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1 **Microencapsulation and controlled release of α -tocopherol by complex coacervation between pea**
2 **protein and tragacanth gum: a comparative study with arabic and tara gums.**

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17

18 **Key Words**

19 Microencapsulation, Spray drying, complex coacervation, pea protein isolate, tragacanth gum, α -
20 tocopherol

21

22

23 **Abstract**

24 The ability of pea protein isolates (PPI) to form complex coacervates with tragacanth gum was used
25 for the microencapsulation of α -tocopherol mixture with pH-dependent release properties. The
26 microcapsules were compared to three other models: PPI alone, PPI and gum arabic (PPI-GAC, known
27 to form microcapsules of complex coacervates), and PPI and tara gum (PPI-TARA, non-ionic
28 polysaccharides). The behaviors of the complex coacervates and microcapsules were studied
29 according to the protein/polysaccharide mixture and protein/polysaccharide ratio. The formation of
30 complex coacervates from the PPI-TRAG and PPI-GAC mixtures impacted the particle size of the
31 liquid suspensions and microcapsules, the efficiency of encapsulation and the active release profile.
32 An interesting gastroprotective behavior was identified for the PPI-TRAG mixture at the 1:1 ratio in
33 simulated digestion media. Overall, the results showed the ability of PPI associated with
34 polysaccharides to form microcapsules with a pH-dependent release behavior, that is promising in
35 the field of gastroprotective microencapsulation.

36

37

1. Introduction

39 α -tocopherol, the major form of vitamin E, is a biologically active hydrophobic antioxidant widely
40 used to reduce the risk of cardiovascular diseases (Pryor, 2000; Yang & McClements, 2013). Although
41 α -tocopherol is beneficial to human health, its poor hydrosolubility and relative sensitivity to light,
42 heat and oxygen limit its bioavailability and its applications in food, pharmaceutical and cosmetic
43 fields (Chen et al., 2013; Chiu & Yang, 1992). Therefore, encapsulation is needed for its protection
44 and targeted release in the digestive system.

45 Microencapsulation is an effective technology used to protect biologically active compounds against
46 environmental factors (light, humidity, oxidation process, chemicals, volatile loses), and also to
47 increase their solubility and bioavailability. Microencapsulation involves the formation of a protective
48 shell to encapsulate the sensitive compound or embed it in a matrix material (Sobel et al., 2014).
49 Among the microencapsulation techniques used in food industry, complex coacervation and spray
50 drying may be cited. Complex coacervation is an effective method for lipophilic active compounds
51 microencapsulation due to high payload, high encapsulation efficiency and mild processing
52 conditions. Complex coacervation consists in forming coacervates from the electrostatic interaction
53 of two oppositely charged biopolymers on the surface of the active compound at a specific condition
54 of pH, temperature and ionic strength (Timilsena et al., 2016). The process includes four main steps:
55 emulsification, coacervation, reticulation (optional) and drying. The complex coacervate -based shells
56 provide microcapsules using emulsification followed by spray-drying with good stability (Barrow et
57 al., 2007). The process prevents the migration of oil to the particle surface and gives greater
58 protection to encapsulated omega-3-oil (Eratte et al., 2014). Complex coacervation has been
59 reported as an effective method for the microencapsulation of lipophilic active compounds (fish oil),
60 using a gelatin/polyphosphate mixture as an encapsulating matrix (Barrow et al., 2007). Gelatin has
61 also proven its efficacy as a cationic compound associated with polyanionic compounds such as gum
62 arabic, pectin, carboxymethylcellulose or alginate in the formation of complex coacervation systems
63 encapsulating lipophilic compounds (Devi et al., 2012; Dong et al., 2011; Duhoranimana et al., 2018;
64 Muhoza et al., 2019). The mechanism implies the interaction between the negatively charge
65 polysaccharide and positively charged proteins below their isoelectric point. However, due to the
66 emergence of new diseases in recent years, in particular prion-related diseases, health and safety
67 regulations have been strengthened, leading to the use of different biopolymer sources (Chourpa et
68 al., 2006). Consequently, proteins extracted from plant sources, especially from vegetables, have
69 been widely developed in the food industry (Bajaj et al., 2017). However, the development of
70 alternative protein-polysaccharide complex coacervates requires optimization of complex
71 coacervation parameters because each protein-polysaccharide couple is able to form complex
72 coacervate at a specific pH value and biopolymer mixing ratio that cannot be replicated to another
73 set of biopolymers (Timilsena et al., 2017). Considerable research has been carried out using plant
74 proteins such as soy, pea, lentil, chia seed proteins to microencapsulate oil (Ducel et al., 2004; Jun-xia
75 et al., 2011; Liu et al., 2010; Timilsena et al., 2016). Among these plant proteins, pea proteins isolate
76 (PPI) present a high nutritional value, non-allergenic properties, are rich in essential amino acids such
77 as lysine, and have shown emulsifying and wall forming properties suitable for microencapsulation
78 (Donsì et al., 2010; Ducel et al., 2005; Roy et al., 2010).

79 Tragacanth gum, described as a bifunctional emulsifier, showed efficient acidic oil-in-water
80 emulsions properties (Balaghi et al., 2010; Farzi et al., 2013), gelling abilities and high mucoadhesive
81 properties (Nur et al., 2016). Largely studied in biomedical field, tragacanth gum was identified as
82 non-toxic, non-teratogenic, non-carcinogenic and non-mutagenic, and can be suitable in wound
83 dressing (Ghayempour et al., 2016). Tragacanth gum forms complex coacervates with casein to

84 efficiently microencapsulate β -carotene (Jain et al., 2016). However, the complex coacervation of
85 tragacanth gum associated with plant proteins is poorly described in the literature and might be
86 consider as an alternative to the widely described gelatin-gum arabic system. Tragacanth gum is an
87 anionic polysaccharide which remains negatively charged for a wide pH range, while pea proteins
88 becomes positively charged below their isoelectric point ($pI = 4,5-5.0$) (Carpentier et al., 2021;
89 Cuevas-Bernardino et al., 2018). Under specific conditions, these oppositely charged biopolymers
90 interact with each other in aqueous solution to form complex coacervates. Because of its stability to
91 acidity, tragacanth gum might be used as a gastroprotective biopolymer (Nazarzadeh Zare et al.,
92 2019).

93 The general objective of this study is to develop a microencapsulation system with pH-dependent
94 properties from plant biopolymers to encapsulate a lipophilic active compound model. Especially, the
95 aim of this study was to develop microcapsules of complex coacervates from a mixture between pea
96 protein isolates (PPI) and tragacanth gum to combine emulsifying properties of PPI, the pH-
97 dependent behavior of protein-polysaccharide complex coacervates and the stability in acid media of
98 tragacanth gum for the pH-dependent release of the encapsulated compound. The model was
99 compared with the association of other polysaccharides (gum arabic or tara gum) at different ratios,
100 according to the optimal parameters determined in a previous study and compared to microcapsules
101 produced from PPI alone used as a vector of a hydrophobic active ingredient (Carpentier et al., 2021).
102 A sunflower oil/ α -tocopherol (90:10) (m/m) mixture was used as an active compound model for the
103 active ingredient to be encapsulated. By varying the protein/polysaccharide ratio, the contribution of
104 each biopolymer was assessed to understand the functionality of the complex coacervates and in
105 particular their pH stability. The formulation conditions of the microcapsules produced at the pilot
106 scale were adapted from a previous work for production purposes. In this context, the different
107 formulations produced during the emulsion and coacervation process in liquid phase (by
108 granulometric monitoring) and the microcapsules produced after drying were characterized, with a
109 physical characterization of the microcapsules and encapsulation efficiency, of the particle
110 morphology by scanning electron microscopy (SEM) and a study of the release of α -tocopherol in
111 buffered and simulated digestion media.

112

113 **2. Material and methods**

114 **2.1. Material and chemicals**

115 The major pea storage proteins of the globulin family are legumin (350-400 kDa) and vicilin (150
116 kDa). Commercially available PPI (80% of protein content) in powder form were purchased from
117 Roquette (Nutralys F85M, Lestrem, FRANCE). The product composition was described as follows: 7%
118 of relative humidity, 80% of proteins, 3% of carbohydrates, 6% of total fat and 4% of ashes. The pea
119 proteins were used without hydrolyzing. Gum arabic was kindly supplied by Nexira (Rouen, FRANCE).
120 Tragacanth gum CEROTRAG 887 was purchased from C.E. Roeper GmbH (Hamburg, GERMANY). Tara
121 gum is a galactomannan extracted from the endosperm of tara shrub (*Caesalpinia spinosa*) seeds.
122 Tara gum was purchased from Starlight Products (Rouen, FRANCE). The gum obtained included $\geq 80\%$
123 of galactomannan. All chemicals used in this study were of reagent grade and purchased from Sigma-
124 Aldrich (Oakville, ON, Canada). Chloroform and acetonitrile used for α -tocopherol extraction and
125 quantification, respectively, were purchased from Carlo Erba.

126 **2.2. Preparation of sunflower oil supplemented with α -tocopherol and biopolymer stock** 127 **solutions**

128 Biopolymer dispersions (PPI, GAC, TRAG or TARA) of varying concentrations were prepared by adding
 129 a known amount of PPI, gum arabic (GAC), tragacanth gum (TRAG) or tara gum (TARA) powders in
 130 distilled water using an IKA RW20 Digital paddle stirrer (IKA, Staufen, Germany) (**Table 1**). Tragacanth
 131 gum and tara gum were hydrated at 45°C for 2 hours.

132 The active compound model consisted in a 90:10 mixture of sunflower oil and α -tocopherol prepared
 133 by adding a known amount of α -tocopherol to sunflower oil under magnetic stirring.

134 **2.3. Preparation of Oil-in-Water (O/W) emulsions**

135 PPI alone and the hydrophobic active mixture were pre-homogenized for 5 minutes at 5,000 rpm
 136 using the Ultra-Turrax (IKA, Staufen, Germany), and then further homogenized using a two-step
 137 MG2-500B homogenizer (BOS Homogenisers, Hilversum, Holland) at 250 bars for two cycles (**Table**
 138 **1**).

139 **2.4. Preparation of complex coacervates between the emulsion and polysaccharides**

140 The process consisted in inducing coacervation between proteins and polysaccharides (**Table 1**).
 141 Briefly, the polysaccharide solution was added to the emulsion under deflocculant paddle stirring at
 142 500 rpm for 10 minutes to limit the introduction of air into the mixture. The pH was then adjusted
 143 with 1M hydrochloric acid (HCl) under the optimal conditions of complex coacervation indicated in
 144 **Table 1**. A pH value corresponding to the highest complex coacervation was selected for a given ratio
 145 for each PPI-polysaccharide selected according to ζ -potential and coacervation yields studied in a
 146 previous work (Carpentier et al., 2021). Stirring was maintained during this step and continued for 10
 147 minutes after addition of HCl, prior to the spray-drying of the suspension.

148 In the case of the emulsion of PPI alone, the pH was maintained at 6.8 after the high-pressure
 149 homogenization step. The aim of the study was to compare the properties of PPI microcapsules at
 150 the initial step to those from protein-polysaccharides complexes after acidification.

151 *Table 1: Composition of the formulations of α -tocopherol microcapsules*

Matrices	Ratio	pH	Active mixture (w/w) (%)	α -tocopherol (w/w) (%)	Matrix (w/w) (%)	Dry matter (%)
PPI	-	6.8	25.0%	2.5%	75.0%	5.0%
PPI-GAC	1:1	3.0	25.0%	2.5%	75.0% (37.5% of PPI – 37.5% of GAC)	5.0%
	2:1	3.5	25.0%	2.5%	75.0% (50% of PPI – 25% of GAC)	5.0%
	5:1	4.2	25.0%	2.5%	75.0% (62.5% of PPI – 12.5% of GAC)	5.0%
	10:1	4.4	25.0%	2.5%	75.0% (68.2% of PPI – 6.8% of GAC)	5.0%
PPI-TRAG	1:1	3.0	25.0%	2.5%	75.0% (37.5% of PPI – 37.5% of TRAG)	5.0%
	2:1	3.2	25.0%	2.5%	75.0% (50% of PPI – 25% of TRAG)	4.7%
	5:1	3.8	25.0%	2.5%	75.0% (62.5% of PPI – 12.5% of TRAG)	5.0%
	10:1	4.2	25.0%	2.5%	75.0% (68.2% of PPI – 6.8% of TRAG)	5.0%

					TRAG)	
	5:1	4.5	25.0%	2.5%	75.0% (62.5% of PPI – 12.5% of	4.5%
					TARA)	
PPI-TARA					75.0% (68.2% of PPI – 6.8% of	5.0%
	10:1	4.5	25.0%	2.5%	TARA)	

152

153 **2.5. Spray drying of complex coacervates in suspension**

154 The spray-drying technology was used to prepare microparticles from feed solutions, composed of
 155 complex coacervates between PPI and polysaccharides. Suspensions were spray-dried using a Niro
 156 Minor pilot scale spray dryer (Niro Atomizer, Copenhagen, Denmark), equipped with a F11 turbine in
 157 order to obtain individualized microcapsules. The suspensions were fed into a drying chamber using a
 158 Masterflex Easy-Load II peristaltic pump (Cole Parmer, Montreal, Canada). The solutions were fed
 159 into an atomizing chamber with an inlet temperature of $140 \pm 2^\circ\text{C}$ and a flow rate of 400 kg/h. The
 160 outlet temperature was controlled between 75 and 80°C by adjusting the feed rate. The rotation
 161 frequency of the turbine was set at 50 Hz. Microcapsule powder was collected from the container,
 162 closed hermetically in opaque packaging and stored at 4°C until analysis. All experiments were
 163 performed in duplicate.

164 The spray-drying yield (SDY) was determined as follows:

$$SDY = \frac{m_f}{m_i} \times 100$$

165 Where m_f is the mass of collected powder and m_i is the initial mass of solid content added to the
 166 emulsion, including PPI, polysaccharides (GAC, TRAG or TARA) and the hydrophobic active mixture.

167 **2.6. Particle size distribution of complex coacervates in suspension**

168 Size distribution of emulsion oil droplets or suspensions of complex coacervates was estimated using
 169 a Malvern Mastersizer 3000 laser particle size analyzer (Malvern Instruments, Worcestershire, UK)
 170 equipped with a Hydro SM liquid feed unit. The particle size detected by the instrument ranged from
 171 $0.1 \mu\text{m}$ to $1,000 \mu\text{m}$. The refractive indices used for particle size calculation were $n_{\text{particle}}=1.456$ and
 172 $n_{\text{water}}=1.33$. As the particle size distribution of the dispersions was multimodal, the results for each
 173 particle population were expressed according to the statistical parameter "mode" corresponding to
 174 the most represented value for this population. The percentage of each population was also
 175 estimated for a multimodal distribution.

176 **2.7. Moisture content**

177 The moisture content of formulated microcapsules was measured using a Halogen Moisture Analyzer
 178 HC103 (Mettler Toledo, Switzerland). Briefly, 2 g of powder were placed in an aluminum pan and
 179 heated at 105°C . Moisture content was measured for each sample after obtaining a stable weight.
 180 Tests were performed in duplicate and the mean values were expressed as a relative humidity
 181 percent (%RH).

182 **2.8. Particle size distribution of microcapsules**

183 The mean diameter of the spray-dried microparticles was determined by laser diffraction using a
 184 Malvern Mastersizer 3000 analyzer (Malvern Instruments, Worcestershire, UK) equipped with an
 185 Aero S dry powder feeder unit. The device operates based on the principle of multi-angular

186 diffraction of the laser red light at 633 nm. Briefly, about 2 g of microparticles were placed in the
187 hopper of the device and the measurement was performed using a refractive index of 1.45 and the
188 “fine particle” mode was activated to improve measurement (air pressure set at 2.5 bars). The
189 measurement was recorded within the range from 0.1 to 1000 μm . From the size distribution, the
190 volume diameter $D(V; 0.5)$ and the dispersion of the data distribution curve (span) of the
191 microparticles were determined by the software (Malvern). The volume particle diameter was
192 calculated as the mean of two measurements per sample.

193 **2.9. Fourier transform infrared (FTIR) spectroscopy**

194 FTIR spectroscopy was used to investigate the functional groups of the spray-dried samples,
195 biopolymer powders (proteins and polysaccharides) and hydrophobic active mixture. FTIR spectra
196 were recorded using a Spectrum 100 FT-IR spectrometer (Perkin Elmer, Waltham, USA). Transmission
197 spectra were obtained within a range from 650 to 4000 cm^{-1} using 32 scans at a resolution of 4 cm^{-1} .
198 The scattering correction procedure was used for baseline correction of the spectra. Mean spectra
199 were calculated from independent measurements performed in triplicate.

200 **2.10. ESEM morphological observations**

201 The morphology of the sample surface was monitored using an environmental scanning electron
202 microscope (ESEM) (QUANTA 200 Environmental Field Effect Gun apparatus, FEI/ Thermo Fischer
203 Scientific, USA). Observations were performed according to the method developed by Conforto *et al.*
204 with some specifications (Conforto et al., 2015). The particles were deposited on conductive carbon
205 double-faced adhesive tape on aluminum SEM stubs. As described by the author, no coating was
206 applied to the samples before observation to avoid any alteration of the particle surface. The
207 analyzes were performed in environmental mode under water vapor pressure with a beam current of
208 0.1 nA and an accelerating voltage of 11 kV. Secondary electron (SE) images were obtained using a
209 large-field detector (LFD). Details of the surface morphology were obtained by adapting the water
210 vapor pressure at 1.30 mbar. The value was suitable for the analysis of all samples under the chosen
211 voltage and current conditions.

212 **2.11. Quantification of α -tocopherol**

213 100 mg of microcapsules (with an initial theoretical concentration of 2,5% of α -tocopherol (w/w))
214 were suspended in 4.5 mL of chloroform in a vial. The solution was stirred vigorously for 10 minutes.
215 1 mL of the chloroform phase was removed and centrifuged for 10 min at 12,000 rpm. The
216 supernatant was diluted by two in acetonitrile and stored in a sealed vial for HPLC analysis.

217 A standard curve with known concentrations of α -tocopherol ranging between 0.1 and 1000 $\mu\text{g}/\text{mL}$
218 was used. α -tocopherol concentration was estimated by calculating the area under the UV detection
219 peak after HPLC separation.

220 α -tocopherol concentration in the samples was measured using a HPLC system (Agilent) equipped
221 with an Uptisphere C18 WOD column (Interchim) and an UV-Vis detector (Agilent). The compound
222 was eluted in a mobile phase consisting in a mix of water (5%) and acetonitrile (95%) in isocratic
223 mode at a flow rate of 0.8 mL/min. The column temperature was set to 30°C and the detection
224 wavelength was 290 nm. The elution time of α -tocopherol was 7.6 min.

225 The theoretical encapsulation efficiency (%EE) was determined as the ratio between the measured
226 concentration and the theoretical concentration of α -tocopherol in the microcapsules:

$$\%EE = \frac{[Tocopherol]_{experimental}}{[Tocopherol]_{theoretical}} \times 100$$

227 The encapsulation efficiency corresponded to α -tocopherol encapsulated within the microcapsule
 228 and the free compound presents on the surface.

229 **2.12. *In vitro* release kinetics assay in simulated gastrointestinal media**

230 α -tocopherol release profile was assessed *in vitro* in simulated digestive media that artificially
 231 mimicked the pH and temperature conditions of the digestive transit. Two media were prepared: the
 232 simulated gastric digestion medium (acidic medium) and the simulated enteric digestion medium
 233 (neutral medium).

234 For the simulated gastric digestion medium, 90 mg of microcapsules were suspended in 4.5 mL of a
 235 buffer solution at pH 2.0 prepared to obtain a suspension of microcapsules at 20 g/L. For the
 236 preparation of 1L of buffer solution at pH 2.0 in a volumetric flask, 2 g of sodium chloride and 7 mL of
 237 HCl 37% were added to ultrapure water. The pH was then adjusted to 2.0 by adding 1M HCl or 1M
 238 sodium hydroxide (NaOH). The solution was adjusted with ultrapure water up to the mark. To test
 239 the effect of the proteolytic activity under gastric digestion conditions, 3.2 g/L of pepsin were added
 240 to the solution.

241 For the simulated enteric digestion medium, 90 mg of microcapsules were suspended in 4.5 mL of a
 242 buffer solution at pH 6.8 prepared to obtain a suspension of microcapsules at 20 g/L. For the
 243 preparation of 1L of buffer solution at pH 6.8 in a volumetric flask, 6.8 g of monobasic potassium
 244 phosphate (KH₂PO₄) and 77 mL of 0.2M NaOH were added to ultrapure water. The pH was adjusted
 245 to 6.8 by adding 1M NaOH or 1M HCl. The solution was adjusted with ultrapure water up to the
 246 mark. To test the effect of the proteolytic activity under intestinal digestion conditions, 10.0 g/L of
 247 pancreatin were added to the solution.

248 The microcapsule/buffer solution mixture was incubated in an oven at 37.5°C with orbital shaking at
 249 180 rpm for a given time. The procedure was repeated for each sampling/analysis timepoint (t= 15,
 250 30, 45, 60 and 90 minutes). The *in vitro* release experiments were performed in triplicate.

251 A liquid/liquid extraction in the presence of chloroform was needed to measure α -tocopherol
 252 released in the aqueous medium. This process used the following protocol: 4.5 mL of chloroform
 253 were added to the microcapsule suspension solutions. The mixture was shaken manually for 10s and
 254 left to phase shift (rapid phase shift <1 min). 1 mL of the chloroform phase was collected and
 255 centrifuged for 10 min at 12,000 rpm. The supernatant was diluted 1:1 with acetonitrile (v/v) and
 256 stored in a sealed vial for further HPLC analysis.

257 To assess the mechanism of α -tocopherol release, kinetic profile data were fitted with the following
 258 mathematical models: zero order, first order, Higuchi and Korsmeyer-Peppas models.

259 Zero order: $Q = Q_0 - K_0t$

260 First order: $\log C = \log C_0 - K_1t/2.303$

261 Higuchi: $Q = K_Ht^{1/2}$ (Higuchi, 1963)

262 Korsmeyer-Peppas: $M_t/M_\infty = K_{KP}t^n$ (Korsmeyer et al., 1983)

263 Where Q is the cumulative amount of drug released at time t, Q_0 is the initial amount of drug (t=0); C
264 is the concentration of drug in solution at time t, C_0 is the initial concentration of drug in solution;
265 M_t/M_∞ is the fraction of drug released at time t, n is the diffusion exponent indicative of the
266 transport mechanism; K_0 , K_1 , K_H and K_{KP} are the release rate constants for zero order, first order,
267 Higuchi and Korsmeyer-Peppas kinetic models, respectively (Estevinho et al., 2013).

268 The correlation coefficient r^2 was used to compare the model applicability.

269 2.13. Statistical analysis

270 All measurements were carried out at least in triplicate using freshly prepared samples and data
271 were reported as mean and standard deviation. The results were analyzed by one-way ANOVA,
272 followed by a student's t-test to determine significant differences ($p < 0.05$) between experimental
273 and control (PPI alone) samples. Graphs and data processing for mathematical models were
274 constructed by Graph Pad Prism® 7 software (H.J. Motulsky, Prism 7 Statistics Guide, GraphPad
275 Software Inc., San Diego, CA, USA).

276 3. Results and discussion

277 3.1. Effect of complex coacervation on the size distribution of emulsion droplets

278 **Figure 1A** shows the evolution of droplet size of emulsions formulated with PPI before and after
279 homogenization at 250 bars. The results showed that the pre-emulsion formed with ultraturrax
280 allowed forming an emulsion with a bimodal population with a mean droplet diameter of 35.8 μm .
281 Homogenization at 250 bars allowed preparing an emulsion with a mean droplet diameter of 5.75
282 μm . Two droplet size populations were observed: a first submicron population accounting for 60% of
283 the total population and a second population centered at 10 μm . The second population was related
284 to a PPI fraction with poor solubility, due to the exposure of hydrophobic amino acid residues,
285 making particle sub-structures difficult to disintegrate (Tsumura et al., 2005). When the distribution
286 of the two particle size populations was compared, it appeared that homogenization allowed
287 obtaining a finer emulsion likely to be more stable (Juttulapa et al., 2017). As an emulsion passes
288 through the valve of the homogenizer, it is subjected to a combination of shear, cavitation forces and
289 turbulent flow conditions, which disrupt the large droplets into smaller ones. Homogenization at 250
290 bars was therefore a key step in stabilizing the emulsion during the process. The second population
291 centered at 10 μm might be reduced by increasing the homogenization pressure. Same studies on pea
292 proteins – oil emulsions at 400 bars homogenization explained that mechanical forces exerted on
293 protein chains during high-pressure homogenization may cause an increase of their flexibilities. At
294 the oil/water interface, proteins were able to unfold and exposed their hydrophobic regions at the
295 interface and stabilize the emulsion by coating the interface (Le Priol et al., 2019).

296 A particle size analysis in liquid dispersions was performed during the different steps of the complex
297 coacervation process of the four studied mixtures: PPI-TRAG, PPI-GAC and PPI-TARA. Only the results
298 obtained at a protein/polysaccharide ratio of 10:1 (m/m) were presented (**Figure 1A, C, E**). The
299 results corresponded to the particle size distribution during the following steps: homogenization of
300 the PPI-oil solution at 250 bars, PPI-polysaccharide mixture and pH adjustment leading to complex
301 coacervation. The curve corresponding to the emulsion by high-pressure homogenization was
302 presented previously. An analysis of the polysaccharide alone in solution was also performed. **Table 1**
303 **supplemental data** describes the size of the different populations observed for each size distribution
304 and the proportion of each population.

305 Regarding the PPI-GAC mixture, a slight increase in the volume of the second population (centered at
306 10 μm) was observed after addition of the polysaccharide. Therefore, it appeared that the addition of

307 gum arabic did not alter emulsion quality (**Figure 1C, Table 1 supplemental data**). According to our
308 previous study of complex coacervation between PPI and GAC, no complex coacervate formed at pH
309 6.8 since the two biopolymers showed a negatively charge density (Carpentier et al., 2021). It can be
310 assumed that polysaccharide was not absorbed to the droplet surfaces because of electrostatic
311 repulsion between the negatively charged droplets and negatively charged polysaccharides,
312 explaining the non-alteration of the emulsion quality (Gharsallaoui et al., 2010). However, after
313 acidification of the medium to the optimal pH for the formation of complex coacervates from the
314 PPI-GAC mixture, a significant change in particle size distribution was observed with a mean particle
315 diameter of 13.1 μm compared to 5.75 μm for the PPI-oil emulsion while the submicron population
316 (0.6 μm) observed in the PPI-oil emulsion disappeared after pH lowering. This increase of the droplet
317 size was explained by the fact that GAC could adsorb to the surface of more than one emulsion
318 droplet by electrostatic interactions between negatively charged GAC and positively charged PPI,
319 acting as a polymeric link that promotes bridging flocculation (Gharsallaoui et al., 2010; Guzey &
320 McClements, 2006). Also, this may be explained by the deposition of the coacervate, which binds
321 together several oil droplets and encapsulates them within the same microcapsules. This
322 phenomenon yields to polynucleated entities (Bordón et al., 2021). This bridging flocculation after
323 complex coacervation can be attributed to formation electrostatic bonds and hydrogen bonds in the
324 complex, which is consistent with FTIR results (Naderi et al., 2020). Lowering the pH seemed to
325 increase the particle size through the formation of complex coacervates. An aggregation of the
326 particles during mixing could explain the change in particle size. Wei *et al.* have investigated PPI
327 stabilization in the presence of different polysaccharides and shown that lowering the pH of the
328 protein from 7 to 3.5 increased the particle size due to protein flocculation (Wei et al., 2020). Since
329 PPI possess both $-\text{NH}_3^+$ and $-\text{COO}^-$ groups, complex coacervates suspensions involved electrostatic
330 interactions during complex coacervation formation but also PPI self-aggregation and counter-ion
331 exchange.

332 Regarding tragacanth gum, a destabilization of the emulsion was observed after adding the
333 tragacanth gum solution to the emulsion (**Figure 1A, Table 1 supplemental data**). The particle size
334 distribution was heterogeneous with the presence of 5 different populations of particles, including a
335 population with a size $<1 \mu\text{m}$ corresponding to the emulsion. This difference in particle size
336 distribution could be due to the particle size distribution of the added tragacanth gum solution.
337 Indeed, the analysis of the particle size distribution of a dispersion containing 2% of tragacanth gum
338 revealed particles with a size of about 129 μm . Farzi *et al.* have shown that in a dispersion containing
339 0.5% of tragacanth gum, particles also had a polydisperse distribution and a $D(4.3)$ of 130 μm for
340 gums from three different species (Farzi et al., 2015). This polydisperse system was due to the
341 presence of two different polysaccharides: tragacanthin and bassorin. Tragacanthin is a branched
342 polymer that is highly soluble in water, while bassorin is considered as an insoluble polysaccharide
343 capable of forming aggregates in solution (López-Franco et al., 2009). Here, the population with a
344 size of 100 μm seemed to correspond to the insoluble fraction of tragacanth gum (bassorin) and the
345 population with a size $>100 \mu\text{m}$ to the soluble fraction (tragacanthin). After pH adjustment, the size
346 distribution was less polydisperse than before pH adjustment. Three populations were obtained: a
347 population (accounting for 47% of the fraction) with a size of 120 μm and a population (accounting
348 for 35% of the fraction) with a size of 4.4 μm , corresponding to the formation of complex coacervates
349 between tragacanth gum and PPI. The last population (accounting for 18% of the fraction)
350 corresponded to the unreacted PPI-oil emulsion. The submicron fraction corresponding to the
351 emulsion was no longer present after pH adjustment and this could be due to the formation of
352 aggregates after complex coacervation.

353 Finally, the addition of tara gum to the PPI-oil emulsion resulted in the formation of particles with a
354 polydisperse distribution, as with the first two polysaccharides (**Figure 1E, Table 1 supplemental
355 data**). The presence of an insoluble fraction in tara gum could explain the presence of the population

356 with a size of 57 μm . The analysis of the distribution of tara gum alone in solution confirmed this
357 hypothesis with the presence of a population with a size of 130 μm . After pH adjustment, the
358 emulsion appeared less destabilized. This could be explained by the fact that tara gum is a non-ionic
359 galactomannan and therefore did not form complex coacervates with PPI. Therefore, pH adjustment
360 only led to protein self-aggregation. This was further confirmed by the fact that the pH of the
361 solution was adjusted to the optimal pH at 4.5, corresponding to the isoelectric point of the protein.
362 The study of the association between PPI and a galactomannan from konjac gum by Wei *et al.* has
363 shown similar results. According to the authors, the small change in particle size could be due to the
364 fact that the galactomannan was neutral and therefore did not aggregate with the protein (Wei *et*
365 *al.*, 2020).

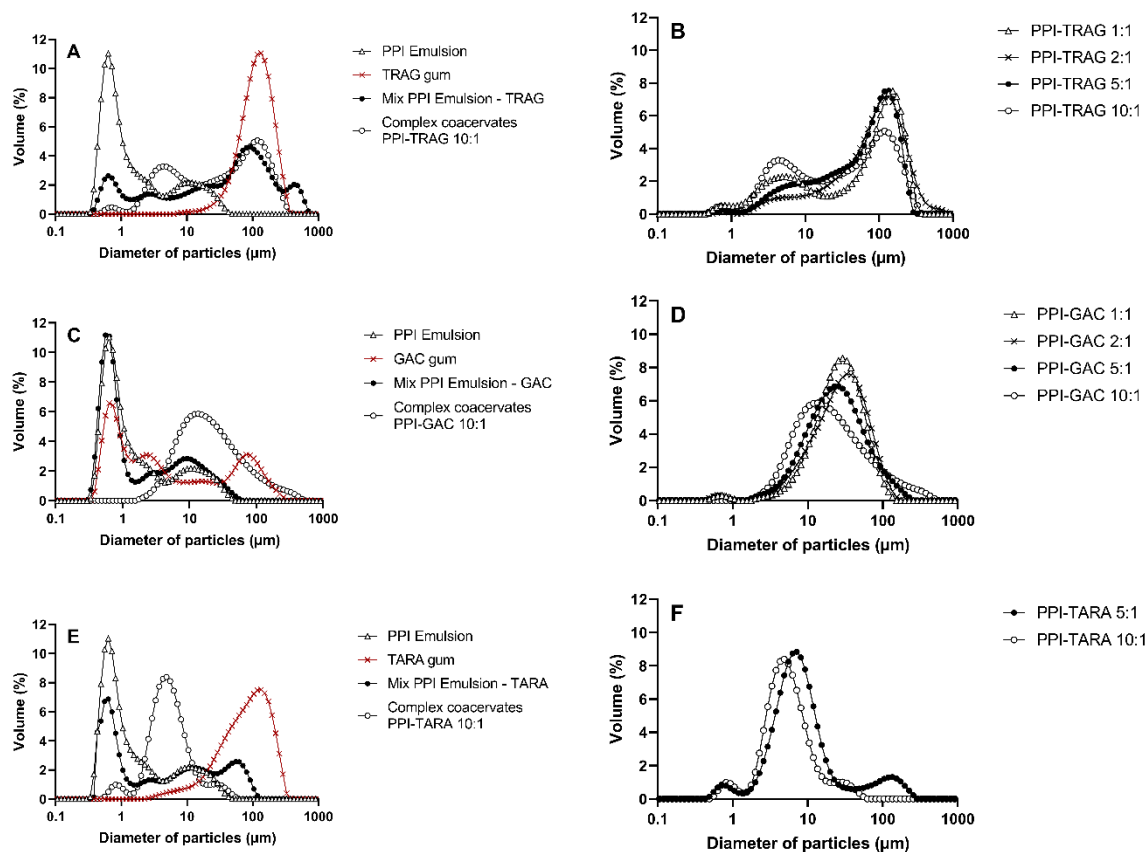
366 When comparing the size distribution of the suspensions after pH adjustment in the presence of gum
367 arabic or tragacanth gum, a monomodal population was obtained in the presence of gum arabic
368 while in the presence of tragacanth gum, a bimodal distribution was observed. Thus, gum arabic
369 could reduce the alteration of the emulsion quality compared to tragacanth gum.

370 We also investigated the change in size distribution of suspended particles depending on the
371 polysaccharide (tragacanth gum, gum arabic or tara gum) and the protein/polysaccharide ratio (1:1,
372 2:1, 5:1 and 10:1) (m/m) used (**Figure 1B, D, F**). **Table 2 supplemental data** shows the size of the
373 different populations observed for each particle size distribution and the percentage of each
374 population. The different solutions were analyzed after pH adjustment, inducing the complex
375 coacervation process.

376 When gum arabic was used, the distribution of suspended particles was monomodal regardless of
377 the ratio used (**Figure 1D**). Apart from the 1:1 ratio, the suspension size decreased with the decrease
378 in gum arabic proportion (the size of the major peak decreased from 34.8 μm at the 2:1 ratio to 13.1
379 μm at the 10:1 ratio) (**Table 2 supplemental data**). It could be assumed that the presence of PPI
380 stabilized the emulsion under high pressure homogenization more than increasing the
381 polysaccharide load. Also, the increase of polysaccharide proportion in the mixture lead to an
382 increase of bridging flocculation between oil droplets by interacting with positively charged proteins
383 – oil droplets.

384 When tragacanth gum was used, a bimodal distribution was found regardless of the ratio used
385 (**Figure 1B**). The size of the main particle population ranged between 40 and 200 μm . The size of the
386 second population ranged between 1 and 20 μm . As for gum arabic, the diameter of the major peak
387 decreased with the decrease in polysaccharide proportion in the mixture with a similar mechanism
388 (**Table 2 supplemental data**).

389 The same observation was made for tara gum (**Figure 1F**). Thus, it was assumed that the presence of
390 the polysaccharide affected the emulsion quality by increasing particle size distribution.



391
 392 *Figure 1: Evolution of droplet/particle size distribution in dispersions during the complex coacervation process for: PPI-TRAG*
 393 *(A), PPI-GAC (C) and PPI-TARA (E), all at the 10:1 ratio. -x- corresponds to the polysaccharide solution alone; -Δ- corresponds*
 394 *to the PPI-oil emulsion after homogenization at 250 bars; -●- corresponds to the PPI-polysaccharide mixture before pH*
 395 *adjustment; -○- corresponds to the PPI-polysaccharide mixture after pH adjustment. Effect of the protein/polysaccharide*
 396 *ratio on the particle size distribution of PPI-TRAG (B), PPI-GAC (D) and PPI-TARA (F) encapsulating α-tocopherol.*

397
 398 **3.2. Characteristics of microcapsules**

399 The influence of the polysaccharide and protein/polysaccharide ratio used on different parameters
 400 such as the moisture content, mean particle diameter, atomization efficiency and encapsulation
 401 efficiency were studied (**Table 2**).

402 Moisture content is a key parameter, which is related to the drying efficiency. A lower moisture
 403 contents limits the ability of water to act as a plasticizer and to reduce the glass transition
 404 temperature (Ferrari et al., 2012). The moisture content obtained for atomized powders was of 3-4%.
 405 The values obtained for the different protein/polysaccharide mixtures were similar to the moisture
 406 content of microcapsules containing only PPI. The nature of the polysaccharide associated with PPI or
 407 the PPI/polysaccharide ratio did not significantly influence the moisture content of the spray-dried
 408 powders. The nature of the polysaccharide associated with PPI or the PPI/polysaccharide ratio did
 409 not seem to significantly influence the moisture content of the spray-dried powders. According to
 410 Botrel *et al.*, the moisture content of spray-dried particles was mainly influenced by the variables of
 411 the drying process such as the process inlet air temperature (Botrel et al., 2014). Changes in the
 412 composition of the encapsulating wall had little effect on the moisture content of the powder.

413 The analysis of spray-dried particle size revealed micrometer-scale particles with a diameter D(V; 0.5)
 414 ranging between 10 and 80 μm in the different mixtures. Drying a PPI emulsion encapsulating a
 415 hydrophobic active ingredient enabled the formation of microparticles with the smallest size (10.6
 416 μm). The results were in accordance with the results usually found in the literature (Costa et al.,
 417 2015; Pereira et al., 2009). In the presence of gum arabic, the microparticle size did not significantly
 418 vary depending on the protein/polysaccharide ratio. In the presence of tragacanth gum, the mean
 419 diameter decreased with the increase in protein/polysaccharide ratio (mean particle size ranging
 420 between 78.3 and 24.4 μm for the 1:1 and 10:1 PPI-TRAG ratios, respectively). This could be
 421 explained by the increase in viscosity with increasing polysaccharide proportion. Xie *et al.* have
 422 shown that decreasing the viscosity of spray-dried preparations reduced microparticle size (Xie et al.,
 423 2010). In the presence of tara gum, a small decrease in particle size was observed with the increase
 424 in protein/polysaccharide ratio. This could also be due to the viscosity of the solutions. In the
 425 presence of Tara gum, particle size significantly increased compared to PPI alone, attributed to the
 426 viscosity of the solutions.

427 The encapsulation efficiency (%EE) of the microcapsules was affected by the ability of the
 428 encapsulating material to form a continuous layer around the oil drops, reducing coalescence
 429 phenomena (Bajaj et al., 2017). The encapsulation efficiencies of microcapsules with a theoretical
 430 load of 25% of hydrophobic active ingredient are presented in **Table 2**. PPI encapsulation efficiency
 431 was of 53.4%. Different trends were observed depending on the nature of the polysaccharide. For
 432 the PPI-GAC interaction, a significant increase in encapsulation efficiency was observed with a
 433 decrease in protein/polysaccharide ratio (77.4% at the 1:1 PPI-GAC ratio *versus* 42.7% at the 10:1
 434 ratio). The addition of carbohydrate to the proteinaceous wall system reduces the diffusion of the
 435 solvent through the matrix, due to the formation of a dry crust and a continuous glass phase in which
 436 proteins are dispersed, increasing the microencapsulation efficiency (Costa et al., 2015; Rosenberg et
 437 al., 1990). This phenomenon was not observed in the case of other protein-polysaccharide
 438 interactions. For tragacanth gum and tara gum, no trend was observed with the various ratios.

439 The atomization efficiency (%SY) of the microcapsules, presented in **Table 2**, varied from 53 to 85%
 440 depending on the mixture. No trend with the spray efficiency was observed between the different
 441 ratios of the same mixture. The spray efficiencies obtained could be related to the presence of
 442 powders adhering to the spray-dryer during the process or to the loss of fine particles through the
 443 spray tower filters. For tragacanth gum and tara gum, the atomization yields were lower. For tara
 444 gum, the yield did not exceed 53%. According to Nesterenko *et al.*, this decrease in spray yield could
 445 be related to the viscosity of emulsions (Nesterenko et al., 2012). High viscosities interfere with the
 446 atomization process and lead to the formation of elongated and large droplets that adversely affect
 447 the drying rate (Rosenberg et al., 1990).

448 *Table 2: Characteristics of the prepared α -tocopherol microcapsules. Statistical significance was assessed by the one-way*
 449 *ANOVA test (n=3). The statistical differences between values obtained with mixture samples and negative control (PPI alone)*
 450 *are indicated by the letters a (p < 0.05), b (p < 0.01), c (p < 0.001) and d (p < 0.0001).*

Matrix	Ratio	Encapsulation efficiency (%)	Moisture content (%)	Particle size D (V ;0,5) (μm)	Spray-drying yield (%)
PPI	-	53.4 \pm 3.4	3.6 \pm 0.1	11.5 \pm 1.3	86
PPI-GAC	1:1	77.4 \pm 0.9 ^d	3.2 \pm 0.3	15.5 \pm 0.7	85

	2:1	54.1 ± 2.9	3.9 ± 0.2	15.2 ± 0.3	59
	5:1	44.6 ± 3.7	3.4 ± 0.1	15.6 ± 1.3	77
	10:1	42.7 ± 1.0 ^a	3.2 ± 0.4	16.6 ± 0.3	57
	1:1	54.1 ± 7.3	4.2 ± 0.4	78.3 ± 6.7 ^d	71
PPI-TRAG	2:1	71.2 ± 4.5 ^b	3.3 ± 0.1	57.5 ± 4.6 ^c	59
	5:1	52.1 ± 2.7	4.2 ± 0.2	14.6 ± 5.6	43
	10:1	61.2 ± 1.9	3.3 ± 0.0	24.4 ± 1.8	55
PPI-TARA	5:1	48.8 ± 1.1	3.8 ± 0.1	75.9 ± 18.4 ^d	53
	10:1	63.1 ± 2.7	3.5 ± 0.3	51.7 ± 23.1 ^b	53

451

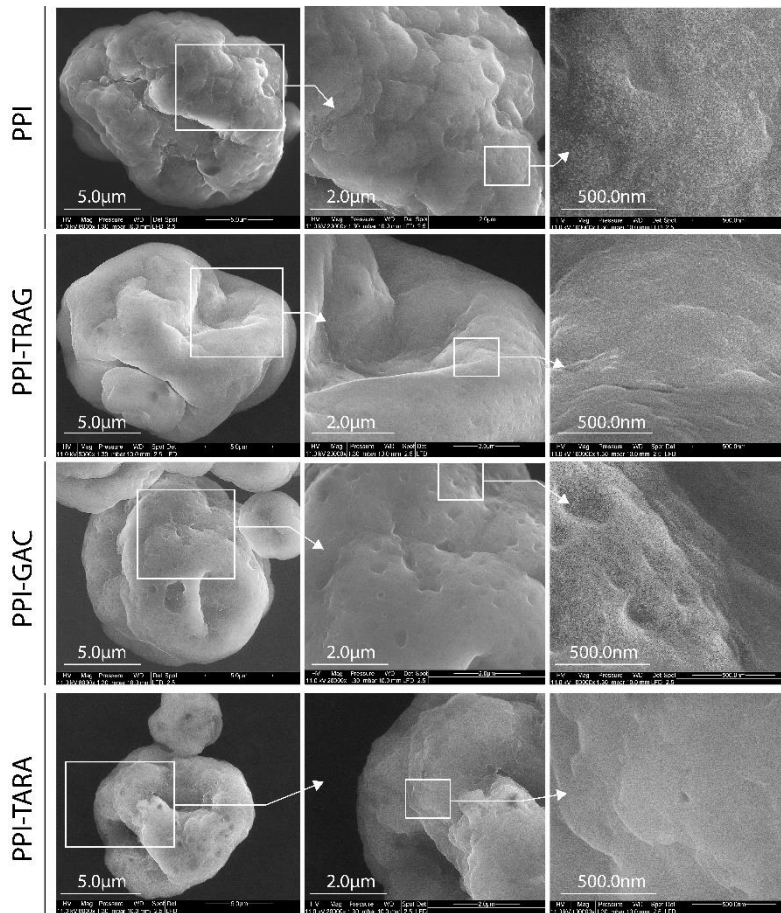
452 3.3. Morphological properties of the microcapsules

453 The microcapsule surface of the PPI-TRAG, PPI-GAC and PPI-TARA microcapsules at the 5:1 ratio and
 454 PPI microcapsules was analyzed (**Figure 2**).

455 The SEM observations showed particles with a size of about 10 μm. The microcapsules presented a
 456 deflated spherical shape characteristic of the spray drying process, in which material shrinkage
 457 occurs due to the rapid water loss during the initial stages of drying (Francisco et al., 2020). Overall,
 458 the topography of the surface of PPI particles encapsulating α-tocopherol showed a smooth surface
 459 and the absence of cracks or pores, protecting α-tocopherol. According to Oliveira *et al.*, the external
 460 surface of particles without cracks or breaks promotes low gas permeability, higher retention and
 461 protection of active ingredients (Oliveira et al., 2007). Spray-dried microcapsules are essentially
 462 spherical but morphological changes may be observed depending on the nature of the carrier used
 463 (Walton & Mumford, 1999). Especially, Microparticles with rough and/or invaginated surfaces
 464 present accelerated release of encapsulated material due to a greater surface area (Walton &
 465 Mumford, 1999). At the highest magnification, the absence of pores was evident, but small
 466 concavities were present, making the particle surface look like a "wrinkled" surface. In the study of
 467 the encapsulation of tuna oil by complex coacervates between milk proteins and gum arabic, the
 468 concavities were mainly related to the presence of the proteins within the wall of the spray-dried
 469 particles (Eratte et al., 2015).

470 The analysis of the surface of the PPI-TRAG microcapsules showed a smooth, pore-free surface as
 471 described in the initial analyzes. For PPI-GAC microcapsules, the surface was also smooth but cavities
 472 were present. At the lowest magnification, these cavities looked like pores. However, an analysis at
 473 the highest magnification confirmed the absence of pores and the presence of cavities. These cavities
 474 could be related to the second phase of spray drying, leading to a deformation of the encapsulating
 475 wall. Finally, for PPI-TARA microparticles, the morphology appeared to be intermediate between the
 476 smooth surface of the PPI-TRAG particles and the rough surface observed for microparticles of PPI

477 alone. This intermediate structure was related to the presence of tara gum, that facilitated particle
 478 smoothing due to the plasticizing properties of the polysaccharide. This phenomenon has been
 479 previously reported, in particular with maltodextrin (Jun-xia et al., 2011).



480
 481 *Figure 2: SEM observations of the different formulations of PPI, PPI-GAC, PPI-TRAG and PPI-TARA microcapsules at the 5:1*
 482 *ratio at x8,000, x20,000 and x100,000 magnifications.*

483 3.4. FTIR analysis

484 **Figure 3** shows the FTIR spectra of spray-dried PPI-TRAG microcapsules at the 2:1 ratio compared to
 485 FTIR spectra of the biopolymers forming the coating material (PPI and TRAG alone) and the
 486 hydrophobic mixture. The other microcapsules presented a similar behavior.

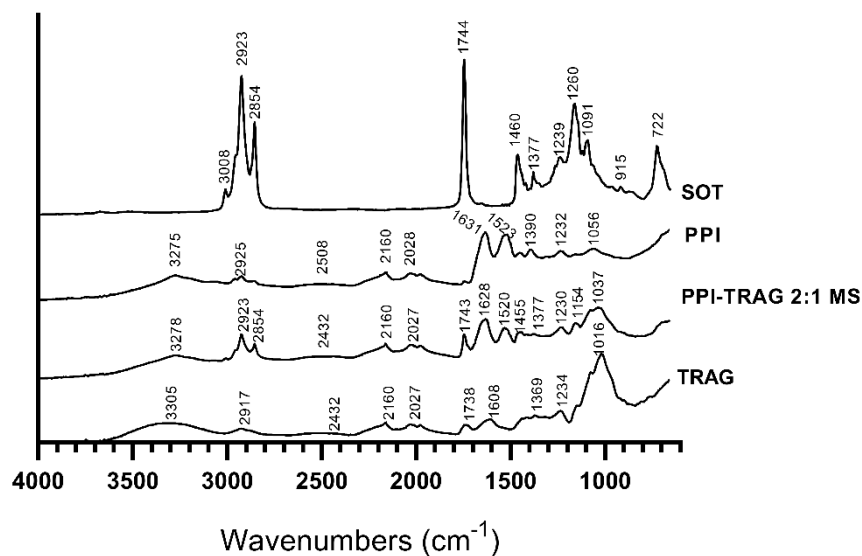
487 The FTIR spectra of PPI and TRAG were characterized in our previous work (Carpentier,2021). The
 488 study showed that the complex coacervate of PPI-TRAG led to a shift of the peaks of amide I, II and III
 489 of pea proteins from 1631, 1523 and 1390 cm^{-1} in PPI to 1628, 1520 and 1377 cm^{-1} in PPI-TRAG. This
 490 shift was attributed to the association of negatively charged TRAG with positively charged PPI during
 491 complex coacervates formation.

492 The spectrum of the mixture of sunflower oil and α -tocopherol (SOT) showed a small peak at 3,008
 493 cm^{-1} associated with the stretching of cis double-bond ($=\text{CH}$). Peaks at 2,923 and 2,854 cm^{-1} were
 494 respectively due to asymmetric and symmetric stretching vibrations of the aliphatic $-\text{CH}_2$ and $-\text{CH}_3$
 495 groups. The peak at 1,744 was known as absorption bands of triglycerides ($\text{C}=\text{O}$ of triglycerides or
 496 esters). The peak at 1,460 cm^{-1} was associated with $\text{C}=\text{C}$ stretching in the phenyl skeletal and methyl
 497 asymmetric bending of α -tocopherol and bending vibrations of the $-\text{CH}_2$ and $-\text{CH}_3$ groups of
 498 sunflower oil. The peak at 1,377 cm^{-1} was associated with rocking vibrations of CH bonds of cis-

499 disubstituted olefins and methyl symmetric bending of α -tocopherol. The peak at $1,091\text{ cm}^{-1}$ was
500 associated with stretching vibrations of the C-O ester group. The peak at 722 cm^{-1} corresponded to
501 rocking vibrations of methylene ($-\text{CH}_2$) and out of plan vibrations of cis-disubstituted olefins (Che
502 Man et al., 2005; Ma et al., 2021; Ozulku et al., 2017; Sim & Ting, 2012; Vlachos et al., 2006).

503 The FTIR spectra of PPI, TRAG, the hydrophobic mixtures and the PPI-TRAG microcapsules at the 2:1
504 ratio showed that the functional groups present in the microcapsules were similar to functional
505 groups of PPI and tragacanth gum.

506 The PPI structure clearly dominated in the spectrum of PPI-TRAG complexes due to the higher protein ratio
507 (2:1), but it was different from that of each individual biopolymer. The spectrum of PPI-TRAG microcapsules
508 at the 2:1 ratio showed all the characteristic peaks of the α -tocopherol/sunflower oil mixture, indicating
509 that the active compound was incorporated into the microcapsules. FTIR measurements did not allow to
510 distinguish the α -tocopherol encapsulated into the microcapsules from the free α -tocopherol present on
511 the surface.



512

513 *Figure 3: FTIR spectra of the PPI-TRAG microcapsules prepared at the 2:1 ratio compared to the spectra of PPI, TRAG and*
514 *SOT (sunflower oil/ α -tocopherol mixture at a 90:10 ratio) alone.*

515 3.5. Influence of the pH on the microcapsule release behavior

516 **Figure 4** shows the release profiles of α -tocopherol in acidic medium (pH 2.0) (**Figure 4 A,C,E**) and in
517 neutral medium (pH 6.8) (**Figure 4 B,D,F**) in the presence of PPI or the complex coacervates from the
518 PPI-GAC, PPI-TRAG and PPI-TARA mixtures at different ratios (1:1, 2:1, 5:1 and 10:1). The percentage
519 of α -tocopherol released was expressed relative to the theoretical α -tocopherol level in microcapsule
520 formulations (25% m/m).

521 For PPI microcapsules alone, a similar release profile was observed regardless of the pH (6.8 or 2.0).
522 At 90 minutes, the release was of 40% at pH 2.0 and 6.8. In both media, the release kinetics can be
523 associated with a two-step biphasic process, with a first step of rapid release (or "burst effect")
524 followed by a second step of lower release (Luo et al., 2011).

525 In the presence of gum arabic, different release profiles were obtained.

526 In acidic medium (pH 2.0), the release profile differed from that of PPI microcapsules alone (**Figure**
527 **4C**). The release of α -tocopherol increased with the increase in protein/polysaccharide ratio, from 15

528 to 54% for the 1:1 and 10:1 ratios, respectively, at 90 minutes. The 1:1 and 2:1 ratios were associated
529 with a similar release profile <20% in the acidic medium. The burst effect observed until t=30
530 minutes was significantly reduced for both ratios compared to the 5:1 ratio. The addition of gum
531 arabic to the mixture improved protection at an acidic pH. However, increasing the ratio (>5:1)
532 increased the release of α -tocopherol, up to release levels greater than those obtained with PPI
533 alone. In neutral medium (pH 6.8), an increased release of α -tocopherol was observed with an
534 increase in protein/polysaccharide ratio, except for the 2:1 ratio (**Figure 4D**). The release profiles at
535 the 5:1 and 10:1 ratios were close to the release profile of PPI alone. The 2:1 ratio was associated
536 with the highest release (release of 39%). The comparison of the release profiles in the two media
537 showed that the presence of gum arabic resulted in a partial protection (15-20%) in the acidic
538 medium and in a release in the neutral medium for ratios <5:1. The difference in release profiles
539 between the acidic and neutral media could be due to a pH-dependent fragility of the microcapsules.
540 At pH 2.0, the electrostatic interactions between PPI and gum arabic were favorable, the
541 biopolymers having opposite charges. On the other hand, in the neutral medium, the pH was higher
542 than the isoelectric point of PPI and the system was dominated by the presence of repellent
543 electrostatic interactions, due to the predominance of negative charges between the two
544 biopolymers (Bastos et al., 2020). Therefore, the complexes were destabilized, resulting in a greater
545 release of α -tocopherol. For a pH-dependent behavior adapted to the protection/release of a
546 hydrophobic active ingredient in a monogastric system, the 2:1 PPI-GAC ratio appeared to be the
547 most effective with a release <20% in acidic medium and a higher release in neutral medium (release
548 of 40%). For higher ratios, the opposite phenomenon was observed with a higher release in acidic
549 medium than in neutral medium (α -tocopherol release of 50% at pH 2.0 *versus* 33% at pH 6.8 after
550 120 minutes for the 5:1 ratio).

551 The release profiles of the PPI-TRAG mixture at the 1:1, 2:1, 5:1 and 10:1 ratios in acidic and neutral
552 media are shown in **Figure 4A, B**. At acidic pH, the release of α -tocopherol increased with the
553 protein/polysaccharide ratio (**Figure 4A**). As with gum arabic, the 1:1 and 2:1 ratios were associated
554 with a low release at acidic pH (release <10%). For ratios >5:1, the release profile was similar to that
555 of PPI alone with release of the active ingredient after 10 minutes. Thus, the presence of tragacanth
556 gum improved protection in acidic environments. A comparison of the release profiles obtained at
557 120 minutes in the two media showed a different behavior according to the protein/polysaccharide
558 ratio tested as demonstrated in PPI-GAC mixtures. As with gum arabic, α -tocopherol release
559 increased at pH 6.8 with increasing protein/polysaccharide ratio, except for the 5:1 ratio (**Figure 4B**).
560 At the 5:1 ratio, the release of α -tocopherol was greater than in the presence of PPI alone. For the
561 5:1 and 10:1 ratios, a biphasic release system similar to that of PPI alone was observed with a first
562 step of rapid release observed until t=60 minutes and a second step of slow release thereafter. This
563 phenomenon has been observed in a study describing the encapsulation of β -carotene by complex
564 coacervates of caseinate and tragacanth gum (Jain et al., 2016). In this study, the rapid release effect
565 was associated with a rapid release of the active ingredient adsorbed at the microcapsule surface or
566 a loss of wall integrity during particle drying. The slower release was related to the mixed
567 mechanisms of penetration, solubilization and diffusion of the lipophilic phase through the polymeric
568 caseinate-gum tragacanth wall.

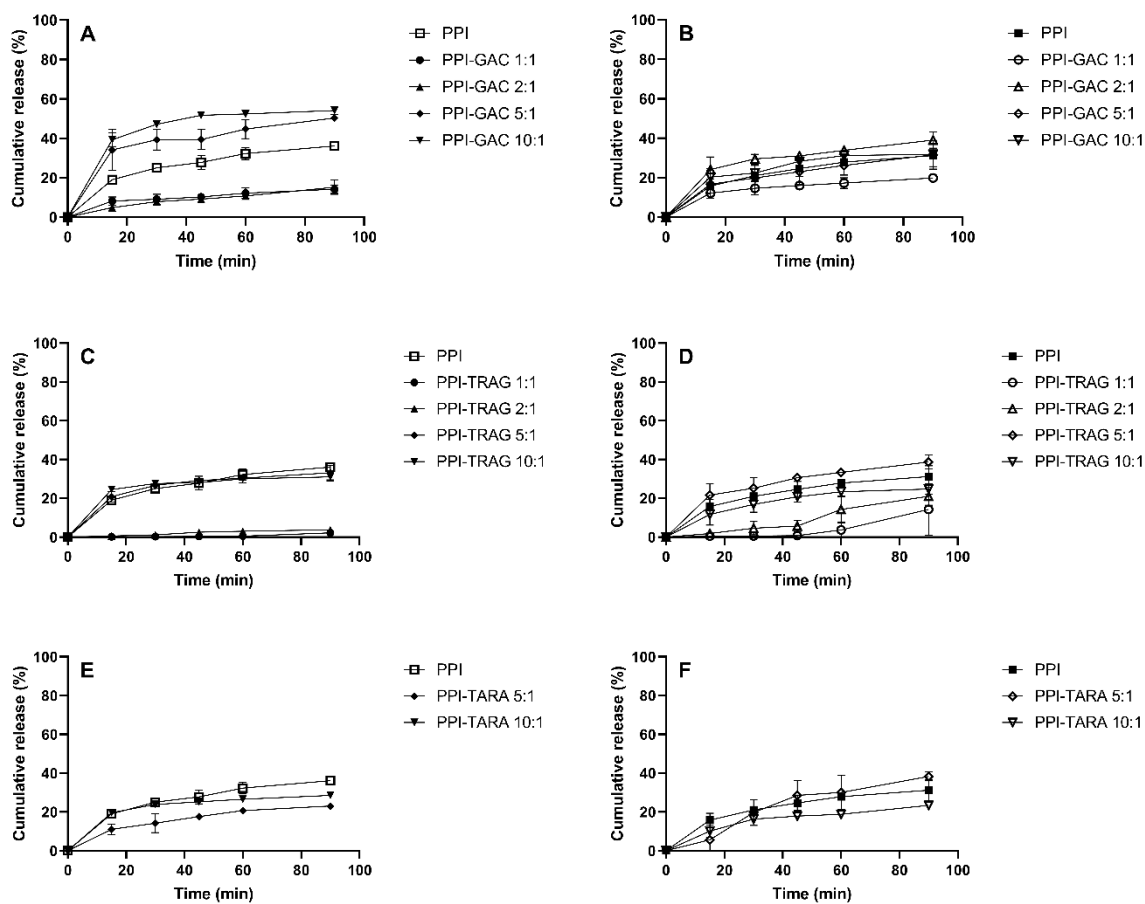
569 **Figure 4 E, F** shows the release profiles of the PPI-TARA mixture at the 5:1 and 10:1 ratios in acidic
570 (pH 2.0) and neutral (pH 6.8) media. In the presence of tara gum, the release of α -tocopherol varied
571 depending on the ratio. At pH 2.0, the release of α -tocopherol was lower in the presence of tara gum
572 (**Figure 4E**). The higher the proportion of tara gum was, the more the mixture reduced the release of
573 the active ingredient in an acidic environment. At pH 6.8, the release of α -tocopherol was greater
574 with the 5:1 PPI-TARA ratio than with PPI alone (40% and 33%, respectively, after 90 minutes of

575 incubation) (**Figure 4F**). For the 10:1 ratio, the release was lower (25% after 90 minutes of
576 incubation). The difference in stability between the 5:1 and 10:1 ratios could be due to the difference
577 in emulsion particle size between the two formulations. The PPI-TARA mixture had a smaller mean
578 emulsion droplet diameter at the 10:1 ratio than at the 5:1 ratio. This difference in emulsion size
579 could improve the stability of PPI-TARA microcapsules at the 10:1 ratio at pH 6.8 and thus limit the
580 release of the active ingredient. In a similar study, the development of emulsions from milk proteins
581 and guar gum delayed the release of vitamins under simulated gastric conditions and led to a release
582 in a simulated enteric environment associated with stronger effects than in the presence of gum
583 arabic (Li et al., 2011). Guar gum has structural similarities with tara gum in terms of galactomannan
584 composition, only the mannose/galactose ratio differs (Wu et al., 2017). However, the comparison of
585 the two release profiles of the PPI-TARA mixture at pH 6.8 and 2.0 did not show any significant
586 difference in active ingredient release profiles and no conclusion on the pH-dependent release
587 properties of the microcapsules could be drawn for the PPI-TARA mixture.

588 Studying the influence of the loaded polysaccharide on the release of α -tocopherol at the 2:1
589 protein-polysaccharide ratio, gum arabic and tragacanth gum appeared to provide the best
590 protection of the active ingredient in acidic medium since they were associated with the lowest
591 release rate (<20% after two hours). Among these two gums, tragacanth gum showed an interesting
592 gastroresistant potential because its associated release rate did not exceed 10%. At pH 6.8, the
593 highest release of α -tocopherol was obtained with gum arabic with a release of the active ingredient
594 of 39% after 90 minutes. Tragacanth gum was again associated with the lowest α -tocopherol release
595 rate after 90 minutes (release of 21%).

596 These profiles highlighted the interesting potential of tragacanth gum associated with PPI for the
597 protection of a lipophilic active ingredient in an acidic environment. Despite a lower release at pH 6.8
598 than with gum arabic, the complex coacervation between PPI and tragacanth gum could be an
599 interesting alternative to gum arabic commonly used for the delivery of liposoluble active ingredients
600 with a pH-dependent controlled release. These formulations were used in the release behavior
601 studies performed under enzymatic digestion conditions described below.

602 In addition, a trend in the change in microparticle release properties was also observed depending on
603 the nature of the polysaccharide and protein/polysaccharide ratio used. For gum arabic and
604 tragacanth gum, in particular, the mixtures appeared to have similar behaviors depending on the
605 ratio. A gastroprotective behavior was observed at the 1:1 and 2:1 ratios and an opposite behavior
606 was observed when the ratio was increased (from the 5:1 ratio) with a higher release in acidic
607 medium than in neutral medium.



608

609 *Figure 4: Influence of the protein/polysaccharide ratio in PPI-TRAG, PPI-GAC and PPI-TARA microcapsules on the time-*
 610 *dependent release profile of α -tocopherol at pH 2.0 (A, C, E) and pH 6.8 (B, D, F).*

611 3.6. Influence of digestive enzymes on the microcapsule release behavior

612 α -tocopherol encapsulated in PPI alone and in complex coacervates between PPI and GAC and
 613 between PPI and TRAG at the 1:1 and 2:1 ratios was incubated at 37°C for 90 minutes under
 614 simulated gastric (pepsin solution at pH 2.0) or intestinal (pancreatin solution at pH 6.8) conditions to
 615 assess its release profile (**Figure 5**). These formulations were selected from the previous release
 616 studies for their gastroprotective properties and compared to the control formulation (PPI alone).

617 α -tocopherol release was increased when the digestive enzymes were added in the simulated gastric
 618 and intestinal media compared to the buffered solutions.

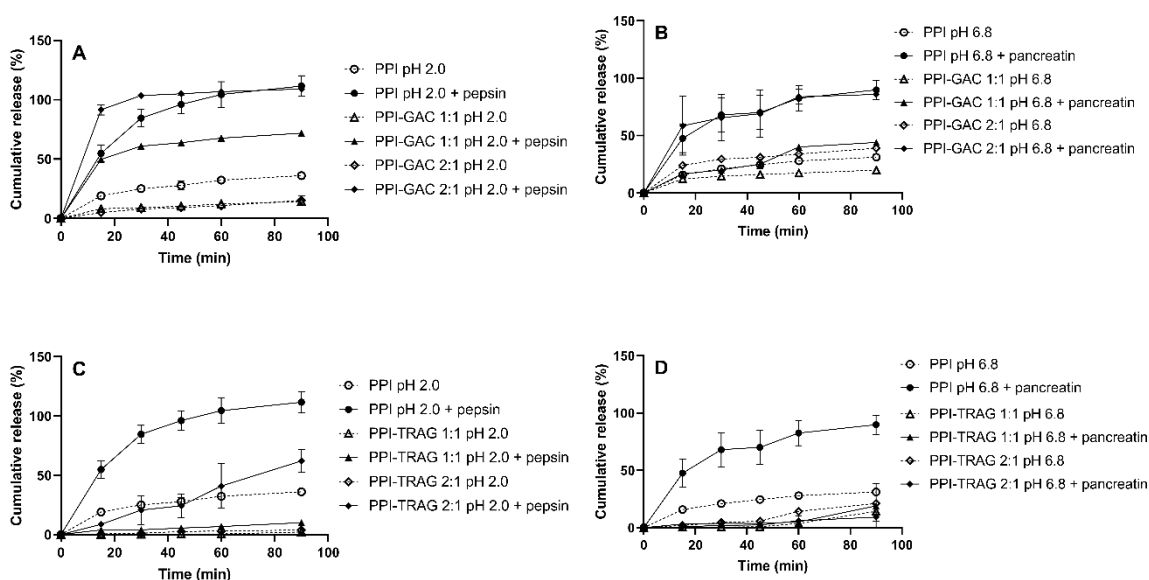
619 For the microcapsules of PPI alone, the change in α -tocopherol release was more rapid under gastric
 620 conditions than under intestinal conditions with a complete release of α -tocopherol (100%) achieved
 621 after 45 and 90 minutes, respectively (**Figure 5 A, B**). The PPI used to form these microcapsules
 622 consist of a heterogeneous mixture of proteins of varying molecular weight and complex assemblies
 623 of subunits linked together by disulfide bridges or low energy bonds. In the presence of pepsin, PPI
 624 were sensitive to hydrolysis, leading to the destabilization of their structure at acid pH (Perrot, 1995).

625 Associated with gum arabic, PPI showed equivalent gastroprotective properties at the 1:1 and 2:1
 626 (m/m) ratios in buffer solutions at pH 2.0. However, the presence of proteolytic enzymes such as
 627 pepsin destabilized the microcapsules since α -tocopherol release varied from 16% in buffer solution
 628 to respectively 72% and 100% at the 1:1 and 2:1 ratios in pepsin solution after 90 minutes of

629 incubation. The release profile with PPI-GAC microcapsules at the 2:1 ratio was slightly faster
 630 compared to PPI alone, with a complete release of α -tocopherol after 30 minutes in pepsin solution.
 631 The gastroprotective properties increased with the increase in polysaccharide amount in the complex
 632 coacervate formulation. Under intestinal conditions, PPI-GAC microcapsules at the 2:1 ratio showed a
 633 profile similar to that of PPI alone. With PPI-GAC microcapsules at the 1:1 ratio, the release rate of α -
 634 tocopherol was lower, showing again the proteolytic resistance of microcapsules in the presence of
 635 polysaccharides. For PPI-GAC microcapsules, the pH-dependent behavior observed in aqueous media
 636 seemed to be lost in presence of proteolytic activity.

637 With the PPI-TRAG mixture, the gastroprotective properties were preserved under proteolytic
 638 conditions, especially for the PPI-TRAG mixture at the 1:1 ratio before 90 minutes. With the increase
 639 in PPI-TRAG ratio from 1:1 to 2:1, a decrease in gastroprotective properties was observed with an α -
 640 tocopherol release rate of 40% after 60 minutes. As with the PPI-GAC mixture, the gastroprotective
 641 properties increased with the increase in polysaccharide amount in the microcapsule matrix. Under
 642 intestinal conditions, the protective properties at both ratios were preserved in the presence of
 643 pancreatin since the release profiles were similar to those obtained in buffer solutions at pH 6.8.
 644 Compared to PPI-GAC, PPI-TRAG showed a better resistance to the proteolytic action of pancreatin
 645 and pepsin. An assumption could be made concerning the organization of the PPI-TRAG matrix
 646 compared to the PPI-GAC matrix. As the PPI-TRAG matrix was associated with a lower release in the
 647 presence of pepsin, it could be assumed that tragacanth gum was organized around PPI during the
 648 formation of complex coacervates, preventing PPI degradation by pepsin. For PPI-GAC, a less well-
 649 organized matrix between PPI and gum arabic could be assumed. This difference in organization
 650 between PPI and polysaccharides could be related to the solubility of the respective gum.

651



652

653 *Figure 5: Influence of the protein/polysaccharide ratio in PPI-TRAG, PPI-GAC and PPI-TARA microcapsules on the time-*
 654 *dependent release profile of α -tocopherol in simulated digestive media at pH 2.0 (A, C) and pH 6.8 (B, D).*

655 The release of an encapsulated active compound may be associated with different mechanisms, such
 656 as the disintegration or erosion of the polymeric skeleton, the diffusion through the wall and pores of
 657 the polymeric matrix or osmosis (Estevinho et al., 2013). The release profiles of α -tocopherol in

658 simulated digestive media were analyzed using different mathematical models: zero order, first
 659 order, Higuchi and Korsmeyer-Peppas model equations to identify the release mechanisms.

660 For PPI alone and PPI-GAC microcapsules, the equation that presented the best correlation
 661 coefficient r^2 with α -tocopherol release profile data was the Korsmeyer-Peppas model (**supplemental**
 662 **data Table 3**). The diffusion exponent n , used to interpret the main release mechanism, was
 663 determined from the model equation (**Table 3**). According to the literature, the transport is
 664 controlled by a Fickian diffusion (case I transport) when n is <0.43 . When n ranges between 0.43 and
 665 0.85, an anomalous transport associated with a non-Fickian diffusion occurs, resulting from a
 666 combination of diffusion mechanisms and swelling releases. When n is >0.85 , the main transport
 667 mechanism is based on matrix swelling ($n=0.85$ case II transport; $n >0.85$ – super case transport)
 668 (Estevinho et al., 2013). For each release kinetics, the estimated n values were <0.43 , indicating that
 669 α -tocopherol release was associated with a Fickian diffusion. This mechanism seemed to be in
 670 contradiction with the non-porous morphology of the microcapsules. A potential hypothesis may be
 671 formulated. As the association between protein and polysaccharide is governed by electrostatic
 672 interactions, the hydration of the capsule matrix in aqueous media destabilizes the electrostatic
 673 interactions between the protein and the polysaccharide, partially destructuring the wall of the
 674 microcapsule. This partial weakness of the wall could lead to the diffusion of α -tocopherol out of the
 675 microcapsule.

676 For PPI-TRAG microcapsules, the Korsmeyer-Peppas model equation also fitted with α -tocopherol
 677 release profile data. Surprisingly, the release mechanism was associated with super case II transport
 678 with $n >0.85$, except for PPI-TRAG at the 1:1 ratio under simulated gastric condition for which the
 679 release mechanism was associated with an anomalous transport. The release rate constant K_k was
 680 very low with these microcapsules, indicating the absence of a burst drug release (Obidike & Emeje,
 681 2011) that suggested the resistance of microcapsules to proteolytic hydrolysis in the presence of
 682 tragacanth gum. The release rate constant and release exponent also suggested an erosion-
 683 controlled release mechanism for a 90-minute incubation time. It was observed in the first 60
 684 minutes the linear and low release of α -tocopherol in both media leading to a n value superior to
 685 0.85. It can be assumed, by pursuing the release profile at several hours, that the release of α -
 686 tocopherol from PPI-TRAG microcapsules will show a similar profile to PPI-GAC microcapsules. In this
 687 case, the release profile will be associated with Fickian diffusion.

688

689 *Table 3: Parameters of the kinetic equations and corresponding correlation coefficients*

		Korsmeyer Peppas		
		K_k	n	r^2
PPI	pH 2.0	24.04	0.3517	0.984
	pH 6.8	20.61	0.3311	0.991
PPI-GAC 1:1	pH 2.0	30.42	0.1939	0.998
	pH 6.8	2.52	0.6418	0.959
PPI-GAC 2:1	pH 2.0	73.58	0.09101	0.998
	pH 6.8	30.13	0.2347	0.990
PPI-TRAG 1:1	pH 2.0	0.45	0.6809	0.964
	pH 6.8	3.5E-4	2.416	0.917
PPI-TRAG 2:1	pH 2.0	0.40	1.121	0.989
	pH 6.8	0.01	1.486	0.969

690

691

692 **4. Conclusion**

693 The interactions between PPI and different polysaccharides were applied to the microencapsulation
694 of α -tocopherol by spray drying of complex coacervates. The formulations, corresponding to
695 different protein/polysaccharide mixtures, were characterized: PPI alone or associated with gum
696 arabic, tragacanth gum or tara gum. The size distribution of emulsion oil droplets and complex
697 coacervate particles in suspension showed an increase of the particles in suspension, correlated with
698 electrostatic interactions between the negatively charged polysaccharide to the positively charged oil
699 droplets. The comparison of the two charged polysaccharides showed that gum arabic allowed
700 obtaining finer particles than tragacanth gum, due to the insoluble fraction of tragacanth gum. An
701 influence of the protein/polysaccharide ratio was also shown as the increase of the polysaccharide
702 proportion increased the size of the particles in suspension due to flocculation bridging by
703 electrostatic interactions. In the presence of an uncharged polysaccharide (tara gum), no influence
704 was observed on the particle size in the mixture, related to the absence of formation of complex
705 coacervates. SEM observations of spray-dried microcapsule surface showed different properties
706 depending on the polysaccharide used and the nature of the interaction between the plant proteins
707 and the polysaccharides. α -tocopherol release in buffer media highlighted the pH-dependent
708 behavior of gum arabic and tragacanth gum depending on the ratio used. An interesting acid stability
709 was identified at the 1:1 and 2:1 protein/polysaccharide ratios in the case of PPI-GAC and PPI-TRAG
710 due to electrostatic interactions between oppositely charged biopolymers. Among the two gums
711 selected, tragacanth gum had the best acid stability. In the presence of proteolytic enzymes, the PPI-
712 TRAG mixture maintained a stronger gastroprotective behavior compared to PPI-GAC matrices. Our
713 results showed the ability of PPI to associate with plant polysaccharides, especially with tragacanth
714 gum, to form an interesting pH-dependent coating for microencapsulation, resistant to gastric
715 digestion and compatible with vegan/vegetarian applications.

716

717 **Declaration of competing interest**

718 The authors are not aware of any affiliations, memberships, funding or financial holdings that could affect
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