

19 **Abstract**

20 Chitosan and fish gelatin, by-products from marine industry, were used to prepare active
21 biobased films containing an antioxidant (coumarin). After drying, the films were irradiated at 40
22 and 60kGy by electron beam accelerator. The effect of irradiation on the film properties as well
23 as the antioxidant release mechanism were investigated and compared with the control. -Electron
24 Spin Resonance (ESR) unravelled free radical formation during irradiation in films containing
25 coumarin. After antioxidant addition and/or irradiation treatment, only a shift of amide A, and
26 amide B peak was observed for all the films, and a shift of amide II band for the control film
27 after 60kGy irradiation dose. Irradiation allowed to improve the thermal stability of the control
28 films. Both addition of coumarin and irradiation increased the surface wettability (increase of the
29 polar component of the surface tension). From the water barrier analysis, neither irradiation nor
30 coumarin addition influenced the permeability at the lower RH gradient used (0-30% RH). Using
31 the higher RH gradient (30-84%) induced a rise of the WVP of all films (containing or not
32 coumarin) after irradiation treatment. At 60kGy, the tensile strength of only the control films
33 increased significantly. Finally, even if functional and structural properties are only weakly
34 affected, it is enough to modify the release kinetics of the antioxidant into aqueous medium. The
35 apparent diffusion coefficient of coumarin is two times reduced after irradiation. Irradiation also
36 allowed to better protecting the encapsulated antioxidant. Indeed, the amount of coumarin in the
37 non-irradiated film was significantly lowered Compared to the initial quantity, which is probably
38 due to degradation. Coumarin in irradiated films is more protected considering this aspect.

39
40 **Key Words:** Electron beam irradiation; coumarin; chitosan-fish gelatin edible film; functional
41 and structural properties, release properties

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43

44 1. Introduction

45 Maintaining food quality, improving safety, and reducing storage losses waste are key objectives
46 of a sustainable food system. Nowadays, the modern food industry is facing new challenges one
47 of which being related to the food packaging to extend shelf life. Currently, a great number of
48 research works are focused on the use of bio-based films with good water and oxygen barrier
49 properties to protect food (Fabra et al., 2011). Since the consumer demand has shifted to safe
50 materials, especially from renewable agriculture by-products and food processing industry
51 wastes (Tharanathan, 2003), natural polymers (proteins, polysaccharides) are the main
52 components of edible films (Gontard & Guilbert, 1994). They are considered as active packaging
53 when they incorporate active compounds, such as antimicrobials, preservatives or antioxidants,
54 which allow to improve food quality and safety (Han, 2002). Chitosan is a natural polymer from
55 fish industry waste obtained by the deacetylation of chitin. It is a nontoxic material,
56 biocompatible, and biodegradable that manifests antibacterial properties. In acidic environment
57 the amino groups are protonated and their positive charges can interact with polyanions such as
58 alginate, carrageenan, gelatin, etc. forming polyelectrolyte complexes (PEC) increasingly used in
59 the encapsulation of various biocomponents. Due to these characteristics, chitosan has been
60 widely used for the production of edible films as well as bio-compatible polymeric materials
61 (Aider, 2010; Rivero et al., 2010). Gelatin is another widely used bio-based material obtained by
62 the controlled hydrolysis of the insoluble fibrous collagen present in the bones and skin
63 generated as waste during animal slaughtering and fish processing. Its excellent film forming
64 ability is well-known (Hoque et al, 2011). Gelatin-based films are used for coating or packaging
65 in order to maintain the quality of foods during storage, due to its good barrier to oxygen, light
66 and prevention of dehydration and lipid oxidation (Jongjareonrak et al, 2006). Most research on
67 gelatin film has focused on gelatin derived from mammalian sources such as bovine and porcine.
68 Recently, there has been more interest in using fish as alternative sources of gelatin, due to

69 religious considerations or fear of bovine spongiform encephalopathy (Pérez-Mateos et al.,
70 2009).

71 Chitosan and gelatin have been shown to be compatible due to the ability to associate through
72 electrostatic and hydrogen bonding. Specifically, when chitosan is positively charged and gelatin
73 is negatively charged under appropriate conditions of pH. This is particularly important to
74 improve the final network properties as compared to those obtained from the pure polymers
75 (Benbettaieb et al., 2015a). Thus, many investigations focused on their possible use as a matrix
76 to obtain bio-packaging materials. In this sense and to make films even more useful, functional
77 edible films that contain active compounds have been developed, to enhance food quality and
78 product shelf-life (Suppakul et al, 2003). The incorporation of antioxidants in these
79 biodegradable and edible polymers is an interesting alternative to food preservation, since
80 oxidation is one of the major problems affecting food quality as well as film biopolymer stability
81 during ageing (Martins et al, 2012). The use of natural, non-toxic antioxidants such as ferulic
82 acid or α -tocopherol to preserve the consumer health has been investigated (Fabra et al, 2011).
83 Several researchers have previously reported on the potential benefits of using naturals
84 antimicrobials and antioxidants compounds in edible and bio-based films for extending food
85 shelf life (Oussalah et al., 2004; Ouattara et al., 2000). However, little information exists about
86 the influence of these compounds on films structural and physicochemical properties.

87 Recently, Tammineni et al.(2014) reported that mechanical and barrier properties of bovine
88 gelatin films were improved after tannic acid incorporation. Furthermore, crosslinking bovine
89 gelatin films with tannic acid results in reduction of film solubility by about 80% (Zhang et al.,
90 2010). Kavooosi et al. (2014) studied antioxidant and antibacterial activity of gelatin films
91 incorporated with *Zataria multiflora* essential oil (2 to 8% w/w of gelatin). They reported that
92 beside their excellent antibacterial properties against both Gram-positive and Gram-negative
93 bacteria, bioactive films have new functional properties. Peng and Li. (2014) demonstrated that

94 water vapour permeability of chitosan films decrease while the tensile strength inversely
95 increases when essential oils are incorporated. Therefore, we were interested in encapsulating
96 these natural compounds into chitosan-gelatin blend edible films. Moreover, physical methods
97 including dehydrothermal treatment, ultraviolet, heat and gamma irradiation (Bigi et al., 1998)
98 help to modify the polymeric network through the cross-linking of the polymer chains and also
99 help to improve the functionality of polysaccharide (Sabato et al., 2000) or protein (Vachon et
100 al., 2000) based films. Indeed, irradiation treatments have been described to enhance water
101 barrier and mechanical properties of protein-polysaccharides complexes (Lacroix et al., 2002;
102 Lee et al., 2004; Jo et al., 2005; Inamura et al., 2013). The structural modifications induced by
103 irradiation could increase the capacity of cross-linked edible films to control the release of
104 embedded active compounds. In the current literature there is a lack of detailed-studies dealing
105 with the effects of polymers structure, in particular chitosan-gelatin films, on the retention and
106 release properties of the added antioxidant compounds (Papadokostaki et al., 1997). Very few
107 studies have been published on the impact of irradiation on the release of active compounds from
108 natural biopolymers. Indeed, Tin Wui et al. (2002) showed that the release-retarding property of
109 alginate and alginate–chitosan beads is significantly enhanced after the beads irradiated by
110 microwave. In the same way, Lacroix et al. (2002) displayed that gamma-irradiation induces
111 cross-links in calcium caseinate edible films and thus allows a better control of enzyme and
112 active compounds release. Previous works displayed that ferulic acid, quercetin or tyrosol
113 addition affected differently the functional properties of gelatin-chitosan films according the
114 irradiation dose (Benbettaieb et al., 2015b). Irradiation accentuated the wettability and the
115 hydrophilicity of the film containing antioxidants whereas oxygen barrier and thermal stability
116 were enhanced.

117 The aim of this study is to further investigate the effect of coumarin addition and electron beam
118 irradiation on the mechanical, thermal, barrier and structural properties of chitosan-fish gelatin

119 edible films. The effect of irradiation on the coumarin release in liquid medium was also
120 investigated.

121

122 **2. Materials and methods**

123 ***2.1. Materials and reagents***

124 Commercial grade chitosan (CS) (France Chitine, , MW=165 kDa, low viscosity, 85%,
125 deacetylation degree, France) and commercial grade fish gelatin (G) (Rousselot 200 FG 8 with a
126 180 Bloom degree, a viscosity of 4 mPa.s at 45°C and with the concentration of 6.67% ,in water,
127 and a pH=5.4) were used as film-forming matrix. Anhydrous glycerol (GLY) (Fluka Chemical,
128 98% purity, Germany) was used as a plasticizer in order to improve the mechanical properties of
129 the films. Glacial acetic acid (Sigma, 99.85% purity) was used to prepare the solvent for chitosan
130 and helped to improve their solubility. Silica gel and potassium chloride saturated salt solution
131 (KCl, Sigma-Aldrich, France) were used to fix the relative humidity at <2% and 84% for water
132 vapour permeability measurements. Coumarin (minimum purity 99%, Sigma Aldrich, molecular
133 weight = 146 g.mol⁻¹, molar volume = 117 cm³.mol⁻¹, melting point = 70°C, LogP = 1.39,
134 solubility in water = 1.9 g.L⁻¹ (at 25°C), boiling point =298°C (à 101325 Pa), surface tension=
135 46.4 mN.m⁻¹ (at 25°C), saturated vapour pressure = 1.33 Pa (at 25°C), data from
136 Chemspider.com,) was used as a model of natural antioxidant molecule.

137

138 ***2.2. Film formation***

139 20 grams of the chitosan powder was dispersed in 1 L of a 1% (v/v) aqueous acetic acid to obtain
140 a 2% (w/v) film forming solution. The solution was homogenized at 1200 rpm with high shear
141 homogeniser (Ultra Turrax (RW16 basic- IKA-WERKE) at 25°C. As a clear film-forming
142 solution was obtained, no more treatment has been applied to the chitosan solution for improving
143 the solubilisation. Then, 2.2 grams of glycerol (10% w/w dry matter) was added to this solution,

144 under stirring. The pH of the chitosan solution was about 4.9 ± 0.2 . A 60 grams of fish gelatin
145 powder was separately solubilized in 1 L of distilled water under continuous stirring and heating
146 at 70°C for 30 min to obtain a 6% w/v solution ($\text{pH}\approx 6.5$). 6.6 grams of Glycerol (10% w/ dry
147 matter) was added to this film forming solution after complete solubilisation of gelatin.
148 Subsequently, equal weight of the respective solution was mixed at 1:1 ratio and stirred for 30
149 min and pH was adjusted to 5.6 with acetic. This condition was specifically designed to obtain a
150 polyelectrolyte complex between chitosan and gelatin since the iso-electric point of gelatin ($\text{Ip}=\text{pI}$
151 4.5-5.2) while the pKa of the amino group ($\text{pH}=6.2\text{-}6.5$) of chitosan. At this condition, therefore,
152 gelatin is negatively charged while chitosan is positively charged thus avoiding any phase
153 separation upon mixing. Coumarin was added to the final film forming solution at a
154 concentration of about 50 mg /g polymer (corresponding to a 47 mg/g total dry weight of film).
155 The aqueous dispersions were homogenized at 1200 rpm using the Ultra Turrax until complete
156 dissolution.

157 30 mL of the film forming solution (FFS) in the presence and absence of coumarin was then
158 poured into plastic Petri dishes (13.5 cm diameter). A minimum of 30 films (ie 30 Petri dishes)
159 have been prepared for each formulation. Aqueous solvent was removed by drying in a
160 ventilated climatic chamber (KBF 240 Binder, ODIL, France) at 25°C and 45% RH for 18 to
161 24h. After drying, films were peeled off from the surface and stored up to equilibrium in a
162 ventilated climatic chamber (KBF 240 Binder, ODIL, France) at 50% RH and 25°C before each
163 measurements.

164

165 ***2.3. Radiation treatment***

166 Irradiation was carried out in the AERIAL pilot plant (Innovation Park, Illkirch, Strasbourg,
167 France), using a linear electron accelerator at ambient temperature ($20\pm 0.5^{\circ}\text{C}$). Thin dried films
168 (70 to $85\mu\text{m}$ thickness) were irradiated for 40 and 60kGy using a 2.2 MeV energy electron beam

169 at a dose rate of 0.3 kGy/sec. One batch of film formation was preserved as non-irradiated
170 reference. We selected a maximum of 60kGy doses that can yield a high density of crosslinking
171 in proteins and in the range of authorized doses in food and packaging. Moreover, according to
172 the Codex General Standard for Irradiated Foods (CAC, 2003), ionizing radiations foreseen for
173 food processing are recommended to be limited to 60kGy. Dosimetry was performed using
174 alanine pellet dosimeters calibrated according to ASTM/ ISO 51607 (2004).

175

176 ***2.4. Film characterizations***

177 ***2.4.1. Thickness measurement***

178 Film thickness was measured with an electronic gauge (PosiTector 6000, DeFelsko Corporation,
179 USA). Five measurements were taken for each film sample, one from the center and four close to
180 the perimeter. Mean value was used in further calculations. The film thickness according the
181 sample and formulation ranges between 70 and 85

182 ***2.4.2. Mechanical properties***

183 A universal traction testing machine (TA.HD plus model, Stable MicroSystems, Haslemere,
184 England) with a 300 N load cell was used to determine tensile strength (TS, MPa), Young's
185 modulus (YM, MPa) and percentage of elongation at breakpoint (E, %), according to ASTM
186 **method D882 (1992)**. Rectangular film samples (2.5×8 cm²) were cut using a special precision
187 sample cutter (Thwing-Albert JDC Precision Sample Cutter) in order to get tensile test piece
188 with an accurate width and parallel sides throughout the entire length. Before testing, all samples
189 were equilibrated for two weeks at 25°C and 50% relative humidity (RH). Equilibrated film
190 samples were then placed in the extension grips of the testing machine and stretched uniaxially at
191 a rate of 50 mm/min until breaking. TS, YM and E were determined from stress–strain curves.
192 Measurements were carried out at room temperature (25±2°C) and five samples for each
193 formulation were tested.

194 **2.4.3. Water vapour permeability**

195 The water vapour permeability (WVP) was determined according to the gravimetric method
196 described in the [ASTM E96-80 \(1980\)](#) and adapted to edible and bio-based films by [Debeaufort,](#)
197 [Martin-Polo and Voilley \(1993\)](#). Two relative humidity gradients were used: 0-30% and 30-84%.
198 Prior to WVP measurements, all film samples were equilibrated at $25 \pm 0.5^\circ\text{C}$ and 30% relative
199 humidity for 72 h. The average value of five thickness measurements per type of film was used
200 in the WVP calculations (statistical error on the film thickness was taken into account in WVP
201 uncertainty). The film samples (6.44 cm² discs) were placed between two teflon rings on the top
202 of the glass cell containing silica gel (0% RH) for the first RH gradient (0-30%) or a salt solution
203 of KCl (84% RH) for the second RH gradient (30-84%). The second RH gradient was selected to
204 obtain an average water content of film at the stationary state of permeation that corresponds to
205 the RH of 50% used for mechanical property characterizations and FTIR analysis. Those
206 permeation cells were then introduced into a climatic chamber (KBF 240 Binder, ODIL, France)
207 maintained at 30% RH and $25 \pm 0.5^\circ\text{C}$ and periodically weighed.
208 The WVP ($\text{g}\cdot\text{m}^{-1}\cdot\text{s}^{-1}\cdot\text{Pa}^{-1}$) calculation was based on the change in the absolute value in weight
209 loss of the permeation cell versus time once the steady state was reached ([Benbettaieb et al,](#)
210 [2015a](#)). Five replicates for each film formulation were performed.

211 **2.4.4. Surface properties**

212 **2.4.4.1. Water contact angle**

213 The sessile drop method was used for contact angle measurement, with a DGD-DX goniometer
214 (GBX, Romans-sur-Isere, France), equipped with the DIGIDROP image analysis software
215 (GBX, Romans-sur-Isere, France), according to [Karbowiak et al. \(2006\)](#). A droplet of liquid (~
216 1.5 μL) was deposited on the film surface with a precision syringe. Then, the method is based on
217 image processing and curve fitting for contact angle measurement from a theoretical meridian
218 drop profile, measuring contact angle between the baseline of the drop and the tangent at the

219 drop boundary. The contact angles with water and diiodomethane at 0 and 20 s were measured
 220 from both sides of the drop and averaged. The measurement was carried on over 120 s. Five
 221 replicates per film were carried out.

222 **2.4.4.2. Surface tension**

223 The surface tension (γ_S) of the film and its polar (γ_S^p) and dispersive (γ_S^d) components were
 224 calculated using diiodomethane ($\gamma_L = 53$ mN/m; $\gamma_L^d = 50.8$ mN/m and $\gamma_L^p = 0$ mN/m) and
 225 water ($\gamma_L = 72$ mN/m ; $\gamma_L^d = 21.8$ mN/m and $\gamma_L^p = 51$ mN/m) knowing their surface
 226 tension (γ_L) and respective dispersive (γ_L^d) and polar components (γ_L^p) given by **Strom et**
 227 **al.(1987)** and according the following equations established by **Owens and Wendt (1969)** :

228
$$\gamma_S = \gamma_S^d + \gamma_S^p \dots\dots\dots \text{Eq.1}$$

229
$$\gamma_{Li}(1 + \cos\theta_i) = 2[\sqrt{\gamma_S^d \times \gamma_{Li}^d} + \sqrt{\gamma_S^p \times \gamma_{Li}^p}] \dots\dots\dots \text{Eq.2}$$

230 where the subscripts S and L refers to the solid (the film surface) and the liquid, respectively. As
 231 only two liquids have been used for the regression, the accuracy of values obtained from this
 232 analysis has been considered at the p-level of 0.01.

233 **2.4.4.3. Swelling index and swelling rate with water**

234
 235 Swelling index was obtained from the water drop volume kinetics using the following equation:

236
$$\text{Swelling index (\%)} = \left(\frac{\Delta V}{V_0} \right) \cdot 100 = \left(\frac{V_{\max} - V_0}{V_0} \right) \cdot 100 \quad \text{Eq.3}$$

237 where ΔV is the droplet volume variation (μL) measured on the film sample (over the first 20s).

238 V_{\max} is the maximal apparent volume (μL) of the droplet and V_0 is the initial volume (μL) of the
 239 droplet.

240 Swelling rate was obtained from the drop volume kinetics using the following equation:

241
$$\text{Swelling rate (\mu L/s)} = \left(\frac{\Delta V}{\Delta t} \right) = \frac{V_{\max} - V_{\text{ini Sw}}}{t_{\max} - t_{\text{ini Sw}}} \quad \text{Eq.4}$$

242 Where ΔV is the droplet volume variation (μL) during Δt time (s) measured on the film sample,
243 assuming a linear regression.

244 V_{max} : maximum swelling volume, $V_{\text{ini Sw}}$: initial volume of swelling, t_{max} : time of maximum
245 swelling, $t_{\text{ini Sw}}$: initial time corresponding to the beginning of swelling

246

247 **2.4.5. GPC-MALLS system**

248 The GPC-MALLS system consisted of a degasser ERC-3215- α (ERC, Japan), a constametric @
249 3200 MS pump (Thermo Separation Products, FL), an injection valve with 100 μL loop
250 (Reodyne 7725i) fitted inside a temperature regulated oven (Gilson, Model 831, UK) maintained
251 at $40^{\circ}\text{C} \pm 1^{\circ}\text{C}$ and a DAWN-DSP multi-angle light scattering photometer (Wyatt Technology,
252 Santa Barbara, CA, USA) equipped with He-Ne laser ($\lambda = 633 \text{ nm}$). Simultaneous concentration
253 detection was performed using a calibrated differential refractometer (RI 2000, Schambek,
254 Germany). A refractive index increment dn/dc value of 0.180 mL/gm was used in the
255 calculations

256 The mobile phase was made to contain 0.15M ammonium acetate, 0.2M acetic acid and 0.1M
257 sodium chloride and was filtered through $0.2 \mu\text{m}$ pore size cellulose nitrate membrane. The
258 samples injected were subjected to prior filtration through a nylon filter of $0.45 \mu\text{m}$ pore size. A
259 set of two columns SB-803HQ and SB-806HQ ($8 \text{ mm} \times 300 \text{ mm}$, Shodex OHpak, Japan,
260 exclusion limits 1×10^5 and $2 \times 10^7 \text{ g/mol}$), housed inside the oven, was used for the separation.

261 The flow rate for the eluent was 0.45 mL/min . The Berry fitting method with linear fit was used
262 for data processing in ASTRA software (Version 4.90.08). For the measurement of molecular
263 weight, about 27mg (30mg of films containing about 12% water content) of chitosan-gelatin film
264 incorporated with coumarin 5% (w/w polymer) was dissolved in 10mL of mobile phase. The
265 solution was then heated in a water bath for 20 min at 45°C and subsequently centrifuged for

266 5min at 25000 rpm at 25°C. The soluble fraction was removed from the respective solution and
267 was heated at 45°C for 5 min prior to injection into the GPC-MALLS system.

268 The entire GPC-MALLS system was maintained at $40 \pm 1^\circ\text{C}$. Temperature control was achieved
269 using the in-house heating methodology provided thermostatic heating to all pipework between
270 the detectors.

271 ***2.4.6. Thermogravimetric analysis***

272 Thermogravimetric analysis (TGA) was used to evaluate the thermal stability of the samples.
273 Measurements were performed using a TA instrument (TA instruments Discovery TGA New-
274 Castle, USA), from 25 to 800°C, at a heating rate of 20°C/min, under nitrogen atmosphere. The
275 weight of the film sample (initially around 8 mg) was constantly measured with an accuracy of
276 0.01 mg. Films were stored at 25°C and 50% RH for two weeks before TGA measurements.

277 ***2.4.7. Attenuated Total Reflectance - Fourier Transforms Infrared (FTIR-ATR) spectroscopy***

278 The Fourier Transform Infrared spectra from each film were obtained using a spectrometer
279 (Perkin-Elmer, Spectrum 65, France) using Attenuated Total Reflectance (ATR) with ZnSe
280 crystal. For each measurement, 32 scans in the wave length range 400-4000 cm^{-1} with a 4 cm^{-1}
281 resolution were co-added before the Fourier transform. The spectra were collected in duplicate.
282 This analysis aimed at determining the modifications at the molecular scale induced between
283 active molecules and polymers after electron beam irradiation.

284 ***2.4.8. Electron spin resonance (ESR)***

285 The ESR technique allows measuring the presence of free radicals. ESR signals were recorded at
286 room temperature on a Bruker EMX spectrometer (Bruker, Berlin, Germany) controlled with a
287 Bruker ER 041 XG microwave bridge operating at X-band (~9 GHz). 50mg of each film was cut
288 and placed in the sampling tube. ESR spectra were carried out using 100 kHz magnetic field with
289 6G modulation amplitude, 20.12 mW microwave power, 9.49 GHz frequency and 5 scans.

290 ***2.5. Release of coumarin in aqueous medium***

291 Prior to release experiments, the real concentration of coumarin in the films was determined. A
292 film sample of about 60 mg (about 5x5 cm²) was immersed up to fully dispersion in 100 mL of
293 acetic acid solution at pH 4 under stirring at 50°C in order to fully solubilized the polymers and
294 the coumarin. The amount of coumarin in the liquid medium was determined by UV-vis
295 spectrophotometry (Biochrom Libra S22) at 278 nm (previously determined from the absorbance
296 spectrum of the pure coumarin in water). A series of standard solutions for this antioxidant (1, 2,
297 4, 5, 10, 25 and 50 mg/L) was used for calibration, according to the Beer-Lambert's law. This
298 concentration was then compared to the theoretical content introduced in the films when
299 prepared. The measured initial concentration was used to calculate the percentage of retention in
300 the film after the release kinetics.

301 The release of the coumarin was carried out in triplicate using the rotating paddle dissolution
302 apparatus (AT7 Smart type II, Sotax, Basel). 600 mg of each film were incorporated at time 0 in
303 1 L of the dissolution medium (water adjusted at pH=7 using 0.1 M of NaOH). 3 mL samples
304 were withdrawn and assayed for antioxidant release periodically up to equilibrium. The amount
305 of coumarin in the release medium was determined by UV-vis spectrophotometry (Biochrom
306 Libra S22) as previously described.

307 The effective diffusion coefficient of coumarin in the film (D) was also determined from the
308 release kinetics assuming a Fick's law (Eq.5), and considering the transient state of the transfer.

309
$$\frac{\partial c}{\partial t} = D \frac{\partial^2 c}{\partial x^2} \quad \text{Eq.5}$$

310 Where C is the concentration of this antioxidant in the film over the time t, x is the thickness of
311 the film and D is the effective diffusion coefficient.

312 The experimental method chosen corresponds to the case of diffusion of the solute from a plane
313 sheet (film) into a stirred solution of limited volume (Crank, 1975). As the solution is constantly
314 stirred, we assumed there is no boundary layer effect on diffusion. Therefore, the concentration
315 of coumarin in the solution, initially zero, is considered to be uniform in the release medium

316 according to Crank (1975). The concentration of this antioxidant in the film is also assumed to be
317 uniformly distributed within the film at time zero. We also consider a unidirectional diffusion of
318 the coumarin in the film, and a D which does not depend on the concentration or on the time.
319 This mass transfer equation (Eq.5) can thus be solved using the following analytic solution to the
320 second Fick's law applied to transient state (Crank, 1975):

$$\frac{C_t}{C_\infty} = 1 - \sum_{n=1}^{\infty} \frac{2\alpha(1 + \alpha)}{1 + \alpha + \alpha^2 q_n^2} \exp\left(-\frac{Dq_n^2 t}{l^2}\right) \quad \text{Eq. 6}$$

321 Where, C_t is the concentration of coumarin determined in the dissolution medium over time, as
322 previously detailed; C_∞ is the maximum concentration of this antioxidant determined in the
323 dissolution medium when equilibrium is achieved; $\alpha = V_s / (K \times V_f)$ with V_s the volume of
324 solution (m^3), V_f the volume of the film (m^3) and K the partition factor; q_n are the non-zero
325 positive roots of $\tan(q_n) = -\alpha q_n$ using n values between 1 and 6.

326 D is the effective diffusion coefficient ($\text{m}^2 \cdot \text{s}^{-1}$), and l is the half thickness of the film (m).

327 This model was applied to the release experimental kinetics (up to equilibrium) in order to
328 determine the effective diffusion coefficient of coumarin in the film, by minimising the sum of
329 the square of the differences between measured and predicted values, using the Levenberg–
330 Marquardt algorithm, and taking D as adjustable parameter. Modeling was performed using
331 Matlab software (The Mathworks, Natick, MA).

332

333 **2.6. Statistical analyses**

334 The data were analyzed using an independent sample t-test with the statistical software SPSS
335 13.0 (SPSS Inc., Chicago, IL). A standard deviation (p-value < 0.05) at the 95% confidence level
336 was used to compare all parameters analysed (water vapour permeability, mechanical properties,
337 surface properties and parameters of the kinetics of release) between irradiated and non-
338 irradiated films in the presence and absence of coumarin.

339

340 **3. Results and discussion**

341 **3.1. How irradiation and coumarin addition influence chemical and structural organisation** 342 **of polymer-blend network**

343 **3.1.1. Free radical generation**

344 Electron spin resonance (ESR) is an appropriate tool to detect and identify the generation of free
345 radicals in a polymer structure. The ESR spectra of chitosan-gelatin blend film incorporated with
346 coumarin before and after irradiation (60kGy) are displayed in **Fig.1**. A very weak ESR signal is
347 detected, at 3365 G, in the case of non-irradiated films. Contrarily, just irradiated film displays a
348 prominent peak at the same position suggesting the presence of free radical species induced by
349 the irradiation treatment. This peak remains visible even after 3 months of storage revealing that
350 free radicals are still present in the films.

351 Recently, we reported that the peak intensity, determined at 3500 G, for irradiated chitosan-
352 gelatin film increases with increasing the irradiation dose [Benbettaieb et al. \(2015a\)](#).

353 [The](#) analysis of the peak to peak amplitude between film containing or not containing coumarin
354 shows that, after irradiation at 60kGy, the peak amplitude is 2 to times higher for irradiated
355 films compared to non-irradiated ones, for film without coumarin and for film containing
356 coumarin, respectively.

357 The detection of free radicals generated within the films may contribute to clarify the role of
358 these reactive species to initiate the reaction sequence inducing change in the chemical and
359 structural organisation of the biopolymer-blend network. A new arrangement in the structure is
360 expected after irradiation, which could favour the linkage between the biopolymers chains or
361 between the biopolymers chains and the active compound. Even if the water content of film was
362 quite low (about 12%), possible hydroxyl-coumarin obtained after hydroxylation reaction by OH
363 radical produced from water radiolysis could react with polysaccharide and protein network. As

364 coumarin is a phenolic substance made of fused benzene and alpha pyrone ring, their phenolic
365 groups can be easily converted to quinone in presence of peroxide radicals (produced by
366 irradiation), via quinone-mediated reactions. Indeed, a slight change in the film colour after
367 irradiation is observed. This could be more probably attributed to quinone generation from
368 antioxidants more than Maillard reaction because the temperature involved in the film making
369 and irradiation process remained lower than 50°C. In addition to providing a source of stable free
370 radicals, quinones are known to complex irreversibly with nucleophilic amino acids in protein.
371 The reaction mechanism involves an initial oxidization of phenolic structures to quinones, which
372 can readily react with nucleophiles from reactive amino acid groups in protein: sulfhydryl
373 group in cysteine, amino group of lysine and arginine, amide group from aspartic and glutamic
374 acids, indole ring of tryptophan and imidazole ring from histidine (Zhang et al., 2010). This tends
375 to be confirmed as our films gain a yellowish colour after irradiation. Sahu et al. (2009) showed
376 the efficiency of microwave irradiation for the oxidation of phenol to quinone after free radicals
377 generation in the presence of hydrogen peroxide. Casimiro et al. (2010) showed that, in acidic
378 medium, NH_3^+ groups (from deacetylated units) of chitosan are able to be involved in some
379 interactions during irradiation. As displayed by Madeleine-Perdrillat et al. (2015), even at low
380 water content, molecular mobility in chitosan films remains quite high and allows the supposed
381 free radical mobility in the film. Knowing free radicals are present after irradiation, they could
382 originate sequences of reactions within and between polymer chains and antioxidant which could
383 in turn affect the structural properties of the final network. These structural and functional
384 properties modifications have been assessed in the followings.

385

386 **3.1.2. Changes in chemical structure by molecular interaction analysis**

387 GPC-MALLS was used to determine the molecular weight parameters of chitosan-gelatin film
388 before after irradiation and in the presence of coumarin. The typical elution profile (15 – 19 mL)

389 of chitosan-gelatin film was monitored by the light scattering (detector 90° degree), refractive
390 index and UV at 280nm detectors (data not shown). The results are tabulated in **Table 1**. The
391 weight average molecular weight for chitosan gelatin film in the presence and absence of
392 coumarin was similar and almost an identical value was obtained following irradiation at 60kGy.
393 The results given in **Table 1** show that there is no significant difference of for the molecular
394 weight, the polydispersity and the z-radius between irradiated (and non-irradiated films are
395 observed for the control). So irradiation induce neither covalent reticulation nor biopolymer
396 degradation under the conditions employed in this study. The incorporation of coumarin has no
397 additional effect on the molecular size of the biopolymers even when coupled with irradiation
398 process

399
400 Fourier transform infrared spectroscopy (FTIR) was used in order to assess the possibility of
401 interactions and the nature of linkage between polysaccharide-protein network and coumarin
402 following irradiation treatment. FTIR spectra of irradiated and non-irradiated chitosan-fish
403 gelatin blend films in the presence and absence of coumarin are displayed in **Fig. 2**. The
404 spectrum of non-irradiated control film showed characteristic peaks: 3300-3360 cm^{-1} , assigned
405 to ν_{OH} stretching of free water and ν_{NH} stretching of amide A, 2915-2935 cm^{-1} , assigned to ν_{CH}
406 asymmetric/symmetric stretching of amide B, 1550-1680 cm^{-1} , assigned to C=C and C=O
407 stretching of primary and secondary amine NH band of amide I, 1550-1610 cm^{-1} , assigned to
408 δ_{NH} of amide II and 1240-1340 cm^{-1} , assigned to aromatic primary amine, CN stretch of amide
409 III (Coates, 2000; Benbettaieb et al., 2015a). The peak observed in 1034 cm^{-1} is related to
410 possible interactions arising between plasticizer (OH group of glycerol) and polymer structure
411 via hydrogen bonds (Cerqueira et al, 2012). The amide B ν_{CH} (2932 cm^{-1}) is slightly shifted to
412 higher wavenumber (2941 cm^{-1}) after incorporation of coumarin. However, no change is
413 observed on amide-I, amide-II and amide-III peak position. Similar results was founded by

414 [Benbettaieb et al.\(2015b\)](#) after incorporation of ferulic acid in chitosan-gelatin film. Contrarily,
415 other author found a shift on amide I band, attributed to interaction between chitosan (amide)
416 and starch or ferulic acid (hydroxyls) in the case of starch-chitosan blend film incorporating
417 ferulic acid ([Mathew and Abraham, 2008](#)); or to caffeic acid oxidation inducing protein
418 crosslinking ([Nuthong et al, 2009](#)); or to crosslinking in gelatin gel after UV irradiation ([Bahat
419 and Karim, 2009](#)). Therefore, FTIR analysis only shows a weak modification in the structure of
420 the final network without new significant linkage. Only the amide A and amide B (and amide II
421 for control film) groups exhibit a shift after both antioxidant addition and irradiation treatment.

422

423 **3.1.3. Thermo-gravimetric analysis (TGA)**

424 Thermo-gravimetric analysis was performed in order to study the effect of coumarin addition
425 before and after irradiation on the thermal stability of blend chitosan-fish gelatin films. **Fig. 3**
426 shows two main stages of weight loss events for all films. The first stage occurs over a
427 temperature range of around 51-121°C and results in a weight loss (Δw_1) of approximately 5.2-
428 7.4%. It is associated with the loss of acetic acid and free water sorbed in the film. These results
429 are in agreement with [Inamura et al. \(2013\)](#), who observed similar behavior for biocomposite
430 films prepared with gelatin and nut wastes as fiber source (46-140°C). [Barreto et al. \(2003\)](#) and
431 [Pena et al. \(2010\)](#) also showed similar results for gelatin film, from 25 to 200°C. These
432 temperature ranges differences can be attributed to the variation of the initial water content as
433 well as the plasticizer used. The second stage of weight loss ($\Delta w_2= 42.3-53.8\%$) occurs in the
434 temperature range from 215 up to 330°C. This is most likely due to the degradation of the
435 polysaccharide and protein backbones as well as the evaporation and thermal degradation of
436 glycerol (from 177-211°C up to 450°C ([Maturana and Pagliuso, 2011](#))) and also structurally
437 bound water evaporation. Pure coumarin (powder) exhibits a single stage in weight loss, with
438 decomposition starting at 160-180°C and finishing at about 230°C (**Fig. 3**). Only weak

439 difference was observed regarding thermal decomposition temperatures and weight loss (Δw_1
440 and Δw_2) when control film is compared to the films with coumarin due to the low coumarin
441 content (47mg/g). Opposite results were observed in the case of skin gelatin after addition of star
442 anise extracts (Hoque et al., 2011) and green tea extract (Wu et al., 2013). They suggested that
443 interactions occurring between phenolic compounds and gelatin yielded to a stronger film
444 network and therefore a higher heat resistance of the films. The above studies are comparable to
445 those we recently reported on the same gelatin- chitosan film which showed improved thermal
446 stability following the addition of quercetin (Benbettaieb et al., 2015b). After irradiation, thermal
447 degradation temperature of the control film is improved, associated with a decrease observed on
448 weight loss (Δw_1 and Δw_2). This result suggests the apparition of new bonds, thermally more
449 resistant to heat than initial bonds existing before irradiation which enhances the thermal
450 properties. Similar result was found by Inamura et al. (2013) in the case of composite gelatin-nut
451 shell fiber after 40 kGy irradiation dose. Inversely, we cannot observe any significant
452 modification of thermal stability for film containing coumarin after irradiation. Benbettaieb et al.
453 (2015b) showed a reverse tendency for chitosan-gelatin film containing ferulic acid after 60kGy
454 irradiation dose. Finally, from the above structural and thermal analysis, we can conclude that
455 the interaction between coumarin and polymer chains is very weak and no covalent or strong
456 linkage occurred following irradiation. For this reason, a complementary analysis must be
457 undertaken to better understand the effect of both irradiation and coumarin addition on functional
458 properties of films.

459

460 **3.2. Impact of both irradiation and coumarin addition on functional film properties**

461 **3.2.1. Surface properties and wettability**

462 The contact angle (θ) value obtained after deposition of a water drop on the film surface
463 indicates the surface hydrophobicity. To estimate the resistance of films to liquid water, the

464 swelling index and swelling rate were also determined from the droplet volume kinetics, along
465 with the contact angle values at the initial time of deposit (0 s) and at a considered metastable
466 equilibrium (20 s). Results for all films are summarized in **Fig. 4**. For untreated films, the contact
467 angles (at 0 and 20s) significantly decrease ($p < 0.05$) after incorporation of coumarin.
468 Furthermore, no swelling is observed for the control film. Swelling index and swelling rate
469 significantly ($p < 0.05$) increase to $52 \pm 4\%$ and to $68 \pm 24 \times 10^{-3} \mu\text{L/s}$, respectively after addition of
470 coumarin. After irradiation, the contact angles (at 0 and 20s) significantly decrease for all films
471 (decreases is not significant only for the contact angle at 20s for coumarin film). Whereas,
472 swelling index and swelling rate tend to increase for all films, but they are only significant
473 ($p < 0.05$) for the control films. To better understand the effect of coumarin on film surface
474 properties under electron beam irradiation, the surface tension was also determined. Surface
475 tension does not show significant modification after incorporation of coumarin. However, a
476 slight increase is observed in the polar component. The presence of this antioxidant seems to
477 slightly contribute to the hydrophilicity of the film. Similar behaviour was recently reported by
478 **Benbettaieb et al.(2015b)** in our study on the same film but in the presence of ferulic acid.
479 Irradiation induces a decrease of the contact angle value with water, concomitant to an increase
480 in the polar component of the surface tension for all films. It can be attributed to a reorientation
481 of polar groups at the film surface, hence increasing the polar component of the surface tension.
482 Thus irradiation increases wettability of the films.

483

484 **3.2.2. Water vapour permeability**

485 **Table 2** displays the WVP of non-irradiated and irradiated films in the presence and absence of
486 coumarin for the two RH gradients studied (0-30 % and 30-84 %). For the 0-30%RH gradient, the
487 WVP of non-irradiated film containing coumarin ($0.47 \pm 0.03 \times 10^{-11} \text{ g.m}^{-1}.\text{s}^{-1}.\text{Pa}^{-1}$) is in the same
488 range to that of control film ($0.52 \pm 0.1 \times 10^{-11} \text{ g.m}^{-1}.\text{s}^{-1}.\text{Pa}^{-1}$). Inversely, **Wu et al. (2013)** noticed a

489 decrease of 16 % in the WVP for films composed of silver carp (*Hypophthalmichthys molitrix*)
490 skin gelatin containing green tea extract (0.7%). Other authors did not observed any change in
491 the water vapour permeability when ferulic acid was added to to gelatin films (Cao et al. 2007),
492 to soy protein films (Ou et al. (2005), or to caseinate based films (Fabra et al., 2011). After
493 irradiation we did not observed any modification in WVP (0-30% RH gradient) for control film
494 and for film containing coumarin. Furthermore, the 0-30% RH gradient correspond to water
495 activity average equal to 0.15, which is in the BET domain (water contained in the film is only
496 involved in the structure organisation and not available for reaction), thus we propose that-only
497 water involved in structure with weak plasticization of the network by water. In this domain,
498 neither irradiation nor coumarin addition affects the water barrier properties of the films. On the
499 other hand, higher RH gradient of 30-84% induces a rise of the WVP of all films (with or
500 without coumarin addition) after irradiation treatment. WVP increases from 2.41 ± 0.44 to
501 $23.06 \pm 0.85 \times 10^{-11} \text{ g.m}^{-1}.\text{s}^{-1}.\text{Pa}^{-1}$ and from 2.23 ± 0.65 to $24.8 \pm 2.11 \times 10^{-11} \text{ g.m}^{-1}.\text{s}^{-1}.\text{Pa}^{-1}$,
502 respectively for control film and film with coumarin after 60kGy irradiation dose. Due to the
503 effect of irradiation, the barrier properties are mainly related to the increasing the water content
504 that induces the plasticization of the film during permeation. The 30-84% RH gradient
505 corresponds to a mean water activity average equal to 0.57, which is in the plasticization domain
506 of the network by water. But the effect of coumarin addition in the network on the transfer
507 phenomena is not significant.

508

509 **3.2.3. Mechanical properties**

510 Mechanical parameters (tensile strength (TS), Young's Modulus (YM) and elongation at break
511 (E)) of all studied films are given in **Table 2**. TS, YM and E of control film (non-irradiated,
512 without coumarin) were 25.9 ± 3.9 MPa, 1523 ± 266 MPa and $2.2 \pm 0.4\%$, respectively. Jridi et al.
513 (2014) found similar value of %E ($2.7 \pm 0.5\%$) but higher value of TS (44.3 ± 1.2 MPa) for

514 chitosan-skin fish gelatin blend film (50:50 w/w) that could be due to film thickness or molar
515 mass of polymer. Compared to the control film, no significant modification is observed on the
516 mechanical parameters (TS, YM and %E) after coumarin addition. An opposite tendency was
517 observed by Benbettaieb et al. (2015b) who showed that TS increases significantly when ferulic
518 acid was added in to the chitosan-gelatin film. Only the TS of control film increases
519 significantly with the increasing dose at a 60kGy. When irradiation doses (40 and 60kGy) were
520 applied on film containing coumarin, no significant ($p < 0.05$) modification of mechanical
521 parameters was observed. This increase of film stiffness and resistance is in accordance with the
522 improvement of thermal stability of control film after 60kGy irradiation dose, previously
523 observed from TGA analysis.

524 Finally, results from functional and structural properties are still less consistent to make any
525 hypothesis related to the crosslinking reaction between polymers chains and coumarin under
526 irradiation. Only few interactions occur (probably modified by hydrogen bonds) between the
527 different reactive compounds and some orientation of polar groups to the surface, which enhance
528 the film wettability and hydrophilicity. The interactions between polymer and coumarin after
529 irradiation, even if they seem to be weak could nevertheless affect the release of the antioxidant
530 into aqueous medium.

531

532 **3.3. Influence of irradiation on the coumarin release in aqueous media**

533 The release experiments were performed three months after film irradiation. All data are
534 summarized in **Table 3**. The amount of coumarin in the non-irradiated film (10.1 ± 1.5 mg/g of
535 film) determined after complete solubilisation of films in acetic acid solution (experimental
536 value) was significantly ($p < 0.05$) lower than the theoretical value (47.1 ± 4.7 mg/g of film)
537 calculated from the film formulation. This means that, about 80% of coumarin disappeared,
538 probably due to oxidation during storage (**Table 3**). After irradiation, measured (real) amount of

539 coumarin in the films is far less reduced, as only 35% is lost. Thus, irradiation protects coumarin
540 against degradation during the time of storage (3 months). It could be that coumarin make some
541 interaction with polymers and/or with free radical and thus is less available to be oxidized during
542 storage period. This result suggests that irradiation may act as a safeguard method of antioxidant
543 when this later is encapsulated in hydrocolloid films. This can be considered as a good way to
544 protect active compound and to ensure its quality until final consumer. As the difference
545 observed between theoretical and experimental content of coumarin in the film, only the
546 experimental concentration is considered for the study of the release. The release kinetics of
547 coumarin from chitosan-gelatin based films (non-irradiated and irradiated at 40 and 60kGy) in
548 water medium (at pH=7) are displayed in **Fig.5**. Release kinetics of coumarin exhibited the
549 typical shape of non-time-dependent and non-concentration-dependent diffusion. The content of
550 coumarin remaining in the film after release significantly ($p < 0.05$) increased from 1.7 ± 0.6 to
551 7.17 ± 0.5 and to 12.6 ± 1.7 mg/g of film, respectively after 40 and 60kGy irradiation doses. This
552 could be explained by the interaction between the polymer chains and this antioxidant, favoured
553 by the irradiation process and because the initial concentration is higher. This could therefore
554 modify the film structure organisation and the release mechanisms of antioxidant from the film.
555 Furthermore, the effective diffusion coefficients (D) of the coumarin in the films were calculated
556 from the release kinetics by fitting experimental data using Eq.6 and are given in **Table 3**. The
557 diffusivity is related to the molecular mobility within the polymeric network and could be related
558 to several factors such as molecular weight, structural characteristics of the matrix and solubility
559 of this antioxidant. We considered here that the partition coefficient did not affect the transfer as
560 the concentrations involved are always much lower than the solubility limit of the coumarin in
561 water media. As it can be observed, the effective diffusion coefficient of coumarin significantly
562 decreased ($p < 0.05$) from 3.26 ± 0.74 to 1.87 ± 0.48 and to $2.04 \pm 0.05 \times 10^{-11} \text{m}^2 \cdot \text{s}^{-1}$ respectively after
563 40 and 60kGy irradiation doses. This is also in agreement with the increase of the coumarin

564 content remaining in the film after the release (by chemical or physical entrapment).This
565 decrease of diffusion coefficient could be related to the irradiation treatment which limits the
566 mobility of coumarin and therefore decreases the apparent diffusivity. [Tin Wui et al. \(2002\)](#),
567 worked on the influence of microwave irradiation on the drug release properties of
568 polysaccharide beads and showed that the release-retarding property of alginate and alginate–
569 chitosan beads was significantly enhanced by subjecting the beads to microwave irradiation.
570 They showed that microwave technology can be employed in the design of solid dosage forms
571 for controlled-release application without the use of noxious chemical agents. In our case,
572 irradiation could favor the interaction between coumarin and biopolymer via free radical
573 mediated mechanism. Hence, coumarin is more linked and consequently, more protected and less
574 mobile. The effect of irradiation also modified the surface properties by increasing its polarity
575 and then swelling phenomenon occurs too quickly to affect the diffusion determination.
576 Despite the swelling, the film remains intact and no dissolution or network structure destruction
577 was observed during the kinetic of release. In non-irradiated film, as the structure is less dense,
578 water can easily enter into the network and favour the polymeric chain mobility and thus the
579 coumarin diffusion through the hydrated films is greater. Irradiation allowed to delay by 50% the
580 release time. So, film irradiation after optimization, would be an effective process for controlled
581 release of active naturals antioxidants in aqueous foods or even for medical applications.

582 583 **4. Conclusions**

584 Chitosan and fish gelatin films encapsulating coumarin were prepared as an active biobased film.
585 After film drying, irradiation using electron beam was applied at 40 and 60kGy. This work
586 aimed to investigate the coupled effect of irradiation and of the presence of the active compound
587 on the structure and functional properties of the films. Electron Spin Resonance (ESR) displayed
588 the free radical formation during irradiation in films. Coumarin did not affect the thermal
589 stability of films whereas irradiation slightly improved it. Both addition of coumarin and

590 irradiation decreased the contact angle with water and increase the polar component of the
591 surface tension of films, as well as the swelling index and rate. This is attributed to a
592 reorientation of polar groups at the film surface. From water barrier analysis, neither irradiation
593 nor coumarin addition affected the water vapour permeability at low RH gradient. However, a
594 higher RH gradient (30-84%) induced a rise of the WVP of all the films after irradiation
595 treatment that is mostly related to the surface properties and film wettability. Incorporation of
596 coumarin did not affect the mechanical properties of films on the contrary to irradiation, but very
597 weakly. The interactions between biopolymers and coumarin after irradiation affected the release
598 of the antioxidant into the aqueous medium. The content of coumarin remaining in the film at
599 equilibrium after release significantly increased when film were irradiated, from 17% to 32%
600 mg/g of film, inversely, the effective diffusion coefficient of coumarin decreased by 1.6 times.
601 Irradiation, also displayed that it is an efficient process to prevent coumarin degradation during
602 the storage of films, as more than 60% of the antioxidant was preserved compared to non-
603 irradiated films.

604

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612

613 **References**

614 Aider, M. (2010). Chitosan application for active bio-based films production and potential in the
615 food industry: review, *LWT-Food Science Technology*, 43, 837–842.

616 ASTM 51607 (2004). Practice for use of the alanine-EPR dosimetry system. ASTM Book of
617 Standards, Philadelphia, PA.

618 ASTM D882-02 (1992). Standard test method for tensile properties of thin plastic sheeting.
619 ASTM Book of Standards, Philadelphia, PA.

620 ASTM E96–80 (1980). Standard test methods for water vapor transmission of materials, ASTM
621 Book of Standards, Philadelphia, PA.

622 Barreto, P. L. M., Pires, A. T. N., & Soldi, V. (2003). Thermal degradation of edible films based
623 on milk proteins and gelatin in inert atmosphere. *Polymer Degradation and Stability*. 79 (1),
624 147–152.

625 Benbettaieb, N., Karbowskiak , T., Bornaz, S., & Debeaufort, F. (2015a). A spectroscopic analyses
626 for understanding the effect of electron beam irradiation doses on mechanical and transport
627 properties and microstructure of chitosan-fish gelatin blend films. *Food Hydrocolloids*, DOI:
628 10.1016/j.foodhyd.2014.09.038.

629 Benbettaieb, N., Karbowskiak , T., Brachais, C.H., & Debeaufort, F. (2015b). Coupling tyrosol,
630 quercetin or ferulic acid and electron beam irradiation to cross-link chitosan-gelatin films: a
631 structure-function approach. *European polymer journal*, 67, 113-127.

632 Bhat, R., & Karim, A. A. (2009). Ultraviolet irradiation improves gel strength of fish gelatin.
633 *Food Chemistry*, 113, 1160–1164.

634 Bigi, A., Bracci, B., Cojazzi, G., Panzavolta, S. & Roveri, N. (1998). Drawn gelatin films with
635 improved mechanical properties. *Biomaterials*, 19, 2335–2340.

636 CAC (Codex Alimentarius Commission). (2003). Codex general standard for irradiated foods.
637 CODEX STAN 106-1983. Rev. 1-2003.

638 Cao, N., Fu, Y., & He, J. (2007). Mechanical properties of gelatin films cross-linked,
639 respectively, by ferulic acid and tannin acid. *Food Hydrocolloids*, 21, 575–584.

640 Casimiro, M. H., Gil, M. H., & Leal, J. P. (2010). Suitability of gamma irradiated chitosan based
641 membranes as matrix in drug release system. *International Journal of Pharmaceutics*, 395,
642 142–146.

643 Cerqueira, M. A., Souza, B.W., Teixeira, J. A., & Vicente, A. A. (2012). Effect of glycerol and
644 corn oil on physicochemical properties of polysaccharide films: a comparative study. *Food*
645 *Hydrocolloids*, 27(1), 175-184.

646 Coates, J. (2000). Interpretation of Infrared Spectra, A Practical Approach. *Encyclopedia of*
647 *Analytical Chemistry*. Ó John Wiley & Sons Ltd, Chichester, 2000. R.A. Meyers (Ed.) pp.
648 10815–10837.

649 Crank, J. (1975). *The mathematics of diffusion*. Brunel University Uxbridge. Second edition.
650 Oxford University Press, Ely House, London W.I.pp. 56-57.

651 Debeaufort, F., Martin-Polo, M.O., & Voilley, A. (1993). Polarity, homogeneity and structure
652 affect water vapor permeability of model edible films. *Journal of Food Science*, 58(2), 426-
653 434.

654 Fabra, M. J., Hambleton, A., Talens, P., Debeaufort, F., & Chiralt, A. (2011). Effect of ferulic
655 acid and α -tocopherol antioxidants on properties of sodium caseinate edible films. *Food*
656 *Hydrocolloids*, 25(6) 1441-1447.

657 Gontard, N., Duchez, C., Cuq, J.L. & Guilbert, S. (1994). Edible composite films of wheat gluten
658 and lipids: water vapour permeability and other physical properties. *International Journal of*
659 *Food Science & Technology*, 29, 39-50.

660 Han, J. H. (2002). Protein-based edible films and coatings carrying antimicrobial agents. In A.
661 Gennadios (Ed.), *Protein-based films and coatings* Boca Raton, FL, CRC Press, 485-499.

662 Hoque, M.S., Benjakul, S., & Prodpran, T. (2011). Properties of film from cuttlefish (*Sepia*
663 *pharaonis*) skin gelatin incorporated with cinnamon, clove and star anise extracts. *Food*
664 *Hydrocolloids*, 25, 1085-1097.

665 Inamura, P.Y., Kraide, F.H., Drumond, W.S., deLima, N.B. Moura, E.A.B & delMastro, N.L.
666 (2013). Ionizing radiation influence on the morphological and thermal characteristics of a
667 biocomposite prepared with gelatin and Brazil nut wastes as fiber source. *Radiation Physics*
668 *and Chemistry*, 84, 66–69.

669 Jo, C., Kang, H., Lee, N. Y., Kwon, J. H. & Byun, M. W. (2005). Pectin- and gelatin-based film:
670 effect of gamma irradiation on the mechanical properties and biodegradation. *Radiation*
671 *Physics and Chemistry*, 72, 745–750.

672 Jongjareonrak, A., Benjakul, S., Visessanguan, W., Prodpran, T. & Tanaka, M. (2006).
673 Characterization of edible films from skin gelatin of brownstripe red snapper and bigeye
674 snapper. *Food Hydrocolloids*, 20, 492–501.

675 Jridi M, Hajji S, Ben Ayed H, Lassoued, I Mbarek, A Kammoun, M Souissi N & Nasri M.
676 (2014). Physical, structural, antioxidant and antimicrobial properties of gelatin–chitosan
677 composite edible films. *International Journal of Biological Macromolecules*, 67, 373–379.

678 Karbowiak, T., Debeaufort, F., Champion, D., & Voilley, A. (2006). Wetting properties at the
679 surface of iota-carrageenan based edible films. *Journal of Colloid and Interface Science*, 294,
680 400–410.

681 Kavooosi, G., Rahmatollahi, A., Mahdi Dadfar, S.M, & Purfard, A.M.(2014). Effects of essential
682 oil on the water binding capacity, physic-mechanical properties, antioxidant and antibacterial
683 activity of gelatin films. *LWT - Food Science and Technology*, 57,556-561.

684 Lacroix, M., Le, T.C., Ouattara, B., Yu, H., Letendre, M., Sabato, S.F., Mateescu, M.A., &
685 Patterson, G. (2002). Use of gamma irradiation to produce films from whey, casein and soy
686 proteins: Structure and functional characteristics. *Radiation Physics Chemistry*, 63, 827–832.

687 Lee, K.Y., Shim, J. & Lee, H.G. (2004). Mechanical properties of gellan and gelatin composite
688 films. *Carbohydrate Polymers*, 56 (2), 251–254.

689 Madeleine-Perdrillat, C., Karbowiak, T., Raya, J., Gougeon, R., Bodart, P.R., Debeaufort, F.
690 (2015). Water-induced local ordering of chitosan polymer chains in thin layer
691 films, *Carbohydrate Polymer*, 118, 107-114. Martins, J.T.,
692 Martins, J.T., Cerqueira, M.A., Vicente A.A. (2012). Influence of a-tocopherol on
693 physicochemical properties of chitosan-based films. *Food Hydrocolloids*, 27, 220-227

694 Mathew, S., & Abraham, T. E. (2008). Characterisation of ferulic acid incorporated starch-
695 chitosan blend films. *Food Hydrocolloids*, 22, 826-835.

696 Maturana, A.Y. & Pagliuso, J.D. (2011). Thermal decomposition behavior of crude glycerin. 21st
697 Brazilian Congress of Mechanical Engineering, October 24-28, 2011, Natal, RN, Brazil.

698 Nuthong, P., Benjakul, S and Prodpran, T. (2009). Characterization of porcine plasma protein-
699 based films as affected by pretreatment and cross-linking agents. *International Journal of*
700 *Biological Macromolecules*, 44, 143–148.

701 Ou, S., Wang, Y., Tang, S., Huang, C., & Jackson, M. G. (2005). Role of ferulic acid in
702 preparing edible films from soy protein isolate, *Journal of Food Engineering*, 70, 205–210.

703 Ouattara, B., Simard, R.E., Piette, G., Begin, A., & Holley, R.A. (2000). Inhibition of surface
704 spoilage bacteria in processed meats by application of antimicrobial films prepared with
705 chitosan. *International Journal Food Microbiology*, 62,139–148.

706 Oussalah, M., Caillet, S., Salmiéri, S., Saucier, L., & Lacroix, M. (2004). Antimicrobial and
707 antioxidant effects of milk protein-based film containing essential oils for the preservation of
708 whole beef muscle. *Journal of Agriculture and Food Chemistry* 52, 5598-5605.

709 Owens, D.K., & Wendt, R.C. (1969). Estimation of the surface free energy of polymers, *Journal*
710 *of Applied Polymer Science*, (13), 1741-1747.

711 Papadokostaki, K.G., Amanratos, S.G., & Petropoulos, J.H. (1997). Kinetics of release of
712 particules solutes incorporated in cellulosic polymer matrices as a function of solute solubility
713 and polymer swellability. I. Sparingly soluble solutes. *J. Appl. Polym. Sci.* 67, 277-287.

714 Pena, C., de la Caba, K., Eceiza, A., Ruseckaite, R., & Mondragon, I. (2010). Enhancing water
715 repellence and mechanical properties of gelatin films by tannin addition.
716 *Bioresource.Technology*, 101(17), 6836–6842.

717 Peng, Y & Li, Y. (2014). Combined effects of two kinds of essential oils on physical, mechanical
718 and structural properties of chitosan films. *Food Hydrocolloids*, 36,287-293.

719 Pérez-Mateos, M., Montero, P., & Gómez-Guillén, M.C. (2009). Formulation and stability of
720 biodegradable films made from cod gelatin and sunflower oil blends. *Food Hydrocolloids*, 23,
721 1, 53–61.

722 Rivero, S., García, M.A. & Pinotti, A. (2010). Crosslinking capacity of tannic acid in plasticized
723 chitosan films. *Carbohydrate Polymers*, 82, 270–276.

724 Sabato, S. F., Ouattara, B., Yu, H., D’Aprano, G., Le Tien, C., Mateescu, M. A., & Lacroix,
725 M.(2000). Mechanical and barrier properties of cross linked soy and whey protein based
726 films. *Journal of Agricultural and Food Chemistry*, 49, 1397–1403.

727 Sahu, A., Rawal, M.K., Sharma, V.K & Parashar, B. (2009) Microwave assisted Fe (III)
728 catalyzed oxidation of phenol by hydrogen peroxide supported on alumina. *Rasayan Journal*
729 *of Chemistry*, 2(2), 536-538.

730 Ström G., Fredriksson M., & Stenius P. (1987). Contact angles, work of adhesion, and interfacial
731 tensions at a dissolving hydrocarbon surface. *Journal of Colloid Interface Science*, 119(2),
732 352–361.

733 Suppakul, P., Miltz, J., Sonneveld, K. & Bigger, S.W. (2003). Active packaging technologies
734 with an emphasis on antimicrobial packaging and its applications. *Journal of Food Science*,
735 68, 408–420.

736 Tammineni,N., Rasco,B., Powers,J., Nindo,C. & Ünlü, G.(2014).Bovine and fish gelatin
737 coatings incorporating tannins: Effect on physical properties and oxidative stability of salmon
738 fillets. *Journal of Food Chemistry and Nutrition*, 2(2). 93-102.

739 Tharanathan, R.N. (2003). Biodegradable films and composite coatings: past, present and future.
740 *Trends in Food Science and Technology*, 14(3), 71- 78.

741 Tin, W., Lai, W.C., Shyan, B.K., & Paul, W.H.(2002). Design of controlled-release solid dosage
742 forms of alginate and chitosan using microwave. *Journal ofcontrolled Release*.(84), 99–114.

743 Vachon, C., Yu, H.L., Yefsah, R., St-Gelais, D. & Lacroix, M.(2000). Mechanical and structural
744 properties of milk protein edible films cross-linked by heating and γ -irradiation. *Journal of*
745 *Agricultural and Food Chemistry*, 48, 3202-3209.

746 Wu, J., Chen, S., Ge, S., Miao, J., Li, J & Zhang, Q. (2013). Preparation, properties and
747 antioxidant activity of an active film from silver carp (*Hypophthalmichthys molitrix*) skin
748 gelatin incorporated with green tea extract. *Food Hydrocolloids*. 32, 42-51.

749 Zhang, F., Xu, S. & Wang, Z. (2010). Pre-treatment optimization and properties of gelatin from
750 fresh water fish scales. *Food and Bioproducts Processing*, doi:10.1016/j.fbp.2010.05.003.

751

1

2 **Figure captions**

3 **Figure 1:** ESR spectra of chitosan-fish gelatin films containing coumarin and irradiated at
4 60kGy at the dose rate of 300Gy/sec. Measurements were performed at 25°C and 50% RH, 3
5 months after irradiation.

6 **Figure 2:** FTIR spectra of non-irradiated and irradiated (60kGy) chitosan-fish gelatin film
7 containing or not coumarin. All films were previously equilibrated at 50% RH and 25°C.

8 **Figure 3:** TGA thermograms of irradiated and non-irradiated chitosan-fish gelatin film with and
9 without coumarin. All films were previously equilibrated at 50% RH and 25°C.

10 **Figure 4:** Water contact angle at 0 and 20s, swelling index, swelling rate and surface tension
11 (γ_S) with dispersive (γ_S^d) and polar components (γ_S^p) of irradiated and non-irradiated chitosan-
12 fish gelatin film with and without coumarin. Measurements were done at room conditions
13 ($\sim 20^\circ\text{C}$, $\sim 50\% \text{RH}$).

14 **Figure 5:** Kinetic release of coumarin in water medium at pH=7 and 25°C, for control (0kGy)
15 and irradiated (40 and 60kGy) chitosan-fish gelatin films. Ct: concentration of coumarin released
16 in the aqueous dissolution medium at time t; C_∞ : the maximum concentration of coumarin
17 released. Symbols are experimental values (mean+standard deviation) and solid line corresponds
18 to a Fickian data modeling using Eq (6).

19

20 **Tables captions**

21 **Table 1:** Number and weight average molar mass (M_n , M_w), polydispersity (M_w/M_n) and z-
22 average mean square radius (R_z) for irradiated and non-irradiated control films and for irradiated
23 coumarin films, determined from SEC-MALLS analysis.

24 **Table 2:** Thickness, water vapour permeability (WVP) and mechanical properties (Tensile
25 strength (TS), Young's Modulus (YM) and elongation at break (% E)) of irradiated and non-

26 irradiated chitosan-fish gelatin film with and without coumarin. Water vapour permeability was
27 measured at 25°C under (0-30) % and (30-84) % RH differentials. Mechanical properties were
28 measured at 25°C and 50% RH.

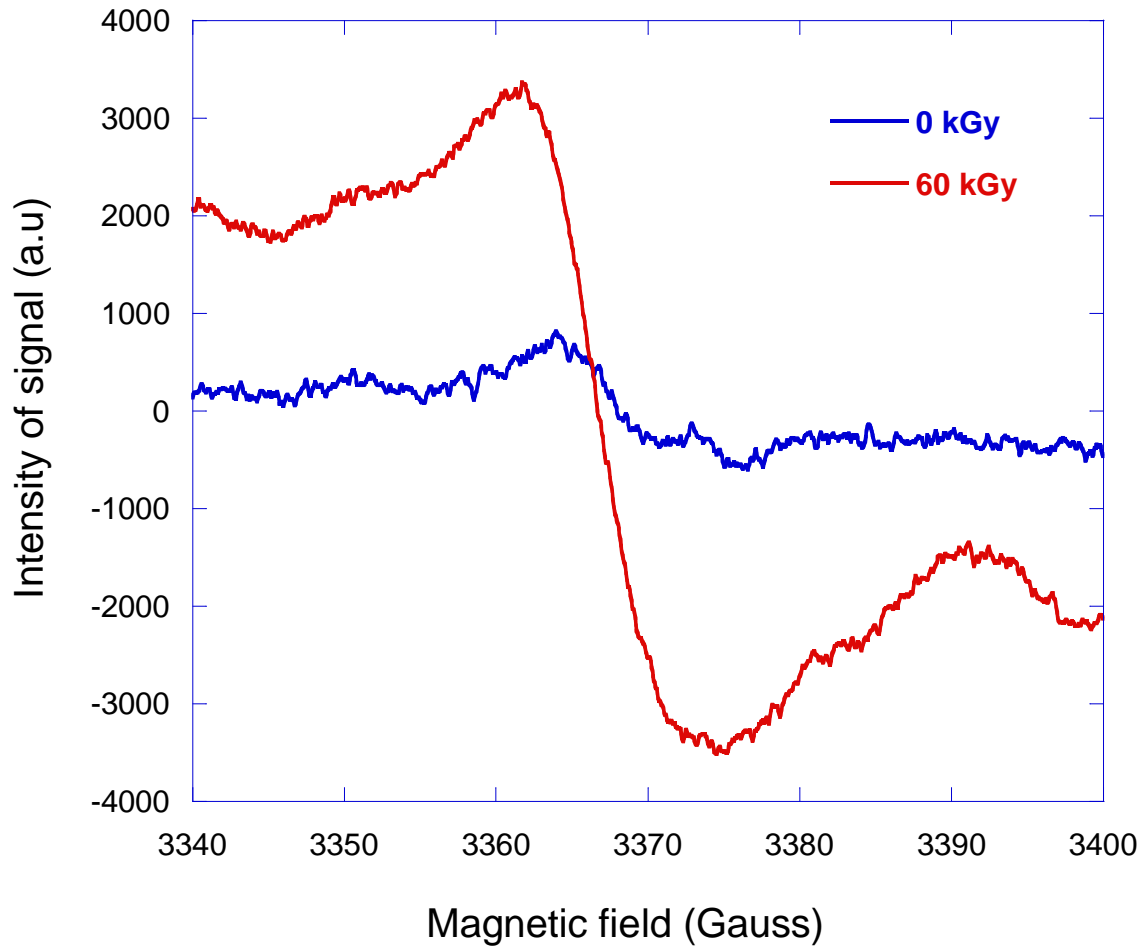
29 **Table 3:** Kinetics release parameters of coumarin from irradiated and non-irradiated chitosan-
30 fish gelatin film. All parameters were determined during release and from release kinetics profile
31 (up to total release) of coumarin from irradiated and non-irradiated films. Dissolution medium is
32 water at pH=7 and 25°C.

33

34

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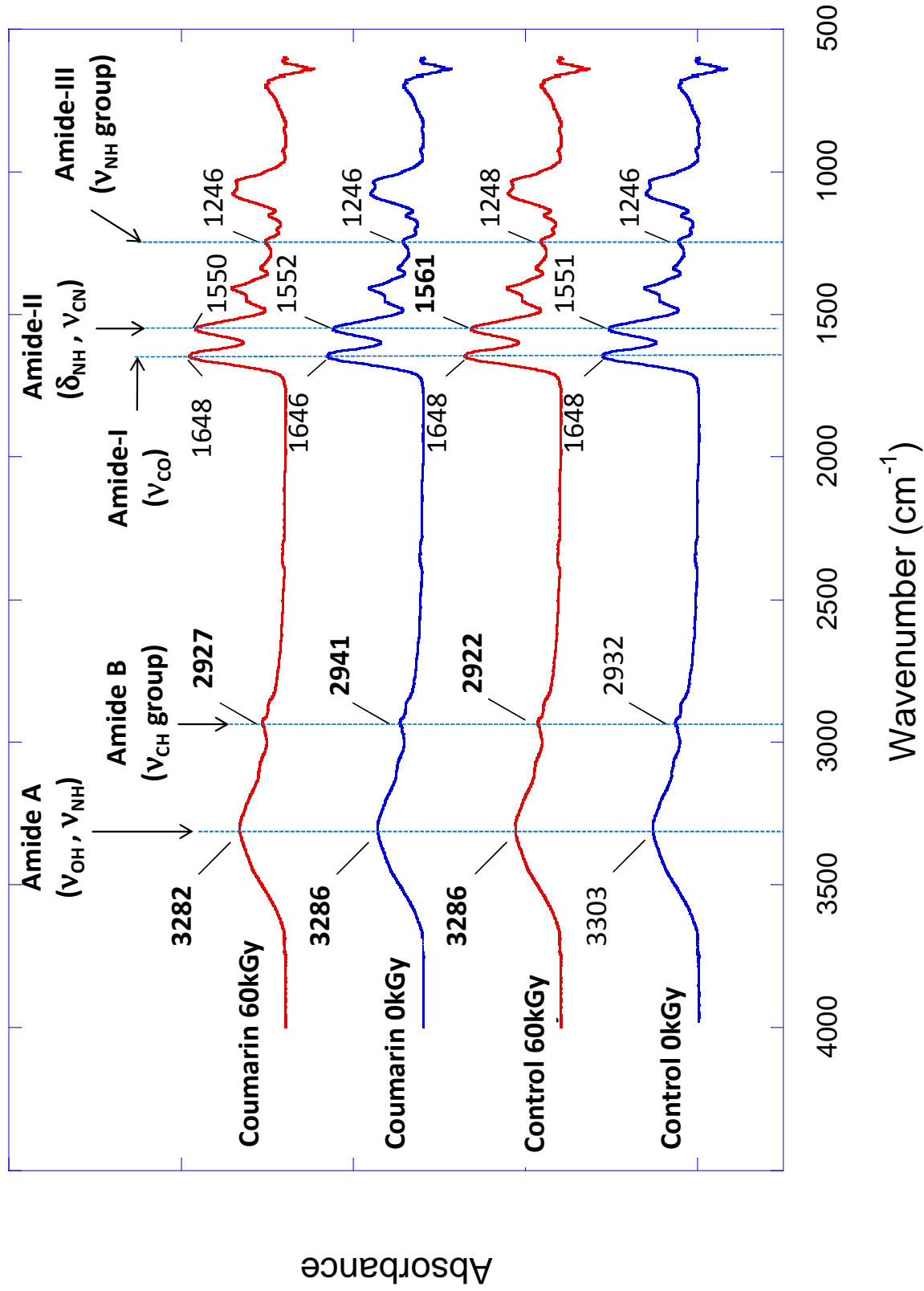
2 **Figure 1**



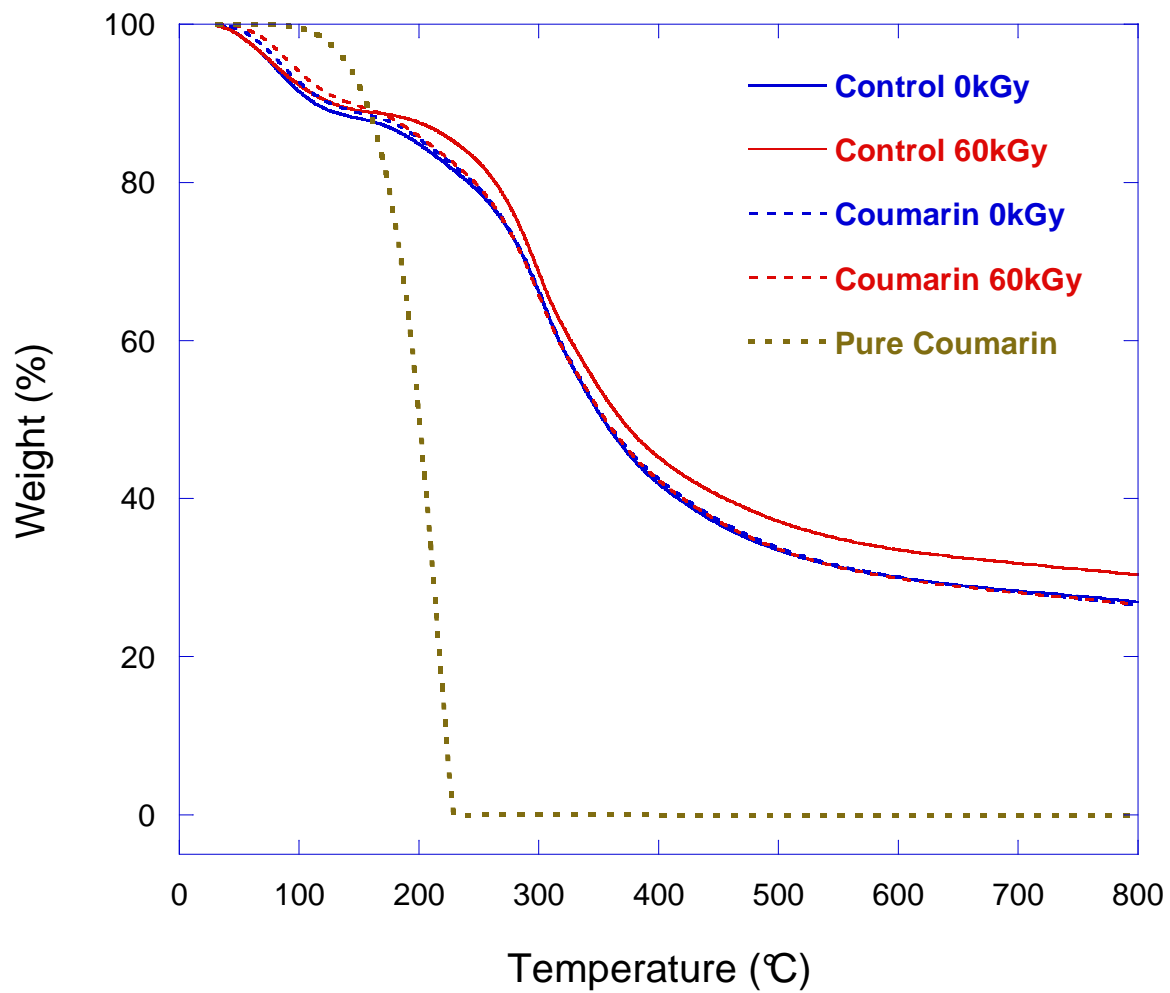
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5 Figure 2

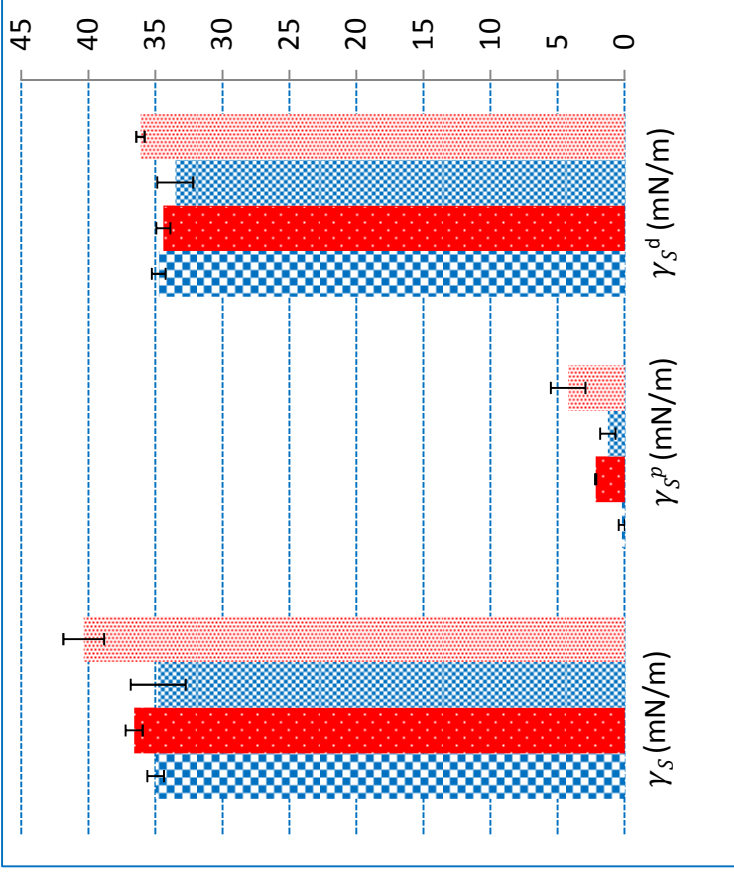
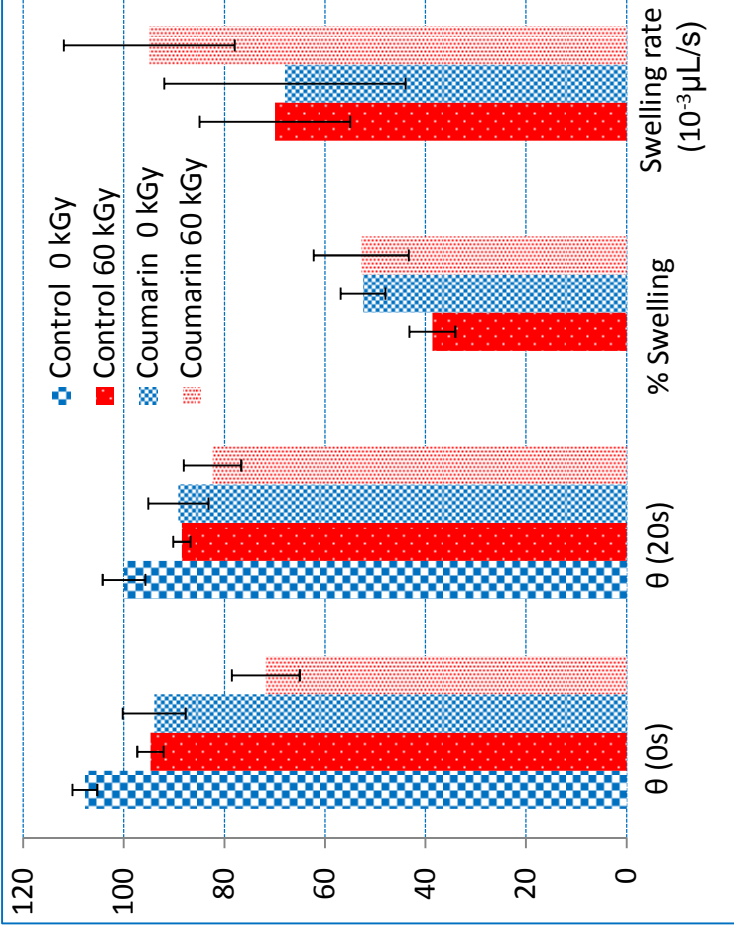


7 **Figure 3**

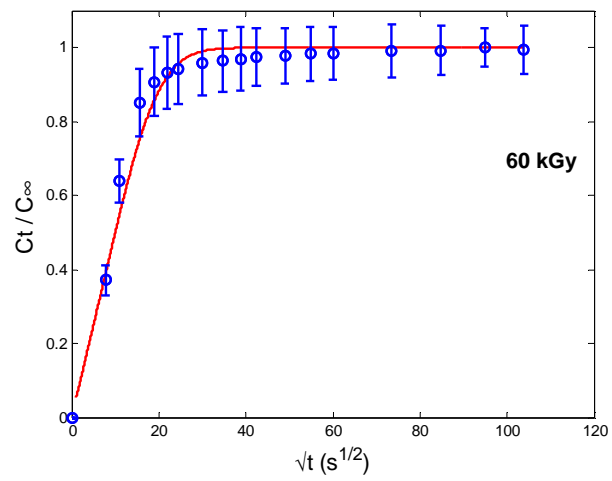
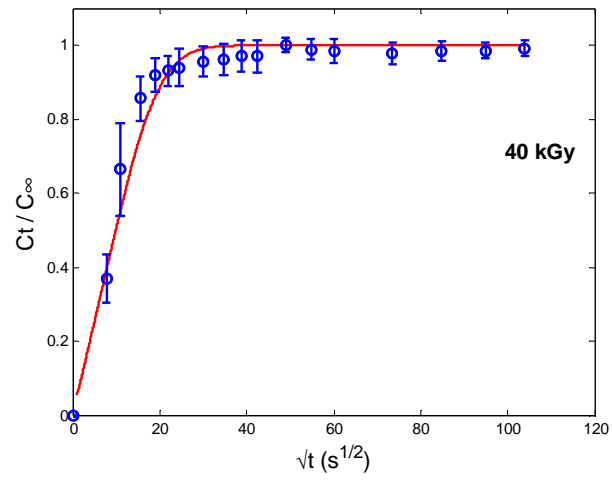
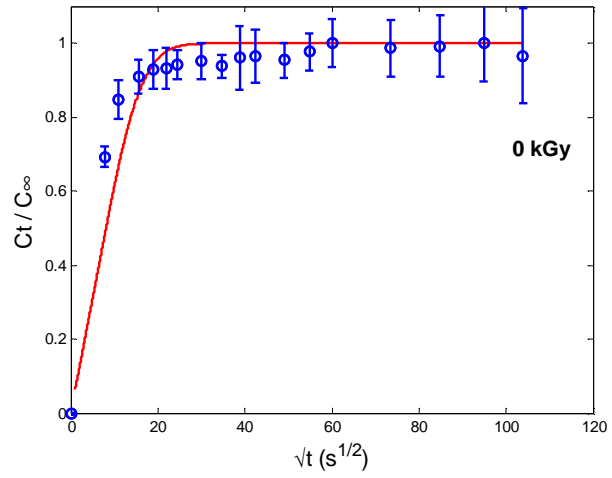


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13 **Figure 5**



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1 **Table 1**

Films	Mw (10⁵ g/mol)	Mn (10⁴ g/mol)	Polydispersity	Rz (nm)
Control 0kGy	1.36 (2%)	8.39 (2.4%)	1.62 (3.2%)	17.3 (24%)
Control 60kGy	1.20 (1.9%)	5.37 (5%)	2.23 (5%)	17.9 (21%)
Coumarin 60kGy	1.35 (2%)	5.09 (6%)	2.66 (7%)	18.1 (22%)

2 Mean (relative error%)

3

4 **Table 2**

5

Films	Irradiation dose (kGy)	Thickness (μm)	WVP ($10^{-11} \text{ g}\cdot\text{m}^{-1}\cdot\text{s}^{-1}\cdot\text{Pa}^{-1}$)		TS (MPa)	YM (MPa)	%E
			$\Delta\text{RH} = (0-30)\%$	$\Delta\text{RH} = (30-84)\%$			
Control	0	65 \pm 8 ^a	0.52 \pm 0.10 ^{a,b}	2.41 \pm 0.44 ^a	25.89 \pm 3.92 ^a	1523 \pm 266 ^a	2.2 \pm 0.4 ^a
	40	65 \pm 8 ^a	0.59 \pm 0.04 ^{b,c}	22.46 \pm 0.7 ^b	28.54 \pm 3.76 ^a	1221 \pm 235 ^{a,b}	3.1 \pm 0.9 ^{a,b}
	60	65 \pm 8 ^a	0.56 \pm 0.03 ^{b,c}	23.06 \pm 0.85 ^{b,c}	39.20 \pm 8.32 ^b	1270 \pm 23 ^{a,b}	4.1 \pm 2.1 ^{a,b}
Coumarin	0	77 \pm 12 ^a	0.47 \pm 0.03 ^{a,d}	2.23 \pm 0.65 ^a	30.95 \pm 0.83 ^{a,b}	1328 \pm 185 ^{a,b}	4.7 \pm 0.5 ^{a,b}
	40	77 \pm 12 ^a	0.47 \pm 0.02 ^{a,d}	25.13 \pm 1.25 ^d	28.65 \pm 5.02 ^a	1006 \pm 115 ^b	3.2 \pm 0.3 ^{a,b}
	60	77 \pm 12 ^a	0.50 \pm 0.04 ^{a,c}	24.80 \pm 2.11 ^{d,c}	31.35 \pm 5.64 ^{a,b}	1187 \pm 201 ^{a,b}	3.0 \pm 0.4 ^{a,b}

6 Values are given as mean \pm standard deviation. Means with the same Arabic letter in the same column are not significantly different at $p < 0.05$.

7

8 **Table 3**

9

	0kGy	40kGy	60kGy
Theoretical content of coumarin in film (mg/g of film)	47.1±4.7	47.1±4.7	47.1±4.7
Initial Content of coumarin in film prior to release (mg/g of film)	10.1±1.5 ^a	31±0.4 ^b	39.2±4 ^c
Content of coumarin remaining in the film after release at equilibrium (mg/g of film)	1.7±0.6 ^a	7.2±0.5 ^b	12.6±1.7 ^c
Diffusion coefficient (10 ⁻¹¹ m ² /s)	3.26±0.74 ^b	1.87±0.48 ^a	2.04±0.05 ^a

10

11

Values are given as mean ± standard deviation. Means with the same Arabic letter in the same line are not significantly different at p<0.05.

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