Interactions between whey protein and inulin in a model system

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Abstract Inulin is a commonly used prebiotic ingredients for functional food formulation. The effect of inulin on the gelation properties of whey protein was investigated using whey protein and inulin (WP/inulin) and polymerized whey protein and inulin (PWP/inulin) mixtures at different levels of protein (4–8%, w/v) and inulin (1–5%, w/v). WP/inulin mixture was prepared by heating protein and inulin together while the latter by heating protein alone and then mixed with inulin. Both mixtures were analyzed for turbidity, zeta potential, particle size, and rheological properties. Dispersions became more opaque with increasing protein but there was no significant difference between the two mixtures. A small shift towards larger size and significantly decreased negative zeta potential with increasing inulin addition (1–5%) were observed for both mixtures. WP/PWP and inulin mixtures exhibited a shear thinning behavior. Transition temperature of whey protein increased with inulin addition. WP/PWP and inulin mixtures were induced into cold-set gels by calcium and the gels were analyzed for hardness. Hardness of WP/PWP and inulin gels increased with the increasing inulin. Results indicated that interactions between whey protein and inulin had impact on the gelation properties of whey protein regardless the way inulin added.

Keywords Whey protein · Polymerized whey protein · Inulin · Gelation property

Abbreviations

WP	Whey protein
PWP	Polymerized whey protein
WPI	Whey protein isolates
OD	Optical density
IP	Isoelectric point
TPA	Texture profile analysis
Ca	Calcium
CaCl ₂	Calcium chloride
ANOVA	One-way analysis of variance
LSD	Least squared differences

Introduction

Inulin is usually a mixture of oligomer and polymer chains with terminal β -D fructose or α -D glucose units. The degree of polymerization of these chains oscillates between 2 and 60 units with an average polymerization of approximate 12 (Gonzaleztomás et al. 2008). Inulin has multiple functions such as promoting healthy bacteria growth in the colon (Gibson and Roberfroid 1995), enhancing calcium absorption and immune function (Staffolo et al. 2004), and lowering serum lipids (Davidson et al. 1998). Besides its nutritional benefits, inulin has also been used to improve food texture, such as fat replacer in low- or non-fat food products due to its similar network to fat crystals, sugar replacer due to its lower calories. Inulin was a good texture modifier in dairy products like fermented milk, milk beverage, kefir, ice cream, and cheese (Meyer et al. 2011; Akalin et al. 2013). It has also been widely used in other food products, such as tofu (Tseng and Xiong 2009), oats-

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based symbiotic beverage (Tseng and Xiong 2009), and pet's food (Van 2007).

β -Lactoglobulin is the major whey protein that accounts for half of the total whey protein (Çelebioğlu et al. 2016). Gelation is an important functional property for whey protein. When heated, β -lactoglobulin dimers in the original whey protein dissociated into monomers, and then denatured at temperature above 60 °C. Denatured β -lactoglobulin molecules aggregated into aggregates above a critical concentration. Polymerized whey protein (PWP) is defined as the soluble whey protein aggregates that are formed when heated at a certain temperature and protein concentration in absence of salt (Vardhanabhuti et al. 2001). The larger aggregates can be induced into gel above a critical concentration under specific conditions. Whey protein gels can be divided into heat-induced gel, cold-set gel (salt-induced gel and acid-induced gel) and pressure-induced gel depending on different conditions. Factors affecting the gelation of whey protein include composition and concentration of the protein, heating conditions, pH, types and concentration of salt, and reducing agents (Hongprasabhas and Barbut 1997a).

Proteins and polysaccharides found in food products, both contribute to food stability, texture, and shelf life (Herceg et al. 2007) and have effect on the gelling property of each other. Substituting lactose with inulin increased the denaturation degree of whey protein (Tobin et al. 2010). Glibowski (2009) studied rheological properties, structure and synergistic interactions of whey proteins and inulin, and found that whey protein above 7% significantly increased storage modulus and loss modulus values of the inulin gels, and a firmer network was formed in presence of whey protein. The effect of α -lactalbumin (1.4%) and β -lactoglobulin (4.2%) on rheological and structural properties of inulin (20%) gels was investigated by Glibowski (2010). α -Lactalbumin did not have significant impact on the properties of gel while β -lactoglobulin significantly increased the storage modulus value of the gel (Glibowski 2010).

Gelation properties of whey protein are ultimately determined by their molecular structure and interactions, as well as by changes in these characteristics caused by environmental conditions (Bryant and McClements 1999). Since whey protein and inulin are both often used in food formulation, it is significant to investigate how inulin (less than 7%) affect aggregation and gelation properties of whey protein in aqueous medium. Currently, no information is available about the possible modifying effect of this non-ionizable polar carbohydrate on whey protein. Therefore, the objective of this study was to investigate effects of inulin on gelation properties of whey protein using whey protein and inulin (WP/inulin) and polymerized whey protein and inulin (PWP/inulin) mixtures. WP/inulin

mixture was prepared by heating protein and inulin together while the latter by heating protein alone and then mixed with inulin. Both mixtures were also induced into cold-set gel by calcium and the gels were analyzed for the hardness.

Materials and methods

Materials

Whey protein isolates (WPI 92.3%) was obtained from Hilmar (California, USA). Native inulin (93%) was purchased from Zhangye Biological Technology Co., Ltd. (Gansu, China). Calcium chloride and all other reagents used were of analytical grade and supplied by Beijing Chemical Works (Beijing, China). Deionized water (18.2 M Ω) was obtained using a Milli-Q deionization reversed osmosis system (Millipore Corp., Bedford, MA, USA).

Sample preparation

Whey protein stock solutions

Whey protein stock solution (20%, w/v) was prepared by dissolving WPI powder slowly into deionized water at room temperature with the help of magnetic stirring for 2 h. When calculating the amount of WPI powder to be added, a correction for the protein content of the powder was taken into account. The stock solution was stored at 4 °C overnight to ensure complete dissolution.

Preparation of WP/inulin mixture

WPI solutions (4, 6, 8%, w/v) with different addition of inulin ranging from 1 to 5% were stirred for at least 2 h. The pH of dispersions was adjusted from the initial value of 6.8–7 with 1 M NaOH. All the alkali-denatured solutions were batch-heated in a water bath at 85 °C for 30 min without stirring. After heat treatment, all dispersions were rapidly cooled down to room temperature in ice water for further analysis. Mixtures where whey protein and inulin were mixed first and then heated were abbreviated as WP/inulin.

Preparation of PWP/inulin mixture

WPI solutions (4, 6, 8%, w/v) at pH 7 were batch-heated in a water bath at 85 °C for 30 min without stirring, and then cooled down to room temperature with ice water. Inulin powder ranging from 1 to 5% was added to polymerized whey protein solution with different concentration while

the dispersion was hot. The mixture was stirred for at least 2 h for the complete interaction between whey protein and inulin. Mixtures where whey protein was heated and then mixed with inulin were abbreviated as PWP/inulin.

Turbidity

The turbidity of all samples was measured using a UV-visible Spectrophotometer (UV2550, Shimadzu, Tokyo, Japan) at a wavelength of 400 nm in a quartz sample cell. Each measurement was replicated three times and each reported value was the average of three consecutive readings. All the measurements were conducted in triplicates for three trials.

Zeta potential and particle size

Freshly prepared polymer dispersions of mixtures of WP/PWP and inulin were analyzed for zeta potential and particle size. All the solutions were determined after agitation for 4 h at room temperature. Solutions used for the particle size determination were diluted with deionized water by tenfolds and determined with scattering angle of 173° . Solution was filtered through a $0.45\ \mu\text{m}$ membrane prior to particle analysis. All the zeta potential and mean size determinations were performed using a Nano-Z model Zetasizer (Malvern Instruments, Malvern, Worcestershire, WR, UK) at 25°C . All the measurements were conducted in triplicates for three trials. Data analysis for particle size were performed according to the Stokes–Einstein equation:

$$d(H) = \frac{KT}{3\eta\pi D} \quad (1)$$

where $d(H)$ diffusion coefficient, K Boltzmann's constant, T absolute temperature, η viscosity, and D hydrodynamic diameter.

The zeta potential was calculated from the measurement of the electrophoretic mobility of particles in an applied oscillating electric field using laser Doppler velocimetry and phase analysis light scattering. Zeta potential was calculated based on the Henry equation:

$$U_E = \frac{2ezf(ka)}{3h} \quad (2)$$

where U_E is the electrophoretic velocity; z is the zeta-potential; e is the permittivity of the biopolymer mixture; h is the dispersion viscosity; $f(ka)$ Henry's function. A value of 1.0 was used as the zeta potential was measured in water solvent.

Rheological properties

Flow behavior and viscoelastic behavior were performed using a rheometer (DHR, TA, USA) equipped with 40 mm diameter parallel-plate. Flow curves were taken on 8% WP/PWP and inulin mixtures at different inulin level (1–5%) at 25°C with shear rate in the range of $50\text{--}1000\ \text{s}^{-1}$. Function of temperature was also conducted on the mixture from 30 to 90°C with the rising rate of $5^\circ\text{C}/\text{min}$ at shear rate of $200\ \text{s}^{-1}$. Sampling mode was 10.0 s/pt and the time was 60 s. Dynamic oscillatory measurements were carried out by heating 15% whey protein solution with different inulin level (1–5%) from 25 to 90°C at a rising rate of $3^\circ\text{C}/\text{min}$, holding at 85°C for 30 min. To ensure the determination was in the linear viscoelastic region, strain was chosen to be 1.0%.

Gel preparation from WP/PWP and inulin mixtures

A CaCl_2 solution at appropriate concentration was prepared and then added very slowly, drop by drop into WP/PWP inulin mixtures at room temperature with stirring. The final concentration of CaCl_2 in the mixture was 10, 30, and 50 mM. Agitation period was deemed to be necessary for uniform distribution of Ca^{2+} at the molecular level. In case of gel setting during CaCl_2 solution addition, the addition was immediately stopped and the sample was rejected. Gel samples induced by CaCl_2 addition were held overnight at 4°C and then analyzed for the large-deformation gel texture. All the gels were prepared in triplicates for three trials.

Texture profile analysis (TPA)

The texture profile of the gel was measured using a CT-3 Texture Analyzer (Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA). Texture profile analysis (TPA) was run with a penetration distance of 30 mm using a cylindrical probe with a diameter of 38.10 and 20 mm in height. The speed of the probe was fixed at $1\ \text{mm s}^{-1}$ during the pre-test, compression and the relaxation of the samples, and the trigger was 4.5 g. All the gels were determined in triplicates for three trials and recorded parameter was hardness.

Statistical analysis

All data obtained from analysis were expressed as mean $\pm$ standard deviation (SD). The significant differences of data between samples and control were calculated using Version SPSS 20 (SPSS Inc. Chicago, IL). The significance level was set at $p < 0.05$ and $p < 0.01$. One-way analysis of variance (ANOVA) and then a least squared

differences (LSD) model was used. All the figures were drawn by origin 8.0 (Origin Lab Corporation, Northampton, USA).

Results and discussion

Effects of inulin on turbidity of whey protein solution

Heat treatment of whey protein solutions above 70 °C under specific conditions resulted in the soluble aggregates suspension. Turbidity reflects the aggregation state in the mixture that remained suspended in the liquid. Optical density (OD) was measured at 400 nm for the reason that OD 400 nm is more sensitive to turbidity differences at lower turbidities and smaller particle size (Ryan et al. 2012). OD values of all samples are shown in Fig. 1. Solutions turn more turbid with increasing protein content, indicating more extensive protein aggregation in the suspension. Heat treatment of whey protein solutions leads to unfolding of proteins and exposure of hydrophobic residues and free sulfhydryl groups to aqueous environment. High concentration of whey protein increased possibility of hydrophobic attractions and formation of disulfide bonds between whey proteins (Ha et al. 2016). Also, there may be electrostatic attraction between the negative groups on one protein and the positive groups on another (Bryant and McClements 1999). Inulin had no obvious effect on the magnitude of OD for both WP and PWP systems. It seemed that the whey protein aggregation (OD 400) was not significantly affected in the presence of inulin. In contrast, Tseng et al. (2009) reported that inulin delayed the soy protein isolate aggregation. No significant difference

between WP/inulin and PWP/inulin at the same protein level was observed ($p > 0.05$). Mixing whey protein with inulin pre- or post-heating had little effects on the aggregation process. Research indicated that whey proteins, mainly β -lactoglobulin, can interact with inulin via hydrogen bonding and hydrophobic interactions (Ha et al. 2016). Compared with strong and permanent chemical bonds, these physical interactions are more transient and weaker (Eissa and Khan 2006) and thus did not influence the properties of polymerized whey protein. Furthermore, it was well accepted that whey protein aggregate was formed mainly via formation of disulfide bond.

Mixtures of protein and neutral polysaccharides could cause phase separation through thermodynamic incompatibility, which arises mainly from the low entropy of mixing and segregate phenomena (Tavares et al. 2005). In this study, all experimental samples remain solution regardless of the type of the mixture. However, white sediment occurs at the bottom of the tube after centrifugation at 15,000 rpm for 20 min at 4 °C. It was difficult to quantify the sediment since it was hard to separate the liquid and the white sediment. Villegas and Costell (2007) found that milk beverages with 5% long chain inulin showed slight sedimentation after storage may due to its recrystallisation. The authors noticed the presence of β -lactoglobulin both in supernatants, where inulin was absent, and in the precipitates, where inulin was present.

Effects of inulin on zeta potential and particle size of whey protein

Zeta potential exists between the particle surface and the dispersing liquid at the slipping plane. The zeta potential indicates whether the particles tend to flocculate. To investigate the possible interaction between whey protein and inulin, zeta potential was determined (Table 1). Around the isoelectric point (IP) of whey protein (~ 5.3), the net charge was around zero (Chen and Subirade 2005). At pH 7, whey protein solution displayed negative charge. Zeta potential values for all experimental samples ranged from -10 to -30 mV, independent of concentration, indicating unstable system (Bourbon et al. 2016). There was no significant difference between samples at different whey protein concentration ($p > 0.05$). Different mixture type did not affect the zeta potential of system. Similar results were reported by Sağlam et al. (2013).

Zeta potential of a system may be affected by pH, electric conductivity and addition of polymer. Addition of inulin (-13.40 ± 1.77 mV for inulin solution) decreased the stability of soluble WP/PWP inulin mixture with the increasing inulin level, indicated by the gradually decreased data. Inulin addition resulted in less negative zeta potential and smaller repulsive force among particles.

Fig. 1 Turbidity of WP/PWP and inulin mixtures measured at 400 nm

Table 1 Zeta potential of WP/PWP and inulin mixtures (mV)

Inulin (%)	4% Protein		6% Protein		8% Protein	
	WP	PWP	WP	PWP	WP	PWP
0	− 23.00 ± 1.42	− 22.1 ± 0.95	− 21.40 ± 1.53	− 22.20 ± 0.52	− 21.70 ± 0.94	− 21.30 ± 1.12
1	− 19.30 ± 0.35*	− 20.8 ± 0.50*	− 19.60 ± 1.15*	− 18.10 ± 1.33*	− 18.80 ± 0.72*	− 19.80 ± 0.60*
2	− 17.60 ± 0.98*	− 18.7 ± 0.88*	− 17.20 ± 0.86*	− 16.10 ± 0.68*	− 15.80 ± 1.62*	− 16.30 ± 1.00*
3	− 16.00 ± 1.04*	− 17.2 ± 0.25*	− 14.20 ± 0.40*	− 16.10 ± 0.65*	− 16.50 ± 1.00*	− 16.70 ± 0.36*
4	− 15.20 ± 1.27*	− 16.2 ± 0.70*	− 13.20 ± 1.28*	− 15.30 ± 0.65*	− 13.40 ± 0.90*	− 14.30 ± 1.11*
5	− 13.70 ± 0.81**	− 13.4 ± 0.75**	− 12.10 ± 0.45**	− 13.40 ± 0.49**	− 12.50 ± 0.15**	− 12.00 ± 0.58**

Compared with control at significant level * $p < 0.05$; ** $p < 0.01$

Aspartic acid and glutamic acid, the main amino acid with negative charge in β -lactoglobulin may interact with inulin. Although interactions between inulin and whey proteins may be concluded from hydrophobicity measurements, the use of an electrophoretic technique did not show any inulin–whey protein complexes (Glibowski 2009). However, interaction of whey protein and inulin might cause coating of inulin on the surface of whey protein, which shielded the surface charge.

Particle size is the diameter of the sphere that diffuses at the same speed as the particle being measured. The tendency to aggregate of the particles is also influenced by particle size. Mean diameter of native WPI was about 5 nm (Zhang et al. 2014) and the denatured β -lactoglobulin was 10–100 nm (del Mar Contreras et al. 2011) with one peak. In our study, three peaks at ~ 40 , ~ 900 , and ~ 5000 nm were observed may due to different whey proteins. Average diameter of PWP ranging from 4 to 8% were 32.45 ± 1.20 , 36.94 ± 3.87 , and 82.56 ± 10.35 (Table 2), respectively. Average size of particles in 8% solution were almost twofold of that of 4–6% ($p < 0.01$). Generally, average diameter increased with the increasing inulin level, however, there was no significant difference between addition levels and the way inulin added ($p > 0.05$).

Effects of inulin on flow behavior and gelation curve of whey protein solution

Shear viscosity/shear stress of WP/PWP and inulin mixtures as a function of shear rate is shown in Fig. 2a, b. The two mixtures exhibited similar flow curves with the shear viscosity in the range of 0–50 mPa s. All the samples belonged to shear-thinning fluid and there was no significant difference in viscosity between inulin levels ($p > 0.05$). All the data were analyzed by software of the equipment and Sisko model was fitted ($R^2 > 0.99$). Figure 2c, d showed the flow behavior of WP/PWP and inulin mixtures as a function of temperature. There was a decrease in viscosity in the temperature range of 30–90 °C. With temperature increased, molecular thermal motion increased and resulted in larger molecular spacing, and then more molecular force hole, resulted in lower molecular inter-atomic forces and lower viscosity.

Effect of inulin on the storage and loss moduli of whey protein is shown in Fig. 3. Samples with inulin showed similar curves with that of single whey protein indicating that whey protein may be the main gelling agent in the system. The gelation temperature can be determined by three methods: (1) cross point of the storage modulus and loss modulus; (2) the onset point where storage modulus

Table 2 Average diameter of dispersions of WP/PWP and inulin mixtures (nm)

Inulin (%)	4% Protein		6% Protein		8% Protein	
	WP _(C1)	PWP _(C2)	WP _(C3)	PWP _(C4)	WP _(C5)	PWP _(C6)
0	32.45 ± 1.20	32.45 ± 1.20	36.94 ± 3.87	36.94 ± 3.87	82.56 ± 10.35	82.56 ± 10.35
1	36.55 ± 2.09	35.89 ± 1.96	37.97 ± 1.81	42.18 ± 2.16	85.26 ± 5.14	85.57 ± 5.29
2	33.44 ± 0.65	38.49 ± 3.17	37.09 ± 0.57	37.45 ± 4.43	88.06 ± 2.10	81.52 ± 7.54
3	33.68 ± 1.43	41.93 ± 3.62	35.63 ± 1.36	40.30 ± 6.91	86.57 ± 11.86	88.92 ± 6.29
4	34.61 ± 1.16	32.96 ± 0.84	34.13 ± 8.16	36.46 ± 2.66	89.64 ± 3.67	89.46 ± 4.97
5	32.3 ± 0.55	36.14 ± 4.52	34.65 ± 2.69	36.96 ± 6.53	86.82 ± 3.56	82.72 ± 3.65

* $p < 0.05$; ** means significant difference between samples at level of $p < 0.01$, C1, C3–C5**, C2, C4–C6**

Fig. 2 Flow behavior of whey protein with different inulin as a function of shear rate and temperature

Fig. 3 Effect of inulin addition on the G' and G'' of whey protein

started to increase sharply; (3) onset point where increasing rate of storage modulus was above $0.5 \text{ Pa}/^\circ\text{C}$ (Van Camp et al. 1997). During $25\text{--}80^\circ\text{C}$, both moduli declined slightly and then increased sharply at around 80°C which can be considered as the transition temperature. Transition temperature of whey protein solution (15%, w/v) was 77.3°C , and those of WP and inulin (1–5%, w/v) systems

were 81.3 , 80.8 , 83.8 , 78.8 , and 84.5°C , respectively. Addition of inulin decreased the storage modulus, suggesting that inulin could lower the springiness. Addition of inulin also decreased the loss modulus, indicating that inulin also decreased the viscosity of whey protein solution. Similar results were reported by others that addition of neutral polysaccharides dextran and galactomannan

reduced modulus of whey proteins (Sun et al. 2012; Gonzalez-toms et al. 2008). However, Tseng et al. (2009) reported that the addition of 5% (w/v) inulin enhanced the acid-induced gel of soy protein isolate (10%, w/v).

Effects of inulin on hardness of whey protein gels induced by calcium

Whey proteins are negatively charged at pH 7 and hence there can be electrostatic repulsions between whey protein molecules. Positive ions, such as calcium, can shield the negative charges on the proteins. The cold-set gels can be induced by adding Ca^{2+} to preheated dispersion. The Ca^{2+} -induced gel of whey protein alone was extensively studied by scholars (Hongprabhas and Barbut 1997b; Barbut 1995; Line et al. 2005). Ca^{2+} concentration was the key factor affecting the texture of formed gel. Ca^{2+} at 10 mM was essential to induce 10% (w/v) preheated whey protein solution (80 °C for 30 min) to form a clear gel (Barbut 1995). However, in this study, it was not sufficient to induce gel for both WP/PWP and inulin mixtures with 10 mM Ca^{2+} while 30 or 50 mM of Ca^{2+} levels can induce all samples into gels.

It is of significant interest to have a comprehensive understanding of protein–polysaccharide mixed gels fabricated under different gelation conditions. Texture of WP/PWP and inulin gels induced by 30 and 50 mM Ca^{2+} were analyzed and the results were shown in Fig. 4. As expected, for both WP/PWP and inulin mixtures, hardness increased significantly with increasing protein concentration ($p < 0.01$). For example, for WP and inulin gel induced by 30 mM Ca^{2+} , hardness was 122.00 ± 15.60 , 1073.60 ± 55.57 , and 2063.00 ± 47.54 g, respectively. Whey protein concentration can affect the degree of denaturation and the size of aggregates and the subsequent gel strength. Generally, hardness of both WP/PWP and inulin mixtures increased with the increasing inulin. Inulin gels (1–10 μm) can be formed by shearing or heating–cooling (Glibowski 2009). The concentration must be above 35% and crystal seeds were necessary for the inulin gels to propagation (Bot et al. 2004). However, the inulin levels in this study are too low to form gel. Therefore, inulin may embed in the whey protein network, enhancing the gel strength. Hardness of gels induced by 30 mM Ca^{2+} was higher than that of gels induced by 50 mM Ca^{2+} . This may be due to the microstructural transition of whey protein gels (Turgeon and Beaulieu 2001). Gels of 4% WP and inulin mixture showed higher hardness values than those of PWP/inulin mixtures, however, the trend reversed when the protein concentration reached 6 and 8%. It can be considered that inulin can embed into the protein network better when co-heated with whey protein at the lower

Fig. 4 Hardness of WP/PWP and inulin gels induced by Ca^{2+}

protein level. However, when the protein reached higher level, this effect was lower.

Conclusion

Interactions between whey protein and inulin had impact on gelation properties of whey protein. Inulin decreased the zeta potential of whey protein solution regardless the way inulin added may due to attachment of inulin on the surface of whey protein aggregates. Transition temperature of whey protein increased while storage and loss moduli decreased by adding inulin. Addition of inulin increased hardness of calcium induced whey protein gels. The results of this study indicated that interactions between inulin and whey protein occurred in the model system.

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