup>-2</sup>·s<sup>-1</sup>·Pa<sup>-1</sup> for C-CE and C-SE films, respectively, which is not significantly  
409 (p<0.05) different from the control with CMC, glycerin and lecithin (C-G-L, 8.4×10<sup>-17</sup>  
410 mol·m·m<sup>-2</sup>·s<sup>-1</sup>·Pa<sup>-1</sup>).

411 The slight tendency for increased O<sub>2</sub>P in active films was not significant except  
412 for the C-SE film in comparison with control of pure CMC (6×10<sup>-17</sup>mol·m·m<sup>-2</sup>·s<sup>-1</sup>·Pa<sup>-1</sup>).  
413 This tendency could be due to the incorporation of liquid lipids into hydrocolloid films,  
414 which usually implies an increase in the oxygen permeability resulting from the  
415 enhancement of the oxygen solubility in the film (Fabra et al., 2012). Conversely, when  
416 antioxidant compounds or oils with antioxidant properties are present in the film matrix,  
417 O<sub>2</sub>P could be lower because of direct chemical trapping of oxygen by antioxidants. In this  
418 sense, CGA present in CE and SE could be able to block the diffusion of oxygen  
419 molecules mediated by lipid components in the active film matrix. However, this effect  
420 of CGA is not sufficient to improve O<sub>2</sub>P barrier property under typical experimental  
421 conditions, i.e., when a strong oxygen flow rate is sweeping the film.

422 The increases in the thickness of active films observed in both previous and  
423 present work after the addition of CE and SE is most likely due to intermolecular  
424 interactions such hydrophobic force and hydrogen bonding of major bioactive compounds  
425 and CMC; however, it does not greatly affect the barrier properties of films compared  
426 with the effect of lipid addition. In our previous work, the addition of CE, SE and GCO  
427 did not modify the water vapor permeability (WVP) of CMC films that averaged 6×10<sup>-12</sup>  
428 mol·m<sup>-2</sup>·s<sup>-1</sup>·Pa<sup>-1</sup> at 25 °C and 90% RH (Table 3).

429           Based on the barrier properties of active films incorporated with CE or SE, we can  
430 say that our films present intermediate water barrier properties, i.e. much lower than good  
431 hydrophobic barrier such as beeswax  $5 \times 10^{-14} \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \cdot \text{Pa}^{-1}$  but similar to values  
432 reported for protein-based – gluten or corn zein,  $7 \times 10^{-12} \text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \cdot \text{Pa}^{-1}$  (Bourlieu et al.,  
433 2009). In addition, films incorporated with CE or SE have quite good oxygen barrier  
434 properties with  $\text{O}_2\text{P}$  twice lower than value reported for instance by Mujica-Paz and  
435 Gontard (1997) for gluten film at 50% RH (24°C), i.e.  $15 \times 10^{-17} \text{mol} \cdot \text{m} \cdot \text{m}^{-2} \cdot \text{s}^{-1} \cdot \text{Pa}^{-1}$ .  
436 Importantly, the resulting active films have a good oxygen barrier at 50% relative  
437 humidity. At higher relative humidity,  $\text{O}_2\text{P}$  values can increase because of the hydrophilic  
438 nature of both CMC and CGA, leading to greater polymer chain mobility (Gontard et al.,  
439 1996).

440           Thus, the films could be used as materials for the packaging of foods products  
441 with low to intermediate water activity and high sensitivity to oxidation. High fat content  
442 products that contain important amount of polyunsaturated fatty acids sensitive to  
443 oxidation such as marine oil or Brazil and Macadamia nuts are thus be good candidates  
444 to be packed.

### 445 *3.5. Light transmission and film transparency*

446           UV–Vis spectra and transparency results in **Table 4** showed low transmittance  
447 and concomitant high absorbance values of C-SE films, which markedly decreased in the  
448 UV region (below 0.0%). In previous work, it was reported very similar values with  
449 higher absorbance of C-SE films in comparison with C-CE ones (Lombo Vidal et al.,  
450 2020); however, these films were incorporated with 40% of SE or CE hydroalcoholic  
451 solution, in contrast with the 1:2 residue extract/polymer ratio (w/w) used in the present  
452 work. An additional sample was prepared by using defatted GCO sediment extract (SE)  
453 into films (named hereafter C-SE def) to eliminate residual oil contribution in the light

454 transmission and film transparency. Defatting SE decreased 2-folds the UV-Vis barrier  
455 capacity in comparison with the non-defatted C-SE films, but it had no impact on  
456 transparency. Interestingly, defatting SE helped to better understand the effect of both  
457 CGA and lipid fraction on light transmission; each respectively contributed evenly and to  
458 half of transmittance roughly. This means that, for rich-polyunsaturated food needing  
459 higher UV-light protection, CMC film with “raw” sediment extract (SE) may be more  
460 suitable than using defatted SE.

461 Transmittance is related to polymer alignment in the film network (Akhtar et al.,  
462 2018). Thus, the very low values can be explained by new intermolecular bounds formed  
463 between CMC and CGA, as well as by the presence of compounds from the lipid fraction,  
464 such as diterpenes (cafestol and kahweol), tocopherols and linoleic acid. Both CGA and  
465 compounds from the lipid fraction are able to absorb UV radiation (Chiari et al., 2014;  
466 Vilela et al., 2017). Transparency values decrease when using defatted SE in contrast with  
467 film containing non-defatted SE raw material, although the values are in the same order  
468 of magnitude (6.2 and 7.0, respectively). This means that fatty acids and other minor  
469 compounds present in the lipid fraction did not greatly impact on film transparency.

### 470 *3.6. Antioxidant activity and release of antioxidant compounds into food simulants*

471 **Table 5** shows the antioxidant activity of raw materials and active films with  
472 added GCO residue extracts. The high antioxidant capacity exhibited by CE and SE (11.0  
473 and 8.7 mmol Trolox equivalents/g, respectively) imparted significant antioxidant  
474 activity for the films formed by using 0.5 g dry residue extracts/g CMC. Importantly, the  
475 raw materials and the films followed different extraction processes, in which the ratio  
476 mass/solvent was different: (1:50, w/v) and (1:10, w/v) for film and raw material  
477 extraction, respectively. In another study (Fabra et al., 2018), films with added green tea  
478 and grape seed extracts at 0.5 g/g alginate, showed 12.1 and 2.95 mmol Trolox/g film

479 d.b., respectively. In this study, films with CE added in the same proportion (0.5 g/g  
480 CMC) had higher antioxidant activity (3.6 mmol Trolox eq/g film d.b.) than alginate film  
481 with added grape seed extract, but lower than alginate films with added green tea extract.

482 GCO did not show the presence of CGA by HPLC analysis; only caffeine was  
483 detected (**Table 2**), and GCO antioxidant activity was low (0.34 mmol Trolox eq/g of  
484 oil). Chiari et al., (2014) reported similar low antioxidant activity in GCO and Esquivel  
485 and Jiménez et al., (2012) associated antioxidant activity with the content of tocopherols  
486 and linoleic acid. GCO has an uncommon content of unsaponifiable matter among natural  
487 oils, reaching up to 19% (w/w). Therefore, this fraction was extracted to characterize its  
488 antioxidant activity owing to the presence of tocopherols, diterpenes (cafestol and  
489 kahweol) and phosphatides (Speer and Kölling-Speer, 2006). It is thus clear that almost  
490 half of the antioxidant activity of GCO is linked to the unsaponifiable fraction that  
491 represented 13% of GCO matter in this study (**Table 1**).

492 In the present work, the C-CE film had higher DPPH reducing abilities than the  
493 C-SE one (**Table 5**). This result can be closely related to the presence of higher  
494 concentrations of CGA in CE than in SE. As a result, the DPPH reducing abilities of C-  
495 CE films were more than 5-fold higher than those of the C-CE-films at the highest  
496 concentration (40%) (0.644 mmol eq. Trolox/g d.b), as reported in previous work, in  
497 which GCO residue extracts were kept in a solution until they were used as a medium to  
498 dissolve the polymer. This previous film preparation method has probably affected CMC  
499 solubility, the distribution of active compounds in the film network and their stability.  
500 Consequently, in the present study, dried/concentrated GCO residue extracts - added to  
501 the aqueous CMC solution - provide better protection of the functionality of antioxidants  
502 compounds during film formation and conditioning, and it boosted their antioxidant  
503 efficiency.

504 To confirm the efficiency of films as active food packaging material, their  
505 antioxidant capacity was explored: 1) either indirectly, through headspace, supposing that  
506 the film can be separated from the food by an inert atmosphere that must be protected by  
507 the film; 2) or directly, supposing that the active film may be in contact with the food and  
508 that such contact will trigger the migration of active compounds released from the film  
509 matrix onto the food surface. The mechanism of release depends largely on the material  
510 and morphology of the film, the partition coefficient between polymer/solvent, as well as  
511 the polarity of the antioxidant compounds in comparison to the packaged or covered food  
512 (Maryam Adilah et al., 2018).

513 Considering firstly the release of antioxidant compounds from both C-CE and C-  
514 SE active films in model solutions (**Fig. 2**), we found that this release was much higher  
515 in water (49.9 and 14.2 mg GAE/g of d.b., for C-CE and C-SE respectively) than in  
516 ethanol (2.0 and 5.4 mg GAE/g of d.b., C-CE and C-SE respectively). More precisely, in  
517 ethanol, the release of antioxidants decreased drastically in active C-CE and C-SE films  
518 (95% and 61%, respectively). In water, the hydrophilic nature of CMC facilitates the  
519 relaxation of polymer chains, resulting in a high swelling degree in which most the active  
520 films were dissolved after 7 days. In this way, the interactions between CGA and  
521 hydrophilic groups of the CMC matrix through the potential hydrogen bonding could be  
522 limited by the competitive binding effect of water molecules. A similar trend was found  
523 in soy protein isolate and fish gelatine films added with mango kernel extract, i.e. high  
524 antioxidant release in water (67.8 and 69.0  $\mu\text{g}$  GAE/g, respectively) and considerable  
525 decreases in ethanol (12.4 and 16.9  $\mu\text{g}$  GAE/g, respectively) (Maryam Adilah et al.,  
526 2018).

527 Importantly, no hydrophobic liquid simulant (which could be a good food system  
528 of fish oil investigated in the food system) was added in this step. However, a low natural

529 content in CGA and other antioxidants in GCO is an indication that the antioxidant  
530 solubility of GCO residues in an oil is probably limited.

### 531 *3.7. Food systems using fish oil indirectly or directly protected by the active film*

532 For a better approach to the active film protection in real conditions, we set up a  
533 test of the active film material in a food system and assessed two types of mechanisms:  
534 1) indirect contact of the film with food but protection of an inert headspace; 2) direct  
535 contact of the film with the food. In addition, as a putative positive control, pure GCO  
536 residue extracts were integrated in direct contact also with the food.

#### 537 *3.7.1. Indirect contact and protective atmosphere:*

538 Primary lipid oxidation results measured by PV in fish oil samples protected with  
539 active films are shown in **Fig 3A**. The acceptability limit of 5 Meq O<sub>2</sub>/kg for fish oil  
540 (Codex Alimentarium Commission, 2017) was reached at day 4, indicating a propagation  
541 stage of lipid oxidation. The C-SE and C-CE films decreased PV values of fish oil  
542 between 80 to 90% in comparison with the control sample covered with aluminum foil  
543 (33.0, 67.2 and 364.2 Meq O<sub>2</sub>/kg, respectively) at the end of the storage period (**Fig 3A**).

544 Moreover, C-SE and C-SE films showed lower PV values ( $p < 0.05$ ) than film  
545 control C-G-L (without extracts addition). Therefore, it seems that GCO residue extracts  
546 provided films with oxygen scavenging ability in a controlled atmosphere, which is a  
547 plausible hypothesis because the oxygen permeability of films with added GCO residue  
548 extracts showed a slight tendency to increase, and it was not significantly different from  
549 film control C-G-L. Bonilla et al. (2013) found satisfactory PV values in sunflower oil  
550 protected with sodium caseinate films with cinnamon or ginger essential oils when  
551 compared with control (aluminum foil); however, the protective effect of films against



552 lipid oxidation was not directly related to the antioxidant capacity of the isolated essential  
553 oils.

554 The extent of secondary lipid oxidation products that are formed by  
555 decomposition of hydroperoxides by means of  $\beta$ -scission reactions was measured by the  
556 TBARS method (**Fig 3C**). Fish oil showed similar TBARS values in samples protected  
557 with C-CE and C-SE at the end of storage period, which were significantly lower than  
558 film control C-G-L and aluminum control (**Fig 3C**). This indicates a decrease in the  
559 formation of secondary oxidation products by 80% (C-SE), 76% (C-CE) and 58% (C-G-  
560 L).

561 The presence of antioxidants can play a key role in protecting fish oil  
562 against oxidation by an  $O_2$  scavenging mechanism. It was found that 1 g of gallic acid can  
563 absorb 400 mg of  $O_2$  (Marino et al., 2014), and very similar values of  $O_2$  consumption  
564 were reported at 38°C for 16 days, still for gallic acid included into bio-based multilayer  
565 films (Pant et al., 2017). More precisely, it was found that in 4 days, 1 g of GA may  
566 absorb 80 mg of  $O_2$  (Pant et al 2017). Supposing that chlorogenic acids behave as gallic  
567 acid and could absorb  $O_2$  with the same rate than the observed for gallic acid, it was  
568 calculated that in the experimental set up conditions of the present work, C-CE and C-SE  
569 could scavenge 0.627 and 0.504 mg of  $O_2$ , respectively in 4 days (see Appendix for all  
570 calculation details). These values are higher than the total amount of both  
571  $O_2$  dissolved into fish oil and  $O_2$  that remained in the headspace after  $N_2$  flushing,  
572 which was estimated equivalent to 0.47 mg of  $O_2$  at day 4.  $O_2$  scavenging capacities of  
573 C-CE and C-SE films would be enough to absorb  $O_2$  residuals obtained after several  
574 opening/ $N_2$  flushing cycles in the film testing process. This can explain the effectiveness  
575 of C-CE and C-SE films against fish oil oxidation observed in the present work, when film  
576 is not in direct contact with the oil. CGA presents hydroxyl groups bonded to the aromatic

577 ring in an *ortho*-position with respect to each other, as gallic acid, and these hydroxyl  
578 groups are chief determinant for the strong antioxidant capacity of phenolic compounds  
579 in general (Badhani et al., 2015). This argument tends to confirm the hypothesis made  
580 about the O<sub>2</sub> scavenging properties of CGA.

581           Moreover, the mixture of two or more different CGA can have a synergistic effect,  
582 resulting in a greater efficiency than individual antioxidants. Apart from hydrogen atom  
583 donation, these acids augment the overall antioxidant capacity by regenerating other  
584 prooxidant phenols via electron donation (Palafox-Carlos et al., 2012).

585           However, both C-CE and C-SE seem to contain quite similar amounts of CGA  
586 and antioxidant; thus, it is difficult to formulate a precise hypothesis that can explain the  
587 highest protection obtained by using C-SE indirectly. Especially because the C-SE film  
588 has higher O<sub>2</sub>P and lower antioxidant-DPPH, but SE has more bioactive compounds from  
589 the lipid fraction and caffeine contents, in addition to a higher release of phenolics to  
590 ethanol 95%.

### 591 3.7.2. *Direct contact without protective atmosphere*

592           In direct contact with fish oil (**Fig 3B**), the pure residue extracts (SE and CE) led  
593 to a greater reduction of PV in comparison with the effect of active films. Indeed, PV  
594 reduction rates were 44.3% and 30.3% in comparison with control (unprotected oil) at the  
595 end of the storage period, for pure residue extracts of SE and CE, respectively.

596           There was a significant tendency to decrease secondary oxidation products only  
597 in fish oil samples with addition of SE (**Fig 3D**), which suggested that lower polarity  
598 compounds from the lipid fraction of green coffee (e.g., tocopherols or diterpenes) and  
599 caffeine could act as antioxidants in the oil phase. The poor effect of active films (both

600 C-CE and C-SE) can be due to the fact that there was no significant migration of  
601 antioxidant compounds from the film matrix into the fish oil. These findings are in line  
602 with those previously reported in the release section (3.7; Fig 2), i.e., migration of  
603 antioxidants was much lower in ethanol than in water. In addition, it would be highly  
604 desirable that the C-CE film, with higher CGA content, could release these polar  
605 compounds at the oil-air interface, where most oxidation occurs. However, this effect was  
606 not found.

607 According to Akhtar et al., (2014), the most appropriate way to control lipid  
608 oxidation may be to decrease light exposure and oxygen by applying suitable O<sub>2</sub>  
609 scavenging-active packaging. Our results with C-SE and C-CE films, in terms of indirect  
610 protection, support this hypothesis. The formation of both primary and secondary  
611 oxidation products in fish oil was greatly delayed along the storage period with the use of  
612 both active films as lids, coupled to inert headspace.

#### 613 **4. Conclusions**

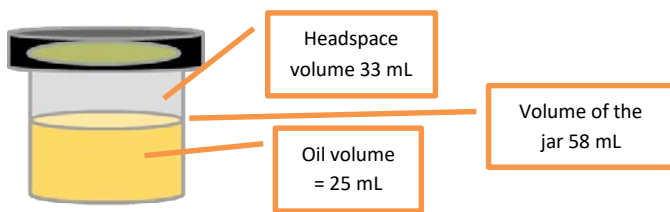
614 The residues of cold-pressed GCO showed an important amount of CGA and  
615 caffeine. The residue extracts of cake (CE) and sediment (SE) provided CMC films with  
616 antioxidant capacity. The active C-CE and C-SE films showed high release of  
617 antioxidants in water (polar simulant), especially C-CE, while antioxidant migration  
618 sharply decreased into the less-polar simulant (ethanol 95%), with C-SE showing a better  
619 performance than C-CE. The incorporation of residue extracts and GCO slightly affect  
620 the oxygen barrier properties of CMC active films. The remarkable UV-Vis absorption  
621 exhibited by active films is due to an additive effect of CGA, caffeine and other non-  
622 identified compounds present in the lipid fraction of green coffee beans. The protective  
623 effect against oxidation observed in fish oil was satisfactory when films were used in  
624 indirect protection over the oil and were coupled with the inert headspace. In this tested

625 food system (fish oil), the C-SE film presented a slightly higher efficacy than C-CE films.  
 626 However, future research should be conducted in a more realistic food packaging system,  
 627 such as Brazil and Macadamia nuts with high fat content, but low water content.

628 **5. Appendix A**

629 *Estimation of the O<sub>2</sub> content absorbed by the active films in the food system with fish oil (§*  
 630 *Indirect contact and protective atmosphere)*

631



632

633 To calculate the quantity of O<sub>2</sub> (in mg) that could be absorbed by the active film, we consider as  
 634 first hypothesis that 1% of O<sub>2</sub> remains in headspace even after N<sub>2</sub> flushing. Some O<sub>2</sub> remains also  
 635 dissolved in oil, this quantity is assumed to be in equilibrium with the O<sub>2</sub> partial pressure in  
 636 headspace (1%).

637 The number of moles of O<sub>2</sub> remaining in headspace is calculated from the perfect gas law as  
 638 follows:

639 
$$n_{O_2,HS} = \frac{p_T V}{RT} \quad \text{Equation (A.1)}$$

640 Where  $p_T$  is the atmospheric pressure ( $1.013 \times 10^5$  Pa),  $V$  is the headspace volume ( $33 \times 10^{-6}$  m<sup>3</sup>),  $R$   
 641 is the universal constant of perfect gas ( $8.32 \text{ J} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$ ) and  $T$  the temperature ( $293^\circ\text{K}$ ).

642 Then, the corresponding quantity of O<sub>2</sub> dissolved in oil is calculated using the Henry's law:

643

644 
$$n_{O_2,oil} = p_{O_2} S_{O_2,in\ oil} \quad \text{Equation (A.2)}$$

645 Where  $p_{O_2}$  is the O<sub>2</sub> partial pressure in headspace ( $p_{O_2} = 0.01 \times 1.013 \times 10^5$ ) and  $S_{O_2,in\ oil}$  is  
 646 the solubility of O<sub>2</sub> in oil at 20°C,  $S_{O_2,in\ oil} = 3.6 \times 10^{-8} \text{ mole} \cdot \text{kg}^{-1} \cdot \text{Pa}^{-1}$  taken from Chaix et al  
 647 (2014).

648

649 According to Equations A.1 and A.2, the total quantity of O<sub>2</sub> to absorb in the system at time 0 (O<sub>2</sub>  
 650 transfer from external atmosphere did not start) is:

651

$n_{O_2,HS}$	$1.37 \times 10^{-5}$	moles $O_2$
$n_{O_2,oil=}$	$3.65 \times 10^{-5}$	moles $O_2$ /kg oil
$n_{O_2,oil=}$	$8.21 \times 10^{-7}$	moles $O_2$ , for 25 ml of oil (supposing density of 0.9 for the fish oil)
$n_{O_2,total(HS+oil)}$	$1.45 \times 10^{-5}$	moles $O_2$ (HS + oil)
$m_{O_2,total(HS+oil)}$	<b>0.47</b>	<b>mg <math>O_2</math> to absorb (HS + oil)</b>

652

653 The films used as lids in the indirect system with fish oil have the following characteristics:

- 654
- C-CE: contains 7.84 mg of chlorogenic acids per film area used as a lid (surface of the lid is 9.6 cm<sup>2</sup>, weight of the lid is 230 mg and thickness of the lid is 140 μm)
- 655
- C-SE: contains 6.3 mg of chlorogenic acids per film area used as a lid (surface of the lid is 9.6 cm<sup>2</sup>, weight of the lid is 200 mg and thickness of the lid is 120 μm).
- 656
- 657
- 658

659 If we consider that chlorogenic acid behaves as gallic acid and can absorb  $O_2$  in the same amount,  
660 i.e. 80 mg of  $O_2$  absorbed per g of gallic acid in 4 days (Pant et al., 2017), we can calculate that:

- 661
- **0.627 mg of  $O_2$**  could be absorbed by C-CE film containing 7.84 mg of (surface of the lid is 9.6 cm<sup>2</sup>, masse of the lid is 230 mg and thickness of the lid is 140 μm)
- 662
- **0.504 mg of  $O_2$**  could be absorbed by C-SE films containing 6.3 mg of chlorogenic acids
- 663
- 664

665 Both quantities are higher than the quantity of  $O_2$  to absorb in the system (47 mg). We conclude  
666 that, assuming that chlorogenic acid behaves as gallic acid, active C-CE and C-SE films may have  
667 protected the oil based on their  $O_2$  scavenging properties.

668 Besides, the maximal  $O_2$  absorption capacity would be about 400 mg/g of active compound (still  
669 based on gallic acid, (Pant et al., 2017). That means that the active films could absorb in total  
670 about 3 mg of  $O_2$ . This correspond to  $O_2$  residuals obtained after several opening cycles in the  
671 film testing process.

672

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## 685 **6. References**

686 AOCS. (2012). American Oil Chemists' Society. Official methods and recommended  
687 practices of the American Oil Chemists' Society. 6th ed. Champaign, USA, AOCS.  
688 [AOCS Official method ISO 18609: 2000, Cd3d – 63, Cd8 – 53].

689 Akhtar, H.M.S., Riaz, A., Hamed, Y.S., Abdin, M., Chen, G., Wan, P., Zeng, X., 2018.  
690 Production and characterization of CMC-based antioxidant and antimicrobial films  
691 enriched with chickpea hull polysaccharides. *Int. J. Biol. Macromol.* 118, 469–477.  
692 <https://doi.org/10.1016/j.ijbiomac.2018.06.090>

693 Andrady, A.L., Neal, M.A., Andrady, A.L., Neal, M.A., 2009. Applications and societal  
694 benefits of plastics Applications and societal benefits of plastics. *Phil. Trans. R.*  
695 *Soc. B.* 364, 1977–1984. <https://doi.org/10.1098/rstb.2008.0304>

696 Arik Kibar, E.A., Us, F., 2013. Thermal, mechanical and water adsorption properties of  
697 corn starch-carboxymethylcellulose/methylcellulose biodegradable films. *J. Food*  
698 *Eng.* 114, 123–131. <https://doi.org/10.1016/j.jfoodeng.2012.07.034>

699 Badhani, B., Sharma, N., Kakkar, R., 2015. Gallic acid: A versatile antioxidant with  
700 promising therapeutic and industrial applications. *RSC Adv.* 5, 27540–27557.  
701 <https://doi.org/10.1039/c5ra01911g>

702 Ballesteros, L.F., Cerqueira, M.A., Teixeira, J.A., Mussatto, S.I., 2018. Production and  
703 physicochemical properties of carboxymethyl cellulose films enriched with spent  
704 coffee grounds polysaccharides. *Int. J. Biol. Macromol.* 106, 647–655.  
705 <https://doi.org/10.1016/j.ijbiomac.2017.08.060>

706 Bitencourt, C.M., Fávaro-Trindade, C.S., Sobral, P.J.A., Carvalho, R.A., 2014. Gelatin-

707 based films additivated with curcuma ethanol extract: Antioxidant activity and  
708 physical properties of films. *Food Hydrocoll.* 40, 145–152.  
709 <https://doi.org/10.1016/j.foodhyd.2014.02.014>

710 Bonilla, J., Talón, E., Atarés, L., Vargas, M., Chiralt, A., 2013. Effect of the  
711 incorporation of antioxidants on physicochemical and antioxidant properties of  
712 wheat starch-chitosan films. *J. Food Eng.* 118, 271–278.  
713 <https://doi.org/10.1016/j.jfoodeng.2013.04.008>

714 Bourlieu, C., Guillard, V., Vallès-Pamiès, B., Guilbert, S., Gontard, N., 2009. Edible  
715 moisture barriers: How to assess of their potential and limits in food products  
716 shelf-life extension. *Crit. Rev. Food Sci. Nutr.* 49, 474–499.  
717 <https://doi.org/10.1080/10408390802145724>

718 Brand-Williams, W., Cuvelier, M.E., Berset, C., 1995. Use of a free radical method to  
719 evaluate antioxidant activity. *LWT – Food Sci. Technol.* 28, 25–30,  
720 [https://doi.org/10.1016/S0023-6438\(95\)80008-5](https://doi.org/10.1016/S0023-6438(95)80008-5).

721 Budryn, G., Nebesny, E., Zyzelewicz, D., Oracz, J., 2014. Properties of model systems  
722 of sunflower oil and green coffee extract after heat treatment and storage. *LWT -*  
723 *Food Sci. Technol.* 59, 467–478. <https://doi.org/10.1016/j.lwt.2014.05.048>

724 Castro, A.C.C.M.C.C.M., Oda, F.B.B., Almeida-Cincotto, M.G.J.G.J., Davanço,  
725 M.G.G., Chiari-Andréo, B.G.G., Cicarelli, R.M.B.M.B., Peccinini, R.G.G., Zocolo,  
726 G.J.J., Ribeiro, P.R.V.R.V., Corrêa, M.A.A., Isaac, V.L.B.L.B., Santos, A.G.G.,  
727 2018. Green Coffee Seed Residue: A Sustainable Source of Antioxidant  
728 Compounds. *Food Chem.* 246, 48–57.  
729 <https://doi.org/10.1016/j.foodchem.2017.10.153>

730 Chaix, E., Guillaume, C., Guillard, V., 2014. Oxygen and Carbon Dioxide Solubility and  
731 Diffusivity in Solid Food Matrices: A Review of Past and Current Knowledge.

732 Compr. Rev. Food Sci. Food Saf. 13, 261–286. <https://doi.org/10.1111/1541->  
733 4337.12058

734 Chiari, B.G., Trovatti, E., Pecoraro, É., Corrêa, M.A., Cicarelli, R.M.B., Ribeiro, S.J.L.,  
735 Isaac, V.L.B., 2014. Synergistic effect of green coffee oil and synthetic sunscreen  
736 for health care application. *Ind. Crops Prod.* 52, 389–393.  
737 <https://doi.org/10.1016/j.indcrop.2013.11.011>

738 Codex Alimentarium Commission, 2017. Standard for Fish Oils. *Codex Stan 329*, 1–6.

739 de Moraes Crizel, T., de Oliveira Rios, A., D. Alves, V., Bandarra, N., Moldão-Martins,  
740 M., Hickmann Flôres, S., 2018. Active food packaging prepared with chitosan and  
741 olive pomace. *Food Hydrocoll.* 74, 139–150.  
742 <https://doi.org/10.1016/j.foodhyd.2017.08.007>

743 Dziki, D., Gawlik-Dziki, U., Pecio, Ł., Różyło, R., Świeca, M., Krzykowski, A., Rudy,  
744 S., 2015. Ground green coffee beans as a functional food supplement - Preliminary  
745 study. *LWT - Food Sci. Technol.* 63, 691–699.  
746 <https://doi.org/10.1016/j.lwt.2015.03.076>

747 Esquivel, P., Jiménez, V.M., 2012. Functional properties of coffee and coffee by-  
748 products. *Food Res. Int.* 46, 488–495.  
749 <https://doi.org/10.1016/j.foodres.2011.05.028>

750 Fabra, M.J., Falcó, I., Randazzo, W., Sánchez, G., López-Rubio, A., 2018. Antiviral and  
751 antioxidant properties of active alginate edible films containing phenolic extracts.  
752 *Food Hydrocoll.* 81, 96–103. <https://doi.org/10.1016/j.foodhyd.2018.02.026>

753 Fabra, M.J., Talens, P., Gavara, R., Chiralt, A., 2012. Barrier properties of sodium  
754 caseinate films as affected by lipid composition and moisture content. *J. Food Eng.*  
755 109, 372–379. <https://doi.org/10.1016/j.jfoodeng.2011.11.019>

756 Farah, A., De Paulis, T., Trugo, L.C., Martin, P.R., 2005. Effect of roasting on the



757 formation of chlorogenic acid lactones in coffee. *J. Agric. Food Chem.* 53, 1505–  
758 1513. <https://doi.org/10.1021/jf048701t>

759 Ganiari, S., Choulitoudi, E., Oreopoulou, V., 2017. Edible and active films and coatings  
760 as carriers of natural antioxidants for lipid food. *Trends Food Sci. Technol.* 68, 70–  
761 82. <https://doi.org/10.1016/j.tifs.2017.08.009>

762 Gawlik-Dziki, U., Świeca, M., Dziki, D., Kowalska, I., Pecio, Ł., Durak, A., Seczyk, Ł.,  
763 2014. Lipoxxygenase inhibitors and antioxidants from green coffee-mechanism of  
764 action in the light of potential bioaccessibility. *Food Res. Int.* 61, 48–55.  
765 <https://doi.org/10.1016/j.foodres.2014.05.002>

766 Ghani, M.A., Barril, C., Bedgood, D.R., Prenzler, P.D., 2017. Measurement of  
767 antioxidant activity with the thiobarbituric acid reactive substances assay. *Food*  
768 *Chem.* 230, 195–207. <https://doi.org/10.1016/j.foodchem.2017.02.127>

769 Gontard, N., Sonesson, U., Birkved, M., Majone, M., Bolzonella, D., Celli, A.,  
770 Angellier-Coussy, H., Jang, G.W., Verniquet, A., Broeze, J., Schaer, B., Batista,  
771 A.P., Sebok, A., 2018. A research challenge vision regarding management of  
772 agricultural waste in a circular bio-based economy. *Crit. Rev. Environ. Sci.*  
773 *Technol.* 48, 614–654. <https://doi.org/10.1080/10643389.2018.1471957>

774 Gontard, N., Thibault, R., Cuq, B., Guilbert, S., 1996. Influence of relative humidity  
775 and film composition on oxygen and carbon dioxide permeabilities of edible films.  
776 *J. Agric. Food Chem.* 44, 1064–1069. <https://doi.org/10.1021/jf9504327>

777 Hu, M., McClements, D.J., Decker, E.A., 2004. Impact of chelators on the oxidative  
778 stability of whey protein isolate-stabilized oil-in-water emulsions containing  $\omega$ -3  
779 fatty acids. *Food Chem.* 88, 57–62.  
780 <https://doi.org/10.1016/j.foodchem.2004.01.022>

781 Palafox-Carlos, H., Gil-Chávez, J., Sotelo-Mundo, R.R., Namiesnik, J., Gorinstein, S.,

782 González-Aguilar, G.A., 2012. Antioxidant interactions between major phenolic  
783 compounds found in “Ataulfo” mango pulp: Chlorogenic, gallic, protocatechuic  
784 and vanillic acids. *Molecules* 17, 12657–12664.  
785 <https://doi.org/10.3390/molecules171112657>

786 Kirschweng, B., Tátraaljai, D., Földes, E., Pukánszky, B., 2017. Natural antioxidants as  
787 stabilizers for polymers. *Polym. Degrad. Stab.* 145, 25–40.  
788 <https://doi.org/10.1016/j.polymdegradstab.2017.07.012>

789 Laguna, O., Barakat, A., Alhamada, H., Durand, E., Baréa, B., Fine, F., Villeneuve, P.,  
790 Citeau, M., Dauguet, S., Lecomte, J., 2018. Production of proteins and phenolic  
791 compounds enriched fractions from rapeseed and sunflower meals by dry  
792 fractionation processes. *Ind. Crops Prod.* 118, 160–172.  
793 <https://doi.org/10.1016/j.indcrop.2018.03.045>

794 Laguna, O., Odinet, E., Bisotto, A., Baréa, B., Villeneuve, P., Sigoillot, J.-C., Record,  
795 E., Faulds, C.B., Fine, F., Lesage-Meessen, L., Lomascolo, A., Lecomte, J., 2019.  
796 Release of phenolic acids from sunflower and rapeseed meals using different  
797 carboxylic esters hydrolases from *Aspergillus niger*. *Ind. Crops Prod.* 139, 111579.  
798 <https://doi.org/10.1016/j.indcrop.2019.111579>

799 Lazouk, M.A., Savoie, R., Kaddour, A., Castello, J., Lanoisellé, J.L., Van Hecke, E.,  
800 Thomasset, B., 2015. Oilseeds sorption isotherms, mechanical properties and  
801 pressing: Global view of water impact. *J. Food Eng.* 153, 73–80.  
802 <https://doi.org/10.1016/j.jfoodeng.2014.12.008>

803 Lombo Vidal, O., Tsukui, A., Garrett, R., Miguez Rocha-Leão, M.H., Piler Carvalho,  
804 C.W., Pereira Freitas, S., Moraes de Rezende, C., Simões Larráz Ferreira, M.,  
805 2020. Production of bioactive films of carboxymethyl cellulose enriched with  
806 green coffee oil and its residues. *Int. J. Biol. Macromol.* 146, 730–738.

807 <https://doi.org/10.1016/j.ijbiomac.2019.10.123>

808 Marino, T., Galano, A., Russo, N., 2014. Radical scavenging ability of gallic acid  
809 toward OH and OOH radicals-reaction mechanism and rate constants from the  
810 density functional theory. *J. Phys. Chem. B* 118, 10380–10389.  
811 <https://doi.org/10.1021/jp505589b>

812 Maryam Adilah, Z.A., Jamilah, B., Nur Hanani, Z.A., 2018. Functional and antioxidant  
813 properties of protein-based films incorporated with mango kernel extract for active  
814 packaging. *Food Hydrocoll.* 74, 207–218.  
815 <https://doi.org/10.1016/j.foodhyd.2017.08.017>

816 Nieto, M.B., 2009. Structure and Function of Polysaccharide Gum-Based Edible Films  
817 and Coatings. In: Huber K., Embuscado M. (eds) *Edible Films and Coatings for*  
818 *Food Applications*. Springer, New York, NY. [https://doi.org/10.1007/978-0-387-](https://doi.org/10.1007/978-0-387-92824-13)  
819 [92824-13](https://doi.org/10.1007/978-0-387-92824-13)

820 Nosari, A.B.F.L., Lima, J.F., Serra, O.A., Freitas, L.A.P., 2015. Improved green coffee  
821 oil antioxidant activity for cosmetical purpose by spray drying microencapsulation.  
822 *Brazilian J. Pharmacogn.* 25, 307–311. <https://doi.org/10.1016/j.bjp.2015.04.006>

823 Oliveira, N.A. de, Cornelio-Santiago, H.P., Fukumasu, H., Oliveira, A.L. de, 2018.  
824 Green coffee extracts rich in diterpenes – Process optimization of pressurized  
825 liquid extraction using ethanol as solvent. *J. Food Eng.* 224, 148–155.  
826 <https://doi.org/10.1016/j.jfoodeng.2017.12.021>

827 Oliveira, R., Carvalho, G.R., Cirillo, M., Queiroz, F., 2019. Effect of ecofriendly bio-  
828 based solvents on oil extraction from green coffee bean and its industrial press  
829 cake. *Brazilian J. Chem. Eng.* 36, 1739–1753. [https://doi.org/10.1590/0104-](https://doi.org/10.1590/0104-6632.20190364s20190102)  
830 [6632.20190364s20190102](https://doi.org/10.1590/0104-6632.20190364s20190102)

831 Pant, A.F., Sangerlaub, S., Muller, K., 2017. Gallic acid as an oxygen scavenger in bio-

832 based multilayer packaging films. *Materials* (Basel). 10.  
833 <https://doi.org/10.3390/ma10050489>

834 Perrone, D., Farah, A., Donangelo, C.M., de Paulis, T., Martin, P.R., 2008.  
835 Comprehensive analysis of major and minor chlorogenic acids and lactones in  
836 economically relevant Brazilian coffee cultivars. *Food Chem.* 106, 859–867.  
837 <https://doi.org/10.1016/j.foodchem.2007.06.053>

838 Piñeros-Hernandez, D., Medina-Jaramillo, C., López-Córdoba, A., Goyanes, S., 2017.  
839 Edible cassava starch films carrying rosemary antioxidant extracts for potential use  
840 as active food packaging. *Food Hydrocoll.* 63, 488–495.  
841 <https://doi.org/10.1016/j.foodhyd.2016.09.034>

842 Ritchie, H., Roser, M., (2018). "Plastic Pollution". Published online at  
843 OurWorldInData.org. Retrieved from: '<https://ourworldindata.org/plastic-pollution>'  
844 [Online Resource]

845 Sahraee, S., Milani, J.M., Regenstein, J.M., Kafil, H.S., 2019. Protection of foods  
846 against oxidative deterioration using edible films and coatings: A review. *Food*  
847 *Biosci.* 32, 100451. <https://doi.org/10.1016/j.fbio.2019.100451>

848 Serrano-León, J.S., Bergamaschi, K.B., Yoshida, C.M.P., Saldaña, E., Selani, M.M.,  
849 Rios-Mera, J.D., Alencar, S.M., Contreras-Castillo, C.J., 2018. Chitosan active  
850 films containing agro-industrial residue extracts for shelf life extension of chicken  
851 restructured product. *Food Res. Int.* 108, 93–100.  
852 <https://doi.org/10.1016/j.foodres.2018.03.031>

853 Singleton, V.L., Orthofer, R., Lamuela-Raventós, R.M., 1999. Analysis of total phenols  
854 and other oxidation substrates and antioxidants by means of Folin-Ciocalteu  
855 reagent. *Methods Enzymol.* 152–178, [https://doi.org/10.1016/S0076-](https://doi.org/10.1016/S0076-6879(99)99017-1)  
856 [6879\(99\)99017-1](https://doi.org/10.1016/S0076-6879(99)99017-1).

857 Speer, K., Kölling-Speer, I., 2006. The lipid fraction of the coffee bean. *Brazilian J.*  
858 *Plant Physiol.* 18, 201–216. <https://doi.org/10.1590/S1677-04202006000100014>

859 Tongnuanchan, P., Benjakul, S., Prodpran, T., Nilsuwan, K., 2015. Emulsion film based  
860 on fish skin gelatin and palm oil: Physical, structural and thermal properties. *Food*  
861 *Hydrocoll.* 48, 248–259. <https://doi.org/10.1016/j.foodhyd.2015.02.025>

862 Vilela, C., Pinto, R.J.B., Coelho, J., Domingues, M.R.M., Daina, S., Sadocco, P.,  
863 Santos, S.A.O., Freire, C.S.R., 2017. Bioactive chitosan/ellagic acid films with  
864 UV-light protection for active food packaging. *Food Hydrocoll.* 73, 120–128.  
865 <https://doi.org/10.1016/j.foodhyd.2017.06.037>

866 Wagemaker, T.A.L., Silva, S.A.M., Leonardi, G.R., Maia Campos, P.M.B.G., 2015.  
867 Green *Coffea arabica* L: Seed oil influences the stability and protective effects of  
868 topical formulations. *Ind. Crops Prod.* 63, 34–40.  
869 <https://doi.org/10.1016/j.indcrop.2014.09.045>

870 Waldhauser, S.S.M., Baumann, T.W., 1996. Compartmentation of Caffeine and Related  
871 Purine of Their Vacuolar Complex Formation With Chlorogenic Acids 42, 985–  
872 996.

873 Wang, W., Yang, H., Johnson, D., Gensler, C., Decker, E., Zhang, G., 2017. Chemistry  
874 and biology of  $\omega$ -3 PUFA peroxidation-derived compounds. *Prostaglandins Other*  
875 *Lipid Mediat.* 132, 84–91. <https://doi.org/10.1016/j.prostaglandins.2016.12.004>

876 Wang, X., Zeng, Q., del Mar Contreras, M., Wang, L., 2017. Profiling and  
877 quantification of phenolic compounds in *Camellia* seed oils: Natural tea  
878 polyphenols in vegetable oil. *Food Res. Int.* 102, 184–194.  
879 <https://doi.org/10.1016/j.foodres.2017.09.089>

880 Wu, H., Lei, Y., Zhu, R., Zhao, M., Lu, J., Xiao, D., Jiao, C., Zhang, Z., Shen, G., Li,  
881 S., 2018. Preparation and characterization of bioactive edible packaging films

882 based on pomelo peel flours incorporating tea polyphenol. *Food Hydrocoll.* 90,  
883 41–49. <https://doi.org/10.1016/j.foodhyd.2018.12.016>

884 Yaradoddi, J.S., Banapurmath, N.R., Ganachari, S. V., Soudagar, M.E.M., Mubarak,  
885 N.M., Hallad, S., Hugar, S., Fayaz, H., 2020. Biodegradable carboxymethyl  
886 cellulose based material for sustainable packaging application. *Sci. Rep.* 10, 1–13.  
887 <https://doi.org/10.1038/s41598-020-78912-z>

888 Yeşilsu, A.F., Özyurt, G., 2019. Oxidative stability of microencapsulated fish oil with  
889 rosemary, thyme and laurel extracts: A kinetic assessment. *J. Food Eng.* 240, 171–  
890 182. <https://doi.org/10.1016/j.jfoodeng.2018.07.021>

891

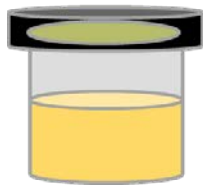
892 **Figure captions**

893 **Fig 1.** Food systems used to assess and compare the oxidative stability of fish oil either  
894 in the indirect mode, protected with active films coupled with inert headspace (N<sub>2</sub>) (1),  
895 or in direct contact with an active film (2) or with the GCO residue extracts – CE and SE  
896 (3). Control systems with aluminium foil were used as a reference for the indirect mode  
897 (1) and control systems without any protection directly exposed to ambient air were used  
898 as a reference for the direct mode (2) and (3).

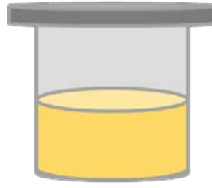
899 **Fig. 2.** Release of antioxidant compounds from active films into food simulant solutions  
900 after 7 days at 25 °C. TPC: Total Phenolic Content; C-G-L: CMC film with glycerol and  
901 lecithin; C-CE and C-SE: CMC films with sediment extract and cake extract, respectively.

902 **Fig. 3.** Peroxide (A, B) and TBARS values (C, D) of fish oil samples storage for 16 days  
903 at 40°C, covered with active films on the top of glass (on the left (A) and (C)) and placed  
904 in direct contact with active films and pure CE and SE, on the right (B) and (D)). CE:  
905 cake extract, SE: sediment extract, C-SE: CMC film with sediment extract; C-CE: CMC  
906 film with cake extract. C-G-L: CMC film with glycerol and lecithin. Control in A and C:  
907 cup covered with aluminium foil and with head space inerted with N<sub>2</sub>. Control in B and  
908 D: open cup of fish oil exposed to ambient air with no film.

909



1. Active film set on perforated screw-on lids and inert headspace (N<sub>2</sub>)



i) Control with aluminium foil on and inert headspace (N<sub>2</sub>)



2. Active film in direct contact with fish oil and open cup



ii) Uncovered control

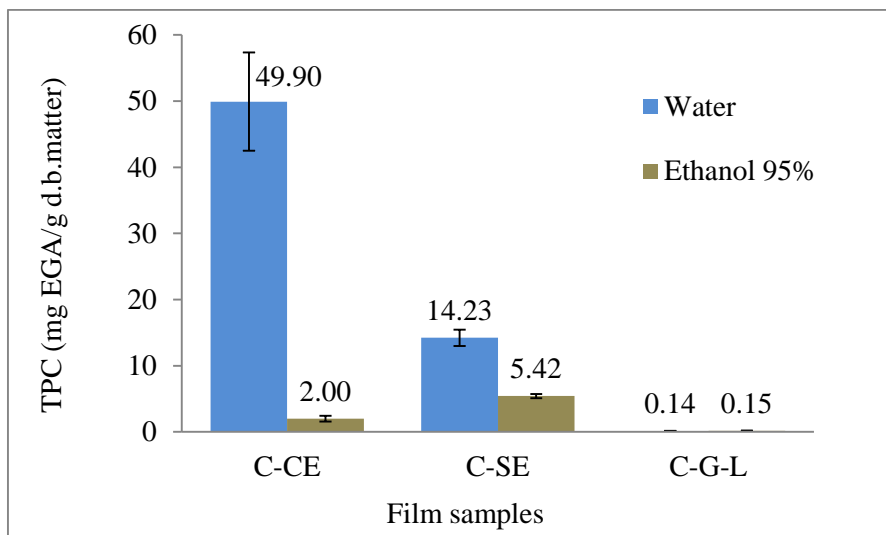


3. Residue extracts in direct contact with fish oil and open cup

910

911 **Fig 1.**





912

913 **Fig. 2.**

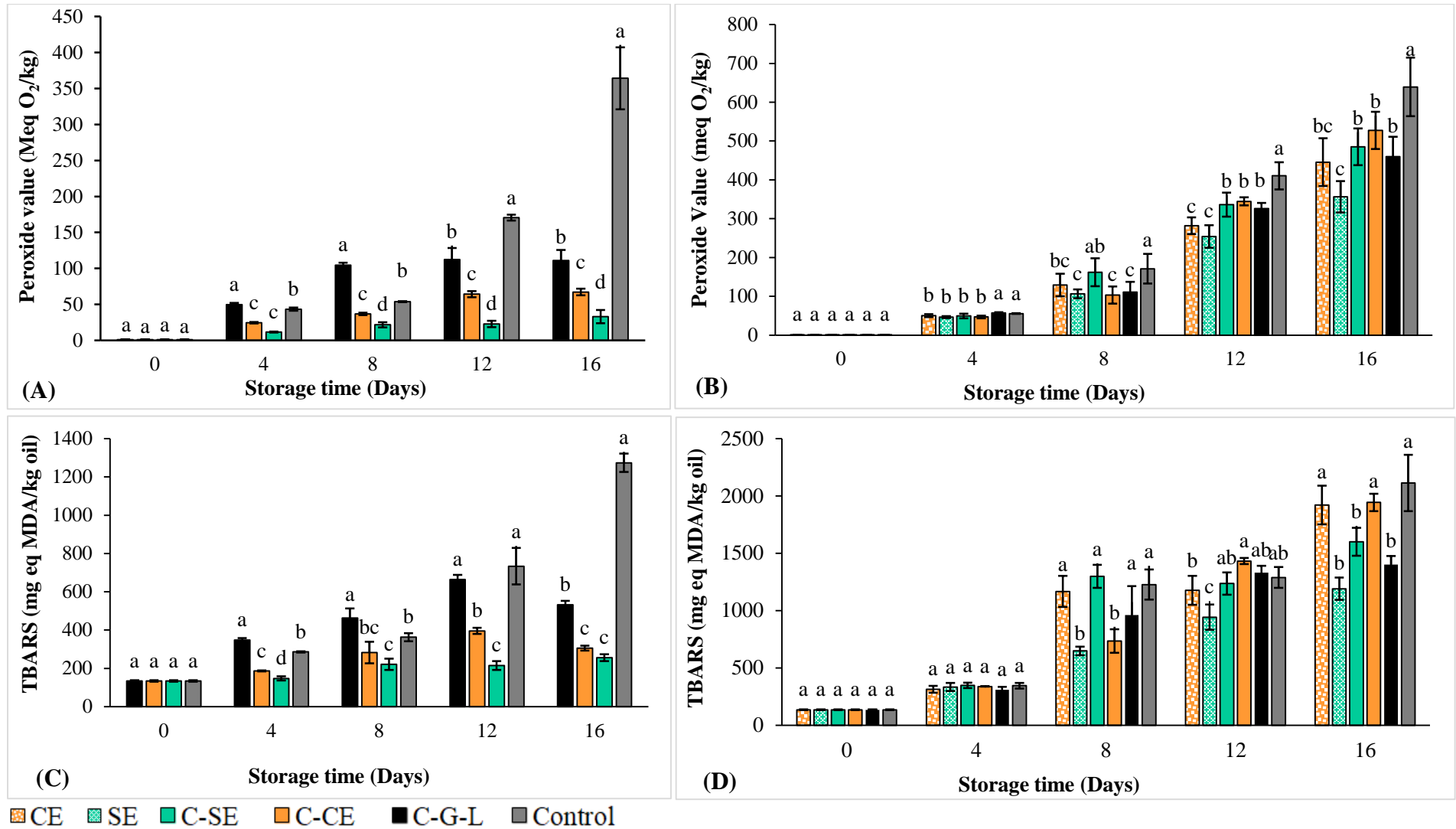


Fig. 3.

915 **Table 1.** Quality parameters for cold-pressed green coffee oil (GCO) and its residues,  
916 cake (CE) and sediment extracts (SE).

<b>Parameters</b>	<b>GCO</b>	<b>CE</b>	<b>SE</b>
Free fatty acid value (% , as oleic acid)	6.0 ± 0.02	-	-
Peroxide value (meq O <sub>2</sub> /kg)	2.5 ± 0.62	-	-
Unsaponifiable matter (%)	13.06 ± 1.04	-	-
Residual oil (%)	-	5.5 ± 0.23	58.1 ± 1.23
Moisture (%)	-	4.38 ± 0.02	4.61 ± 0.02
Yield (%)	4.2 ± 0.15	91 ± 1.68	4.6 ± 0.74

917

918 Data are reported as average and standard deviation (n=3); - stands for not determined.

919 **Table 2.** Chlorogenic acid content and caffeine in green coffee oil and its residues after  
 920 pressing.

	Chlorogenic acids and caffeine content (mg/g d.b.)			Caffeine
	CQA	FQA	diCQA	
Green coffee oil (GCO)	n.d.	n.d.	n.d.	2.4 ± 0.0*
Cake extract (CE)	74.2 ± 0.2 <sup>a</sup>	6.9 ± 0.1 <sup>a</sup>	17.4 ± 0.2 <sup>a</sup>	36.6 ± 0.2 <sup>b</sup>
Sediment extract (SE)	68.1 ± 0.4 <sup>b</sup>	4.5 ± 0.1 <sup>b</sup>	18.5 ± 0.3 <sup>a</sup>	44.9 ± 0.2 <sup>a</sup>

921 Results are shown as the means of extractions in triplicates ± SD, expressed as mg/g of  
 922 green coffee of dry matter basis (d.b). \*Caffeine in GCO is expressed as mg/g of oil.  
 923 Different superscript letters in the same column indicate significant differences (p<0.05).  
 924 n.d. stands for not detected. Caffeoylquinic acids (3-, 4- and 5-CQA); feruloylquinic acids  
 925 (4- and 5-FQA); and dicaffeoylquinic acids (3,5-diCQA and 4,5-diCQA).

926 **Table 3.** Total solids of filmogenic solutions and thickness, moisture content, oxygen  
 927 permeability (O<sub>2</sub>P), permeance and water vapor permeability (WVP) of active films.

Film sample	Total solids (mg/100 mL)	Thickness (μm)	Moisture (%)	O <sub>2</sub> P (10 <sup>-17</sup> mol·m·m <sup>-2</sup> ·s <sup>-1</sup> ·Pa <sup>-1</sup> )	O <sub>2</sub> permeance (10 <sup>-13</sup> mol·m <sup>-2</sup> ·s <sup>-1</sup> ·Pa <sup>-1</sup> )	WVP (10 <sup>-12</sup> mol·m <sup>-2</sup> ·s <sup>-1</sup> ·Pa <sup>-1</sup> )
CMC	1.82 ± 0.03 <sup>c</sup>	94 ± 11 <sup>c</sup>	21.0 ± 0.6 <sup>a</sup>	5.94 ± 1.09 <sup>b</sup>	5.84 ± 0.54 <sup>b</sup>	6.64 ± 1.08 <sup>a*</sup>
C-G-L	2.23 ± 0.03 <sup>b</sup>	114 ± 5 <sup>b</sup>	16.5 ± 0.1 <sup>b</sup>	8.40 ± 1.07 <sup>ab</sup>	7.12 ± 0.86 <sup>ab</sup>	6.48 ± 0.77 <sup>a*</sup>
C-CE	3.42 ± 0.02 <sup>a</sup>	119 ± 8 <sup>ab</sup>	14.7 ± 0.6 <sup>b</sup>	8.28 ± 0.12 <sup>ab</sup>	7.46 ± 0.17 <sup>ab</sup>	5.86 ± 0.62 <sup>a**</sup>
C-SE	3.35 ± 0.03 <sup>a</sup>	133 ± 9 <sup>a</sup>	15.0 ± 0.3 <sup>b</sup>	9.92 ± 0.72 <sup>a</sup>	7.80 ± 0.20 <sup>a</sup>	5.56 ± 0.62 <sup>a**</sup>

928 CMC: carboxymethyl cellulose film control; C-G-L: CMC film with glycerol and lecithin  
 929 (25 and 4wt% based on CMC, respectively); C-CE and C-SE: CMC films with sediment  
 930 extract and cake extract, respectively, 1:2 extract/CMC ratio. O<sub>2</sub>P measured at 23 °C and  
 931 50% RH. (\*) WVP refers to control films and (\*\*) films with cake and sediment extracts,  
 932 respectively, at 40% (v/v) and GCO (10wt% based on CMC) in previous work (Lombo  
 933 Vidal et al., 2020). Different superscript letters in the same column indicate significant  
 934 differences (p<0.05). Data reported as average and standard deviation.

935 **Table 4.** UV and visible light transmittance (%) and transparency (%) of active films.

Film samples	Transmittance (%)					Transparency at 600 nm
	200-300 nm	400 nm	500 nm	600 nm	800 nm	
CMC	< 0.00	2.29 ± 0.03	2.43 ± 0.02	2.45 ± 0.02	89.7 ± 0.7	0.82 ± 0.06 <sup>c</sup>
C-SE def*	< 0.00	0.03 ± 00	0.38 ± 0.01	0.54 ± 0.01	31.7 ± 1.7	6.22 ± 0.02 <sup>b</sup>
C-SE	< 0.00	0.02 ± 00	0.17 ± 0.01	0.23 ± 0.01	16.3 ± 0.8	7.02 ± 0.2 <sup>a</sup>

936 Values are given as mean ± SD (n=3). \* def: defatted raw material.

937 **Table 5.** Antioxidant capacity of the developed active films and raw materials.

Raw materials / films	DPPH (mmol Trolox equivalents/g of dry basis or oil)
CE	$11.05 \pm 0.0^A$
SE	$8.73 \pm 0.01^B$
Unsaponifiable fraction	$0.14 \pm 0.0^C$
GCO	$0.34 \pm 0.0^*$
CMC film	n.d.
C-G-L film	n.d.
C-CE film	$3.61 \pm 0.01^a$
C-SE film	$2.03 \pm 0.01^b$

938 CE: cake extract; SE: sediment extract; GCO: green coffee oil; CMC: carboxymethyl  
 939 cellulose film control; C-G-L: CMC film with glycerol and lecithin (25 and 4wt% based  
 940 on CMC, respectively); C-CE and C-SE: CMC films with sediment extract and cake  
 941 extract, respectively, 1:2 extract/CMC ratio. The mass/solvent ratios for films and raw  
 942 material were 1:50 and 1:10 w/v, respectively, during extraction. Different superscript  
 943 letters (uppercase for raw materials, lowercase for films and (\*) for GCO expressed as g  
 944 of oil) indicate significant differences ( $p < 0.05$ ). Data are reported as average  $\pm$  SD (n=3).  
 945 n.d. stands for not detected.

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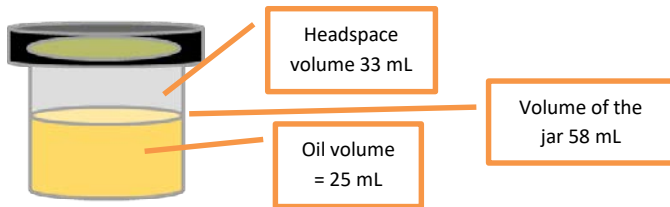
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950

951 **Appendix A.**

952 *Estimation of the O<sub>2</sub> content absorbed by the active films in the food system with fish oil (§*  
 953 *Indirect contact and protective atmosphere)*

954



955

956

957 To calculate the quantity of O<sub>2</sub> (in mg) that could be absorbed by the active film, we consider  
 958 as first hypothesis that 1% of O<sub>2</sub> remains in headspace even after N<sub>2</sub> flushing. Some O<sub>2</sub> remains  
 959 also dissolved in oil, this quantity is assumed to be in equilibrium with the O<sub>2</sub> partial pressure  
 960 in headspace (1%).

961 The number of moles of O<sub>2</sub> remaining in headspace is calculated from the perfect gas law as  
 962 follows:

963 
$$n_{O_2,HS} = \frac{p_T V}{RT} \quad \text{Equation (A.3)}$$

964 Where  $p_T$  is the atmospheric pressure ( $1.013 \times 10^5$  Pa),  $V$  is the headspace volume ( $33 \times 10^{-6}$  m<sup>3</sup>),  
 965  $R$  is the universal constant of perfect gas ( $8.32$  J.mol<sup>-1</sup> K<sup>-1</sup>) and  $T$  the temperature (293 °K).

966 Then, the corresponding quantity of O<sub>2</sub> dissolved in oil is calculated using the Henry's law:

967

968 
$$n_{O_2,oil} = p_{O_2} S_{O_2,in\ oil} \quad \text{Equation (A.4)}$$

969 Where  $p_{O_2}$  is the O<sub>2</sub> partial pressure in headspace ( $p_{O_2} = 0.01 \times 1.013 \times 10^5$ ) and  $S_{O_2,in\ oil}$  is  
 970 the solubility of O<sub>2</sub> in oil at 20°C,  $S_{O_2,in\ oil} = 3.6 \times 10^{-8}$  mole kg<sup>-1</sup> Pa<sup>-1</sup> taken from Chaix et al  
 971 2014 (Chaix et al., 2014).

972

973 According to Equations A.1 and A.2, the total quantity of O<sub>2</sub> to absorb in the system at time 0  
 974 (O<sub>2</sub> transfer from external atmosphere did not start) is:

975

$n_{O_2,HS}$	$1.37 \times 10^{-5}$	moles O <sub>2</sub>
$n_{O_2,oil} =$	$3.65 \times 10^{-5}$	moles O <sub>2</sub> /kg oil
$n_{O_2,oil} =$	$8.21 \times 10^{-7}$	moles O <sub>2</sub> , for 25 ml of oil (supposing density of 0.9 for the fish oil)
$n_{O_2,total(HS+oil)}$	$1.45 \times 10^{-5}$	moles O <sub>2</sub> (HS + oil)



$m_{O_2, total (HS+oil)}$	<b>0.47</b>	<b>mg O<sub>2</sub> to absorb (HS + oil)</b>
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976

977

978 The films used as lids in the indirect system with fish oil have the following characteristics:

- 979
- 980 • C-CE: contains 7.84 mg of chlorogenic acids per film area used as a lid (surface
  - 981 of the lid is 9.6 cm<sup>2</sup>, weight of the lid is 230 mg and thickness of the lid is 140
  - 982 μm)
  - 983 • C-SE: contains 6.3 mg of chlorogenic acids per film area used as a lid (surface
  - 984 of the lid is 9.6 cm<sup>2</sup>, weight of the lid is 200 mg and thickness of the lid is 120
  - 985 μm).

986 If we consider that chlorogenic acid behaves as gallic acid and can absorb O<sub>2</sub> in the same

987 amount, i.e. 80 mg of O<sub>2</sub> absorbed per g of gallic acid in 4 days (Pant et al., 2017), we can

988 calculate that:

- 989 • **0.627 mg of O<sub>2</sub>** could be absorbed by C-CE film containing 7.84 mg of (surface
- 990 of the lid is 9.6 cm<sup>2</sup>, masse of the lid is 230 mg and thickness of the lid is 140
- 991 μm)
- 992 • **0.504 mg of O<sub>2</sub>** could be absorbed by C-SE films containing 6.3 mg of
- 993 chlorogenic acids
- 994

995 Both quantities are higher than the quantity of O<sub>2</sub> to absorb in the system (47 mg). We

996 conclude that, assuming that chlorogenic acid behaves as gallic acid, active C-CE and C-SE films

997 may have protected the oil based on their O<sub>2</sub> scavenging properties.

998 Besides, the maximal O<sub>2</sub> absorption capacity would be about 400 mg/g of active compound

999 (still based on gallic acid, (Pant et al., 2017)). That means that the active films could absorb in

1000 total about 3 mg of O<sub>2</sub>. This correspond to O<sub>2</sub> residuals obtained after several opening cycles in

1001 the film testing process.

1002

1003

1004 Chaix, E., Guillaume, C., Guillard, V., 2014. Oxygen and Carbon Dioxide Solubility and Diffusivity

1005 in Solid Food Matrices: A Review of Past and Current Knowledge. Compr. Rev. Food Sci.

1006 Food Saf. 13, 261–286. <https://doi.org/10.1111/1541-4337.12058>

1007 Pant, A.F., Sangerlaub, S., Muller, K., 2017. Gallic Acid as an Oxygen Scavenger in Bio-Based

1008 Multilayer Packaging Films. Materials (Basel). 10. <https://doi.org/10.3390/ma10050489>

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