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Short communication

The effect of pH on gel structures produced using protein–polysaccharide phase separation and network inversion

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ABSTRACT

Forming heat-induced gels through combined effects of micro-phase separation of whey protein isolate (WPI; 5%, w/v, 100 mM NaCl) by pH change (5.5, 6.0, and 6.5), and addition of κ-carrageenan (0–0.3%, w/w), were evaluated. The microstructure of WPI gels was homogeneous at pH 6.0 and 6.5 and micro-phase separated at pH 5.5. Addition of 0.075% κ-carrageenan to WPI solutions caused the microstructure of the gel to switch from homogeneous (pH 6.0 and 6.5) to micro-phase separated; and higher concentrations led to inversion of the continuous network from protein to κ-carrageenan. Protein solutions containing 0.075% (w/w) κ-carrageenan produced gels with increased storage modulus (G') at pH 6.5 and decreased G' at pH 5.5. All gels containing 0.3% (w/w) κ-carrageenan had κ-carrageenan-continuous networks. It was shown that microstructural and rheological changes were different in WPI and κ-carrageenan mixed gels when micro-phase separation was caused by pH rather than ionic strength.

1. Introduction

The ability to design food structures is important to producing healthy foods (Norton & Norton, 2010). One of the most common ways to adjust food structure and produce a wide range of textures is by changing the amount and types of proteins and polysaccharides (van den Berg, Rosenberg, van Boekel, Rosenberg, & van de Velde, 2009). This is because biopolymer mixtures can be formulated to produce single-phase or phase separated conditions (Tolstogouzov & Braudo, 1983).

Solution conditions determine the degree and type of phase separation and rheological behavior of whey proteins–κ-carrageenan mixed gels (de Jong, Klok, & van de Velde, 2009; de Jong & van de Velde, 2007). In our previous investigation, we increased ionic strength to cause protein micro-phase separation at pH 7.0 (Çakır & Foegeding, 2011). Increasing κ-carrageenan concentration in WPI solutions under single-phase conditions caused progressive microstructural transitions from homogeneous to protein continuous to bicontinuous to carrageenan continuous. Alternatively, microstructure moves from particulate to coarse stranded to carrageenan continuous under micro-phase separated conditions (Çakır & Foegeding, 2011). The microstructures were associated with distinctive sensory texture properties (Çakır et al., 2012).

Micro-phase separation of whey proteins is also caused when the pH approaches the isoelectric point (Ako, Nicolai, Durand, & Brotons, 2009). Ould Eleya and Turgeon (2000) investigated rheological properties of heat-set β-lactoglobulin and κ-carrageenan mixed gels between pH 4.0 and 7.0; suggesting similarity between pH 5.0 and 7.0, but a different gel at pH 4.0. Their study was limited to one polymer combination (10% β-lactoglobulin + 1% κ-carrageenan) and did not measure changes in microstructure.

The objective of this study was to determine how pH-induced micro-phase separation of whey proteins alters the effect of κ-carrageenan on gel structure and rheological properties.

2. Materials and methods

2.1. Materials

Whey protein isolate (WPI) Bipro™ (93.37% protein, dry basis, nitrogen × 6.38) was obtained from Davisco Foods International, Inc. (Le Sueur, MN, USA). GENUGEL® κ-carrageenan (CHP-2) was donated by CP Kelco Inc. (Lille Skensved, Denmark). Respective WPI and κ-carrageenan mineral contents (w/w), determined by inductively coupled plasma atomic emission spectroscopy, were (i) 0.08% P, <0.005% K, 0.07% Ca, <0.005% Mg, 1.70% S, and 0.0008% Na (w/w), and (ii) 0.002% P, 16.93% K, 0.06% Ca, 0.12% Mg, 0.0006% S, and 0.50%

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Na. Sodium chloride and HCl were purchased from Sigma–Aldrich (St. Louis, MO, USA). Rhodamine B was obtained from Invitrogen (Eugene, OR, USA).

2.2. Sample preparation

WPI and κ-carrageenan mixed solutions were prepared at three pH levels (5.5, 6.0, or 6.5), a constant protein of 5% (w/v), and varied κ-carrageenan concentrations (0, 0.075, 0.15, and 0.3%, w/w), according to the procedure of Çakır and Foegeding (2011). Stock solutions of WPI and κ-carrageenan contained 100 mM NaCl to allow for maximum final gel rigidity and strength. The stock solutions were equilibrated and mixed at 45 °C to prevent κ-carrageenan gelation. Each treatment was replicated three times.

For microstructure imaging, gels were formed in glass tubes (19 mm diameter × 180 mm long) by heating solutions in a water bath at 90 °C for 30 min and cooling at 22 ± 2 °C for 1 h. Gels were held at 4 °C until analyzed.

2.3. Confocal laser scanning microscopy imaging

Confocal laser scanning microscopy (CLSM) was used to observe microstructure after gelation according to the method of Çakır and Foegeding (2011). The system consisted of an inverted (model Zeiss Axio Observer Z1) microscope (Carl Zeiss LSM 710, Thornwood, NY, USA), 40× objective lens (LD C-Apochromat 40×/1.1 W Korr M27), and a multiline argon laser light source. The excitation was performed at 514 nm and the emission recorded between 531 and 703 nm.

2.4. Small strain rheological properties

Rheological properties were determined using a stress controlled rheometer (StressTech, Rheologica Instruments AB, Lund, Sweden) and serrated couette geometry (CCE25) with cup (27 mm diameter) and bob (25 mm diameter, 45 mm length). Solutions were covered with mineral oil to prevent evaporation. The temperature regime was: heating (45 °C–90 °C at 2 °C min^{-1}), holding (90 °C for 30 min), cooling (to 20 °C at 2 °C min^{-1}), holding (20 °C for 10 min). Measurements were at a frequency of 1 Hz and a stress of 1 Pa during heating/holding and 5 Pa during cooling. These conditions were within the linear viscoelastic region as determined by stress sweeps at 90 °C and 20 °C. Dynamic stress sweep tests were also carried out at 1 Hz with a stress range from 0.5 to 500 Pa. The critical stress value was the point when the complex modulus (G^*) decreased consistently.

3. Results and discussion

3.1. Microstructure

Whey protein gels at pH 6.0 and 6.5 formed homogenous networks (i.e., network strands were not visible at this length scale) (Fig. 1). At pH 5.5, under micro-phase separation (Ako et al., 2009), a particulate network formed. The addition of 0.075% κ-carrageenan to WPI caused a mild degree of phase separation in the gels, resulting in a coarsening of the protein network, with structures similar to a particulate network when ionic strength is used to induce micro-phase separation (Çakır & Foegeding, 2011). Similar

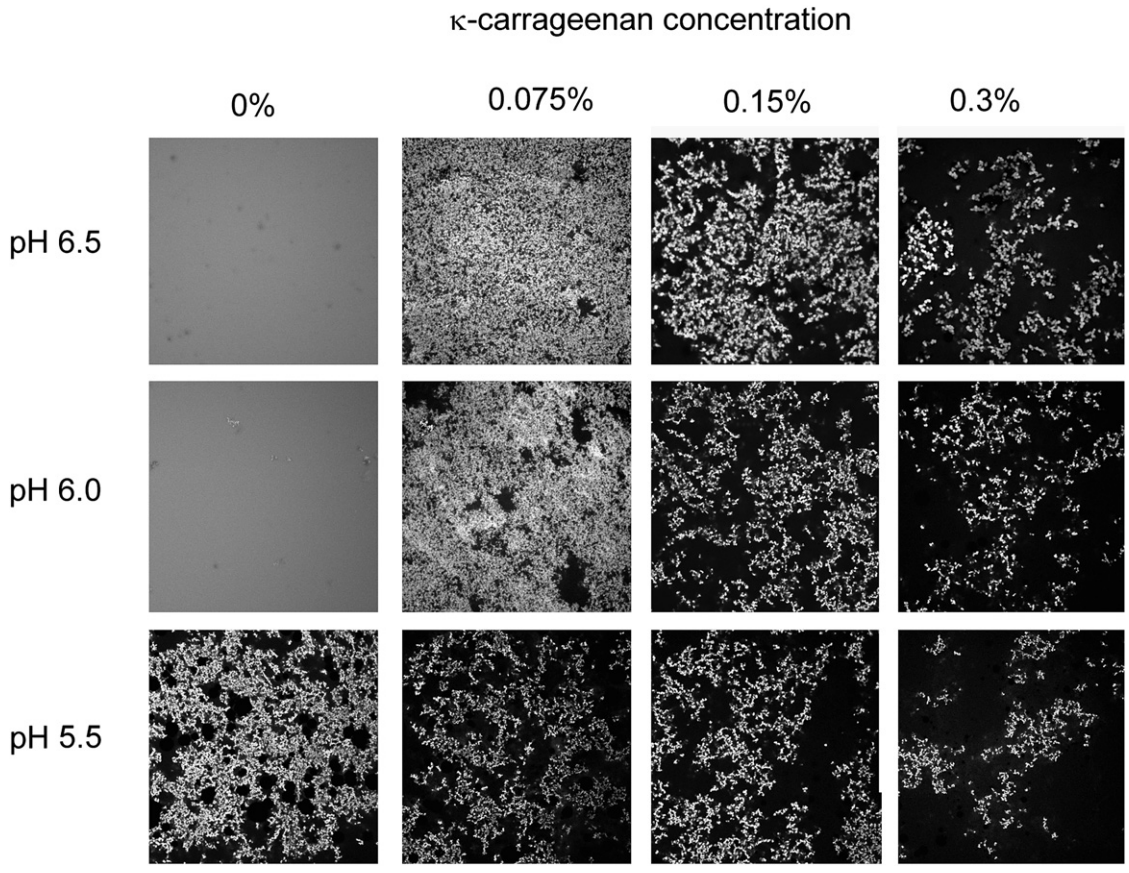

Fig. 1. Microstructure of whey protein isolate and whey protein isolate/κ-carrageenan mixed gels. The images represent a total surface of 212 μm × 212 μm.

changes in bovine serum albumin (BSA) gel networks are observed at pH 6.4 with addition of κ-carrageenan (Neiser, Draget, & Smidsrød, 2000). However, protein continuous and bicontinuous intermediate structures, seen when κ-carrageenan was mixed with WPI at pH 7.0 (Çakır & Foegeding, 2011), were not observed at any pH. At pH 5.5, the structure of the particulate network did not change with different levels of κ-carrageenan, except that the water-carrageenan rich phase increased with increasing κ-carrageenan concentration (Fig. 1).

3.2. Small strain rheological properties of WPI/κ-carrageenan mixed gels

Three phases of G' (storage modulus) transitions were observed in all treatments: 1) heating and holding at 90 °C, 2) cooling to 20 °C, and 3) holding at 20 °C (Fig. 2). For all gels, heating and holding at 90 °C produce a slight increase in G'; followed by a major increase during cooling to 20 °C. The relative magnitude of G' after cooling depended on pH and κ-carrageenan concentration. Gels at pH 6.5 and 6.0, with 0.075% κ-carrageenan, had similar microstructures (Fig. 1) and G' values equal or above the protein network alone (Fig. 2a, b). This was likely caused by κ-carrageenan inducing some degree of segregative phase separation and increasing gel strand density (Gaaloul, Turgeon, & Corredig, 2010). It is also possible that the structure of the gel network was altered. The G' was decreased by adding any level of κ-carrageenan at pH 5.5, or 0.15% κ-carrageenan at pH 6.5 or 6.0 (Fig. 2). This suggests a loss of connectivity among strands or a decreased stiffness of individual strands.

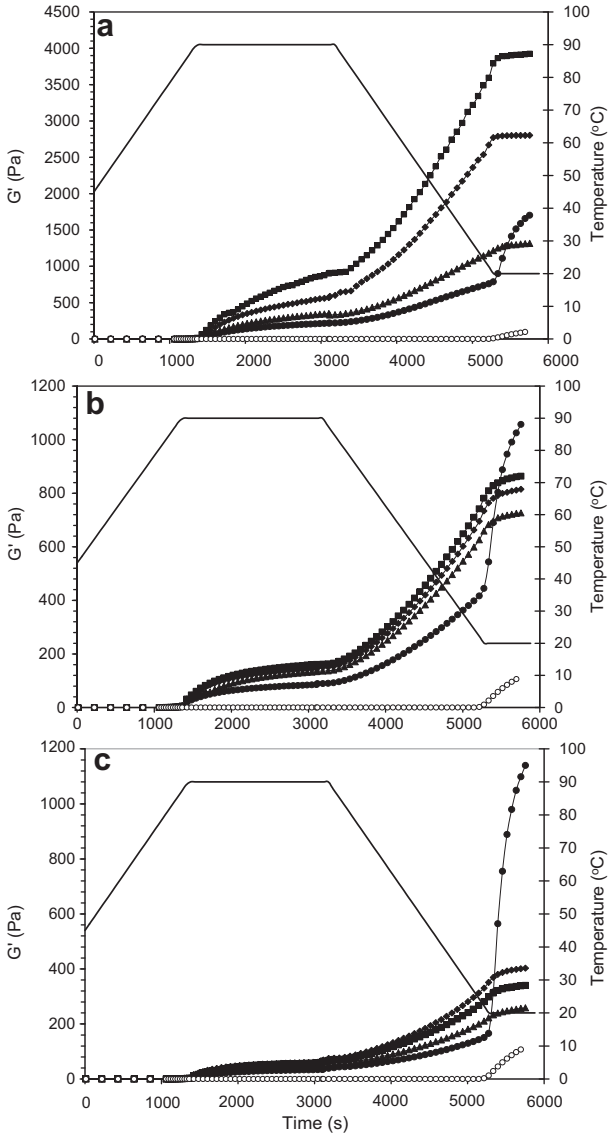

Fig. 2. Change in storage modulus during gel formation of solutions containing whey protein isolate (5%, w/v) at (a) pH 6.5, (b) pH 6.0 and (c) pH 5.5 with 0% (◆), 0.075% (■), 0.15% (▲), and 0.3% (●) κ-carrageenan. The open symbol (○) indicates the κ-carrageenan alone gel at 0.3%. The straight line indicates the temperature profile where samples were heated from 45 °C to 90 °C at 2 °C min^{-1}, held for 30 min, then cooled to 20 °C at the same scan rate and held for 10 min.

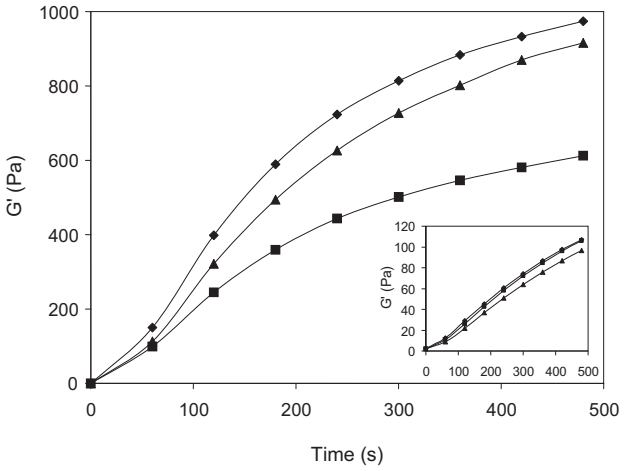

Fig. 3. The normalized development of storage modulus (G') of network inverted mixed gels (5%, w/v, WPI and 0.3%, w/w, κ-carrageenan) at pH 5.5 (◆), 6.0 (■), and 6.5 (▲) during holding at 20 °C. Values were normalized by using the beginning of the holding period at 20 °C as time zero for each pH. The value of G' at time zero for each pH was subtracted from each data point to show the temporal differences. κ-Carrageenan only gels at 0.3% (w/w) are shown in the lower right hand side corner.

During holding at 20 °C, all gels containing 0.3% κ-carrageenan had a dramatic increase in G'; very different than the slight increases seen in other gels. This also corresponded to the increase in G' in κ-carrageenan gels and indicated a κ-carrageenan-continuous network. Increased G' during cooling to 20 °C suggests the coexistence of a WPI network so the precise arrangement is unclear. The development of G' in the κ-carrageenan-continuous networks was normalized for a comparison (Fig. 3). κ-Carrageenan-continuous gels at pH 5.5 displayed the greatest increase in G' at 20 °C. This does not appear to be due to a pH effect on κ-carrageenan network structure, as little to no effect of pH was seen on gel firmness of carrageenan gels (insert in Fig. 3).

The critical stress values further differentiated among gel structures (Table 1). Gels with a WPI continuous network had critical stresses ranging from 46 to 94 Pa; with the exception of pH 6.5 gels that had values >190 Pa. In contrast, gels with κ-carrageenan-continuous networks were weak (7–29 Pa) with

Table 1
Critical stress values[a] (Pa) of single and mixed WPI/κ-carrageenan gels (5%, w/v, WPI and varying concentrations of κ-carrageenan) at pH 5.5, 6.0, and 6.5.

pH	Single gels		Mixed gels		
	WPI only (5%)	κ-Carrageenan only (0.3%)	Carrageenan concentration (%)		
			0.075	0.15	0.3
5.5	59	7	46	46	245
6.0	94	18	94	94	394
6.5	>500	29	>500	193	>500

[a] The values were determined based on the dynamic stress sweep test conducted at 1 Hz from 0.5 to 500 Pa at 20 °C. The critical stress value indicates the point that the complex modulus (G^*) decreased consistently.

κ-carrageenan alone but became stronger (245 to >500 Pa) when whey proteins were present.

4. Conclusions

Micro-phase separation of whey proteins is caused by decreasing electrostatic repulsion among molecules due to solution pH and ionic strength (Ako et al., 2009). This investigation showed that microstructural and rheological properties are different if pH, rather than ionic strength (Çakır & Foegeding, 2011), is used to induce micro-phase separation when combined with effects of κ-carrageenan. Two mechanisms were involved in reinforcement of gel rigidity: a segregative phase separation at high pH and low κ-carrageenan concentration, and a network inversion at low pH and high κ-carrageenan concentration.

Acknowledgments

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