THE INCORPORATION OF PREHEATED WHEY PROTEIN ISOLATE AND PECTIN COMPLEXES AS NATURAL EMULSIFIERS AND STABILIZERS IN PROCESSED CHEDDAR CHEESE SAUCE

A Thesis

Presented to

the Faculty of the Graduate School

at the University of Missouri

In Partial Fulfillment of the Requirements for the Degree

Master of Science

By

BRITTANY N. SIEVERS

Dr. Bongkosh Vardhanabhuti, Thesis Supervisor

JULY 2019

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

THE INCORPORATION OF PREHEATED WHEY PROTEIN ISOLATE AND PECTIN COMPLEXES AS NATURAL EMULSIFIERS AND STABILIZERS IN PROCESSED CHEDDAR CHEESE SAUCE

Presented by Brittany N. Sievers

A candidate for the degree of Master of Science,

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

Bongkosh Vardhanabhuti, Ph.D., Department of Food Science

Andrew D. Clarke, Ph.D., Department of Food Science

Catherine Peterson, Ph.D., Department of Nutrition and Exercise Physiology

ACKNOWLEDGEMENTS

I would like to express my appreciation to the Department of Food Science for accepting me into the program. As an alumna, it has been a great experience as an undergraduate student in food science and I am blessed to be accepted into the food science master's program and pursue my education. I am thankful to know such supportive faculty and students at the University of Missouri.

I would also like to thank my advisor, Dr. Vardhanabhuti for providing me guidance in my graduate studies and encouraging me to succeed. I'm thankful that my co-advisor, Dr. Clarke helped me grasp important concepts regarding creating a thesis and how to succeed in graduate school in the Research Methods 8402 class. Likewise, I am thankful that he provided me with guidance and support through my research project. I would also like to thank Dr. Peterson for providing feedback regarding the nutritional aspect of my research project. Overall, I am thankful for my committee for providing useful feedback on my graduate research project.

Next, I would like to thank my friends Taylor Harper, Akkasubba Kotchabhakdi, Yanni Bullock, Sargun Malik, Ava Lin, and Lin Sun for providing me support and guidance in graduate school. I am thankful to have such wonderful friends and colleagues in my life that encourage me to succeed. Likewise, I would like to thank my parents and my boyfriend Dan Avila for encouraging me to succeed and achieve my goals.

TABLE OF CONTENTS

LIST OF FIGURES

Figure

LIST OF TABLES

Table

THE INCORPORATION OF PREHEATED WHEY PROTEIN ISOLATE AND PECTIN COMPLEXES AS NATURAL EMULSIFIERS AND STABILIZERS IN PROCESSED CHEDDAR CHEESE SAUCE

ABSTRACT

Processed products that contain no artificial ingredients are becoming a new trend and there is a need to develop emulsifiers and stabilizers for clean label application. Whey protein isolate (WPI) has excellent emulsifying properties and has been a widely used natural emulsifier in food application. However, WPI does not provide long term stability and its functions are minimized when the pH is at or near its isoelectric point (pI). At pI, protein has no net charge and therefore, protein-stabilized emulsions become unstable. In order to improve the emulsification properties of WPI, the incorporation of additional ingredients such as polysaccharides are needed.

Previous studies showed that heated WPI and pectin soluble complexes (HCPX) can be developed to have improved emulsification and stabilization properties in model emulsions. In this study, the effects of HCPX as emulsifier and stabilizer in cheese sauce were investigated. HCPX were made by heated 3% (w/w) WPI with 0.45% or 0.6% pectin at pH 5.5 or 6.2. All model cheese sauces were prepared from cheddar cheese (25% w/w), vegetable oil (7.7% w/w), and corn starch (3% w/w). The effects of HCPX (1.5% protein from HCPX in the final sauces) on cheese properties were compared with 0.3% pectin (PT), heated whey protein isolate (HWPI), HWPI with added 0.3% pectin (HWPI + PT), emulsifier (ES) (0.3% w/w), and the controlled formulation (CRT) with only starch. Cheese sauces were cooked using a multifunctional laboratory-scale

cooker. Properties of the freshly made cheese sauces were characterized by dynamic rheology and fat droplet size analysis. Stability of the sauces was determined by measuring droplet size and syneresis after three-week storage. Five samples that showed a significant difference in storage stability were selected for freeze/thaw stability and microstructural analysis.

As shown by the rheological properties results, cheese sauces containing HWPI and PT had increased solid-like behavior (e.g., elastic modulus or G′) compared to CRT while the sample with ES had decreased G′. Among samples with pectin, those made with HWPI + pectin (HWPI + PT) and HCPX were firmer (e.g., higher G′) compared to HWPI or pectin alone. Sauces with the HCPX formed with 0.6% pectin at pH 6.2 exhibited the largest elastic property. Mean droplet particle sizes for CRT and PT were significantly larger ($p<0.05$) compared to the other treatments. After 3-week storage, CRT and ES samples had the largest syneresis. Adding pectin and/or HWPI alone led to reduced syneresis; however, samples with the mixtures at higher pectin concentration were most stable. When subjected to freeze/thaw cycle, all cheese sauces containing pectin were significantly more stable than CRT with the most stable samples being HCPX-0.6-6.2 and HWPI+PT. Finally, microstructural analysis revealed that sauces containing HCPX-0.6-5.5 and HCPX-0.6-6.2 had the smallest droplet sizes which were in agreement with other results.

In conclusion, HCPX can be applied to foods such as cheese sauce and replace synthetic emulsifiers and stabilizers. Pectin concentration and pH during HCPX formation could affect the function of the HCPX in the resulting cheese sauces. HCPX can be utilized in various oil in water food emulsions.

CHAPTER 1

INTRODUCTION

Over time, food industries have begun to modernize its ingredients and processes to produce a cleaner labeled food product. Finding these natural or "clean" ingredients are essential for meeting this growing demand (McClements and Gumus 2016).Some processed products in the market may be less natural because of the addition of synthetic emulsifiers (Asioli et al. 2017). Natural, or "clean" emulsifiers and stabilizers are essential for meeting the growing need of consumers (McClements and Gumus 2016). Today, consumers are more involved with how food is processed, the ingredients added, and are becoming more interested in organic foods because they are perceived as healthy and natural. Though there is no clear definition of "clean label", the best understanding is that food products are free from artificial ingredients (Asioli et al. 2017). Some ingredients in foods may be viewed as unnatural because of the addition of synthetic emulsifiers and stabilizers.

Many foods in the dairy industry such as cheese sauces, salad dressings, and butter contain different emulsifiers that may or may not be considered "clean label". These emulsions occur in two phases: oil in water (o/w) and water in oil (w/o). Oil in water emulsions, such as a cheese sauce have oil (dispersed phase) suspended in water (continuous phase). For water in oil emulsions, such as butter, water is the disappeared phase and oil is the continuous phase. Emulsifiers are added to bring the two immiscible fluids together. Lecithin, mono and diglycerides, and dairy proteins such as whey are considered emulsifiers that lower interfacial tension due to the presence of hydrophilic (water loving) and hydrophobic (water fearing) components. An emulsifier adsorbs at the oil-water interface to prevent the newly formed droplets from flocculation

and/or coalescence. Synthetic emulsifiers such as mono and diglycerides are commonly used in processed cheese sauces. However, the food industry is seeking to develop natural emulsifiers with improved emulsification and emulsion stabilization properties in order to replace these synthetic emulsifiers and promote clean label products.

Whey protein isolate (WPI) has been a widely used natural emulsifier, but the protein alone does not achieve long term stability. This dairy protein does have great emulsifying properties due to the fact that it has the ability to rapidly adsorb at the oil- water interface and form a protective layer around oil droplets in order to reduce the droplets from coalescing (Demetriades and Coupland 1997). Whey protein has an isoelectric point at around pH 5.2, which is around the pH of many foods such as cheese sauce (e.g., pH 5.1 – 6.6; (Lightfield and Malone 2005). At isoelectric point, there is minimum net charge resulting in weak electrostatic repulsion (Demetriades and Coupland 1997; Chanamai and McClements 2002; Yan Huan et al. 2016)). Therefore, protein has limited ability as an emulsifier in this pH range.

Research showed that the heating of whey protein isolate above the denaturation temperature (70^{o}C) leads to the unfolding of the molecular structure. It has been reported that heating whey protein solutions at 75^{o}C and 85^{o}C had turned translucent and opaque respectively (Kotchabhakdi 2018). This was due to the aggregation and denaturation of WPI heated above 70^{o}C (Dybowska 2011). Pre-heating whey protein before emulsification could improve the functional properties of protein in emulsion stability. Dybowska (2011) reported that pre-heating whey protein between 80-90^{o}C improved emulsion stability compared to unheated whey protein. This is because when protein is heated above the denaturation state, it unfolds and forms many charged groups. These forms of heating may increase the functional properties of the whey protein isolate, but is also important to note that emulsifying properties are dependent on many other factors like

temperature, time, and other ingredients present (Koupantsis and Kiosseoglou 2009).

Polysaccharides, such as pectin, are anionic biopolymers and are often used to enhance the functional properties of proteins especially near their isoelectric point (pI) (Wagoner,Vardhanabhuti, Foegeding, 2016). A few studies had focused on the formation of soluble whey protein and polysaccharide complexes via electrostatic interaction above the pI of the protein. At pH > pI, both molecules carry a net negative charge, however, there are still some positive charged groups on the protein which can bind to anionic groups on the polysaccharides forming soluble complexes (Dickinson 2003; Vardhanabhuti et al. 2009;Huan et al. 2016). Heating these complexes above protein denaturation temperature can lead to the formation of heated protein-polysaccharide soluble complexes (HCPX) with improved stability against pH. Previous research showed that emulsions stabilized by HCPX at 3% protein with 20% oil at pH 5.5 formed at 85^{o}C had shown high stability (Kotchabhakdi 2018). This study has also stated that HCPX formed at 6.2 had a less negative charge compared to HCPX at pH 5.5 and 5.8. A lower charge potential would have the inability to prevent flocculation and coalescence during emulsification which can result in large droplet size (Cho and McClements 2009). This is due to the lipid and pectin molecules both exhibiting anionic nature near neutral pH. Studies from (Kotchabhakdi 2018) showed that increasing pectin from 0.2-0.3% produced the most stable emulsions prepared at pH 5.5, 0.60% pectin (>30 days) due to small droplet sizes with higher surface charge (Kotchabhakdi 2018). The formation of soluble complexes is highly focused on the electrostatic interaction between protein and polysaccharides. These heated protein-polysaccharide soluble complexes have the potential to be used as a clean label emulsifier and stabilizer in food emulsions such as sauces. Therefore, the objective of this study are:

1.1 Objectives

1. To determine the effects of heated whey protein and pectin complex (HCPX) as a natural emulsifier and stabilizer in cheese sauce and to evaluate these complexes as a viable replacement for synthetic emulsifiers.

2. To determine the effects of HCPX at 3% WPI with 0.45%, and 0.60% pectin at pH 5.5 or 6.2 on the emulsion stability of processed cheddar cheese sauce.

3. To determine the key factors that led to optimum emulsion stability cheddar cheese sauce.

CHAPTER 2

LITERATURE REVIEW

2.1 Cheese Sauce in the Market

Commercially processed cheese sauces can be packaged in many different forms such as shelf-stable, frozen, or as a dry mix (Childs et al. 2009). Cheese sauce can be used for many purposes including being incorporated in processed meat products, poured on vegetables and pasta, or as a dip. Though cheese is nutrient-dense and contains 9% protein and 27% calcium, it is known to be high in sodium and fat. Individuals who suffer from hypertension and have coronary heart disease may stay away from consuming processed cheese products due to the high amount of sodium present in processed cheese sauce. The food industry is trying to incorporate omega- 3-fatty acids for the replacement of saturated fats such as butter in order to improve processed cheese products (Childs et al. 2009). Clean label products are also becoming a current trend.

There is no clear definition of "clean label," but it implies that the product is free from artificial colors, flavors, and binding agents (Williams et al. 2009). Since 1994, consumers have been concerned with ingredients in their foods in terms of safety (Brewer and Prestat 2002). Consumers since then have become interested in food products that list the words "natural" and "organic". Food manufacturing has a crucial role in increasing the shelf life of food products which has immensely decreases food waste and lost. However, ingredients that improve quality or shelf-life of the products could be undesirable if they are not perceived as "clean-label" ingredients by consumers. The goal in the food industry is to manufacture safe healthy foods and extend the shelf life of "clean label" products.

2.2 Ingredient Functions in Processed Cheddar Cheese Sauce

2.2.1 Vegetable Oil

Vegetable, also known as soybean oil, serves as the fat (dispersed phase) in oil in water emulsions. Soybean oil is known to be the most preferred oil consumed in the world (Gunstone 2002). There are also high amounts of polyunsaturated and linolenic acids in soybean oil (Gunstone 2002). Fat is known to be an essential component to human health. Vegetable oil, or soybean oil, is widely used in various food emulsions such as dressings, sauce, and sausages due to its potential in increasing the linoleic fatty acid lipid profile in the product. In the Physicians Health Study, results showed that individuals who consumed high amounts of polyunsaturated fatty acids had a 90% reduction cardiac death, compared to those who have very low polyunsaturated fatty acids present in their blood (Albert et al. 2002). This may be due to the effects of polyunsaturated fatty acids (PUFAs) on reducing cardiac arrhythmias and increasing atherosclerotic plaque stability, thus preventing plaque rupture and reducing risk of coronary disease (Siriwardhana et al. 2012).

2.3.2 Corn Starch

The most widely used native starches are corn (maize), waxy maize, potato, wheat, and tapioca starch. Corn starch is used widely in the food industry especially for sauces and gravies. The major functions of starch in food applications are to bind water, contribute to the texture of the product, and exhibit with freeze/thaw stability. Starch acts as a hydrocolloid and swells by taking up water during processing. Corn starch at high concentrations with water are known to exhibit shear thickening behavior under shear (Fall et al. 2009). When starch is dispersed in water,

the functional properties arise. Starch begins to swell as temperature increases to the gelatinization temperature (Pomeranz Y 2012).

Gelatinization is defined as the breakage of intermolecular bonds of starch molecules when immersed into water during heating. This allows more hydrogen bonding sites which in turn allows starch molecules to readily dissolve in water and causes them to swell (Wang et al. 2015). The gelatinization temperature of corn starch is 62-72^{0}C (Pomeranz 2012). Studies have investigated the gelatinization and pasting characteristics of starch. Work by Tsai et al. (1997) showed that corn starch characterized by temperature sweep gelatinized at 61.7$\pm$0.56 to 73.5$\pm$0.2. When starch is heated while stirring, the starch at the gelatinized state forms a paste, which shows an increase in viscosity of the water and starch mixture. This paste consists of swollen starch granules and fragments.

Retrogradation occurs in cooked gelatinized starch. Starch retrogradation is a reaction that takes places when the amylose and amylopectin form an ordered structure as the cooling process of the starch takes place after heating (Wang et al. 2015). Changes that starch undergoes during retrogradation determine the quality, acceptability, and shelf life of the food. The initial process of retrogradation starts with the rapid recrystallization of amylose. The amylose retrogradation determines the initial firmness of the gel and the stickiness of processed foods. Long term crystallinity takes place in the staling of breads due to the retrogradation of amylopectin (BeMiller and Huber 2015). During long term storage, sauces stored in the fridge that contain starch may show syneresis inducing moisture loss of the starch granules. Other ingredients, such as salt, dairy proteins, and fats such as vegetable oil alters the function of starch resulting in changes in the final food product. Studies have demonstrated the roles of protein-starch, lipid-starch, salt-starch, and

polysaccharide-starch complexes on starch properties. (Yang H et al. 2004) states that the addition of whey protein isolate (WPI) to starch increased gel strength due to the introduction of hydrophobic protein-protein interactions during cooling and due to the dilution effect of WPI on the formation of hydrogen bonds with starch and water molecules.

When lipids are added during the gelatinization of starch, the amylose chains interact and the hydroxyl groups derived from the amylose are presented in the outer surface of the helix, while the lipid groups are present in the inter portion of the helix which demonstrate the hydrophobic complexing reagents. The hydrophobic reagents, or lipids are present in the inter portion of the helix because of van-der Waals forces between the lipid (Zhen F et al. 2015). Therefore, the mobility of the amylose molecules is restricted in the starch granule and the amylose dissolving in the aqueous phase is reduced. In other words, adding lipids to starch could positively restrict amylose retrogradation. Salts are known to be strong electrolytes that can be readily ionized into cations and anions in water. This can promote hydrogen bonding in water-starch system. Work shown by Zhen F et al. (2015) revealed that the addition of salt affects the retrogradation as well as the pH of starch-water systems. The greater the addition of salt, the more amylose leached out of starch granules leading to reducing the rate of starch retrogradation. Divalent cations, such as Ca^{2+} and Mg^{2+} have been shown to reduce starch retrogradation more than Na^{2+} and K^{+} (Beck et al. 2011). Adding salt does decrease the pH of starch-water systems because the cations replace the hydrogen ions within the starch molecules and the hydrogen ions move to the aqueous phase. The addition of cations in the starch molecules leads to less recrystallization of starch. Adding ions to starch can also affect the solubility of starch due to the interaction of hydroxyl starch molecules which can lead to differences in charge.

The addition of polysaccharides influences the hydrogen bonding and retrogradation of starch-water systems. One factor that affects starch-polysaccharide interactions is the molecular weight of the polysaccharide. For example, in a starch-guar gum mixture, the higher the molecular weight of guar gum, the greater the interactions between the polysaccharide and amylopectin due to the presence of large amounts of hydroxyl groups in both polymers. The interactions are mainly via hydrogen bonding. Polysaccharides such as xanthan and guar gum could also alter the water distribution of the starch and water system because they have the ability to bind water. Since polysaccharides have the ability to bind water, they promote usable water for amylose and amylopectin for crystallization (Katsuta et al. 1992). The addition of polysaccharides such as pectin enhances short-term retrogradation due to their ability to increase the concentration of amylose and amylopectin through thermal thickening in the continuous phase (Funami et al. 2005).

2.2.3 Whey Protein Isolate.

Milk proteins are currently being added to dairy products, such as yogurt, cheese spreads and sauces to improve nutritional availability, flavor, texture, and rheological properties, such as flow and shear stress. Whey protein contains immunoglobulins, serum albumins, and lactoferrin (Law and Leaver 2000). Furthermore, whey proteins are rich in essential branched chain amino acids which are required for human health (Devries and Phillips 2015). The major amino acids in whey are leucine, isoleucine and valine. These amino acids serve as an important role in metabolism, maintaining blood glucose levels, and promoting neural function. Whey protein is also rich in bioactive peptides which support dietary management of chronic disease (Patel 2015). Whey protein isolate contains 90% protein (Hayes and Cribb 2008).

Whey proteins are widely used in foods due to their nutritional and functional properties. Whey protein can enhance flavor, contribute to rheological properties such as viscosity, texture, and stability of the products. In food emulsion, such as ice cream or processed cheese sauce, whey protein can function as emulsifier. The surface-active property of whey proteins allows them to adsorb at the oil-water interface and form layer around the oil droplet. The droplets are stabilized via electrostatic repulsion due to charge property of the proteins.

When heated to the thermal denaturation stage, whey proteins unfold and form aggregates. When whey proteins unfold, they can form hydrogen ionic bonds. The protein chains are able to link hydrogen bonds and gaps are formed (Rodrigues et al. 2015). The gaps have the capability to hold water and form a three-dimensional network. Whey proteins begin to denature at 70^{o}C (Dybowska 2011). The ideal temperature for protein denaturation is 85^{o}C. Whey proteins completely denature at 90^{o}C after five minutes. Beta-lactoglobulin, the main protein fraction of whey, determines the degree of protein denaturation.

One important factor affecting protein functional properties is pH. The isoelectric point (pI) of whey protein is around 5.2. When the pH is near pI, the protein will precipitate out of solution. At isoelectric point (pI), the net charge of whey proteins is zero and has the least solubility (Kakalis and Regenstein 1986; Mann and Malik 1996; Wong et al. 1996). At pH at or near pI, protein functional properties, especially emulsification properties will be minimized which limits the use of protein in certain applications. Modification or interactions with other ingredients such as polysaccharides could improve protein functional properties.

2.2.4 Low-Methoxyl Pectin and Whey Protein Interaction

Polysaccharides, such as pectin are used as a stabilizer in food emulsions such as sauces (Wang et al. 2015). Polysaccharides, such as pectin, are known as a gelling a thickening agent in food emulsions. Protein and polysaccharide interactions are derived from the oppositely charged biopolymers (Schmitt and Turgeon 2011). The pH has an immense factor on protein and polysaccharide interactions. When the pH is below pI of protein, the positively charged protein can form strong bonds with anionic, or negatively charged polysaccharides. This results in the formation of soluble complexes (Ye 2008). However, at pH values above pI of whey protein, there is a net negative charge, but there are still some localized patches on the surface of whey protein that can interact with anionic polysaccharides (Dickinson 2003).

2.3 Concept of Emulsion

By definition, an emulsion is a mixture of two immiscible liquids with one liquid being in the dispersed phase, while the other being in the continuous phase (McClements 2005). There are two types of emulsions: water in oil (W/O) emulsions, and oil in water (O/W) emulsions. For example, cheese sauce has aqueous continuous phase and oil droplets in the dispersed phase, meaning it is an O/W emulsion (McClements 2005). Butter, for example, is an example of a W/O emulsion due to the fact that that water is in the dispersed phase.

Due to the immiscibility of the dispersed and continuous phases, mechanical energy and an emulsifier are needed to form an emulsion. One common method to form food emulsions is homogenization which brings the oil phase and the liquid phase into one phase by extreme agitation. During homogenization, emulsifiers work to reduce surface tension by aiding the

disturbance of emulsion droplets. This reduces the size of droplets to facilitate the formation of emulsions (McClements 2007). Proteins are examples of emulsifiers due to the fact that they are known to be amphiphilic organic compounds.

Overtime, the emulsion breaks due to physiochemical mechanisms, such as phase inversion and gravitational separation (McClements 2005). Emulsions are known to be thermodynamically unstable even with the help of added emulsifiers and excessive agitation due to differences in density of the oil and water phases. Emulsifiers and stabilizers do in fact facilitate emulsion stability, but the emulsion will break overtime.

Many foods are considered emulsions such as cheese sauce, milk, mayonnaise, and sausage. Emulsion stability by definition means an emulsion has the ability to withstand the physicochemical properties overtime (McClements 2005). Food emulsions, such as cheddar cheese sauce, can become unstable due to gravitational separation. Creaming, or sedimentation is caused by gravitational separation. Creaming occurs when droplets move upwards due to their low density and sedimentation happens when droplets move downwards due to the fact that they have higher density than the surrounding liquid. Flocculation can also occur due to emulsion instability. This defect occurs when droplets move close together to form aggregates. Coalescence is another emulsion instability mechanism. This is a process when two or more droplets merge together to form into a large droplet.

Whey protein isolate can act as an emulsifier and stabilizer in food emulsion systems. The major stabilization mechanism is via electrostatic repulsion. The limitation of using WPI is the lack of long-term stability due to the small size of the protein. Modification by heating could alter emulsification properties. When being heated, whey proteins begin to unfold at 55-65 degrees

Celsius, denature and form aggregates. The ideal temperature for protein denaturation is 85 degrees Celsius (Wijayanti et al. 2014b). Beta-lactoglobulin, the main protein fraction of whey, determines the degree of protein denaturation. Protein aggregates can form viscoelastic films that are more resistant to mechanical stresses compared to native unheated protein. The films formed with larger aggregates also provide steric stabilization, which prevents flocculation and coalescences.

2.4 Cheese Sauce

Cheese sauce is known to be a shelf stable product, has an acceptable mouth feel, and is tolerant to heat (Spanier 1986). A shelf stable cheese sauce consists of about 5-15% cheese, 0.5-5% natural cheese flavor (Spanier 1986) and 2-6% starch (Lightfield and Malone 2005). Natural cheese is known to be a perishable food item and must be stored in the refrigerator to prevent mold growth. The main function of starch in cheese sauce is to provide flow characteristic of the sauce. Starch also provides heat, cold, and storage stability properties. The pH and the addition of stabilizers are also important in terms of the functional properties of commercial processed cheddar cheese sauce.

Acetic acid is added to decrease the pH of the cheese mixture (Lightfield and Malone 2005). Phosphoric and lactic acid are also used to regulate the pH of commercial processed cheese sauce as well. Hydrocolloids control the viscosity and prevent separation in sauces. There are various physiochemical transformations in sauces which include protein aggregation, interactions between hydrocolloids, starch gelatinization, and fat globule distribution (Guardeno et al. 2011)

Cheese sauce must be free from lumps, separation between the oil and aqueous phase, and can be reheated to obtain acceptable quality (Visee 2001). The cheese must be minced prior to processing to create a homogenous mass in the sauce (Visee 2001). A roux is the initial step in producing commercial sauces. In this step, fats and starch form a mixture in order to bind other ingredients added later in the process. The melting point of fats is between 10 to 40^{o}C (Visee 2001). This is important in cheese sauce processing because natural cheese contains fat and the addition oil serves as the fat component.

2.5 Processing Cheese Sauce

Processed cheese sauce is traditionally prepared by heating and stirring a mixture of natural cheese, chelating salts, water, and fat at high temperatures between 70-95^{o}C. The ingredients are processed at high temperatures for a short amount of time to prevent the growth of spoilage bacteria. During the processing of cheese sauce, water is added so that calcium chelating salts and proteins are dissolved, and a smooth stable emulsion is produced. Water is also added to reduce cost and it attributes to meltability. Polysaccharides such as guar and xanthan gums are added to cheese sauce or spreads to aid texture, reduce cost, and extend shelf-life (Arocas et al. 2009). All ingredients to make a cheese sauce are added to a multi-functional cooker, such as a Thermomix (Lee et al. 2004). This multi-functional cooker works with homogenizing and pasteurizing the cheese sauce.

The Thermomix cooker has a four-blade rotor. Two blades are facing upward, and two are facing down. The two lower blades are used for grating cheese, milling, grinding and mixing. This multi-functional food processor has the ability to keep heat at a constant temperature, steam food, emulsify, cook, grate, mill, grind, puree, blend, and boil. Due to the fact that the Thermomix has many functions, it is becoming more used in the food industry (Aguilera 2018).

2.6 Rheological Measurements of Foods

The word rheology is defined as the study of flow ("rheo") and deformation of a material in a response to a mechanical force (Bayod 2008). Most foods are classified as complex fluids. These complex fluids have mechanical properties between those that are liquid and solid. In dynamic oscillatory tests, foods are deformed sinusoidally, or in a smooth periodic oscillation by the application of small-amplitude, or maximum distance/displacement on a sinusoidal wave in a basic shear field. The linear viscoelastic region can be determined by strain sweep tests. Strain sweep tests include increasing the magnitude of strain, while keeping a constant frequency at 1 Hz. This test can be useful to determine when the material begins to deform. In testing foods, it is necessary to keep strains below deformation limit to avoid any non-linear effects. The linear viscoelastic region determined by strain sweep is further used in frequency sweep tests. Frequency tests further investigate the physical properties and phase changes of a material overtime at a constant strain. For semi-liquid or semi solid foods, such as tomato pastes, are found to be known as weak gels , G′(elastic modulus) > G″(loss modulus, or viscous) over all frequencies (0.01-10 Hz). In a low frequency range, G″shows a minimum, which is common for weak gels.

CHAPTER 3

WHEY PROTEIN AND PECTIN COMPLEXES AS NATURAL EMULSIFIERS AND STABILIZERS IN CHEESE SAUCE

Abstract

There is a need to develop emulsifiers and stabilizers for clean label application. Whey protein isolate (WPI) has excellent emulsifying properties except when the pH is at or near its isoelectric point. The objective of this study was to determine the effect of heated whey protein and pectin complex (HCPX) as a natural emulsifier and stabilizer in cheese sauce and to evaluate these complexes as a viable replacement for synthetic emulsifiers. Previous studies showed that HCPX can be developed to have improved emulsification and emulsion stabilization properties. In this study, the effects of HCPX as an emulsifier and stabilizer in cheese sauce were investigated. HCPX were made by heated 3% (w/w) WPI with 0.45% or 0.6% pectin at pH 5.5 or 6.2. All model cheese sauces were prepared from cheddar cheese (25%), vegetable oil (7.7%), and corn starch (3%). The effects of HCPX (1.5% protein from HCPX in the final sauces) on cheese properties were compared with 0.3% pectin (PT), heated whey protein isolate (HWPI), and HWPI with added pectin (HWPI+ PT). Samples with starch only (CTR) or with 0.3% emulsifier (ES) were also included. Cheese sauces were cooked using a laboratory-scale cooker. Samples were characterized by dynamic rheology, particle size analysis, stability after three-week storage, freeze/thaw stability, and microscopy. All cheese sauces containing pectin had increased solid like behavior compared to CTR and ES as shown by strain sweep and frequency sweep results. Among samples with pectin, those made with HCPX at higher pectin concentration and at pH 6.2 were firmer as shown by G′. Due to the lack of emulsifying agent, significantly larger particle size (d_{43}) ($p<0.05$) was observed with CRT and PT compared to other treatments. All samples containing pectin were

more stable with cheese sauces containing HCPX or HWPI+PT being most stable as shown by significantly lower syneresis after 3-week storage under refrigeration and after one freeze/thaw cycle. Microscopy images of also revealed smallest fat droplet sizes and more compact protein network from cheese sauces stabilized by HCPX. Therefore, sauces containing HCPX can be used a viable replacement as a natural emulsifier in cheddar cheese sauce. HCPX preparation conditions also influenced their functions and the resulting cheese sauce properties.

3.1 Introduction

There is an increasing consumer demand for clean label products. Natural, or "clean" emulsifiers and stabilizers are essential for meeting the growing need of consumers (McClements and Gumus 2016). Today individuals are more involved with how food is processed, the ingredients added, and are becoming interested in organic foods because they are perceived as healthy and natural. Synthetic emulsifiers and stabilizers are considered unnatural and consumers believe them to not be healthy.

Whey protein isolate is known to be a clean label emulsifier for food application. During homogenization, whey protein lowers interfacial tension and creates small lipid droplets. Protein, such as whey, has the ability to absorb on fat droplets and forms a protective layer. This protective layer provides electrostatic repulsion between droplets to help maintain emulsion stability (Ozturk and McClements 2016). Although whey protein is a widely used emulsifier, it cannot provide long term stability especially under environmental stresses such as pH (Mangino 1984). Proteins surface charges are highly dependent on the pH of the surrounding environment. Protein carries a net negative charge at pH>pI and a net positive charge at pH<pI (Wagoner et al. 2016).Whey protein has an isoelectric point at pH 5.2, where the net charge is zero and the protein is least soluble and

their functional properties are at minimized (Kakalis and Regenstein 1986; Hall 1996; Mann and Malik 1996; Wong et al. 1996). Dairy sauces have a pH between 5.1-6.6 (Lightfield and Malone 2005). Cheese sauce, in particular, has a pH around 5, and therefore it is a challenge to solely use WPI as an emulsifier. Modification such as heating above the denaturation temperature and interacting with polysaccharides have been used to improve its emulsification properties.

Heating whey protein alone above 70ºC changed the molecular structure (Dybowska 2011). The heating of whey protein is considered reversible with the loss of helical structure, and when the aggregation process occurs involving disulfide (S-S) or sulfhydryl groups (S-H) intermolecular interaction is present is considered to be irreversible (Wijayanti et al. 2014a). These intermolecular interactions include electrostatic and hydrophobic interactions. The S-S groups (most active is Cs 6-Cs120in α-Lactalbumin), is located on the surface and once cleaved by heating, it's -SH groups are accessible for aggregation and they participate in a positive charge distribution (Kuwajima et al. 1990; Wijayanti et al. 2014a). These positive charges can react with anionic polysaccharides and form soluble complexes.

Examples of food emulsions that consist of polysaccharides include cheese sauce, salad dressings, and meat products (Stephen et al. 2006). Natural surface-active polysaccharides include gum Arabic and pectin (Ozturk and McClements 2016). Polysaccharides, such as pectin, are known to stabilize emulsions by increasing the viscosity of the aqueous phase and therefore inhibiting droplet movement (McClements 2015). Adsorbed polysaccharide can also promote steric stabilization. Steric stabilization is when two interfacial layers interact and create a temporary repulsive interaction. This interaction increases in strength as the hydrophilicity and thickness of the interfacial layers increase. Pectin can coat lipid droplets and create steric

stabilization due to their large hydrophilic groups that are present in the aqueous phase. Since polysaccharides create steric stabilization, they tend to be less prone to environment stresses such as pH and ionic strength, compared to electrostatic repulsion. Therefore, steric stabilization derived from pectin can stabilize protein and prevent it from undergoing emulsion destabilization due to environmental stresses.

Protein and polysaccharide interactions can be incorporated to improve the properties and stability in food emulsions. The protein and pectin soluble complexes are driven mainly due to the electrostatic interactions between the opposite charges of the different molecules (Jones and McClements 2011). Protein and polysaccharides soluble complexes can be formed from cationic protein surfaces and anionic polysaccharides, or vice versa. There have been studies that focus on the formation of protein and polysaccharide soluble complexes at pH<pI, where there is high electrostatic interaction between positive charge on the protein surface and negative charge on from the anionic polysaccharide. However, heating protein and polysaccharides had been shown to improve emulsion stability.

Heating proteins above the denaturation state has been shown to improve emulsion stability. When protein is heated above the denaturation state, the molecular structure of protein changes and forms sulfhydryl bonds and thus creating more oppositely charged groups that can interact with anionic polysaccharides during pH adjustment near pI (Jones et al. 2010). Research showed that at pH<pI of heated WPI (0.5% w/w) and low-methoxyl pectin resulted in a number of cationic groups which lead to stronger electrostatic interactions between protein and polysaccharide complexes (Salminen and Weiss 2014). Studies from our laboratory showed that heating WPI with a negatively charged polysaccharide at pH above pI leads to the formation of

heated complexes with improved emulsification properties. The formation of soluble complexes is focused on the positive charges localized on the protein surface which can bind to anionic groups of polysaccharides (Vardhanabhuti et al. 2009). Work had shown that emulsions with 20% oil stabilized by heated whey protein and pectin complex (HCPX) formed by heating 3% WPI with 0.3% pectin at pH 5.5 to 6.2 at 85^{o}C had smaller mean droplet sizes and higher negative charges compared to those stabilized by heated protein alone (Kotchabhakdi 2018). Increasing pectin in the HCPX to 0.3% also produced the most stable emulsions (>30 days) due to small droplet sizes with higher surface charge.

Milk proteins, such as whey are some of the most commonly used emulsifiers in cheese sauce. The stability of oil in water food emulsions, such as cheese sauce is dependent on many factors including oil content, type and concentration of emulsifiers, thickening agents, homogenization method, salts and cryoprotectants. The initial particle size of lipid droplets relates to the stability of a desirable sauce (Degner et al. 2014). One application of cheese sauces is in frozen ready to eat meals. Oil in water emulsions are highly susceptible to physical instability during freezing and thawing, therefore focusing on delivering desirable quality is crucial (Sherman 1968; Friberg and Larsson 1997; Dickinson 2001; van Aken et al. 2003; Cramp et al. 2004; McClements 2005). Due to the fact that food emulsions are highly prone to breaking down during freezing and thawing, the sample may appear to look spoiled due to phase separation which appears to be undesirable to consumers. It is crucial for manufacturer to produce products with good properties and stability by carefully selecting processing conditions, emulsifier and stabilizer.

The objective of this study was to apply HCPX as a clean label emulsifier and stabilizer in order to replace synthetic emulsifiers in cheese sauce. The effect of HCPX formation conditions

(e.g., pH and pectin concentration) on the properties and stability of cheese sauce were measured. In this study, the sauces were characterized by testing the rheological properties, mean fat particle size (d_{43}), storage stability, microscopy, and freeze/thaw in order to determine the emulsifier system that led to an acceptable cheddar cheese sauce. An acceptable cheddar cheese sauce would be classified as thick, homogenous, and free of lumps.

3.2 Materials and Methods

3.2.1 Materials

Whey protein isolate was provided by Grande Custom Ingredients Group (Lomira Wisconsin). WPI contained 88.1% protein and 2.5% ash on a dry basis according to the manufacturer. Low methoxylated pectin (LM-12-CG) was provided by CP Kelco Inc. (Lille Skensved, Denmark). Natural cheddar cheese (Crystal Farms, Wisconsin Sharp Cheddar Natural Cheese) used for each replication was from the same lot number. Vegetable oil (100% soybean oil, Great Value), nonfat dry milk powder (NFDM, Great Value), salt (Iodized, Great Value), sugar (Great Value), vinegar (5% acidity, Great Value), and native corn starch (Argo) were purchased from the local grocery store. Food-grade potassium sorbate (Fisher Scientific), phosphoric acid, and sodium hydroxide were used as preservative and for pH adjustment, respectively. See Appendix A for specific amounts of ingredients.

Table 3.1: Additional ingredients in cheese sauces

Treatment	WPI (%)	Pectin (%)	Emulsifier (%)
CRT			
PT		0.3%	
HWPI	1.5%		
ES			0.3%
HWPI + PT	1.5%	0.3%	
HCPX-0.45-5.5	1.5%	0.225%	
HCPX-0.6-5.5	1.5%	0.3%	
HCPX-0.45-6.2	1.5%	0.225%	
HCPX-0.60-6.2	1.5%	0.3%	

3.2.2 Preparation of Whey Protein and Pectin Stock Solutions

WPI stock solution (10% w/w) was prepared by slowly dissolving the protein power into deionized (DI) water with continuously stirring for 2 h at ambient temperature (22±2°C). Pectin stock solution (2% w/w) was prepared by slowly adding LM-12 pectin powder into deionized water at 65°C and continuously stirring for 2 h using a magnetic stir plate. The stock solutions were stored at 4° C overnight to allow full hydration.

3.2.3 Preparation of Heated Whey Protein Isolate (HWPI) and Pectin Solutions

For preparing HWPI, WPI stock solution was diluted with deionized water and the pH was adjusted to 6.8 before final weight adjustment with water such that the final solution contained 8% protein. WPI solution was heated at 85°C for 30 min and cooled. For pectin solutions used to add in cheese sauce formulation, the stock solution was diluted with deionized water and pH was adjusted to 5.2 before final weight adjustment such that the final solutions contained 0.9% pectin.

3.2.4 Preparation of Heated Whey Protein Isolate and Pectin Complex

Heated whey protein isolate and pectin complex (HCPX) was prepared by diluting and adjusting the stock solutions to pH 5.5 or 6.2. Certain amounts of WPI and pectin stock solutions were mixed. Deionized water was added after pH adjustment so that the solutions contained 3% w/w protein and 0.45% to 0.60% w/w pectin and at pH 5.5 or 6.2. The mixed solutions were gently stirred for 30 minutes at ambient temperature (22±2°C) at room temperature before heating in a water bath set to 85°C for 30 minutes. The solutions were immediately cooled after heating.

3.2.5 Cheese Sauce Processing Method

A multifunctional cooker (ThermomixTM 5) was used to process the cheese sauce. Base formulation of all samples is shown in Appendix A. In this experiment, the natural cheddar cheese was initially cut into 2 x 2 cm squares and then grated/minced in the Thermomix™. The Thermomix was set to a high speed of 8 for a short time of 5 seconds to finely grate the cheese. After the cheese finished being grated, the Thermomix dial was then set for 12 minutes, 90ºC, speed 1, and vegetable oil (100% soybean oil, Great Value) was added as the grated cheese stirred and melted in the Thermomix. Once the vegetable oil was added, the remaining ingredients (non-fat dry milk, starch, complex, salt, sugar, deionized water, salt sugar, and vinegar) were added to the thermo mix. When the temperature reached 90ºC, the speed was increased to 4.

3.2.6 Measuring Rheological Properties

A Kinexus Malvern Rheometer (Malvern Instruments Ltd., Worcestershire, UK)was used to measure the oscillatory viscoelastic properties of the processed cheddar cheese sauces. The rheometer was equipped with a plate- and-plate geometry (40 mm). All samples were cooled to ambient temperature for one hour before they were placed on the rheometer (Lee, Anema and Klostermeyer 2004). The upper geometry was descended to a 2 mm gap. A plastic spatula was used to trim the excess sample. Mineral oil was applied around the edge and a solvent trap was used to prevent the samples from drying during tests. Strain sweep was performed from 0.01-100% at 1 Hz, 20ºC. Frequency sweep was performed at 0.1 to 100 Hz at 0.1% strain and 20ºC. For temperature sweep, sample was heated from 20ºC-80ºC at 1.5ºC/min while the viscoelastic properties were measured at 0.1% strain and 1 Hz.

3.2.7 Fat Particle Size Measurement

A laser diffraction particle size analyzer was used in the study to measure the fat particle size of the processed cheddar cheese sauces. Sample preparation and measuring method from (Lee SK et al. 2004) were used with modification. Cheese sauce was equilibrated to room temperature prior to dissolving. Sample (0.5g) was dispersed in DI water containing Tween 20 (0.06g) and EDTA (0.1875g). The solution was adjusted to a pH of 10, re-weighed and DI water was used to bring the total weight of the solution to 50g. The solution was stirred and stored at 4^{o}C overnight. The next day, the sample was stirred and equilibrated to room temperature for one hour before measurement (Lee SK et al. 2004). About 40ml of solution was suspended to the wet dispersion unit (Hydro LV/MV) of a Mastersizer 3000™ (Malvern Panalytical Ltd.) (Worcestershire, UK).The fat particle size obtained, d_{43}, was the volume-to- mean distribution of the particles and was used as a measure of the relative size of the fat particles in the processed cheese sauces. Each sample was measured in duplicate.

3.2.8 Storage Stability

A centrifuge (Eppendorf, Centrifuge 5804 R) was used to determine the storage stability of the processed cheddar cheese sauces. The processed cheddar cheese sauces (15g) was stored in 50mL centrifuge tubes at 4^{o}C for three weeks. The forced syneresis method was demonstrated in this study to determine the shelf stability (Wolfschoon-Pombo, Dang, Chiriboga 2018). This method consists of re-weighing the samples and serum with increasing the rpm or g-force per step. In this study, the 15g sample was centrifuged at 6,000 rpm, 9,000 rpm, and 12,000 rpm, each at 10^{o}C for 10 minutes. After each step, the centrifuge tube was tilted on an approximately 45^{o} angle,

and serum was siphoned out of the tube and weighed on a weigh boat. The total serum released was calculated by adding up the serum in each step, then expressed as a percentage of the total sample weight (Eq. 1).

$$\text{The total serum released (TRS) \%} = (\sum \text{serum per step (g)}/15\text{g}) \times 100 \quad (1)$$

3.2.9 Determination of Freeze/Thaw Stability

To investigate the effect of freeze/thaw stability, the processed cheddar cheese sauce was cooled to 20°C and 15g sample was stored in 50 ml centrifuge tubes at 0°C. After four-day storage, the samples were thawed to ambient temperature, re-heated in a thermally-controlled water bath at 30°C for 30 min before centrifugation. The centrifugation method followed the forced syneresis method as previously described (Wolfschoon-Pombo et al. 2018).

3.2.10 Confocal Laser Scanning Microscopy

For CLSM imaging, the cheese sauce samples were stained with Rhodamine B (20µL of 0.2 w/w% solution/mL of sample) (Zhang et al. 2014) and Nile Red (30µL of 0.2 w/w% solution/mL of sample). The dyes were mixed with 1g of sample and placed on a laboratory well-made slide with a 0.17mm coverslip. Samples were imaged using a Lecia SP8 confocal microscope equipped with a 63x/1.2NA water immersion objective. Nile Red and Rhodamine B were excited with a 405nm laser and their emissions were detected using 450-500nm and 560-590nm wavelengths, respectively. To minimize crosstalk between two emission channels, images were acquired in a sequential mode. Digital image files were acquired in 1024 x 1024 resolution and the size of 92.26µm x 96.26µm at zoom 2.

3.2.11 Statistical Analysis

All analyses were repeated at least twice. ANOVA using Minitab software (Version 17.1.0) was used to determine significant differences between treatments ($P<0.05$). Tukey's pairwise comparison was used to determine the differences between means between treatments and replications. The treatments that do not share the same letter show significant difference.

3.3 RESULTS AND DISCUSSION

3.3.1 Effect of Cheese Sauce Processing on Rheological Properties

Strain sweep was used to determine the linear viscoelastic region and to compare the viscoelastic properties of the cheese sauces. The complex modulus (G*) explains the overall viscoelastic characteristics. and was used to determine the critical strain of the sample. Under the critical strain the sample remains in the linear viscoelastic region which means that sample maintains its structure during testing without any deformation. The critical strain values of all samples ranged from 0.1 to 0.5%. The lowest critical stain was used in frequency sweep and temperature sweep experiment.

In addition to determination of critical strain, strain sweep is also used to compare the viscoelastic properties of the samples. Elastic modulus (G′) is a measure of material deformation that relates to sensory attributes such as mouth coating sensation. Phase angle represents the relative extent of elastic and viscous response. The phase angle below 45^{o} indicates predominantly solid-like behavior. All sauces had a phase angle less than 45^{o} (data not shown), which indicated that they all remained solid-like property at 20^{o}C.

CTR had low viscosity at room temperature which could be due the lack of hydrocolloid typically used in the formulation. Interestingly, ES sauce was weaker than CTR as shown by lower G′ across all strain values (Figure 3.1). It was observed that full fat processed cheese containing 0.05-0.2% lecithin had decreased elasticity (Drake et.al 2006). Another study reported that lecithin reduced the hardness of reduced and low-fat feta cheese (Sipahioglu et.al 1999). This is due the fact that the structures created by the proteins in cheese are weakened with lecithin through hydration (Haumann 1986). As expected, the addition of HWPI or pectin led to higher G′; however,

the sauces prepared with HCPX-0.6-6.2 and HWPI with pectin were firmest as shown by highest G′.

The addition of stabilizers such as pectin are known to increase the firmness and the viscosity of the continuous phase in emulsions (Akhtar et al. 2002). Previous finding showed that processed cheeses with basil seed gum (BSA) had a more elastic structure due to the formation of the BSA network throughout the casein matrix (Hosseini-Parvar et al. 2015). Other studies reported that cheese analogues containing pectin gel resulted in a more compact product and as a result had a high storage modulus (G′) (Liu et al. 2008; Macků et al. 2008). The results in this study showed that sauces containing pectin had higher elasticity than the control and the sauce with the emulsifier. However, sauces containing mixed HWPI and pectin as in HWPI+PT and HCPX resulted in a firmer texture (e.g., higher G′ and lower phase angle). When compared among sauces stabilized by HCPX, higher G′ was observed in those with HCPX formed with higher pectin concentration (e.g., HCPX-0.6-5.5 and HCPX-0.6-6.2) or with HCPX formed at pH 6.2.

Frequency sweeps of moduli (G′, G″) are presented in (Fig. 3.3). All treatments showed G′ > G″ and both values were frequency dependent, indicating that these treatments were weak gels. When compared the moduli across treatments the results were in agreement with strain sweep results. The sample with the emulsifier had a lower G′ and G″ across all frequencies compared to CTR while samples with pectin (PT), HWPI or mixed whey and pectin (HWPI + PT) showed increased in G′ and G″. Note that the addition of stabilizers such as pectin are known to increase the firmness and the viscosity of the continuous phase in emulsions (Akhtar et al. 2002). A study showed the behavior of stabilizers in processed cheeses. The findings showed that processed cheeses with basil seed gum (BSA) had a more elastic structure due to the formation of the BSA network throughout the casein matrix (Hosseini-Parvar et al. 2015). Other studies reported that

cheese analogues containing pectin gel resulted in a more compact product and as a result had a high storage modulus (G′) (Liu et al. 2008; Macků et al. 2008). In this study, sauces stabilized with HWPI+ PT and HCPX showed higher G′ and G″ at across all frequencies. The highest G′ and G″ were observed in sauces containing higher pectin concentration (e.g., HWPI+PT and HCPX formed with 0.6% pectin). The HCPX formed at pH 6.2 led to the formation of firmer cheese sauces (e.g., higher G′ and G″) compared to HCPX formed at pH 5.5.

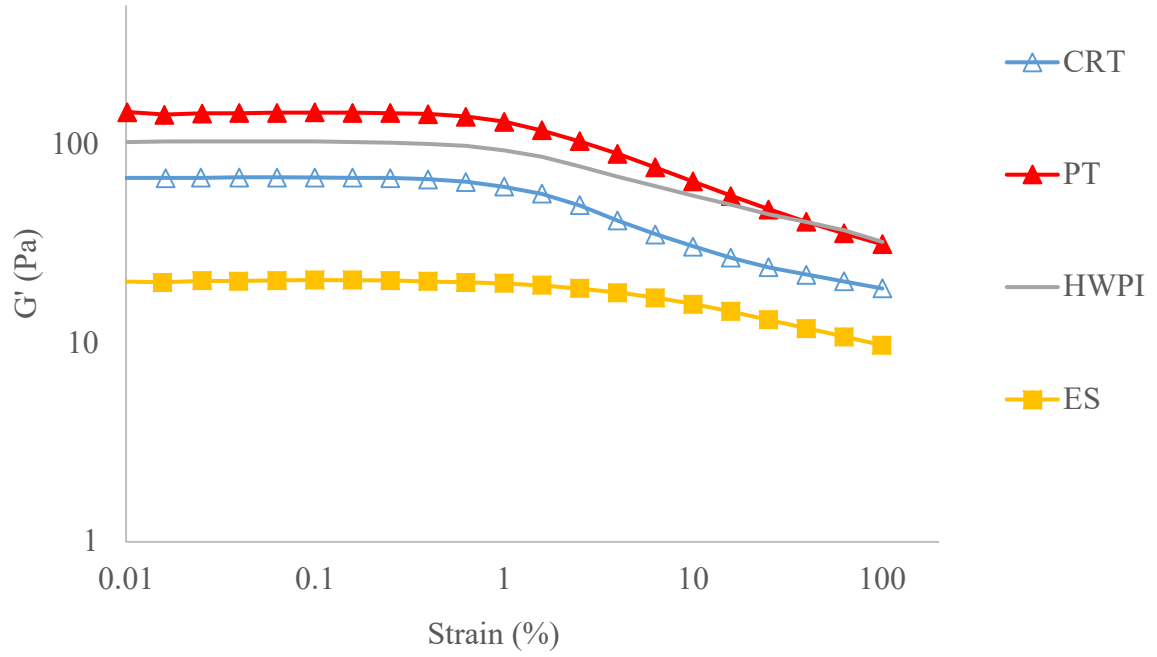
The processed cheddar cheese samples were subjected to a temperature sweep and the rheological properties were measured as temperature increased from 20-80^{o}C (Fig. 3.3). The results showed that the elasticity (G′) increased from 20-40^{o}C. Work from Eliasson (1986) described the results in temperature sweep tests and explained that the initial increase in the elastic modulus (G′) could be attributed to the starch granule swelling. Thus, an increase in elastic modulus from all samples indicated that starch granule swelling occurred at 20-40^{o}C. When temperature was increased from 40-80^{o}C a rapid decrease in G′ was observed among all treatments. Work from Eliasson (1986) also explained that a rapid decrease in G′ showed that heating altered the deformation of starch granules and the degree of molecular entanglement decreased with increasing temperatures. Therefore, the granule swelling decreased above 40^{o}C among the treatments. Results had shown that CTR and ES samples had the lowest firmness among all treatments. However, sauces with pectin had a higher firmness across 20-80^{o}C. This suggested that sauces containing pectin (stabilizer) created a more compact cheese sauces at low and high temperatures (Liu et al. 2008; Macků et al. 2008). It should be noted that the temperature at maximum G′ for PT was lower than the other treatments. It is not clear what caused this change and will be further investigated. A study showed that the cheese analogue with pectin gel melted at 25^{o}C and the control full-fat cheese analogue melted at 30^{o}C. This may be due to the fact that

hydrocolloids, such as pectin, are able to hold water and this causes swelling with the matrix (Liu H, Xu Xm, Guo Sd 2008).

Similar to previous results, HCPX and HWPI+PT had higher G′ during heating with HCPX-0.6-6.2 showing the highest G′. Interaction between WPI with pectin during HCPX formation creates a more structured network which also influenced the cheese sauce network (Ye 2008).

The ratio of the elastic (G′) and viscous (G″) modulus was expressed as the phase angle to show the physical characteristics of the sauces with increasing temperature. A phase angle of >45^{o} with G″ exceeding the G″ shows that the material is liquid-like, and at 90^{o} is shown to be completely liquid. The results in this study indicated that the phase angle was <45^{o} , and G′ exceed G″, which indicated that the samples were solid-like at low and high temperatures (data not shown).

A



B

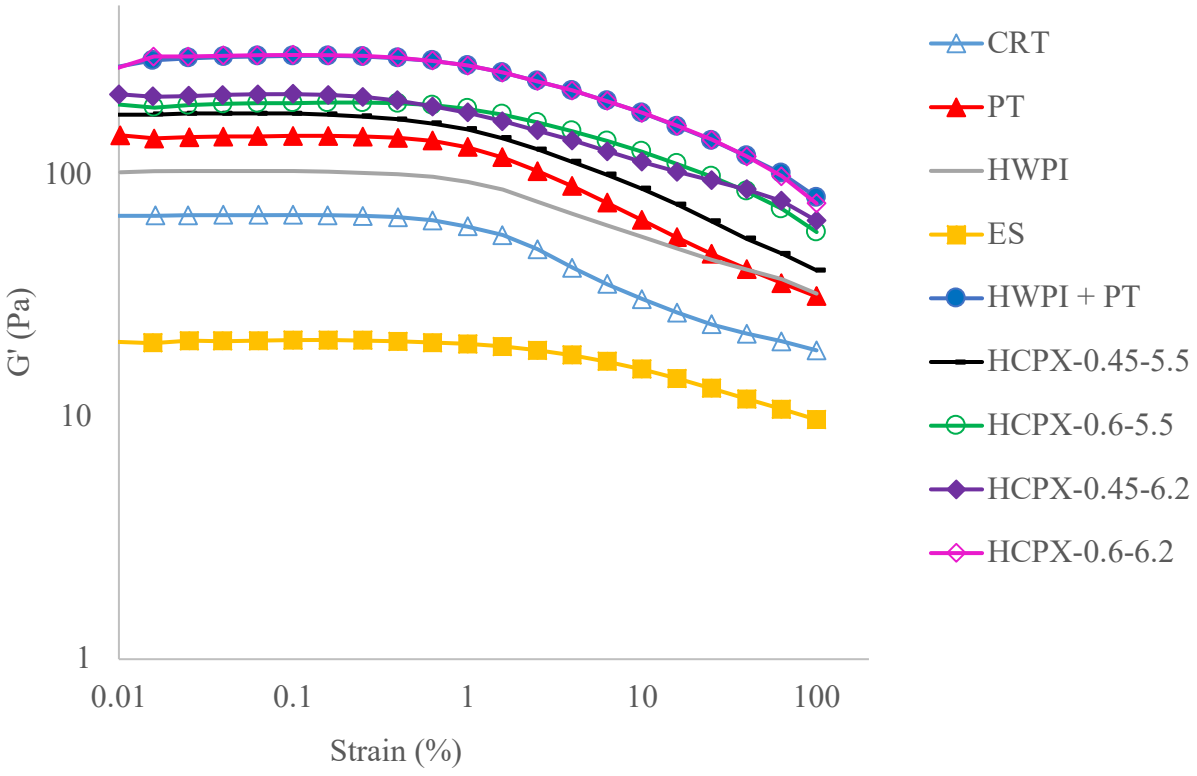


Figure 3.1: Strain sweep of processed cheddar cheese sauces at 1 Hz and 20°C: (A) Control (CRT), Emulsifier (ES), Pectin (PT), and Heated Whey Protein (HWPI). (B) All treatments.

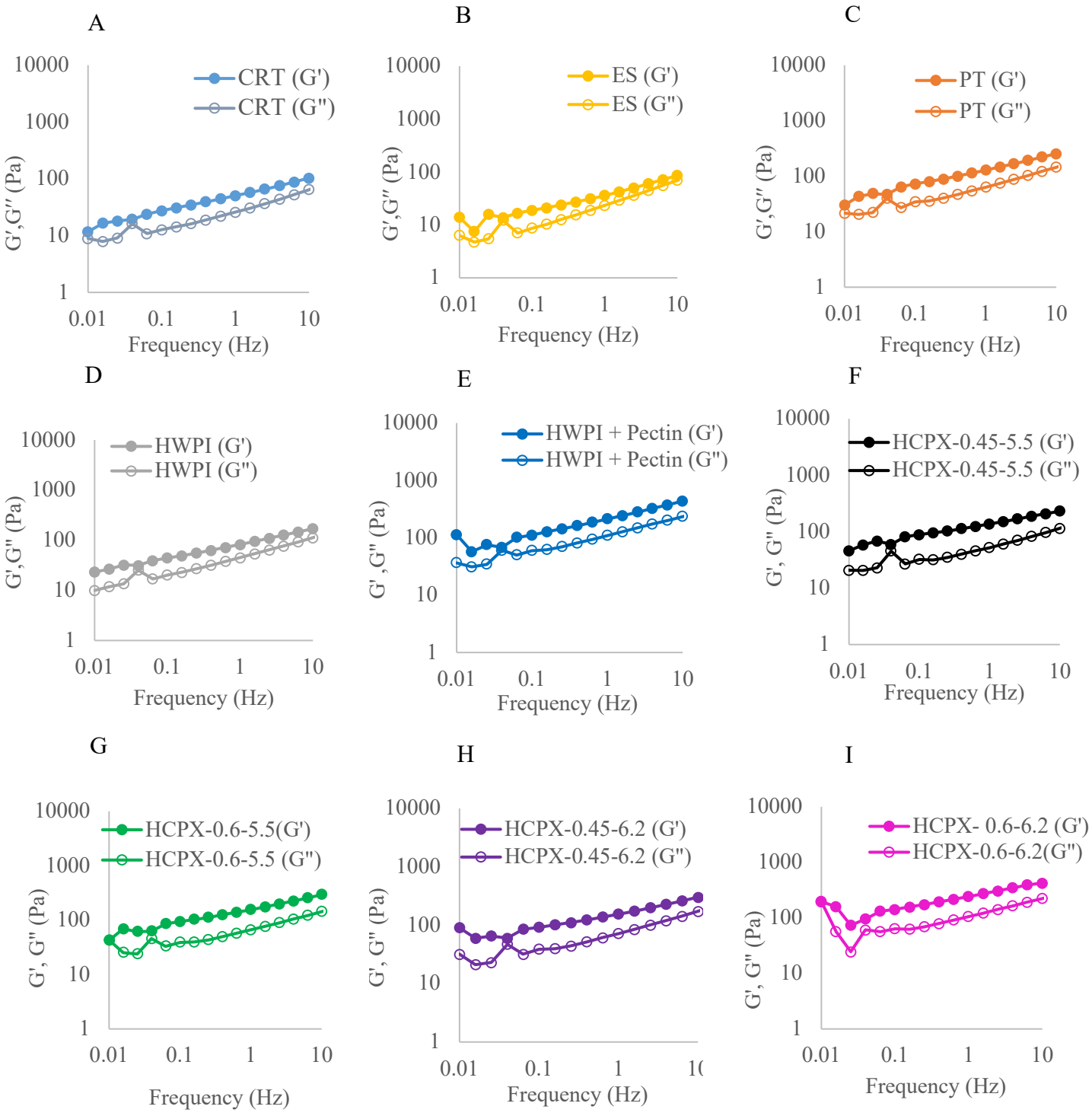


Figure 3.2: Frequency sweeps of cheese samples at 20°C at 0.1% strain: (A) Control (CRT), (B) Emulsifier (ES), (C) Pectin (PT), (D) Heated Whey Protein (HWPI), (E) Heated Whey Protein and Pectin (HWPI + PT), (F) HCPX-0.45-5.5, (G) HCPX-0.6-5.5, (H) HCPX-0.45-6.2, (I) HCPX-0.6-6.2.

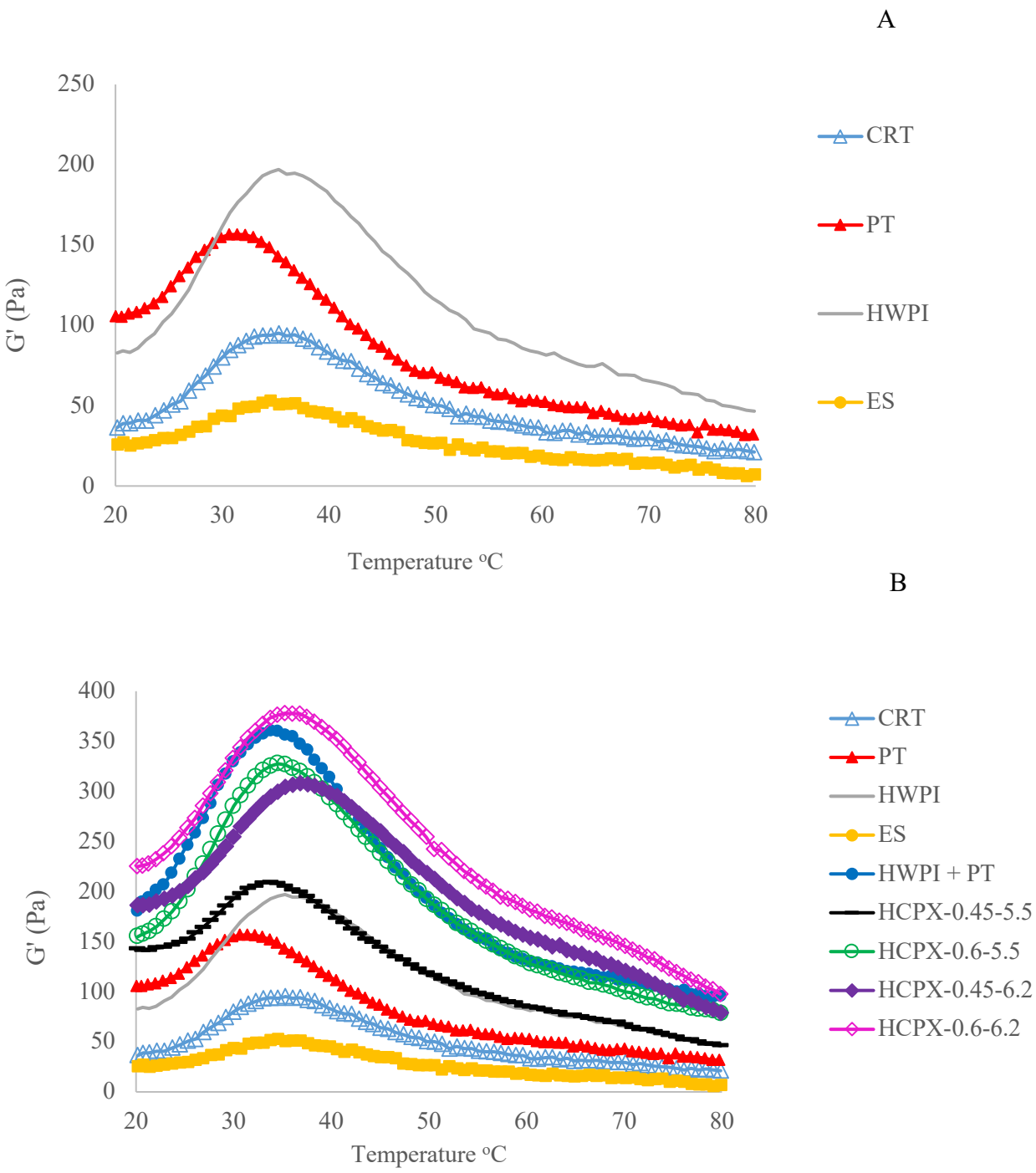


Figure 3.3: Temperature sweeps of cheese sauces from 20-80°C: (A) Control (CRT), Emulsifier (ES), Pectin (PT), Heated Whey Protein (HWPI), and (B) All treatments.

3.3.2 Mean Diameter of Oil Droplets in Cheese Sauce

As shown in Table 3.2, mean particle size (d_{43}) of freshly made CRT cheese sauce was 107±3.5 μm which was not significantly different ($p>0.05$) from PT (137±17 μm), indicating that the presence of pectin alone could not stabilize the cheese sauce. This is because these treatments did not contain emulsifier. Addition of HWPI, HWPI + Pectin or HCPX systems resulted in significant lower ($p<0.05$) in d_{43}. Emulsifiers, such as whey protein, is known to be a surface-active macromolecule containing a mixture of hydrophilic and hydrophobic amino acids along their polypeptide chain (Ozturk and McClements 2016). Proteins, such as whey protein can rapidly adsorb at the oil and water interface and lower the interracial tension as well as creating charge and generating electrostatic repulsion. Sauces stabilized by HCPX also had decreased d_{43} compared to HWPI though the differences were not significant ($p > 0.05$). This is due to the combined electrostatic and steric repulsion between droplets. Polysaccharides are known to promote steric stabilization, which can stabilize protein and prevent droplets from coalescing. (Ozturk and McClements 2016). Complexation of protein and pectin on the droplet interface increased the stability of the droplets (Salminen and Weiss 2014). Work has shown that emulsions (20% oil, 2% protein, pH 5.2) stabilized by HCPX formed with 0.60% pectin had reduced mean droplet sizes and increased stability compared to those stabilized by protein alone (Kotchabhakdi 2018). The lack of statistical differences between HWPI and HCPX could be due to the high standard deviation which is a limitation of this method. In conclusion, sauces stabilized by HCPX and HWPI resulted in better emulsions as shown by smaller mean oil droplet diameters.

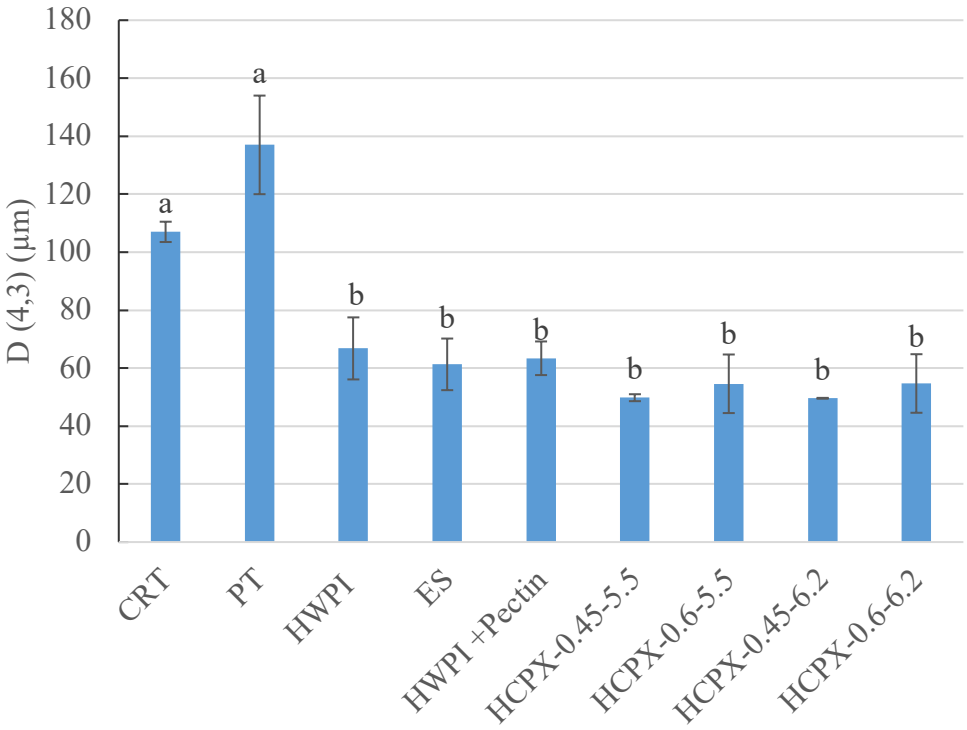


Figure 3.4: The mean diameter (d_{43}) of oil droplets results for the cheese sauce treatments.

Results are the mean of determinations. Different letters indicate significant difference ($p<0.05$) between samples.

Table 3.2: Mean Fat Particle Size Distribution (d_{43}) of all Treatments

Treatment	d_{43} [a]
CRT	107± 3.5[a]
PT	137 ± 17[a]
HWPI	66.8±10.7[b]
ES	61.3± 8.9[b]
HWPI + PT	63.4±5.8[b]
HCPX-0.45-5.5	49.8±1.2[b]
HCPX-0.6-5.5	54.6±10.1[b]
HCPX-0.45-6.2	49.6±0.14[b]
HCPX-0.6-6.2	54.7±10.1[b]

[a] Results are the mean of determinations. Different letters indicate significant difference ($p<0.05$) between samples.

3.3.3 Storage Stability

Syneresis or released water from the sauce structure observed after refrigeration or the freeze/thaw process is a negative quality factor. The water release negatively affects the appearance and textural attributes. All freshly made sauces did not exhibit syneresis (data not shown) indicating an initial stability of the sauces which can be due to the high-water absorption capacity of corn starch as well as dairy proteins (Yang et al. 2004) . After 3-week storage under refrigeration condition, CRT exhibited 28.7±1.4 % syneresis (Figure 3.6). This phenomenon is due to the reorganization of starch molecules during cold storage. Addition of ES did not affect syneresis ($p > 0.05$). Incorporation of HWPI, HWPI+PT, as well as all the HCPX resulted in significantly lower syneresis ($p < 0.05$). The effect of hydrocolloids such as pectin has been known to reduce starch retrogradation (Witczak et al. 2014). Similarly, HWPI could improve water holding capacity in several gel systems (Hongsprabhas and Barbut 1997). Lowest syneresis was observed in HCPX formed with 0.6% pectin and HWPI + PT. Thus, pectin concentration as well as pectin and protein interaction play important role in the stability of the sauces.

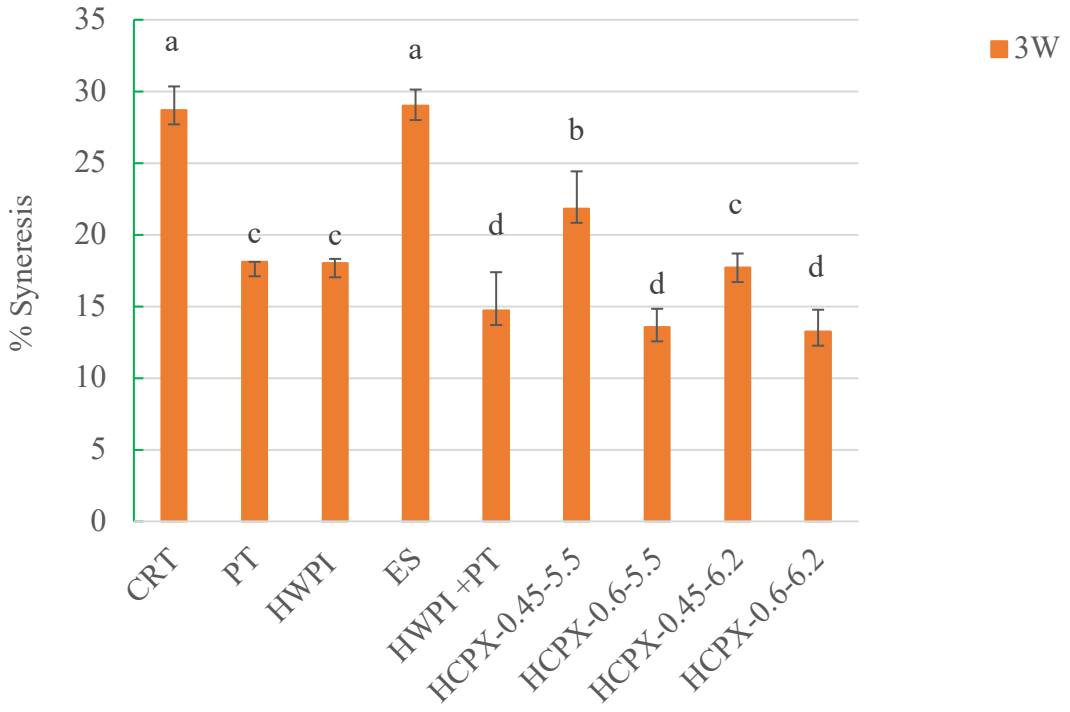


Figure 3.5: Total % syneresis of treatments after 3-week storage at 4°C.

Results are the mean of determinations. Different letters indicate significant difference ($p<0.05$) between samples.

Table 3.3: Stability after 3 weeks at 4°C.

Treatment	WPI (%)	Pectin (%)	% Serum [a]
CRT			28.7 ± 1.4[a]
PT		0.3%	18.1 ±0.3[c]
HWPI	1.5%		18.0 ±0.5[c]
ES		0.3%	29.2 ±1.3[a]
HWPI + PT	1.5%	0.3%	11.9±0.1[d]
HCPX-0.45-5.5	1.5%	0.225%	21.6±2.4[d]
HCPX-0.6-5.5	1.5%	0.3%	14.0±1.7[d]
HCPX-0.45-6.2	1.5%	0.225%	17.7±1.2[c]
HCPX-0.6-6.2	1.5%	0.3%	13.3±1.3[d]

[a] Results are the mean of determinations. Different letters indicate significant difference ($p<0.05$) between samples.

3.3.4 Freeze/Thaw Stability

Syneresis after freeze/thaw process is a common characteristic of frozen foods especially those containing starch. Stability against freeze/thaw process is an important property for frozen products. Since all of these treatments contained native corn starch, syneresis was expected to be present after one freeze/thaw cycle. Similar results were reported by Arocas et al. (2009) that white sauces containing 6% native corn and potato starch showed syneresis after one freeze/thaw cycle. Syneresis was observed among all treatments after the freeze/thaw cycle.

The CTR sample exhibited the highest amount of syneresis among all five treatments. This is since this treatment only contained starch and lacked additional protein and/or polysaccharide in order to improve the freeze thaw stability. Syneresis of PT was significantly lower ($p<0.05$) than the control sample. Polysaccharides are known to increase the viscosity of the non-frozen aqueous solution in frozen products, which inhibits the movement of fat droplets (Degner et al. 2014). In addition, it has been reported that polysaccharides such as xanthan gum, formed a protective coating around fat droplets which protects them from coalescence during freezing and thawing (Mun et al. 2008). Protein such as whey, is known to attribute to thick layers around oil droplets which can prevent fat crystals from penetrating into one oil droplet into another (Degner et al. 2014). Milk proteins, such as whey with the presence of polysaccharides such as xanthan gum were shown to reduce ice recrystallization (Degner et al. 2014). Sauces containing both HWPI and PT showed further reduction in syneresis. However, HCPX-0.6-6.2 and HWPI+PT exhibited the least syneresis compared to HCPX-0.6-5.5 though the three samples contained a similar amount of WPI and pectin. It should be noted that HCPX-0.6-6.2 and HWPI+PT also showed higher G' compared to HCPX-0.6-5.5. Thus, these results indicate that the method to formed mixed WPI and pectin plays a role in their functions.

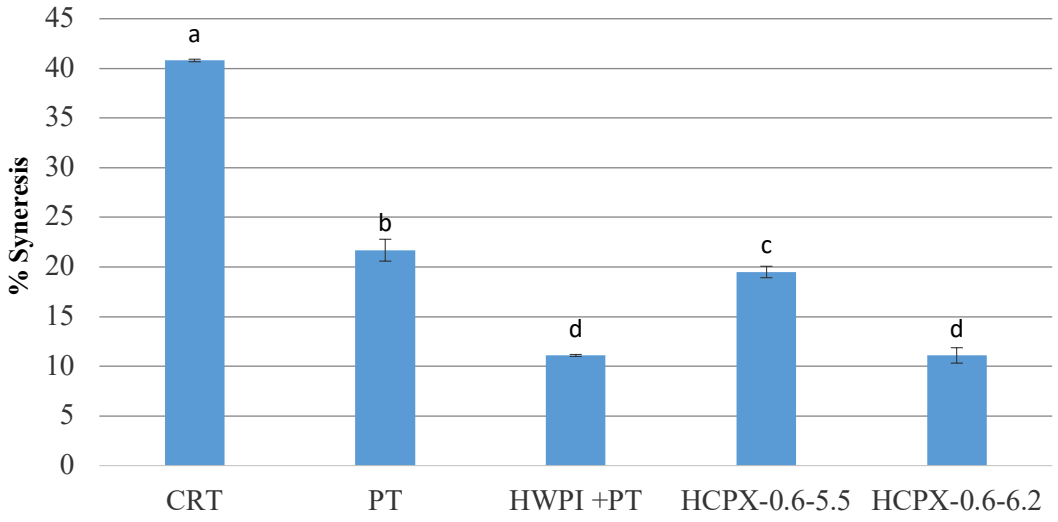


Figure 3.6: Total % syneresis after one freeze/thaw cycle after 4-day storage.

Table 3.4:Freeze/thaw stability

Treatment	WPI (%)	Pectin (%)	% Serum [a]
CRT			40.8±0.2[a]
PT		0.3%	21.7±1.2[b]
HWPI + PT	d 1.5%	0.3%	11.1±0.1[d]
HCPX-0.6-5.5	1.5%	0.3%	19.5±0.6[c]
HCPX-0.6-6.2	1.5%	0.3%	11.1±0.8[d]

Sauces after one freeze/thaw cycle. The % serum was shown after one freeze/thaw cycle after 4-day storage among the five treatments that showed a significant difference ($p<0.05$).

[a] Results show % serum in the 3 –week storage stability results. According to the table showed above, control and pectin are significantly different from sauces stabilized with HCPX.

3.3.5 Microstructure

Treatments showing highest stability which include HWPI+PT, HCPX-0.6-5.5 and HCPX-0.6-6.2 were selected for microstructural analysis. CTR and PT treatments were also included as comparison. Oil droplets are shown with the Nile red staining (Figure 3.8) while protein network is shown with Rhodamine B staining (Figure 3.9). The CLSM images showed that the fat globules were in the protein matrix of the samples. CRT clearly exhibited the largest fat globule size distribution compared to the other treatments indicating that this treatment had poor emulsion stability (Figure 8-a1). PT showed flocculated droplets though the individual droplets were small. Treatment with HWPI+PT showed smaller fat globule sizes compared to the control; however, there were few large droplets. Sauces with HCPX both exhibited smaller droplet sizes and more even size distribution with HCPX-0.6-5.5 showing the smallest sizes overall. Stabilizers, such as pectin, are known to stabilize vegetable-oil-in-water emulsions and therefore promote smaller lipid globules (Akhtar et al. 2002). Proteins and polysaccharides interactions upon heating have been known to stabilize emulsions droplets from coalescing by creating electrostatic repulsion between molecules. Smaller and more even size distribution of the fat droplets suggest that the emulsion is and less prone to coalescence and flocculation.

When Rhodamine B is used the structure of the protein network can be observed. The sauce containing pectin (PT) clearly showed more porous structure compared to sauces with HWPI+PT or HCPX. Sample with HCPX were the least porous. The results are in-line with the stability results which showed higher stability with the HCPX and HWPI+PT compared to CTR and PT samples. A previous study from our laboratory has shown that HCPX had improved emulsification and emulsion stabilization properties in oil in water emulsions with 20% and at pH 5.2. Our results

showed that these HCPX could be used to emulsify and stabilize the emulsion and reduce syneresis in cheese sauce.

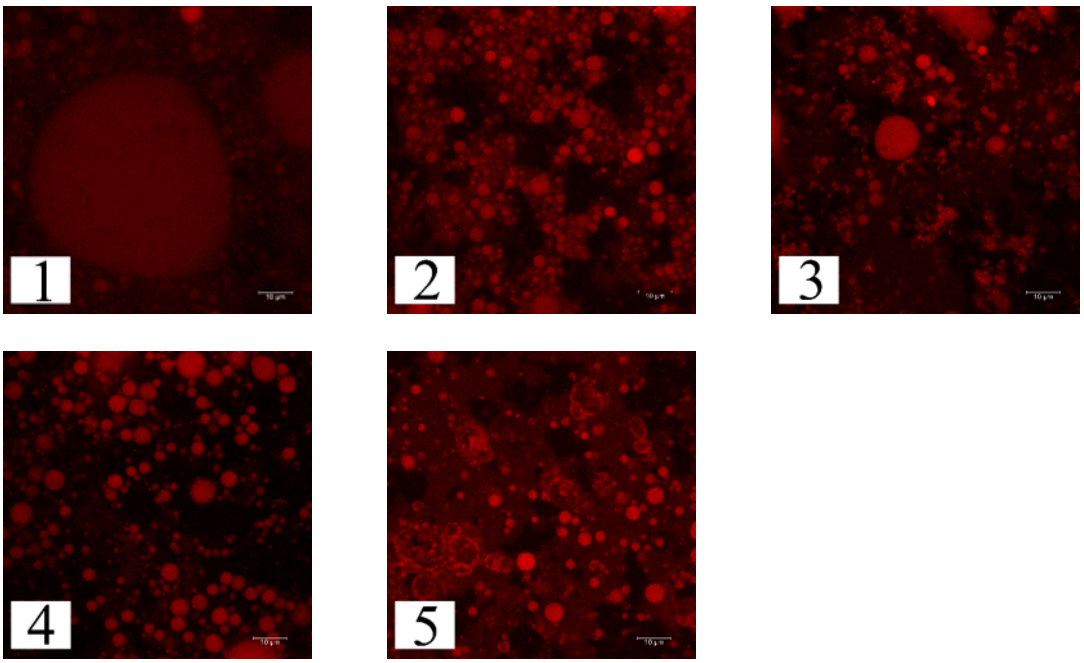


Figure 3.7: Confocal micrographs of microstructure: (1) CRT, (2) PT, (3) HWPI + PT, (4) HCPX-0.60-5.5, and (5) HCPX-0.60-6.2. The Nile red stained fat appears red. The scale bars are 10µm in length.

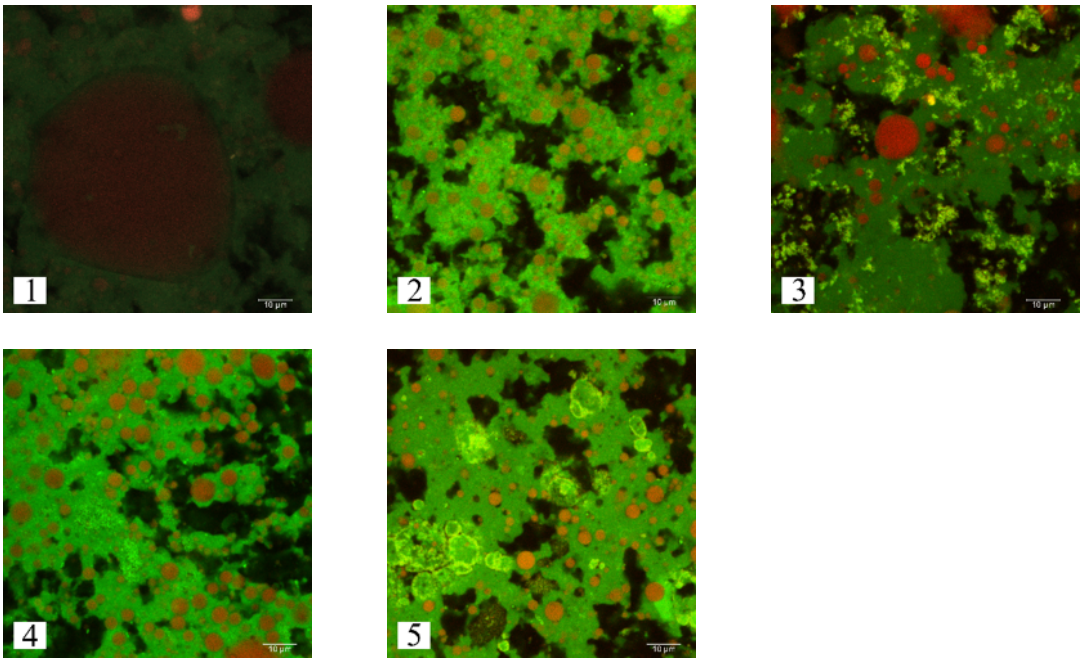


Figure 3.8: Confocal micrographs microstructure of (1) CTR, (2) PT, (3) HWPI+PT, (4) HCPX-0.60-5.5, and (5) HCPX-0.60-6.2. The Nile red stained fat appears red and the Rhodamine B stained protein stains the green. The scale bars are 10µm in length.

3.3.6 Conclusion

Cheese sauce containing no emulsifiers and/or stabilizers (CTR) was shown to have large oil droplets as shown by mean lipid droplet sizes (d_{43}) and microstructural images, weak elasticity (G′) as shown by rheological properties, poor stability during cold temperature storage as well as poor stability after one freeze/thaw cycle. The addition of the emulsifier decreased oil droplet sizes of freshly made sauce but weaken the sauce structure and did not improve storage stability. The use of HWPI alone led to firmer sauce and decreased syneresis and decreased oil droplet sizes. The PT treatment showed large d_{43}, similar to CTR, and flocculated droplets in CLSM images; however, decreased syneresis was observed after refrigeration storage and one freeze/thaw cycle. Overall, the sauces with mixtures of whey protein and pectin were the firmest with small oil droplet sizes and more stable under refrigeration or freeze/thaw. The method of preparing mixed whey protein and pectin played important role in the cheese sauce properties. Higher pectin concentration resulted in firmer sauce and improved stability. Formation of HCPX at higher pH led to a firmer sauce and improved freeze/thaw stability. In comparison to HWPI+PT, sauces stabilized by HCPX showed smaller oil droplet sizes and less porous structure. In conclusion, HCPX has great potential to replace synthetic emulsifiers and stabilizers.

Table 3.5: Summary of Results

Treatment	Average Particle Size	% Syneresis (3 Wk at 4°C)	% Syneresis (Freeze Thaw)
CRT	107± 3.5[a]	28.7 ± 1.4[a]	40.8±0.2[a]
PT	137 ± 17[a]	18.1 ±0.3[c]	21.7±1.2[b]
HWPI	66.8±10.7[b]	18.0 ±0.5[c]	
ES	29.2 ±1.3[a]	29.2 ±1.3[a]	
HWPI + PT	63.4±5.8[b]	11.9±0.1[d]	19.5±0.6[c]
HCPX-0.45-5.5	49.8±1.2[b]	21.6±2.4[d]	
HCPX-0.6-5.5	54.6±10.1[b]	14.0±1.7[d]	19.5±0.6[c]
HCPX-0.45-6.2	49.6±0.14[b]	17.7±1.2[c]	
HCPX-0.60-6.2	54.7±10.1[b]	13.3±1.3[d]	11.1±0.8[d]

CHAPTER 4

CONCLUSION

4.1 Summary

The overall objective of this study was to apply HCPX as a clean label emulsifier and stabilizer in order to replace synthetic emulsifiers in cheese sauce. The cheese sauce treatments with HCPX showed increased firmness, smaller average oil droplet diameters, and lower syneresis after cold storage or freeze/thaw. These results indicated improved emulsification properties and stability. HCPX, which is clean label, can replace synthetic emulsifiers and stabilizers in cheddar cheese sauce. The method used to form HCPX also plays important roles on the properties and stability of the cheese sauces.

4.2 Future Work

Consumers today are not only interested in clean label products, but also the flavor and appearance of foods. Running a sensory panel and comparing control and sauces stabilized by HCPX can be usefully in order to determine whether they will be accepted by consumers.

REFERENCES

Aguilera JM. 2018. Relating food engineering to cooking and gastronomy. Compr Rev Food Sci Food Saf 17:1021–1039. doi: 10.1111/1541-4337.12361.

Akhtar M, Dickinson E, Mazoyer J, Langendorff V. 2002. Emulsion stabilizing properties of depolymerized pectin. Food Hydrocoll 16:249–256. doi: 10.1016/S0268-005X(01)00095-9.

Albert CM, Campos H, Stampfer MJ, Ridker PM, Mason JE, Willett WC, Ma J. 2002. Blood levels of long chain n-3 fatty acids and the risk of sudden death. N Engl J Med 346:1113–1118. doi: 10.1056/NEJMoa012918.

Arocas A, Sanz T, Fiszman SM. 2009. Clean label starches as thickeners in white sauces. Shearing, heating and freeze/thaw stability. Food Hydrocoll 23:2031–2037. doi: 10.1016/j.foodhyd.2009.04.006.

Asioli D, Aschemann-Witzel J, Caputo V, Vecchio R, Annunziata A, Næs T, Varela P 2017. Making sense of the "clean label" trends: a review of consumer food choice behavior and discussion of industry implications. Food Res Int 99:58–71. doi: 10.1016/j.foodres.2017.07.022.

Bayod E. 2008. Microstructural and Rheological Properties of Concentrated Tomato Suspensions during Processing. Dissertation.

Beck M, Jekle M, Becker T. 2011. Starch re-crystallization kinetics as a function of various cations. Starch/Staerke 63:792–800. doi: 10.1002/star.201100071.

BeMiller JN, Huber KC. 2015. Physical modification of food starch functionalities. Annu Rev Food Sci Technol 6:19.

Brewer MS, Prestat CJ. 2002. Consumer Attitudes Toward Food Safety Issues. J Food Saf 22:67–83. doi: 10.1111/j.1745-4565.2002.tb00331.x.

Chanamai R, McClements D J. 2002. Comparison of gum arabic, modified starch, and whey protein isolate as emulsifiers: Influence of pH, CaCl2 and temperature. J Food Sci 67:120–125. doi: 10.1111/j.1365-2621.2002.tb11370.x.

Childs J l., Yates MD., Drake M. 2009. Sensory properties and consumer perception of wet and dry cheese sauces. J Food Sci 74:S205–S216. doi: 10.1111/j.1750-3841.2009.01187.x

Cho Y-H, McClements DJ. 2009. Theoretical stability maps for guiding preparation of Emulsions Stabilized by Protein-Polysaccharide Interfacial Complexes. Langmuir 25:6649–6657. doi: 10.1021/la8006684.

Cramp GL, Docking AM, Supratim Ghosh, Coupland JN. 2004. On the stability of oil-in-water emulsions to freezing. Food Hydrocoll 18:899–905. doi: 10.1016/j.foodhyd.2003.10.007.

Degner BM, Chung C, Schlegel V, Hutkins R, McClements JD. 2014. Factors influencing the freeze-thaw stability of emulsion-based foods. Compr Rev Food Sci Food Saf 13:98–113.

Demetriades K, Coupland NJ, McClements JD. 1997. Physiochemical properties of whey protein-stabilized emulsions as affected by heating and ionic strength. J Food Sci Wiley-Blackwell 62:462. doi: 10.1111/j.1365-2621.1997.tb04407.x.

Dickinson E. 2003. Hydrocolloids at interfaces and the influence on the properties of dispersed systems. Food Hydrocoll 17:25–39. doi: 10.1016/S0268-005X(01)00120-5.

Dickinson E. 2001. Milk protein interfacial layers and the relationship to emulsion stability and rheology. Colloids Surf B Biointerfaces 20:197–210. doi: 10.1016/S0927-7765(00)00204-6.

Drake AM, Truong V.D, Daubert RC. 2006. Rheological and Sensory Properties of Reduced-Fat Processed Cheeses Containing Lecithin. J Food Sci 64:744-747.

Dybowska BE. 2011. Whey protein-stabilized emulsion properties in relation to thermal modification of the continuous phase. J Food Eng 104:81–88. doi: 10.1016/j.jfoodeng.2010.11.030.

Eliasson A-C. 1986. Viscoelastic behavior during the gelatinization of starch in comparison of wheat, maize, potato and waxy-barley starches. J Texture Stud 17:253–265. doi: 10.1111/j.1745-4603.1986.tb00551.x.

Fall A, Bertrand F, Ovarlez G, Bonn D. 2009. Yield stress and shear-banding in granular suspensions. doi: 10.1103/PhysRevLett.103.178301.

Friberg S, Larsson K (eds). 1997. Food emulsions, 3rd ed., rev. and expanded. Marcel Dekker, New York.

Funami T, Kataoka Y, Omoto T, Goto Y, Asai I, Nishinari K. 2005. Effects of non-ionic polysaccharides on the gelatinization and retrogradation behavior of wheat starch☆. Food Hydrocoll 19:1–13. doi: 10.1016/j.foodhyd.2004.04.024.

Guardeno LM, Sanz T, Fiszman SM, Quiles A, Hernando I. 2011. Microwave heating effect on rheology and microstructure of white sauces. J Food Sci 76:E544–E552. doi: 10.1111/j.1750-3841.2011.02339.x.

Hall GM (ed). 1996. Methods of Testing Protein Functionality. Springer US.

Haumann, B. F. 1986. Getting the fat out. Journal of American Oil Chemists Society 63: 278-288.

Hongsprabhas P, Barbut S. 1997. Ca2+-Induced cold gelation of whey protein isolate: effect of two-stage gelation. Food Res Int 30:523–527. doi: 10.1016/S0963-9969(98)00010-6.

Hosseini-Parvar SH, Matia-Merino L, Golding M. 2015. Effect of basil seed gum (BSG) on textural, rheological and microstructural properties of model processed cheese. Food Hydrocoll 43:557–567. doi: 10.1016/j.foodhyd.2014.07.015.

Huan Y, Zhang S, Vardhanabhuti B. 2016.Effect of CMC molecular weight on acid-induced gelation of heated WPI-CMC soluble complex. J Food Sci 81:N502–N507. doi: 10.1111/1750-3841.13209.

Jones OG, Decker EA, McClements DJ. 2010.Comparison of protein–polysaccharide nanoparticle fabrication methods: Impact of biopolymer complexation before or after particle formation. J Colloid Interface Sci 344:21–29. doi: 10.1016/j.jcis.2009.12.017.

Jones OG, McClements DJ.2011.Recent progress in biopolymer nanoparticle and microparticle formation by heat-treating electrostatic protein–polysaccharide complexes. Adv Colloid Interface Sci 167:49–62. doi: 10.1016/j.cis.2010.10.006.

Kakalis LT, Regenstein JM.1986.Effect of pH and salts on the solubility of egg white protein. J Food Sci 51:1445. doi: 10.1111/j.1365-2621.1986.tb13830.x.

Katsuta K, Miura M, Nishimura A.1992.Kinetic treatment for rheological properties and effects of saccharides on retrogradation of rice starch gels. Food Hydrocoll 6:187–198. doi: 10.1016/S0268-005X(09)80359-7.

Kotchabhakdi A.2018.Developing Clean Label Emulsifier on Whey Protein and Pectin Complexes. University of Missouri. Thesis

Koupantsis T, Kiosseoglou V.2009.Whey protein–carboxymethylcellulose interaction in solution and in oil-in-water emulsion systems. Effect on emulsion stability. Food Hydrocoll 23:1156–1163. doi: 10.1016/j.foodhyd.2008.09.004.

Kuwajima K, Ikeguchi M, Sugawara T, Hiraoka Y, Sugai S.1990.Kinetics of disulfide bond reduction in alpha-lactalbumin by dithiothreitol and molecular basis of superreactivity of the Cys6-Cys120 disulfide bond. Biochemistry 29:8240–8249.

Law AJR, Leaver J.2000.Effect of pH on the Thermal Denaturation of Whey Proteins in Milk. J Agric Food Chem 48:672–679. doi: 10.1021/jf981302b.

Lee SK, Huss M, Klostermeyer H, Anema SG.2013.The effect of pre-denatured whey proteins on the textural and micro-structural properties of model processed cheese spreads. Int Dairy J 32:79–88. doi: 10.1016/j.idairyj.2013.04.006.

Lightfield, KD, Malone, MM.2005.Acidified Food Sauces.US Patent 0058761.

Liu H, Xu XM, Guo SD.2008.Comparison of full-fat and low-fat cheese analogues with or without pectin gel through microstructure, texture, rheology, thermal and sensory analysis. Int J Food Sci Technol 43:1581–1592. doi: 10.1111/j.1365-2621.2007.01616.x.

Macků I, Buňka F, Pavlínek V, Leciánová P, Hrabě J.2008.The effect of pectin concentration on viscoelastic and sensory properties of processed cheese. Int J Food Sci Technol 43:1663–1670. doi: 10.1111/j.1365-2621.2008.01734.x.

Mann B, Malik RC.1996.Studies on some functional characteristics of whey protein-polysaccharide complex. J Food Sci Technol India 33:202–206.

McClements DJ.2005.Food emulsions: principles, practices, and techniques, 2nd ed. CRC Press, Boca Raton.

McClements DJ.2015. Food Emulsions : Principles, Practices, and Techniques, Third Edition. CRC Press, Boca Raton.

McClements DJ, Gumus CE.2016.Natural emulsifiers - Biosurfactants, phospholipids, biopolymers, and colloidal particles: Molecular and physicochemical basis of functional performance. Adv Colloid Interface Sci 234:3–26. doi: 10.1016/j.cis.2016.03.002.

Mun S, Cho Y, Decker EA, McClements DJ.2008.Utilization of polysaccharide coatings to improve freeze–thaw and freeze–dry stability of protein-coated lipid droplets. J Food Eng 86:508–518. doi: 10.1016/j.jfoodeng.2007.11.002.

Ozturk B, McClements DJ.2016.Progress in natural emulsifiers for utilization in food emulsions. Curr Opin Food Sci 7:1–6. doi: 10.1016/j.cofs.2015.07.008.

Rodrigues RM, Martins AJ, Ramos OL, Malcata XF, Teixeira AJ, Vicente AA, Pereira NR.2015.Influence of moderate electric fields on gelation of whey protein isolate. Food Hydrocoll 43:329–339.

Salminen H, Weiss J.2014.Effect of pectin type on association and pH stability of whey protein-pectin complexes. Food Biophys 9:29–38.

Schmitt C, Turgeon SL.2011.Protein/polysaccharide complexes and coacervates in food systems. Adv Colloid Interface Sci 167:63–70. doi: 10.1016/j.cis.2010.10.001.

Sherman P.1968.Emulsion science. Academic Press, London, New York.

Siew Kim Lee, Anema S, Klostermeyer H.2004.The influence of moisture content on the rheological properties of processed cheese spreads. Int J Food Sci Technol 39:763–771. doi: 10.1111/j.1365-2621.2004.00842.x.

Sipahioglu O, Alvarez BV, Solano-Lopez C.1999.Structure, physico-chemical and sensory properties of feta cheese made with tapioca starch and lecithin as fat mimetics. Int Dairy Journal783-789.

Siriwardhana N, Kalupahana NS, Moustaid-Moussa N.2012.Health benefits of n-3 polyunsaturated fatty acids: eicosapentaenoic acid and docosahexaenoic acid. Adv Food Nutr Res 65:211–222. doi: 10.1016/B978-0-12-416003-3.00013-5.

Spanier HC.1986.Cheese sauce US Patent 4568555

Stephen AM, Phillips GO, Williams PA (eds).2006.Food Polysaccharides and their Applications, 2nd ed. CRC/Taylor & Francis, Boca Raton, FL.

Tsai M-L, Li C-F, Lii C-Y.1997.Effects of Granular Structures on the Pasting Behaviors of Starches. Cereal Chem J 74:750–757. doi: 10.1094/CCHEM.1997.74.6.750.

van Aken G, Blijdenstein T, E Hotrum N.2003.Colloidal destabilisation mechanisms in protein-stabilised emulsions. Curr Opin Colloid Interface Sci 8:371–379. doi: 10.1016/S1359-0294(03)00098-0.

Vardhanabhuti B, Yucel U, Coupland JN, Foegeding EA.2009.Interactions between β-lactoglobulin and dextran sulfate at near neutral pH and their effect on thermal stability. Food Hydrocoll 23:1511–1520. doi: 10.1016/j.foodhyd.2008.09.006.

Visee FL.2001.Cheese sauce and method for producing it. US Patent 0041209.

Wagoner T, Vardhanabhuti B, Foegeding EA.2016.Designing whey protein-polysaccharide particles for colloidal stability. Annu Rev Food Sci Technol 7:93–116. doi: 10.1146/annurev-food-041715-033315.

Wang S, Li C, Copeland L, Niu Q, Wang S.2015.Starch Retrogradation: A Comprehensive Review. Compr Rev Food Sci Food Saf. 14:568–585. doi: 10.1111/1541-4337.12143.

Wijayanti HB, Bansal N, Deeth HC.2014a.Stability of Whey Proteins during Thermal Processing: A Review:. Compr Rev Food Sci Food Saf 13:1235–1251. doi: 10.1111/1541-4337.12105.

Wijayanti HB, Bansal N, Sharma R, Deeth HC.2014b.Effect of sulphydryl reagents on the heat stability of whey protein isolate. Food Chem 163:129–135. doi: 10.1016/j.foodchem.2014.04.094.

Williams PG, Markoska J, Chachay V, McMahon A. 2009. ‘Natural’ claims on foods: a review of regulations and a pilot study of the views of Australian consumers. Food Australia 61(9):383-389.

Witczak T, Witczak M, Ziobro R.2014.Effect of inulin and pectin on rheological and thermal properties of potato starch paste and gel. J Food Eng 124:72–79. doi: 10.1016/j.jfoodeng.2013.10.005.

Wolfschoon-Pombo AF, Dang BP, Chiriboga BC.2018.Forced syneresis determination results from commercial cream cheese samples. Int Dairy J 85:129–136. doi: 10.1016/j.idairyj.2018.05.006.

Wong DWS, Camirand WM, Pavlath AE.1996.Structures and functionalities of milk proteins. Crit Rev Food Sci Nutr 36:807–844. doi: 10.1080/10408399609527751.

Yang H, Irudayaraj J, Otgonchimeg S, Walsh M.2004.Rheological study of starch and dairy ingredient-based food systems. Food Chem 86:571–578. doi: 10.1016/j.foodchem.2003.10.004.

Ye A.2008.Complexation between milk proteins and polysaccharides via electrostatic interaction: principles and applications – a review. Int J Food Sci Technol 43:406–415. doi: 10.1111/j.1365-2621.2006.01454.x.

Zhang S, Fu-Hung Hsieh, Vardhanabhuti B.2014.Acid-induced gelation properties of heated whey protein-pectin soluble complex (Part I): effect of initial pH. Food Hydrocoll 36:76–84.

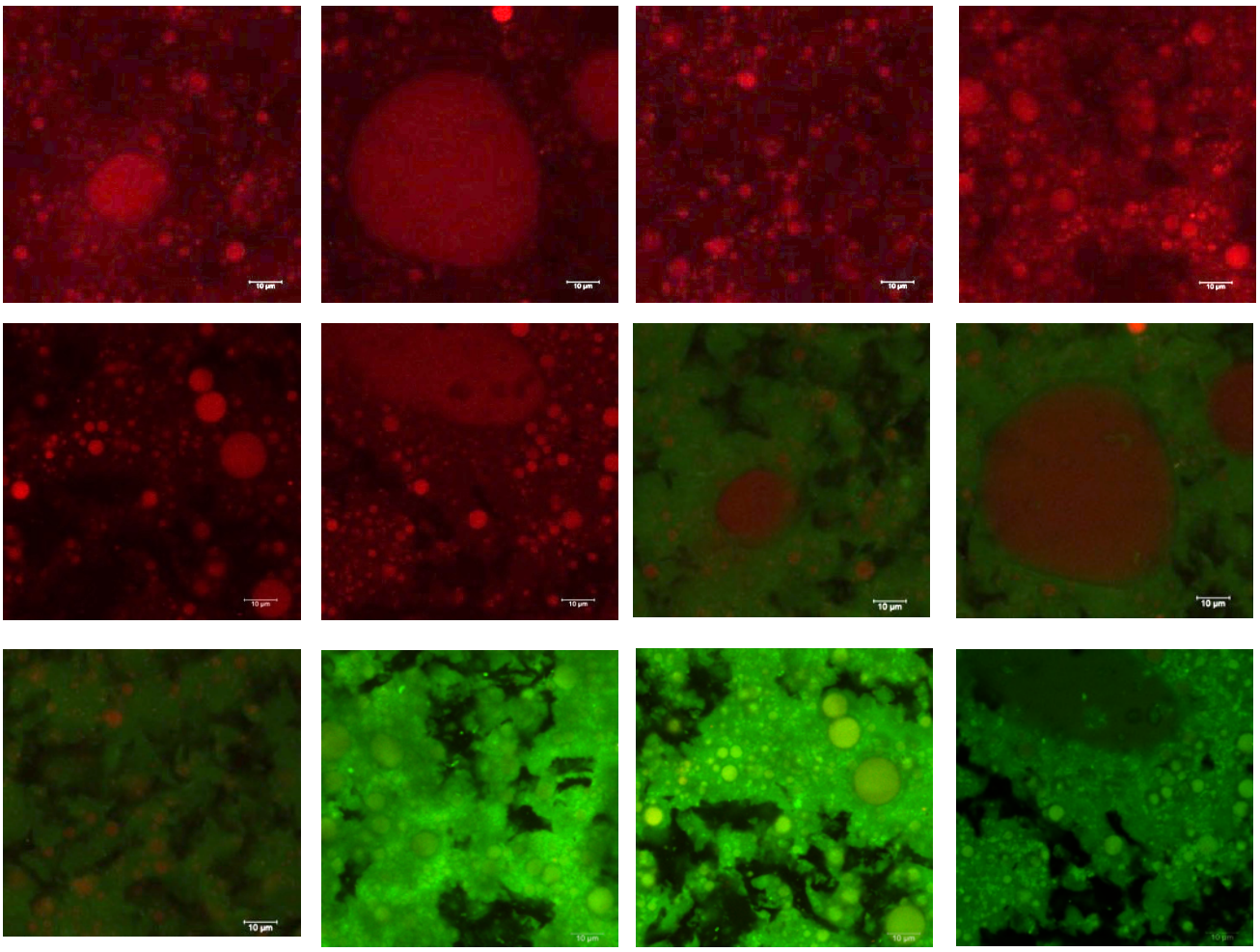
Zhen Fu, Jun Chen, Shun-Jing Luo, Liu M-C, Liu W.2015.Effect of food additives on starch retrogradation: a review. Starch/Staerke 67:69–78.

APPENDIX A: AMOUNTS OF INGREDIENTS

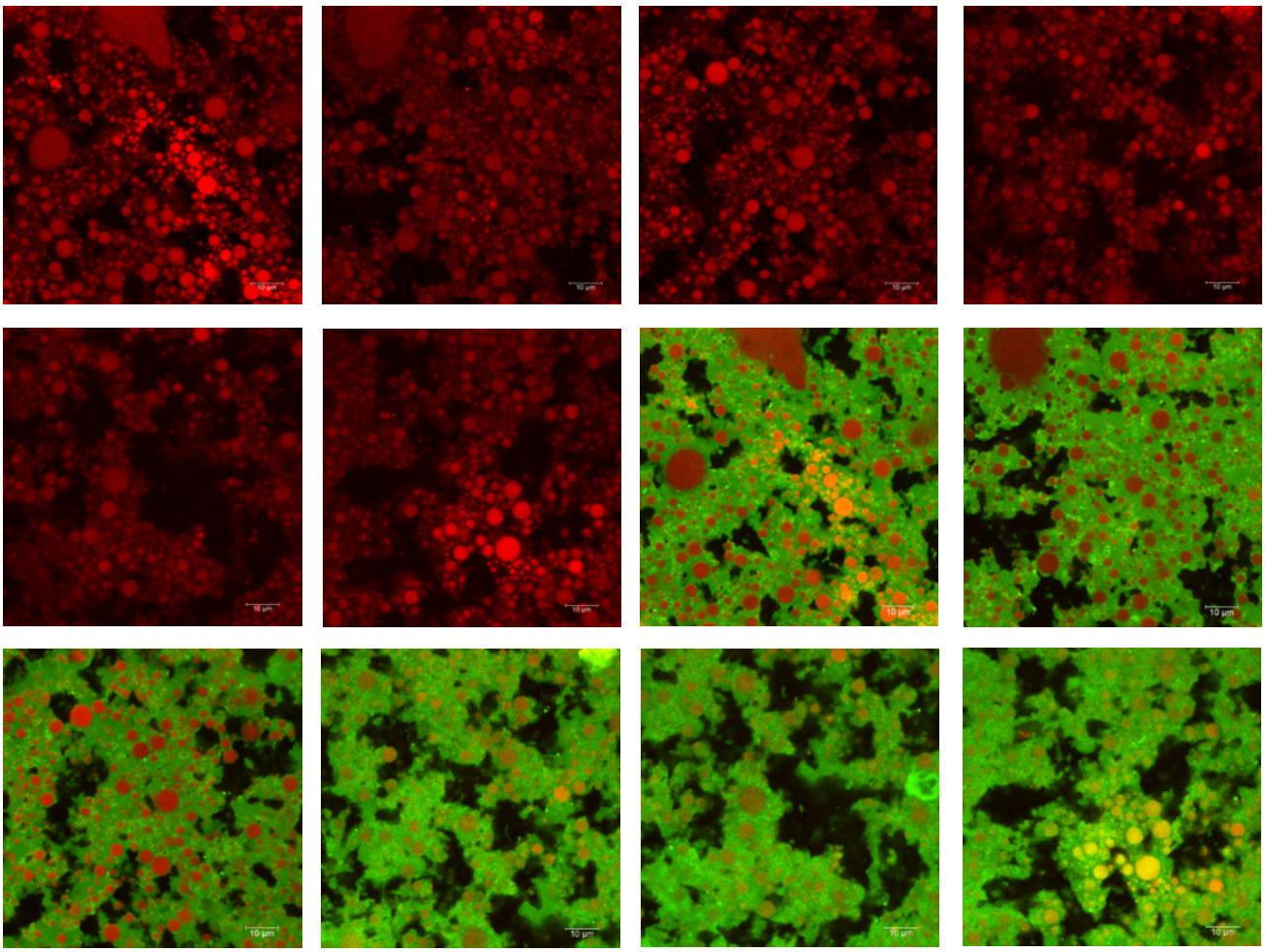
Natural Cheddar Cheese (Crystal Farms, Wisconsin Sharp Cheddar Natural Cheese)	25%
Vegetable Oil (100% soybean oil, Great Value)	7.7%
Nonfat Dry Milk Powder (NFDM, Great Value)	1%
Salt (Iodized, Great Value)	1%
Sugar (Great Value)	1.5%
Vinegar (5% acidity, Great Value)	0.5%
Potassium Sorbate (Fisher Scientific)	0.1%
Corn Starch (Argo)	3%

APPENDIX B: CLSM IMAGES

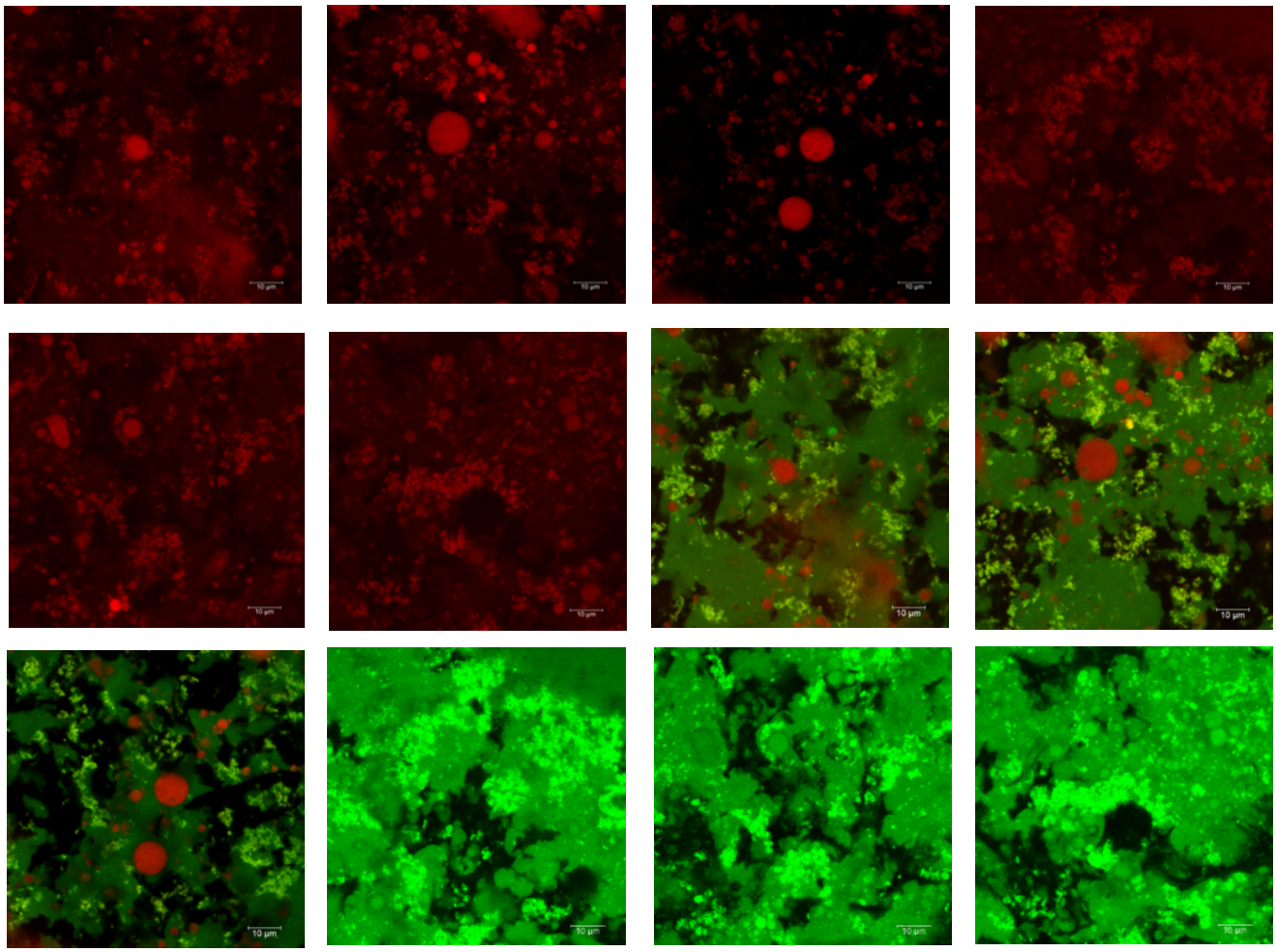
Control (CRT)



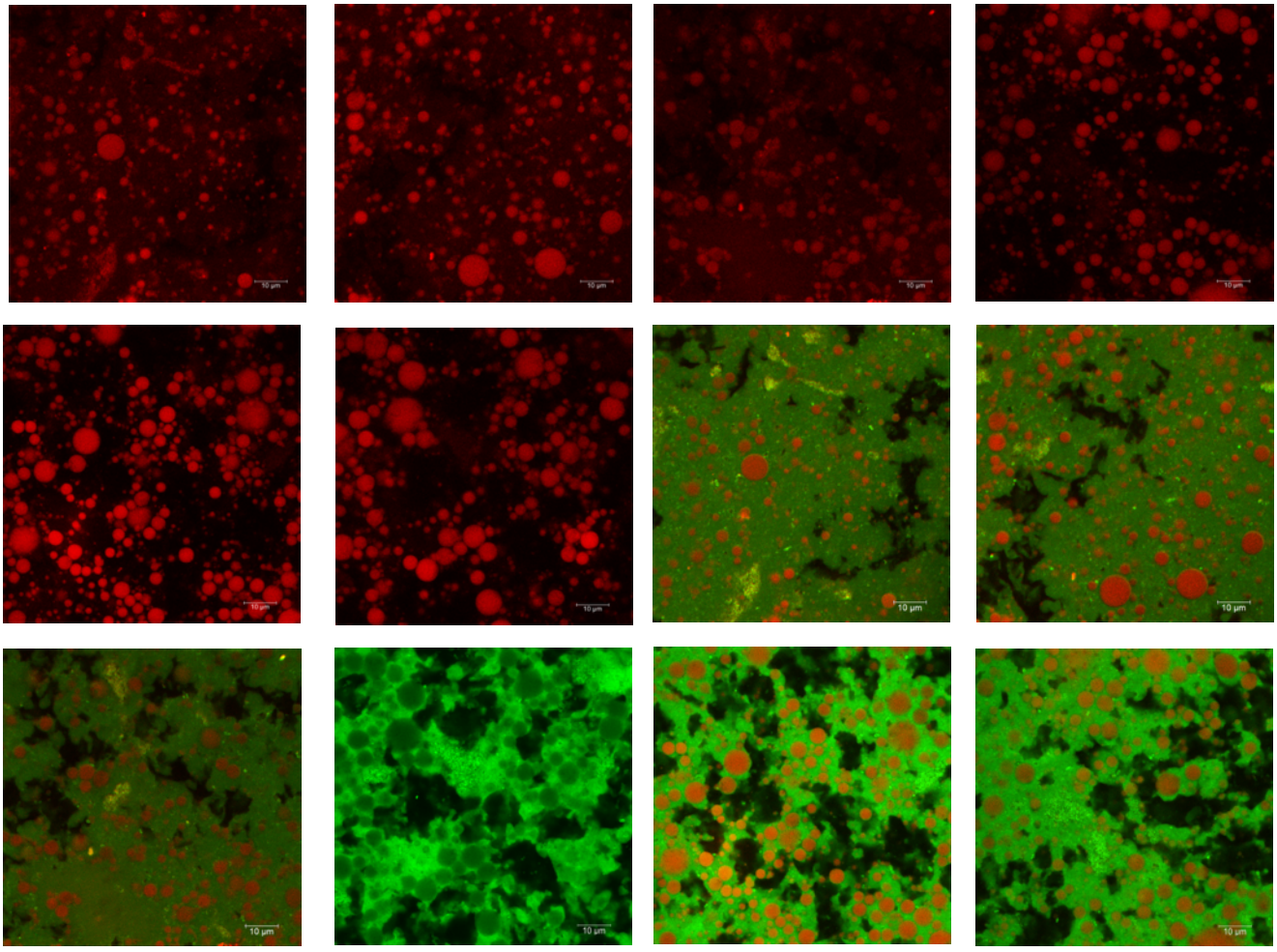
Pectin (PT)



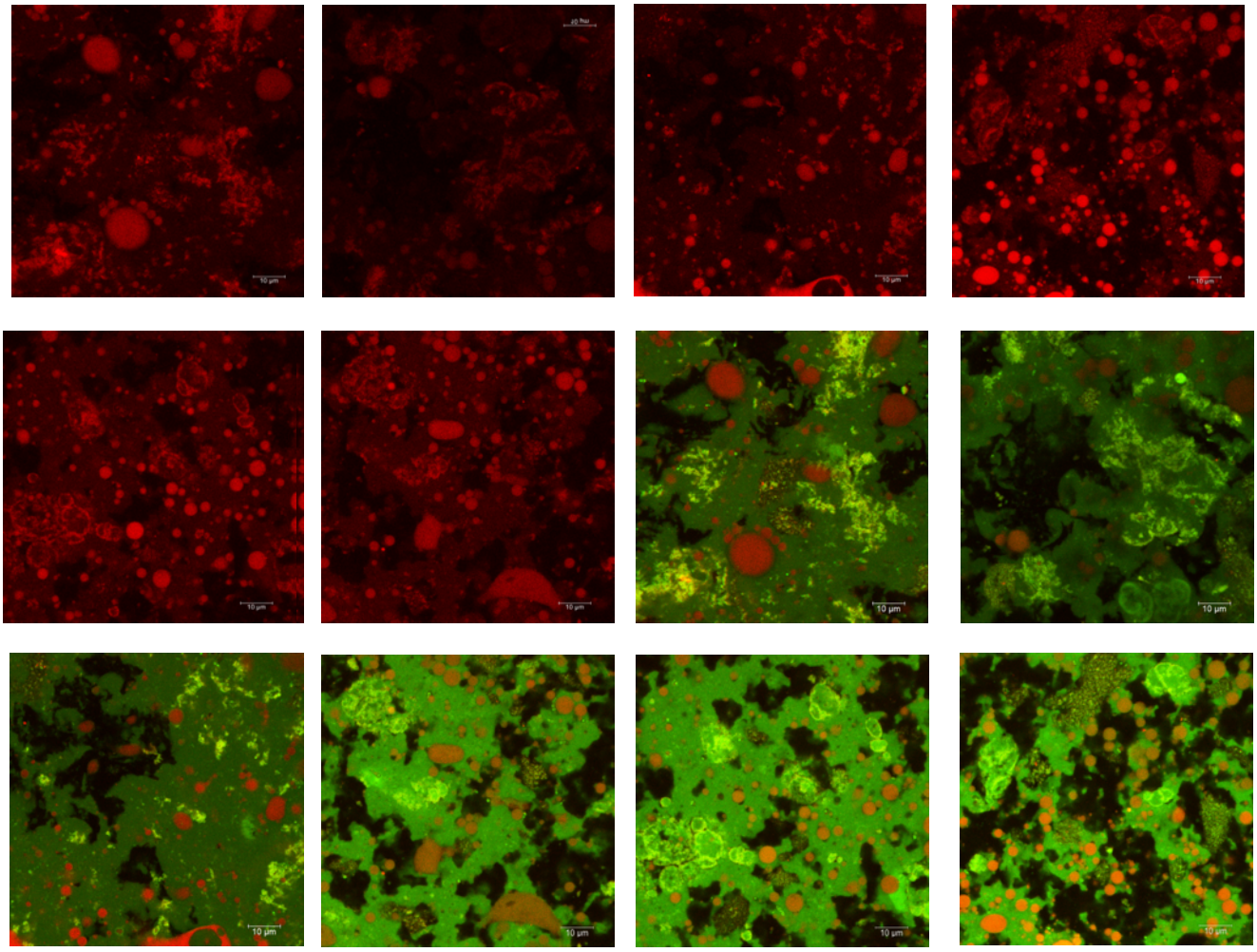
Heated Whey Protein and Pectin (HWPI + PT)



Heated Whey Protein and Pectin Complex, pH 5.5, 0.60% pectin (HCPX-0.60-5.5)



Heated Whey Protein and Pectin Complex, pH 6.2,0.60% Pectin (HCPX-0.6-6.2)



APPENDIX C: MINITAB PROCEDURE OUTPUT

One-way ANOVA: Fat Particle Size D(4,3) versus TRT

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
TRT	9	1, 2, 3, 4, 5, 6, 7, 8, 9

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TRT	8	14112.1	1764.0	16.78	0.000
Error	9	945.9	105.1		
Total	17	15058.0			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
10.2518	93.72%	88.13%	74.87%

Means

TRT	N	Mean	StDev	95% CI
CRT	2	107.50	3.54	(91.10, 123.90)
PT	2	137.0	17.0	(120.6, 153.4)
HWPI	2	66.85	10.68	(50.45, 83.25)
ES	2	61.30	8.91	(44.90, 77.70)
HWPI + PT	2	63.40	5.80	(47.00, 79.80)
HCPX-0.45-5.5	2	49.600	0.141	(33.201, 65.999)
HCPX-0.60-5.5	2	63.9	17.7	(47.5, 80.2)
HCPX-0.45-6.2	2	49.850	1.061	(33.451, 66.249)
HCPX-0.60-6.2	2	54.65	10.11	(38.25, 71.05)

Pooled StDev = 10.2518

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

TRT	N	Mean	Grouping

PT	2	137.0	A	
CRT	2	107.50	A	
HWPI	2	66.85		B
HCPX-0.60-5.5	2	63.9		B
HWPI + PT	2	63.40		B
ES	2	61.30		B
HCPX-0.60-6.2	2	54.65		B
HCPX-0.45-6.2	2	49.850		B
HCPX-0.45-5.5	2	49.600		B

Means that do not share a letter are significantly different.

One-way ANOVA: % Syneresis 3-week versus TRT

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
TRT	9	1, 2, 3, 4, 5, 6, 7, 8, 9

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TRT	8	1256.06	157.008	84.36	0.000
Error	27	50.25	1.861		
Total	35	1306.31			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.36425	96.15%	95.01%	93.16%

Means

TRT	N	Mean	StDev	95% CI
CRT	4	28.680	1.394	(27.280, 30.080)
PT	4	18.083	0.310	(16.684, 19.483)
HWPI	4	18.033	0.470	(16.634, 19.433)
ES	4	29.167	1.347	(27.767, 30.566)
HWPI +PT	4	11.983	0.998	(10.584, 13.383)
HCPX-0.45-5.5	4	17.683	1.187	(16.284, 19.083)
HCPX-0.60-5.5	4	13.267	1.263	(11.867, 14.666)

HCPX-0.45-6.2	4	21.60	2.39	(20.20, 23.00)
HCPX-0.60-6.2	4	14.017	1.727	(12.617, 15.416)

Pooled StDev = 1.36425

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

TRT	N	Mean	Grouping		
ES	4	29.167	A		
CRT	4	28.680	A		
HCPX-0.45-6.2	4	21.60		B	
PT	4	18.083			C
HWPI	4	18.033			C
HCPX-0.45-5.5	4	17.683			C
HCPX-0.60-6.2	4	14.017			D
HCPX-0.60-5.5	4	13.267			D
HWPI+PT	4	11.983			D

Means that do not share a letter are significantly different.

One-way ANOVA: % Syneresis Freeze Thaw versus TRT

Method

Null hypothesis	All means are equal
Alternative hypothesis	Not all means are equal
Significance level	α = 0.05

Equal variances were assumed for the analysis.

Factor Information

Factor	Levels	Values
TRT	5	1, 2, 3, 4, 5

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
TRT	4	2244.24	561.060	891.10	0.000
Error	15	9.44	0.630		
Total	19	2253.69			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
0.793492	99.58%	99.47%	99.25%

Means

TRT	N	Mean	StDev	95% CI

CRT	4	40.8833	0.1262	(40.0377, 41.7290)
PT	4	21.733	1.098	(20.888, 22.579)
HWPI+PT	4	11.983	0.998	(11.138, 12.829)
HCPX-0.60-5.5	4	19.517	0.582	(18.671, 20.362)
HCPX-0.60-6.2	4	11.950	0.769	(11.104, 12.796)

Pooled StDev = 0.793492

Tukey Pairwise Comparisons

Grouping Information Using the Tukey Method and 95% Confidence

TRT	N	Mean	Grouping			
CRT	4	40.8833	A			
PT	4	21.733		B		
HCPX-0.60-5.5	4	19.517			C	
HWPI + PT	4	11.983				D
HCPX-0.60-6.2	4	11.950				D

Means that do not share a letter are significantly different.