

Investigation of the fabrication and subsequent emulsifying capacity of potato protein isolate/k-carrageenan electrostatic complexes

O'Sullivan, Jonathan; Kurukji, Daniel; Norton, Ian T.; Spyropoulos, Fotios

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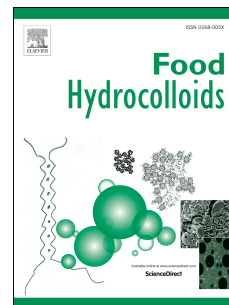
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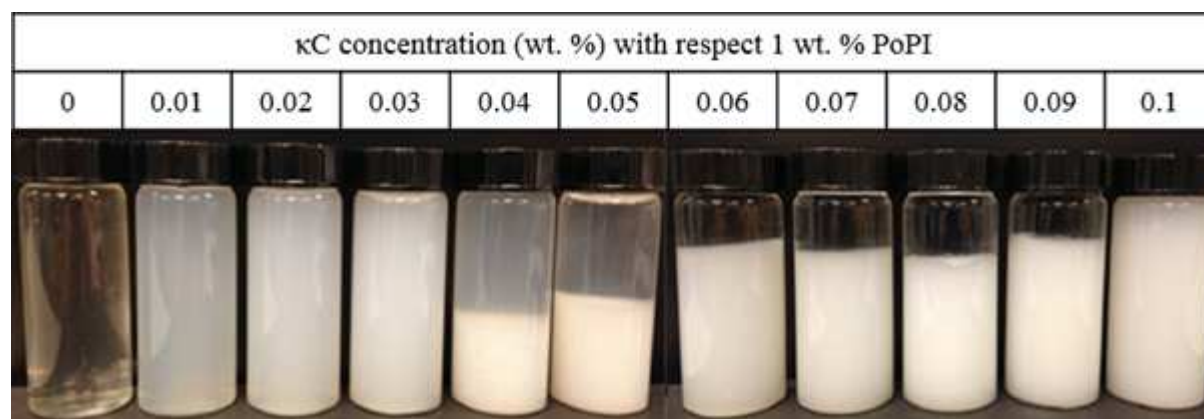
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ACCEPTED MANUSCRIPT

1 **Investigation of the fabrication and subsequent emulsifying capacity of potato protein**
2 **isolate/ κ -carrageenan electrostatic complexes**

3

4 Jonathan O'Sullivan*, Daniel Kurukji, Ian Norton, Fotis Spyropoulos

5 School of Chemical Engineering, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK

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7 * Corresponding author. *Email address:* j.j.osullivan@bham.ac.uk

8 Abstract

9 The fabrication of protein-polysaccharide complexes via electrostatic interactions was investigated
10 with a naturally cationic protein, potato protein isolate (PoPI), and an anionic polysaccharide, κ -carrageenan
11 (κ C), at unadjusted pH conditions. Moreover, the emulsifying capacity of these electrostatic complexes (PoPI-
12 κ C) was assessed. PoPI- κ C complexes were prepared with a fixed concentration of PoPI (1 wt. %), and varying
13 concentrations of κ C (0.01 – 0.5 wt. %), using gentle agitation, followed by sonication to fabricate the
14 complexes. The physicochemical properties of PoPI- κ C complexes was assessed in terms of size and surface
15 charge, measured using light scattering techniques and electrokinetic potential, respectively. The emulsifying
16 performance of emulsions prepared with PoPI- κ C complexes was assessed as a function of κ C, and to PoPI,
17 with respect to initial emulsion droplet size, emulsion stability, interfacial tension and optical microscopy.

18 Addition of κ C to a 1 wt. % PoPI solution yielded the formation of submicron (~120 nm) electrostatic
19 complexes up to a κ C concentration of ≤ 0.0375 wt. %. Higher concentrations of κ C yielded micron sized
20 complexes ($> 10 \mu\text{m}$). Emulsions prepared with PoPI- κ C complexes yielded comparable emulsion droplet sizes
21 to that of PoPI alone, with the exception of complexes prepared with κ C in the range of 0.05 – 0.07 wt. %.
22 Larger emulsion droplets were observed, as these complexes possessed an electrokinetic potential close to the
23 isoelectric point, resulting in aggregation. Emulsions prepared with PoPI- κ C complexes possessed marginally
24 enhanced long-term stability in comparison to emulsions prepared with PoPI alone.

25

26 **Keywords:** *Solanum tuberosum*, Potato protein isolate, κ -carrageenan, Complexes,
27 Coacervates, O/W emulsions

28 1. Introduction

29 Emulsions are mixtures of two immiscible fluids, whereby one fluid manifests as
30 spherical droplets dispersed within the other fluid (Walstra, 1993). Emulsions are employed
31 within a myriad of food formulations (*e.g.*, salad dressings, yoghurt, margarine, etc.) and the
32 fluids in these systems are typically oil and water (McClements, 2005). Invariably, there are
33 two main classes of simple emulsion: oil-in-water (O/W) emulsions whereby oil droplets are
34 dispersed within an aqueous continuous phase, and water-in-oil (W/O) emulsions whereby
35 water droplets are dispersed within an oil continuous phase (McClements, 2009). By their
36 very nature, emulsions are thermodynamically unstable systems, which are stabilised by a
37 class of chemical entities known as emulsifiers. There are three main categories of
38 emulsifying agents: (1) low molecular weight surfactants (*e.g.* sodium dodecyl sulphate,
39 polysorbates, etc.), (2) high molecular weight biopolymers (*e.g.* sodium caseinate, gelatin,
40 etc.), and (3) solid particles, known as Pickering particles (*e.g.* colloidal silica, starch
41 granules, etc.) (Kurukji *et al.*, 2013; O'Sullivan *et al.*, 2014; O'Sullivan *et al.*, 2015; Rayner
42 *et al.*, 2012; Walstra & Smulders, 2000).

43 Proteins and polysaccharides are biopolymers utilised within the food,
44 pharmaceutical, and agrochemical industries for a myriad of applications, such as emulsion
45 stabilisation by proteins and viscosity enhancement of solutions by high molecular weight
46 polysaccharides (Foegeding & Davis, 2011; Morris *et al.*, 1981). Studies investigating the
47 interactions between proteins and polysaccharides are numerous in the research literature, and
48 it is well known that different biopolymers can interact via electrostatic interactions to form
49 colloidal or supramolecular entities, referred to as complexes (Dickinson, 2006; Kurukji *et al.*,
50 2015). The electrostatic complexation of proteins and polysaccharides have also been
51 considered as a possible fabrication route to food grade Pickering particles, whereby
52 emulsions stabilised with Pickering particles typically exhibit enhanced emulsion stability in

53 comparison to those stabilised with surfactants or biopolymers (Kurukji *et al.*, 2015; Pichot *et*
54 *al.*, 2010; Vignati *et al.*, 2003). Electrostatic complexation between proteins and
55 polysaccharides usually occurs when each of these respective biopolymers possess opposite
56 charges, and this is often achieved through controlling the pH and/or ionic conditions of the
57 serum phase (Kurukji, *et al.*, 2015; Rodríguez Patino & Pilosof, 2011). In this work, a
58 simplified method for the fabrication of electrostatic complexes is reported, precluding the
59 necessity for pH adjustments. To achieve this, a naturally cationic protein at an unadjusted
60 pH (*i.e.*, after solubilisation; potato protein isolate (PoPI)) was complexed with a naturally
61 anionic polysaccharide at an unadjusted pH (κ -carrageenan (κ C)). More broadly, this adds a
62 novel and unique biopolymer combination to the research tool-box and moves away from the
63 necessity of reducing the pH below the isoelectric point of proteins to promote electrostatic
64 complexation, as is the case for dairy protein based complexes. Be that as it may, many
65 industrially relevant formulations possess a wide range of ingredients, some of which may
66 alter, deliberately or unintentionally, the pH within the final product (O'Sullivan &
67 O'Mahony, 2016). Thus, it is essential to consider to pH sensitivity of such electrostatic
68 complexes for their incorporation within food systems.

69 Potato protein isolate (PoPI), extracted from *Solanum tuberosum*, is a highly
70 functional ingredient readily capable of emulsification, foaming and gelation (Holm &
71 Eriksen, 2007; O'Sullivan & O'Mahony, 2016; Ralet & Guéguen, 2000; van Koningsveld *et*
72 *al.*, 2002, 2006). There are three main protein fractions within PoPI: (1) patatin (41 kDa), a
73 glycoprotein, (2) protease inhibitors (5 – 25 kDa) and other minor fractions (higher molecular
74 weight species) (Løkra *et al.*, 2008; Snyder & Desborough, 1980). Upon solubilisation,
75 commercially available PoPI (section 2.1) exhibits cationic behaviour at unadjusted pH
76 conditions (pH 3.6; section 2.1). Systems possessing cationic characteristics are of particular
77 interest for targeted delivery in humans, through mechanisms including enhanced

78 mucoadhesiveness and interactions with bioreceptors (Grabovac *et al.*, 2005). This makes
79 PoPI an interesting biopolymer to study.

80 κ -carrageenan (κ C) is a polysaccharide of particular interest to the food industry, for
81 both the enhancement of viscosity at comparatively low concentrations, due to its high
82 molecular weight and hydrodynamic structure, and the development of a gelled network in
83 the presence of alkali metal counterions (*e.g.* Na^+ , K^+ , Rb^+) (Gładkowska–Balewicz, *et al.*,
84 2014; Hermansson, *et al.*, 1991; Millane, *et al.*, 1988; O’Sullivan & O’Mahony, 2016). κ C,
85 extracted from Rhodophyta (*e.g.* *Chondrus crispus*), is a sulphated D-galactan, with one
86 sulphate group (*i.e.* SO_4^{2-}) on each disaccharide monomer unit (Millane, *et al.*, 1988).

87 In the present work, protein-polysaccharide complexes were fabricated for the first
88 time with potato protein isolate (PoPI), a naturally cationic biopolymer at an unadjusted pH
89 conditions (pH 3.6; section 2.1), and κ -carrageenan (κ C), an anionic polysaccharide at an
90 unadjusted pH conditions (pH 3.55; section 2.1), in order to assess the ability of these
91 biopolymers to form electrostatic complexes. The objective of this research was to assess the
92 effect of κ C concentration, with a fixed concentration of PoPI, on the fabrication of
93 electrostatic protein-polysaccharide complexes, discerned in terms of initial complex size,
94 complex stability and electrokinetic potential. Moreover, the emulsifying performance of
95 these electrostatic complexes was probed in terms of initial emulsion droplet size, emulsion
96 stability and interfacial tension. Electrostatic complexes were prepared with a fixed
97 concentration of PoPI and increasing concentrations of κ C, and subsequently investigated for
98 their capacity to form emulsions.

99 **2. Materials and methodology**

100 **2.1. Materials**

101 Potato protein isolate (PoPI) was kindly provided by Solanic B.V. (Veendam, the
102 Netherlands), and the protein, moisture and ash content was 90 wt. %, 6 wt. % and 4 wt. %,
103 respectively. The composition of PoPI was acquired from material specification form from
104 the supplier. Sodium azide and κ -carrageenan (κ C) were purchased from Sigma Aldrich
105 (UK). The unadjusted pH of PoPI and κ -C was 3.6 and 3.55, respectively, measured at a
106 temperature of 20 °C and a biopolymer concentration of 1 wt. %. The oil used was
107 commercially available rapeseed oil. All materials were used without further purification.
108 The water was passed through a double distillation unit (A4000D, Aquatron, UK).

109 **2.2. Methods**

110 **2.2.1. Preparation of protein and polysaccharide solutions**

111 Potato protein isolate (PoPI) and κ -carrageenan (κ C) solutions were prepared by
112 dispersion in distilled water, whereby a 5 wt. % and 1 wt. % stock solutions of PoPI and κ C,
113 respectively, were prepared. Both biopolymers were completely soluble at these
114 concentrations. Sodium azide (0.02 wt. %) was added to the solutions to mitigate against
115 microbial activity.

116 **2.2.2. Preparation of protein-polysaccharide complexes**

117 Protein-polysaccharide complexes were prepared with a fixed concentration of PoPI
118 (1 wt. %) and by varying the concentration of κ C (0.01 – 0.5 wt. %), as detailed in Table. 1,
119 whereby the total biopolymer concentration (TBC) in each system is an addition of the
120 concentration of PoPI and κ C. PoPI- κ C insoluble complexes were formed spontaneously by
121 careful addition of specific masses of κ C solution to a known quantity of PoPI solution under
122 gentle agitation (*i.e.* on a magnetic stirrer).

123 Owing to the presence of large aggregates, these suspensions were treated with an
124 ultrasonic processor (Viber Cell 750, Sonics, USA) to minimise their size, with a 12 mm
125 diameter stainless steel sonotrode with a frequency of 20 kHz and an ultrasonic amplitude
126 95% (wave amplitude of 108 μm at 100% amplitude) for 2 min, in an ice bath to reduce heat
127 gain. This yielded an acoustic intensity of $\sim 34 \text{ W cm}^{-2}$, which was determined by measuring
128 the temperature rise of the sample as a function of treatment time, under adiabatic conditions.
129 The acoustic intensity, I_a (W cm^{-2}), was determined as follows (Margulis & Margulis, 2003;
130 O'Sullivan *et al.*, 2015):

$$131 \quad I_a = \frac{P_a}{S_A}, \text{ where } P = m \cdot c_p \left(\frac{dT}{dt} \right) \quad (1)$$

132 Where, P_a (W) is the acoustic power, S_A is the surface area of the ultrasound emitting
133 surface (1.13 cm^2), m is the mass of ultrasound treated solution (g), c_p is the specific heat of
134 the medium (4.18 kJ/gK) and dT/dt is the rate of temperature change with respect to time,
135 starting at $t = 0$ ($^{\circ}\text{C/s}$). The temperature of the biopolymer mix solutions was measured before
136 and after sonication by means of a digital thermometer (TGST3, Sensor-Tech Ltd., Ireland),
137 with an accuracy of ± 0.1 $^{\circ}\text{C}$.

138 **2.2.3. Characterisation of protein-polysaccharides complexes**

139 **2.2.3.1. Microstructure characterisation**

140 The size of either one biopolymer (*i.e.* PoPI or κC) or mixtures of both biopolymer
141 with varying ratios of polysaccharide with respect to protein to a fixed concentration of
142 protein (*cf.* Table 1) was measured either by dynamic light scattering (DLS) using a Nano
143 Series ZS (Malvern Instruments, UK), or by laser diffraction using the Mastersizer 2000
144 (Hydro 2000SM, Malvern Instruments, UK). DLS was employed for systems whereby the
145 size of the species in question was $< 1 \mu\text{m}$ and samples for DLS analysis were diluted using

146 deionised water to a solids concentration of 0.1 wt. %, whereas laser diffraction was utilised
147 for entities exhibiting micron sized ($> 1 \mu\text{m}$) entities, using a refractive index of 1.45 for size
148 measurement of complexes (Kurukji *et al.*, 2015). Size values for either biopolymers or
149 electrostatic complexes are reported as z-average diameter (D_z). The reported size values are
150 the average and standard deviation of three repeat measurements.

151 **2.2.3.2. Electrokinetic potential characterisation**

152 The electrokinetic potential, more commonly referred to as zeta-potential (ζ -
153 potential), of an aqueous phase containing either one biopolymer or mixtures of both
154 biopolymer with varying ratios of polysaccharide with respect to protein to a fixed
155 concentration of protein (*cf.* Table 1), was measured by electrophoretic mobility using a
156 Zetasizer Nano Series ZS (Malvern Instruments, UK). Zeta-potential measurements were
157 conducted at a solids concentration of 0.1 wt. %, by careful dilution of the aforementioned
158 systems with distilled water, and added to a specialised disposable capillary cell for
159 measurement. Zeta-potential measurements are reported as the average and standard
160 deviation of three repeat measurements.

161 **2.2.4. Preparation of oil-in-water emulsions**

162 10 wt. % dispersed phase (rapeseed oil) was added to the aqueous continuous phase
163 containing either solely PoPI or PoPI- κC complexes, whereby the concentration of PoPI is
164 fixed at 1 wt. % with an increasing concentration of κC (0.01 – 0.5 wt. %) for the electrostatic
165 complexes. Oil-in-water emulsions were prepared by emulsifying this mixture at 6,000 rpm
166 for 3 min using a high shear mixer (L4RT, Silverson, UK).

167 **2.2.5. Characterisation of oil-in-water emulsions**

168 **2.2.5.1. Droplet size measurements**

169 The droplet size and droplet size distribution (DSD) of emulsions was measured by
170 laser diffraction using a Mastersizer 2000 (Malvern Instruments, UK) immediately after
171 emulsification, using a refractive index of 1.47 for the dispersed phase (O'Sullivan, Park, &
172 Beevers, 2016). Emulsion droplet size values are reported as the volume-surface area mean
173 diameter (Sauter diameter; $d_{3,2}$). The stability of emulsions was assessed by droplet size
174 measurements over 28 days, where emulsions were stored under refrigeration conditions (4
175 °C) throughout the duration of this stability study. The droplet sizes and error bars are
176 reported as the mean and standard deviation, respectively, of measured emulsions prepared in
177 triplicate.

178 **2.2.5.2. Interfacial tension measurements**

179 The interfacial tension between the aqueous phase (distilled water, protein solution or
180 protein-polysaccharide complexes) and oil phase (rapeseed oil) was measured using an
181 optical tensiometer on an Easydrop Goniometer (Krüss, Germany). Pendant drop method was
182 used to determine the interfacial tension, whereby a drop of aqueous phase, initially
183 contained within a microsyringe (Hamilton 1750 TLLX, 500 μ L) equipped with a 1.8 mm
184 diameter needle, was formed with a volume of 12 μ L in the oil phase placed within an optical
185 glass cuvette (40 x 40 x 30 mm). The investigated systems are presented within Table 1. The
186 interfacial tension test was conducted over 1,200 s and the temperature was maintained at 20
187 °C in a temperature controlled laboratory throughout the duration of the test. The interfacial
188 tension values and error bars are reported as the mean and standard deviation, respectively, of
189 three repeat measurements.

190 2.2.5.3. Emulsion visualisation

191 Optical microscopy (Brunel Microscopes Ltd SP300F, UK), equipped with a camera
192 (Canon EOS 1000D, Japan), was used to visualise emulsion, stabilised by either PoPI or
193 PoPI- κ C complexes, microstructure. A drop of emulsion was placed on a glass slide with a
194 cover slip and then visualised.

195 2.3. Statistical analysis

196 Student's t-test with a 95% confidence interval was used to assess the significance of
197 the results obtained. t-test data with $P < 0.05$ were considered statistically significant.

198 3. Results and discussions

199 3.1. Effect of PoPI- κ C ratio on the fabrication of electrostatic complexes

200 The effect of increasing concentration of κ -carrageenan (κ C) to a fixed concentration
201 of potato protein isolate (PoPI) was initially investigated. Pre-determined concentrations and
202 masses of κ C solutions were carefully added to a fixed mass and concentration of PoPI
203 solution, in order to achieve a specific ratio of the aforementioned biopolymers, whereby the
204 final concentration of PoPI in all instances was 1 wt. % (with increasing concentrations of
205 κ C, ranging from 0.01 – 0.5 wt. % (*cf.* Table 1)). These biopolymer mixtures were
206 subsequently treated with ultrasound with an ultrasonic amplitude of 95% for 2 min, yielding
207 an acoustic intensity of $\sim 34 \text{ W cm}^{-2}$ (*cf.* section 2.2.2.), in order to reduce the initial size of
208 the PoPI- κ C electrostatic complexes. Complex size (D_z) as a function of increasing κ C
209 concentration from 0.01 – 0.5 wt. % with a fixed concentration of PoPI (1 wt. %) is shown in
210 Fig. 1. The size of PoPI and κ C are $69 \pm 4 \text{ nm}$ and $648 \pm 63 \text{ nm}$, respectively, as measured by
211 DLS.

212 For the case of PoPI, it should be noted that the reported proteins size represent
213 aggregates of protein molecules rather than discrete protein fractions. Native patatin has a
214 hydrodynamic radii (R_h) of approximately 5 nm (Pots *et al.*, 1999), in comparison to size data
215 presented in this study for PoPI. This disparity in size is due to the formation of molecular
216 associations of protein in solution. Proteins in aqueous solutions associate together to form
217 aggregates due to hydrophobic and electrostatic interactions (O'Connell *et al.*, 2003).

218 Fig. 1 shows that upon addition of κ C to PoPI, there is initially a significant ($P < 0.05$)
219 increase in the size to 125 ± 7 nm from that of solely PoPI (69 ± 4 nm). This initial increase
220 in size is attributed to the formation of submicron PoPI- κ C complexes, due to differences in
221 the electrokinetic potential between the respective biopolymers investigated (*i.e.*, PoPI is
222 cationic, whereas κ C is anionic, at unadjusted pH conditions; *cf.* Fig. 2), within a
223 concentration range of 0.01 and 0.375 wt. % κ C, with respect to 1 wt. % PoPI. Despite the
224 significantly ($P < 0.05$) larger size of κ C with respect to either the formed complexes or PoPI,
225 it is thought that the κ C uncoils in the presence of PoPI associates, surrounding them and
226 forming a compact interfacial layer, accounting for the formation of submicron electrostatic
227 complexes.

228 Our results are in agreement with those of Kurukji, *et al.*, (2015), who showed that
229 submicron electrostatic complexes were formed between sodium caseinate and chitosan
230 (~ 500 nm), and bovine serum albumin and chitosan (~ 700 nm), under specific pH and
231 concentration conditions. At concentrations > 0.0375 wt. % κ C, with respect to 1 wt. % PoPI,
232 there is a further significant ($P < 0.05$) increase in size to the micron sized entities (> 10 μ m),
233 and is ascribed to an excess of κ C leading to depletion flocculation interactions between
234 PoPI- κ C complexes, rather than reduced electrostatic interactions between the two
235 biopolymers. These hypotheses were explored by electrokinetic potential measurements,
236 more commonly referred to as zeta potential (*i.e.*, ζ -potential), of biopolymer mixtures,

237 prepared with increasing concentrations of κ C, ranging from 0.01 – 0.5 wt. %, with respect to
238 1 wt. % PoPI, as detailed in Table 1. Electrokinetic potential as a function of increasing κ C
239 concentration from 0.01 – 0.5 wt. % with a fixed concentration of PoPI (1 wt. %), as
240 measured at a solids concentration of 0.1 wt. % (achieve through dilution with distilled
241 water), is shown in Fig. 2.

242 The ζ -potential of PoPI and κ C as measured by electrophoretic mobility (*cf.*, section
243 2.2.3.2.), was 28.9 ± 1.1 mV and -52.3 ± 2.4 mV, respectively, at unadjusted pH conditions
244 (*cf.*, section 2.1.). Initially, addition of κ C to a fixed concentration of PoPI (1 wt. %) yielded a
245 decrease in the cationic value ζ -potential to a value of 0 mV at a κ C concentration of ~ 0.058
246 wt. %. Further increases in the concentration of κ C increased the anionic value of ζ -potential,
247 tending to a value of that of solely a κ C solution (-52.3 ± 2.4 mV). These ζ -potential results
248 confirm the hypothesis that the formation of micron-sized electrostatic complexes ($> 10 \mu\text{m}$)
249 was due to an excess of polysaccharide (*i.e.*, depletion flocculation interactions), rather than a
250 minimisation of electrostatic interactions between the complexes, as the ζ -potential at a κ C
251 concentration of 0.04 wt. % was 6.8 ± 0.8 mV. Furthermore, it is thought that the excess of
252 κ C in the bulk associates with the κ C at the surface of the electrostatic complexes, achieving
253 the formation of these larger flocculated structures. Hosseini, *et al.*, (2013) reported a
254 comparable trend, whereby increasing the concentration of κ C with respect to a fixed
255 concentration of β -lactoglobulin yielded a reduction in ζ -potential to a value of 0 mV,
256 followed by a further increase in the anionic ζ -potential value.

257 Furthermore, the addition of κ C to PoPI minimally altered the pH of that of single
258 biopolymer solutions. The unadjusted pH of κ C and PoPI was 3.55 and 3.6, respectively,
259 measured at a concentration of 1 wt. % (*cf.*, section 2.1.). The pH of the biopolymer mixtures
260 was consistently within a pH range of 3.55 ± 0.25 .

261 Images of PoPI- κ C complexes samples were captured after 30 min after preparation at
262 20 °C in order to assess the separation behaviour of PoPI- κ C complexes with respect to
263 increasing concentration of κ C (0.01 – 0.1 wt. %, with an increment of 0.01 wt. %) to a fixed
264 concentration of PoPI (1 wt. %).

265 As can be seen in Fig. 3, the initial addition κ C up to a concentration of 0.03 wt. %
266 yields the formation of submicron non-sedimenting entities (*cf.* Fig. 1), observed due to the
267 noticeable increase in turbidity (*cf.* Fig. 3). Concentrations \geq 0.04 wt. % yield electrostatic
268 complexes possessing sizes within the micron range (*cf.* Fig. 1), and thus sediment under
269 gravitational forces due to their large size (*cf.* Fig. 3). However, at concentrations \geq 0.1 wt. %
270 this sedimentation behaviour is no longer observed (*cf.* Fig. 3), as the viscosity of the
271 mixture, predominately dictated by κ C, is sufficient to maintain stability with respect to
272 gravitational separation (Hermansson, *et al.*, 1991).

273 **3.2. Comparison of the emulsifying performance of complexes fabricated with varying** 274 **ratios of PoPI and κ C**

275 A series of oil-in-water emulsions were produced with 10 wt. % rapeseed oil and an
276 aqueous continuous phase containing either PoPI- κ C complexes (as per Table 1) or solely
277 PoPI (1 wt. %). The emulsions were prepared via high shear mixing at 6,000 rpm for 3 min.
278 Emulsion droplet size measurements obtained by laser diffraction are shown in Fig. 4. The
279 emulsion droplet size was measured immediately after emulsification, and all exhibited
280 unimodal droplet size distributions.

281 Emulsions prepared with PoPI- κ C complexes yielded comparable ($P > 0.05$) emulsion
282 droplet sizes to that prepared with solely PoPI (1 wt. %), with the exception of emulsions
283 prepared with κ C concentrations within a range of 0.05 - 0.07 wt. %, and at concentrations $>$
284 0.09 wt. %, whereby significantly larger ($P < 0.05$) emulsion droplets were observed for both

285 of these instances. The large emulsion droplet sizes, in comparison to that of solely PoPI
286 emulsions, within a κ C concentration range of 0.05 - 0.07 wt. %, with respect to 1 wt. %
287 PoPI, was ascribed to the proximity of these PoPI- κ C complexes to the pH of the neutralised
288 complex charge (*cf.* Fig. 2), whereby electrostatic repulsive interactions were reduced
289 yielding greater interactions between emulsion droplets and consequently the formation of
290 significantly larger ($P < 0.05$) emulsion droplets. The large emulsion droplet sizes exhibited
291 within close proximity to the pH of the neutralised complex charge in this study are in
292 agreement with results obtained by Demetriades, *et al.*, (1997), for emulsions prepared with
293 whey protein (2 wt. %) in close proximity to the isoelectric point (pH 5), whereby larger
294 emulsion drops were achieved in comparison to emulsions prepared at pH conditions
295 distanced from the isoelectric point (pH 3 and 7). Furthermore, emulsions prepared with κ C
296 concentrations > 0.09 wt. %, with respect to 1 wt. % PoPI, yielded a significant increase ($P <$
297 0.05) in emulsion droplet size in comparison to emulsions prepared solely with PoPI (1 wt.
298 %). A comparable trend with respect to PoPI- κ C complex size and high concentrations of κ C
299 (> 0.1 wt. %) was observed in Fig. 1, whereby a notable increase in complex size was
300 exhibited. This behaviour is attributed to an access in biopolymer concentration yielding
301 depletion flocculation interactions.

302 Differences in emulsion microstructure were examined utilising optical microscopy
303 for emulsions prepared with solely PoPI (1 wt. %) and PoPI- κ C complexes (0.01, 0.04, 0.07,
304 0.1 and 0.5 wt. % of κ C with respect to 1 wt. % PoPI), and is presented in Fig. 5.

305 The microstructure of emulsions prepared with solely PoPI (*cf.* Fig. 5a) exhibited
306 discrete emulsion droplets, predominately possessing a size < 40 μ m, with some exceptions
307 where larger droplets were observed. As the concentration of κ C is increased (0.01 – 0.07 wt.
308 %) for emulsion stabilised with PoPI- κ C complexes (*cf.* Fig. 5b – d), it appears that the
309 droplet size distribution is more uniform, with slightly larger emulsion droplets. However, for

310 emulsions stabilised with elevated concentrations of κ C (0.1 and 0.5 wt. %) within the PoPI-
311 κ C complexes (*cf.* Fig. 5e and f), larger emulsion droplets were observed, appearing to have a
312 broader droplet size distribution and a flocculated microstructure. These observations are
313 consistent with the previously discussed PoPI- κ C complex (*cf.* Fig. 1) and emulsion droplet
314 size (*cf.* Fig. 4) data.

315 The interfacial tension between water and rapeseed oil of the studied systems is
316 presented in Fig. 6, for PoPI (1 wt. %) and PoPI- κ C complexes (0.03, 0.06 and 0.1 wt. % of
317 κ C with respect to 1 wt. % PoPI). The oil used in this study, commercially available rapeseed
318 oil, was assessed for the presence of surface active impurities in the works of O'Sullivan, *et al.*,
319 *al.*, (2014, 2016), whereby the interfacial tension between distilled water and rapeseed oil
320 was measured, in addition to an aqueous phase containing a wide range of proteins (*e.g.*,
321 sodium caseinate, whey protein isolate, pea protein isolate, bovine gelatin, etc.). It was shown
322 that the interfacial tension of all systems decreases continually as a function of time. Based
323 on this, the decrease in interfacial tension with time was ascribed primarily to the nature of
324 the dispersed phase employed, and to a lesser extent the type of emulsifier (O'Sullivan, *et al.*,
325 2014, 2016; O'Sullivan, *et al.*, 2015). Gaonkar, (1989, 1991) explained that the time
326 dependant nature of interfacial tension of commercially available vegetable oils against water
327 was due to the adsorption of surface active impurities present within the oils at the oil-water
328 interface.

329 Significant differences ($P < 0.05$) were observed in the interfacial tension between
330 PoPI alone and PoPI- κ C complexes (at all concentrations of κ C), whereby a greater decrease
331 in the rate of interfacial tension and equilibrium value were observed for PoPI (*cf.* Fig. 6).
332 This behaviour is ascribed to the smaller size of PoPI (69 ± 4 nm) in comparison to that of the
333 PoPI- κ C complexes (> 120 nm in all cases), allowing for increased rates of molecular
334 mobility and enhanced packing at the oil-water interface. O'Sullivan, *et al.*, (2016) observed

335 comparable behaviour, whereby the interfacial properties (*i.e.*, initial interfacial tension
336 value, rate of decrease of interfacial tension and equilibrium value of interfacial tension) of
337 egg white protein (1.6 μm) were better than that of larger aggregated proteins, such as either
338 pea protein isolate (5.2 μm). Furthermore, as the concentration of κC was increased within
339 PoPI- κC complexes, the rate of decrease in interfacial tension significantly decreased ($P <$
340 0.05; *cf.* Fig. 6), attributed to a combination of increases in complex size with respect to
341 increasing concentration of κC (*cf.* Fig. 1) and the increased bulk viscosity as a function of
342 increasing κC . In addition, comparable equilibrium interfacial tension values were observed
343 for all PoPI- κC complexes, yet significantly greater ($P < 0.05$) than PoPI alone, owing to a
344 combination of their larger size (*cf.*, Fig. 1) and lower electrokinetic potential (*cf.*, Fig. 2). It
345 is thought that this behaviour is due to improved interfacial packing of PoPI in comparison to
346 PoPI- κC complexes at the oil-water interface, due to aforementioned size differences.

347 The stability of oil-in-water emulsions prepared with PoPI- κC complexes was
348 assessed over a 28 day period. Fig. 7 shows the development of emulsion droplet size ($d_{3,2}$) as
349 a function of time for emulsions prepared with PoPI- κC complexes as emulsifiers, with
350 varying contents of κC (0.03, 0.06, 0.9 and 0.5 wt. %, with respect to 1 wt. PoPI), as well as
351 PoPI alone (1 wt. %).

352 Emulsions prepared with solely 1 wt. % PoPI (*cf.* Fig. 7) demonstrated a marginal
353 growth in emulsion droplet size throughout the duration of the stability study (28 days).
354 However, emulsions prepared with PoPI- κC complexes containing 0.03, 0.09 and 0.3 wt. %
355 κC (*cf.* Fig. 7a, c and d) yielded a marginal increase in emulsion stability, as negligible
356 change in emulsion droplet size was observed throughout the duration of the stability study.
357 This behaviour was attributed to an improved, thicker interfacial layer, due to the
358 significantly larger size of the PoPI- κC complexes in comparison to solely PoPI (*cf.* Fig. 1),
359 inhibiting emulsion coalescence. In addition, emulsions stabilised with PoPI- κC complexes

360 containing concentrations of $\kappa\text{C} \geq 0.1$ wt. % (data not shown for 0.1 – 0.4 wt. % κC) were
361 thought to be more stable due to elevated viscosity of these systems owing to the high content
362 of κC , significantly reducing the mobility of emulsion droplets through the continuous phase
363 through increased bulk viscosity of said phase (Hermansson, *et al.*, 1991). Furthermore,
364 emulsions prepared with PoPI- κC complexes containing 0.06 wt. % κC (*cf.* Fig. 7b) yielded
365 emulsions with reduced emulsion stability, demonstrating growth in emulsion droplet size.
366 This reduction in emulsion stability for PoPI- κC complexes containing 0.06 wt. % κC was
367 ascribed to the proximity of these complexes to the isoelectric point, reducing electrostatic
368 stabilisation, enhancing drop-drop interactions and consequently, coalescence of adjacent
369 emulsion droplets.

370 4. Conclusions

371 This study showed that biopolymer mixtures of potato protein isolate (PoPI), a
372 naturally cationic protein at unadjusted pH (3.6), and κ -carrageenan (κC), a naturally anionic
373 polysaccharide at unadjusted pH (3.55), yielded the formation of electrostatic complexes
374 without the necessity for pH adjustment. Submicron (~ 120 nm) protein polysaccharide (PoPI-
375 κC) were produced with ≤ 0.0375 wt. % κC with respect to 1 wt. % PoPI, whereas κC
376 concentrations ≥ 0.04 wt. % yielded the formation of micron sized entities (> 10 μm). This
377 significant increase ($P < 0.05$) in size is attributed to an excess of κC yielding depletion
378 flocculation interactions, as the system still possessed an overall positive charge allowing for
379 the electrostatic repulsive forces between complexes, within a κC concentration range of 0.04
380 – 0.055 wt. %.

381 Emulsions prepared with PoPI- κC complexes yielded comparable emulsion droplet
382 sizes to those prepared with PoPI alone, with the exceptions of emulsions prepared with
383 concentration of κC within a concentration range of 0.05 – 0.07 wt. % (proximity to the

384 isoelectric point), and at κC concentrations > 0.1 wt. % (excessive polysaccharide), whereby
385 larger emulsion droplets were achieved ($> 20 \mu\text{m}$) in these instances. Emulsions prepared
386 with PoPI- κC complexes, with a desirable ratio of κC (0.01 – 0.04 wt. %, and 0.08 – 0.1 wt.
387 %) with respect to PoPI (1 wt. %), yielded emulsions possessing enhanced emulsion stability
388 in comparison to emulsions prepared solely with PoPI.

389 Electrostatic interactions between proteins and polysaccharides can thus yield
390 complexes, whereby these protein-polysaccharide complexes yield emulsions, with both,
391 comparable emulsion droplet size and, enhanced emulsion stability in comparison to those
392 prepared with solely protein.

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- 518

Table. 1 Ratio of PoPI-to- κ -C used for the fabrication of protein-polysaccharide complexes, whereby the concentration of PoPI was maintained at 1 wt. % in all instances.

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κ C (wt. %)	Total biopolymer concentration (wt. %)
0.01	1.01
0.02	1.02
0.03	1.03
0.04	1.04
0.05	1.05
0.06	1.06
0.07	1.07
0.08	1.08
0.09	1.09
0.1	1.1
0.2	1.2
0.3	1.3
0.4	1.4
0.5	1.5

Fig. 1. Effect of increasing κ C concentration (0.01 – 0.5 wt. %) with a fixed concentration of PoPI (1 wt. %) on the size of PoPI- κ C electrostatic complexes.

Fig. 2. Effect of increasing κ C concentration (0.01 – 0.5 wt. %) with a fixed concentration of PoPI (1 wt. %) on the electrokinetic potential of PoPI- κ C electrostatic complexes.

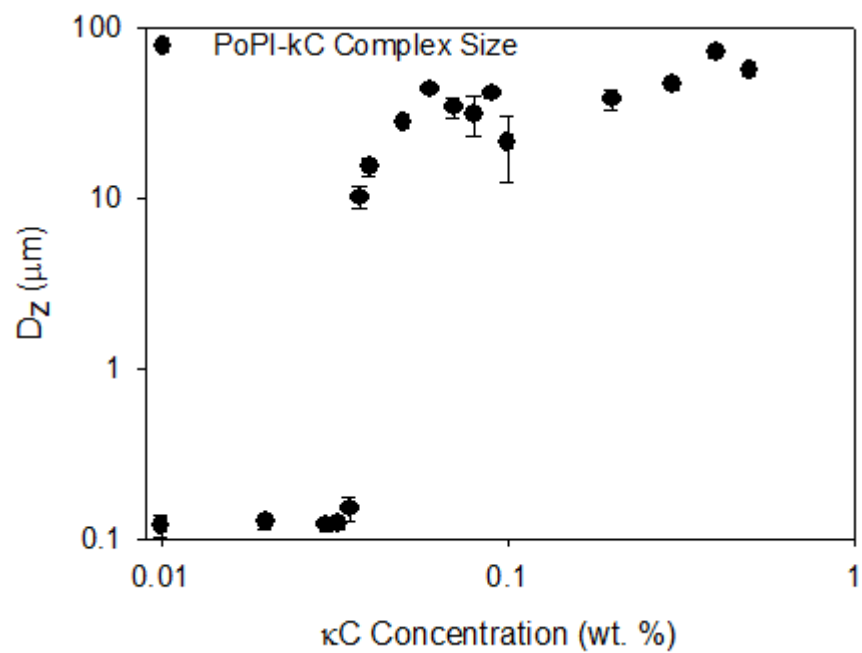
Fig. 3. Representative images of PoPI- κ C electrostatic complexes with an increasing of κ C from 0 to 0.1 wt. % at an increment of 0.01 wt. %, with a fixed concentration of PoPI (1 wt. %).

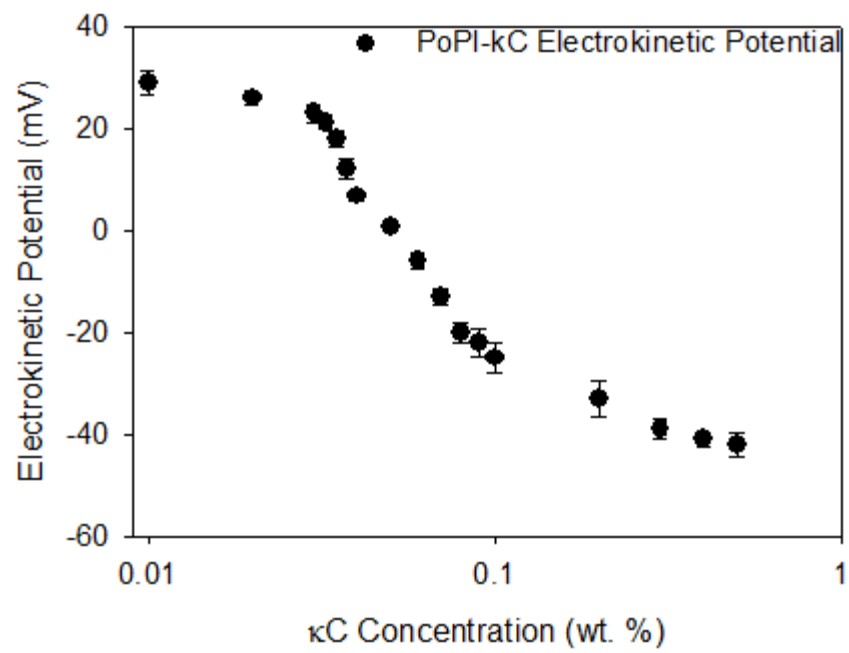
Fig. 4. Emulsion droplet size ($d_{3,2}$) of emulsions prepared with PoPI- κ C complexes as a function of increasing concentration of κ C (0.01 – 0.5 wt. %).

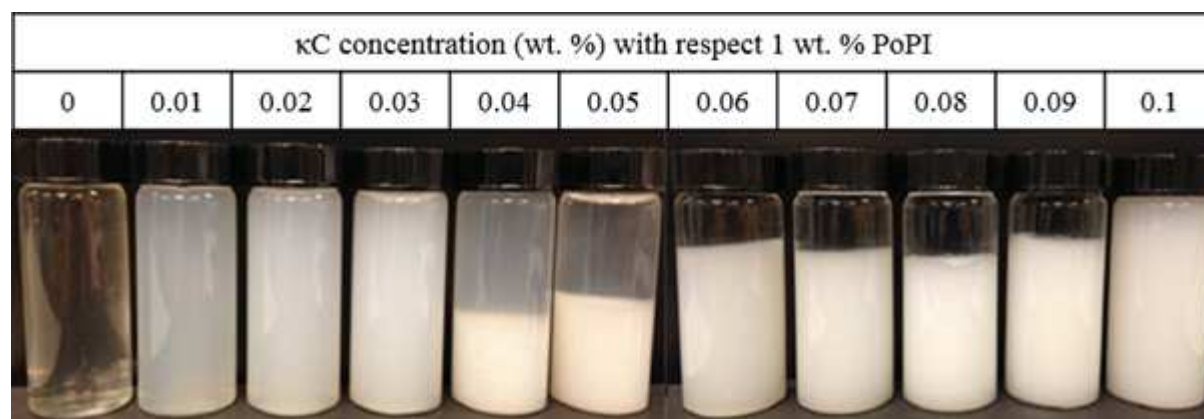
Fig. 5. Optical micrographs of PoPI and PoPI- κ C complex stabilised O/W emulsions, whereby the concentration of PoPI was fixed at 1 wt. %: (a) 0% κ C, (b) 0.01% κ C, (c) 0.04% κ C, (d) 0.07% κ C, (e) 0.1% κ C and (f) 0.5% κ C. Scale bar is 40 μ m in all instances.

Fig. 6. Interfacial tension between water and rapeseed oil as a function of emulsifier type: 1% PoPI (\bullet), 1% PoPI-0.03% κ C complexes (\circ), 1% PoPI-0.06% κ C complexes (\blacktriangledown), and 1% PoPI-0.1% κ C complexes (Δ).

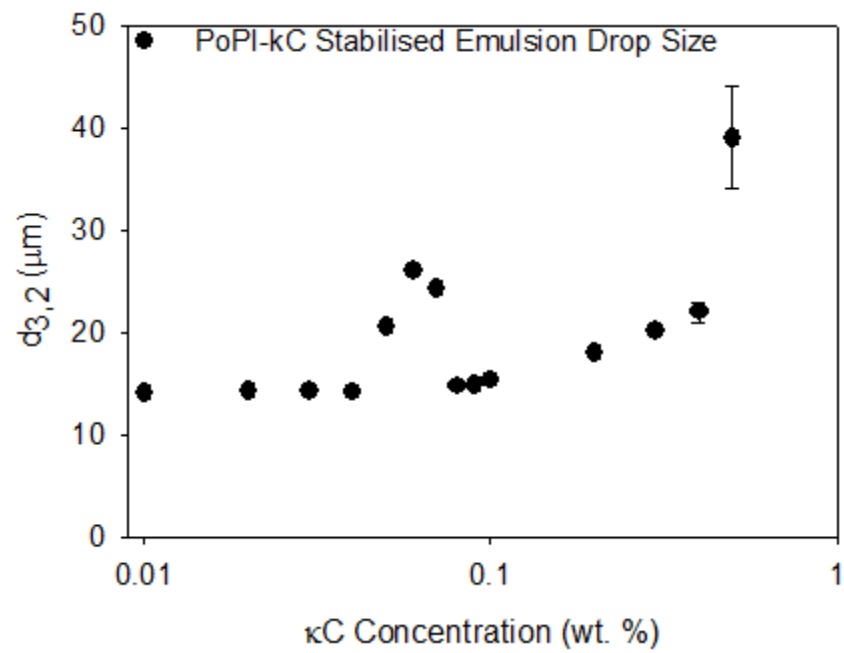
Fig. 7. Effect of κ C content within PoPI- κ C complexes on droplet size ($d_{3,2}$) as a function of time for O/W emulsions stabilised by: (a) 1% PoPI and 1% PoPI-0.03% κ C complexes, (b) 1% PoPI and 1% PoPI-0.06% κ C complexes, (c) 1% PoPI and 1% PoPI-0.09% κ C complexes, and (d) 1% PoPI and 1% PoPI-0.5% κ C complexes.

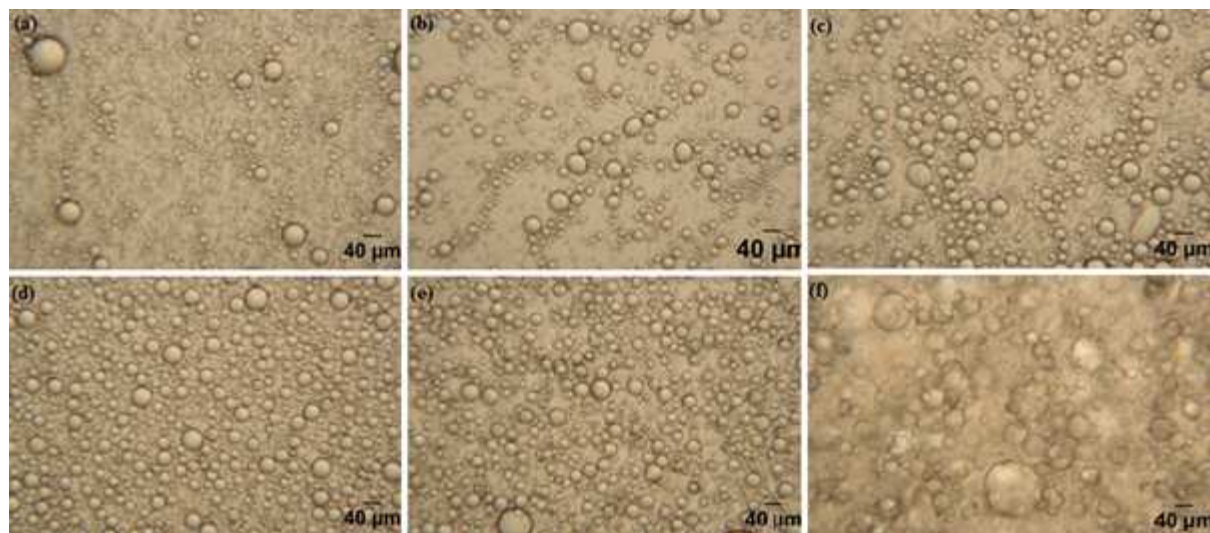




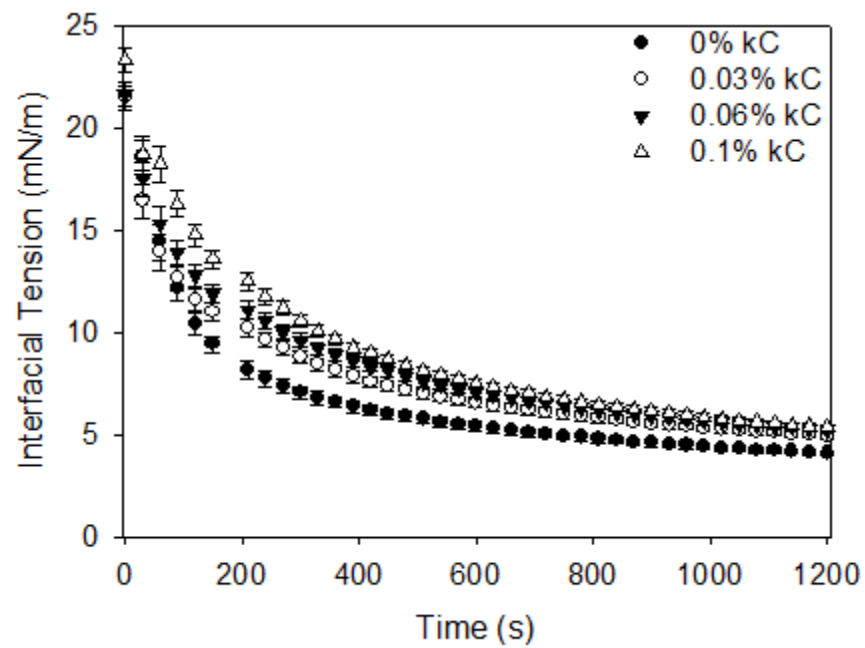


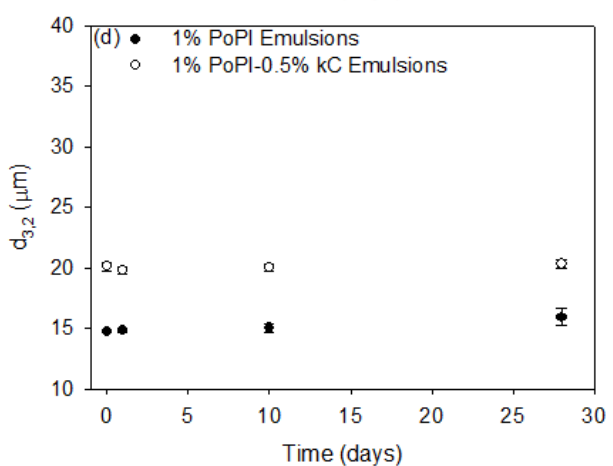
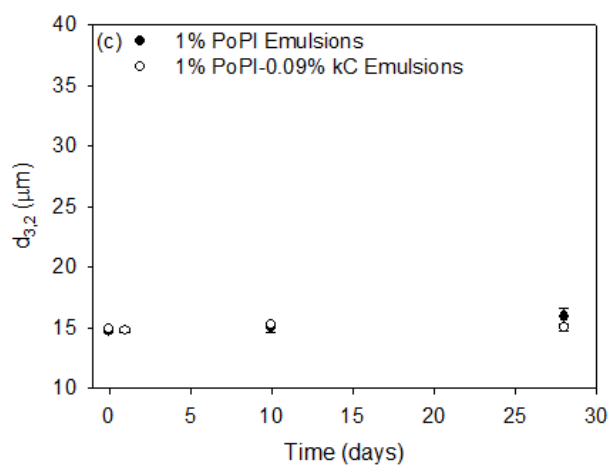
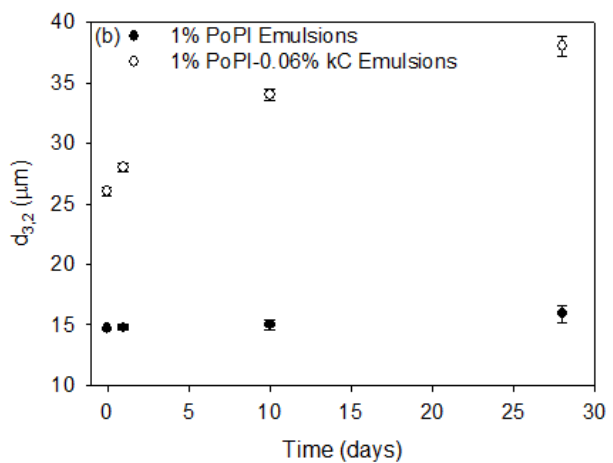
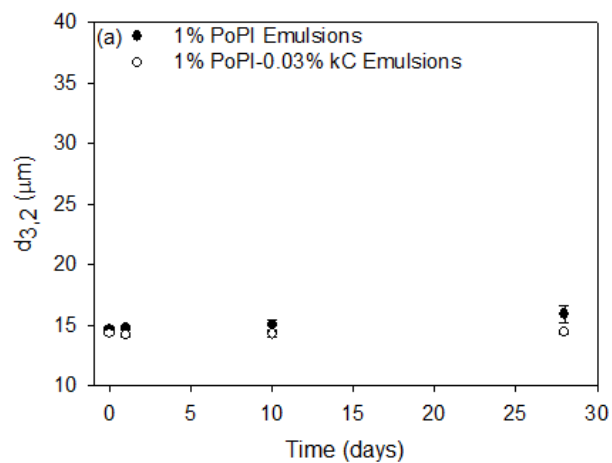
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Highlights:

- Electrostatic complexes were formed between potato protein and κ -carrageenan (κ C).
- Submicron complexes (< 150 nm) were formed at κ C concentrations $\leq 0.0375\%$.
- Micron-sized complexes (> 1 μ m) were formed at κ C concentrations $> 0.0375\%$.
- Complex stabilised emulsions possessed enhanced long-term stability.