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

3

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 ± 0.01 and
28 2.03 ± 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 O₂

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, O₂ 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 (± 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 Δx 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 m². After a nitrogen flush was applied on both sides of the films to remove all surrounding
259 O₂, the lower chamber was exposed to an oxygen flow (50 mL/min), while the upper
260 chamber was hermetically closed. By permeation, the O₂ 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 (O₂P) of films was calculated using the following equation

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

265 Where, O₂P is the O₂ permeability (mol·m·m⁻²·s⁻¹·Pa⁻¹), J is the transmission rate
266 of O₂ (mol/s), Δx is the film thickness (m), A is the surface area of the film (m²), and p
267 is the differential partial pressure of the permeant gas across the film (Pa).

268 O₂ Permeance of films were calculated as the ratio of oxygen permeability (O₂P)
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 cm²) 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 N₂ to get rid of internal residual O₂ 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, N₂ 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 μ M). The result was then expressed as meq O₂/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 μ L) was weighed and diluted in 1 mL of a methanol: *n*-butanol solution (30:70, v/v); 25
339 μ L was taken out and diluted in a 1:4 ratio (v/v) with ultrapure water to react with 200
340 μ 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 O₂/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 O₂P 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 9.9×10⁻¹⁷
408 mol·m·m⁻²·s⁻¹·Pa⁻¹ 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, 8.4×10⁻¹⁷
410 mol·m·m⁻²·s⁻¹·Pa⁻¹).

411 The slight tendency for increased O₂P in active films was not significant except
412 for the C-SE film in comparison with control of pure CMC (6×10⁻¹⁷mol·m·m⁻²·s⁻¹·Pa⁻¹).
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 O₂P 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 O₂P 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 6×10⁻¹²
428 mol·m⁻²·s⁻¹·Pa⁻¹ 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 O_2P 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, O_2P 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 μg GAE/g, respectively) and considerable
525 decreases in ethanol (12.4 and 16.9 μg 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 O₂/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 O₂/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 β -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 O₂ 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 O₂P 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 O₂
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**

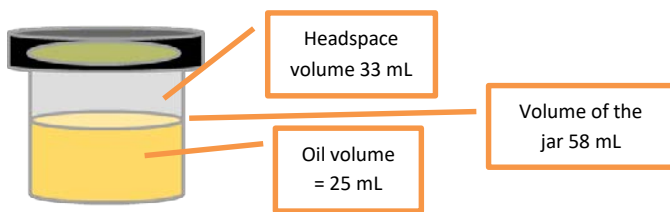
614 The residues of cold-pressed GCO showed an important amount of CGA and
615 caffeine. The residue extracts of cake (CE) and sediment (SE) provided CMC films with
616 antioxidant capacity. The active C-CE and C-SE films showed high release of
617 antioxidants in water (polar simulant), especially C-CE, 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 O₂ 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 O₂ (in mg) that could be absorbed by the active film, we consider as
 634 first hypothesis that 1% of O₂ remains in headspace even after N₂ flushing. Some O₂ remains also
 635 dissolved in oil, this quantity is assumed to be in equilibrium with the O₂ partial pressure in
 636 headspace (1%).

637 The number of moles of O₂ 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 (1.013×10^5 Pa), V is the headspace volume (33×10^{-6} m³), 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°K).

642 Then, the corresponding quantity of O₂ 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 O₂ 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 O₂ 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 O₂ to absorb in the system at time 0 (O₂
 650 transfer from external atmosphere did not start) is:

651

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

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 cm², 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 cm², 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 cm², 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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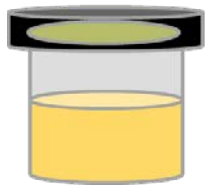
892 **Figure captions**

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 (N₂) (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).

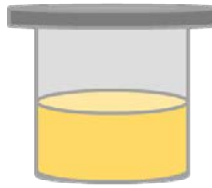
899 **Fig. 2.** Release of antioxidant compounds from active films into food simulant solutions
900 after 7 days at 25 °C. TPC: Total Phenolic Content; C-G-L: CMC film with glycerol and
901 lecithin; C-CE and C-SE: CMC films with sediment extract and cake extract, respectively.

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 N₂. Control in B and
908 D: open cup of fish oil exposed to ambient air with no film.

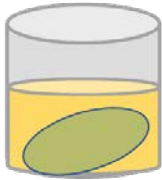
909



1. Active film set on perforated screw-on lids and inert headspace (N₂)



i) Control with aluminium foil on and inert headspace (N₂)



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



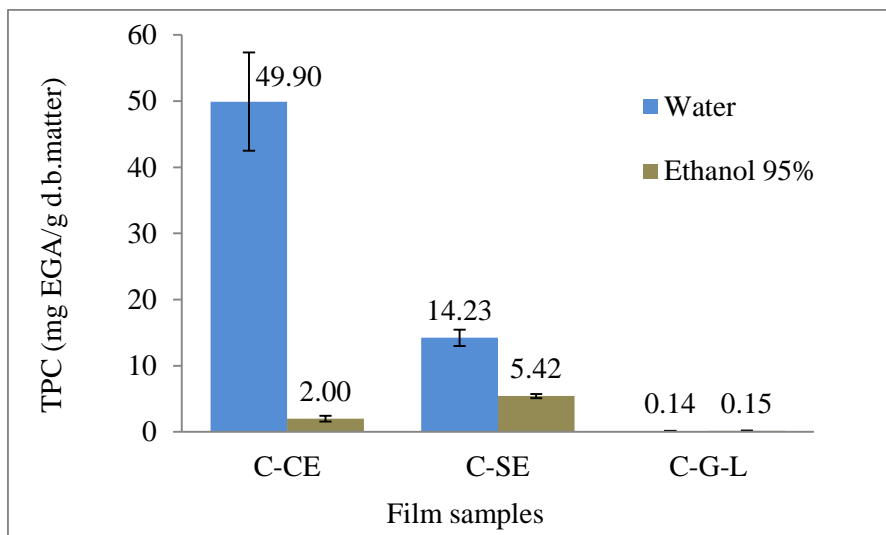
ii) Uncovered control



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

910

911 **Fig 1.**



912

913 **Fig. 2.**

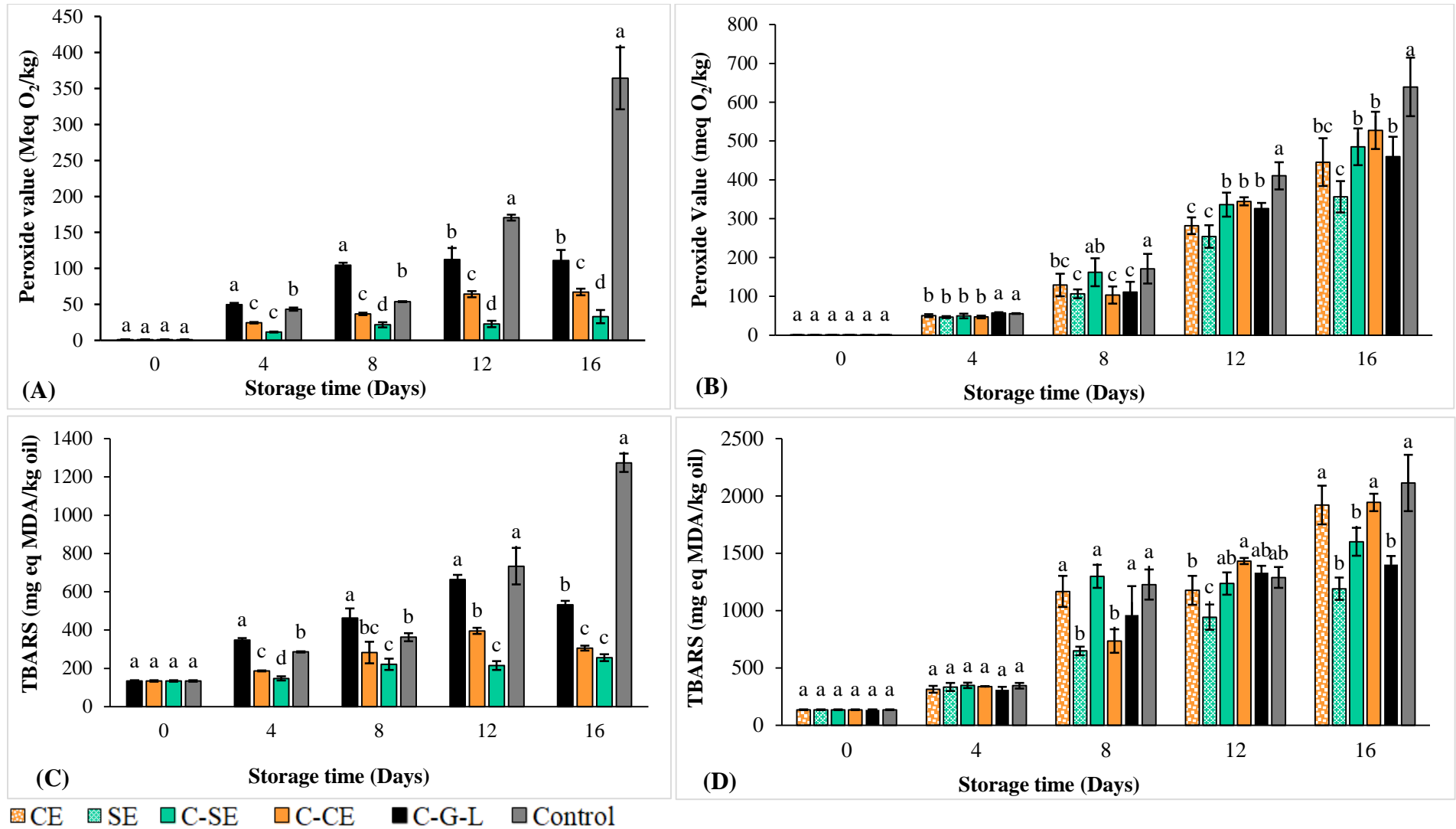


Fig. 3.

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

Parameters	GCO	CE	SE
Free fatty acid value (% , as oleic acid)	6.0 ± 0.02	-	-
Peroxide value (meq O ₂ /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 ^a	6.9 ± 0.1 ^a	17.4 ± 0.2 ^a	36.6 ± 0.2 ^b
Sediment extract (SE)	68.1 ± 0.4 ^b	4.5 ± 0.1 ^b	18.5 ± 0.3 ^a	44.9 ± 0.2 ^a

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 (O₂P), permeance and water vapor permeability (WVP) of active films.

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

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. O₂P 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 ^c
C-SE def [*]	< 0.00	0.03 ± 00	0.38 ± 0.01	0.54 ± 0.01	31.7 ± 1.7	6.22 ± 0.02 ^b
C-SE	< 0.00	0.02 ± 00	0.17 ± 0.01	0.23 ± 0.01	16.3 ± 0.8	7.02 ± 0.2 ^a

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 ± 0.0^A
SE	8.73 ± 0.01^B
Unsaponifiable fraction	0.14 ± 0.0^C
GCO	$0.34 \pm 0.0^*$
CMC film	n.d.
C-G-L film	n.d.
C-CE film	3.61 ± 0.01^a
C-SE film	2.03 ± 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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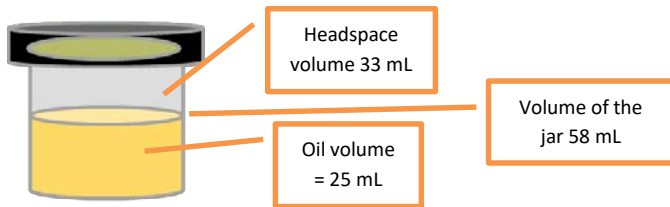
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950

951 **Appendix A.**

952 *Estimation of the O₂ 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 O₂ (in mg) that could be absorbed by the active film, we consider
 958 as first hypothesis that 1% of O₂ remains in headspace even after N₂ flushing. Some O₂ remains
 959 also dissolved in oil, this quantity is assumed to be in equilibrium with the O₂ partial pressure
 960 in headspace (1%).

961 The number of moles of O₂ 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 (1.013×10^5 Pa), V is the headspace volume (33×10^{-6} m³),
 965 R is the universal constant of perfect gas (8.32 J.mol⁻¹ K⁻¹) and T the temperature (293 °K).

966 Then, the corresponding quantity of O₂ dissolved in oil is calculated using the Henry's law:

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

969 Where p_{O_2} is the O₂ 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 O₂ in oil at 20°C, $S_{O_2,in\ oil} = 3.6 \times 10^{-8}$ mole kg⁻¹ Pa⁻¹ taken from Chaix et al
 971 2014 (Chaix et al., 2014).

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973 According to Equations A.1 and A.2, the total quantity of O₂ to absorb in the system at time 0
 974 (O₂ transfer from external atmosphere did not start) is:

975

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

$m_{O_2, total} (HS+oil)$	0.47	mg O₂ to absorb (HS + oil)
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978 The films used as lids in the indirect system with fish oil have the following characteristics:

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- C-CE: contains 7.84 mg of chlorogenic acids per film area used as a lid (surface of the lid is 9.6 cm², weight of the lid is 230 mg and thickness of the lid is 140 μm)
 - C-SE: contains 6.3 mg of chlorogenic acids per film area used as a lid (surface of the lid is 9.6 cm², weight of the lid is 200 mg and thickness of the lid is 120 μm).

986 If we consider that chlorogenic acid behaves as gallic acid and can absorb O₂ in the same
 987 amount, i.e. 80 mg of O₂ absorbed per g of gallic acid in 4 days (Pant et al., 2017), we can
 988 calculate that:

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- 990
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- 994
- **0.627 mg of O₂** could be absorbed by C-CE film containing 7.84 mg of (surface of the lid is 9.6 cm², masse of the lid is 230 mg and thickness of the lid is 140 μm)
 - **0.504 mg of O₂** could be absorbed by C-SE films containing 6.3 mg of chlorogenic acids

995 Both quantities are higher than the quantity of O₂ 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 O₂ scavenging properties.

998 Besides, the maximal O₂ 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 O₂. This correspond to O₂ residuals obtained after several opening cycles in
 1001 the film testing process.

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