

EMULSIFICATION PROPERTIES OF HEATED WHEY
PROTEIN-PECTIN FORMED AT NEUTRAL pH

A Thesis

Presented to

The Faculty of the Graduate School
At the University of Missouri-Columbia

In Partial Fulfillment
of the Requirements for the Degree
Master of Science

By
YEYANG WANG

Dr. Bongkosh Vardhanabhuti, Thesis Supervisor

July 2018

The undersigned, appointed by the dean of the Graduate School, have examined the
thesis entitled

EMULSIFICATION PROPERTIES OF HEATED WHEY PROTEIN-
PECTIN FORMED AT NEUTRAL pH

Presented by Yeyang Wang,

A candidate for the degree of Master of Science,

And hereby certify that, in their opinion, it is worthy of acceptance.

Dr. Bongkosh Vardhanabhuti, Department of Food Science

Dr. Ingolf Gruen, Department of Food Science

Dr. Fu-Hung Hsieh, Department of Bioengineering

ACKNOWLEDGEMENTS

I would like to express my gratitude to my advisor Dr. Vardhanabhuti, whose expertise, understanding, and patience, added considerably to my graduate experience. Without her guidance and support, my thesis cannot be completed. She taught me how to be patient, hardworking and independent thinking.

I would like to thank Dr. Gruen and Dr. Hsieh for your willingness to become my committee members, in spite of a busy schedule. I am grateful for their support and advice.

I would like to thank my colleagues in the lab, Bami, George, Minhua, Yafan, Yun. Thank you for giving me support and for helping to create a harmonious working atmosphere. I also want to thank my friends, Lin, Yun, Zhilong, for their support in my hard time.

Outside of the lab, many people helped me along the way. I would like to thank Dr. Mustapha and Dr. Clarke for the enlightening and interesting class; Jennifer, for her constant help.

Finally, I would like to express my special thanks to my parents for their love and support. Without them, I cannot finish my career. I love you.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS	ii
LIST OF TABLES	vi
LIST OF FIGURES	vii
ABSTRACT.....	ix
CHAPTER	
1. INTRODUCTION	1
2. REVIEW OF LITERATURE	4
2.1 Whey protein.....	4
2.2 Pectin.....	5
2.3 Protein-polysaccharides interaction	7
2.3.1 Protein-polysaccharide complexes.....	8
2.3.2 Factors influence protein-polysaccharide interaction	9
2.3.2.1 pH.....	10
2.3.2.2 Ionic strength	10
2.3.2.3 Biopolymer ratio and concentration.....	11
2.3.2.4 Nature of biopolymers	11
2.3.2.5 Processing	12
2.4 Emulsion	12
2.4.1 Introduction of emulsion.....	12
2.4.2 Protein-polysaccharides mixture stabilized emulsion.....	14
2.4.2.1 Emulsion preparation	14
2.4.2.2 Factors affect emulsion stability	15
2.4.3 Heated protein-polysaccharides complex stabilized emulsion	18

3. MATERIAL AND METHODS	19
3.1 Material	19
3.2 Sample preparation	19
3.2.1 Formation of heated WPI-pectin complex	19
3.2.2 Formation of heated WPI and pectin mixture	20
3.2.3 Emulsion preparation	20
3.4 Particle size and ξ -potential measurement	21
3.5 Rheological properties measurement	21
3.6 Creaming index measurement	21
3.7 Statistical analysis	22
4. RESULTS AND DISCUSSION	23
4.1 Emulsification properties of heated complex	23
4.1.1 Particle sizes and surface charge of complex	23
4.1.2 Formation of WPI-Pectin Complex on Oil Droplets	29
4.1.2.1 Surface charges of the oil droplets	29
4.1.2.2 Mean particle diameter of the oil droplets	31
4.1.2.3 Rheological properties of emulsions	32
4.1.2.4 Emulsion stability	37
4.2 Effect of different mixed systems	40
4.2.1. Particle size and zeta-potential of biopolymers	40
4.2.2. Emulsions	42
4.2.2.1 Surface charge and mean particle diameters of the oil droplets	42
4.2.2.2 Rheological properties of emulsions	45

4.2.2.3 Creaming stability	47
5. CONCLUSION	49
5.1 Conclusion	49
5.2 Future study	50
REFERENCE.....	51
APPENDIX.....	57

LIST OF TABLES

Table	Page
Table 1 Power Law model parameters of emulsions stabilized by Cpx formed at different pH and pectin concentrations	36
Table 2 Power law model parameters for emulsions with different biopolymers	46

LIST OF FIGURES

Figure	Page
Figure 1 Mean particle sizes of complexes formed at pH 6.0 (black), pH 6.5 (grey), and pH 7.0 (white) and at different pectin concentrations.....	26
Figure 2 Effect of heating pH and pectin concentration on the aggregation of WPI (3 wt%) heated at pH 6 (a), pH 6.5 (b), and pH 7 (c). From left to right (0, 0.15, 0.30, 0.45, 0.60 wt% pectin).	27
Figure 3 ζ -potential of Cpx formed at pH 6.0 (black), pH 6.5 (grey), and pH 7.0 (white) and different pectin concentration.	28
Figure 4 ζ -potentials of emulsions stabilized by Cpx formed at pH 6.0 (black), heated at pH 6.5 (grey), heated at pH 7.0 (white) and different pectin concentration.	30
Figure 5 Mean particle sizes of emulsion stabilized by Cpx formed at pH 6.0 (black), heated at pH 6.5 (grey), heated at pH 7.0 (white) and with different pectin concentration.....	32
Figure 6. Apparent viscosity of fresh emulsions stabilized by Cpx formed at pH 6 (a), pH 6.5 (b) and pH 7 (c) with different pectin concentrations;.....	35
Figure 7 Creaming stability of emulsions prepared with Cpx formed at pH 6.0 (black bar), pH 6.5 (grey bar), and pH 7.0 (white bar) at different pectin concentrations after day 1 (a), day 5 (b), and day 15 (c).....	39
Figure 8 Mean particle sizes of unheated protein (UH ptn), heated WPI (H ptn), heated WPI with added pectin (H mix) and Cpx.....	41
Figure 9 ζ -potentials of heated WPI (H ptn), heated WPI with added pectin (H mix) and Cpx.....	42

Figure 10 ζ -potential of emulsion droplets stabilized by biopolymers..... 44

Figure 11 Mean droplets size of emulsion stabilized by biopolymers..... 44

Figure 12 Apparent viscosity of fresh emulsions stabilized by ○=UH ptn; ×= H ptn; □=
H mix; +=Cpx. 46

Figure 13 Creaming stability of emulsions stabilized by different mixed WPI and pectin
systems at room temperature for 15 days. 48

Emulsification properties of heated whey protein and pectin formed at neutral pH

Yeyang Wang

Dr. Bongkosh Vardhanabhuti, Thesis supervisor

ABSTRACT

Interactions between protein and polysaccharides could lead to improved protein functional properties including emulsification properties. Most studies focus on complex coacervates which are formed at $\text{pH} < \text{pI}$. Much less attention has been given to interactions at $\text{pH} > \text{pI}$, especially when the mixtures are heated. The objective of this study was to investigate the emulsification properties of heated whey protein isolate (WPI) and pectin complexes formed at near neutral pH.

The effect of pH during heating and pectin concentration in heated complex (Cpx) were studied. Cpx were formed by heating WPI (3.0 wt% protein) and pectin (0 to 0.60 wt%) together at pH 6.0, 6.5, or 7.0 at 85°C for 30 min. Emulsions (5.0 wt% oil and 0.5 wt% protein) were obtained by homogenizing oil and aqueous solution, followed by ultrasonic processing. The emulsions were acidified to pH 5.5. Droplet size, ζ -potential, rheological properties and creaming stability of emulsions were measured. Stability against creaming was determined for 15 days.

Results showed that heated WPI-stabilized emulsions were unstable and separated into two layers within 24 hours. Regardless of heating pH, Cpx formed more stable emulsions with significantly smaller droplet size, more negative charge and less shear-

thinning behavior ($P < 0.05$). At fixed pectin concentration, emulsions stabilized by Cpx formed at pH 7.0 were the most stable. At optimum heating pH, increasing pectin concentration led to a decrease in mean droplet size and an increase in negative charge. Maximum stability (> 30 days) was achieved with emulsion stabilized by Cpx formed with 0.60 wt% pectin at pH 7.0.

In order to confirm the effectiveness of Cpx, emulsification properties of Cpx were compared to heated protein with added pectin (H mix). H mix was formed by mixing heated 3.0 wt% WPI with 0.60 wt% pectin at pH 7.0 (e.g., only WPI was heated). Emulsions stabilized by H mix and Cpx both showed improved emulsification properties. Compared to those with Cpx, emulsions with H mix showed more Newtonian behavior and lower viscosity. However, emulsions stabilized by Cpx had higher stability, which could be due to differences in interfacial layer (e.g., thicker layer and different structure).

This study demonstrates that heat complexation of whey protein with pectin at near neutral pH could improve emulsification properties at pH near pI of the protein. Heated WPI-pectin complexes could be utilized as emulsifier and stabilizer in food applications.

CHAPTER 1

INTRODUCTION

1.Introduction

Whey protein is the major co-product of fermented dairy products. It is widely used for a variety of applications in the food industry. It could be used to alter non-protein ingredient, enhance the functional properties and provide additional nutrients in the food product. However, other components and processing procedures have a huge impact on the stability of whey proteins and influence quality of food products (Foegeding, Davis, Doucet, & McGuffey, 2002). To improve the functionality of whey proteins, several modification methods were proposed. One of them is forming complexes with polysaccharides through non-covalent interaction.

Protein-polysaccharide interactions have been the subject of investigation since the early 20th century (Beijerinck, 1910). Due to their polyelectrolyte nature, proteins and anionic polysaccharides readily associate via electrostatic interactions to form complexes. Complexation between proteins and polysaccharides may result in improvement in functional properties compared with individual macromolecules, including thermal stability (Mishra, Mann, & Joshi, 2001; S.Zhang, Zhang, Lin, & Vardhanabhuti, 2012), solubility, emulsification properties (Surh, Decker, & McClements, 2006), and foaming properties (Wang, Zhang, & Vardhanabhuti, 2015). The nature and strength of the interactions strongly depend on molecular properties biopolymers concentration, pH, and ionic strength (de Kruif, Weinbreck, & de Vries, 2004; Jones & McClements, 2011; Schmitt, Sanchez, Desobry-Banon, & Hardy,

1998; Wagoner, Vardhanabhuti, & Foegeding, 2016). Most studies have focused on protein-polysaccharides complex or coacervates formed at pH lower than pI (Cooper, Dubin, Kayitmazer, & Turksen, 2005; C. G. de Kruif & Tuinier, 2001; Jones & McClements, 2011; Turgeon, Schmitt, & Sanchez, 2007), where proteins carry positive charges and can form strong electrostatic interaction with anionic polysaccharides. However, recent studies have shown that complexation still occurs at pH above pI where interactions can exist between chain segments of anionic polysaccharides and the positively charged residue ($-\text{NH}_3^+$) groups on protein (Huan, Zhang, & Vardhanabhuti, 2016; Zhang, Hsieh, & Vardhanabhuti, 2014). These interactions can be enhanced by heating at conditions where the mixed biopolymer solution eventually forms soluble aggregates (Girard, Turgeon, & Gauthier, 2002; Zhang, Hsieh, & Vardhanabhuti, 2014).

Emulsion is one of the most common systems in food products. It is relatively unstable due to energetically unfavorable contact between oil droplets and water (McClements, 2015). Formation of kinetically stable emulsions can be achieved by adding emulsifiers such as small molecular weight surfactants and proteins. Due to its high surface activity, whey protein isolate (WPI) can rapidly absorb onto the oil droplet surfaces, lower the surface tension, and prevent the droplets from aggregation. However, the emulsification properties of whey protein are highly influenced by pH, ionic strength and temperature.

Studies found that complexation between protein and polysaccharides significantly improved protein emulsification properties compared with single molecules (Bouyer, Mekhloufi, Rosilio, Grossiord, & Agnely, 2012; Dickinson, 1998;

Dickinson, 2009). Emulsions stabilized by complexes have smaller droplet sizes, higher surface charge and higher viscosity compared to those stabilized by protein (Gu, Decker, & McClements, 2004; Huan, Zhang, & Vardhanabhuti, 2016). Since interaction between two biopolymers is mainly driven by electrostatic interaction, emulsification properties of the complexes are influenced by pH, salt environment and the nature of biopolymers (Gu, Decker, & McClements, 2004, 2005; Huan, Zhang, & Vardhanabhuti, 2016; Neiryneck et al., 2007; Surh, Decker, & McClements, 2006). Polysaccharides can adsorb on the oil droplets primarily emulsified with proteins through electrostatic interaction and increases the thickness as well as charge potential of the interfacial layer between the oil droplets. Subsequently, the steric and electrostatic repulsions between the oil droplets are enhanced, leading to improved emulsion stability (Surh, Decker, & McClements, 2006). Like studies related to complex, very few studies focus on emulsification properties related to soluble complex. One study (Salminen & Weiss, 2014) fabricated heated complex formed at pH 4.75 and found the emulsions with complex showed notable improvement of thermal and salty stability. However, there is no study related to emulsions with heat soluble complex formed at neutral pH.

In this study, WPI and low-methoxyl pectin were used to form heated soluble complexes at pH higher than pI. Their emulsification properties were evaluated at pH 5.5, where the emulsification and stabilizing properties of protein may be diminished. The effects of pectin concentration and heating pH, complex formation methods on emulsification properties and stability of emulsions were investigated.

CHAPTER 2

REVIEW OF LITERATURE

2.1 Whey protein

Whey is a major co-product of fermented product in the dairy industry. The main composition of whey protein is β -lactoglobulin (β -lg, 65%), α -lactalbumin (α -la, 25%), and serum albumin (8%). There are three main forms of whey protein depending on processing techniques and separation methods: whey protein powder (WPP), whey protein concentrate (WPC) and whey protein isolate (WPI) (Hoffman & Falvo, 2004). WPP contains 11-14.5% protein by weight, and the major component is lactose from 63 to 75%. It can be used as an additive in food product. WPC contains 29 to 89% protein, due to removal of the water, lactose, ash, and some minerals compared to WPP. Compared to WPI, WPC contains more biologically active components and proteins that make it an ideal supplement for athletes. WPI is the purest protein source available, the protein concentration is higher than 90%. Among different types of protein powders, the WPC and WPI are most widely used (Dissanayake & Vasiljevic, 2009).

Whey proteins have the highest biological value (BV) among edible proteins (Smithers, 2008). BV refers to how well and quickly the body can utilize the protein consumed. As an ideal source of high quality protein, the physiological function of whey protein is of interest. Whey proteins have potential as a functional food component by reducing short-term food intake, affecting satiation and participating in

intake regulation system (Luhovyy, Akhavan, & Anderson, 2007). Whey proteins are widely used in various food applications, they are highly soluble within a broad range of pH (from 3 to 7), making them a good quality of foaming (Jambrak, Mason, Lelas, Herceg, & Herceg, 2008), emulsifying and gelling agent (Foegeding, Davis, Doucet, & McGuffey, 2002).

However, whey proteins are relatively thermal and salt unstable. They easily undergo denaturation by heat at high temperature (above 72 °C). The denaturation of whey proteins affects their functionality. It is influenced by several factors: temperature, heating time, pH and ionic strength. This vulnerability of whey proteins limits their application. Thus, nowadays, modification of whey protein is of interest (Ryan, Zhong, & Foegeding, 2013; Wijayanti, Bansal, & Deeth, 2014).

2.2 Pectin

Pectin is a linear chain polysaccharide, it is extracted from plant cell wall, especially from citrus peel and sugar beet pulp (Leroux, Langendorff, Schick, Vaishnav, & Mazoyer, 2003). Pectin has a complex structure, depending on its source and extraction process. Basically, it consists of α -D-galacturonic acid with 1-4 linkages. In natural pectin, some of the carboxyl groups are in the methyl ester form, but the esterified groups can be chemically modified. The ratio of esterified D-galacturonic acid units to total units is called the degree of esterification (DE) (BeMiller, 1986). If DE is greater than 50%, it is called high methoxyl (HM) pectin; If DE is lower than 50%, it is low methoxyl (LM) pectin. The nonesterified carboxyl groups give LM pectin a higher overall charge density (de Jong & van de Velde,

2007; Wagoner, Vardhanabhuti, & Foegeding, 2016), which greatly influences functional properties of pectin such as solubility and gelling ability.

Due to its ubiquity structure, pectin is widely utilized in the food industry as gelling, thickening, or stabilizing agent, especially in beverages or in semi-solids area. During the past several decades, pectin has been perceived as a stabilizing agent in emulsions, mainly by increasing the viscosity to prolong a product's shelf life (Eric Dickinson, 2003, 2009). Studies reported that emulsification properties of pectin highly depend on intrinsic characteristics of a system, such as ionic strength, pH, presence of bivalent cation, as well as pectin properties (conformation, charge density and molecular mass) (Ngouémazong, Christiaens, Shpigelman, Van Loey, & Hendrickx, 2015; Williams et al., 2005). Leroux and others (Leroux, Langendorff, Schick, Vaishnav, & Mazoyer, 2003) proposed a "loop and tail" absorption model to elucidate the emulsification mechanism of pectin. Small proportion of pectin and proteins residual in pectin absorbed onto oil droplets as strong point of anchor, then rest pectin attached to protein. The carbohydrate portion of pectin extended to the aqueous phase as the tail, providing additional steric barrier and electrostatic repulsion against droplets coalescence and flocculation (Akhtar, Dickinson, Mazoyer, & Langendorff, 2002). Besides, the ability of pectin to increase viscosity of the continuous phase contributes to improving emulsion stability. However, emulsification activity of pectin is highly related to the proteinaceous moiety in pectin. Funami and others (Funami et al., 2007) found that with an enzymatic modification using proteinase, sugar beet pectin showed significantly decreased emulsification activity and stability, suggesting protein in pectin played an important

role to explain emulsification properties in pectin. The result indicates pure pectin is not a suitable emulsifying agent. Combination of pectin with other biopolymers like protein seems to be a better idea.

2.3 Protein-polysaccharides interaction

Electrostatic interaction between macromolecules, like proteins and polysaccharides, has fundamental biological significance, which is related to various biological process, like protein transcription, sensory perception or enzymatic channeling (Turgeon, Schmitt, & Sanchez, 2007). The interaction between two kinds of biopolymers is also important in industry. Studies found that combination of polysaccharides with protein could significantly improve functionalities of two macromolecules.

The combination of protein and polysaccharides generates two kinds of particles: conjugate and complex. The former is achieved by Maillard reaction, first reported by Maillard in 1912. It is a series of non-enzymatic browning reaction, occurring between amino acids in proteins and the reducing end of sugars via covalent bonds through dry or wet heating methods (Akhtar & Ding, 2017). Complexation between two biopolymers are mainly through electrostatic interaction in aqueous phase. It is entropy-driven and reversible, but bonding is relatively weak compared to covalent bond. Both types enhanced solubility and functionalities of protein (Akhtar & Ding, 2017; Cooper, Dubin, Kayitmazer, & Turksen, 2005; Turgeon, Schmitt, & Sanchez, 2007). Due to the brown color, conjugates, further application is limited. In this study, we will focus on complexation between protein and polysaccharides through electrostatic interaction.

2.3.1 Protein-polysaccharide complexes

Complexation between protein and polysaccharides have fundamentally biological meaning in nature. The main driven force is electrostatic interaction, along with hydrogen bonding and hydrophobic interaction. Based on biopolymer type and intrinsic condition, complex of protein and polysaccharides can be classified into two categories: coacervates and soluble aggregates. Typically, soluble complexes form when the electrostatic interaction is relatively weak; coacervate form when the electrostatic interactions between two is strong.

Studies of protein-polysaccharides complex have been carried out at ambient temperature. Protein and polysaccharides are mixed in aqueous solutions under different conditions (pH, ionic strength, etc.) (Cooper, Dubin, Kayitmazer, & Turksen, 2005; Turgeon, Schmitt, & Sanchez, 2007; Aiqian Ye, 2008). The improvement of functional properties of the electrostatic complex are well established. Complexation between protein and polysaccharides improved thermal stability (Burova et al., 2007; Ibanoglu, 2005), gelling properties (Braga & Cunha, 2004; de Jong, Klok, & van de Velde, 2009), foaming ability (Ganzevles, Cohen Stuart, Vliet, & de Jongh, 2006; Miquelim, Lannes, & Mezzenga, 2010; Wang, Zhang, & Vardhanabhuti, 2015) and emulsifying properties (Bouyer, Mekhloufi, Rosilio, Grossiord, & Agnely, 2012; McClements, 2004; Mishra, Mann, & Joshi, 2001).

While recently, numbers of studies found that biopolymer nanoparticles or microparticles can be formed by controlling heating of electrostatic complexes of globular proteins and ionic polysaccharides (Jones & McClements, 2011). There are two ways to fabricate heat-treating protein-polysaccharides complex: 1. Form protein aggregates above denaturation temperature and then complex with polysaccharides (Jones, Decker, & McClements, 2010); 2. Form complex directly by heating mixed solution of protein and polysaccharides (Jones, Lesmes, Dubin, & McClements, 2010; Wagoner & Foegeding, 2017). A study (Jones, Decker, & McClements, 2010) compared nanoparticles fabricated from two methods and type 2 showed better stability to heat and salt, suggesting they have different structures. To explain the formation of heated protein-polysaccharide complex, the core shell theory was proposed (Jones & McClements, 2011). Protein first aggregated and formed a core, then polysaccharides surround protein core and formed a shell outside. Most studies were carried at pH lower than pI, where protein and polysaccharides have opposite charges and have strong attractive interaction. Few Studies found when at neutral pH, where proteins and anionic polysaccharides both carry net negative charges, heating of protein and polysaccharides can still form soluble complex (Wagoner & Foegeding, 2017) and showed improved foaming properties (Wang, Zhang, & Vardhanabhuti, 2015), gelling ability (Zhang, Hsieh, & Vardhanabhuti, 2014) and had potential to prolong satiety (Zhang, Zhang, & Vardhanabhuti, 2014).

2.3.2 Factors influence protein-polysaccharide interaction

Factors that influence compatibility and complex formation mainly are biopolymer ratio, pH, ionic strength and nature of biopolymers (molecular weight, net

charge, structure and length of chains). Pretreatment of biopolymers also affects complex formation. High pressure and temperature have been reported to affect final functional properties of formed complex (Cooper, Dubin, Kayitmazer, & Turksen, 2005).

2.3.2.1 pH

Electrostatic interaction play a prominent role in complex formation, and this interaction is mainly controlled by the charge density of biopolymers, which is directly influenced by pH. At pHs below the isoelectric point of protein, protein and an anionic polysaccharide carry opposite charges, resulting in strong electrostatic attraction (Aiqian Ye, 2008). At pHs above pI, both protein and anionic polysaccharides carry negative charges, thus have weak attraction and strong repulsion. Transition of complexation between protein and polysaccharides have been carried out in numerous systems (Turgeon, Schmitt, & Sanchez, 2007). There are two important pH values involved: pH_c : initial pH for soluble complex formation and pH_ϕ : initial pH to form coacervates. When $pH_c < pH < pH_\phi$, strong interaction between two macromolecules occurred and soluble aggregate complex were formed. When $pH > pH_c$, small soluble aggregates and cosoluble solution of protein and polysaccharides can be found (Sanchez, Mekhloufi, & Renard, 2006).

2.3.2.2 Ionic strength

At high ionic strength, interaction between proteins and polysaccharides can be reduced by a screening effect, thus affect the formation of complex (Aiqian Ye, 2008). Jones (Jones & McClements, 2010) found turbidity of heat-treated β -lactoglobulin and pectin mixture increased with increasing salt concentration. While

unheated mixture was relative insensitive. The results showed that increasing ionic strength diminished the repulsion force between similar charged molecules during heat treatment. However, when the ionic strength is relatively low, there is no influence on complexation. The addition of 0 to 200 mM NaCl had little effect on turbidity of β -lactoglobulin-beet pectin complex formed at pH 5 (Jones & McClements, 2008).

2.3.2.3 Biopolymer ratio and concentration

For mixture of proteins and polysaccharides, the optimum complexation is achieved at a specific ratio of protein to polysaccharides, which depends on conformation, charge density and flexibility of biopolymers (Jones & McClements, 2011; Ye, 2008). Study (Singh et al., 2007) used gelatin and agar to investigated the transition between soluble and insoluble complex. The ratio of gelatin to agar affected value of pH_c at low ratio ($r < 1$; abundance of agar), soluble complex could be formed at pH around 7.5 while at high ratio, the same could be achieved at pH around 9. At high concentration of biopolymers, no complexation occurred due to high charge screen and occurrences of phase separation (A. Ye, Flanagan, & Singh, 2006). The ratio of protein to polysaccharides also affect protein self-association at low pH (Jones, Lesmes, Dubin, & McClements, 2010).

2.3.2.4 Nature of biopolymers

Proteins and polysaccharides from different sources and processing procedures have different molecular characteristics, such as molecular weight, conformation, hydrophobicity and charge density (Jones & McClements, 2011). These differences will influence the interaction between two biopolymers. When they

form complex at pH value between pH 4.5 to 7.0, β -lactoglobulin complexed more with LM pectin than with HM pectin, which is due to more carboxyl groups in LM pectin and carried higher charge density (Girard, Turgeon, & Gauthier, 2002). When heating β -lactoglobulin with other anionic polysaccharides, smaller particles were formed with the presence of carrageenan across entire pH range compared with other polysaccharides (Jones & McClements, 2011).

2.3.2.5 Processing

Processing factors like heating temperature, heating time, shear rate and pressure can affect the formation and stability of protein-polysaccharides complex (Aiqian Ye, 2008). At high temperature, denaturation of protein cause the exposure of more reactive site, enhance the complexation between protein and polysaccharides. When they form soluble complex through heating, pH, holding temperature, holding time, ionic strength need to be controlled to obtain desirable biopolymers particles (Jones & McClements, 2011).

2.4 Emulsion

2.4.1 Introduction of emulsion

Emulsion is one of the most common systems in food, it is a mixture of two or more immiscible liquids. Emulsions can be classified into two categories: simple emulsion and multiple emulsions. Simple emulsions can be divided into two class: oil-in-water (O/W), and water-in-oil (W/O). Multiple emulsions consist of a more sophisticated system. The simplest example is oil-in-water-in-oil (O/W/O) or water-in-oil-in- water (W/O/W) double emulsions (Bouyer, Mekhloufi, Rosilio, Grossiord,

& Agnely, 2012). Emulsions can also be classified into three categories according to droplets size: macroemulsions (from 0.1-100 μ m), microemulsions (from 2nm to 50 nm), and nanoemulsions (from 10 to 100 nm). Macroemulsions usually show white color due to light scattering, both microemulsions and nanoemulsions show optically transparent, but some of nanoemulsions tend to be slightly turbid (McClements, 2011).

During emulsification, the interfacial free energy between the continuous and the dispersed phases increased significantly. The dispersed phase, like oil droplets tend to fuge into large droplets to increase interfacial area and minimize interfacial energy. This instability show up by various mechanisms: flocculation, coalescence, creaming or sedimentation, and Ostwald ripening (Bouyer, Mekhloufi, Rosilio, Grossiord, & Agnely, 2012; McClements, 2015). Coalescence and flocculation are the major mechanisms of emulsion destabilization. Coalescence is the process by which small droplets fuse into larger droplets. Flocculation refers to the process by which droplets are caused to clump together into a floc rather than fuse into larger droplets. There are two types of flocculation: bridging and depletion. In some cases, the flocculation is reversible in that simple stirring can break down flocs. In other cases, it is irreversible since the droplets coalesced. Creaming and sedimentation involve the migration of globules to the top or bottom of the dispersion. Finally, Ostwald ripening is an observed phenomenon in solid solution or liquid sols. Small particles dissolved in continuous phase and redeposition on larger particles, driven by a higher Laplace pressure. Ostwald ripening generally occurred in water in oil emulsions.

2.4.2 Protein-polysaccharides mixture stabilized emulsion

The studies found that combination of protein and polysaccharides in the same system improved both their functionalities in emulsions. Proteins can quickly absorb onto oil surfaces, while polysaccharides provide additional steric repulsion and enhanced viscosity.

Protein-polysaccharides complexation from non-covalent interactions which mainly are driven by attractive electrostatic interaction, which is commonly showed in the food system. Numerous studies have shown that combination of two macromolecules improved emulsification properties. There are many review papers well discussed the understanding of the nature of different protein polysaccharides complex (Bouyer, Mekhloufi, Rosilio, Grossiord, & Agnely, 2012; Dickinson, 2009; Evans, Ratcliffe, & Williams, 2013; McClements, 2011).

2.4.2.1 Emulsion preparation

There are two methods to form stable emulsions based on electrical interaction between protein and polysaccharides (Evans, Ratcliffe, & Williams, 2013; Guzey & McClements, 2006). Traditionally, emulsions are prepared by using mixture of two biopolymers. Proteins and polysaccharides are premixed, then final emulsions were formed at pH that two macromolecules carry opposite charges and have strong attractive interaction. The second method is named the layer-by-layer (LBL) electrodeposition technique. Complexation occurs directly at the surface of droplets. Primary emulsion is prepared by homogenizing oil and protein solution. Then the polysaccharide solution is mixed with the primary emulsion to form secondary emulsion. The secondary emulsion can be mixed into another solution containing

electrolytes that have opposite charge to the previous one if needed. The formation of the second layer allows charges reversal and additional steric repulsions. The LBL technique could be utilized in encapsulation of sensitive food components, control lipid releasing. And this method can be easily adapted to high-scale production due to fastness and simplicity (Bouyer, Mekhloufi, Rosilio, Grossiord, & Agnely, 2012). However, there are still some limitations. It needs careful control of the system composition and preparation condition to obtain stable multilayer particles. Insufficient polysaccharides can cause bridging flocculation; while when the concentration of polysaccharides exceeds critical point, depletion flocculation will be presented. One study compared two methods by using sodium caseinate and dextran sulfate to form mixed emulsions and LBL emulsions (Jourdain, Leser, Schmitt, Michel, & Dickinson, 2008). The stability of mixed emulsion is greater than a bilayer emulsion with same biopolymer constitute. However, another study used whey protein isolate and tragacanthin to form mixed and LBL emulsions and found LBL emulsions showed better stability against various ionic strengths and freeze–thaw treatments (Azarikia, Abbasi, Scanlon, & McClements, 2017). The difference conclusion could due to different systems and emulsion preparation conditions.

2.4.2.2 Factors affect emulsion stability

The factors affecting emulsifying properties of protein-polysaccharides complexes are similar to those affecting the complex, like nature of biopolymers, biopolymers ratio, intrinsic factors (pH and ionic strength) and preparation methods.

In the presence of polysaccharides, emulsion stability generally depends on the biopolymer ratio. With addition of polysaccharides, the surface charge density of

droplets significantly increased, providing additional repulsion forces and layer between droplets to prevent flocculation and coalescence. For example, 0.5% WPI mixed with 0.05%- 0.5% carboxymethylcellulose (CMC) significantly decreased droplets size and ζ -potential of emulsion at pH 5.2. (Huan, Zhang, & Vardhanabhuti, 2016). Similar results were reported that stability of emulsions was improved when carrageenan was added into emulsions coated by 0.5% whey protein (Gu, Decker, & McClements, 2004, 2005). However, relative low and high concentration of polysaccharides can induce droplets flocculation, especially with LBL methods. Large aggregates were observed in β -lactoglobulin coated emulsion with small amount of ι -carrageenan (Gu, Decker, & McClements, 2004). It could be due to the bridging flocculation that single carrageenan molecule absorbed onto two or more protein coated droplets. When biopolymer concentration is too high, the depletion flocculation occurs. Emulsions stabilized by 0.5% β -lactoglobulin with κ -carrageena were very unstable to creaming when carrageenan concentration $\geq 0.04\%$, which was induced by a steric-exclusion effect. Besides biopolymer ratio, the construction of polysaccharides affects emulsification properties. Huan (Huan, Zhang, & Vardhanabhuti, 2016) found that emulsions contain higher molecule weight CMC were more stable than those with low molecular weight CMC. In another study, Gu and coworkers examined influence of carrageenan type on the stability of β -lactoglobulin stabilized emulsions. At pH 3 and 5, extensive droplets aggregation and creaming were observed with addition of λ - and ι - carrageenan (Gu, Decker, & McClements, 2005). The charge density, chain length, charge distribution and degree of branching can influence polysaccharides ability to interact with protein and absorption (Guzey & McClements, 2006; Surh, Decker, & McClements, 2006).

Electrostatic interaction comes from electrical attraction between ions carrying different charges, so pH of emulsions highly affect the interactions between two biopolymers and the final condition of emulsions. Neiryneck (Neiryneck et al., 2007) compared emulsions formed at pH 4 and 5.5 from whey protein and pectin mixtures. The amount of pectin required is larger at pH 5.5 to reach optimum condition. The interaction between two molecules is relatively weak at pH 5.5 when compared to pH 4. In another study, Gu (Gu, Decker, & McClements, 2004) compared emulsifying properties of β -lactoglobulin (β -lg) stabilized emulsions with ι -carrageenan at different pH. At pH 7 and 8, the droplets charge did not change with addition of carrageenan, which is due to weak interaction between β -lg and carrageenan. While at pH 3-5, droplets charge decreased with increasing carrageenan density and showed higher stability.

The ionic strength determines the strength and range of electrostatic interaction intra- and inter-molecular, hence affects layer structure and thickness (Guzey & McClements, 2006). A study (Guzey & McClements, 2007) found that when using the Layer-By-Layer method to form emulsion, stable polysaccharides-proteins stabilized emulsion could be formed if salt added after pectin absorbed onto protein coated droplets, while adding salt before pectin adsorption led to droplets flocculation.

2.4.3 Heated protein-polysaccharides complex stabilized emulsion

Recently, some studies investigated the emulsion stabilized by heated protein-polysaccharides complex. Salminen and Weiss (Salminen & Weiss, 2014) fabricated heated whey protein-pectin complex at pH 4.75 by heating at 85 °C for 20 min. Emulsion stabilized by the complex showed notable thermal and salt stability at low pH. Xu and coworkers (Xu, Wang, Jiang, Yuan, & Gao, 2012) reported that conjugates of WPI-beet pectin formed at pH 7 had improved emulsifying properties compared with unheated WPI-beet pectin mixtures. Emulsions with conjugate showed much smaller droplets size, more Newtonian flow behavior, improved freeze-thaw stability and reduced degradation of β -carotene in emulsions. However, there is no study related to heated protein-polysaccharides complex in emulsions.

CHAPTER 3

MATERIAL AND METHODS

3.1 Material

Whey Protein isolate (BiPro) was kindly provided by Davisco Foods International Inc. (Le Sueur, MN). According to the manufacturer, the powdered WPI contained 97.9 wt% protein and 2.1 wt% ash and 0.3 wt% fat (dry weight basis) and 4.7 wt% moisture. Pectin (LM-12 CG) was kindly donated by CP Kelco Inc (Atlanta, GA). All chemicals used were of analytical grade.

3.2 Sample preparation

WPI stock solution (10 wt% protein) and pectin stock solutions (2 wt%) were prepared by slowly dissolving the protein or polysaccharide powder in the deionized (DI) water (>17 MΩ). WPI and pectin solutions were stirred for 2 h at room temperature and at 65°C, respectively. Both stock solutions were left overnight in the refrigerator (4°C) for complete hydration. On the next day, the solutions were warmed to room temperature for 2 h.

3.2.1 Formation of heated WPI-pectin complex

Stock solutions of WPI and pectin were mixed in appropriate amounts and their pH was adjusted to 7.0, 6.5 or 6.0. DI water was added to reach the final protein concentration of 3.0 wt% and the final pectin concentration of 0 to 0.60 wt%. The mixtures were gently stirred at room temperature for 2 h before being heated in a

temperature-controlled water bath at 85 °C for 30 min and cooled. Samples were kept in at 4 °C for 18 h.

3.2.2 Formation of heated WPI and pectin mixture

For the heated mixture (H mix), WPI polymers was prepared by heating the WPI solution with an initial concentration of 3 wt% at pH 7.0 at 85 °C for 30 min. After cooling, pectin stock solution was added at appropriate amount and the pH was adjusted to pH 7.0. DI water was added to reach the final protein concentration of 2.0 wt% and the final pectin concentration was 0.40 wt%.

3.2.3 Emulsion preparation

All emulsions (5 wt% oil, 0.5 wt% protein, 0- 0.10 wt% pectin and pH 5.5) were obtained by a two-step process emulsification. Coarse emulsions were prepared by blending oil and aqueous solution together using a laboratory homogenizer, Ultra Turrax T-25 (IKA Instruments, Germany) at 12,000 rpm for 15 seconds at room temperature. Final emulsion samples were obtained by using an ultrasonic processor (Sonics VC 505, power 500 W, frequency 24 kHz) with a sonotrode (6 mm, approx. length 142 mm, titanium) for 3 minutes (40% amplitude of maximum power). Sodium azide (0.02 wt%) was added as an antimicrobial agent. After emulsification, the emulsions were slowly acidified to pH 5.5. Then emulsions were stirred for 1 h before final sonication for 30 s using similar condition as described above.

3.4 Particle size and ξ -potential measurement

Particle size distribution and ξ -potential of aqueous solutions and emulsions were determined with a Zetasizer Nano ZS (Malvern Instruments Ltd., Worcestershire, UK) equipped with a 633-nm laser and 173° detection optics at 25°C. Each aqueous solution was diluted to 0.3 wt% protein with 5 mM phosphate buffer at respective pH. Emulsion samples were diluted in a 1:100 ratio with 5 mM phosphate buffer at pH 5.5. All measurements were carried out in triplicate.

3.5 Rheological properties measurement

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

3.6 Creaming index measurement

Fresh emulsion samples (10 mL) were 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 at ambient temperature (~22°C) for 15 days. Emulsion stability was determined by measuring the height of a distinctive clear

or semi-transparent bottom serum phase layer on day 5, 10, and 15 after emulsion preparation. The extent of creaming was characterized by creaming index (CI, %) = $(HS/HI) \times 100\%$, where HS is the height of the serum layer and HI is the initial height of the emulsion. Creaming measurements were conducted in triplicate.

3.7 Statistical analysis

Minitab (Minitab Inc., version 18) was used to analyze significant differences ($p < 0.05$) between treatments by one-way ANOVA. The comparisons between the mean values were evaluated by the Tukey HSD test.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Emulsification properties of heated complex

4.1.1 Particle sizes and surface charge of complex

Effects of heating pH and pectin concentration on formation of heated WPI-pectin complexes (Cpx) were determined by measuring the aggregates size and surface charge. The Z-average mean diameter of Cpx solution is shown in Figure 1. Similar to what was reported by (S. Zhang, Zhang, Lin, & Vardhanabhuti, 2012), the mean diameters of unheated WPI and pectin were 5 nm and 700 nm (data not shown), respectively. Mean particle sizes significantly increased after heating due to the formation of heated soluble aggregates. Mechanism of whey protein aggregation has been well established (Nicolai, Britten, & Schmitt, 2011; Roefs & De Kruif, 1994; Wijayanti, Bansal, & Deeth, 2014). During heating, the folded hydrophobic groups and thiol groups become exposed and interact with other protein molecules through disulfide bonds, non-covalent interactions and hydrophobic interaction (Ryan, Zhong, & Foegeding, 2013). Protein heated at pH 6.0 showed significantly larger particle size than those heated at pH 6.5 and pH 7.0. A Study found that the different mechanism involved in whey protein aggregation below and above pH 6.5 (Xiong, 1992). At neutral pH, the disulfide bond is highly responsible for the formation of large aggregates, whereas non-covalent interactions are more prominent at lower pH (Hoffmann & vanMil, 1997). The opaque color (Figure 2) and size data at pH 6.0

indicate the formation of large aggregates. It is likely due to the relatively weak repulsion between protein molecules which cannot prevent self-association of protein.

When WPI and pectin were heated together, the particle size distribution showed a single peak in all treatments, whereas peaks of pectin and protein disappeared, indicating that soluble complexes were formed between two biopolymers. Studies reported that soluble complexes could be formed at neutral pH (Dickinson, 1998; Girard, Turgeon, & Gauthier, 2002; Zhang, Hsieh, & Vardhanabhuti, 2014). At $\text{pH} > \text{pI}$, the interaction still occurs between chain segments of anionic polysaccharides and the positively charged residue ($-\text{NH}_3^+$) groups on the protein. At pH 6, the z-average decreased from 81.1 ± 4.7 nm to 62.7 ± 2.5 nm when heated with 0.15 wt% pectin. Complexation with pectin can suppress interaction between protein molecules by providing additional electrostatic repulsive force (Jones, Decker, & McClements, 2009; Wagoner & Foegeding, 2017; Zhang, Zhang, Lin, & Vardhanabhuti, 2012). Addition of 0.30 wt% pectin led to further decrease in size to a minimum (59.0 ± 6.5 nm). When pectin concentration exceeded 0.45 wt%, size started increasing again, indicating that phase separation became more dominant. The effect of pectin on protein aggregate sizes showed a different pattern at pH 6.5 and 7.0. Increasing pectin concentration led to increased mean particle sizes when mixed biopolymers were heated at pH 6.5 or 7.0. At higher pH value, repulsion between protein molecules was enhanced with additional pectin, resulting in micro phase separation and larger aggregates (Zhang, Hsieh, & Vardhanabhuti, 2014). It should be noted that despite differences in mean diameters the z-average values ranged from 34.6 to 81 nm. Solutions became opaque after heating at pH 6.0, whereas

those heated at pH 6.5 and 7.0 remained translucent. The difference in appearance suggests that different shape of aggregates were formed (Jones & McClements, 2008). Aggregates that are large and more spherical appear opaque while those with more linear shape appear more clear or translucent (Vardhanabhuti & Foegeding, 2008; Vardhanabhuti, Yucel, Coupland, & Foegeding, 2009).

Since the main interaction between whey protein and pectin is electrostatic, change in surface charge density is an ideal tool to investigate the interaction between two biopolymers. ξ -potentials of Cpx formed at different pH and pectin concentration are shown in Figure 3. All heated WPI carried a net negative charge at pH above pI, and became more negative with increasing pH, from -25.1 ± 1.2 mV at pH 6.0 to -27.9 ± 1.5 mV at pH 7.0. At all pH, the value of ξ -potentials became more negative as pectin concentration increased, indicating the existing interaction between protein and pectin. At high pectin concentration, e.g., 0.45 wt% to 0.60 wt%, there is no significant difference in ξ -potential (e.g., plateau around -34 mV), indicating that proteins were fully covered by pectin molecules. Similar trend was reported for heated WPI-pectin complex at pH 4.75 and 6.0-7.0 (Jones, Decker, & McClements, 2009; Zhang, Hsieh, & Vardhanabhuti, 2014) and whey protein-xanthan gum conjugate formed at pH 7 (Benichou, Aserin, Lutz, & Garti, 2007). It is notable that the effect of pH on the change in surface charge was not as prominent as pectin concentration. Interestingly, similar ξ -potentials were observed among different pH at the same pectin concentration ($P > 0.05$). It is possible that the interaction between protein and pectin was still relatively limited at high pH value and more interactions were allowed at lower pH (Salminen & Weiss, 2013) also reported similar surface

charge for heated whey protein-pectin complex formed at pH 6.0-7.0. Particle size and ξ -potential results in this study demonstrate the interactions between protein and pectin through thermal treatment at neutral. Pectin concentration and pH have different effects on particle size and surface charge.

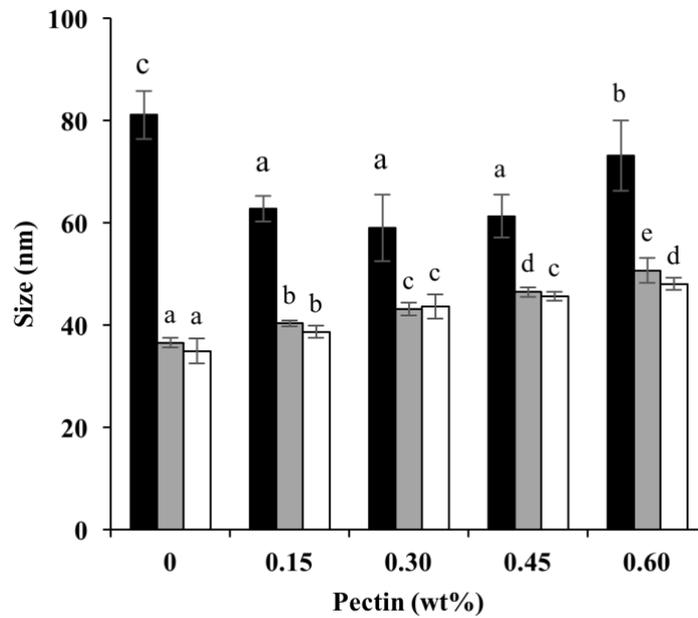
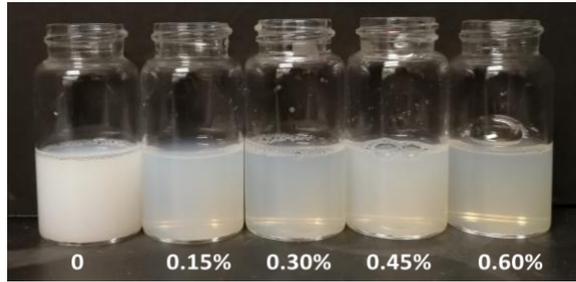
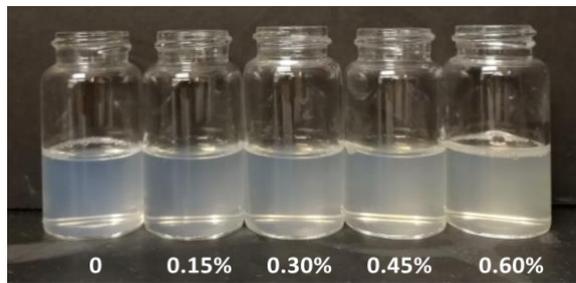


Figure 1 Mean particle sizes of complexes formed at pH 6.0 (black), pH 6.5 (grey), and pH 7.0 (white) and at different pectin concentrations. Different letters indicate significant difference ($p < 0.05$) between samples at the same heating pH.

(a)



(b)



(c)

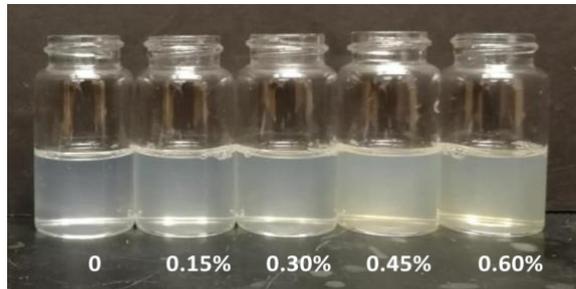


Figure 2 Effect of heating pH and pectin concentration on the aggregation of WPI (3 wt%) heated at pH 6 (a), pH 6.5 (b), and pH 7 (c). From left to right (0, 0.15, 0.30, 0.45, 0.60 wt% pectin).

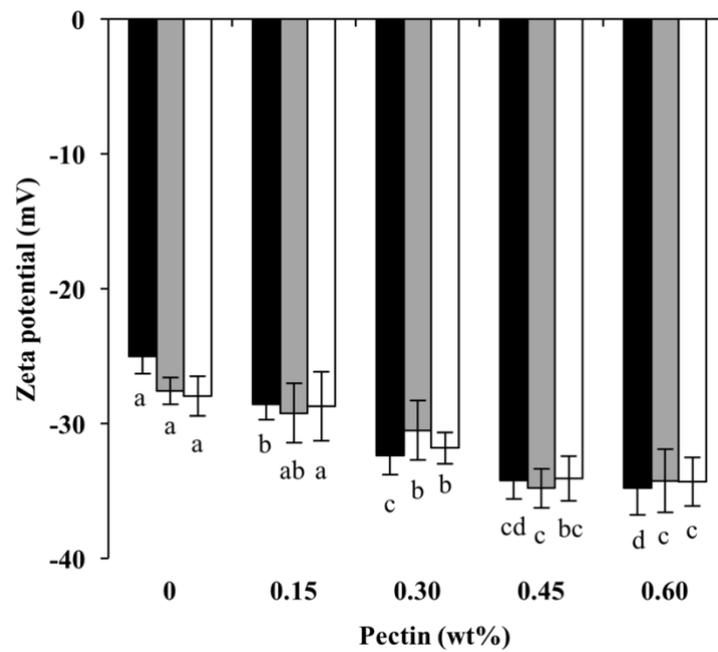


Figure 3 ζ -potential of Cpx formed at pH 6.0 (black), pH 6.5 (grey), and pH 7.0 (white) and different pectin concentration. Different letters indicate significant difference ($p < 0.05$) between samples at the same heating pH.

4.1.2 Formation of WPI-Pectin Complex on Oil Droplets

4.1.2.1 Surface charges of the oil droplets

Heated WPI and heated complex (Cpx) solutions were used as aqueous phase during emulsion formation. The final emulsions contained 5.0 wt % Oil, 0.5 wt% protein and were at pH 5.5. The final concentration of pectin in emulsions was 0, 0.025, 0.050, 0.075 and 0.10 wt%, which corresponded to 0, 0.15, 0.30, 0.45 and 0.60 wt% pectin, respectively in the Cpx solutions.

The influences of formation pH and pectin concentration on the surface charge of fresh emulsions were shown in Figure 4. In the absence of pectin, the net charge of emulsion droplets remained negative at pH 5.5, from -27.1 ± 2.0 mV to -25.6 ± 2.8 mV. Generally, an absolute ζ -potential of at least 30 mV is required to form a stable dispersion. Introducing of pectin caused a noticeable change in z-potential. For emulsions prepared with Cpx formed at pH 6.0, surface charge changed from -27.1 ± 2.0 mV to -30.6 ± 1.5 mV with addition of 0.025 wt% pectin. Anionic groups of pectin bound with cationic patches on the protein through electrostatic interaction, increasing negative charges on the droplet surface. However, z-potential appeared to be constant with further addition of pectin, suggesting the absorption of Cpx or pectin onto the droplet surface was saturated at low pectin concentration (Huan, Zhang, & Vardhanabhuti, 2016). A similar trend has been reported with emulsions stabilized by 0.5 wt% β -lactoglobulin with 0-0.15 wt% pectin at pH 3 and 5 (Gu, Decker, & McClements, 2005). High repulsion on the droplets may prevent additional adsorption

of pectin or Cpx at the interface (Salminen & Weiss, 2014). Interestingly, the saturated concentrations were at 0.075 wt% and 0.10 wt% for pH 7.0 and 6.5, respectively. Aggregate size and the amount of soluble aggregates vs native protein could play a role on the absorption of pectin to protein at the interface. A study found that heating pH significantly affected yield of soluble aggregates from pH 6 to 7 (Schmitt, Bovay, Rouvet, Shojaei-Rami, & Kolodziejczyk, 2007).

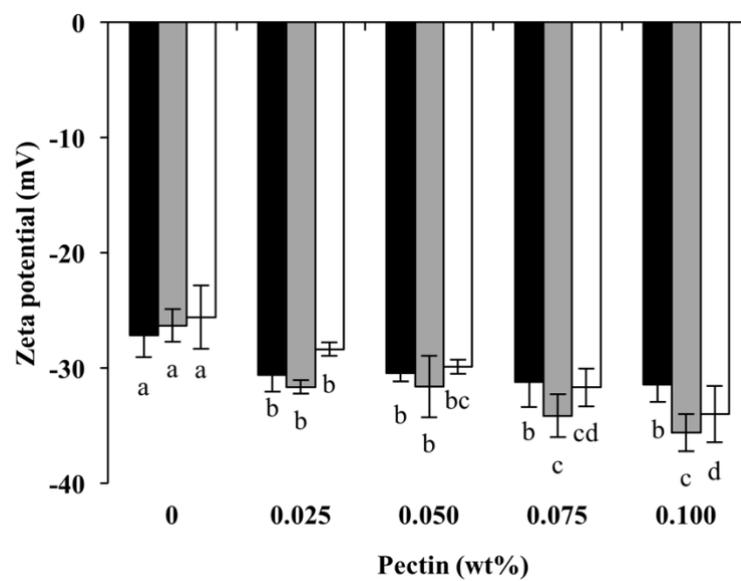


Figure 4 ζ -potentials of emulsions stabilized by Cpx formed at pH 6.0 (black), heated at pH 6.5 (grey), heated at pH 7.0 (white) and different pectin concentration. Different letters indicate significant difference ($p < 0.05$) between samples at the same heating pH.

4.1.2.2 Mean particle diameter of the oil droplets

The combined influence of pectin concentration and heating pH on the mean diameter of droplets is shown in Figure 5. In the absence of pectin, the mean diameter of droplets ranged from $2.80 \pm 0.95 \mu\text{m}$ when WPI was heated at pH 6.0 to $3.37 \pm 0.28 \mu\text{m}$ when WPI was heated at pH 7.0. Since the final pH of emulsions was close to the isoelectric point, the lack of charge repulsion at the interface can promote droplets aggregation (McClements, 2004). Low electrostatic repulsion between oil droplets at pH near pI was too small to prevent flocculation (Huan, Zhang, & Vardhanabhuti, 2016; McClements, 2015; Surh, Decker, & McClements, 2006). Aggregation and flocculation during emulsion preparation could also result in large polydispersity as shown by large standard deviation. Addition of pectin even at 0.025 wt% significantly decreased droplets sizes with Cpx at pH 7.0 due to the additional electric repulsion and steric hindrance from adsorbed pectin. With increasing pectin concentration, the mean droplet sizes continued to decrease, indicating lower degree of flocculation and coalescence due to higher negative charges and steric hindrance from pectin (Akhtar & Dickinson, 2003; Jones, Decker, & McClements, 2009; Turgeon, Schmitt, & Sanchez, 2007; Wagoner, Vardhanabhuti, & Foegeding, 2016).

Influence of heating on emulsion formation was also investigated. The mean droplet sizes of emulsions stabilized by heated protein showed no significant effect ($p < 0.05$) of heating pH. However, heating pH appeared to be one of the major factors in the emulsification properties of Cpx. Emulsions prepared with Cpx formed at pH 7.0 showed the smallest sizes. A study found that 1 wt% WPI heated at pH 7 formed fibrillar aggregates while those heated at pH 6 were mainly compact and spherical.

An intermediate mixture of both morphologies was observed at the intermediate pH of 6.6 (Schmitt, Bovay, Rouvet, Shojaei-Rami, & Kolodziejczyk, 2007). Smaller size and filamentous allowed faster interfacial adsorption of particles formed at higher pH, resulting in the formation of droplets with smaller sizes.

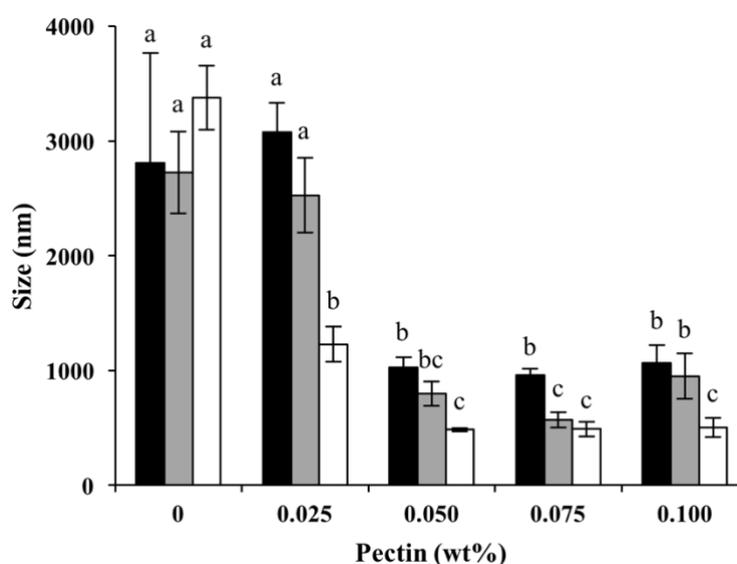


Figure 5 Mean particle sizes of emulsion stabilized by Cpx formed at pH 6.0 (black), heated at pH 6.5 (grey), heated at pH 7.0 (white) and with different pectin concentration. Different letters indicate significant difference ($p < 0.05$) between samples at the same heating pH.

4.1.2.3 Rheological properties of emulsions

The rheological properties were measured immediately after emulsion preparation. Flow behavior curves of fresh emulsions are shown in Figure 6. Power law model was applied to describe the rheological properties. The values of consistency coefficient (K) and flow behavior index (n) are listed in Table 1. Consistency coefficient is a parameter related to viscosity. The flow behavior index

indicates flow behavior of liquid. Generally, flow behavior is divided into three categories: shear-thinning ($n < 1$), shear-thickening ($n > 1$), and Newtonian ($n = 1$).

In this study, all emulsions showed shear-thinning behavior with n less than 1. The shear thinning behavior could be due to the flow characteristic of hydrocolloids in the aqueous phase and flocs been gradually breaking up of flocs under increased shear rate (Quemada & Berli, 2002).

In the absence of pectin, emulsions stabilized by heated WPI exhibited highly shear-thinning behavior with n values of 0.525, 0.520 and 0.448 and K values of 0.047, 0.066 and 0.098 for pH 6.0, pH 6.5 and pH 7.0, respectively. These emulsions also showed a deflection point which might be due to the disruption of flocs, thus reducing the effective volume fraction and viscosity with increasing shear rate (Franco, Berjano, Guerrero, Muñoz, & Gallegos, 1995; Huan, Zhang, & Vardhanabhuti, 2016; Surh, Decker, & McClements, 2006).

The rheological behavior of fresh emulsions was highly influenced by complex formation. The effect of heating pH was pronounced. Emulsions stabilized by Cpx formed at pH 6.0 all showed a deflection point. Generally, at the same pectin concentration, emulsions stabilized by Cpx formed at pH 6.5 had higher K and lower n values compared to those stabilized by Cpx formed at pH 7.0, indicating that these emulsions were more viscous and shear thinning. Emulsions stabilized by Cpx formed at pH 7 were less viscous and less shear thinning with increasing pectin concentration from 0 to 0.075%. The lower viscosity and more Newtonian behavior could be due to the smaller droplet sizes. However, at 0.1% pectin, the emulsion became more viscous and higher shear thinning which could be due to the effect of

unadsorbed pectin on the continuous phase (Evans, Ratcliffe, & Williams, 2013; Huan, Zhang, & Vardhanabhuti, 2016; Sun, Gunasekaran, & Richards, 2007). It should be noted that, at lower pectin concentrations, emulsion stabilized by Cpx formed at pH 6.0 showed the lowest n and the highest K value ($p < 0.05$), which corresponded to large droplet sizes as previously shown. The presence of large droplets due to coalescence in emulsion can cause an increase in viscosity and shear-thinning behavior (McClements, 2007). Since droplet size measurement was made on diluted emulsion, differences in rheological properties suggest that emulsions stabilized with Cpx at pH 6.0 and 6.5 could have some degree of flocculation but flocs dissociated with dilution.

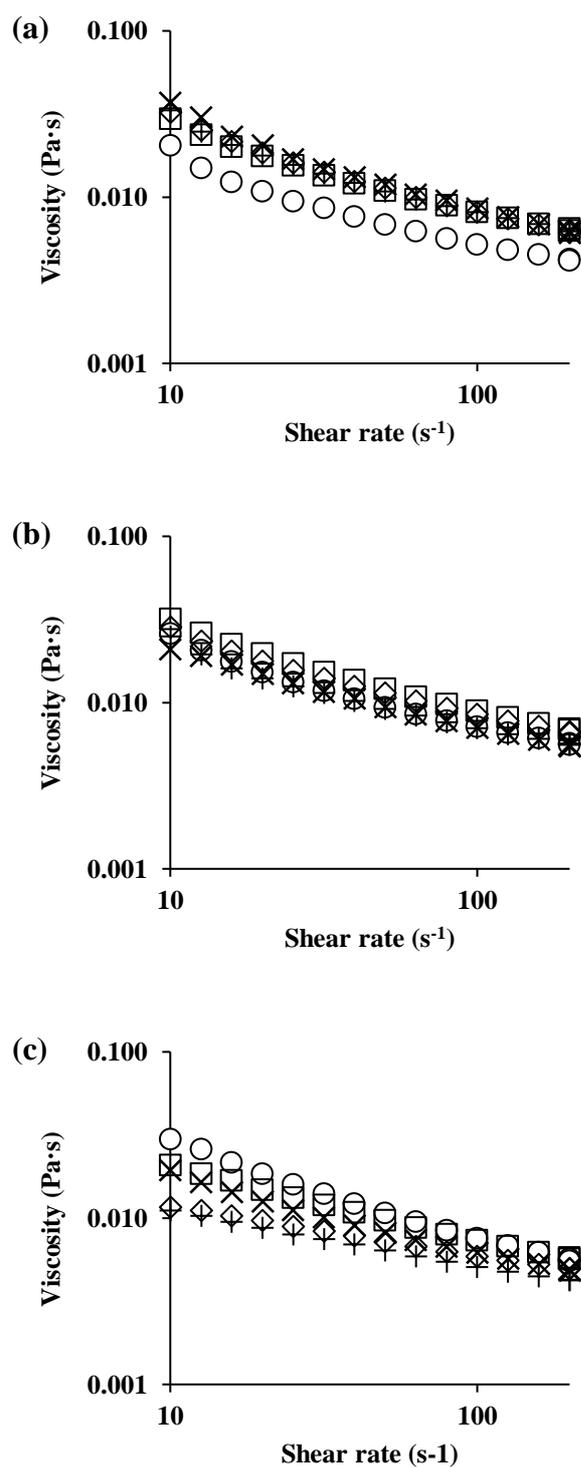


Figure 6. Apparent viscosity of fresh emulsions stabilized by Cpx formed at pH 6 (a), pH 6.5 (b) and pH 7 (c) with different pectin concentrations; \circ = 0; \times = 0.025%; $+$ = 0.050%; \diamond = 0.075%; \square = 0.100%. The viscosity in flow curve is the average of three samples at corresponding shear rate.

Table 1 Power Law model parameters of emulsions stabilized by Cpx formed at different pH and pectin concentrations.

Heating pH	Pectin wt%	K (Pa·s ⁿ)	n
6.0	0*	0.047	0.520
	0.025*	0.067	0.528
	0.050*	0.085	0.494
	0.075*	0.091	0.482
	0.100*	0.078	0.518
6.5	0*	0.066	0.529
	0.025	0.057	0.552
	0.050	0.058	0.544
	0.075	0.074	0.541
	0.100	0.088	0.564
7.0	0*	0.099	0.448
	0.025	0.049	0.556
	0.050	0.023	0.679
	0.075	0.023	0.708
	0.100	0.054	0.566

Consistency coefficient (K) and flow behavior index (n) were determined by fitting the flow curve to the Power Law model. * indicates the presence of a deflection point at low shear rate (<10 s⁻¹) in the flow behavior curve.

4.1.2.4 Emulsion stability

Figure 7 shows the effect of pectin concentration and Cpx formation pH on emulsion stability against creaming during 15 days of storage. It should be noted that emulsion formed with unheated WPI separated into two layers within one day. Separated emulsions showed two layers: opaque cream layer at the top and a transparent layer at the bottom, suggesting that all of the droplets were aggregated and rapidly moved upwards due to gravity (Surh, Decker, & McClements, 2006). In the absence of pectin, emulsions stabilized by heated protein showed separation within 1 day. The high viscosity shows in emulsions with heated protein did not decrease the creaming velocity of flocs sedimentation, indicating that part of the flocculation occurred during emulsification. Large droplet size and insufficient electrostatic repulsion in protein stabilized emulsion caused a high degree of flocculation and coalescence.

Cpx clearly improved emulsion stability and the effects of heating pH and pectin concentration were prominent. Regardless of complex formation pH, increasing pectin concentration led to improved stability. The improvement of creaming stability is in line with smaller droplets and increased negative charges. During pH adjustment to 5.5, more binding sites on the proteins were available to interact with pectin. Free excess pectin in the aqueous phase could interact with protein-covered droplets (Guzey & McClements, 2006). At low pectin concentration, insufficient charge repulsion and steric stabilization as well as bridging flocculation could lead to demixing of emulsions (Jourdain, Leser, Schmitt, Michel, & Dickinson, 2008). Stabilization from both charge and steric effect could be sufficient at high

pectin concentration. In addition, cross-linking between pectin molecules formed relatively weak gel-like network, increasing the viscosity and enhancing stability as seen with emulsions stabilized by Cpx formed at pH 7.0 (Gu, Decker, & McClements, 2005).

Across the pectin concentrations, emulsions stabilized by Cpx formed at pH 6.0 were the least stable, and creaming was observed within one day even at the highest pectin concentration. Emulsions stabilized by Cpx formed at pH 7.0 were the most stable. No creaming was observed after 15-day storage when the emulsions contained at least 0.05 wt% pectin. The effect of heating pH could be supported by the size, ζ -potential and viscosity results.

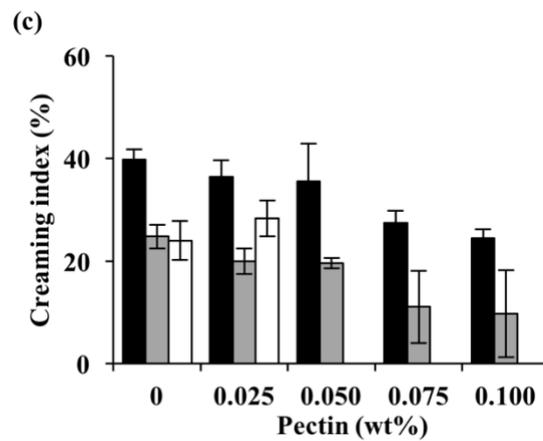
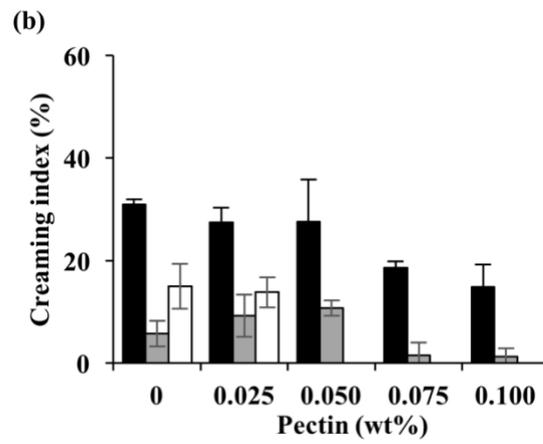
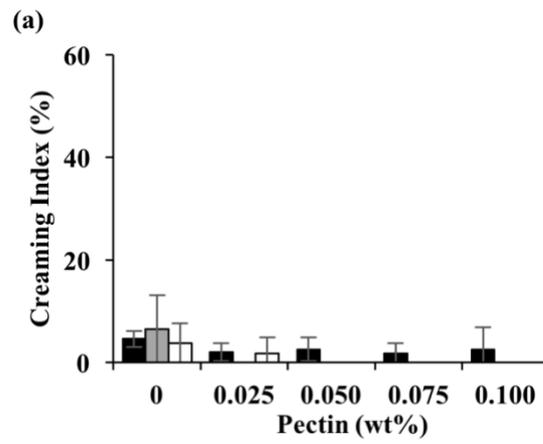


Figure 7 Creaming stability of emulsions prepared with Cpx formed at pH 6.0 (black bar), pH 6.5 (grey bar), and pH 7.0 (white bar) at different pectin concentrations after day 1 (a), day 5 (b), and day 15 (c).

4.2 Effect of different mixed systems

4.2.1. Particle size and zeta-potential of biopolymers

To confirm the effectiveness of heated complex, three different complex systems were compared. Unheated protein (UH ptn) was transparent at pH 7.0, indicating no large aggregates in the solution. Heated protein (H ptn) remained transparent after heating, suggesting the formation of small and more linear aggregates.

The mean particle size of biopolymer solutions was measured to confirm the formation of soluble complex (Figure 8). The result of unheated protein were >200 nm (data not shown), while the volume distribution showed the majority of particles' size were < 6.0 nm, suggesting a small number of large particles in the solution or that the structure of UH ptn is not suitable for DLS measurement. Addition of pectin led to the formation of larger particles for both H mix and Cpx. The particle size of Cpx is significantly smaller than H mix ($P < 0.05$), indicating the interaction between protein and pectin was different in the two emulsions. Comparing the sizes of heated protein with added pectin and heated mixture of protein and pectin at pH 5.8 (Jones, Decker, & McClements, 2010) also reported that two types of protein-pectin complexes having different structures were formed.

ζ -potential of biopolymers was measured at pH 7.0 (Figure 9). UH ptn and H ptn had a ζ -potential of -26.6 ± 0.5 mV and -27.9 ± 1.5 mV, respectively. A slight increase in negative charge of WPI after heating was reported (Ryan et al., 2012). H

mix and Cpx had a ζ -potential of -28.6 ± 2.3 mV and -34.3 ± 1.8 mV. Higher negative charge of Cpx suggests that the interactions between protein and pectin could be enhanced during heating. A similar result was reported by Zhang (Zhang, Hsieh, & Vardhanabhuti, 2014).

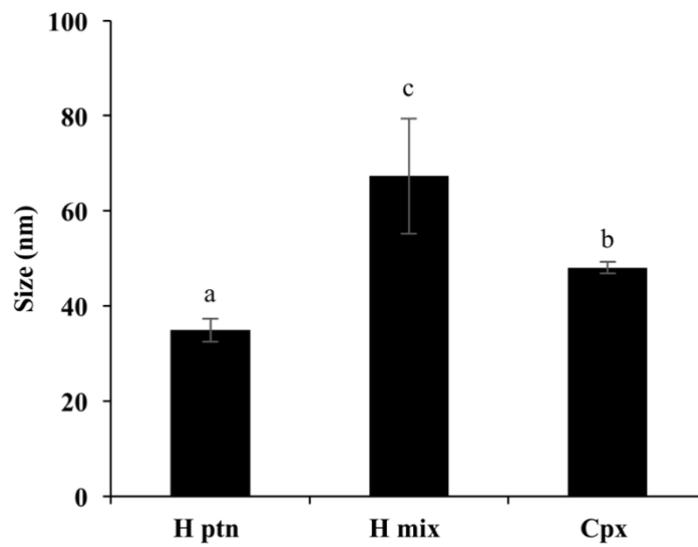


Figure 8 Mean particle sizes of unheated protein (UH ptn), heated WPI (H ptn), heated WPI with added pectin (H mix) and Cpx. Different letters indicate significant difference ($p < 0.05$) between samples.

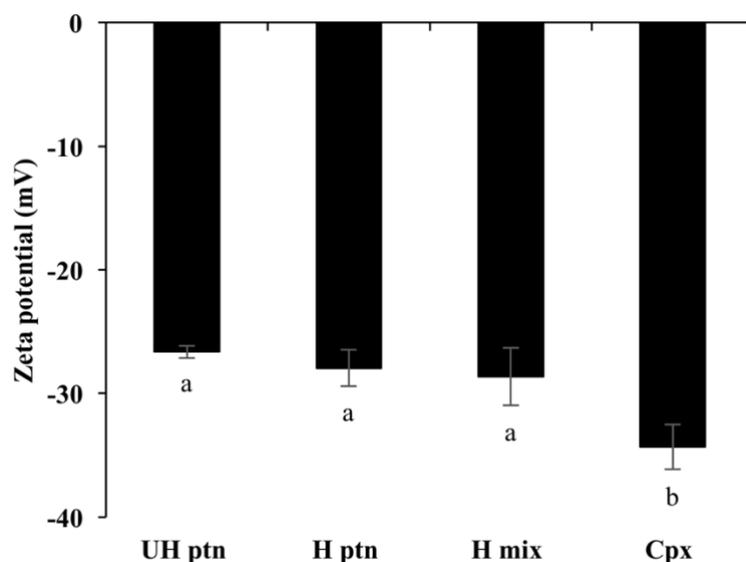


Figure 9 ζ -potentials of heated WPI (H ptn), heated WPI with added pectin (H mix) and Cpx. Different letters indicate significant difference ($p < 0.05$) between samples.

4.2.2. Emulsions

4.2.2.1 Surface charge and mean particle diameters of the oil droplets

Biopolymers were used as emulsifiers to form emulsions at pH 7.0. The final emulsions contained 5.0 wt% oil, 0.5 wt% protein, and the final pH was adjusted to 5.5. The final pectin concentrations in the emulsions were 0 or 0.10 wt%. In the absence of pectin, the ζ -potential of emulsions stabilized by UH ptn and H ptn was -22 ± 2.5 and -25.6 ± 2.8 mV (Figure 10). As shown previously, heating exposes more charged residues on the protein and enhances the interaction leading to increased net negative charges in Cpx sample. Emulsion stabilized with H mix showed slightly lower ζ -potentials than those with Cpx, indicating more pectin absorbed.

The mean droplets size of emulsions is shown in Figure 11. In the absence of pectin, mean droplets size of emulsions with UH ptn and H ptn were 1256 ± 91 nm

and 3377 ± 280 nm. Aggregation and flocculation occurred due to insufficient repulsion force at pH near pI. Addition of pectin significantly decreased droplets size to less than 600 nm. Emulsions with H mix had the lowest size of 407 ± 13 nm. More absorbed pectin provided more repulsion force and steric hindrance between droplets, preventing them from forming large aggregates (Neiryneck et al., 2007).

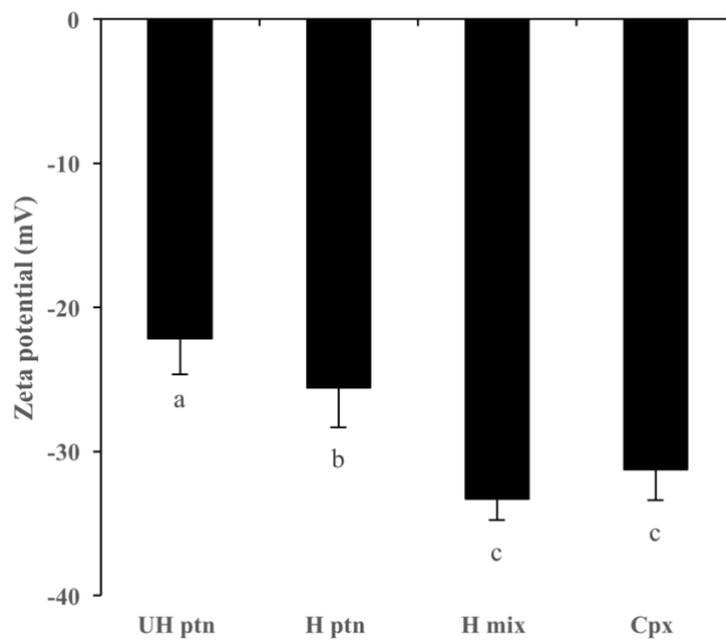


Figure 10 ζ -potential of emulsion droplets stabilized by biopolymers. Different letters indicate significant difference ($p < 0.05$) between samples.

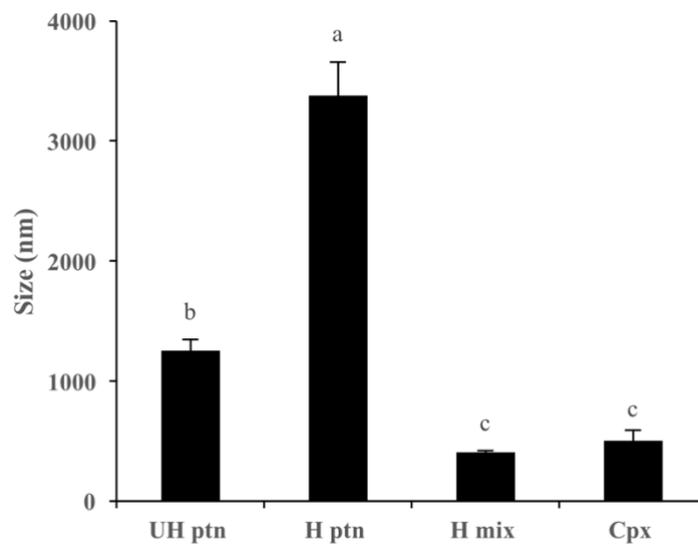


Figure 11 Mean droplets size of emulsion stabilized by biopolymers. Different letters indicate significant difference ($p < 0.05$) between samples.

4.2.2.2 Rheological properties of emulsions

Flow behaviors of emulsions are shown in Figure 12. Consistency coefficient and flow behavior index were obtained by fitting flow curve with Power law model (Table 2). All emulsions exhibited a shear-thinning behavior with n value less than 1. Emulsions with protein alone showed low n values and high K values with a deflection point, suggesting that protein-stabilized emulsions were relatively unstable (Huan, Zhang, & Vardhanabhuti, 2016; Surh, Decker, & McClements, 2006). The deflection point was not observed for emulsions with H mix and Cpx. Introduction of pectin significantly decreased K value and increased n value ($p < 0.05$) in biopolymer mixtures. Emulsions with H mix exhibited more Newtonian behavior, while emulsions with complex showed significant higher K value and lower n value. The difference is likely due to the different structure of biopolymers on the surface of oil droplets.

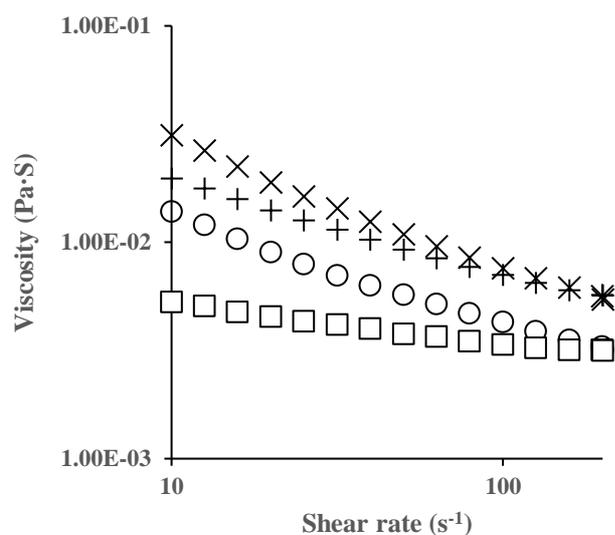


Figure 12 Apparent viscosity of fresh emulsions stabilized by \circ =UH ptn; \times = H ptn; \square = H mix; $+$ =Cpx. The viscosity in flow curve is the average of three samples at corresponding shear rate.

Table 2 Power law model parameters for emulsions with different biopolymers.

Treatment	Pectin (%)	K (Pa·s ⁿ)	n
UH ptn	0*	0.0377 ^c	0.528 ^b
H ptn	0*	0.0990 ^a	0.448 ^b
H mix	0.10	0.0077 ^c	0.937 ^a
Cpx	0.10	0.0538 ^b	0.422 ^b

Consistency coefficient (K) and flow behavior index (n) were determined by fitting power flow curve to the Power Law model. * indicate there is a deflection point at low shear rate ($<10 \text{ s}^{-1}$) in the flow curve. Different letters indicate significant difference between samples.

4.2.2.3 Creaming stability

Creaming stability of emulsions during 15-day storage is shown in Figure 13. Emulsion stabilized by unheated protein showed separation within one day. The weak repulsion and large droplets size favored flocculation and coalescence (Gu, Decker, & McClements, 2004). H ptn-stabilized emulsion showed slightly improved stability, but creaming was still observed in 24 hr. Improvement of emulsion stability with heated protein could be due to the thicker layer and higher electrostatic repulsion force. Heating may help generate stable nanoparticles or aggregates which may help improve emulsion stability (Dybowska, 2011). In the presence of pectin, the stability of emulsions showed significant improvement. Adsorbed pectin on the surface of protein-stabilized oil droplets provided extra repulsion force and steric hindrance. However, separation still occurred in emulsions with H mix after 15 days. Emulsion stabilized by Cpx was the most stable, though emulsion stabilized by H mix had lower surface charge and smaller size. This could be due to different interfacial structures of the adsorbed layer and higher viscosity of the emulsion (e.g., Cpx sample).

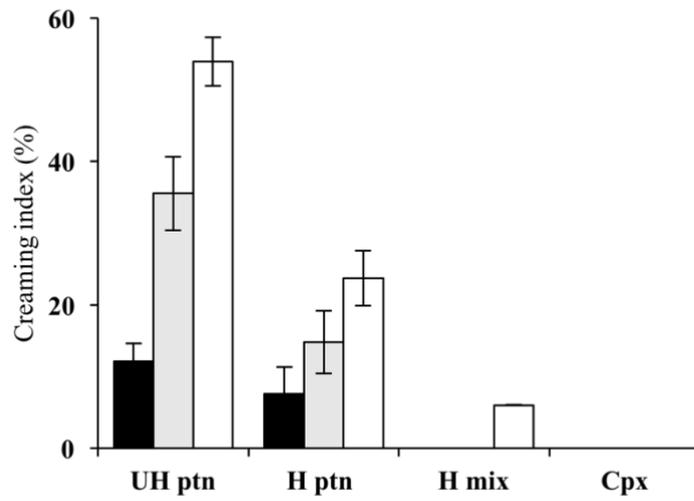


Figure 13 Creaming stability of emulsions stabilized by different mixed WPI and pectin systems at room temperature for 15 days. Day 1 (black bar), day 5 (grey bar) and day 15 (white bar).

CHAPTER 5

CONCLUSION

5.1 Conclusion

The objective of the thesis was to investigate the emulsification properties of heated soluble complex formed at pH above pI. The effect of pectin concentration, heating pH, and complex formation methods were investigated. Complexation between protein and pectin led to changes in surface charge and structure of the heated aggregates. Results showed that Cpx formed stable emulsions at pH near pI. Stability of emulsions improved with increasing pectin concentration. With 0.025 wt% pectin addition in emulsions, mean particle diameters and surface charge of droplets decreased significantly ($p < 0.05$). Added pectin provided extra electrostatic and steric repulsion between droplets. Cpx formed at pH 7.0 and pH 6.5 showed different size change trend compared to Cpx heated at pH 6.0, suggesting heating pH affected final structure of the aggregates. Improved stability of emulsions was observed with Cpx formed at pH 6.5 and pH 7.0, while stability was not achieved with Cpx formed at pH 6.0. Maximum stability was achieved when Cpx was formed at pH 7.0 and with 0.60 wt% pectin.

The benefit of Cpx was tested by comparing its emulsification properties with WPI and heated WPI with added pectin. Both H mix and Cpx showed improved emulsification properties compared to heated WPI, and emulsions stabilized by Cpx had higher viscosity and was more stable. These results confirmed our hypothesis that

the method of complex formation played an important role in the emulsification properties of mixed WPI and pectin.

5.2 Future study

Further study may focus on i) applying Cpx in food emulsions, ii) stability of Cpx-stabilized emulsion against salt and oxidation, and ii) formation of heated Cpx based on other biopolymer systems.

REFERENCE

- Akhtar, M., & Dickinson, E. (2003). Emulsifying properties of whey protein–dextran conjugates at low pH and different salt concentrations. *Colloids and Surfaces B: Biointerfaces*, 31(1-4), 125-132. doi:10.1016/s0927-7765(03)00049-3
- Akhtar, M., Dickinson, E., Mazoyer, J., & Langendorff, V. (2002). Emulsion stabilizing properties of depolymerized pectin. *Food Hydrocolloids*, 16(3), 249-256. doi:[https://doi.org/10.1016/S0268-005X\(01\)00095-9](https://doi.org/10.1016/S0268-005X(01)00095-9)
- Akhtar, M., & Ding, R. (2017). Covalently cross-linked proteins & polysaccharides: Formation, characterisation and potential applications. *Current Opinion in Colloid & Interface Science*, 28, 31-36. doi:10.1016/j.cocis.2017.01.002
- Azarikia, F., Abbasi, S., Scanlon, M. G., & McClements, D. J. (2017). Emulsion stability enhancement against environmental stresses using whey protein–tragacanthin complex: Comparison of layer-by-layer and mixing methods. *International Journal of Food Properties*, 1-12. doi:10.1080/10942912.2017.1362651
- Beijerinck, M. W. (1910). Ueber Emulsionsbildung bei der Vermischung wässriger Lösungen gewisser gelatinierender Kolloide. *Zeitschrift für Chemie und Industrie der Kolloide*, 7(1), 16-20. doi:10.1007/bf01503954
- BeMiller, J. N. (1986). An Introduction to Pectins: Structure and Properties *Chemistry and Function of Pectins* (pp. 2-12).
- Benichou, A., Aserin, A., Lutz, R., & Garti, N. (2007). Formation and characterization of amphiphilic conjugates of whey protein isolate (WPI)/xanthan to improve surface activity. *Food Hydrocolloids*, 21(3), 379-391. doi:10.1016/j.foodhyd.2006.04.013
- Bouyer, E., Mekhloufi, G., Rosilio, V., Grossiord, J. L., & Agnely, F. (2012). Proteins, polysaccharides, and their complexes used as stabilizers for emulsions: alternatives to synthetic surfactants in the pharmaceutical field? *Int J Pharm*, 436(1-2), 359-378. doi:10.1016/j.ijpharm.2012.06.052
- Braga, A. L. M., & Cunha, R. L. (2004). The effects of xanthan conformation and sucrose concentration on the rheological properties of acidified sodium caseinate–xanthan gels. *Food Hydrocolloids*, 18(6), 977-986. doi:10.1016/j.foodhyd.2004.04.002
- Burova, T. V., Grinberg, N. V., Grinberg, V. Y., Usov, A. I., Tolstoguzov, V. B., & Kruif, C. G. (2007). Conformational changes in iota- and kappa-carrageenans induced by complex formation with bovine beta-casein. *Biomacromolecules*, 8(2), 368-375. doi:10.1021/bm060761f
- Cooper, C. L., Dubin, P. L., Kayitmazer, A. B., & Turksen, S. (2005). Polyelectrolyte–protein complexes. *Current Opinion in Colloid & Interface Science*, 10(1-2), 52-78. doi:10.1016/j.cocis.2005.05.007
- de Jong, S., Klok, H. J., & van de Velde, F. (2009). The mechanism behind microstructure formation in mixed whey protein–polysaccharide cold-set gels. *Food Hydrocolloids*, 23(3), 755-764. doi:10.1016/j.foodhyd.2008.03.017
- de Jong, S., & van de Velde, F. (2007). Charge density of polysaccharide controls microstructure and large deformation properties of mixed gels. *Food Hydrocolloids*, 21(7), 1172-1187. doi:10.1016/j.foodhyd.2006.09.004

- de Kruif, C. G., & Tuinier, R. (2001). Polysaccharide protein interactions. *Food Hydrocolloids*, 15(4), 555-563. doi:[https://doi.org/10.1016/S0268-005X\(01\)00076-5](https://doi.org/10.1016/S0268-005X(01)00076-5)
- de Kruif, C. G., Weinbreck, F., & de Vries, R. (2004). Complex coacervation of proteins and anionic polysaccharides. *Current Opinion in Colloid & Interface Science*, 9(5), 340-349. doi:<https://doi.org/10.1016/j.cocis.2004.09.006>
- Dickinson, E. (1998). Stability and rheological implications of electrostatic milk protein-polysaccharide interactions. *Trends in Food Science & Technology*, 9(10), 347-354. doi:Doi 10.1016/S0924-2244(98)00057-0
- Dickinson, E. (2003). Hydrocolloids at interfaces and the influence on the properties of dispersed systems. *Food Hydrocolloids*, 17(1), 25-39. doi:[https://doi.org/10.1016/S0268-005X\(01\)00120-5](https://doi.org/10.1016/S0268-005X(01)00120-5)
- Dickinson, E. (2009). Hydrocolloids as emulsifiers and emulsion stabilizers. *Food Hydrocolloids*, 23(6), 1473-1482. doi:10.1016/j.foodhyd.2008.08.005
- Dissanayake, M., & Vasiljevic, T. (2009). Functional properties of whey proteins affected by heat treatment and hydrodynamic high-pressure shearing. *J Dairy Sci*, 92(4), 1387-1397. doi:10.3168/jds.2008-1791
- Dybowska, B. E. (2011). Whey protein-stabilized emulsion properties in relation to thermal modification of the continuous phase. *Journal of Food Engineering*, 104(1), 81-88. doi:10.1016/j.jfoodeng.2010.11.030
- Evans, M., Ratcliffe, I., & Williams, P. A. (2013). Emulsion stabilisation using polysaccharide–protein complexes. *Current Opinion in Colloid & Interface Science*, 18(4), 272-282. doi:10.1016/j.cocis.2013.04.004
- Foegeding, E. A., Davis, J. P., Doucet, D., & McGuffey, M. K. (2002). Advances in modifying and understanding whey protein functionality. *Trends in Food Science & Technology*, 13(5), 151-159. doi:[https://doi.org/10.1016/S0924-2244\(02\)00111-5](https://doi.org/10.1016/S0924-2244(02)00111-5)
- Franco, J. M., Berjano, M., Guerrero, A., Muñoz, J., & Gallegos, C. (1995). Flow behaviour and stability of light mayonnaise containing a mixture of egg yolk and sucrose stearate as emulsifiers. *Food Hydrocolloids*, 9(2), 111-121. doi:[https://doi.org/10.1016/S0268-005X\(09\)80273-7](https://doi.org/10.1016/S0268-005X(09)80273-7)
- Funami, T., Zhang, G., Hiroe, M., Noda, S., Nakauma, M., Asai, I., . . . Phillips, G. O. (2007). Effects of the proteinaceous moiety on the emulsifying properties of sugar beet pectin. *Food Hydrocolloids*, 21(8), 1319-1329. doi:10.1016/j.foodhyd.2006.10.009
- Ganzevles, R. A., Cohen Stuart, M. A., Vliet, T. v., & de Jongh, H. H. J. (2006). Use of polysaccharides to control protein adsorption to the air–water interface. *Food Hydrocolloids*, 20(6), 872-878. doi:10.1016/j.foodhyd.2005.08.009
- Girard, M., Turgeon, S. L., & Gauthier, S. F. (2002). Interbiopolymer complexing between β -lactoglobulin and low- and high-methylated pectin measured by potentiometric titration and ultrafiltration. *Food Hydrocolloids*, 16(6), 585-591. doi:[https://doi.org/10.1016/S0268-005X\(02\)00020-6](https://doi.org/10.1016/S0268-005X(02)00020-6)
- Gu, Y. S., Decker, E. A., & McClements, D. J. (2004). Influence of pH and ι -Carrageenan Concentration on Physicochemical Properties and Stability of β -Lactoglobulin-Stabilized Oil-in-Water Emulsions. *Journal of Agricultural and Food Chemistry*, 52(11), 3626-3632. doi:10.1021/jf0352834

- Gu, Y. S., Decker, E. A., & McClements, D. J. (2005). Influence of pH and carrageenan type on properties of β -lactoglobulin stabilized oil-in-water emulsions. *Food Hydrocolloids*, 19(1), 83-91. doi:10.1016/j.foodhyd.2004.04.016
- Guzey, D., & McClements, D. J. (2006). Formation, stability and properties of multilayer emulsions for application in the food industry. *Adv Colloid Interface Sci*, 128-130, 227-248. doi:10.1016/j.cis.2006.11.021
- Guzey, D., & McClements, D. J. (2007). Impact of Electrostatic Interactions on Formation and Stability of Emulsions Containing Oil Droplets Coated by β -Lactoglobulin–Pectin Complexes. *Journal of Agricultural and Food Chemistry*, 55(2), 475-485. doi:10.1021/jf062342f
- Hoffman, J. R., & Falvo, M. J. (2004). Protein – Which is Best? *Journal of Sports Science & Medicine*, 3(3), 118-130.
- Hoffmann, M. A. M., & vanMil, P. J. J. M. (1997). Heat-induced aggregation of beta-lactoglobulin: Role of the free thiol group and disulfide bonds. *Journal of Agricultural and Food Chemistry*, 45(8), 2942-2948. doi:DOI 10.1021/jf960789q
- Huan, Y., Zhang, S., & Vardhanabhuti, B. (2016). Influence of the molecular weight of carboxymethylcellulose on properties and stability of whey protein-stabilized oil-in-water emulsions. *J Dairy Sci*, 99(5), 3305-3315. doi:10.3168/jds.2015-10278
- Ibanoglu, E. (2005). Effect of hydrocolloids on the thermal denaturation of proteins. *Food Chemistry*, 90(4), 621-626. doi:10.1016/j.foodchem.2004.04.022
- Jambrak, A. R., Mason, T. J., Lelas, V., Herceg, Z., & Herceg, I. L. (2008). Effect of ultrasound treatment on solubility and foaming properties of whey protein suspensions. *Journal of Food Engineering*, 86(2), 281-287. doi:10.1016/j.jfoodeng.2007.10.004
- Jones, O. G., Decker, E. A., & McClements, D. J. (2009). Formation of biopolymer particles by thermal treatment of β -lactoglobulin–pectin complexes. *Food Hydrocolloids*, 23(5), 1312-1321. doi:10.1016/j.foodhyd.2008.11.013
- Jones, O. G., Decker, E. A., & McClements, D. J. (2010). Comparison of protein-polysaccharide nanoparticle fabrication methods: impact of biopolymer complexation before or after particle formation. *J Colloid Interface Sci*, 344(1), 21-29. doi:10.1016/j.jcis.2009.12.017
- Jones, O. G., Lesmes, U., Dubin, P., & McClements, D. J. (2010). Effect of polysaccharide charge on formation and properties of biopolymer nanoparticles created by heat treatment of β -lactoglobulin–pectin complexes. *Food Hydrocolloids*, 24(4), 374-383. doi:10.1016/j.foodhyd.2009.11.003
- Jones, O. G., & McClements, D. J. (2008). Stability of Biopolymer Particles Formed by Heat Treatment of β -lactoglobulin/Beet Pectin Electrostatic Complexes. *Food Biophysics*, 3(2), 191-197. doi:10.1007/s11483-008-9068-5
- Jones, O. G., & McClements, D. J. (2010). Biopolymer nanoparticles from heat-treated electrostatic protein-polysaccharide complexes: factors affecting particle characteristics. *J Food Sci*, 75(2), N36-43. doi:10.1111/j.1750-3841.2009.01512.x
- Jones, O. G., & McClements, D. J. (2011). Recent progress in biopolymer nanoparticle and microparticle formation by heat-treating electrostatic protein-

- polysaccharide complexes. *Adv Colloid Interface Sci*, 167(1-2), 49-62.
doi:10.1016/j.cis.2010.10.006
- Jourdain, L., Leser, M. E., Schmitt, C., Michel, M., & Dickinson, E. (2008). Stability of emulsions containing sodium caseinate and dextran sulfate: Relationship to complexation in solution. *Food Hydrocolloids*, 22(4), 647-659.
doi:10.1016/j.foodhyd.2007.01.007
- Leroux, J., Langendorff, V., Schick, G., Vaishnav, V., & Mazoyer, J. (2003). Emulsion stabilizing properties of pectin. *Food Hydrocolloids*, 17(4), 455-462. doi:10.1016/s0268-005x(03)00027-4
- Luhovyy, B. L., Akhavan, T., & Anderson, G. H. (2007). Whey Proteins in the Regulation of Food Intake and Satiety. *Journal of the American College of Nutrition*, 26(6), 704S-712S. doi:10.1080/07315724.2007.10719651
- McClements, D. J. (2004). Protein-stabilized emulsions. *Current Opinion in Colloid & Interface Science*, 9(5), 305-313. doi:10.1016/j.cocis.2004.09.003
- McClements, D. J. (2007). Critical review of techniques and methodologies for characterization of emulsion stability. *Crit Rev Food Sci Nutr*, 47(7), 611-649. doi:10.1080/10408390701289292
- McClements, D. J. (2011). Edible nanoemulsions: fabrication, properties, and functional performance. *Soft Matter*, 7(6), 2297-2316.
doi:10.1039/c0sm00549e
- McClements, D. J. (2015). *Food Emulsions: Principles, Practices, and Techniques, Third Edition*: CRC Press.
- Miquelím, J. N., Lannes, S. C. S., & Mezzenga, R. (2010). pH Influence on the stability of foams with protein-polysaccharide complexes at their interfaces. *Food Hydrocolloids*, 24(4), 398-405. doi:10.1016/j.foodhyd.2009.11.006
- Mishra, S., Mann, B., & Joshi, V. K. (2001). Functional improvement of whey protein concentrate on interaction with pectin. *Food Hydrocolloids*, 15(1), 9-15.
doi:10.1016/S0268-005x(00)00043-6
- Neiryneck, N., Van der Meeren, P., Lukaszewicz-Lausecker, M., Cocquyt, J., Verbeken, D., & Dewettinck, K. (2007). Influence of pH and biopolymer ratio on whey protein-pectin interactions in aqueous solutions and in O/W emulsions. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 298(1-2), 99-107. doi:10.1016/j.colsurfa.2006.12.001
- Ngouémazong, E. D., Christiaens, S., Shpigelman, A., Van Loey, A., & Hendrickx, M. (2015). The Emulsifying and Emulsion-Stabilizing Properties of Pectin: A Review. *Comprehensive Reviews in Food Science and Food Safety*, 14(6), 705-718. doi:10.1111/1541-4337.12160
- Nicolai, T., Britten, M., & Schmitt, C. (2011). β -Lactoglobulin and WPI aggregates: Formation, structure and applications. *Food Hydrocolloids*, 25(8), 1945-1962. doi:10.1016/j.foodhyd.2011.02.006
- Quemada, D., & Berli, C. (2002). Energy of interaction in colloids and its implications in rheological modeling. *Adv Colloid Interface Sci*, 98(1), 51-85.
doi:10.1016/S0001868601000938
- Roefs, S. P., & De Kruif, K. G. (1994). A model for the denaturation and aggregation of beta-lactoglobulin. *Eur J Biochem*, 226(3), 883-889.
- Ryan, K. N., Vardhanabhuti, B., Jaramillo, D. P., van Zanten, J. H., Coupland, J. N., & Foegeding, E. A. (2012). Stability and mechanism of whey protein soluble

- aggregates thermally treated with salts. *Food Hydrocolloids*, 27(2), 411-420. doi:10.1016/j.foodhyd.2011.11.006
- Ryan, K. N., Zhong, Q., & Foegeding, E. A. (2013). Use of whey protein soluble aggregates for thermal stability—a hypothesis paper. *J Food Sci*, 78(8), R1105-1115. doi:10.1111/1750-3841.12207
- Salminen, H., & Weiss, J. (2013). Effect of Pectin Type on Association and pH Stability of Whey Protein—Pectin Complexes. *Food Biophysics*, 9(1), 29-38. doi:10.1007/s11483-013-9314-3
- Salminen, H., & Weiss, J. (2014). Electrostatic adsorption and stability of whey protein–pectin complexes on emulsion interfaces. *Food Hydrocolloids*, 35, 410-419. doi:10.1016/j.foodhyd.2013.06.020
- Sanchez, C., Mekhloufi, G., & Renard, D. (2006). Complex coacervation between beta-lactoglobulin and Acacia gum: a nucleation and growth mechanism. *J Colloid Interface Sci*, 299(2), 867-873. doi:10.1016/j.jcis.2006.02.031
- Schmitt, C., Bovay, C., Rouvet, M., Shojaei-Rami, S., & Kolodziejczyk, E. (2007). Whey protein soluble aggregates from heating with NaCl: physicochemical, interfacial, and foaming properties. *Langmuir*, 23(8), 4155-4166. doi:10.1021/la0632575
- Schmitt, C., Sanchez, C., Desobry-Banon, S., & Hardy, J. (1998). Structure and Technofunctional Properties of Protein-Polysaccharide Complexes: A Review. *Critical Reviews in Food Science and Nutrition*, 38(8), 689-753. doi:10.1080/10408699891274354
- Singh, S. S., Siddhanta, A. K., Meena, R., Prasad, K., Bandyopadhyay, S., & Bohidar, H. B. (2007). Intermolecular complexation and phase separation in aqueous solutions of oppositely charged biopolymers. *Int J Biol Macromol*, 41(2), 185-192. doi:10.1016/j.ijbiomac.2007.02.004
- Smithers, G. W. (2008). Whey and whey proteins—From ‘gutter-to-gold’. *International Dairy Journal*, 18(7), 695-704. doi:10.1016/j.idairyj.2008.03.008
- Sun, C., Gunasekaran, S., & Richards, M. P. (2007). Effect of xanthan gum on physicochemical properties of whey protein isolate stabilized oil-in-water emulsions. *Food Hydrocolloids*, 21(4), 555-564. doi:10.1016/j.foodhyd.2006.06.003
- Surh, J., Decker, E., & McClements, D. (2006). Influence of pH and pectin type on properties and stability of sodium-caseinate stabilized oil-in-water emulsions. *Food Hydrocolloids*, 20(5), 607-618. doi:10.1016/j.foodhyd.2005.07.004
- Turgeon, S. L., Schmitt, C., & Sanchez, C. (2007). Protein–polysaccharide complexes and coacervates. *Current Opinion in Colloid & Interface Science*, 12(4-5), 166-178. doi:10.1016/j.cocis.2007.07.007
- Vardhanabhuti, B., & Foegeding, E. A. (2008). Effects of dextran sulfate, NaCl, and initial protein concentration on thermal stability of β -lactoglobulin and α -lactalbumin at neutral pH. *Food Hydrocolloids*, 22(5), 752-762.
- Vardhanabhuti, B., Yucel, U., Coupland, J. N., & Foegeding, E. A. (2009). Interaction between β -lactoglobulin and Dextran Sulfate at Near Neutral pH and their Effect on Thermal Stability. *Food Hydrocolloids*, 23(Journal Article), 1511-1520.

- Wagoner, T., Vardhanabhuti, B., & Foegeding, E. A. (2016). Designing Whey Protein-Polysaccharide Particles for Colloidal Stability. *Annu Rev Food Sci Technol*, 7, 93-116. doi:10.1146/annurev-food-041715-033315
- Wagoner, T. B., & Foegeding, E. A. (2017). Whey protein–pectin soluble complexes for beverage applications. *Food Hydrocolloids*, 63, 130-138. doi:10.1016/j.foodhyd.2016.08.027
- Wang, Z., Zhang, S., & Vardhanabhuti, B. (2015). Foaming Properties of Whey Protein Isolate and lambda-Carrageenan Mixed Systems. *J Food Sci*, 80(8), N1893-1902. doi:10.1111/1750-3841.12940
- Wijayanti, H. B., Bansal, N., & Deeth, H. C. (2014). Stability of Whey Proteins during Thermal Processing: A Review. *Comprehensive Reviews in Food Science and Food Safety*, 13(6), 1235-1251. doi:10.1111/1541-4337.12105
- Williams, P. A., Sayers, C., Viebke, C., Senan, C., Mazoyer, J., & Boulenguer, P. (2005). Elucidation of the Emulsification Properties of Sugar Beet Pectin. *Journal of Agricultural and Food Chemistry*, 53(9), 3592-3597. doi:10.1021/jf0404142
- Xiong, Y. L. L. (1992). Influence of Ph and Ionic Environment on Thermal Aggregation of Whey Proteins. *Journal of Agricultural and Food Chemistry*, 40(3), 380-384. doi:DOI 10.1021/jf00015a004
- Xu, D., Wang, X., Jiang, J., Yuan, F., & Gao, Y. (2012). Impact of whey protein – Beet pectin conjugation on the physicochemical stability of β -carotene emulsions. *Food Hydrocolloids*, 28(2), 258-266. doi:10.1016/j.foodhyd.2012.01.002
- Ye, A. (2008). Complexation between milk proteins and polysaccharides via electrostatic interaction: principles and applications – a review. *International Journal of Food Science & Technology*, 43(3), 406-415. doi:10.1111/j.1365-2621.2006.01454.x
- Ye, A., Flanagan, J., & Singh, H. (2006). Formation of stable nanoparticles via electrostatic complexation between sodium caseinate and gum arabic. *Biopolymers*, 82(2), 121-133. doi:10.1002/bip.20465
- Zhang, S., Hsieh, F.-H., & Vardhanabhuti, B. (2014). Acid-induced gelation properties of heated whey protein–pectin soluble complex (Part I): Effect of initial pH. *Food Hydrocolloids*, 36, 76-84. doi:10.1016/j.foodhyd.2013.07.029
- Zhang, S., Zhang, Z., Lin, M., & Vardhanabhuti, B. (2012). Raman spectroscopic characterization of structural changes in heated whey protein isolate upon soluble complex formation with pectin at near neutral pH. *J Agric Food Chem*, 60(48), 12029-12035. doi:10.1021/jf303780c
- Zhang, S., Zhang, Z., & Vardhanabhuti, B. (2014). Effect of charge density of polysaccharides on self-assembled intragastric gelation of whey protein/polysaccharide under simulated gastric conditions. *Food Funct*, 5(8), 1829-1838. doi:10.1039/c4fo00019f

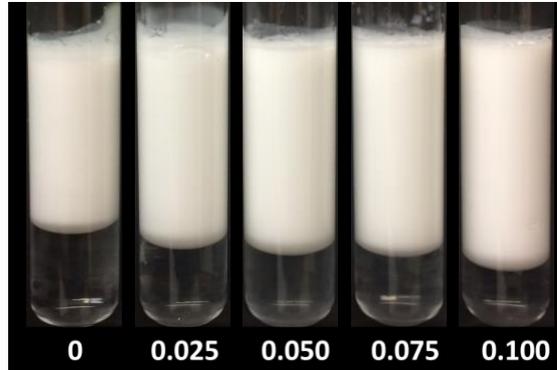
APPENDIX

Table 1 Power Law model parameters of emulsions with different pectin concentration and heating pH.

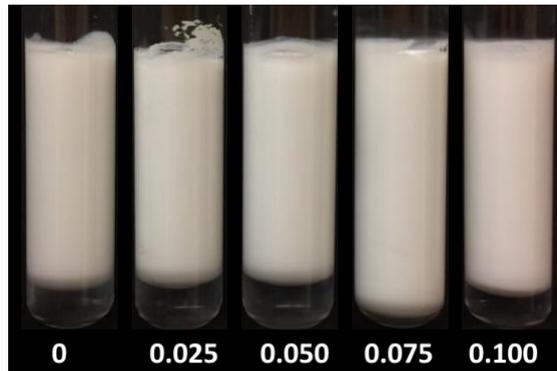
Pectin%	pH	K	n
0	6	0.127±0.03 ^A	0.288±0.06 ^C
	6.5	0.076±0.01 ^A	0.478±0.03 ^A
	7	0.118±0.01 ^A	0.405±0.02 ^B
0.025	6	0.133±0.01 ^A	0.378±0.09 ^B
	6.5	0.091±0.03 ^{AB}	0.367±0.02 ^B
	7	0.053±0.00 ^B	0.533±0.01 ^A
0.050	6	0.213±0.03 ^A	0.262±0.04 ^C
	6.5	0.076±0.02 ^B	0.477±0.06 ^B
	7	0.027±0.01 ^B	0.633±0.04 ^A
0.075	6	0.156±0.02 ^A	0.334±0.01 ^B
	6.5	0.160±0.02 ^A	0.312±0.06 ^B
	7	0.029±0.00 ^B	0.639±0.05 ^A
0.10	6	0.146±0.03 ^A	0.316±0.03 ^B
	6.5	0.113±0.04 ^A	0.391±0.06 ^A
	7	0.082±0.01 ^A	0.422±0.02 ^A

Different letters indicate significant difference ($p < 0.05$) with same pectin concentration. The K and n value is calculated by fitting in Power law model with shear rate between (0.1 to 200 s⁻¹)

(a)



(b)



(c)

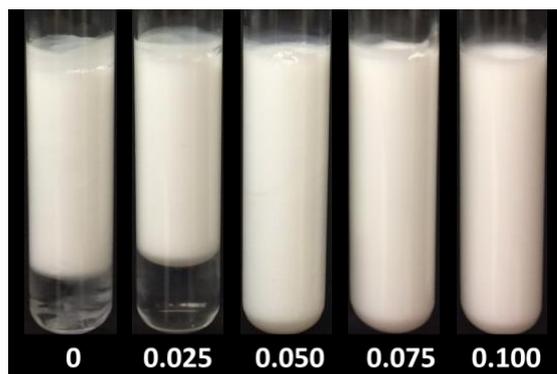


Figure 1 Emulsions stabilized by Cpx with different pectin concentration after 15 days; (a) pH 6.0, (b) pH6.5, (c) pH 7.0; From left to right (0,0.025,0.050,0.075,0.100 wt% pectin in emulsion); with ((a) pH 6, (b) pH6.5, (c) pH 7.