Characterization of Whey Protein Isolate-Polysaccharide Complexes as Antifreezing Agents in Commercial Cream Cheese

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Celeste A. Chadwick
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ABSTRACT

Freezing foods can result in texture and quality loss, especially in high-moisture products like cream cheese. There is a need for food-safe antifreeze agents for long-term storage to address this issue. This study aimed to develop a cryoprotective agent using dairy protein-polysaccharide complexes, assess their ice recrystallization inhibition effect, and reduce freezing-induced damage by incorporating them into commercial cream cheese. Complexes were formed between whey protein isolate and either locust bean gum or lambda carrageenan, including complexes made from their hydrolysates. Analysis of molecular weight, particle size, zeta potential, surface hydrophobicity, and Fourier transform infrared spectroscopy confirmed complexation. The cream cheese with complex addition at 4% dry-weight and after repeated freeze-thaw treatments were evaluated using oscillatory rheology and texture analysis and visualized by confocal laser scanning microscopy. Results indicate moderate anti-freezing activity of the complexes in model systems. Moreover, rheological measurements, texture analysis, and confocal images showed that complex treatments containing carrageenan gum were effective at reducing the freezing-induced damage of cream cheese.

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NOMENCLATURE

AFP	Antifreeze protein
AFGP	Antifreeze glycoprotein
ANOVA	Analysis of variance
CG	Carrageenan gum
CFR	Code of federal regulations
CLSM	Confocal laser scanning microscopy
CMC	Carboxymethyl cellulose
DH	Degree of hydrolysis
FTIR	Fourier-transform infrared microscopy
G’	Storage modulus
G”	Loss modulus
GG	Guar gum
λCG	Lambda carrageenan gum
λCG-0	Unhydrolyzed lambda carrageenan gum
λCG-6	Lambda carrageenan gum hydrolyzed 6 hr
LBG	Locust bean gum
LBG-0	Unhydrolyzed locust bean gum
LBG-6	Locust bean gum hydrolyzed 6 hr
LVR	Linear viscoelastic region
MFG	Milkfat globule
PBS	Phosphate buffer solution

PEG	Polyethylene glycol
pI	Isoelectric point
SA	Sodium alginate
USDA	United States Department of Agriculture
WλCG-0	Unhydrolyzed WPI-λCG complex
WλCG-6	Complex of WPI hydrolysate and 6-hr λCG hydrolysate
WLBG-0	Unhydrolyzed WPI-LBG complex
WLBG-6	Complex of WPI hydrolysate and 6-hr LBG hydrolysate
WPC	Whey protein concentrate
Xanthan gum	XG
Z0	Zero cycles of freezing and thawing
Z1	One cycle of freezing and thawing
Z4	Four cycles of freezing and thawing
Z10	Ten cycles of freezing and thawing

CHAPTER 1. RESEACH JUSTIFICATION AND LITERATURE REVIEW

Research justification

There is a need for effective antifreeze agents within the food industry, especially for dairy products, where optimizing freezing conditions presents a significant challenge. Freezing can negatively impact the texture, flavor, and overall quality of cream cheese by promoting the formation and growth of ice crystals (Fu and Labuza, 1997). These ice crystals can cause undesirable changes, including a grainy texture and syneresis, where the liquid is expelled from the gel network (Tribst et al., 2018). Thus, ensuring the stability and quality of cream cheese during storage and transportation in frozen conditions is crucial for maintaining consumer satisfaction and reducing waste.

Protein-polysaccharide complexes have shown improved surface functionalities indicating a potential to serve as antifreeze agents (Benichou et al., 2007; Benna-Zayani et al., 2008; Yi et al., 2011). These complexes often consist of naturally occurring compounds, which are effective and safe for consumption. Whey protein isolate (WPI), a byproduct of cheese production, is a well-known and widely utilized protein in the food industry due to its excellent functional properties, including solubility, gelation, and emulsification (Fachin and Viotto, 2005; Barbut and Drake, 1997). Polysaccharides such as lambda carrageenan (λCG) and locust bean gum (LBG) are commonly used as thickening, gelling, and stabilizing agents (Hotchkiss et al., 2016). This widespread use and long history of safe use in food products further justifies their use and consumer acceptability in protein-polysaccharide complexes (Woodward, 2021).

Despite the extensive research on the formation, characterization, and application of protein-polysaccharide complexes in various industries, there is a notable gap in the literature

regarding their potential as ice recrystallization inhibition (IRI) agents in the food industry. Ice recrystallization is a critical process in freezing, where larger ice crystals grow at the expense of smaller ones, leading to detrimental changes in the texture of frozen foods (Voets, 2017). Effective IRI agents can inhibit this process, maintaining the quality and structural integrity of frozen products.

The present study aims to address this gap by developing and characterizing WPI-polysaccharide complexes as potential IRI agents specifically for use in commercial cream cheese. By forming complexes with either whole or hydrolyzed forms of λCG or LBG, we can investigate the impact of these biopolymers on the antifreeze properties of WPI. The hydrolyzed forms of these polysaccharides may offer different interaction dynamics and effectiveness compared to their whole counterparts, providing a comprehensive understanding of how these complexes function.

Ultimately, the optimized development of these WPI-polysaccharide complexes as antifreeze agents could lead to significant advancements in the food industry. This includes improved texture and quality of frozen dairy products, extended shelf-life, and increased consumer satisfaction (Alinovi and Mucchetti, 2021). Moreover, using familiar and naturally derived compounds aligns with the growing consumer demand for clean-label ingredients, enhancing the marketability of products utilizing these innovative solutions (McClements and Gumus, 2016).

Chapter 1 provides the rationale for the study and offers a comprehensive literature review to highlight the problem and underscore the necessity for a solution. Chapter 2 narrows the focus, defining the objectives of the research project. Chapter 3 details the materials, their

sources, and the research methodologies used. Chapter 4 presents the research results and contextualizes the findings within the framework of existing literature. Finally, Chapter 5 summarizes the key findings, discusses limitations, and suggests directions for future research.

High moisture cheese and need for freezing

Structure and processing of cream cheese

Cream cheese has been described as an acid-induced gel created from high-fat milk that has undergone various processing steps such as shearing, heating, and dewatering that ultimately convert the acid gel into a complex cheese product (Brighenti et al., 2018). It has also been described in the literature as a soft, fresh, acid-coagulated cheese product that has been acidified by mesophilic lactic acid bacteria (Phadungath, 2005). According to the United States (U.S.) government, cream cheese is a colloidal system consisting of milk, cream, salt, one or more gum stabilizers, and cheese starter culture (21 CFR 133.134). Various cream cheese types are recognized by the United States Department of Agriculture (USDA) including full-fat cream cheese, Neufchâtel cheese, reduced-fat cream cheese, and light cream cheese. These products differ in moisture content, milkfat content, pH range, and salt concentration. Specific requirements can be seen in Table 1.1. Despite the vast cream cheese variations available, the focus of this work was on full-fat cream cheese. Per the code of federal regulations (CFR), full-fat cream cheese produced in the U.S. must contain a minimum milk fat of 33% with a maximum moisture content of 55%. Stabilizers may be added but must not exceed 0.5% of the finished product (CFR). Additionally, the USDA outlines that cream cheese should fall within a pH range of 4.4-4.9 and not exceed a salt concentration of 1.4% (USDA, 1994).

Table 1.1. Compositional requirements of cream cheese per USDA regulations.

Product	Moisture content (%)	Milkfat content (%)	pH range	Salt content (%)
Full-fat cream cheese	≤ 55	≥ 33	4.4 - 4.9	≤ 1.4
Neufchâtel cheese	≤ 65	20 - 33	4.4 – 5.0	≤ 1.4
Reduced-fat cream cheese	≤ 70	16.5 – 20	4.4 – 5.1	≤ 1.4
Light cream cheese	≤ 70	≤ 16.5	4.4 – 5.2	≤ 1.4

*Adapted from USDA, 1994, and Phadungath, 2005.

Structurally, cream cheese contains protein aggregates about 0.5 µm in diameter and embedded fat globules around 1 µm on average but up to 15 µm in diameter that can be observed under electron microscopy (Kalab and Modler, 1985). The rheological behavior of cream cheese is evidence of a 3-dimensional gel-like structure (Sanchez et al., 1994) that exhibits a partially thixotropic behavior with dynamic viscoelastic properties like that of pharmaceutical creams (Sanchez et al., 1994). The temperature at which rheological measurements are collected will affect the resulting properties exhibited. For example, increased elasticity and viscosity can be measured when tested between 5-50°C due to fat crystallization compared to analysis done when fat is melted at elevated temperatures (Bruckner and Senge, 2007; Sanchez et al., 1996).

Various factors during production can ultimately impact the cream cheese's 3-dimensional structure, rheological properties, and sensory properties. For example, relative amounts of casein and whey protein, milk fat content, homogenization and pasteurization conditions, final pH, addition of one or more gum stabilizers, and many others. Despite these many factors, the commercial manufacturing process of cream cheese is similar across brands.

The process begins with fat standardization from homogenized, pasteurized milk to a final fat content of 8-14% as-is (Brighenti et al., 2018). The size of fat globules is reduced through homogenization, thereby increasing their surface area, such that the fat globules are coated in casein protein (Walstra et al., 1999). Homogenization is typically done at two pressures with the first between 15-25 MPa and the second at 3-5 MPa meanwhile pasteurization normally occurs between 72-75°C for 30-60 seconds (Guinee and Hickey, 2009). After cooling to around 20-30°C, mesophilic lactic acid bacteria are added to begin fermentation (Lucey, 2002). Typically, a D-type starter culture is added including *Lactococcus lactis* subsp. *lactis* or *L. lactis*

subsp. *cremoris* (Guinee and Hickey, 2009). Fermentation is allowed to continue at this temperature until the pH reaches ~4.5-4.8 (Guinee and Hickey, 2009).

After fermentation, the gel is agitated, heated, and the whey is removed. Early processing methods of cream cheese featured 24-hr gravity draining of whey from curd in cheesecloth (Phadungath, 2005). Modern methods, however, include the use of a centrifugal separator at 70-85°C which allows for the continuous whey removal from the hot cheese curd ultimately resulting in a faster and more sterile separation contributing to a longer shelf-life (Guinee and Hickey, 2009).

The final stage includes heating the curd to ~80-85°C and simultaneous addition of salt (NaCl) and hydrocolloid gum stabilizers (Guinee and Hickey, 2002). Most often, salt is added between 0.5-1% as a flavor enhancer (Guinee and Hickey, 2002). The addition of gum is optional; however, it is generally added between ~0.2-0.5% (Guinee and Hickey, 2002). Most often, a proprietary blend of gums including guar gum (GG), traganth, xanthan gum (XG), LBG, CMC, carrageenans (CG), or pectins is added (Hunt and Maynes, 1997; Guinee and Hickey, 2002). Each gum type and the amount used will contribute to the final texture and sensory properties of the cream cheese; therefore, companies will develop their own blend according to desired qualities (Guinee and Hickey, 2002). In the case of flavored cream cheese and cream cheese products, condiments or spices may be added at this stage as well (Guinee and Hickey, 2002). Homogenization may be performed once more after inclusion of additional flavorings and stabilizers, imparting a more uniform consistency on the finished product (Guinee and Hickey, 2002). The overall production flow of commercial cream cheese can be seen in Figure 1.1.

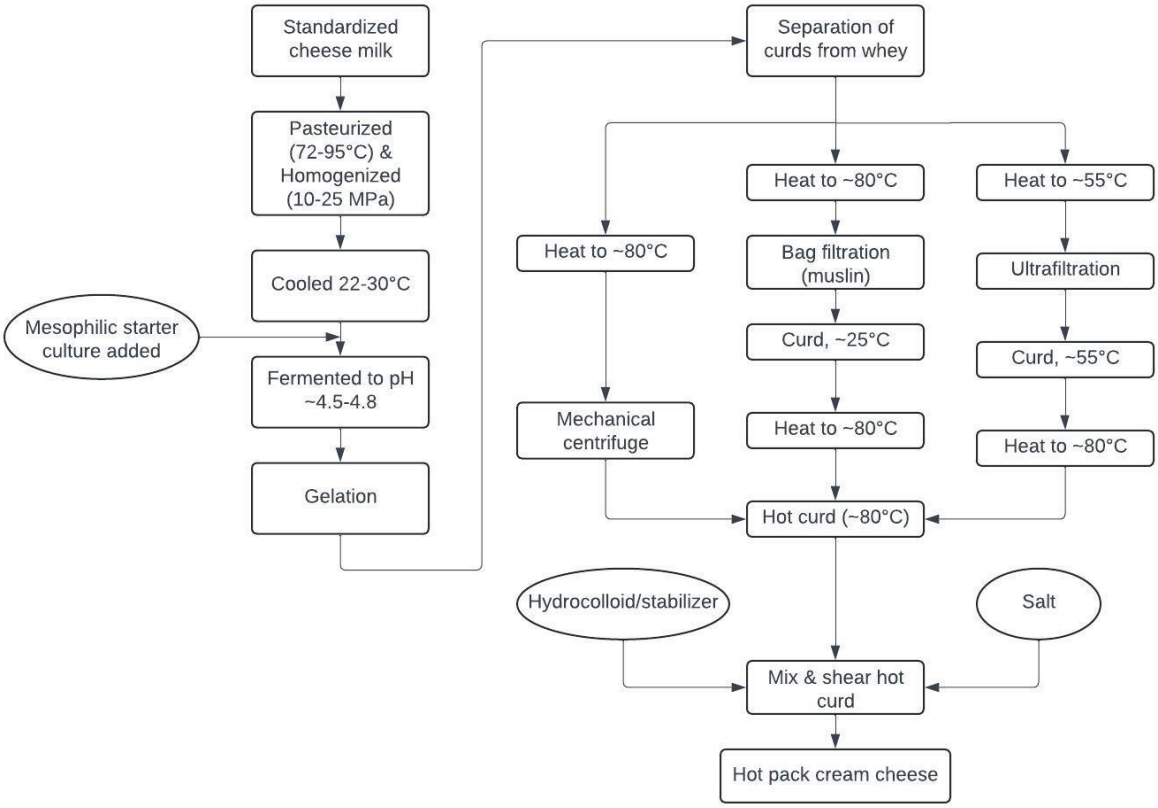


Figure 1.1. Process flow diagram of commercial manufacturing of cream cheese (Adapted from Guinee and Hickey, 2002).

CG specifically are commonly incorporated into dairy products such as ice cream and chocolate milk to better stabilize the milk fat emulsions. Interactions between CG and casein micelles have been of research interest and have been consistently shown to have a unique interaction due to electrostatic interactions between CG chains and k-casein (Snoeren, 1976). Moreover, all three forms of CG have been shown to adsorb onto the casein micelles at temperatures below the coil-helix transition temperature. Conversely, at temperatures above the coil-helix transition, depletion flocculation has been reported (Asakura and Oosawa, 1958).

Freezing process and its effects on the microstructure of high moisture cheeses

Freezing is one of the most common methods of food preservation and is used to store a variety of food products. The process of freezing is a phase change in which liquid water begins to nucleate when lowered to a temperature below the freezing point of the system, allowing ice crystals to form (Alinovi et al., 2020). Freezing cheeses is of interest to prolong the shelf-life of the product, and provide a greater supply year-round (Alinovi et al., 2020). Additionally, freezing cream cheese is of particular interest due to its practical applications in cheesecake, a commonly created dessert in North America, particularly in the U.S. (Brighenti et al., 2018). Several methods of freezing have been adopted to freeze various cheese types and dairy products including conventional air-still freezing (Simov and Ivanov, 2005), blast freezing (Diefes et al., 1993), deep immersion freezing in brine solutions (Ribero et al., 2009), and preservation in cryogenic liquids (Bertola et al., 1996). Air-still freezing is by far the most used conventional method of freezing, however given the variability between regions and challenges in optimization between product types, this method can have major effects on the final texture and sensory products of the food (Alinovi and Mucchetti, 2020).

Ice crystals can cause severe damage to the milk fat globule (MFG) membrane. MFG rupture is the primary concern as this can ultimately lead to increased rate of lipolysis and fat oxidation (Antifantakis et al., 1980), destabilized emulsions, and fat globule coalescence (Tribst et al., 2020). For this reason, it is critical to optimize freezing conditions for each specific cheese type.

During the freezing process of cheese, unbound water is frozen into ice crystals throughout the cheese matrix. As these ice crystals form, solutes, including casein, are excluded, thereby resulting in an increased concentration of casein in the unfrozen regions (Alinovi et al., 2020). This increased volume fraction of solids in the unfrozen phase increases the relative viscosity and colloidal interactions (Corredig et al., 2019). Moreover, structural changes of casein during frozen storage have been documented (Xiong, 1997), due to water migration into the ice crystals. This migration leaves the hydrophobic portions of the protein exposed and able to participate in hydrophobic and protein-protein interactions resulting in protein aggregates (Xiong, 1997) and ultimately changes in the texture and quality of cream cheese.

Given the complex nature of freezing cheese, most commercial producers will advise consumers not to freeze cream cheese due to the resulting negative effects on the texture and quality. Nonetheless, most producers will incorporate one or more gum stabilizers into their product, most often LBG, GG, XG, sodium alginate, or CG (Phandungath, 2005). These gums serve a variety of functions in cream cheese, one of which being increased viscosity of the aqueous phase thereby enhancing texture and providing resistance against freezing-induced damage to the microstructure.

Numerous studies have been conducted on the effect of freezing on the microstructure of other cheeses, namely effect on mozzarella's stretchability (Diefes et al., 1993; Guinee et al., 2002; Reid and Yan, 2004; Alinovi and Mucchetti 2020). Moreover, the addition of antifreeze agents to food products is very much an emerging research area, and their effect on the complex cheese matrix (beyond a model testing system) is unknown. This indicates a gap in the current literature and a strong need for further research surrounding the effect of freezing on the cream cheese matrix and the effect of adding antifreeze agents on these cheeses.

Variation among cream cheese brands and potential effect on freezing-induced damage

While freezing effect will vary between cheese types, it is reasonable to assume that differences in nutritional components and different processing conditions will also affect the eventual damage from freezing. Table 1.2 provides percent of nutritional components (based on dry matter) of the Tillamook cream cheese spread used in this study compared with the traditional brick style of the same brand and both the spread and brick style of the "gold-standard," Philadelphia brand. Tillamook cream cheese spread, and brick-style were similar in nutritional composition with the only difference being in sodium content with spread containing 0.8% and brick-style containing 0.7%. The Philadelphia brand, however, had greater variability in nutritional composition. Philadelphia cream cheese products showed variation in nearly every nutritional component apart from cholesterol which was calculated to be 0.2% in each instance. While freezing-induced damage was not evaluated for Philadelphia brand products in this study, we can reasonably assume that the effect from freezing would be different in both the spread and brick-style compared to the Tillamook spread considering nutritional composition alone. Comparing brands of the same style cream cheese product, differences for nearly every

Table 1.2. Differences in percent nutrient components based on solid content between Philadelphia and Tillamook cream cheese spread and brick-style cream cheese.

Nutritional component	Percent (%) based on solid content	Nutritional component	Percent (%) based on solid content
Tillamook spread-style cream cheese		**Tillamook brick-style cream cheese**	
46.1% solids	53.9% moisture	51.0% solids	49.0% moisture
Fat	69.9	Fat	69.9
Carbohydrate	14.0	Carbohydrate	14.0
Protein	14.0	Protein	14.0
Sodium	0.8	Sodium	0.7
Cholesterol	0.2	Cholesterol	0.2
Ash	0.9	Ash	0.9
Total (%)	99.8	**Total (%)**	99.7
Philadelphia spread-style cream cheese		**Philadelphia brick-style cream cheese**	
36.1% solids	63.9% moisture	46.8% solids	53.2% moisture
Fat	62.5	Fat	76.1
Carbohydrate	17.9	Carbohydrate	7.6
Protein	17.9	Protein	15.2
Sodium	1.2	Sodium	0.8
Cholesterol	0.2	Cholesterol	0.2
Ash	Unknown	Ash	Unknown
Total (%)	99.6	**Total (%)**	99.9

Note: Nutrition facts retrieved from Tillamook and Philadelphia nutrition facts panels.

nutritional component were calculated. This would again likely lead to observable and measurable differences in freezing-induced damage.

Overall, the Philadelphia brick style contained higher fat content and lower protein content than the spread of the same brand. Meanwhile, the Philadelphia spread style contained lower fat and higher protein content than the Tillamook spread style and the Philadelphia brick-style contained lower fat and lower protein content than the Tillamook brick style. Fat content relative to other solids within cream cheese will impact the degree of freezing-induced damage observed. Cream cheese containing relatively more fat content will typically experience a greater degree of freezing-induced damage if all other factors are held constant (Webb and Hall, 1935). Moreover, those containing higher protein and carbohydrate solid content would be less susceptible to freezing-induced damage owing to the lowered freezing point (Webb and Hall, 1935; Damodaran et al., 2007).

Differing moisture content among brands and styles must also be considered. As moisture content is increased, the susceptibility to freezing-induced damage also increases (Alonso et al., 2011). The incorporation of gum stabilizers will also influence the role played by moisture content, however. Both Tillamook cream cheese types contain no hydrocolloids, while both Philadelphia types do. Philadelphia spread contains guar gum, meanwhile, the brick style contains locust bean gum. GG produces the highest viscosity of all naturally derived commercial gums (Damodaran et al., 2017). GG was proven to have similar rheological properties to LBG, however, when evaluated by frequency sweep at temperature ranging from 5 °C to 80 °C (Brighenti et al., 2020). Additionally, both gums ranked equivalent for difficulty to spread as determined by a sensory panel (Brighenti et al., 2020). It follows then that both could be used

successfully in cream cheese to obtain similar properties. Indeed, these gums are routinely added across the dairy industry, with about 85% of commercially used LBG being used in dairy and frozen dessert products (Damodaran et al., 2017). A key difference between these two gum types is that LBG requires heating in water to 90 °C to be fully soluble (Damodaran et al., 2017). Given the higher moisture content (~10%) of Philadelphia spread, heating to 90 °C to solubilize LBG is undesirable as this could lead to more severe changes to the matrix. Therefore, the incorporation of GG, which does not require heat treatment is ideal for addition to Philadelphia cream cheese spread.

Food-Grade Antifreeze Agents

A variety of products have been shown to have IRI activity and therefore have been added to foods to control ice recrystallization including antifreeze proteins (AFP) (Marshall et al., 2003) and hydrocolloids (Bahramparvar and Mazaheri Tehrani, 2011). IRI agents attracted early interest in ice cream manufacturing due to the high degree of ice recrystallization due to fluctuations in temperature during consumer storage (Ustun and Turhan, 2015). Presently, a wide array of hydrocolloids is added to ice cream mix including gelatin, GG, sodium CMC, LBG, CG, XG, alginates, and microcrystalline cellulose (Bahramparvar and Tehrani, 2011).

Each stabilizer imparts unique properties to the ice cream; however, stabilizers are often added in combination to gain some synergistic functionalities (Bahramparvar and Tehrani, 2011). For example, GG is known in part for its stability at high temperatures and is frequently used in high-temperature short-time pasteurization (Bahramparvar and Tehrani, 2011).

Meanwhile, CG is effective at preventing phase separation and improving protein stability in various conditions; therefore, a synergistic benefit could be observed from the inclusion of both stabilizers (Bahramparvar and Tehrani, 2011). Currently, there is no reported effect of these hydrocolloids used in cream cheese to prevent freezing-induced damage.

Proteins and polysaccharides as IRI agents

Slowing of ice recrystallization in foods from proteins and hydrocolloids

During the freezing of biological materials, damage can occur within the structure due to the recrystallization of ice. For this reason, research has been heavily conducted over the past several decades on finding IRI active molecules for use in agricultural, pharmaceutical, and food industries as well as others. Various types of molecules can exhibit IRI activity, including proteins, protein hydrolysates, and hydrocolloids. Antifreeze proteins (AFPs) can come from a variety of sources and can function through different mechanisms. Specifically, there are numerous articles published on the use of protein hydrolysates as IRI agents. Various protein hydrolysates have been shown to be effective as IRI agents including soy protein hydrolysates (Wan et al., 2022; Fomich et al., 2023) and zein and gelatin hydrolysates (Yuan et al., 2023). In the study by Fomich et al. (2023), a variety of protease enzymes were used to obtain soy protein hydrolysates. Enzyme type did show an effect on IRI activity meanwhile hydrolysis time did not have a significant effect on the measured IRI activity of active hydrolysates (Fomich et al., 2023). In another study (Yuan et al., 2023), chemical modification of zein and gelatin hydrolysates with succinic anhydride and octenyl succinic anhydride proved effective at increasing IRI activity. Through succinylation, the ratio of hydrophobic and hydrophilic groups was altered, therefore resulting in a more balanced amphiphilicity than the unmodified

compounds. This amphiphilicity has been shown in literature to be related to increased IRI activity through ice binding by methyl groups and through a stabilization effect by the hydrophilic portion. Amphiphilicity is also a common characteristic among well-reported IRI active molecules such as AFPs, antifreeze glycoproteins (AFGPs), and polyvinyl alcohol (Mitchell et al., 2017; Mitchell and Gibson, 2015; Biggs et al., 2019).

Likewise, several hydrocolloids have been shown to have IRI effects in a variety of systems as well. Hydrocolloids are commonly used in ice cream specifically to inhibit ice recrystallization (Gaukel et al., 2014). IRI activity of some hydrocolloids may have to do with ice crystal morphology. Gaukel et al. (2014) examined the ice crystal morphology of 6.2 mg/mL sodium alginate and 2.5 mg/mL κ-CG in 49% sucrose solution. The slightly cryoprotective sodium alginate exhibited similar round-shaped ice crystals as the model solution. The more IRI active κ-CG meanwhile resulted in elongated and angular ice crystals compared to the model solution (Gaukel et al., 2014).

Changes in ice crystal morphology occur due to the binding of AFPs/AFGPs at specific parts of the ice crystal (Gaukel et al., 2014). This effect is likely due to AFPs causing water to freeze only in small spaces between the AFP molecules on the ice, resulting in smaller, more curved surfaces (Raymond and DeVries, 1977). The result is local freezing point depression and no further crystal growth. Overall, a synergistic effect between sodium alginate and AFPIII resulted in an improved cryoprotective effect (Gaukel et al., 2014). The authors discussed a potential for greater interaction between the AFP and the ice surface in the presence of other molecules in the water phase through the hydration of sodium alginate next to the AFP resulting

in weaker AFP interactions with the water molecules and enhanced AFP interactions with the ice crystal (Gaukel et al., 2014; Hayakari and Hagiwara, 2012).

Another study conducted by Chun et al. (2012) also considered ice crystal morphology in 40% sucrose solution with WPI alone, and WPI and κ-CG interacting synergistically. When up to 3% WPI was added alone, both round and rectangular ice crystals were observed (Chun et al., 2012). Meanwhile, the ice crystal morphology with the addition of both biopolymers was pH dependent with larger, and more round ice crystals at low pH (3-5) and both round and rectangular shapes at higher pH (7-9) (Chun et al., 2012). While a cryoprotective effect was observed with WPI alone in sucrose solution, the effectiveness was enhanced with the addition of κ-CG with the greatest IRI effect at 0.1% addition (Chun et al., 2012). The increased cryoprotective effect through addition of both WPI and κ-CG was attributed to induced interactions and conformational changes between biopolymers (Chun et al., 2012).

Although these two studies examined the cryoprotective effects of combining polysaccharides and proteins, they did not investigate them as pre-formed complexes, as we have in our current study. To the best of our knowledge, no research has explored the IRI effect of pre-formed protein-polysaccharide complexes. Therefore, this study is necessary to fill that gap.

Chemistry of whey protein, LBG, and λCG

Whey proteins make up about 20% by weight of all protein in milk (Damodaran et al., 2017). It is a globular protein comprised primarily of β-lactoglobulin and α-lactalbumin with these constituents making up approximately 80% of whey protein (Damodaran et al., 2017). Their globular structure containing a hydrophobic interior and hydrophilic exterior gives it unique functional properties, such as improved solubility in water, making it ideal for a variety

of food applications (Damodaran et al., 2017). Because it is a natural byproduct of the cheesemaking process, this protein was selected for use in this study as a naturally occurring protein in cream cheese to improve consumer acceptability of the resulting complexes.

Physical and enzymatic treatment of proteins including WPI are common due to their improved functional properties and overall food safety. These functional properties include solubility, viscosity, water binding, gelation, emulsification, foaming, and others (Je, 1985). Hydrolysis is a commonly practiced method in the food industry where the final hydrolysate and degree of hydrolysis (DH) will depend on the specificity and selectivity of the enzyme used (Jeewanthi et al., 2015). It is important to control DH as over-hydrolysis can have the undesirable effect of reducing functionality (Spellman et al., 2009; Fomich et al., 2023). Therefore, to improve IRI activity, WPI was hydrolyzed in this study for 5 min by Alcalase protease.

Alcalase is a commonly used enzyme for commercial hydrolyses given its relatively low cost (Adamson and Reynolds, 1996). It is derived from *Bacillus licheniformis.* The specificity of Alcalase is broad, but it shows a preference for cleaving peptide bonds adjacent to hydrophobic amino acids (Adamson and Reynolds, 1996). Given that WPI is primarily made up of β-lactoglobulin and α-lactalbumin, hydrophobic-rich components, it is readily hydrolyzed by this enzyme. Depending on various factors, including the extent of protein folding, different regions of WPI will vary in susceptibility to enzymatic attack, resulting in hydrolysates of various molecular weights (MW). This range of hydrolysate sizes is key to improved protein functionality (Spellman et al., 2009).

Likewise, consideration must be given to the chemical structure of the polysaccharides used in this study as this will also contribute to the observed IRI effect in cream cheese. LBG is a galactomannan consisting of a main chain of β-D-mannopyranosyl units linked by (1→4) bonds with the presence of a single α-D-(1→6) galactopyranosyl (Damodaran et al., 2017). The chemical structure of LBG can be seen in Figure 1.2. Galactomannans such as LBG are known for their ability to form highly viscous solutions owing to their hydrophilic nature and their large, rigid structure (Damodaran et al., 2017).

Carrageenans are sulfated galactans that are extracted from red seaweed (Damodaran et al., 2017). They are linear structures made up of D-galactopyranosyl units linked together by (1→3)-α-D and (1→4)-β-D-glycosidic linkages (Damodaran et al., 2017). There are three forms of carrageenans including kappa (κ), iota (ι), and lambda (λ). These forms differ in the number of sulfate groups, with κ containing 1, ι containing 2, and λ containing 3 (Damodaran et al., 2017). The structure of λCG can also be seen below in Figure 1.2. Carrageenans are commonly used in the dairy industry due to their ability to form gels with casein and their high freeze-thaw stability, making them ideal candidates for inclusion in ice cream (Damodaran et al., 2017). Gelation will occur with heating and subsequent cooling of κ and ι type carrageenans due to the formation of a three-dimensional network of helical structures; λCG however, will not form gels due to its high sulfation preventing it from forming helical structures (Damodaran et al., 2017). Due to its lack of gel-forming abilities and its negative stability across a wide range of pH values, λCG was ultimately chosen for this study.

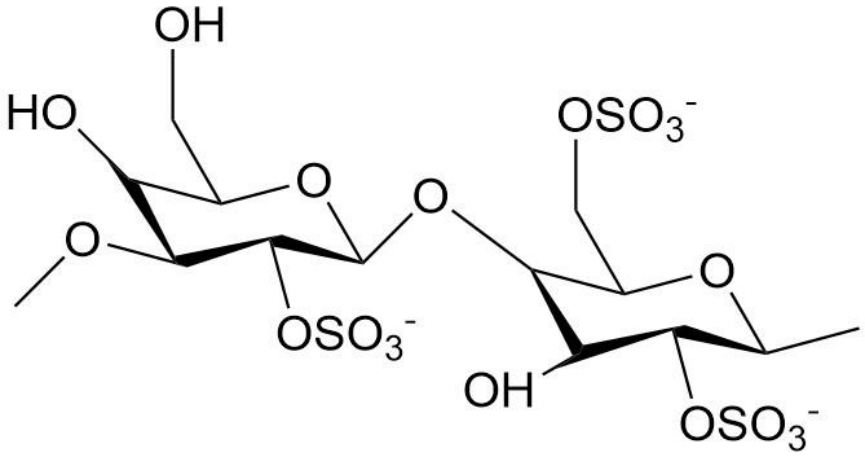
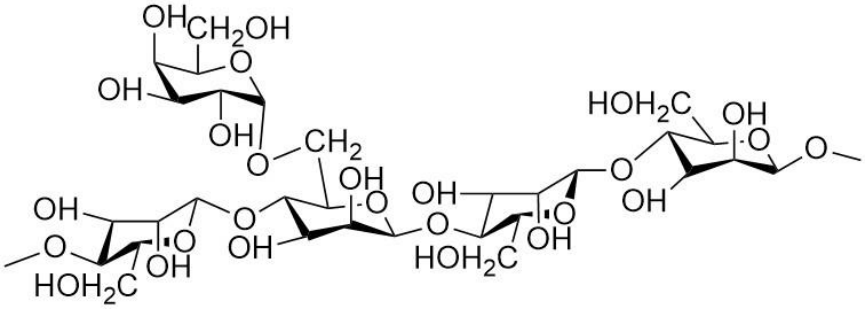
Gum name	Chemical structure
λCG	
LBG	

Figure 1.2. Chemical structure of λCG and LBG.

For both polysaccharides, cellulase was selected for enzymatic hydrolysis due to its specificity for β-(1→4) glycosidic linkages (Sharrock, 1988). Given the repeating sugar units and linkages of LBG, as previously mentioned, this gum was expected to achieve a higher degree of hydrolysis; the presence of α-D-(1→6) galactopyranosyl would hinder this process somewhat, however. Given the alternating α-(1→3) and β-(1→4) linkages between galactose groups, the two gums were expected to respond differently to the enzyme.

CHAPTER 2. INTRODUCTION

The demand for high-quality dairy products with extended shelf life is continually growing in the food industry. Cream cheese, a popular dairy product, often faces challenges related to storage, particularly when subjected to freezing temperatures. Freezing, while essential for extending shelf life and creating cream cheese products such as cheesecake, can induce physical and physicochemical changes in cream cheese, leading to quality degradation and food loss. The high moisture content of commercial cream cheese (~50%) makes commercial cream cheese more susceptible to freezing-induced damage (Phadungath, 2005). These changes are predominantly attributed to the formation of large ice crystals and ice recrystallization, which disrupt the smooth and creamy consistency characteristic of high-quality cream cheese, leading to a gritty and undesirable texture (Alinovi et al., 2021). Moreover, the phase separation caused by freezing and thawing cycles can result in syneresis, where liquid whey is expelled, further compromising product integrity.

These adverse effects reduce consumer acceptability and marketability, particularly in food service industries. Traditional methods to mitigate these problems often include the addition of gums and stabilizers such as locust bean gum (LBG), carrageenan gum (CG), or whey protein. While these products are used to preserve the quality of the cream cheese with storage, they do not target frozen storage specifically. Therefore, the need persists for the development and inclusion of an antifreeze agent for commercial cream cheese. Proteins and hydrocolloids have been shown to be effective in slowing ice recrystallization, which is crucial for maintaining the quality of frozen dairy products. Proteins such as whey protein isolate (WPI) can interact with ice crystal surfaces, thereby reducing the rate of ice crystal growth (Bianco et al., 2020). Hydrocolloids such as lambda carrageenan (λCG), a sulfated polysaccharide, can form networks

that trap water and limit water mobility, thus reducing ice recrystallization. When combined, these substances may potentially enhance each other's effects, resulting in a more pronounced inhibition of ice recrystallization. Numerous protein hydrolysates have demonstrated effectiveness as ice recrystallization inhibition (IRI) agents. Notably, soy protein hydrolysates have been highlighted in recent studies for their efficacy (Wan et al., 2022; Fomich et al., 2023). Additionally, hydrolysates derived from zein, and gelatin have also been identified as potent IRI agents (Yuan et al., 2023). These findings suggest that a balanced amphiphilicity and improved mobility of the molecules may have played an important role in the IRI activity. Studies like this offer the possibility of identifying effective IRI agents for potential applications in food, cryopreservation, and for other industries where controlling ice formation is crucial.

The interaction between proteins and polysaccharides can result in synergistic effects that enhance their functional properties (Benichou et al., 2007; Benna-Zayani et al., 2008; Li et al., 2011). In the context of IRI, protein-polysaccharide complexes may increase IRI activity because of conformational changes to protein structure. Specific changes in surface hydrophobicity have been shown to influence the degree of IRI activity achieved for certain IRI active molecules. An increase in surface hydrophobicity resulted in greater IRI activity as shown in one study conducted by Li et al. (2020). Facial amphiphilicity is known to be of critical importance to the activity of IRI agents. Specifically, the hydrophobic domains of these compounds are essential in their function to repel water molecules during recrystallization (Tachibana et al., 2004). In the context of protein-polysaccharide complexes, the modulation of the protein's hydrophobicity or amphiphilicity by complexing with polysaccharides could play an important role in enhancing IRI activity. Therefore, the formation of complexes between WPI and λCG may thus offer a

promising approach to enhancing IRI activity and reducing freezing-induced damage in cream cheese.

Specific changes in surface hydrophobicity also have been shown to influence the degree of IRI activity achieved for certain IRI active molecules. An increase in surface hydrophobicity resulted in greater IRI activity as shown in one study conducted by Li et al. (2020). Facial amphiphilicity is known to be of critical importance to the activity of IRI agents. Specifically, the hydrophobic domains of these compounds are essential in their function to repel water molecules during recrystallization (Kurnaz et al., 2023). In the context of protein-polysaccharide complexes, the modulation of hydrophobicity could play an important role in enhancing IRI activity. By strategically altering the hydrophobic regions of these protein-polysaccharide complexes, it may be possible to improve their effectiveness in preventing ice recrystallization.

The lower molecular weight of natural polymers is another key attribute of IRI-potent molecules. This characteristic is attributed to increased molecular flexibility along the backbone and increased mobility in an aqueous system, allowing for greater interaction at the ice-water interface (Balcerzak et al., 2014). Numerous proteins such as zein, hemp, and soy proteins, have been shown to have increased IRI activity following hydrolysis reactions to various degrees (Wan et al., 2022; Yuan et al., 2024; Ollis et al., 2024; Fomich et al., 2023). This suggests that modifying the molecular size and flexibility of WPI protein could further enhance its IRI capabilities, especially when complexed with polysaccharides such as λCG and LBG and their hydrolysates. These findings suggest that a balanced amphiphilicity and improved mobility of the molecules may play an important role in the IRI activity.

While numerous studies have explored the individual effects of proteins and polysaccharides on IRI activity, there is a notable lack of literature describing the synergistic effects that arise specifically from the formation of protein-polysaccharide complexes. This study aims to address these gaps by investigating the effect of WPI-λCG and WPI-LBG complexes on IRI activity and their effectiveness in reducing freezing-induced damage in commercial cream cheese. We hypothesize firstly that the complexes will demonstrate significantly greater IRI activity compared to the effects of the individual components in a model system, and secondly that these complexes will be effective in preserving the textural and rheological properties of commercial cream cheese during freezing and thawing cycles. By exploring these hypotheses, this research seeks to provide a foundation for developing superior antifreeze formulations for the dairy industry.

This study aims to address these gaps by investigating the combined effect of WPI-λCG and WPI-LBG as pre-formed complexes on IRI activity and their effectiveness in reducing freezing-induced damage in commercial cream cheese. We hypothesize firstly that the complexes will demonstrate significantly greater IRI activity compared to the effects of the individual components, and secondly that these complexes will be effective in preserving the textural and rheological properties of commercial cream cheese during freezing and thawing cycles. By exploring these hypotheses, this research seeks to provide a foundation for developing superior antifreeze formulations for the dairy industry.

CHAPTER 3. MATERIALS AND METHODS

3.1. Materials

WPI was purchased from Bulk Supplements (Nevada, USA), and the Alcalase enzyme from *Bacillus licheniformis* (3.03 U/mL) was purchased from EMD Millipore Corp. (Massachusetts, USA). λCG and LBG were purchased from Modernist Pantry (Maine, USA) and cellulase was from Fisher Scientific (Massachusetts, USA). Tillamook cream cheese spread (70.0% fat, 14.0% carbohydrate, 14.0% protein, 0.02% cholesterol, 0.84% sodium, and 0.9% ash, all determined on a dry weight basis) having a solid content of 46.1% was purchased from a local grocery store in Knoxville, TN.

3.2. Sample preparation

3.2.1. Preparation of peptide by Alcalase hydrolysis and determination of average molecular weight of the hydrolysate

A 10% dispersion of WPI was created with deionized (DI) water. pH was then adjusted to ~8.0 with 2 N NaOH. Alcalase enzyme was added at an enzyme-to-substrate ratio of 0.176 Anson units/g WPI, and the mixture was subsequently shaken for 5 min at 50 ^{o}C. Following hydrolysis, the hydrolysate was boiled for 10 min to inactivate the enzyme. Finally, the hydrolysate was collected and refrigerated for future use in complexation with polysaccharide hydrolysate and incorporation into cream cheese.

High-performance liquid chromatography by size-exclusion chromatography (HPLC-SEC) was done to determine the average molecular weight of the WPI hydrolysate. Hydrolysate mixture and native WPI were prepared at 1 mg/mL concentration in HPLC-grade water and subsequently filtered through 4 mm nylon membrane filters (GE Healthcare Life Sciences).

Filtered samples were then analyzed with 1200 Agilent HPLC (Agilent Technologies, Santa Clara, CA). The HPLC system consisted of an autosampler (G1329A), quaternary pump (G1311A), vacuum degasser (G1322A), a temperature-controlled column oven (G1316A), and a diode array detector (G1315D). The column used for separation was a BioSep-SEP-S2000 column (300x7.80 mm, Phenomenex, Torrance, CA). A flow rate of 1 mL/min was used for a mobile phase consisting of 45% aqueous acetonitrile with 0.1% trifluoroacetic acid. A standard curve was created from the known molecular weight protein standards consisting of albumin, aprotinin, glucagon, bradykinin, glutathione, and glycine. The linear regression equation from the standard curve was used to calculate the molecular weight of the native WPI and hydrolysate. Average molecular weight and relative quantity were calculated by finding the percent area of each peak and its corresponding molecular weight compared with the standard curve.

3.2.2. Preparation of gum hydrolysates by cellulase and determination of degree of hydrolysis

A 1% dispersion of λCG and LBG was prepared in DI water, followed by hydration at 4 °C overnight (~14 hr) under stirring. The pH was adjusted to approximately 6.0 using 1N HCl, and cellulase was added at 2% relative to the gum quantity. The mixture was then shaken for 6 hr at 55 °C in a water bath. After hydrolysis, the samples were boiled for 10 min to inactivate the enzyme, and the complete hydrolysate was collected and refrigerated for future use in complexation with peptides.

The degree of hydrolysis (DH) was quantified by Somogyi method according to Somogyi and Nelson (1944) with modification. Reagents A, B, and C were prepared as described. Reagent A was a solution consisting of copper (II) sulfate, reagent B was made of sodium potassium tartrate and sodium carbonate dissolved in water, and reagent C was made of

potassium thiocyanate. Reagent D was freshly prepared with 25 mL reagent A and 1.0 mL Reagent B. A glucose standard curve was created with concentrations from 0 to 100 µg/mL. To quantify reducing sugar, 1 mL of sample was placed in a test tube with 1 mL of reagent D and samples were boiled for 20 min. The test tubes were then cooled in a water bath for 5 min. Then, 1 mL of reagent C was added to each tube and the tubes were shaken until the bubbles dissipated. After 20 min, all samples were diluted to 25 mL volumetrically with DI water, and absorbance measurements were taken at 520 nm. For each of the complexes, three measurements were taken. The DH was calculated using the following equation with reducing sugar determined:

$$DH\ (\%) = \frac{hydrolyzed\ concentration\ \left(\frac{mg}{mL}\right) - unhydrolyzed\ concentration\ \left(\frac{mg}{mL}\right)}{10\ mg/mL}\ X\ 100$$

3.2.3. Preparation of protein-gum complexes

Samples of gum were hydrated as described above. WPI was also hydrated at room temperature for at least 4 hr at 10%. Complexes of protein-gum were prepared according to the pH shifting method described by Li and Zhong (2021) with slight modification. WPI was adjusted to alkaline pH 11.3 with 1 N NaOH and respective gum was then added at a 5:1 mass ratio of protein to polysaccharide, which has previously been shown to form complexes (Souza and Garcia-Rojas, 2017). The mixtures were then stirred at room temperature for 1 hr before adjusting pH to 7.0 with 1 N HCl. Dispersions were subsequently heated at 50 ^{o}C and stirred for 30 min. Samples were then cooled, and pH was adjusted to pH 4.5 and stirred at room temperature for at least 1 hr. Finished complexes were stored at 4 ^{o}C for further analysis and use in cream cheese (in the form of freeze-dried powder). The flow diagram shown in Figure 3.1 illustrates the process of

creating complexes and their incorporation into the cheeses. Hydrolysate complexes were prepared with the same procedure following respective hydrolysis methods as previously described. One batch of protein-polysaccharide or hydrolysate complexes was created and freeze-dried. Each batch of complexes and controls was sampled twice for analytical characterization and was used in two different batches of commercial cream cheese to test the effect of treatments in two cheese replicates.

3.3. Analytical methods

3.3.1. Determination of surface hydrophobicity of protein-gum complexes

Surface hydrophobicity of protein-polysaccharide whole (denoted as unhydrolyzed) and hydrolysate complexes, and the native protein and its hydrolysate was determined by fluorescence spectroscopy using a 1-ani-lino-8-naphthalene sulfonate (ANS) probe (Kato et al., 1980). Samples were mixed with 1 mL of 1xPBS and vortexed for 1 hr at room temperature, then centrifuged at 10000 x g for 30 min at 4 ^{o}C. The supernatants were collected, with minimal pellet removed, and diluted with PBS to obtain a range of concentrations from 0.0156 and 0.5 mg/mL. Diluted samples were then mixed with ANS reagent on a black polystyrene 96-well microplate and allowed to incubate in the dark for 10 min. Fluorescence intensity was then measured at the excitation and emission wavelengths of 390 and 479 nm, respectively (Synergy H1, BioTek, USA). The slope of the line of fluorescence intensity as a function of protein concentration was obtained as the surface hydrophobicity of the sample. Two treatment replicates were analyzed, and each sample was measured three times.

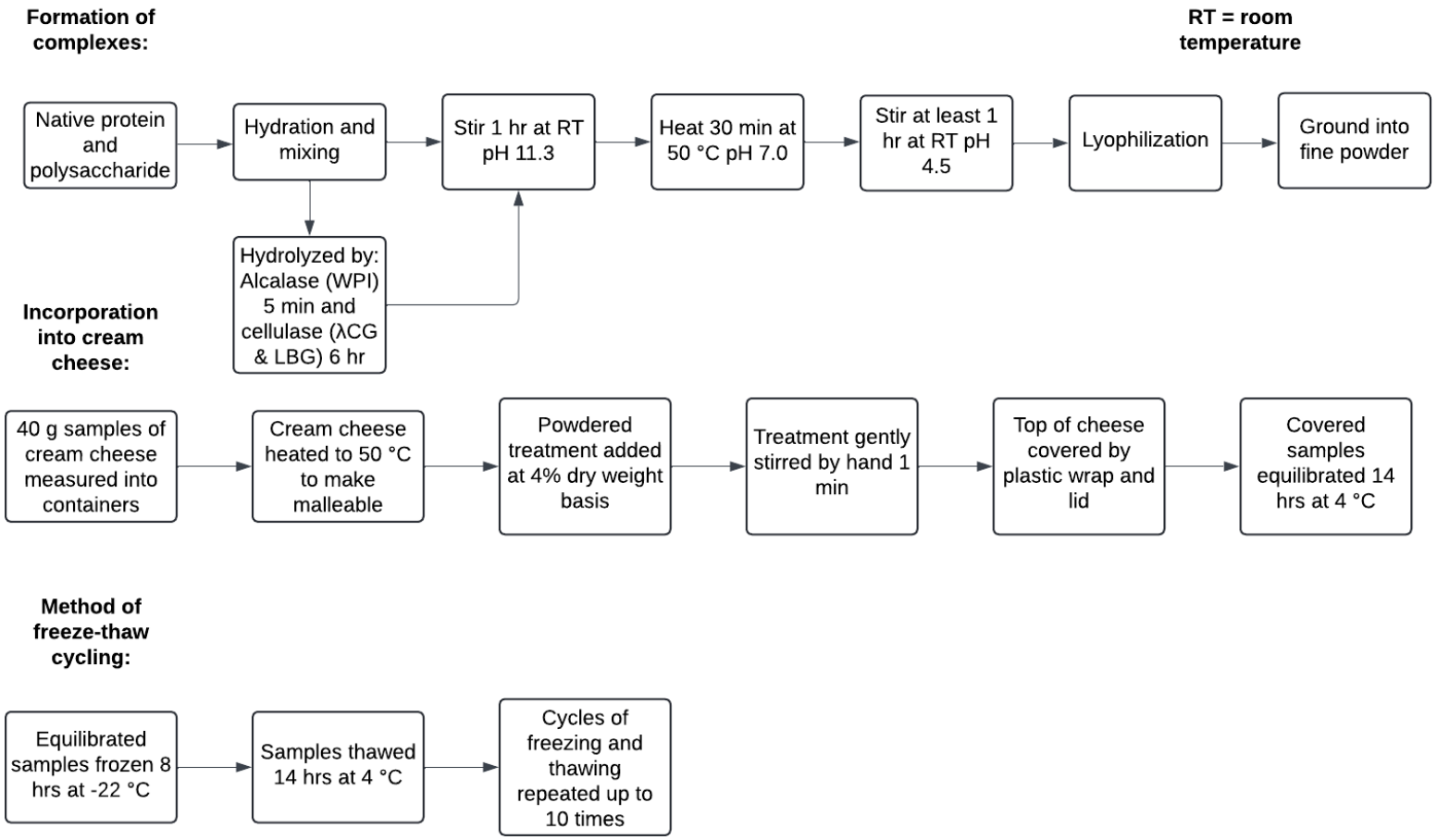


Figure 3.1. Flow diagram illustrating the process of forming protein-polysaccharide complexes and hydrolysate complexes, incorporation into cream cheese, and process of freezing and thawing for the evaluation of additives' effect on reducing freeze-induced damage.

3.3.2. Quantifying zeta potential and particle size distribution of protein-gum complexes

Zeta potential and particle size distribution measurements were performed using Malvern Zetasizer, model 3000, Malvern Instruments (Worcestershire, England). A 1 mg/mL dilution of sample was created in 100 mM NaCl at neutral pH and the other was adjusted to pH 4.5 and placed in the electrophoretic cell (DTS1070). Particle size distribution was reported as a percentage of the total volume of particles in a particular size range. For both zeta potential and particle size distribution, two treatment replications were conducted, and three measurements made for each.

3.3.3. Changes in protein's secondary structure by Fourier Transform Infrared spectroscopy (FTIR)

The secondary structure of protein and complexes was analyzed by FTIR using a Spectrum Two FT-IR Spectrometer (PerkinElmer Inc., Waltham, MA, USA). Individual biopolymers and their complexes were prepared in 1xphosphate buffer solution (PBS) at 60 mg/mL concentration. Spectra was collected with 32 scans in wavelength region between 3000 and 300 cm^{-1} at a resolution of 4 cm^{-1}. Data was then analyzed with OriginLab 2021 (Northampton, MA, USA). The specific band of interest was the amide I band (1600-1700 cm^{-1}). Spectra was collected from two treatment replicates with three measurements of each.

3.3.4. IRI activity determined by splat assay

IRI activity of the biopolymers and their complexes was determined in 1xPBS and 100 mM NaCl. The method described by Bredow and Walker (2017) was used with slight modification. The assay was performed using 4% of the compounds in 1x PBS at pH 7.0 and

100mM NaCl solution at pH 4.5 and 7.0 to test the effect of salt and pH. Polyethylene glycol (PEG) at 4% was used as a negative control as described by Biggs et al. (2019). One drop of approximately 10 µL was released from a syringe at a height of 1.5 m onto a pre-chilled microscope slide (-80 °C). The sample was then annealed at -8 °C for 30 min using a cold stage HCS 302 (Instec Instruments, Boulder, CO, USA) when pictures were taken using a Leica DMC 2700M microscope (Wetzlar, Germany). Three drops were done for each sample and three pictures were taken for each drop from which the Feret's maximum diameter was averaged by ImageJ software (Saad et al., 2023).

3.4 Preparation, storage, and measurements of cream cheese with the complexes incorporated

Tillamook commercial cream cheese was heated to 50 °C in a water bath to make it soft and malleable to allow the addition of the complexes. Complexes (5:1 of protein to polysaccharide) of dry form at 4% of the cream cheese mixture on a dry weight basis (1.8 % as-is basis) were incorporated. Controls containing the individual gum, protein, and their hydrolysates were also created in amounts proportionate to their ratio in the complexes. Commercial cream cheese controls, with no treatment or heating procedure done to it, and a heat control (50 °C) were created as well. Once added, the mixture was gently hand stirred for ~1 min to fully incorporate the complex into the cream cheese matrix. Modified samples were allowed to equilibrate at 4 °C overnight (~14 hr) to allow for complete hydration of complexes in the cheese matrices. Samples of 40 g ± 1g were measured and stored in airtight plastic containers with plastic wrap resting on top of the cheese samples to prevent moisture loss. Following the 14-hr equilibration period at 4 °C, freezing treatment was performed by cycling 10 times of the cycle

of 8 hr storage at -22 °C followed by overnight thawing at 4 °C. Rheological and textural analyses were done following overnight thawing at cycle 0, 1, 4, and 10. Two batches of cream cheese were used as duplicated treatments.

3.4.1. Viscoelastic properties of modified cheese by rheological measurements

The viscoelastic characteristics of the treated cream cheese were evaluated using a small amplitude oscillatory test. A strain sweep test was performed using a T.A. Instruments Discovery HR-2 Rheometer (Delaware, USA) fitted with a 40-mm cross-hatched parallel plate at a gap of 2 mm to determine the storage and loss moduli over a set strain range that included the linear viscoelastic region (LVR). The samples first remained at 4 °C for 2 min to equilibrate. The analysis was performed at a frequency of 1.0 Hz at strain from 0.01 to 300% at 4 °C. This provided both the elastic properties and the viscous properties of the cream cheese systems, obtained as storage modulus (G') and loss modulus (G"). Yield stress was also calculated as a 10% drop in G'. Measurements were collected from two replicate treatments with three measurement replicates for each.

3.4.2. Hardness, consistency, and adhesiveness measurement of modified cream cheeses by single compression texture analysis

A single penetration test was conducted on the cream cheese at room temperature (21 °C) according to the method outlined by Brighenti et al. (2008) with modification. A T.A.TXT2 texture analyzer with a 50-kg load cell (Texture Technologies Corp., South Hamilton, MA) was used to perform analyses. A 45° stainless-steel conical probe (TA-15) was used to penetrate the sample to a depth of 15 mm with 2.0 g trigger force. Pre-test, test, and post-test speed were set at

1.00 mm/sec. Hardness was determined as the maximum peak force for a single compression. Consistency was determined as the area of the positive peak resulting from a single penetration. Adhesiveness was measured as the peak of negative force following a single penetration. Two treatment replicates were collected, and each treatment replicate was measured three times.

3.4.3. Observation of cream cheese aggregation using confocal laser scanning microscopy (CLSM)

A 1:9 dilution of cream cheese samples was created by diluting 1 g of the sample in 9 mL of deionized water with gentle hand mixing. Nile Red dye for lipids and Fast Green dye for protein were created in 1 mg/mL concentration dissolved in methanol and DI water respectively. To cream cheese dilutions, 0.1 mL of each dye solution was added and gently mixed by hand until both dyes were apparently fully incorporated into dispersions. Samples remained in the refrigerator overnight at 4 °C so that the dyes could fully incorporate into the samples.

The Nile Red and Fast Green dyes were excited at wavelengths 488 nm and 633 nm respectively. The emission ranges of the two dyes were set according to Ong et al. (2018), which were 520-590 nm for Nile Red and 660-750 nm for Fast Green. Samples were observed under an inverted CLSM (Leica Microsystems, Baden-Wurttemberg, Germany) at 63x magnification with oil immersion.

3.5. Statistical analysis

All experiments were performed in duplicate treatments, with each replicate measured three times unless otherwise stated. For cream cheese samples, duplicates came from the same batch of complexes applied to different batches of cream cheese. Results were presented as the

mean ± standard deviations (SD). An ANOVA test was conducted using JMP v12.0 (SAS Institute, Cary, NC, USA). Statistical significance was determined as $P<0.05$. Differences among the means were determined using a Tukey HSD post-hoc test.

CHAPTER 4. RESULTS AND DISCUSSION

4.1. Degree of hydrolysis (DH) of biopolymers

To create smaller gum molecules, λCG and LBG were hydrolyzed by cellulase for six hr to achieve a reduction in MW. Somogyi-Nelson was done to determine the concentration of reducing sugar after hydrolysis. As glycosidic bonds were cleaved, more reducing ends were exposed and thus there was an increase in reducing sugar concentration. For the gums hydrolyzed in this study, cellulase-catalyzed hydrolysis was more effective for LBG than for λCG as shown in Table 4.1. This can be attributed to the linkages of the biopolymers because LBG is made of mannose and galactose units linked by β-1,4 glycosidic bonds (Damodaran et al., 2007). λCG, however, lacks the dominance of these linkages, instead containing sulfated and nonsulfated galactose and 3,6 anhydrogalactose units linked by alternating α-1,3 and β-1,4 glycosidic linkages. Preliminary studies showed a slight increase in DH for both polysaccharides when hydrolyzed for a longer duration (up to 48 hr). Ultimately, only a 6 hr hydrolysis was selected to prove the concept.

Changes in the viscosity of gum solutions was another indication of a successful hydrolysis reaction. Following hydrolysis of each gum for 6 hr, an obvious reduction in the viscosity was observed. While the DH itself was low as calculated and presented in Table 4.1, the change in reducing end concentration itself can also be indicative of a successful hydrolysis reaction. Following hydrolysis, the λCG achieved a 2.3x increase in reducing end concentration meanwhile the LBG achieved a 4.8x increase. This indicates that while the hydrolysis may not have been extensive in breaking up the polysaccharides, the reaction produced an average of 2.3- and 4.8 times smaller molecules for the two gums.

Table 4.1. DH and average MW of gums and hydrolysates by cellulase for 6 hr

Sample	Reducing sugar concentration (mg/mL)		DH (%)
	Unhydrolyzed	**6-hr hydrolysates**	
λCG	0.08 ± 0.00	0.18 ± 0.00	1.0 ± 0.03
LBG	0.24 ± 0.02	1.15 ± 0.37	9.1 ± 0.66

***Values are shown as the mean ± standard deviation for three replicate measurements.*

Higher levels of hydrolysis have been reported in literature, such as nearly 99.9% hydrolysis of LBG by Chen et al. (2018) after 48 hr hydrolysis with β-D-mannanase, apparently due to the specificity of the enzyme used. If a specific enzyme for λCG, i.e. λ-carrageenase, was used, various molecular weights with a range of degrees of hydrolysis could be achieved (Chauhan and Saxena, 2016). Most gum hydrolysis studies use acid hydrolysis at 60 °C for several hours at strong acidity to achieve a reduction in molecular weight (Ávila-Fernández et al., 2011; Du et al., 2011). Initial attempts were made to hydrolyze LBG and λCG under varying high heat and acidic conditions, but no measurable and consistent DH was achieved.

Hydrolysis of WPI by Alcalase resulted in peptides of various molecular weights. From preliminary studies, 5 min was determined to be sufficient to significantly reduce WPI's molecular weight. To prove the concept that smaller molecules may perform better as IRI agents, only one protein hydrolysate was created for complexation with the two gum hydrolysates. From HPLC-SEC analysis, native WPI had an average MW of 13.6 kDa. After 5 min of hydrolysis, the average MW was reduced to ~0.6 kDa. MW distribution of WPI and hydrolysate was examined and is shown in Table 4.2.

These results are comparable with findings from a study conducted by Li-jun et al (2008) which found that following a 7 hr Alcalase hydrolysis of whey protein concentrate, most peptides were less than 1 kDa with a range from 0.6-1 kDa. Additionally, when soy protein isolate was hydrolyzed by Alcalase under the same conditions as used in the present research, ~50% of the molecules were less than 1 kDa, with only ~10% greater than 6.5 kDa (Fomich et al. 2023). This is consistent with the distribution obtained in the current study where the majority were below 1 kDa and only 1.4% of the molecules were above 6.5 kDa.

Table 4.2. MW distribution of WPI and 5-min Alcalase hydrolysate

Sample	<1 kDa	1-4.9 kDa	5-9.9 kDa	10-19.9 kDa	>20 kDa
WPI-0	4.8	16.2	0	24.2	43.2
WPI-5	74.7	8.9	0	0.8	0.6

Fomich et al. (2023) also found that SPI hydrolyzed by Alcalase produced more IRI-active peptides compared to those by other enzymes. However, increasing the hydrolysis time did not lead to higher IRI activity. The study also suggested that larger MW peptides in the hydrolysate were necessary to give strong IRI activity. Therefore, this the 5 min hydrolysis time for WPI should was expected to be sufficient to achieve the appropriate MW distribution for IRI activity.

peptides compared to those by other enzymes. However, increasing the hydrolysis time did not lead to higher IRI activity. The study also suggested that larger MW peptides in the hydrolysate were necessary to give strong IRI activity. Therefore, this 5-min hydrolysis time for WPI should be sufficient to achieve the appropriate MW distribution for IRI activity.

4.2. Surface hydrophobicity of protein-gum complexes

Surface hydrophobicity of WPI, and its hydrolysate, and complexes are presented in Figure 4.1. Individual polysaccharides and their hydrolysates were not evaluated as they do not contain hydrophobic regions that can interact with the ANS probe. WPI had the highest surface hydrophobicity and it decreased drastically with hydrolysis. In general, the molecular weight was decreased during hydrolysis, and increased solubility was achieved, as observed by Schröder et al. (2017), who found a nearly 50% reduction in surface hydrophobicity of whey protein following hydrolysis due to increased molecular charge caused by peptide bond cleavage. A reduction of hydrophobicity following hydrolysis of pea protein was also reported (Shuai et al., 2022), and this was attributed to increased hydrophobic interaction and protein-protein aggregations due to the exposure of the internal hydrophobic moieties by hydrolysis, thus leading to a decrease in surface hydrophobicity.

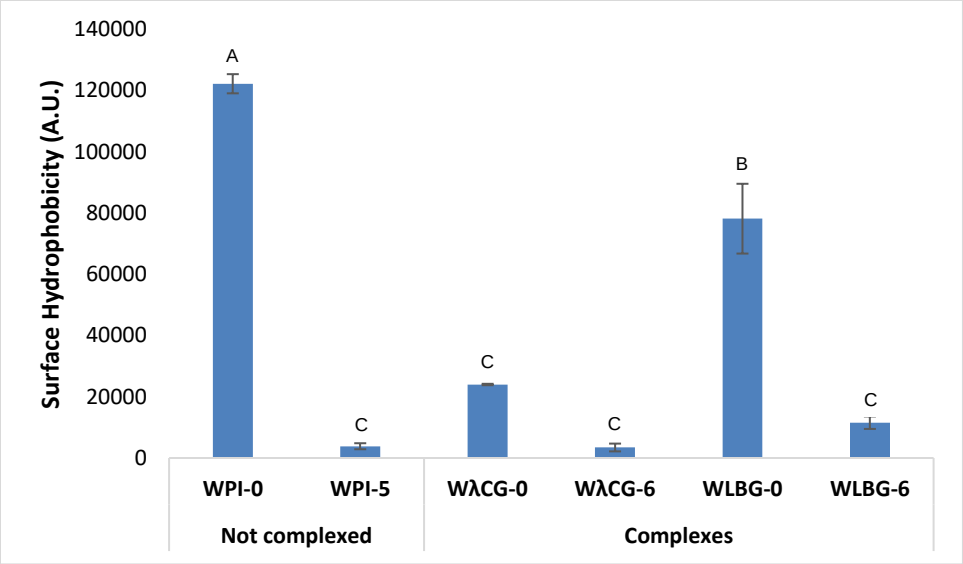


Figure 4.1. Surface hydrophobicity of whole and hydrolysate complexes, and their respective controls. Each column indicates the mean of two treatment replicates, each measured three times with error bars indicating standard deviations of the mean. Different letters indicate significant differences ($p<0.05$). For WPI, 0 and 5 indicate the number of min of hydrolysis of the biopolymer. For the complexes, 0 and 6 refer to the number of hr of hydrolysis for the respective gums that were complexed with either unhydrolyzed or 5-min WPI hydrolysates.

Following complexation with polysaccharides, a further reduction in surface hydrophobicity was observed in both whole and hydrolysate complexes of both gum types. This can be attributed to surface coverage or binding of the hydrophobic regions by polysaccharides. Additionally, given the hydrophilic nature of both gums, their complexes would also be expected to exhibit greater solubility and reduced hydrophobicity. Or, during the complexing with gum, WPI protein or hydrolysate unfolded, allowing for hydrophobic interactions where exposed hydrophobic residues clustered together, minimizing their exposure to a hydrophilic environment, as reported by Li et al (2024). For each treatment, the hydrolysate complex recorded a lower surface hydrophobicity than the whole complexes, which can be attributed to improved solubility from a reduction of MW. Since the λCG is more charged than LBG, its complexes also showed much lower surface hydrophobicity as expected.

Changes in surface hydrophobicity or amphiphilicity are expected to impact IRI activity, as suggested in the literature. However, Hydrophobicity itself may not directly correlate with the IRI activity (Ollis et al., 2024). Ultimately, it was suggested that a balance of hydrophobic and hydrophilic characteristics is needed to increase IRI activity. This surface hydrophobicity measurement was not quantitatively correlated with the IRI activity as discussed below, but it simply served as an indicator of the complex formation.

Statistical analysis by a one-way ANOVA indicated a significant reduction ($P=0.0001$) in surface hydrophobicity for WPI hydrolysate compared to whole protein. A significant reduction ($P=0.0001$) was also obtained for the LBG hydrolysate complex compared to the whole complex. No significant difference was observed for the λCG whole complex compared to the hydrolysate complex.

4.3. Particle size of protein-gum complex as characterized by particle size analysis

The mean particle diameter data are shown in Table 4.3. There are four major findings or trends. First, WPI hydrolysate had a much bigger particle size than that of the unhydrolyzed WPI dispersion. During hydrolysis, the hydrophobic interior regions of the WPI were exposed as peptide bonds were cleaved. This can ultimately lead to aggregation of hydrolysates by hydrophobic interaction, which would lead to an increase in particle size. Secondly, the whole complexes with both gums were much bigger than their individual dispersions, indicating complex formation. Thirdly, for the hydrolysate complexes particularly with the λCG-6, the particle size was greatly reduced, indicating the gum polysaccharide was able to break up the WPI hydrolysate's aggregates. Fourthly, the effect of pH was not as significant as expected. At pH above the pI of WPI (~5), the protein will carry a net negative charge, allowing for improved solubility (Damodaran et al., 2017). The particles of protein and hydrolysate at pH 4.5 seemed to be bigger due to the reduced dispersibility near the pI of the protein.

Shifts in the particle size distribution curves can also provide an indication of complexation between biopolymers. A monomodal curve was observed following complexation when measured at pH 7.0. When measured at pH 4.5, however, a lagging peak can be seen for most of the complexes. Typically, a monomodal peak is indicative of more uniform particles within a dispersion, which can be associated with the formation of complexes. Bimodal peaks, however, do not necessarily indicate a lack of complexation, but rather a different degree of complex and uncomplexed components in the mixtures.

Overall, the particle size and changes are an indication of complex formation.

Table 4.3. Mean particle diameter in 100mM NaCl solution by zetasizer (nm).

Sample	Mean particle diameter (nm)
Not complexed pH 7.0	
WPI-0	717 ± 22
WPI-5	6402 ± 51
λCG-0	855 ± 50
λCG-6	721 ± 56
LBG-0	1367 ± 28
LBG-6	1031 ± 445
5:1 complexes	
WλCG-0	8399 ± 188
WλCG-6	2260 ± 112
WLBG-0	5722 ± 2128
WLBG-6	6803 ± 873
pH 4.5 Not complexed	
WPI-0	958 ± 247
WPI-5	8309 ± 1214
λCG-0	549 ± 138
λCG-6	656 ± 54
LBG-0	1491 ± 96
LBG-6	979 ± 387
5:1 complexes	
WλCG-0	8001 ± 82
WλCG-6	1686 ± 484
WLBG-0	8008 ± 3140
WLBG-6	4809 ± 714

4.4 Zeta potential of protein-gum complexes as characterized by zetasizer

Measuring charges of the complexes in comparison to the uncomplexed components is another indication of complex formation. The Zeta potential of whole and hydrolysate complexes measured in 100 mM NaCl at neutral pH and pH 4.5 are shown in Figure 4.2. The two sets of measurements at pH 7 and 4.5 showed similar profiles. The general observations are as follows: Even though large standard deviations were observed, the WPI hydrolysate carried more negative charge than WPI. A more negative zeta potential was produced following hydrolysis of WPI, because of the release of free amine and carboxylic acid groups and the strong acidity of the acidic group.

Zeta potential was most negative for λCG-0 and λCG-6, which can be attributed to the sulfate groups along the backbone of the polysaccharide. A strong indication of complex formation is that after a 5:1 ratio of protein:gum complex, the charge of the products was more similar to that of the gum than to protein or hydrolysate, opposite of the trend expected if the two molecules were in a simple physical mixture.

From the literature and due to the pI of WPI around 5, it was expected that when measured at this lower pH, zeta potential should be less negative and approach neutral values as WPI approaches neutrality at pH 4.5 (Li et al., 2024). A high zeta potential value is indicative of strong repulsion between particles, which would ultimately prevent aggregation of ice crystals and vice versa, as suggested in one study (Zhu et al., 2019) that showed high zeta potential and increased IRI activity.

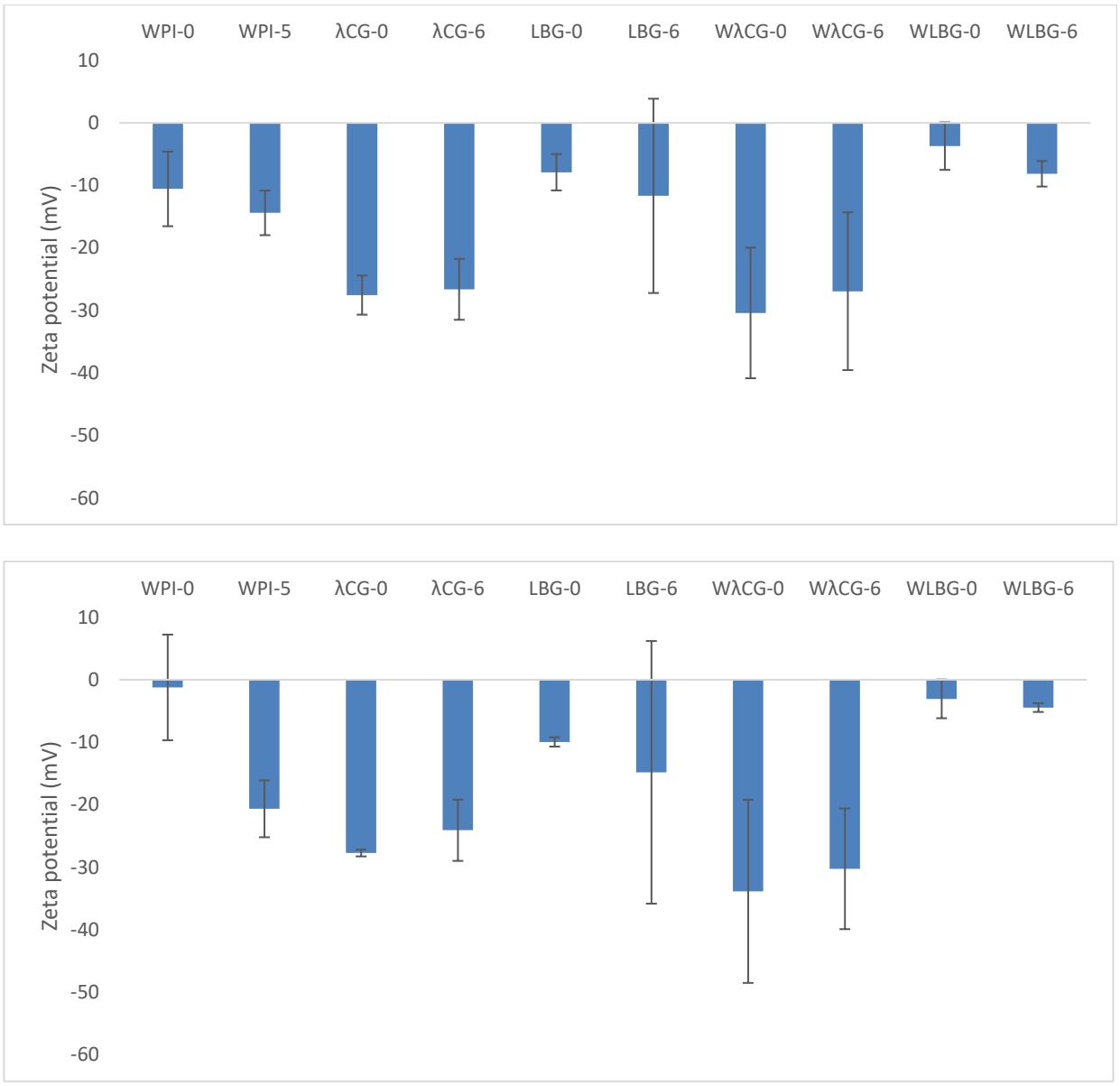


Figure 4.2. Zeta potential of whole and hydrolysate complexes at in 100 mM NaCl at pH 7.0 (top) and at pH 4.5 (bottom).

4.5. Secondary structure of protein-gum complexes as determined by FTIR analysis

FTIR spectra should allow for further confirmation of complexation between biopolymers and can be used to understand non-covalent interactions between our two biopolymers (Guerrero et al., 2014). The FTIR spectra of native WPI, λCG, LBG, and their respective hydrolysates, as well as whole and hydrolysate complexes were collected and shown in Figure 4.3. For these protein-polysaccharides complexes specifically, the bands of interest include the amide I and II band regions. In each sample, a clear peak can be seen in the region between 1500-1700 cm^{-1}, which is commonly reported in the literature as the amide I and II band region (Miller and Dumas, 2010; Pooja et al., 2014; Zhang et al., 2023). This band and its minor shifts represent the stretching vibrations of the carbonyl (C=O) groups and C-N stretching and bending vibrations. A change in intensity for this peak from both gum types was seen after complexing. A more distinct increase in intensity was observed for the whole complex compared to that of hydrolysate.

Another peak between the 3000-3600 cm^{-1} region is associated with the hydroxyl group's stretching vibrations (Ping et al., 2001). In the case of the whole complexes, both gum types resulted in peak broadening compared to WPI alone. This is an indication of complex formation. All the profiles of the hydrolysate complexes were very similar, possibly because these were mixtures of molecules with different degrees of polymerization or degree of hydrolysis. These molecules may have interacted among themselves, minimizing the differences caused by the treatments.

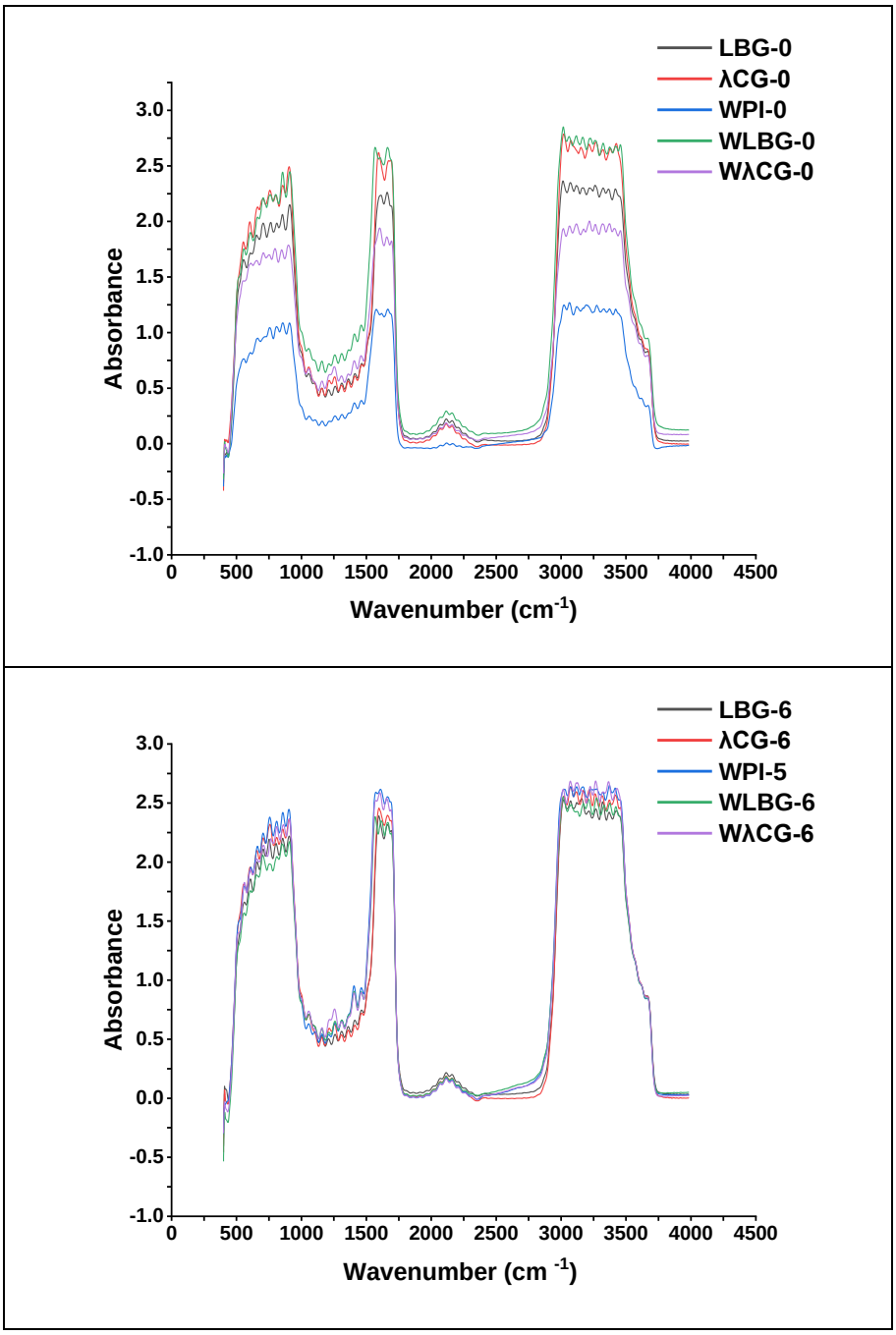


Figure 4.3. FTIR spectra of various complexes and their respective controls measured at 40 mg/mL concentration from 4000 to 400 cm $^{-1}$. Spectra is shown as the mean of two sample replicates with three replicate measurements.

4.6. IRI activity as determined by splat assay

Because salt type and concentration can affect IRI activity (Olijve et al., 2016; Surís-Valls and Voets, 2019), IRI activity of the complexes and controls was evaluated in both 1xPBS and 100 mM NaCl that had neutral and pH of 4.5. With the objective to add complexes to cream cheese that have a typical pH of 4.5, and about 100 mM NaCl concentration, the test at low pH would mimic the conditions of common cream cheese.

The maximum Feret's diameter of the ice crystals was determined and the percent Feret's diameter relative to an equal concentration of PEG negative control under the identical conditions was calculated. A high percentage value indicates a less IRI-active compound. Three replicates of each treatment were done, and the mean and standard deviations are shown in Table 4.4.

Variations in IRI activity were observed in both 1xPBS and 100 mM NaCl at both pH conditions. Overall, all controls, except for λCG-6, showed no IRI activity under all conditions. Complex treatments, however, showed much increased IRI activity in PBS and NaCl at both pH, except for the WPI-6 hydrolysate complex. The whole λCG complex showed a consistent IRI effect with ~66% IRI activity in both NaCl and PBS at neutral pH, but 80% activity at pH 4.5. This pH effect could be attributed to the differences in solubility at each pH. As the pI of WPI is ~5, poor solubility was expected at pH 4.5. With better solubility or dispersibility at neutral pH, a more uniform distribution and effective interaction of complex with water around ice crystal surfaces would be expected, thus preventing ice growth and fusion.

Table 4.4. Ice crystal reduction in % relative to PEG tested at 4% in 1xPBS and 100 mM NaCl.

Treatment	IRI activity (%)	Treatment	IRI activity (%)
1xPBS pH 7.0			
Not complexed controls		5:1 Complexes	
λCG-0	96.4 ± 4.2	WλCG-0	65.7 ± 1.4
λCG-6	81.2 ± 2.9	WλCG-6	80.8 ± 1.5
LBG-0	140.4 ± 8.7	WLBG-0	71.2 ± 6.5
LBG-6	144.8 ± 1.8	WLBG-6	102.3 ± 2.6
WPI-0	108.2 ± 2.8		
WPI-5 min	98.4 ± 3.1		
100 mM NaCl at pH 7.0			
Not complexed controls		5:1 Complexes	
λCG-0	109.4 ± 3.0	WλCG-0	66.1 ± 5.7
λCG-6	114.4 ± 8.4	WλCG-6	84.8 ± 0.8
LBG-0	162.7 ± 1.5	WLBG-0	53.7 ± 7.2
LBG-6	145.6 ± 9.0	WLBG-6	104.5 ± 3.1
WPI-0	119.8 ± 2.7		
WPI-5	112.9 ± 2.4		
100 mM NaCl at pH 4.5			
Not complexed controls		5:1 Complexes	
λCG-0	165.4 ± 5.0	WλCG-0	79.7 ± 3.0
λCG-6	84.0 ± 3.0	WλCG-6	89.3 ± 0.7
LBG-0	170.3 ± 12.5	WLBG-0	103.5 ± 1.6
LBG-6	105.3 ± 6.1	WLBG-6	96.2 ± 2.5
WPI-0	102.2 ± 2.0		
WPI-5	117.1 ± 4.5		

*IRI activity is reported as the average of 3 replicate treatments with 3 pictures taken for each treatment. PEG negative control was obtained under the exact conditions. An IRI activity of 100 would indicate the treatment is not effective compared to PEG control.

It was hypothesized that by reducing the MW of the polymers, the complexes would be more mobile and flexible in their chemical structure, and thus would have an increased IRI activity. However, the data disapproved of the hypothesis. The complexes of the hydrolysates of both gums were less IRI active than their unhydrolyzed whole complexes under almost all conditions, as shown in Table 4.

Overall, the protein-gum complexes showed significant IRI activity compared to their inactive individual compounds. The WPI- λCG complex showed potential to be used in food applications.

4.7. Rheological and textural properties of freeze-thawed cream cheese

Rheological measurements as shown in Figures 4.4 and 4.5 illustrate how the freeze-thawing cycles impacted cream cheese quality. Large standard deviations were observed due to the two batches of cheeses used, the heterogeneity of the product matrices, and the manual stirring and incorporation of the treatments into the matrix without further homogenization treatment due to the laboratory equipment limitations. Therefore, only general trends are presented and discussed.

As shown in the graphs, the heat control showed a measurable difference compared to the blank control. This is indicative of initial damage to the cream cheese microstructure from the mild heating treatment aimed to allow for the incorporation of treatments. This disrupted the already pre-established cream cheese structure. To address this problem, we made cream cheese in the laboratory, but the large variability and the lack of proper processing, such as homogenization, led to large batch-to-batch differences.

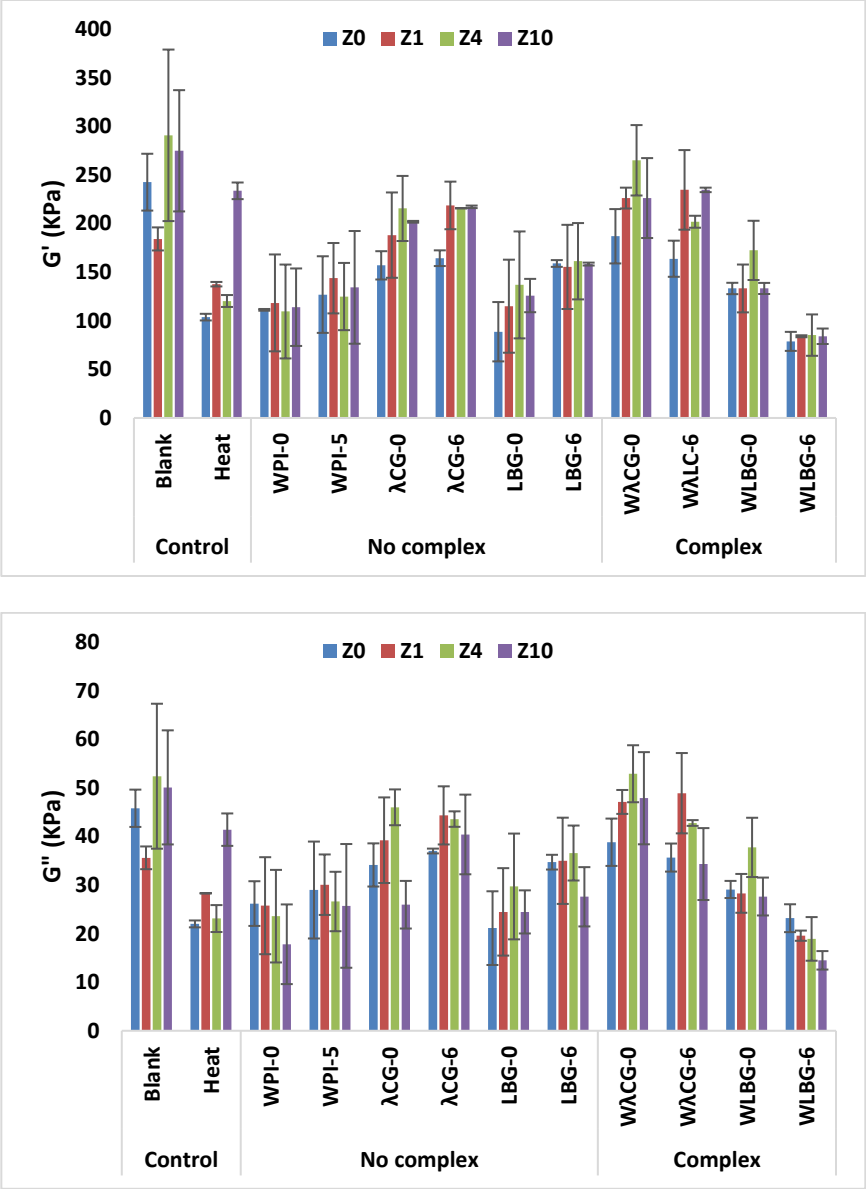


Figure 4.4. Storage modulus (G'), top, and loss modulus (G"), bottom, obtained by oscillation strain sweep of original and modified commercial cream cheese with increasing freeze-thaw cycles (Z0-10). Values are shown as the mean of 2 treatment replicates, each measured 3 times and error bars indicate the standard deviations of the mean.

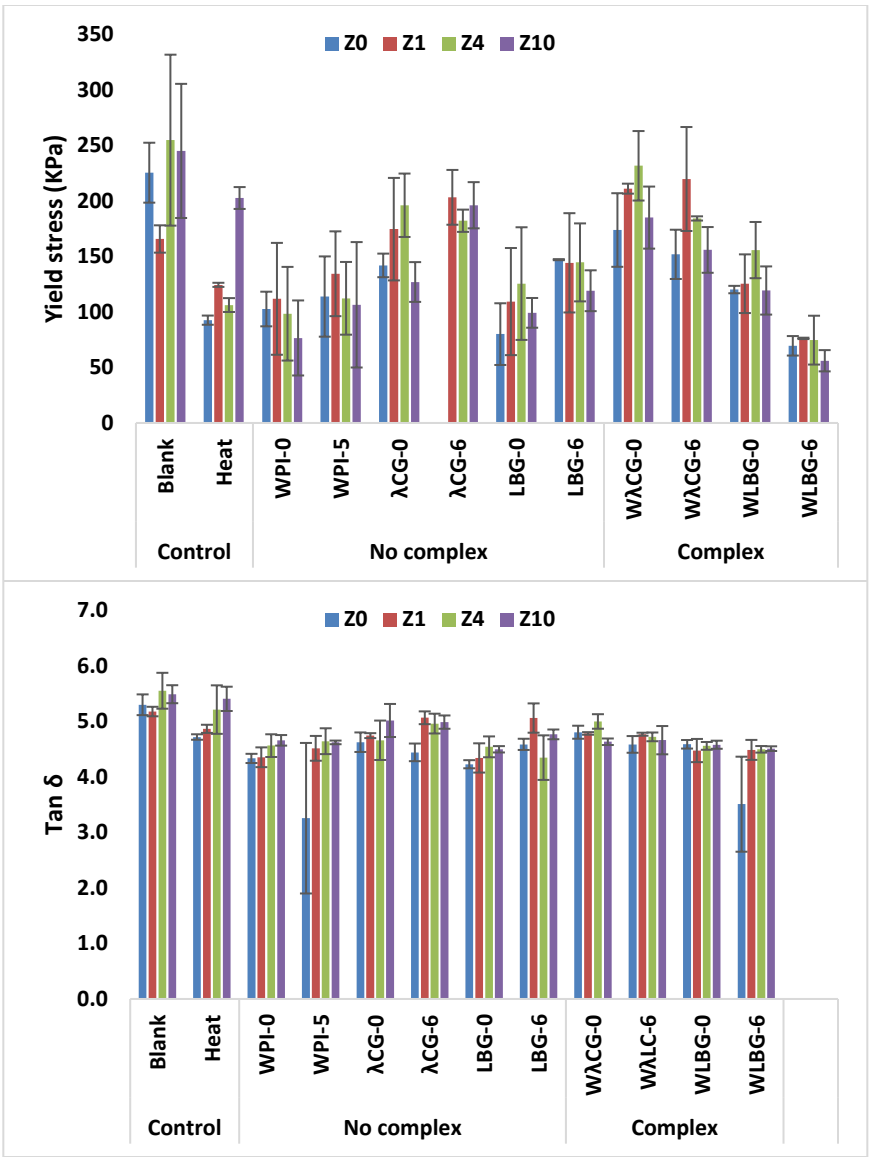


Figure 4.5. Yield stress (top) and tangent delta (bottom) obtained by oscillation strain sweep of original and modified commercial cream cheese with increasing freeze-thaw cycles (Z0-Z10). Values are shown as the mean of 2 treatment replicates, each measured 3 times and error bars indicate the standard deviations of the mean.

Visually, an obvious difference was observed between the blank and heat controls after each freezing cycle. While both controls were much crumblier than any of the treatments, the heat control was consistently crumblier than the blank control and appeared more cracked when removed from the freezer after each cycle of freezing and thawing. Because this commercial cream cheese had no added gum stabilizers, high crumbliness due to freezing damage was expected.

Unsurprisingly, all treatments resulted in some degree of reduction in observable crumbliness. This can be attributed to the increased viscosity from addition of solids and subsequent increased bound water, in addition to the expected IRI activity and less freezing damage. Furthermore, while syneresis was observed for both blank and heat control cheeses, it was not observed for any of the treatments which can again be attributed to a reduction of unbound water in the system. It should be noted that efforts were made to quantify the changes in crumbliness and syneresis throughout the study. Unfortunately, a consistent and quantitative method could not be established.

4.7.1. Rheological properties as affected by freezing cycle and IRI agents

A preliminary 3-month freezing study was conducted to determine at which point freezing-induced damage could be measured and when it would reach a plateau. After this, another preliminary study was done to determine if freezing and thawing cycles can create a similar effect of freezing-induced damage, but with less time requirement. It was determined that 10 cycles were sufficient to be comparable to the 3-month time study. Therefore, two different batches of cream cheese were created for the evaluation of each complex and corresponding controls.

Treatments containing λCG consistently had a seemingly higher storage modulus (G') that was similar to the blank control compared to the rest, indicating more retained elasticity and solid-like properties. So, λCG treatments were the most effective treatment from rheological evaluation. This is unsurprising given that λCG is known to have excellent water-binding ability.

However, the changes are not as consistent as what was observed in the preliminary experiment which showed 10 cycles was equivalent to 3 months of constant frozen storage and they both induced damage with decreasing G', G", and yield stress. This can be explained by the difference in cream cheese and gum type. The treatments, however, had varying effects. From rheological measurement, λCG treatments when added as whole and hydrolyzed biopolymers and their complexes showed the greatest similarity to the blank control, indicating retained structure over time and being the most effective treatment from rheological evaluation.

In general, each treatment had an overall slight increase in rheological properties with increasing freezing and thawing cycles. In each treatment for all parameters, rheological testing showed G' dominance over G", with increasing oscillation until a yield stress was eventually reached. This is consistent with gel-like behavior and is comparable to what similar cream cheese studies have shown (Brighenti et al., 2020). Overall, the rheology results of this study showed greater variability and higher standard deviation than the preliminary three-month trial. An important note is Tillamook cream cheese spread used in this study did not contain any hydrocolloids. The cream cheese that contained gum stabilizers from the manufacturer exhibited an overall decrease in hardness and rheological properties with increased freezing time. This is the opposite of what was observed in the present study where there was a general increase. With manufacturer-added gums, the cream cheese has an initial stabilized structure. This structure was

then disrupted upon freezing storage such that texture was degraded due to the disruption of the gel network by ice crystals. The non-stabilized commercial cream cheese meanwhile had a less-than-optimal network. Upon freezing this cream cheese, this matrix was inherently less prone to ice recrystallization damage due to the simpler, less structured gel network.

Moreover, protein dehydration and subsequent hardening can play a role in rheological properties. As ice crystals are formed from the water in the cream cheese, water was removed from the surrounding protein matrix, leading to localized dehydration of the proteins. There was also a concentration of solids in the system as the water was frozen and the relative solids in the unfrozen phase increased. This removal of water can ultimately lead to protein denaturation whereby the hydrophobic regions of the proteins were more exposed, leading to aggregation. Ultimately, this may have resulted in increased firmness and rheological parameters as seen in the present research.

For this reason, tan δ was also considered. Tan δ was determined as G'/G" and thus would be expected to show reduced variability as the value is proportional to each individual treatment's rheological properties. A similar overall trend was observed with this parameter as well with a general increase in value with increasing freezing and thawing cycles. Ultimately, this parameter did not show much distinction between treatments, and all appeared to be nearly as ineffective as the blank control. Because tan δ is the ratio between viscous and elastic properties, a lack of change indicates an overall balance of elastic and viscous behavior. Therefore, while the absolute values of both elastic and viscous behavior are changes with increased freezing and thawing cycles, they appear to be doing so proportionally, maintaining the overall balance. Overall, G' and G" indicate more specific information about the materials'

response to deformation, while tan δ provides a more general sense of overall viscoelastic balance, ultimately meaning that despite the higher variability, G' and G" provide a more detailed understanding of treatment effect with increased freezing cycles.

4.7.2. Textural properties as affected by freezing cycle and IRI agents

Figure 4.6 shows hardness, consistency, and adhesiveness measured by textural analysis. Large variability was observed as explained in the above section. Overall, hardness showed a different profile from the rheological measurement. Generally, with increasing freezing and thawing cycles, hardness values seemed to decrease, particularly for controls before cycle 10. Given the nature of this testing method, water was retained in the sample, and may have served as a lubricant for the moving penetration probe. Just as with rheological measurements previously discussed, λCG gum alone and in complexes of whole and their hydrolysate forms retained the greatest hardness with freezing and thawing, suggesting the most effectiveness at reducing freezing-induced damage.

Textural consistency recorded as the positive area resulting from single penetration was also evaluated. Similarly to hardness and rheological measurements, the λCG treatments showed the highest consistency. This parameter for all treatments remained relatively constant regardless of the number of freezing and thawing cycles.

Adhesiveness was measured as the negative area from a single penetration test. All treatments containing λCG behaved the most similar to the blank and heat controls, with less stickiness to the probe when exiting from the sample. The exact reason for the greatly increased stickiness from the other treatments is not known, but this may be related to the internal cohesive force vs interfacial orientation of the functional groups of the dispersed molecules.

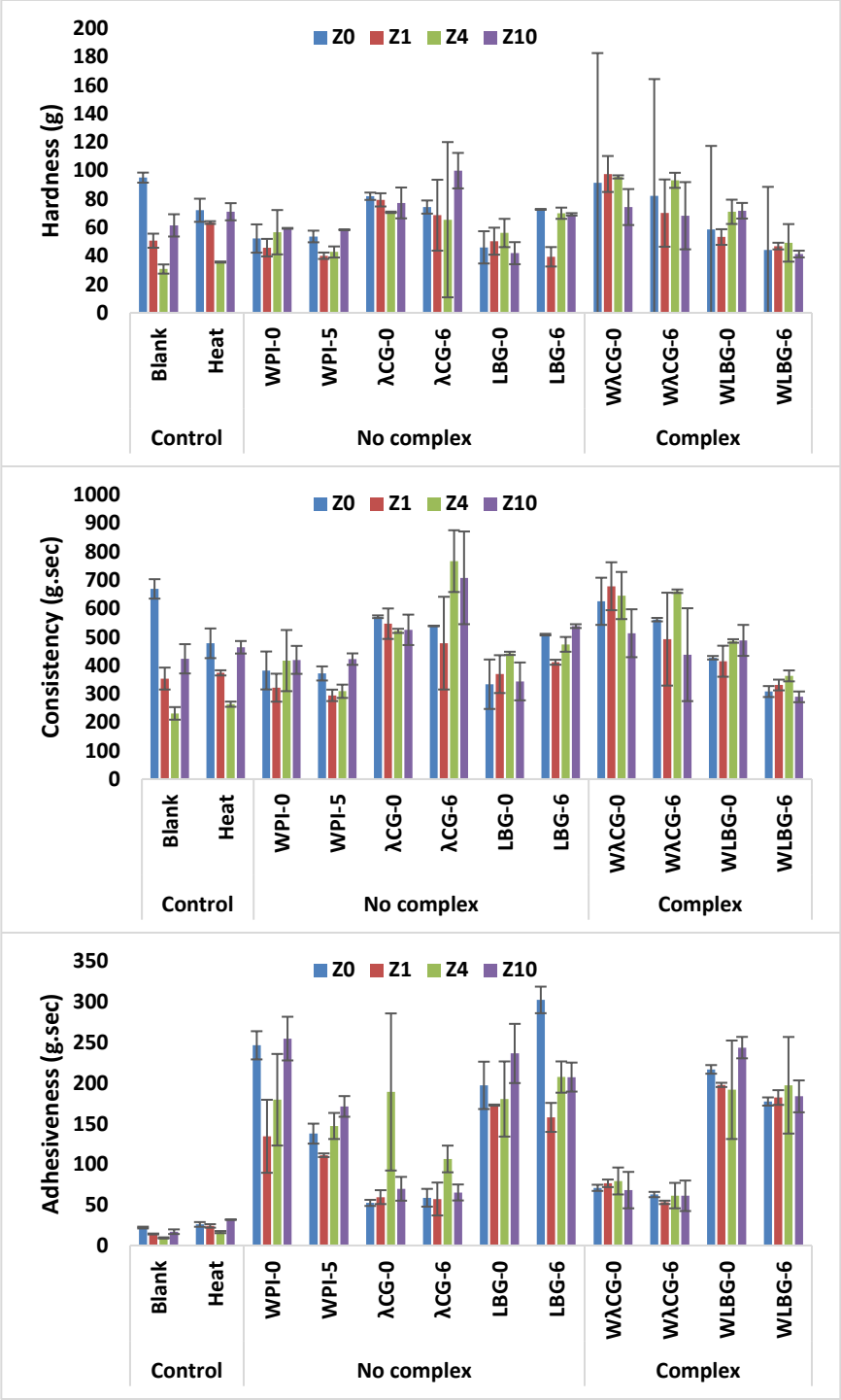


Figure 4.6. Texture measurements as determined by a single penetration test of commercial cream cheese treatments with increasing freeze-thaw cycles (Z0-10). Values shown are the mean of 2 treatment replicates, each measured 3 times, and error bars indicate the standard deviations of the mean.

Adhesiveness was measured as the negative area from a single penetration test. All treatments containing λCG behaved the most similar to the blank and heat controls, with less stickiness to the probe when exiting from the sample. The exact reason for the greatly increased stickiness from the other treatments is not known, but thus may be related to the internal cohesive force vs interfacial orientation of the functional groups of the dispersed molecules.

4.7.3. Relationship between the two instrumental measurements

From rheological measurement, each parameter collected showed a similar change resulting from increased freezing and thawing cycles. Interestingly, its controls exhibited the opposite trend compared to the hardness measurement obtained from texture analysis. During rheological measurement, water did escape from the solid sample of both blank and heat controls due to the nature of the geometry having an open edge compared to the container applied in texture analysis. While samples were gently mixed before measurement, the water was not tightly bound and therefore was able to easily separate once normal and shear forces were applied. For each of the treatments, syneresis was not observed before rheological measurement, however, some heterogeneity was visible in each sample given the freezing damage of the matrices, leading to high standard deviations in many treatments.

There is a key distinction to make between the nature of these two instrumental analysis methods. Rheology applies a shear force, whereas texture analysis only applies a normal force. Texture analysis provides insight into the macrostructure of the system, indicating how the structure responds to localized deformation. Rheology indicates how the system responds to small, oscillatory deformations, providing insight into the material's viscoelastic properties. Both methods are essential given their complementary nature and ability to provide different and more

complete insights into the changes within the cream cheese matrix with increasing freeze-thaw cycles. Since textural analysis measures the entire system without the loss of water and changes of solid content in the sample this method may therefore be more indicative of the true nature of the texture properties of the cream cheese. Texture analysis is also frequently correlated to the sensory properties of foods by providing quantitative values, such as hardness, consistency, and adhesiveness.

4.7.4. General treatment effectiveness by statistical analysis

Through an ANOVA test, the number of freezing and thawing cycles did not have a significant effect (P=0.27) on the ability of treatments to reduce freezing-induced damage in cream cheese. A significant difference (P=0.01) was measured however for treatment effect on ability to reduce freezing-induced damage. In other words, WλCG-0 and WλCG-06 were significantly different from WLBG-0 and WLBG-6 respectively, in their ability to reduce freezing-induced damage in cream cheese. The ANOVA test also showed, however, that λCG-0 and λCG-6 when used on their own, were not significantly different from their respective complexes of the whole biopolymers and hydrolysates. In other words, both treatments were equally as effective at preserving the texture and rheological properties of cream cheese. While whole complexes showed a seeming greater retention of rheological and textural properties with increased freeze-thaw cycles compared to hydrolysate complexes, this was not statistically significant when evaluated at the 5% significance level.

4.7.5. The effectiveness of treatments in the IRI model system versus in cream cheese

The results of textural and rheological analysis were in partial agreement with the results from the model Splat assay for IRI activity. From the Splat assay, λCG complexes and hydrolysate complexes were effective IRI agents. However, the LBG whole complexes showed no consistent IRI effect. These were in general agreement with the cream cheese test results. However, unlike the cream cheese system, no biopolymers alone showed IRI effect in Splat assay. These results suggest the model IRI evaluation may not always translate to the real system performance due to the complex interaction of matrix components. For practical impact, IRI screened agents need to be validated in targeted applications.

Although the hardness and rheological parameters collected in this study can be related to several other textural properties including creaminess, spreadability, grittiness, and others, it did prove to be somewhat limiting. As discussed, visual graininess and crumbliness were observed after the freezing treatment, however, quantification of this property proved to be challenging. Attempts were made to quantify this through centrifugation to measure the degree of syneresis, microscopic observation to quantify light transmitted through a sample on a predetermined greyscale, and others. Ultimately, each attempt was futile.

4.8. Observation of cream cheese microstructure by confocal laser scanning microscopy (CLSM)

Figure 4.7 shows confocal microscopy images of various treatments to illustrate the effect of increasing freezing and thawing cycles on the cream cheese matrix. It provides a visual representation of changes in protein aggregate size and free fat outside of milk fat globules (MFG) caused by freezing damage. Blank and heat controls are shown to illustrate the damage

done by mild heating alone. Additionally, an effective treatment (WλCG-6) was contrasted with an ineffective treatment (WLBG-6).

Before freezing-induced damage of the blank control, fat was not fully visible as bright red as it was contained within the MFG membrane, surrounded by protein. As a result of freezing and thawing, however, ice crystals can rupture this membrane, allowing free fat to escape. The result was more visible, as larger fat globules were densely dyed.

In each treatment sample, some fat can be seen leaching out at all stages. To a minimal extent, some damage was observed in the unfrozen samples. This can be attributed to the mild heating and mixing during complex incorporation as these actions would disrupt the MFG membrane and cause some damage. Following the freezing cycle treatment, the amount of free fat visible increased. The WLBG-6 treatment seemed to have very little stabilizing effect as fat was seen dispersed throughout the image and proteins were densely packed.

Meanwhile, the WλCG-6 treatment did appear to somewhat stabilize the MFG, with smaller fat droplets and a more loosely packed protein network. λCG is known to be used in cream cheese specifically due to its ability to bind water effectively and to strengthen the gel network (Damodaran et al., 2007). This action along with the IRI activity would have helped reduce the matrix damage induced by freezing.

These confocal images serve as a helpful tool to observe qualitatively how the cream cheese matrix was affected by freezing and thawing cycles with different treatments. While alone, they are insufficient to conclude which treatments are effective; they can be coupled with other methods of characterization to obtain answers to proposed research questions.

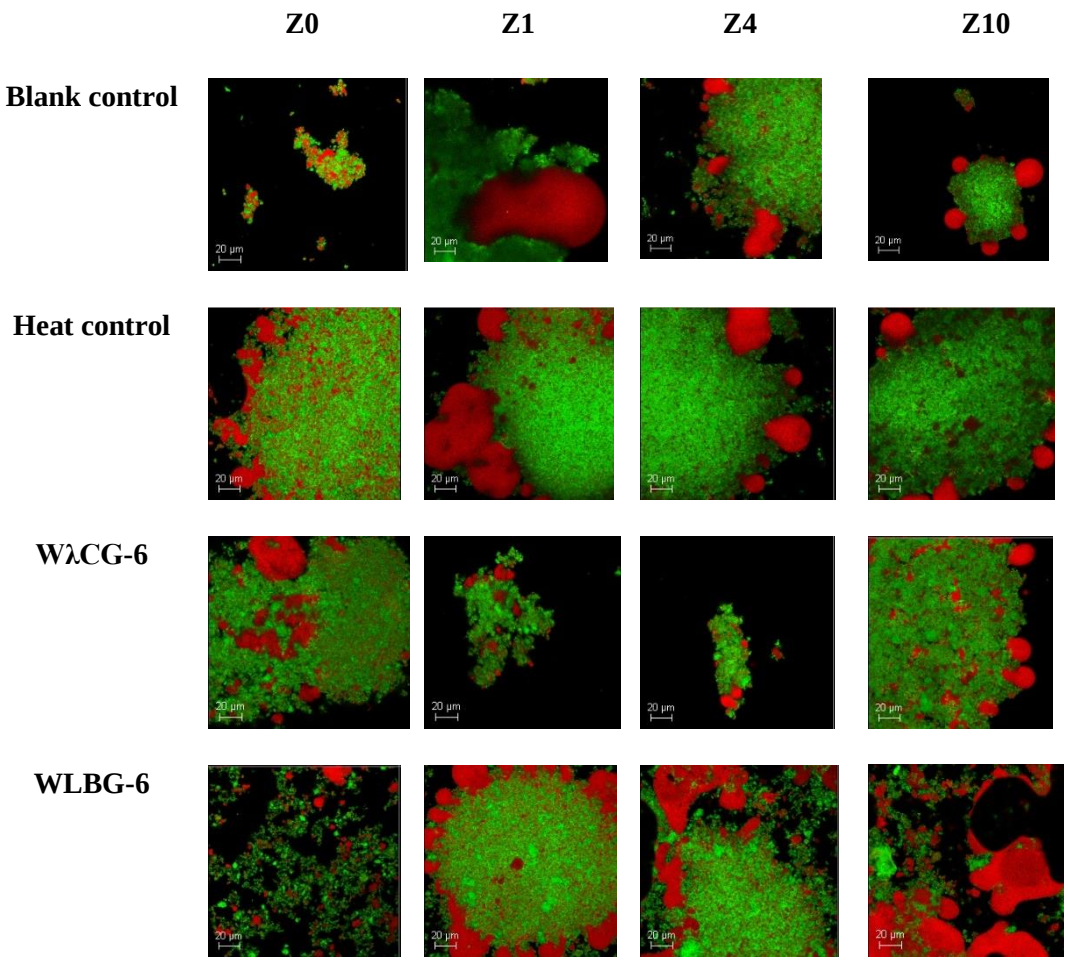


Figure 4.7. Observation of cream cheese samples (controls and hydrolysate complexes) by CLSM under 63x magnification with oil immersion. Nile Red stains fat in sample and Fast Green stains protein in sample.

CHAPTER 5. CONCLUSION

5.1. Conclusion

A dairy protein-polysaccharide complex was made for use as an antifreeze agent in commercial cream cheese. Locust bean gum (LBG) or lambda carrageenan (λCG) were shown to complex with whey protein isolate (WPI), and the complexes were characterized using several methods, and their ability to reduce ice crystal growth and freezing-induced damage was evaluated using a model aqueous system and in cream cheese. The complexes containing λCG were proven effective as anti-freezing agents in the model system. The quantitative rheological and textural measurement, as well as visual observation, of the cream cheese with the treatments containing λCG showed an increased resistance to freezing induced damage.

5.2. Limitations

In preliminary trials, an attempt was made to create cream cheese in the laboratory for use in this study, so the treatments could be incorporated at the desired step. Unfortunately, the lack of access to proper equipment to mimic production at the commercial scale made this impossible. For this reason, the decision was ultimately made to use readily available commercial cream cheese spread for the incorporation of our complexes. As previously discussed, cream cheese is a complex matrix, as such any processes done after production can easily alter or damage its structure. While a heat control was used to account for the additional mild heating and agitation during the incorporation of complexes, there would likely be a different outcome of the antifreeze effect had it been possible to add complexes in during the final homogenization stage as occurs in the commercial production just before hot packing.

Although different brands of cream cheese follow the same general procedure in the production process, there is some variability in formulation and processing. Each brand may

differ slightly, for example in final pH obtained after fermentation, temperatures used at each stage of production, pressure used during homogenization, or inclusion of stabilizers and flavorings. These differences could ultimately impact the application and antifreeze effect of complexes in the cheese system.

The hydrolysis of the polysaccharides proved challenging as well. While protein hydrolysis is routinely done in research and the food industry, hydrolysis of hydrocolloids is not as commonly used. As such, procedures are not well established and specific enzymes are difficult to obtain. In this study, cellulase was used for the hydrolysis of both gums with different sugar monomers and glycosidic linkages. LBG achieved a higher degree of hydrolysis (DH) than λCG. The maximum DH achieved, however, in all preliminary work was ~9% for LBG and ~1% for λCG, and the hydrolysate's molecular profiles are unknown. Hydrolysis of these gums is reported in the literature, but those studies reported the use of specific enzymes, carrageenase for carrageenans (CG) and β-mannanase for LBG, with much higher DH. We will consider using such enzymes in future related studies now with successful proof of the concept.

Finally, across literature related to IRI active agents, various mechanisms have been shown to give their IRI activity. For these specific complexes, however, the mechanism is not known. Future studies therefore could perform molecular dynamic simulations to gain further insight into the mechanism of action for these complexes.

5.3. Future direction

WPI was selected for use in this study as it is already commonly used in commercial cream cheese or other dairy products; a variety of other proteins have been used in the formation of protein-gum complexes, however. Therefore, complexes of other protein and gum

combinations could be investigated for use as antifreeze agents in different food systems where they may be more readily accepted by consumers.

Two logical next steps to expand on this project are to conduct pilot scale cream cheese production and inclusion of the complex at the proper processing step, and to evaluate consumer acceptability by sensory panel for the modified cream cheese after freezing treatment. To do this, a large quantity of the complexes would have to be made with food-safe ingredients. The most likely stage to include these complexes would be during the final step when the hot curd is agitated for inclusion of gum stabilizers, salt, or additional flavorings. The addition of these complexes into commercial cream cheese products are not anticipated to negatively alter consumer acceptance or the palatability of cream cheese. Currently, brands such as Tillamook (Tillamook, OR, USA) and Philadelphia (Kraft Heinz, Chicago, IL, USA) already have WPC added to their cream cheese spreads. Meanwhile, brands such as Publix (Lakeland, FL, USA) contain a variety of gums including LBG, guar gum (GG), and xanthan gum (XG). Given the acceptability of these cream cheese brands, it was expected that the proposed complexes would be similarly accepted by consumers.

A shelf-life freezing test (under more realistic conditions, such as freezing for 3 or 6 months as this is when the industry has identified crumbliness) could also be done to see how the storage of cream cheese containing these additives compares to control products. For application, frozen cream cheese treated as such, along with a control without such additive, should be used in cheesecake making. Rheological and sensory evaluation of the cheesecake product should be evaluated to show commercial success.

Finally, the Code of Federal Regulations (CFR) for cream cheese and cream cheese products must be considered as well. In this study, complexes were added such that added protein made up ~1.5% of the total cheese sample and added gum of ~0.3%. Per the CFR however, stabilizers may not be added to cream cheese more than 0.5% of the finished product. Therefore, additional tests would need to be conducted to evaluate the reduced freezing-induced damage effect of these complexes in cream cheese when used in lower concentrations.

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APPENDIX

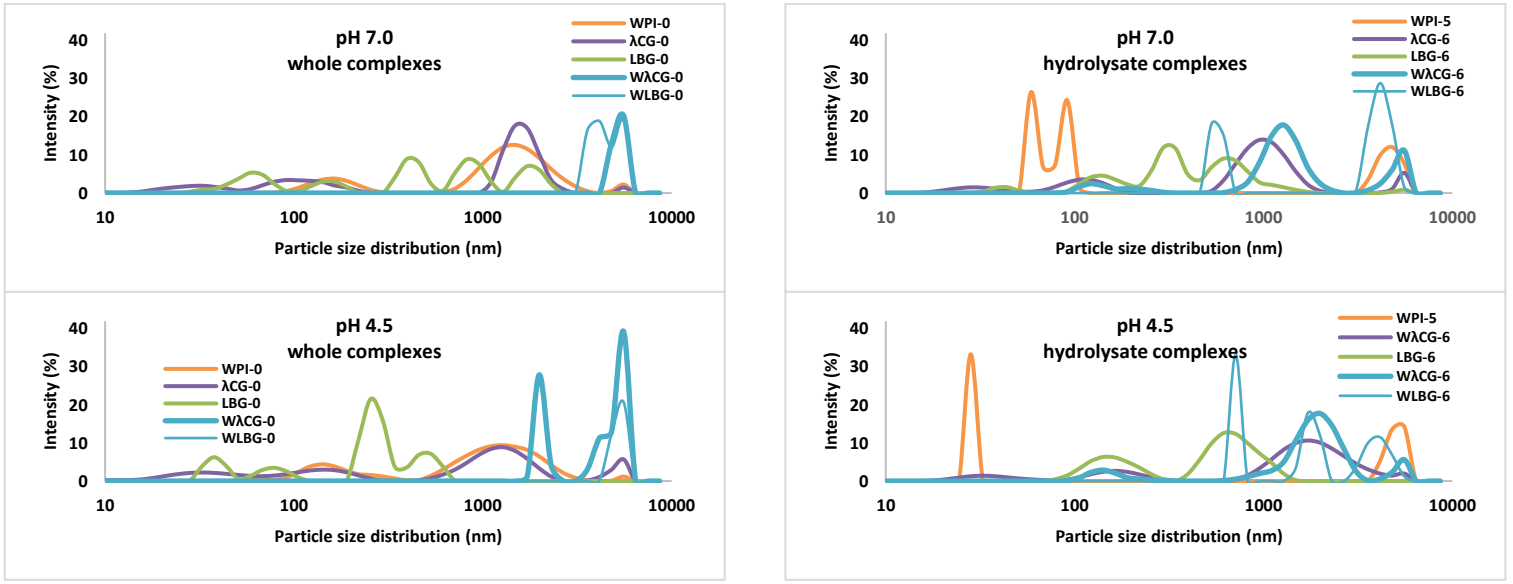

Figure A. Particle size distribution by zetasizer in 100 mM NaCl of whole and hydrolysate complexes at pH 7.0 and 4.5.

VITA

Celeste Alexandra Chadwick was born in San Antonio, Texas in 1999. She moved to Iowa City, IA at 2 years of age where she attended schools in the Iowa City Community School District and graduated from Iowa City High School in May 2017. The following August, she entered Iowa State University and in May 2022 received a Bachelor of Science in Food Science and a Bachelor of Arts in French Language and Culture. During her time at Iowa State, she received various awards and honors including Dean's list, being selected as a Ronald E. McNair Postbaccalaureate Scholar, and being inducted in Pi Delta Phi National French Honor Society. In her final year, Celeste travelled abroad to France for 3 weeks as part of the Cyclone Scholars program through the food science department. She entered the University of Tennessee in August 2022 to pursue a master's degree under the guidance of Dr. Toni Wang.