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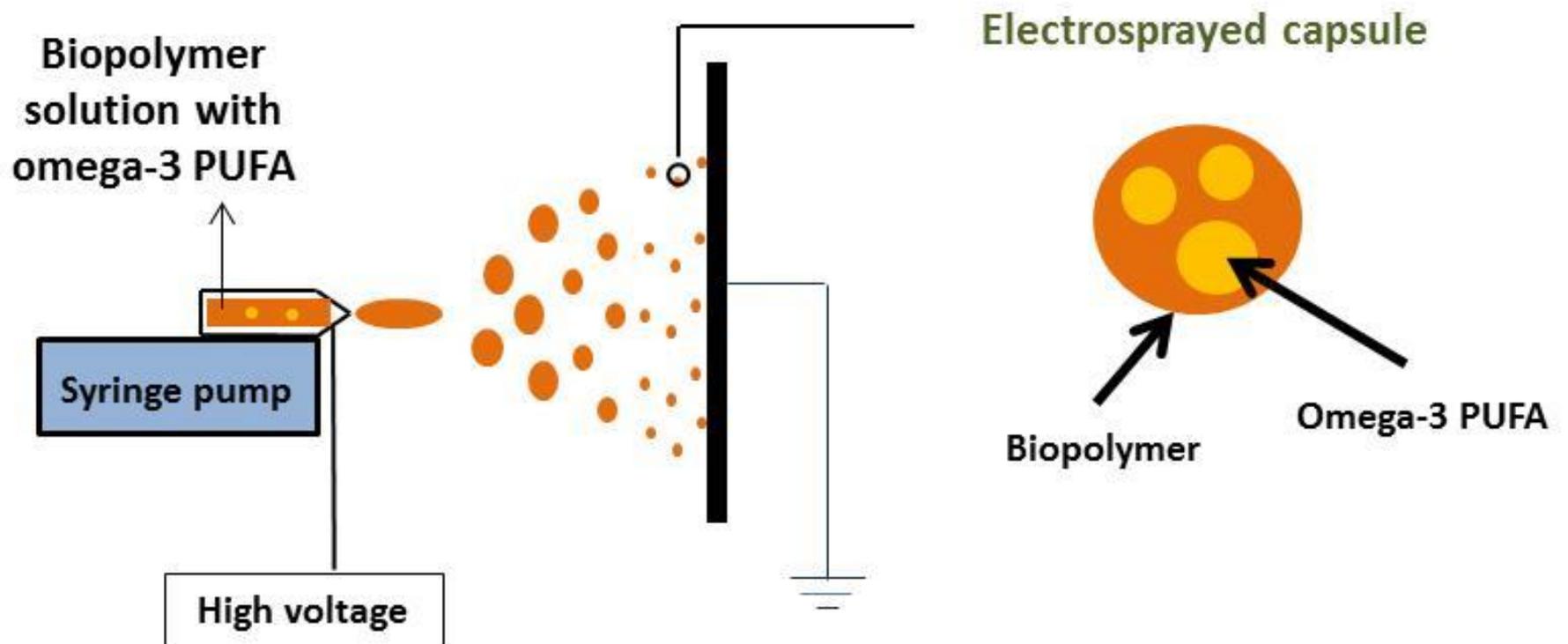
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# 1 Physicochemical characterization and oxidative stability of fish oil-loaded 2 electrospayed capsules: combined use of whey protein and 3 carbohydrates as wall materials

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## 9 **ABSTRACT**

10 The encapsulation of fish oil in electrospayed capsules using whey protein and carbohydrates  
11 (pullulan and dextran or glucose syrup) mixtures as glassy wall materials was studied. Capsules with  
12 fish oil emulsified by using only a rotor-stator emulsification exhibited higher oxidative stability than  
13 capsules where the oil was emulsified by high-pressure homogenization. Moreover, glucose syrup  
14 capsules (with a peroxide value, PV, of 19.7±4.4 meq/kg oil and a content of 1-penten-3-ol of  
15 751.0±69.8 ng/g oil) were less oxidized than dextran capsules after 21 days of storage at 20 °C (PV of  
16 24.9±0.4 meq/kg oil and 1-penten-3-ol of 1161.0±222.0 ng/g oil). This finding may be attributed to  
17 differences in oxygen permeability between both types of capsules. These results indicated the  
18 potential of both combinations of whey protein, pullulan, and dextran or glucose syrup as shell  
19 materials for the encapsulation of omega-3 PUFA in nano-microcapsules obtained by electrospaying.

20 **Keywords:** Omega-3; Lipid oxidation; Electrospaying; Nano-microencapsulation; Dextran; Glucose  
21 syrup

## 22 **1. INTRODUCTION**

23 Long chain omega-3 polyunsaturated fatty acids (PUFA) such as eicosapentaenoic (C20:5n-3) and  
24 docosahexaenoic (C22:6n-3) acids, which are mainly extracted from fish, krill or microalgae biomass,

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25 have numerous beneficial health effects on humans (Calder, 2017). Hence, and due to the low  
26 consumption of fish, krill or algae-based products by Western populations, the development of food  
27 fortified with omega-3 PUFA is still having an increasing interest for the food industry (GOED, 2015).  
28 Nevertheless, these nutritionally beneficial lipids are highly prone to oxidation (i.e. due to their high  
29 content of bis-allylic hydrogens), which limit their successful incorporation into complex food systems  
30 (e.g. containing prooxidants such as metal ions) (Jacobsen, 2015).

31 In this regard, encapsulation of omega-3 PUFA is an approach generally used to avoid their oxidative  
32 deterioration (i.e. formation of secondary volatile oxidation products which are responsible for  
33 undesirable off-flavours) (García-Moreno et al., 2016). An emerging encapsulation technique for  
34 producing omega-3 nano-microencapsulates is electrospraying (Torres-Giner et al. 2010). Contrary to  
35 spray-drying (the most employed encapsulation technique), electrospraying can be carried out at room  
36 temperature, which should result in a better stability of thermo-sensitive bioactives (Lim, 2015). The  
37 process uses a high-voltage electrostatic field to charge the surface of a biopolymer solution droplet at  
38 the end of a capillary tube. When the surface tension of the droplet is overcome by the electric field, a  
39 charged jet is ejected from the tip of the Taylor cone (formed at the end of the capillary tube) to a  
40 grounded collector. Due to the low viscoelasticity of the biopolymer solution, the jet destabilizes due to  
41 varicose instability forming fine highly charged droplets. On the way to the collector, the droplets are  
42 further disrupted due to electrostatic repulsion, which favors solvent evaporation resulting in solids  
43 particles (Ghorani & Tucker, 2015). Electrosprayed encapsulates, which present high encapsulation  
44 efficiency and large surface-to-volume ratio, are of special interest for the food industry for the  
45 encapsulation of unstable bioactive compounds such as vitamins, probiotics, antioxidants and omega-3  
46 fatty acids. Furthermore, due to their reduced size, these novel encapsulates exhibit a higher  
47 bioaccessibility than traditional capsules (Jacobsen et al., 2018).

48 To the best of the authors' knowledge, omega-3 fatty acids have only been encapsulated by  
49 electrospraying when using proteins such as zein, whey protein concentrate, soy protein isolate, and  
50 gelatin as shell material (Gómez-Mascaraque & López-Rubio, 2016; Moomand & Lim, 2015; Torres-  
51 Giner et al., 2010). In the authors' previous work, the potential of dextran as a biopolymer shell to

52 produce fish oil-loaded electrospayed capsules was reported. However, further optimization of  
53 dextran solutions was required to improve the physical stability of the emulsion as well as the oil  
54 entrapment within the capsules (García-Moreno et al., 2017a). To this end, an interesting approach to  
55 be evaluated is the combination of both carbohydrates, which usually act as filler or matrix-forming  
56 material, and proteins, which exhibit emulsifying properties and are effective film-formers (Augustin  
57 & Oliver, 2014). Dairy proteins (e.g. whey protein or casein), which also exhibit antioxidant properties  
58 (Adjonu et al. 2014), are usually combined with carbohydrates (i.e. glucose syrup, lactose,  
59 maltodextrin, starch) in order to obtain fish oil-loaded microencapsulates by spray-drying with  
60 enhanced properties (Encina et al. 2016). For instance, Aghbashlo et al. (2012) reported the production  
61 of microcapsules by spray-drying with significantly higher encapsulation efficiencies using mixtures  
62 of skim milk powder and lactose or sucrose (70% and 30%, respectively) when compared to the use of  
63 only skim milk powder. Likewise, Ramakrishnan et al. (2013) found that the replacement of part of  
64 whey protein by maltodextrin as wall materials increased the oxidative stability of fish oil-loaded  
65 microcapsules. This was attributed to lower oxygen permeability of the shell material composed of  
66 maltodextrin. Furthermore, the incorporation of high-molecular weight carbohydrates (e.g. starch,  
67 maltodextrin, dextran) also increases the glass transition temperature of the wall material, which  
68 implies that the shell material will be in glassy state in a broader range of temperature (Schutyser et al.  
69 2012). Glassy state of the protein-carbohydrate matrix is preferred to rubbery state due to its lower  
70 free volume, which restricts diffusion of oxygen and other prooxidants (i.e. trace of metals) enhancing  
71 the oxidative stability of the encapsulates (Hu, 2016). In addition, the use of carbohydrates as  
72 encapsulating material, which are not digested in the stomach, will allow a more targeted delivery of  
73 omega-3 PUFA (e.g. in the small intestine where most absorption occurs) (Fathi et al. 2014).

74 In the light of the above, this work aimed at investigating the encapsulation of fish oil by  
75 electrospaying using combinations of whey protein and carbohydrates as biopolymers. Dextran and  
76 glucose syrup were selected as carbohydrates due to their appropriate properties to form  
77 electrospayed capsules (García-Moreno et al., 2017a) and to their successful use in spray-dried  
78 capsules loaded with oils rich in omega-3 PUFA (Tamm et al. 2016), respectively. First, the influence

79 of total concentration of biopolymers and carbohydrate to protein ratio on oil droplet size,  
80 electro spraying flow rate, and morphology of the capsules was assessed in lab scale. Secondly, the  
81 approach used to emulsify the oil (i.e. high pressure homogenization or rotor-stator emulsification) in  
82 the optimized biopolymers solution was studied. Particularly, the ability to entrap the oil and the oil  
83 distribution of capsules produced by a high-throughput electro spraying process in pilot-plant scale was  
84 investigated. Finally, the protective effect against oxidative degradation of the different encapsulating  
85 matrices used was investigated during storage of the fish oil-loaded nano-microcapsules.

## 86 **2. MATERIALS AND METHODS**

### 87 **2.1 Materials**

88 Dextran (molecular weight = 70,000 Da, dextran70) was generously provided by Pharmacosmos A/S  
89 (Holbaek, Denmark). Glucose syrup (DE38, C\*Dry 1934) was kindly provided by Cargill Germany  
90 GmbH (Krefeld, Germany). Pullulan (molecular weight = 200,000 Da) was donated by Hayashibara  
91 Co., Ltd. (Okayama, Japan). Whey protein concentrate (WPC), under the commercial name of  
92 Lacprodan® DI-8090, was provided by ARLA Food Ingredients (Viby, Denmark). Citrem (citric acid  
93 ester without antioxidants) was provided from Danisco (Copenhagen, Denmark). The peroxide value  
94 (PV) of the citrem used was  $2.3 \pm 0.1$  meq/kg oil. Commercial cod liver oil was donated by Maritex  
95 A/S, subsidiary of TINE, BA (Sortland, Norway) and stored at  $-40$  °C until use. The fatty acid  
96 composition of the fish oil was determined by fatty acid methylation (AOCS, 1998a) followed by  
97 separation through GC (AOCS, 1998b). It was (major fatty acids only) as follows: C16:0, 9.5%;  
98 C16:1, 8.7%; C18:1, 16.3%; C20:1, 12.6%; C20:5, 9.2%; and C22:6, 11.4%. The tocopherol content  
99 of the fish oil was:  $\alpha$ -tocopherol,  $200 \pm 3$   $\mu\text{g/g}$  oil;  $\beta$ -tocopherol,  $5 \pm 1$   $\mu\text{g/g}$  oil;  $\gamma$ -tocopherol,  $96 \pm 3$   $\mu\text{g/g}$   
100 oil; and  $\delta$ -tocopherol,  $47 \pm 1$   $\mu\text{g/g}$  oil (AOCS, 1998c). PV of the fish oil used was  $0.4 \pm 0.1$  meq/kg oil.  
101 All other chemicals and solvents used were of analytical grade.

## 102 **2.2 Preparation of biopolymer solutions containing fish oil**

### 103 **2.1 For optimization of capsules morphology in lab scale**

104 Electro spraying solutions containing fish oil (20 wt.% with respect to biopolymer), WPC (1 wt.%),  
105 and carbohydrates (pullulan and dextran or glucose syrup) at different concentrations (1-5 wt.%  
106 pullulan and 15 or 20 wt.% dextran or 15 wt.% glucose syrup) were tested in lab scale in order to  
107 optimize capsule morphology. First, WPC, pullulan, and dextran or glucose syrup were dissolved in  
108 distilled water by stirring overnight at 500 rpm. Secondly, fish oil was added slowly to the  
109 biopolymers solution during mixing at 16,000 rpm using an Ystral mixer (Ystral GmbH, Ballrechten-  
110 Dottingen, Germany). The fish oil was added during the first minute of mixing, and the total mixing  
111 time was 3 min. Further homogenization was done on a microfluidizer (M110L Microfluidics,  
112 Newton, MA, USA) equipped with a ceramic interaction chamber (CIXC, F20Y, internal dimension  
113 75  $\mu\text{m}$ ). Emulsions were homogenized at a pressure of 9000 psi, running 3 passes. Samples were used  
114 immediately after production for electro spraying processing in lab scale and for droplet size analysis.

### 115 **2.2 For production in pilot plant**

116 Biopolymer solutions containing fish oil for processing in pilot plant were prepared following two  
117 different approaches to emulsify the oil. In the first approach, fish oil was emulsified by using high  
118 pressure homogenization. Briefly, pullulan and dextran or glucose syrup were dissolved in distilled  
119 water under constant stirring (500 rpm) at room temperature. Fish oil was added as 10 wt.% fish oil-  
120 in-water emulsion stabilized with 1 wt.% WPC and 1 wt.% citrem at pH 7. The homogenization  
121 process was carried out by using an Ystral mixer followed by microfluidizer (M110L Microfluidics,  
122 Newton, MA, USA) as described above. The biopolymer solutions and the fish oil-in-water emulsion  
123 were mixed under nitrogen atmosphere by using magnetic stirring (500 rpm) for 30 min at 5 °C in the  
124 dark. Finally, the resulting emulsion was passed 3 times through microfluidizer (M110L  
125 Microfluidics, Newton, MA, USA) at a pressure of 9,000 psi. The resulting electro spraying solutions  
126 contained 20 wt.% fish oil (with respect to biopolymer). The samples were subsequently characterized  
127 for droplet size analysis and dried by electro spraying assisted by pressurized air using the  
128 Fluidnatek<sup>TM</sup> LE500 Capsultek pilot tool by Bioinicia S.L. (Valencia, Spain). Dextran solution was

129 electrospayed two days after production, whereas glucose syrup was processed after three days. Both  
130 samples were kept at 4 °C until electrospaying was carried out.

131 In the second approach, fish oil was emulsified by only using a rotor-stator emulsification. In brief,  
132 carbohydrates (pullulan and dextran or glucose syrup) together with WPC were dissolved in distilled  
133 water and stirred overnight using magnetic stirrer at 500 rpm. Then, the biopolymer solution was  
134 passed through microfluidizer (M110L Microfluidics, Newton, MA, USA) 3 times at 9,000 psi.  
135 Citrem and fish oil were added slowly, under nitrogen atmosphere, into the resulting biopolymer  
136 solution during mixing at 17,500 rpm using an Ultraturrax T-25 homogenizer (IKA, Staufen,  
137 Germany). The fish oil was added during the first 5 min of mixing, and the total mixing time was 8  
138 min. The resulting electrospaying solutions also contained 20 wt.% fish oil (with respect to  
139 biopolymer). Samples were used immediately after production for processing in pilot plant equipment  
140 LE500 Capsultek (Bioinicia and Fluidnatek®, Valencia, Spain).

### 141 **2.3 Droplet size distribution of solutions**

142 Droplet sizes were measured by laser diffraction in a Mastersizer 2000 (Malvern Instruments, Ltd.,  
143 Worcestershire, UK). Solutions were diluted in recirculating water (3000 rpm), until it reached an  
144 obscuration of 12%. The refractive indices of sunflower oil (1.469) and water (1.330) were used as  
145 particle and dispersant, respectively. Results were given in surface area mean diameter ( $D_{3,2}$ ) and 90%  
146 percentile ( $d_{0,9}$ ). Measurements were made in triplicate.

### 147 **2.4 Electrospaying process**

148 In lab scale, the electrospaying process was carried out at room temperature by adding the biopolymer  
149 solutions containing the fish oil to a syringe, which was placed in a syringe pump (New Era Pump  
150 Systems, Inc., USA). A 16 G needle (Proto Advantage, Canada) was used. A high-voltage electrostatic  
151 field was applied between the spinneret of the syringe and a 15 × 15 cm collector plate made of  
152 stainless by using a high voltage power supply (Gamma High Voltage Research, USA). An horizontal  
153 conformation was selected and the distance between the syringe tip and the collector plate was 15 cm.

154 The flowrate, ranging from 0.003 to 0.012 mL/min, and applied voltage, varying from 17 to 20 kV,  
155 were optimized in order to stabilize the Taylor cone and avoid dripping or droplets in the collector.  
156 In pilot plant scale, biopolymers solutions containing fish oil were subjected to electro spraying  
157 assisted by pressurized air using the patent pending Fluidnatek™ LE500 Capsultek pilot tool  
158 (Bioinicia S.L., Valencia, Spain). Solutions were processed using flowrates between 1.5 and 1.8  
159 mL/min and voltages between 10 and 15 kV. The collection of the encapsulated powder was carried  
160 out in a grounded cyclonic collector as a free flowing powder. Temperature was maintained at 24 °C  
161 and relative humidity at 40 %. The production batches had a duration of 40 min. The powder collected  
162 in the different batches (for the same type of capsule) were blended together in order to have a  
163 homogeneous final sample. Dextran and glucose syrup capsules were coded as D-HPH or G-HPH and  
164 D-RSE or G-RSE when the oil was emulsified by using high-pressure homogenization of rotor-stator  
165 emulsification, respectively.

## 166 **2.5 Characterization of electro sprayed capsules**

### 167 **2.5.1 Morphology**

168 The morphology of the different types of electro sprayed capsules produced was investigated using  
169 scanning electron microscopy (SEM) (Phenom Pro, Phenom-World B.V., Eindhoven, The Netherlands).  
170 Approximately 0.5×0.5 cm of the capsules aluminium sheet was placed on carbon tape and sputter  
171 coated with gold, 8 s, 40 mA utilizing a Q150T Quorum Coater (Quorum Technologies Ltd, East Sussex,  
172 UK). The capsule diameter distribution was determined from the micrographs by using the open  
173 source image processing program ImageJ (National Institutes of Health). One hundred random  
174 capsules were measured.

### 175 **2.5.2 Encapsulation efficiency (EE)**

176 The efficiency of the encapsulation was determined by measuring the non-encapsulated oil, which was  
177 extracted according to Westergaard (2004) with some modifications. Briefly, 1 g of electro sprayed  
178 capsules was immersed in 5 mL heptane and shaken at 100 rpm for 2 min. Then, the suspension was  
179 filtered and the retained solid washed three times with 2 mL heptane. From the recovered liquid, the  
180 heptane was evaporated and the amount of extracted oil was weighted. Analyses were carried out in

181 triplicate. Results are expressed in wt.% of the oil encapsulated mass against the total oil content of the  
182 capsules.

### 183 **2.5.3 Oil distribution**

184 The droplet size distribution of the fish oil-loaded electrospayed capsules after re-dispersion in  
185 distilled water was measured as previously described in section 2.3. For that purpose, 1 g of capsules  
186 was dissolved in 15 mL of distilled water at room temperature under magnetic stirring (100 rpm) for  
187 30 min. The resulting dispersion was filtered in order to remove the possible rest of capsules.

188 Moreover, oil distribution in the capsules was also analyzed by fluorescence microscopy.  
189 Electrospaying solutions were stained with Nile red and fluorescein isothiocyanate to visualize fish  
190 oil and WPC, respectively. The nano-microcapsules were directly electrospayed in a coverslip and  
191 then covered with a glass slide. Then, the distribution of fish oil and WPC within the nano-  
192 microcapsules was analyzed with a fluorescence microscope Olympus BX53 fitted with a Retiga-6000  
193 monochrome camera (Olympus Danmark A/S, Ballerup, Denmark). Fluorescence was measured after  
194 irradiation at 580 nm or 488 nm. Images were captured using the Olympus cellSens Dimension V1  
195 software and further processed in Adobe Photoshop CS6.

### 196 **2.5.4 Glass transition temperature**

197 Glass transition temperature ( $T_g$ ) of the capsules was determined using a Discovery DSC (TA  
198 Instruments, New Castle, USA). For each scan, 2-5 mg of capsules were hermetically sealed in an  
199 aluminium pan and tested against an identical empty pan. Samples were cooled and held isothermally  
200 at -80 °C for 10 min, then heated to 200 °C with a ramp rate of 10 °C/min under nitrogen atmosphere  
201 (50 mL/min). TRIOS software (TA Instruments, New Castle, USA) was used to determine  $T_g$  from  
202 the midpoint of the heat flow derivative.

### 203 **2.5.5 Oxidative stability**

204 For lipid oxidation measurements, immediately after receiving the samples from Bioinicia and  
205 Fluidnatek® (Valencia, Spain), the capsules were stored in 10 mL vial at 20 °C in the dark for 21  
206 days. Each vial contained approximately 2.2 g of capsules in order to maintain a similar headspace.  
207 Samples were taken at day 0, 3, 8, 14 and 21 for analysis.

#### 208 2.5.5.1 Attenuated total reflection – Fourier transform infrared (ATR-FTIR)

209 ATR-FTIR spectra of the capsules were obtained by using Spectrum 100 FT-IR Spectrometer  
210 (PerkinElmer, Waltham, USA). Approximately 10 mg of capsules were placed on top of the  
211 ZnSe/diamond crystal and good contact was assured by using the Universal ATR Sampling Accessory  
212 (PerkinElmer, Waltham, USA). All spectra were recorded within the wavenumber range of 4000-600  
213  $\text{cm}^{-1}$  by averaging 20 scans at 4  $\text{cm}^{-1}$  resolution. Measurements were performed in triplicate. Results  
214 were normalized to the initial intensity for a better comparison among the different capsules.

#### 215 2.5.5.2 Determination of oil content and peroxide value

216 Oil was extracted from approximately 0.5 g of capsules according to Bligh and Dyer method using a  
217 reduced amount of the chloroform/methanol (1:1, wt.%) solvent (Bligh & Dyer, 1959). Two  
218 extractions were made from each sample. Peroxide value was determined on lipid extracts using the  
219 colorimetric ferric-thiocyanate method at 500 nm as described by Shantha and Decker (1994). Results  
220 were expressed as milliequivalents of peroxides per kg of oil.

#### 221 2.5.5.3 Volatiles secondary oxidation products – Dynamic headspace GC-MS

222 Approximately 0.4 g of capsules and 10 mg internal standard (4-methyl-1-pentanol, 30  $\mu\text{g/g}$  water)  
223 were weighted out in a 100 mL purge bottle, to which 5 mL of distilled water and 1 mL antifoam  
224 (Synperonic 800  $\mu\text{L/L}$  water) were added. The bottle was heated to 45°C in a water bath while purging  
225 with nitrogen (flow 250 mL/min, 30 min). Volatile secondary oxidation products were trapped on  
226 Tenax GR tubes. The volatiles were desorbed again by heating (200°C) in an Automatic Thermal  
227 Desorber (ATD-400, Perkin Elmer, Norwalk, CN), cryofocused on a cold trap (-30°C), released again  
228 (220°C), and led to a gas chromatograph (HP 5890IIA, Hewlett Packard, Palo Alto, CA, USA;  
229 Column: DB-1701, 30 m x 0.25 mm x 1.0  $\mu\text{m}$ ; J&W Scientific, CA, USA). The oven program had an  
230 initial temperature of 45°C for 5 min, increasing with 1.5°C/min until 55°C, with 2.5°C/min until  
231 90°C, and with 12.0°C/min until 220°C, where the temperature was kept for 4 min. The individual  
232 compounds were analyzed by mass-spectrometry (HP 5972 mass-selective detector, Agilent  
233 Technologies, USA; electron ionization mode, 70 eV; mass to charge ratio scan between 30 and 250).  
234 The individual compounds were identified by both MS-library searches (Wiley 138 K, John Wiley and

235 Sons, Hewlett-Packard) and by authentic external standard and quantified through calibration curves.  
236 The external standards employed were 2-ethyl-furan, 1-penten-3-one, pentanal, 1-penten-3-ol, (*E*)-2-  
237 pentenal, hexanal, 2-hexenal, heptanal, 2-pentyl-furan, (*E,E*)-2,4-heptadienal, and nonanal (Sigma-  
238 Aldrich, Brøndby, Denmark). Samples were analyzed in triplicate.

## 239 **2.6 Statistical analysis**

240 Statgraphics Centurion XV (Statistical Graphics Corp., Rockville, MD, USA) was used for data  
241 analysis. Data were expressed as mean  $\pm$  standard deviation. Firstly, multiple sample comparison  
242 analysis was performed to identify significant differences between samples. Secondly, mean values  
243 were compared by using the Tukey's test. Differences between means were considered significant at  $p$   
244  $< 0.05$ .

## 245 **3. RESULTS AND DISCUSSION**

### 246 **3.1 Optimization of biopolymer solutions**

247 The properties of the biopolymer solutions (e.g. viscosity, conductivity, and surface tension) together  
248 with the processing variables (i.e. voltage and flowrate) have a high influence on the electrospaying  
249 process (e.g. stability of Taylor cone and morphology of capsules). The solution properties are mainly  
250 determined by the type of biopolymers used (i.e. molecular weight and concentration) as well as by  
251 the type of solvent (Drosou et al. 2017). Taking this into account, the composition of dextran and  
252 glucose syrup solutions containing fish oil were first optimized in the lab before scaling-up. Pullulan  
253 was added to both type of solutions as thickening agent since it allowed to increase the stability of the  
254 Taylor cone, avoiding dripping and droplets in the collector while also working at higher flow rate.  
255 Both high solid content in solutions (wt.% of biopolymers and oil) as well as high flow rate are desired  
256 in order to increase the throughput of electrospaying process. With a similar objective, Pérez-Masiá et  
257 al. (2014) employed gums (e.g. guar and xanthan gum) in order to increase the viscosity of  
258 carbohydrate (i.e. resistant starch or maltodextrin) solutions, which allowed the formation of capsules  
259 by electrospaying. However, these authors also observed the formation of a continuous film together  
260 with the capsules, which was attributed to the ability of gums to retain water leading to an incomplete

261 drying of the droplets. This phenomenon was not observed in this study when using pullulan (Fig. 1),  
262 which may be attributed to a lower retention of water by pullulan when compared to gum facilitating  
263 the drying process.

264 Fig. 1 shows the morphology of the nano-microstructures obtained when varying the concentration of  
265 pullulan (1-2 wt.%) and dextran (15-20 wt.%) in an aqueous solution also containing 1 wt.% WPC and  
266 20 wt.% of emulsified fish oil (with respect to biopolymers). It was observed that when using 2 wt.%  
267 pullulan and 20 wt.% dextran, some capsules were obtained, but they were interconnected with  
268 abundant fibers (Fig. 1a). Nevertheless, reducing the concentration of biopolymers, especially pullulan  
269 which has been reported to have an extraordinary spinnability leading to fiber formation (García-  
270 Moreno et al. 2017), considerably decreased the polymer chain entanglements avoiding the formation  
271 of fibril defects. For instance, decreasing the concentration of dextran to 15 wt.% (2 wt.% pullulan)  
272 significantly reduced the formation of fibers (Fig. 1b). Likewise, fibers were almost not observed  
273 when reducing the content of pullulan to 1 wt.% (20 wt.% dextran) (Fig. 1c), and no fibril defects  
274 were found in the capsules obtained when using a solution of 1wt.% pullulan and 15 wt.% dextran  
275 (Fig. 1d). Capsules are preferred to fibers as delivery systems due to their ability to easily disperse in  
276 the food matrix (Pérez-Masiá et al. 2015). The dispersion of fibers is more challenging due to their  
277 continuous and interconnected morphology. As expected, decreasing pullulan concentration from 2 to  
278 1 wt.% slightly reduced the electrospraying flow rate (from 0.012 to 0.010 mL/min) (Table 1).  
279 Moreover, decreasing dextran concentration in the solution led to a significantly ( $p<0.05$ ) more  
280 effective droplet disruption in the high pressure homogenizer (Table 1), mainly due to a lower  
281 viscosity of the solution. Smaller droplet sizes are desired in order to enhance the entrapment of the oil  
282 within the wall material matrix (Ramakrishnan et al. 2013).

283 For the glucose syrup solution, only the pullulan content was varied from 2 to 5 wt.%, while the  
284 content of the glucose syrup was kept constant at 15 wt.% in order to have the same concentration as  
285 for dextran in the previous solution. Fig. 2 shows that only capsules were obtained when using either 2  
286 or 4 wt.% pullulan (Fig. 2a,b), whereas fibril defects appeared between the capsules due to more  
287 polymer chain entanglements when increasing pullulan concentration to 5 wt.% (Fig. 2c). In order to

288 select between 2 and 4 wt.% pullulan, flow rate and oil droplet size values were considered. Although  
289 increasing pullulan content from 2 to 4 wt.% led to a significantly ( $p < 0.05$ ) larger droplet size, it also  
290 allowed a considerable improvement of the electro spraying flow rate (from 0.003 to 0.007 mL/min)  
291 (Table 1). It is worth noting the lower electro spraying flow rate for the glucose syrup solution when  
292 compared to the dextran solution, despite the higher content of pullulan employed (4 vs. 1 wt.%,  
293 respectively). This might be attributed to the lower number of polymer chain entanglements in the  
294 glucose solution compared to dextran, as a consequence of the different molecular weights of the  
295 carbohydrates used (70 kDa for dextran and 12.5 kDa for glucose syrup, as reported by Pharmacosmos  
296 A/S and Cargill Germany GmbH respectively) (Pérez-Masiá et al., 2014).  
297 Finally, the replacement of half of the WPC used as emulsifier by an efficient surfactant such as citrem  
298 led to significant ( $p < 0.05$ ) smaller oil droplet size when compared to electro spraying solutions  
299 containing only WPC, both in dextran and glucose syrup solutions (Table 1). This should favor both  
300 the physical stability of the solution until drying as well as the efficiency of the encapsulation process.  
301 Therefore, taken together, optimum solutions for further production in pilot-plant scale were selected  
302 as: i) 0.5 wt.% WPC, 0.5 wt.% citrem, 1 wt.% pullulan, and 15 wt.% dextran, and ii) 0.5 wt.% WPC,  
303 0.5 wt.% citrem, 4 wt.% pullulan, and 15 wt.% glucose syrup.

## 304 **3.2 Physicochemical properties of the capsules**

### 305 **3.2.1. Morphology**

306 Fig. 3 shows that the capsules obtained in pilot-plant for the two types of carbohydrates (dextran or  
307 glucose syrup) and for the two approaches used to emulsify the oil (high pressure homogenization or  
308 rotor-stator emulsification) had a spherical shape with no fibril defects. In general, dextran capsules  
309 showed no clear cracks or fissures, although small holes could be observed at their surface, especially  
310 for capsules where the oil was incorporated by using high pressure homogenization (Fig. 3a,b).  
311 Glucose syrup capsules presented a less smooth surface when compared to dextran capsules, as  
312 indicated by the presence of some fissures and larger holes on the surface. This phenomenon was also  
313 observed to a higher extent for capsules with oil emulsified by high pressure homogenization (Fig.

314 3c,d). These holes may be explained by the presence of surface oil, which is discussed below in  
315 section 3.2.2.

316 The four type of capsules produced presented a broad size range, varying from submicron particles to  
317 microcapsules up to 10  $\mu\text{m}$  (Fig. 3). Nonetheless, most of the capsules (approximately 70 %) had a  
318 size lower than 3  $\mu\text{m}$ . Although no significant differences were observed between G-HPH and G-RSE  
319 capsules, D-HPH capsules showed a larger diameter when compared D-RSE capsules (Fig. 3a,b).  
320 This may be attributed to the higher flow rate and voltage used to produce D-RSE capsules compared  
321 to D-HPH (1.8 vs. 1.5 mL/min and 15 vs. 10 kV), which favoured the formation of satellite droplets  
322 (Hartman et al. 2000).

323 Overall, the capsules produced in pilot-plant presented a larger diameter when compared to capsules  
324 produced in lab scale, where approximately 60-70% of the capsules were below 1  $\mu\text{m}$  (see Fig. S1 of  
325 the Supplementary material). In order to increase throughput, in pilot-plant electro spraying the  
326 solution is impelled into the electric field by pressurized air, hence the solution droplet size cannot be  
327 reduced to the level obtained by conventional electro spraying process in lab scale. In any case, the  
328 electro sprayed capsules obtained in this study showed a reduced size compared to microcapsules  
329 loaded with fish oil and obtained by spray-drying, which have been reported to have diameters  
330 between 14.2-18.1  $\mu\text{m}$  (Drusch, 2007) or 17.9-23.0  $\mu\text{m}$  (Carneiro et al. 2013). Capsules with a reduced  
331 size are preferred for incorporation into a food matrix since they might be easier to disperse and could  
332 have a lower effect on product quality (e.g. texture). Moreover, particles with a lower diameter present  
333 a larger specific surface area, which might enhance the release profile of the bioactive compound. On  
334 the other hand, a larger surface-to-volume ratio also implies an increase of the contact surface between  
335 lipids and prooxidants, which negatively affects oxidative stability of the capsules (Jacobsen et al.  
336 2018).

### 337 **3.2.2 Oil encapsulation and distribution**

338 Oil encapsulation and distribution within the shell material determines the accessibility of prooxidants  
339 to the oil as well as the available contact surface, which might have a great importance on the  
340 oxidative stability of the capsules (Drusch & Berg, 2008). The longer time spent before the drying of

341 the parent emulsions produced by high-pressure homogenization compared to emulsions produced by  
342 rotor-stator emulsification (which were produced in situ in the electro-spraying pilot-plant) explain the  
343 significantly ( $p < 0.05$ ) higher  $D_{3,2}$  values of the reconstituted capsules obtained from the former  
344 emulsions (Table 2). However, the correlation between droplet size and EE is not clear since EE  
345 values were higher for the capsules containing oil emulsified by rotor-stator emulsification, besides  
346 the significantly higher  $D_{0,9}$  value of these capsules (Table 2).

347 Dextran capsules showed significantly higher EE values than glucose syrup capsules for both type of  
348 emulsification approaches, which correlated well with the lower  $D_{3,2}$  and  $D_{0,9}$  values of the dextran  
349 capsules when compared to glucose syrup capsules (Table 2). Glucose syrup nano-microcapsules  
350 presented large holes on the surface (Fig. 3a,b), which in fish oil-loaded microcapsules produced by  
351 spray-drying has been related to the presence of non-encapsulated oil droplets (Drusch & Berg, 2008).  
352 Fig. 4 shows how fish oil is distributed in electro-sprayed nano-microcapsules containing oil emulsified  
353 by high-pressure homogenization and produced in lab scale. Fig.4a1,b1 show the location of fish oil  
354 (in red) and Fig.4a2,b2 show the location of WPC (in green). It was observed that oil droplets were  
355 entrapped within the shell material but both dextran and glucose syrup capsules also presented non-  
356 encapsulated oil, since oil droplets were located at the capsule surface or very close to the surface  
357 (marked as white arrows in Fig. 4-a3,b3). In any case, it is worth noting that the EE values obtained  
358 for fish oil-loaded dextran and glucose syrup nano-microcapsules (20 wt.% oil load) were higher than  
359 EE values reported for gelatin, whey protein concentrate and soy protein isolate nano-microcapsules  
360 loaded with 10 wt.% of  $\alpha$ -linoleic acid (ALA) and produced by electro-spraying (23-67 % EE based on  
361 intact ALA) (Gómez-Mascaraque & López-Rubio, 2016). Nevertheless, they were in the same range  
362 as fish oil-loaded capsules (40 wt.% oil load) produced by spray-drying using caseinate and glucose  
363 syrup (13 wt.% extractable oil) or sugar beet pectin and glucose syrup (25.9 wt.% of extractable oil) as  
364 wall materials (Drusch et al., 2007).

### 365 **3.2.3 Glass transition temperature**

366 A glassy shell is desired in order to prevent oxygen diffusion through the capsule and to avoid caking  
367 (Huang et al., 2014). Fig. 5 shows the DSC heating curves of the nano-microcapsules containing oil

368 emulsified by high-pressure homogenization and produced in pilot-plant scale. For both types of  
369 capsules, three endothermic peaks were found in the range from -75 °C to 10 °C, which indicated the  
370 range of melting temperature for the different triglycerides in the fish oil. In the case of dextran  
371 capsules no  $T_g$  could be detected in the range of temperature assessed (Fig. 5a), which implies that the  
372 shell material will be in glassy state minimum up to 200 °C. Nevertheless, glucose capsules showed a  
373  $T_g$  at 94.2 °C (Fig. 5b). This difference between both types of capsules could be explained by the  
374 different  $T_g$  of the carbohydrates used as wall materials. The dextran employed had a  $T_g$  at 143.3 °C,  
375 whereas the  $T_g$  of the glucose syrup used was at 59.3 °C (see Fig. S2ab of the Supplementary  
376 material). These values differed from those previously reported for glucose syrup (DE36) and dextran  
377 (74.3 kDa) in the literature,  $T_g$  at 31 °C and 223 °C respectively (Drusch et al., 2007; Scandola et al.  
378 1991). This might be due to the different type of biopolymer used (Mw and DE) and to possible  
379 differences in their residual water levels. In regard to pullulan, no  $T_g$  was found in the range of  
380 temperature studied (see Fig. S2c of the Supplementary material). This is in line with the findings of  
381 Scandola et al. (1991), who did not observe any melting or  $T_g$  for pullulan below its thermal  
382 decomposition temperature (300 °C). It is worth mentioning that fish oil-loaded dextran and glucose  
383 capsules had a higher  $T_g$  than skim milk powder and lactose ( $T_g$  at 67 °C) or sucrose ( $T_g$  at 50 °C)  
384 capsules containing fish oil and obtained by spray-drying (Aghbashlo et al., 2012).

### 385 **3.3 Oxidative stability of capsules**

#### 386 **3.3.1 ATR-FTIR**

387 Firstly, and in accordance with previous studies (Gómez-Mascaraque & López-Rubio, 2016; Torres-  
388 Giner et al., 2010), the oxidative stability of the capsules was evaluated by ATR-FTIR. Many of the  
389 characteristic bands of fish oil overlapped with the infrared bands of the biopolymers used as wall  
390 materials (WPC, dextran, pullulan and glucose syrup). Nevertheless, the characteristic absorption band  
391 of omega-3 PUFA at 3012  $\text{cm}^{-1}$ , which corresponds to the stretching of *cis*-alkene (-HC=CH-) groups  
392 (Guillén & Cabo, 1999), did not overlap with the vibrational modes of the protein and carbohydrates  
393 employed (see Fig. S3 of the Supplementary material). As a result, the intensity of this band, which  
394 indicated the disappearance or not of *cis* double bonds due to oxidation, was monitored during the

395 storage of the capsules. Fig. 6a shows that the normalized absorbance obtained for the different  
396 capsules did not significantly decrease during storage, apart from D-HPH capsules which slightly  
397 decreased at day 21. This may imply that the capsules were not oxidized during storage due to the fact  
398 that the *cis*-alkene groups were not degraded. Nonetheless, it should be noted that, although the  
399 generation of hydroperoxides changes the conformation of some double bonds due to the formation of  
400 conjugated *cis*-/*trans*- or *trans*-/*cis*- dienes, a reduction in the frequency of the band at 3006-3012  $\text{cm}^{-1}$   
401 is generally associated with advanced stages of lipid oxidation (Guillén & Cabo, 2000). However, and  
402 opposite to what we observed, other studies have reported a decrease in the intensity of this band  
403 during storage. For instance, Moomand and Lim (2014) observed a reduction of the absorption at 3012  
404  $\text{cm}^{-1}$  for ultrathin zein fibers containing fish oil during storage at different temperatures (4, 25, and 60  
405 °C) for 14 days. Likewise, Gomez-Mascaraque and López-Rubio (2016) found a reduction in the  
406 intensity of the same band for gelatin, whey protein, and soy protein electrosprayed capsules loaded  
407 with ALA during storage at 80 °C for 5 days.

### 408 3.3.2 PV and volatiles

409 The oxidative stability of the capsules was further evaluated by measuring the formation of primary  
410 and secondary volatile oxidation compounds. Fig. 6b shows the PV of the different capsules during  
411 storage. It was observed that the PV of the fish oil-loaded capsules after production, which ranged  
412 from  $7.4 \pm 0.6$  to  $10.3 \pm 0.1$  meq/kg oil, was significantly higher than the PV of the initial fish oil  
413 ( $0.4 \pm 0.1$  meq/kg oil). This might be attributed to lipid oxidation during: i) emulsion preparation due to  
414 oxygen inclusion and increase in specific surface area (as reported by Serfert et al., 2009), and ii)  
415 encapsulation process as a result of the exposure of the surface oil to atmospheric air during  
416 production (as reported by Drusch et al., 2006).

417 Although the PV of the capsules at day 0 was not significantly affected by the carbohydrate or oil  
418 emulsification approach used, different trends in hydroperoxides content were observed during storage  
419 for the capsules studied (Fig. 6b). Independently of the carbohydrate used, a longer lag phase was  
420 found for the capsules with oil emulsified by rotor-stator equipment when compared to those  
421 containing oil emulsified by high-pressure homogenization (8 days vs. 3 days). Furthermore, the PV of

422 the capsules with oil emulsified by high-pressure homogenization was significantly higher during  
423 storage than the PV of the capsules with oil incorporated by rotor-stator emulsification (apart from  
424 glucose syrup capsules at day 21). PV of the electrosprayed capsules with oil (20 wt.%) emulsified  
425 only by rotor-stator equipment was similar to those reported by Morales-Medina et al. (2016) for  
426 microcapsules containing 14 wt.% fish oil and produced by spray-drying using fish protein  
427 hydrolysates and glucose syrup as wall materials (PV of ca. 20 meq/kg oil after 20 days storage at 20  
428 °C); and lower than those found by Drusch & Berg (2008) for spray-dried microcapsules loaded with  
429 30 wt.% fish oil and containing n-OSA starch and glucose syrup as shell materials (PV of ca. 30  
430 meq/kg oil after 21 days storage at 20 °C). Nevertheless, lower hydroperoxide concentrations (PV<10  
431 meq/kg oil after 50 days storage at 20 °C) were obtained for Serfert et al. (2009) for microcapsules  
432 with a shell matrix composed of n-OSA starch and glucose syrup (fish oil load of 40 wt.%), which  
433 were stabilized with specific combinations of natural antioxidants ( $\alpha$ - and  $\delta$ -tocopherols, ascorbyl  
434 palmitate, citric acid, lecithin or citrem and rosemary extract). In line with PV results, the  
435 concentration of secondary volatile oxidation products was also higher for capsules with oil emulsified  
436 by high-pressure homogenization compared to capsules where the oil was emulsified using only a  
437 rotor-stator equipment (Fig. 7). For instance, significantly lower concentration of 1-penten-3-ol during  
438 storage was observed for G-RSE capsules compared to G-HPH capsules (Fig. 7a). Similarly, D-RSE  
439 and G-RSE showed a lower content of (*E*)-2-pentenal up to 14 days of storage than D-HPH and G-  
440 HPH capsules, respectively (Fig. 7b). Both 1-penten-3-ol and (*E*)-2-pentenal are volatiles derived from  
441 the oxidation of omega-3 PUFA and they have low odour threshold values (0.001-3 ppm and 0.04-25  
442 ppm, respectively) and undesired sensory attributes (milky, butter and sweet or oily, soapy, pungent,  
443 glue, green and grassy, respectively) (Hartvigsen et al. 2001; Venkateshwarlu et al. 2004). Likewise,  
444 lower content of volatiles products derived from oxidation of omega-9 fatty acids (i.e. heptanal and  
445 nonanal) were also found for D-RSE and G-RSE capsules compared to D-HPH and G-HPH capsules  
446 (Fig. 7c,d). Both heptanal and nonanal have also low odour threshold values (0.014-1 ppm) and  
447 unacceptable sensory characteristics for the consumer such as chemical and burnt or green plant-like,  
448 compost-like and rancid, respectively (Hartvigsen et al., 2000; Shahidi, 2001; Venkateshwarlu et al.,

2004). The higher oxidative stability of D-RSE and G-RSE capsules could be explained due to their higher EE values when compared to D-HPH and G-HPH capsules, respectively (Table 2). Oil on the surface of the capsules oxidized rapidly due to the lack of protection by any interfacial layer of biopolymers, which implies that a larger amount of surface oil might have reduced the oxidative stability of D-HPH and G-HPH capsules (Drusch et al., 2007). Furthermore, the parent emulsions of D-RSE and G-RSE capsules were produced in situ in the pilot plant just right before electrospaying, which reduced the time elapsed from emulsification to drying and minimized physical destabilization of the emulsions. On the contrary, a more pronounced physical destabilization was observed in the parent emulsions of D-HPH and G-HPH capsules (Table 2), which may have led to unprotected oil droplets by modification of the interfacial layer.

Although no significant effect of the shell matrices evaluated (dextran vs. glucose syrup) was observed in PV (Fig. 6b), significant differences were found in terms of secondary volatile oxidation products (Fig. 7). In general, glucose capsules showed a significantly ( $p < 0.05$ ) lower concentration of volatiles compared to dextran capsules. This trend was clearly observed for: i) 1-penten-3-ol when comparing D-RSE and G-RSE capsules (Fig. 7a), ii) (*E*)-2-pentenal when comparing both D-HPH and G-HPH or D-RSE and G-RSE capsules up to day 14 of storage (Fig. 7b), and iii) heptanal when comparing D-HPH and G-HPH capsules at day 21 of storage, or D-RSE and G-RSE capsules up to 14 days of storage. An opposite trend was found for nonanal, with glucose syrup capsules presenting a higher concentration of this volatile during storage (Fig. 7d). However, this is attributed to the higher content of pullulan in glucose syrup capsules (4 wt.%) compared to dextran capsules (1wt.%), since pure pullulan electrospun fibers have been reported to present high concentration of nonanal (García-Moreno et al., 2017a). These results indicated that the highest oxidative stability of G-RSE capsules cannot be solely explained by their extractable oil content, since D-RSE capsules presented significantly ( $p < 0.05$ ) higher EE values than G-RSE capsules (Table 2). This is in agreement with previous studies in the literature which indicated that: i) extractable oil also contains oil droplets surrounded by interfacial layer, which could offer some protection against oxidation (Drusch et al., 2007), and ii) surface oil could protect other fractions of the extractable oil from oxidation (e.g. oil

droplets close to the surface) (Drusch & Berg, 2008). Besides, a previous study on encapsulation of fish oil in microcapsules by spray-drying stated the importance of oxygen diffusivity on autooxidation of omega-3 PUFA (Drusch et al., 2009). Therefore, a plausible explanation for the higher oxidative stability of G-RSE compared to D-RSE might be the result of a reduced free volume for the glucose syrup matrix compared to the dextran shell. Glucose syrup had a lower molecular weight than dextran (12.5 vs. 70 kDa), which may allow a more dense packaging within the glassy wall, limiting oxygen permeability; and thus, reducing oil oxidation. Similarly, Drusch et al. (2009) demonstrated the presence of larger free volume elements for glassy carbohydrates matrices with higher molecular weight, which correlated well with the lower oxidative stability of fish oil encapsulated in these matrices (i.e. maltodextrin with DE 18 and maltose with estimated DE 50). Therefore, the authors suggested that the reduced oxygen diffusivity in fish oil-loaded microcapsules containing low molecular weight carbohydrates (e.g. maltose with estimated DE 50) explained the enhanced oxidative stability of these capsules.

#### 4. CONCLUSIONS

Biopolymer solutions containing fish oil (20 wt.% with respect to biopolymers), WPC (0.5 wt.%), citrem (0.5 wt.%), and pullulan (1wt.%) plus dextran (15wt.%) or pullulan (4wt.%) plus glucose syrup (15 wt.%) led to electrosprayed capsules without any fibril defects, both when producing in lab and pilot-plant scale. To the best of the authors' knowledge, this is the first study reporting the production of electrosprayed capsules by using combinations of whey protein and carbohydrates. Moreover, electrosprayed capsules were developed for the first time by using glucose syrup as the main wall material. This is of special importance due to the low cost of this biopolymer.

The ATR-FTIR method was not sensitive enough to study oxidative stability of the fish oil-loaded capsules, since no changes in the normalized absorbance of the band at  $3012\text{ cm}^{-1}$  (indicating the stretching of cis-alkene  $-\text{HC}=\text{CH}-$  groups) was observed during storage. Nevertheless, the hydroperoxide and volatiles content of the capsules increased during storage. Capsules with oil emulsified by using only a rotor-stator equipment showed higher oxidative stability than capsules

502 where the oil was incorporated by high-pressure homogenization, as evaluated by hydroperoxide and  
503 volatiles (1-penten-3-ol, (*E*)-2-pentenal, heptanal, and nonanal) content. This was explained by a  
504 higher encapsulation efficiency and a shorter time span between emulsification and drying which  
505 reduced physical destabilization of emulsions. Glucose syrup capsules presented higher oxidative  
506 stability than dextran capsules. This finding was attributed to the lower molecular weight of glucose  
507 syrup, which led to lower free volume in the glassy matrix reducing oxygen diffusivity. Finally, it has  
508 to be mentioned that the oxidative stability of the electrosprayed capsules needs to be further improved  
509 (i.e. by reducing surface oil).

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Table 1. Oil droplet size and electrospaying flow rate for the different biopolymers solutions

Solutions	Oil droplet size, $\mu\text{m}$		Electrospaying flow rate, mL/min	
	$D_{3,2}$	$D_{0,9}$		
Dextran	1% WPC + 2% pullulan + 20% dextran	0.684 <sup>a</sup>	1.894 <sup>a</sup>	0.012
	1% WPC + 2% pullulan + 15% dextran	0.327 <sup>b</sup>	1.009 <sup>b</sup>	0.012
	1% WPC + 1% pullulan + 20% dextran	0.600 <sup>a,*</sup>	1.647 <sup>a,*</sup>	0.010
	1% WPC + 1% pullulan + 15% dextran	0.280 <sup>b,*</sup>	0.909 <sup>b,*</sup>	0.010
	0.5% WPC + 0.5% citrem + 1% pullulan + 15% dextran	0.129 <sup>†</sup>	0.334 <sup>†</sup>	0.010
Glucose syrup	1% WPC+2% pullulan + 15% glucose syrup	0.163 <sup>a</sup>	0.485 <sup>a</sup>	0.003
	1% WPC+4% pullulan + 15% glucose syrup	0.189 <sup>b</sup>	0.581 <sup>b</sup>	0.007
	1% WPC+5% pullulan + 15% glucose	0.212 <sup>c</sup>	0.614 <sup>c</sup>	0.010
	0.5% WPC + 0.5% citrem + 4% pullulan + 15% glucose syrup	0.112 <sup>†</sup>	0.259 <sup>†</sup>	0.007

Standard deviations for oil droplet size measurements were  $< 0.008 \mu\text{m}$ . No deviations were observed for flow rate.

For dextran samples, different letters (a-b) indicate statistical significant differences ( $p < 0.05$ ) between samples containing different concentration of dextran but same concentration of pullulan. \* indicates statistical significant differences ( $p < 0.05$ ) between samples with same dextran concentration but different concentration of pullulan.

For glucose syrup samples, different letters (a-b) indicate statistical significant differences ( $p < 0.05$ ) between samples containing different concentration of pullulan.

† indicates statistical significant differences ( $p < 0.05$ ) between samples with and without citrem.

Table 2. Oil droplet size of redispersed nano-microcapsules and encapsulation efficiency

Electrosprayed capsules		Oil droplet size, $\mu\text{m}$		Encapsulation efficiency (EE), %
		D <sub>3,2</sub>	D <sub>0,9</sub>	
Dextran	HPH	0.414±0.013 <sup>a</sup>	1.762±0.102 <sup>a</sup>	86.9±1.5 <sup>a</sup>
	RSE	0.388±0.006 <sup>b</sup>	2.548±0.176 <sup>b</sup>	91.7±0.9 <sup>b</sup>
Glucose syrup	HPH	0.605±0.009 <sup>x,*</sup>	3.008±0.079 <sup>x,*</sup>	78.1±3.2 <sup>x,*</sup>
	RSE	0.461±0.061 <sup>y,ns</sup>	3.960±0.355 <sup>y,†</sup>	85.7±0.3 <sup>y,†</sup>

HPH: high-pressure homogenization; RSE: rotor-stator emulsification

Letters (a-b) indicate statistical significant differences ( $p < 0.05$ ) between dextran samples.

Letters (x-y) indicate statistical significant differences ( $p < 0.05$ ) between glucose syrup samples.

\* indicates significant differences ( $p < 0.05$ ) between dextran and glucose samples with oil emulsified by high-pressure homogenization.

ns indicates no significant differences ( $p > 0.05$ ) between dextran and glucose samples with oil emulsified by rotor-stator emulsification.

† indicates significant differences ( $p < 0.05$ ) between dextran and glucose samples with oil emulsified by rotor-stator emulsification.

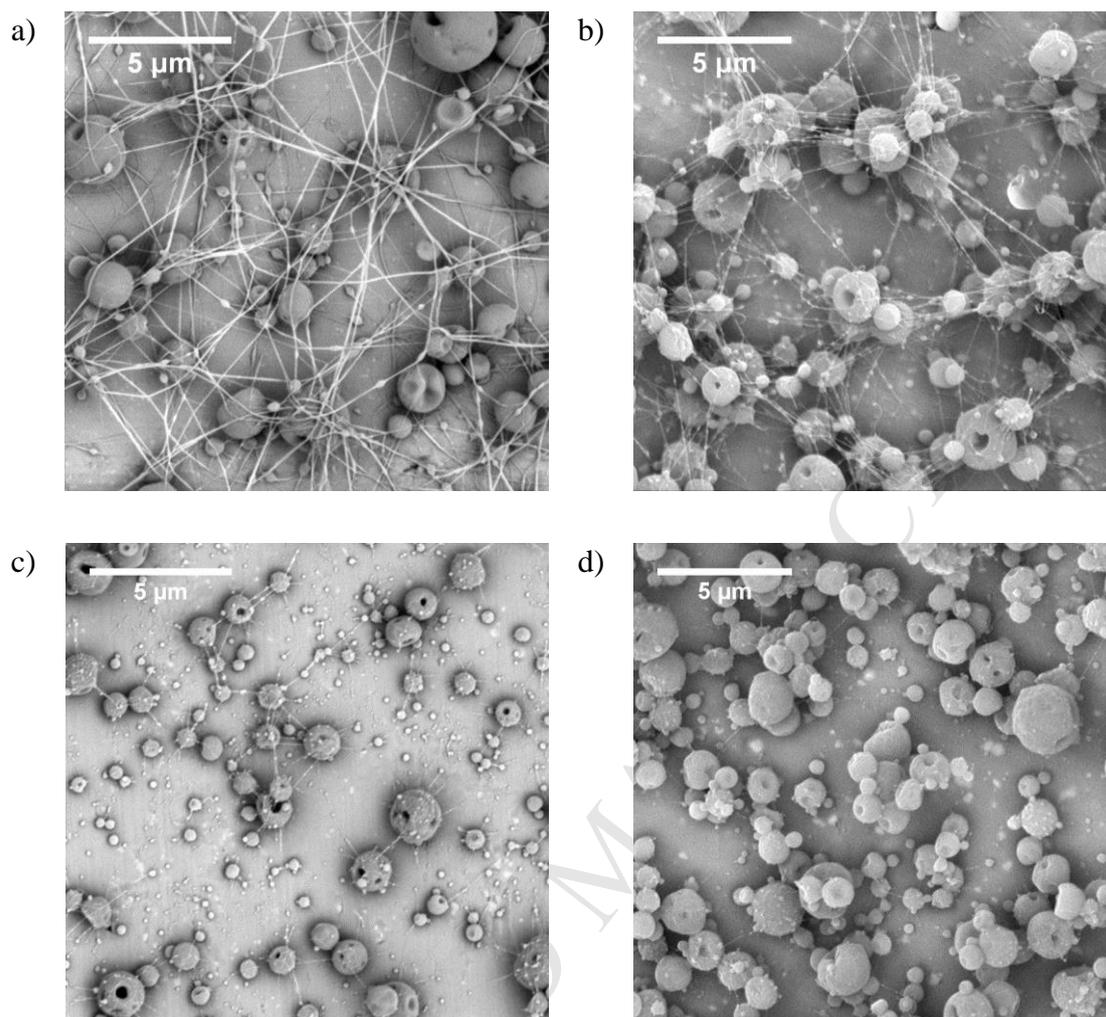


Figure 1. SEM images of nano-microstructures obtained by electrospinning of WPC (1 wt.%), pullulan and dextran solutions containing emulsified fish oil (20 wt.% oil with respect to biopolymers): (a) 2 wt.% pullulan + 20 wt.% dextran, (b) 2 wt.% pullulan + 15 wt.% dextran, (c) 1 wt.% pullulan + 20 wt.% dextran, and (d) 1 wt.% pullulan + 15 wt.% dextran.

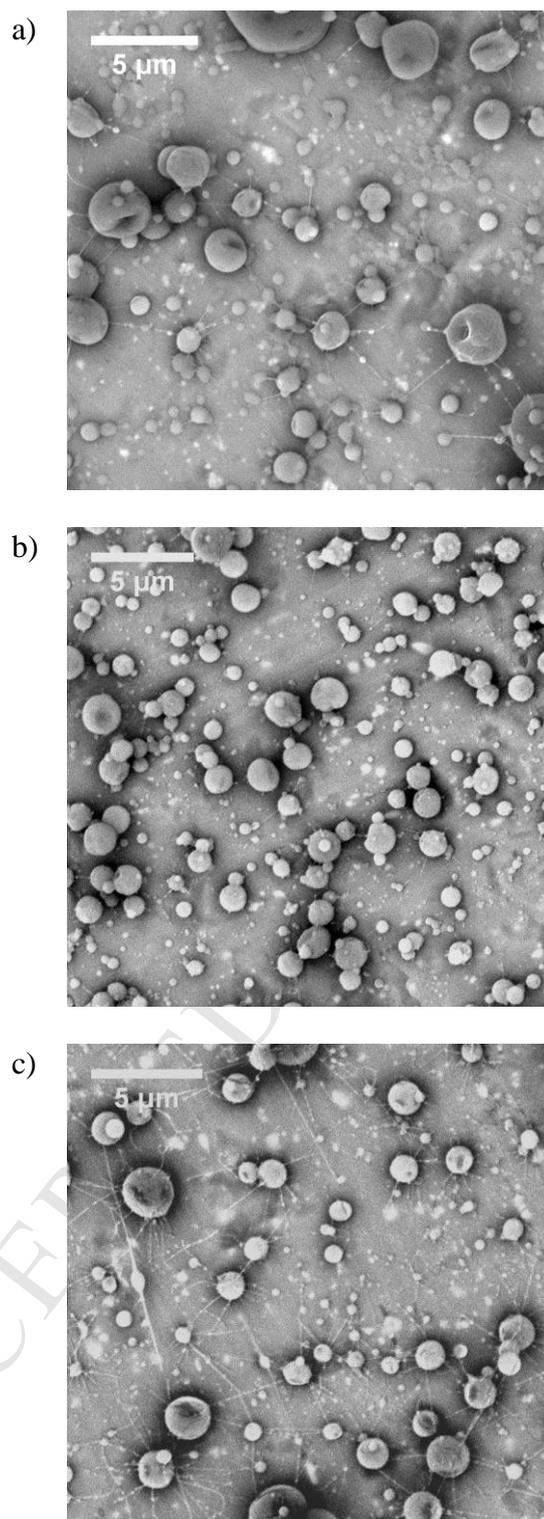


Figure 2. SEM images of nano-microstructures obtained by electrospaying of WPC (1 wt.%), pullulan and glucose syrup (15 wt.%) solutions containing emulsified fish oil (20 wt.% oil with respect to biopolymers): (a) 2 wt.% pullulan, (b) 4 wt.% pullulan, and (c) 5 wt.% pullulan.

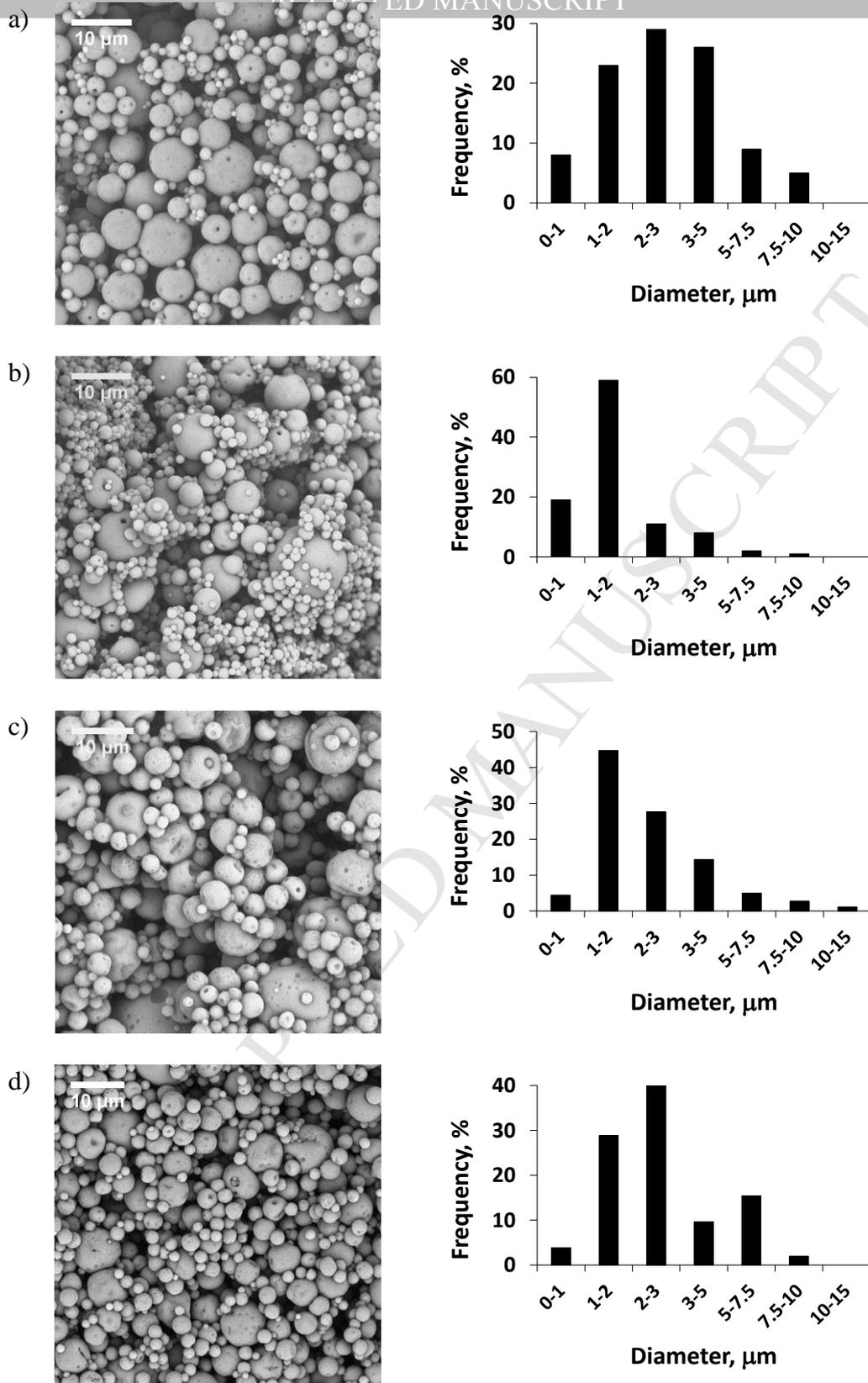


Figure 3. SEM images and diameter distribution of electrosprayed capsules: (a) D-HPH, (b) D-RSE, (c) G-HPH, and (d) G-RSE.

D: dextran; G: glucose syrup; HPH: high-pressure homogenization; RSE: rotor-stator emulsification.

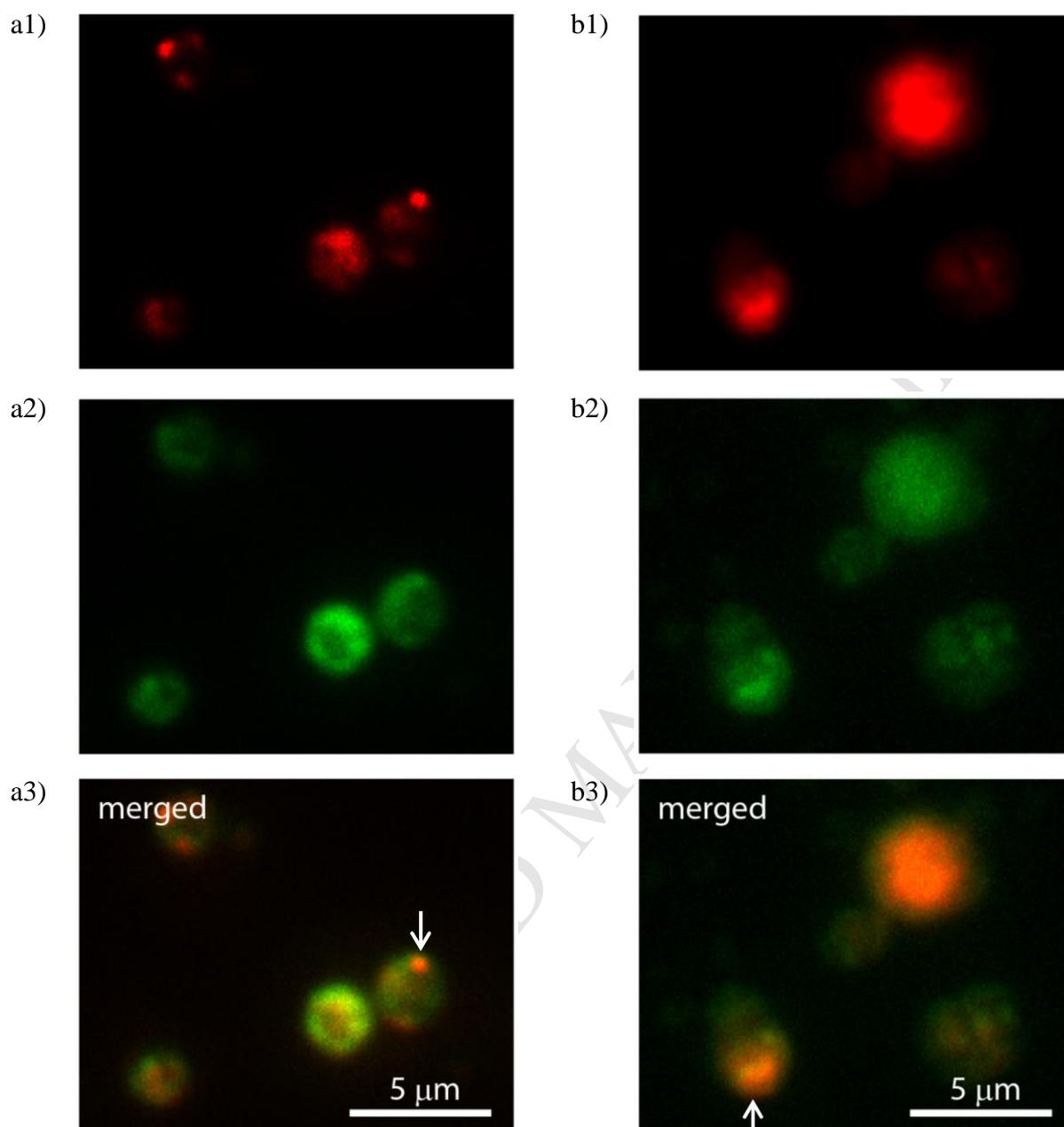


Figure 4. Fluorescence microscopy images of electrospayed capsules produced in lab scale using high-pressure homogenization for incorporating the oil: a) dextran-based capsules, and b) glucose syrup-based capsules. a1) and b1) show the location of fish oil (in red); a2) and b2) show the location of WPC (in green); and a3) and b3) show the simultaneous location of fish oil and WPC.

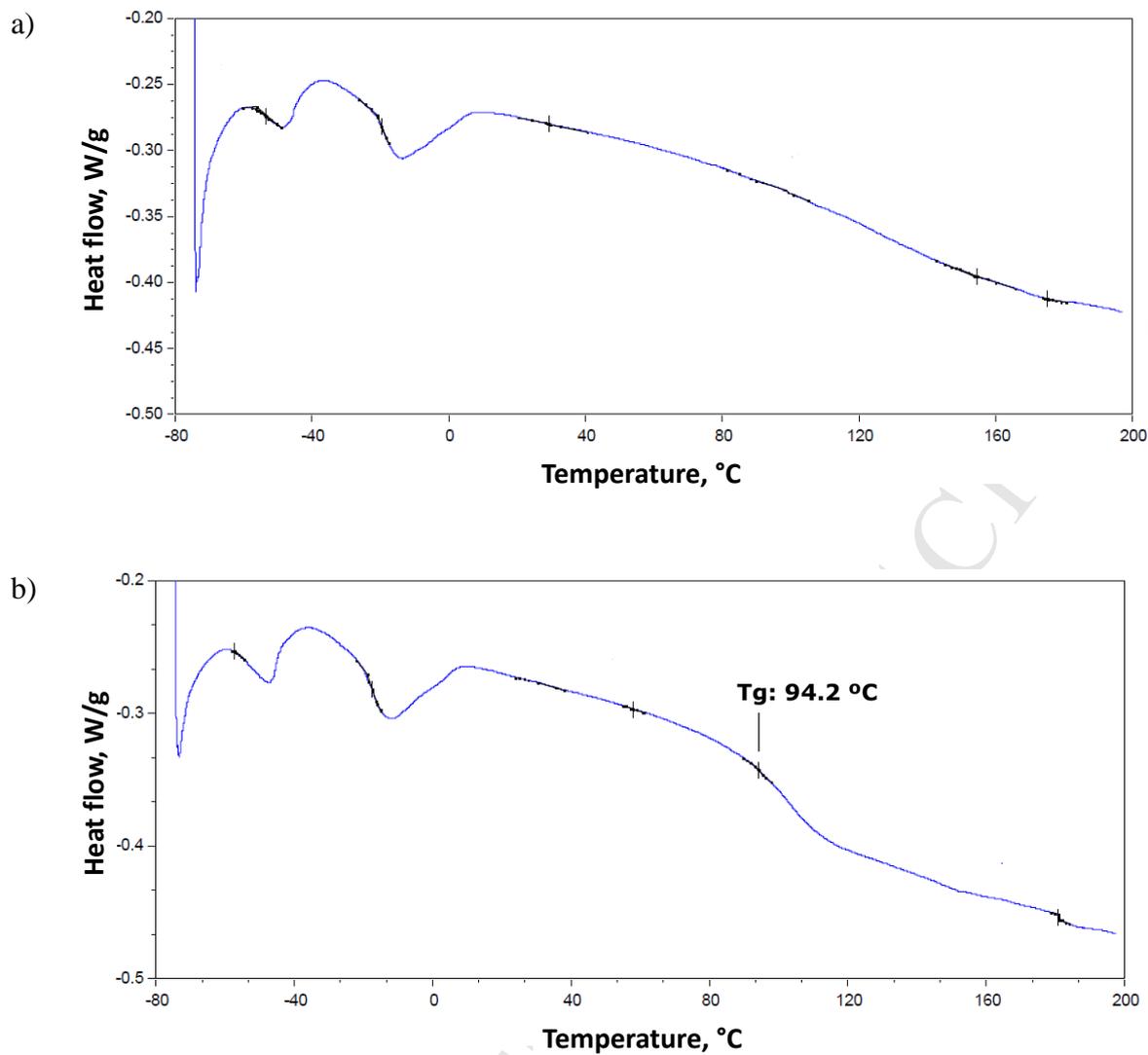


Figure 5. DSC heating curves of electrospayed capsules produced in pilot-plant scale using high-pressure homogenization for incorporating the oil: a) dextran-based capsules, and b) glucose syrup-based capsules.

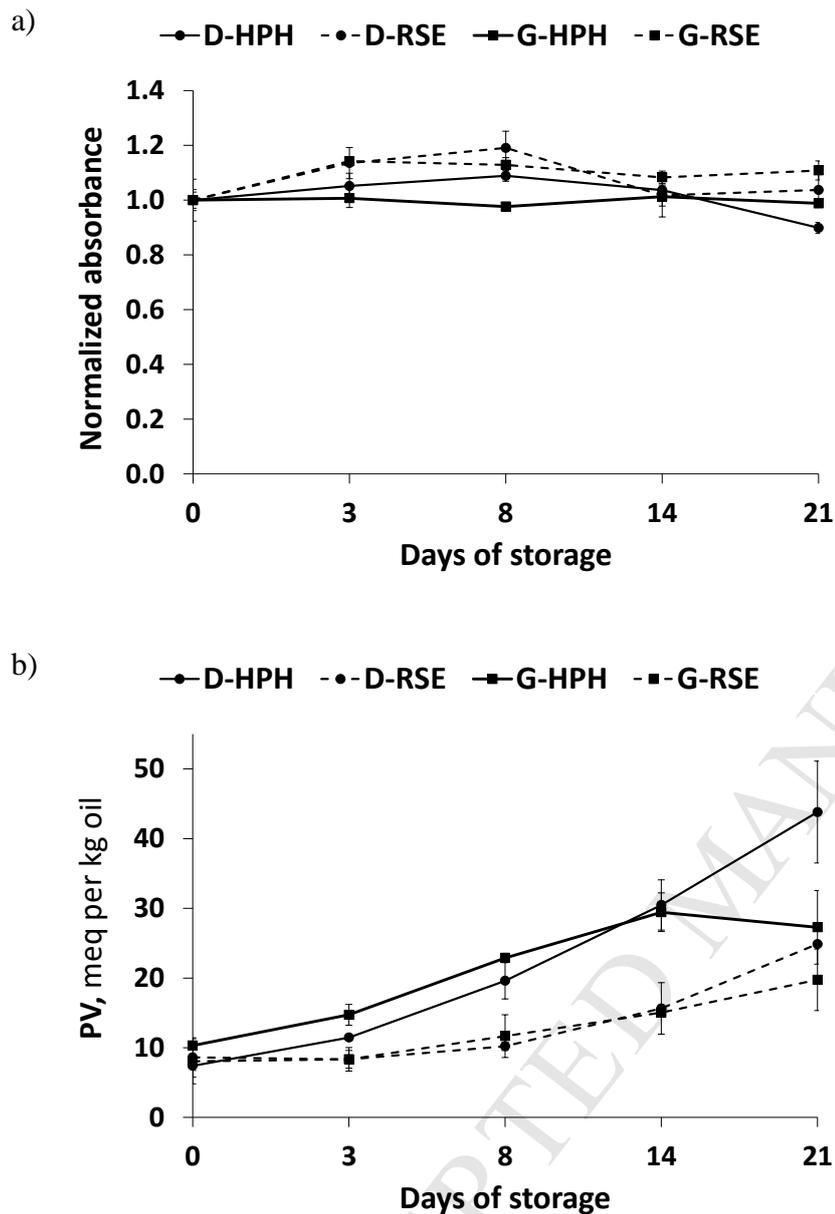


Figure 6. Oxidative stability of electrosprayed capsules loaded with fish oil during storage at 20 °C: a) ATR-FTIR, and b) Peroxide value (PV).

D: dextran; G: glucose syrup; HPH: high-pressure homogenization; RSE: rotor-stator emulsification

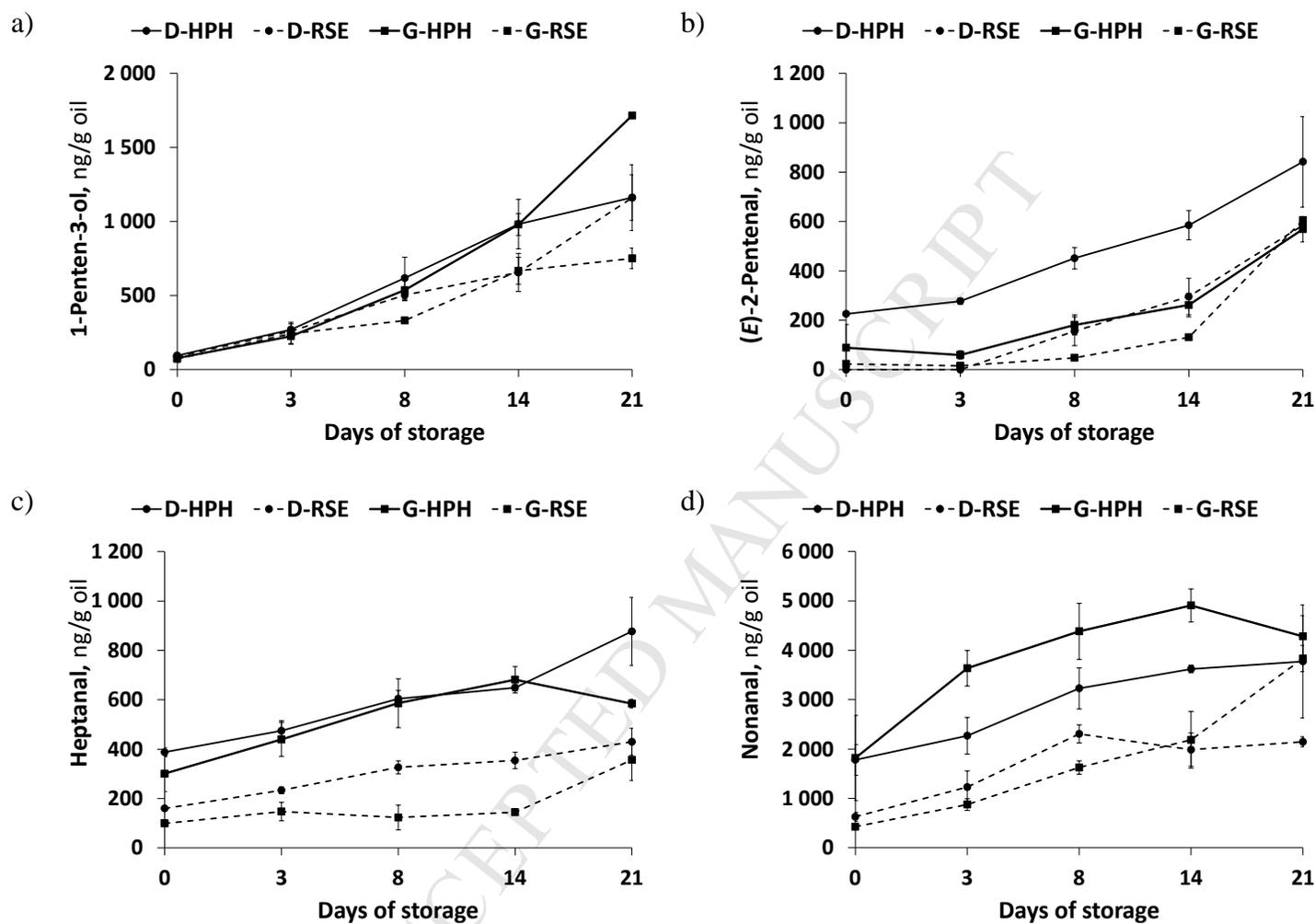


Figure 7. Secondary volatiles oxidation products of electro sprayed capsules loaded with fish oil during storage at 20 °C: a) 1-penten-3-ol, b) D: dextran; G: glucose syrup; HPH: high-pressure homogenization; RSE: rotor-stator emulsification.

**Highlights**

- Fish oil-loaded nano-microcapsules were produced by electrospraying
- Whey protein, pullulan and dextran or glucose syrup were used as wall materials
- Rotor-stator emulsification led to capsules with higher oxidative stability
- The glucose syrup matrix prevented more efficiently oxidation of encapsulated oil
- Electrosprayed capsules are promising omega-3 nano-delivery systems