Polyphenol-Mediated Protein and Fat Structure Development in a Frozen Dessert Model

by

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A dissertation submitted in partial fulfillment of

the requirements for the degree of

Doctor of Philosophy

(Food Science)

at the

UNIVERSITY OF WISCONSIN-MADISON

2024

Date of final oral examination: 6/7/2024

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Abstract

Polyphenol sources are often incorporated into frozen desserts to enhance flavor, color, and nutritional content. When optimized, these ingredients can result in a product that melts slower than conventional ice cream. This is achieved by creating a matrix that resists collapse and impedes the flow of diluted serum. Although many have speculated about the underlying mechanism, it has not been fully investigated. This study aims to examine the impact of polyphenols on the meltdown behavior of ice cream and which structural components are the primary drivers of this unique effect.

This work is divided into four studies. The first study investigated the interaction of tannic acid (TA) in cream to understand how a pure polyphenol affects milk fat and protein. At higher concentrations of TA, a significant increase in viscosity was observed, leading to the formation of a gel-like product. Microscope imaging and particle size measurements revealed that the network of protein-mediated fat aggregates, facilitated by TA complexation, was responsible for this outcome. It was hypothesized that these aggregates could potentially reduce the melting rate in an ice cream system by increasing its viscosity and forming a network that restricts the flow of diluted serum during the melting process. This hypothesis inspired three additional ice cream studies.

The addition of various polyphenol sources to ice cream was investigated to determine their influence on melting and rheological properties, starting with TA. In a standard ice cream formula, the melting rate decreased at a high TA concentration, which was attributed to protein-mediated fat aggregates facilitated by TA, as previously observed in cream/TA samples. The resulting matrix also demonstrated an ability to inhibit ice recrystallization during a shelf-life study. The importance of fat was confirmed

when TA was added to two other formulas, including one with elevated fat and the other with elevated protein. The high fat formula with TA showed a decrease in melting rate, while the high protein formula with TA had little effect on melting behavior.

The following experiments shifted focus from pure polyphenols to other sources of varying phenolic content. Green tea and grapeseed extracts ice creams, which contain high levels of polyphenols (2.4-2.9%), were found to decrease the melting rate and produce a product with adequate shape retention. After four hours, only 10% of these extract-enriched products were lost during a drip-through melting test. Green tea extract was found to be an effective substitute for a common stabilizer blend in terms of slowing melting and inhibiting ice crystal growth by hindering the mobility of the serum phase.

In contrast, other polyphenol sources did not significantly impact the melting rate when added to ice cream. However, the crude extracts with moderate phenolic content (1.2-1.4% polyphenols) displayed a slightly lower melting rate and higher complex viscosity than the samples with freeze-dried powders and juice concentrate, which contained less than 0.05% polyphenols.

Overall, this study provided deeper knowledge into the effect of polyphenols on the structural component of ice cream that can impact meltdown. This information allows for the development of a novel product with unique melting properties that also improves product quality during storage.

Acknowledgment

I would like to express my deepest gratitude to my research professor, Dr. Rich Hartel, for his guidance throughout this journey. Your expertise and dedication have been instrumental in the completion of this dissertation. Thank you for instilling in me the mindset of a scientist. I also extend my heartfelt thanks to Dr. Brad Bolling for his support and assistance throughout this project.

I appreciate my other committee members, Drs. Audrey Girard, Mark Richards, and Arnoldo Lopez-Hernandez, for their insightful feedback and direction, which have significantly enhanced the quality of this work. A special thanks to the United States Department of Agriculture and SciMed for their financial support, which made this research possible.

To my parents and family, your unwavering love and patience have been a guiding light. Your belief in me has been a constant source of strength and motivation. I am forever grateful.

I appreciate the support and encouragement from my friends and peers in Babcock Hall. The countless memories will always be dear to my heart. I would also like to thank the staff of the Department of Food Science for helping me access the resources I needed.

Thank you to everyone who has invested in my success and future. Your support has been invaluable. I am excited about what the future holds. Dreams do come true.

Dedication

Momma and Dad,

Your unconditional love, countless sacrifices, and unwavering support have been the foundation of my success. Thank you for everything. We cross this finish line together.

“For nothing will be impossible with God.”

Luke 1:37 (ESV)

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1. Introduction

Ice cream is a complex system comprised of multiple phases, including ice, air, fat, and serum, which together determine its three-dimensional structure and properties such as melting behavior and sensory attributes. Due to its consumer acceptability, ice cream is frequently used to deliver health-promoting ingredients, such as polyphenols. Studies focused on enhancing the antioxidant capacity of ice cream have observed that polyphenol addition can reduce the melting rate. However, the mechanism behind this phenomenon has not been thoroughly investigated.

This study aims to understand the effect polyphenols have on the ice cream matrix and their potential to influence characteristics such as melting. Experiments were designed to investigate how polyphenols interact with ice cream components and what formulation considerations and polyphenol sources can decrease the melting rate. An additional aim was to uncover the necessary parameters to create a product with great shape retention during melting over an extended period of time, known as “no melt” or “no collapse” ice cream.

The first study examined the influence of tannic acid (TA) on the milk fat and proteins in cream, providing a model to evaluate polyphenols without the complexity of an ice cream matrix. The hypothesis was that TA will increase cream viscosity by promoting either partial coalescence and/or protein-mediated fat aggregation. The second study assessed the effect of TA concentration on a standard ice cream formula, relating previous findings about the fat aggregation mechanism to a potential reduction in melting rate. TA was also incorporated into a high fat and high protein ice cream to determine which component more significantly impacted meltdown behavior. The third

study evaluated high phenolic content extracts (green tea and grapeseed) for their potential to replace stabilizers and decrease the melting rate by comparing extract-enriched ice creams with and without stabilizers. The final study explored whether crude extracts, freeze-dried powders, and a juice concentrate could influence ice cream melting characteristics. This experiment aimed to replicate a previous study that asserted berry freeze-dried powders could reduce the melting rate of ice cream, highlighting a potential cost-effective option. Both the second and third studies included a shelf-life experiment that investigated the capacity of TA, green tea extract, and grapeseed to inhibit ice recrystallization. It was hypothesized that a matrix hindering serum mobility during melting could also prevent ice crystal growth during storage.

Ultimately, the goal of this research is to determine which polyphenols and polyphenol sources can achieve the desired melting characteristics in ice cream. This information will be beneficial for the formulation of novel frozen desserts and may also inspire the utilization of these mechanisms to enhance other dairy and non-dairy products.

2. Literature Review

2.1 Ice cream composition

Ice cream is a frozen food product that contains milk fat, milk-solids-non-fat (MNSF), sweeteners, emulsifiers, stabilizers, water, and flavoring agents. Following churning and freezing, these ingredients work together to create multiple phases of individual and partially-coalesced fat globules, air cells, ice crystals, and freeze-concentrated serum that impact overall quality (Goff & Hartel, 2013). Legal standards for ice cream, such as minimum percentages of milk fat, total solids, and overrun, are

established by the Code of Federal Regulation (21 CFR 135.110). Industry standards, on the other hand, are typically determined by quality, with “premium” or “super-premium” products having higher fat content, lower overrun, and stabilizers. The following sections provide an overview of the ingredients required to create this frozen desert.

2.1.1 Milk fat

The source of milk fat in ice cream is typically derived from cream, butter, or anhydrous milk fat. One of the unique properties of milk fat is its wide range of melting rates from milk fat triglycerides (Waldron et al., 2020). This component is crucial to the structure of the product and serves as a matrix that stabilizes many other phases. Milk fat influences the texture and flavor by contributing to creaminess, richness, and smoothness (Roland et al., 1999).

2.1.2 MSNF

MSNF contains milk proteins, lactose, minerals, vitamins, and enzymes. This ingredient contributes to various structural aspects, like freezing point depression, air cell stability, and serum phase viscosity, and sensory attributes, like flavor, body, and texture (Goff & Hartel, 2013). Casein and whey are the two primary classes of milk proteins. Casein constitutes 80% of the total milk protein content and is present in large colloidal micelles with calcium phosphate. Whey proteins are globular, more sensitive to heat, and account for the remaining 20% of the proteins in milk (Fox et al., 2015). The protein content in ice cream is generally around 3-4%.

2.1.3 Sweeteners

Common sweeteners include sucrose and corn syrup. Sweeteners not only provide sweetness but also have a significant impact on the ice phase. The concentration of sweeteners relative to water can affect the freezing point depression. A higher concentration can decrease the ice phase and increase the viscosity of the unfrozen serum, which can influence textural attributes (Miller-Livney & Hartel, 1997).

2.1.4 Stabilizers

In ice cream, hydrocolloid stabilizers impart much functionality. These polysaccharides are derived from many sources, such as gums, seaweed extracts, pectin, etc. The hydration of this ingredient is important since it swells and takes up space in the aqueous phase, leading to an increase in viscosity. Recrystallization of ice due to temperature abuse can be hindered by this increase in viscosity due to the freeze concentration of the polysaccharide in the serum phase (Bolliger et al., 2000). Some stabilizers, like locust bean gum, can create a gel-like network that can achieve this result (Goff et al., 1999). Stabilizers also contribute to creating a product that is uniform, smooth, and resistant to melting.

2.1.5 Emulsifiers

The function of an emulsifier is to create a stable blend of two liquids that do not combine naturally. However, in the context of ice cream, emulsifiers are generally utilized to destabilize the fat globules, leading to increased partial coalescence. Emulsifiers contribute to the smooth texture of ice cream by improving its whippability

and yielding a drier and stiffer consistency (Goff & Hartel, 2013). They also increase resistance to shrinkage and meltdown. Mono- and diglycerides and sorbitan esters, such as polysorbate 80 (PS80), are common ingredients used at usage levels of 0.1-0.2% and 0.02-0.04%, respectively. Different types of emulsifiers are combined to achieve a range of functionalities. Emulsifiers help reduce the interfacial tension between fat and water phases in ice cream mixes, causing the displacement of proteins from the surface of fat globules. This allows the fat globules to partially coalesce while being mixed during freezing. Mono- and diglycerides are more effective at stabilizing air bubbles, while sorbitan esters are better suited for promoting fat destabilization.

2.2 Ice cream processing

Process conditions also influence the structures formed in the final product. Blending, pasteurizing, homogenizing, cooling, and aging are key stages of the manufacturing process. The mix is then frozen using a batch or continuous freezer. Finally, the product is hardened in preparation for storage.

2.2.1 Mixing and pasteurization

Liquid ingredients, such as cream, milk, water, and syrups, are combined with dry ingredients, such as powdered dairy products, sugars, emulsifiers, and stabilizers. These ingredients are blended together using gentle agitation until the mixture reaches a temperature of 50℃. This preliminary heating process ensures the dry powders are dispersed and dissolved. Following this, the mix is pasteurized to minimize the bacterial load and inactive pathogens. Pasteurization not only helps eliminate harmful bacteria

but also aids in the hydration of proteins and stabilizers. One type of pasteurization commonly used is high temperature short time (HTST), which involves heating the mix to 80 °C (175 °F) for 25 seconds (Goff & Hartel, 2013).

2.2.2 Homogenization

Immediately after pasteurization, while the mixture is still hot, a two-stage homogenizer is employed to homogenize the mix. Homogenization results in the formation of small fat globules and increases the interfacial area available for proteins to adsorb to, thus yielding a uniform sample. This process also allows for the stability of the fat droplets during aging and results in a smoother final product (Goff, 1997).

2.2.3 Aging

After the ice cream is cooled to 10°C under gentle agitation, the product is aged in the refrigerator (~4°C) for 4-24 hours (Goff & Hartel, 2013). This aging process allows for the stabilizers in the formulation to fully hydrate and creates an environment conducive to shear-induced partial coalescence. This occurs because the solid fat in the globules returns to its crystalline state due to the lower temperature, and milk proteins at the interface are displaced due to the presence of emulsifiers.

2.2.4 Freezing

Two conventional methods for freezing ice cream mix are batch or continuous freezing. Continuous freezing utilizes pumps to continuously feed mix throughout the system, pressurized air is added, and then dispensed for packaging and storage. In a

batch freezer, a distinct amount of mix is added to the barrel of the machine, where it is whipped with a dasher, commonly at 100-200 rpm. Ice is scraped from the barrel surface by the dasher blades and incorporated into the mix. The freezing rate of the ice cream and the temperature of the barrel must be balanced to prevent the formation of large ice crystals and allow for proper air incorporation. Unlike continuous freezers, batch freezers tend to have higher draw temperatures and longer residence times in the freezing barrel (Goff & Hartel, 2013). However, they are optimal for small batches and laboratory use.

2.2.5 Hardening and storage

The product removed from the freezer is semi-solid and must be hardened to ensure future quality. To harden, ice cream is typically stored at -25 to -30℃ until the center reaches -18℃, around 12-24 hours (Goff & Hartel, 2013). Achieving the target temperature quickly is essential for preserving favorable microstructural components. While the sample is subjected to elevated temperatures, air cells can expand in size via various coalescence mechanisms (Chang & Hartel, 2002b). Furthermore, as the temperature decreases, an increase in ice crystal size can be expected as a greater amount of water is frozen (Cook & Hartel, 2010). Storage temperature of ice cream can encompass a large range (-9˚C to -26˚C), which affects the quality of the finished product (Ben-Yoseph & Hartel, 1998).

2.3 Ice Cream structural components and their effect on melting

The creation and arrangement of ice cream's structural components are influenced by its composition and manufacturing parameters. Ice cream consists of fat globules (individual and partially-coalesced), air cells, ice crystals, and a freeze-concentrated serum phase (Goff & Hartel, 2013). Changes in the size and distribution of any of these parameters can affect quality, rheological, sensory, and melting attributes. The following sections emphasize the significance of each component and illustrate its impact on the meltdown process.

2.3.1 Fat

The fat matrix of ice cream can contain a mixture of single, partially-coalesced, and fully-coalesced fat globules. The size of individual fat globules in ice cream mix can range from 0.5 to 3 μm (Goff & Hartel, 2013). The displacement of proteins by emulsifiers and the presence of solid fat promotes partial coalescence in ice cream. Partial coalescence occurs when the merger of fat globules is initiated but is hindered before complete coalescence. Typically, the collision of two droplets would be driven by Laplace pressure to assume the energetically favorable result of one large globule that minimizes the interfacial area. However, the internal crystalline fat and/or particles on the interfacial surface can prevent fat coalescence (Thiel et al., 2016). This process is caused by the shear of the dasher during freezing, which initiates collisions between fat globules. In commercial products, the extent of fat destabilization was seen to range from 2.6 to 55.3% (Warren & Hartel, 2014). Various process and formulation factors influence the extent of fat destabilization (Warren & Hartel, 2018). Emulsifier type and

concentration can significantly decrease the amount of adsorbed proteins, which in turn will increase fat destabilization (Bolliger et al., 2000; Muse & Hartel, 2004). The fat matrix also facilitates the incorporation and stabilization of air in the ice cream (Zhang & Goff, 2005).

Factors such as fat content, resulting fat aggregate size, and degree of fat destabilization all play a role in meltdown behavior. Liu et al. (2022) found that there is an optimal size of the fat aggregates to create a 3D network in the system. They also observed how increasing fat content can impact the shape retention of the residual foam after melting. Higher fat contents have also been shown to increase the viscosity of the resultant product (Li et al., 1997; Mostafavi, 2019), which may inadvertently slow the flow of melted ice cream.

2.3.2 Air cells

The presence of air in ice cream has an impact on its texture, stability, sensory properties, rheological behavior, and melting characteristics. Air may be incorporated by the shear of a dasher in a batch freezer or injected into the mix by a continuous freezer. The size distribution for air cells can be from a few microns up to >100 μm (Goff & Hartel, 2013). The measurement, overrun, is used to describe the volume of air present. As the dasher in the batch freezer incorporates air, the size of the air cells decreases.

According to Chang & Hartel (2002), this trend is unique to the combination of the whipping and freezing processes of ice cream, and not just the whipping of the mix under similar conditions. This is because the increase in serum/ice crystal viscosity during freezing leads to more force being applied to the system, which is sufficient to

overcome the interfacial tension of the air and serum phases. The air cell interface is stabilized by emulsifiers, absorbed proteins, and fat globules (both individual and partially-coalesced) (Goff et al., 1999). Formulation and temperature conditions control the susceptibility of the air cells to be disrupted by various mechanisms, such as coalescence, drainage, or disproportionation (Chang & Hartel, 2002b), especially during hardening.

Some researchers have observed that ice cream with high levels of overrun has a greater resistance to melting (Sakurai et al., 1996; Warren & Hartel, 2018). They hypothesize that air is an effective insulating medium against the heat needed to induce melting. VanWees et al. (2020) proposed that increased overrun samples generate a matrix of air cells, which, when combined with a network of coalesced fat, can impact the path that the diluted serum must follow in order to drain, resulting in a slower melting profile. Wu et al. (2019) found that overrun only significantly affected the melting rate of ice cream when there were no stabilizers present.

2.3.3 Ice crystals

Ice crystals are created during the freezing process and continue to grow and develop during the hardening and storage stages. Ice crystals can be found in a range of sizes (1-50 μm), but the average size is between 35-45 μm (Goff & Hartel, 2013). Ice crystal distributions can impact the smoothness and hardness of the resultant product (Muse & Hartel, 2004). Sensory testing has revealed that samples with ice crystals over 50 μm are perceived to have a coarse texture (Arbuckle, 1966). Various formulation and processing parameters can be optimized to reduce this quality defect.

The life cycle of an ice crystal is comprised of three stages: nucleation, growth, and recrystallization (Goff & Hartel, 2013). Dendritic ice crystals are formed at the freezer barrel surface because of the favorable cooling conditions, creating nuclei. These small crystals are scraped off the barrel by the dasher blades and are incorporated into the warmer mix. This leads to some partial melting, but crystal growth can be influenced by temperature fluctuations in the mix. When the final product is removed from the freezer, small crystals are present. They can increase in size as the product hardens and the core temperature decreases.

Donhowe et al. (1991) observed a reduction in average ice crystal size as total solids increased by decreasing the amount of water in the mix. Sweeteners, such as lactose, mono-and disaccharides, and milk salts, affect the solute concentration, which depresses the freezing point and the resulting percentage of water that is frozen (Goff & Hartel, 2013). Depending on ingredients and draw temperatures, about half of the water present is frozen after being removed from the freezer and approximately 75% after the hardening step (Cook & Hartel, 2010). As for process parameters, increasing the dasher speed has been shown to aid the creation of larger ice crystals in the end product due to the excess of frictional energy displaced into the freezing mix, increasing the temperature and melting smaller crystals (Russell et al., 1999). Recrystallization generally refers to modifications in the size and distribution of ice crystals, which are typically attributed to the melting of small crystals and their subsequent transformation into larger ones. Mechanisms of recrystallization include: Ostwald ripening (small crystals melting into larger ones), isomass rounding (reduction of rough surfaces), and accretion (bridging between two ice crystals) (Hartel, 1998). The primary cause of

recrystallization is improper storage from temperature fluctuations, normally attributed to home freezers and distribution systems. Stabilizers are commonly used to stop the development of a coarse texture due to storage by increasing the viscosity and reducing water mobility (Huynh et al., 2014).

Ice crystal size and volume can influence the melting properties of ice cream. Muse & Hartel (2004) found that samples with larger ice crystals had higher melting rates. Smaller ice crystals can encumber the flow of unfrozen serum, by creating obstacles and hindering drainage. The total amount of ice in the system, controlled by the total solids and water content, also play a role. Ice cream with higher total solids tends to melt quicker (Li et al., 1997) from the drop in freezing point depression. Less frozen water increases the volume of unfrozen phase available to flow while eliminating the tortuous path created by an abundance of small ice crystals. In order to understand how various components work together, Liu et al. (2023) developed a model ice cream that featured ice crystals as the predominant structure with a limited degree of partial coalescence. They found that when this system was paired with high levels of overrun, the sample melted faster at the beginning. However, after the connectivity between ice crystals was lost from the displaced serum phase, stabilized air bubbles hindered the melting rate at the later stages of the process.

2.3.4 Serum phase

The serum phase contains components of the mix that can be dissolved, such as sweeteners, proteins, and stabilizers. As the temperature decreases during freezing, the ice phase increases, and these ingredients freeze concentrate. This creates a liquid

unfrozen continuous phase that surrounds all other microstructure components (ice crystals, air cells, and fat globules), which becomes more viscous as more ice freezes. Freezing point depression, a parameter calculated using the solute concentration of mono-disaccharides and salts in the mix, influences the viscosity of the serum phase by regulating the amount of ice at a given temperature (Goff & Hartel, 2013). The stabilizers and proteins in the serum phase can also increase the viscosity of the mix. (Alvarez et al., 2005; Amador et al., 2017). By limiting the mobility of the system, serum phase viscosity can inhibit ice recrystallization and improve sensory qualities such as iciness.

The viscosity and composition of the serum phase are important to melting behavior in ice cream. Researchers have found that ice creams made with stabilizers have higher serum phase viscosity, which can slow the drainage of the melted fraction of ice cream, decreasing the melting rate.(Amador et al., 2017; Wu et al., 2019). Proteins in this phase can also affect the viscosity. According to VanWees (2024), milk protein concentrate and sodium caseinate resulted in a greater apparent viscosity in ice cream than whey proteins. Despite this relationship, the ice cream fortified with whey proteins showed more melting resistance than the other variables. This may be related to whey proteins creating a thinner protein film than caseins at the oil/water interface, making the product more susceptible to partial coalescence (Daw & Hartel, 2015).

2.4 Ice Cream Meltdown Process

Melting characteristics of ice cream are investigated to ensure optimal quality and sensory attributes, like balancing melting resistance with a desirable consumption

experience and mouthfeel. As previously discussed, many formulation parameters and structural components can affect how an ice cream melts. Meltdown tests at room temperature are commonly used to categorize this behavior. For testing, a sample is placed on a mesh screen over a beaker on a scale that sends outputs to a computer. The computer collects the amount of product that has dripped through the mesh over time. An example output is shown in **Figure 2.1**. The meltdown process can be divided into three stages.

Lag phase: Heat penetrates the product as the temperature rises above its freezing point. The ice melts and begins to dilute the freeze-concentrated serum phase, causing it to flow. This phase is defined by the induction time, the time the first drop of ice cream falls into the beaker, signifying the beginning of the next phase.

Melting phase: The flow of the ice cream increases as more diluted serum escapes the system due to the force of gravity. The rate of drainage can depend on the structural barriers to flow that the serum must overcome. The extent of fat destabilization and air volume/stability can create a tortuous path for the melted liquid. This is considered the linear portion of the drip-through versus time graph and from which the melting rate is calculated.

Stationary phase: As the serum phase flows through the mesh, the partially destabilized fat and air cells (that do not get caught in the flow into the beaker) begin to stack to create a remnant foam. Once there is no more serum dripping through, the graph enters the stationary phase at an equilibrium point with a horizontal line. This phase helps identify the complete melting time (from the x-axis) and the residual weight left on the screen (from the y-axis).

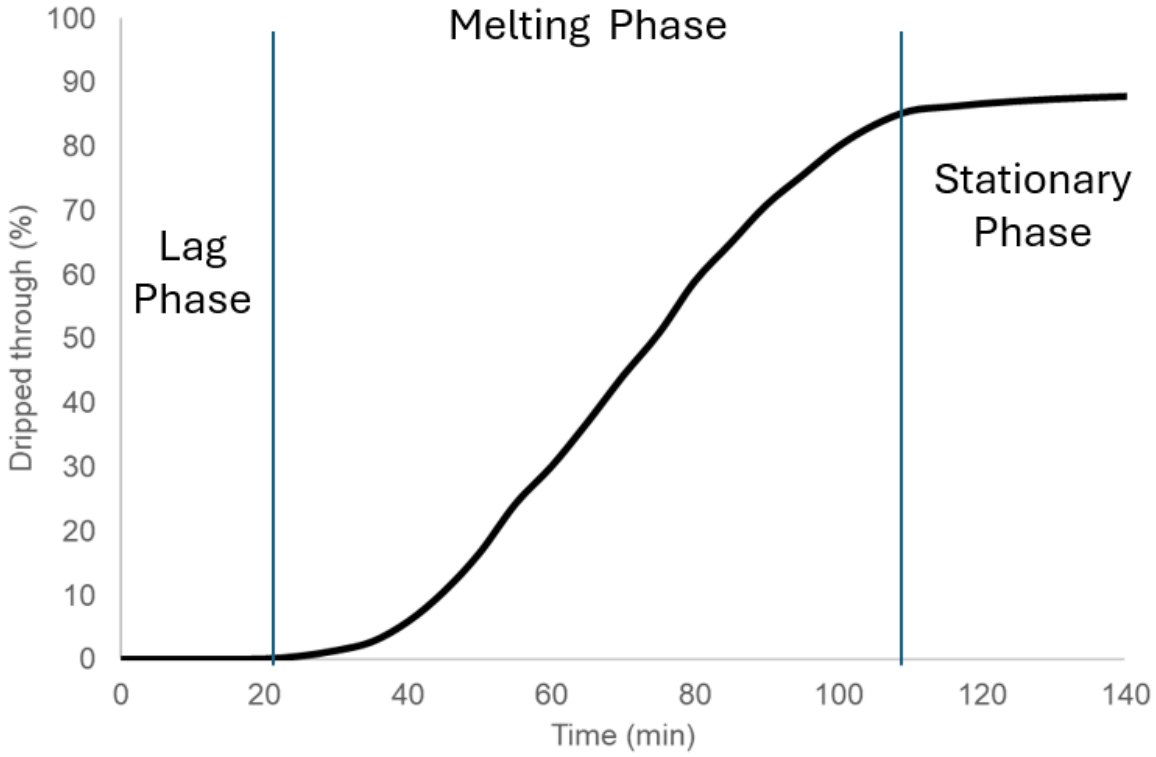

Figure 2.1 Example melting profile of a 12% fat/ 3% protein ice cream

2.4.1 "No-melt" ice cream

Scientists at Japan's Biotherapy Development Research Center have used the polyphenols in strawberry extract to influence the melting rate of their "no-melt" ice cream, making their products more resistant to melting and shape deformation for extended amounts of time at elevated temperatures (Bilbao-Sainz, Sinrod, et al., 2019). They observed that adding this extract instantaneously increased the viscosity of dairy cream. The creator, Tomihisa Ota, stated that, "Polyphenol liquid has properties to make it difficult for water and oil to separate so that a popsicle containing it will be able to retain the original shape of the cream for a longer time than usual and be hard to melt" (Huen, 2018). The creator patented the use of strawberry extract for this product.

This innovation spotlighted the use of polyphenol sources in ice cream systems. Common ice cream goes through the complete meltdown process quickly, within two hours. Goff & Hartel (2013) define an ice cream that does not melt as a sample that maintains its shape after 15 minutes. Anecdotally, many consumers of this “no-melt” ice cream have reported that this product far exceeds these parameters. “No collapse” is a more fitting description of the behavior of this product since the ice eventually melts but has enhanced shape retention. This highlights the need for a modified definition that encompasses this extreme behavior.

2.5 Ice cream rheology

Ice cream is categorized as a semi-solid food, having elastic and viscous characteristics. Rheology is used to categorize the structure and flow behavior of its complex microstructure. This information can help design optimal formulas when correlated with desirable quality and sensory attributes (Whaley et al., 2019).

2.5.1 Complex Viscosity

Viscosity measures a material’s ability to resist flow and deformation by shear stress (Tan, 2019). Small amplitude oscillatory shear (SAOS) can be used to accurately study viscosity and other rheological properties in ice cream due to its semisolid properties (Yu & Gunasekaran, 2001). This method limits the magnitude of deformation applied to the sample, ensuring that the underlying structure remains relatively unchanged (Whaley et al., 2019). To determine the optimal strain, the linear viscoelastic region is established through an amplitude sweep of the material. When using SAOS,

complex viscosity is calculated, as it represents the total resistance to flow as a function of frequency, unlike steady-state viscosity, which measures resistance to flow as a function of shear stress.

2.5.2 Thixotropy

Thixotropy is a time-dependent shear thinning behavior, where the viscosity decreases over time at a constant shear rate to a steady state viscosity. The viscosity is recovered when flow is stopped. In a colloidal system, a colloidal aggregate is often the source of a sample's viscoelastic properties. Particles can align to create a gel-like system but disperse into smaller structures when shear is applied. When a new shear rate is applied suddenly to a material, three phases occur. First, there is an instantaneous response, resulting in the realignment of the aggregates. Second, the extent of aggregate deformation is facilitated by the relaxation time. Finally, the structure of the aggregates adapts to the new shear rate. The last two parts happen simultaneously, which then translates into stress (Pa) measured (Labanda & Llorens, 2006).

Ice cream has thixotropic properties, like many other colloidal systems (Goff & Hartel, 2013). This highlights ice cream's internal structures and the physical energy needed to overcome the product's resistance to breakdown and rebuild those structures. At 0°C, ice cream is similar to a weak gel from the presence of proteins, fat globules and foam structure despite the melted ice (Goff & Hartel, 2013). Thixotropy can be evaluated at this temperature to analyze these structures without an ice phase (Freire, 2020). Thixotropy is measured using the hysteresis area between the up and

down curves of the material's stress response when shear is linearly increased and decreased over time (Labanda & Llorens, 2006). Strong thixotropic fluids are expected to result in a larger hysteresis area.

2.6 Polyphenols

Polyphenols are secondary metabolites of plants characterized by the presence of at least one aromatic ring with one or more hydroxyl and functional subunits (Skinner et al., 2013). They have been widely researched for their benefits against diseases that are linked to oxidative stress due to their antioxidant properties (Manach et al., 2004). In the food industry, these compounds have many uses, including preservatives, colorants, and nutritional enrichment (Bravo, 1998). Flavonoids, tocopherols, phenolic acids, stilbenes, and tannins are a few of the polyphenolic classes (Skinner et al., 2013). When adding polyphenols to foods, it is important to consider their bioavailability and how they interact with macromolecules (Jakobek, 2015). Researchers have shown interest in using ice cream as a vehicle for these compounds due to matrix compatibility, high consumer acceptability, and increased demand for healthier, functional foods (Soukoulis et al., 2014).

2.6.1 Source and Extracts

Many researchers have added various plant sources to ice cream, including waste products (Hwang et al., 2009; Sharma et al., 2015), unpalatable plants (Yuksel, 2015), and polyphenol-rich commodities (Çakmakçı et al., 2016; Erkaya et al., 2012; Naeem et al., 2019). Different source preparations can have different effects on the ice

cream matrix. They can add additional water, sugar, and other complex carbohydrates such as pectin and fiber. Therefore, it is important to understand how polyphenols are impacted due to processing. A simple process, like peeling, can greatly decrease the polyphenol content of a food source because of the higher concentrations found in that fraction. (Manach et al., 2004). Enzymatic processes initiated by chopping and cutting can lead to polyphenol degradation and browning. Thermal processing is a common method used to preserve polyphenol-rich materials despite their sensitivity to heat (Debelo et al., 2020). Balancing temperature with duration is important to ensure a quality end product with high antioxidant activity (Bustos et al., 2018). In a study searching for the optimal processing method for incorporating persimmon peels as a functional ingredient in ice cream, freeze-drying was shown to be an effective method of polyphenol preservation, when compared to other methods like oven, vacuum oven, and microwave drying (Yosefiyan et al., 2024). Due to the small quantity of polyphenols that can be found in a given source, extraction is a tool to help concentrate various phenolic profiles for utilization. However, many factors can impact its efficacy like solvent type, solid:solvent ratio, temperature, and particle size (Sridhar et al., 2021)

2.6.2 Polyphenols in ice cream

Although many studies have added polyphenol-rich ingredients to ice cream to enhance its color, taste, and nutritional value, few have examined how these sources can affect structural, melting, and rheological characteristics.

2.6.2.1 Effect of polyphenols on melting rate of ice cream

Many researchers have observed that incorporating polyphenol-rich sources into ice cream results in a reduction of the melting rate. Many articles attribute the mechanism of this trend to the ability of polyphenol source components to absorb water (Erkaya et al., 2012; Kavaz et al., 2016; Salem et al., 2014; Yuksel, 2015). Some credit the presence of polysaccharides, like fiber (Karaman et al., 2014) and pectin (Bilbao-Sainz, Sinrod, et al., 2019) that are present in the polyphenol source. An increase in mix viscosity is a common trend among polyphenol-enriched ice creams (Erkaya et al., 2012; Ürkek et al., 2019; Yuksel, 2015) and forms the basis for many hypotheses regarding the melting rate of these products. Goraya and Bajwa (2015) saw that a range of different preparations of Indian gooseberry (shreds, pulp, candy, powder, and preserves) all reduced the melting rate. The source preparations that caused an increase in water content, like purees and pastes, decreased the viscosity, which did not significantly prolong the melting time (Çakmakçı et al., 2016; Karaman et al., 2014).

A few studies (Naeem et al., 2019; Pundhir et al., 2018) attribute this decrease in melting rate to an increase in the fat destabilization index with increasing polyphenol content, causing a matrix of destabilized fat to hinder drainage. This test measures the percent increase in the absorbance of the melted ice cream relative to the ice cream mix. While Hwang et al. (2009) also observed an increase in fat particle size along with this trend, no study confirmed their results with microscope images. As a result, the true cause of the increase in fat globule size remains unknown.

2.6.3 Potential phenolic interactions with ice cream macromolecules

Various macromolecular structures contribute to the development of a quality ice cream product. Certain polyphenols can interact with fats, proteins, and carbohydrates in distinct ways. When manufacturers incorporate polyphenols into ice cream, it is crucial to comprehend how their properties influence the components of frozen desserts.

2.6.3.1 Protein

Polyphenols can complex with various proteins, including both classes of milk proteins, casein and whey. These reactions are mostly facilitated by non-covalent hydrophobic interactions and hydrogen bonding working together (Yildirim-Elikoglu & Erdem, 2018). Several factors affect the prevalence and efficacy of this complexation. The proline content of casein (Arts et al., 2002) and the pH-dependent configurations of whey proteins (Riihimäki et al., 2008) allow them to react favorably with various polyphenols. According to Hasni et al. (2011), tea polyphenols form stronger complexes with β-casein because of their hydrophobic nature compared to α-casein, although this trend is not widely accepted (Bohin et al., 2014). Whey proteins have been shown to significantly change their secondary structure as phenolic acid content increases (Zhang et al., 2014) and have better binding affinity when heated (Shpigelman et al., 2010). The structure and type of polyphenol also play a role in this reaction, such as the presence of hydroxylation, hydrogenation, glycosylation, and methylation in the molecule (Ozdal et al., 2013). Both β-casein and β-lactoglobulin have been shown to

favorably bind with epigallocatechin gallate (EGCG) over other tea polyphenols due to the large quantities of -OH groups present (Hasni et al., 2011; Kanakis et al., 2011)

The binding process (depicted in **Figure 2.2**) of these complexes, specifically proline-rich salivary proteins and tannic acid, occurs in three stages (Charlton et al. 2002). The first stage includes the binding of a single, soluble peptide molecule with multiple polyphenols at the available sites. The second stage requires the presence of another polyphenol-coated peptide and a weak intermolecular interaction to create a new, insoluble complex. The last stage is the spontaneous aggregation of these insoluble complexes to create larger aggregates. The formation of these large aggregates was also observed by Han et al. (2011) in β-casein and tannic acid samples.

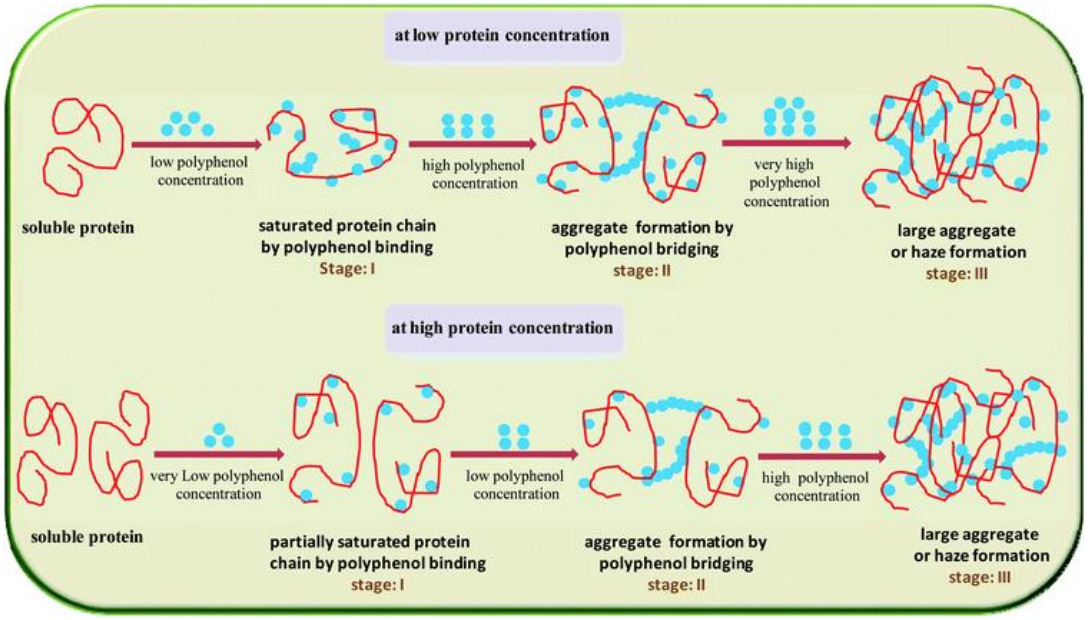

Figure 2.2 Proposed mechanisms of interaction between proline-rich human salivary protein and polyphenols. From Charlton et al. (2002)

The ratio of polyphenols to protein can affect this binding mechanism. The above explanation is based on a low protein concentration system, where the polyphenol content is large enough to saturate the peptide. However, at higher protein contents, bridges between proteins can form before polyphenol saturation occurs, allowing for lower phenolic concentrations (Pascal et al., 2007; Yildirim-Elikoglu & Erdem, 2018). Complexation can also be optimized with a pH environment close to the isoelectric point due to the reduction in electrostatic forces.

2.6.3.2 Fat

Polyphenols are known to interact with lipids by preventing oxidation and promoting encapsulation for enhanced delivery, stability, and bioavailability (Lu et al., 2016; von Staszewski et al., 2014). However, in an ice cream system, the mechanism of interest is how polyphenols interact in a protein-stabilized emulsion, which is the initial state of the ice cream mix. EGCG has been shown to reach up to 90% absorption (of total added polyphenol to the system) to a sodium caseinate stabilized soybean oil interface by binding to the protein (Sabouri et al., 2015). Certain complexes possess better emulsifying capacity compared to the proteins themselves and can contribute to enhanced emulsion stability (strength to resist phase separation) (Li et al., 2020). When rutin was incorporated into soy protein-stabilized emulsion, it not only formed complexes with the protein but also decreased interfacial tension and competitively displaced proteins at the oil interface. This process resulted in improved physical and oxidative stability for the emulsion (Atarés et al., 2012). Fat droplets can also aggregate into clusters at elevated concentrations of polyphenols, as seen by Fuhrmann et al. (2019).

Large fat clusters (>100 μm) were observed in a whey protein-stabilized emulsion containing high amounts of grape seed extract (see **Figure 2.3**). Polyphenol bridging between proteins at the interface was credited as the mechanism, leading to an increase in the viscosity of the sample. Bock et al. (2022) also observed this bridging of whey proteins and polyphenols at the fat globule interface. This work highlighted the importance of pH environment, protein/polyphenol ratio and phenol ring content of the phenolic acids on the efficacy of this reaction.

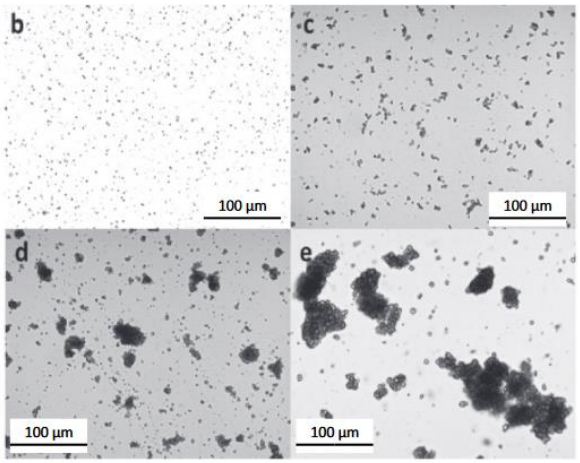

Figure 2.3 Micrographs of whey protein isolate/ sunflower oil emulsions with grape seed extract. The images show a non-clustered o/w emulsion (b), an emulsion clustered using 25% grape seed extract (c), an emulsion clustered using 50% grape seed extract (d), and an emulsion clustered using 75% grape seed extract (e). From Fuhrmann et al. (2019)

2.6.3.3 Carbohydrates

Sugars and polysaccharide stabilizers are crucial components in the production of ice cream and are known to interact with phenolic compounds. Although polyphenols, such as gallotannins, have the ability to bind to sugars, they exhibit significantly lower binding affinities to sugars than to proteins or phospholipids. This lower binding affinity is attributed to hydrogen bonding being the primary driver of this interaction (He et al.,

2006). It has been demonstrated that sugars can disrupt protein-polyphenol complexes. In a wine model, increasing amounts of glucose, fructose, and sucrose were shown to inhibit tannin/bovine serum albumin complexes. Two mechanisms have been proposed to explain these results: either the sugars prevent protein-polyphenol interactions, or the sugars cause the complexes to be more soluble (Harbertson et al., 2013). The use of xanthan gum, pectin, and gum arabic can also interrupt tannin-bovine serum albumin aggregates. Mateus et al. (2004) discovered that the structure of polysaccharides and polyphenols played a significant role in these results. Xanthan gum was the most effective in disrupting these complexes due to its high polarity. The study also found that grapeseed procyanidin fractions with low degrees of polymerization were more prone to being disrupted by these gums. Unlike polyphenol interactions with fat and/or protein that form aggregate systems, sugars and gums can inhibit and solubilize these complexes. The type and usage level of these ingredients must be understood in order to effectively develop polyphenol-enriched ice creams.

2.7 Summary and direction of research

The structural components of ice cream work together to influence the meltdown behavior. Various polyphenols have been shown to interact with many molecules that make up these structural elements. Understanding polyphenol interactions and their impact on an ice cream matrix is crucial to discovering new ways to utilize phenolic sources as functional ingredients and enhance properties, such as melting rate, to create innovative, high quality products.

3. Materials and Methods

These experiments were designed to understand the impact of polyphenols on ice cream structure and characteristics. The analytical methods, such as pH, rheology, microscopy, and particle size, used to characterize a cream/TA system were discussed in Section 3.1. Sections 3.2, 3.3, and 3.4 outlined meltdown testing and rheological measurements employed to investigate the effects of different polyphenol sources on various ice cream formulations. Shelf-life studies in Sections 3.2 and 3.3 were used to examine the impact of polyphenols on the ice crystal distribution of enriched samples during storage.

3.1 Effect of Tannic Acid in Dairy Cream

In this study, tannic acid (TA) was added to commercial dairy cream at three different concentrations. The aim was to categorize the rheological properties and understand the underlying mechanism for formation of the fat clusters found in the system.

3.1.1 Materials

Pasteurized dairy cream (~37% fat) was purchased from Sassy Cow Creamery (Columbus, WI, USA). TA (98% purity) was acquired by Cayman Chemical (Ann Arbor, MI, USA). Sodium dodecyl sulfate (SDS) and HCl were procured from Sigma Aldrich, Inc. (St. Louis, MO, USA). Ethylenediaminetetraacetic acid (EDTA) was purchased from IBI Scientific (Peosta, IA, USA). Citric Acid was purchased from Tate and Lyle (London, UK). Deionized (D.I.) water was used throughout all experiments.

3.1.2 Sample Preparation

TA was dissolved into D.I. water to create 7.5%, 15%, and 30% (wt/wt) solutions. TA solutions were then loaded into a syringe for injection into the cream. Cream was stirred with a paddle attachment at 180 rpm, and the appropriate amount of TA solution was injected evenly. The samples were mixed for an additional 20 s to ensure homogeneity. The final TA concentrations of the cream samples were 0.75%, 1.5%, and 3% (wt/wt) (**Table 3.1**). The same amount of solution was added to the cream sample to ensure similar dilutions regardless of TA level. The samples were stored at 4°C for 24 h prior to analysis. Each sample was made in triplicate.

Table 3.1 Composition of cream samples at different tannic acid (TA) concentrations

TA Concentration (%)	TA powder (g)	Water (g)	Cream (g)
0	0	5	45
0.75	0.375	4.625	45
1.5	0.75	4.25	45
3	1.5	3.5	45

3.1.3 Analyses

3.1.3.1 Rheology

A DHR-2 rheometer (TA instruments, Delaware, USA) with parallel plate geometry (dia. 40 mm) was used to measure the complex viscosity of the cream/TA samples. Plain cream (0% TA) was not measured due to its inability to produce consistent, sinusoidal torque and displacement waveforms. This sample may have been out of the range of this geometry. The 0.75% TA sample, which was a viscous liquid, was deposited on the plate using a 1 mL syringe. Small disks of the thicker, gelled, 1.5% and 3% (wt/wt) cream/TA samples were cut from a cylindrical mold and placed in

the center of the Peltier plate for analysis. The samples were measured at 4°C using a recirculating chiller (Thermos Cube, Solid State Cooling System, Wappingers Falls, NY, USA) connected to the bottom plate. The geometry was moved vertically to reach the geometry gap (1 mm) and equilibrated at 4 °C for 90 s. An amplitude sweep test to confirm the linear viscoelastic region (LVR) was performed at a strain range of 0.01-3% and 1 Hz frequency. After the strain (0.019%) was properly adjusted for each sample, they were oscillated for 60 s, and the complex viscosity was measured in triplicate.

3.1.3.2 pH

The pH was measured with a FiveEasy Plus pH/mV meter with InLab® Viscous Pro-ISM probe (Mettler Toledo, Hampton, Schwerzenbach, Switzerland). The electrode was calibrated at pH four and seven. Samples were held at ~25°C for 1 h before measuring pH in triplicate. To test the effect of pH on cream, solutions of citric acid and HCl at pH 3 and 3.5 were made and then added to cream at the same concentration as the tannic acid solutions. After the samples were stored at 4°C for 24 h, their pH was measured using the parameters above. Each sample was made and measured in triplicate.

3.1.3.3 Particle size distribution

Particle size distributions of cream/TA samples (1/10 dilution) were measured in triplicate using laser light scattering (Malvern Mastersizer 2000, Malvern Instruments Ltd., Worcestershire, UK). A refractive index of milkfat, 1.46, was used for these measurements. D.I. water was used as the dispersant with a refractive index of 1.33.

Diluted samples were added dropwise into the attached Hydro 2000S liquid sampler until a laser obscuration value of 13–15% was reached. Mean particle size distributions and $d_{4,3}$, the weight-average mean diameters of the particles, were calculated by the Mastersizer.

3.1.3.4 Optical light microscopy

Cream/TA samples were lightly mixed with D.I. water to create a 1:10 dilution. One drop of diluted sample was placed on a glass slide and covered with a cover slip. Samples were imaged at 200x magnification with a Nikon Eclipse FN1 optical microscope (Nikon Instruments Inc., Melville, NY, USA) and a Nikon Digital Sight DS-U3 camera control unit attached (ver. 1). NIS-Elements D Imaging Software (ver. 4) was used to capture images.

3.1.3.5 SDS and EDTA treatments

The preparation method as described in Méndez-Velasco and Goff (2012) was used to differentiate between the presence of partial coalescence or protein-mediated aggregation in these systems. A SDS solution (4% wt/wt) was prepared using D.I. water, and a Na_2H_2EDTA solution (3.7% wt/wt) was prepared using 0.1 M phosphate buffer (pH 6.8) as a solvent. The cream/TA samples were diluted at 1:1 ratio with each solution separately, then compared with a sample diluted with only D.I. water. Treated samples were mixed lightly for 2 min, then stored at 4°C for 2 h. Optical microscopy and particle size measurements were conducted in triplicate for each treated sample.

3.1.3.6 Statistical Analysis

Analysis of variance (one-way ANOVA) and Tukey's honest significant difference (HSD) test were used to compare means of the data taken. Analysis was performed using JMP® Pro version 15.0.0 (SAS Institute Inc., Cary, NC, USA). The level of significance was determined at $p < 0.05$.

3.2 Effect of Tannic Acid on Ice Cream

In this study, TA was added to three different ice cream formulations to examine its effects on melting behavior and structural components of the system.

3.2.1 Materials

Pasteurized dairy cream was purchased from Sassy Cow Creamery (Columbus, WI, USA). TA was purchased from Cayman Chemical (Ann Arbor, MI, USA). Sugar (United Sugars, Edina, MN, USA) and nonfat dry milk (NFDM) (Dairy America, Fresno, CA, USA) were acquired from the University of Wisconsin-Madison's dairy plant (Madison, WI, USA). A blend of stabilizers (locust bean gum, guar gum, and carrageenan) from Germantown Premium I.C and mono- and diglycerides (MDG) from Grinsted® were purchased from Danisco USA (New Century, KS, USA).

3.2.2 Experimental design

A standard ice cream formula (12% fat /3% protein) was evaluated at five different TA concentrations (0-2.5%). In addition, high fat (15% fat/ 3% protein) and high protein (12% fat/5% protein) formulas were used to investigate the effects of elevated

protein and fat on the system at 0% and 2.5% TA. The ingredients of each formula can be found in **Table 3.2**. All formulas had a freezing point depression between -2.6°C and -2.5°C, optimized using the sugar content. Each formula was made in duplicate.

Table 3.2 Composition of the standard (12% fat /3% protein), high fat (15% fat/ 3% protein), and high protein (12% fat/5% protein) ice cream samples at different tannic acid (TA) concentrations.

	Standard						High Protein		High Fat	
Ingredients (%)										
Cream	32	32	32	32	32	32	32	32	40.1	40.1
Nonfat dry milk	6.35	6.35	6.35	6.35	6.35	6.35	12.2	12.2	6.35	6.35
Sugar	17.7	17.6	17.45	17.3	17.15	17	13.1	12.4	16.9	16.2
Mono- and diglycerides	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12
Stabilizers	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Water	33.63	33.73	33.88	34.03	34.18	34.33	32.38	33.08	26.33	27.03
TA solutions (%)										
TA	0	0.5	1	1.5	2	2.5	0	2.5	0	2.5
Water	10	9.5	9	8.5	8	7.5	10	7.5	10	7.5

3.2.3 Ice cream production

All ice cream mix ingredients (excluding the TA solution) were mixed and heated to 85ºC in a jacketed mixer (Stephan Food Processing Machinery, Hamelin, Germany). The mix went through a two-stage homogenizer at 2500 psi (Manton-Gaulin MFG, Co. Inc., Everett, MA, USA). The homogenized ice cream mix was returned to the jacketed mixer and cooled to 10˚C. TA and water were mixed to make a solution. This solution was blended into the ice cream mix with a Waring Commercial WSB50 Big Stix Heavy Duty Immersion Blender (Waring Commercial, Stamford, CT, USA). Following TA addition, the mix was aged at 4˚C for 24 hrs. A batch freezer (Stoelting VB120-109A Telme Batch Freezer, The Vollrath Company, Kiel, WI, USA) was used to freeze 4 kg of

ice cream mix per formula, where the product was frozen to -6 ˚C. The finished samples were placed in sample cups, then hardened and stored in a walk-in freezer (-25˚C).

3.2.4 Analyses

3.2.4.1 pH

The pH was measured with a FiveEasy Plus pH/mV meter with InLab® Viscous Pro-ISM probe (Mettler Toledo, Hampton, Schwerzenbach, Switzerland). The electrode was calibrated at pH two, four, and seven. Mix samples were held at approximately 22°C for 1 h before measuring pH in triplicate.

3.2.4.2 Meltdown Test

The melting characteristics of the ice cream samples were analyzed using a drip through test adapted from Bolliger et al. (2000). The samples were tempered in a -20°C freezer for 12 hrs prior to testing. To begin the test, the sample's weight was recorded, then the sample cup was carefully removed and weighed. The ice cream was placed on a wire mesh screen suspended above a beaker. The beaker collected the dripped-through fraction of the sample while on top of a scale that recorded the weight every 5 mins. Testing was conducted for 180 mins at ambient temperatures (~22°C). Each sample was measured in triplicate.

The percentage of sample that dripped through was plotted against time to create a melting profile. The melting rate was calculated from the linear region of the graph. Induction time was calculated from the first scale measurement >0 g. The residual weight on the screen (%) was determined by the following equation:

$$Residual\ weight\ on\ the\ screen\ (\%) = 100 - total\ dripped\ through\ sample\ (\%)$$

where the total dripped through sample is taken from the stationary phase of the melting profile or the maximum amount collected after testing is concluded.

3.2.4.3 Rheology

To prep the rheology samples, ice cream was placed into custom molds directly after freezing. This mold shaped the ice cream into thin cylindrical coins (25mm inner diameter x 2.5mm height). After hardening, they were demolded, placed in individual sample cups, and stored at -25˚C until testing. A DHR-2 rheometer (TA instruments, Delaware, USA) with crosshatched parallel plate geometry (dia. 25 mm) was used for measurement. The samples were measured at 0°C using a recirculating chiller (Thermos Cube, Solid State Cooling System, Wappingers Falls, NY, USA) connected to the bottom plate. An amplitude sweep test to confirm the linear viscoelastic region (LVR) was performed at a strain range of 0.01-3% and 1 Hz frequency. After the strain (0.1%) was properly adjusted for each sample, they were oscillated for 10 mins, and the complex viscosity was measured in triplicate. Next, a flow ramp was conducted. The shear rate was increased from 0.001 to 100 s^{-1}, capturing 10 points per decade for 10

minutes. This was followed by decreasing the shear rate from 100 to 0.001 s^{-1} for an additional 10 minutes. The TRIOS software (2019 TA Instruments–Waters LLC, New Castle, USA) calculated the area between the up and down shear stress curves using the thixotropy function. Each sample was evaluated in triplicate.

3.2.4.4 Overrun

Overrun was measured by weighing the ice cream mix (prior to freezing) and the final frozen product in a fixed-volume container. The value was calculated with the following equation in triplicate:

$$Overrun(\%) = \frac{ice\ cream\ mix\ (g) - frozen\ ice\ cream\ (g)}{frozen\ ice\ cream\ (g)} \times 100$$

3.2.4.5 Optical light microscopy

Melted ice cream samples were lightly mixed with D.I. water to create a 1:10 dilution. One drop of diluted sample was placed on a glass slide and covered with a cover slip. Samples were imaged at 200x magnification with a Nikon Eclipse FN1 optical microscope (Nikon Instruments Inc., Melville, NY, USA) and a Nikon Digital Sight DS-U3 camera control unit attached (ver. 1). NIS-Elements D Imaging Software (ver. 4) was used to capture images.

3.2.4.6 Shelf-life storage

Samples from each formula were put in a refrigerated dipping cabinet for the shelf-life study. The temperature of the cabinet oscillated from -9.4°C to -14.3°C twice

an hour. Samples were removed at 2 and 4 weeks of storage for ice crystal analysis in triplicate.

3.2.4.7 Ice crystal size analysis

This methodology of observing ice crystals using optical microscopy in a refrigerated glovebox is outlined by Donhowe et al. (1991). Samples are tempered at -20°C for 12 hrs before being placed in the glovebox set at -15°C and held for an additional 30 minutes prior to testing. While working in the glovebox, a small piece of sample is placed on a chilled microscope slide. In order to disperse the ice crystals, a chilled solvent of pentanol and kerosene (1:1 ratio) is added to the sample, covered with a cover slide, and gently agitated with tweezers. Prepped samples are loaded on an optical microscope (FX-35DX, Nikon, Inc., Garden City, NY, USA). Around 20-30 images were captured to ensure each sample had at least 300 representative ice crystals for analysis. Each ice crystal was traced in Microsoft Paint and measured with Image Pro Plus (Version 7.0 Media Cybernetics 2009, Rockville, MD). Measurements were performed in triplicate.

3.2.4.8 Statistical Analysis

Analysis of variance (one-way ANOVA) and Tukey's honest significant difference (HSD) test were used to compare means of the data taken. Analysis was performed using JMP® Pro version 17.0.0 (SAS Institute Inc., Cary, NC, USA). The level of significance was determined at $p < 0.05$.

3.3 Effect of high phenolic content extracts on ice cream and their ability to replace commercial stabilizers

In this section, green tea and grapeseed extract were added to ice cream to investigate their ability to reduce the melting rate, replace stabilizers, and inhibit ice recrystallization.

3.3.1 Materials

Pasteurized dairy cream was purchased from Sassy Cow Creamery (Columbus, WI, USA). Sugar (United Sugars, Edina, MN, USA) and NFDM (Dairy America, Fresno, CA, USA) were purchased from the dairy plant at the University of Wisconsin-Madison (Madison, WI, USA). A blend of stabilizers (locust bean gum, guar gum, and carrageenan) from Germantown Premium I.C and mono- and diglyceride from Grinsted® were purchased from Danisco USA (New Century, KS, USA). High fructose corn syrup (HFCS) (42% fructose) was acquired from the Archer Daniels Midland Company (Decatur, IL, USA). Green tea and grapeseed extract were purchased from Ethical Naturals, Inc. (Novato, CA, USA).

3.3.2 Experimental Design

Green tea and grapeseed-enriched ice creams were made with and without stabilizers. The ingredients for control and extract formulas can be found in **Table 3.3**. These formulas are 15% fat and 3% protein. The addition of high fructose corn syrup was used to balance the freezing point depression across formulas due to the sugar

content of the extracts. The calculated freezing point depression values for these formulas were between -2.62°C and -2.52°C.

Table 3.3 Composition of control and extract (green tea and grapeseed) ice creams with and without stabilizers.

	Control		Extract	
Stabilizer (%)	0	0.2	0	0.2
Ingredients (%)				
Cream	40.1	40.1	40.1	40.1
Nonfat dry milk	5.8	5.8	5.8	5.8
Sugar	11	11	11	11
High fructose corn syrup	6	6	3	3
Mono- and diglycerides	0.12	0.12	0.12	0.12
Stabilizers	0	0.2	0	0.2
Water	26.98	26.78	29.98	29.78
Extract Solution (%)				
Extract	0	0	3	3
Water	10	10	7	7

3.3.3 Ice cream production

Ice cream samples were prepared according to the procedure outlined in Section 3.2.3**.** Mix ingredients (excluding the extract solution) were mixed and heated to 85ºC in a jacketed mixer (Stephan Food Processing Machinery, Hamelin, Germany). The mix went through a two-stage homogenizer (Manton-Gaulin MFG, Co. Inc., Everett, MA, USA), then returned to the jacketed mixer and cooled to 10˚C. Extract and water were mixed to make a solution (only water was added for control.). A Waring Commercial Heavy Duty Immersion Blender (Waring Commercial, Stamford, CT, USA) blended the extract solution into the mix. The enriched mix was aged at 4˚C for 24 hr. Ice cream mix (4kg) was frozen with a batch freezer (Stoelting VB120-109A Telme Batch Freezer, The

Vollrath Company, Kiel, WI, USA). The finished product was hardened and stored in a walk-in freezer (-25˚C). Each sample batch was made in duplicate.

3.3.4 Analyses

3.3.4.1 pH

The pH was measured with a FiveEasy Plus pH/mV meter with InLab® Viscous Pro-ISM probe (Mettler Toledo, Hampton, Schwerzenbach, Switzerland). The electrode and samples were prepared according to the method in Section 3.2.4.1.

3.3.4.2 Meltdown Test

Samples were prepared based on the method outlined in Section 3.2.4.2. The ice cream was placed on a wire mesh screen above a beaker, where the dripped through fraction of the sample was collected on a scale, recording the weight every minute. Testing was conducted for 240 mins at ambient temperatures (~22°C). Each sample was measured in triplicate.

3.3.4.3 Rheology

Samples were prepared based on the methods outlined in Section 3.2.4.3. A DHR-2 rheometer (TA instruments, Delaware, USA) with crosshatched parallel plate geometry (dia. 25 mm) was used for measurement. The samples were measured at 0°C using a recirculating chiller (Thermos Cube, Solid State Cooling System, Wappingers Falls, NY, USA) connected to the bottom plate. Complex viscosity was measured in triplicate. Next, a flow ramp was conducted, where the shear rate was increased (0.001

to 100 s^{-1}) and then decreased (100 to 0.001 s^{-1}), capturing 10 points per decade for 10 minutes for each ramp. The TRIOS software (2019 TA Instruments–Waters LLC, New Castle, USA) calculated the thixotropy. Each sample was evaluated in triplicate.

3.3.4.4 Overrun

Overrun was measured by weighing the ice cream mix (prior to freezing) and the final frozen product in a fixed-volume container. The value was calculated using the equation from Section 3.2.4.4 in triplicate.

3.3.4.5 Optical light microscopy

Samples were prepared based on the methods outlined in Section 3.2.4.5. Images were taken at 200x magnification with a Nikon Eclipse FN1 optical microscope (Nikon Instruments Inc., Melville, NY, USA).

3.3.4.6 Shelf-life storage

Samples were prepared based on the methods outlined in Section 3.2.4.6. The temperature of the cabinet oscillated from -9.4°C to -14.3°C twice an hour. Samples were removed at four weeks of storage for ice crystal analysis in triplicate.

3.3.4.7 Ice crystal size analysis

Samples were prepared based on the methods outlined in Section 3.2.4.7. Prepped samples were loaded on an optical microscope (FX-35DX, Nikon, Inc., Garden City, NY, USA) where around 20-30 images were captured. Each ice crystal was traced

in Microsoft Paint and measured with Image Pro Plus (Version 7.0 Media Cybernetics 2009, Rockville, MD). Measurements were performed in triplicate.

3.3.4.8 Statistical Analysis

Analysis of variance (one-way ANOVA) and Tukey's honest significant difference (HSD) test were used to compare means of the data taken. Analysis was performed using JMP® Pro version 17.0.0 (SAS Institute Inc., Cary, NC, USA). The level of significance was determined at $p < 0.05$.

3.4 Effect of fruit extracts, freeze-dried powders, and juice concentrates on ice cream

In this section, different crude polyphenol sources were added to ice cream to assess their capacity to reduce the melting rate and influence rheological properties.

3.4.1 Materials

Pasteurized dairy cream was purchased from Sassy Cow Creamery (Columbus, WI, USA). Sugar (United Sugars, Edina, MN, USA) and NFDM (Dairy America, Fresno, CA, USA) were purchased from the dairy plant at the University of Wisconsin-Madison (Madison, WI, USA). A blend of stabilizers (locust bean gum, guar gum, and carrageenan) from Germantown Premium I.C. and MDG from Grinsted® were purchased from Danisco USA (New Century, KS, USA). HFCS (42% fructose) was acquired from the Archer Daniels Midland Company (Decatur, IL, USA). Blueberry extract and cranberry extract were purchased from Ethical Naturals, Inc. (Novato, CA,

USA). Strawberry juice concentrate was supplied by TreeTop, Inc. (Selah, WA, USA). Blueberry freeze-dried powder was purchased from Nuts.com (Cranford, NJ, USA). The freeze-dried strawberry powder was purchased from Micro Ingredients (Montclair, CA, USA). Strawberry extract was produced by the Bolling Lab at the University of Wisconsin-Madison (Madison, WI, USA).

3.4.2 Experimental Design

Crude polyphenol sources with varying levels of phenolic content were added to ice cream at a 3.5% concentration. The ingredients for control and test formulas can be found in **Table 3.4**. These formulas are 15% fat and 3% protein. The addition of high fructose corn syrup was used to balance the freezing point depression across formulas due to the sugar content of the extracts. The calculated freezing point depression values for these formulas were between -2.62°C and -2.52°C.

3.4.3 Ice cream production

Ice cream samples were prepared according to the procedure outlined in Section 3.2.3**.** Mix ingredients (excluding the extract solution) were mixed and heated to 85ºC in a jacketed mixer (Stephan Food Processing Machinery, Hamelin, Germany). The mix went through a two-stage homogenizer (Manton-Gaulin MFG, Co. Inc., Everett, MA, USA), then returned to the jacketed mixer, and cooled to 10˚C. The cranberry, blueberry, and strawberry extract were mixed with water to make a solution. A Waring Commercial Heavy Duty Immersion Blender (Waring Commercial, Stamford, CT, USA) blended the extract solution into the mix.

Table 3.4 Composition of ice creams made with crude polyphenol sources.

	Control	Cranberry extract	Blueberry extract	Strawberry freeze-dried	Strawberry extract	Blueberry freeze-dried	Strawberry juice concentrate
Ingredients (%)							
Cream	40.1	40.1	40.1	40.1	40.1	40.1	40.1
Nonfat dry milk	5.8	5.8	5.8	5.8	5.8	5.8	5.8
Sugar	11	11	11	11	11	11	2.5
High fructose corn syrup	6	3	3	0	0	1	0
Mono- and diglycerides	0.12	0.12	0.12	0.12	0.12	0.12	0.12
Stabilizers	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Water	26.78	29.78	29.78	39.28	39.28	38.28	31.28
Extract Solution (%)							
Extract	0	3.5	3.5	3.5	3.5	3.5	20
Water	10	6.5	6.5	0	6.5	0	0

The freeze-dried powders and juice concentrate were slowly added directly to the mix while blending. The enriched mix was aged at 4˚C for 24 hr. Ice cream mix (4kg) was frozen with a batch freezer (Stoelting VB120-109A Telme Batch Freezer, The Vollrath Company, Kiel, WI, USA). The finished product was hardened and stored in a walk-in freezer (-25˚C). Each batch was made in duplicate.

3.4.4 Analyses

3.4.4.1 pH

The pH was measured with a FiveEasy Plus pH/mV meter with InLab® Viscous Pro-ISM probe (Mettler Toledo, Hampton, Schwerzenbach, Switzerland). The electrode and samples were prepared according to the method in Section 3.2.4.1.

3.4.4.2 Meltdown Test

Samples were prepared based on the method outlined in Section 3.2.4.2. The ice cream was placed on a wire mesh screen above a beaker, where the dripped through fraction of the sample was collected on a scale, recording the weight every minute. Testing was conducted for 240 mins at ambient temperatures (~22°C). Each sample was measured in triplicate.

3.4.4.3 Rheology

Samples were prepared based on the methods outlined in Section 3.2.4.3. A DHR-2 rheometer (TA instruments, Delaware, USA) with crosshatched parallel plate geometry (dia. 25 mm) was used for measurement. The samples were measured at 0°C using a recirculating chiller (Thermos Cube, Solid State Cooling System, Wappingers Falls, NY, USA) connected to the bottom plate. Complex viscosity was measured in triplicate. Next, a flow ramp was conducted, where the shear rate was increased (0.001 to 100 s^{-1}) and then decreased (100 to 0.001 s^{-1}), capturing 10 points per decade for 10 minutes for each ramp. The TRIOS software (2019 TA Instruments–Waters LLC, New Castle, USA) calculated the thixotropy. Each sample was evaluated in triplicate.

3.4.4.4 Overrun

Overrun was measured by weighing the ice cream mix (prior to freezing) and the final frozen product in a fixed-volume container. The value was calculated using the equation from Section 3.2.4.4 in triplicate.

3.4.4.5 Optical light microscopy

Samples were prepared based on the methods outlined in Section 3.2.4.5. Images were taken at 200x magnification with a Nikon Eclipse FN1 optical microscope (Nikon Instruments Inc., Melville, NY, USA).

3.4.4.6 Statistical Analysis

Analysis of variance (one-way ANOVA) and Tukey's honest significant difference (HSD) test were used to compare means of the data taken. Analysis was performed using JMP® Pro version 17.0.0 (SAS Institute Inc., Cary, NC, USA). The level of significance was determined at $p < 0.05$.

4. Results and Discussion

The objective of this dissertation was to analyze the effect of polyphenols on an ice cream system and their impact on melting behavior. In Section 4.1, cream and TA were used to examine the interactions between the polyphenol, milk fat, and milk protein, which led to the discovery of the mechanism behind the fat clustering observed in the sample. Ice creams made with TA (Section 4.2), high phenolic extracts (Section 4.3), and other crude polyphenol sources (Section 4.4) were analyzed for their melting characteristics. The use of microscope imaging and rheological measurements helped characterize the resultant structure from the addition of polyphenol sources. Section 4.2 further explored the impact of increasing the fat and protein content of TA-enriched ice cream formulas to determine which was more favorable for decreasing the melting rate. High phenolic extracts were assessed for their ability to replace a common stabilizer

blend in ice cream by including formulas that excluded the ingredient in the Section 4.3 experiments. Ice creams made with TA (Section 4.2) and high phenolic extracts (Section 4.3) were subjected to a 4-week shelf-life study to analyze the change in ice crystal size over storage time.

4.1 Effect of tannic acid on dairy cream

The objective of this study was to investigate the mechanisms that drive gelation in cream due to the presence of a polyphenol, tannic acid (TA). The hypothesis is that this resulting gelation is facilitated through either partial coalescence of fat globules or protein-mediated fat aggregation, or both. Since polyphenols can also interact with many carbohydrates and hydrocolloids that are present in more complex systems, like an ice cream mix (Mateus et al., 2004), a simple cream and polyphenol system was utilized to reduce potential confounding variables. TA was chosen due to its purity, easy procurement, and known interactions with cream.

4.1.1 Characteristics of cream/TA sample

4.1.1.1 Viscosity

Visually, the addition of TA solutions to cream caused an increase in viscosity, leading to gelation at higher TA addition levels. At 0.75%, the sample resembled a slightly thickened cream. The 1.5% and 3% (wt/wt) samples were significantly more viscous and had gelled. These gels were sufficiently solid that, when inverted, no sample fell out of the cup. The 3% sample had a cuttable texture, which differed from

the other samples. Although the viscosity change was almost instantaneous upon TA addition, samples were analyzed quantitatively after 24 h at 4°C.

Complex viscosity is the total resistance to flow of a viscous liquid (Dimitreli & Thomareis, 2008). **Figure 4.1** shows that as the TA% increased, the complex viscosity increased, which correlates with the visual observations. Note that no reading was possible for pure cream since it was too thin (low viscosity) for this analysis. Only the 3% TA sample was significantly different from the other samples ($P < 0.05$). Since this study used a pure polyphenol source without any other confounding components (e.g., fiber, pectin, etc.), it is possible to attribute this viscosity change to the presence of the TA and its impact on the fat and protein found in cream.

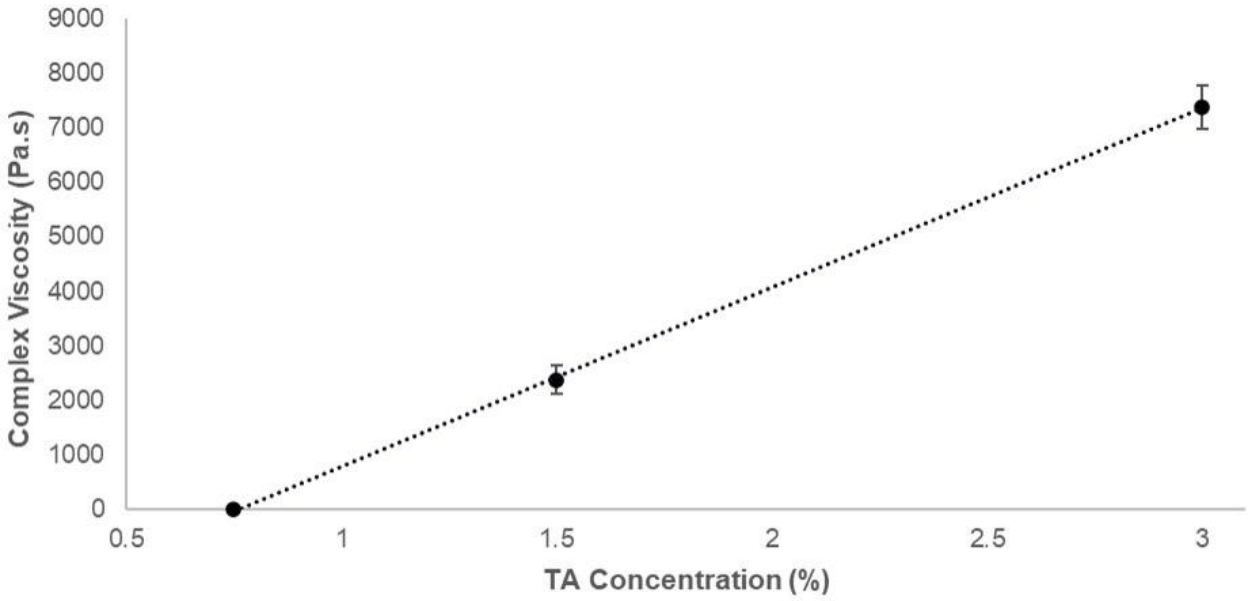

Figure 4.1 Effect of tannic acid (TA) concentration on the complex viscosity of cream. The error bars represent standard error between sample and measurement replicates. (R value = 0.9998)

4.1.1.2 pH

To understand the mechanism behind the gelation of these samples, the role of pH cannot be overlooked. The pH of the regular dairy cream was 6.67. At the isoelectric points (pI) of casein (pH 4.6) and whey proteins (pH 4.5–5.2), protein coagulation occurs as the surface charges are reduced (Abugoch, 2009). The pH of the TA solutions (prior to their addition to cream) were around 3.39-3.43. **Table 4.1** lists the pH of the final cream/TA samples at each concentration. While the TA solutions were acidic, the resulting cream samples at 0.75% and 1.5% (wt/wt) TA remained above the pI for both proteins. The 3% (wt/wt) cream/TA sample had a pH below the pI of whey, which might suggest that whey protein coagulation had some potential impact on the sample's structure at this high TA concentration. However, since 1.5% and 3% samples both created a gelled product, a pH below the isoelectric point was not required for gelation. Diaz et al. (2020) also studied the role of pH while investigating the effect of polyphenol-rich berry juices on WPI solutions. For their control, they created imitation juices that contained comparable pH and sugar contents with no polyphenols. The berry juice caused more whey protein aggregates than the imitation juice, indicating the importance of polyphenols on the system beyond the pH they impart. Harbourne et al. (2011) observed that while tannic acid did not significantly change the pH of the system, it did cause significantly faster gelation times in an acid milk gel system. This was attributed to the effect of tannic acid on proteins. These results suggest that, while pH does affect the overall environment of the sample, it was not the main driver of the viscosity and texture changes observed at these TA concentrations.

To test the effects of pH versus polyphenols on cream, solutions of HCl or citric acid were added (**Table 4.1**). The pH values of both acid solutions (pH 3 and 3.5) were chosen to mimic the pH range of the TA solutions. Viscosity data were assessed visually for these systems. The objective was to observe if the TA gelled cream product could be replicated using a solution at a similar pH (without any polyphenols present) at a constant weight added and preparation. When both HCl solutions were added to cream, there was little to no effect on the pH and the resulting viscosity did not change (similar to pure cream viscosity). There was a greater decrease in pH and increase in viscosity for the citric acid samples. Despite this sharper pH decrease, these samples were still semi-fluid, with a loose texture and did not create an invertible gel like the 1.5% and 3% (wt/wt) TA samples. Further, the citric acid samples did not cause an instantaneous viscosity change, as observed with TA addition, the effect required longer time. Lastly, another visual test was conducted that included adding citric acid into cream until a final pH of 5.20 was reached (similar to the final pH of 3% TA sample), and still a gelled product was not created despite pH below pI of some whey proteins. These experiments highlight that there are factors beyond pH that caused gelation of the cream/TA system at 1.5 and 3 % (wt/wt) TA addition. While the role of pH cannot be completely disregarded, especially for the 3% TA sample, these results document that pH is not the primary mechanism in the viscosity changes observed in **Figure 4.1**.

Table 4.1. pH of resulting cream samples with 0.75%, 1.5% and 3% tannic acid (TA) addition, as well as the addition of HCl and citric acid solutions at pH 3 and 3.5

Cream Sample	pH
0.75% TA	6.08 ± 0.05^{b}
1.5% TA	5.75 ± 0.03^{c}
3.0% TA	5.18 ± 0.03^{d}
pH 3 HCl solution	6.70 ± 0.02^{a}
pH 3.5 HCl solution	6.75 ± 0.02^{a}
pH 3 citric acid Solution	3.78 ± 0.02^{f}
pH 3.5 citric acid Solution	4.21 ± 0.06^{e}

Mean ± SE are shown. Values sharing a letter are not significantly different at α=0.05.

4.1.1.3 Particle size distribution and micrographs

The fat globule particle size of these samples was analyzed to gain deeper insight into the structure of the cream/TA systems. **Figure 4.2** shows how the particle size of cream changed with the addition of TA. Regular cream had a singular peak around 5 µm, representative of the original emulsion droplets. As the concentration of polyphenols increased, a bimodal distribution was created with a secondary peak formed near the 50 µm range.

The intensity of the first peak decreased with added TA because of the depletion of individual globules to create larger aggregates (Note, aggregation and clustering are used interchangeably in this work when describing a grouping of fat globules held together by various interactions).

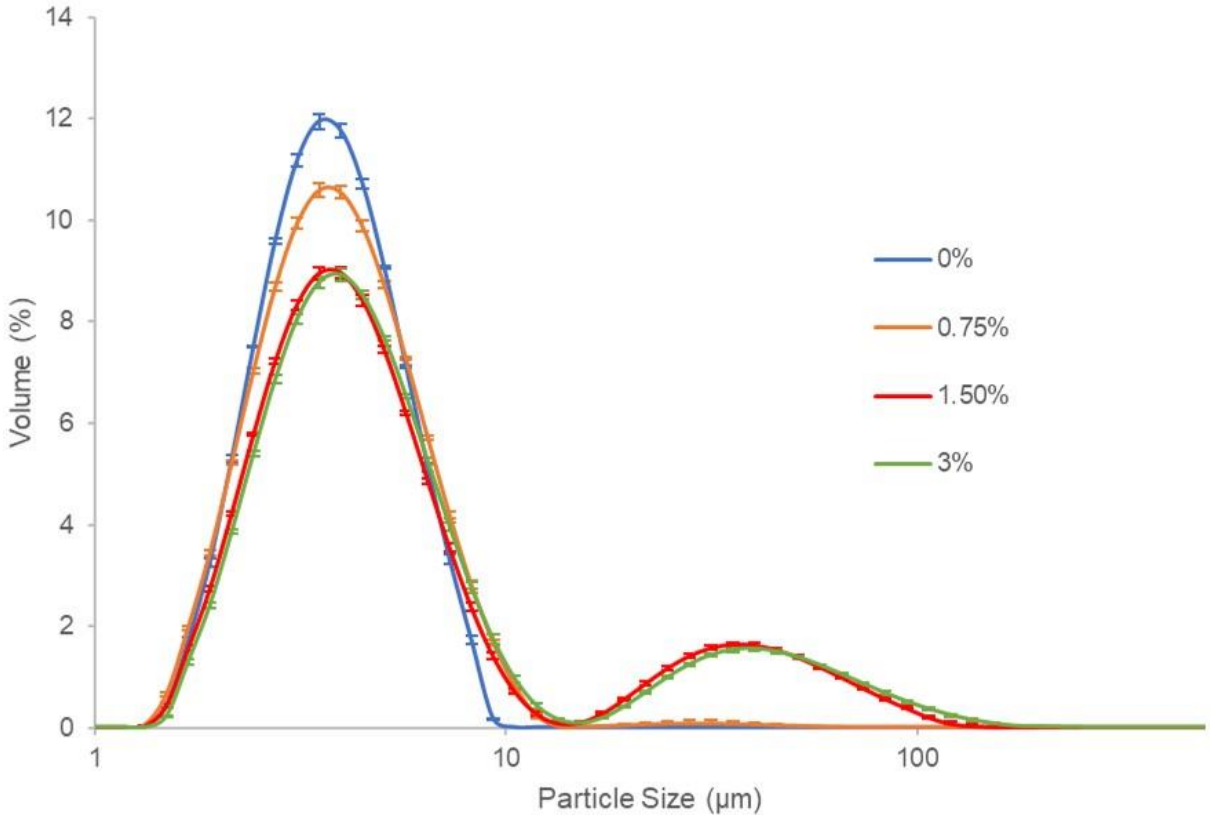

Figure 4.2 Particle size distribution of cream at increasing concentrations of tannic acid, diluted 1:1 with D.I. water. The error bars represent standard error between sample and measurement replicates.

To better understand this shift to larger particles, microscope images were taken of each sample (**Figure 4.3**). The samples with added TA showed visible aggregates of fat globules (**Figure 4.3B-D**), although some individual fat globules remained, but with decreasing amount as TA increased. These aggregates began to form a gel network at 1.5% and 3% (wt/wt) TA. Average particle size and microscope images together suggest that, as TA levels increased, the presence and size of the fat globule clusters increased.

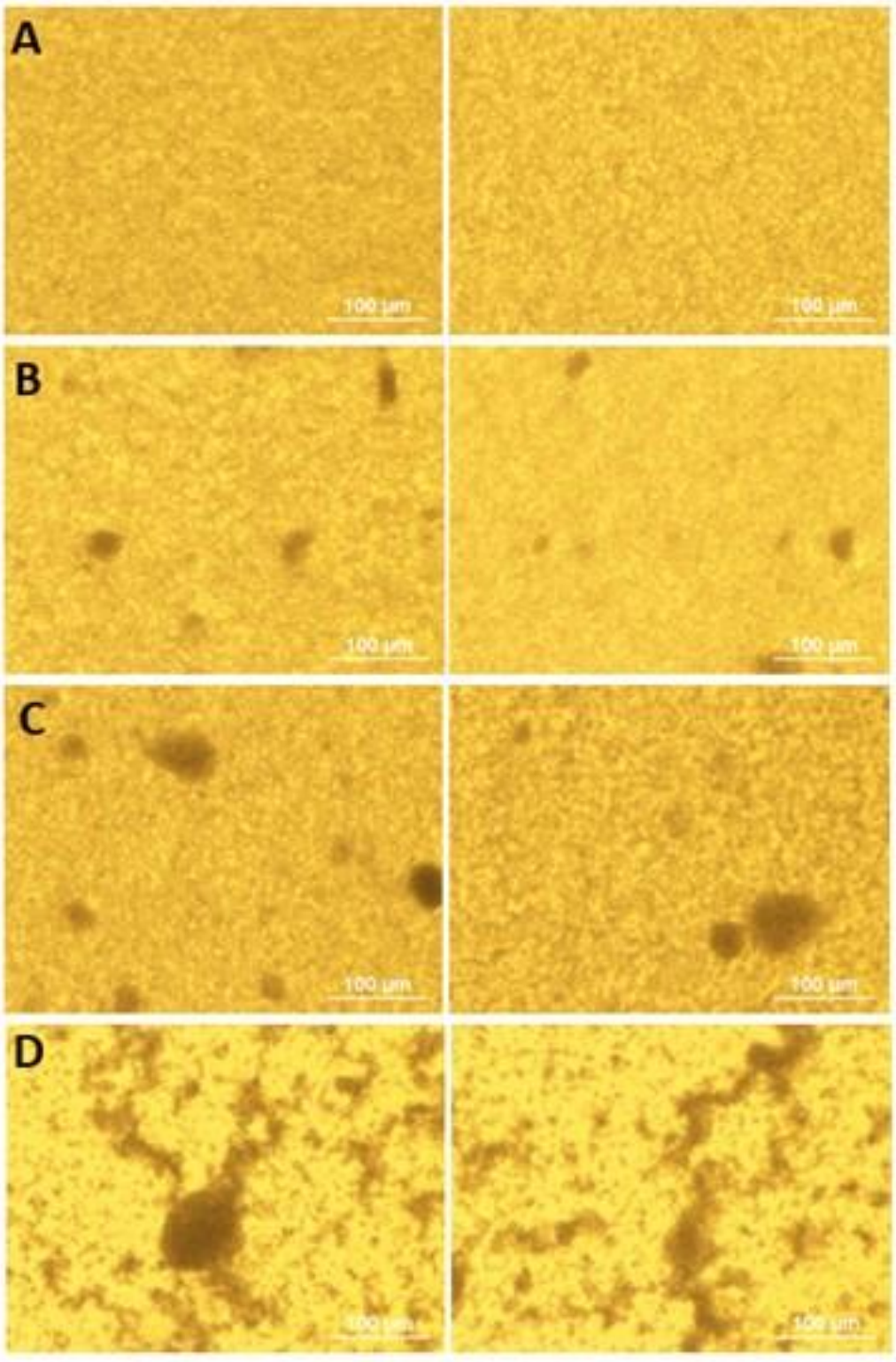

Figure 4.3 Microscope images of cream samples with addition of (A) 0%, (B) 0.75%, (C) 1.5%, and (D) 3.0% tannic acid. Samples diluted 10-fold with D.I. water.

To validate the importance of milkfat in this observed cream gelation and fat clustering, two tests were conducted. First, TA was added to skim milk at the same TA concentrations (0.75%,1.5%, 3% wt/wt), using the same procedure. No visual viscosity change was observed. Second, diluted samples similar to those shown in **Figure 4.3** were heated on the microscope (data shown later). Upon warming to 55°C, melted fat globules were clearly observed, along with coalescence of some droplets in the same proximity. Clearly, both protein and fat play important roles in the formation of these aggregates.

4.1.2 Investigating partial coalescence and protein-mediated fat aggregation

While these experiments highlight the appearance of fat clustering, they do not confirm the mechanism of aggregation. Due to the composition of the cream system, it might be expected that clustering is driven by either fat aggregation mediated by protein interactions or by partial coalescence of the fat globules, or some combination of the two mechanisms. These expectations are informed by the likely presence of protein-polyphenol complexes and solid fat in this system, as discussed above. Protein-TA complexes could potentially act as a bridge between fat globules, as they have an affinity to the fat globule interface and can connect with other protein-polyphenol complexes. Polyphenols can potentially impact the fat globule interface through displacing proteins and influencing other surface-active properties. Based on the partially-crystalline milkfat, these changes could allow the fat globules to be more susceptible to partial coalescence as a method of fat clustering. Further analysis is needed to help understand these complicated interactions.

4.1.2.1 SDS and EDTA treated cream/TA sample

By utilizing SDS and EDTA solutions, the binding mechanism of this observed colloidal network was investigated, along the lines of the work of Mendez-Velasco and Goff (2011). SDS can change the wettability of fat crystals present at the interface of a partially-coalesced sample and cause the expulsion of fat crystals into the aqueous phase, thus redispersing the fat globules (Méndez-Velasco and Goff, 2011). However, SDS is also used to break hydrophobic bonds between proteins (Hou et al., 2020), a critical point since polyphenols are thought to form aggregates with proteins through hydrophobic interactions (Yildirim-Elikoglu & Erdem, 2018). EDTA, by sequestering solution calcium, removes colloidal calcium from the casein micelle, which causes the micelle to separate. By disrupting the micelles, casein-mediated fat aggregates can also be redispersed. Both treatments are necessary to distinguish between fat aggregation and partial coalescence.

The $d_{4,3}$ or volume-weighted mean is a measure of the average particle size (McClements, 2015). **Figure 4.4** shows $d_{4,3}$ of the fat globules in cream with increasing levels of TA, treated with water (control), SDS, and EDTA solutions to dilute samples for Mastersizer analysis. These values were analyzed via one-way ANOVA ($P < 0.01$) and Tukey's HSD test. At 0.75% TA, only the SDS treatment was significantly different than the control (dilution with D.I. water). However, all treatments were significantly different at 1.5% and 3% (wt/wt) TA. At 1.5% TA, the control had a $d_{4,3}$ of 10.3 µm, and the EDTA and SDS samples were 4.5 µm and 3.6 µm, respectively. A similar trend can be seen at 3% TA for the control (10.3 µm), EDTA (5.1 µm), and SDS (3.7 µm) samples.

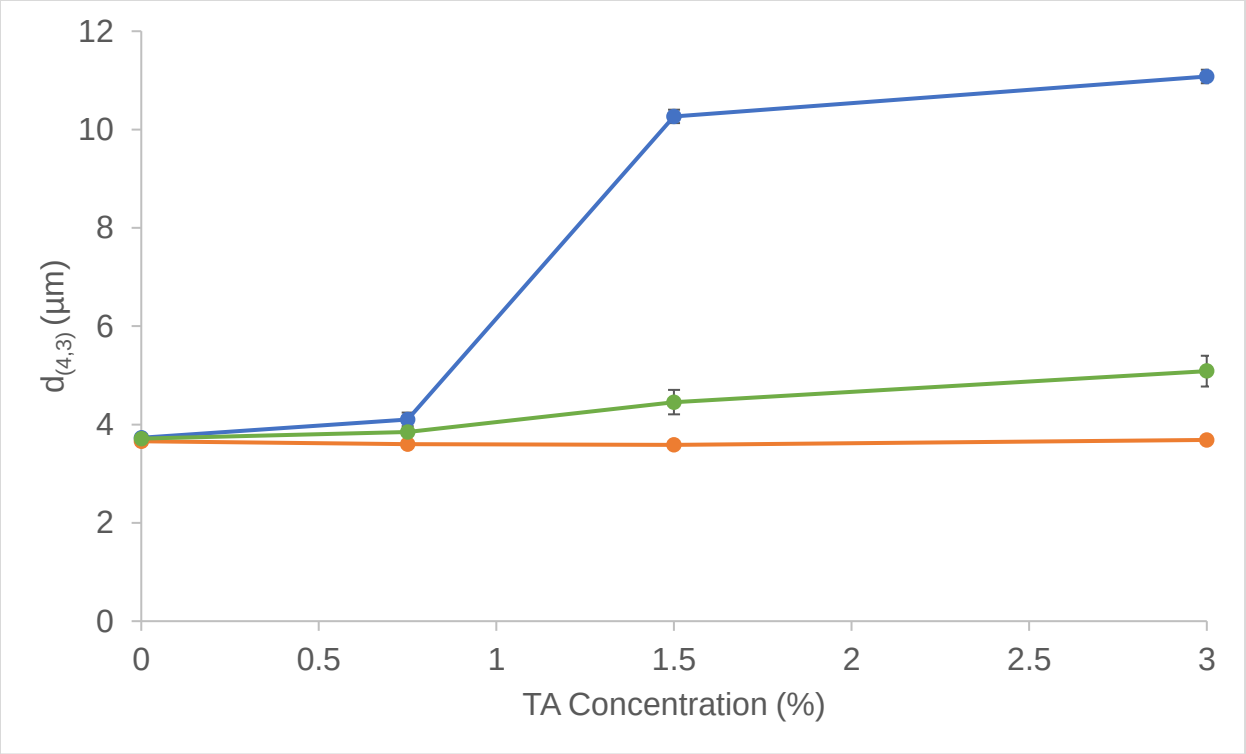

Figure 4.4 Average fat globule diameter $d_{4,3}$ of cream with increasing levels of tannic acid (TA) concentration after 1:1 dilution with water (control) (blue), 4% SDS (orange), and 3.7% EDTA (green). The error bars represent standard error.

These trends are generally supported by the microscope images. **Figure 4.5** depicts the water, SDS, and EDTA treated cream samples at 3% (wt/wt) TA. The SDS sample (**Figure 4.5B**), shows an almost complete redispersion of the aggregated fat network found in the control (**Figure 4.5A**), while the EDTA sample still has some fat clusters present (**Figure 4.5C**). The $d_{4,3}$ value of the 3% EDTA sample is smaller than the clusters that appear in the microscope images. This may be attributed to a combination of the required mechanical agitation needed for particle size measurements and a weakened cream/TA matrix from EDTA treatment. Since image preparation needs only light mixing, there is less potential for disrupting a fragile network. To further confirm that these clusters contained fat, all treated samples (**Figure**

4.5) were heated on a microscope stage and, once again, some coalescence of neighboring fat globules was observed (**Figure 4.6**).

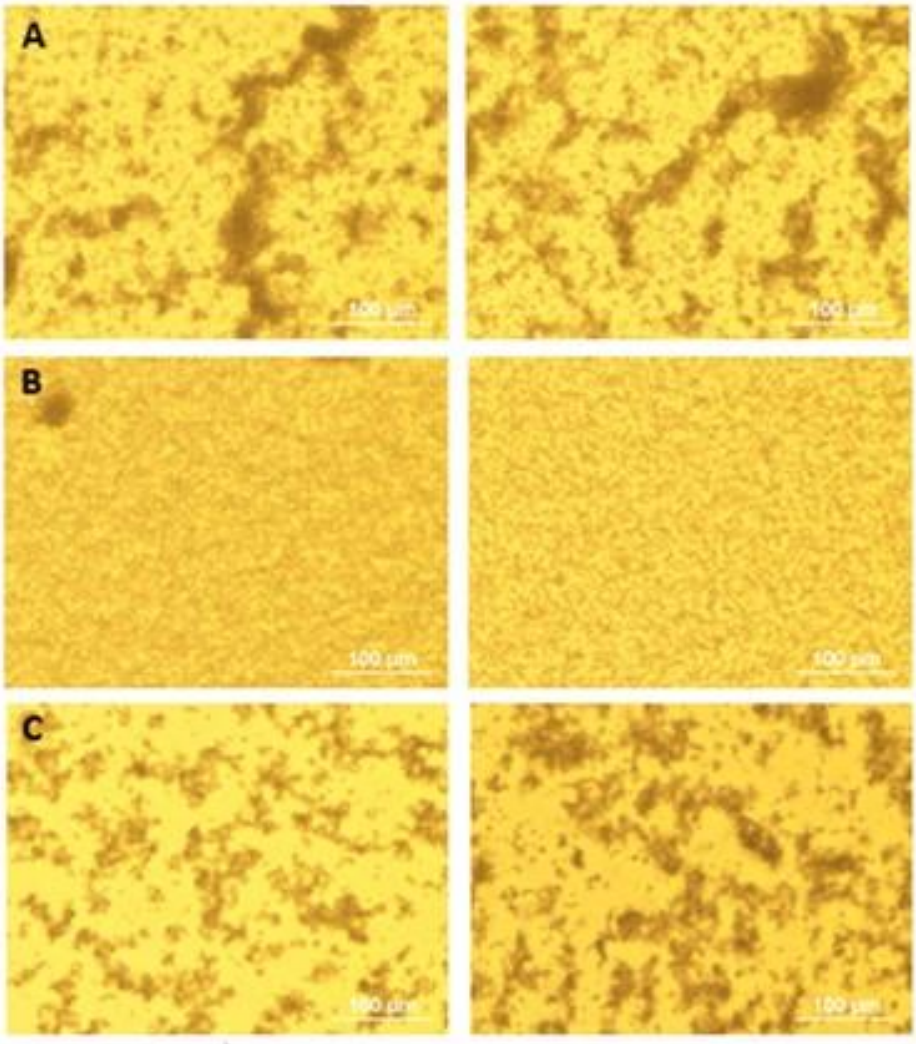

Figure 4.5 Microscope images of 3% tannic acid in cream after a 1:1 dilution with (A) water – control, (B) 4% SDS and (C) 3.7% EDTA

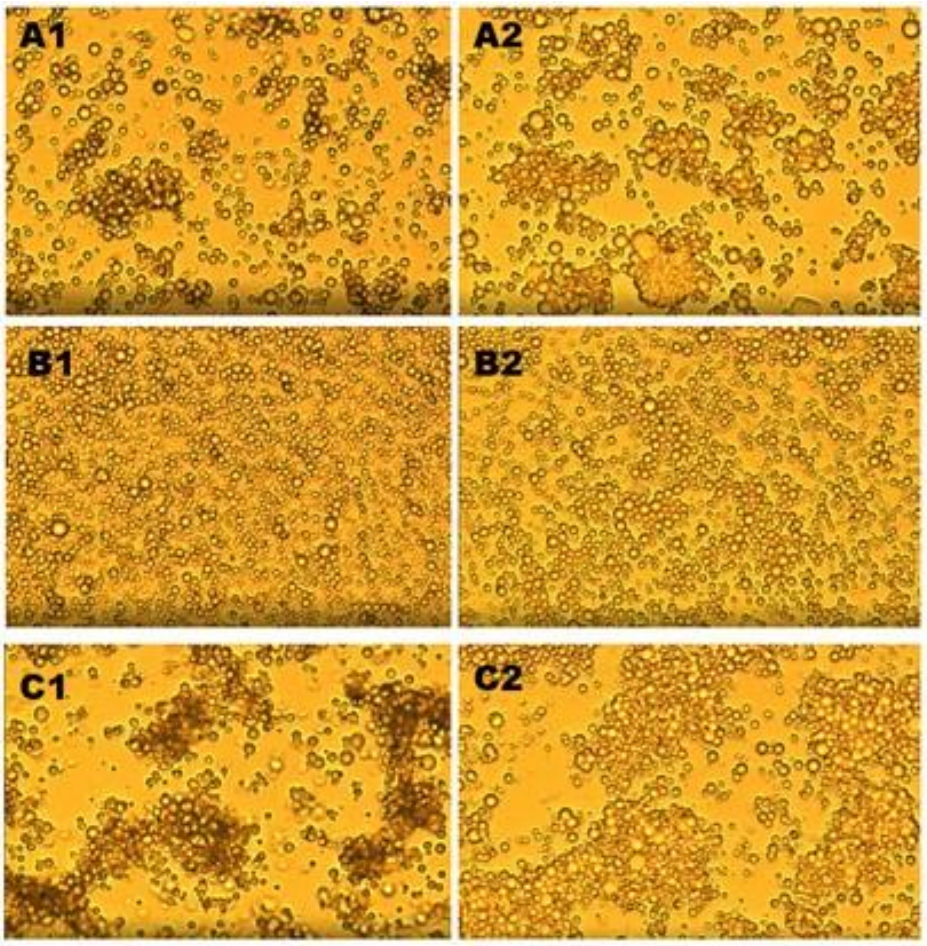

Figure 4.6 Microscope images of 3% tannic acid in cream after a 1:1 dilution with (A1) water – control, (B1) 4% SDS, and (C1) 3.7% EDTA before and after heating at 55°C (A2, B2, C2, respectively)

These treatments help us understand the components of the observed aggregate network. The redispersion of the SDS sample was attributed to the surfactant's ability to disrupt noncovalent bonds (within proteins and protein interactions with the fat globule membrane and polyphenols) and reverse partial coalescence. The EDTA treatment disrupted casein micelles and any casein micelle-TA complexes from the control. This SDS and EDTA identification method works if partial coalescence and/or casein-mediated fat aggregation are the only two fat clustering mechanisms involved in the

system. Since the cream/TA system also contained fat aggregation mediated by whey proteins, this method was ineffective at identifying partial coalescence. However, the statistically significant decrease in average particle size between the control and EDTA samples highlights the importance of casein micelles on the fat aggregation of this system. After the casein micelles were reversed in the EDTA sample, the remaining aggregates found in microscope images suggest that whey-TA complexes are also present in the system. Potential impact of partial coalescence on these samples cannot be confirmed because protein interactions were not completely reversed during the EDTA treatment. These data suggest that TA complexation with both types of milk proteins facilitates fat clustering, which leads to cream gelation. This could be attributed to the number of available binding sites on both the TA and proteins, the ratio of each present in the sample, and the ability to utilize these parameters to create bridges between globules by sharing TA binding sites between two or more proteins bound to the fat globule membrane (and vice versa) (Charlton et al., 2002; Siebert, 1999; Wei et al., 2020).

4.1.3 Summary

This work provides a basis to understand how TA interacts with pure cream to increase viscosity and promote gelation. The pH data revealed that the added acidity from TA was not the dominant driver of aggregation and increased viscosity in the samples. Average particle size and microscopic images confirmed that a stable network of protein and fat aggregates was created in the presence of TA. SDS and EDTA treatments document that both casein and whey proteins are involved in clustering of fat

globules through protein aggregation. Partial coalescence of fat globules did not seem to factor into formation of the clusters.

4.2 The effect of tannic acid on ice cream

In the following section, TA was added to ice cream to understand its impact on this matrix. The objective was to determine whether the fat clusters observed in the previous section had a significant presence and influence on the properties of ice cream, specifically meltdown characteristics. A standard formula (12% fat / 3% protein) with increasing amounts of TA was used to observe general trends. A high fat (15% fat/ 3% protein) and high protein (12% fat /5% protein) formula were used to further unlock which aspect (protein or fat) is more critical for achieving lower melting rate. This study also examined the potential of TA to affect the quality parameter of ice recrystallization by means of a shelf-life study.

4.2.1 pH

Analyzing the pH of ice cream mixes was crucial to ensure that variations in acidity were not solely responsible for the results in subsequent sections. The control ice cream mix had a pH of 6.5, which falls within the normal range for regular ice cream (Goff & Hartel, 2013). The pH decreased with increasing TA across all formulations (**Table 4.2**). However, similar to Section **4.1,** the standard and high fat 2.5% TA samples approach the isoelectric point of whey protein (4.5–5.2). Particle size of whey-polyphenol complexes can grow larger as they approach the isoelectric point of the protein (von Staszewski et al., 2012). Although the hypothesis is that polyphenols play a

primary role in the following results due to the outcomes of Section 4.1 and the findings of Diaz et al. (2020), the potential impact of whey-tannic acid complexes on structure and viscosity cannot be dismissed.

Table 4.2 pH of different ice cream formulations made with increasing concentrations of tannic acid (TA)

Formula Type	Fat (%)	Protein (%)	TA (%)	pH
Standard	12	3	0	6.5 ± 0.0^{a}
			0.5	6.1 ± 0.1^{ab}
			1	5.7 ± 0.1^{bc}
			1.5	5.65 ± 0.1^{bc}
			2	5.5 ± 0.0^{c}
			2.5	5.3 ± 0.0^{c}
High Protein	12	5	0	6.3 ± 0.0^{a}
			2.5	5.7 ± 0.1^{bc}
High Fat	15	3	0	6.45 ± 0.2^{a}
			2.5	5.4 ± 0.1^{c}

Mean ± SE are shown. Values sharing a letter are not significantly different at α=0.05.

4.2.2 Meltdown

This study aims to determine whether an increase in TA% will lead to a reduction in the melting rate while simultaneously enhancing other melting parameters, such as an increase in induction time and residual weight on the screen. As stated above in Section 2.4, an ice cream begins in the lag phase of the melting profile, where no serum has fallen through the mesh. The induction time, the time the first drip of ice cream falls through the mesh, signifies the beginning of the melting phase. The melting rate is derived from the linear portion of the output found in this phase. As the melting process

ends, the melting profile comes to a plateau in the data, where there is no more sample flowing through the mesh, also known as the stationary phase. The residual weight on the screen is the percentage of the original sample remaining after the sample reaches this phase.

Figure 4.7 illustrates that the standard formula has a slight increase in melting rate as TA increased from 0 to 2%, before a distinct reduction at 2.5% TA. **Table 4.3** also shows that the 2.5% TA sample from the standard formula has an induction time exceeding one hour and a significant amount of residual foam remaining after melting. **Figure 4.8** compares the relationship between these melting characteristics and TA% in the standard formula, providing another way to visualize this data. Induction time and residual weight showed significant increases starting at 1.5% and 2% TA, respectively. Melting rate has an inverse relationship, with a steep decline at 2.5% TA. These trends suggest that a specific quantity of TA (threshold amount) is required to produce a significant change. After this threshold was reached, the induction time and residual weight parameters exhibited a strong positive relationship.

Looking across formulas, high fat ice cream at 2.5% TA had a slower melting profile (**Figure 4.9**) and drip-through rate than the high protein ice cream at 2.5% TA. **Table 4.3** also shows that high fat ice cream had the highest induction time and residual weight when TA was incorporated. Comparatively, the 2.5% TA high protein samples did not exhibit a decline in the melting rate and left a similar quantity of residual foam on the screen as all control formulas.

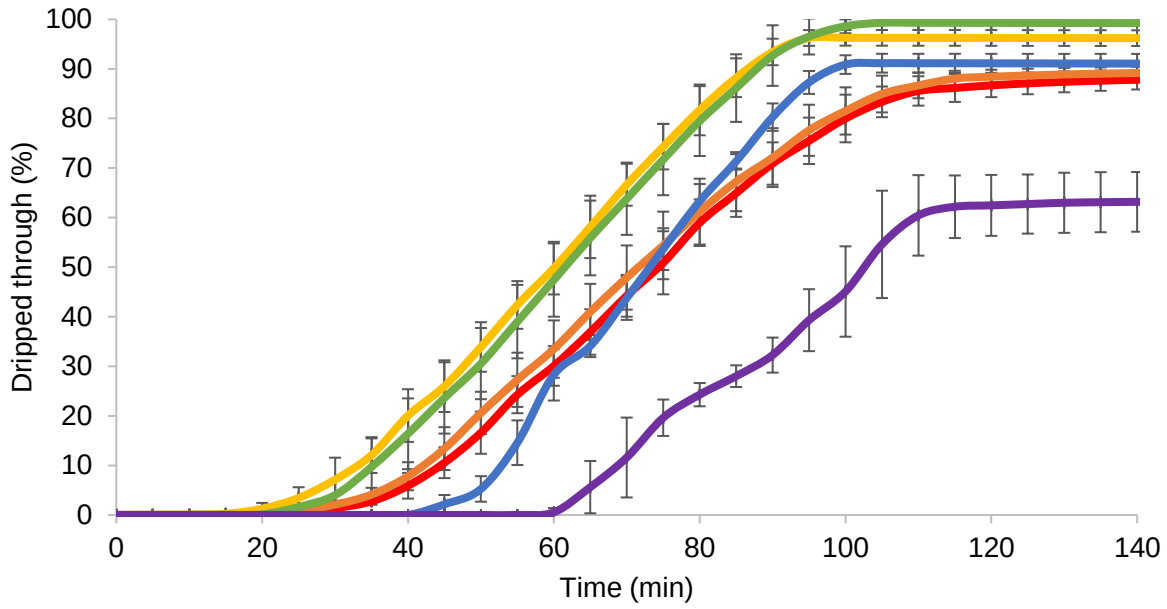

Figure 4.7 Melting profiles of the standard (12% fat/ 3% protein) ice cream formulation with 0% (red), 0.5% (orange), 1% (yellow), 1.5% (green), 2% (blue), and 2.5% (purple) tannic acid. Error bars represent the standard deviation of mean values measured in triplicate.

Table 4.3 Melting characteristics for different ice cream formulas with increasing tannic acid (TA) content.

Formula Type	Fat (%)	Protein (%)	TA (%)	Melting Rate (%/min)	Induction Time (min)	Residual Weight on Screen (%)
Standard	12	3	0	1.29 ± 0.07^{bc}	16 ± 0.0^{c}	10.9 ± 0.7^{b}
			0.5	1.38 ± 0.04^{ab}	20 ± 0.8^{c}	9.8 ± 0.5^{b}
			1	1.55 ± 0.02^{ab}	13 ± 4.2^{c}	3.7 ± 0.1^{b}
			1.5	1.55 ± 0.02^{ab}	22 ± 4.2^{c}	0.7 ± 0.2^{b}
			2	1.75 ± 0.09^{a}	44 ± 0.0^{b}	8.8 ± 0.6^{b}
			2.5	0.72 ± 0.03^{a}	78 ± 3.3^{a}	36.2 ± 4.1^{a}
High Protein	12	5	0	1.43 ± 0.01^{ab}	18 ± 0.0^{c}	7.4 ± 1.4^{b}
			2.5	1.74 ± 0.07^{a}	51.7 ± 2.5^{b}	9.2 ± 2.9^{b}
High Fat	15	3	0	1.39 ± 0.05^{ab}	25.8 ± 0.0^{c}	10.5 ± 0.6^{b}
			2.5	0.91 ± 0.15^{cd}	79.2 ± 1.7^{a}	42.9 ± 4.0^{a}

Mean ± SE are shown. Values in the same column sharing a letter are not significantly different at α=0.05.

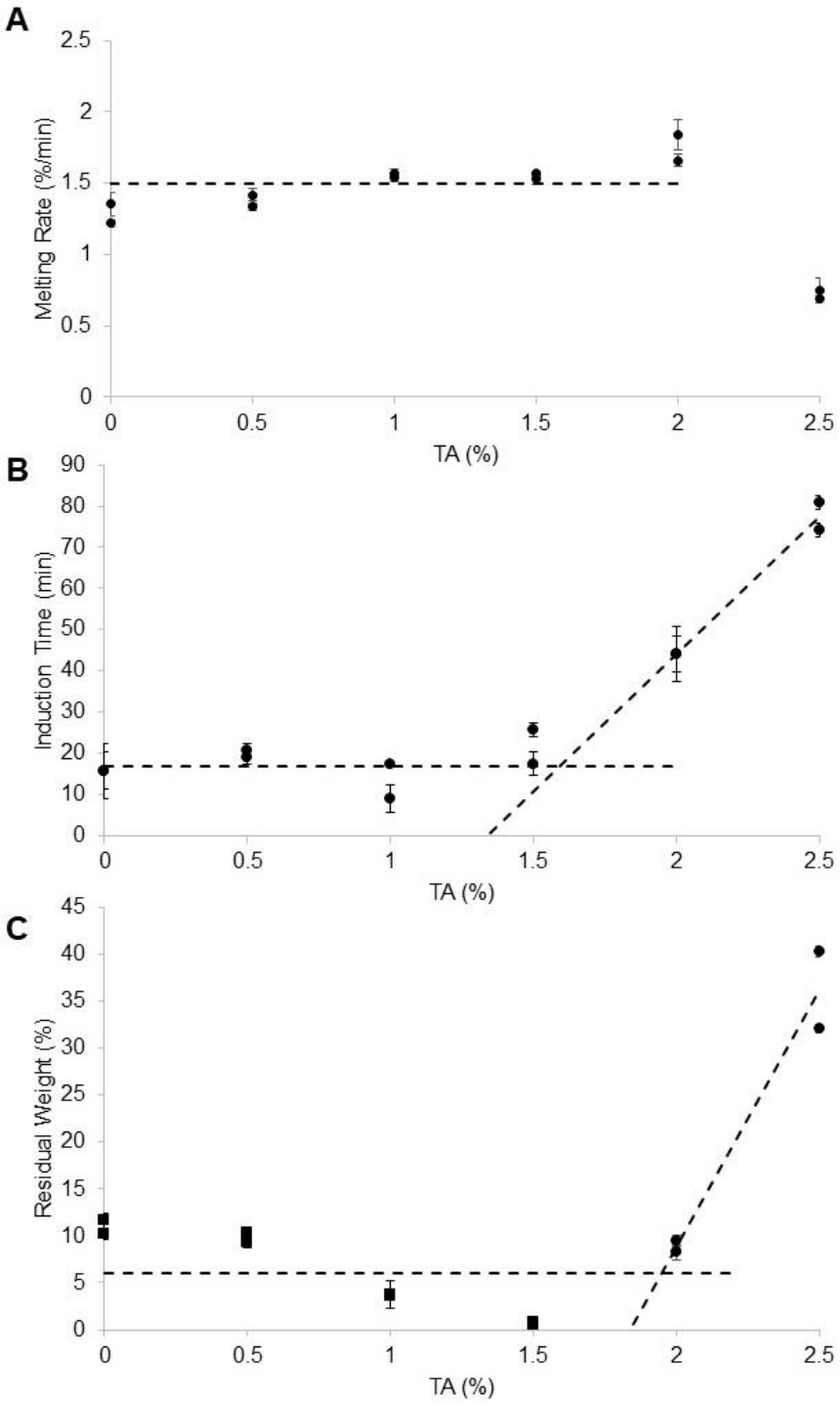

Figure 4.8 Plots of melting rate (**A**), induction time (**B**), and residual weight (**C**) against tannic acid (TA) concentration for the standard (12% fat/3% protein) ice cream formulation. Lines simply indicate trends. Error bars represent the standard error of mean values measured in triplicate.

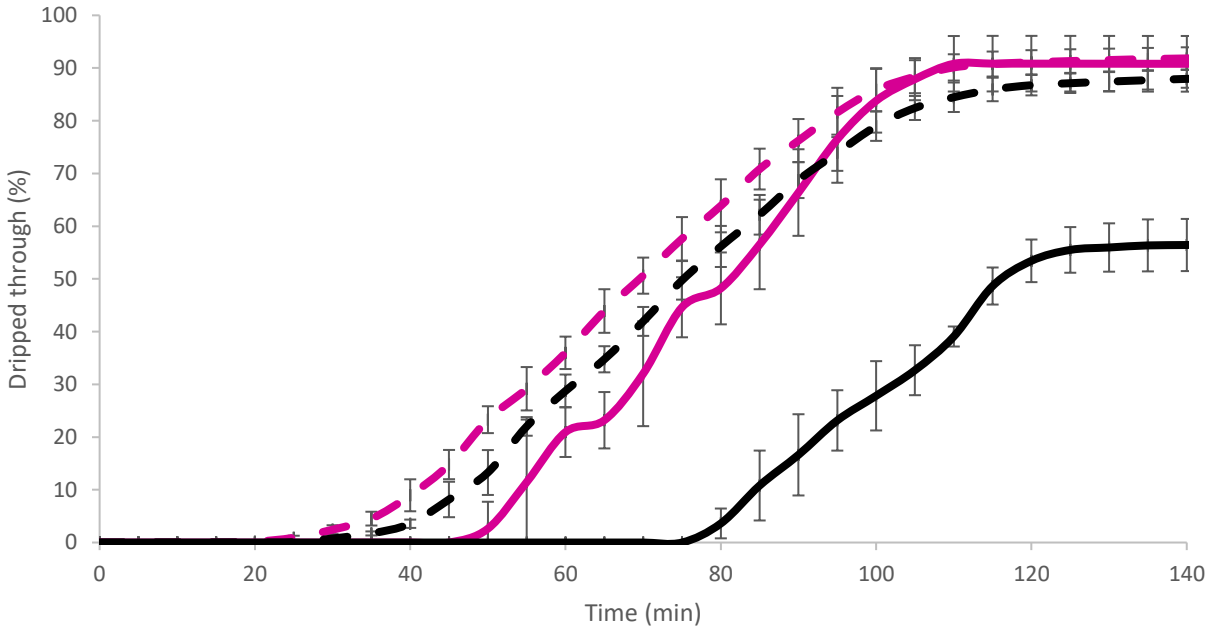

Figure 4.9 Melting profiles of the high protein (12% fat/ 5% protein - magenta) and high fat (15% fat/ 3% protein - black) ice cream formulations with 0% (dashed line) and 2.5% (solid line) tannic acid. Error bars represent the standard deviation of mean values measured in triplicate.

Figure 4.10 depicts the images of the control and 2.5% TA samples for each formula as they melt on a mesh screen. The control samples at 60 minutes show that the sample transitioned from its initial cylindrical form to a semi-spherical shape, which suggests considerable melting and a decline in shape retention. At the same 60 minute time point, the 2.5% TA samples for the standard and high fat formula exhibited greater shape retention. In contrast, the high-protein sample (2.5% TA) displayed obvious signs of melting, as evidenced by its rounded edges and pooling at the base. After 120 min, each sample reached its stationary phase (**Figure 4.7**), and differences in the residual foam were apparent.

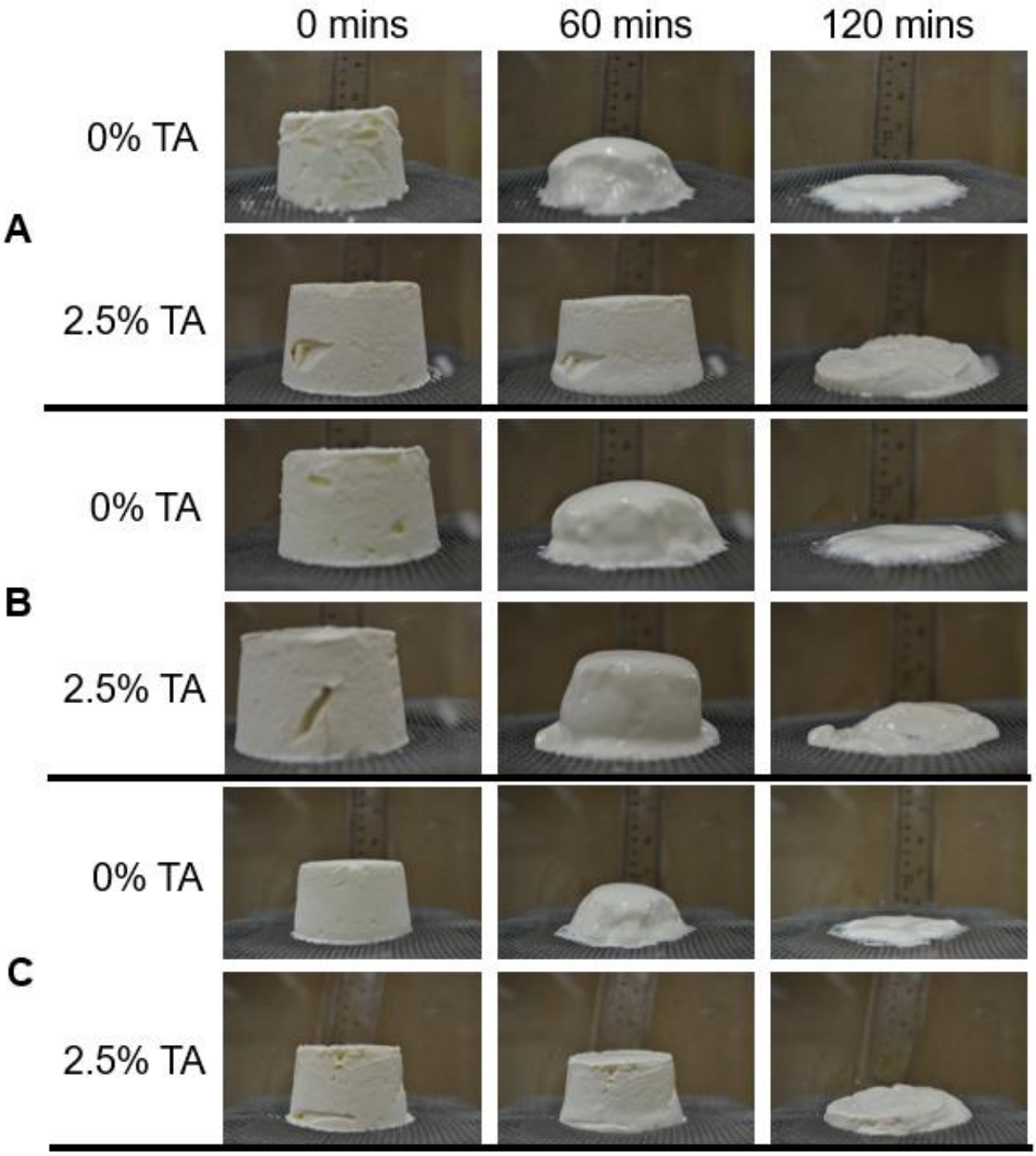

Figure 4.10 Melting images of the standard (12% fat/3% protein) (A), high protein (12% fat/5% protein) (B), and high fat (15% fat/3% protein) (C) ice cream samples on the screen at different tannic acid (TA) concentrations (0 and 2.5%) at 0, 60, and 120 mins.

These results indicate that an elevated protein concentration does not significantly improve the melting behavior of the standard ice cream formula through TA interactions. This aligns with the findings presented in Section 4.1, which emphasize the critical role of fat in enabling TA to form a flow-resistant matrix. In subsequent sections, the underlying reasons for these occurrences are explored using methodologies that delve into the microstructure.

4.2.3 Rheology

The rheological properties of melted ice cream (measured at 0°C) were investigated to understand the effects of TA addition on the numerous inherent structures present in the system and to correlate it to the meltdown process. After the addition of TA to the ice cream mix, a visual increase in viscosity was observed, with a gel-like consistency at 2.5% TA across all formulations. Complex viscosity of the melted ice cream, the ratio of the deformation of the sample (complex modulus) to the frequency (Tunick, 2011), was used to characterize the effects. In the standard formula, the complex viscosity was similar to the control up to 1.5% TA addition, then significantly increased at 2% and 2.5% TA (**Table 4.4**). When graphed in **Figure 4.11**, that distinct increase starting at 2% TA corresponds with a positive trend for higher TA concentrations. This observation supports the previous assertion that a threshold TA concentration is needed to reach the targeted outcomes on the system. The high fat formula at 2.5% TA had the highest viscosity among all formulas, while the high protein formula at 2.5% TA was not significantly different from any control in the study, highlighting the differential impact of fat on the system.

Table 4.4 Rheological data from different ice cream formulations with increasing tannic acid (TA) concentrations.

Formula Type	Fat (%)	Protein (%)	TA (%)	Complex Viscosity (Pa*s)	Thixotropy (Pa/s)
Standard	12	3	0	3.4 ± 0.5^{d}	440 ± 18^{cd}
			0.5	6.5 ± 0.6^{d}	427 ± 14^{cd}
			1	6 ± 0.5^{d}	390 ± 5^{cd}
			1.5	11 ± 0.3^{d}	546 ± 29^{c}
			2	178 ± 13^{c}	779 ± 57^{b}
			2.5	601 ± 19^{b}	1039 ± 76^{a}
High Protein	12	5	0	6.5 ± 1.9^{d}	331 ± 9.7^{d}
			2.5	54 ± 3.1^{cd}	1037 ± 31^{a}
High Fat	15	3	0	6.4 ± 0.9^{d}	439 ± 22^{cd}
			2.5	905 ± 86^{a}	1051 ± 94^{a}

Mean ± SE are shown. Values in the same column sharing a letter are not significantly different at α=0.05.

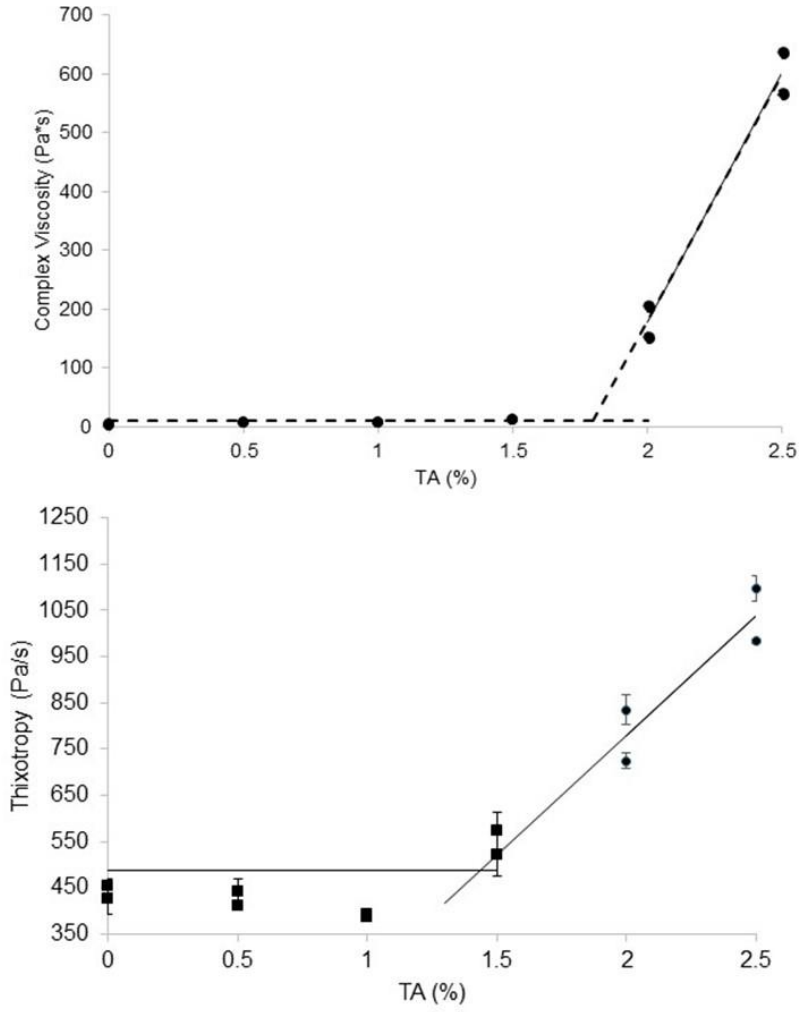

Figure 4.11 Plot of complex viscosity and thixotropy against tannic acid (TA) concentration. Lines simply indicate trends. Error bars represent the standard error of mean values measured in triplicate.

Thixotropy is a measurement that defines the physical energy needed to overcome a material's resistance to breakdown and to build up internal structures (Labanda & Llorens, 2006). This helps to characterize samples that have time-dependent, reversible changes in viscosity when shear is present. Similar to Section 4.1, the hypothesis is that there is a network of particles that form structures due to the presence of TA. When the TA concentration exceeded 2%, the thixotropy values in this

study significantly increased across all formulas (**Table 4.4**). **Figure 4.11**, which plots thixotropy against TA%, also supports the claim that there is a threshold concentration of TA needed to cause an effect. Freire (2020) reported a correlation between ice cream samples with extensive internal structures and high thixotropy values. **Figure 4.12** shows the hysteresis loops between the control and 2.5% TA across all formulas. Each formula that contains 2.5% TA has a large initial peak in the upward shear ramp of the hysteresis loop. This may be because the low initial shear rate is not sufficient to cause the sample to flow, and there is a higher minimum requirement, unlike in the control. This is confirmed by the return to normal shear thinning behavior of the high TA samples at shear rates of approximately 0.5-1 s^{-1}. The high protein sample with 2.5% TA was not expected to have such a high thixotropy because of its poor melting characteristics and low viscosity.

The potential of using rheological data to predict various melting characteristics is an area of interest that warrants further exploration. Complex viscosity and thixotropy values were strongly correlated with the induction time, but the melting rate and residual weight left on the screen showed no discernable trend with the rheological data (**Figure 4.13**). The first drip, indicating induction time, occurs when the sample is unable to withstand the flow of diluted serum out of the matrix. The complex viscosity of a sample, which serves as a measure of its overall resistance to flow, is a significant factor that influences the duration of this measurement. It is reasonable to assume that samples with higher flow resistance will take a longer time for the first drip to fall. However, it is important to consider that the correlation between a sample's resistance to flow and the

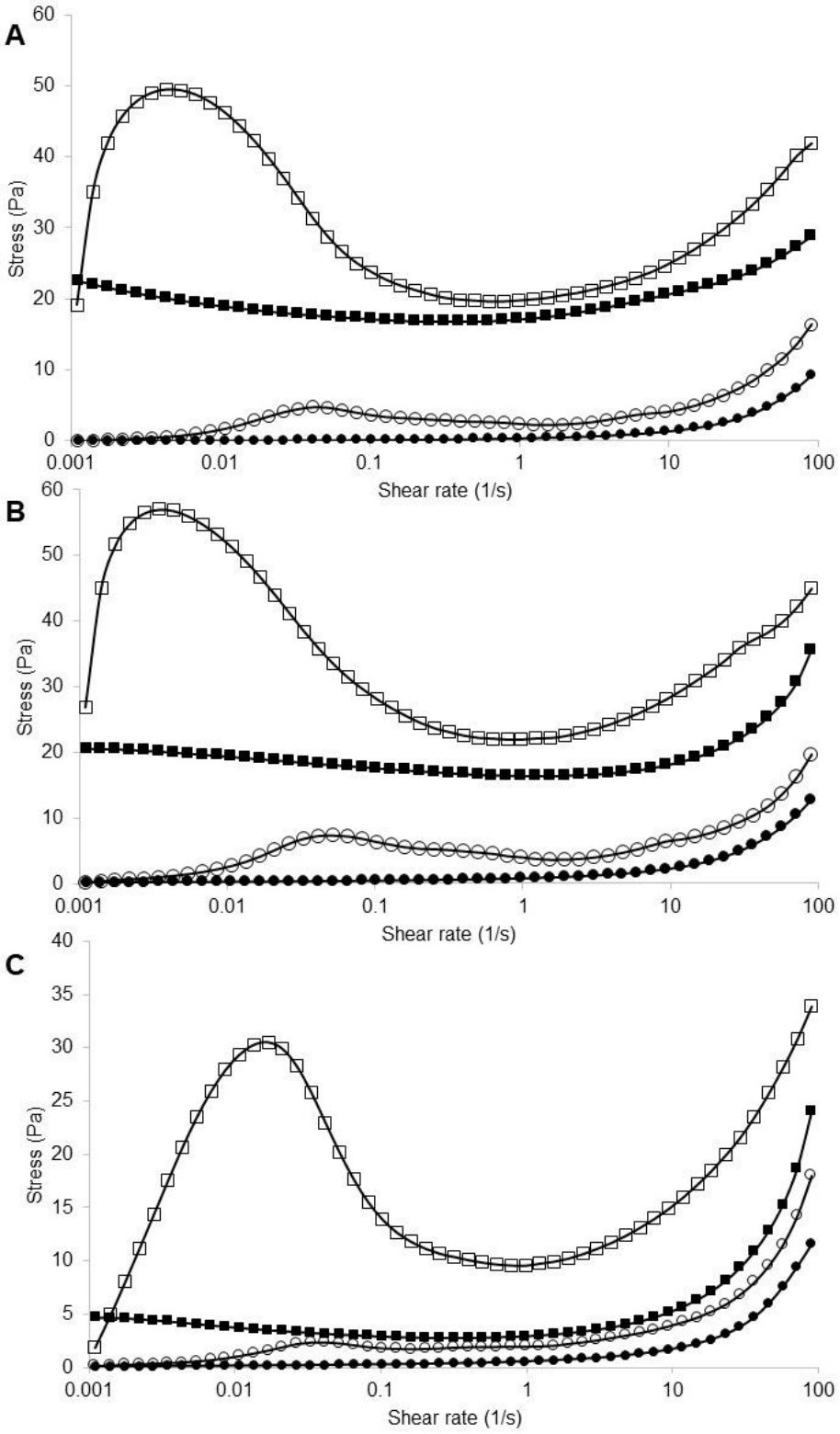

Figure 4.12 Averaged hysteresis graphs for up (open) and down (filled) curves of shear stress against shear rate for standard (12% fat/3% protein) (A), high fat (15% fat/3% protein) (B), and high protein (12% fat/5% protein) (C) ice cream samples at 0% (○) and 2.5% (□) tannic acid.

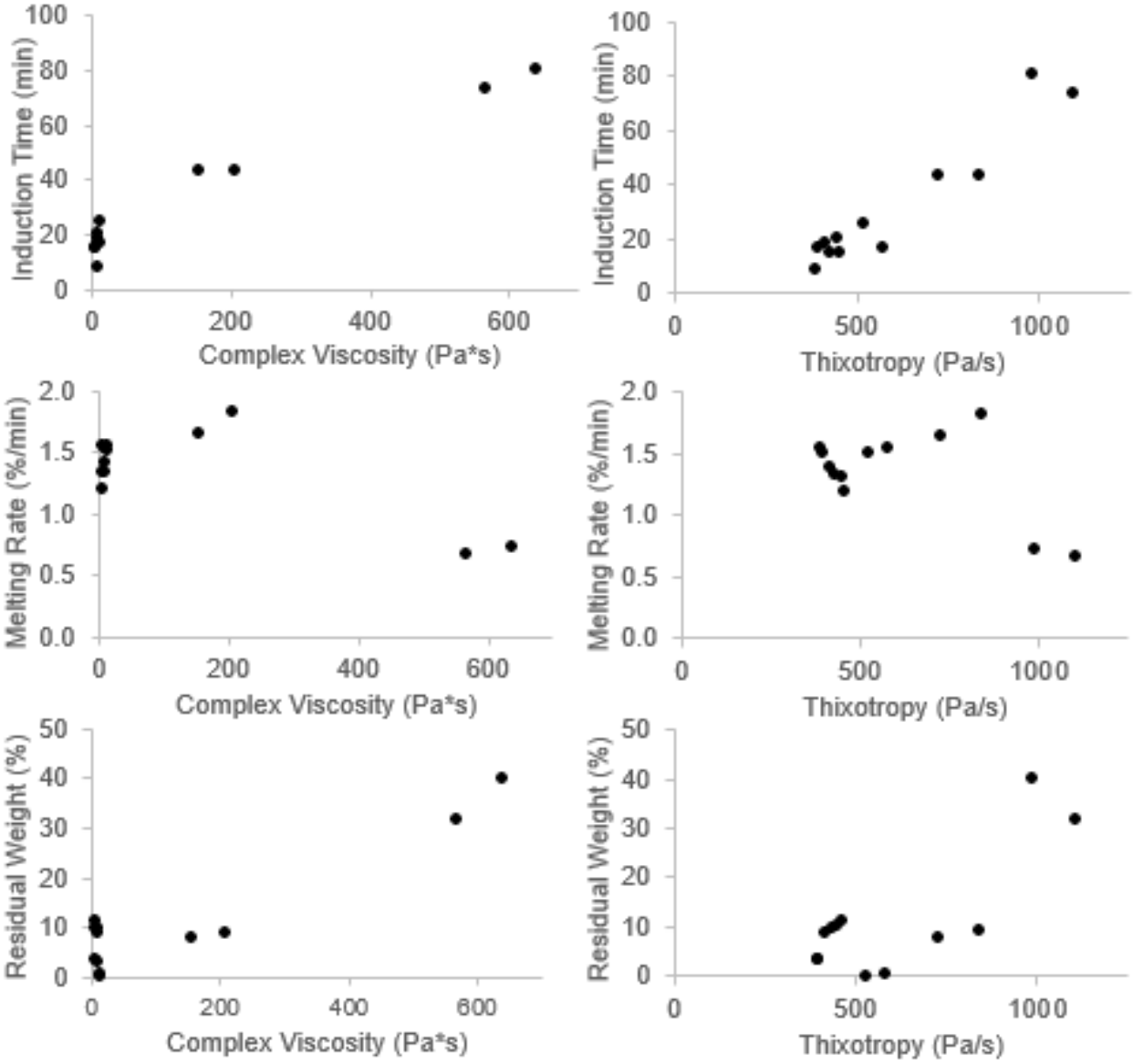

Figure 4.13 Dependence of melting characteristics (induction time, melting rate, and residual weight on screen) on rheological factors (complex viscosity and thixotropy) for the standard (12% fat/3% protein) ice cream samples.

energy necessary to break down its internal structures may not be the only factors that apply to other melting parameters (like melting rate or the leftover sample on the screen after melting). These variables are influenced by a range of parameters, including thermal diffusivity and the presence/size of the stabilizing structures, which can impact the overall outcome.

4.2.4 Overrun

Overrun is a measure of the air content of ice cream, calculated using the percent increase in volume from mix to final product. The overrun decreased with increasing TA% across all formulas (**Table 4.5**). It is particularly important to investigate overrun because of its potential effect on the melting rate of ice cream. Some researchers have stated that high overrun ice creams melt more slowly due to a lower rate of heat transfer from the large volume of incorporated air, the complicated flow path of the melting liquid, and the formation of stabilized air cells that stack into a foam, preventing drip through and complete collapse (Sakurai et al., 1996; Sofjan & Hartel, 2004; Warren & Hartel, 2018). Recent studies by our research group have shown similar correlations under certain conditions (Freire, 2020; Wu, 2023). The high protein formula results agreed with these prior findings since the lower overrun sample melted faster. However, for the standard and high fat formulas, this study found that TA-enriched samples with low overruns melted slower, not faster.

Table 4.5 Overrun values for different ice cream formulations at increasing concentrations of tannic acid (TA)

Formula Type	Fat (%)	Protein (%)	TA (%)	Overrun (%)
Standard	12	3	0	40 ± 0.1^{a}
			0.5	31 ± 2.9^{ab}
			1	32 ± 1.1^{abc}
			1.5	23 ± 0.1^{abc}
			2	23 ± 3.4^{bcd}
			2.5	21 ± 1.2^{bcd}
High Protein	12	5	0	38 ± 0.7^{a}
			2.5	22 ± 0.2^{bcd}
High Fat	15	3	0	34 ± 3.2^{ab}
			2.5	16 ± 4.3^{d}

Mean ± SE are shown. Values in the same column sharing a letter are not significantly different at α=0.05.

The parameter that could potentially connect these variables is viscosity. Higher viscosity ice cream mixes can restrict the amount of air that can be whipped into the final product, leading to a reduction in overrun, especially in a batch freezer, where air is folded into the mix as it freezes. All formulas followed this trend, where a lower overrun was observed in the samples with higher viscosity. **Figure 4.14A** shows a distinct positive increase in melted ice cream viscosity at low overrun values. Since there was no definite relationship between melting rate and overrun (**Figure 4.14B**), the results confirm that the presence of TA can create a matrix that increases the viscosity, making it harder for air to be incorporated. Even though overrun can affect foam structure and flow path of melted liquid due to the volume of air cells, Wu et al. (2019) found that in the presence of a stabilizer, ice cream mix viscosity had a greater impact on meltdown than other characteristics, such as fat destabilization and overrun. The results of this study are

consistent with Wu's observations, as TA exhibits several properties similar to those of a stabilizer.

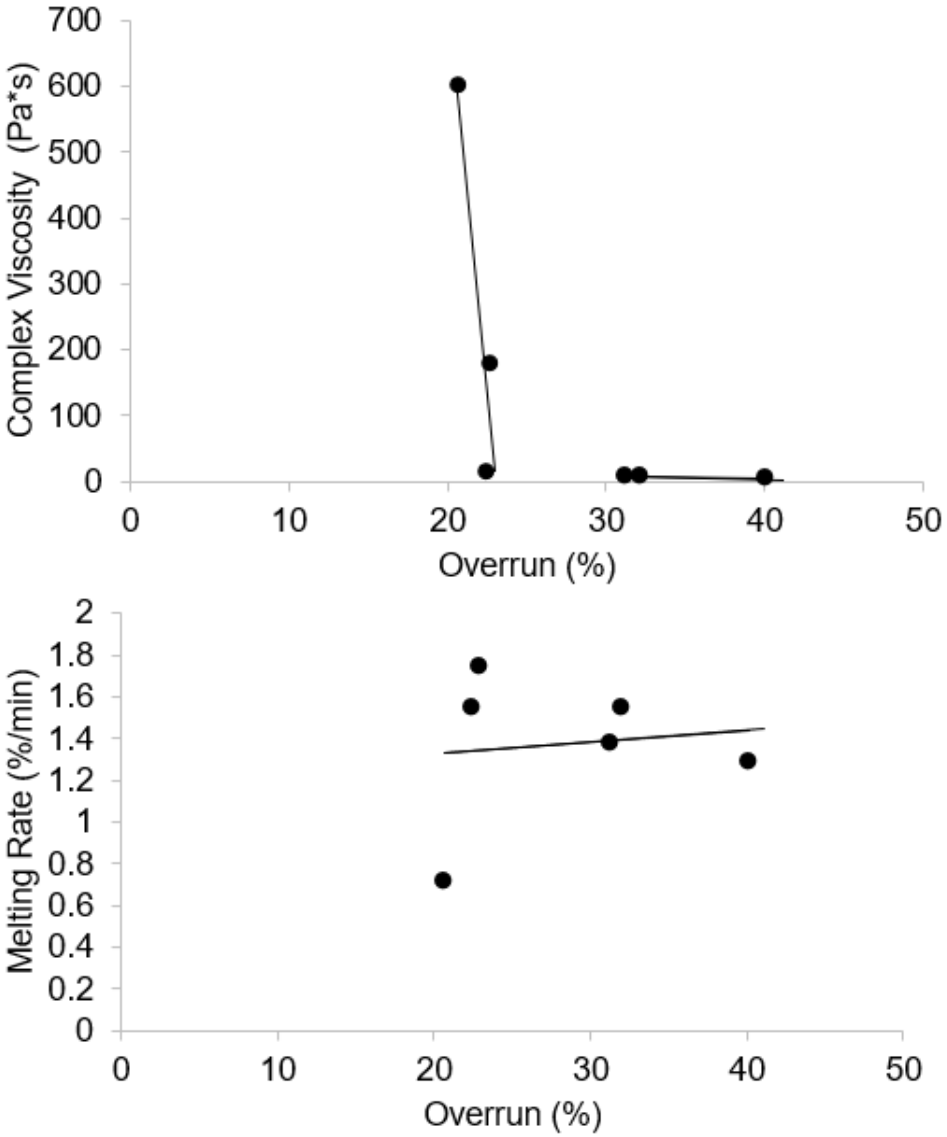

Figure 4.14 Dependence of complex viscosity and melting rate on overrun for the standard (12% fat/3% protein) ice cream samples. Lines simply indicate trends.

4.2.5 Microscope images

In Section 4.1, fat clusters were found in cream with high concentrations of TA, which led to a gel-like matrix that had greater resistance to flow. In the ice cream mix, clusters were detected in the standard formula at 2% and 2.5% TA, as well as in the high fat formula at 2.5% TA. However, no clusters were observed in the standard formula with <2% TA or the high protein ice cream mix at 2.5% TA. In the melted ice cream, clusters were present in all samples with 1% TA or higher, with their size and frequency increasing as the TA% in the standard formula rose. The high fat and high protein samples at 2.5% TA also exhibited clusters (**Figure 4.15**). Any samples without clusters showed no evidence of matrix formation in the emulsion phase, consisting primarily of numerous individual fat globules and a small number of partially-coalesced fat clusters.

The control and 2.5% TA melted samples for each formulation were placed on a heated stage and exposed to elevated temperatures (**Figure 4.16**). The absence of coalescence after ten minutes at 65°C suggests that these structures were being held together by proteins and not due to partial coalescence, which is similar to the findings in Section 4.1. The high-protein formula at 2.5% showed some coalescence of the fat globules after heating, which was unexpected.

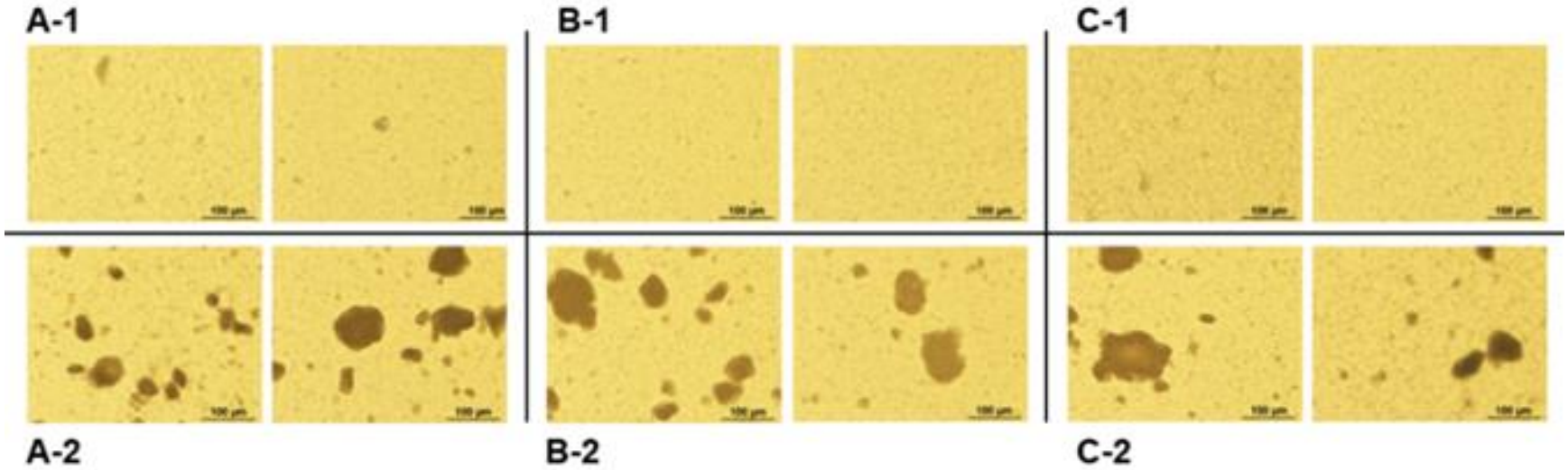

Figure 4.15 Duplicate microscope images of melted ice cream samples with tannic acid (1:10 dilution) at the following formulations: standard (12% fat/3% protein) at 0% TA (A-1) and 2.5% TA (A-2), high protein (12% fat/5% protein) at 0% TA (B-1) and 2.5% TA (B-2), and high fat (15% fat/ 3% protein) at 0% TA (C-1) and 2.5% TA (C-2)

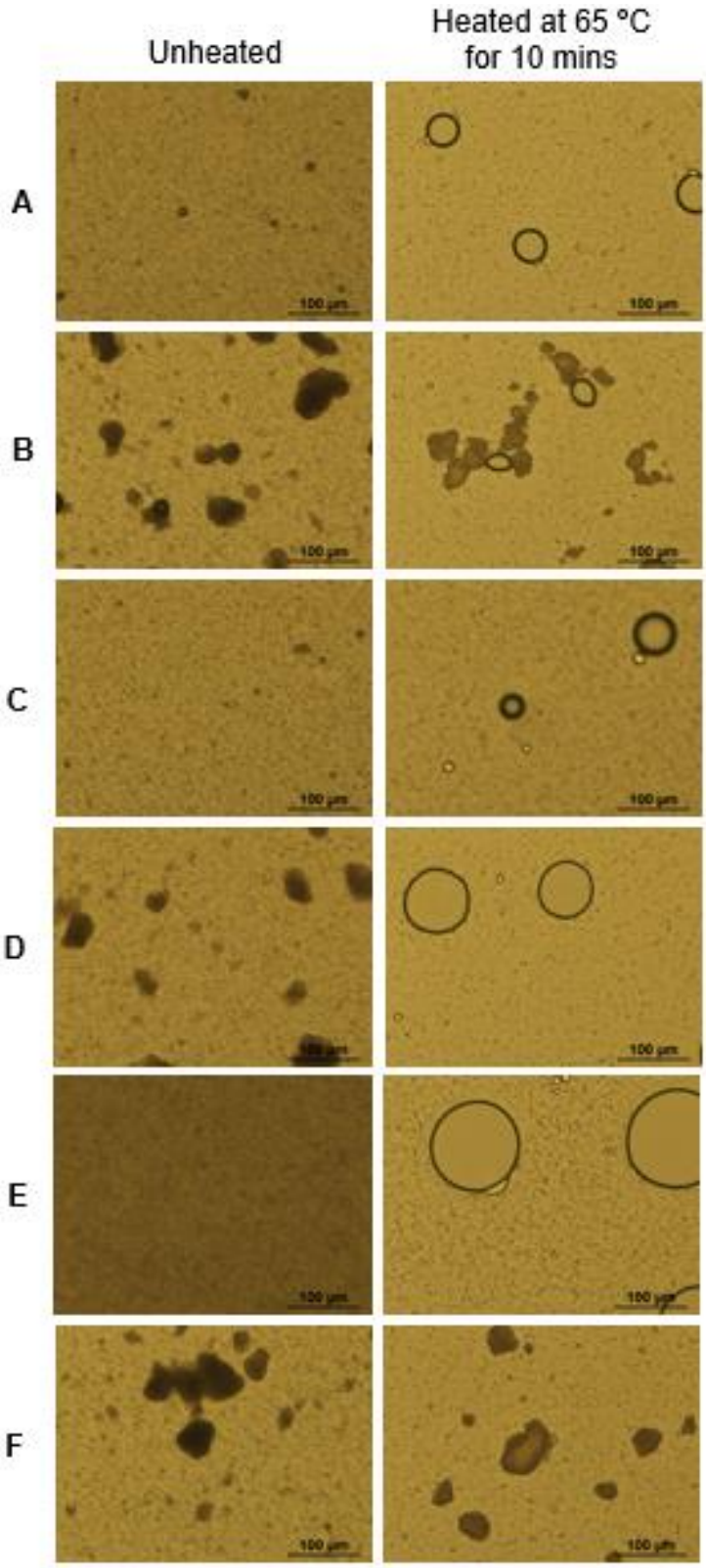

Figure 4.16 Microscope images of the melted ice cream control (standard [A], high protein [C], and high fat [E]) and 2.5% tannic acid (standard [B], high protein [D], and high fat [F]) formulas before and after 10 mins heated at 65ºC

Normally, microscope analysis is paired with particle size distributions in order to quantify the structures that are observed. However, these structures were not able to withstand the shear needed for particle size measurements in the Mastersizer. Although quantitative data is necessary to draw more definitive conclusions, certain qualitative observations can still be made. First, **Figure 4.15** displayed fat clusters in each TA-enriched sample, which supported the trends shown by the thixotropy values (where all 2.5% TA samples showed a significant increase in thixotropy from the control). These structures help explain why the high protein sample at 2.5% TA had a high thixotropy value despite having poor melting characteristics. Another interesting observation is that only the samples with significantly different residual weights after melting (compared to the control) showed evidence of structures in their ice cream mix through microscopy. Notably, the high protein sample at 2.5% TA had no clusters present in the mix and no significant difference in residual weight from its control. Overall, these structures are likely to exert some influence on the system, even if the mechanism is not completely understood.

4.2.6 Ice recrystallization

The purpose of this shelf-life study was to assess whether the addition of TA has the capacity to retard ice crystal growth during storage. The mean ice crystal sizes of the initial ice cream samples (which did not undergo shelf-life abuse) were consistent with the expected ice crystal size of regular ice cream (Goff & Hartel, 2013) (**Table 4.6**). At week two, the sample made with 1.5% TA from the standard formula as well as those made with 2.5% TA across all formulas were not significantly different from their week 0

mean sizes. At week four, only the samples made with 2.5% TA from each formula had not changed from their week 0 size, indicating that no significant growth was observed.

Table 4.6 Ice crystal size of different ice cream samples (standard [12% fat/3% protein], high protein [12% fat/ 5% protein], and high fat [15% fat/3% protein]) at increasing tannic acid (TA) concentrations over a four week period

		Ice Crystal Size (μm)		
Formula Type	TA (%)	Week 0	Week 2	Week 4
Standard	0	36.4 ± 3.7^{DEFGH}	53.2 ± 5.7^{ABCDE}	67.9 ± 9.4^{A}
	0.5	31.6 ± 0.0^{FGH}	51.7 ± 0.0^{ABCDEFG}	58.3 ± 0.0^{AB}
	1.5	33.8 ± 4.9^{EFGH}	42.9 ± 1.4^{BCDEFGH}	52.0 ± 2.1^{ABCDEF}
	2.5	29.8 ± 2.2^{H}	41.1 ± 2.5^{BCDEFGH}	42.7 ± 0.7^{BCDEFGH}
Higher Protein	0	33.9 ± 0.9^{EFGH}	56.4 ± 4.5^{ABCD}	71.1 ± 7.0^{A}
	2.5	31.3 ± 3.1^{FGH}	32.7 ± 0.6^{EFGH}	36.8 ± 3.3^{CDEFGH}
Higher Fat	0	30.6 ± 3.0^{GH}	57.9 ± 4.5^{ABC}	67.6 ± 3.3^{A}
	2.5	32.5 ± 4.0^{EFGH}	34.8 ± 0.2^{EFGH}	38.7 ± 0.7^{BCDEFGH}

Mean ± SE are shown. Values sharing a letter are not significantly different at α=0.05.

It is well established that many polysaccharide stabilizers in ice cream can slow down ice recrystallization and changes in mean ice crystal size (Hagiwara & Hartel, 1996; Sutton & Wilcox, 1998). Regand and Goff (2002) stated that an effective stabilizer would possess the following attributes: increase microviscosity of the serum phase, decrease water diffusion through steric hindrance, and have sufficient visco-elastic properties that are stable at elevated temperatures. Rheological data and micrographs suggest that this TA-rich ice cream may meet these requirements. It is interesting to note that despite not decreasing the melting rate, the high protein formula at 2.5% TA reduced recrystallization, similarly to the other 2.5% TA formulas. This may be attributed to TA's ability to enhance rheological properties, which allows this polyphenol to

stabilize the system against ice crystal growth, but not against melting. Elevated protein content in ice cream is known to interact at the air-water interface and freeze concentrate in the serum phase, which may lead to recrystallization inhibition, though this phenomenon is not universally observed (Wu, 2023)

4.2.7 Discussion

In conventional ice cream, partial coalescence forms a stable fat network that provides structure, which contributes to shape retention and reduced melting rate. This study's findings support the assertion that incorporating polyphenols into ice cream creates a similar fat network capable of reducing the melting rate. As discussed in Section 4.1, these clusters consist of protein-mediated fat aggregates that are bridged together with TA. The microscope images from this experiment clearly show distinct fat aggregates that do not coalesce when heated.

Previous research indicates that TA can interact with both casein and whey at an oil-water interface to stabilize an emulsion (Chen et al., 2022; Yi et al., 2023). The interactions between milk protein and polyphenols are attributed to non-covalent interactions, specifically hydrophobic interactions and hydrogen bonding. Factors that control the creation and strength of these interactions include protein/polyphenol structure, pI, solubility, hydrophobicity, thermal stability, and other environmental factors (Tosif et al., 2021). One hypothesis suggests that, while hydrophobic interactions are the primary driver, hydrogen bonds strengthen the system (Prigent, 2005).

The distinction between partial coalescence and protein/polyphenol-mediated fat aggregation lies in the strength of these networks. Non-covalent bonds are reversible

and can be influenced by numerous factors, while partial coalescence is only reversible at high temperatures or in the presence of specific chemicals, such as SDS. The weakness of the fat clusters observed in **Figure 4.15** is evident in various aspects of this experiment. Unlike in Section 4.1, particle size distributions were unmeasurable for these TA ice cream samples. All melted ice cream samples, regardless of the TA%, exhibited a unimodal peak from the Malvern Mastersizer in the same size range as the mixes, which did not accurately reflect the differences between the samples or the aggregates observed through microscope images. This may be attributed to the agitation applied by the Mastersizer's liquid sampler before measurement, which disrupts these weak clusters.

According to Bock et al. (2022), whey protein-stabilized emulsions transitioned from small oil droplet sizes to larger flocculated particles at higher concentrations of phenolic acids. This was attributed to increased protein aggregation at the oil/water interface, which was facilitated by the reduction in electrostatic forces caused by conformational modifications of proteins and/or the presence of a favorable weakly acidic environment close to the isoelectric point. They also found that the protein/polyphenol ratio is also important to protein-mediated fat aggregates. Samples with a 1:1 ratio showed enhanced emulsion stability, with a homogeneous emulsion cream after 15 days of storage. However, the samples with 1:10 and 1:50 ratios of whey and phenolic acids had high volumes of flocculated particles, with some creaming observed. The study concluded that the decrease in electrostatic repulsion enabled the close proximity of particles, which allowed for bridging flocculation between the polyphenols and the interfacial protein films of the fat globules. Similar results were

observed by Li et al. (2020), who found a shift from a unimodal peak of protein-stabilized emulsion droplets to a bimodal distribution at high concentrations of polyphenols. These findings relate to this section's results by highlighting the importance of various environmental factors in achieving these fat aggregates.

The ratio of proteins to polyphenols influences the properties of a system, and could help explain the threshold effect observed in this study across various variables. Lower concentrations of TA may not have provided the necessary ratio for aggregation. With the goal of creating a fat matrix similar to the partial coalescence found in common ice creams, a lower TA% may have resulted in a more homogenous, single-particle emulsion phase. However, this was not sufficient to cause the targeted changes in the system (i.e., decreasing the melting rate) until the appropriate conditions were met to create a complex network of protein-mediated fat aggregates.

4.2.8 Summary

The incorporation of TA, a pure polyphenol, at varying concentrations in an ice cream model demonstrated its capacity to effectively reduce the melting rate, which was previously thought to be caused by impurities in a polyphenol source rather than the polyphenol itself. The importance of fat was clearly established by the favorable melting outcomes of the TA-enriched high fat ice cream when compared to the high protein formulation. The ability of TA to limit ice recrystallization at high concentrations during storage highlighted a potential new use for phenolic-rich ingredients.

4.3 The effect of high phenolic extracts on ice cream and their ability to replace commercial stabilizers.

While the previous section's results showed that tannic acid functioned somewhat like a stabilizer, this experiment aimed to expand upon these findings by investigating the potential of polyphenol-rich extracts (>85% polyphenols) to reduce the melting rate, increase viscosity, and hinder ice recrystallization. These extracts were not only assessed for their ability to imitate but also replace a common stabilizer blend (locust bean gum, guar gum, and carrageenan at 0.2% concentration in ice cream) by analyzing these enriched ice cream samples with and without stabilizers present. Green tea extract is rich in catechins, while grapeseed extract Is high in condensed tannins. The higher fat formula (15% fat/3% protein) from section 4.2 was modified for use in these experiments, and extracts were incorporated at a concentration of 3% into the ice creams. The phenolic content of the extract and resulting ice creams are found in **Table 4.7**.

4.3.1 pH

It is important to verify the pH of these extract-enriched samples since extracts can contain organic acids (Das et al., 2019). The pH of ice cream mixes (**Table 4.8**) were not below the isoelectric point of the milk proteins found in ice cream. These results support our previous hypothesis that polyphenols, and not acidity, are crucial in forming aggregates that are resistant to flow, leading to an ice cream with a low melting rate.

Table 4.7 Total polyphenols and proanthocyanidin (PAC) in extract and final ice cream samples with 3% extract added.[1]

Extract	Total polyphenols in extract (%)	Total PAC in extract (%)	Total polyphenols in ice cream (%)	PAC content in ice cream (%)
green tea extract	97.6 ± 8.0	38.7 ± 7.4	2.9	1.2
grape seed extract	80.7 ± 7.0	13.8 ± 0.3	2.4	0.4

Mean ± SE are shown.

[1]Data supplied by Bolling Lab (University of Wisconsin-Madison)

Table 4.8 pH of extract-enriched ice cream mixes (control [yellow], grapeseed [purple], green tea [green]) with and without stabilizer.

	Stabilizer (%)	
	0	0.2
pH	6.3 ± 0[a]	6.3 ± 0.1[a]
	5.75 ± 0.05[b]	5.5 ± 0[b]
	5.55 ± 0.05[b]	5.65 ± 0.05[b]

Mean ± SE are shown. Values sharing a letter are not significantly different at α=0.05.

4.3.2 Microscope Images

Microscope imaging was able to confirm the presence of fat aggregates that resemble the ones observed in the previous TA studies. Every extract-enriched sample had large aggregates in the ice cream mix and melted ice cream (**Figure 4.17** and **Figure 4.18**). After slides were heated to 65°C on a heated stage and held for 10 mins, these aggregates did not fully coalesce, and some individual fat globules can be distinguished in the clustering, implying these are also protein-mediated fat aggregates (**Figure 4.19**). Some large fully-coalesced fat globules were also present, originating from the other individual and partially-coalesced fat globules also in the matrix. This

aggregation was most likely facilitated by the polyphenols in green tea and grapeseed extract, which are known to interact with milk proteins. Fuhrmann et al. (2019) also observed large clusters of fat at high concentrations of grapeseed extract when added to a protein-stabilized emulsion.

4.3.3 Melting Characteristics

After identifying these extract-induced fat aggregates, their impact on the ice cream system was examined with meltdown testing. **Table 4.9** illustrates that these extracts had a significant influence on the melting characteristics of these ice creams. In particular, both green tea extract samples resulted in a substantial decrease in the melting rate and an increase in the induction time. The melting profiles in **Figure 4.20A-B** show that these green tea samples failed to reach the stationary phase of the melting process during the four-hour testing period.

After four hours, the samples began to dry out on the outer surface (as depicted in **Figure 4.21**), leading to the termination of the test. This drying and subsequent moisture loss to the atmosphere makes the melting profile less accurate. After four hours at ambient temperature, both green tea samples released minimal liquid, leaving ≥90% of the sample on the screen. Notably, the fluid that dripped into the beaker was nearly transparent. These findings demonstrate that green tea extract successfully reduced the melting rate. The stabilizer had a negligible impact on these outcomes, as the results were similar with and without the stabilizer, suggesting that the extract effectively stabilized the system against flow during melting.

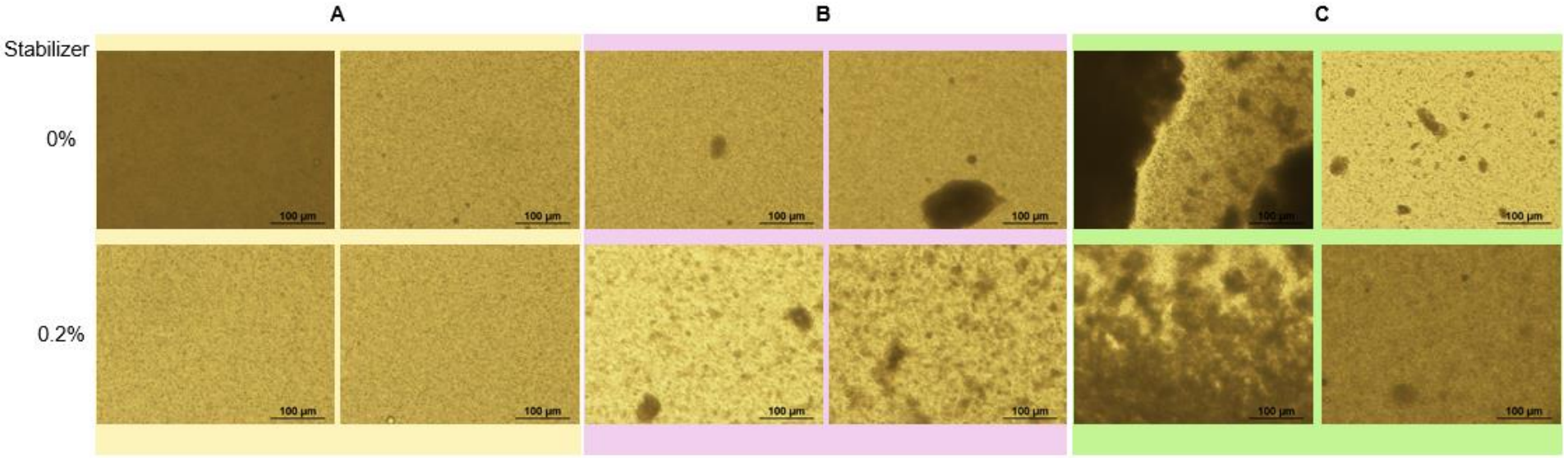

Figure 4.17 Microscope images of extract-enriched ice cream mixes (control [A], grapeseed [B], green tea[C]) with and without stabilizer.

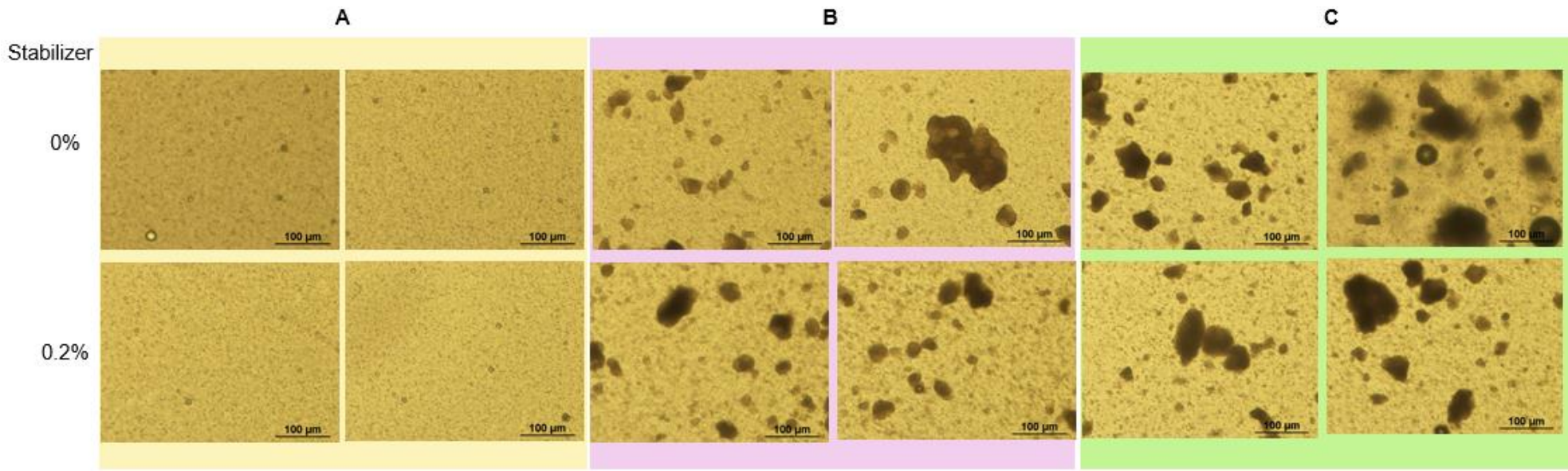

Figure 4.18 Microscope images of melted extract-enriched ice cream (control [A], grapeseed [B], green tea [C]) with and without stabilizer.

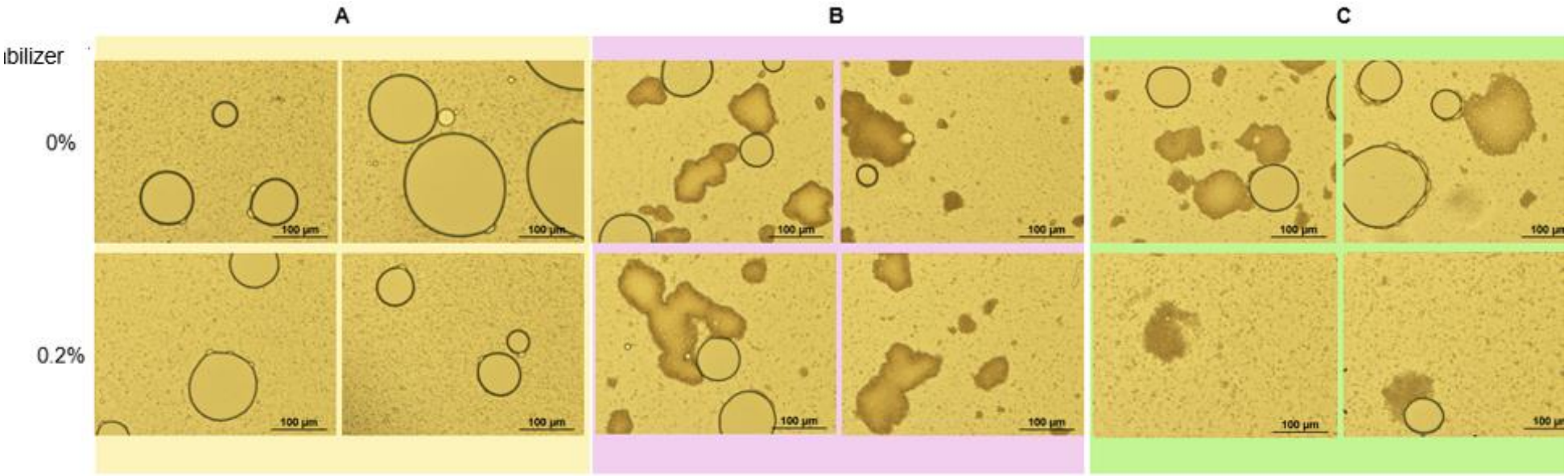

Figure 4.19 Microscope images of melted extract-enriched ice cream heated to 65°C for 10 mins on a heated stage (control [A], grapeseed [B], green tea [C]) with and without stabilizer.

Table 4.9 Melting characteristics of extract-enriched ice creams (control [yellow], grapeseed [purple], green tea [green]) with and without stabilizer.

	Stabilizer (%)	
	0	0.2
Melting rate (%/min)	0.71 ± 0.02[a]	0.63 ± 0.03[a]
	0.51 ± 0.01[b]	0.03 ± 0[c]
	0.04 ± 0.01[c]	0.025 ± 0.01[c]
Induction time (min)	17 ± 3[c]	30 ± 5[c]
	67 ± 2[b]	107 ± 2[a]
	116 ± 7[a]	112 ± 5[a]
Complete melting time (min)	141 ± 3	140 ± 5
	130 ± 6	N/A[1]
	N/A[1]	N/A[1]
Residual weight on the screen (%)	3 ± 1[a]	6 ± 0[a]
	21 ± 2.4[b]	93 ± 0.8[c]
	90 ± 2.4[c]	93 ± 1.2[c]

Mean ± SE are shown. Variables sharing a letter within a measurement are not significantly different at α=0.05.

[1]N/A was assigned to samples that reached 4 hours of testing without reaching the stationary phase. The testing was terminated because the sample was showing signs of drying.

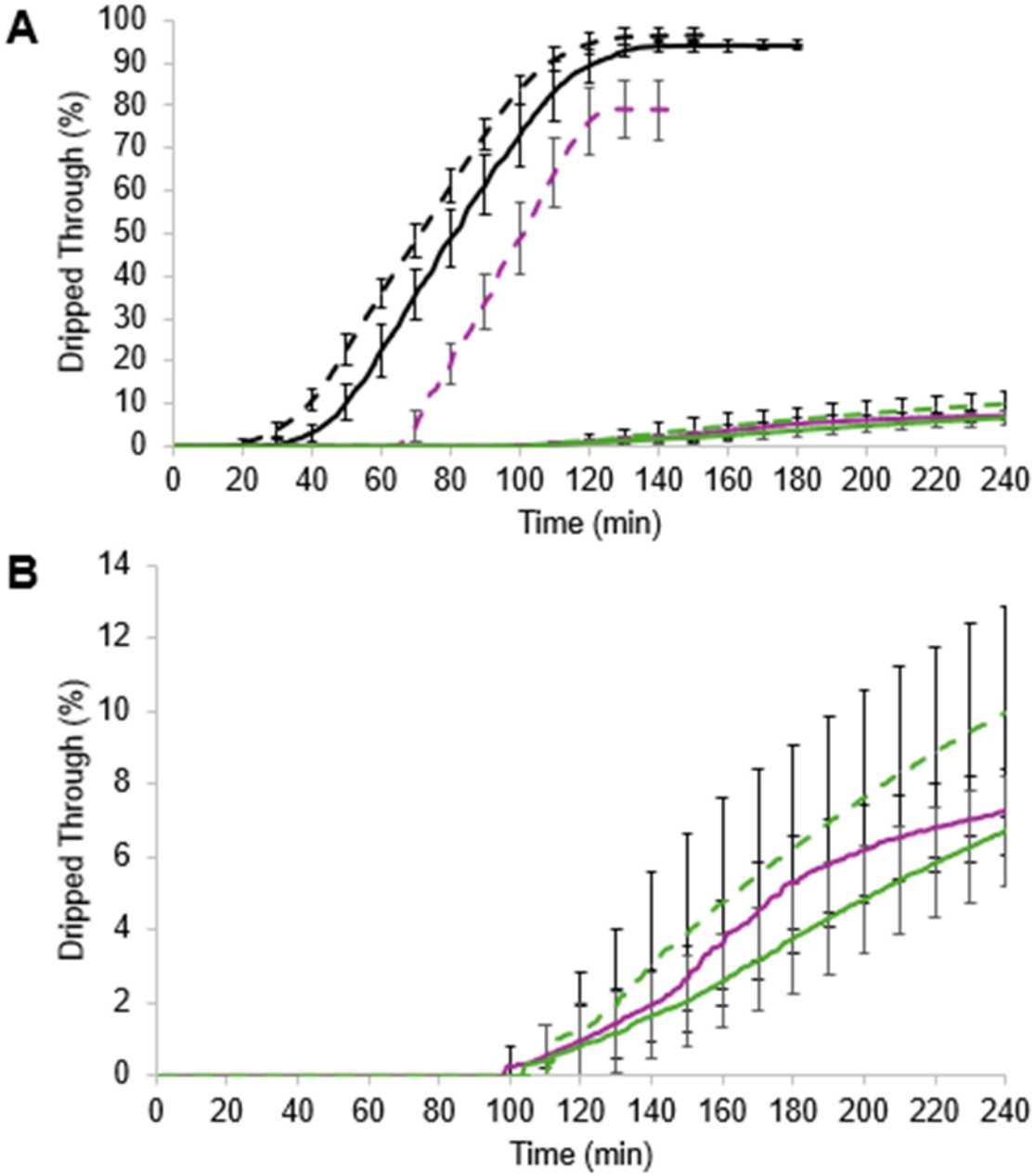

Figure 4.20 (A) Melting profiles of extract-enriched ice creams (control [black], grapeseed [purple], green tea [green]) with (solid line) and without (dashed line) stabilizer. (B) A closer look at the melting profiles of a few extract-enriched samples [green tea with stabilizer (green, solid), green tea with no stabilizer (green, dashed), and grapeseed with stabilizer (purple, solid)]. Error bars represent the standard deviation of mean values measured in triplicate.

Figure 4.21 Image of surface drying on an extract-enriched ice cream sample after four hours at ambient temperature.

The grapeseed extract sample with stabilizers produced results similar to those of both green tea samples in all measurements. However, the grapeseed extract ice cream without stabilizer had a much faster melting profile (**Figure 4.20A**). Although the grapeseed extract sample without stabilizers resulted in a slight decrease in the melting rate and a small increase in the induction time compared to the control, this formula had a minimal effect on the total melting time and residual weight on the screen.

The images in **Figure 4.22** illustrate the progression of the control and extract ice cream samples melting on the screen. Although the green tea samples and the grapeseed extract with stabilizer sample maintained their shape better than the other samples, they still exhibited some signs of collapse (such as loss of height and slouching). The samples initially had ice crystals to provide structure. However, as the

ice crystals melted, the samples began to lose shape while still resisting flow, as demonstrated by the minimal drip-through observed.

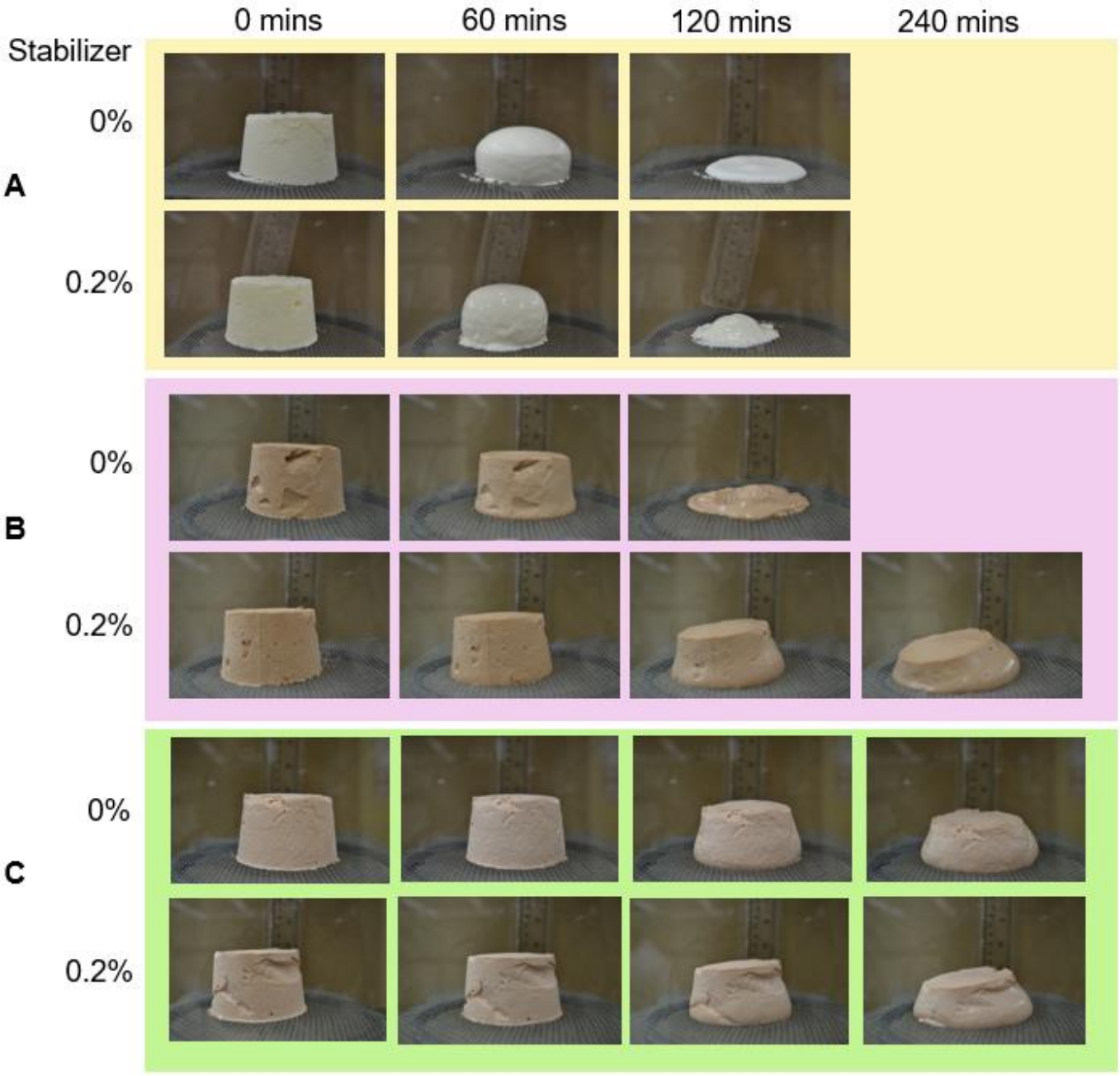

Figure 4.22 Melting images of extract-enriched ice cream samples (control [yellow], grapeseed [purple], green tea [green]) with and without stabilizer over 240 minutes.

When reviewing the literature to understand the implications of these meltdown results, it is evident that existing studies support the results of the green tea extract samples, whereas the grapeseed extract samples appear to contradict several previously established trends. Green tea polyphenols are well-known for their interactions with both casein and whey (Hasni et al., 2011; Kanakis et al., 2011), as well as their ability to bind to milk proteins in protein-stabilized emulsions (Sabouri et al., 2015). Tian et al. (2021) reported that increased levels of green tea polyphenols in a whey protein-stabilized emulsion resulted in the aggregation of fat droplets facilitated by protein/polyphenol interaction. This supports the existence of a threshold effect of polyphenols in creating these fat clusters, as previously observed in Section 4.2. One study observed that adding green tea powder to ice cream prolonged the induction time, although the underlying mechanism remained unclear (Kavaz et al., 2017). These findings provide valuable insights into the microstructure of green tea extract-enriched ice creams that successfully reduced the melting rate.

However, the outcomes of the grapeseed extract samples diverged from previously established trends. The grapeseed extract sample without stabilizer had a much higher melting rate than the green tea extract sample without stabilizer. This implies that the polyphenols in grapeseed extract interacted with milk proteins to form fat clusters differently than the polyphenols in green tea extract, despite both samples displaying aggregates (**Figure 4.18**). However, Liu et al. (2024) recently found that proanthocyanidins with a higher degree of polymerization, more commonly present in grape seed extract, increased the viscosity and particle size of protein-mediated fat aggregates in cream. Grapeseed extract has also demonstrated the ability to create

protein-mediated fat aggregates at high concentrations (Fuhrmann et al., 2019), and other grape-derived polyphenols have been shown to decrease the melting rate (Hwang et al., 2009; Pundhir et al., 2018; Salem et al., 2014; Sharma et al., 2015). It is unknown why the targeted fat clustering was observed without the resultant decrease in melting rate for the grapeseed extract ice cream without stabilizers.

Moreover, there may be some interaction between the stabilizer blend and the grapeseed extract proanthocyanidins, as the sample with stabilizers successfully decreased the melting rate. Several studies have confirmed that not only do polysaccharide stabilizers complex with polyphenols, but these aggregates can also impact the rheology of the system and decrease the gum's ability to hold water (Tudorache & Bordenave, 2019). However, like many interactions, the efficacy of this aggregation depends on molecular structure and concentration (Dridi & Bordenave, 2021). Further investigation is warranted to determine whether the type and concentration of gums utilized in this experiment were impacted by polyphenol-polysaccharide interactions. Another area of research has revealed that carbohydrates, such as polysaccharide stabilizers, can interfere with protein-polyphenol interactions. One study discovered that grapeseed procyanidin fractions with low degrees of polymerization were more susceptible to disruption by polysaccharide gums (Mateus et al., 2004). These findings contradict the observations in this study since this could potentially hinder the polyphenol interactions targeted by this work, highlighting the need for additional research.

4.3.4 Rheology

Rheological testing is used to relate the effect of fat aggregates, as seen in the micrographs of **Figure 4.18**, to the melting characteristics. As shown in **Table 4.10**, both green tea extract ice creams and the grapeseed extract with stabilizer sample exhibited the highest complex viscosity and thixotropic values in this experiment. This highlights these sample's resistance to flow and extensive structure present after the ice was melted since measurements were taken at 0°C.

Table 4.10 Rheological data from extract-enriched ice creams (control [yellow], grapeseed [purple], green tea [green]) with and without stabilizer.

	Stabilizer (%)	
	0	0.2
Complex Viscosity (Pa*s)	2 ± 0.3^{c}	4.2 ± 1.8^{c}
	579 ± 3.3^{b}	622 ± 62^{b}
	1921 ± 154^{a}	808 ± 75^{b}
Thixotropy (Pa/s)	310 ± 3.4^{b}	378 ± 3.3^{b}
	462 ± 24^{b}	877 ± 17^{a}
	843 ± 62^{a}	947 ± 108^{a}

Mean ± SE are shown. Variables sharing a letter within a measurement are not significantly different at α=0.05.

The ice creams with higher rheological measurements, such as the grapeseed with stabilizer and all green tea samples, demonstrated the lowest melting rates, longest induction and complete drip time, and highest residual weight on the screen. These findings, similar to Section 4.2, assert that the aggregate structures found in these samples were able to impact the rheological behavior of the ice cream, making the product more resilient to melting than normal ice cream. Despite their drastically different melting profiles, an intriguing observation was the lack of a significant difference in the complex viscosity of the grapeseed extract samples. This trend contradicts the expectation that slower melting rates are associated with ice creams with higher viscosities, further highlighting that these samples do not conform to expected outcomes.

4.3.5 Overrun

Unlike Section 4.2, the overrun measurements for this experiment were not significantly different from each other (**Table 4.11**). Previously, a relationship was established between high viscosity samples and lower overrun levels, which may have contributed to the slower melting rate of tannic acid ice cream samples. However, these polyphenol-rich ice creams exhibited a wide range of viscosities (~2 to 2000 Pa*s) yet still had minimal differences in overrun, which may be attributed to the type of batch freezer used and its ability to whip the high viscosity ice cream mixes. This experiment allowed for the observation of the impact of polyphenols on the melting rate of an ice cream system without the confounding variable of overrun.

Table 4.11 Overrun values of extract-enriched ice creams (control [yellow], grapeseed [purple], green tea [green]) with and without stabilizer.

	Stabilizer (%)	
	0	0.2
Overrun	34 ± 4.9[a]	29 ± 9.2[a]
	31 ± 3.7[a]	29 ± 0.2[a]
	21 ± 6.4[a]	28 ± 2.8[a]

Mean ± SE are shown. Values sharing a letter are not significantly different at α=0.05.

4.3.6 Ice recrystallization in storage

Another way to assess these extracts' ability to replace stabilizers is by analyzing their capacity to maintain a favorable ice crystal distribution during storage. A shelf-life study revealed that both green tea and grapeseed extracts were effective in impeding ice crystal growth. After four weeks of storage, these extract-enriched samples (with and without stabilizers) showed little to no change in ice crystal size (**Table 4.12**). This may be attributed to the high viscosity of these samples, which inhibited the mobility of the serum phase and the growth of ice crystals. These results showcase the potential utilization of these extracts in enhancing the quality of ice cream over storage time.

Table 4.12 Ice crystal sizes of extract-enriched ice creams (grapeseed and green tea) with and without stabilizer during a four-week shelf-life study.

		Mean Size (µm)	
Extract	Stabilizer (%)	Week 0	Week 4
Control	0	36.2 ± 0.2[B]	86.4 ± 14[A]
	0.2	39.5 ± 4.3[B]	82.5 ± 13[A]
Grapeseed	0	34.2 ± 1.7[B]	33.2 ± 3.8[B]
	0.2	31.1 ± 3.2[B]	35.7 ± 0.1[B]
Green Tea	0	32.7 ± 0.2[B]	34.9 ± 0.9[B]
	0.2	32.9 ± 1.3[B]	37.6 ± 0.7[B]

Mean ± SE are shown. Values sharing a letter are not significantly different at α=0.05.

4.3.7 Summary

The primary objectives of this study were to assess the efficacy of polyphenol-rich extracts in producing a low melting rate ice cream and replacing ice cream stabilizers. The green tea and grapeseed extracts created an ice cream matrix that could resist the flow of diluted serum for over four hours with minimal dripping, had good shape retention, and inhibited ice recrystallization. These are characteristics of a successful “no-melt” or “no-collapse” ice cream, particularly given typical consumer expectations. The success of these extracts is likely due to their phenolic profiles and compatibility with milk proteins.

4.4 The effect of fruit extracts, freeze-dried powders, and juice concentrates on ice cream

This experiment aimed to investigate whether various fruit preparations could influence the melting and rheological properties of ice cream. Polyphenol sources were added to the ice cream mix at a concentration of 3.5%, with the final percentage of polyphenols in the ice cream from each source shown in **Table 4.13**. This addition level was inspired by Bilbao-Sainz et al. (2019), who observed that adding this quantity of freeze-dried powder resulted in a frozen dessert that remained stable and did not drip or deform after 2 hours.

Table 4.13 Total polyphenols and proanthocyanidin (PAC) in extract and final ice cream samples with 3.5% polyphenol source added.[1]

Crude Extract	Total polyphenols in source (%)	Total PAC in source (%)	Total polyphenols in ice cream (%)	PAC content in ice cream (%)
Blueberry extract	39 ± 1.3	5.3 ± 0.4	1.4	0.2
Cranberry extract	36 ± 0.2	3.4 ± 0.3	1.2	0.1
Strawberry freeze-dried powder	0.3 ± 0.01	0.02 ± 0.0	0.01	0.001
Blueberry freeze-dried powder	0.8 ± 0.06	0.06 ± 0.001	0.03	0.002
Strawberry juice concentrate	1.1 ± 0.1	0.042 ± 0.001	0.04	0.001

Mean ± SE are shown.

[1]Data supplied by Bolling Lab (University of Wisconsin-Madison)

4.4.1 pH and overrun

All pH values of ice cream mixes made with fruit extracts and freeze-dried powders were above the isoelectric point of milk proteins (**Table 4.14**), except for the strawberry juice concentrate sample, which corresponds to the high acidity of the ingredient (~pH 3.3). Acid gelation of milk occurs when aggregated proteins form a continuous network of structures throughout a system. At low pH, the surface charge of caseins decreases, resulting in a reduction of electrostatic repulsion and solubilization of colloidal calcium phosphate, leading to the disruption of the casein micelle structure (Lucey, 2004; Wang & Zhao, 2022). Whey proteins, especially when denatured by heat treatment, can aggregate due to the exposure of hydrophobic groups and contribute to the gel matrix (Vasbinder et al., 2004). It is possible that the high acidity of this sample might influence certain sensory and textural attributes.

In terms of overrun, no significant differences were observed among the samples, similar to section 4.3, which suggests that it can be excluded as a variable when assessing melting values.

Table 4.14 pH and overrun values of ice cream mixes made with various polyphenol sources.

Formula	pH	Overrun (%)
Control	6.3 ± 0.1^{a}	28.5 ± 9.2^{a}
Blueberry extract	5.6 ± 0^{bc}	36.6 ± 0.9^{a}
Cranberry extract	5.6 ± 0^{bc}	42.55 ± 0.55^{a}
Strawberry freeze-dried powder	5.4 ± 0.1^{c}	32 ± 0.1^{a}
Blueberry freeze-dried powder	6 ± 0.1^{ab}	38.8 ± 6.3^{a}
Strawberry juice concentrate	3.6 ± 0.1^{d}	31.4 ± 9.1^{a}

Mean ± SE are shown. Variables within a measurement sharing a letter are not significantly different at α=0.05.

4.4.2 Microscope images

As depicted in **Figure 4.23**, no aggregates were detected in the ice cream mixes made with crude extract or freeze-dried powder samples. On the other hand, **Figure 4.24** reveals that the melted ice cream samples made with crude extract (blueberry and cranberry) have fat clusters, while the freeze-dried samples did not. The addition of freeze-dried powders to the ice cream contributed less than 0.05% polyphenols to the final product. As previously discussed, the absence of clusters in low phenolic content samples may be attributed to the need for a threshold polyphenol concentration in this ice cream system. Since there were no aggregates in the ice creams made with freeze-dried powders, they looked similar to the control when heated on a heated stage, containing mostly individual fat globules with many large, fully-coalesced globules

(**Figure 4.25**). When the aggregates found in the crude extract samples were heated, the cranberry extract ice cream aggregates were heat stable, suggesting that they are protein-mediated fat aggregates. However, the blueberry extract ice cream did not perform as expected, showing many large, fully-coalesced fat globules. Although this difference may be associated with a characteristic of the blueberry extract, such as its phenolic profile, it could merely be due to a low number of clusters that were not captured by the sample preparation process.

The strawberry juice concentrate sample is unique compared to other samples, showing an aggregate protein network in the mix and melted ice cream due to its low pH. As expected, these protein structures are stable at elevated temperatures (**Figure 4.25**). In the melted ice cream, the pH-induced aggregates of the strawberry juice concentrate look different from the polyphenol-induced aggregates of the crude extracts. However, there are some visual similarities between the heated aggregates across samples, as they contain some intact single fat globules dispersed within the aggregates. These similarities may translate into similar melting and rheological parameters in the following sections.

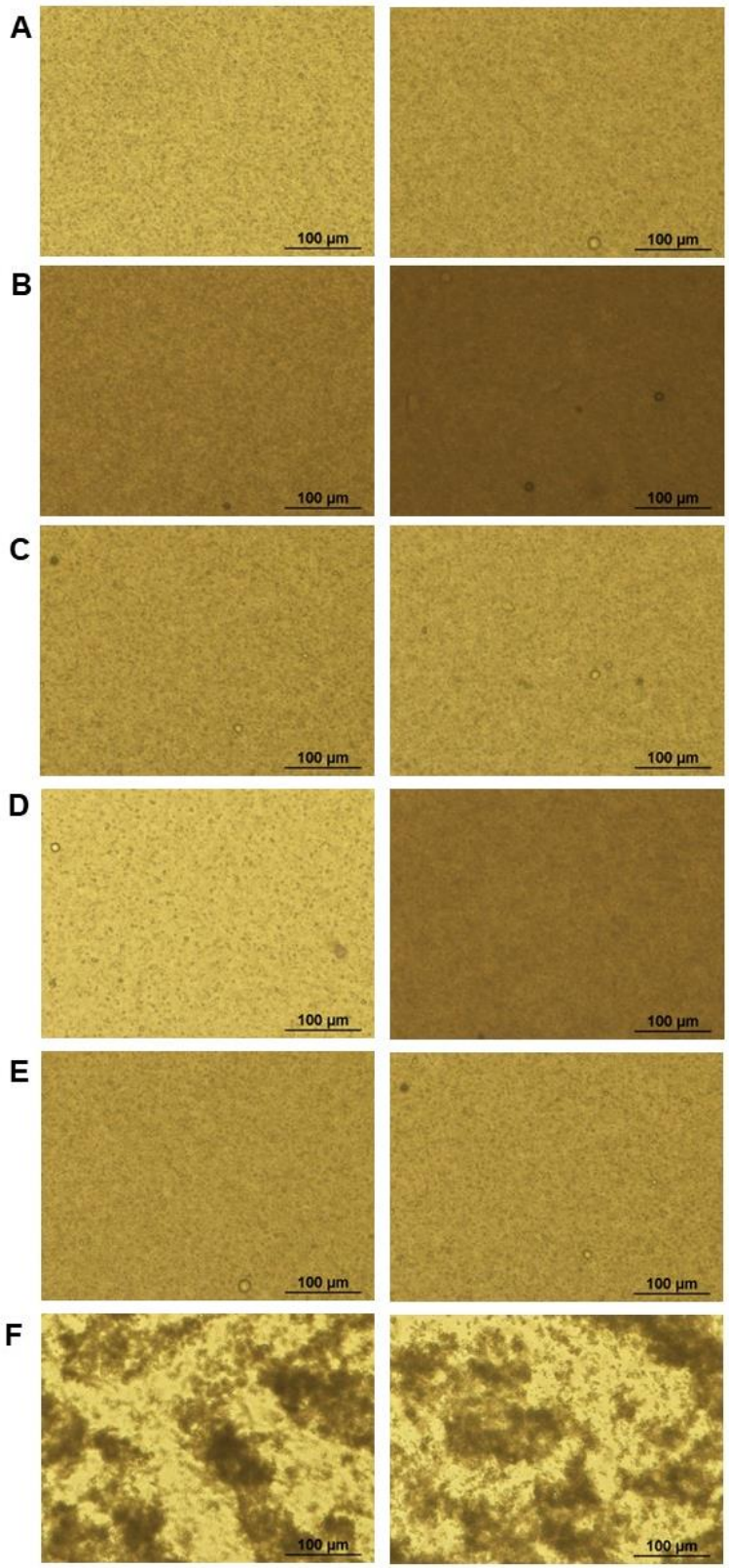

Figure 4.23 Microscope images of ice cream mixes with various polyphenol sources (control [A], blueberry extract [B], blueberry free-dried powder [C], cranberry extract [D], strawberry freeze-dried powder [E], and strawberry juice concentrate [F]).

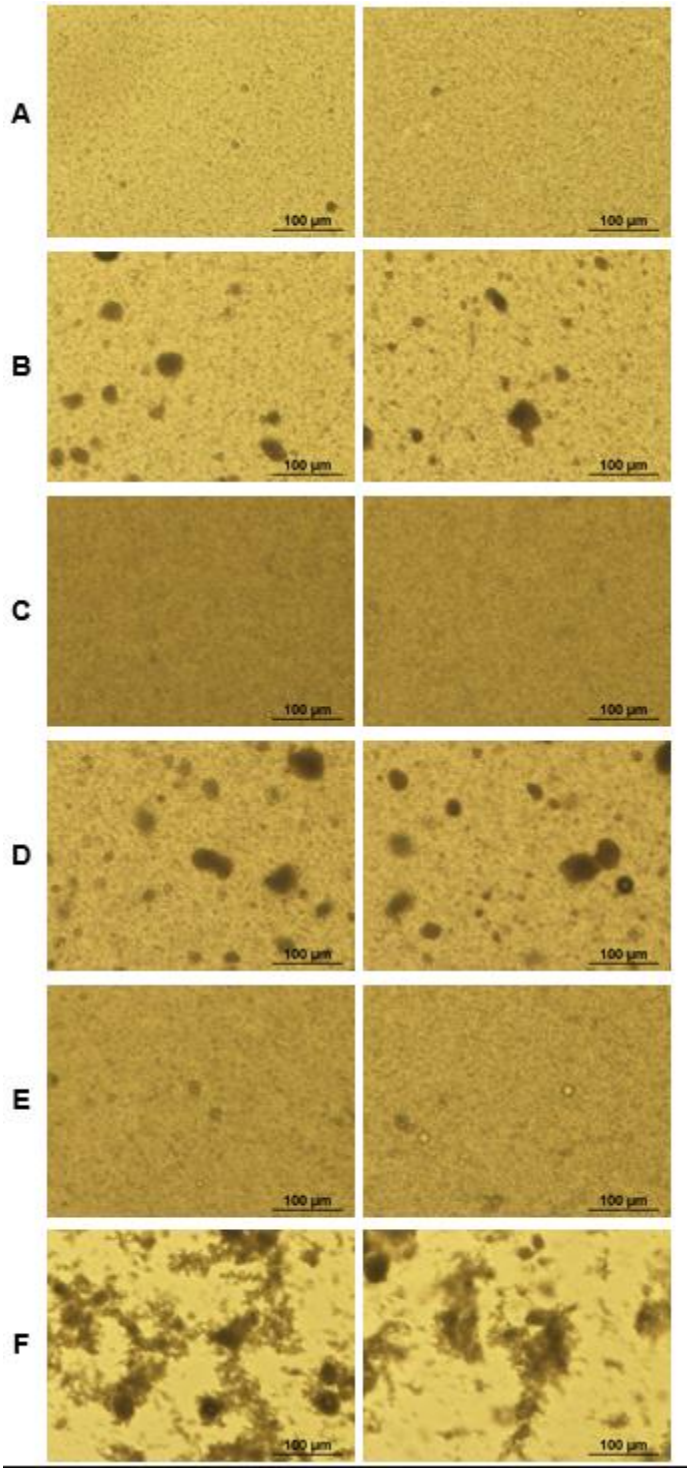

Figure 4.24 Microscope images of melted ice cream with various polyphenol sources (control [A], blueberry extract [B], blueberry free-dried powder [C], cranberry extract [D], strawberry freeze-dried powder [E], and strawberry juice concentrate [F]).

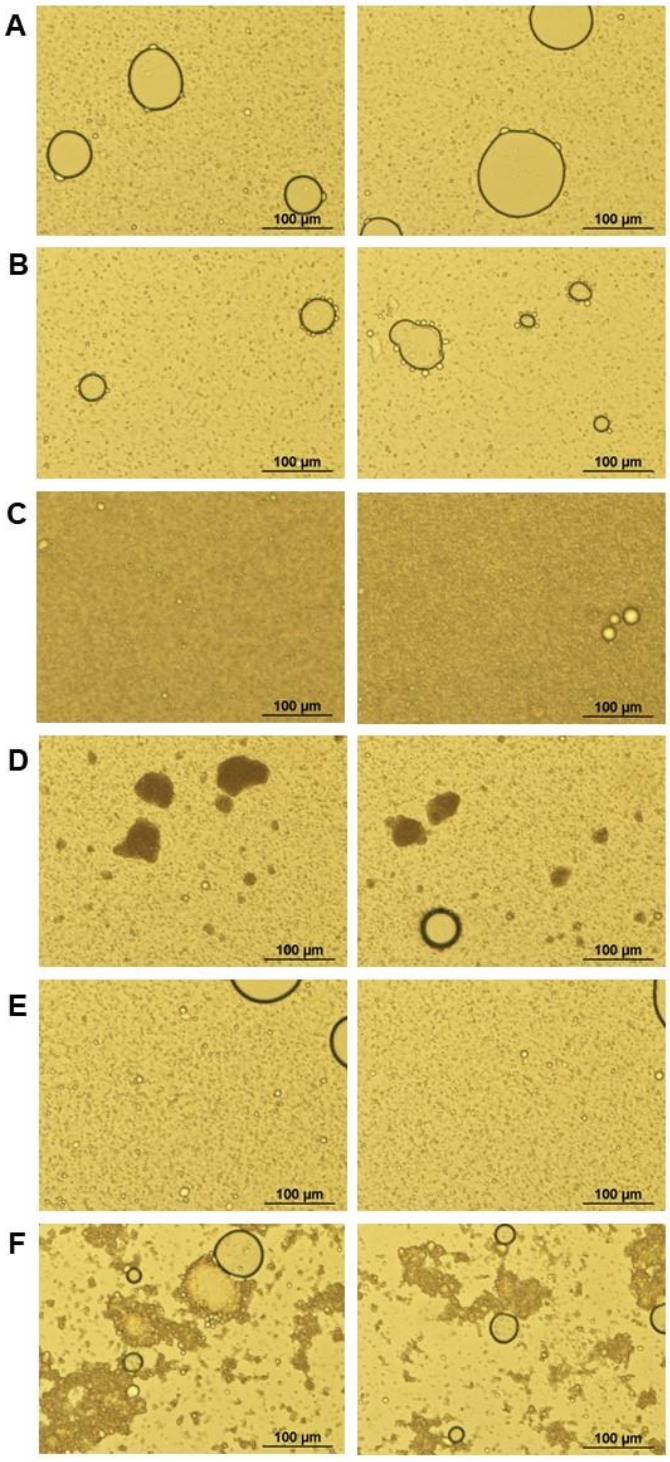

Figure 4.25 Microscope images of melted ice creams with various polyphenol sources ((control [A], blueberry extract [B], blueberry free-dried powder [C], cranberry extract [D], strawberry freeze-dried powder [E], and strawberry juice concentrate [F]) heated to 65°C for 10 mins.

4.4.3 Melting Characteristics

Table 4.15 demonstrates that the melting rates for all samples were not significantly different from the control, although the crude extracts resulted in slightly lower values. Unlike the polyphenol extracts in Section 4.3, samples reached the stationary phase of the melting profile within 150 mins (**Figure 4.26**). The total melting time and residual weight measurements were also not statistically different among the various fruit preparations. However, the induction times for the ice creams with crude extracts and juice concentrate were significantly longer, by approximately 30 minutes, compared to the freeze-dried powders.

The crude extracts (blueberry and cranberry) used in this experiment were less effective in reducing the melting rate of ice cream when compared to the polyphenol-rich extracts (green tea and grapeseed) in Section 4.3. When examining total polyphenols (%) and PAC content (%) from both experiments, the green tea and grapeseed extracts delivered over twice the amount of polyphenols to the final ice cream, even when added at a lower concentration (3%). This demonstrates that polyphenol concentration plays a predominant role in driving the melting characteristics targeted by this work. Another critical factor is the phenolic profile. The blueberry and cranberry extract samples highlight the potential effectiveness of another phenolic class, anthocyanins, in influencing the melting properties of ice cream.

Table 4.15 Melting characteristics of ice cream samples made with various polyphenol sources.

Formula	Melting rate (%/min)	Induction time (min)	Complete melting time (min)	Residual weight on the screen (%)
Control	0.63 ± 0.03[a]	30 ± 4.5[b]	140 ± 5[a]	6 ± 0[a]
Blueberry extract	0.55 ± 0.04[a]	56 ± 1.5[a]	120 ± 3.5[a]	7 ± 1.3[a]
Cranberry extract	0.69 ± 0.03[a]	31 ± 2.5[b]	117 ± 9[a]	4 ± 0.3[a]
Strawberry freeze-dried powder	0.55 ± 0.01[a]	61 ± 1.5[a]	126 ± 1[a]	10 ± 0.8[a]
Blueberry freeze-dried powder	0.63 ± 0.03[a]	34 ± 5[b]	151 ± 11[a]	10± 3.4[a]
Strawberry juice concentrate	0.57 ± 0.01[a]	56 ± 1.5[a]	126 ± 10[a]	9 ± 2.4[a]

Mean ± SE are shown. Variables sharing a letter within a measurement are not significantly different at α=0.05.

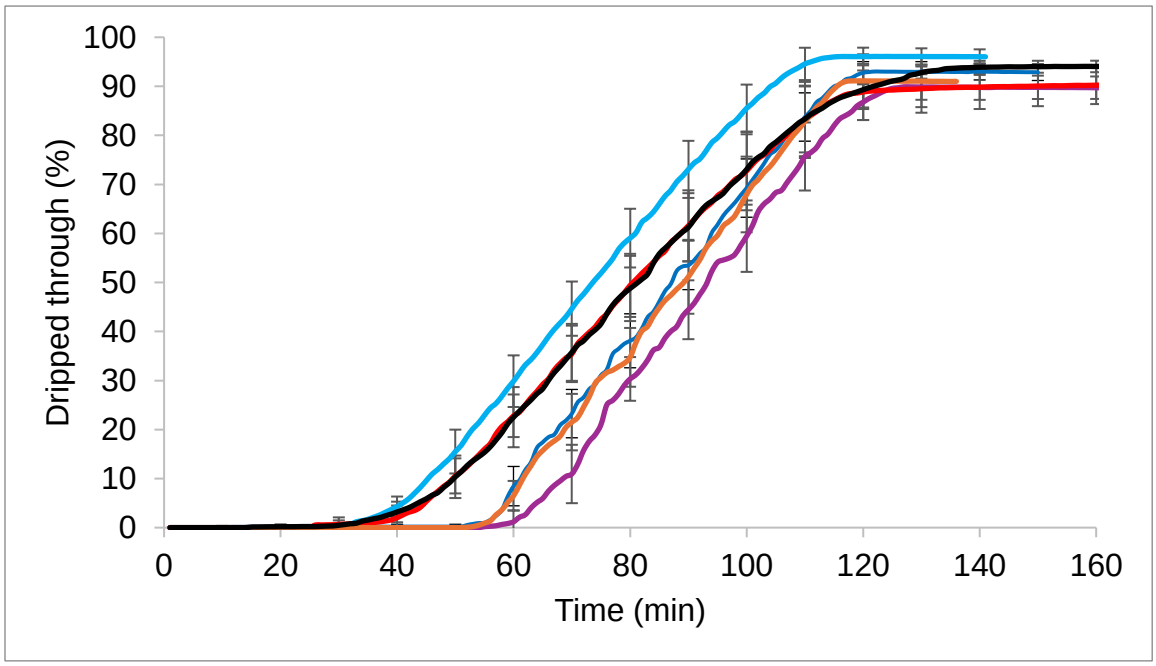

Figure 4.26 Melting profiles of various polyphenol sources in ice cream (control[black], blueberry extract [dark blue], blueberry freeze-dried [light blue], cranberry [purple], strawberry freeze-dried [red], strawberry juice concentrate [orange]). Error bars represent the standard deviation of mean values measured in triplicate.

The use of freeze-dried powder was inspired by Bilbao-Sainz et al. (2019), who added 3.5% of different berry freeze-dried powders to frozen desserts and observed a melting rate of 0%/min after two hours. In contrast, the freeze-dried powder samples in this study had melted entirely within two hours. This discrepancy may be attributed to differences in experimental design and procedures, particularly that 21% milk fat was used in the Bilbao-Sainz et al. (2019) frozen dessert mix. The different ice cream makers used for freezing might have also caused differences. Additionally, the absence of overrun values in the Bilbao-Sainz et al. (2019) study hindered the evaluation of potential interactions between overrun and low melting rates, which is important to consider when using a freezer with a low dasher speed. They also attributed the low melting rate to the fiber and pectin content of the berry powders. Despite adding the

same amount of powder and potentially having comparable amounts of these polysaccharides, our study did not produce a “no-melt” product.

4.4.4 Rheology

The addition of cranberry extract, blueberry extract, and strawberry juice concentrate to ice cream resulted in higher complex viscosity and thixotropy values than other samples, whereas the freeze-dried powder samples showed little difference from the control (**Table 4.16**). Similar to Section 4.2, the samples with the highest rheological properties (crude extracts and strawberry juice concentrate) also had the highest induction time, making the initial drip time a reasonable predictor of resistance to flow for these polyphenol-enriched ice creams. The elevated rheological values for the crude extract samples can be attributed to the presence of protein-mediated fat aggregates in these samples (**Figure 4.24**). Conversely, the favorable melting performance and viscosity of strawberry juice concentrate ice cream can be attributed to its low pH, which caused protein conformational changes. High thixotropy values illustrated the pH-induced protein structures in the strawberry juice concentrate sample. Although these aggregates did not affect the viscosity, they did enable the sample to exhibit a similar induction time to the crude extracts (blueberry and cranberry) with higher phenolic content. Further investigation is needed to quantify the extent to which pH-induced protein aggregation can impact these melting and rheological characteristics without deleterious consequences, like taste and texture.

The samples with freeze-dried powder were expected to have higher viscosities due to the introduction of dry matter, fiber, sugar, organic acids, and pectin from the source,

yet they did not have values significantly different from the control. The low concentration of powders incorporated into the ice cream might be responsible for this outcome.

Table 4.16 Rheological data of ice cream samples made with various polyphenol sources.

Formula	Complex Viscosity (Pa*s)	Thixotropy (Pa/s)
Control	4.2 ± 1.8^{d}	378 ± 3.3^{b}
Blueberry extract	67 ± 5.1^{b}	619 ± 20^{a}
Cranberry extract	5.8 ± 2.2^{d}	412 ± 14^{b}
Strawberry freeze-dried powder	98 ± 4.2^{a}	773 ± 16^{a}
Blueberry freeze-dried powder	4.7 ± 1.1^{d}	381 ± 52^{b}
Strawberry juice concentrate	36 ± 5^{c}	749 ± 46^{a}

Mean ± SE are shown. Measurement variables sharing a letter are not significantly different at α=0.05.

4.4.5 Summary

The results of this study did not reveal a crude polyphenol extract or source that could reproduce the low melting rate and shape retention of the grapeseed and green tea ice creams discussed in Section 4.3. Nevertheless, the importance of phenolic content was observed, as the crude extracts still exhibited a slight impact on various measurement variables. Furthermore, the aggregated protein network induced by low pH, observed in the ice cream made with strawberry juice concentrate, requires further exploration of its influence on the ice cream matrix.

5. Conclusions and Recommendations

5.1 Conclusion

This research revealed that polyphenols can decrease the melting rate of ice cream, and this effect is not exclusively dependent on the fiber, pectin, dry matter, and organic acids that are commonly found in polyphenol sources, as suggested by previous researchers. Based on particle size measurements and other chemical analyses, an overall mechanism was discovered using a simplified cream/TA system. Then, a series of experiments were conducted to determine the applicability of that mechanism to a complex ice cream system under different formula and polyphenol source conditions. The primary focus of the ice cream studies was evaluating melting characteristics, such as melting rate induction time, and residual weight on the screen. Various measurements, like rheological testing and micrographs, were examined to better understand the structural factors that contribute to the meltdown behavior.

Protein-mediated fat aggregates were found to be the primary factor contributing to the reduction in the melting rate of ice creams enriched with polyphenols. Treating enriched samples with EDTA and heat confirmed that milk proteins at the fat globule interface were involved in the fat clustering. Although polyphenols are known to interact with proteins to form complexes, the importance of fat in facilitating this reaction was shown when no reaction was observed after TA was added to skim milk. In cream, the quantity of fat clusters increased with TA concentration and led to an increase in viscosity.

When TA was incorporated into ice cream, a decrease in the melting rate was observed in a standard ice cream formula at a high concentration of TA. In order to

reduce the melting rate of ice cream, the protein-mediated fat aggregates had to effectively impede the flow of the serum phase during melting. Rheological analysis of the melted ice cream revealed that these fat clusters increased the thixotropic behavior of the system, highlighting the creation of an extensive fat network. An ice cream formula with elevated fat content and 2.5% TA decreased the melting rate, whereas the high protein formula with 2.5% TA did not, also emphasizing the role of fat. The efficacy of this reaction was based not only on the phenolic profile of the source but also on the concentration of polyphenols added, and a threshold concentration was necessary to elicit noticeable changes in melting characteristics. Green tea and grapeseed extract-enriched ice creams, which have high phenolic content, were able to promote shape retention and limit the loss of serum phase, leaving over 90% of the sample still on the screen after a 4-hour drip-through melting test. The samples with added freeze-dried powders were unsuccessful in reducing the melting rate due to their low phenolic content.

TA and high phenolic extracts were also highly effective in impeding ice recrystallization during a shelf-life study. These phenolic sources achieved this by decreasing the mobility of the serum phase due to the increased viscosity caused by the fat aggregates. Many polyphenol sources tested in this study exhibited attributes similar to a polysaccharide stabilizer when added to ice cream, including increasing the microviscosity of the serum phase and promoting viscoelastic properties that are stable at elevated temperatures (Regand & Goff, 2002). Green tea extract was shown to be a sufficient replacement for a stabilizer blend by imparting favorable melting, rheological, and storage stability parameters when stabilizers were omitted from the extract-

enriched ice cream formula. Overall, this study provides insight into the potential uses of polyphenols in modifying the structural properties of frozen desserts to improve melting characteristics and quality attributes.

5.2 Recommendation

This study lays the groundwork for extensive future research. There is much more to explore about the mechanism, optimization, and consumer acceptability of incorporating polyphenol sources in frozen desserts.

Additional protein/polyphenol complexation research is needed to fully understand the mechanism behind protein-mediated fat aggregation facilitated by polyphenols found in ice cream. Future studies should compare the differences in binding capacities of casein and whey to various polyphenols and examine which protein is more favorable for creating fat aggregates in a protein-stabilized emulsion when a polyphenol source is present. This would uncover how proteins can be properly optimized for this reaction. The stabilizer replacement study demonstrated the need for more research that investigates the effect of carbohydrates on polyphenol/protein interactions. Since ice cream can contain many carbohydrates, polysaccharide stabilizers and sugars commonly used in ice cream should be investigated for their capacity to impede protein/polyphenol complexation at the typical ice cream addition levels. The next step in studying polyphenol’s ability to act like a stabilizer would be removing mono-and diglycerides from the ice cream formulas and assessing similar parameters.

With the foundational knowledge of polyphenols' impact on ice cream evaluated, further research can optimize and promote the practical application of this functional ingredient. This study only investigated a few different types of polyphenols, necessitating more work in identifying additional cost-effective phenolic classes that can decrease the melting rate. Subsequent research should also focus on developing the optimal method for processing polyphenol-enriched ice cream at scale. This includes determining the optimal time and approach for incorporating a polyphenol source into ice cream while prioritizing the heat sensitivity of the phenolic compounds. At high polyphenol concentrations, the ice cream mix instantaneously increases in viscosity after addition, which needs to be considered when setting processing conditions.

To gain more insights into the melting behavior of ice cream containing polyphenols, it would be valuable to study this product's performance at elevated temperatures and humidities, simulating various climates in which this frozen dessert could be enjoyed. Additional shelf-life studies are needed, particularly ones that replicate the transportation life cycle of ice cream across different countries with their varying refrigerated transportation systems, to determine whether it is possible to maintain high-quality ice cream with a desirable ice crystal distribution during transport.

Consumer expectations and acceptability could be used to help prioritize the above recommendations. Adding polyphenols to ice cream alters many attributes that may affect the consumer experience. Conducting sensory testing is crucial to determine the optimal amount of polyphenol source that must be incorporated to reduce the melting rate while ensuring that the taste remains acceptable, especially due to the astringency of some polyphenols. Sensory evaluations would also provide insight into

consumer acceptance of new textures and whether the increased viscosity of a polyphenol-enriched product changes aspects such as mouthfeel and flavor delivery. It would also be interesting to study the acceptability over storage time and after sitting at ambient temperature for extended periods. Consumers' willingness to accept a product that deviates from their expectations will also need to be surveyed. Historically, products that do not deform and melt when left at ambient temperature are considered "fake" and less desirable. To combat the negative stigmas surrounding this reaction, it is important to emphasize the health benefits of polyphenols. This highlights the need for studies that assess the bioavailability of polyphenols in ice cream when added at an optimized level for this reaction.

The outcome of this research is truly remarkable, as its potential applications extend far beyond the realm of ice cream. Other products that contain milk fat and protein could also be impacted by protein-mediated fat aggregates, in order to change texture and quality attributes. Polyphenols can also interact with other non-dairy proteins, which suggests that this technology could be optimized to enhance the quality of non-dairy frozen desserts and any other matrix containing the appropriate components.

6. References

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