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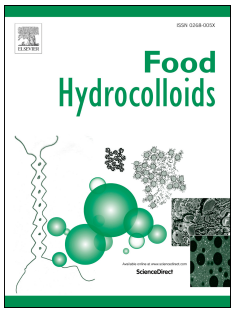

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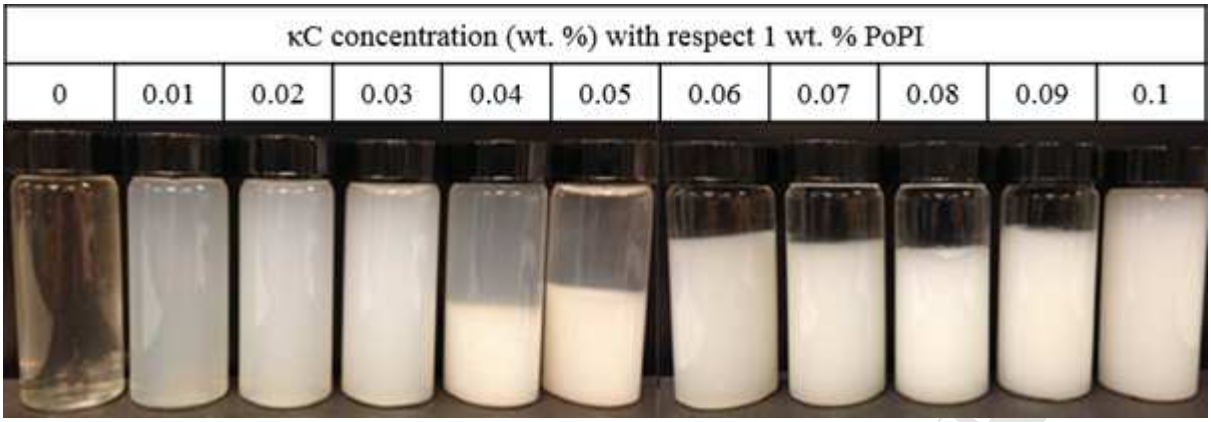

Investigation of the fabrication and subsequent emulsifying capacity of potato protein isolate/κ-carrageenan electrostatic complexes

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Abstract

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

Addition of κC to a 1 wt. % PoPI solution yielded the formation of submicron (~120 nm) electrostatic complexes up to a κC concentration of ≤ 0.0375 wt. %. Higher concentrations of κC yielded micron sized complexes (> 10 μm). Emulsions prepared with PoPI-κC complexes yielded comparable emulsion droplet sizes to that of PoPI alone, with the exception of complexes prepared with κC in the range of 0.05 – 0.07 wt. %. Larger emulsion droplets were observed, as these complexes possessed an electrokinetic potential close to the isoelectric point, resulting in aggregation. Emulsions prepared with PoPI-κC complexes possessed marginally enhanced long-term stability in comparison to emulsions prepared with PoPI alone.

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

1. Introduction

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

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

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

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

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

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

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

2. Materials and methodology

2.1. Materials

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

2.2. Methods

2.2.1. Preparation of protein and polysaccharide solutions

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

2.2.2. Preparation of protein-polysaccharide complexes

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

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

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

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

2.2.3. Characterisation of protein-polysaccharides complexes

2.2.3.1. Microstructure characterisation

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

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

2.2.3.2. Electrokinetic potential characterisation

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

2.2.4. Preparation of oil-in-water emulsions

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

2.2.5. Characterisation of oil-in-water emulsions

2.2.5.1. Droplet size measurements

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

2.2.5.2. Interfacial tension measurements

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

2.2.5.3. Emulsion visualisation

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

2.3. Statistical analysis

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

3. Results and discussions

3.1. Effect of PoPI-κC ratio on the fabrication of electrostatic complexes

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

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

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

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

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

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

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

Images of PoPI-κC complexes samples were captured after 30 min after preparation at 20 ^{o}C in order to assess the separation behaviour of PoPI-κC complexes with respect to increasing concentration of κC (0.01 – 0.1 wt. %, with an increment of 0.01 wt. %) to a fixed concentration of PoPI (1 wt. %).

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

3.2. Comparison of the emulsifying performance of complexes fabricated with varying ratios of PoPI and κC

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

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

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

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

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

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

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

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

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

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

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

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

4. Conclusions

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

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

isoelectric point), and at κC concentrations > 0.1 wt. % (excessive polysaccharide), whereby larger emulsion droplets were achieved (> 20 μm) in these instances. Emulsions prepared with PoPI-κC complexes, with a desirable ratio of κC (0.01 – 0.04 wt. %, and 0.08 – 0.1 wt. %) with respect to PoPI (1 wt. %), yielded emulsions possessing enhanced emulsion stability in comparison to emulsions prepared solely with PoPI.

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

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References

Beverung, C. J., Radke, C. J., & Blanch, H. W. (1999). Protein adsorption at the oil/water interface: characterization of adsorption kinetics by dynamic interfacial tension measurements. *Biophysical Chemistry*, *81*(1), 59–80. JOUR. http://doi.org/http://dx.doi.org/10.1016/S0301-4622(99)00082-4

Demetriades, K., Coupland, J. N., & McClements, D. J. (1997). Physical Properties of Whey Protein Stabilized Emulsions as Related to pH and NaCl. *Journal of Food Science*, *62*(2), 342–347. http://doi.org/10.1111/j.1365-2621.1997.tb03997.x

Dickinson, E. (2006). Colloid science of mixed ingredients. *Soft Matter*, *2*(8), 642. http://doi.org/10.1039/b605670a

Foegeding, E. A., & Davis, J. P. (2011). Food protein functionality: A comprehensive approach. *Food Hydrocolloids*, *25*(8), 1853–1864. JOUR. http://doi.org/http://dx.doi.org/10.1016/j.foodhyd.2011.05.008

Gaonkar, A. G. (1989). Interfacial tensions of vegetable oil/water systems: Effect of oil purification. *Journal of the American Oil Chemists' Society*, *66*(8), 1090–1092. http://doi.org/10.1007/BF02670090

Gaonkar, A. G. (1991). Surface and interfacial activities and emulsion characteristics of some food hydrocolloids. *Food Hydrocolloids*, *5*(4), 329–337. http://doi.org/10.1016/S0268-005X(09)80045-3

Gladkowska–Balewicz, I., Norton, I. T., & Hamilton, I. E. (2014). Effect of process conditions, and component concentrations on dynamic viscosity of κ-carrageenan and pregelatinised cross-linked waxy maize starch mixed fluid gels. *Food Hydrocolloids*, *42*, 355–361. http://doi.org/10.1016/j.foodhyd.2014.03.003

Grabovac, V., Guggi, D., & Bernkopschnurch, A. (2005). Comparison of the mucoadhesive properties of various polymers. *Advanced Drug Delivery Reviews*, *57*(11), 1713–1723. http://doi.org/10.1016/j.addr.2005.07.006

Hermansson, A.-M., Eriksson, E., & Jordansson, E. (1991). Effects of potassium, sodium and calcium on the microstructure and rheological behaviour of kappa-carrageenan gels. *Carbohydrate Polymers*, *16*(3), 297–320. http://doi.org/10.1016/0144-8617(91)90115-S

Holm, F., & Eriksen, S. (2007). Emulsifying properties of undenatured potato protein concentrate. *International Journal of Food Science & Technology*, *15*(1), 71–83. http://doi.org/10.1111/j.1365-2621.1980.tb00920.x

Hosseini, S. M. H., Emam-Djomeh, Z., Razavi, S. H., Moosavi-Movahedi, A. A., Saboury, A. A., Mohammadifar, M. A., … Van der Meeren, P. (2013). Complex coacervation of β-lactoglobulin - κ-carrageenan aqueous mixtures as affected by polysaccharide sonication. *Food Chemistry*, *141*(1), 215–22. http://doi.org/10.1016/j.foodchem.2013.02.090

Kurukji, D., Norton, I., & Spyropoulos, F. (2015). Fabrication of sub-micron protein-chitosan electrostatic complexes for encapsulation and pH-Modulated delivery of model hydrophilic active compounds. *Food Hydrocolloids*. http://doi.org/10.1016/j.foodhyd.2015.02.021

Kurukji, D., Pichot, R., Spyropoulos, F., & Norton, I. T. (2013). Interfacial behaviour of sodium stearoyllactylate (SSL) as an oil-in-water pickering emulsion stabiliser. *Journal of Colloid and Interface Science*, *409*, 88–97. http://doi.org/10.1016/j.jcis.2013.07.016

Løkra, S., Helland, M. H., Claussen, I. C., Strætkvern, K. O., & Egelandsdal, B. (2008). Chemical characterization and functional properties of a potato protein concentrate prepared by large-scale expanded bed adsorption chromatography. *LWT - Food Science and Technology*, *41*(6), 1089–1099. http://doi.org/10.1016/j.lwt.2007.07.006

Margulis, M. A., & Margulis, I. M. (2003). Calorimetric method for measurement of acoustic power absorbed in a volume of a liquid. *Ultrasonics Sonochemistry*, *10*(6), 343–345. JOUR. http://doi.org/http://dx.doi.org/10.1016/S1350-4177(03)00100-7

McClements, D. J. (2005). *Food Emulsions: Principles, Practices, and Techniques* (2nd ed.). CRC Press.

McClements, D. J. (2009). *Biopolymers in Food Emulsions*. *Modern Biopolymer Science* (First Edit). Elsevier Inc. http://doi.org/10.1016/B978-0-12-374195-0.00004-5

Millane, R. P., Chandrasekaran, R., Arnott, S., & Dea, I. C. M. (1988). The molecular structure of kappa-carrageenan and comparison with iota-carrageenan. *Carbohydrate Research*, *182*(1), 1–17. http://doi.org/10.1016/0008-6215(88)84087-4

Morris, E. R., Cutler, A. N., Ross-Murphy, S. B., Rees, D. A., & Price, J. (1981). Concentration and shear rate dependence of viscosity in random coil polysaccharide solutions. *Carbohydrate Polymers*, *1*, 5–21.

O'Connell, J. E., & Flynn, C. (2007). The manufacture and application of casein-derived ingredients. In Y. H. Hui (Ed.), *Handbook of Food Products Manufacturing* (1st ed., pp. 557–593). New Jersey: John Wiley & Sons.

O'Connell, J. E., Grinberg, V. Y., & de Kruif, C. G. (2003). Association behavior of β-casein. *Journal of Colloid and Interface Science*, *258*(1), 33–39. http://doi.org/10.1016/S0021-9797(02)00066-8

O'Sullivan, J., Arellano, M., Pichot, R., & Norton, I. (2014). The Effect of Ultrasound Treatment on the Structural, Physical and Emulsifying Properties of Dairy Proteins. *Food Hydrocolloids*, *42*(3), 386–396.

O'Sullivan, J., Beevers, J., Park, M., Greenwood, R., & Norton, I. (2015). Comparative assessment of the effect of ultrasound treatment on protein functionality pre- and post-emulsification. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*. http://doi.org/10.1016/j.colsurfa.2015.07.065

O'Sullivan, J. J., & O'Mahony, J. A. (2016). Food Ingredients. In *Reference Module in Food Science* (pp. 1–3).

O'Sullivan, J., Murray, B., Flynn, C., & Norton, I. (2015). Comparison of batch and continuous ultrasonic emulsification processes. *Journal of Food Engineering*, *167(B)*, 141–121.

O'Sullivan, J., Murray, B., Flynn, C., & Norton, I. T. (2016). The effect of ultrasound treatment on the structural, physical and emulsifying properties of animal and vegetable proteins. *Food Hydrocolloids*, *53*, 141–154.

O'Sullivan, J., Park, M., & Beevers, J. (2016). The effect of ultrasound upon the physicochemical and emulsifying properties of wheat and soy protein isolates. *Journal of Cereal Science*.

Pichot, R., Spyropoulos, F., & Norton, I. T. (2010). O/W emulsions stabilised by both low molecular weight surfactants and colloidal particles: The effect of surfactant type and concentration. *Journal of Colloid and Interface Science*, *352*(1), 128–35. http://doi.org/10.1016/j.jcis.2010.08.021

Pots, A. M., ten Grotenhuis, E., Gruppen, H., Voragen, A. G. J., & de Kruif, K. G. (1999). Thermal Aggregation of Patatin Studied in Situ. *Journal of Agricultural and Food Chemistry*, *47*(11), 4600–4605. JOUR. http://doi.org/10.1021/jf9901901

Ralet, M.-C., & Guéguen, J. (2000). Fractionation of Potato Proteins: Solubility, Thermal Coagulation and Emulsifying Properties. *LWT - Food Science and Technology*, *33*(5), 380–387. http://doi.org/10.1006/fstl.2000.0672

Rayner, M., Timgren, A., Sjöö, M., & Dejmek, P. (2012). Quinoa starch granules: a candidate for stabilising food-grade Pickering emulsions. *Journal of the Science of Food and Agriculture*, *92*(9), 1841–7. http://doi.org/10.1002/jsfa.5610

Rodríguez Patino, J. M., & Pilosof, A. M. R. (2011). Protein–polysaccharide interactions at fluid interfaces. *Food Hydrocolloids*, *25*(8), 1925–1937. http://doi.org/10.1016/j.foodhyd.2011.02.023

Snyder, J. C., & Desborough, S. L. (1980). Total protein and protein fractions in tubers of Group Andigena and Phureja-Tuberosum hybrids. *Qualitas Plantarum Plant Foods for Human Nutrition*, *30*(2), 123–134. http://doi.org/10.1007/BF01099050

van Koningsveld, G. A., Walstra, P., Gruppen, H., Wijngaards, G., van Boekel, M. A. J. S., & Voragen, A. G. J. (2002). Formation and stability of foam made with various potato

504 protein preparations. *Journal of Agricultural and Food Chemistry*, *50*(26), 7651–9.
505 Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12475285

506 van Koningsveld, G. A., Walstra, P., Voragen, A. G. J., Kuijpers, I. J., van Boekel, M. A. J.
507 S., & Gruppen, H. (2006). Effects of protein composition and enzymatic activity on
508 formation and properties of potato protein stabilized emulsions. *Journal of Agricultural*
509 *and Food Chemistry*, *54*(17), 6419–27. http://doi.org/10.1021/jf061278z

510 Vignati, E., Piazza, R., & Lockhart, T. P. (2003). Pickering Emulsions: Interfacial Tension,
511 Colloidal Layer Morphology, and Trapped-Particle Motion. *Langmuir*, *19*(17), 6650–
512 6656. http://doi.org/10.1021/la034264l

513 Walstra, P. (1993). Principles of emulsion formation. *Chemical Engineering Science*, *48*(2),
514 333–349. JOUR. http://doi.org/http://dx.doi.org/10.1016/0009-2509(93)80021-H

515 Walstra, P., & Smulders, P. (2000). Emulsion Formation. In B. . Binks (Ed.), *Modern Aspects*
516 *of Emulsion Science* (1st ed., pp. 56–99). Cambridge, UK: The Royal Society of
517 Chemistry.

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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.

κC (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 κC concentration (0.01 – 0.5 wt. %) with a fixed concentration of PoPI (1 wt. %) on the size of PoPI-κC electrostatic complexes.

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

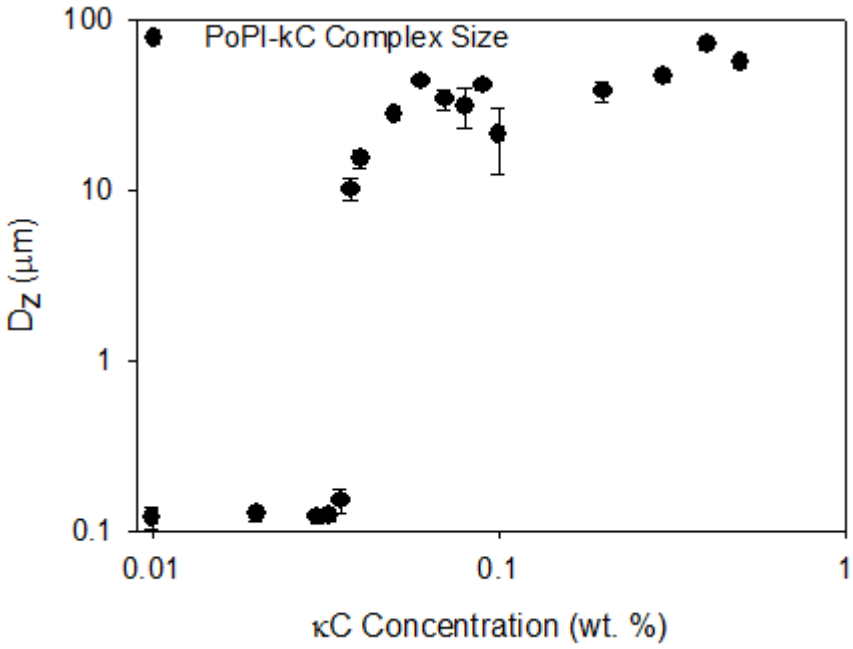
Fig. 3. Representative images of PoPI-κC electrostatic complexes with an increasing of κC from 0 to 0.1 wt. % at an increment of 0.01 wt. %, with a fixed concentration of PoPI (1 wt. %).

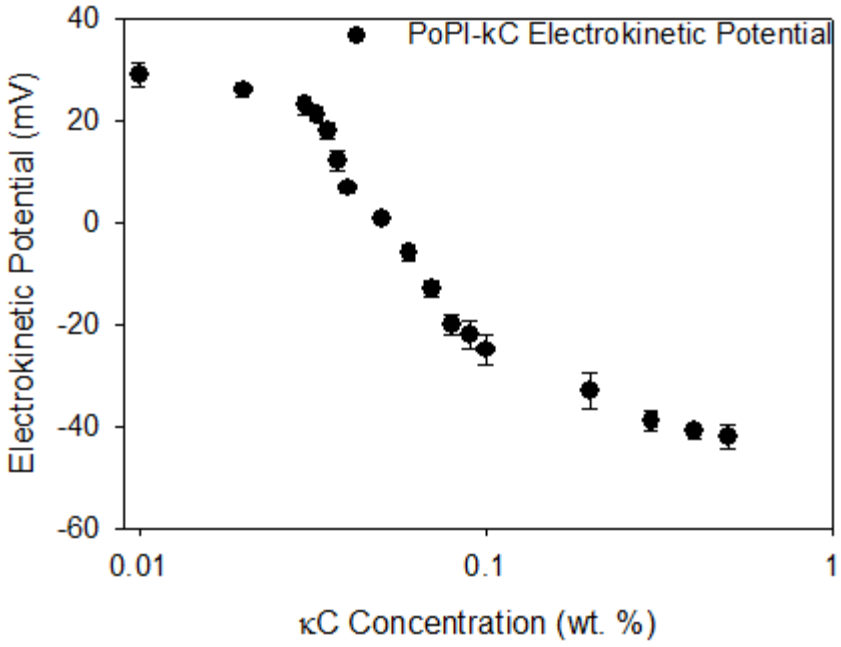
Fig. 4. Emulsion droplet size ($d_{3,2}$) of emulsions prepared with PoPI-κC complexes as a function of increasing concentration of κC (0.01 – 0.5 wt. %).

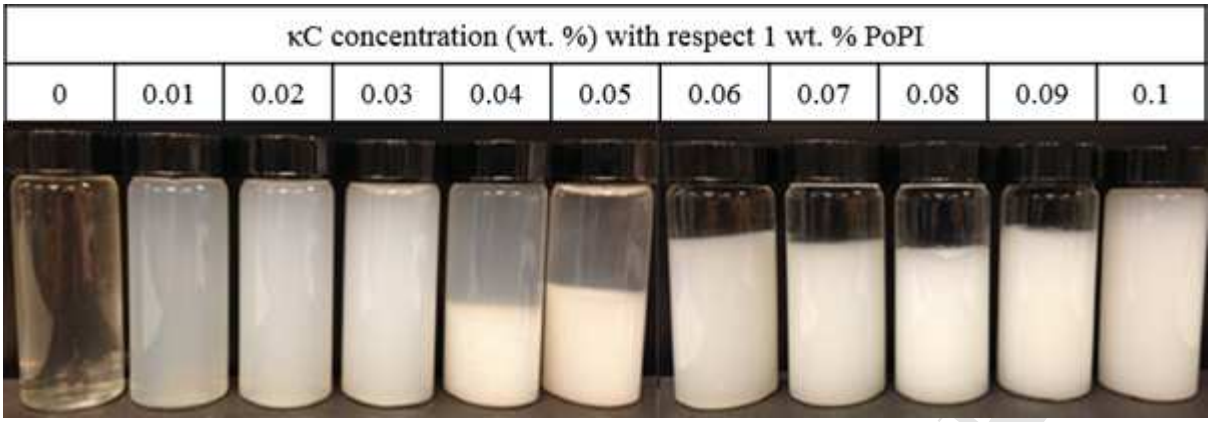
Fig. 5. Optical micrographs of PoPI and PoPI-κC complex stabilised O/W emulsions, whereby the concentration of PoPI was fixed at 1 wt. %: (a) 0% κC, (b) 0.01% κC, (c) 0.04% κC, (d) 0.07% κC, (e) 0.1% κC and (f) 0.5% κC. Scale bar is 40 µm in all instances.

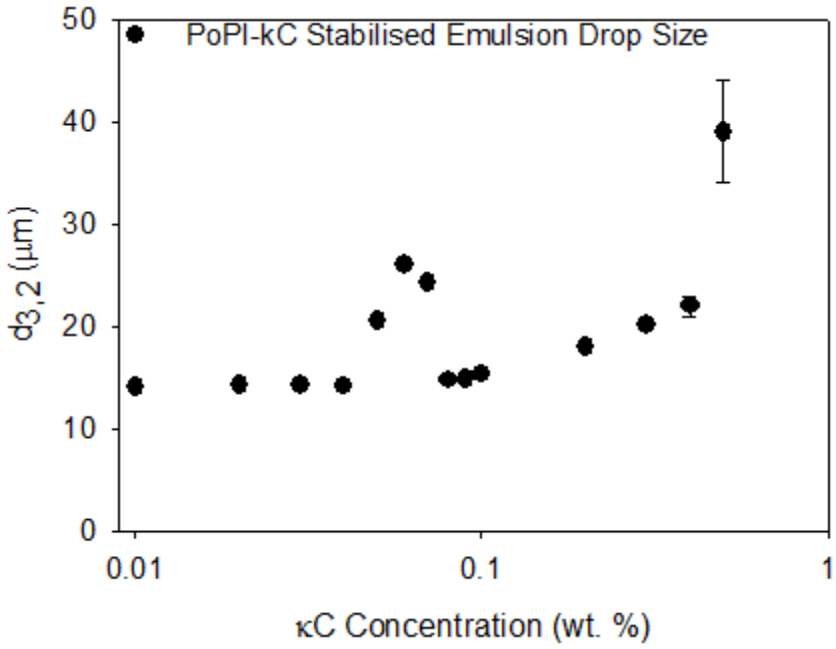
Fig. 6. Interfacial tension between water and rapeseed oil as a function of emulsifier type: 1% PoPI (●), 1% PoPI-0.03% κC complexes (○), 1% PoPI-0.06% κC complexes (▼), and 1% PoPI-0.1% κC complexes (Δ).

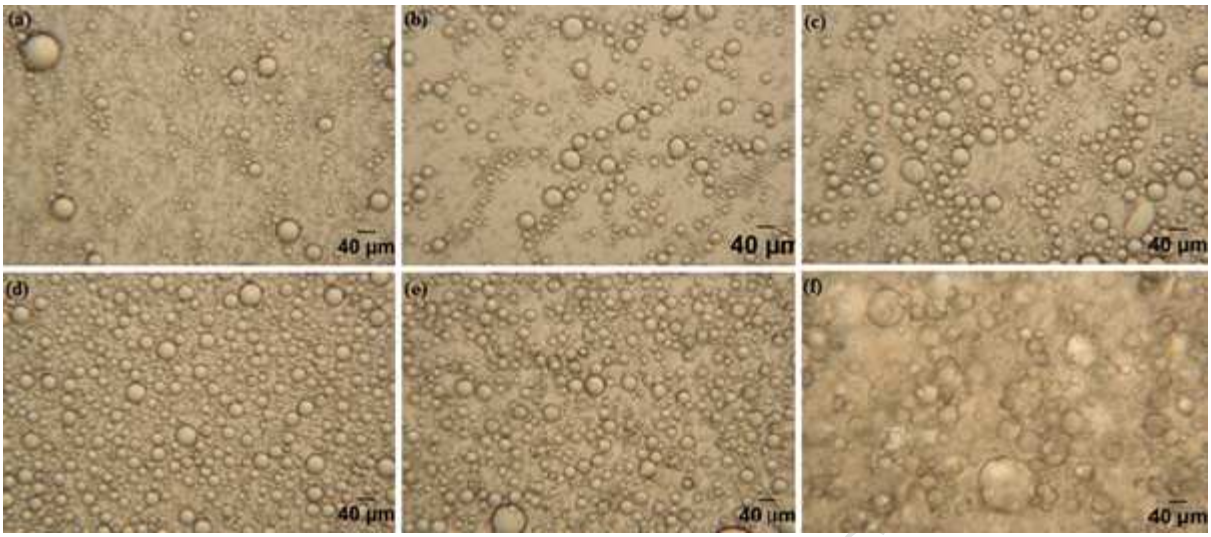
Fig. 7. Effect of κC content within PoPI-κC complexes on droplet size ($d_{3,2}$) as a function of time for O/W emulsions stabilised by: (a) 1% PoPI and 1% PoPI-0.03% κC complexes, (b) 1% PoPI and 1% PoPI-0.06% κC complexes, (c) 1% PoPI and 1% PoPI-0.09% κC complexes, and (d) 1% PoPI and 1% PoPI-0.5%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

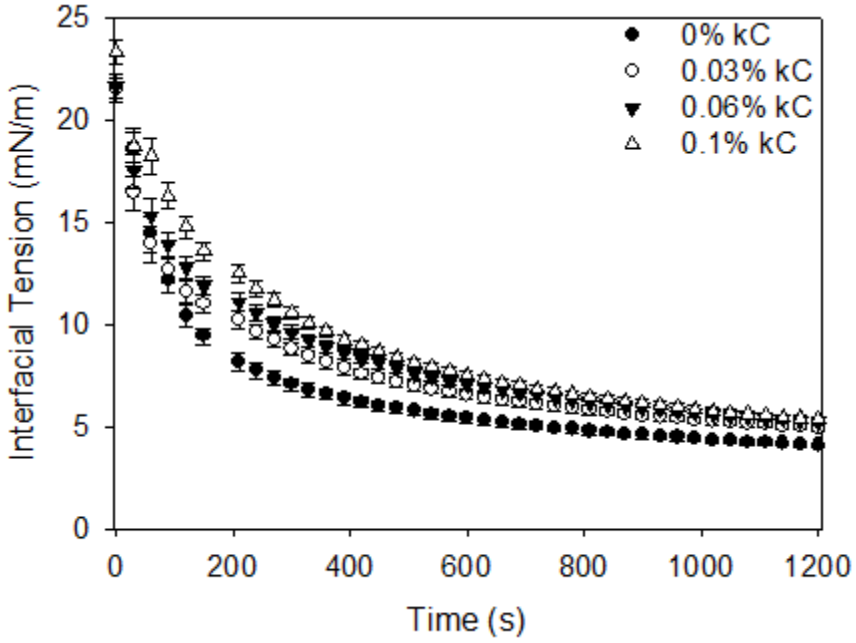


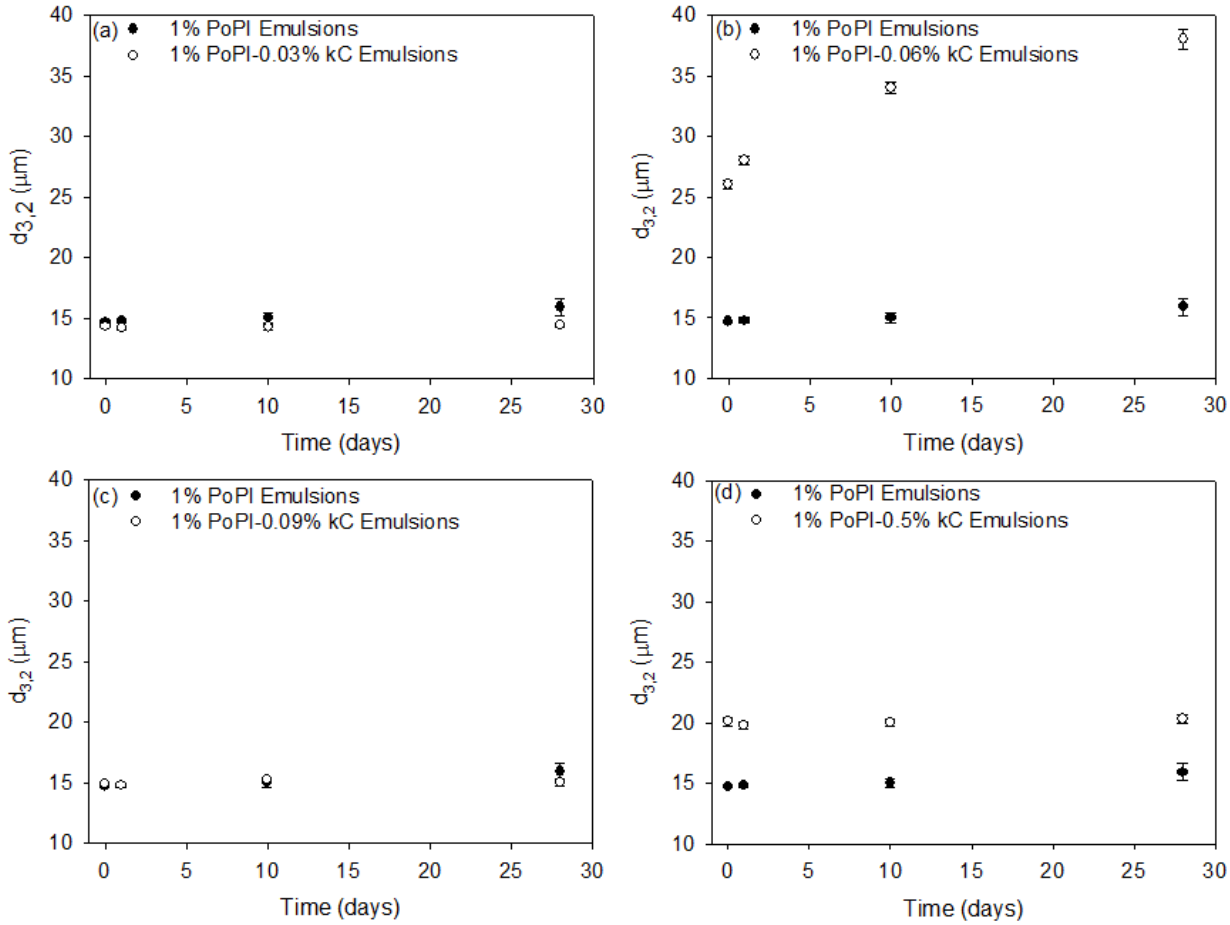











(a)
1% PoPI Emulsions
1% PoPI-0.03% kC Emulsions
(b)
1% PoPI Emulsions
1% PoPI-0.06% kC Emulsions
(c)
1% PoPI Emulsions
1% PoPI-0.09% kC Emulsions
(d)
1% PoPI Emulsions
1% PoPI-0.5% kC Emulsions
d3,2 (µm)
Time (days)

Highlights:

- Electrostatic complexes were formed between potato protein and κ-carrageenan (κC).
- Submicron complexes (< 150 nm) were formed at κC concentrations ≤ 0.0375%.
- Micron-sized complexes (> 1 μm) were formed at κC concentrations > 0.0375%.
- Complex stabilised emulsions possessed enhanced long-term stability.