Formation of heated whey protein isolate-pectin complexes at pH greater than the isoelectric point with improved emulsification properties

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ABSTRACT

In this study, heated whey protein isolate and pectin complexes (HCPX) formed at pH > isoelectric point (pI) were used to stabilize oil-in-water emulsions containing 5% oil and 1.5% (wt%) protein at pH 5.5. The effects of pectin concentration and heating temperature on emulsification and emulsion stabilization properties were determined. The HCPX were produced by heating mixed 3% (wt) whey protein isolate and pectin (0.1 or 0.3 wt%) at pH 6.2 and 75 or 85°C for 15 min. Aggregate sizes significantly increased with increasing heating temperature but decreased with the addition of pectin. The HCPX became more negatively charged with increasing pectin concentration; however, the effect of heating temperature was significant only at 0.1% pectin. Unheated complexes and HCPX successfully adsorbed at the oil-in-water interface and improved the emulsification properties as shown by higher negative charge and smaller droplet sizes. Despite the presence of pectin, rheological properties of the emulsions were not significantly different. All complexes showed increased emulsion stability; however, HCPX made at 85°C formed emulsions that were the most stable against creaming and heating.

Key words: emulsification properties, whey protein isolate, pectin, protein polysaccharide interaction

INTRODUCTION

With increasing consumer demand for clean label products, there is a growing need for “clean” ingredients including emulsifiers and stabilizers (Surh et al., 2006; McClements and Gumus, 2016; Ozturk and McClements, 2016). Protein is a natural emulsifier with excellent emulsifying properties at pH away from the isoelectric point (pI; Dickinson, 2008). However, using protein alone may not sufficiently prolong emul-

sion stability during environmental stress such as pH, temperature, ionic strength, and storage (McClements, 2004; Surh et al., 2006). Among different approaches that have been developed, the use of polysaccharides to improve the emulsification and emulsion stabilization properties of protein via electrostatic interactions has been extensively studied (Dickinson and Pawlowsky, 1997; Gu et al., 2004; Guzey and McClements, 2007; Jones et al., 2010b; Xu et al., 2012; Salminen and Weiss, 2014b; Huan et al., 2016). Protein and polysaccharide complexes can stabilize emulsion droplets via 2 methods. In a bilayer emulsion, a primary emulsion is prepared with the protein as an emulsifier, and the charged polysaccharide is added to produce a secondary emulsion with droplets having a protein-polysaccharide layer-by-layer surface coating (Raei et al., 2017). Alternatively, a mixed emulsion is made by first preparing a solution of protein-polysaccharide complex at pH > pI before homogenizing with oil and acidifying to desirable pH (Jourdain et al., 2008; Huan et al., 2016). At pH > pI, both biopolymers carry a net negative charge that could typically lead to segregative phase separation (Tolstoguzov, 1986; Dickinson, 1998; Turgeon et al., 2003); however, interactions still exist between the positively charged patches on the protein and negatively charged polysaccharides, resulting in the formation of soluble complex (Galazka et al., 1999; Vardhanabhuti et al., 2009; Zhang et al., 2012). In mixed emulsion, the adsorption of protein-polysaccharide complex as well as additional interactions between the biopolymers at the interface could alter interfacial properties, leading to improved emulsification properties (Jourdain et al., 2008, 2009; Huan et al., 2016). In addition, protein and polysaccharide electrostatic complexes used to produce emulsion or double emulsions have exhibited improved efficiency in encapsulation and delivery of bioactive compounds (Mohammadi et al., 2016; Faridi Esfanzani et al., 2017; Ghasemi et al., 2017; Raei et al., 2017).

Heating solutions of biopolymer complexes above the denaturation temperature of the protein creates heated protein and polysaccharide complexes or particles (Gentès et al., 2010; Jones and McClements, 2011; Zhang et al., 2012). During heating, protein will

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unfold and expose some charged groups, which are available for further reactions. Physical and functional properties as well as stability of these complexes depend on the biopolymer ratio, polysaccharide type, pH, ionic strength, and heating conditions (Jones et al., 2009; Gentès et al., 2010; Jones et al., 2010c; Jones and McClements, 2010; Zhang et al., 2012). Jones and McClements (2008) reported that heating β -LG and beet pectin complexes at pH 5 at 83°C for 15 min could stabilize these complexes over pH 3 to 7. Gentès et al. (2010) also demonstrated that severe heating conditions (85 and 90°C) allowed the stabilization of WPI and low methoxyl pectin complexes against pH change (Gentès et al., 2010). The majority of research in this field has focused on complexation conditions at pH < pI where strong electrostatic attractions are present between the biopolymers. Heated whey protein isolates (WPI) and polysaccharides at pH > pI were previously studied by our group. The potential use of these heated complexes is justified because they show improved functional properties as compared with the individual macromolecules. Potential applications include foams, acid-induced gels, and aerated gels (Zhang et al., 2014; Zhang and Vardhanabhuti, 2014; Wang et al., 2015; O'Chiu and Vardhanabhuti, 2017). An additional advantage of forming heated protein and polysaccharide complexes in this pH region is that they can be manufactured at higher protein concentration (Zhang et al., 2014), allowing the technology to be applicable in the industry.

To the best of our knowledge, no reports have fully investigated the properties of heated protein and polysaccharide complexes in mixed emulsion. In this study, heated complexes were formed by heating WPI with low methoxyl pectin at pH > pI and their emulsification properties were investigated. The effects of pectin concentration and heating temperature were studied. Whey protein can undergo molecular changes above a temperature of 70°C (Millqvist-Fureby et al., 2001; Dybowska, 2011); thus, heating temperatures at 75 and 85°C were selected and compared with unheated complexes. Finally, since most food emulsions become destabilized during thermal heating due to kinetic instability (Gu et al., 2005; Raikos, 2010), the heat stability of emulsions was also determined.

MATERIALS AND METHODS

Materials

Whey protein isolate was provided by Grande Custom Ingredients Group (Lomira, WI). The WPI contained 92.8% protein, 0.19% fat, and 2.6% ash on a dry basis.

Low methoxyl pectin (LM-12 CG) was provided by CP Kelco Inc. (Atlanta, GA). Phosphate buffer (5 mM, pH 5.5 and 6.2) and all solutions were made with Milli-Q water (>18.2 M Ω /cm; Millipore, Billerica, MA). Commercial vegetable oil was purchased from a local supermarket and all other reagents were of analytical grade.

Preparation of Stock Solutions

The WPI stock solution (10% wt/wt) was prepared by slowly dissolving protein powder into water and stirred at room temperature for at least 2 h. Pectin stock solution (1% wt/wt) was prepared by slowly dissolving pectin powder into water at 60°C for 1 h under continuous stirring. Both stock solutions were stored at 4°C overnight for complete hydration.

Preparation of Heated WPI-Pectin Complexes

The WPI and pectin stock solutions were warmed up to room temperature ($25 \pm 1^\circ\text{C}$) for at least 1 h. The WPI stock solution (9 mL), pectin stock solution (0, 3, or 9 mL), and water (18, 15, or 9 mL) were mixed, and the pH was adjusted to 6.2 with 0.1 M NaOH (50 μL at a time). Water was added such that the final solutions (30 mL) contained 3% protein and 0, 0.1, or 0.3% pectin. Samples were stirred for at least 2 h at room temperature before being heated at 75 or 85°C for 15 min in a temperature-controlled water bath. For unheated WPI or unheated WPI-pectin complexes, solutions were prepared in a similar manner without heating.

Preparation of Emulsions

All oil-in-water emulsions containing 5% (wt/wt) oil, 1.5% (wt/wt) protein, and 0 to 0.15% (wt/wt) pectin were obtained by emulsification of oil with an aqueous solution [e.g., WPI, heated WPI (HWPI), unheated WPI-pectin complex (CPX), or heated WPI-pectin complex (HCPX)], through a 2-stage process involving coarse emulsification followed by ultrasonic processing. Aqueous solution and water were mixed, and the pH of the mixture was adjusted to 7.0 (Guzey et al., 2004). After stirring for 1 h, oil was added. Coarse emulsion was prepared by blending the mixture at 12,000 rpm for 15 s using a laboratory homogenizer, Ultra Turrax T-25 (IKA Instruments, Staufen, Germany). Final emulsion was obtained with an ultrasonic processor (Sonics VC 505, power 500 W, frequency 24 kHz; Sonics, Newtown, CT) with a sonotrode (3 mm, approximate length = 100 mm, titanium) at 40% amplitude for 3 min. Sodium azide (0.02%) was added as an antimicrobial agent.

After emulsification, the emulsion was slowly acidified to pH 5.5 by adding 0.1 M HCl (50 μ L at a time). The acidified emulsions were stirred for at least 1 h before analysis.

Particle Size, Droplet Size, and Zeta-Potential Measurement

Particle size and droplet size distributions as well as zeta-potential were determined using the Zetasizer Nano ZS (Malvern Instruments Ltd., Malvern, UK) equipped with a 633-nm laser and 173° detection optics at 25°C. Aqueous solution was diluted to 0.3% (wt/wt) protein using 5 mM phosphate buffer at pH 6.2. The emulsion sample was diluted at a ratio of 1:1,000 for size and 1:100 for zeta-potential using 5 mM phosphate buffer at pH 5.5 to prevent multiple scattering effects. A refractive index of 1.46 was used for both protein and oil droplets. An individual measurement was determined from the average of 5 readings taken on the same sample. All treatments were carried out in triplicate.

Rheological Properties Measurement

Rheological properties of fresh emulsions were measured using a Kinexus Pro Rheometer (Malvern Instruments Ltd.) equipped with a cone (40-mm diameter, 4° angle) and plate geometry. Emulsion sample was loaded on a lower plate and the upper cone geometry was gently lowered to a gap of 0.05 mm. Flow behavior of the sample was conducted under a shear rate ramp from 0.1 s⁻¹ to 200 s⁻¹ at 25°C and under a solvent trap setting to prevent evaporation. The flow behavior index and consistency coefficient were calculated using the power law model. Each treatment was measured in triplicate.

Creaming Stability of the Emulsion

The fresh emulsion sample (10 mL) was pipetted into a cylindrical glass tube (internal diameter = 16 mm, height = 100 mm). Subsequently, the tubes were sealed with Parafilm M film (Pechiney Plastic Packaging Company, Chicago, IL) to prevent evaporation. Emulsion samples were stored quiescently at ambient temperature (~25°C) for 40 d. Emulsion stability evolution was determined by measurement of height (millimeter units) of a distinctive clear or semitransparent bottom serum phase layer on d 7, 14, 21, and 30 after emulsion preparation. The extent of creaming was characterized by creaming index (%) = (HS/HT) $\times$ 100%, where HS is the height of the serum layer and HT is the initial height of the emulsion. Each creaming index of an emulsion sample was recorded in triplicate.

Heat Stability of Emulsions

Freshly made emulsions were heated at 85°C for 15 min. Heated emulsions were cooled to room temperature for 2 h before analysis.

Statistical Analysis

Significance differences between treatments ($P < 0.05$) were determined by ANOVA using Minitab (version 17.1.0, State College, PA). Differences between means were determined using Tukey's honestly significant difference test.

RESULTS AND DISCUSSION

Physical Characteristics of HCPX

Appearance. We examined the effect of heating temperature (e.g., no heating, 75 and 85°C) and pectin concentration (e.g., 0, 0.15, and 0.3%) during HCPX formation while protein concentration (3%) and pH (6.2) were kept constant. The WPI and CPX appeared transparent without any precipitation (Figure 1a). Presumably, this was due to the electrostatic repulsion among negatively charged protein molecules, the formation of soluble complexes between protein and pectin, or both (Jones et al., 2009). Though both WPI and pectin carry a net negative charge (pH > pI), the attraction between localized positively charged patches on the protein can interact with anionic pectin (Dickinson, 1998; Vardhanabhuti et al., 2009). The WPI solutions turned translucent and opaque after heating at 75 and 85°C, respectively, due to denaturation and aggregation of WPI at >70°C (Jones et al., 2009). In the presence of pectin, HCPX formed at 75°C showed decreasing turbidity with increasing pectin concentration, whereas all heated samples at 85°C appeared opaque without apparent difference.

Zeta-potential. Zeta-potential results showed that, at pH 6.2, WPI and HWPI at 75 and 85°C had particle net charges of -19 ± 2.7 , -21 ± 1.2 , and -23 ± 1.9 mV, respectively (Figure 1b). A slight increase in negative charge potential of WPI after heating has been reported (Ryan et al., 2012; Salminen and Weiss, 2014a). In all heated or unheated samples, addition of pectin led to a decrease in zeta-potential. At 0.3% pectin, CPX and HCPX heated at 75 and 85°C had a zeta-potential of -25 ± 3.5 , -25 ± 2 and -28 ± 1.2 , respectively. Change in zeta-potential was likely due to binding of negatively charged pectin molecules to positively charged patches on the proteins before heating (e.g., CPX samples). During heating thermal energy could disturb the interactions, but the presence of pec-

tin molecules could alter protein aggregation as shown by the reduction in turbidity and particle sizes. At the same time, unfolding could expose hidden charged groups that could interact with pectin (Gentès et al., 2010). During cooling, pectin could then absorb to protein aggregates, resulting in aggregates being more negatively charged (e.g., HCPX samples). It should be noted that changes in zeta-potential between samples with 0.1 and 0.3% pectin were smaller, indicating a limitation of additional binding at higher pectin concentration.

Particle Size. Unheated WPI exhibited a monomodal distribution with a mean diameter of 4 nm (Figure 2a), which agreed with the result from Zhang et al. (2014). Addition of pectin led to the formation of soluble complexes as shown by the appearance of large particles and the decrease in the WPI peak area, indicating that a certain amount of WPI was involved in complex formation with pectin. At 75°C, the z-average

diameter of HWPI was 281 ± 18 nm, whereas addition of 0.1 and 0.3% pectin led to the formation of smaller aggregates with multimodal distributions (Figure 2b). It should be noted that the majority of the aggregates in HCPX with 0.3% pectin had the average diameters <100 nm and some native WPI was still present (e.g., peak ~4 nm), which corresponded to its translucent appearance (Figure 1a). This result was in agreement with the study from Donato et al. (2009) that reported that heated β -LG aggregates (70°C) at pH 5.9 were composed of small aggregates between 50 and 70 nm diameter.

At 85°C, size distributions of all heated samples showed less polydispersity compared with those heated

Figure 1. Appearance (a) and zeta-potential (b) of whey protein isolate with different heating temperatures and different pectin concentrations. Means within the same heating temperature with different letters (a, b) differ ($P < 0.05$). Error bars represent SD.

Figure 2. Particle size distribution of whey protein isolate with different heating temperatures [unheated (a), heated at 75°C (b), and heated at 85°C (c)] and different pectin concentrations [0% ($\square$), 0.1% ($\circ$), and 0.3% (Δ)].

at 75°C (Figure 2C). The z-average diameters decreased from 557 ± 51 at 0% pectin to 311 ± 14 and 421 ± 19 nm in the presence of 0.1 and 0.3% pectin, respectively. These results indicate that interactions between WPI and pectin altered protein aggregation and heated soluble complexes had smaller sizes compared with HWPI. Similar results were reported by Vardhanabhuti et al. (2009). The changes in turbidity and particle sizes with temperature indicate changes in the structure of the aggregates or complexes. The slight increase in turbidity observed at 75°C was due to the unfolding and aggregation of the native protein molecules with increased thermal energy. The drastic increase in turbidity and particle sizes at 85°C was attributed to an increased number of protein molecules engaging in heat aggregation and formation of larger aggregates (Figures 1a and 2). Complexation with pectin altered aggregation of protein as shown by decreased turbidity and mean diameters. Nonetheless, HCPX at 85°C formed large particles that strongly affected the scattering light (Jones et al., 2010a). This implies that the structure and number of protein aggregates differed by the effect of heating between 75 and 85°C (Donato et al., 2009; Dybowska, 2011). It could be concluded from both particle size and zeta-potential results that, compared with WPI and complexes, the electrostatic interactions between protein and pectin were enhanced by forming heated complexes.

Zeta-Potential of Emulsions

The WPI, HWPI, CPX, or HCPX were used as aqueous phase during emulsion formation. The final emulsions contained 5% (wt/wt) oil, 1.5% (wt/wt) protein, and were at pH 5.5. The final pectin concentrations in the emulsions were 0, 0.05, or 0.15% pectin, which corresponded to the protein solutions (before emulsification) having 0, 0.1, and 0.3% pectin, respectively. In the absence of pectin, the zeta-potential of emulsions stabilized by WPI and HWPI ranged from -23 ± 1.3 to -28 ± 1.3 mV (Figure 3a). The effect of heating showed a significant increase in droplet net charge when emulsions were stabilized by HWPI ($P < 0.05$).

For mixed emulsions, the zeta-potentials were significantly more negative ($P < 0.05$) and were all below -30 mV. Higher negative charge was due to the interfacial adsorption of CPX or HCPX themselves being more negatively charged. In addition, it is likely that the decreased charge of protein during pH adjustment to 5.5 allowed the additional adsorption of free pectin in the aqueous phase that did not bind to the protein. Similar results showing the charge properties of mixed emulsions have been reported (Jourdain et al., 2008;

Huan et al., 2016). It should be noted that increasing pectin concentration in CPX and HCPX did not change the emulsion droplet charge, suggesting that the droplet surface was saturated when emulsions contained 1.5% WPI and 0.05% pectin. At the same pectin concentration, there was no significant effect ($P > 0.05$) of complex formation temperature (unheated, heated at 75 and 85°C) on the emulsion surface charges. However, overall, the trend showed a slightly lower zeta-potential in samples stabilized by HCPX formed at 85°C.

Mean Droplet Size of Emulsions

The effect of pectin concentration and complex formation temperature on the mean droplet sizes of emulsions is presented in Figure 3b. In the absence of

Figure 3. Zeta-potential and mean droplet sizes of emulsion stabilized by whey protein isolate with different concentrations of pectin and heating temperatures; unheated (white), heated at 75°C (gray), and heated at 85°C (black). Means within the same heating temperature with different letters (a, b) differ ($P < 0.05$). Error bars represent SD.

pectin, mean droplet sizes of emulsions stabilized by WPI and HWPI at 75 and 85°C were 393.4 ± 17.9 , 434.3 ± 37.5 , and 533 ± 34.7 nm, respectively. The effect of heating temperature was significant as shown by larger droplet sizes ($P < 0.05$) in emulsion stabilized by HWPI at 85°C. It should be noted that these values were smaller than anticipated. In other studies, emulsions stabilized by unheated or heated WPI at pH near pI showed larger mean droplet diameters (Demetriades and McClements, 1998; Chanamai and McClements, 2002; Ruffin et al., 2014; Huan et al., 2016). This could be due to the different sources of protein used and the higher protein content in the emulsion. In addition, the emulsions were made at pH 7.0 before acidification; thus, the higher net charge could prevent droplet flocculation during emulsification, resulting in initial small droplet sizes. Droplet size measurement was conducted on freshly made emulsions; thus, coalescence could have been at a minimum.

The presence of pectin in mixed emulsions clearly reduced the mean droplet sizes and increased surface charge potential. At 0.15% pectin, those formed by CPX had smaller mean droplet sizes ($P < 0.05$) compared with WPI without pectin. Similarly, HCPX stabilized emulsions also showed significantly decreased in mean droplet sizes ($P < 0.05$) compared with HWPI. This is attributed to the adsorbed layer of higher negatively charged complexes on the droplet surface, which provided sufficient electrostatic and steric repulsion that could prevent droplet aggregation. At the same pectin concentration, the effect of heating temperature on size showed that HWPI and HCPX heated at 85°C led to emulsions with larger size ($P < 0.05$) compared with those from HWPI or HCPX at 75°C. As previously discussed, HWPI and HCPX at 75°C were composed of smaller aggregates and may retain some native proteins (Simmons et al., 2007). Presumably, the presence of different types of particles (e.g., native protein, aggregates, CPX, and HCPX) competed to form layers surrounded the oil droplets. Thus, differences in the types and properties of the aggregates resulted in differences in emulsion properties (Donato et al., 2009).

Rheological Properties of Emulsions

As obtained from the power law model, consistency coefficient (K) and flow behavior index (n) are used to describe rheological properties of freshly made emulsions (Table 1). All emulsions exhibited low viscosity with a Newtonian or slightly shear thinning behavior. The K and n values ranged from 2.44 to 5.90 mPa.s^{*n*} and 0.818 to 1.064, respectively. In general, an emulsion with $n < 1$ is determined as having shear thinning

Table 1. Power law model parameters for emulsions stabilized by whey protein isolates (WPI) or WPI-pectin complexes formed at different pectin concentrations and heating temperatures¹

Heating temperature	Pectin (%)	K^2 (Pa.s ^{<i>n</i>})	n^2
Unheated	0	5.17 ± 1.03^a	0.939 ± 0.061^a
	0.05	2.44 ± 0.41^a	0.994 ± 0.006^a
	0.15	3.92 ± 1.76^a	1.012 ± 0.116^a
Heated at 75°C	0	5.90 ± 2.85^a	0.818 ± 0.256^a
	0.05	3.72 ± 0.86^a	0.987 ± 0.019^a
	0.15	5.54 ± 1.73^a	1.011 ± 0.061^a
Heated at 85°C	0	3.47 ± 2.40^a	0.866 ± 0.129^a
	0.05	1.81 ± 0.35^a	1.056 ± 0.086^a
	0.15	3.53 ± 0.45^a	1.064 ± 0.061^a

^aMeans $\pm$ SD ($n = 3$) with the same superscript do not differ ($P > 0.05$).

¹Emulsions contained 5% oil, 1.5% protein, and 0 to 0.15% pectin at pH 5.5.

² K = consistency coefficient; n = flow behavior index.

behavior, which could be interpreted as the disruption of flocs and decreased volume viscosity at increasing shear rate (Huan et al., 2016). Our results showed no significant differences among samples for both n and K values. This could be due to the fact that the measurements were conducted with freshly made emulsions, which showed relatively small droplet sizes as described above.

Typically, addition of hydrocolloids increases the viscosity of the continuous phase and results in decreased n and increased K values of the emulsions (Xu et al., 2012). In this study we found that the presence of pectin in unheated and heated complex samples did not lead to any changes in the rheological properties of the emulsions ($P > 0.05$). These results suggest that pectin did not significantly affect the viscosity of the continuous phase. The results were also in agreement with the observed droplet sizes and particle charge.

Creaming Stability

Figure 4 shows the effect of complex formation temperature and pectin concentration on creaming stability of emulsions during 30-d storage. Emulsions stabilized by WPI showed separation within 24 h (data not shown) despite the initial small droplet sizes. This could be due to weak electrostatic repulsion, which is favorable for droplet flocculation and coalescence (Gu et al., 2004). The HWPI-stabilized emulsions had improved stability but creaming was observed on d 7 and 14 for HWPI at 75 and 85°C, respectively. Improved emulsion stability of heated protein could be due to the formation of thicker adsorbed layer consisting of protein aggregates (Dybowska, 2011; Liang et al., 2017). Larger aggregates

Figure 4. Creaming stability of emulsions stabilized by whey protein isolate with different concentrations of pectin and heating temperatures. Emulsions contained 5% oil, 1.5% protein, and 0 to 0.15% pectin at pH 5.5; unheated (a), heated at 75°C (b), and heated at 85°C (c). Error bars represent SD.

in HWPI formed at 85°C could provide better stability compared with HWPI at 75°C.

In the presence of 0.05% pectin in emulsions, CPX and HCPX at 75°C formed emulsions with improved stability; however, creaming was observed on d 30 and 7, respectively. This indicated that whey protein and pectin complex formed membranes that could prevent the droplet rupture. It has been known that polysaccharides increase the emulsion stability by their thickening and steric stabilizing effect (Faridi Esfanzani et al., 2017). No creaming was observed in HCPX at 85°C. Similar to the above, due to their larger sizes, the HCPX at 85°C could potentially form a stronger adsorbed layer on the oil droplets. In addition, HCPX at 85°C also showed lower zeta-potential (though the difference was not significant). The approach to produce mixed emulsion by complexation of unheated protein and polysaccharide has already been shown to improve emulsion stability. Thus, improved creaming stability of unheated complexes was expected. The significant finding in this study is that, at lower pectin concentration, using HCPX formed at 85°C resulted in the most stable emulsions.

Heat Stability of Emulsions

Due to the lack of stability against creaming, emulsions stabilized by WPI or HWPI without pectin were not tested for their heat stability. Regardless of pectin concentration, emulsions stabilized by CPX as well as HCPX formed at 75°C all turned into weak gels after heating. In emulsions containing CPX, unfolding of native protein during heating expose the hydrophobic surface, which promotes the interaction and aggregation between droplet and nonadsorbed protein (Euston et al., 2000; McClements, 2004; Çakır-Fuller, 2015). Extensive protein-protein interactions led to flocculation and eventually gelation. For HCPX at 75°C, some undenatured protein could be present, whereas complete denaturation occurred in HCPX at 85°C. Thus, unfolding as well as protein-protein interactions and gelation could occur in emulsions stabilized by HCPX at 75°C. These samples were not further characterized.

Emulsions stabilized by HCPX formed at 85°C maintained their fluid properties after heating. Size, zeta-potential, and rheological properties of these samples were characterized and compared with their properties before heating (Table 2). All emulsions stabilized by HCPX at 85°C showed no significant changes ($P > 0.05$) in zeta-potential and rheological properties (e.g., η and K) after the emulsions were heated, whereas mean droplet sizes increased slightly ($P < 0.05$). It has been suggested that large mean droplet sizes are protein

Table 2. Effects of heating (85°C, 15 min) on emulsions stabilized by heated whey protein isolate and pectin complexes (HCPX) formed at 85°C¹

HCPX at 85°C	Pectin (%)	Zeta-potential (mV)	z-average diameter (nm)	K ² (mPa·s ⁿ)	n ²
Emulsion	0.05	-35.9 ± 1.4 ^a	419 ± 12 ^b	1.81 ± 0.36 ^a	1.056 ± 0.086 ^a
	0.15	-36.0 ± 0.3 ^a	380 ± 7 ^b	3.53 ± 0.45 ^a	1.064 ± 0.061 ^a
Heated emulsion	0.05	-34.3 ± 1.2 ^a	562 ± 4 ^a	2.64 ± 0.92 ^a	0.942 ± 0.159 ^a
	0.15	-35.3 ± 1.0 ^a	516 ± 6 ^a	4.15 ± 1.64 ^a	1.019 ± 0.117 ^a

^{a,b}Means ± SD (n = 3) with different superscripts differ ($P < 0.05$).

¹Emulsions contained 5% oil, 1.5% protein, and 0 to 0.15% pectin at pH 5.5.

²K = consistency coefficient; n = flow behavior index.

aggregates from heating (Euston et al., 2000). However, the increase in droplet size was not enough to cause any significant changes in the rheological properties of emulsion. As shown by n and K values, emulsions were stable against flocculation and maintained Newtonian behavior with low viscosity. The apparent viscosity at 50 s⁻¹ of all samples showed low viscosity and ranged from 0.002 to 0.004 mPa·s.

Creaming stability experiment indicated that all emulsions were stable for more than 30 d (data not shown). These results suggested that HCPX at 85°C could form a relatively strong interaction between whey protein and pectin that improved emulsifying activity and provided steric force (Gu et al., 2005; Setiowati et al., 2017).

CONCLUSIONS

Formation of CPX or HCPX led to an increase in negative charge potential and, in the case of HCPX, altered the formation of protein aggregates. Pectin concentration was the major factor affecting the charge of the complexes, whereas heating temperature mainly influenced the sizes of the heated complexes. Using CPX or HCPX to stabilize the oil-in-water emulsions in a mixed emulsion approach resulted in emulsions having smaller droplet sizes, increased negative charge, and improved stability against creaming compared with WPI or HWPI alone. Emulsion properties and stability depended on pectin concentration as well as heating temperature. Only emulsions stabilized by HCPX formed at 85°C were stable after heating. The HCPX formed at 85°C with 3% WPI and at least 0.1% pectin are promising ingredients to be used in emulsions close to the isoelectric point.

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