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MORPHOLOGY AND STABILITY OF EDIBLE LYCOPENE-CONTAINING MICRO- AND NANOCAPSULES PRODUCED THROUGH ELECTROSPRAYING AND SPRAY DRYING

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Abstract:	In this work, lycopene was encapsulated through electrospraying and spray drying (using a microporous membrane cap) within different edible biopolymeric matrices. Specifically, dextran, a whey protein concentrate (WPC) and chitosan were used as matrix materials. As a strategy to incorporate the hydrophobic bioactive within the hydrophilic matrices, emulsion electrospraying and spray drying from emulsion were carried out. Moreover and, for comparison purposes, coaxial electrospraying was also performed. The electrospraying solutions properties were studied, since they do not only affect the success of the electrohydrodynamic process, but also influence the morphology of the capsules. Apart from characterizing the morphology and molecular organization of the developed capsules, the encapsulation efficiency and the lycopene stability under moisture and heating conditions were also evaluated. Results showed that even though encapsulation structures were obtained from all the matrices assayed through both processing technologies, spray drying, as a consequence of the high temperatures needed in this process, affected lycopene stability. It was also seen that WPC presented the greatest encapsulation efficiency, probably ascribed to the interactions between the biopolymer and the lycopene. Furthermore, WPC capsules were able to better protect lycopene against moisture and thermal degradation.

1 MORPHOLOGY AND STABILITY OF EDIBLE LYCOPENE-CONTAINING MICRO- AND
2 NANOCAPSULES PRODUCED THROUGH ELECTROSPRAYING AND SPRAY DRYING

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10 Abstract

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27 23 consequence of the high temperatures needed in this process, affected lycopene stability **and**
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29 24 **very poor encapsulation efficiencies were found in this case.** It was also seen that WPC
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47 29 Keywords: lycopene, encapsulation, electrospraying, electrospinning, spray drying
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1. Introduction

33 Carotenoids are some of the most common pigments in nature, the most abundant being β -
34 carotene, lycopene, lutein and zeaxanthin. These compounds are incorporated into foods, not
35 only due to their colorant properties, but also to their nutraceutical uses. Specifically, lycopene
36 is a natural red pigment mainly found in tomato and other red fruits, like watermelon or
37 papaya, and it is one of the main responsible of the antioxidant capacity of these products. It
38 has received great interest in recent years because of its activity in the prevention of chronic
39 diseases such as atherosclerosis, skin cancer, and prostate cancer (Rao and Agarwal, 1999; Xue
40 et al. 2013). However, lycopene presents several unsaturated bonds in its molecular structure,
41 which makes it very susceptible to oxidants, light and heat. This fact makes that lycopene
42 could lose its beneficial properties during processing and storage of foodstuffs. Moreover, it
43 has a very low water solubility, which limits its industrial applicability in aqueous-based
44 systems. A possible approach to overcome these limitations is the micro/nanoencapsulation of
45 lycopene. Some technologies have been already used to prepare lycopene microcapsules. For
46 instance, many authors have prepared lycopene microcapsules through the spray drying
47 methodology. However, the resulted capsules did not present an optimum stability at room
48 temperature (Matioli and Rodriguez-Amaya 2002; Shu et al. 2006; Rocha et al. 2012). Blanch et
49 al. (2007) prepared lycopene microcapsules by supercritical fluid extraction method. The use of
50 supercritical fluids in food is recommended, because it avoids the employment of large
51 amounts of organic solvents. However, the costs of equipment and operation are too high and
52 the technological parameters need further optimization (Guo et al. 2012). Other techniques
53 such as complex coacervation have been used, (Rocha-Selmi et al. 2013; Silva et al. 2012) but
54 in this case either organic solvents (not convenient for food applications), high temperature or
55 acid conditions are needed, which could affect lycopene stability.

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In this study, the use of electrohydrodynamic processing for obtaining micro/nanocapsules of lycopene was proposed as a novel encapsulation technology. This technique was compared to the spray drying methodology, since it is the most common encapsulation technology used in the food industry nowadays. Nevertheless, in this work, a novel spray drying device with a microporous membrane cap able to obtain smaller encapsulation structures was used. The electrohydrodynamic process uses electrostatic forces to produce electrically charged jets from viscoelastic polymer solutions which on drying, by the evaporation of the solvent, produce ultrathin structures (Li and Xia 2004). In particular, the technique is referred to as electrospinning when ultrathin continuous fibres are obtained. When size-reduced capsules are obtained, the technique is called electrospraying. For food and nutraceutical applications, capsules are generally preferred rather than fibres, since apart from facilitating handling and subsequent incorporation into different products, they usually present greater surface/volume ratio and, thus, are expected to have better release profiles than fibres (Hong et al. 2008). It is important to note that electrospraying does not require the use of high temperatures and, thus, temperature-sensitive ingredients, such as carotenoids, may be encapsulated using this processing technique without suffering from any activity loss (Fernandez et al. 2009). Moreover, electrosprayed capsules can be produced from some biopolymers using aqueous solutions, mainly by changing the solution properties through the addition of proper additives (Pérez-Masiá et al. 2014). This issue has special interest in food applications, where the use of organic solvents could lead to toxic effects due to the presence of remaining solvents in the structures. However, lycopene presented very low water solubility, thus electrospraying and subsequent encapsulation of this compound from water-based solutions is not simple. Nevertheless, emulsion electrospraying, as well as coaxial electrospraying can be used for the encapsulation of immiscible compounds. In the emulsion electrospraying, an immiscible liquid phase is dispersed into a polymer solution until both components form a stable emulsion. As a result, the immiscible phase forms the core material and the polymer constitutes the shell of

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82 the capsules (Angeles et al. 2007). In the coaxial methodology, the polymer and the core
83 material are introduced into the electro spraying equipment from separated solutions, thus the
84 immiscibility problem is solved.

85 In this work, lycopene was encapsulated through electro spraying and spray drying in various
86 food hydrocolloid matrices for potential food applications. Specifically, a whey protein
87 concentrate from milk (WPC), and two polysaccharides (dextran and chitosan) were evaluated.
88 It is worth noting that WPC and dextran are water dispersible polymers and, thus, emulsion
89 and coaxial electro spraying were used in these cases. In the case of the spray drying
90 methodology, an emulsion was also formed with these materials. For chitosan, an acetic acid
91 solution was used, where lycopene could be easily dissolved and, thus, uniaxial electro spraying
92 or spray drying from the acid solution were carried out. Acetic acid is allowed for food contact
93 applications and would not generate toxicity problems in case some solvent remained within
94 the capsules. The morphology and the molecular organization of the capsules obtained were
95 evaluated. Moreover, the stability of encapsulated lycopene was carried out taking into
96 account different situations that can be found in the food industry, such as the incorporation
97 of the capsules into aqueous-based foods and exposure to thermal treatments.

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105 2. Materials and Methods

106 2.1. Materials

107 Whey protein concentrate (WPC) was kindly donated by ARLA (ARLA Food Ingredients, Viby,
108 Denmark). Under the commercial name Lactrodan® DI-8090, the composition per 100 g of
109 product consisted of ~80 g of protein, ~9 g of lactose, and ~8 g of lipids, being the rest water
110 and minerals like sodium and potassium. Dextran (Mw ~70000), low molecular weight chitosan
111 (~50000-190000), lycopene, Tween-20 and soybean oil were supplied by Sigma-Aldrich (Spain)
112 and they were used as received, without further purification.

113 2.2. Preparation of the encapsulation solutions

114 For the uniaxial electrospaying, 2% (w/v) of chitosan and 1.5% (w/w) of lycopene with respect
115 to the chitosan weight were dissolved in a 90% (v/v) aqueous/acetic solution. For the coaxial
116 electrospaying, the shell solutions were prepared by dissolving 30% (w/v) of WPC or 20%
117 (w/v) of dextran and 5% (w/v) of Tween-20 in distilled water. The core solution was prepared
118 by dissolving 1.25% (w/v) of lycopene in soybean oil. For the emulsion electrospaying, an
119 aqueous surfactant solution was prepared by dissolving 10% (w/v) of Tween-20 in water.
120 Afterwards, 16% (v/v) of lycopene/soybean oil solution [1.25 % (w/v)] was added to the
121 surfactant solution to prepare an emulsion premix. The premix was ultrasonicated at 10% of
122 amplitude and 20 kHz of process frequency for 120s using an ultrasonic homogenizer (Bandelin
123 electronic, Germany). These conditions led to an oil-in-water (O/W) emulsion with an average
124 particle size of around 150 nm. Finally, 30% (w/v) of WPC or 20% (w/v) of dextran were added
125 to the emulsion and they were mechanically stirred until homogeneous emulsions were
126 obtained.

127 For the spray drying solutions, 0.04 % (w/v) of chitosan and 1.5% (w/w) of lycopene with
128 respect to the chitosan weight were dissolved in a 0.4% (v/v) aqueous/acetic solution. For the

129 preparation of the water dispersible biopolymer solutions, Tween-20 was dissolved in distilled
130 water and afterwards, 0.3% (v/v) of lycopene/soybean oil solution [1.25 %(w/v)] was added to
131 the surfactant solution to prepare an emulsion premix. The premix was ultrasonicated at 10%
132 of amplitude and 20 kHz of process frequency for 120 s using an ultrasonic homogenizer
133 (Bandelin electronic, Germany) in order to obtain an O/W emulsion with an average particle
134 size of around 150 nm. Finally, 0.4% (w/v) of dextran or WPC was added to the emulsion and
135 they were mechanically stirred until homogeneous emulsions were obtained.

136 2.3. Emulsion particle size characterization

137 The mean particle size and size distribution of the O/W emulsion attained were determined via
138 dynamic light scattering (DLS) with a Zetasizer Nano-ZS 90 (Malvern Instruments Corp., UK). All
139 measurements were performed at 25°C in triplicate and intensity-weighted results were
140 reported.

141 2.4. Characterization of the electrospraying solutions

142 The apparent viscosity (η_a) of the polymeric solutions at 100 s^{-1} was determined using a
143 rotational viscosity meter Visco Basic Plus L from Fungilab S.A. (San Feliu de Llobregat, Spain).
144 The surface tension of the solutions was measured using the Wilhemy plate method in an
145 EasyDyne K20 tensiometer (Krüss GmbH, Hamburg, Germany). The conductivity of the
146 solutions was measured using a conductivity meter XS Con6 (Labbox, Barcelona, Spain). All
147 measurements were made in triplicate at 25°C.

148 2.5. Encapsulation through electrospraying

149 The electrospraying apparatus, equipped with a variable high-voltage 0-30 kV power supply,
150 was a Fluidnatek® L-10 assembled and supplied by Bioinicia S.L. (Valencia, Spain). Solutions
151 were introduced in a 5 mL plastic syringe and were electrosprayed under a steady flow-rate
152 using a stainless-steel needle. For the coaxial electrospraying, two concentric needles were

153 used. The inner one was used for the core material and the outer one for the shell solution.
154 The needle was connected through a PTFE wire to the syringe. The syringe was lying on a
155 digitally controlled syringe pump while the needle was in horizontal towards a stainless-steel
156 plate attached to a copper grid used as collector. More details of the electro-spraying
157 equipment and the different uniaxial and coaxial configurations can be found elsewhere
158 (Pérez-Masiá et al. 2013). The flow rate was 0.15 mL/h, the voltage varied from 12 to 18 kV
159 and the distance between the tip and the collector varied from 9-20 cm.

160 2.6. Encapsulation through spray drying

161 The solutions with and without lycopene were spray-dried using a Spray-dryer B-90 (Büchi,
162 Switzerland) with a 7.0 μm spray cap. The solutions were introduced into the equipment
163 through a silicone wire, which was connected to the spraying head of the equipment. The air
164 flow was ~ 140 L/h with an inlet and outlet temperatures of 90°C and 45°C, respectively.

165 2.7. Scanning electron microscopy (SEM)

166 SEM was conducted on a Hitachi microscope (Hitachi S-4100) at an accelerating voltage of 10
167 KV and a working distance of 12-16 mm. The capsules were sputtered with a gold-palladium
168 mixture under vacuum before their morphology was examined using SEM. Capsule diameters
169 were measured by means of the Adobe Photoshop CS3 extended software from the SEM
170 micrographs in their original magnification.

171 2.8. Attenuated total reflectance infrared spectroscopy (ATR-FTIR)

172 ATR-FTIR spectra were recorded in a controlled chamber at 24°C and 40% RH using a Bruker
173 FT-IR Tensor 37 equipment (Rheinstetten, Germany) coupled with the ATR accessory
174 GoldenGate of Specac Ltd. (Orpington, UK) in the 4000–600 cm^{-1} range. The spectra were
175 collected in the different materials by averaging 20 scans at 4 cm^{-1} resolution. The experiments
176 were repeated twice to verify that the spectra were consistent between individual samples.

177 2.9. Encapsulation efficiency

178 The lycopene encapsulation efficiency was calculated by dividing the lycopene concentration
179 found in the capsules by the initial lycopene concentration added to the different solutions.
180 Lycopene was extracted from the capsules by dissolving the capsules in water and afterwards,
181 adding dodecane to the solution in order to separate the lycopene from the rest of the
182 components. The capsules' concentration was evaluated from the UV absorbance of the
183 capsules using a SP-2000UV spectrophotometer (Spectrum, Shanghai, China). As lycopene has
184 an absorption maximum at a wavelength of 476 nm, the bioactive concentration in the
185 capsules was calculated according to a calibration curve obtained for different known lycopene
186 concentrations and the corresponding absorbance at this wavenumber:

187 $y = 0.0187x - 0.0008$ ($R^2 = 0.99$) (Equation 1)

188 Where "y" was the lycopene concentration and "x" was the UV absorbance at 476 nm.

189 2.10. Water sorption analysis

190 Water sorption of different capsules was evaluated at 70%RH, by introducing petri dishes
191 containing the materials inside a desiccator with saturated sodium chloride. Weight gain of the
192 capsules vs. time curves at 24°C was studied until constant weight. Afterwards, lycopene was
193 again extracted from the capsules and the bioactive concentration was studied through the UV
194 absorbance of lycopene at 476 nm using the previously obtained calibration curve (cf. Equation
195 1).

196 2.11. Thermal stability of lycopene

197 Thermal stability of lycopene in WPC capsules was studied through the exposure of the
198 structures and the raw lycopene to heating conditions and the evaluation of the antioxidant
199 activity of the samples before and after the heating experiment. Specifically, samples were

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200 stored at 70°C in darkness during 4 h, since it was observed that degradation of non-
201 encapsulated lycopene occurred in these conditions. Antioxidant activity of lycopene was
202 evaluated according to the ABTS radical cation (ABTS^{•+}) methodology developed by Re et al.
203 (1999). To this aim, ABTS^{•+} was produced by reacting ABTS solution with potassium persulfate
204 and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. For
205 the study of the antioxidant activity of different samples, the ABTS^{•+} solution was diluted with
206 ethanol to an absorbance of 0.70 (±0.02) at 734 nm. Afterwards, capsules and raw lycopene
207 were dissolved in dichloromethane and an aliquot of these solutions was added into the
208 ABTS^{•+} solution in order to inhibit the ABTS^{•+} absorbance. The percentage inhibition of
209 absorbance at 734 nm after 4 minutes was calculated for each sample, since it was previously
210 observed that reaction between carotenoids and ABTS^{•+} was complete after this time (Re et al.
211 1999). A Trolox curve in the same solvent was used as reference (Equation 2) and results were
212 reported as a function of equivalent Trolox concentration.

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213 $y = 6.7579x + 2.0433(R^2 = 0.99)$ (Equation 2)

214 Where “y” was the percentage inhibition of ABTS^{•+} absorbance at 734 nm and “x” was the
215 corresponding Trolox concentration (μM).

216 2.12. Statistical analysis

217 Statistical analysis of data was performed through analysis of variance (ANOVA) using
218 Statgraphics Centurion XV (Manugistics Corp., Rockville, MD). Homogeneous sample groups
219 were obtained by using LSD test (95% significant level).

220 3. Results and Discussion

221 As briefly mentioned in the introduction, the aim of this work was to compare between the
222 traditional spray drying technique and the most novel electrospraying method for the
223 encapsulation and protection of lycopene. One of the strategies used to incorporate the

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224 hydrophobic bioactive within the hydrocolloidal-based matrices (whey protein concentrate-
225 WPC-, dextran and chitosan) was to form O/W emulsions using a soybean oil (containing the
226 lycopene) dispersed within the aqueous phase which had a surfactant. Later on, the
227 encapsulating matrices were added to the solutions and the emulsions were processed using
228 both processing techniques.

229 3.1. Emulsion particle size characterization and electro spraying solution properties

230 Initially, the emulsions obtained were characterized. Figure 1 shows the particle size
231 distribution of the O/W emulsion attained before adding the biopolymers. From this figure it
232 was observed that only one curve was seen with the maximum peak at ~150 nm. This result
233 indicated that there was only one population of particles with an average size of ~150 nm. It
234 was not possible to measure the particle size distribution of the emulsion containing the
235 biopolymers, as upon their incorporation at the concentration used for electro spraying, the
236 turbidity of the solution became too high for a correct characterization.

237 INSERT FIGURE 1 ABOUT HERE

238 The physical properties of the electro spraying solutions containing the biopolymers were
239 characterized as they strongly affect the stability of the process and the successful
240 development of structures through this technology. Moreover, capsules morphology is also
241 influenced by solution properties. Table 1 compiles the viscosity, surface tension and electrical
242 conductivity of the electro spraying solutions assayed. These final compositions were obtained
243 after a previous optimization process in which the concentration of the various compounds
244 was adjusted until stable emulsions and electro spraying jets were obtained. Nevertheless, it
245 was seen that a phase separation always occurred in dextran emulsions after a few hours. On
246 the contrary, lycopene-containing WPC emulsions were stable during days. From Table 1 it can
247 be observed that the incorporation of lycopene to the dextran and WPC solutions through the
248 emulsion process, significantly affected the solution properties. Specifically, it was seen that

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249 viscosity considerably increased for both solutions because, in this case, lycopene was
250 incorporated through soybean oil which was rather viscous. It is worth noting that the viscosity
251 increase was greater for the WPC solution, which could indicate some kind of interaction
252 between the protein and the lycopene in solution. Regarding the surface tension and electrical
253 conductivity, it was observed that for the dextran solution these parameters slightly increased
254 when compared to the solution without lycopene. However, in the case of WPC, the presence
255 of the soybean oil/lycopene in the solution significantly decreased the electrical conductivity.
256 This again could be ascribed to the interaction between the protein and the lycopene, thus,
257 resulting in the neutralization of some of the protein charges. In contrast with the previous
258 results, the chitosan solution properties were not modified upon lycopene incorporation. It is
259 important to highlight that, in this case, lycopene was not incorporated through soybean oil,
260 but it was directly added to the acid chitosan solution. Nevertheless, these data suggested that
261 there were no interactions between chitosan and lycopene.

INSERT TABLE 1 ABOUT HERE

263 3.2. Morphology of the lycopene-containing capsules

264 SEM was used in order to analyse capsules' size and morphology. Figure 2 shows the SEM
265 images of the different biopolymer/lycopene capsules obtained. Emulsion electro spraying of
266 the dextran-based solution led to very homogeneous capsule sizes, while coaxial
267 electro spraying produced more aggregated particles. This fact could be due to a worse
268 entrapment of the soybean oil/lycopene solution when coaxial electro spraying was used,
269 probably because of the non-continuous nature of the capsules, which complicated the
270 incorporation of the antioxidant into the structures. In this case, part of the oil could leak from
271 the dextran matrix, thus, causing capsules aggregation. It was also observed that spray drying
272 gave rise to bigger structures with a broader capsule size distribution. In contrast, when WPC
273 was used as encapsulating matrix, more heterogeneous structures were obtained through

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274 electro spraying, while smoother and more regular size capsules were produced through spray
275 drying. This result might be explained by the greater electrical conductivity of the protein
276 solutions, which could destabilize the electro spraying jet, thus producing different particle
277 sizes. In this case, aggregated particles were also seen when coaxial electro spraying was used,
278 probably because of oil leakages. Regarding the structures made up of chitosan, from Figure 2
279 it can be observed that very small capsules were obtained through electro spraying and spray
280 drying. This fact was attributed to the use of the acid solvent (pH ~ 2.5), which generated
281 strong electrostatic repulsions along the chitosan chains. These repulsive interactions hindered
282 the formation of chain entanglements and, thus, small capsules were attained with this
283 polymer (Torres-Giner et al. 2008).

284 INSERT FIGURE 2 ABOUT HERE

285 3.3. Infrared spectra of the bioactive capsules

286 ATR-FTIR analyses were carried out in order to confirm the presence of the antioxidant in the
287 different structures, as well as to characterize the molecular organization of the capsules and
288 detect interactions between the matrix and the core material. Figure 3 shows the ATR-FTIR
289 spectra of the different materials assayed. From Figure 3A it was seen that FTIR spectra of
290 dextran encapsulation structures showed some bands attributed to both soybean oil (SBO) and
291 lycopene (cf. arrows in Figure 3A). Specifically, it was seen that the C-H stretching vibration
292 bands, centered around 2930 cm^{-1} , which appeared as a single broad spectral band for the as-
293 received dextran, became narrower and better defined in the dextran capsules. These could be
294 related to a greater molecular order due to the formation of the capsules, as well as to the
295 presence of the SBO and lycopene in these capsules, since it was seen that both components
296 presented sharp bands in this area. Moreover, the C=O group of the triglycerides of SBO found
297 at 1743 cm^{-1} (Vlachos et al. 2005) and the lycopene band attributed to the C=C trans bond
298 found at 957 cm^{-1} (Rubio-Díaz et al., 2011) also appeared in the dextran capsules, especially in

299 those obtained through spray drying. Therefore, FTIR results suggested that lycopene was
300 incorporated in all the structures, but spray drying seemed to result in a greater encapsulation
301 yield. It is also important to note that spectral bands of dextran were shifted depending on the
302 encapsulation technology used. This shift was attributed to the process conditions, i.e. the
303 solution preparation and subsequent drying of the carbohydrate, which could led to a
304 rearrangement of the molecules, which was also probably influenced by the presence of
305 surfactant. Figure 3A shows the details of the spectral region from 800 to 1200 cm^{-1} , in which
306 the characteristic vibrational bands from carbohydrates arise, and it shows that the maximum
307 of the most intense carbohydrate band at around 1005 cm^{-1} varied depending on the
308 encapsulation technology used, i.e. this band arose at $\sim 1006 \text{ cm}^{-1}$, $\sim 1004 \text{ cm}^{-1}$ and $\sim 1012 \text{ cm}^{-1}$
309 in the capsules obtained through emulsion electrospraying, coaxial electrospraying and spray
310 drying, respectively. The shift towards higher wavenumbers has been normally related to
311 shorter and more attractive bonds (Wolkers et al. 2004). Therefore, this result suggested that
312 the molecular conformation of capsules was affected by the encapsulation technology and
313 spray drying, probably as a consequence of the high temperatures used in this process,
314 produced more compact structures.

315 Regarding the WPC capsules, Figure 3B shows that the C=O band of SBO at 1743 cm^{-1} and the
316 lycopene band at 957 cm^{-1} also appeared in the spectra from all the capsules, thus confirming
317 the encapsulation of the lycopene through all the techniques used. Moreover, it was observed
318 that both amide bands of the WPC/lycopene capsules were shifted in comparison to the pure
319 protein. In this case the shifts could be attributed not only to the process conditions, but also
320 to chemical or physico-chemical interactions between the protein and the bioactive, since the
321 previous results about the viscosity and the emulsion stabilization suggested that some kind of
322 interaction occurred between the protein and the lycopene, as already reported in previous
323 works (Zhang and Zhong, 2013; Mensi et al. 2013). Specifically, it was seen that amide I band
324 ($\sim 1630 \text{ cm}^{-1}$) was displaced towards lower wavenumbers, indicating new β -sheets

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325 arrangements (Eissa et al. 2006) and the amide II band ($\sim 1530\text{ cm}^{-1}$) of WPC/lyc capsules was
326 displaced towards greater wavenumbers when compared to the as-received WPC powder. This
327 latter shift was related to changes in the in-plane N-H and C-N vibrations (Kong and Yu 2007).
328 Again, it is interesting to highlight that these shifts were different depending on the kind of
329 encapsulation technique, being the spray dried capsules those which again presented the most
330 noticeable shifts.

331 Concerning the chitosan materials, from Figure 3C it was observed that the chitosan spectra
332 completely changed after both encapsulation processes. This fact was attributed to the
333 acetylation of chitosan due to the dissolution of the biopolymer in acetic acid media.
334 Specifically, the main changes in the chitosan spectra occurred in the amide I and II regions
335 (from 1700 to 1500 cm^{-1} aprox.). In this area the as-received chitosan shows the amide I and
336 amide II bands at around 1650 and 1590 cm^{-1} respectively. However, after processing, the
337 capsules presented two dominant bands centered at around 1546 and 1405 cm^{-1} associated to
338 carboxylate ions (NH_3^+ and COOH). The amine groups in this chemical form are referred to as
339 protonated or activated amine groups. These results suggested that capsules could exhibit
340 antimicrobial performance (Fernandez-Saiz et al., 2006), even though the free acetic acid band
341 at $\sim 1706\text{ cm}^{-1}$ was not evident in the capsules spectra, indicating that solvent was completely
342 evaporated during the encapsulation process. However, lycopene signal was not apparent in
343 these capsules, suggesting that the bioactive could be degraded in the presence of the
344 concentrated acetic acid or that it was not properly encapsulated in this case (maybe as a
345 consequence of the smaller size of these structures). As with the other encapsulation matrices,
346 it is interesting to note that, depending on the encapsulation technology used, the
347 characteristic spectral bands arose slightly at different wavenumbers, thus confirming different
348 molecular conformations depending on the technology used.

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350 3.4. Encapsulation efficiency

351 The encapsulation efficiency of the different structures developed was evaluated through UV
352 spectroscopy. Figure 4 shows the UV spectra from 350 to 650 nm of the raw lycopene, as well
353 as of the lycopene extracted from the capsules. It was observed that lycopene presented 3
354 characteristic bands at 449, 476 and 509 nm, being the band at 476 nm the most intense one.
355 Therefore, the intensity of the band at 476 nm was used to evaluate lycopene concentration
356 according to Equation 1 (cf. materials and methods, section 2.8). From Figure 4 it can be
357 observed that lycopene extracted from the capsules presented a similar spectrum than that
358 from raw lycopene. However, lycopene extracted from chitosan capsules presented very low
359 absorbance values probably because of a greater degradation of the carotenoid in this case
360 due to the acid conditions used in the formation of these capsules. From this figure it is also
361 seen that the high temperature employed during spray drying also affected to the stability of
362 the bioactive. Indeed, if comparing the spectra of extracted lycopene from the electrosprayed
363 and spray dried capsules (cf. Figure 4A vs. 4B), it is clear that the high temperature involved in
364 the spray drying technique led to a certain degradation of the bioactive, as reflected by the
365 lower spectral intensity. It was previously seen that degradation kinetics of lycopene during
366 hot air drying strongly increased at temperatures above 70°C (Demiray et al. 2013). However,
367 as inferred from the literature, it appears that the degradation temperature of lycopene is
368 strongly dependent on its degree of purity and extraction method used (Goula and
369 Adamopoulos, 2005; Shu et al., 2006). A thermal stability analysis of the lycopene used in this
370 work was done before the spray drying experiment and it was seen that the compound was
371 degraded after 3h at 70°C. However, temperatures below 90°C were not enough for
372 completely eliminating the solvent and, thus, producing the dried capsules.

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375 Table 2 shows the initial lycopene concentration added to the solutions and the lycopene
376 concentration remaining in the capsules obtained through Equation 1, expressed as the
377 percentage of lycopene with respect to the total solids in the capsules. The encapsulation
378 efficiency is also included in this table and it was calculated by dividing the initial lycopene
379 concentration by the lycopene concentration found in the capsules. From this table it was seen
380 that capsules obtained through spray drying presented very low encapsulation efficiencies for
381 all the matrices, probably because the high temperature needed for solvent evaporation
382 during capsule formation affected lycopene stability. This data seems to be in disagreement
383 with FTIR results, which suggested that spray drying led to higher encapsulation yield.
384 However, FTIR spectra showed the soybean oil (SBO) signal which was not affected by
385 temperature. Thus, although higher amount of SBO/lycopene solution was probably
386 encapsulated through spray drying, lycopene was possibly degraded due to heat exposure and,
387 thus, the bioactive signal could not be detected through UV spectroscopy. This result is in
388 accordance with previous works which have studied lycopene loss during heating or drying of
389 tomato products (Rocha et al. 2012; Goula and Adamopoulos, 2005). Table 2 also shows that
390 for dextran, emulsion electrospaying also led to low encapsulation efficiency, due to the
391 unstable emulsion formed with this polymer. Therefore, for this material it was better to use
392 the coaxial methodology, which led to an encapsulation yield around 55-60%. For WPC
393 capsules it was seen that emulsion electrospaying led to proper encapsulation efficiency,
394 since WPC and SBO/lycopene solution produced a stable emulsion which did not separate
395 during the electrospaying experiment. Regarding the coaxial electrospaying very high
396 variability was found, probably because this technique led to a worse encapsulation process
397 with oil leakages, which made that some capsules presented very high lycopene contents,
398 while other structures presented very low encapsulation yields. Finally, it was observed that
399 lycopene was poorly encapsulated in chitosan capsules through uniaxial electrospaying.

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400 Moreover, the lycopene found in these capsules was probably partially degraded due to the
401 acid conditions used in this case.

402 INSERT TABLE 2 ABOUT HERE

403 3.5. Water sorption analysis

404 If considering the developed structures for food applications, and taking into account that
405 most food products have high water activity, it is important to know the water sorption
406 behavior of the different capsules and, what is more important, the bioactive stability in the
407 presence of moisture. To this aim, water sorption was evaluated through the exposure of the
408 capsules and also of the raw lycopene (for comparison purposes) at ambient temperature ($T =$
409 $21\text{ }^{\circ}\text{C}$) and high relative humidity ($\sim 70\%$). Their weight gain was evaluated until a constant
410 value was reached. Afterwards, lycopene was extracted from the capsules and evaluated
411 through UV spectroscopy in order to study the efficiency of the capsules for stabilizing the
412 antioxidant compound under moisture conditions. Table 3 shows the weight gain of all the
413 capsules developed, the initial lycopene concentration in the capsules previously obtained (cf.
414 Table 2), the lycopene concentration after moisture exposure obtained through Eq. 1 and the
415 total lycopene loss. From this table it can be clearly seen that after moisture exposure, in all
416 cases, lycopene absorbance decreased. This effect was attributed to spatial rearrangements of
417 the double bonds and partial degradation of the bioactive (Tan and Soderstrom, 1989).
418 Specifically, it was seen that $\sim 65\%$ of non-encapsulated lycopene was degraded, even when
419 the raw lycopene hardly increased its weight after moisture exposure. Nevertheless, the
420 oxygen presence and ambient temperature (21°C) in the moisture chambers could also alter
421 the molecular structure of the lycopene. Regarding the capsules it was seen that water uptake
422 of capsules was generally greater than those of raw lycopene, probably because capsules'
423 matrices were hydrophilic materials which favoured water sorption. Specifically, it was seen
424 that dextran capsules absorbed a greater amount of water than WPC and chitosan, ascribed to

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425 its higher water solubility. This fact affected lycopene stability, since dextran capsules were
426 partially dissolved and lycopene was thus exposed to moisture, oxygen and temperature
427 conditions. Thus, it was seen that the lycopene absorbance decreased in these capsules after
428 moisture exposure, probably because of the degradation of part of the antioxidant. On the
429 contrary, WPC is not water soluble, but it is water dispersible, thus water sorption was lower
430 for these capsules than for dextran capsules. In this case, it was seen that WPC capsules
431 obtained through emulsion electrospraying and spray drying were able to better protect the
432 bioactive, which was not considerably affected after moisture exposure. However, a
433 considerable decrease of the lycopene content was seen for the WPC capsules obtained
434 through coaxial electrospraying. This was probably because, as discussed above, non-
435 continuous capsules hindered the proper incorporation of the bioactive through the coaxial
436 methodology and, thus, there was some leakage from these capsules. Therefore, part of the
437 lycopene could be on the surface of the structures exposed to humidity and, thus, more prone
438 to degradation. Regarding chitosan, it is important to highlight that it was partially acetylated
439 due to the solution preparation procedure with acetic acid as the solvent. This fact resulted in
440 increased water solubility of chitosan, affecting the water uptake of these capsules (Pillai et al.
441 2009). Even though water uptake was limited in the chitosan matrices, in comparison with the
442 other encapsulating materials, lycopene was completely degraded in these capsules, not only
443 due to moisture exposure, but also probably because of the acid conditions needed for
444 capsules formation, which led to a rapid degradation of the antioxidant. From Table 3 it can
445 also be observed that water uptake was greater for electrosprayed capsules than for capsules
446 obtained through spray drying. As it was previously seen from ATR-FTIR results, molecular
447 organization of matrices was different in electrosprayed than in spray dried capsules.
448 Particularly, it was seen that spray dried capsules presented more attractive bonds and, thus,
449 more compact structures than electrosprayed capsules. This fact could be hindering water

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450 uptake in the spray dried capsules. Moreover, the greater specific surface of the
451 electrospayed materials could be also favouring water sorption.

452 INSERT TABLE 3 ABOUT HERE

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454 3.6. Thermal stability

455 Thermal treatments are commonly employed for food processing and, thus, lycopene stability
456 under heating conditions is an important attribute to evaluate. Specifically in this work, the
457 stability of non-encapsulated lycopene and lycopene encapsulated in WPC capsules obtained
458 through emulsion electrospaying was studied, since it was previously seen that these
459 structures provided the best encapsulation efficiency and protection under moisture
460 conditions. To this end, the antioxidant activity of lycopene was evaluated according to the
461 ABTS methodology. It is important to note that WPC capsules also contained SBO and, thus,
462 the antioxidant activity of the solution lycopene/SBO was taken as the control value. Table 4
463 shows the antioxidant activity of lycopene before and after the thermal treatment (70°C
464 during 4h). From this data it was observed that, initially, both systems presented similar
465 antioxidant activity, which confirmed that lycopene was not degraded after encapsulation in
466 the WPC matrix. However, after the thermal treatment, it was seen that the ABTS inhibition
467 percentage of the non-encapsulated lycopene/SBO solution considerably decreased, when
468 compared to the lycopene/SBO extracted from the WPC capsules. This data indicated that
469 lycopene lost its antioxidant activity and, thus, its beneficial effects when it was exposed to
470 heating conditions. In contrast, WPC capsules were able to significantly reduce the bioactive
471 degradation and, thus, while the antioxidant activity of non-encapsulated lycopene was
472 reduced by ~80%, more than 60% of the antioxidant activity was retained when encapsulated
473 in WPC. These promising results seem to indicate that encapsulation using adequate matrices

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474 and encapsulation technologies would effectively have an impact in the commercial life of the
475 antioxidant.

476 INSERT TABLE 4 ABOUT HERE

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479 4. Conclusions

480 In this work it has been demonstrated, for the first time, that lycopene can be properly
481 encapsulated through electrospraying using aqueous biopolymer solutions. These results are
482 very interesting for food-related applications in which organic solvents could lead to toxicity
483 problems. The antioxidant-containing electrosprayed capsules were compared with those
484 obtained through spray drying and results showed that poor encapsulation efficiencies were
485 obtained through the latter technique, probably because of the high temperature required in
486 this case which led to a partial degradation of the antioxidant. On the contrary, emulsion
487 electrospraying could be used for lycopene encapsulation, especially when using protein
488 matrices which create more stable emulsions that do not phase separate during the
489 encapsulation process. In this case, around 75% of the antioxidant was incorporated within the
490 capsules. Coaxial electrospraying could also be used for lycopene encapsulation, although
491 some leakages were found in this case probably because of the non-continuous nature of the
492 structures developed which resulted in a worse entrapment of the core material. Chitosan was
493 also used as a matrix to encapsulate lycopene through both techniques. However, the acid
494 conditions needed in this case in order to dissolve the polymer affected lycopene stability and
495 very poor encapsulation efficiencies were found in this case (~2%). Nevertheless, other
496 bioactive ingredients with higher acid resistance could be potentially encapsulated using this
497 matrix. Finally, it was seen that WPC-based capsules were able to protect lycopene from both

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498 moisture and heating conditions and, thus, this kind of capsules could be used to increase
499 lycopene shelf life when incorporated within different food products.

500

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Figure Captions

Figure 1. Particle size distribution of the O/W emulsion without the biopolymers.

Figure 2. Selected SEM images of dextran/lycopene capsules obtained through emulsion (A), coaxial electro spraying (B) and spray drying (C); WPC/lycopene capsules obtained through emulsion (D), coaxial electro spraying (E) and spray drying (F); chitosan/lycopene capsules obtained through uniaxial electro spraying (G) and spray drying (H).

Figure 3. ATR-FTIR spectra of the different materials assayed: (A) dextran-based materials, (B) WPC-based materials and (C) chitosan-based materials. Arrows point out the most remarkable spectral changes as a consequence of lycopene incorporation. Images on the left side show the complete spectra of the materials from 4000 to 600 cm^{-1} . Images on the right side show the lycopene contribution in the spectra of the different materials.

Figure 4. UV spectra from 350 to 650 nm of lycopene extracted from electro sprayed capsules (A) and spray-dried capsules (B).

Figure 1
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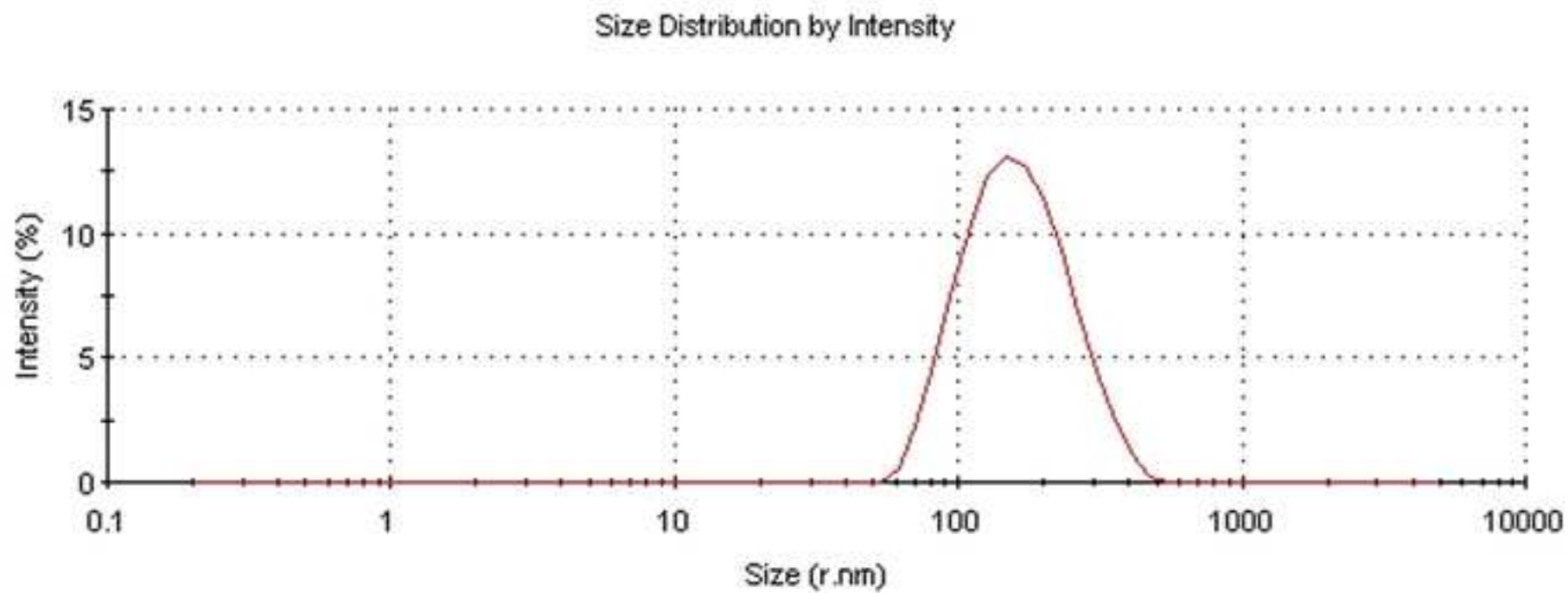


Figure 2
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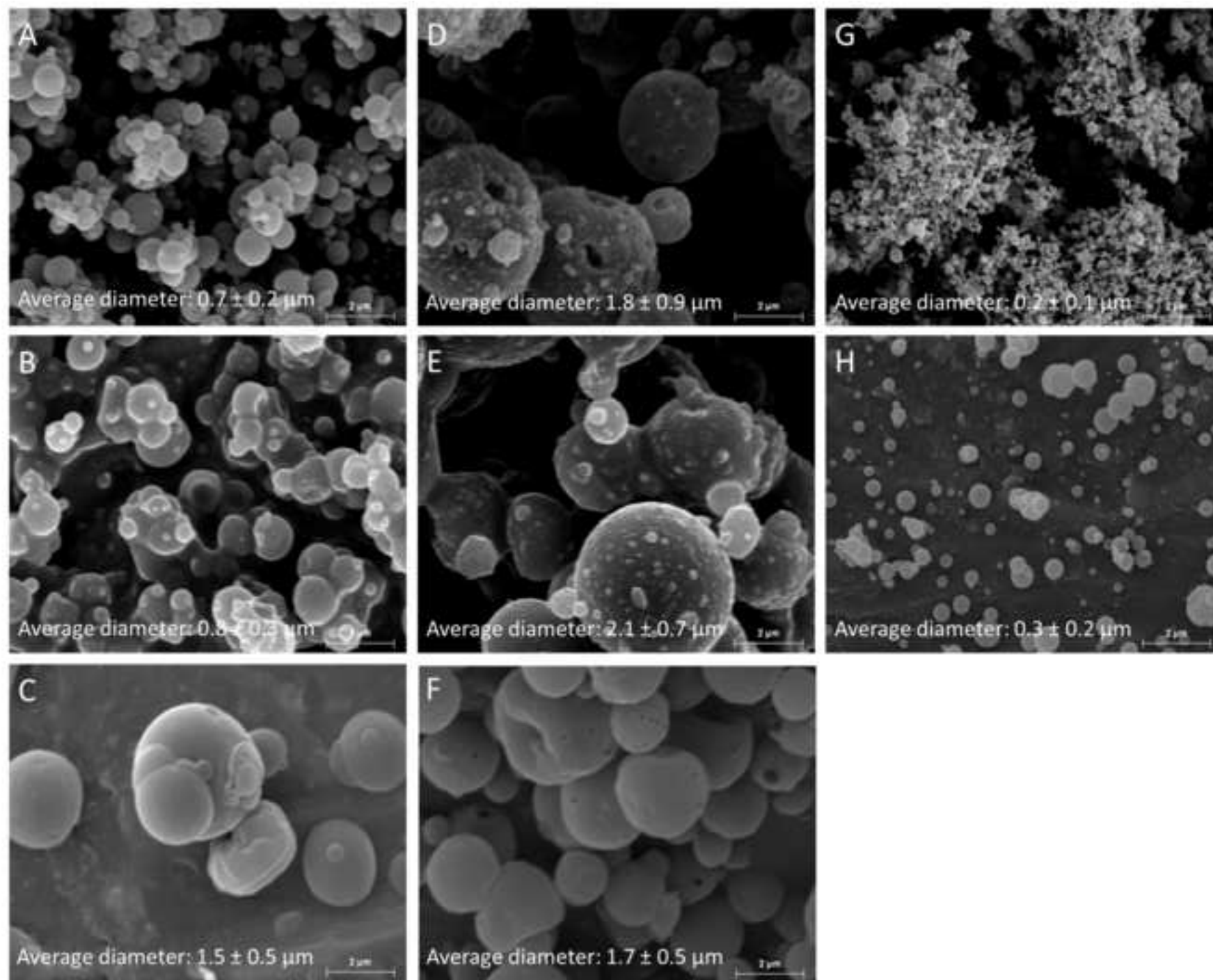


Figure 3
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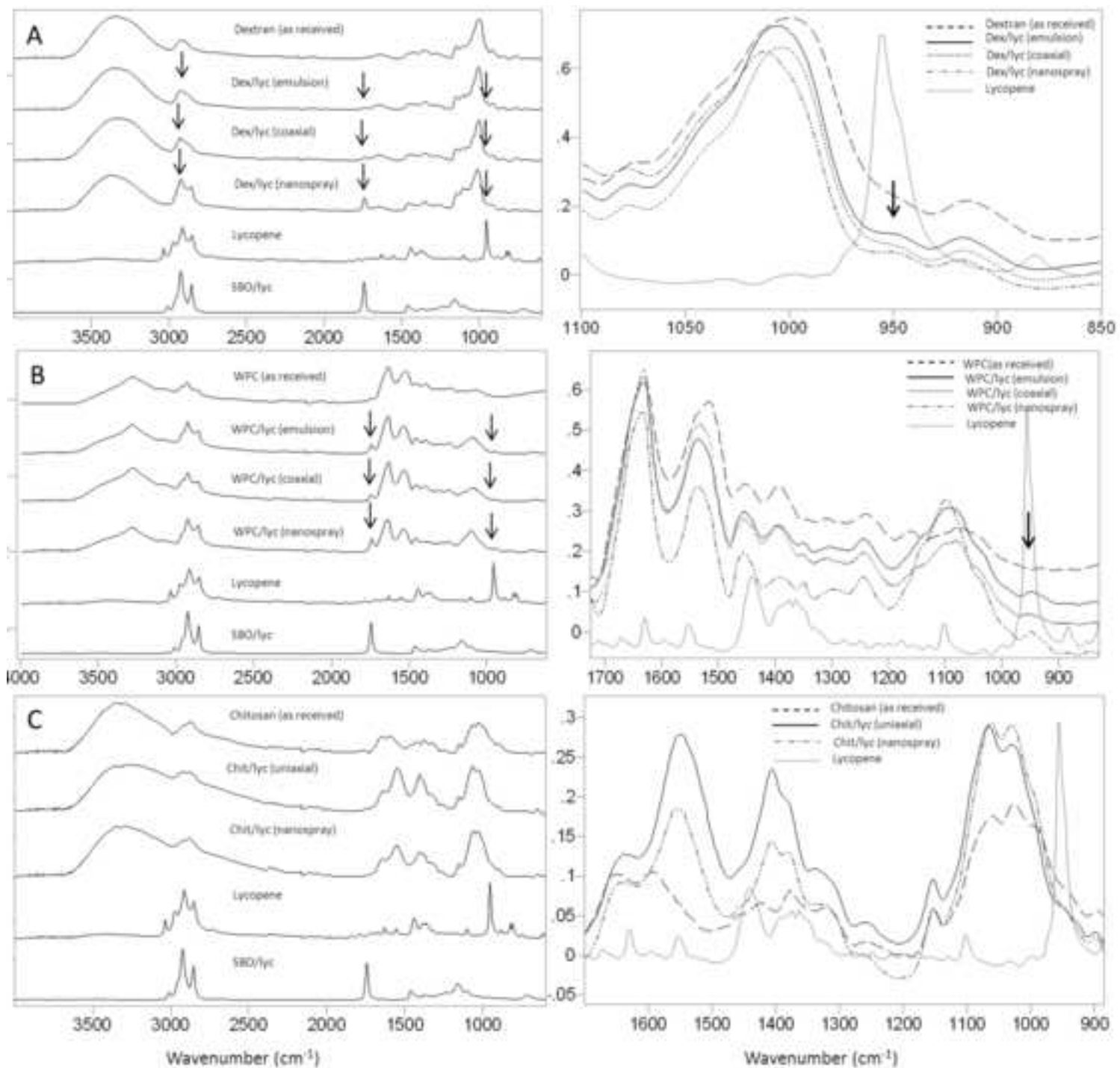


Figure 4

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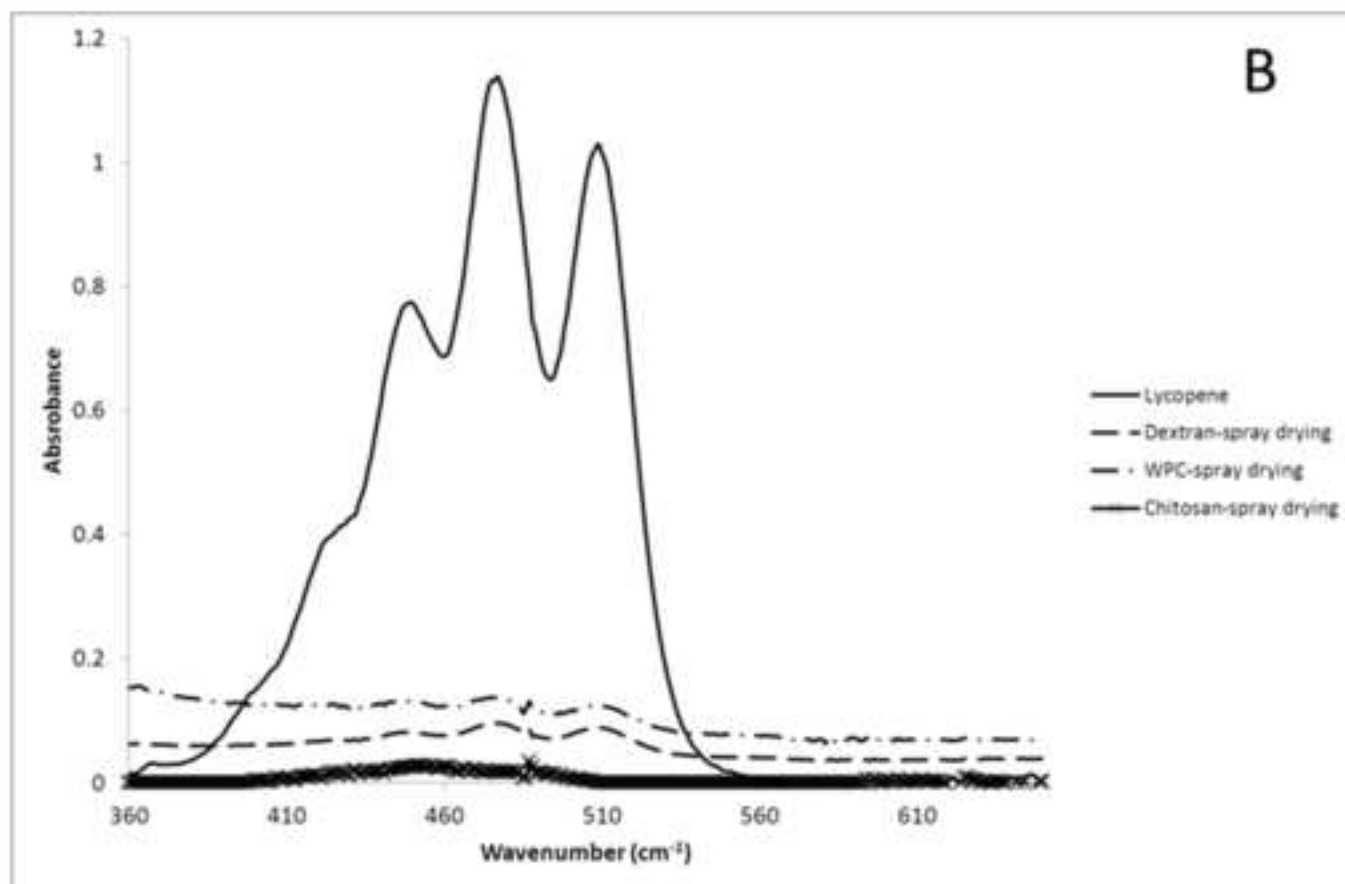
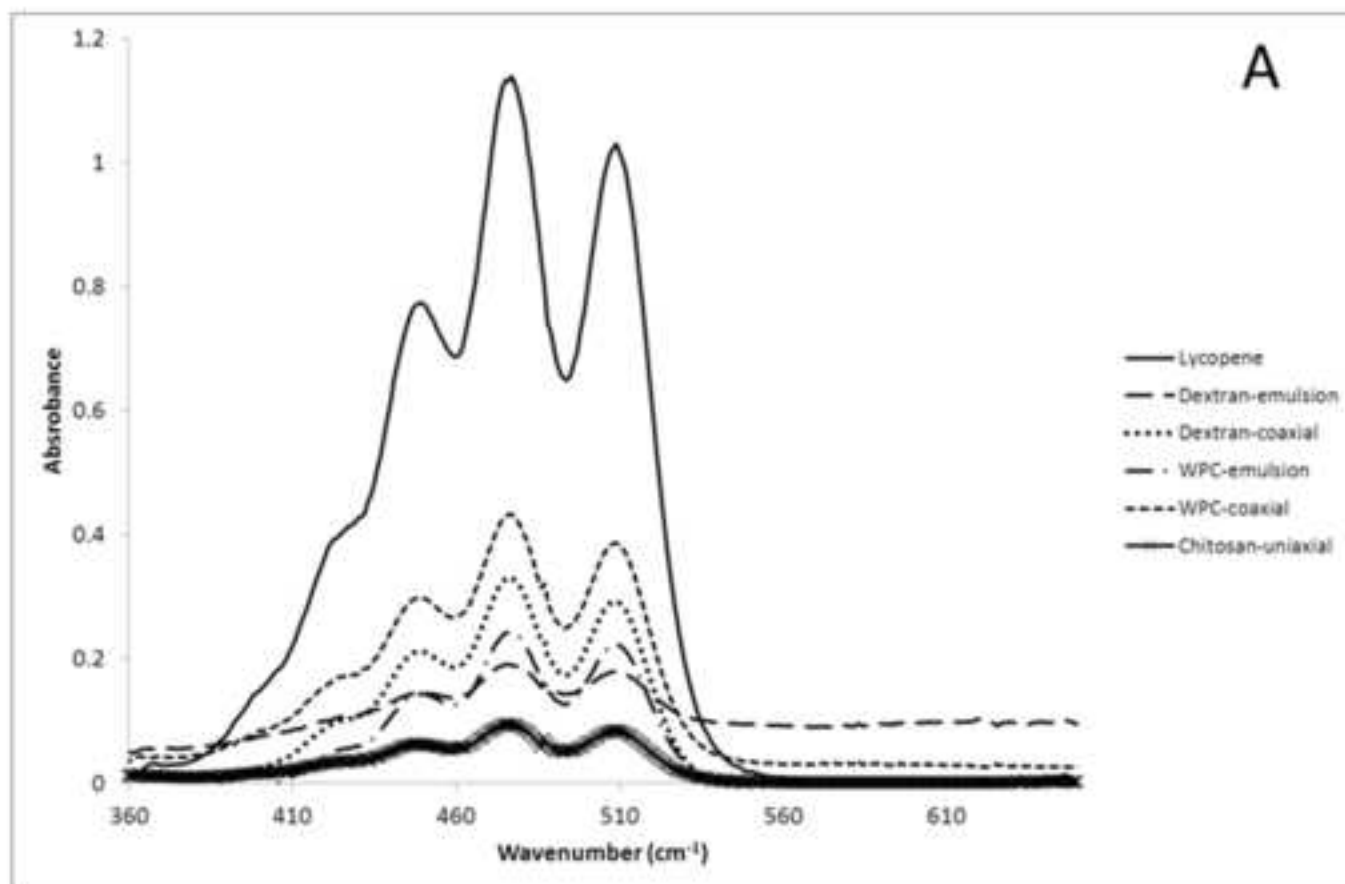


Table 1. Properties of the different electrospraying solutions

	Viscosity (cP)	Surface Tension (mN/m)	Electrical conductivity (μS)
Dextran	29.6 ± 0.2^a	35.6 ± 0.2^a	91.7 ± 0.8^a
WPC	19.0 ± 0.9^b	35.2 ± 0.2^a	2056.7 ± 35.1^b
Chitosan	49.0 ± 2.0^c	39.9 ± 0.8^b	392 ± 4.7^c
Soybean Oil/lycopene	58.9 ± 0.7^d	33.8 ± 0.1^c	0.2 ± 0.1^d
Dextran/lycopene	73.4 ± 1.1^e	36.7 ± 0.5^d	103.2 ± 1.0^e
WPC/lycopene	110.7 ± 1.3^f	36.7 ± 0.2^d	1578.3 ± 11.2^f
Chitosan/lycopene	49.3 ± 2.3^c	39.1 ± 1.3^b	398 ± 7.1^c

a-f: Different superscripts within the same column indicate significant differences among different solutions ($p < 0.05$)

Table 2. Lycopene concentration and encapsulation efficiency of the different capsules developed through electrospraying (e-sp.) and spray drying.

		Lycopene concentration		
		Solution concentration (%)	Capsules concentration (%)	Encapsulation efficiency (%)
Dextran/lycopene	Emulsion e-sp.	0.5	0.13 ± 0.04 ^a	26.0 ± 8.7 ^a
	Coaxial e-sp.	0.4	0.23 ± 0.07 ^b	57.5 ± 17.7 ^b
	Spray Drying	0.5	0.08 ± 0.02 ^a	16.0 ± 4.3 ^a
WPC/lycopene	Emulsion e-sp.	0.4	0.29 ± 0.03 ^b	72.5 ± 7.1 ^b
	Coaxial e-sp.	0.3	0.23 ± 0.06 ^b	75.2 ± 33.5 ^b
	Spray Drying	0.4	0.11 ± 0.01 ^a	27.5 ± 2.1 ^a
Chitosan/lycopene	Uniaxial e-sp.	1.5	0.04 ± 0.02 ^c	2.7 ± 4.2 ^a
	Spray Drying	1.5	0.02 ± 0.01 ^c	1.3 ± 2.3 ^c

a-c: Different superscripts within the same column indicate significant differences among lycopene capsules ($p < 0.05$)

Table 3. Equilibrium water uptake of as-received lycopene and capsules after exposure to 70% RH, lycopene concentration before (initial) and after (final) water sorption, and total lycopene loss after water sorption.

		Lycopene concentration			
		Water uptake (%)	Initial (%)	Final (%)	Lycopene loss (%)
Lycopene	raw material	2.5 ± 0.7 ^a	100 ± 0.0 ^a	36.2 ± 10.6 ^a	63.8 ± 10.6 ^a
Dextran/lycopene	emulsion e-sp.	10.6 ± 1.4 ^b	0.13 ± 0.04 ^b	0 ^b	100 ^b
	coaxial e-sp.	15.1 ± 0.8 ^c	0.23 ± 0.07 ^{bc}	0.09 ± 0.02 ^c	56.6 ± 13.6 ^a
	spray drying	6.0 ± 1.8 ^d	0.08 ± 0.02 ^b	0.06 ± 0.01 ^c	19.8 ± 15.2 ^c
WPC/lycopene	emulsion e-sp.	6.1 ± 1.0 ^d	0.29 ± 0.03 ^c	0.26 ± 0.01 ^d	11.6 ± 7.3 ^c
	coaxial e-sp.	5.2 ± 0.9 ^d	0.23 ± 0.06 ^c	0.11 ± 0.06 ^c	52.1 ± 23.6 ^{ac}
	spray drying	2.3 ± 0.6 ^a	0.11 ± 0.01 ^b	0.10 ± 0.02 ^c	16.7 ± 23.3 ^{cd}
chitosan/lycopene	uniaxial e-sp.	3.3 ± 1.2 ^a	0.41 ± 0.02 ^d	0 ^b	100 ^b
	spray drying	4.8 ± 0.2 ^d	0.04 ± 0.01 ^e	0 ^b	100 ^b

a-d: Different superscripts within the same column indicate significant differences among different lycopene capsules ($p < 0.05$)

Table 4. Antioxidant activity, according to the ABTS methodology, of non-encapsulated lycopene/SBO and encapsulated lycopene/SBO before and after the thermal treatment (70°C during 4 hours).

		% inhibition/ μg antioxidant	μM Trolox/ μg antioxidant
Lycopene/SBO	Before thermal exposure	$25.6 \pm 6.2^{\text{a}}$	$3.5 \pm 0.9^{\text{a}}$
	After thermal exposure	$5.2 \pm 0.6^{\text{b}}$	$0.5 \pm 0.1^{\text{b}}$
Lycopene/SBO from WPC capsules	Before thermal exposure	$24.1 \pm 1.8^{\text{a}}$	$3.3 \pm 0.3^{\text{a}}$
	After thermal exposure	$14.6 \pm 1.8^{\text{c}}$	$1.9 \pm 0.3^{\text{c}}$

a-c: Different superscripts within the same column indicate significant differences among antioxidant activity ($p < 0.05$)

MORPHOLOGY AND STABILITY OF EDIBLE LYCOPENE-CONTAINING MICRO- AND NANOCAPSULES PRODUCED THROUGH ELECTROSPRAYING AND SPRAY DRYING

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Abstract

In this work, lycopene was encapsulated through electrospraying and spray drying (using a microporous membrane cap) within different edible biopolymeric matrices. Specifically, dextran, a whey protein concentrate (WPC) and chitosan were used as matrix materials. As a strategy to incorporate the hydrophobic bioactive within the hydrophilic matrices, emulsion **electrospraying** and spray drying from emulsion were carried out. Moreover and, for comparison purposes, coaxial electrospraying was also performed. The electrospraying solutions properties were studied, since they do not only affect the success of the electrohydrodynamic process, but also influence the morphology of the capsules. Apart from characterizing the morphology and molecular organization of the developed capsules, the encapsulation efficiency and the lycopene stability under moisture and heating conditions were also evaluated. Results showed that even though encapsulation structures were obtained from all the matrices assayed through both processing technologies, spray drying, as a consequence of the high temperatures needed in this process, affected lycopene stability **and very poor encapsulation efficiencies were found in this case**. It was also seen that WPC presented the greatest encapsulation efficiency (**around 75%**), probably ascribed to the interactions between the biopolymer and the lycopene. Furthermore, WPC capsules were able to better protect lycopene against moisture and thermal degradation.

Keywords: lycopene, encapsulation, electrospraying, electrospinning, spray drying