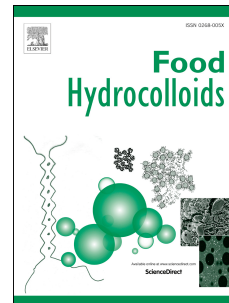


Accepted Manuscript

Crosslinked electrospun zein-based food packaging coatings containing bioactive chilito fruit extracts

María Alejandra Moreno, María Eugenia Orqueda, Laura G. Gómez-Mascaraque, María Inés Isla, Amparo López-Rubio



PII: S0268-005X(19)30405-9

DOI: <https://doi.org/10.1016/j.foodhyd.2019.05.001>

Reference: FOOHYD 5100

To appear in: *Food Hydrocolloids*

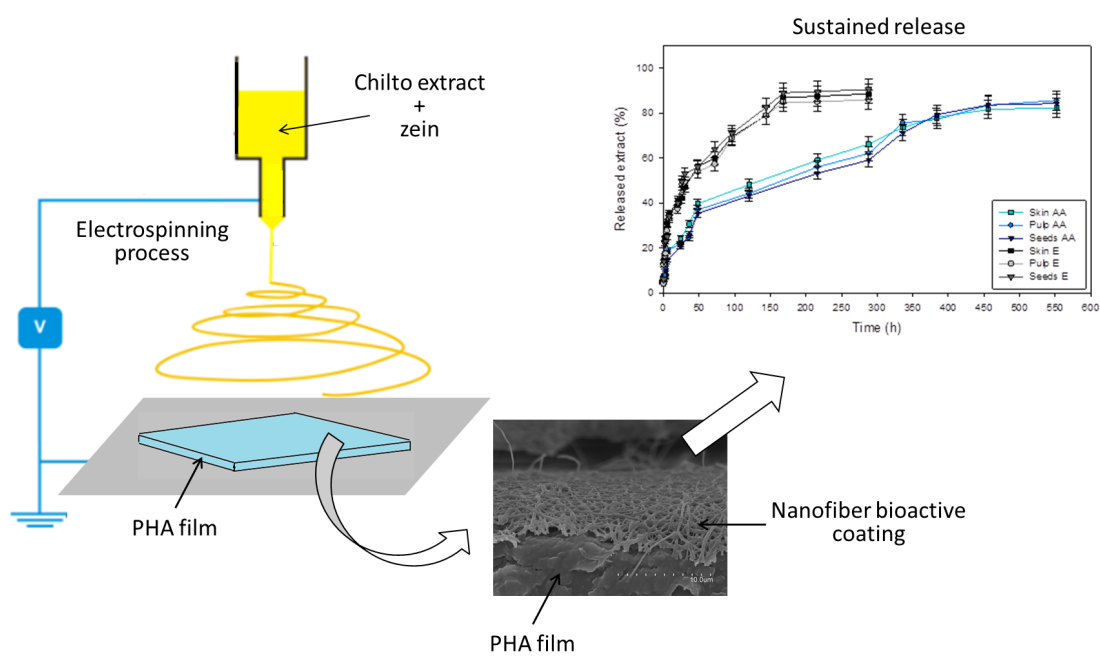
Received Date: 20 February 2019

Revised Date: 30 April 2019

Accepted Date: 1 May 2019

Please cite this article as: Moreno, Marí.Alejandra., Orqueda, Marí.Eugenia., Gómez-Mascaraque, L.G., Isla, Marí.Iné., López-Rubio, A., Crosslinked electrospun zein-based food packaging coatings containing bioactive chilito fruit extracts, *Food Hydrocolloids* (2019), doi: <https://doi.org/10.1016/j.foodhyd.2019.05.001>.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.



1 **Crosslinked electrospun zein-based food packaging coatings containing bioactive**
2 **chilto fruit extracts**

3
4 María Alejandra Moreno¹, María Eugenia Orqueda¹, Laura G. Gómez-Mascaraque²,
5 María Inés Isla¹#, Amparo López-Rubio³*#

6
7
8 ¹*Laboratorio de Investigación de Productos Naturales (LIPRON), Instituto de*
9 *Bioprospección y Fisiología Vegetal (INBIOFIV-CONICET-UNT), Facultad de*
10 *Ciencias Naturales e Instituto Miguel Lillo, Universidad Nacional de Tucumán, San*
11 *Lorenzo 1469. San Miguel de Tucumán, Tucumán, Argentina.*

12 ²*Food Chemistry and Technology Department, Teagasc Food Research Centre,*
13 *Moorepark, Fermoy, Co. Cork, Ireland.*

14 ³*Instituto de Agroquímica y Tecnología de Alimentos (IATA-CSIC) Avda. Agustín*
15 *Escardino 7, 46980 Paterna, Valencia, España.*

16
17 #Both authors contributed equally to this work.

18
19 *Corresponding author: Tel.: +34 963900022; fax: +34 963636301

20 E-mail address: amparo.lopez@iata.csic.es (A. López-Rubio)

21
22
23
24
25
26
27
28
29 **Keywords:** *Solanum betaceum*; electrospinning; zein fibers; crosslinking; food
30 packaging.

31
32
33
34
35

36 **Abstract**

37

38 In this work, zein fibers loaded with phenolic-enriched extracts from pulp, seed and skin
39 of orange chilito were collected on polyhydroxyalkanoate (PHA) films through the
40 electrospinning technique, for their potential use as bioactive internal coatings for food
41 packaging applications. The zein fibers were characterized by scanning electron
42 microscopy (SEM), Fourier transform infrared (FTIR) spectroscopy and
43 thermogravimetric analysis (TGA). The water stability of the zein fibers was improved
44 by crosslinking with glutaraldehyde vapors. The encapsulation efficiency of all
45 bioactive phenolic-enriched extracts was greater than 90%. Encapsulation in the zein
46 fibers improved the thermostability of the extracts. Two food simulants (50% ethanol
47 and 3% acetic acid) were used to evaluate the release of the extracts from the
48 crosslinked zein fibers. It was observed that crosslinking delayed the release of phenolic
49 compounds (rosmarinic acid, caffeic acid and its derivatives) in both solvents (80%
50 released after 7 days of contact in 50% ethanol and 23 days in 3% acetic acid) and their
51 antioxidant properties were kept. Therefore, this work demonstrates the potential of the
52 developed zein-based encapsulation structures containing chilito extracts to be applied as
53 antioxidant coatings in food packaging structures to contribute to the preservation of
54 both hydrophilic and lipophilic food products.

55

56

57

58

59

60

61

62

63

64

65

66

67

68

69

70

71 **1. Introduction**

72 Chilto (*Solanum betaceum* Cav) is a native fruit that grows in the Northwest region of
73 Argentina. These fruits are popularly consumed (in salads, juices, jams, liquors, and
74 other regional products) by different aboriginal and rural communities and their virtues
75 are currently being rediscovered (Orqueda et al., 2017).

76 *Solanum betaceum*, known as “tamarillo”, “chilto” or “tree tomato”, produces edible
77 fleshy fruits with a growing market in its native Andean countries, as well as in North
78 America, Europe and Oceania (Prohens & Nuez, 2000; Samuels, 2015). The main types
79 of fruit are recognized depending on the color of the skin, with dark purple, orange and
80 red fruits (Prohens, Ruiz, & Nuez, 1996). Currently, the sustainable cultivation of
81 orange chilto in Argentina is taking place in the Yungas region (Orqueda et al., 2017).

82 In a previous work, the interest of the pulp, seed and skin of chilto to produce functional
83 foods was demonstrated. Specifically, the nutritional and phytochemical composition,
84 as well as the antioxidant activity and the inhibitory capacity of fruit fractions on key
85 enzymes involved in the metabolic syndrome (α -glucosidase, α -amylase and pancreatic
86 lipase) were described (Orqueda et al, 2017). In fact, these bioactive properties were
87 demonstrated both before and after simulated gastroduodenal digestion and were
88 ascribed to the phenolic compounds present in the fruit biomass (Orqueda et al, 2017).

89 The main phenolic compounds of chilto seed, pulp and skin extracts were rosmarinic
90 acid and its derivatives (Orqueda et al., 2017). Rosmarinic acid (RosA), a naturally
91 water-soluble phenolic compound is an ester of caffeic acid and 3, 4-
92 dihydroxyphenyllactic acid. RosA has gained a great deal of attention due to its various
93 biological activities, such as antibacterial, antiviral, antioxidant and anti-inflammatory
94 activities (Ngo, Lau, & Chua, 2018; Gonçalves et al., 2019).

95 Moreover, the safety of the polyphenol-rich extracts from chilto were evaluated,
96 showing no toxic and mutagenic effect on *Artemia salina* and *Salmonella typhimurium*
97 strains, respectively (Orqueda et al., 2017).

98 The interesting health-promoting properties of chilto, make these fruits excellent
99 sources of natural functional food ingredients. For this reason, pulp, skin and seed
100 extracts of chilto, previously characterized by Orqueda et al. (2017), were considered
101 for inclusion in bioactive food packaging structures.

102 Electrospinning has recently gained significant interest in the fields of bioactive
103 encapsulation and functional food development as it is a relatively simple, versatile, low

104 cost, non-thermal process and does not require the use of organic solvents as aqueous-
105 based solutions have shown their potential as starting solutions for electrospinning
106 (Quek, Hadi, & Tanambell, 2019). This non-mechanical technique involves the use of a
107 high voltage electrostatic field to charge the surface of a polymer solution droplet,
108 thereby inducing the ejection of a liquid jet through a spinneret (Mendes, Stephansen, &
109 Chronakis, 2017).

110 Zein has demonstrated to be an excellent material for electrospinning and has been
111 successfully used to encapsulate diverse bioactive compounds (Gómez-Mascaraque et
112 al., 2017; Gómez-Mascaraque, Pérez-Masiá, González-Barrio, Periago, & Lopez-Rubio,
113 2017; Gomez-Mascaraque, Tordera, Fabra, Martínez-Sanz, & López-Rubio, 2019; Quek
114 et al., 2019) and to generate biodegradable food packaging layers (Fabra, López-Rubio,
115 & Lagaron, 2016). Zein is a plant protein that has found use in adhesive and coating
116 materials for pharmaceutical, biomedical, and food applications, because of its non-
117 toxicity, biodegradability, biocompatibility and economic reasons (Moradkhannejhad,
118 Abdouss, Nikfarjam, Mazinani, & Heydari, 2018; Schmidt, Hamaker, & Wilker, 2018).

119 It is a hydrophobic protein (prolamin) with high thermal stability and oxygen-barrier
120 properties (Neo et al., 2013). Electrospun zein nanofibers are easily produced giving
121 raise to homogeneous and flexible structures, but with poor mechanical properties and
122 poor water stability, fact which restricts their applications. When immersed in water,
123 zein fibers swell and collapse into films with a considerable decrease in surface area,
124 also decreasing the number of interconnected pores and tensile strength (Jiang, Reddy,
125 & Yang, 2010), fact which could hamper its use as bioactive food packaging coating.

126 However, this can be significantly improved by applying crosslinking processes (Quek
127 et al., 2018). Glutaraldehyde is a crosslinking agent that has been widely used to modify
128 biodegradable films based on polyvinyl alcohol (Mansur, Sadahira, Souza, & Mansur,
129 2008), polyvinyl alcohol/methyl cellulose (Park, Park, & Ruckenstein, 2001), gelatin
130 and zein (Fan, Duquette, Dumont, & Simpson, 2018; Matsuda, Iwata, Se, & Ikada,
131 1999; Shahbazi, Ahmadi, Seif, & Rajabzadeh, 2016). Some advantages of this
132 crosslinking agent are the low cost, fast reaction time, and ability to crosslink many
133 amino acid groups present in the protein molecules (Huang et al., 2014). Glutaraldehyde
134 is more effective than other aldehydes as a crosslinking agent and its application to
135 crosslink nanofibers upon exposure to its vapors has demonstrated lower or no cytotoxic
136 effect (Destaye, Lin, & Lee, 2013). In fact, the use of glutaraldehyde in the vapor phase
137 as crosslinking agent of natural and artificial polymer blends has proven to be an

138 effective procedure that avoids the presence of toxic residues into the materials, as no
139 effect on cell viability and proliferation, neither increase in the cytoplasmic lactate
140 dehydrogenase release, nor damages on mitochondrial and lysosomal functions were
141 observed (Ramires & Milella, 2002; Shahbazi et al., 2016).

142 Therefore, the aim of this work was to develop and characterize crosslinked electrospun
143 zein fibers loaded with phenolic-rich orange chilito extracts with potential as bioactive
144 food packaging coatings. The base material chosen in this work was a commercial
145 polyhydroxyalkanoate, given the increased interest of these materials for biodegradable
146 food packaging applications.

147

148 **2. Materials and methods**

149 **2.1. Reagents**

150 Zein from corn (grade Z3625), with reported molecular weight of 22–24 kDa, was
151 purchased from Sigma-Aldrich (Spain). 96% (v/v) acetic acid was supplied by Scharlab
152 (Spain). Absolute ethanol (> 99.9%) was purchased from VWR (UK). Glutaraldehyde
153 solution and hydrochloric acid were obtained from Sigma-Aldrich.

154 The polyhydroxyalkanoate (PHA) film used as base film to deposit the electrospun zein
155 layer was a polyhydroxybutyrate/polyhydroxyvalerate 8% - Biopolymer PHB92/PHV 8
156 film produced by Goodfellow GmbH (Germany) and distributed by MicroPlanet
157 Laboratorios, S.L. (Spain).

158 2,2'-azino-bis (3-ethylbenzo thiazoline-6-sulphonic acid) (ABTS), 2,2'-azo-bis(2-
159 amidinopropane) dihydrochloride (AAPH) were purchased from Sigma-Aldrich (St.
160 Louis, MO, USA).

161

162 **2.2. Plant material**

163 Fruits of *Solanum betaceum* Cav. (orange cultivar) were collected in Parque Sierra de
164 San Javier, Tucumán, Argentina, during February and March 2014 and 2015. The fruits
165 were harvested at the ripening stage in which they are consumed and were transported
166 immediately to the laboratory at 4°C. The fresh fruits were washed with water. Skin,
167 pulp and seeds (without jelly portion) were separated, freeze-dried and powdered.

168

169 **2.3. Phenolic enriched extracts preparation**

170 The powders of seed, pulp and skin were extracted at room temperature with 95°
171 ethanol (1 g of powder per 5 mL of ethanol) assisted by ultrasound during 30 min under

172 stirring (40 cycles/min). Subsequently, the samples were centrifuged at 12,000 g for 10
173 min. The supernatants were taken to dryness under reduced pressure to give the
174 phenolic-enriched extracts (PEE).

175

176 **2.4. Preparation of zein fibers through electrospinning**

177 Zein solutions 20% (w/v) were prepared by dissolving the protein in 80% (v/v) ethanol
178 at room temperature under magnetic stirring. PEE (10% w/w of the total solids content)
179 were added to the zein solutions and the mixtures were stirred until complete
180 dissolution. Then, the solutions were electrospun following a procedure adapted from
181 Gómez-Mascaraque, Sanchez, & López-Rubio (2016), using an electrospinning
182 apparatus assembled in-house, equipped with a variable high-voltage 0-30 kV power
183 supply, at a steady flow-rate of 0.15 mL/h, an applied voltage of 11 kV and a syringe
184 tip-collector distance of 10 cm. The zein fibers were directly collected onto the PHA
185 films for 40 min. The amount of electrospun extract loaded zein was calculated by
186 weighing the film before and after the collection of fibers (Fabra, Sánchez, López-
187 Rubio, & Lagaron, 2014).

188

189 **2.5. Zein fibers Post-Treatment**

190 Once the zein fibers were collected onto PHA films, they were exposed to 25% v/v
191 glutaraldehyde (GA) vapors as a crosslinker and to 35% v/v hydrochloric acid (HCl)
192 solution to create the necessary conditions for catalysis (Lee, Li, Chen, & Park, 2016).
193 The crosslinking container was divided into two parts by a grid. On the bottom, a Petri
194 dish was placed with the glutaraldehyde solution and another with the HCl solution. On
195 top, PHA films containing the zein fibers were placed inside Petri dishes. In this way,
196 the films were exposed to the vapors, in the closed container. The crosslinking was
197 performed for 5h (Treatment A) and 24 h (Treatment B). All the crosslinked samples
198 were rinsed for 30 min in phosphate buffer saline (PBS) to eliminate the residual
199 glutaraldehyde and then they were dried in a desiccator at 23 ± 2 °C (Ramires &
200 Milella, 2002).

201 Then, the films were spread between two Teflon layers and compressed in a hot press
202 (Carver 4122, USA) at 45 °C for 2 min. The two hot plates from the press were put into
203 contact at the specified conditions, but no extra pressure was applied. These conditions
204 were sufficient to guarantee the adhesion between the zein coating and the PHA film.

205

206 **2.6. Characterization of films**

207 **2.6.1. Morphological characterization of the particles**

208 Samples were sputter-coated with a gold-palladium mixture under vacuum and analyzed
209 by scanning electron microscopy (SEM) on a Hitachi microscope (Hitachi S-4800),
210 following the method described in Gómez-Mascaraque et al. (2016). Particle diameters
211 were measured from the SEM micrographs using the ImageJ software. Size
212 distributions were obtained from a minimum of 200 measurements.

213

214 **2.6.2. Analysis of the particles by attenuated total reflectance Fourier transform** 215 **infrared spectroscopic (ATR-FTIR) and Fourier transform infrared (FT-IR)**

216 FT-IR spectra were collected following the method described in Gómez-Mascaraque et
217 al. (2016). PEE (ca. 1–2 mg) were dispersed in about 130 mg of potassium bromide and
218 a pellet was formed by compressing at ca. 150 MPa.

219 FT-IR spectra were collected in the transmission mode using a Bruker (Rheinstetten,
220 Germany) FT-IR Tensor 37 equipment. Zein fibers with and without PEE were
221 analyzed without further processing in ATR mode. All spectra were obtained by
222 averaging 10 scans at 1 cm^{-1} resolution.

223

224 **2.6.3. Thermogravimetric analysis (TGA)**

225 Thermogravimetric analysis (TGA) was performed with a TG-STDA Mettler Toledo
226 model TGA/STDA851e/LF/1600 analyzer. The samples (ca. 10 mg) were heated from
227 $25\text{ }^{\circ}\text{C}$ to $600\text{ }^{\circ}\text{C}$ at a heating rate of $10\text{ }^{\circ}\text{C}/\text{min}$ under dynamic nitrogen flow.
228 Thermogravimetric curves express the weight of the sample as a function of
229 temperature.

230

231 **2.7. Fiber stability assessment**

232 Stability of the films containing zein fibers with and without PEE, was assessed using a
233 protocol adapted from Kiechel & Schauer (2013). Briefly, the zein fibers collected on
234 PHA films were cut into pieces of $5\text{ x }5\text{ mm}$. Each piece was immersed in 20 mL of
235 distilled water. After different time intervals, the zein coatings were removed from the
236 tubes and dried in a desiccator (0% relative humidity). The surface morphology of the
237 dried zein coating was analyzed by SEM as described in Section 2.6.1. The test was
238 carried out using films with and without crosslinking and with and without press
239 treatment. All experiments were performed in triplicates.

240

241 **2.8. Phenolic enriched extract encapsulation efficiency**

242 The total amount of each PEE incorporated within the fibers was estimated by UV–Vis
243 spectroscopy according to a protocol adapted from Atay et al. (2018). For this purpose,
244 zein fibers (10 mg/mL) were dissolved in ethanol 80% (v/v). The absorbance at 280 nm
245 was measured using a NanoDrop ND1000 spectrophotometer (Thermo Fisher
246 Scientific, USA). Calibration curves for each PEE in ethanol 80% were previously
247 obtained by its absorbance at 280 nm ($R^2 > 0.997$). The contribution of zein to the
248 absorbance at 280 nm was also considered.

249 The encapsulation efficiency (EE) was then calculated according to Eq. (1). Three
250 independent replicates of each sample were analyzed.

$$251 \text{ EE (\%)} = \frac{\text{Total extract content in the fibers}}{\text{Theoretical extract content in the fibers}} \times 100 \quad (1)$$

252

253 **2.9. *In vitro* release assays**

254 The release of phenolic compounds from the zein fibers was assessed in two different
255 food simulants following a method adapted from Alehosseini, Gómez-Mascaraque,
256 Martínez-Sanz, & López-Rubio (2019). 50% ethanol and 3% acetic acid (w/v) were
257 selected as food simulants, according to the Commission Regulation 10/2011 EU on
258 plastic materials and articles intended to come into contact with food (10/2011/EC). For
259 this analysis, the fibers (20 mg / mL) were immersed in the release medium during
260 different times. The absorbance of each system was measured at 280 nm using a
261 NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, USA), according to
262 an already established methodology (Moreno et al., 2018). With the absorbance values,
263 the concentration of phenolic components released over time was determined using
264 calibration curves (considering that the fibers contained 10% extract) of each PEE in
265 both media ($R^2 > 0.9975$).

266 The contribution of zein to the absorbance at 280 nm was also considered and
267 subtracted from the total absorbance of the release medium. Experiments were
268 performed at room temperature (20 °C) in independent duplicates.

269

270 **2.10. High performance liquid chromatography (HPLC) analysis**

271 The phenolic compounds of each extract and the phenolic components released from
272 zein fibers (2 mg DW/mL) were identified by HPLC-DAD. The HPLC system

273 consisting of a Waters 1525 Binary HPLC Pumps system with a 1500 Series Column
274 Heater, a manual injection valve with a 20 μ L loop (Rheodyne Inc., Cotati, CA) and a
275 Waters 2998 photodiode array detector (PDA) were used to analyze the extracts eluted
276 from the zein fibers and the individual extracts. An XbridgeTM 135 C18 column (4.6 x
277 150 mm, 5 μ m; Waters Corporation, Milford, MA). The solvent system for the
278 separation of components was composed of 0.1% acetic acid in water (A) and 0.1%
279 methanol in acetic acid as follow: 10% to 57% B over 45 min and increasing to 100% B
280 at 60 min. The flow rate was 0.5 mL/min and the volume injected was 20 μ L. The
281 compounds were monitored at 254 nm.

282 The identification of phenolic compounds was carried out by comparing the retention
283 times and spectral data (220–600 nm) of each peak with those of standards from Sigma-
284 Aldrich (MO, USA) and Fluka Chemical Corp. (USA). Polyphenols quantification was
285 based on external calibration curves from available phenolic standards. Plots were built
286 by comparison of the area and concentration in the range of 1-500 ppm. Results were
287 expressed as μ g equivalents of the standard compounds per milligram of dry weight.
288 Experiments were performed in independent triplicates.

289

290 **2.11.1. Antioxidant activities**

291 In order to determine if the encapsulated extracts in fibers retained antioxidant activity,
292 ABTS and AAPH assays were performed. For these tests, equilibrium concentrations of
293 phenolic compounds previously eluted from the zein fibers (between 0.2 and 20 μ g
294 GAE/mL) were used.

295

296 **2.11.1.1. Total antioxidant capacity assay:** The antioxidant power of the extracts loaded
297 fibers was assayed by the improved ABTS radical cation (ABTS^{•+}) method as described
298 by Re et al. (1999). Results were expressed as SC₅₀ (concentration of extract necessary
299 to scavenge 50% of ABTS radical). In all cases, the antioxidant capacity of zein was
300 considered.

301

302 **2.11.1.2. Protection of oxidative hemolysis assay:** The method reported by Mendes, de
303 Freitas, Baptista, & Carvalho (2011) was used to determine the protective activity of
304 oxidative hemolysis of red blood cells (RBC) using AAPH as reagent generator of
305 peroxy radicals. The reaction mixture (RBC, APPH and phenolic compounds

306 previously eluted from the zein fibers) was incubated at 37°C for 1 h in water bath, and
307 then was centrifuged (4000xg) for 3 min. The absorbance of supernatant was measured
308 at 545 nm in spectrophotometer, and the percent of oxidative hemolysis was calculated.
309 IC₅₀ values were determined as the concentration of extracts necessary to protect the
310 RBC from oxidative hemolysis by 50%. The antioxidant capacity of zein was also
311 determined.

312

313 **2.12. Statistical analysis**

314 Each experimental value is expressed as the mean ± standard deviation (SD). The
315 statistical analysis of experimental data was performed using InfoStat software (Student
316 Version, 2011). The one-way ANOVA with Tukey post-test at a confidence level of
317 95% was used to evaluate the significance of differences between groups. The criterion
318 of statistical significance was taken as $p \leq 0.05$.

319

320 **3. Results and discussion**

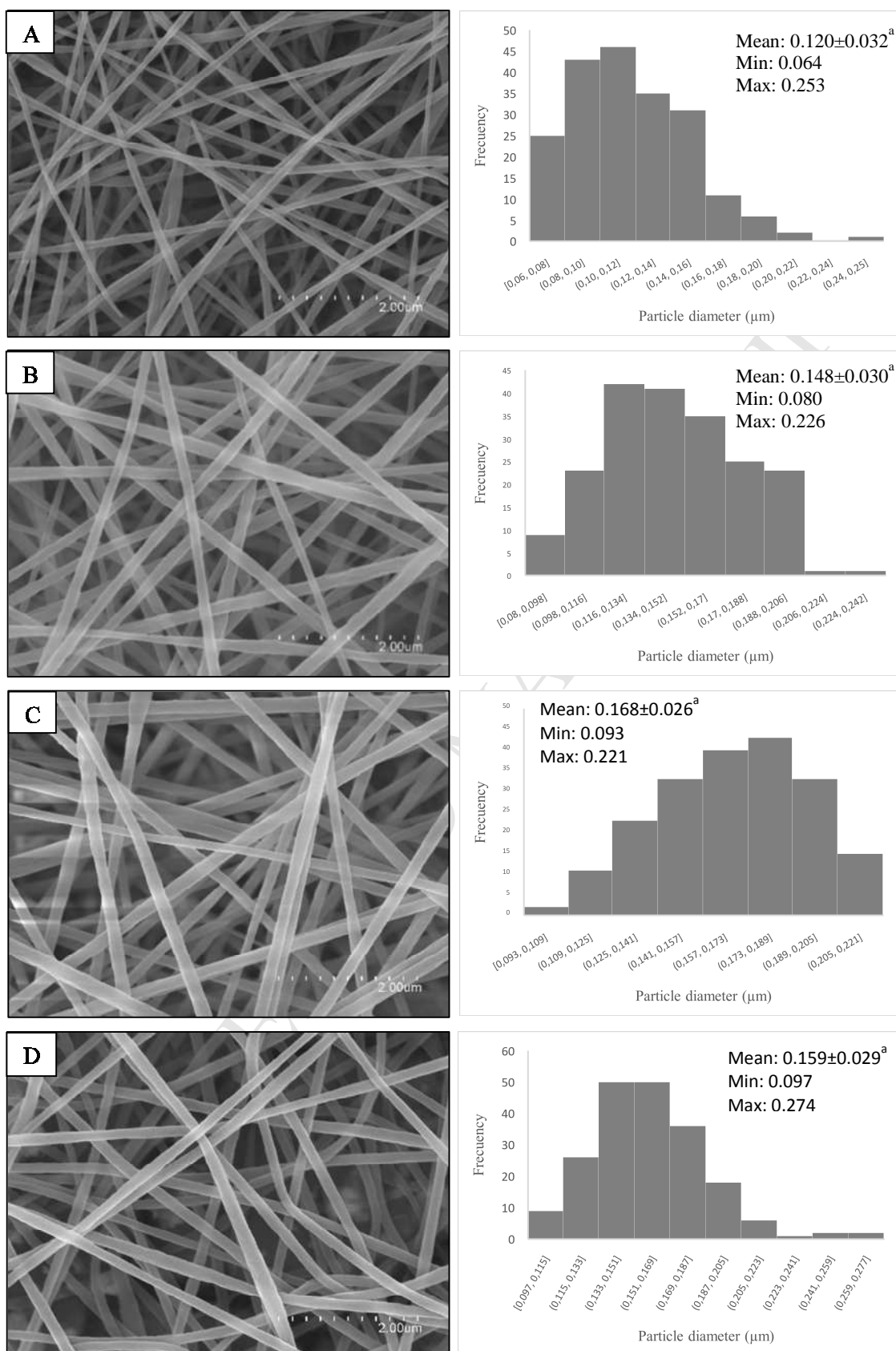
321 The aim of this work was to develop bioactive food packaging coatings through
322 electrospinning containing PEE of different parts from orange chilito fruits. The low
323 compatibility of hydrocolloids like zein and hydrophobic materials such as
324 polyhydroxyalcanoates (PHAs) used in this work, prevented the use of casting methods
325 to develop multilayer systems or to combine both materials through compression
326 moulding, since adhesion between the layers was very poor and partial or even complete
327 delamination between the different layers occurred, as previously reported for similar
328 systems (Martínez-Sanz, López-Rubio, & Lagaron, 2013; Fabra, López-Rubio, &
329 Lagaron, 2016). Thus, in this work, the polyphenolic extract of pulp and even of waste
330 material such as seed, and skin were used. These extracts mainly contained caffeoyl
331 derivatives, RosA and its derivatives and showed several functional properties principally
332 antioxidant, and antimicrobial that could be useful for active food packaging
333 applications (Orqueda et al., 2017). A preliminary optimization of the electrospinning
334 parameters was carried out in order to select the best conditions for zein fibers
335 production. Subsequently, the chilito PEE loaded zein fibers were produced and
336 characterized.

337

338 **3.1. Characterization of films**

339 **3.1.1. Morphological characterization of the fibers**

340 The SEM images and particle size distribution graphs of zein fibers (with and without
341 PEE from seed, pulp and skin of orange chilito) are displayed in **Fig. 1**. This figure
342 shows the average, maximum and minimum diameter values for the different
343 electrospun fibers (μm). The diameter of the zein fibers without extracts was similar to
344 those previously reported for electrospun zein fibers (Alehosseini et al., 2019; Li, Lim,
345 & Kakuda, 2009; Neo et al., 2013), showing an ultrathin fibrillar structure, of
346 cylindrical shape and homogeneous morphology (**Fig. 1**). No significant differences
347 were observed in the average diameter between the extract-free zein fibers and those
348 loaded with PEE. In all cases, the fiber diameters were between 0.064-0.274 μm . The
349 obtained medium values were similar for all the developed structures, independently of
350 the type of extract encapsulated.



351

352 **Figure 1.** SEM images and particle size distributions graphs of zein fibers containing353 phenolic enriched extracts from: **A.** Orange chilito seeds; **B.** Orange chilito pulp; **C.**354 Orange chilito skin; and **D.** without extract.

355

356 **3.1.2. Analysis of the fibers by FT-IR**

357 FT-IR analysis was performed to evaluate the interactions between zein and the
358 phenolic compounds from chilto PEE. **Fig. 2** shows, as an example, the FT-IR spectra
359 of the zein fibers containing chilto seed extract together with the spectrum of the zein
360 fibers without extracts, analyzed in ATR mode. The FT-IR spectrum of the chilto seed
361 extract is also shown in **Fig. 2**. The spectra of the pulp and skin extracts and zein fibers
362 with and without extracts are included in the **Supplementary Material (Fig. S1)**.
363 Typical zein bands were present in the different fiber coatings at 3290, 2950, 1640,
364 1530, 1440 and 1245 cm^{-1} that are derived from Amide A (N-H stretching vibration),
365 Amide B (asymmetric stretching vibration of $=\text{C}-\text{H}$ and $-\text{NH}_3^+$), Amide I band (80%
366 $\text{C}=\text{O}$ stretching, 10% $\text{C}-\text{N}$ stretching), Amide II band (60% N-H bending, 30% $\text{C}-\text{N}$
367 stretching and 10% $\text{C}-\text{C}$ stretching), and Amide III band (complex band resulting from
368 several coordinate displacements), respectively (Alehosseini et al., 2019; Costamagna et
369 al., 2017; Deng, Kang, Liu, Feng, & Zhang, 2018; Sivam, Sun-Waterhouse, Perera, &
370 Waterhouse, 2012).

371 The spectra of the PEE were characterized by the presence of absorption bands at 3325-
372 3395 cm^{-1} , 2920 cm^{-1} , 1740-1700 cm^{-1} and 1600–800 cm^{-1} region attributed to the
373 stretching, bending and deformation vibrations of polyphenolic compounds (Gannasin,
374 Adzahan, Hamzah, Mustafa, & Muhammad, 2015; Moreno et al., 2018; Sivam et al.,
375 2012). The peak at 1710 cm^{-1} is assigned to the stretching vibration of carbonyl groups
376 (Stehfest, Boese, Kerns, Piry, & Wilhelm, 2004). The peaks at 1612 cm^{-1} , 1520 cm^{-1}
377 and 1463 cm^{-1} are ascribed to benzene ring stretching vibrations (Stehfest, et al., 2004).
378 The spectral vibrations of the PEE loaded zein fibers and unloaded zein fibers showed
379 small spectral variations. For instance, a displacement to greater wavenumbers (~ 10 -30
380 cm^{-1}) was observed in the Amide I and Amide II bands. In addition, a slight narrowing
381 in the Amide A band was observed, which is generally due to changes in the hydrogen
382 bonding structure of the protein (Doyle, Bendit, & Blout, 1975). These changes could
383 be attributed to possible intermolecular interactions between the zein and the hydroxyl
384 groups of rosmarinic acid and caffeic acid and its derivatives present in the chilto
385 extracts or between free carboxyl group of phenolic compounds with the free ϵ -amino
386 groups from the protein to form amide bands.

387 **Fig. 3** also shows a magnification of the regions of interest in which the arrows point
388 out to the spectral differences. Moreover, a characteristic absorption peak of the extract

389 can be observed at 1050 cm^{-1} (see arrow), which confirms its incorporation into the zein
 390 fibers.

391

392

393

394

395

396

397

398

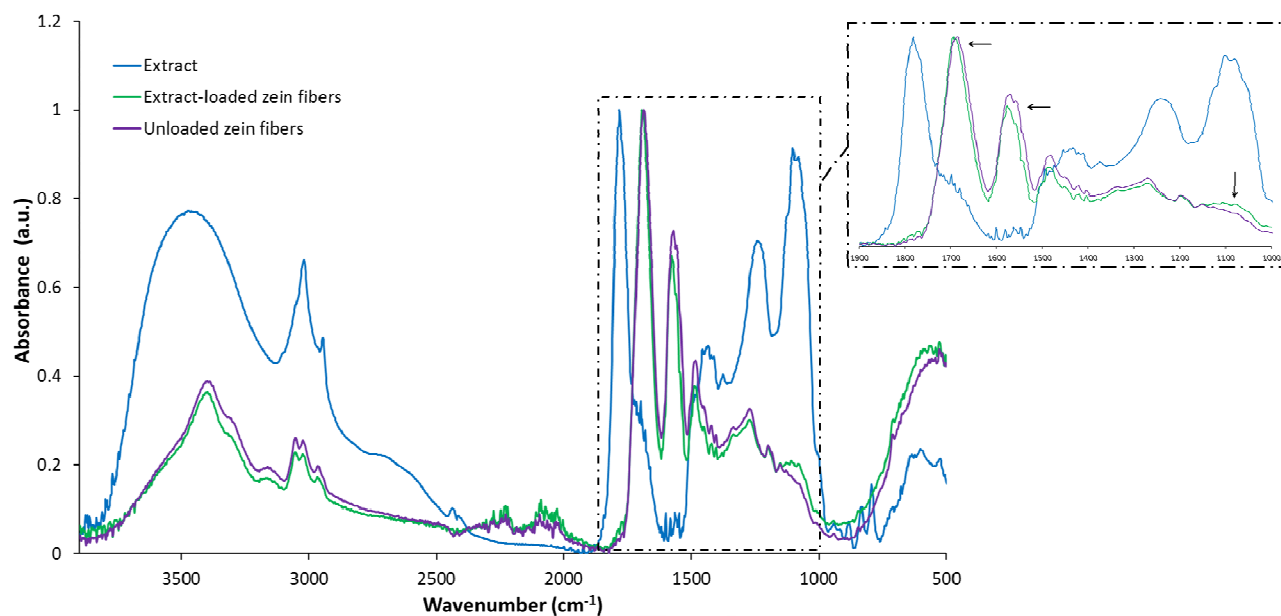
399

400

401

402

403



404

Figure 2. FT-IR spectra of chilto seeds extract, together with unloaded and extract-
 405 loaded zein fibers. Arrows in the magnified spectral range point out to the main spectral
 406 changes as a consequence of extract incorporation.

407

408

3.1.3. Thermal stability of PEE-loaded zein fibers

409

The thermal stability of the extracts-loaded zein fibers was also determined. **Fig. 3**
 410 shows, as an example, the thermogravimetric curves obtained for PEE from orange
 411 chilto skin and those for zein fibers with and without PEE.

412

The zein fibers were stable at 45°C , temperature used in the heat press treatment. An
 413 initial weight loss of 2-4% was observed in the zein fibers between 60° and 150°C ,
 414 probably due to water evaporation. The main mass loss of zein fibers took place around
 415 300°C , which could be ascribed to the thermal degradation of the polymer structure
 416 (Erdogan, Demir, & Bayraktar, 2015), only retaining around 17% of the initial mass.

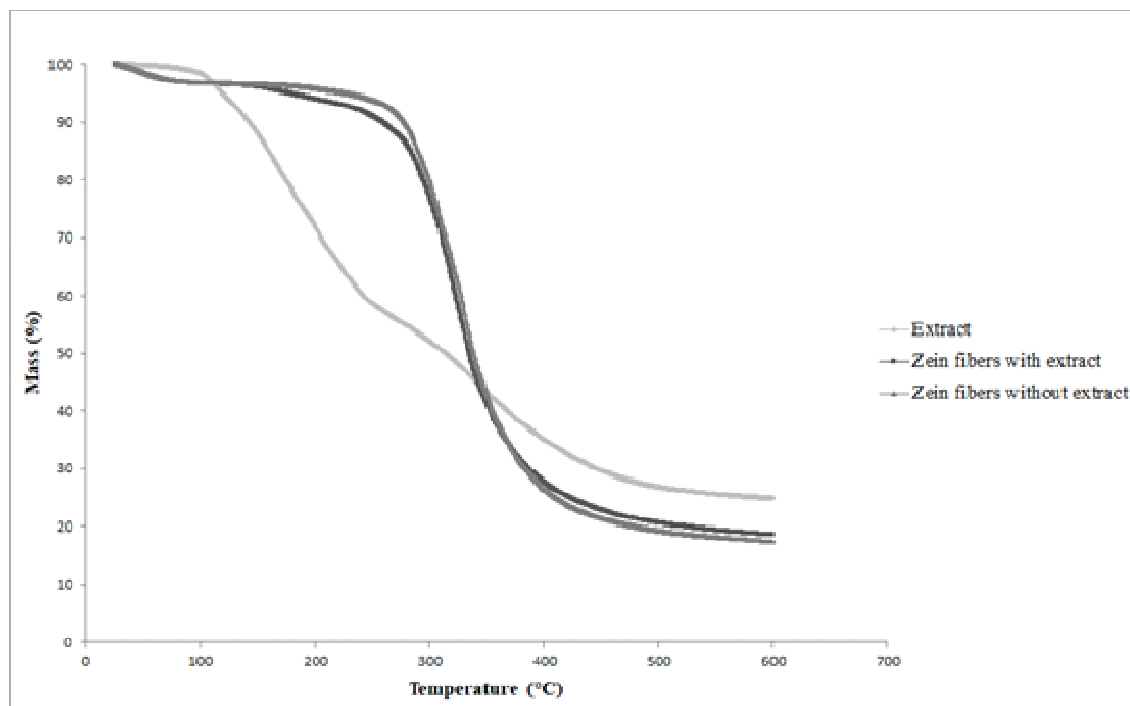
417

The thermal degradation of the PEE from the chilto skin started at a lower temperature
 418 and showed a more gradual mass loss in the temperature range from $\sim 100^\circ\text{C}$ to 450°C .

419

When comparing the thermal stability of the electropun fibers containing the three
 420 different extracts from chilto, with that from the unloaded zein fibers, a very similar
 421 degradation profile was seen, which normally is ascribed to a thermal stabilization of

422 the encapsulated compounds. However, taking into account the small contribution of the
 423 extracts to the total mass of material, from **Figure 3** (as a representative example), it can
 424 only be stated that the incorporation of the PEE did not affect the thermal stability of the
 425 zein fibers.



426
 427 **Figure 3.** Thermogravimetric curves of PEE orange chulto skin and zein fibers with and
 428 without PEE.

429 430 **3.2. Water resistance properties of the zein fibers**

431 As most food products contain relatively high water contents, the integrity of the
 432 packaging coating upon water exposure was evaluated. The surface morphology of the
 433 coated PHA films was thus analyzed by SEM and the effect of crosslinking on the water
 434 resistance of the coatings was evaluated. The changes in the surface morphology at
 435 different water immersion time periods, are shown in **Fig. 4** for zein fibers containing
 436 orange chulto skin extract as an example.

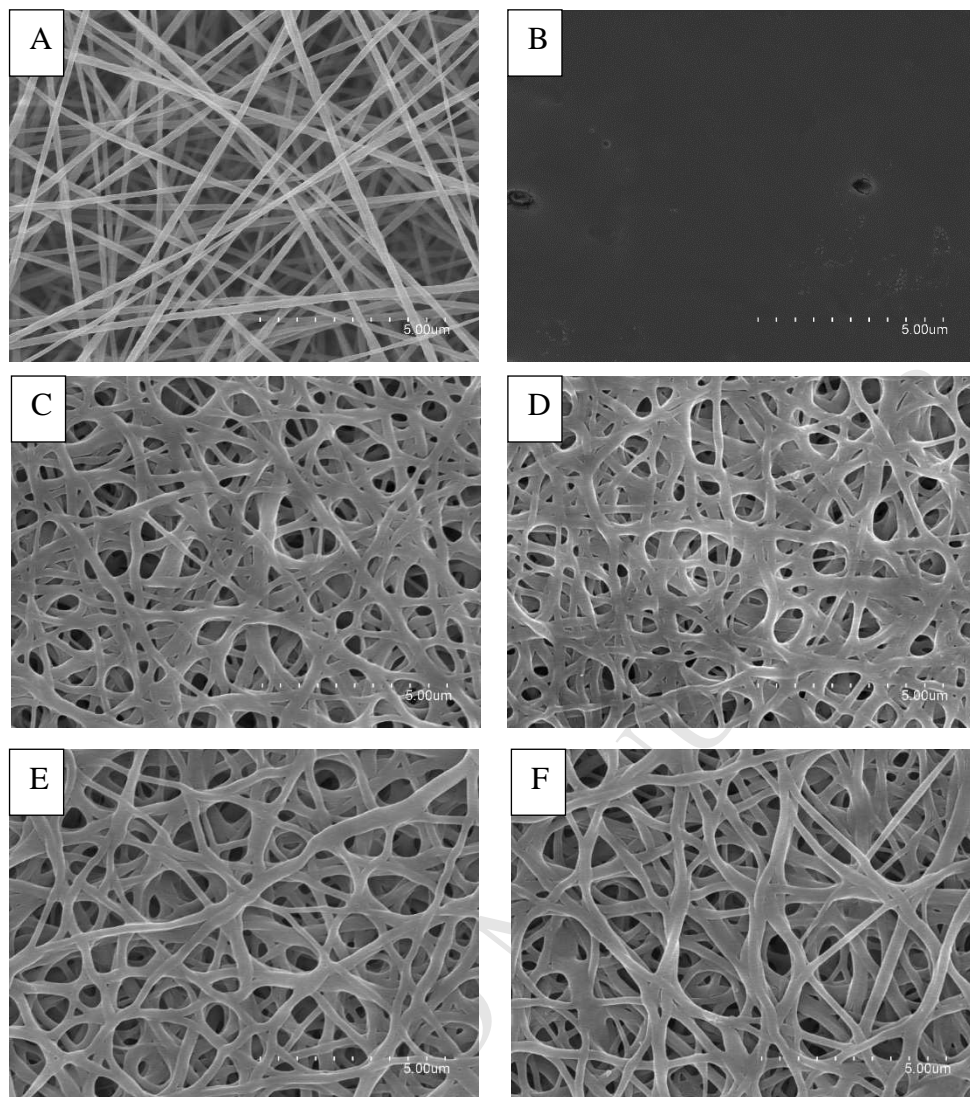
437 When no crosslinking was applied, the fibrillar morphology of the electrospun zein
 438 coatings was completely lost after 6 h of immersion in water (**Fig 4A** and **Fig 4B**)
 439 similarly as previously reported by other authors (Jiang et al., 2010; Li et al., 2009). The
 440 fibers collapsed in the water and were released from the PHA base film.

441 In contrast, a better stability was observed for the coatings crosslinked using
 442 glutaraldehyde vapors in an acid environment. Glutaraldehyde has been previously used
 443 as a crosslinking reagent to produce electrospun zein fibers with improved physical

444 properties and solvent resistance (Selling, Woods, Sessa, & Biswas, 2008) and for the
445 modification of biodegradable films (Shahbazi et al., 2016), demonstrating that the
446 films crosslinked by this method did not display any cytotoxic effects on fibroblast
447 cells. Ramirez & Milella (2002) evaluated the harmful effects of glutaraldehyde residues
448 released from crosslinked membranes through *in-vitro* cytotoxicity and
449 cytocompatibility tests, showing that these do not have toxic effects. **Fig. 4C** and **4E**
450 show the morphology of the fibers after the crosslinking treatment A and B,
451 respectively, before water immersion, while **Fig. 4D** and **4F** show the same materials
452 after 24 h immersion in water. It can be observed that while the shorter treatment A led
453 to a certain degree of swelling after this immersion time, the fibers crosslinked for a
454 longer period (treatment B), kept the integrity without significant morphological
455 changes after being 24h in water (**Fig. 4F**). For this reason, treatment B was selected to
456 check how the integrity of the fibers upon water immersion was kept at longer time
457 periods (**Fig. S2, Supplementary Material**). It could be seen that the crosslinking
458 treatment was very effective in protecting the integrity of the coating even after 12 days
459 of immersing the coated PHA film in water, although from day 9 a significant swelling
460 occurred which led to a partial collapse of the fibers.

461 Glutaraldehyde interacts with amino, carboxy, amido and other protein groups
462 (Jayakrishnan, & Jameela, 1996). These bonds formed during crosslinking should be the
463 main responsible for the best water stability of the crosslinked samples. In addition,
464 crosslinking reduces the interstitial spaces between the protein chains, thus decreasing
465 molecular motion and preventing swelling of the fibers (Fan et al., 2018; Jiang et al.,
466 2010).

467 The cross-sections of the coated films were also analysed. **Fig. 5A** shows, as an
468 example, the cross-section of coated PHA films, in this case with the zein fibers
469 containing the chilo skin extract. A clear delamination of the coating was observed and,
470 thus, a hot press treatment was applied to improve adhesion of the coating to the PHA
471 base film. This treatment effectively improved the adhesion of the fibers to the PHA
472 film, being even better when the fibers were previously crosslinked with treatments A
473 and B (**Figs. 5B, 5C and 5D**).



474

475

476 **Figure 4.** SEM micrographs of electrospun coatings containing orange chilito skin

477 extract after immersion in water for different time periods: As-obtained fibers without

478 crosslinking, before (A) and after immersion in water during 6 h (B); Fibers crosslinked

479 for 5 h, before (C) and after immersion in water during 24 h (D.); Fibers crosslinked for

480

481

482

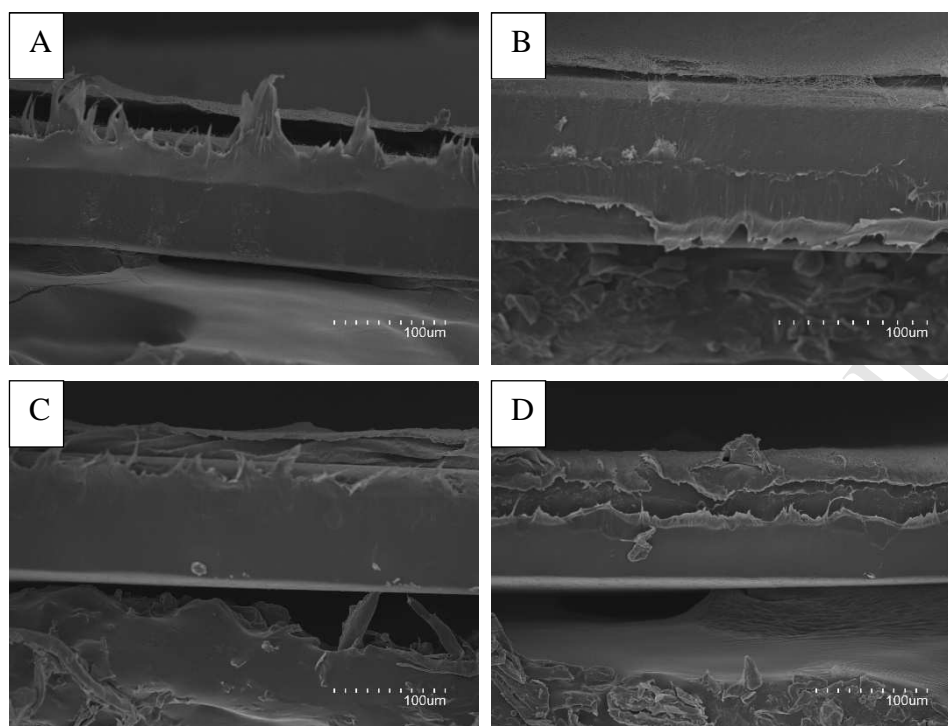
483

484

485

486

487



489

490

491 **Figure 5.** SEM Cross-section images of PHA films with the zein fiber coatings
492 containing the chulto skin extract: **A.** Non-crosslinked coating without hot press
493 treatment (delamination is clearly seen); **B.** Non-crosslinked coating after hot press
494 treatment (better adhesion); **C.** Coating crosslinked for 5 h with hot press treatment; **D.**
495 Coating crosslinked for 24 h with hot press treatment.

496

497 **3.3. Extracts encapsulation efficiency**

498 In order to estimate the amount of each extract effectively incorporated within the
499 developed coatings, the encapsulation efficiency was calculated according to Eq. (1)
500 and the results are summarized in **Table 1**.

501 All extracts showed similar encapsulation efficiencies (90.2 - 94.3%). These values
502 were considerably high and similar to those previously reported for the
503 microencapsulation of other natural compounds through electrohydrodynamic
504 processing, using zein as an encapsulation matrix (Alehosseini et al., 2019; Fabra et al.,
505 2016; Neo et al., 2013; Wang et al., 2017). The high encapsulation efficiency values
506 obtained highlight the interest of the electrospinning technique for encapsulation
507 purposes, not only derived from the high efficiency obtained, but also to additional
508 advantages, such as the use of aqueous solutions and mild conditions (not requiring high
509 temperatures for drying out the structures obtained). In this specific work, it is also

510 demonstrated that these type of structures can themselves constitute an alternative form
 511 of delivering bioactive compounds to foods in the form of inner coatings of packaging
 512 materials.

513

514 **Table 1.** Extracts encapsulation efficiency (%) for the developed coatings.

		Zein fibers
<i>Solanum betaceum</i>	Seeds	90.2±6.1 ^a
Cav. (orange)	Pulp	94.3±4.2 ^a
	Skin	92.4±4.4 ^a

515

516 Values are reported as mean ± standard deviation of triplicates. The values in the same
 517 column with a common letter are not significantly different according to Tukey's test (p
 518 ≤ 0.05).

519

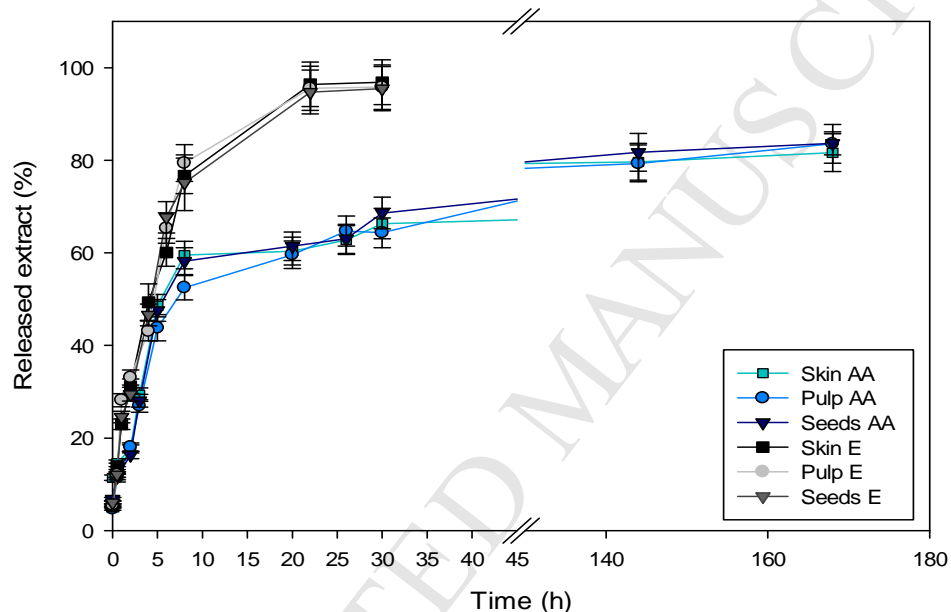
520 **3.4. Release of PEE from the electrospun coatings**

521 The objective of the developed coatings, as already mentioned, was to gradually release
 522 the extracts to the actual food product from the packaging structure. Therefore, the
 523 diffusion of the encapsulated bioactive compounds from the zein coatings into 50%
 524 ethanol and 3% acetic acid solutions used as food simulants was evaluated. The aqueous
 525 ethanolic solution can be used to simulate fatty food products, foods capable of
 526 extracting lipophilic substances, as well as alcoholic foods with an alcoholic strength
 527 greater than 20% or oil- in-water emulsions. On the other hand, 3% acetic acid is
 528 normally used as a simulant of hydrophilic foods or foods capable of extracting
 529 hydrophilic substances, with pH less than 4.5. Both solvents can simulate food type
 530 turbid drinks, juices, nectars, canned meats, yogurt, cream, cheese, etc (10/2011 / CE).

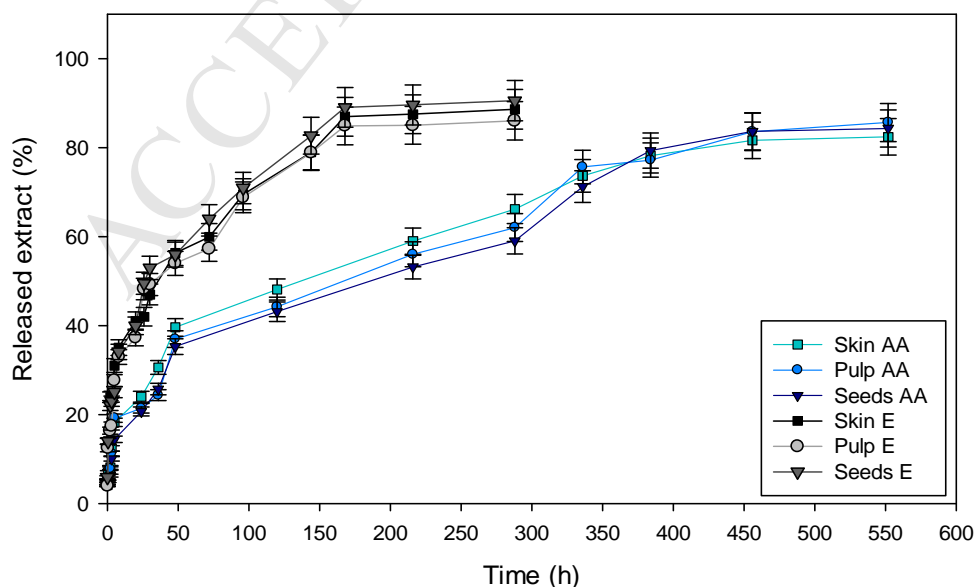
531 Given the previous results obtained, only the crosslinked materials were used for the
 532 release experiments. **Fig. 6** shows the release profiles obtained for the different
 533 formulations in both food simulants. The release was greater and faster with 50%
 534 ethanol, being superior to 90% after 24 h in the case of the coating crosslinked for lower
 535 time period (treatment A, **Fig. 6A**), and over 80% after 7 days of experiment for the
 536 different coatings crosslinked for 24h (treatment B, **Fig. 6B**). When 3% acetic acid was
 537 used as a release medium, a release higher than 80% was obtained for the crosslinking

538 treatment A after 7 days (**Fig. 6A**), while for treatment B, release values over 70% were
 539 obtained after 14 days, reaching 80-86% after 23 days of experiment (**Fig. 6B**).
 540 The longer crosslinking (treatment B) effectively delayed the release of the extracts in
 541 both simulating solvents, which is desirable for the intended application. The release of
 542 phenolic compounds in ethanol 50% is faster because in this medium both the zein and
 543 the extracts are more soluble and thus, a partial dissolution of the fibers is expected,
 544 thus promoting a greater and quicker release. In contrast, only swelling took place when
 545 using 3 % acetic acid, thus allowing a slower extract release.

546 **A**



564 **B**



580 **Figure 6.** Release of PEE from the electrospun fibers after crosslinking during 5 h (A)
581 and during 24 h (B) in 50% ethanol (E) and 3% acetic acid (AA).

582

583 **3.5. HPLC analysis**

584 HPLC-DAD was used to analyze the individual phenolic compounds released from the
585 fibers, as well as to evaluate the recovery of phenolic compounds after the release
586 experiments. The chromatography profile of each of them was similar to each extract
587 used in this study.

588 **Fig. 3 (Supplementary Material)** shows the profile obtained for the eluate from the
589 orange chilto skin fibers with the crosslinking treatment B, after 7 days of release in
590 50% ethanol. Peaks 1 and 2 were identified as 3-caffeoyl quinic acid and rosmarinic
591 acid hexoside derivative, respectively. Previously, Orqueda et al. (2017) showed that the
592 main phenolic compounds found in the chilto extracts were RosA and caffeic acid
593 derivatives.

594 Under our experimental conditions, the percent recovery of the compounds 3-
595 caffeoylquinic acid and rosmarinic acid hexoside derivative was 98 and 54%,
596 respectively in 50% ethanol and 78% and 43%, respectively in 3% acetic acid (**Fig. 3,**
597 **Supplementary Material**). Orqueda et al. (2017) reported for the orange chilto skin
598 extract a content of 1724.1 ± 80.7 and 871.8 ± 3.5 mg / 100 g of dry weight of 3-
599 caffeoylquinic acid and rosmarinic acid, respectively. Previously, Espin et al. (2016)
600 reported values between 25.04 and 163.62 mg / 100 g of dry weight for 3-O-
601 caffeoylquinic acid (CaQA) and between 12.22 and 121.89 mg / 100 g of dry weight for
602 RosA, respectively, for samples of chilto from Ecuador. CaQA and RosA and its
603 derivatives, are characterized as natural antioxidants and potential natural anti-diabetic
604 and anti-obesity compounds, because they are glucosidase and amylase inhibitors
605 (McCue & Shetty, 2004; Chen et al., 2014; Ngo et al., 2018; Gonçalves et al., 2019) and
606 lipase inhibitors (Mohamed, 2014). Therefore, it is also feasible that the effect of chilto
607 extracts on enzyme related to metabolic syndrome could be attributed to CaQA and
608 RosA and its derivatives present in the extracts (Orqueda et al., 2017).

609

610 **3.6. Antioxidant activity of extracts released from PEE loaded zein fibers**

611 The antioxidant activity of orange chilto seed, skin and pulp extracts in cell free systems
612 was previously reported (Orqueda et al., 2017). In this work, the antioxidant capacity of
613 PEE was compared with the activity of the phenolics released from the fiber coatings.

614 The released phenolics were able not only to reduce ABTS but also to prevent and limit
 615 the release of lysosomal enzymes from human red blood cell to the extracellular matrix.
 616 In agreement with the results obtained for the non-encapsulated extracts, the antioxidant
 617 capacity of the phenolic compounds from skin released from the coatings was higher
 618 than that from the coatings containing seeds and pulp extracts (**Table 2**). In general, no
 619 significant differences were observed between the antioxidant activity of free and
 620 released extracts, thus confirming that encapsulation through electrospinning does not
 621 affect the bioactivity of the compounds.

622 **Table 2.** Antioxidant activity of *S. betaceum* polyphenolic extracts before and after
 623 encapsulating procedure.

Sample		SC ₅₀ (µgGAE/mL)	IC ₅₀ (µgGAE/mL)
<i>S. betaceum</i>		ABTS	AAPH
Seeds	PEE	1.38±0.05 ^a	0.91 ± 0.05 ^a
	PEE loaded fiber	2.69±0.01 ^b	1.12±0.05 ^a
Pulp	PEE	1.09±0.10 ^A	0.40 ± 0.03 ^A
	PEE loaded fiber	0.90±0.02 ^A	0.53±0.01 ^A
Skin	PEE	0.80±0.10 ^{II}	0.50 ± 0.00 ^I
	PEE loaded fiber	0.49±0.002 ^I	0.78±0.02 ^I

624

625 SC₅₀: Concentration of polyphenolic extract necessary to scavenge 50% of ABTS.

626 IC₅₀: Concentration of polyphenolic extract necessary to inhibit 50% of oxidative hemolysis. Different
 627 letters or number in the same column for each extract indicated significant differences in the antioxidant
 628 activity according to Tukey's test ($p \leq 0.05$).

629

630 4. Conclusions

631 In this work, bioactive coatings consisting on zein fibers with orange chilito PEE were
 632 collected onto PHA films through the electrospinning technique, with encapsulation
 633 efficiencies greater than 90%.

634 Zein electrospun nanofiber coatings showed poor mechanical properties and stability in
 635 aqueous environments, which were improved by crosslinking with glutaraldehyde
 636 vapors.

637 The crosslinked zein fibers showed better morphological stability and were able to
638 maintain their fibrous structure after immersion in water for more than 12 days.
639 In addition, the crosslinking of the zein fibers for 24h (treatment B), allowed for a
640 gradual release of the encapsulated extract in two different food simulants, showing a
641 slower and more limited release in 3% acetic acid, with a total polyphenolic release of
642 80-86% after 23 days. Given the similar composition of the three different extracts
643 evaluated, they all showed similar release behavior, and, thus, the proven health benefits
644 ascribed to chilto fruits (skin, pulp and seeds) can be exploited for inclusion in bioactive
645 packaging structures. This work has demonstrated that it is possible to develop bioactive
646 coating structures based on zein fibers containing chilto PEE, which could result in
647 added value applications of these fruits that are currently commercially underexploited,
648 as well as in the valorization of the skin and the seeds from these fruits that are usually
649 discarded. These coatings can be optimized for packaging structures in contact with
650 more hydrophilic or lipophilic food products.

651

652 **Acknowledgements**

653 This research was supported by grants from Secretaría de Ciencia, Arte e Innovación
654 Tecnológica de la Universidad Nacional de Tucumán, Argentina (SCAIT-UNT),
655 Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina (CONICET), the
656 Spanish MINECO project AGL2015-63855-C2-1, Agencia Nacional de Promoción
657 Científica y Tecnológica (ANPCyT), ARCOR Award to Technological Innovation
658 (Argentina) in Edition 2015 and EMHE-CSIC (Grant MHE-200038). The authors thank
659 the Central Support Service for Experimental Research (SCSIE) of the University of
660 Valencia for the electronic microscopy service.

661

662 **References**

- 663 Alehosseini, A., Gómez-Mascaraque, L. G., Martínez-Sanz, M., & López-Rubio, A.
664 (2019). Electrospun curcumin-loaded protein nanofiber mats as active/bioactive
665 coatings for food packaging applications. *Food Hydrocolloids*, 87, 487–496.
- 666 Atay, E., Fabra, M. J., Martínez-Sanz, M., Gómez-Mascaraque, L. G., Altan, A., &
667 Lopez-Rubio, A. (2018). Development and characterization of chitosan/gelatin
668 electrosprayed microparticles as food grade delivery vehicles for anthocyanin
669 extracts. *Food Hydrocolloids*, 77, 699–710.

- 670 Chen, J., Mangelinck, S., Ma, L., Wang, Z., Li, W., & De Kimpe, N. (2014).
671 Caffeoylquinic acid derivatives isolated from the aerial parts of *Gynura divaricata*
672 and their yeast α -glucosidase and PTP1B inhibitory activity. *Fitoterapia*, *99*, 1–6.
- 673 Costamagna, M. S., Gómez-Mascaraque, L. G., Zampini, I. C., Alberto, M. R., Pérez, J.,
674 López-Rubio, A., & Isla, M. I. (2017). Microencapsulated chañar phenolics: A
675 potential ingredient for functional foods development. *Journal of Functional Foods*,
676 *37*, 523–530.
- 677 Deng, L., Kang, X., Liu, Y., Feng, F., & Zhang, H. (2018). Characterization of
678 gelatin/zein films fabricated by electrospinning vs solvent casting. *Food*
679 *Hydrocolloids*, *74*, 324–332.
- 680 Destaye, A. G., Lin, C.-K., & Lee, C.-K. (2013). Glutaraldehyde vapor cross-linked
681 nanofibrous PVA mat with in situ formed silver nanoparticles. *ACS Applied*
682 *Materials & Interfaces*, *5*(11), 4745–4752.
- 683 Doyle, B. B., Bendit, E. G., & Blout, E. R. (1975). Infrared spectroscopy of collagen
684 and collagen-like polypeptides. *Biopolymers*, *14*(5), 937–95.
- 685 Erdogan, I., Demir, M., & Bayraktar, O. (2015). Olive leaf extract as a crosslinking
686 agent for the preparation of electrospun zein fibers. *Journal of Applied Polymer*
687 *Science*, *132*, 41338.
- 688 Espin, S., Gonzalez-Manzano, S., Taco, V., Poveda, C., Ayuda-Durán, B., Gonzalez-
689 Paramas, A. M., Santos-Buelga, C. (2016). Phenolic composition and antioxidant
690 capacity of yellow and purple-red Ecuadorian cultivars of tree tomato (*Solanum*
691 *betaceum* Cav.). *Food Chemistry*, *194*, 1073–1080.
- 692 Fabra, M. J., López-Rubio, A., & Lagaron, J. M. (2016). Use of the
693 electrohydrodynamic process to develop active/bioactive bilayer films for food
694 packaging applications. *Food Hydrocolloids*, *55*, 11–18.
- 695 Fabra, M. J., Sánchez, G., López-Rubio, A., & Lagaron, J. M. (2014). Microbiological
696 and ageing performance of polyhydroxyalkanoate-based multilayer structures of
697 interest in food packaging. *LWT - Food Science and Technology*, *59*(2), 760–767.
- 698 Fan, H. Y., Duquette, D., Dumont, M.-J., & Simpson, B. K. (2018). Salmon skin
699 gelatin-corn zein composite films produced via crosslinking with glutaraldehyde:
700 Optimization using response surface methodology and characterization. *International*
701 *Journal of Biological Macromolecules*, *120*(Pt A), 263–273.

- 702 Gannasin, S. P., Adzahan, N. M., Hamzah, M. Y., Mustafa, S., & Muhammad, K.
703 (2015). Physicochemical properties of tamarillo (*Solanum betaceum* Cav.)
704 hydrocolloid fractions. *Food Chemistry*, *182*, 292–301.
- 705 Gómez-Mascaraque, L. G., Sanchez, G., & López-Rubio, A. (2016). Impact of
706 molecular weight on the formation of electrosprayed chitosan microcapsules as
707 delivery vehicles for bioactive compounds. *Carbohydrate polymers*, *150*(Supplement
708 C), 121–130.
- 709 Gómez-Mascaraque, L. G., Hernández-Rojas, M., Tarancón, P., Tenon, M., Feuillère,
710 N., Vélez Ruiz, J. F., Fiszman, S., & López-Rubio, A. (2017). Impact of
711 microencapsulation within electrosprayed proteins on the formulation of green tea
712 extract-enriched biscuits. *LWT-Food Science and Technology*, *81*, 77–86.
- 713 Gómez-Mascaraque, L. G., Pérez-Masiá, R., González-Barrio, R., Periago, M. J., &
714 López-Rubio, A. (2017). Potential of microencapsulation through emulsion-
715 electrospraying to improve the bioaccessibility of β -carotene. *Food Hydrocolloids*, *73*,
716 1–12.
- 717 Gómez-Mascaraque, L. G., Tordera, F., Fabra, M. J., Martínez-Sanz, M., & López-
718 Rubio A. (2019). Coaxial electrospraying of biopolymers as a strategy to improve
719 protection of bioactive food ingredients. *Innovative Food Science & Emerging*
720 *Technologies*, *51*, 2–11.
- 721 Gonçalves, G. A., Corrêa, R. C. G., Barros, L., Dias, M. I., Calhelha, R. C., Correa, V.
722 G., Bracht, A., Peralta, R. M., Ferreira, I. C. F. R. (2019). Effects of in vitro
723 gastrointestinal digestion and colonic fermentation on a rosemary (*Rosmarinus*
724 *officinalis* L) extract rich in rosmarinic acid. *Food Chemistry*, *271*, 393–400.
- 725 Huang, G. P., Shanmugasundaram, S., Masih, P., Pandya, D., Amara, S., Collins, G., &
726 Arinzeh, T. L. (2014). An investigation of common crosslinking agents on the
727 stability of electrospun collagen scaffolds. *Journal of Biomedical Materials Research*
728 *Part A*, *103*(2), 762–771.
- 729 Jayakrishnan, A., & Jameela, S. R. (1996). Glutaraldehyde as a fixative in bioprotheses
730 and drug delivery matrices. *Biomaterials*, *17*(5), 471–484.
- 731 Jiang, Q., Reddy, N., & Yang, Y. (2010). Cytocompatible cross-linking of electrospun
732 zein fibers for the development of water-stable tissue engineering scaffolds. *Acta*
733 *Biomaterialia*, *6*(10), 4042–4051.
- 734 Kiechel, M. A., & Schauer, C. L. (2013). Non-covalent crosslinkers for electrospun
735 chitosan fibers. *Carbohydrate Polymers*, *95*(1), 123–133.

- 736 Lee, I. W., Li, J., Chen, X., & Park, H. J. (2016). Electrospun poly(vinyl alcohol)
737 composite nanofibers with halloysite nanotubes for the sustained release of sodium
738 d-pantothenate. *Journal of Applied Polymer Science*, 133(4), 42900.
- 739 Li, Y., Lim, L. T., & Kakuda, Y. (2009). Electrospun zein fibers as carriers to stabilize
740 (-)-epigallocatechin gallate. *Journal of Food Science*, 74(3), C233–C240.
- 741 Mansur, H. S., Sadahira, C. M., Souza, A. N., & Mansur, A. A. (2008). FTIR
742 spectroscopy characterization of poly (vinyl alcohol) hydrogel with different
743 hydrolysis degree and chemically crosslinked with glutaraldehyde. *Materials Science
744 and Engineering: C*, 28(4), 539–548.
- 745 Martínez-Sanz, M., López-Rubio, A., & Lagaron, J.M. (2013). High-barrier coated
746 bacterial cellulose nanowhiskers films with reduced moisture sensitivity.
747 *Carbohydrate Polymers*, 98(1), 1072-1082.
- 748 Matsuda, S., Iwata, H., Se, N., & Ikada, Y. (1999). Bioadhesion of gel films crosslinked
749 with glutaraldehyde. *Journal of biomedical materials research*, 45(1), 20–27.
- 750 McCue, P., & Shetty, K. (2004). Inhibitory effects of rosmarinic acid extracts on
751 porcine pancreatic amylase in vitro. *Asia Pacific Journal of Clinical Nutrition*, 13,
752 101–106.
- 753 Mendes, L., de Freitas, V., Baptista, P., & Carvalho, M. (2011). Comparative
754 antihemolytic and radical scavenging activities of strawberry tree (*Arbutus unedo* L.)
755 leaf and fruit. *Food and Chemical Toxicology*, 49, 2285–2291.
- 756 Mendes, A. C., Stephansen, K., & Chronakis, I. S. (2017). Electrospinning of food
757 proteins and polysaccharides. *Food Hydrocolloids*, 68, 53–68.
- 758 Mohamed, S. (2014). Functional foods against metabolic syndrome (obesity, diabetes,
759 hypertension and dyslipidemia) and cardiovascular disease. *Trends in Food Science
760 & Technology*, 35, 114–128.
- 761 Moradkhannejhad, L., Abdouss, M., Nikfarjam, N., Mazinani, S., & Heydari, V.
762 (2018). Electrospinning of zein/propolis nanofibers; antimicrobial properties and
763 morphology investigation. *Journal of Materials Science: Materials in Medicine*,
764 29(11), 165.
- 765 Moreno, M. A., Gómez-Mascaraque, L. G., Arias, M., Zampini, I. C., Sayago, J. E.,
766 Ramos, L. L. P., Schmeda-Hirschmann, G., López-Rubio, A., & Isla, M. I. (2018).
767 Electrospayed chitosan microcapsules as delivery vehicles for vaginal
768 phytoformulations. *Carbohydrate Polymers*, 201, 425–437.

- 769 Neo, Y. P., Ray, S., Jin, J., Gizdavic-Nikolaidis, M., Nieuwoudt, M. K., Liu, D., &
770 Quek, S. Y. (2013). Encapsulation of food grade antioxidant in natural biopolymer
771 by electrospinning technique: A physicochemical study based on zein–gallic acid
772 system. *Food Chemistry*, *136*(2), 1013–1021.
- 773 Ngo, Y. L., Lau, C. H., & Chua, L. S. (2018). Review on rosmarinic acid extraction,
774 fractionation and its anti-diabetic potential. *Food and Chemical Toxicology*, *121*,
775 687-700.
- 776 Orqueda, M. E., Rivas, M., Zampini, I. C., Alberto, M. R., Torres, S., Cuello, S.,
777 Sayago, J., Thomas-Valdes, S., Jiménez-Aspee, F., Schmeda-Hirschmann, G., & Isla,
778 M. I. (2017a). Chemical and functional characterization of seed, pulp and skin
779 powder from chilto (*Solanum betaceum*), an Argentine native fruit. Phenolic
780 fractions affect key enzymes involved in metabolic syndrome and oxidative stress.
781 *Food Chemistry*, *216*, 70–79.
- 782 Park, J.-S., Park, J.-W., & Ruckenstein, E. (2001). Thermal and dynamic mechanical
783 analysis of PVA/MC blend hydrogels. *Polymer*, *42*(9), 4271–4280.
- 784 Prohens, J., & Nuez, F. (2000). The tamarillo (*Cyphomandra betacea*): a review of a
785 promising small fruit crop. *Small Fruits Review*, *2*, 43–68.
- 786 Prohens, J., Ruiz, J.J., & Nuez, F. (1996). Advancing the tamarillo harvest by induced
787 postharvest ripening. *Horticultural Science*, *31*, 109–111.
- 788 Quek, S. Y., Hadi, J., & Tanambell, H. (2019). Application of Electrospinning as
789 Bioactive Delivery System. *Reference Module in Food Science*, 145-149.
- 790 Ramires, P. A., & Milella, E. (2002). Biocompatibility of poly(vinyl alcohol)-
791 hyaluronic acid and poly(vinyl alcohol)-gellan membranes crosslinked by
792 glutaraldehyde vapors. *Journal of Materials Science: Materials in Medicine*, *13*(1),
793 119–123.
- 794 Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999).
795 Antioxidant activity applying an improved ABTS radical cation decoloration assay.
796 *Free Radical Biological Medicinal*, *26*, 1231–1237.
- 797 Samuels, J. (2015). Biodiversity of food species of the *Solanaceae* family: a preliminary
798 taxonomic inventory of subfamily *Solanoideae*. *Resources*, *4*, 277–322.
- 799 Schmidt, G., Hamaker, B. R., & Wilker, J. J. (2018). High strength adhesives from
800 catechol cross-linking of zein protein and plant phenolics. *Advanced Sustainable*
801 *Systems*, *2*(3), 1700159.

- 802 Selling, G. W., Woods, K. K., Sessa, D., & Biswas, A. (2008). Electrospun zein fibers
803 using glutaraldehyde as the crosslinking reagent: effect of time and temperature.
804 *Macromolecular Chemistry and Physics*, 209(10), 1003–1011.
- 805 Shahbazi, M., Ahmadi, S. J., Seif, A., & Rajabzadeh, G. (2016). Carboxymethyl
806 cellulose film modification through surface photo-crosslinking and chemical
807 crosslinking for food packaging applications. *Food Hydrocolloids*, 61, 378–389.
- 808 Sivam, A. S., Sun-Waterhouse, D., Perera, C. O., & Waterhouse, G. I. N. (2012).
809 Exploring the interactions between blackcurrant polyphenols, pectin and wheat
810 biopolymers in model breads; A FTIR and HPLC investigation. *Food Chemistry*,
811 131(3), 802–810.
- 812 Stehfest, K., Boese, M., Kerns, G., Piry, A., & Wilhelm, C. (2004). Fourier transform
813 infrared spectroscopy as a new tool to determine rosmarinic acid in situ. *Journal of*
814 *Plant Physiology*, 161(2), 151–156.
- 815 Wang, H., Hao, L., Wang, P., Chen, M., Jiang, S., & Jiang, S. (2017). Release kinetics
816 and antibacterial activity of curcumin loaded zein fibers. *Food Hydrocolloids*, 63,
817 437–446.
- 818 Yao, C., Li, X. S., & Song, T. Y. (2007). Electrospinning and crosslinking of zein
819 nanofiber mats. *Journal of Applied Polymer Science*, 103–380.
- 820
- 821

Highlights

- Bioactive coatings for food packaging were obtained by electrospinning
- Chilto extracts with antioxidant capacity were included in the coatings
- The encapsulation efficiency of extracts within the coating was greater than 90%
- Cross-linking of zein fibers improved coating integrity upon water contact
- A sustained release of the extracts in food simulants was observed