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Effect of the Biopolymer Mixing Ratio on the Formation of Electrostatically Coupled Whey Protein−κ- and ι-Carrageenan Networks in the Presence and Absence of Oil Droplets

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ABSTRACT: The rheological properties of 1.0% (w/w) whey protein isolate (WPI)−κ-/ι-carrageenan (CG) mixtures were investigated during a slow acidification process by glucono-δ-lactone from pH 7.00 to ∼4.20 as a function of biopolymer mixing ratio and in the presence and absence of oil droplets. In all cases, electrostatic coupled biopolymer and emulsion gel networks were formed at pH values corresponding to where attractive interactions between WPI and CG began. Formed WPI−CG complexes were found to be surface active, capable of lowering interfacial tension and forming viscoelastic interfacial films within emulsion-based systems. Both biopolymer and emulsion-based gels increased in strength and elasticity as the CG content increased, regardless of the type of CG present. However, WPI−ι-CG coupled networks were stronger than WPI−κ-CG networks, presumably due to the higher number of sulfate groups attracting the WPI molecules.

KEYWORDS: *emulsion, electrostatic coupled gel, coacervation, whey proteins, carrageenan*

INTRODUCTION

Whey protein and carrageenan (CG) are commonly used as food ingredients in many applications (e.g., baked goods, beverages, desserts, confectionary and meat products) due to their gelling, emulsifying,or thickening abilities.[1] Whey proteins represent a mixture of proteins that are dominated by α-lactalbumin and β-lactoglobulin. These proteins have been intensively studied over the years for their functional attributes such as gelation[2−4] and their capacity to stabilize emulsions.[5−7] In contrast, carrageenan polysaccharides are extracted from red seaweeds (*Rhodophyta* species)[8] and consist of disaccharide repeating units composed of D-galactose and 3,6-anhydro-D-galactose residues, with one, two, or three sulfate groups associated each repeat unit for κ-, ι-, and λ-type carrageenans, respectively.[9] Although other minor types of CG have been identified by Knutsen and co-workers,[10] the present research will focus only on the κ- and ι-types due to their abilities to form strong gels.[11−13]

Mixtures of proteins and polysaccharides are known to undergo associative liquid/liquid phase separation (also known as complex coacervation) under conditions where biopolymers are oppositely charged and/or experiencing a significant amount of electrostatic attraction.[14] Whey protein−carrageenan mixtures have been explored previously in this respect by de Kruif and co-workers,[14] Weinbreck and co-workers,[9] and Stone and Nickerson.[15] During coacervation, the pH is lowered until a negatively charged protein (i.e., pH > p*I*) becomes neutral (i.e., at p*I*) and then positively charged (i.e., pH < p*I*), when it then can interact with a negatively charged polysaccharide. In the case of whey protein and CG, the initial interaction typically occurs at pH > p*I*, when both biopolymers carry similar net charges due to the interaction between CG and positively charged "patches" on a protein surface.[9] This initial interaction is denoted pH_c and is evident by a slight increase in the absorption or light scattering in the protein−polysaccharide solution.[14] As the pH continues to decline, a sharp rise in the absorbance or light scattering of the biopolymer solution (designated $pH_{\phi 1}$) follows as the solution becomes turbid.[14] This sustained increase in absorbance and light scattering continues until a maximum is reached (designated pH_{opt}).[14] Beyond this point, continual acidification causes the complex to dissociate and dissolve.[14] The area of complex coacervation has been well studied with a number of reviews in the area.[14,16,17]

Recently, researchers have sought to use complexes as a means to stabilize emulsions.[18−24] Emulsions are formed when two immiscible liquids are mechanically agitated until one is dispersed as tiny droplets within the other. Because emulsions are thermodynamically unstable, amphiphilic biopolymers (e.g., proteins) can be used to inhibit emulsions from phase separation by reducing the surface tension between the droplets and the continuous solvent. Depending on the level of interactions, the biopolymers involved, and the processing conditions, systems may become stable or unstable. For instance, in dilute systems, the addition of a polysaccharide to a protein-stabilized oil droplet could lead to the formation of a viscoelastic bilayer where the polysaccharide complexes to the interfacial film, leading to increased emulsion stability[25] or causing instability via flocculation by the depletion interaction or bridging flocculation of neighboring droplets.[26] In more concentrated systems, various mixed protein−polysaccharide systems may take on a gel-like structure.[27] The nature of the gel may be considered as an "emulsion-filled protein gel, whereby oil droplets are simply encased within a gelled protein or a protein-stabilized emulsion gel", whereby the gel structure

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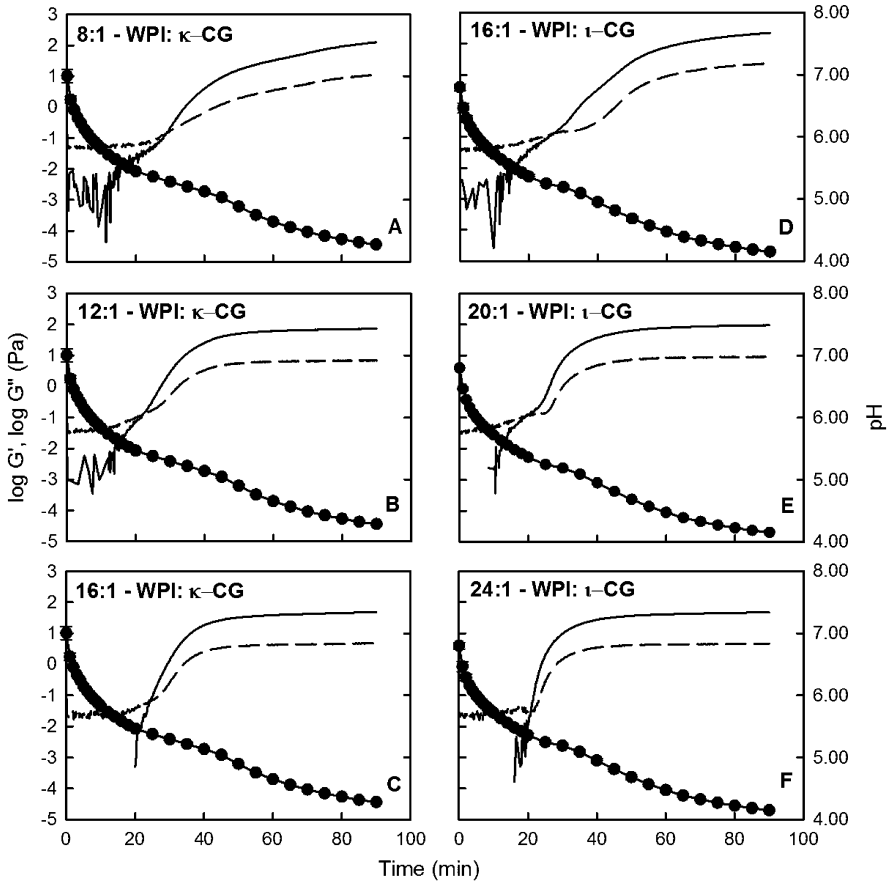

Figure 1. Dynamic viscoelastic storage (G', solid line) and loss (G'', dashed line) moduli measured at 1 Hz with 1.0% constant strain for mixtures of WPI–κ-CG (A–C) and WPI–ι-CG (D–F) at various mixing ratios as a function of pH (solid circles) and time (min). Each curve is the mean ± one standard deviation of three separate time sweep runs on separate samples. All solutions had a total biopolymer concentration of 1.0% (w/w) and were acidified using 0.5% (w/w) GDL.

consists of an interconnected flocculated network of oil droplets stabilized by an interfacial coacervate film.[27]

The inclusion of polysaccharides to protein-based emulsion systems has recently been researched as a means to reduce the oxidation of the emulsified oil for bioactive delivery[28] and for developing novel applications in controlled-release systems.[29] This body of work will continue to enhance our understanding of protein–polysaccharide interactions in emulsified systems.[30] The overall goal of the present study is to examine the underlying mechanisms driving the formation of electrostatically coupled biopolymer and emulsion gel networks, as a function of CG type (κ- and ι-types) and mixing ratio between whey protein isolate (WPI) and the CG molecules. Stone and Nickerson investigated the formation of electrostatic complexes involving WPI and CG molecules as a function of CG type under dilute conditions (0.25% w/w) and reported the optimal mixing ratio to occur at a 12:1 ratio for WPI–κ-CG mixtures and at a 20:1 ratio for the WPI–ι-CG system.[15] Information arising from this study will aid in controlling and optimizing their use in food or controlled delivery-type applications where gelation is important.

MATERIALS AND METHODS

Materials. Whey protein isolate used in this study was generously donated by Davidson Foods International, Inc. (Le Sueur, MN, USA). The chemical composition of the commercial WPI powder was determined according to AOAC[31] methods; 925.10 (moisture), 923.03 (ash), 920.87 (crude protein), and 920.85 (lipid). Carbohydrate content was determined on the basis of present differential from 100%. The WPI powder was composed of 89.78% protein (%N × 6.38), 0.10% lipid, 4.92% moisture, 2.06% ash (including 0.08% Ca^{2+}, 0.01% Mg^{2+}, 0.02% K^+, and 0.66% Na^+), and 3.13% carbohydrate (wet basis). Carrageenan (CG) (κ- and ι-) and glucono-δ-lactone (GDL) were purchased from Sigma-Aldrich (Oakville, ON, Canada). For the two types of CG, the supplier information indicated that κ-CG was composed of 66.50% carbohydrate, 10.65% moisture, and 22.86% ash (including 2.4% Ca^{2+}, 0.16% Mg^{2+}, 5.4% K^+, 0.49% Na^+) and that ι-CG contained 64.40% carbohydrate, 10.82% moisture, and 24.97% ash (including 3.4% Ca^{2+}, 0.18% Mg^{2+}, 3.2% K^+, 1.2% Na^+). Canola oil used in this study was purchased from a local supermarket. All other chemicals used in this study were of reagent grade and purchased through Sigma-Aldrich. The water used in this research was filtered using a Millipore Milli-Q water purification system (Millipore Corp., Milford, MA, USA). WPI and CG were used without further purification and were corrected for protein and polysaccharide content, respectively.

Sample Preparation. Biopolymer solutions (1.0% w/w biopolymer) were prepared by dispersing WPI and CG powders in water by mechanical stirring (500 rpm) at room temperature (21–23 °C) for 60 min. Afterward, 0.5% (w/w) of GDL was added to the biopolymer solution and stirred for 1 min prior to analysis to slowly acidify the solution. WPI and CG were mixed at ratios above and below the optimal ratio determined by Stone and Nickerson.[15] Mixing ratios of 8:1, 12:1, and 16:1 were tested for WPI–κ-CG, and ratios of 16:1, 20:1, and 24:1 were tested for WPI–ι-CG. A total of 1.0% (w/w) biopolymer into the aqueous phase was used for all experiments. All WPI and CG powder weights used were corrected for both protein

and carbohydrate concentrations, respectively. Changes in pH upon GDL addition was monitored for each mixture using a Fisher Scientific Accumet pH meter equipped with an Ag/AgCl pH probe (Fisher Scientific, Ottawa, ON, Canada) over time.

Emulsion Preparation. Initially, biopolymer solutions were prepared as previously mentioned. Upon addition of the GDL, and equal ratio (1:1) of the aqueous biopolymer solution and canola oil was mixed to a total weight of 10 g. The mixture was subsequently homogenized at 7200 rpm for 5 min using an Omni Macro Homogenizer (Omni International Inc., Marietta, GA, USA) equipped with a 20 mm sawtooth generating probe.

Interfacial Tension. The interfacial tension for each WPI−CG mixture was measured using a Lauda TD 2 tensiometer (Lauda-Königshofen, Germany) equipped with a Du Nüoy ring (20 mm diameter). Within a 40 mm diameter glass sample cup, a 20 g biopolymer solution was added and then stirred for 1 min after the addition of 0.5% w/w GDL, followed by the immersion of the Du Nüoy ring and then the addition of the upper canola oil layer (20 g). The ring was then pulled upward to stretch the interface to measure the maximum force without breaking into the oil phase. Interfacial tension was measured over time (50 min) every 600 s, where the mean of three replicates ± one standard deviation was reported. Interfacial tension was calculated from the maximum force (F_{max}; units of mN; instrument measures mg × gravity) using equation 1)

$$\gamma = \frac{F_{max}}{4\pi R\beta} \quad (1)$$

where γ is the interfacial tension (mN/m), R is the radius of the ring (20 mm), and β is a correction factor that depends on the dimensions of the ring and the density of the liquid involved.[32] All measurements are reported as the mean ± one standard deviation (n = 3).

Oscillatory Shear Deformation of the Bulk Solution. Oscillatory shear deformation of the bulk biopolymer and emulsion solutions during a GDL acidification was monitored over time using an AR-G2 rheometer (TA Instruments, New Castle, DE, USA) equipped with a 40 mm diameter 2° acrylic cone. A time sweep was performed over a 90 min period to monitor changes in the dynamic storage (G') and loss (G'') moduli. Measurements were made within the linear viscoelastic region at a frequency of 1 Hz every 10 s, where the maximum amplitude for strain was set at 1.0%. Following the time sweep, a frequency and strain sweep was performed on the same material to better characterize the formed gel network. During frequency sweeps, frequencies were increased continuously from 0.05 to 100 Hz with 15 points per logarithmic decade being captured, with maximum strain set at 1%. In contrast, strain sweeps were conducted by increasing the applied strain continuously from 0.01 to 500% with 15 data points per logarithmic decade captured at a frequency of 5 Hz. Each sample was prepared in triplicate.

Oscillatory Shear Deformation at the Oil−Water Interface. Interfacial oscillatory shear deformation was applied at the oil−water interface using the AR-G2 rheometer equipped with their interfacial bicone fixture. Interfacial oscillatory shear was performed in time sweep mode for 90 min at a frequency of 1 Hz and maximum strain set at 1.0%. Biopolymer solutions with GDL comprised the denser phase beneath the bicone, whereas canola oil comprised the less dense phase above the bicone. All measurements were made within the linear viscoelastic regimen. Each sample was prepared in triplicate.

RESULTS AND DISCUSSION

Formation of Electrostatic Coupled Biopolymer Networks. The rheological properties of WPI−CG mixtures as a function of CG type and mixing ratio during a slow acidification from pH 7.00 to ∼4.20 using GDL are given in Figure 1. In all cases, gel-like properties ($G' > G''$) were evident once the solution pH was reduced to induce complexation between the WPI and CG molecules. In the absence of CG, WPI solutions did not exhibit gel-like properties (data not shown). In the case of the WPI−κ-CG mixture, the crossover point (denoted as the gel point[33]) of G' with G'' was found to correspond to pH 5.37, 5.33, and 5.29 for the 16:1, 12:1, and 8:1 WPI−κ-CG mixing ratios, respectively (Figure 1a-c). The pI of WPI has been reported to be at pH 4.6,[34] suggesting that complexation is occurring where both biopolymers are carrying a negative net charge and most likely involve CG interactions with positively charged patches on the protein's surface.[9] At pH above the gel point, biopolymers were assumed to be cosoluble and noninteracting, leading to fluid-like flow behavior ($G' < G''$). Afterward, G' increased substantially indicating a rise in network strength. In the case of the 8:1 mixing ratio, G' approached a plateau regime whereas for the 12:1 and 16:1 ratio a plateau was reached (Figure 1A−C). The magnitude of G' was found to increase as the CG concentration increased within the ratio, where G' was found to be 45.4, 73.1, and 108.0 Pa for 16:1, 12:1, and 8:1 WPI−κ-CG mixing ratios, respectively (Table 1). It was hypothesized that the lower pH at the gel point for the 8:1 mixing ratio reflects a greater viscosity imparted by the higher CG content. Similarly, Perez and co-workers reported that an increase in polysaccharide content led to slower mobility of whey protein−polysaccharide complexes.[35] Therefore, it was hypothesized that the reduced mobility of WPI−CG complexes led to a slower gelation time due to the high viscosity imparted by the high CG content of the system. In the case of the WPI−ι-CG mixture, the gel point was found to correspond to pH 5.34, 5.34, and 5.20 for the 24:1, 20:1, and 16:1 mixing ratios, respectively (Figure 1D,E). A similar increase in the maximum G' was seen with increasing CG content, where the magnitude was found at 52.9, 93.1, and 256.9 Pa for mixing ratios 24:1, 20:1, and 16:1, respectively (Table 1), suggesting it was following a similar mechanism of network formation.

Table 1. Dynamic Storage (G') and Loss (G'') Moduli for Electrostatic Coupled Networks Involving WPI−CG Mixtures As a Function of Mixing Ratio and in the Absence and Presence of Oil after a 90 min Time Sweep Measured at 1 Hz while a 1.0% Constant Strain Was Applied[a]

	biopolymer gel		emulsion gel	
mixing ratio	G' (Pa)	G'' (Pa)	G' (Pa)	G'' (Pa)
WPI−κ-Carrageenan				
8:1	108.0 ± 15.6	10.0 ± 0.9	522.9 ± 49.8	67.5 ± 0.7
12:1	73.1 ± 2.2	6.9 ± 0.7	435.3 ± 16.2	54.5 ± 0.8
16:1	45.4 ± 6.8	4.5 ± 0.6	257.3 ± 28.5	36.2 ± 5.3
WPI−ι-Carrageenan				
16:1	256.8 ± 29.8	26.6 ± 2.9	658.5 ± 60.0	91.4 ± 9.3
20:1	93.1 ± 1.6	9.1 ± 0.2	320.4 ± 17.4	45.9 ± 3.3
24:1	52.9 ± 12.5	5.1 ± 1.0	70.8 ± 16.6	15.8 ± 1.2

[a]All solutions had a total biopolymer concentration of 1.0% (w/w) and were acidified using 0.5% (w/w) GDL to pH 4.28 and 4.15 for the WPI−κ-CG and WPI−ι-CG mixtures, respectively. Data represent the mean value ± one standard deviation.

An increase in the G' or the stiffness of the network has been previously reported by Tavares and co-workers, where the stiffness of whey protein−galactomannan networks was found with an increase in the polysaccharide content.[36] In the absence of CG, acidified WPI solutions did not form a network (data not shown). Therefore, it is assumed that the formation of WPI−CG networks is attributed to the formation of WPI−CG electrostatic complexes due to GDL acidification, which act as junction zones within the gel. Complex formation has been previously reported by others[9,14,15] and is a well-known

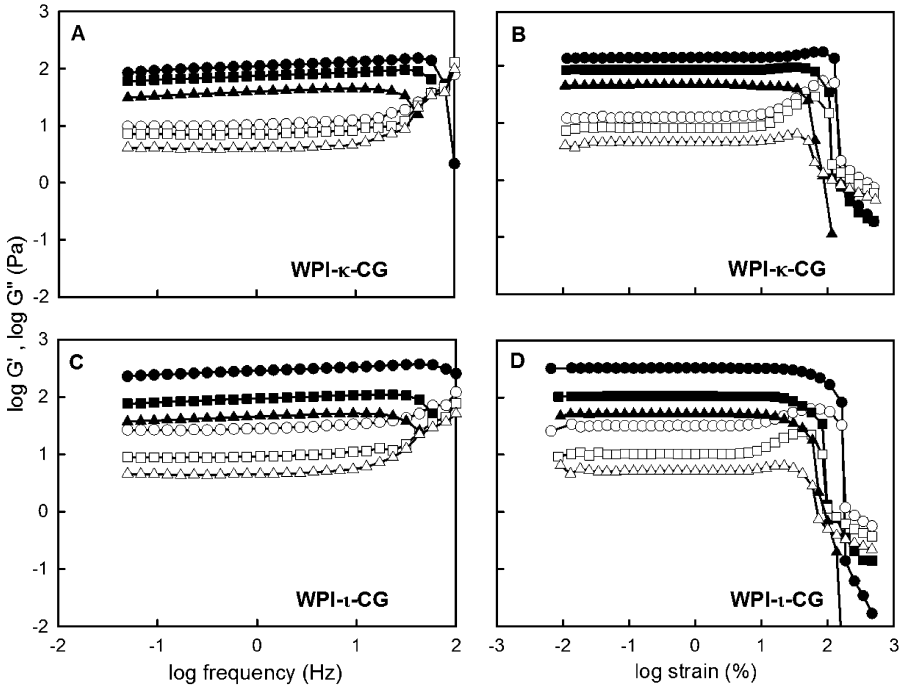

Figure 2. Dynamic viscoelastic storage (G', solid symbols) and loss (G'', open symbols) moduli for mixtures of WPI−κ-CG (A, B) and WPI−ι-CG (C, D) at various mixing ratios as a function of frequency (A, C) and strain (B, D). WPI−κ-CG (A, B) mixtures were mixed at ratios of 8:1 (circles), 12:1 (squares), and 16:1 (triangles), whereas WPI−ι-CG (C, D) mixtures were mixed at 16:1 (circles), 20:1 (squares), and 24:1 (triangles). Each curve is representative of three separate sweep runs on separate samples. All solutions had a total biopolymer concentration of 1.0% (w/w) and were measured at a pH of 4.28 or 4.15 for WPI−κ-CG and WPI−ι-CG mixtures, respectively.

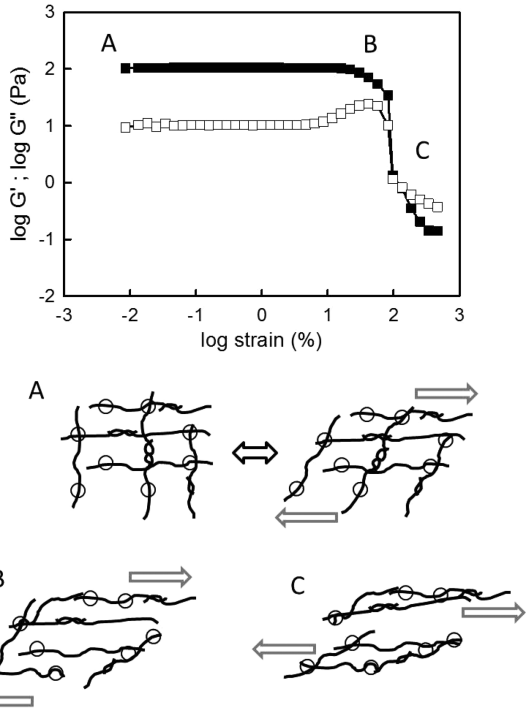

Figure 3. Schematic of a sample WPI−ι-CG biopolymer gel at the ratio of 20:1 in response to strain (G', solid symbols; G'', open symbols) where gelled networks composed of WPI (open circles) coupled with CG (lines) are elastic at low strains (A) and rearrange in their molecular bonding at higher strains (B), which leads to the breakdown of the network (C). The gray arrows indicate the direction of the applied shear.

phenomenon and therefore is beyond the scope of this paper. In part, coacervation requires the phase separation of the networks, where it is presumed that as proteins approach their p*I*, the precipitation of the proteins and their coacervation with polysaccharides enhance the strength of these networks. In a study of the effects of β-glucans complexed onto egg yolk stabilized oil-in-water emulsions, Santipanichwong and Suphantharika reported that insoluble β-glucans compared to soluble β-glucans exhibited a greater degree of structuring on their own.[37] To gain greater insights into the nature of these networks and to uncover their structural mechanism, they were physically characterized using frequency and strain sweeps.

Oscillatory frequency sweeps for both WPI−κ-CG and WPI−ι-CG systems after the 90 min time sweep indicated that all materials exhibited gel-like features, regardless of the mixing ratio. Within this regimen, both moduli were slightly frequency dependent, and the magnitude of G' was greater than that of G'' (Figure 2A,C). Without acidification, frequency sweeps of these WPI−CG biopolymer solutions were strongly frequency dependent, suggesting a noninteracting mixed biopolymer solution (data not shown). The slight dependence of these GDL acidified WPI−CG mixtures indicates a weak gel structure. Similarly, whey protein−galactomannan networks were also found to be slightly frequency dependent.[36]

Critical strains are helpful in characterizing the breakup of these gels under strain. In a previous study on pure CG gels, Azevedo and co-workers reported that in low ionic content CG mixtures, the critical strain was dependent on the CG content.[38] Similar to the present study, the critical strain decreases with increasing CG content.[38] CG content allowed for greater elasticity within these networks such that critical strains increased with CG content. Critical strain at break for WPI−κ-CG was found at ∼58.83, ∼73.41, and ∼116.4% for

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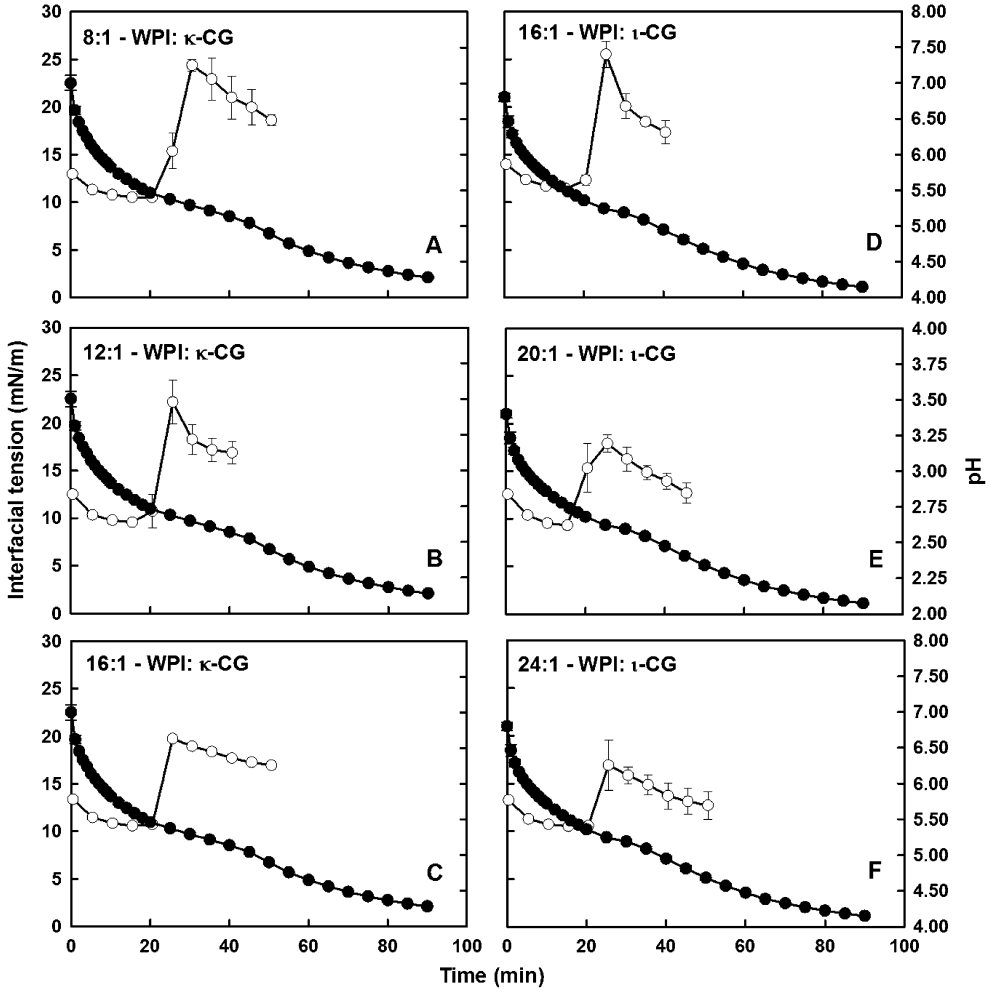

Figure 4. Interfacial properties of WPI/CG mixtures at the canola oil−water interface measured by interfacial tension (open circles) reported as the mean ± one standard deviation for mixtures of WPI−*κ*-CG (A−C) and WPI−*ι*-CG (D−F) at various mixing ratios as a function of pH (solid circles) and time (min). All solutions had a total biopolymer concentration of 1.0% (w/w) and were acidified using 0.5% (w/w) GDL.

mixing ratios of 16:1, 12:1, and 8:1, respectively, whereas for WPI−*ι*-CG, critical strain at the break occurred at ∼46.67, ∼74.52, and ∼98.25% for mixing ratios of 24:1, 20:1, and 16:1 respectively (Figure 2B,D). For both systems, the most elastic networks were formed at the highest CG content (i.e., lowest WPI/CG ratios). Networks were also more brittle (i.e., lower critical strain) when *κ*-CG was present relative to *ι*-CG, possibly due to its lower linear charge density, which reduces the number of potential bonds while allowing it to have greater conformational entropy (e.g., flexibility) and possibly more flexible cross-links between WPI molecules within the electrostatic coupled network. In contrast, *ι*-CG would have a higher number of sulfate groups present, leading to a stiffer polysaccharide molecule between the WPI molecules, which builds a stronger network.

The breakdown behavior in the strain sweep reveals the nature of these networks (Figure 2B,D) where the strain-independent behavior observed initially is a result of the elasticity exhibited by the network under low strains. At higher strains, the loss modulus increases slightly before decreasing along with the storage modulus. This strain behavior has been previously characterized as weak strain overshoot behavior by Hyun and co-workers.[39] In weak strain overshoot behavior, although frequency sweeps demonstrate gel-like behavior, their anionic polysaccharide (e.g., xanthan gum in a study by Hyun et al.[36] and CG in this study) was explained to possess large conformational entropy due to electrostatic repulsion within its structure.[39] This allows for alignment and association between biopolymers allowing for a network to form.[39] Hyun and co-workers later reported that weak strain overshoot behavior which results in a local G'' maximum is the result of junctions both breaking and forming new ones within the network under high strains.[40] Others have suggested that this localized G'' maximum is due to structural rearrangements due to the shear imposed onto the network.[41] Weak strain overshoot behavior was observed for all biopolymer networks formed with WPI−*ι*-CG mixtures and for WPI−*κ*-CG mixtures at a ratio of 16:1. In the case of WPI−*κ*-CG mixtures at ratios of 8:1 and 12:1, strong overshoot behavior was observed,[39] which is caused by the strong interactions formed by the interacting biopolymers. Under strong overshoot behavior, a local maximum for both G' and G'' is observed before both rapidly decrease at higher strains. The decrease in both moduli is attributed to the breakdown of the network by shearing. This strain overshoot phenomenon is not unique to these WPI−CG systems but has also been previously reported in other associative biopolymer solutions.[42,43] WPI−CG networks within the present study are formed as a result of GDL acidification causing coacervation.

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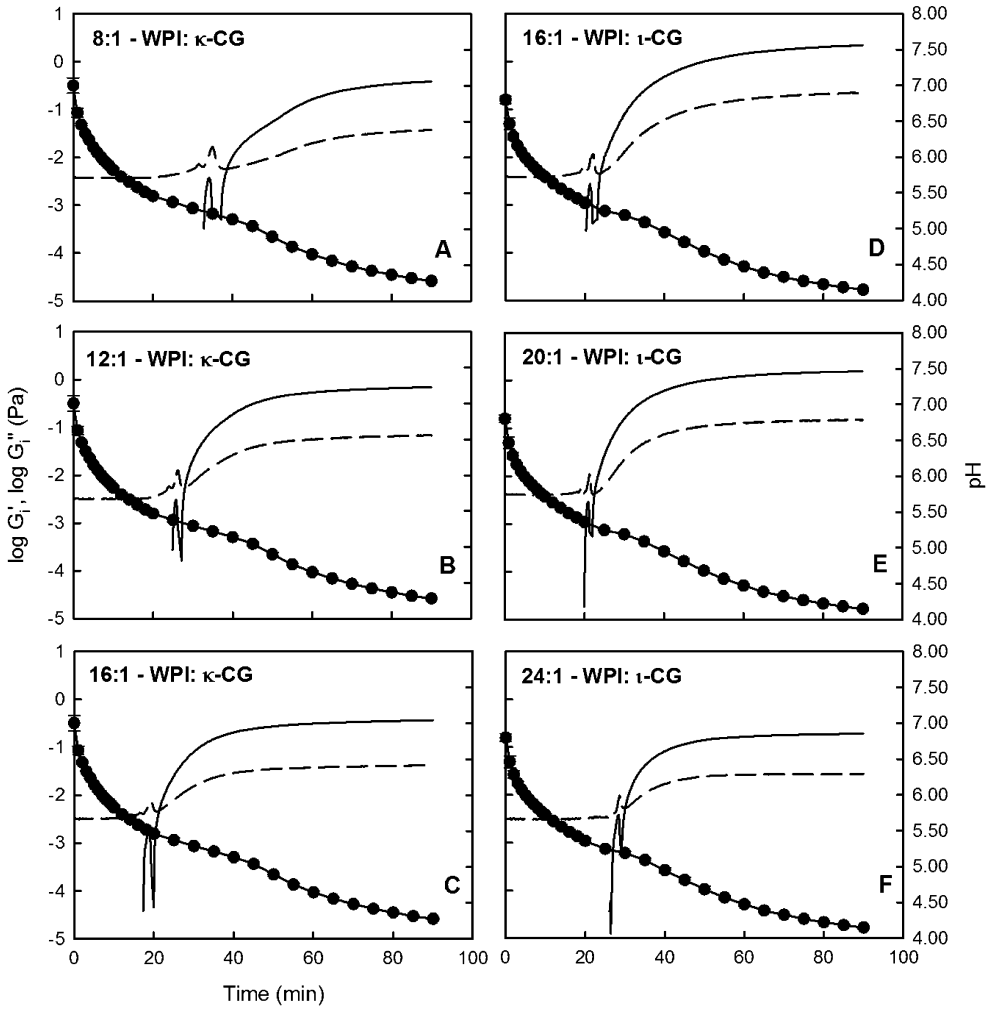

Figure 5. Interfacial rheological properties of WPI/CG mixtures at the canola oil−water interface measured by interfacial storage moduli (solid line) and interfacial loss moduli (dashed line) for mixtures of WPI−*κ*-CG (A−C) and WPI−*ι*-CG (D−F) at various mixing ratios as a function of pH (solid circles) and time (min). All solutions had a total biopolymer concentration of 1.0% (w/w) and were acidified using 0.5% (w/w) GDL.

The conformational entropy associated with CG allows for junction zones to be formed with either other CGs or WPIs present in solution (i.e., WPI−WPI, CG−CG, and WPI−CG interactions). These interactions, formed due to acidification over time, allow for a continuous network to be formed as captured by time sweeps under oscillatory stress measurements. Although a continuous network is formed, it is by no means uniform. Figure 3 provides a visual representation of the breakdown of networks under strain which are linked together via CG chains with other CG or WPI biopolymers. These networks are elastic under low-strain amplitudes (Figure 3A) but undergo stress overshoot behavior at higher strains (Figure 3B), which eventually leads to their breakdown (Figure 3C).

Interfacial Properties of WPI−CG Complexes at the Water-and-Oil Interface. Due to the amphiphilicity of WPI, the surface activity of WPI−CG mixtures during acidification was measured at the canola oil−water interface. In the absence of biopolymers, the interfacial tension between canola oil and water was found to be ∼28 mN/m, where the addition of WPI alone lowered the interfacial tension to ∼12 mN/m, where interfacial tension remains relatively constant between ∼10 and ∼12 mN/m for the duration for GDL acidification. Interfacial tension and interfacial rheology for WPI−CG mixtures as a function of mixing ratio during a slow acidification with GDL are given in Figures 4 and 5, respectively. Interfacial tension for all systems was found to decrease until reaching a pH where WPI and CG formed electrostatic complexes (Figures 4 and 5). This decline in tension is presumed to be caused by a highly surface active WPI molecule, which is presumed to be integrating alone into the interface rather than the CG molecule over this pH range (from 7.00 to 5.46 and 5.36 for WPI−*κ*-CG and WPI−*ι*-CG mixtures, respectively). At ∼20 min (pH 5.46 and 5.36 for WPI−*κ*-CG and WPI−*ι*-CG mixtures, respectively), a dramatic increase in tension was seen believed to be associated with the electrostatic interactions between WPI and CG. The rise in tension suggests that, possibly, WPI adhering to the interface is being pulled away by electrostatic attractive forces with CG molecules in solution, reducing its ability to lower the interfacial tension. Upon electrostatically interacting with CG molecules, WPI−CG complexes can readhere to the interface to form a stable film, to lower the interfacial tension once again. The decrease in interfacial tension can only be measured until ∼45 min/pH ∼5.00, after which the Du Nüoy ring punctures through the interface due to the formation of an electrostatic coupled gel network within the continuous phase, which would restrict the mobility of the interfacial film (Figure 4). However, it is presumed that free WPI and WPI−CG complexes not involved

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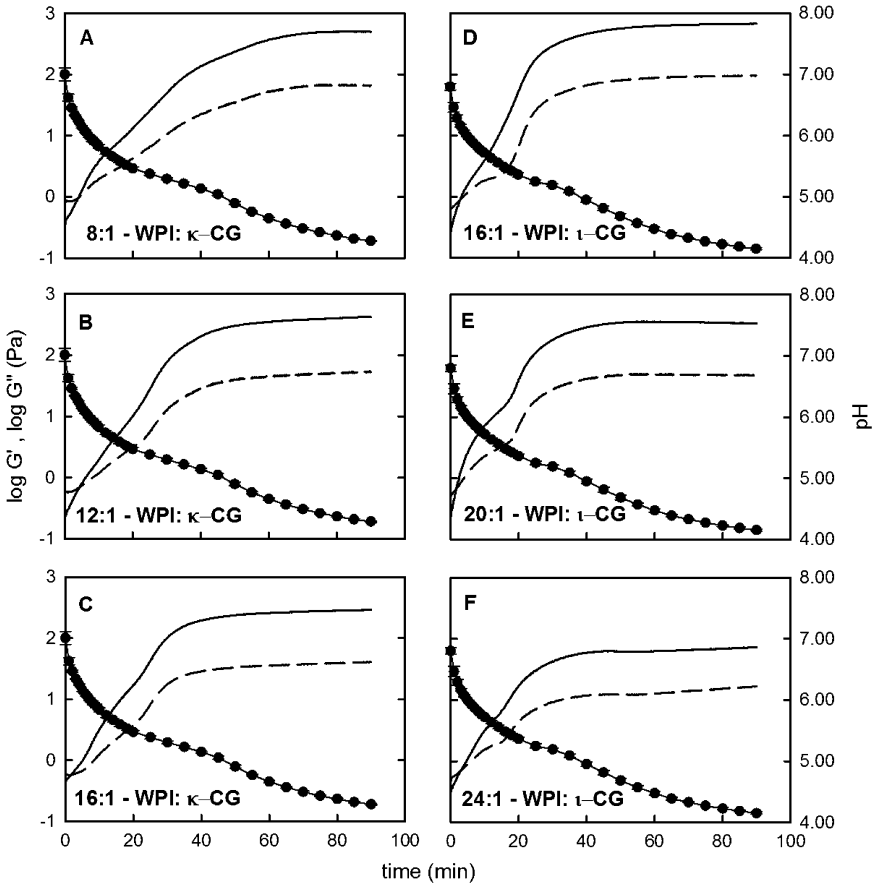

Figure 6. Dynamic viscoelastic storage (G', solid line) and loss (G'', dashed line) moduli for 1:1 water/canola oil emulsions stabilized with WPI–κ-CG (A–C) and WPI–ι-CG (D–F) at various mixing ratios measured at 1 Hz with 1.0% constant strain as a function of pH (solid circles) and time (min). Each curve is representative of three separate time sweep runs on separate samples. All solutions had a total biopolymer concentration of 1.0% (w/w) and were acidified using 0.5% (w/w) GDL.

within the network may still adhere to the interface to form a viscoelastic interfacial film.

The interfacial G_i' and G_i'' values (Figure 5) within the pH range (7.00–5.41) where the two biopolymers were non-interacting (electrostatically) remained relatively constant. Because the interfacial tension decreased during this interval (Figure 4), it is possible that only WPI molecules were adhering to the oil–water interface. Near the time/pH corresponding to the rise in interfacial tension, small fluctuations or peaks in the G_i' and G_i'' data were evident for all materials (Figure 5). These small peaks were found to be dependent upon the mixing ratio, suggesting they might be related to biopolymer interactions and complex formation. In the case of WPI–κ-CG mixtures, a small peak was observed in both G_i' and G_i'' near pH ~5.22, ~5.33, and ~5.42 for the mixing ratios of 8:1, 12:1, and 16:1, respectively (Figure 5A–C). Similarly, for WPI–ι-CG mixtures, a small peak was observed in both G_i' and G_i'' within the pH range of ~5.37, ~5.33, and ~5.27 for the mixing ratios of 16:1, 20:1, and 24:1, respectively (Figure 5D,E). As the pH is reduced further, a rise in G_i' values (i.e., so that they become greater than G_i'') occurs, until a plateau is reached corresponding to the formation of a viscoelastic interfacial film (Figure 3). For the WPI–κ-CG mixtures at pH ~4.2, the magnitude of G_i' values was found to be 366.5, 729.6, and 424.2 mPa for the mixing ratios of 16:1, 12:1, and 8:1, respectively (Figure 5A–C), whereas for the WPI–ι-CG mixtures at pH ~4.2, the magnitude of G_i' values was found to be 173.6, 1358, and 2149 mPa for the mixing ratios of 24:1, 20:1, and 16:1, respectively (Figure 5D,E). Differences in the interfacial rheological properties of these two WPI–CG mixtures are attributed to the nature of their coacervation and their sensitivity toward changes in mixing ratio. Whereas WPI–κ-CG mixtures were reported by Stone and Nickerson to be sensitive toward changes in mixing ratio such that a peak optical density was reached when a 12:1 ratio was used, in contrast, WPI–ι-CG mixtures were not found to be as sensitive to changes in mixing ratio, whereas at the ratio of 20:1 the optical density approached its plateau region.[15] This would account for the viscoelastic maxima at the interface observed for 12:1 WPI–κ-CG mixtures and the increasing moduli values observed as a function of CG content in WPI–ι-CG mixtures.

Formation of Electrostatically Coupled Biopolymer Emulsion Gels. The rheological properties of WPI–CG mixtures as a function of CG type and mixing ratio during a slow acidification from pH 7.00 to ~4.20 using GDL, after being homogenized with canola oil to make an O/W emulsion, are given in Figure 6. For all materials, electrostatically coupled networks were formed as evident by a constant plateau region where G' was greater than G'' (Figure 6). For both biopolymer mixtures, the magnitude of G' at pH ~4.2 was found to increase as the mixing ratio decreased, corresponding to an increasing CG content indicating an increase in network strength (Figure 6). For instance, G' was found to be 257.3, 435.3, and 522.9 Pa for the 16:1, 12:1, and 8:1 mixing ratios,

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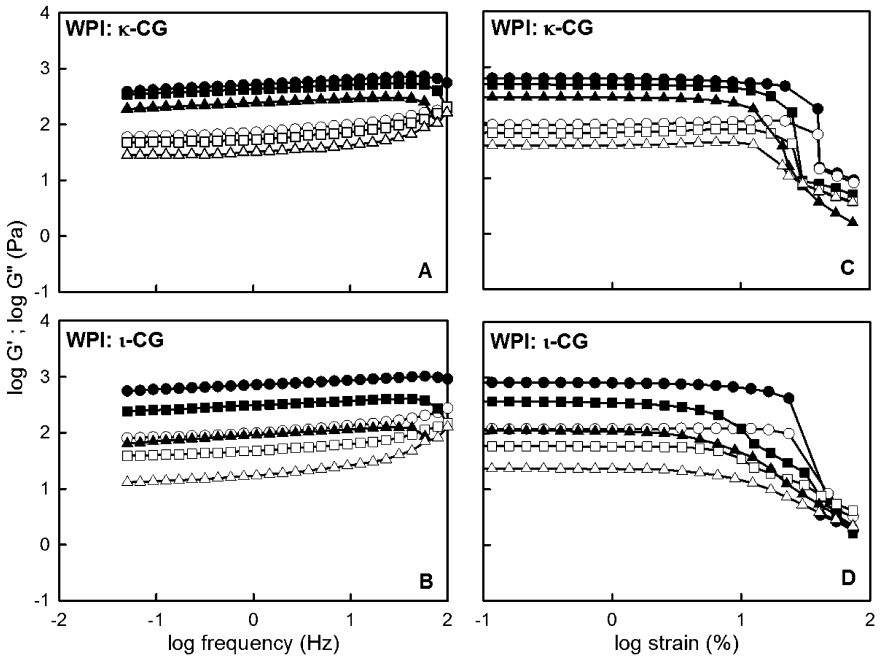

Figure 7. Dynamic viscoelastic storage (G', solid symbols) and loss (G'', open symbols) moduli for 1:1 water/canola oil emulsions stabilized with WPI−κ-CG (A, C) and WPI−ι-CG (B, D) at various mixing ratios as a function of frequency (A, B) and strain (C, D). WPI−κ-CG (A, C) mixtures were mixed at ratios of 8:1 (circles), 12:1 (squares), and 16:1 (triangles), whereas WPI−ι-CG (B, D) were mixed at 16:1 (circles), 20:1 (squares), and 24:1 (triangles). Each curve is representative of three separate sweep runs on separate samples. All solutions had a total biopolymer concentration of 1.0% (w/w) and were measured at a pH of 4.28 or 4.15 for WPI−κ-CG and WPI−ι-CG mixtures, respectively.

respectively, for the WPI−κ-CG mixture (Table 1). Similarly, G' was found to be 70.8, 320.4, and 658.5 Pa for the 24:1, 20:1, and 16:1 mixing ratios, respectively for the WPI−ι-CG mixture (Table 1). Similar to their biopolymer counterparts, CGs are known for their gelling ability and capacity to form coacervates with WPI. CGs are speculated to form bonds among themselves and with WPI. The conformational entropy associated with CGs allow for a greater number of bonds. It is presumed that three intermolecular interactions are present in this system: CG−CG, WPI−CG coacervate, and CG−CG. Therefore, an increase in CG content leads to better coverage of CG polymers throughout the solvent, leading to a greater number of junction zones and a stronger network. Therefore, the primary difference between mixtures containing κ-CG and ι-CG is that the latter contains a greater number of sulfated groups allowing for a greater number of bonds, leading to its comparatively stronger networks.

After the 90 min time sweep, the frequency and strain dependence of the viscoelastic moduli were measured to characterize these networks (Figure 7). For both the WPI−κ-CG and WPI−ι-CG mixtures, moduli were relatively frequency independent with $G' > G''$ (Figure 7A,B). The slight frequency dependence seen suggests that these networks may be considered as weak gels (Figure 7A,B). In the absence of acidification by GDL, these emulsions were found to be largely frequency dependent (data not shown). This suggests that the coacervation of WPI with CG led to the formation of a continuous gel-like network. Therefore, the strain response of these networks was captured to understand the nature of these networks to propose how these emulsion networks are structured.

Critical strain at the break was found to be ∼26.22, ∼17.77, and ∼8.83% for WPI−κ-CG with mixing ratios of 8:1, 12:1, and 16:1, respectively, and ∼27.35, ∼9.33, and ∼2.41% for WPI−ι-CG with mixing ratios of 16:1, 20:1, and 24:1, respectively (Figure 7C,D). Findings from this study indicate that as the CG content is higher (i.e., lower mixing ratios) within the sample, a more brittle network is formed. Compared to their biopolymer counterparts, these emulsion networks are much more brittle in nature. These emulsion networks were found to be elastic under low strain stresses and broke down when exposed to higher strains. These networks exhibited strain thinning behavior,[40] where structures within the network reorient in the direction of the applied strain. At higher strains, these networks begin to break in the direction of the applied strain and the viscous drag of the system decreases.[40] Beyond this point, increasing strains further break down the network.

Emulsion-based gels also were found to have higher network strength than the corresponding material in the biopolymer-only gels (Figures 1 and 6; Table 1). It is presumed that the greater stiffness of the network is attributed to the incompressibility of droplets and interdroplet interactions occurring within the continuous phase. The critical strains were found to increase with increasing CG content similar to the biopolymer-only networks; however, the strain needed for the emulsion-based gels to induce breakage was much less than the biopolymer gel counterparts, suggesting that they were more brittle in nature. These networks are more brittle in nature in part due to the presence of WPI−CG coacervates at the water−oil interface. Interfacial properties for WPI-CG coacervates reveal their ability to form a viscoelastic film at the water−oil interface. This does not fully explain why increasing CG content leads to stronger networks and does not explain why a similar trend was not observed in interfacial viscoelasticity. At interface, the rheological properties are sensitive to mixing ratio due to the limited space at the surface for bond formation, whereas in a continuous network, network strength is enhanced depending on the availability and

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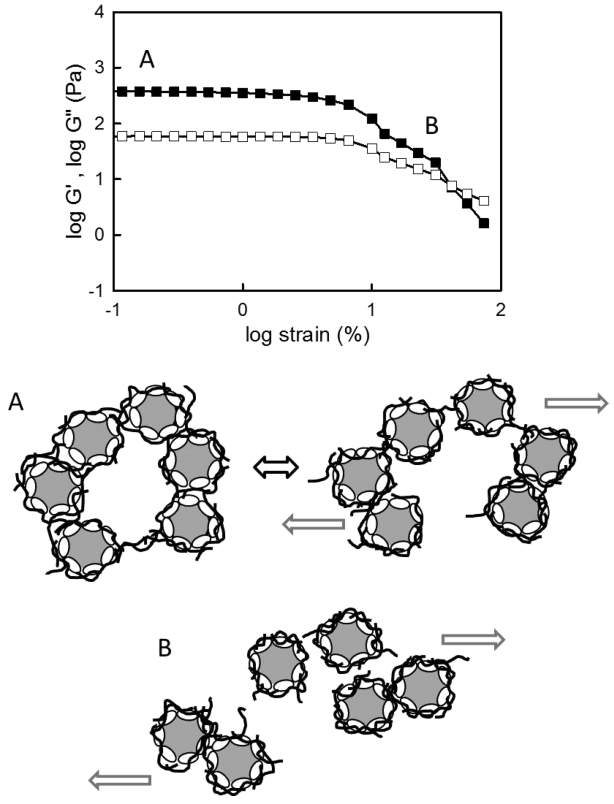

Figure 8. Schematic of a sample WPI–*ι*-CG emulsion gel at the biopolymer ratio of 20:1 and an aqueous/oil ratio of 1:1 in response to strain (G', solid symbols; G'', open symbols) where the gelled network composed of WPI (open ovals) coupled with CG (lines) stabilizing oil droplets (gray circles) is elastic at low strains (A) and its breakdown at higher strains (B). The gray arrows indicate the direction of the applied shear.

opportunity to form bonds, which create junction zones that give the network strength.

In a study by Santipanichwong and Suphantharika, *β*-glucans were found to enhance the stability of egg yolk emulsions.[37] They were speculated to enhance emulsion stability by either increasing the viscosity of the continuous phase or creation of a three-dimensional network of flocculated droplets.[37] Similar to results reported here, frequency sweeps revealed that Santipanichwong and Suphantharika formed weak gels as well and that the addition of *β*-glucans to egg yolk emulsions had improved their creaming stability.[37] Furthermore, an addition of the polysaccharides to the emulsions had increased the storage modulus (i.e., stiffness) of the networks formed.[37] Kontogiorgos and co-workers, who also studied the influence of *β*-glucans on egg yolk stabilized emulsions, reported that the mechanism for stability depended on the molecular weight of the polysaccharide.[44] Polysaccharides containing lower molecular weights tended to form a network in the continuous phase, whereas higher molecular weight polysaccharides increased the viscosity of the continuous phase.[44] Therefore, enhanced emulsion stability in systems containing multiple biopolymers is reliant on the interactions between the biopolymers involved.

It is speculated that as WPI adheres to the water–oil interface, GDL acidification leads to a coacervate formed at the interface whereby the CG on the outside could bind and cause flocculation with other WPI or CG molecules bound to other droplets. This allows for a continuous network of droplets to form, which becomes the primary means for structuring these emulsion networks. Their brittleness may be attributed to the limited surface interactions between flocculated droplets. This reduces the number of available bonds between the biopolymers, which are needed to stabilize the network. A schematic of these emulsion systems is presented in Figure 8, which represents the breakdown of these emulsion networks under strain. It is speculated that any biopolymers not bound to the water–oil interface becomes available for interacting in the continuous phase, which provides additional strength to the network.

In conclusion, WPI–CG mixtures were found to form an electrostatically coupled continuous network during a slow acidification process using GDL. Networks formed with WPI–*ι*-CG complexes were found to be stronger than those with WPI–*κ*-CG. Although a similar mechanism is proposed for network formation, the difference is attributed to the number of sulfated bonds whereby *ι*-CG may form a greater number of bonds compared to *κ*-CG. Frequency sweeps determined that the networks formed were relatively frequency independent and gel-like. An increase in the CG content led to increased stiffness of these gels. Strain sweeps revealed that gels became increasingly elastic with rising CG levels within the mixing ratio. WPI–CG complexes were found to be surface active and capable of forming viscoelastic films at the water–oil interface. Furthermore, as WPI–CG formed electrostatic complexes, they had a profound impact on the interface, which was observed both by interfacial tension and by interfacial rheology. Due to the surface active properties of the WPI–CG complexes, they were used to stabilize the formed emulsions during gelation. Emulsion gels made with WPI–CG complexes were stiffer compared to their biopolymer counterparts but were comparatively much more brittle. The comparative difference in brittleness is attributed to the differences in the structure of these two networks. It is speculated that the large conformational entropy associated with CGs enabled their capacity to form a three-dimensionsal network as a biopolymer solution. Overall, the surface activity of WPI and their coacervation with CGs enabled a WPI-stabilized, flocculated emulsion interlinked by CGs.

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