The Role of Saliva and Hydrocolloids in Texture Perception of Semisolid Foods: Using Rheological, Tribological and Sensory Evaluation Techniques

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Maryam Baniasadidehkordi

Major Professor: Helen Joyner, Ph.D.
Committee Members: Carolyn Ross, Ph.D.; Gulhan Unlu, Ph.D.; Girish Ganjyal Ph.D.
Department Administrator: Barbara Rasco Ph.D.

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Authorization to Submit Dissertation

This dissertation of Maryam Baniasadidehkordi submitted for the degree of Doctor of Philosophy with a Major in Food Science and titled "The Role of Saliva and Hydrocolloids in Texture Perception of Semisolid Foods: Using Rheological, Tribological and Sensory Evaluation Techniques," has been reviewed in final form. Permission, as indicated by the signatures and dates below, is now granted to submit final copies to the College of Graduate Studies for approval.

Major Professor: ______________________________ .Date: ______________
Helen Joyner, Ph.D.

Committee Members: ______________________________ Date: ______________
Carolyn Ross, Ph.D.

______________________________ Date: ______________
Gulhan Unlu, Ph.D.

______________________________ Date: ______________
Girish Ganjyal, Ph.D.

Department Administrator: ______________________________ Date: ______________
Barbara Rasco, Ph.D.

Abstract

The most common way to optimize the texture attributes of semisolid foods is to use hydrocolloids as texture modifiers. However, addition of hydrocolloids to a food system may cause adverse texture effects. These unpleasant effects can be avoided by selecting the right type and concentration of hydrocolloid for the food system. Rheometry and tribometry are popular techniques that can be beneficial in characterizing texture-related attributes. However, prediction of texture attributes by instrumental testing has its own restrictions, e.g. the lack of saliva effects on semisolid food. As a result, addition of saliva to semisolid foods during instrumental testing has been used for better clarification of oral texture perception. Combining different techniques for mimicking oral processing can frame a more realistic picture of how hydrocolloids and saliva application may affect texture perception of semisolid foods. Therefore, the objectives of this work were to 1) determine the structural, rheological, tribological, and sensory properties of semisolid foods that incorporated hydrocolloids as texture modifiers, 2) evaluate the effect of human whole saliva (HWS) on these properties, and 3) determine correlations among the different properties. For this project, twenty-four acid milk gels were used as a model system. Additionally, formulations for twelve yogurts were selected as a representative sample of the acid milk gel formulations. Acid milk gels and yogurts were prepared by mixing skim milk with different milkfat contents and dry powders including skim milk powder (SMP), sweet whey protein isolate (WPI), and non-based protein hydrocolloids (locust bean gum, carboxymethyl cellulose (CMC), potato starch, and corn starch). In addition to providing functional properties, SMP and WPI were also used to adjust protein and solids non-fat. For all samples, shear rate, strain, and frequency sweeps were carried out at 8˚C and 25˚C to evaluate rheological behaviors. Tribometry was also performed at 25˚C to measure frictional behaviors. All samples were tested with and without HWS (1:6 ratio of HWS: sample). Sensory evaluation was performed using descriptive analysis. Subsequently, confocal imaging was used to determine sample microstructural differences for different formulations and upon addition of water and HWS. ANOVA, principal component analysis (PCA), and partial least squares (PLS) were used to analyze the results. Overall, the viscosity, viscoelastic, frictional, microstructural, and sensory profiles of the formulations were significantly different for different formulations and with addition of HWS. Combinations of hydrocolloids in yogurts, including CMC and PS individually and all hydrocolloids together, were correlated with desirable textural attributes i.e. viscosity, firmness, smoothness, and spoon viscosity. These samples also showed negative correlations with grittiness, chalkiness, astringency, and graininess. These correlations also were found in acid milk gels made with all hydrocolloids, as well as samples including CMC individually or in combination with a gum (e.g. PS) or with WPI. Acid milk gel and yogurt formulas made with either CMC alone or with all hydrocolloids showed similar rheological, tribological, and microstructural properties. Samples with PS had the most drastic structural changes after addition of HWS. Rheological, tribological, and sensory properties showed significant correlations for multiple parameters. Mouthfeel attributes from sensory results correlated well with acid milk gel friction behaviors, viscoelastic parameters, and viscosity parameters. Undesirable sensory attributes, i.e. grittiness, graininess, chalkiness, and astringency, were positively correlated with friction coefficients within sliding speeds of 15-30 mm/s and

negatively correlated with positive sensory attributes such as smoothness, firmness, and viscosity related attributes. Friction coefficient at 30 mm/s was correlated with all textural attributes except spoon lumpiness. G* was positively correlated with viscosity and friction coefficients; γ_c was negatively correlated with friction coefficients. Thus, friction increased with increased resistance to permanent deformation and less stiff microstructure. The results of this work highlighted the beneficial role of rheological and tribological techniques in studying texture perception of semisolid foods. Therefore, they can be used to assist in design of reduced or non-fat semisolid foods with an acceptable texture to provide healthier options to meet consumer demands.

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Dedication

I would like to dedicate this dissertation to my kind, loving, and incredible mother who never got a chance to see my success. My dedication also goes to the rest of my family, who have always been helpful and supportive in all periods of my life.

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CHAPTER 1: INTRODUCTION

Texture plays a key role in consumer's acceptance of semisolid foods (Bourne, 2002, Grygorczyk et al., 2013), including yogurt, a semisolid food that is popular among many different cultures around the world. Reduced-fat and fat-free yogurts are becoming increasingly popular due to their lower caloric value and improved health benefits compared to full-fat yogurts (Da Silva and Rudkowska, 2014, Pei et al., 2017). The texture of reduced and non-fat yogurts, however, might not be as palatable as their full-fat counterparts, since fat is one of the most important components in yogurts that contributes to the textural and mouthfeel properties (Peng and Yao, 2017).

Different factors, including starter cultures, type of hydrocolloids, amount of milkfat, processing variables such as heat treatment, homogenization pressure, incubation time, and storage conditions, can affect the texture and quality of yogurt (Keogh and O'kennedy, 1998, Lee and Lucey, 2004, Soukoulis et al., 2007, Ciron et al., 2010). In particular, hydrocolloid type and concentration are two key factors that can affect the texture and sensory properties of reduced-fat and non-fat yogurts (Marcotte et al., 2001, Gallardo-Escamilla et al., 2007). The hydrocolloids in yogurt systems need to be chosen carefully since they can impact the texture of the yogurt, as well as the consumer acceptability of the product (Routray and Mishra, 2011). Using improper hydrocolloids as fat replacers can result in differences in flavor perception and distribution of flavor molecules (Routray and Mishra, 2011).

Rheology and tribology can help better understand different texture-related characteristics of semisolid food along with sensory measurements. Rheology relates to the food breakdown and ingestion at the early stages of oral processing, and tribology is more important at later stages when rheology is less dominant (Chen and Stokes, 2012, Stokes et al., 2013). Rheology is a popular technique for measuring food mechanical properties such as flow and deformation. It can provide information about the strength and stability of the food structure as well as flow behavior of foods in the mouth (Chen and Stokes, 2012). There are various tests for measuring different rheological behaviors; common tests include rotational tests, e.g. shear rate sweep; oscillatory tests, e.g. strain and frequency sweep tests (Steffe, 1996).

Tribometry measures the frictional behaviors of foods, which can be correlated to friction-related sensory attributes for studying the texture perception in the mouth. The lubrication and friction behaviors of semisolid foods can be measured by mimicking the conditions between different oral surfaces such as the tongue and palate (Chen and Stokes, 2012). Friction coefficient has been linked with some sensory attributes such as creaminess, smoothness, lumpiness, and astringency (De Wijk et al., 2006a, Debon et al., 2013, Sonne et al., 2014) Similarly, some rheological properties has been related to other sensory attributes e.g. viscosity, smoothness (Nguyen et al., 2017). Combining rheology and tribology evaluations of food products can provide a fuller understanding of drivers behind their textural attributes.

Microstructural imaging is a complementary tool for developing structure–function–texture relationships of semisolid foods when used in combination with rheology, tribology, and sensory measurements (Pereira et al., 2003, Guggisberg et al., 2009, Abhyankar et al., 2014). For example, imaging the structure of proteins and fat in yogurts and acid milk gels prepared with other ingredients, such as hydrocolloids, can be useful for understanding both instrumental properties and sensory attributes of these foods. For instance, acid milk gel samples with added potato starch showed a denser protein network with higher storage modulus and higher viscosity of the aqueous phase due to leaching of amylose during starch gelatinization compared to samples with no starch (Oh et al., 2007). Both changes in viscosity and storage modulus of acid milk gels were observed in combination with a stronger and denser protein network through the confocal images (Oh et al., 2007).

In addition to formulation and manufacturing procedure affecting yogurt texture, yogurts can show notably different oral behaviors after incorporating with saliva. Saliva is composed of various components such as water, proteins, enzymes, and electrolytes (Humphrey and Williamson, 2001). It has been found that proteins and enzymes such as α-amylase have an underlying role in texture perception (Engelen et al., 2007). Accordingly, adding saliva to semisolid foods can change their rheological and tribological behavior. This can be due to dilution or lubrication effects of saliva or other interactions between food and saliva components such as precipitation of proteins by tannins, resulting in perception of astringency, and primary breakdown of food (Green, 1993, Engelen et al., 2003a). For example, samples with added starch had the most significant changes due to starch breakdown, which was attributed to interaction with the α-amylase in saliva (Joyner (Melito) et al., 2014, Morell et al., 2016).

Although some research has been done correlating rheological, tribological, and sensory measurements, there is no published research using all these techniques in combination with microstructural imaging and saliva addition. The combination of all these techniques along with the contribution of saliva addition will provide a better understanding of semisolid food textures. Hence, the goal of this study was to determine the relationships among the rheological, tribological, microstructural, and sensory characteristics of acid milk gels as a model system and apply these relationships to a yogurt system. The results of this study will allow more fundamental development of healthier low-fat yogurts with acceptable textures.

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CHAPTER 2: LITERATURE REVIEW

Although yogurt is generally considered a healthy food, containing probiotics and other essential nutrients, a large portion of yogurt consumed in the USA contains high fat levels. Substitution of high-fat food products with reduced-fat ones can be an effective way to control obesity in the USA. Therefore, it is desirable to replace full-fat foods with their reduced-fat or fat-free counterparts. One potential obstacle with the introduction of these reduced fat or fat-free yogurt varieties is that of texture. In general, full-fat foods have desirable palatability and better mouthfeel than their lower-fat counterparts.

Texture plays a critical role in the consumer acceptance of semisolid foods such as yogurt. However, the thickeners and stabilizers used in reduced-fat yogurts can significantly affect the texture and mouthfeel of yogurt. Furthermore, in the quest for improving texture, one also needs to consider the different behavior of semisolid foods after incorporating saliva during consumption of the food. The effect of different hydrocolloids and saliva on the behavior of yogurts and their sensory textures needs to be carefully evaluated. Determining the mechanical properties, frictional behavior, and texture attributes of semisolid foods can be helpful for the evaluation of these effects. These characteristics are measured through rheology, tribology and sensory evaluation methods, respectively.

2.1 Yogurt and acid milk gel compositions

Yogurt is produced by adding two lactic acid bacteria (LAB), *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, to some combination of milk, cream, and skim milk. Other ingredients could be added to the yogurt to increase the solid non-fat content. According to Code of Federal Regulations, these components include nonfat dry milk, concentrated skim milk, whey or whey protein, buttermilk, lactose, lactoglobulins, and lactalbumins (FDA, 2017).

Another way to acidify the milk is to add acidifiers such HCl and glucono-delta-lactone (GDL) to the milk (De Kruif, 1997, Lucey and Singh, 1997). Over time, GDL is hydrolyzed to gluconic acid, which will cause the pH reduction. Chemical acidification is used to prepare acid milk gels, but the product is not a yogurt since it does not contain live bacteria. Acid milk gels are used as a model system for studying the gel formation and structure of yogurts (Lucey and Singh, 1997). The advantage of GDL compared to other acidulants is due to its gradual pH reduction, which is similar to the growth of starter culture activity in the fermentation of yogurt. Another advantage of using GDL is due to a better control of pH reduction compared to live bacteria. The final pH of acid milk gels depends on the amount of GDL, but the pH in fermented yogurts is dependent on microbial growth, which can continues to a pH below 4.0 before the acidity suppresses further growth of LAB (Lucey and Singh, 1997). GDL is also preferred over other acids because direct addition of organic acids such as acetic acid can result in immediate coagulation of casein micelles and separation of the two phases (casein and whey proteins).

2.1.1 Yogurt composition

Full-fat yogurt is composed of water, sugar, fat, proteins, starter cultures, vitamins and minerals. The amount of water in yogurts varies with different milkfat content, but is approximately 88.5% in full-fat yogurts, ~91.25% in non-fat yogurts, and 89.75 to 91.25 % in low-fat yogurts (USDA, 2001). Aside from differences in moisture content, the variety of yogurt formulations has made this product a unique food.

2.1.1.1 Fat

According to the US Code of Federal Regulations, yogurts can be divided into three groups based on their fat content. Full-fat yogurts contain at least 3.25% milkfat, non-fat yogurts have milkfat content <0.5%, and low-fat yogurt fat contents is between 0.5% and 2% milkfat (FDA, 2017). Lipids in bovine milk are mostly present as fat globules as an oil-in-water emulsion. Homogenization is a necessary procedure in manufacturing yogurt to break down the fat globules for an even dispersion throughout the yogurt system. This procedure also helps prevent the formation of a cream layer on top of yogurt (Cioranescu and Donato, 2000). Additionally, homogenization improves the sensation of smoothness in dairy products (Richardson et al., 1993). Fat content is the main reason for the perception of creaminess and smoothness in yogurts (Richardson et al., 1993, De Wijk et al., 2006b). Fat molecules can also affect the texture of yogurt by interacting with the protein network; homogenized fat globules are partly covered with casein, facilitating protein–protein interactions. Fat becomes trapped within this protein network where it results in a smooth, creamy mouthfeel and spoonable consistency of full-fat yogurts (Richardson et al., 1993).

2.1.1.2 Proteins

Yogurt is a good source of protein (Buttriss, 1997): there are about 6 grams of protein per typical serving size (6 oz). The amount of protein required in non-strained yogurts is 3.5% of the solid content of milk (USDA National Nutrient Database for Standard Reference (May

2016)). Most yogurts have higher protein content due to fortification with milk powders and protein powder (from whole or skim milk, whey protein or casein) during yogurt manufacturing.

There are two primary types of protein found in yogurt: casein and serum protein. About 80% (w/w) of the total protein in yogurt is casein and 20% (w/w) are serum proteins (Farrell et al., 2004). The word “serum” is used rather than “whey” for the water-soluble protein fraction to differentiate these proteins from the proteins in cheese whey. αs_1-, αs_2-, β-, and κ-caseins are the four types of casein that exist in approximate amount of 4:1:3.5:1.5, respectively, in milk. Caseins along with calcium phosphate form an aggregated protein molecule, called a micelle, with a diameter of 150-200 nm ((De Kruif, 1998)Needs et al., 2000). κ-casein is mainly located on the surface of the micelle, β-caseins exist in the interior and, along with α_s-caseins, are present throughout the micelle (Figure 2.1) (Swaisgood, 2003, Farrell Jr et al., 2004). Because they are on the surface of micelles and are able to stabilize the surface level of micelle and minimize then aqueous area inside the casein micelles, κ-caseins can be indicative of micelle size. Micelles that have higher amounts of κ-caseins are smaller due to increased surface stabilization; κ-caseins act as a connector between the aqueous environment and hydrophobic caseins in the micelle interior (Creamer et al., 1998). The structure of casein normally remains stable with moderate heating or cooling, but it can easily get disrupted and coagulate by acidification, proteolytic enzymes (Creamer et al., 1998), or salts such as sodium chloride (Lucey et al., 1997b, Zhao and Corredig, 2015).

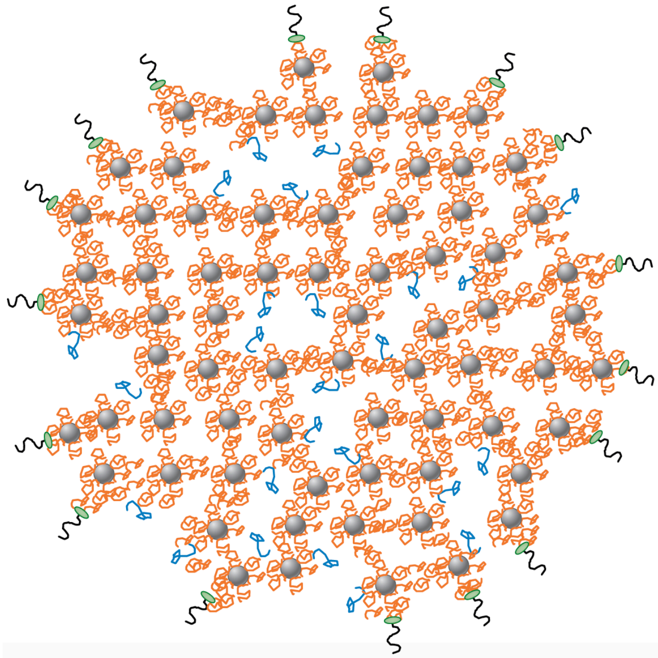

Figure 2.1. A schematic image of casein micelles
Key: β-caseins (orange color); Calcium phosphate nanoclusters (grey spheres); β-caseins (blue color): removable by cooling and can attach to other casein hydrophobically; Para-κ-casein (green color); Casein hairy layers (black color) (Dalgleish and Corredig, 2012).

Major serum proteins include β–lactoglobulin, α-lactalbumin, serum albumin, immunoglobulins and lactoferrin with the approximate mass proportions of 60%, 20%, 3%, 10%, and <0.1%, respectively (Kinsella and Whitehead, 1989). Heating above 70°C leads to large serum protein aggregates, but heating to below 70°C generally results in reversible aggregation (Iametti et al., 1996). Serum protein aggregation can also happen at the isoelectric point of serum proteins (pH=5.2) and inducing $CaCl_2$ salt (Lucey, 2002). Heat treatment in yogurt processing, which normally is above 70°C, results in the denaturation of serum proteins and solubilization of colloidal calcium phosphate (Lucey, 2002). Denatured serum proteins interact with κ-casein on the surface of casein micelles. Additionally, casein–casein attraction increases as the pH of milk decreases from native milk pH (typically: 6.6 to 6.8) to 4.5-4.6 during yogurt fermentation. On the other hand, at casein's isoelectric point (pH=4.6), there is no net charge on casein, which decreases electrostatic repulsion between charged groups, including the phosphoserine residues that are exposed when the colloidal calcium phosphate (CCP) is solubilized (Lee and Lucey, 2004). Electrostatic attraction increases and protein–protein attraction also increases through enhanced hydrophobic interactions at casein's isoelectric point (pH=4.6) (Lee and Lucey, 2004). These increased attractions cause gelation as caseins approach their isoelectric point at pH of 4.6, and the higher the number of interactions are, the stronger the gel network gets (Lucey, 2002).

Caseins and serum proteins are considered a rich source of amino acids. The availability of nitrogen has been shown as high as 93% (Bissonnette et al., 1994, BOULEY and TOMÃ, 1994). However, the proteins in yogurt have a better biological quality compared to milk due to a higher number of free amino acids like proline and glycine. The amount of free amino acids increases for up to 21 days of storage time since proteases and peptidases are still active in the yogurt system even after completion of fermentation time. As a result, the protein from fermented products like yogurt, including both native proteins in the yogurt milk and added milk protein powder, is digested more easily due to the pre-digestion activities by yogurt bacterial cultures (Lipatov et al., 1978, Shahani and Chandan, 1979, Ebringer et al., 2008, Agarwal et al., 2015). On the other hand, during fermentation, both acid production and heat treatment from lactic acid bacteria result in greater coagulation of casein compared to unheated or acid milk gels; this coagulation results in higher protein digestibility of yogurts compared to milk.

In addition to nutritional value, milk proteins also have various functional roles in the structure of dairy products and other foods. The structure of milk proteins (casein and serum proteins) can be manipulated by different processing conditions such as temperature, biochemical response, and high pressure. During heat treatment of yogurt manufacturing, serum proteins denature. These denatured proteins interact with each other and also with caseins, mainly with disulfide and non-covalent bonds (Considine et al., 2007). The interaction of κ-caseins and β-lactoglobulin is also strongly pH-dependent. For example, increasing pH from 6.5 to 6.7 can decrease the association between the denatured serum proteins from 80% to 30% (Anema et al., 2004).

Under pressurized conditions, most of β-lactoglobulin and some of α-lactalbumin denature. The number of κ-caseins in pressurized milk were increased and their particle size decreased (Considine et al., 2007). On the other hand, serum proteins denature under pressure, and it has been suggested that caseins and serum proteins can also denature as heat treated acid milk gels. For these reasons, pressurized milk can yield an acid milk gel with greater rigidity and less syneresis, similar to acid milk gels produced from heated milk (Harte et al., 2003, Anema et al., 2004). Physicochemical properties of food, such as the interaction with other components, ionic strength, and pH, can also be adjusted to produce various protein-based foods with desirable textures (Aliste and Kindstedt, 2005).

2.1.1.3 Carbohydrates

Carbohydrates in yogurt are in the form of sugars. The main milk sugar is lactose and is present between 4.8 and 5.1% in fresh bovine milk (Goodenough and Kleyn, 1976). The amount of carbohydrate varies among different commercial plain yogurts based on formulation but generally totals 4.8-5.2%. Carbohydrate sources in plain yogurts include unfermented lactose (3.8–4.0 %), galactose (1.0–1.2 %), and trace amounts of glucose (Goodenough and Kleyn, 1976). Lactic acid bacteria convert lactose to galactose and glucose via lactase (Leroy and De Vuyst, 2004). In a study, 4.8% w/w of lactose in fermented dairy products decreased to about 2.3% w/w after 11 days of storage. On the other hand, the amount of galactose increased to 1.3% from trace amounts in milk (Alm, 1982). For this reason, yogurt can be tolerated by lactose-intolerant individuals, and side effects of lactose malabsorption like diarrhea can be significantly reduced due to the hydrolysis of lactose by the lactase (Shah, 2006).

Sugars such as sucrose or fructose can also be added in yogurt mix before heat treatment and homogenization (Tamime and Robinson, 1985). Added sugars can increase the viscosity of the serum phase in yogurt due to their water binding capacity. The type of sugar used can also change the sensory characteristics of the product. When lactose is hydrolyzed to glucose and galactose or when sucrose is substituted with fructose, a higher viscosity is observed (Tamime and Robinson, 1985). Monosaccharides can increase the viscosity of serum phase more than disaccharides such as sucrose, resulting in a higher water holding capacity. Added sugars can also increase fermentation time by reducing production of lactic acid and increasing osmotic pressure (Fernández-Garía et al., 1998).

2.1.1.4 Vitamins and minerals

Among the micronutrients naturally present in milk, including calcium, protein, phosphorus, potassium, magnesium, zinc, and multiple B vitamins, calcium is the critical mineral in yogurts both nutritionally and functionally (Buttriss, 1997). Calcium phosphate plays a vital element in casein micelle contribution to the strength of yogurt gel formation (Lee and Lucey, 2004). An imbalanced concentration of calcium in milk causes lower heat stability and results in fouling during milk processing (Jeurnink and De Kruif, 1995). In the absence of calcium, the structure of micelles dissociates to αs1-, αs2-, β-, and κ-caseins. Adding calcium to the system causes submicelles to re-associate through salt bridges formed between calcium and protein side-chains (Chu et al., 1995). Calcium–protein interactions occur through carboxylic and phosphate groups (Farrell Jr, 1988). Neutralizing the negatively charged residues on casein micelles results in better aggregation of casein micelles and gel firmness, buffering during the acidification of acid milk gels and cheese, and decreased rennet coagulation time of milk (Lucey and Fox, 1993).

2.1.2 Acidification

2.1.2.1 Chemical acidification of milk

As previously discussed, milk can be acidified by bacterial fermentation of lactose to lactic acid or direct addition of acidulants, such as GDL (Lucey et al., 1997b), hydrochloric acid, acetic acid, citric acid, lactic acid, and formic acid.

GDL is widely used in dairy products (Ramachandran et al., 2006). This acidulant is a neutral cyclic ester of gluconic acid and typically used in the form of a white crystalline powder. When GDL is added to an aqueous solution, it dissolves rapidly and becomes hydrolyzed to gluconic acid (Lucey and Singh, 1997, 2003). It can be added to milk at a wide range of temperatures and it allows an excellent control and reproducibility of pH reduction in milk (Hatami et al., 2012). GDL also does not cause significant organoleptic changes in dairy products. It gives an initial slight sweet taste to the solution that later changes to a mildly acidic taste. It is GDL's slow rate of acidification and mild taste that differentiate it from other acidulants and favor its use in applications requiring a controlled decrease of pH and/or a neutral flavor profile (Ramachandran et al., 2006).

2.1.2.2 Starter cultures

Two traditional lactic acid bacteria, *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, are required for yogurt manufacturing (Clark and Plotka, 2004, Özer, 2010). There is a synergic relation between these two bacteria, which can be considered a binary feedback loop. *Streptococcus thermophilus* produces pyruvic acid, formic acid, and CO_2 that will stimulate the growth of *Lactobacillus delbrueckii* subsp. *bulgaricus* (Kosikowski and Mistry, 1977, Tamime and Robinson, 1999). It has been also proven that *Streptococcus thermophilus* progresses faster than *Lactobacillus bulgaricus* through lag-phase, reducing both pH from 6.7 to 5.7 and reduction potential.(Oliveira et al., 2009, Oliveira et al., 2011).

The optimal temperature for *Streptococcus thermophilus* growth is 35°C to 42°C; *Lactobacillus bulgaricus* grows best between 43°C to 46°C (Radke-Mitchell and Sandine, 1984). Thus, the typical temperature used for fermentation of the two species together is 42-43°C. This temperature gives the best associative and symbiotic growth when using the ratios of *Lactobacillus bulgaricus* to *Streptococcus thermophilus* between 2:1 and 1:5 (Aryana and Olson, 2017).

One important factor that affects yogurt quality is the type of starter culture (Sodini et al., 2004). Different strains of starter cultures can influence yogurt rheological and sensory properties (Beal et al., 1999, van de Velde et al., 2015). Bacteria cells can produce either neutral or charged EPS's; the interaction of milk proteins and EPS are based on their respective net charges (Hassan et al., 1996, van de Velde et al., 2015). Additionally, exopolysaccharide (EPS)-producing cultures can significantly affect the texture of yogurts. They can increase instrumental viscosity and improve sensory properties by a similar mechanism as polysaccharides (van de Velde et al., 2015). Yogurts made with EPS-producing bacteria were smoother and thicker compared to the inhomogeneous and thin yogurt yogurts from moderately ropy cultures. Confocal imaging of these three types of yogurts showed the number and size of the pores for the yogurts with non-ropy cultures were greater than the yogurts with ropy cultures (Van Marle, 1999). The smaller particle size of stirred yogurts has been related to a smoother and creamier texture (Sonne et al., 2014). EPS-producing starter cultures can also affect the viscoelastic properties of yogurt. Yogurts with lower amounts of EPS had firmer structure and higher viscosity (Hassan et al., 1996, Marshall and Rawson, 1999). These cultures contribute to a polymer-like behavior of the serum phase, which might have the ability to bind water and increase yogurt viscosity (Tamime and Robinson, 2007b).

2.1.3 Yogurt processing

There are 5 types of yogurts: set, stirred, drinking, frozen, and concentrated style yogurts, each with their own specific manufacturing process and structural properties (Lee and Lucey, 2010). Because the focus of this project is on stirred yogurts since it is the most common yogurt in the US, only the manufacturing procedure of stirred yogurt will be reviewed in this section (Figure 2.2). The first step of manufacturing yogurts is blending different ingredients to make the yogurt mix (White et al., 2008).

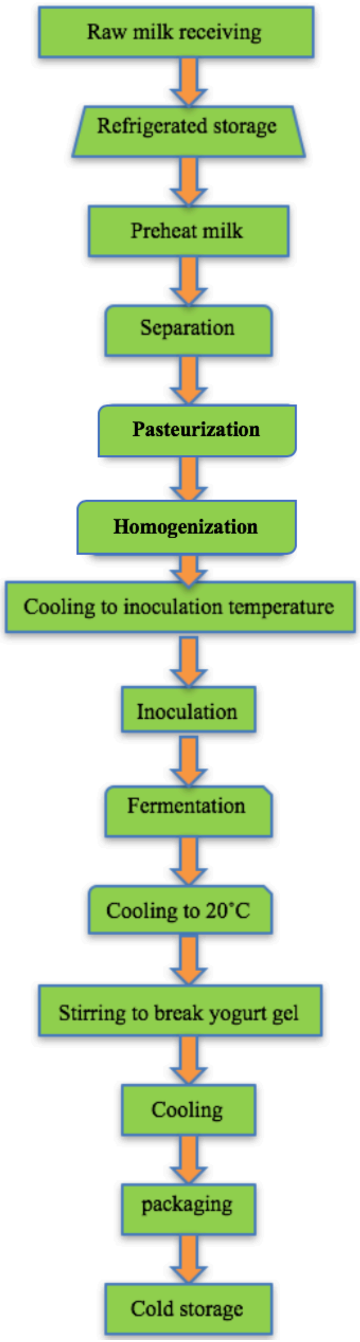

Figure 2.2. Stirred yogurt manufacturing procedure

In this step, fluid components such as skim milk, low-fat milk, and cream are pumped into a processing tanks, then dried materials such as protein and carbohydrate powders are added to the liquid phase. Both phases are blended using a high-shear blender to obtain a homogenous mix (White et al., 2008).

The next step is pasteurization and heat treatment of the yogurt mix. Pasteurization is performed to kill pathogens and inactivating inherent enzymes (Motarjemi et al., 2014). The most common pasteurization method is high temperature short time (HTST). Different combinations of temperature and time and their impact on the denaturation of serum proteins is shown in Table 2.1 (White et al., 2008). The heat treatment in yogurt processing is more intense than that of legal milk pasteurization (72°C (161.5°F) for 15 s) (Services, 2011). Temperatures above 70°C will denature serum proteins and optimize functionality such as water holding capacity and gel formation (Lucey et al., 1997).

Table 2.1. The effects of time and temperature in denaturation of serum proteins

Temperature	Holding time	Denaturation of serum proteins
85.0°C (185°F)	20–30 min	85–90%
85.0–90.6°C	30 min	85–90%
90.6°C (195°F)	15 min	85–90%
90.6–93.3°C (195–200° F)	2 min	70–75%
95.0°C (203°F)	8–10 min	90–95%

Serum proteins, e.g. β-lactoglobulin, interact with the κ-casein on the casein micelle surface and any soluble κ -casein molecules through disulfide bridging, resulting in increased gel firmness and yogurt viscosity (Lucey et al., 1997). Heating above 95.0°C leads to excessive serum protein denaturation and may cause a weaker gel due to syneresis. The typical optimum time-temperature combinations for yogurt manufacturing are 85°C for 30 min and 90-95°C for 5 min (Tamime and Robinson, 1999)The pasteurized yogurt mix is then homogenized. The aim of homogenization after pasteurization is to break down the fat globules and emulsify the fat droplets in the yogurt mix (Tamime and Robinson, 1999). Homogenization prevents separation of milkfat during incubation. High-pressure-high-temperature homogenization also improves the viscosity of the yogurt by breaking up the casein micelles (White et al., 2008). The increased hydrophilicity of the casein micelles increases their interaction with the serum proteins. and make a stronger protein network and a higher viscosity in yogurts (White et al., 2008).

After homogenization, the yogurt mix is cooled to 42-43°C to add starter cultures, then incubated at 42-43°C until the pH reaches 4.5-4.6 (Lee and Lucey, 2004). The isoelectric point of casein occurs at pH=4.6. Gelation of yogurts occurs at the pI of caseins; at this pH, casein has fewer bonds to calcium phosphate, and casein particles have little net charge to repel them from each other (White et al., 2008). When the yogurt reaches the desired pH (4.55-4.6), it is cooled to prevent bacterial growth, formation of a weaker gel, and undesirable texture (Lee and Lucey, 2010). Partial cooling to 20°C is followed by breaking the gel through agitation in fermentation tanks with a low-shear blender. Although the agitation of yogurt after incubation causes significant changes in its rheological properties, the changes from agitation can be minimized by stirring at low shear for a short time; rigorous and long stirring disrupt the yogurt structure and cause a weak texture and body (White et al., 2008). The stirred yogurt is usually pumped through a screen which gives the product a smooth and viscous texture (Tamime and Robinson, 1999). The pumps also move the stirred yogurts from fermentation tanks, through a plate cooler, and to a filling machine (White et al., 2008). Plastic cups are normally used for yogurt packaging. After filling the containers with yogurt, either a die-cut foil or a plastic lid is sealed on top of the containers (White et al., 2008). The stirred yogurt will then be blast-chilled to 7-13°C (45-55°F) (Lee and Lucey, 2010) and stored at 4°C.

The other types of yogurts are produced in the same manner as stirred yogurt; the min differences are in fermentation and post-fermentation steps. Set-style yogurts are incubated and cooled in their final packaging, resulting in a more gel-like product than stirred yogurts. Concentrated yogurts, also called Greek or strained yogurt, have an extra concentration and cooling stepbefore packaging. Drinking yogurts are blended after fermentation until the gel is broken down to a liquid. The yogurt mix used for frozen yogurts is incubated in tanks and frozen like ice cream. Depending on type of the fruit used, fruits can be either be blended with the fermented base or put into the bottom of the containers. For the latter yogurts, fruits are dispensed from the bottom of the containers, yogurt mix is added on the top, fermented or cultured, in the container, and cooled.

2.2 Yogurt microstructures

The microstructure of acid milk gels either from live bacteria or with GDL has been studied with confocal laser scanning microscopy (CSLM) for the last 4 decades. The protein network of acid milk gels is the main component observed by this technique (Lucey and Singh, 1997, Lucey et al., 1998a, Lucey et al., 1998b, Lee and Lucey, 2004, Guggisberg et al., 2009, Ciron et al., 2010, Krzeminski et al., 2011, Pang et al., 2015). In a protein network, casein micelles are linked by protein clusters, strands, and chains that are distributed in a serum phase with void pores or pores in which the aqueous phased is trapped (Lee and Lucey, 2010)

Addition of milkfat and hydrocolloids can significantly alter the microstructural properties of yogurts. Fat globules from milkfat are embedded throughout the protein matrix. By increasing fat, the density of the network increases. Structural changes from hydrocolloids are dependent on the type used (Hansen, 1993, Everett and McLeod, 2005, Gentès et al., 2013, van de Velde et al., 2015). For instance, adding carrageenan to acid milk gels showed that gels prepared with low carrageenan concentration had microstructures that were highly flocculated with large aggregates (Arltoft et al., 2007). In another study on the impact of different total solid non-fat (10-20% w/v) and fat contents (0-4% w/v), CLSM showed that mean pore size decreased and mean cluster size increased with fat addition (Pereira et al., 2006).

CSLM images of acid milk gels are also used to interpret the results from rheology, tribology and sensory evaluation for a better understanding of the behaviors from these methods (Ozer et al., 1999, Pereira et al., 2003, Lee and Lucey, 2004, Pereira et al., 2006, Guggisberg et al., 2007). For example, CSLM images can be used to determine the microstructure of the protein network e.g., pores size, cross-linking concentration, and length of the casein chains; yogurt texture is related to the physical properties of the yogurt, which are defined by the protein network microstructure (Lee and Lucey 2010).

2.3 Factors influencing yogurt structure and texture

Manufacturers can alter the body and texture of yogurts by manipulation of the composition of the yogurt mix, e.g. addition of hydrocolloids and other additives, heat treatment of the mix prior to incubation, starter culture selection, and incubation conditions (Bouzar et al., 1997).

2.3.1 Hydrocolloids

A wide variety of hydrocolloids can be used in yogurts. Hydrocolloids encompass a wide range of long polymers that can be dispersed completely, are partially soluble, or swell in the presence of water. The can change the physicochemical properties of foods by thickening, gelling, stabilizing, and emulsifying (Nishinari et al., 2000). They are also able to alter the rheological behaviors of yogurts like viscosity without a significant change in other attributes when a properly selected hydrocolloid is used in right (Sandoval-Castilla et al., 2004, Alakali et al., 2008, GONÇALVEZ et al., 2009). The major categories of hydrocolloids are proteins and polysaccharides. Starches (e.g., corn starch, potato starch), gums (e.g., locust bean gum, xanthan gum, guar gum, cellulose gum), carrageenan, and pectin are good examples of polysaccharide hydrocolloids used in reduced-fat yogurt manufacturing (van de Velde et al., 2015, Peng and Yao, 2017) . Protein-based hydrocolloids used to modify yogurt textures include whey protein isolate (WPI), whey protein concentrate (WPC) and added as milk powder, typically skim milk powder (SMP) or whole milk powder (WMP).

Hydrocolloids such as starch, gelatin, and cellulose gum have been shown to influence rheological and physicochemical and sensory properties of yogurts (Sandoval-Castilla et al., 2004, Alakali et al., 2008, GONÇALVEZ et al., 2009). Using gelatin and starch can significantly increase viscosity and alter mouthfeel, although gelatin is shown to be more effective than starch for syneresis prevention (Fiszman et al., 1999, GONÇALVEZ et al., 2009). In these studies, sensory differences were more significant for texture than for the other perceptions including taste and aroma. Therefore, determination of the texture (both mechanically and sensory) differences is the main focus in reduced or non-fat semisolid foods with added hydrocolloids. Overall, using the right type and quantity of the additives can improve the physicochemical, sensory and rheological properties of yogurt.

Hydrocolloids have different structures and properties, which can result in different behaviors in yogurt systems. Anionic hydrocolloids (polyelectrolytes) such as cellulose gum, pectin, and carrageenan have a negative charge on their hydrophilic end and interact with the positive charges on the surface of casein micelles to strengthen the casein network and decrease syneresis (Everett and McLeod, 2005). Neutral hydrocolloids such as xanthan gum, guar gum, and LBG stabilize the protein network through a different mechanism: increasing the viscosity of the continuous phase (Hansen, 1993). There are no electrostatic interactions between neutral gums and casein micelles due to lack of charge. As a result, depletion flocculation causes an increase in casein micelles aggregation, which is counterbalanced by the neutral gum molecules, resulting in a stable system. Neutral hydrocolloids in low concentration have been shown to have lower apparent viscosity compared to no-added polysaccharides samples (Everett and McLeod, 2005). Low concentrations of neutral hydrocolloids may not fill up enough aqueous space to increase the viscosity of the continuous phase. On the other hand, they do increase apparent viscosity and decrease phase angle of yogurts at higher concentrations (5 g/L) (Everett and McLeod, 2005). The stronger aggregation at higher concentrations would prevent the yogurt from breaking under applied strain and result in solid-like behavior, as opposed to yogurts with low concentration of LBG and guar gum, which showed viscous dominant behavior (Everett and McLeod, 2005).

2.3.1.1 Inulin

Inulin is a carbohydrate that is extracted from chicory (Corcoran et al., 2004). The effect of inulin as a fat replacer in dairy products has been studied (Paseephol et al., 2008, Guggisberg et al., 2009). Inulin can be added as long-, medium- or short-chain inulin where only long-chain inulin with the right concentration (4% w/v) can achieve rheological characteristics comparable to the results from fat (Guggisberg et al., 2007, Paseephol et al., 2008, Guggisberg et al., 2009). Inulin can also significantly increase the consistency of set yogurts, which was in agreement with observations made using CLSM (Guggisberg et al., 2007). The casein network was not negatively influenced by inulin addition; moreover, inulin can support the structure of set yogurts by building up a second network (Guggisberg et al., 2009). There was also a good correlation between sensory firmness and yield stress (Guggisberg et al., 2009).

2.3.1.2 Pectin

Pectin is a polysaccharide that is extracted from peel of citrus fruits. Pectin usually is more effective in presence of Ca^{2+} and low pH (Thakur et al., 1997). This characteristic of pectin will make it a suitable stabilizer in dairy products. There are two main categories of pectin, low-methoxyl pectin and high-methoxyl pectin. In low-methoxyl pectin, the ionic linkage of calcium bonds between two carboxyl groups of two different chains will cause gelation. In high-methoxyl pectin, the molecules of pectin are cross-linked through hydrophobic interactions and hydrogen bonds (Thakur et al., 1997).

Pectin addition to yogurt can significantly impact yogurt rheological and sensory properties. Pectin increases the viscosity and acidity of yogurt and will also improve other rheological properties of yogurt, such as adhesiveness and cohesiveness (Arioui et al., 2017). It can also prevent serum release during storage and will positively affects the growth of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (Arioui et al., 2017).

2.3.1.3 Starch

Starch is a polymeric carbohydrate which is considered as the main source of human diet's carbohydrate. Major sources of food starch include potato, rice, corn, wheat, and tapioca (Eliasson, 2004). Starch is made up of two polymeric glucose chains, amylose and amylopectin; amylopectin has a molecular size 100 times larger than amylose. The ratio of amylose and amylopectin is about 1:4 in a typical starch molecule (Eliasson, 2004).

One of reasons of heat treatment is used during yogurt manufacturing is to fully disperse the hydrocolloids, including starch. The structure of starch changes in presence of water and high temperature between 55-85°C; this change is called gelatinization. Briefly, the diameter of starch granules swells to many times its size, and the Maltese crosses between starch molecules are disrupted at a critical gelatinization temperature (55-85°C) in the presence of water. Additionally, amylose leaks out of the swollen granules, and the viscosity of the yogurts increases through this mechanism (Whistler and BeMiller, 1997, Oh et al., 2007).

Starch can be used as a thickener in a wide range of foods such as soups and dairy products (Mason, 2009). Addition of starch impacts acid milk gel and yogurt rheological and physicochemical properties. Using potato starch increased gelation time but did not affect the temperature and frequency-related viscoelastic behavior compared to acid milk gels without added starch (Oh et al., 2007). Confocal imaging showed that acid milk gels prepared with potato starch had swollen starch granules embedded in a protein network, and the structure was denser than acid milk gels without potato starch (Oh et al., 2007). Addition of corn starch was shown to increase lactic acid production and improved acid milk gel sensory attributes such as mouthfeel, consistency and appearance (Alakali et al., 2008). It was also reported that a combination of modified waxy starch and maltodextrin obtained by enzymatic conversion of potato starch can be used to produce non-fat yogurt with a creamy, smooth texture and rich mouthfeel (Wang, 2000). The addition of modified tapioca starch resulted in higher firmness compared to full fat yogurt (Sandoval-Castilla et al., 2004). Yogurts with a combination of tapioca starch and protein-based additives were more cohesive and less firm and adhesive than full fat yogurt (Sandoval-Castilla et al., 2004).

2.3.1.4 Cellulose gum

Cellulose is a polymeric substance that mainly exists in the cell walls of plants. It is considered as a dietary fiber since it is not digested by humans, who are not able to synthesize cellulase (Holloway et al., 1978). Cellulose derivatives include microcrystalline cellulose, powdered cellulose, methylcellulose, carboxymethyl cellulose, and hydroxypropyl methylcellulose (Peng and Yao, 2017). Cellulose gum has a variety of applications in the food industry, such as use in dairy products, sauces, baked products, frozen desserts, and salad dressings (Cho and Prosky, 1999, Ognean et al., 2006). Various functions have been linked to cellulose gum, including syneresis control, viscosity, body, consistency and mouthfeel improvement, spooning quality, pouring improvement, creaminess, moisture retention, and appearance improvement (Cho and Prosky, 1999, Ognean et al., 2006).

Sodium carboxymethylcellulose, also known as cellulose gum, exists in different particle size, viscosity, and hydration ability (Cho and Prosky, 1999). Cellulose gums are highly viscous and are suitable for promoting gel formation. Cellulose gum in powdered form is the result of chemical depolymerization of different plant sources (Ognean et al., 2006). As an insoluble powder, they can be added to foods as a dietary fiber or used to prevent stickiness and improve freshness in shredded cheeses or tortillas (Peng and Yao, 2017). Powdered cellulose gum can also be used to stabilize proteins in milk products; it is considered more effective at isoelectric pH (Walstra, 1996). Thus, cellulose gum can prevent casein precipitation and form a stronger gel that results in increased viscosity and firmness compared to control yogurts. Yogurts with cellulose gum were also more acceptable in terms of consistency and mouthfeel compared to yogurts containing gelatin. However, this result was not significantly different for the samples with corn starch (Alakali et al., 2008, Andiç et al., 2013).

2.3.1.5 Locust bean gum

Locust bean gum (LBG), also known as carob gum or carob bean gum, is extracted from the seeds of the carob tree (Lazaridou et al., 2001). LBG is a galactomannan consisting of a 1-4- linked-beta-D-mannan backbone with 1-6-linked-α-D-galactose side groups (Lazaridou et al., 2001). The galactose content and its unit distribution along the main chain has a great impact on LBG physicochemical properties (Dea & Morrison, 1975). LBG is used as a thickener and gelling agent in various foods, including as dairy, beverages, baked foods, and processed fruit products (Barak and Mudgil, 2014) . It has a strong ability to form hydrogen bonds with water molecules and interact with protein structures, forming stronger gels in yogurts (Barak and Mudgil, 2014). LBG can also be added to low-fat yogurt to increase viscosity, firmness and water-holding capacity, and reduce syneresis compared to control yogurt samples with no LBG (Ünal et al., 2003).

2.3.1.6 Xanthan gum

Xanthan gum is an extracellular polysaccharide produced by fermentation of the bacterium *Xanthomonas campestris* (Garcıa-Ochoa et al., 2000). It has a backbone with glycosidic links of β1-4, but it does not crystalize like cellulose gum due to its trisaccharide chain that comprises two mannoses and one glucuronic acid (Nussinovitch, 1997). The carboxylic acids in the side chains are negatively charged, which help the molecule stay linear due to electrostatic repulsion. This linear structure helps xanthan gum stay stable in acid, high-temperature, and alkali environments (Whistler and BeMiller, 1997).

Xanthan gum is a popular polysaccharide in the food industry for its unique properties. It can increase viscosity even at low concentrations and improve water solubility of foods (Williams, 2008). It can be added individually or in combination with other polysaccharides such as guar gum, starch, or cellulose gum to achieve different textures (Williams, 2008).

Xanthan gum increased the consistency, viscosity, and firmness in yogurts without developing negative sensory attributes such as gumminess or brittleness. It has also been shown to prevent serum separation and syneresis in yogurts (El-Sayed et al., 2002, Soukoulis et al., 2007).

2.3.2 Fat content

Fat is the primary determinant of creamy and smooth texture in dairy products. Full fat semisolid foods such as yogurt have a creamier, smoother, and richer mouthfeel compared to their corresponding reduced-fat products (De Wijk et al., 2006b, Janhøj et al., 2006). The perception of creaminess from fat can be categorized in two ways. First, fat droplets in yogurt act like fillers and contribute to smoothness perception (Janhoj et al., 2006). Second, the perception of fat is a result of both flavor and mouthfeel attributes (Bult et al., 2007). In general, a better understanding of the mechanism of fat-correlated sensory attributes, e.g. creaminess, can help during formulation of palatable reduced-fat yogurts. Fat content can also impact the rate of gelation and rigidity of the final gel (Xu et al., 2008). The first reason for this effect is the formation of casein–casein clusters due to interaction of phospholipids and casein micelle–serum protein crosslinks, the latter of which becomes a dominant interaction at high temperatures. These interactions can increase at higher temperatures because attracting forces are mainly hydrophobic at gelation temperatures (Xu et al., 2008). Another effect of higher fat content is decreased gelation time and increased gel strength as shown by higher viscoelastic moduli values (Xu et al., 2008). Fat reduction can lead to a weak texture, although it may be possible to produce reduced-fat yogurt with an identical texture to full fat yogurt using fat replacers like gelatin and starch (Tavakolipour et al., 2014). This can be explained by microparticulation of polysaccharides in dispersions of protein particles with diameters close to that of fat globules, which might be able to mimic the sensory effects of fat (Cheftel and Dumay, 1993).

2.4 Yogurt processing temperatures and times

Fermentation time and temperature are two factors that can highly affect the texture, rheological, microstructural, and sensory characteristics of yogurt (Schellhaass and Morris, 1985, Beal et al., 1999, Haque et al., 2001, Lee and Lucey, 2004, Xu et al., 2008, Saffon et al., 2013, Trejo et al., 2014). Different studies have illustrated the relationship between these two factors and their influence on the final product quality. Lower incubation temperature and longer fermentation time can lead to firmer gels (Beal et al., 1999, Skriver et al., 1999, Lee and Lucey, 2006, Oliveira et al., 2006, Damin et al., 2008). Accordingly, yogurt gels demonstrated a higher storage modulus and apparent viscosity when higher preheating temperature and lower incubation temperature with a longer set time were used for yogurt manufacturing (Lee and Lucey, 2006). This procedure also resulted in increased oral viscosity and perceived mouth coating attributes, as well as decreased chalkiness for stirred yogurt due to more even protein–protein crosslinks and smaller pores (shown through CLSM) throughout the protein matrix (Beal et al., 1999, Skriver et al., 1999, Lee and Lucey, 2006). Slow acidification may provide a better condition for protein interaction, strengthening the gel and increasing the viscosity (Beal et al., 1999, Skriver et al., 1999, Lee and Lucey, 2006).

Heat treatment and its duration in yogurt manufacturing play a crucial role in final yogurt firmness, consistency and viscosity (Dannenberg and Kessler, 1988, Lucey et al., 1997b, Lee and Lucey, 2004, Lee and Lucey, 2006). In general, when milk is heated at >70°C, the majority of the serum proteins, such as β-lactoglobulin, are denatured (Lucey et al., 1997). During denaturation, β-lactoglobulin interacts with κ-casein by disulfide bridging, which results in increased gel firmness and yogurt viscosity (Lucey et al., 1997). This effect has been studied over multiple time-temperature combinations. Heat treatment of milk for 15 min at ≥ 80° C significantly increased denaturation of β-lactoglobulin and gel strength compared to milk heated at 75° C for 15 min (Lucey et al., 1997).

2.4.1 Storage time

Changes during storage, e.g. yogurt acidity, one of the key aspects of consumer acceptance, can significantly affect yogurt structure and rheological behavior (Beal et al., 1999). Acidification of yogurt can also lead to variations in yogurt gel structure due to changes in bacterial activity and pH; high viscosity is related to slow acidification (Beal et al., 1999). Acidification is a result of lactic acid gained through both incubation and postacidification during storage (Beal et al., 1999). Postacidification is mainly affected by the strains of the culture used and storage temperature and time (Beal et al., 1999). Longer storage times result in higher viscosity, with the changes being especially notable between 1 and 7 d storage (Beal et al., 1999).

Storage time can also impact rheological properties. Yogurt viscoelastic moduli (G', G") and viscosity increased after 35 d storage at 4°C. This was attributed to continuing casein interactions with other proteins, forming a denser protein matrix (Damin et al., 2008).

2.5 Sensory evaluation

Sensory evaluation is a scientific discipline that uses the five human senses in different tests and statistical analysis to characterize different attributes of foods (Meilgaard et al., 2006). It is considered a multi-disciplinary science because human subjects are used to measure and describe the sensory properties of foods and other materials (Meilgaard et al., 2006). One of the most important roles of sensory evaluation is to provide the food industry a better understanding of consumer acceptance through evaluation of their products' sensory attributes. There are two main cateogriesof sensory evaluation tests: product-oriented tests and consumer-oriented tests. Product-oriented tests are able to measure presence and/or intensity of specific attribute in the product. Consumer-oriented tests determine consumer acceptability and measure opinions about an emotional reaction to a product such as preference or acceptability (Meilgaard et al., 2006).

The attributes that are perceived through the five senses of humans (sight, hearing, taste, small and touch) are appearance, odor/aroma/fragrance, texture and consistency, and flavor (aromatic, chemical feelings and taste) (Meilgaard et al., 2006). Most attributes are multi-modal (Meilgaard et al., 2006). For instance, flavor is the combined impression perceived via the chemical senses from a product in the mouth and does not include appearance and texture (Meilgaard et al., 2006).

2.5.1 Appearance

The appearance of the product or the packaging is usually the first attribute for customers use to choose and purchase food products. Hence, every aspect of the appearance requires attention during sensory evaluation (Kotler et al., 1983). Key appearance features are surface texture, size and shape, color, carbonation, and clarity. Color is a phenomenon involving both physical and psychological aspects (Meilgaard et al., 2006). Size and shape are associated with length, thickness, width, particle size, and geometric shape. Surface texture is related to many factors, including shininess/dullness, evenness/roughness, soft or hard, and crispy or tough (Meilgaard et al., 2006). In yogurts, uneven texture such as lumpy or grainy, uneven color, and serum separation, as well as excess or lack of fruit in fruit-flavored yogurts, are considered appearance defects (Clark et al., 2009).

2.5.2 Texture and mouthfeel

Texture is a multi-parameter sensory attribute that can only be described and quantified by humans (Hyldig and Nielsen, 2001, Saint-Eve et al., 2004, Engelen et al., 2005, Janssen et al., 2007, Pascua et al., 2013, Sonne et al., 2014). While the mechanical structure and surface properties of foods are detectable through vision, hearing, touch, and kinesthetic senses, the most important senses for texture perception are those of touch and pressure (Meilgaard et al., 2006). Common texture characteristics (mechanical parameters of texture) are shown in Table 2.2.

Mouthfeel is a sensation perceived through physical and chemical interactions in the mouth (Meilgaard et al., 2006). It is related with rheological behaviors and sensory attributes for tactile behaviors perceived during oral processing from first bite to swallow (Guinard and Mazzucchelli, 1996). Mouthfeel terms are shown in Table 2.3.

Table 2.2. Definitions of mechanical texture attributes
(Civille and Szczesniak, 1973)

Attributes	Instrumental	Sensory
	Primary properties	
Hardness	**Force necessary to attain a given deformation**	**Force required to compress a substance between molar teeth (in the case of solids) or between tongue and palate (in the case of semisolids).**
Cohesiveness	**Extent to which a material can be deformed before it ruptures.**	**Degree to which a substance is compressed between the teeth before it breaks.**
Viscosity	**Rate of flow per unit force.**	**Force required to draw a liquid from a spoon over the tongue.**
Springiness	**Rate at which a deformed material goes back to its undeformed condition after the deforming force is removed**	**Degree to which, product returns to its original shape once it has been compressed between the teeth**
Adhesiveness	**Work necessary to overcome the attractive forces between the surface of the food and the surface of the other materials with which the food comes in contact.**	**Force required to remove the material that adheres to the mouth (generally the palate) during the normal eating process.**
	Secondary properties	
Fracturability	**Force with which a material fractures: a product of high degree of hardness and low degree of cohesiveness.**	**Force with which a sample crumbles, cracks, or shatters.**
Chewiness	**Energy required to masticate a solid food to a state ready for swallowing: a product of hardness, cohesiveness and springiness**	**Length of time (in sec) required to masticate the sample, at a constant rate of force application, to reduce it to a consistency suitable for swallowing.**
Gumminess	**Energy required to disintegrate a semisolid food to a state ready for swallowing: a product of a low degree of hardness and a high degree of cohesiveness.**	**Denseness that persists throughout mastication; energy required to disintegrate a semisolid food to a state ready for swallowing.**

Afterfeel refers to the residual mouthfeel after the food is swallowed or expectorated; these terms include astringency and mouthcoating (Guinard and Mazzucchelli, 1996). Some attributes for desirable texture for yogurt have been introduced by USDA product judging. These attributes include yogurt that is thick and firm, is not-gel-like, and has a smooth and homogeneous texture and appearance (Tribby, 2008). In sensory studies of yogurts, sensory attributes typically include viscosity, smoothness, thickness, sliminess, lumpiness, graininess,

Table 2.3. Classification of mouthfeel terms
(Civille and Szczesniak, 1979)

Number	Category	Typical words
1	Viscosity-related terms	Thin, thick, viscous
2	Feel on soft tissue surfaces	Smooth, pulpy, creamy
3	Carbonation-related terms	Bubbly, tingly, foamy
4	Body-related terms	Heavy, watery, light
5	Chemical effect	Astringent, burning, sharp
6	Coating of oral cavity	Mouthcoating, clinging, fatty, oily
7	Resistance to tongue movement	Slimy, syrupy, pasty, sticky
8	Afterfeel-mouth	Clean, drying, lingering, cleansing
9	Afterfeel-physiological	Refreshing, warming, thirst-quenching, filling
10	Temperature-related	Cold, hot
11	Wetness-related	Wet, dry

grittiness, and chalkiness (Karagul-Yuceer and Drake, 2006, Tribby, 2008, Ozcan, 2013). Overall liking can be related to these attributes. For example, the overall liking score of yogurts decreased significantly when the yogurts were either too thick, too thin or lacking in smoothness (Lovely and Meullenet, 2009).

2.5.3 Flavor, aroma and taste

Flavor is a sensory attribute that is perceived as the sum of the perceptions resulting from stimulation of receptors in the alimentary and respiratory tracts (Figure 2.3) (Meilgaard et al., 2006). Flavor includes 1) aromatics, e.g. olfactory perceptions resulted through volatile compounds released from a food in the mouth; 2) tastes, e.g. gustatory perceptions from soluble compounds in the mouth; and 3) chemical or trigeminal feeling factors that stimulate nerve ends in the soft membranes of buccal and nasal cavities including. Chemical sensations can be perceived as astringency, spice heat, cooling, bite, metallic flavor and umami taste (Meilgaard et al., 2006).

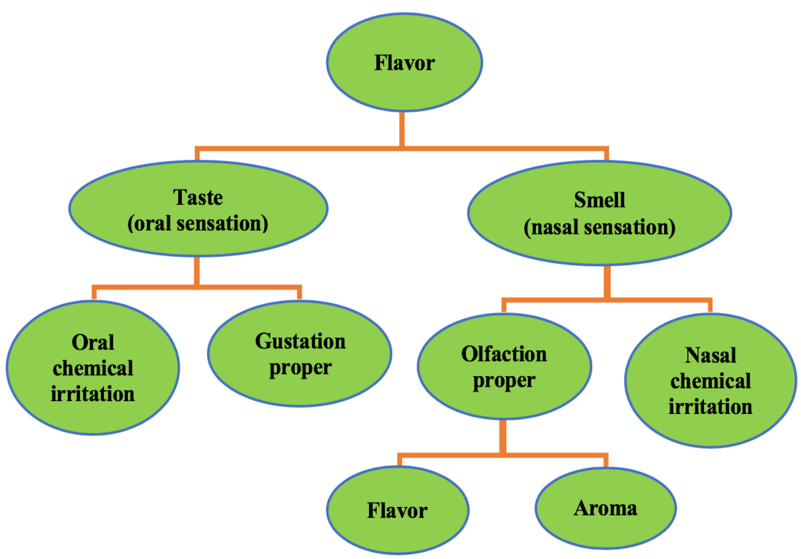

Figure 2.3. The summary of flavor perception
(Meilgaard et al., 2006)

As previously mentioned, addition of hydrocolloids can significantly affect the flavor and aroma of plain yogurt (Decourcelle et al., 2004, Alakali et al., 2008, Routray and Mishra, 2011). This can be due to different interactions with the yogurt network and other flavor and taste components in the yogurt. A significant reduction of aroma compounds was seen upon adding starch and pectin to yogurts. The decrease in aroma release may be due to the interactions of aroma compounds with the hydrocolloids or helical starch chains. The amylose in starch interacts with aroma compounds and prevents aroma release into the yogurt headspace. On the other hand, LBG resulted in an increase in yogurt flavor and guar gum had no significant impact on aroma and yogurts flavor (Decourcelle et al., 2004). CMC and corn starch were shown to positively impact the appearance, texture (mouthfeel and consistency) and flavor of yogurts at 0.75% of concentration compared to gelatin. The mild acidic environment of yogurts combined with the heat treatment in yogurt manufacturing can hydrolize corn starch to D-glucose, which can enhance sweetness (Alakali et al., 2008).

Taste is another food attribute perceived through taste receptor cells located on the taste buds in the oral cavity (Meilgaard et al., 2006). There are six basic tastes: salty, sour, bitter, sweet, umami, and fatty (Meilgaard et al., 2006, Besnard et al., 2016). Different factors such as temperature, viscosity, rate, duration, area of applications of stimulus, and differences in individuals can affect taste perception. (Meilgaard et al., 2006). As taste is a complex perception, it has mostly been investigated along with other attributes like texture, aroma, and flavor in yogurt. Starter culture strains, fat content, flavor additives, stabilizers, temperature, and storage conditions are the most important factors in yogurt taste (Kähkönen et al., 1997, Martin et al., 1999, Chee et al., 2005, Higgins and Scholer, 2009, Routray and Mishra, 2011, Cruz et al., 2013). For example, addition of hydrocolloids such as pectin and LBG reduced the aroma flavors by measuring these in the headspace of yogurts (Decourcelle et al., 2004). The decrease in aroma release may be due to the interactions of aroma compounds with the hydrocolloids or helical starch chains. In samples with LBG, LBG decreased available water content and increased "salting-out" phenomenon (Decourcelle et al., 2004).

2.6 Rheology of semisolid foods

Rheology is the science of deformation and flow of matter and has been used to study food behaviors for decades. Rheological behaviors of foods have been widely used to understand and describe food in-mouth flow properties and associated sensory attributes. Materials that show both viscous and elastic behaviors are called viscoelastic materials. An ideal elastic material stores all imposed deformation energy and will in return totally recover upon release of the stress. An ideal viscous fluid is unable to store any deformation energy. Hence, it is irreversibly deformed when subjected to stress; it flows, and the deformation energy is dissipated as heat, resulting in a rise of temperature. Gases and simple fluids are normally described as viscous fluids. Viscoelastic materials store some of the deformation energy (elastic portion) in their structure, while other energy is lost as the material flows (viscous portion). In other words, when they are deformed, they may not return to their original shape. Most semisolid foods are viscoelastic, e.g. yogurt, starch-based puddings, mayonnaise, tomato purées and sweet jelly (Zargaraan et al., 2013).

There are two categories of fluids: Newtonian and non-Newtonian. The viscosity of the Newtonian materials does not change with shear rate. Water, mineral and vegetable oils, and pure sucrose solutions are good examples of Newtonian fluids. The viscosity of non-Newtonian fluids is dependent on shear rate, time, or both. Non-Newtonian materials that are time independent are defined as shear-thinning or shear-thickening. Non-Newtonian materials that are time-dependent are defined as thixotropic (thinning over time) or rheopectic (thickening over time) (Steffe, 1996). Cream, juice concentrates, and salad dressings are examples of thixotropic fluid foods (Paredes et al., 1989, Ramos and Ibarz, 1998, HU and JIN, 2008). Yogurt is a thixotropic and shear-thinning semisolid food (Steffe, 1996).

2.6.1 Rheological tests for semisolid foods

2.6.1.1 Rotational tests

Parallel plate, cone and plate, and concentric cylinder (cup and bob) are three types of rotational attachments for measuring viscosity (Steffe, 1996). Measuring cells are chosen according to food properties such as particle size and viscoelastic behaviors. The concentric cylinder system is suitable for foods with low viscosity such as milk or fruit juice. Cone and plate is used for semisolid homogenous foods with small particle size since the cone angle is less than 5 degrees and larger particles may become trapped between the cone and plate, causing measurement errors (Steffe, 1996). During testing with a cone and plate, the apex of cone is held just above the plate and the sample fills the gap. The parallel plate geometry can be used for non-Newtonian semisolid foods such as yogurt (Tabilo-Munizaga and Barbosa-Cánovas, 2005), as well as solid foods. Parallel plates are suitable for heterogeneous systems; both parallel plate and cone and plate systems can be used for temperature-dependent tests and small sample volumes.

Yogurt and acid milk gels are non-Newtonian fluids meaning their viscosity depends on shear rate (Steffe, 1996). Additional factors that affect yogurt viscosity include temperature, time, pressure, and physicochemical behavior of foods. Depending on the impact on texture attributes, yogurt viscosity changes can cause both desirable and non-desirable texture. One desirable viscosity effect is the dispersion stability due to high viscosity when the product is at rest.

2.6.1.2 Transient tests

Common transient tests used for viscoelastic foods include stress relaxation, creep, oscillatory, and start-up flow (Steffe, 1996). Oscillatory tests are generally used to determine yogurt gel strength and resistance to deformation. Stress relaxation tests apply an instantaneous strain and measure the relaxation of stress in the material as a function of time. Elastic materials have no relaxation, viscous materials relax instantly, and viscoelastic material relax over time (Steffe, 1996). In creep recovery testing, an instantaneous stress is applied to the material, then removed after a certain time period, and the change in strain (creep) is recorded over time. Materials will show different responses in creep testing. Ideal elastic materials return to their original shape when the stress is removed and show complete recovery. Viscous materials do not recover any deformation after removing the stress. Viscoelastic materials are able to recover some of the deformation caused by applying stress. In a start-up flow test, a shear rate is applied to a viscoelastic material that has been held at rest. The shear stress from this deformation can show a primary overshoot (maximum in the stress response) before material reaches to a steady-flow state. This overshoot stress can be used to obtain a shear stress growth function.

2.6.1.3 Oscillatory tests

Oscillatory tests are the most common tests used to determine food viscoelastic behaviors (Steffe, 1996). They can provide information on gel strength, protein denaturation, and curd formation, and can be used in correlation of food rheological behaviors to sensory attributes for more targeted food product development (Steffe, 1996). Either shear or compressive strain can be used in oscillatory tests. Shear strain is preferred in the food industry because it can measure the deformation threshold of foods to determine material strength (Steffe, 1996). In oscillatory testing, an oscillating shear strain is applied to the food and the stress response measured or vice versa. The strain or stress used in these tests can be small or large, depending on the type of food and the information desired from the test (Steffe, 1996).

Two key parameters measured in oscillatory tests are storage modulus (G’) and loss modulus (G”). When G’ (storage modulus) is bigger than G” (loss modulus), this indicates that the material stores more energy than it dissipates, resulting in elastic-dominant (solid-like) behavior. When G’ is less than G”, the material dissipates more energy than it stores, resulting in viscous-dominant (fluid-like) behavior. Both G’ and G” provide information about structural stability and gel strength of yogurt and acid milk gels and yogurts (Rohm and Kovac, 1994, Lucey, 2002, Dello Staffolo et al., 2004, Guggisberg et al., 2009, Laneuville and Turgeon, 2014).

Strain or stress sweep tests can be used to measure the viscoelastic behavior of semisolid foods. These tests determine the linear viscoelastic region (LVR) through a gradually increasing oscillatory strain or stress at constant frequency. As the applied strain or stress increases, it causes disruption of the food microstructure, resulting in irreversible deformation and a notable decrease in the viscoelastic moduli (end of LVR). The critical strain, or the strain at which permanent deformation occurs, marks the end of the LVR. Usually, the rheological properties of a viscoelastic material are independent of strain up to a critical strain (Steffe, 1996).

In frequency sweeps, the amplitude of strain or stress is held constant and frequency increased. Viscoelastic foods normally show elastic-dominant behaviors at higher frequencies as long as their structure is not damaged by the test (Steffe, 1996). By determining the sample's LVR via a strain sweep, the structure of foods can be examined by frequency sweeps at a strain below the critical strain to evaluate gel strength and viscoelastic behaviors (Tunick, 2010). Using a strain within the LVR prevents disruption of the structure of the food. Frequency sweep results can be used to determine how different factors, e.g. addition of saliva or hydrocolloids, affect yogurt microstructural strength. Yogurt gel strength has also been related to its physical properties, e.g., the type of bonds (covalent or non-covalent), and protein network conformation (Lee and Lucey, 2010).

2.7 Tribology of foods

Tribology is the study of interacting surfaces in relative motion and concerns friction, wear and lubrication (Bhushan, 2000). Tribology originated from the old Greek word "tribos", which means "to rub". The Egyptians were using water or oil as a lubricant for wooden sledges used to move huge statues in 2400 B.C (Hahner and Spencer, 1998). Although tribology is a very old science, it has a short history in food science.

Oral tribology has been introduced as a key concept in food texture perception during the later stages of food oral processing, when food rheological behaviors become less dominant (van Aken, 2010). When food is chewed, its structure changes as it absorbs moisture, broken into small particles by mechanical forces and salivary enzymes, and forms a bolus. The perception of food texture at later stages of oral processing is largely dominated by a combination of fluid flow and surface characteristics. Hence, rheology behaviors become less relevant and surface friction and lubrication tend to dominate texture sensation and perception (Chen and Stokes, 2012). Oral processing conditions can be simulated in tribological experiments (Malone et al., 2003).

Tribometry is used along with rheometry to obtain a better understanding of food oral processing, since rheological parameters only provide mechanical properties of food during the first stage of oral processing (Chen and Stokes, 2012). Friction behavior is generally represented in the form of a Stribeck curve, where the coefficient of friction is given either as a function of entrainment speed or film thickness (Figure 2.4) (De Vicente et al., 2006, Chen and Stokes, 2012).

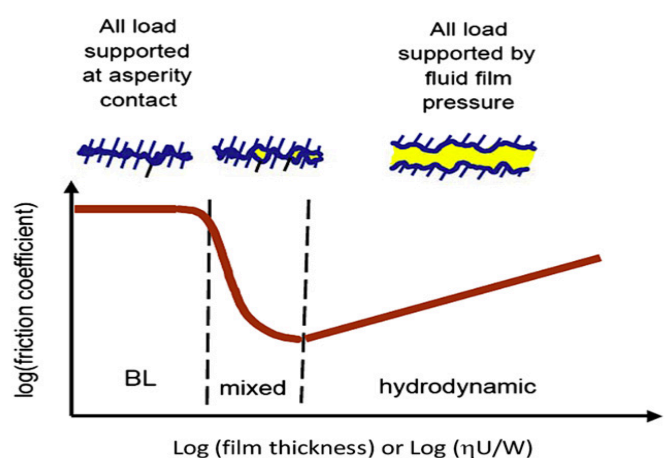

Figure 2.4. Typical Stribeck curve as a function of entrainment speed and film thickness. (De Vicente et al., 2006)

According to the Stribeck curve, there are three different regimes for tribological behavior: hydrodynamic, boundary and mixed. At high sliding speeds, the rate of entrainment of the lubricant into the contact space due to surface motion results in an adequately high fluid pressure to fully separate the surfaces. This is called hydrodynamic lubrication. The friction obtained in this type of lubrication is dependent on the viscosity of the lubricant (Cassin et al., 2001). If the hydrodynamic fluid pressure is insufficient to separate the palate and tongue or any two other sliding surfaces, then the lubrication properties of the food or lubricant depend on the ability of the lubricant or food's components to form boundary films (Prakash et al., 2013). The boundary regime occurs in low sliding speeds where the pressure of pressure of fluid is not enough to separate the two sliding surfaces, resulting in significant asperity contact. The boundary regime has been noted as the regime closely related to human perception of astringency and slipperiness (Cassin et al., 2001, Dresselhuis, 2008). Boundary lubrication is characterized by the presence of an immobile fluid layer on tongue and palate surfaces. The mixed lubrication regime lies between the boundary and the hydrodynamic regime. In this regime, the food entrainment into the tongue–palate contact zone is sufficient to partly separate the two rubbing surfaces. However, the lubricant film thickness and the height of the asperities of the substrate surfaces are of similar dimensions, so the contact load is borne in part by fluid pressure and in part by asperity contact pressure (Cassin et al., 2001). The friction coefficient reaches a minimum in this regime and, with either increased asperity contact or increased thickness of the lubricant layer, the friction coefficient will increase (Dresselhuis, 2008, Gabriele et al., 2010). Boundary and mixed regimes are the dominant regimes in foods (Dresselhuis et al., 2007b). The hydrodynamic regime is often used in lubrication processing in industry; it is not typically applicable in food tribology. Both oral processing speeds and contact pressures are relatively low compared to other industries that involve tribology, such as material engineering industries (Dresselhuis et al., 2007b). In general, all three regimes may be observed in the material is tested over a wide enough range of sliding speeds. The sliding speed ranges used for food tribological tests can differ based on the material and its application (Selway and Stokes, 2013).Oral sliding speeds have been suggested to be between 5 and 60 mm/s (Chojnicka et al., 2009).

Tribometry can be used to indicate mouthfeel attributes of fluid and semisolid foods (Malone et al., 2003, Dresselhuis et al., 2007b). Thin film-related properties are thought to be related to friction-dominated mouthfeel attributes, including creaminess, smoothness, slipperiness, astringency, and stickiness (De Wijk et al., 2006b, Stokes et al., 2013, Sonne et al., 2014).Boundary and mixed regimes have been shown to be the dominant regimes in oral processing of fluid and semisolid foods (Selway and Stokes, 2013, Sonne et al., 2014). Sensory attributes, e.g., astringency, dry, and gritty, have been reported to be perceived during boundary sliding. The may be due to high friction and lower sliding speeds between the two surfaces (van Aken, 2010). One of the reasons for dry sensations have found to be due to stimulation of mechanoreceptors by rubbing the two surfaces (De Wijk et al., 2003). Upon increasing the oral or instrumental sliding speed, a thin film of food or aggregated fat globules forms between the two surfaces, causing lower friction coefficients and potentially a smoother mouthfeel (Selway and Stokes, 2013, Sonne et al., 2014).

2.7.1 Tribological surfaces

The surface chemistry and mechanical and physicochemical properties of the plate and ball materials can be used to explain the physicochemical interactions between the surfaces and lubricant (Cassin et al., 2001). For instance, using steel and hydrophobic elastomer contacts showed higher friction compared to steel and hydrophilic elastomers in both dry contacts or lubricated with either water or gum solutions. These results were attributed to differences in friction profiles caused by different surface interactions (De Vicente et al., 2005).

For a better comparison with food oral processing, the surfaces used in tribological testing of foods should mimic the conditions of the oral cavity (palate and tongue). However, the complexity of the oral cavity makes straightforward selection of sliding surfaces difficult (Cassin et al., 2001). Knowing the physicochemical characteristics of the oral cavity is required for designing similar surfaces for tribology tests. Gastric and salivary mucins have shown to be adsorbed on oral surfaces, making them hydrophilic (Cassin et al., 2001). Mucin has non-polar amino-acids and will adsorb to hydrophobic surfaces like oral tissues. On the contrary, guar gum did not adsorb on hydrophobic surfaces. As a result, guar gum showed a higher friction in boundary regime of the Stribeck curve compared to mucin solutions (De Vicente et al., 2006), indicating that guar gum is not a suitable substance to use when mimicking oral conditions. A soft PDMS surface has been used to emulate realistic physical parameters for soft bio-tribological contacts (Cassin et al., 2001, De Vicente et al., 2005, 2006). Hard surfaces, such as steel, resulted in a higher pressure than those present in the oral cavity during mastication (Chojnicka et al., 2008, De Vicente et al., 2005). Other factors that make steel surfaces inappropriate for food tribology include its large Young's modulus and its different surface chemistry from that of oral surfaces (Bongaerts et al., 2007a, Chojnicka et al., 2008). Steel surfaces are also not able to deform in the manner of the soft surfaces in the mouth, especially the tongue.

2.7.2 Tribology of semisolid foods

Mouthfeel and texture are considered important attributes for yogurt acceptance by consumers. Defects such as chalkiness and graininess may be influenced by factors such as processing conditions, additives, and starter culture selection (Tribby, 2008). Tribological tests can measure friction and lubrications behaviors, and this information may be related to attributes such as fatty mouthfeel, astringency, smoothness, roughness, and slipperiness (Malone et al., 2003, Dresselhuis et al., 2007b). These attributes are key for consumer acceptance when selecting semisolid foods like yogurt, mayonnaise, and mousse (Malone et al., 2003, Dresselhuis et al., 2007b). The most important interacting surfaces during food texture perception are tongue–palate and tongue–food (Chen and Stokes, 2012). The degree of lubrication between these two surfaces can significantly influence food texture perceptions. This in-mouth lubrication is formed by the interaction between the oral mucosa and the food product, as well as between the tongue and palate, even in the presence of a food-saliva combination as a lubricant (Prakash et al., 2013).

Tribological studies of acid milk gels has shown that the general pattern of the Stribeck curve was boundary to mixed regime for this food (Chojnicka-Paszun et al., De Vicente et al., 2005, Dresselhuis et al., 2007a, Chojnicka et al., 2008, Zinoviadou et al., 2008, Zinoviadou et al., 2012). These regimes are also the dominant regimes in oral cavity. Both addition of saliva and different ingredients affected the rheological and tribological behaviors of acid milk gels. Friction coefficients were more affected when saliva was added, specifically for the formulations with starch (Joyner (Melito) et al., 2014). This result was attributed to breakdown of starch due to amylase from saliva (Humphrey and Williamson, 2001); it was not a dilution effect based on comparison of samples mixed with deionized water (Joyner (Melito) et al., 2014). Different correlations were found among acid milk gel viscosity and tribological behaviors and sensory attributes. Sensory attributes related to viscosity, such as spoon, and mouth viscosity and spoon drip, were highly correlated to instrumental viscosity results. Increased chalkiness was correlated with increased friction at lower sliding speeds. Friction coefficient was also correlated with other sensory attributes such as lumpiness and smoothness. Stronger correlations were found when using friction coefficient multiplied by viscosity, which indicates that both rheological and tribological data were related to texture perception of acid milk gels (Joyner (Melito) et al., 2014(Janssen et al., 2007, Pascua et al., 2013, Selway and Stokes, 2013).

As can be seen from the previous discussion, multiple studies have measured semisolid food rheological, tribological, and sensory properties and correlated these behaviors to achieve a better understanding of food stricture–function–texture relationships for design of reduced-fat yogurt and other semisolid products (Hewitt and Bancroft, 1985, Krzeminski et al., 2013, Ozcan, 2013, Krzeminski et al., 2014, Sonne et al., 2014). Application of saliva in these studies along with determining correlation of these properties to each other may be useful in future oral processing studies. Mixing saliva with the sample results in a better understanding of what happens during later stages of mastication and swallowing (Malone et al., 2003, Janssen et al., 2007, Joyner (Melito) et al., 2014(Morell et al., 2016).

2.8 Important factors in tribological properties of acid milk gels and yogurts

2.8.1 Formulation

The shape of the Stribeck curve depends on different factors, including formulation (Chojnicka-Paszun et al., De Vicente et al., 2005, Dresselhuis et al., 2007a, Chojnicka et al., 2008, Zinoviadou et al., 2008, Zinoviadou et al., 2012). Hydrocolloids can significantly impact food tribological behaviors (Chojnicka-Paszun et al., Selway and Stokes, 2013, Sonne et al., 2014, Morell et al., 2016, Laiho et al., 2017) Friction coefficients have been shown to decrease with increasing fat and protein, and decreasing proportion of serum protein to casein (Sonne et al., 2014). Using high amounts of hydrocolloids (up to 4% w/w), including pectin, xanthan gum, locust bean gum, carrageenan, and gelatin; and up to 9% (w/w) WPI resulted in a chalky, gritty or powdery texture, which might increase friction coefficients. These hydrocolloids are all neutral or negative charges and are extensively used as thickeners in foods e.g., desserts, sauces, soups, or bakery products (Chojnicka-Paszun et al.). Molecule/particle size, material stiffness, and material homogeneity are additional factors that can influence friction. For example, in one study on protein-fortified milk, the slope of the mixed regime changed with different concentrations of WPI (Chojnicka-Paszun et al., Chojnicka et al., 2008). Additionally, at low concentrations of WPI, friction decreased more rapidly in the mixed regime. In the same study, dairy products incorporating different hydrocolloids, such as pectin, LBG and xanthan gum, showed different Stribeck curves. LBG showed the highest friction and was the only hydrocolloid that showed a boundary regime. Xanthan gum had the lowest friction among three and showed the mixed regime along with pectin. Food properties has been also correlated with oral slippery perception in the mixed regime for guar gum solutions (Malone et al., 2003, Selway and Stokes, 2013).

2.8.2 Temperature

Temperature can significantly affect friction behaviors (Iqbal and Fitzpatrick, 2006) . This can be attributed to thermal expansion. As the free space between the molecules becomes greater and molecular movement increased, relaxation times shorten. This phenomenon can vary in different materials since increased temperature can affect both physical and rheological properties of materials. Since temperature is one of the main factors affecting friction behavior in the oral cavity, it should be considered in tribological tests (Iqbal and Fitzpatrick, 2006). Although a limited range is considered for food tribological testing, typically between room temperature (25°C) and body temperature(37°C), maintaining constant temperature is important for these tests (Chojnicka et al., 2009). Tests may be run at body temperature to mimic conditions in the oral cavity; testing at room temperature mimics the approximate temperature that refrigerated semisolid foods reach at the end of oral processing since they are not held long enough in the mouth to reach body temperature (Chojnicka et al., 2009). Temperatures higher or lower than this range may not present results comparable with sensory attributes.

2.8.3 Particle size

The effect of particles on perceived texture and lubrication properties of semisolid food has been studied (de Wijk and Prinz, 2005). Large or irregular particles showed higher friction compared to small or round ones. Larger particle size and higher concentration reduce creaminess (Kilcast and Clegg, 2002). Additionally, higher friction in semisolid foods was typically associated with a decreasing sensation of creaminess, fattiness, stickiness, or smoothness and increasing sensation of roughness (de Wijk and Prinz, 2005). Another study related particle size with oral perception and found strong correlations between presence of the particles and texture perception of semisolid custard desserts (Engelen et al., 2005). This relation was described by the lubricative behaviors of the food relative to oral tissues.

2.9 The effect of saliva composition on food texture perception

One of the most important factors that can impact food texture perception is saliva. Saliva is secreted mainly by the contra-lateral major glands and minor salivary glands present in the mucosa of the tongue, cheeks, lips and palate (Young and Van Lennep, 1978). There

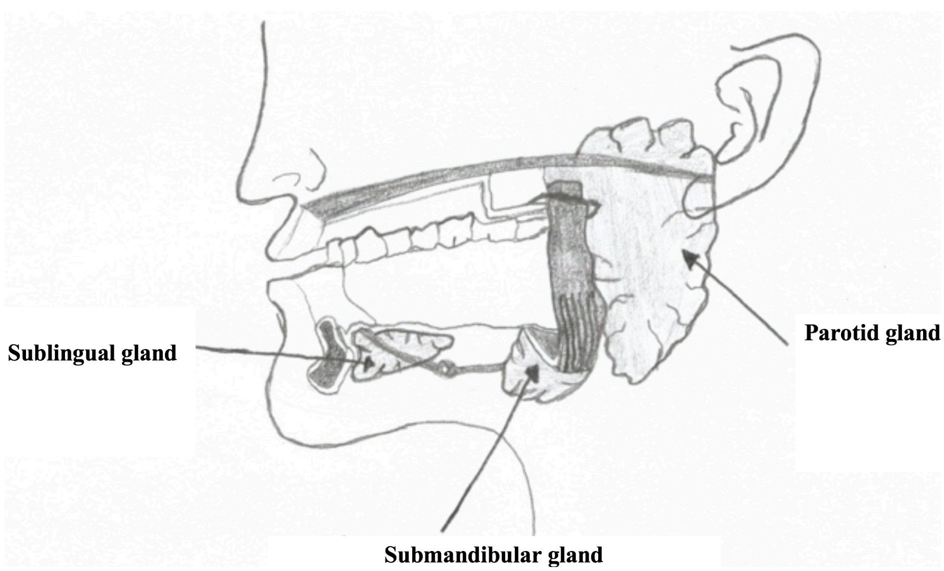

Figure 2.5. Salivary glands in humans

are three contra-lateral major salivary glands in humans (Figure 2.5). The parotid gland is the largest and contributes the greatest amount of saliva (approximately 60% of the total flow) when stimulated by eating or tasting but contributes a smaller amount to resting salivary flow (Matsuo, 2000). The parotid glands are situated just below and in front of each ear and secrete no mucins; the saliva from these glands is high in amylase and proline-rich proteins. The other salivary glands are submandibular and sublingual. The pair of submandibular glands is below the jaw and sublingual glands are under the tongue (Young and Van Lennep, 1978).

The main constituents of human saliva are water (97-99.5%), various electrolytes (sodium, potassium, calcium, chloride, magnesium, bicarbonate, phosphate), proteins, numerous enzymes, immunoglobulins and other antimicrobial components and mucosal glycoproteins (Stack and Papas, 2001, Nagler, 2004). Saliva forms a seromucosal coat (a secretion of serous to mucous glandular cells) that lubricates and protects the oral tissues against irritating agents (Stack and Papas, 2001, Nagler, 2004). The coat is mainly provided by mucins, which are responsible for lubrication, protection against dehydration, and maintenance of salivary viscoelasticity. Mucin is reported to be the main salivary protein to be responsible for lubrication effects (Cárdenas et al., 2007).

The most important functions of saliva are lubrication of the oral cavity and bolus formation. Saliva protects against various irritants such as hydrolytic and proteolytic enzymes, desiccation from breathing, and carcinogens from chemicals or smoking (Louis, 1988). As previously mentioned, mucin, secreted by submandibular and sublingual glands, is the best lubricating component in saliva. Lubricant effects of the proteins in saliva assist with mastication, speech, and deglutition (Humphrey and Williamson, 2001, Stack and Papas, 2001, Amerongen and Veerman, 2002, Nagler, 2004). Mucins have different features that help mastication, swallowing, and speech (Tabak, 1990) . The lubricating properties of mucin include high elasticity and viscosity, low solubility, and adhesiveness (Edgar, 1989).

The second function of saliva is its buffering effect. Components that help the buffering action include bicarbonate, urea, phosphate, and amphoteric proteins and enzymes. Bicarbonate is the most important buffering component. It neutralizes the acids in tooth plaque and also produces ammonia to form amines with additional buffering action (Mandel, 1989). The third function of saliva is to keep the oral cavity clean and maintain tooth integrity through remineralization (Mandel, 1987, Edgar, 1989). The fourth function is the antibacterial feature of saliva. Different components are secreted through salivary glands to protect the oral cavity, including IgA, IgG, and IgM immunologic agents and proteins such as lactoferrin, peptides mucins, and enzymes such as lysozyme. Finally, saliva helps with food digestion and perception (Humphrey and Williamson, 2001). Saliva starts the digestion process with starch breakdown. Amylase that primarily breaks down starches into sugar is secreted by parotid gland (Mandel, 1987, MOSS, 1995). Saliva helps in texture perception of food products, primarily thickness (van Vliet et al., 2009).

The effect of saliva and its different components on food texture perception have been studied (Guinard et al., 1997, Engelen et al., 2003a, Engelen et al., 2003b, De Wijk et al., 2006a, Engelen et al., 2007, Kupirovič et al., 2017). Saliva composition showed significant changes when stimulated with odor, parafilm, or citric acid compared to an unstimulated saliva as the control. Mucin was at highest level in unstimulated saliva (rest); α-amylase increased for stimulated saliva chewed with parafilm, since during mastication saliva is mostly secreted from parotid gland and α-amylase is secreted by parotid gland. Buffer capacity of mechanically stimulated saliva was also higher than unstimulated saliva. Other studies have found that protein concentration and α-amylase activity had the highest correlation with semisolid food texture perception compared to mucin level and buffer capacity (Engelen et al., 2007).

2.10 Conclusion

Food texture and mouthfeel are two of the most important parameters for consumer acceptability of semisolid foods like yogurt. Texture perception is a complex process due to the different factors in the mouth, such as saliva, and movement of the jaw and tongue. Fat content and different formulation can also significantly influence the perceived texture of semisolid foods such as yogurt or acid milk gels. Using instrumental evaluation can provide a good understanding of what happens in the mouth during mastication. An abundance of information exists regarding the effects of different fat replacers on the perception of yogurt texture as well as the impact on yogurt microstructural, mechanical, and physicochemical measurements. However, the role of saliva along in combination with yogurt microstructure, rheological and tribological behaviors, and sensory attributes has not been well-studied. Therefore, the objective of this study is to determine the effect of various hydrocolloids and saliva application on acid milk gel and yogurt rheological, tribological, and microstructural properties, and how these properties relate to texture perception of the acid milk gels and yogurts. Having a thorough understanding of semisolid food structure–function–texture relationships with and without added saliva can contribute to designing high-quality semisolid foods with higher consumer acceptance. Formulating the desired and palatable semisolid foods like yogurt with less fat could also be an important step toward preventing obesity.

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CHAPTER 3: THE EFFECTS OF HUMAN WHOLE SALIVA ANDHYDROCOLLOIDS ON TEXTURE PERCEPTION OF ACID MILKGELS: RELATIONSHIPS AMONG RHEOLOGICAL, TRIBOLOGICAL, AND MICROSTRUCTURAL PROPERTIES

3.1 Abstract

Reduction or removal of fat in yogurts can negatively affect their textural properties. Hydrocolloids can improve the texture of reduced or non-fat semisolid foods through different mechanisms. As a result, they will demonstrate different functionality when added to the foods. Also, incorporation of human whole saliva (HWS) with food in the mouth can alter the texture characteristics of foods. Thus, the objective of this study was to determine the effects of HWS and hydrocolloids on rheological, tribological and microstructural behaviors of acid milk gels as a model system. Glucono-delta-lactone (GDL) was used as an acidifier in acid milk gels to reach the pH close to yogurts (4.55-4.6). The advantage of GDL application in the model system compared to live bacteria is an easier control of pH during testing. For this project, 24 acid milk gels were prepared using skim milk, cream, and hydrocolloids (locust bean gum, cellulose gum, corn starch and potato starch, whey protein isolate, and skim milk powder). Shear rate sweeps, strain sweeps, and frequency sweeps were carried out for all samples with or without HWS at 8°C and 25°C. Tribometry was done at only 25°C with and without HWS. Samples were also imaged by confocal laser scanning microscopy. Overall, the protein aggregation seen in microstructural imaging increased by addition of saliva and hydrocolloid application made thicker chains and clusters of proteins. Viscosity and viscoelastic moduli (G' and G") decreased when samples were mixed with HWS and tested at higher temperature, but the specific effect was dependent on the type of hydrocolloids used. Cross, Cross-Williamson, and Herschel-Bulkley models were fit to the averaged viscosity curves of acid milk gels, based on their formulations, with $R^2>0.720$, $R^2>0.813$, and $R^2>0.692$, respectively. Friction profiles were significantly different among formulations, and friction coefficient decreased with addition of HWS mainly for samples with potato starch. Samples prepared with gums showed less of a decrease in friction coefficient with addition of saliva. The protein structures of samples prepared with an anionic hydrocolloid and the starch with larger granule size were stronger than the formulations with neutral hydrocolloids and the starch with smaller granule size. Confocal images were useful for explaining some of the rheological and tribological behaviors of acid milk gels. Microstructures that had a more open network and aggregated protein were linked to higher viscosity and higher friction in non-fat samples prepared with hydrocolloids. Relating rheology, tribology, and microstructural imaging aided in determining the effect of hydrocolloids and HWS in acid milk gels for a fuller illustration of texture perception; these results can be used to design palatable reduced-fat semisolid products by understanding the impacts of hydrocolloids and HWS on the non-fat acid milk gels textures.

Key words: Rheology, tribology, hydrocolloids, semisolid foods

3.2 Introduction

Rheology and tribology are techniques that have been used to indicate food texture attributes. Rheology is a well-known method to measure mechanical properties of foods, e.g. flow and deformation, that are important to functionality and texture attributes. For example, viscosity is an important rheology concept that has been correlated with several textural attributes of semisolid foods (Chojnicka-Paszun et al., Stanley and Taylor, 1993, Malone et al., 2003). Rheological behavior of foods can be related to the manipulation of food under the shear and pressure of the oral surfaces at different sliding speeds after ingestion (Janssen et al., 2007). Nevertheless, food rheological behaviors are not sufficient to completely predict the perception of certain textural attributes such as graininess, smoothness, and chalkiness. For this reason, tribology has been introduced as an effective tool to measure lubrication, friction and wear properties of foods.

Tribology is the science of friction, lubrication, and wear and has become popular in oral processing studies due to its similarity to sensations in the mouth. These sensations are the result of rubbing two oral surfaces and producing a friction or lubrication that results in perceiving different textural attributes e.g., astringency, creaminess (de Wijk and Prinz, 2005, Engelen et al., 2005, Selway and Stokes, 2013, Sonne et al., 2014). Stribeck curves, which are plots of friction coefficient versus sliding speed, can be used to represent tribological behaviors of foods. These curves have 3 different regimes. First is the boundary regime, in which contacting surfaces have a minimal gap and there is almost no space for the lubricant between surfaces. Thus, the boundary regime has high constant friction coefficients compared to the other regimes due to significant surface–surface contact (Cassin et al., 2001). Second is the mixed regime. In this regime, the friction coefficient decreases to a minimum with increased sliding speed. The amount of lubricant between the contact surfaces increases and results in increased surface separation, but the surfaces are still in contact. Third is the hydrodynamic regime. In this regime, the pressure from the lubricant becomes sufficient to completely separate the sliding surfaces. Understanding the frictional properties of semisolid foods in a Stribeck curve plot is the first step to a better illustration of oral processing and texture perception. This can be explained by mimicking the oral processing by creating surfaces (palate-tongue) with as similar as possible to their characteristics e.g. chemistry, roughness, hydrophilic or hydrophobic properties. Creating a Stribeck curve profile with a friction coefficient vs. sliding speed can help predicting mouthfeel or after mouthfeel properties of semisolid foods during oral processing. For instance, it is shown that astringency and slipperiness are mostly related to boundary regime (Prakash et al., 2013).

Besides mechanical and frictional properties, microstructural imaging can help describe the textural differences in semisolid foods formulated with different hydrocolloids. For instance, addition of starch to semisolid food can increase the viscosity as well as the viscoelastic moduli. These results were in accordance with confocal images of acid milk gels with added potato starch (Oh et al., 2007). Confocal imaging results showed that the swollen potato starch granules were embedded in the protein network of the acid milk gels, and the density of the protein matrix increased with increased concentration of potato starch (Oh et al., 2007).

Different types and concentrations of hydrocolloids can be added to reduced or non-fat semisolid foods as texture enhancers (Ognean et al., 2006, Peng and Yao, 2017). The mechanism of their behavior in a food system depends on their physicochemical properties as well as their origin. The effect of hydrocolloids on semisolid food has received significant attention in the literature (Chojnicka-Paszun et al., Janssen et al., 2007, Oh et al., 2007, Milani and Maleki, 2012). Whey protein powder is known to increase the viscosity of semisolid foods such as yogurt (Huc et al., 2016). However, it can have an adverse effect on sensory attributes such as astringency, chalkiness, and grittiness (Lucey and Singh, 1997, Lee and Lucey, 2010, Morell et al., 2016). Cellulose gum is known to mimic fat functionality but can also increase food viscosity (Cho and Prosky, 1999). Starches are another category of hydrocolloids that are used as fat replacers in semisolid foods. They can improve the rheological properties of foods (Cho and Prosky, 1999, Peng and Yao, 2017), but they may show significantly different oral behavior compared to the original food due to amylose hydrolysis after interaction with human whole saliva (HWS) (Janssen et al., 2007). Thus, considering the effect of HWS in predicting texture perception of semisolid foods is key for better interpretation of the results.

HWS, secreted during all the stages of oral processing, can significantly impact texture perception of solid foods. The most important role of HWS is lubrication. It softens food, helps move the formed bolus in the mouth under oral pressure and shear, and contributes to the initial breakdown of food components, mainly starch (Andrewes et al., 2011). In addition, HWS complexes with smaller food particles in the mouth and forms a thin lubricating layer between the palate and tongue, and the oral surfaces and food bolus during mastication (Chen and Engelen, 2012). HWS can disrupt food structure by enzymes and protein complexation as well as its dilution effects. Salivary proteins, mainly mucin, can alter food structures by altering the net charge, potentially resulting in particle precipitation (Chen and Engelen, 2012). α-amylase and protein concentration were shown to have the greatest effect on texture perception of semisolid foods, particularly starched-based ones (Engelen et al., 2007).

Temperature change is also another factor that can affect texture perception of semisolid foods and needs to be considered during *in vitro* testing. Addition of HWS and controlling temperature plus mimicking the oral surfaces in tribological testing can provide a better picture of how food structure changes during oral processing and contributes to texture perception of foods. Therefore, the objectives of this study were to determine how hydrocolloids and HWS impact semisolid food structure, rheological, and tribological behaviors of acid milk gels at 25°C and 8°C. These results can be applied to formulation of reduced or non-fat semisolid foods with desirable textural properties.

3.3 Materials and Methods

3.3.1 Materials

Skim milk (WinCo Foods) was obtained from a local supermarket (Moscow, ID., U.S.A.). Low heat skim milk powder (SMP) and Darigold brand heavy cream (40% fat) were provided by the WSU Creamery (Pullman, WA., U.S.A.). Locust bean gum (LBG) and carboxymethyl cellulose (CMC) (pre-hydrated Ticalose CMC 2500 powder) were donated by TIC Gums (TIC Gums, Inc., Belcamp, Md., U.S.A.). Corn starch (CS) and modified potato starch were donated by Ingredion (Bridgewater, N.J., U.S.A.). Whey protein isolate (WPI) (Provon 190, 89.4% protein) was donated by Glanbia Nutritionals (Fitchburg, Wis., U.S.A.). Glucono-delta-lactone (GDL) was donated by Jungbunzlauer (Jungbunzlauer, Inc., MA., U.S.A.). The protein assay kit (Quick Start Bradford) used for protein measurement of HWS was purchased from Bio-Rad laboratories (Bio-Rad laboratories, Inc. CA., U.S.A.). Teflon balls (6 mm) for tribometry were purchased from McMaster-Carr (Atlanta, Ga., U.S.A.). GluconoFluorescein Isothiocyanate (FITC) dye and cavity slides were purchased from Sigma

(Sigma-Aldrich, St. Louis, MO., U.S.A.), and Nile red dye was purchased from TCI America (Portland, OR., U.S.A.).

3.3.2 Sample preparation

Twenty-four different formulations of acid milk gels were prepared with skim milk (89.15-97.2% w/w), SMP (0-2.8% w/w), cream (0-3.5% w/w), WPI (0-2.8% w/w), and hydrocolloids, including LBG (0-1.8% w/w), corn starch, potato starch, and CMC (0-1.55% w/w) (Table 3.1). These values were determined based on the total solid of acid milk gels which was equal in all samples (13% w/w). After adding all the powders and cream to the skim milk at room temperature (22 ± 2°C), the mixture was stirred with a spatula to disperse the dry powders for 3 min in the water bath (Precision, Thermo Fisher Scientific, Waltham, MA, U.S.A.) at pasteurization temperature (85°C) for complete dissolution. The heat treatment was done at 85°C for an additional 30 min without stirring. The mixture was homogenized at 5,000 rpm for 1 min using a stand homogenizer (Polytron, Kinematica AG, NY, U.S.A.), and cooled to 42.2°C for addition of GDL (1.1%-1.55% w/w, Table 3.1). The mixture was incubated at 42.2°C for 4 hours to reach a pH of 4.55-4.6. The gel was then broken with a metal laboratory spatula and stored in the refrigerator at 4°C overnight.
Acid milk gels were blended at 350 rpm for 10 seconds the next day before testing. Each sample was made in duplicate in different days and they were stored overnight for the next day testing.

Table 3.1. Experimental design of acid milk gels

Formula number	SMP (w/w)	Sweet WPI (w/w)	LBG (w/w)	CMC (w/w)	Potato starch (w/w)	Corn starch (w/w)	Skim milk (w/w)	Cream (w/w)	GDL (w/w)
1	2.8	0	0	0	0	0	97.2	0	1.1-1.55
2	2.83	0	0	0	0	0	95.96	1.21	1.1-1.55
3	2.89	0	0	0	0	0	92.26	4.85	1.1-1.55
4	2.95	0	0	0	0	0	89.15	7.9	1.1-1.55
5	1.8	1	0	0	0	0	97.2	0	1.1-1.55
6	1.8	0	1	0	0	0	97.2	0	1.1-1.55
7	1.8	0	0	1	0	0	97.2	0	1.1-1.55
8	2.1	0	0	0	0.7	0	97.2	0	1.1-1.55
9	2.1	0	0	0	0	0.7	97.2	0	1.1-1.55
10	0	1.25	1.55	0	0	0	97.2	0	1.1-1.55
11	0	1.25	0	1.55	0	0	97.2	0	1.1-1.55
12	0	1.25	0	0	1.55	0	97.2	0	1.1-1.55
13	0	1.25	0	0	0	1.55	97.2	0	1.1-1.55
14	0.5	0.8	0	0.75	0.75	0	97.2	0	1.1-1.55
15	0.5	0.8	0.75	0.75	0	0	97.2	0	1.1-1.55
16	0	2.8	0	0	0	0	97.2	0	1.1-1.55
17	0	0	0	1.4	1.4	0	97.2	0	1.1-1.55
18	0	0	1.8	0	0	1	97.2	0	1.1-1.55
19	0	1.15	0.55	0.55	0.55	0	97.2	0	1.1-1.55
20	0	1.15	0	0.55	0.55	0.55	97.2	0	1.1-1.55
21	0	0	0.7	0.7	0.7	0.7	97.2	0	1.1-1.55
22	0.55	0.75	0.5	0.5	0.5	0	97.2	0	1.1-1.55
23	1	0	0.45	0.45	0.45	0.45	97.2	0	1.1-1.55
24	0.2	0.8	0.45	0.45	0.45	0.45	97.2	0	1.1-1.55

3.3.3 Proximate analysis

All proximate analysis was carried out in duplicate. Protein content was determined using a Leco FP-528 nitrogen analyzer (Leco Corp., St. Joseph, MI, USA) according to the manufacturer’s instructions (Kjeldahl conversion factor 6.38). Moisture contents were determined using a DKN 400 oven (Yamato Scientific America, INC., Santa Clara, CA., U.S.A.), according to the method of (AOAC, 1999). Fat contents were determined only for samples with added cream using the Mojonnier method 989.05 (AOAC, 1995). Skim milk with zero fat content was used for preparation of other samples. There was also no trace of fat in other hydrocolloids according to their specification sheets that were provided by their manufacturers. Therefore, fat content was considered zero for no-added cream samples in this study. Ash contents were determined by using the method of (AOAC, 1995) based on dry basis. Carbohydrate content was calculated by difference.

3.3.4 HWS collection

The approval for collecting HWS was received from University of Idaho Institutional Review Board (protocol 17-196). HWS collection was done according to a modified method of (Bongaerts et al., 2007b). HWS was collected from 5 healthy people (3 females and 2 males, ages 20-35) with normal saliva flow according to the method of (Bongaerts et al., 2007b). Panelists were asked to refrain from eating and drinking anything except water 2 hr prior to the expectoration. At the start of collection, they were required to rinse their mouth twice with deionized water and expectorate into a waste cup. They were given a disposable plastic pipette to chew for the HWS stimulation and expectorate their HWS into a 2-oz. cup. HWS was collected freshly after every two hours. HWS was used for both rheological and tribological testing within two hours of collection for the testing and the excess was discarded.

3.3.5 Rheometry

Rheological properties of acid milk gels were measured with an Anton Paar MCR 302 rheometer (Anton Paar, Graz., Austria) using a 50 mm diameter parallel plate with a measuring gap of 1 mm. The sensor force was set at 5 N for the zero gap of the upper plate.

Zero gap was done for the rheological tests before loading the samples. All tests were carried out at 25°C and 8°C with and without addition of human whole saliva (HWS) collected per Section 3.4 Samples were equilibrated to the test temperature prior to the testing for 60 s. Each sample was tested in triplicate and results were averaged for data analysis. Shear rate sweeps (shear rate of 0.01 to 100 s^{-1}) were carried out to measure viscosity of acid milk gels. Oscillatory tests were performed to measure the viscoelastic properties of the acid milk gels. Strain sweep tests were done at 0.01-100% and a frequency of 1 Hz. Frequency sweep tests were done at 0.1-100 rad/s and 75% of the lowest critical strain calculated from strain sweep tests to remain in linear viscoelastic region (LVR). Critical strain was calculated by determining the strain at which G* deviated by more than 1% from the previous value within the LVR (Steffe, 1996, Tunick, 2010).

3.3.6 PDMS plate production

Polydimethylsiloxane (PDMS) gel plates were used for tribometry using the method reported by (Bongaerts et al., 2007a). Briefly, plates were made by mixing a curing agent and a base (Dow Corning Corporation, Midland, MI, U.S.A.) in a proportion of 1:10 in a beaker, then the mixture was poured into an aluminum mold (4 mm height and 60 mm diameter). Air bubbles were removed by a cabinet vacuum desiccator (Bel-Art Products, Wayne, N.J., U.S.A) under a pressure of -90 kPag. This action was repeated up to 10 times until all bubbles were removed. The PDMS plates were cured at 55°C for 2 hr in a DKN 400 oven (Yamato Scientific America, INC., Santa Clara, CA., U.S.A.), then stored overnight at room temperature (22 ± 2°C) to complete curing. The plates were removed and stored at room temperature (22 ± 2°C) until used for testing.

3.3.7 Tribometry

Tribometry was performed using an Anton Paar MCR 302 (Anton Paar, Graz., Austria) with a three ball (Teflon, 6 mm diameter) geometry on a 60-mm diameter PDMS plate. These surfaces were selected to mimic the oral surfaces (palate-tongue) (Johnson et al., 1993, Prakash et al., 2013). The normal force used was 1 N to mimic the in-mouth force during swallowing, which is between 0.01 and 10 N (Miller and Watkin, 1996). The PDMS plate was placed on top of the original stainless plate of the rheometer and pressed firmly to adhere the two surfaces. A line was marked on both the PDMS plate and stainless steel using a laboratory pen to provide a visual indicator that the PDMS plate did not move during testing. Friction coefficient was measured at sliding speeds of 0.01-1000 mm/s. Samples were tested at 25°C with and without addition of human whole HWS. The collected HWS from panelists was mixed thoroughly before being used for testing. For testing samples with HWS, 0.5 ml of HWS was pipetted and mixed with 3 g of sample and held at room temperature (22 ± 2°C) for 5 min for complete digestion (Joyner (Melito) et al., 2014). At least three replicates for each sample duplicate were performed with and without HWS. The PDMS plate was cleaned after each run with 70% ethanol and laboratory wipes for non-fat samples; 70% ethyl ether was used for the samples with fat to prevent fat film build-up on the surface of PDMS plates and balls and then rinsed with 70% ethanol. Both plates and balls were changed after every 6 runs to prevent wear from affecting the results.

3.3.8 HWS composition analysis

The composition of HWS can significantly affect the texture perception of semisolid foods. Protein concentration and α-amylase activity were reported to have the greatest impact among other components of HWS (Engelen et al., 2007). HWS was collected from five healthy panelists (Section 3.4) for measuring these two components. Collected HWS was centrifuged at 10,000 rpm (14087 *g*) for 5 min to remove buccal cells and oral microorganisms. The clear supernatants were stored at -18°C for further measurements and were thawed at room temperature (22 ± 2°C) 30 min before testing (Engelen et al., 2007).

3.3.9 HWS protein analysis

A Bradford protein kit was used to determine the protein concentration in whole HWS (Quick Start™ Bradford Protein Assay, Bio-Rad). Eight samples were collected from 5 healthy panelists within two weeks. Samples were tested in triplicates. The measurement was performed according to the manufacturer's guidelines (Bio-Rad Laboratories, Inc. CA., U.S.A.). A microplate standard assay using bovine serum albumin (BSA) as the standard protein was used for this test.

3.3.10 HWS α-amylase activity

A modified Somogyi-Nelson assay was performed to determine α-amylase activity

(Shao and Lin, 2018). 1 mM maltose in different concentrations was used as the reducing sugar for creating the standard curve for the assay; maltose is one of the sugars produced by α-amylase from amylose and amylopectin by cleaving the starch chain. The curve was plotted based on the absorbance as a function of sugar concentration with a linear relationship of (R^2=0.9715). The procedure and mechanisms of this test were explained in detail by (Shao and Lin, 2018).

For measuring reducing sugars in HWS, a soluble starch solution was prepared by adding 0.05 g soluble starch to 5 ml water in a Falcon tube and gelatinized in a boiling water bath for 30 min; the tube was shaken every 5 min. After heating, 50 µl of the starch solution was micropipetted into each of 15 microtubes with 1.7 ml capacity (Sorenson, BioScience, Inc., Salt Lake City, UT, U.S.A.); a dilution of 1:250 was made for HWS with DI water samples due to high α-amylase activity. 50 µl of the diluted HWS was also added in the microtubes containing starch solution; each sample of HWS had 15 microtubes in total. Numbered microtubes with the mix of starch and HWS were incubated at 37 °C for 0, 3, 5, 7, and 9 min. Samples were diluted again with DI water at a ratio of 1:5 sample: water for the reaction time of 3 and 5 min, and 1:7 sample: water for the 7 and 9 min. Blank samples of HWS and no soluble starch were created to have zero reaction time. The mix of HWS and soluble starch was pipetted into a polypropylene 96-well microplate (Corning Company, NY, U.S.A) in triplicate, then enzymes were inactivated by boiling the microplate covered with a silicon mat and foil (Shao and Lin, 2018). The covered microplate was cooled for 5 min under cold water, then 45 µl of arsenomolybdate color reagent, prepared via the method of (Nelson, 1944) was added to each well. The microplate was held at room temperature (22 ± 2°C) for 15 min, then the absorbance was at 600 nm with a microplate reader (Spectra Max 190 Microplate Reader, Molecular Devices, CA, U.S.A.). The slope of the amount of reducing sugar of the HWS was determined by using a linear regression of the maltose concentration and absorbance from Somogyi-Nelson assay. Considering the slope values and protein concentration, α-amylase activity was determined as the quantity of enzyme required to produce 1 μM of maltose in 1 min per 1 mg of protein (U/mg).

3.3.11 Confocal imaging

Microstructural properties of the acid milk gels were analyzed using confocal laser scanning microscopy (CLSM). GluconoFluorescein isothiocyanate (FITC) dye and Nile red were used to stain the proteins and fat globules, respectively. 500 μL of ethanol was added to 8 mg of FITC in a 1 mL vial and was vortexed for 10 s, then another 500 μL of deionized water was transferred to the FITC solution and vortexed for another 10 s. The same procedure was repeated for making the Nile red dye, except 5 mg of Nile red was added. Both dyes were used for the samples containing fat and FITC was used for all the samples. Dyes were added to 120 g of the acid milk gel. Samples were then stirred with a spatula to mix the dyes evenly throughout the acid milk gels. Samples were incubated for 4 hr to reach the pH of 4.55-4.6 and was refrigerated overnight and the microscopy analysis was done the next day. 500 μL of the sample was transferred on a cavity slide followed by covering with a glass coverslip. The magnification for imaging the samples was 20X and the temperature was 4-8°C. The excitation wavelength was 559 nm for FITC and 488 nm for Nile red.

3.3.12 Data analyses

Rheology and tribology results were plotted using Origin 8 software (OriginLab;

Northampton, MA, USA). The error bars of each sample for both rheology and tribology tests were calculated using the standard deviation of the samples and their duplicates (6 data points per formulation). The average of the full viscosity profile for each formulation was calculated and the average curves were fitted to three models: Cross-Williams (Equation 1), Cross (Equation 2), and Hershel Bulkley (Equation 3) using TRIOS software (TA Instruments; New Castle, Delaware, USA). These models can provide useful information about flow and deformation properties of acid milk gels.

$$\eta = \frac{\eta_o}{[1+(c\gamma\cdot)^{1-n}]} \qquad (1)$$

$$\eta = \eta\infty + \frac{\eta_o - \eta_\infty}{1+(k\gamma\cdot)^n} k\gamma^{n-1} \qquad (2)$$

$$\sigma = \sigma_o + K\dot{\gamma}^n \qquad (3)$$

In the Cross-Williams model (Equation 1), $\boldsymbol{\eta_o}$ is the zero-shear rate viscosity (Pa s). This parameter can be helpful for determining gel stability and comparing the polymer molecular weight. c is the time constant (s); $1/c$ can be indicative of a critical shear rate for the onset shear rate when shear thinning starts. n is the flow behavior index (unitless), and it is indicative of the level of viscosity dependence on shear rate. For instance, the value of n is one for Newtonian materials. In the Cross model (Equation 2) $\boldsymbol{\eta_o}$ is the zero-shear rate viscosity (Pa s), η_∞ is infinite viscosity (Pa s); η_∞ can show material flow behavior under high shear conditions such as processing with blades or knives; c is the time constant (s), and c is flow behavior index (unitless). In Herschel Bulkley model (Equation 3), σ_o is the yield stress (Pa), the minimum force needed to induce flow. k is the consistency coefficient (Pa s^{1-n}) and

n is the flow behavior index (unitless). In shear-thinning materials ($0<n<1$), pseudoplastic behavior increases as n approaches zero. These parameters were used for statistical analysis or comparison of viscosity properties of acid milk gels.

All statistical analyses were performed with SAS version 9.1 (SAS; Cary, NC). From the rheological results, γ_c (critical strain, %), G^* (complex modulus, Pa), and tan δ (phase angle, rad) were selected for statistical analysis, including three-way ANOVA for determining the impacts of added hydrocolloids, HWS, and temperature on these parameters. viscoelastic properties of acid milk gels, including critical strain (γ_c), tan δ, and G^* (complex modulus), were used to obtain F-values from a three-way ANOVA to determine the effects of added hydrocolloids, HWS, and temperature on these parameters. Friction coefficients at 10, 15, 20, 25, and 30 mm/s of sliding speeds were used for tribological analysis including three-way ANOVA for determining the influence of added hydrocolloids, HWS, and temperature on the friction coefficients measured at these sliding speeds. This selection of speeds was to mimic the oral speed, reported to be 10–30 mm/s for semisolid foods (De Wijk and Prinz, 2006). ANOVA (α=0.05) followed by Tukey's HSD (Honest Significant Difference) test was used to determine significant differences among acid milk gel rheological, tribological, and proximate analysis results.

3.4 Results and Discussion

3.4.1 Proximate analysis results

The proximate composition analysis of the acid milk gels showed significant differences for moisture, protein, ash, fat, and carbohydrate contents (Table 3.2). The total solids content was kept constant for all formulations at 13% w/w. Protein content of the formulations ranged from 3.78% to 6.78%. Sample 23 had the lowest amount of protein since it contained only non-protein hydrocolloids. Sample 16 had the highest concentration, as expected due to WPI addition.

Table 3.2. Acid milk gel proximate composition[1]

Samples	Protein (%)	Moisture (%)	Fat (%)	Ash (%)	Carbohydrate (%)[2]
1	4.51±0.045^{de}	86.22±0.043^{abcd}	0	0.63±0.009^{bcde}	8.64^{efgh}
2	4.4±0.054^{defgh}	85.87±0.206^{abcde}	0.49±0.008^{a}	0.58±0.001^{cdef}	8.67^{defgh}
3	4.02±0.01^{l}	82.79±0.213^{i}	1.92±0.142^{b}	0.59±0.02^{cdef}	10.68^{b}
4	4.24±0.009^{hijk}	83.88±0.404^{hg}	3.36±0.064^{c}	0.53±0.03^{f}	8.00^{ij}
5	6.13±0.02^{b}	84.17±0.015^{hg}	0[3]	0.72±0.002^{a}	8.98^{defghi}
6	4.46±0.04^{defg}	86.72±0.29^{a}	0	0.62±0.009^{cde}	8.21^{ghij}
7	4.29±0.014^{ghij}	81.79±0.198^{j}	0	0.69±0.019^{ab}	13.23^{a}
8	4.38±0.031^{efghi}	85.52±0.191^{cde}	0	0.64±0.006^{bc}	9.47^{cdef}
9	4.49±0.014^{de}	85.52±0.149^{cde}	0	0.59±0.033^{cdef}	9.41^{cdef}
10	5.42±0.065^{c}	85.36±0.085^{fde}	0	0.56±0.007^{ef}	8.66^{efghij}
11	4.51±0.003^{de}	84.47±0.198^{fg}	0	0.63±0.013^{bcd}	10.39^{bc}
12	5.49±0.119^{c}	86.3±0.022^{abcd}	0	0.56±0.002^{def}	7.66^{j}
13	4.26±0.014^{hijk}	82.72±0.043^{ij}	0	0.59±0.024^{cdef}	12.43^{a}
14	4.57±0.014^{d}	86.14±0.12^{abcde}	0	0.64±0.007^{bc}	8.66^{efghij}
15	4.3±0.055^{fghij}	86.62±0.177^{ab}	0	0.61±0.002^{cde}	8.48^{fghij}
16	6.87±0.011^{a}	83.33±0.149^{hi}	0	0.58±0.021^{cdef}	9.23^{defgj}
17	4.07±0.092^{kl}	86.22±0.001^{abcd}	0	0.72±0.001^{a}	9.00^{defghj}
18	4.39±0.06^{defgh}	86.82±0.029^{a}	0	0.57±0.014^{def}	8.22^{ghij}
19	4.49±0.009^{def}	86.74±0.085^{a}	0	0.62±0.003^{bcde}	8.15^{hij}
20	4.46±0.06^{defg}	85.47±0.269^{cde}	0	0.62±0.008^{cde}	9.45^{cdef}
21	4.19±0.067^{ijkl}	86.4±0.015^{abc}	0	0.62±0.002^{cde}	8.79^{defghi}
22	4.37±0.012^{efghi}	85.2±0.686^{ef}	0	0.73±0.001^{a}	9.71^{bcd}
23	3.78±0.014^{m}	86.45±0.142^{abc}	0	0.59±0.047^{cdef}	9.18^{defgh}
24	4.12±0.014^{jkl}	85.67±0.481^{bcde}	0	0.62±0.002^{bcde}	9.59^{cde}

[1]Different letters in a given column indicate significant differences among yogurts for different components at p-value ≤0.05.
[2]Carbohydrates were calculated by difference.
[3]The fat content for non-fat samples were considered zero based on the nutrition facts of ingredients rather than fat analysis.

Moisture content of the formulations with LBG (sample 6, 18) and sample 19, prepared with all hydrocolloids except CS, was the greatest amount among other acid milk gels. These results may have been due to the weak structure of the gel that LBG builds with the milk proteins. The dispersion of LBG throughout the gel structure may have increased its water holding capacity and make it more difficult to escape during the moisture measurement. Sample 7 had the lowest moisture content. Differences in moisture content may be due to the differences in the number of available molecules in the system for interaction with the protein network; more interactions would trap additional water and increase the retained moisture.

The fat content of acid milk gels with no added cream was considered zero since there was negligible fat in their formulations from the ingredients. Ash and carbohydrate contents of acid milk gels showed significant differences based on the type and quantity of the hydrocolloids. Moisture and carbohydrate contents appeared to have opposite trends. Sample 7 had the lowest moisture content and the most amount of carbohydrate. This effect was inversed for sample 6, which contained LBG. Full-fat acid milk gels had the lowest amount of ash and samples 5 (low level of WPI), 17 (CG and PS), and 22 (all hydrocolloids but CS) showed the highest ash content. The range of ash content in the non-fat milk powders e.g. SMP and WPI was higher than the ones with fat. Additionally, different gums and starches have different ash contents, which would explain the differences in ash content.

3.4.2 The effect of hydrocolloids and HWS on microstructural properties of acid milk gels

Overall, all acid milk gel microstructures comprised a particulate protein network containing serum and the specific conformation of the protein network structure was dependent on acid milk gel formulation (Figure 3.1., 3.2., 3.3.). The control sample showed a more homogenous protein network with smaller pores sizes (Figure 3.1.). The branches of protein network became thicker and the size of openings (aqueous phase) increased with addition of hydrocolloids. The microstructure conformation was different for CS and PS.

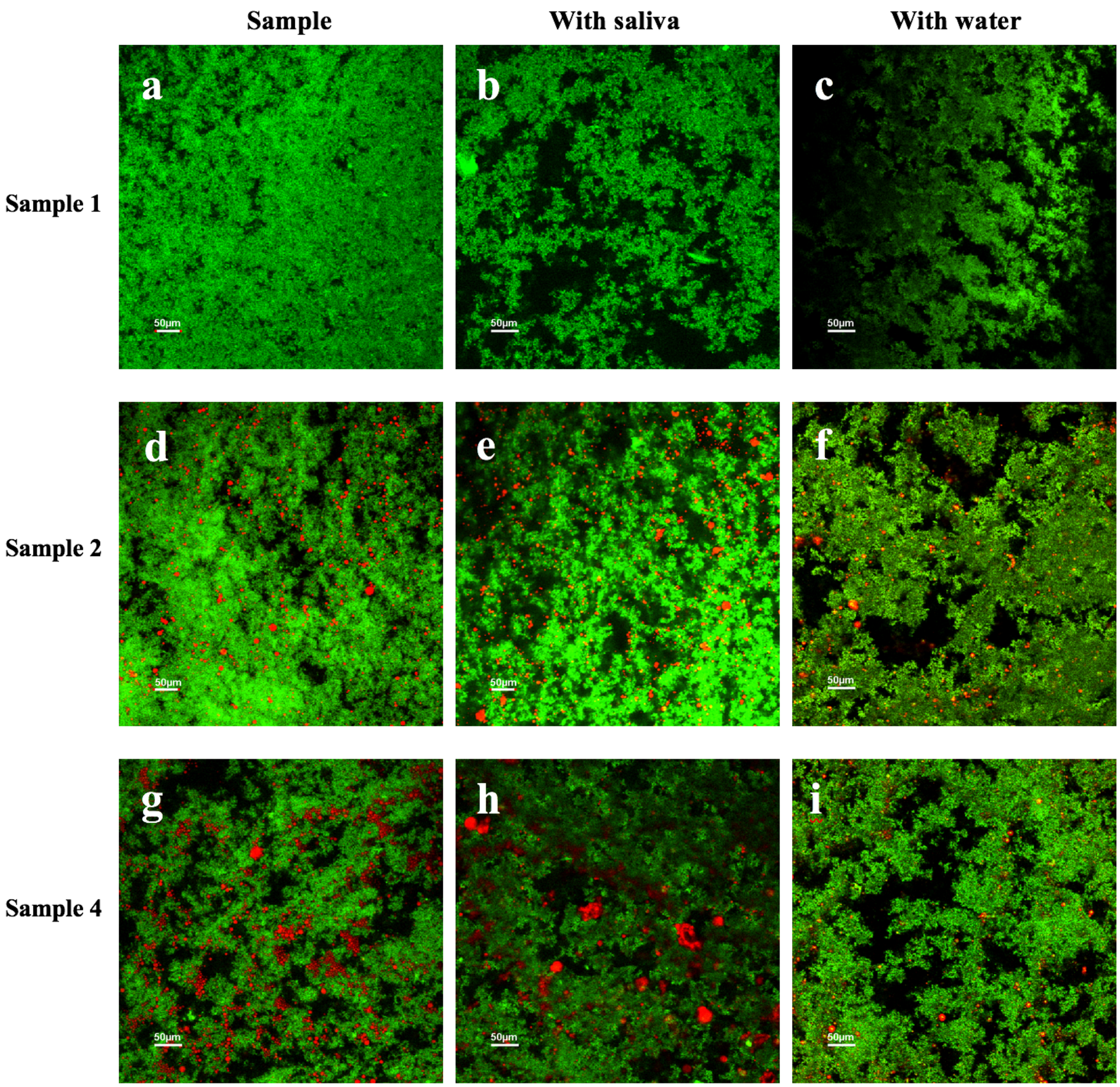

Figure 3.1. CLSM results of acid milk gels;
a) sample 1; b) sample 1: with HWS; c) sample 1: with water; d) sample 2; e) sample 2: with HWS; f) sample 2: with water; g) sample 4; h) sample 4: with HWS; i) sample 4: with water. The protein network, fat globules, and serum pores are shown in green, red, and black, respectively.

However, the specific microstructures differed with the specific hydrocolloid used. The density of the protein structure for the sample with PS (sample 8) decreased and the aqueous phase increased compared to the control. However, the sample with CS (sample 9)

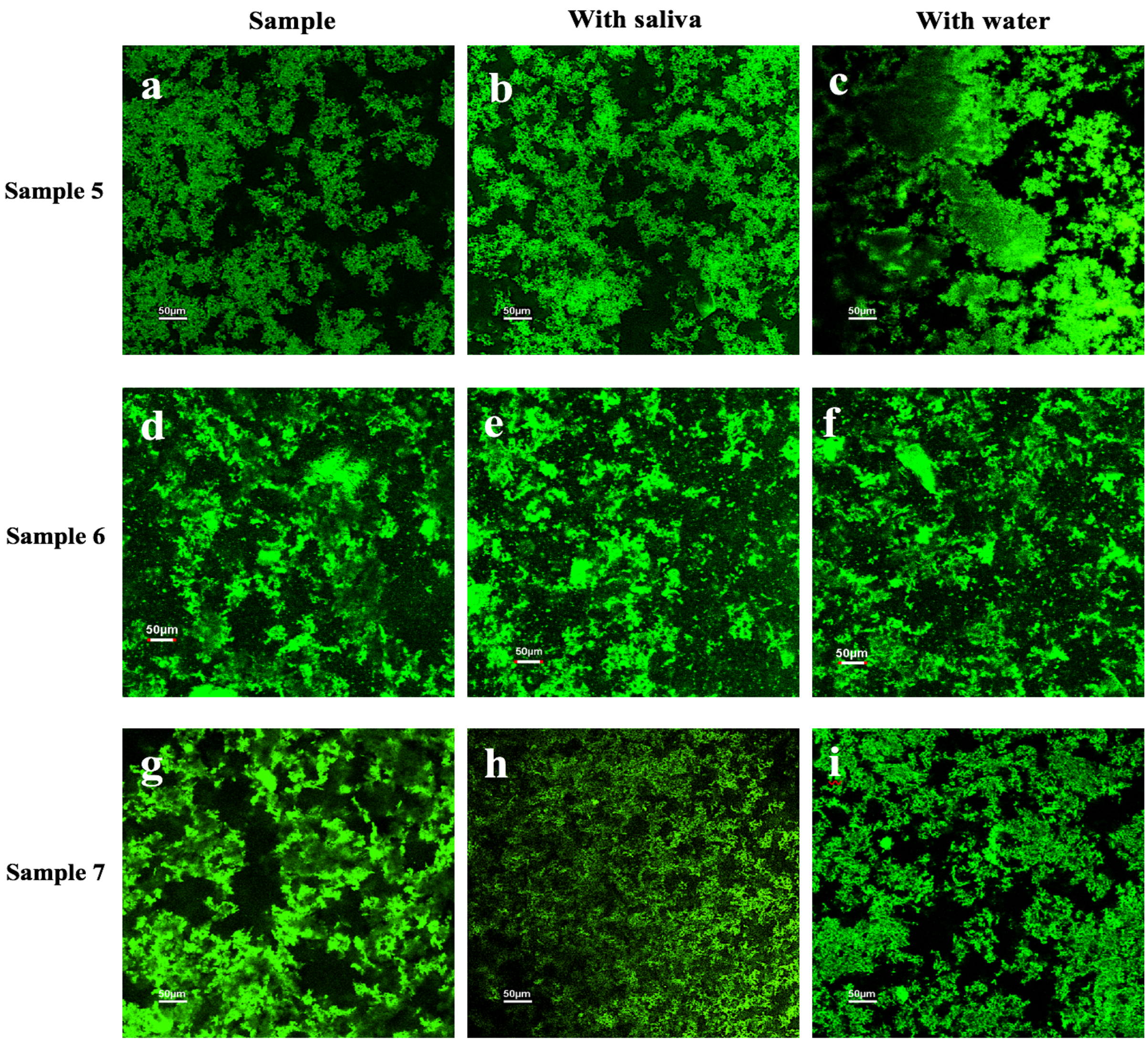

Figure 3.2. CLSM results of acid milk gels;
a) sample 5; b) sample 5: with HWS; c) sample 5: with water; d) sample 6; e) sample 6: with HWS; f) sample 6: with water; g) sample 7; h) sample 7: with HWS; i) sample 7: with water. The protein network and serum pores are shown in green and black, respectively.

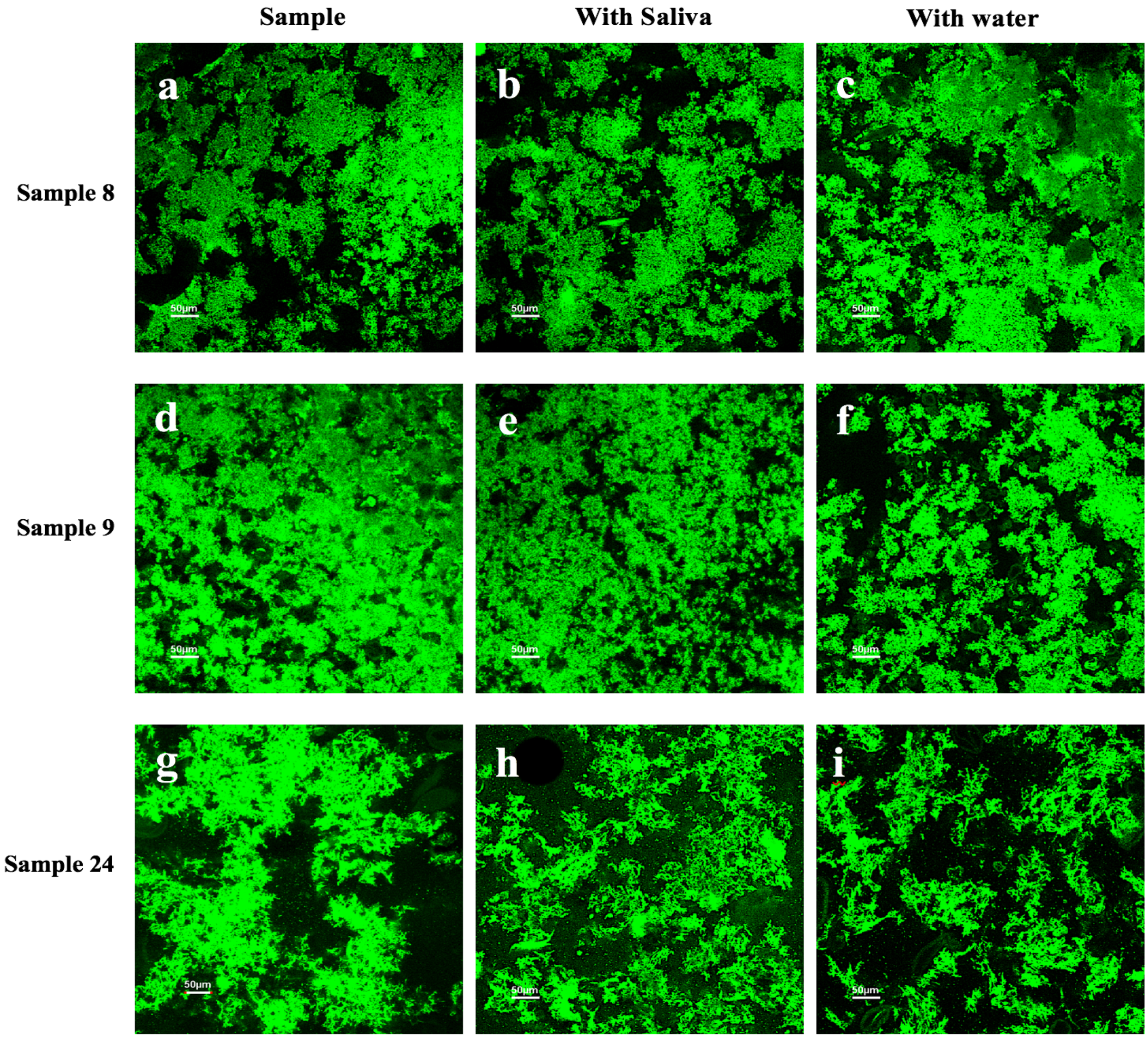

Figure 3.3. CLSM results of acid milk gels;
a) sample 8; b) sample 8: with HWS; c) sample 8: with water; d) sample 9; e) sample 9: with HWS; f) sample 9: with water; g) sample 24; h) sample 24: with HWS; i) sample 24: with water.
The protein network and serum pores are shown in green and black, respectively.

had smaller pores and was more homogenous and similar to the control. Although the branches of the protein network for the sample with CMC (sample 7) were very large and thick, the void area was larger than the void area for the sample with LBG (sample 6). This effect was reflected in the moisture content of these two samples (Figure 3.2, Table 3.2). The thickest and largest clusters in the protein network was shown in the sample with all hydrocolloids (sample 24), indicating that addition of all hydrocolloids caused the most notable differences in the protein matrix due to different interactions of hydrocolloids with protein network. In general, addition of HWS increased protein aggregation, regardless of formulation. The density of the protein branches was higher for most of the samples with added HWS, and addition of water caused a more uneven protein chain with larger void spaces. In other words, samples with added HWS showed more distinct protein clusters than samples with water. This irregularity of the protein network structure was very clear for the sample containing WPI with added water (sample 5). This may have been due to the solubility of the added WPI in water, bearing in mind that free WPI can significantly decrease by protein denaturation during the heat treatment of the acid milk gels preparation. HWS can change the entire food structure through mucin–food interactions, allowing the food particles to form a cohesive bolus. Additionally, in the later stages of oral processing, salivary α-amylase breaks down starch molecules to simple sugars. These effects were visible in samples prepared with starch (samples 8 and 9) when mixed with HWS.

HWS also had a significant impact on fat globule size: addition of HWS caused fat globules to coalesce. Fat coalescence was most visible in the full-fat acid milk gel with added HWS (sample 4). This result was attributed to depletion flocculation due to the osmotic pressure from salivary proteins, mainly proline-rich mucins (Chen, 2015). Fat coalescence as well as protein aggregation in the protein matrix resulted in larger serum pores. HWS showed the least effect on the sample with LBG (sample 6). This minimal effect can also be observed in rheological and tribological results in accordance with (Zinoviadou et al., 2008).

In general, addition of water resulted in a more homogenous protein network, except for sample 9 (containing CS), with smaller protein chains and branches. This result was attributed to the digestive, dissolving, and coalescence effects of HWS caused by enzymes, salivary proteins and electrolyte presented in HWS but not water. Amylose content can change the enzymatic digestion to minimal from salivary α-amylase due to its high linear amylose content and its crystalline structural conformation after gelatinization and retrogradation. This compact amylose structure can cause difficult conditions for HWS to travel throughout the system. This might be a possible reason for less effects of HWS on the samples with CS since the amylose content can be slightly higher in CS compared to PS.

3.4.3 The effect of hydrocolloids and HWS on acid milk gel flow behaviors

All samples showed pseudoplastic behavior regardless of formulation or addition of HWS (Table 3.3., 3.4., 3.5., 3.6.) based on their profile fitting to non-Newtonian viscosity models and their shear-rate dependent viscosity behavior. Pseudoplastic behavior occurs when the rate of external forces dominates the formation of internal entanglements, reducing the number of internal molecular interactions and resulting in decreased viscosity (Morris et al., 1981).

The averaged viscosity profiles from 6 replicates of each formulation were individually fitted to non-Newtonian viscosity models. The selected models were Cross-

Williams, Cross, and Herschel Bulkley with R^2>0.813, R^2>0.720, and R^2>0.692, respectively. The Cross model is a popular model that is extensively used in food dispersions and polymers

Table 3.3. Viscosity profiles for acid milk gels (n=24) at 8°C without added HWS

Formula	Model	η_o (Pa s)	η_∞ (Pa s)	n	c (s)	k (Pa s^{1-n})	σ_y (Pa)	R^2
1	Cross	595	0.122	0.949	13.4	N/A	N/A	0.878
2	Herschel-Bulkley	N/A	N/A	0.992	N/A	0.096	13.9	0.709
3	Herschel-Bulkley	N/A	N/A	0.730	N/A	0.492	11.1	0.918
4	Cross	2944	0.319	0.972	47.3	N/A	N/A	0.911
5	Cross-Williamson	3251	N/A	0.923	86.7	N/A	N/A	0.888
6	Cross-Williamson	88.1	N/A	0.697	4.51	N/A	N/A	0.999
7	Cross	1238	0.135	0.893	38.5	N/A	N/A	1.000
8	Cross	385	0.214	0.910	98.7	N/A	N/A	0.832
9	Herschel-Bulkley	N/A	N/A	0.866	N/A	0.330	15.559	0.826
10	Cross-Williamson	312	N/A	0.867	2.64	N/A	N/A	0.999
11	Cross-Williamson	382	N/A	0.864	4.59	N/A	N/A	1.000
12	Cross	4180	1.15	0.922	26.5	N/A	N/A	1.000
13	Herschel-Bulkley	N/A	N/A	0.605	N/A	1.00	12.3	0.937
14	Cross-Williamson	288	N/A	0.864	3.96	N/A	N/A	1.000
15	Cross-Williamson	183	N/A	0.825	6.25	N/A	N/A	1.000
16	Cross-Williamson	4836	N/A	0.923	19.33	N/A	N/A	0.909
17	Cross-Williamson	1053	N/A	0.875	9.60	N/A	N/A	1.000
18	Cross-Williamson	275	N/A	0.812	2.44	N/A	N/A	0.999
19	Cross-Williamson	325	N/A	0.841	6.41	N/A	N/A	1.000
20	Cross-Williamson	786	N/A	0.795	78.3	N/A	N/A	1.000
21	Cross-Williamson	374	N/A	0.871	4.03	N/A	N/A	1.000
22	Cross-Williamson	159	N/A	0.827	4.35	N/A	N/A	1.000
23	Cross-Williamson	288	N/A	0.839	6.45	N/A	N/A	1.000
24	Cross-Williamson	267	N/A	0.819	8.94	N/A	N/A	1.000

η_o (zero-shear viscosity) is related to the lower region of Newtonian plateau. This parameter can be a good criterion to track changes in food formulations over time. η_∞ (infinite viscosity) can determine the food or material behavior under high shear conditions. This parameter is considered zero in Cross-Williams model. For the formulations modeled with the

Table 3.4. Viscosity profiles for acid milk gels (n=24) at 8°C with added HWS

Formula	Model	η_o (Pa s)	η_∞ (Pa s)	n	c (s)	k (Pa s^{1-n})	σ_y (Pa)	R^2
1	Herschel-Bulkley	N/A	N/A	0.572	N/A	0.956	14.4	0.901
2	Herschel-Bulkley	N/A	N/A	0.549	N/A	1.062	7.45	0.963
3	Herschel-Bulkley	N/A	N/A	0.414	N/A	0.403	3.95	0.992
4	Herschel-Bulkley	N/A	N/A	0.964	N/A	0.119	18.4	0.692
5	Cross-Williamson	1917	N/A	0.906	75.2	N/A	N/A	0.947
6	Cross-Williamson	41.2	N/A	0.648	3.37	N/A	N/A	0.998
7	Cross	521	0.145	0.857	34.3	N/A	N/A	1.000
8	Cross	92.9	0.091	0.564	48.7	N/A	N/A	0.922
9	Herschel-Bulkley	N/A	N/A	0.481	N/A	1.72	7.374	0.956
10	Cross-Williamson	204	N/A	0.831	2.55	N/A	N/A	0.999
11	Cross-Williamson	232	N/A	0.837	4.50	N/A	N/A	1.000
12	Cross	1163	0.32	0.822	13.3	N/A	N/A	1.000
13	Herschel-Bulkley	N/A	N/A	0.355	N/A	0.393	1.45	0.994
14	Cross-Williamson	146	N/A	0.832	2.97	N/A	N/A	1.000
15	Cross-Williamson	65.4	N/A	0.777	3.34	N/A	N/A	1.000
16	Cross-Williamson	2910	N/A	0.878	19.78	N/A	N/A	0.895
17	Cross-Williamson	209	N/A	0.821	5.17	N/A	N/A	0.999
18	Cross-Williamson	150	N/A	0.799	1.86	N/A	N/A	1.000
19	Cross-Williamson	103	N/A	0.791	5.01	N/A	N/A	1.000
20	Cross-Williamson	132	N/A	0.784	23.4	N/A	N/A	0.999
21	Cross-Williamson	224	N/A	0.834	3.64	N/A	N/A	1.000
22	Cross-Williamson	69.1	N/A	0.793	3.14	N/A	N/A	1.000
23	Cross-Williamson	108	N/A	0.799	5.00	N/A	N/A	1.000
24	Cross-Williamson	70.6	N/A	0.762	5.04	N/A	N/A	1.000

Cross model, η_o and η_∞ decreased with increasing temperature and addition of HWS. Interestingly, the decrease in η_o due to application of HWS was greater that caused by increased temperature.

Table 3.5. Viscosity profiles for acid milk gels (n=24) at 25°C without added HWS

Formula	Model	η_o (Pa s)	η_∞ (Pa s)	n	c (s)	k (Pa s^{1-n})	σ_y (Pa)	R^2
1	Cross	380	0.080	0.908	11.1	N/A	N/A	0.720
2	Herschel-Bulkley	N/A	N/A	0.503	N/A	0.485	7.74	0.612
3	Herschel-Bulkley	N/A	N/A	0.740	N/A	0.346	4.65	0.969
4	Cross	2241	0.172	0.962	N/A	N/A	N/A	0.799
5	Cross-Williamson	1072	N/A	0.907	74.2	N/A	N/A	0.865
6	Cross-Williamson	42.6	N/A	0.604	5.32	N/A	N/A	0.998
7	Cross	517	0.106	0.823	30.7	N/A	N/A	1.000
8	Cross	105	0.114	0.833	68.6	N/A	N/A	0.737
9	Herschel-Bulkley	N/A	N/A	0.979	N/A	0.10	9.518	0.769
10	Cross-Williamson	205	N/A	0.822	2.60	N/A	N/A	0.999
11	Cross-Williamson	231	N/A	0.855	3.96	N/A	N/A	1.000
12	Cross	3447	0.50	0.860	56.5	N/A	N/A	0.999
13	Herschel-Bulkley	N/A	N/A	0.584	N/A	0.407	6.15	0.952
14	Cross-Williamson	151	N/A	0.844	2.76	N/A	N/A	1.000
15	Cross-Williamson	96.7	N/A	0.802	4.50	N/A	N/A	1.000
16	Cross-Williamson	2298	N/A	0.827	16.20	N/A	N/A	0.813
17	Cross-Williamson	467	N/A	0.873	5.96	N/A	N/A	1.000
18	Cross-Williamson	212	N/A	0.792	2.19	N/A	N/A	0.999
19	Cross-Williamson	161	N/A	0.805	4.65	N/A	N/A	1.000
20	Cross-Williamson	307	N/A	0.748	66.0	N/A	N/A	1.000
21	Cross-Williamson	190	N/A	0.843	2.98	N/A	N/A	1.000
22	Cross-Williamson	75.1	N/A	0.778	3.42	N/A	N/A	1.000
23	Cross-Williamson	143	N/A	0.794	5.18	N/A	N/A	1.000
24	Cross-Williamson	136	N/A	0.781	6.64	N/A	N/A	1.000

This may indicate a greater reduction of protein entanglements in acid milk gels caused by HWS. Samples with WPI (samples 5, 12, and 16) had the highest η_o values. This was attributed to the high interactions between whey protein and caseins throughout the protein network, which would result in a strong structure resistant to initial flow. The flow behavior index (n) and time constant (c) of acid milk gels decreased with addition of saliva and increasing temperature. In other words, their pseudoplastic behavior increased under these conditions. c has been attributed to the extent of entanglement density in a system (Bourbon et al., 2010). Acid milk gels structures were denser with more entanglements in the protein network before applying saliva and/or increasing temperature. As a result, the freedom of movement for individual strands becomes more restricted by increasing time, so the strands would require longer time to form new entanglements to replace the ones depleted by the external force (Bourbon et al., 2010). This is reflected in the higher c values for samples tested at lower temperature and without saliva.

The same trends for the viscosity parameters applied to the Cross-Williams results, with the exception of η_∞, which was always zero per the model assumptions. η_o values for the samples containing WPI (samples 5, 12, and 16) were significantly greater than those of the other samples. This may have been due to the heat treatment of acid milk gels at 85°C for 30 min.

Table 3.6. Viscosity profiles for acid milk gels (n=24) at 25°C with added HWS

Formula	Model	η_o (Pa s)	η_∞ (Pa s)	n	c (s)	k (Pa s^{1-n})	σ_y (Pa)	R^2
1	Herschel-Bulkley	N/A	N/A	0.643	N/A	0.520	6.10	0.942
2	Herschel-Bulkley	N/A	N/A	0.600	N/A	0.563	3.61	0.983
3	Herschel-Bulkley	N/A	N/A	0.407	N/A	0.936	2.71	0.992
4	Herschel-Bulkley	N/A	N/A	0.926	N/A	0.118	9.37	0.791
5	Cross-Williamson	728	N/A	0.816	68.6	N/A	N/A	0.924
6	Cross-Williamson	26.4	N/A	0.550	6.71	N/A	N/A	0.998
7	Cross	290	0.098	0.804	34.6	N/A	N/A	1.000
8	Cross	52.8	0.074	0.827	36.7	N/A	N/A	0.996
9	Herschel-Bulkley	N/A	N/A	0.622	N/A	0.642	4.285	0.961
10	Cross-Williamson	114	N/A	0.783	2.18	N/A	N/A	0.998
11	Cross-Williamson	123	N/A	0.853	2.96	N/A	N/A	1.000
12	Cross	787	0.26	0.804	51.4	N/A	N/A	1.000
13	Herschel-Bulkley	N/A	N/A	0.350	N/A	0.340	1.23	0.996
14	Cross-Williamson	73.9	N/A	0.802	2.16	N/A	N/A	0.9996
15	Cross-Williamson	34.0	N/A	0.731	2.89	N/A	N/A	1.000
16	Cross-Williamson	1773	N/A	0.721	36.55	N/A	N/A	0.821
17	Cross-Williamson	120	N/A	0.824	3.67	N/A	N/A	0.999
18	Cross-Williamson	79.2	N/A	0.782	1.18	N/A	N/A	0.999
19	Cross-Williamson	54.0	N/A	0.746	3.977	N/A	N/A	1.000
20	Cross-Williamson	45.6	N/A	0.712	18.6	N/A	N/A	1.000
21	Cross-Williamson	103	N/A	0.839	2.06	N/A	N/A	1.000
22	Cross-Williamson	30.6	N/A	0.720	2.61	N/A	N/A	1.000
23	Cross-Williamson	43.0	N/A	0.731	3.91	N/A	N/A	1.000
24	Cross-Williamson	49.3	N/A	0.705	6.85	N/A	N/A	1.000

During heat treatment above 70°C, whey protein, specifically β–lactoglobulins, denature. The interaction of denatured whey proteins with κ-casein on the surface of casein micelles leads to greater protein aggregation, cross-linking throughout the gel network, and increased water-holding capacity (Lucey et al., 1997a). Full-fat samples (sample 4) also had a notably high value for η_o. Fat globules interact with protein network and make a stronger gel. η_o increased in full-fat acid milk gels (sample 4) compared to the control acid milk gel (sample 1) with no added HWS in the Cross model; σ_o in the Herschel Bulkley models for these samples with added HWS was noticeably higher in the full-fat acid milk gel compared to the control.

The bigger size and greater number of fat globules in the full-fat sample compared to the control sample would cause a resistance to flow and increased viscosity (Chojnicka-Paszun et al., Chojnicka et al., 2009, Chojnicka-Paszun et al., 2012, Nguyen et al., 2017). These results are visually shown in microstructural images of acid milk gels (Figure 3.2).

Samples 7, 8, and 12 were fit to the Cross model and had $\eta_\infty > 0$; these results were attributed to greater gel strength in conditions with higher shear application compared to the other samples. These formulations incorporated CMC, PS, and PS and WPI, respectively. CMC is an anionic polysaccharide (polyelectrolyte). This gum, which has a negative charge on its hydrophilic end, interacts with the positive charges on the surface of casein micelles, strengthening the protein network (Everett and McLeod, 2005). Another reason for these results may be the effectiveness of CMC at casein's isoelectric point, which prevents casein perception and maintains a higher viscosity (Alakali et al., 2008, Andiç et al., 2013).

In general, formulation (hydrocolloids used), HWS, temperature, and interaction effects of formulation and HWS and formulation and temperature showed significant differences at $p \leq 0.001$ for all viscosity parameters except n for temperature. Interaction of HWS and temperature showed significant influence at $p \leq 0.05$ for c and n and at $p \leq 0.01$ for η_o. The significant effect of hydrocolloids can be mainly attributed to the electrostatic bonds between oppositely charged molecules of anionic hydrocolloids with casein micelles, swelling starch granules throughout the system, and dispersion of large particles of neutral hydrocolloids in the continuous phase as well as depletion flocculation phenomenon. These factors can significantly change the structure of protein network and overall conformation of acid milk gels (Figure 3.1, 3.2, and 3.3). The significant effects from HWS can be explained by digestive, dissolving, and coalescence properties of HWS resulted mainly by enzymes, salivary proteins and electrolyte presented in the HWS. Temperature can weaken the intermolecular bonds in a semisolid food system, decrease resistance to flow and lower the viscosity (Berk, 2018). The significant impacts of hydrocolloids and applied HWS on the flow

Table 3.7. Effect of main sources of variations on viscosity parameters of acid milk gels (n=24) determined by F-values obtained from three-way ANOVA[1].

Source of variation	ηo	n	c
Formulations	79.2***	97.5***	70.6***
HWS	156.7***	339.1***	41.9***
Temperature	60.7***	172.5***	3.5
HWS*Temperature	11.5**	5.3*	4.7*
Formulation*HWS	18.3***	18.3***	7.1***
Formulation*Temperature	7.3***	6.9***	3.9***

[1] *, **, and *** indicate significant differences at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively.

behaviors of acid milk gels as a model system for the semisolid foods, not only help formulating semisolid with better rheological properties (related to the texture quality), but also determine the importance of considering HWS in instrumental testing.

The greatest effect of HWS was seen in sample 8 and 12. The η_o value in sample 12 decreased to more than 60% of the original value when HWS was added at 8°C and 25°C. This decrease was more than one third in sample 8 at 8°C and approximately half at 25°C, when HWS was added. The key component for this result is PS. α-amylase in amylase breaks down starch to smaller monosaccharides (Humphrey and Williamson, 2001). The greater impact of PS on the viscosity parameter compared to CS (sample 9) after addition of saliva may be due to the larger granule size of PS and its higher swelling power, higher solubility and the possible lower number of amylose molecules (Bird et al., 2000, Li and Yeh, 2001, Singh et al., 2003).

Acid milk gels with a yield stress (σ_0) were fitted to the Herschel-Buckley model. As expected, K (consistency coefficient) decreased with both addition of saliva and increased temperature for all samples. These results were expected because a smaller stress is needed to deform the acid milk gels under these conditions since the higher temperature and/or saliva addition disrupted their structure. The value of k has been related to viscosity but since only a few samples have been fitted to Herschel-Buckley, using this parameter to determine their viscosity may not be a good criterion.

In summary, all acid milk gels showed non-Newtonian behavior. The mechanical forces applied during testing can resemble the shear forces during oral processing; increasing oral movements would result in decreased viscosity of the acid milk gels in the mouth. Addition of hydrocolloids and HWS significantly affected the viscosity parameters determined for acid milk gel viscosity profiles. These parameters can provide useful information about viscosity, consistency, shear thinning and shear properties. Because these rheological properties can be related to oral processing actions as well as correlated with sensory attributes such as thickness, the rheological properties of acid milk gels can be used to create semisolid foods with structures that break down and flow in a desired manner.

3.4.4 The effect of hydrocolloids and HWS on acid milk gel viscoelastic properties

Formulations, HWS, temperature, and all their interactions showed significant differences at $p \leq 0.001$ for G* (Table 3.8.). Formulation and HWS had significant effects on tan δ at $p \leq 0.001$. Subsequently, temperature and Formulation showed significant impacts on γ_c, at $p \leq 0.001$ and at $p \leq 0.05$ for HWS. This result was attributed to an increased stability and rigidity of acid milk gels when hydrocolloids were added to the samples compared to the control sample. Gums (CMC and LBG) and starches (PS and CS) improve gel stability by increasing the number of internal molecular interactions as well as promoting stronger bonds through different mechanisms. HWS can disrupt the structure of semisolid foods through digestion, osmotic pressure, dilution, or altering polymer net charges. Increasing temperature alters the thermodynamic condition of materials. Internal molecules can move faster with increased heat energy, and the strength of molecular bonds may decrease. As a result, the acid milk gels can lose their initial structure and become more susceptible to deformation when mechanical force is applied. The interaction of formulation (hydrocolloid) with HWS and temperature significantly increased their impact on G*. Aside from formulation, no other parameters showed significant effects for γ_c and tan δ.

Table 3.8. Effect of main sources of variations on viscoelastic properties of acid milk gels (n=24) determined by F-values obtained from three-way ANOVA[1].

Source of variation	γ_c	G*	tan δ
Formulations	65.4***	589.9***	19.4***
HWS	4.7*	798.5***	19.8***
Temperature	27***	302.5***	3
HWS*Temperature	0.1	16.2***	1.5
Formulation*HWS	1.9	100.6***	1.8
Formulation*Temperature	1.1	27.4***	1

[1] *, **, and *** indicate significant differences at p-value ≤ 0.05, p-value ≤ 0.01, and p-value ≤ 0.001, respectively.

Overall, G' < G" for all formulations under the strain sweeps parameters tested (0.01-100% strain, constant frequency of 1 Hz) (Table 3.7.). Meaning, solid (elastic)-like behavior of the acid milk gels was the dominant behavior up to the critical strain at which permanent deformation happened to the acid milk gels structure and viscous (liquid)-like behavior became greater after this point. Accordingly, tan $\delta<1$ for all samples except for samples 6 (containing LBG) and 23 (containing all hydrocolloids but WPI). γ_c values varied with formulation. The critical strain of formulations with PS and CS and their combination with WPI (samples 12 and 13, respectively) either decreased or remained constant after addition of HWS. This may have been due to the digestion of starch with α-amylase, which can disrupt the gel structure and decrease the stability and strength of the acid milk gels. Formulations with an individual gum (samples 6 and 7) did not show any difference in critical strain upon addition of HWS. Interestingly, the critical strain of sample 18 increased with HWS. It is possible that LBG molecules were evenly dispersed in the continuous phase, allowing HWS to mix into this phase but not interact with casein micelles (flocculated due to osmotic pressure) since both LBG and HWS have hydrophobic areas. These hydrophobic forces can provide stability to the matrix. G* (complex modulus) values for all samples decreased with addition of HWS, increased temperature or both. Tan δ results showed viscoelastic solid behaviors for most samples with or without HWS (tan $\delta<1$). However, tan δ was >1 for samples 6 (containing LBG) and 22 (containing all hydrocolloids but CS) when HWS was added. Increasing temperature and addition of HWS to the formulations increased the value of tan δ. Thus, acid milk gels had elastic-dominant behavior, indicating that the mechanical forces applied at strains below the critical strain were not sufficient to overcome the microstructural forces among the molecules within the acid milk gels, so samples store more energy than they dissipated at strains below critical strain. In sample 18 (containing LBG and corn starch), CS likely contributed to depletion as well, since it has a neutral charge.

Complex modulus (G*) decreased with increasing temperature, HWS addition, or both. Applying both higher temperature and HWS resulted in the lowest values of G*. The decrease in viscoelastic moduli with increased temperature may have been due to increasing kinetic energy in the intermolecular structure of acid milk gels. At higher temperatures, molecules can move faster, and the energy they produce can overcome the intermolecular forces allowing the samples to flow more easily. Addition of HWS can also disturb the food structure due to dilution, interaction with mucins, and enzymatic breakdown of amylose in starch by amylase (Janssen et al., 2007, Vingerhoeds et al., 2009).

The most viscous samples were those prepared with LBG (6,10,18 and 23). These results were likely caused by depletion flocculation between LBG and milk proteins, as previously discussed. It is noteworthy that the decrease of viscoelastic moduli for samples with starches (samples 8 and 9) was not significantly different from other hydrocolloids.

Table 3.9. Viscoelastic parameters of acid milk gels

Formula number	γc (%) 8°C NS	γc (%) 8°C S	γc (%) 25°C NS	γc (%) 25°C S	G* (Pa) 8°C NS	G* (Pa) 8°C S	G* (Pa) 25°C NS	G* (Pa) 25°C S	Tan δ (radians) 8°C NS	Tan δ (radians) 8°C S	Tan δ (radians) 25°C NS	Tan δ (radians) 25°C S
1	0.411^{j}	0.417^{k}	0.411^{k}	0.555^{m}	494^{de}	1820^{a}	181^{d}	208^{def}	0.300^{mn}	0.325^{h}	0.329^{nop}	0.346^{n}
2	0.976^{f}	0.73^{h}	0.975^{h}	0.975^{j}	250^{efgh}	783^{b}	94.4^{d}	101^{ef}	0.307^{lm}	0.333^{h}	0.339^{mno}	0.365^{l}
3	0.730^{i}	0.411^{l}	0.975^{l}	1.30^{h}	88.8^{h}	560^{c}	58.1^{d}	55.7^{f}	0.311^{klm}	0.316^{h}	0.362^{m}	0.371^{l}
4	0.411^{j}	0.547^{i}	0.731^{i}	0.411^{o}	700^{cd}	317^{d}	284^{d}	317^{d}	0.319^{kl}	0.314^{h}	0.334^{mno}	0.329^{no}
5	0.731^{h}	0.731^{g}	0.731^{g}	0.731^{k}	998^{c}	265^{de}	693^{c}	7840^{b}	0.299^{mno}	0.320^{h}	0.321^{op}	0.326^{no}
6	1.74^{e}	0.73^{h}	1.74^{h}	0.73^{l}	100^{h}	224^{def}	64.4^{d}	50.2^{f}	0.951^{a}	1.11^{a}	1.18^{a}	1.29^{a}
7	0.731^{h}	0.731^{g}	0.731^{g}	0.73^{l}	361^{efgh}	208^{def}	174^{d}	166^{def}	0.36^{1j}	0.413^{g}	0.403^{l}	0.450^{k}
8	0.731^{h}	0.547^{j}	0.975^{j}	0.975^{j}	156^{fgh}	166^{def}	118^{d}	60.5^{f}	0.316^{klm}	0.331^{h}	0.355^{mn}	0.375^{l}
9	0.731^{h}	0.411^{l}	0.975^{l}	0.73^{l}	224^{efgh}	124^{def}	130^{d}	124^{def}	0.326^{k}	0.314^{h}	0.355^{mn}	0.354^{lmn}
10	1.74^{e}	1.74^{e}	2.33^{e}	2.33^{f}	429^{defg}	116^{def}	296^{d}	265^{de}	0.618^{e}	0.71^{e}	0.745^{g}	0.815^{g}
11	10.0^{a}	10.0^{a}	10.0^{a}	13.4^{a}	172^{fgh}	115^{def}	112^{d}	101^{ef}	0.518^{g}	0.606^{f}	0.561^{i}	0.692^{i}
12	0.975^{g}	0.548^{i}	0.975^{i}	0.975^{i}	1720^{b}	101^{ef}	1180^{b}	560^{c}	0.248^{p}	0.293^{i}	0.250^{q}	0.301^{o}
13	0.411^{j}	0.411^{l}	0.73^{l}	0.730^{l}	145^{gh}	101^{ef}	73.2^{d}	63.8^{ef}	0.280^{o}	0.321^{h}	0.316^{op}	0.359^{l}
14	5.59^{b}	7.48^{b}	10.0^{b}	7.48^{c}	182^{fgh}	100^{ef}	111^{d}	115^{def}	0.669^{c}	0.754^{d}	0.802^{de}	0.901^{d}
15	2.33^{d}	2.33^{d}	4.18^{d}	4.18^{d}	130^{gh}	85.5^{ef}	73.0^{d}	75.3^{ef}	0.666^{c}	0.740^{d}	0.815^{cd}	0.905^{d}
16	0.975^{f}	0.976^{f}	1.30^{f}	1.74^{g}	3600^{a}	78.1^{ef}	2640^{a}	1820^{a}	0.288^{no}	0.293^{i}	0.302^{p}	0.312^{o}
17	4.18^{c}	7.48^{b}	5.59^{b}	10.0^{b}	379^{efgh}	75.3^{ef}	250^{d}	100^{ef}	0.386^{i}	0.606^{f}	0.436^{k}	0.677^{i}
18	1.74^{e}	2.33^{d}	3.12^{d}	4.18^{d}	455^{def}	63.9^{ef}	315^{d}	224^{def}	0.681^{bc}	0.859^{b}	0.843^{bc}	1.08^{b}
19	2.33^{d}	1.74^{e}	3.12^{e}	4.18^{d}	219^{efgh}	63.8^{ef}	130^{d}	85.5^{ef}	0.621^{de}	0.713^{e}	0.758^{fg}	0.866^{f}
20	0.411^{j}	0.232^{m}	0.411^{m}	0.547^{n}	179^{fgh}	60.5^{f}	87.9^{d}	31.7^{f}	0.433^{h}	0.609^{f}	0.506^{j}	0.541^{j}
21	5.59^{b}	7.48^{b}	7.48^{b}	7.48^{c}	233^{efgh}	59.5^{f}	150^{d}	116^{def}	0.598^{f}	0.752^{d}	0.733^{g}	0.874^{f}
22	2.33^{d}	4.18^{c}	4.18^{c}	4.18^{d}	139^{gh}	55.7^{f}	66.4^{d}	63.8^{ef}	0.692^{b}	0.852^{b}	0.864^{b}	1.04^{c}
23	1.74^{e}	2.33^{d}	2.33^{d}	3.12^{e}	201^{efgh}	50.2^{f}	115^{d}	78.1^{ef}	0.635^{de}	0.775^{c}	0.779^{ef}	0.922^{d}
24	1.74^{e}	1.74^{e}	2.33^{e}	2.33^{f}	143^{gh}	31.7^{f}	98.1^{d}	59.4^{f}	0.638^{d}	0.750^{d}	0.692^{h}	0.759^{h}

NS: no HWS added; S: HWS added.

These results indicated that the protein network was the dominant contributor to viscoelastic behavior rather than HWS addition. Crossover at above 10% strain was also observed in strain sweep results. Before the crossover point, G'>G"; the crossover indicated a switch from viscoelastic solid to viscoelastic fluid behavior. These results were attributed to reduced gel stability, which would result in disruption and breakdown of the protein network at higher strains. Interestingly, sample 6 (containing LBG) did not show this phenomenon. Rather, G">G' for these samples with addition of HWS 8°C and 25°C and also at 25°C. This viscous-dominant behavior of LBG has been also reported by (Perrechil et al., 2009). Additionally, these results were expected since sample 6 was a weak gel at 8°C; the additional energy at increased temperatures altered the network from a soft gel with an entangled matrix system to a weak physical gel with non-covalent linkages (Stading and Hermansson, 1990, Tunick, 2010, Tang and Liu, 2013). The interaction of HWS and its gel weakening mechanism was explained in Section 4.3. The length that a formulation remained as solid dominant was also different (Appendix A) with addition of various hydrocolloids due to their rate of gel stability.

Frequency sweep results were dependent on formulation (selected results shown in Figure 3.4). Overall, G' and G" decreased with increasing temperature, HWS addition, or both. Increasing temperature and application of HWS decreased G' and G". Several acid milk gel formulations showed a crossover between G' and G" within the frequency range of 0.1 to 100 rad/s (Table 3.10). The crossover of sample 14 at 8°C and 25°C with addition of HWS showed viscous-dominant behavior (G">G') at low frequencies but showed solid-like behavior (G'>G") at higher frequencies. Crossovers for samples were attributed to the different responses of the microstructures at different timescales. At low frequencies (long timescales), the protein molecules had time to relax and slide past each other, resulting in dissipation of energy. At higher frequencies (short timescales), the oscillation time was faster than the material's relaxation time, so the polymers tended to stretch and store energy rather than relax and dissipate energy. Sample 16 (containing high levels of WPI) had the highest G' and G" values. This sample showed little frequency-dependent behavior compared to other formulations, indicating it was a stronger gel. The properties of strong gels have been reported by (Lee and Lucey, 2010, Tunick, 2010). Other formulations with this behavior included samples 5, 7, 13, 24, and all samples with fat (samples 2, 3, and 4). This comparison shows that addition of WPI both had a significant effect on acid milk gel structural rigidity and can change the rheological behavior of the samples. Additional available whey proteins would increase the number of interactions with both other polysaccharides and caseins in the system, resulting in a stronger gel (Laneuville et al., 2000).

Table 3.10. Crossover frequencies for acid milk gels.

Formula number	8°C-S (rad/s)	8°C-NS (rad/s)	25°C-S (rad/s)	25°C-NS (rad/s)
14	0.398	N/A	1	N/A
22	0.158	N/A	2.5	0.1
21	0.1	0.1	0.25	0.63
6	6.31	2.51	10	3.98
10	0.398	0.631	0.398	1
18	3.98	1	3.98	1.58

NS: no HWS added; S: HWS added.

Addition of CMC (sample 7), WPI (sample 16), and fat (samples 2, 3, and 4) significantly increased the values of G' and G" compared to the control sample (sample 1) via the same mechanism explained earlier for the strain sweep results. The gel strength of these samples showed similar behavior as observed for strain sweeps, and the change of frequency did not affect this result.

Addition of WPI (sample 16) resulted in the highest values of G' and G". This significant increase of viscoelastic moduli was in accordance with the results of (Lucey et al., 1998, 2010). Addition of WPI increases the amount of bound, denatured whey proteins due to heat treatment at 80°C for 30 min. Subsequently, non-associated, denatured whey proteins can also interact with the bound, denatured whey proteins, forming a stronger gel (Lucey et al., 1998). The viscoelastic moduli decreased when WPI was used in combination with SMP (sample 5), likely because the protein content of WPI was higher than SMP, with an approximate ratio of 3:1. Because the total solid content of both formulations was equal, there would be less protein in samples 5, which was shown in the proximate results (Table 3.2).

Briefly, the effects of HWS, hydrocolloids, and temperature on acid milk gel viscoelastic parameters were significant. The impact of hydrocolloids appeared greater compared to the other parameters. For example, in frequency sweeps of the formulation with WPI only (sample 16), its viscoelastic moduli were almost independent of frequency as opposed to the sample made with LBG (sample 6), which showed a G">G' at frequencies below the crossover point.

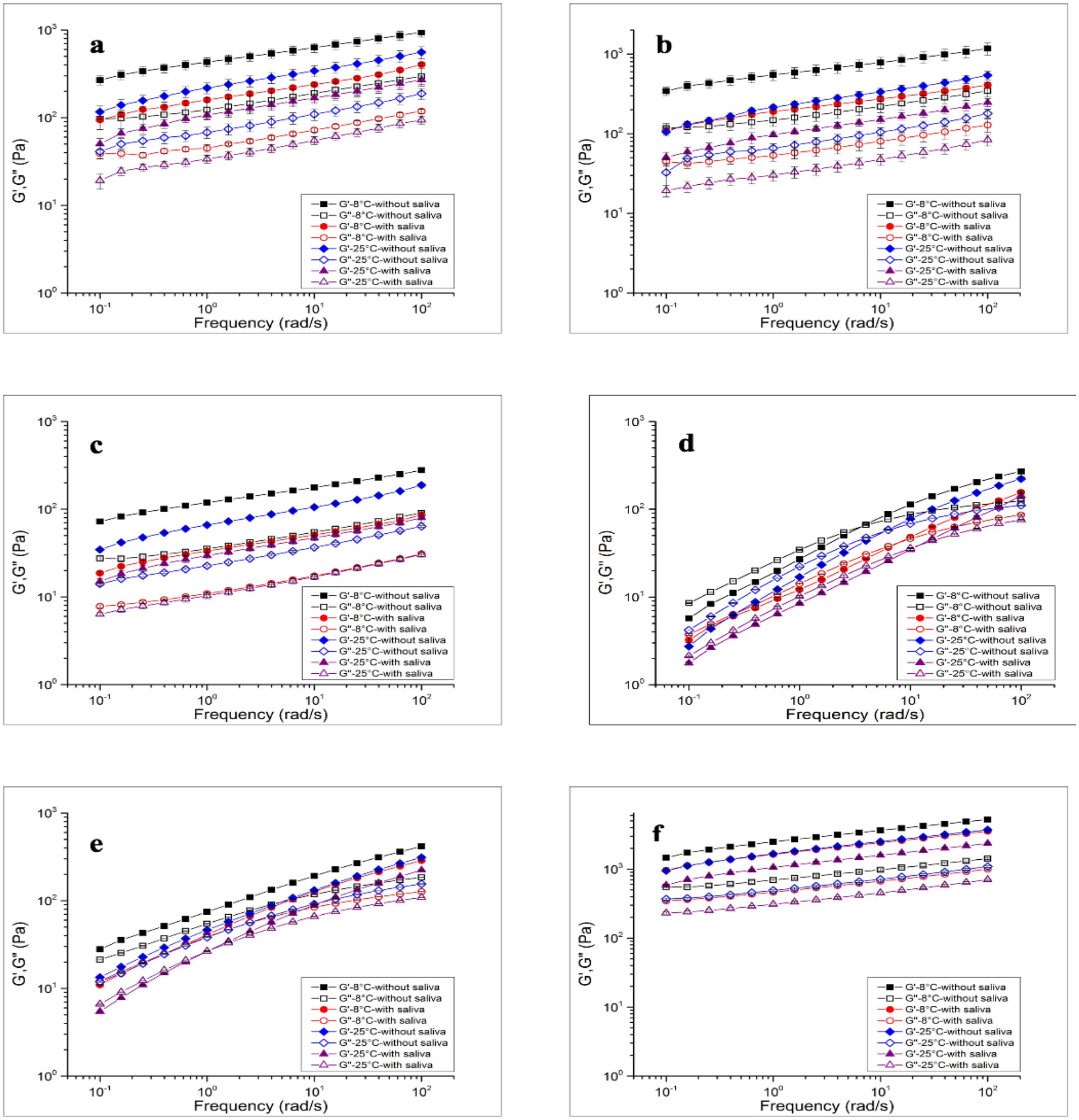

Figure 3.4. Selected frequency sweep results of acid milk gels;

a) sample 1; b) sample 4; c) sample 8; d) sample 6; e) sample 14; f) sample 16;

The explanation of this significant difference among formulations was most likely the difference in strength and quantity of the electrostatic interactions as well as differences hydrophobicity properties of internal molecules. Additionally, the conformation of protein networks was shown to be notably different in different formulations from microstructural (confocal) imaging of in acid milk gels (Figures 3.1, 3.2, and 3.3.).

The effect of HWS was significant for samples with PS due to enzymatic digestion of large granules of PS with α-amylase. This effect was particularly notable when PS was used alone or in combination with 1 to 2 other hydrocolloids. However, when PS was used with more than 2 hydrocolloids, this effect was not observed. This may have been because the quantity of PS used in formulations with more than 2 hydrocolloids would decrease to balance the total solid (13% in all samples), resulting in less potato starch available for digestion. An example of the significant impact of PS was observed when the crossover occurred for the sample 14 when HWS was added to the system regardless of temperature. The disruption of digestion has been enough to disrupt the sample in a way it solid portion becomes equal to its viscous portion. However, this crossover was not shown when HWS was not applied. Additionally, the interactions among the proteins and polysaccharides may have changed based on which ones were present in the system. Overall, this information about viscoelastic properties of semisolid foods provides an understanding of how acid milk gel formulation and testing parameters result in different degrees of structural stability and viscoelastic behavior. This information can be used to assist in proper selection of hydrocolloids during formulation development to generate structures that create desirable textures.

3.4.5 The effect of different hydrocolloids and HWS on tribological properties of acid milk gels

The effects of formulation, HWS, sliding speed, and the interaction of formulation with temperature and HWS were significant at $p \leq 0.001$ (Table 3.11.). The interaction of sliding speed and HWS on friction coefficients was significant at $p \leq 0.05$. The significant effect of formulation was attributed to the drastically different friction behaviors of the hydrocolloids used. These differences were mainly due to differences in their electrical charges, molecular size. For instance, addition of WPI may result in a larger particle size that can increase the friction coefficient. The significant impact of HWS can be explained by disruption of acid milk gels structures by digestion, osmotic pressure, dilution or altering their net charges mainly caused by enzymes, salivary proteins, electrolytes, and water in HWS. The significant impact of sliding speed can be most likely due to the changes of lubricant (food) position in between the two surfaces during tribometry which can profoundly impact the outcome friction.

Table 3.11. Effect of main source of variations on frictional properties of acid milk gels (n=24) determined by F-values obtained from three-way ANOVA[1].

Source of variation	Friction coefficient
Formulations	264.1***
Sliding speed	92.8***
HWS	596.1***
Sliding speed*HWS	3.5*
Formulation*HWS	39.2***
Formulation * Sliding speed	3.7***

[1] *, **, and *** indicate significant differences at p-value ≤ 0.05, p-value ≤ 0.01, and p-value ≤ 0.001, respectively.

Stribeck curves for most acid milk gels showed startup behaviors from 0.01 mm/s to 1 mm/s (Figure, 3.5). These results were due to deformation of PDMS plate rather than sliding behavior at low sliding speeds (Zinoviadou et al., 2008). The boundary and mixed regime were the dominant regimes for all samples (Figure 3.5). These regimes have been shown for semisolid food with added hydrocolloids during tribological testing (De Vicente et al., 2006, Dresselhuis et al., 2007a, Chojnicka et al., 2008, Chojnicka-Paszun et al., 2012, Morell et al., 2016).

Samples with fat (samples 2, 3, and 4) had low friction coefficients compared to the other samples; full-fat samples (sample 4) had the lowest friction coefficients among all samples. The fat globules can decrease in friction coefficient; as the number of fat globules increases, friction decreases. One possible reason for this behavior is that fat globules can be trapped in the contact area and form a thin film of fat due to fat coalescence or flocculation, acting as a lubricant (Huc et al., 2016).

Hydrocolloids, including WPI, LBG, and SMP (samples 6, 16, and 18) notably increased the friction coefficient within the boundary regime. Samples with WPI (sample 16) showed the highest friction coefficients. This effect of WPI may have been due to the larger particle size of protein molecules compared to the rest of the hydrocolloids (Chojnicka-Paszun et al., Huc et al., 2016). Combining WPI with CMC (sample 11) significantly reduced the friction coefficient, but the combination of WPI with LBG (sample 10) did not affect the high frictional behaviors contributed by WPI. Stribeck curves for samples with LBG (sample 16) and WPI and LBG (sample 10) were similar, which was in agreement with the results for the viscoelastic properties of these two formulations. The friction results may have been due to incompatibility of the LBG with milk proteins. Although LBG can stabilize food systems, phase separation between casein micelles and LBG on the microscopic scale can occur due to depletion flocculation and thermodynamic incompatibility (Thaiudom and Goff, 2003). drastically decreased, but addition HWS to samples with PS individually (sample 8) showed little impact on friction behaviors. Sample 9 with CS showed a greater decrease in friction coefficient when mixed with HWS. This result was not in accordance with the viscosity and viscoelastic results for sample 8 and 9. α-amylase in the HWS breaks down amylose in starch, and the mechanism for the friction behaviors is likely similar to that for the drastic transformations of viscosity curves for samples with PS (Sections 4.3 and 4.4).

Friction coefficient of all samples either decreased or was unchanged with addition of HWS. Samples with PS in combination with other hydrocolloids (samples 12, 14, 17, and 24)

Samples containing LBG (sample 6) had small changes in friction coefficient within the boundary regime. This effect was also shown by (Zinoviadou et al., 2008). The friction profiles of samples 6 and 7 (containing CG) were drastically different, and the effect of HWS on the friction of sample 7 was greater than for sample 6. The main reason for the decrease in friction coefficient for the remaining samples appeared to be mainly due to the lubricating effect of the proteins in HWS (mainly proline-rich mucin) and the dilution provided by saliva (Janssen et al., 2007, Vingerhoeds et al., 2009), since HWS is 95% water (Humphrey and Williamson, 2001).

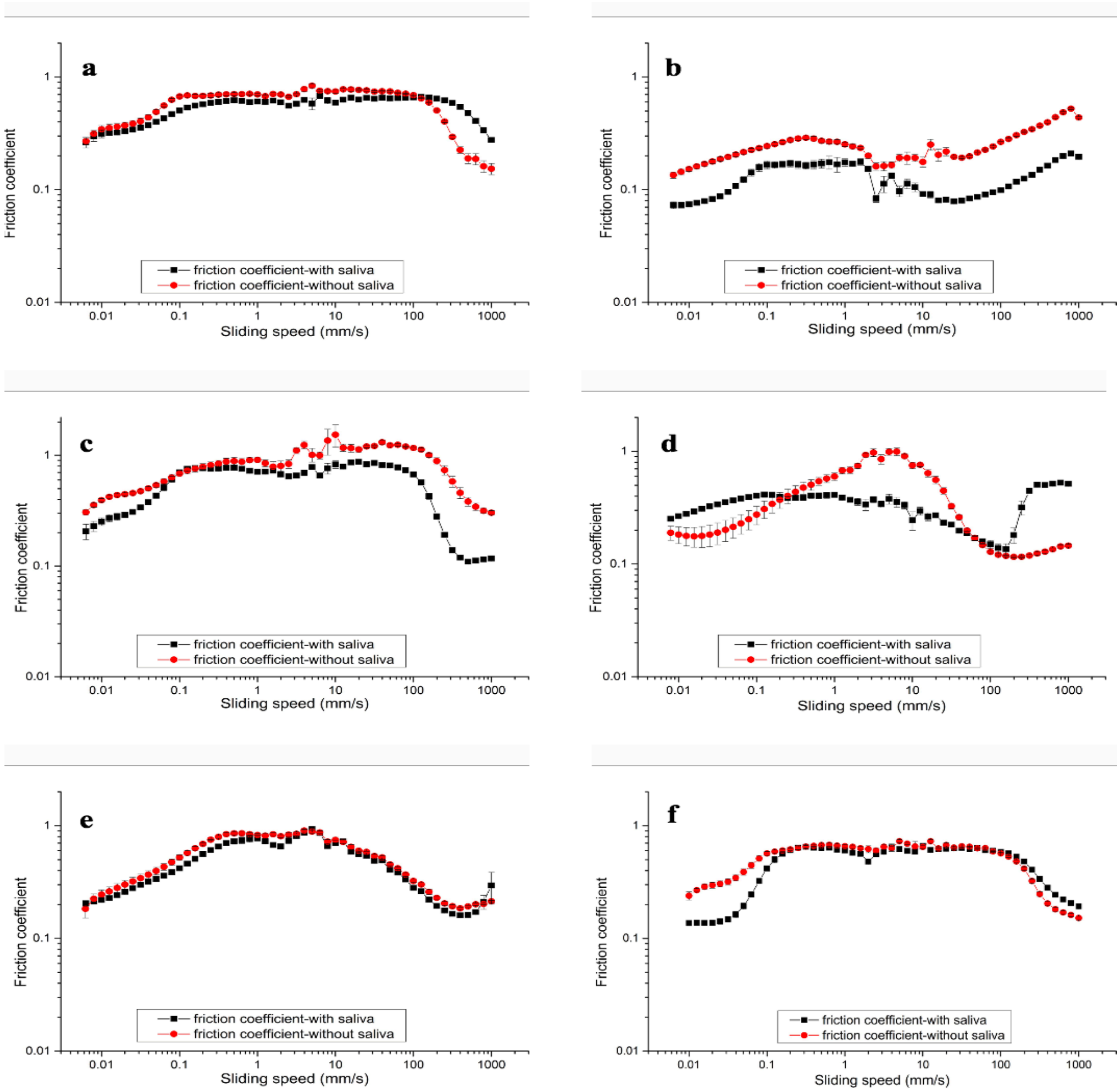

Figure 3.5. Tribology results of acid milk gels;
sample 1; b) sample 4; c) sample 16; d) sample 7; e) sample 22; f) sample 8.

The composition analysis of 8 HWS samples showed no significant differences among α-amylase activity (U/mg) at p-value ≤ 0.05. However, significant differences were found for protein concentration at p-value ≤ 0.05 (Appendix B). The effect of these significant differences of protein concentration was difficult to be shown in rheological, tribological, and microstructural imaging most likely due to the sufficient time considered for the samples to be interacted and digested by HWS for 5 min.

3.5 Conclusions

Addition of HWS and hydrocolloids significantly affected the microstructural, rheological, and tribological properties of acid milk gels. Samples with hydrocolloids had thicker clusters and bigger chains in their microstructures compared to the control sample, which was more homogenous with smaller pores. Samples with HWS had a distinct, more homogeneous protein network compared to the samples were imaged by water. HWS also caused visible fat coalescence for samples containing fat. The notable effects of hydrocolloids and HWS on acid milk gel microstructures as observed in confocal imaging was also observed in the differences in their viscosity and viscoelastic properties. For instance, thicker clusters and bigger chains after addition of hydrocolloids was associated with greater mechanical viscosity. This result can be attributed of the greater resistance of those larger clusters to the mechanical force, causing increased viscosity. Addition of HWS to the acid milk gels resulted in decreased viscosity, viscoelastic moduli, and frictional coefficients. A decreased in the void area after addition of saliva in confocal images can be in agreement with this finding. The most notable differences were for samples with either potato starch individually or in combination with other hydrocolloids. However, these results were not found for corn starch formulations, indicating that the effect may be starch-specific. WPI and LBG significantly increased friction coefficients. These results provide important information on factors that alter acid milk gel rheological and tribological behaviors, as well as how structural changes due to formulation and HWS incorporation contribute to those differences in mechanical behaviors. Illustration of the structural changes with consideration of the mechanisms during oral processing will lead to more realistic results for designing ideal textures for the human palate.

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CHAPTER 4: THE RELATIONSHIP BETWEEN SENSORY, RHEOLOGICAL, AND TRIBOLOGICAL PROPERTIES OF ACID MILK GELS WITH VARIOUS HYDROCOLLOIDS

4.1 Abstract

Sensory evaluation is a useful technique to optimize the textural properties of semisolid foods. Although, this method may not be time- and cost effective for the development of new healthy semisolid foods with palatable texture for consumer acceptance. Rheometry can determine semisolid food mechanical behaviors that have been correlated to sensory attributes. Tribometry is a complementary measurement for texture perception of these foods since some textural attributes, e.g. smoothness and astringency, may be related to friction behaviors rather than mechanical behaviors. Accordingly, the objective of this study was to determine the relationships among rheological, tribological, and sensory behaviors of semisolid foods, as well as how addition of human whole saliva (HWS) during instrumental testing impacted these relationships. The textural attributes of 24 formulations of acid milk gels were evaluated using descriptive analysis. Rheological and tribological behaviors of the acid milk gels were evaluated with and without the addition of HWS. The sensory results were correlated with acid milk gel rheological and tribological properties using partial least square (PLS) analysis. Overall, several sensory attributes were correlated with the viscosity, viscoelastic, and tribological behaviors of acid milk gels. Most correlations among rheological and tribological properties with sensory attributes were with viscoelastic parameters, including critical strain, tan δ, and complex modulus. Sensory attributed that correlated with acid milk gel viscosity profiles (zero shear viscosity and flow index) were mouthcoat, mouth viscosity, melting, smoothness, firmness, astringency, grittiness, and graininess. Friction coefficient at sliding speed of 30 mm/s provided the best correlation to sensory attributes. However, chalkiness, graininess, and grittiness were correlated with friction coefficients at sliding speeds in a range of 10-30 mm/s. Changes in rheological and tribological behavior due to addition of HWS during instrumental testing did have an impact on the correlations. The results of this study provide a better understanding of the relationships among acid milk gel rheological, tribological, and sensory relationships. This understanding can be helpful to develop textures of reduced or non-fat semisolid foods that are similar to their full-fat counterparts.

Key words: Sensory, tribology, rheology, semisolid foods, texture perception

4.2 Introduction

The textural optimization of reduced or non-fat semisolid foods has been a challenge to the food industry. The use of hydrocolloids is the most popular way to enhance the textural properties of semisolid foods, e.g. yogurt. However, improving functional properties of these products may negatively impact the consumer acceptability due to an unpleasant texture. Both protein and polysaccharide hydrocolloids are used in yogurts. They have different effects in a food system based on their net charge, molecular size, degree of modification, and overall structure (Thaiudom and Goff, 2003, Engelen et al., 2005, Lee and Lucey, 2010, Morell et al., 2015, van de Velde et al., 2015, Peng and Yao, 2017). Their type and concentration need to be chosen based on the final favored texture of a food system to minimize the unwanted effects.

One popular sensory method for evaluating the food texture perception and correlating sensory data to instrumental results is descriptive analysis. Specific attribute descriptors are chosen based on physical properties of foods. For instance, initial attributes that are related to the first bite perception is not used for some of viscoelastic foods, e.g. yogurt. On the other hand, rheological and tribological properties of semisolid foods have been reported to correlate with sensory data (Chojnicka-Paszun et al., Malone et al., 2003, Chen and Engelen, 2012, Stokes et al., 2013, Sonne et al., 2014). Viscosity and viscoelastic properties of semisolid foods have been mostly corelated to textural attributes evaluated by a trained panel e.g. smoothness, thickness, creaminess, and sliminess (Malone et al., 2003). The shear-thinning properties of yogurt from viscosity profiles were positively correlated with smoothness and sliminess. Additionally, viscoelastic properties of semisolid foods from small and large deformation have also shown correlations with sensory attributes (Malone et al., 2003, Ozcan, 2013). Tribology, the study of friction, lubrication and wear, has been found to be a good addition to rheology and sensory tests for texture perception studies. Food friction behaviors have been correlated with a different set of sensory attributes compared to those correlated to rheological behaviors (de Wijk and Prinz, 2005, Stokes et al., 2013). Astringency might be the most popular sensory attribute which has been correlated with tribological properties. The main reason for perceiving this attributes is the participation of salivary proteins when interact with substances like tannins, polyphenol, and whey proteins in milk (de Wijk and Prinz, 2005, Stokes et al., 2013).

Human whole saliva (HWS) can be added to the samples while testing these properties to reduce food breakdown due to HWS incorporation with food during oral processing (de

Wijk and Prinz, 2005, Stokes et al., 2013, Morell et al., 2016). HWS has shown to reduce the viscosity and friction profiles of semisolid foods. The components in HWS can greatly affect the perception of friction-related attributes e.g. astringency. The cause of this has been attributed to the presence of larger particles from either precipitation of salivary proteins or interactions of saliva proteins with food components. On the other hand, salivary amylase breaks down food starch, which can result in a decrease in friction (Stokes et al., 2013, Chen, 2015, Morell et al., 2016, Engelen et al., 2003a, Janssen et al., 2007). There is a current lack of information in the literature of how addition of HWS impacts food rheological and tribological behaviors, and whether addition of HWS during instrumental testing results in stronger correlations of the resulting data with sensory texture data. Therefore, the objective of this study was to determine the relationships among rheological, tribological, and sensory behaviors of semisolid foods, as well as how addition of HWS during instrumental testing impacted these relationships since HWS significantly affected rheological and tribological properties of acid milk gels in the previous study (chapter 3).

4.3 Materials and Methods

4.3.1 Materials

Skim milk (WinCo Foods brand) was purchased from a local supermarket (Moscow, ID., U.S.A.). Locust bean gum and carboxymethyl cellulose (pre-hydrated Ticalose CMC 2500 powder) were donated by TIC Gums (TIC Gums, Inc., Belcamp, Md., U.S.A.). Low-heat skim milk powder and Darigold brand heavy cream (40% fat) were provided by the Washington State University Creamery (Pullman, WA., U.S.A.). Whey protein isolate (Provon 190, 89.4% protein) was donated by Glanbia Nutritionals (Fitchburg, Wis., U.S.A.).

Glucono-delta-lactone was donated by Jungbunzlauer (Jungbunzlauer, Inc., MA., U.S.A.). Corn starch and modified potato starch were donated by Ingredion (Bridgewater, N.J., U.S.A.).

4.3.2 Acid milk gel preparation

Stirred acid milk gels were used as a model system for yogurt. The advantage of GDL application in the model system compared to live bacteria is an easier control of pH during testing. Glucono-delta-lactone (GDL) was used for acidification of the acid milk gels because it has a slow rate of acidification and a mild taste, providing a controlled pH reduction and neutral flavor. Twenty-four formulations (see Table 4.1. for specific ingredient amounts in each

formulation) of including locust bean gum (LBG), carboxymethyl cellulose (CMC), corn starch (CS), and potato starch (PS). Several samples were also made with cream to provide fat. Whey protein isolate (WPI) and skim milk powder (SMP) were utilized as protein sources. SMP was used in control samples to adjust total solids non-fat. Addition of SMP is a standard practice in yogurt manufacturing (Karam et al., 2013) and it also improves the texture and decreases syneresis in yogurts (Modler et al., 1983). 2% SMP has been shown to enhance the textural properties of yogurts (Soukoulis et al., 2007, Tamime and Robinson, 2007a). All samples had a total solids content of 13% w/w.

The milk base for each acid milk gel formulation was prepared adding the designated amount of dry powders and fat to the skim milk at room temperature (22 ± 2°C).

The mixture was stirred with a lab spatula to achieve full dispersion of hydrocolloids in a water bath (Precision, Thermo Fisher Scientific, Waltham, MA, U.S.A.) at 85˚C for 3 min. Next, milk bases were pasteurized in the water bath at 85˚C for 30 min.

Table 4.1. Experimental design of acid milk gels

Formula number	SMP (w/w)	WPI (w/w)	LBG (w/w)	CMC (w/w)	Potato starch (w/w)	Corn starch (w/w)	Skim milk (w/w)	Cream (w/w)	GDL (w/w)
1	2.8	0	0	0	0	0	97.2	0	1.1-1.55
2	2.83	0	0	0	0	0	95.96	1.21	1.1-1.55
3	2.89	0	0	0	0	0	92.26	4.85	1.1-1.55
4	2.95	0	0	0	0	0	89.15	7.9	1.1-1.55
5	1.8	1	0	0	0	0	97.2	0	1.1-1.55
6	1.8	0	1	0	0	0	97.2	0	1.1-1.55
7	1.8	0	0	1	0	0	97.2	0	1.1-1.55
8	2.1	0	0	0	0.7	0	97.2	0	1.1-1.55
9	2.1	0	0	0	0	0.7	97.2	0	1.1-1.55
10	0	1.25	1.55	0	0	0	97.2	0	1.1-1.55
11	0	1.25	0	1.55	0	0	97.2	0	1.1-1.55
12	0	1.25	0	0	1.55	0	97.2	0	1.1-1.55
13	0	1.25	0	0	0	1.55	97.2	0	1.1-1.55
14	0.5	0.8	0	0.75	0.75	0	97.2	0	1.1-1.55
15	0.5	0.8	0.75	0.75	0	0	97.2	0	1.1-1.55
16	0	2.8	0	0	0	0	97.2	0	1.1-1.55
17	0	0	0	1.4	1.4	0	97.2	0	1.1-1.55
18	0	0	1.8	0	0	1	97.2	0	1.1-1.55
19	0	1.15	0.55	0.55	0.55	0	97.2	0	1.1-1.55
20	0	1.15	0	0.55	0.55	0.55	97.2	0	1.1-1.55
21	0	0	0.7	0.7	0.7	0.7	97.2	0	1.1-1.55
22	0.55	0.75	0.5	0.5	0.5	0	97.2	0	1.1-1.55
23	1	0	0.45	0.45	0.45	0.45	97.2	0	1.1-1.55
24	0.2	0.8	0.45	0.45	0.45	0.45	97.2	0	1.1-1.55

This step also assisted in protein denaturation for yielding a stronger gel. The milk bases were homogenized for 1 min at 5,000 rpm using a stand homogenizer (Polytron, Kinematica AG, NY, U.S.A.) and cooled to 42.2˚C. After addition of GDL (1.1%-1.55% w/w, see Table 4.1 for precise quantities) under constant stirring, the milk bases were incubated for 4 hr at 42.2˚C to reach a pH of 4.55-4.6. Subsequently, samples were removed from the water bath, and the gel was broken with a spatula. Samples were then stored in a refrigerator at temperature of 4-8˚C overnight. The next day, each sample was blended at 350 rpm for 10 s to remove possible lumps and achieve a homogenous texture prior to testing, which was performed immediately after blending. All samples were prepared in duplicates.

4.3.3 Descriptive sensory evaluation

Sensory evaluation of acid milk gels was performed under the approval of the University of Idaho's Institutional Review Board (protocol 17-195). Ten panelists were recruited from the Washington State University and the University of Idaho School of Food Science via electronic communication and social media. Panelists were 100% female ranging in age from 25 to 55 yr with an average age of 33.9 yr. They were trained for 11 hr before performing formal evaluations over a total of 8 hr.

Thirteen textural attributes were introduced to the panelist for describing the texture of the acid milk gels (Table 4.2) according to previous related studies (Saint-Eve et al., 2004, Pascua et al., 2013, Joyner (Melito) et al., 2014). The panelists also verbally agreed on type and intensity of the references. The panelists profiled each yogurt individually using a 15-cm line scale to indicate the intensity of each attribute present in the samples. Hard copies of the

13 attributes description along with a 15-cm line scale for each attribute were provided for the training sessions.

Table 4.2. Texture attributes and reference products used for sensory evaluation of yogurts

Sensory terms, definitions, and references obtained from (Saint-Eve et al., 2004, Pascua et al., 2013) and (Joyner (Melito) et al., 2014).

Attribute	Definition	Reference (scale 0 to 15)
Visual terms		
Lumpiness	Presence of lumps observed in yogurts after being stirred	Yoplait vanilla yogurt=1 Jell-O tapioca pudding=15
Spoon viscosity	Thickness of food after being stirred back and forth for 10 times	Water=1 Jell-O pudding=10.5
Mouthfeel terms		
Grainy	Perception of food granules (small particles) on tongue after expectorating	ReddiWip whipped cream=1 Baby rice cereal (Gerber)=12
Mouthcoating	Force required to clear sample adhered to the mouth/with the tongue during eating	Cream cheese=10 ReddiWip whipped cream =1
Mouth Viscosity	Force needed to draw food from a spoon over the tongue	Water=1 Chocolate Jell-O pudding=12
Firmness	Firmness of food in the mouth when food is compressed up and down via tongue motions	ReddiWip whipped cream =1 Cream cheese (Philadelphia)=14
Lumpiness in-mouth	Feeling of lumps in the mouth during eating	Yoplait yogurt=1 Jell-O tapioca pudding=15
Smooth	Lack of individual food particles, opposite of grainy and lumpy attributes	Yoplait yogurt=13 Baby rice cereal (Gerber)=1
Melting	Food spreads out in the mouth at different rates	ReddiWip whipped cream =1 Jell-O pudding=1
Grittiness in- mouth	Feeling of gritty/chalky particles in the oral cavity during eating	Walmart non-fat Greek yogurt =10 ReddiWip whipped cream =1
After-feel mouth terms		
Astringent	Astringent/dry sensation in the mouth after food is swallowed or expectorated	Atkins strawberry protein drink=10 ReddiWip whipped cream =1
Chalky/Gritty after-feel	Feeling of chalk-like particles in the mouth after food is swallowed or expectorated	Walmart non-fat Greek yogurt =10 ReddiWip whipped cream =1
Slimy	Difficulty of clearing the mouth after food is swallowed or expectorated	Banana baby food Gerber=7 ReddiWip whipped cream=1

During the last two training sessions, panelists practiced with the sensory software (Compusense Cloud, Guelph, Ontario, Canada) to become familiar with its operation before formal sample evaluations. Formal sample evaluations were carried out in individual sensory facility booths under white light. Six acid milk gels (from total n=24) with their duplicates (12 samples in total) were evaluated per session by the panelists in a completely randomized balanced design. All samples were evaluated in duplicate. Samples were served in 4 oz. plastic soufflé cups and randomly coded with 3-digit numbers. All samples were prepared the day before evaluation.

During evaluation, panelists were provided the references upon requests. To minimize fatigue, panelists were asked to rinse their mouths with filtered water, expectorate samples after each evaluation, and cleanse their palate with unsalted crackers after evaluation of each sample. Additionally, a 5 min enforced rest period was held after finishing 6 samples to minimize fatigue and errors during evaluation. All samples were evaluated on a 15-cm line scale with anchors at 1.5 cm for low intensity and 13.5 cm for high intensity. The results of the evaluations were collected from Compusense software.

4.3.4 Rheological and tribological results used for correlations

The results from rheometry and tribometry performed on acid milk gels during a previous study (Chapter 3) were used for correlation with the sensory attribute data collected in this study. Viscosity results selected for correlation included zero-shear rate viscosity (η_{o},, Pa s), infinite viscosity (η_{∞}, Pa s), time constant (c, s), and flow behavior index (n, unitless). Viscoelastic parameters selected included critical strain (γ_c, %), complex modulus (G^*, Pa), and phase angle (tan δ, rad). Selected tribological results included friction coefficients at sliding speeds of 10, 15, 20, 25, and 30 mm/s from. All selected parameters are summarized in Table 4.3.

4.3.5 Data analysis

Analysis of Variance (ANOVA) followed by Tukey's HSD (Honest Significant Difference) test using SAS version 9.1 (SAS; Cary, NC, U.S.A.) was used to determine significant differences for three main variables (panelists, replicates, samples, and their interactions) of the sensory results.

Table 4.3. Selected rheological and tribological parameters for correlation analysis (Chapter 3)[1,2]

	Viscosity parameters				Viscoelastic parameters				Friction coefficients	
Formula	ηo (Pa s) 25°C, NS	n 25°C, NS	ηo (Pa s) 25°C, S	n 25°C, S	γc (%) 25°C, NS	γc (%) 25°C, S	G* (Pa) 25°C, NS	G* (Pa) 25°C, S	μ, 25°C, (10 mm/s) NS	μ, 25°C, (10 mm/s) S
1	380	0.908	0	0.643	0.547	0.555	179	119	0.745	0.592
2	0	0.503	0	0.5	0.975	0.975	94.5	56.6	0.609	0.521
3	0	0.414	0	0.407	0.975	1.300	58.1	38.2	0.152	0.101
4	2241	0.962	0	0.926	0.731	0.411	284	119	0.177	0.091
5	1072	0.907	728	0.816	0.731	0.731	698	503	1.004	0.761
6	42.6	0.604	26.4	0.55	1.74	0.73	64.4	30.4	1.435	0.907
7	517	0.823	290	0.804	0.730	0.730	362	78.3	0.965	0.751
8	105	0.833	52.8	0.427	0.975	0.975	119	37.7	0.662	0.662
9	0	0.679	0	0.422	0.975	0.731	130	76.6	1.328	0.764
10	205	0.822	114	0.783	2.33	2.33	288	185.8	1.320	1.320
11	231	0.855	123	0.853	10.000	13.400	113	61.7	0.305	0.218
12	3447	0.86	787	0.804	0.975	0.976	1178	295	1.767	0.646
13	0	0.584	0	0.35	0.730	0.730	73.3	42.2	1.078	0.878
14	151	0.844	73.9	0.802	10	7.48	112	75.5	0.387	0.530
15	96.7	0.802	34	0.731	4.180	4.180	73.0	45.7	0.556	0.732
16	2298	0.827	1773	0.721	1.3	1.74	2638	879	1.536	0.826
17	467	0.873	120	0.824	5.6	10	250	61	0.927	0.352
18	212	0.792	79.2	0.782	3.120	4.180	315	141	0.722	0.833
19	161	0.805	54	0.746	3.120	4.180	131	45.9	0.705	0.404
20	307	0.748	45.6	0.712	0.41	0.547	87.8	28.3	1.051	0.795
21	190	0.843	103	0.839	7.480	7.480	150	77.1	0.700	0.521
22	75.1	0.778	30.6	0.72	4.18	4.18	64.6	35.2	0.747	0.705
23	143	0.794	43	0.731	2.330	3.120	115	43.6	0.871	0.635
24	136	0.781	49.3	0.705	2.33	2.33	97.9	44.2	0.957	0.595

[1] ηo: zero-shear viscosity; n: flow index; γc: critical strain; G*: complex modulus; μ: friction coefficient; [2]S: HWS; NS; without WHS.

4.4 Results and Discussion

4.4.1 The effect of different hydrocolloids on texture perception of acid milk gels

Formulations and panelists had significant influence on textural attributes of acid milk gels at $p \leq 0.001$ (Table 4.4). The control sample, samples with added fat (samples 2, 3, and 4), CS (sample 9), or both WPI and CS (sample 13) showed the greatest spoon lumpiness (Table 4.5). Samples with WPI and gums (samples 10 and 11) and samples formulation with more than three hydrocolloids had the least spoon lumpiness.

Table 4.4. Effect of different formulations (hydrocolloids) on texture attributes of acid milk gels (n=24) determined by F-values obtained from three-way ANOVA[1].

Textural attributes	panelist	Formulation	replicate	Formulation*replicate
Spoon viscosity	20.9***	137.5***	2.3	1.7
Graininess	10.1***	9.6***	0.3	0.7
Mouthcoat	16.1***	59.5***	1.5	0.8
Firmness	20.7***	60.8***	2.2	0.9
Mouth viscosity	31.1***	79.3***	0.4	0.4
Lumpiness	21.4***	36.8***	3	1.7
Lumpiness-in-mouth	13.8***	18.3***	1.6	1
Smoothness	30.1***	18.4***	0.5	0.5
Melting	15.5***	28.6***	0.1	0.9
Grittiness-in-mouth	14.3***	8.8***	0.2	0.5
Astringency	27.6***	14.4***	1.2	0.8
Chalkiness-Afterfeel	19.4***	8.5***	1.7	0.6
Sliminess	21.8***	62.6***	2.4	0.4

[1] *, **, and *** indicate significant differences at p-value ≤ 0.05, p-value ≤ 0.01, and p-value ≤ 0.001, respectively.

Addition of whey powder has been linked to the formation of lumps, grits, or grains (Morell et al., 2015). This effect significantly improved when WPI was used with CMC. Additional proteins from WPI and ionic charged CMC can form a strong casein network which minimize structure irregularity by forming a high number of cross-linking and aggregations throughout the system (Ibrahim et al., 2010, van de Velde et al., 2015).

Mouthcoat, spoon viscosity, firmness, and viscosity in mouth attributes were higher for samples with a combination of two or more hydrocolloids (samples 19-24) compared to when hydrocolloids were used individually (samples 1-9). Interestingly, addition of fat (samples 2, 3, and 4) did not have a significant effect on these attributes compared to the control sample (sample 1). These results can be due to the associative interactions between the oppositely charged portions of the polysaccharides and the proteins from the milk, SMP, and WPI. Network stabilization can also occur because of hydrophobic interactions and hydrogen bridging among the polymers in the system (Doublier et al., 2000, Bertrand and Turgeon, 2007).

As expected, lumpiness in mouth and smoothness were inversely related, as smoothness is related to the texture with minimum food particles without lumps, grains, and grits. The least smooth samples were the control sample (sample 1), samples with added fat (samples 2, 3, and 4), samples with added starch (samples 8 and 9), and samples with added CS and WPI (sample 13) (Table 4.4.).

The intensities for spoon viscosity and in-mouth-viscosity were very similar, which was unsurprising (Table 4.5.).

Table 4.5. Acid milk gels sensory attributes as evaluated by trained panelists

Formula number	Spoon lumpiness	Spoon viscosity	Graininess	Mouthcoating	Mouth viscosity	Firmness	Lumpiness in mouth	Smoothness	Melting	Grittiness in mouth	Astringency	Chalkiness afterfeel	Sliminess
1	7.93^{ab}	2.81^{k}	$4.08^{abcdefg}$	2.78^{h}	2.45^{h}	2.60^{g}	4.52^{bcd}	5.23^{g}	9.63^{a}	5.12^{ab}	5.86^{a}	4.65^{a}	1.97^{f}
2	7.96^{ab}	3.29^{k}	5.18^{ab}	3.115^{h}	3.04^{gh}	2.64^{g}	5.64^{ab}	5.67^{fg}	8.91^{ab}	$4.20^{abcdefghi}$	5.00^{abc}	4.05^{abc}	1.98^{f}
3	9.42^{a}	3.10^{k}	$3.94^{abcdefg}$	3.15^{h}	2.93^{gh}	2.72^{g}	4.78^{bc}	6.25^{efg}	9.56^{a}	$3.48^{cdefghij}$	5.16^{ab}	3.34^{abcd}	1.94^{f}
4	7.86^{ab}	3.31^{k}	$3.87^{abcdefg}$	3.17^{h}	2.98^{gh}	2.96^{g}	4.52^{bcd}	6.38^{defg}	8.99^{ab}	$3.61^{bcdefghij}$	4.91^{abcd}	3.35^{abcd}	2.000^{f}
5	8.21^{ab}	3.01^{k}	$4.01^{abcdefg}$	2.69^{h}	2.65^{h}	2.47^{g}	4.56^{bcd}	5.26^{g}	9.27^{ab}	4.96^{abcd}	5.69^{a}	4.52^{a}	1.91^{f}
6	5.11^{cdef}	8.30^{cde}	4.33^{abcdef}	6.43^{bcde}	7.16^{bc}	7.79^{bcde}	3.49^{cde}	8.52^{abcde}	3.36^{ef}	3.16^{fghij}	3.61^{cde}	2.84^{cd}	4.78^{bcde}
7	4.32^{defg}	5.02^{hi}	2.57^{fgh}	4.41^{fg}	4.30^{gf}	4.95^{f}	3.17^{cde}	8.68^{abcd}	6.84^{bcd}	3.26^{fghij}	4.7^{bcde}	3.51^{abcd}	4.00^{e}
8	6.90^{bc}	3.48^{jk}	4.47^{abcde}	3.35^{gh}	2.84^{h}	2.98^{g}	4.71^{bc}	6.23^{efg}	7.88^{abc}	4.61^{abcdef}	5.43^{ab}	4.25^{ab}	2.12^{f}
9	10.1^{a}	2.92^{k}	5.39^{ab}	3.01^{h}	2.87^{h}	2.80^{g}	7.37^{a}	4.80^{g}	8.50^{ab}	4.82^{abcde}	5.25^{ab}	4.65^{a}	1.93^{f}
10	2.26^{gh}	6.23^{fg}	4.79^{abcd}	5.38^{ef}	5.72^{de}	6.34ef	2.35^{e}	7.96^{bcdef}	5.34^{cde}	$4.26^{abcdefgh}$	3.72^{cde}	3.78^{abcd}	4.16^{de}
11	3.17^{fgh}	9.51^{ab}	2.35^{gh}	7.31^{ab}	7.85^{ab}	9.02^{ab}	2.16^{e}	10.16^{ab}	3.36^{ef}	2.93^{hij}	3.33^{e}	2.50^{d}	6.02^{a}
12	5.03^{cdef}	4.48^{ij}	5.88^{a}	3.36^{gh}	3.40^{gh}	3.36^{g}	3.17^{cde}	5.64gf	9.09^{ab}	5.19^{a}	4.87^{abcd}	4.59^{a}	2.43^{f}
13	9.57^{a}	3.08^{k}	4.86^{abc}	2.93^{h}	2.705^{h}	2.53^{g}	5.67^{ab}	5.12^{g}	8.21^{ab}	4.82^{abcde}	5.65^{a}	4.47^{a}	2.21^{f}
14	3.61^{efgh}	7.54^{de}	2.33^{gh}	6.11^{bcde}	5.76^{cde}	7.08^{de}	2.16^{e}	9.59^{ab}	4.17^{ef}	2.83^{hij}	3.55^{de}	2.97^{bcd}	4.68^{cde}
15	6.07^{bc}	7.82^{cde}	2.49^{fgh}	6.91^{abc}	7.03^{bcd}	8.36^{abcd}	2.6^{e}	9.40^{ab}	3.84^{ef}	2.64^{ij}	2.84^{e}	2.79^{cd}	5.28^{abc}
16	5.58^{cde}	2.92^{k}	3.13^{cdefgh}	2.45^{h}	2.14^{h}	2.26^{g}	3.34^{cde}	6.79^{cdefg}	10.04^{a}	5.02^{abc}	5.76^{a}	4.525^{a}	1.92^{f}
17	2.96^{fgh}	9.85^{a}	2.10^{h}	7.69^{a}	8.93^{a}	9.79^{a}	2.1^{e}	9.78^{ab}	2.26^{f}	2.72^{hij}	3.04^{e}	2.49^{d}	5.77^{ab}
18	2.29^{gh}	5.88^{gh}	2.94^{defgh}	5.41^{ef}	5.06^{ef}	6.58^{e}	2.9^{e}	8.94^{abc}	5.42^{cde}	$4.52^{abcdefg}$	3.36^{e}	4.05^{abc}	4.54^{cde}
19	3.17^{fgh}	8.13^{cde}	3.11^{cdefgh}	6.23^{bcde}	6.63^{bcd}	7.64^{bcde}	2^{e}	9.71^{ab}	4.17^{ef}	$3.39^{defghij}$	3.59^{cde}	2.96^{bcd}	5.24^{abc}
20	1.59^{h}	8.38^{cde}	2.73^{efgh}	6.68^{abcd}	6.66^{bcd}	8.13^{bcd}	1.89^{e}	10.92^{a}	4.52^{def}	3.00^{ghij}	3.20^{e}	2.83^{cd}	5.23^{abc}
21	3.77^{efgh}	8.69^{bc}	2.60^{efgh}	7.15^{ab}	8.01^{ab}	8.83^{abc}	2.36^{e}	10.44^{a}	3.45^{ef}	2.98^{ghij}	3.13^{e}	3.09^{bcd}	5.47^{abc}
22	2.47^{gh}	7.52^{de}	2.33^{gh}	5.69^{de}	5.84^{cde}	7.18^{de}	1.91^{e}	10.02^{ab}	4.23^{ef}	2.62^{j}	3.43^{e}	3.01^{bcd}	5.07^{abcd}
23	2.00^{h}	7.32^{ef}	2.58^{fgh}	5.87^{cde}	5.95^{cde}	7.35^{cde}	2.1^{e}	9.11^{abc}	4.31^{def}	2.87^{hij}	3.27^{e}	2.83^{cd}	4.93^{bcde}
24	4.83^{cdef}	8.49^{bcd}	$3.75^{bcdefgh}$	6.66^{abcd}	6.76^{bcd}	7.47^{cde}	2.88^{de}	9.02^{abc}	2.97^{ef}	$3.46^{cdefghij}$	3.43^{e}	3.36^{abcd}	5.09^{abcd}

Letters that are different in each column indicate significant differences ($p<0.05$).

Additionally, samples with high viscosity, smoothness, and sliminess, made with more than two hydrocolloids, had low graininess, chalkiness afterfeel, and grittiness. Hydrocolloids can stabilize the protein network in dairy products by strong interactions with casein micelles, and this phenomenon gets more effective at a lower Partial Least Square (PLS) analysis was used to correlate the rheological and sensory, tribological and sensory, and rheological and tribological results to determine relationships among mechanical–sensory, frictional–sensory, and mechanical–frictional properties of acid milk gels, respectively using SAS version 9.1 (SAS; Cary, NC, U.S.A.).pH value (Walstra, 1996). This stabilization will decrease the casein precipitation and improve the viscosity and gel strength (Walstra, 1996). Neutral hydrocolloids improve the texture properties of the system with increasing the continuous phase viscosity (Walstra, 1996). The mechanism for starches is different. Starch granules can swell in the solution in the presence of water and heat to alter texture properties of semisolid foods. Sensory results showed, the control sample (sample 1), samples with fat (samples 2, 3, and 4), samples with added starch (samples 8 and 9), and samples with a combination of starch and protein (samples 12 and 13) had the lowest mouthcoating, mouth viscosity, firmness, smoothness, and sliminess. These samples also had the highest graininess, chalkiness afterfeel, astringency, and grittiness. Addition of milkfat may have caused an incompatibility with SMP due to the solubility level of SMP in the milk when milkfat is added. Starches can reduce smoothness and increase graininess, chalkiness, and grittiness due to the retrogradation effect when the gelatinized starch cools (Bird et al., 2000). Addition of milk powders like WPI and SMP can have a similar effect (Isleten and Karagul-Yuceer, 2006). Milk proteins, mainly those in whey, participate at their isoelectric points when mixed with HWS. This effect generally results in increased astringency (Sano et al., 2005, Andrewes et al., 2011). In addition, the particle size of whey proteins increases upon addition to yogurt due to increased interactions between whey proteins and the binding sites of κ-casein (Beaulieu et al., 1999, Puvanenthiran et al., 2002). Furthermore, the interaction of κ-casein with the larger and irregularly-shaped whey protein particles can cause some protein aggregation (Puvanenthiran et al., 2002, Engelen et al., 2005). The larger particles can be felt during oral processing, resulting in a sensation of roughness and dryness (Cayot et al., 2008) on the oral surfaces including tongue, palate and surrounding soft oral tissues (Engelen et al., 2007).

Samples formulated with more than two hydrocolloids (samples 14, 15, and 19-24) generally had the lowest amount of astringency, likely because the hydrocolloids prevented HWS from interacting with whey proteins, which is known to cause an astringent sensation (Andrewes et al., 2011). In yogurt and acid milk gels, the particle size of the whey protein increases once they are added to the yogurt due to the higher interaction of whey proteins with the binding sites of κ-casein (Beaulieu et al., 1999, Puvanenthiran et al., 2002). These large particles result in an astringent sensation. If the whey proteins interact with hydrocolloids, they are more likely to remain as part of the network instead of existing as free particles, reducing astringency.

Overall, addition of gums significantly improved desirable attributes e.g. firmness, viscosity, smoothness, lumpiness, and sliminess (manifesting as a ropy texture, which is pleasing to consumers) (van de Velde et al., 2015, Han et al., 2016), and decreased negative attributes such as grittiness, graininess, astringency, and chalkiness afterfeel (Alakali et al., 2008). Addition of gums can significantly change the microstructural, textural, and rheological properties of acid milk gels by two major mechanisms: segregative and aggregative interactions. Aggregative interactions are generally related to the hydrocolloids with net charges, e.g. CMC. The counterions from protein and charged hydrocolloids create strong aggregation throughout the protein matrix and improve textural properties such as firmness and smoothness. Segregative interactions are used to describe interactions between proteins and neutral gums, e.g. LBG. These hydrocolloids improve textural properties by increasing the viscosity of the continuous phase and resulting in increased gel firmness (Thaiudom and Goff, 2003).

4.4.2 Principal Component Analysis

Principle component analysis (PCA) was performed to visualize sensory texture attributes (Figure 4.1.). Using PCA also helped eliminate descriptor redundancies from textural attributes and obtain possible latent variables by fitting dependent variables into major factor (component) groups. Component 1 explained 85.54% of the variance observed, while component 2 explained 5.87%, indicating that the majority of the variation among the samples was described by these components.

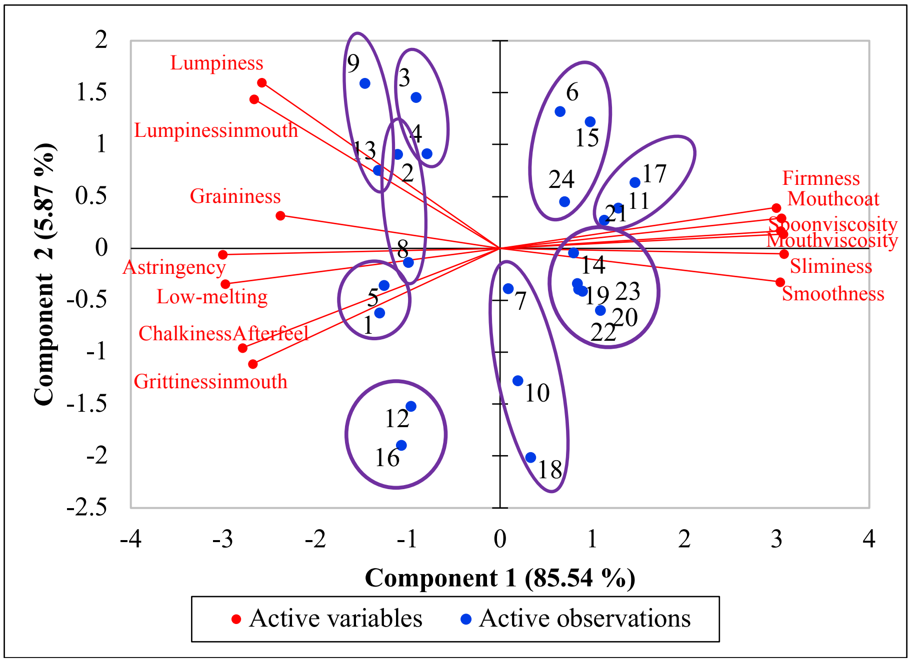

Figure 4.1. Principle Component Analysis (PCA) biplot for acid milk gels
(n=24 formulations composition in table 4.1.)
Clusters have been circled based on cluster analysis (Figure 4.2.)

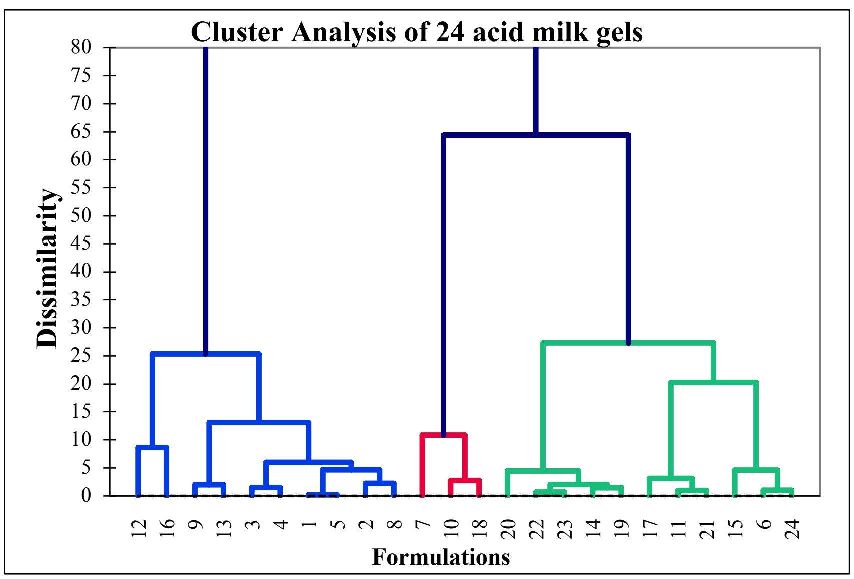

Figure 4.2. Cluster Analysis for acid milk gels (n=24)
(n=24 formulations composition in table 4.1.)

Component 1 was primarily and positively defined by sliminess, spoon viscosity, mouth viscosity, mouthcoat, smoothness, and firmness; and negatively with astringency, low-melting, and graininess. It should be noted that a lower intensity of the low-melting attribute was considered to be more desirable. For instance, ReddiWip whipped cream, a reference product for this attribute had a low intensity score since it needed a very short time to melt away in the mouth. Component 2 was mostly described by lumpiness, lumpiness in mouth, grittiness in mouth, and chalkiness afterfeel. Samples with CMC and WPI (sample 11); SMP, WPI, CMC, and PS (sample14), CMC and PS (sample 17), WPI, LBG, CMC, and PS (sample 19), WPI, CMC, PS, and CS (sample 20), LBG, CMC, PS, and CS (sample 21) were most positively related to component 1. The control sample (sample 1), samples with low levels of WPI (sample 5), 0.5% milkfat content (sample 2), PS (sample 8), and both WPI and CS (sample13) were most negatively related to component 1. The sample with LBG and CS (sample 18) was most negatively related to component 2. Overall, the PCA plot showed that samples prepared with more than two hydrocolloids that included at least one gum (CMC or LBG) were associated with more desirable texture attributes, which was in line with the descriptive sensory results.

Acid milk gels formulations were divided into three large clutters based on the sum of the differences between the initial two object cluster (Figure 4.2.). The first large cluster (green) consisted of three sub-clusters including 1) samples 19, 20, 22, 23, and 14; 2) samples 17, 11, and 21; and 3) samples 6, 24, and 15. This cluster (green) was closest to smoothness, sliminess, mouth viscosity, spoon viscosity, mouthcoat, and firmness. These similarities were also shown in the descriptive analysis data (Table 4.3). These samples all had PS and CMC in their formulations; most formulations also included WPI. The correlation of CMC and smoothness has been shown in a yogurt study (Alakali et al., 2008). CMC are capable of making strong ionic interactions with casein micelles at pH=4.6. Additionally, PS granules can swell in the continuous phase and improve the evenness and uniformity of the texture (Roller, 1996). Additionally, the combination of WPI and CMC (sample 11) appeared to form similar texture to when CMC was used with PS (sample 17) as well as using both gums and starches without addition of SMP and WPI (sample 21) or all hydrocolloids (sample 24). These results indicated that CMC had a major contribution to textural attributes, regardless of other hydrocolloids used.

The samples in cluster 3 (blue) included those that contained milk fat and SMP (samples 2, 3, and 4), CS and SMP (sample 9), PS and SMP (sample 8), WPI and CS (sample 13), WPI and PS (sample 12), WPI and SMP (sample 5), and WPI (sample 16). This cluster was broken into five sub-clusters including samples 1) 12 and 16, 2) 9 and 13, 3) 3 and 4, 4) control and 5, and 5) 2 and 8. Texture attributes most closely related to this cluster included chalkiness afterfeel, grittiness in mouth, astringency, low-melting, both in mouth and spoon lumpiness. Addition of SMP and WPI is known to increase these attributes (Isleten and Karagul-Yuceer, 2006); the presence and large particles size of PS may have also contributed to these attributes, particularly in sample 8. Cluster 2 (red) included samples 7, 10, and 18. These samples were most positively related to the attributes from Cluster 1, and negatively to the attributes from Cluster 3. The intensity of desirable textural attributes such as smoothness and viscosity were greater than the intensity of the undesirable ones, e.g. graininess and astringency.

These results showed that use of protein and starches as hydrocolloids individually or in combination without addition of gums can increase the intensity of texture defects in acid milk gels, such as astringency and chalkiness afterfeel. On the other hand, the intensity of desirable texture attributes can be increased by addition of one or more gums in combination with other hydrocolloids.

4.4.3 Correlations among acid milk gel textural attributes

The correlation matrix of textural attributes showed spoon viscosity was positively correlated with mouthcoating, mouth viscosity, firmness, smoothness, and sliminess and negatively correlation with low-melting and astringency (Table 4.6).

Table 4.6. Correlation matrix for acid milk gel textural attributes[1,2,3]

Attribute	Spoon viscosity	Mouth -coat	Mouth viscosity	Firmness	Smoothness	Melting	Astringency	Chalkiness afterfeel	Sliminess
Spoon viscosity	1	0.989*	0.988*	0.986*	0.923*	-0.973*	-0.934*		0.979*
Mouthcoat		1	0.995*	0.990*	0.925*	-0.980*	-0.957*		0.981*
Mouth viscosity			1	0.988*	0.934*	-0.976*	-0.961*		0.989*
Firmness				1		-0.968*	-0.933*		0.965*
Smoothness					1		-0.928*		0.953*
Melting						1	0.937*		-0.970*
Astringency							1		-0.960*
Grittiness-in-mouth								0.967*	

[1] Coefficients that were not statistically significant (p>0.05) and repetitive correlations were removed from cells and columns

[2] **p*-value ≤ 0.001

[3] Non-significant attributes have been removed from the table.

Similarly, mouthcoating had positive correlations with mouth viscosity, firmness, smoothness, and sliminess and was negatively correlated with low-melting and astringency. Mouthcoating correlations with firmness and sliminess were also found in yogurts by (Janiaski et al., 2016). Measurement of mouthcoating in custards showed that its intensity can be related to thickness, viscosity, smoothness, and graininess (Prinz et al., 2006).

Sliminess has been correlated with the use of exopolysaccharide LAB in yogurt production (van de Velde et al., 2015). In this study, sliminess was related to addition of gums, particularly CMC. Exopolysaccharides produced by LAB can have neutral or negative charges, similar to hydrocolloids (van de Velde et al., 2015). The strong interaction of negatively charged polysaccharides produced from LAB with positively charged casein micelles at $pH<4.6$ results in a stronger protein network with longer chains, similar to the structure produced by CMC interactions with milk proteins. CMC is an anionic polysaccharide that can form strong interactions with casein micelles. CMC and this type of LAB due to similar net charges can form the same structure to improve the texture of yogurts (van de Velde et al., 2015). In particular, the longer chains contribute to a slick, potentially slimy mouthfeel.

Firmness was positively correlated with sliminess. Smoothness was positively correlated with sliminess and negatively correlated with low-melting and astringency. Grittiness was positively correlated with chalkiness afterfeel, which was not surprising as both of these attributes can be attributed to particle size. Similarly, chalkiness-in-mouth was positively correlated with grittiness-in-mouth due to the perception of bigger particle sizes on the tongue, especially in samples formulated with WPI, SMP, starch, or a combination of these (Puvanenthiran et al., 2002, Engelen et al., 2005).

Astringency was negatively correlated with sliminess and positively correlated with low-melting. This positive correlation between low-melting and astringency was in agreement with (Morell et al., 2016). Astringent samples did not melt away as rapidly in the mouth as those with lower astringency. According to the American Society for Testing of Materials, the sensory definition of astringency is “the complex of sensations due to shrinking, drawing or puckering of the epithelium as a result of exposure to substances such as alums or tannins” (Materials, 2004). Astringency is a common defect in dairy products due to interactions among whey proteins, caseins, and calcium phosphates, or whey proteins with astringent compounds. These interactions result in aggregation that disrupts the salivary film and reduces lubricity in the mouth (Josephson et al., 1967, Andrewes et al., 2011, Gibbins and Carpenter, 2013). Astringency has also been attributed to the production of γ-caseins from β-casein by breaking of the peptide bonds between 28 and 29, 105 and 106, and 107 and 108 amino acids in the β-casein chain (Harwalkar et al., 1993, Lemieux and Simard, 1994). Another explanation for the astringency perception was believed though to be the aggregation and precipitation of salivary proteins, which results in loss of saliva lubricity (Jöbstl et al., 2004). The dry sensation is due to precipitation of salivary proteins after complexation with astringent molecules from alums, tannins, and polyphenols (Green, 1993). The precipitation causes direct contact of two oral surfaces (Gibbins and Carpenter, 2013).

4.4.4 Correlations among acid milk gel sensory and rheological properties

Statistical correlations of rheological and textural properties were determined by preforming Partial Least Square (PLS). Viscosity parameters with statistically significant correlations were η_0 and n (Table 4.7). The greatest number of correlations for η_0 were at 8°C when no HWS was added; n showed the most correlations at both 25°C and 8°C and when HWS was added. This effect was in accordance with those observed in a previous study (Chapter 3): HWS showed significant impact on the flow behavior index of acid milk gels based on F-values. While the effects of HWS were not significant on η_0 and c, the effect of formulation was significant for η_0 (Chapter 3).

η_0 at 8°C without HWS was positively correlated with low-melting, grittiness in mouth and astringency, and negatively correlated with mouthcoat, mouth viscosity, and sliminess. Interestingly, addition of HWS resulted fewer correlations for η_0 at 8°C: only grittiness in mouth and chalkiness showed correlations. This can be explained by the differences in particle sizes among the formulations. Addition of HWS at 8°C would make the effect of particle size more prominent due to HWS-induced depletion flocculation of protein aggregates in acid milk gels. Temperature may have influenced these effects since this correlation was not found for η_0 at 25°C. Conversely, n at 8°C with HWS was negatively correlated with graininess, spoon lumpiness, and lumpiness in mouth and positively correlated with smoothness. This result indicated that as graininess and lumpiness increase, the shear thinning behavior of the acid milk gels decrease, which may also be related to increased smoothness since the material does not become thin but would maintain a constant layer over the oral surfaces. n showed more correlations at 25°C when HWS was added compared to 8°C. This result implied that the extent of shear thinning was an important parameter for food texture perception of semisolid foods. This parameter was negatively correlated with lumpiness and graininess and positively correlated with spoon viscosity, mouth viscosity, smoothness, and sliminess. However, n at 25°C was not correlated with any of the tests when HWS was not added. This result implies that HWS application is important for understanding the effects of flow behavior index on oral texture attributes. It should be noted that, in general, more correlations were found for parameters with added HWS than without.

Table 4.7. Correlations between sensory and viscosity results for acid milk gels (n=24 formulations)[1]

Attribute	η_0 at 8°C without HWS	n at 8°C with HWS	η_0 at 8°C with HWS	η_0 at 25°C without HWS	η_0 25°C with HWS	n at 25°C with HWS
Spoon lumpiness		-0.553**				
Lumpiness in mouth		-0.667***				-0.614**
Spoon viscosity						0.438*
Mouthcoat	0.435*					
Mouth viscosity	-0.407*					0.445*
Firmness						
Smoothness		0.471*				0.500*
Melting	0.491*			0.464*		
Grittiness in mouth	0.440*		0.442*	0.414*		-0.520*
Graininess		-0.543**				
Chalkiness			0.416*		0.410*	
Astringency	0.418*					
Sliminess	-0.408*					0.460*

[1] Non-significant coefficients and repetitive correlations were removed from cells and columns.
**p*-value ≤ 0.05; ***p*-value ≤ 0.01; ****p*-value ≤ 0.001;

This result points to the importance of instrumental testing with HWS when investigating relationships between behaviors found through instrumental testing and textural attributes. This finding was supported by significant effects of HWS (for n) and interaction of HWS by formulations (for n, c, andη_0) with viscosity parameters in a previous study (Chapter 3). A large number of correlations were found between viscoelastic and textural properties of acid milk gels (Table 4.8.). Among viscoelastic parameters, critical strain and tan δ had the highest numbers of correlations with other sensory attributes. Critical strain values at 8°C and 25°C with or without HWS were correlated with all sensory attributes. The same correlations were observed for tan δ except at 8°C without HWS: tan δ was not correlated with graininess. Most critical strain and tan δ correlations were positive for with ideal attributes such as spoon viscosity, firmness, mouth viscosity and smoothness. Some of these correlations were also found by Joyner (Melito) et al. (2014) for acid milk gels. One potential reason for these results is that high critical strain indicates a stronger intermolecular structure that requires a greater force to make it flow or break. This would likely manifest as increased viscosity and firmness. tan δ indicates the degrees of elastic- versus viscous- type behavior, with larger values indicating more viscous-type behavior. Samples with less elastic-type behavior (i.e. gel-like samples) would likely be perceived as smoother and with a greater degree of mouthcoating due to the increased viscous flow.

Table 4.8. Correlation between sensory results and viscoelastic parameters for acid milk gels (n=24 formulations)[1]

Attribute	γ_c (%) 8°C NS	γ_c (%) 8°C S	γ_c (%) 25°C NS	γ_c (%) 25°C S	G* (Pa) 8°C NS	G* (Pa) 8°C S	G* (Pa) 25°C NS	G* (Pa) 25°C S	Tan δ (radians) 8°C NS	Tan δ (radians) 8°C S	Tan δ (radians) 25°C NS	Tan δ (radians) 25°C S
Lumpiness	-0.438*	-0.485*	-0.469*	-0.498*					-0.625**	-0.604**	-0.628**	-0.589**
Spoon viscosity	0.659***	0.674***	0.669***	0.695***					0.747***	0.619**	0.731***	0.723***
Graininess	-0.530**	-0.627**	-0.605**	-0.632***						-0.412*	-0.466*	-0.408*
Mouthcoat	0.649***	0.669***	0.673***	0.693***	-0.437*	-0.456*		-0.454*	0.763***	0.615**	0.752***	0.732***
Mouth viscosity	0.643***	0.671***	0.667***	0.697***	-0.405*	-0.423*		-0.421*	0.769***	0.640***	0.762***	0.747***
Firmness	0.657***	0.680***	0.660***	0.704***	-0.412*	-0.433*		-0.429*	0.730***	0.596**	0.718***	0.724***
Lumpiness in mouth	-0.485*	-0.532**	-0.539**	-0.554**					-0.629***	-0.550**	-0.630***	-0.578**
Smoothness	0.585**	0.622**	0.632***	0.630***					0.722***	0.581**	0.724***	0.669***
Melting	-0.605**	-0.637***	-0.645***	-0.649***	0.479*	0.486*	0.441*	0.482*	-0.814***	-0.671***	-0.803***	-0.781***
Grittiness in mouth	-0.518**	-0.567**	-0.574**	-0.547**	0.503*	0.503*	0.477*	0.535**	-0.629***	-0.565**	-0.634***	-0.601**
Astringency	-0.540**	-0.574**	-0.595**	-0.595**	0.431*	0.460*		0.463*	-0.793***	-0.665***	-0.791***	-0.743***
Chalkiness afterfeel	-0.576**	-0.592**	-0.591**	-0.609**	0.461*	0.469*	0.440*	0.498*	-0.644***	-0.589**	-0.640***	-0.634***
Sliminess	0.640***	0.658***	0.661***	0.687***	-0.397	-0.412*	-0.357	-0.411*	0.775***	0.642***	0.768***	0.735***

NS: no HWS added; S: HWS added.

G* had the lowest number of correlations with sensory results compared to the other two parameters. G* with and without HWS at 8°C and with HWS at 25°C was positively correlated with low-melting, grittiness-in-mouth, and astringency, and negatively correlated with mouthcoat, mouth viscosity, and firmness. At 25°C and without HWS, G* was positively correlated with low-melting, grittiness in mouth, and chalkiness afterfeel, and negatively correlated with sliminess. The repeating of the correlations for the tests with and without HWS was indicative that strain sweep can be a good tool for the prediction of the texture perception of semisolid foods. Additionally, HWS improved the strength of the correlations found for G*, which implies that HWS may not be needed for correlation of strain sweep parameters with sensory texture attributes. The significant correlations of HWS and its interaction with formulation for G* (Chapter 3) provide support for the increased correlations with G*.

4.4.5 Correlations among acid milk gel tribological and sensory properties

Partial Least Square (PLS) analysis was used to determine correlations between the sensory and tribological results from 24 formulations of acid milk gels with and without addition of HWS at 25°C (Table 4.9.). Graininess, grittiness in mouth, and chalkiness afterfeel were positively correlated to friction coefficients (μ) at 10 mm/s and15 mm/s. These attributes have been shown to be perceived in the boundary regime (Cassin et al., 2001, Dresselhuis et al., 2008), which is in agreement with the friction profiles observed for these samples (Chapter 3). A negative correlation of smoothness to frictional coefficient of acid milk gels was observed at sliding speeds of 20, 25, and 30 mm/s, which was not surprising: higher friction would result in a less smooth feeling in the mouth. Additionally, this result points to development of an interfacial film at these speeds. As the friction coefficients decreased with increased sliding speed, increased perception of attributes such as smoothness, mouth viscosity, mouthcoating, melting, and sliminess may occur. These attributes are related to "smooth mouthfeel," (De Wijk et al., 2006b). The correlation strength of chalkiness afterfeel, grittiness-in-mouth and graininess to friction coefficient increased with increasing sliding speed, potentially because the more rapid movement would make any particulates more noticeable.

Table 4.9. Correlations between sensory results and friction coefficients of acid milk gels without HWS addition at 25°C (n=24). [1]

Sensory attribute	μ at 10 mm/s	μ at 15 mm/s	μ at 20 mm/s	μ at 25 mm/s	μ at 30 mm/s
Graininess	0.428*	0.529**	0.570**	0.560**	0.590**
Mouthcoating					-0.438*
Mouth viscosity					-0.419*
Lumpiness in mouth					0.404*
Smoothness			-0.420*	-0.446*	-0.491*
Melting					0.402*
Grittiness in mouth	0.454*	0.540**	0.597**	0.618**	0.655***
Astringency				0.434*	0.493*
Chalkiness afterfeel	0.428*	0.524**	0.590**	0.611**	0.642***
Sliminess					-0.436*

**p*-value ≤ 0.05; ***p*-value ≤ 0.01; ****p*-value ≤ 0.001;
[1]μ: friction coefficient

Friction behavior was also positively correlated with astringency at speeds of 25 and 30 mm/s. The lumpiness-in-mouth was positively correlated only at the sliding speed of 30 mm/s. The correlations number of correlations found for these data indicated that a sliding speed range of 10-30 mm/s was appropriate for evaluating friction-related textural attributes with instrumental measurements.

Correlating tribological results with added HWS with sensory attributes showed significant differences (Table 4.10) compared to when salvia was not used in tribometry. No correlations were found for friction coefficients at sliding speeds of 10 mm/s. Friction coefficients at 25 and 30 mm/s were correlated to most sensory attributes.

Table 4.10. Correlations between sensory results and friction coefficients of acid milk gels with HWS addition at 25°C (n=24). [1]

Sensory attribute	μ at 15 mm/s	μ at 20 mm/s	μ at 25 mm/s	μ at 30 mm/s
Spoon viscosity			-0.418*	-0.509**
Graininess		0.407*	0.481*	0.542**
Mouthcoating			-0.439*	0.541**
Mouth viscosity			-0.413*	-0.518**
Firmness			-0.434*	-0.526**
Lumpiness in mouth				0.432*
Smoothness			-0.460*	-0.569**
Melting				0.444*
Grittiness in mouth	0.497*	0.555**	0.631**	0.709***
Astringency			0.443*	0.570**
Chalkiness afterfeel	0.518**	0.588**	0.656**	0.730***
Sliminess				-0.504*

**p*-value ≤ 0.05; ***p*-value ≤ 0.01; ****p*-value ≤ 0.001;
[1]μ: friction coefficient

The correlation for graininess was not shown at 10 and 15 mm/s for friction coefficients for samples with added HWS, whereas this attribute was correlated with friction coefficients at all selected sliding speeds when samples were tested without HWS. These results suggested that HWS resulted in a decrease of granules or food particles that would increase friction coefficients at those speeds. This may have been due to the lubrication effect of salivary proteins, mainly proline-rich mucins (Chen and Stokes, 2012).

Mouthcoating and mouth viscosity were correlated for friction coefficients at 25 mm/s and 30 mm/s for samples tested with HWS (Table 4.10), showing that these attributes can be perceived at lower sliding speeds when mixed with HWS. Spoon viscosity and lumpiness in mouth were not correlated with tribological data for samples tested without HWS but were for samples tested with HWS. Smoothness correlations occurred at a lower sliding speeds when samples were tested without HWS. The appearance of correlations between friction coefficients and low-melting, grittiness in mouth, astringency, chalkiness afterfeel, and sliminess were not affected by addition of HWS during tribological analysis. However, the correlation coefficients were higher for friction coefficients for samples tested with added HWS.

4.4.6 Correlations among acid milk gel tribological and rheological properties

The only correlations found between friction coefficients and viscosity parameters η_o (zero-shear viscosity, Pa s), n (flow index, Pa s), and c (time constant, s) were between 1) η_o at 8°C and friction coefficient at a sliding speed of 30 mm/s when HWS was not added to the samples, 2) η_o at 8°C and friction coefficient at a sliding speed of 30 mm/s when HWS was added to the samples and 3) n at 8°C and friction coefficient at sliding speeds of 25 and 30 mm/s when HWS was added to the samples (results not shown in tables). These results indicated that viscosity and friction were not strongly related for these samples. Additionally, incorporation of HWS during instrumental testing may improve viscosity and friction coefficient correlations.

There was no correlation between friction coefficient at 10-30 mm/s and tan δ (radians) at 8°C or 25°C with or without addition of HWS, so these correlation coefficients are not presented. γ_c showed significant negative correlations with friction coefficient at sliding speeds between 15-30 mm/s for samples without addition of HWS (Table 4.11), and at all sliding speeds for samples with added HWS for both strain sweeps and tribometry (Table 4.12). These negative correlations implied that increased acid milk gel resistance to permanent deformation resulted in decreased friction coefficients, and that HWS caused this effect at lower sliding speeds. The number of correlations between friction coefficients and G* decreased when HWS was added to during measurement of G* values. Correlations between friction coefficients and G* were positive, indicating that increased friction coefficients were related increased structural stiffness.

Table 4.11. Correlations between viscoelastic results and friction coefficients of acid milk gels with no HWS added (n=24). [1]

Attribute	μ at 10 mm/s at 25°C	μ at 15 mm/s at 25°C	μ at 20 mm/s at 25°C	μ at 25 mm/s at 25°C	μ at 30 mm/s at 25°C
γc (%) at 8°C		-0.423*	-0.427*	-0.442*	-0.422*
γc (%) at 25°C		-0.480*	-0.489*	-0.503*	-0.486*
G* (Pa) at 8°C	0.493*			0.437*	0.475*
G* (Pa) at 25°C	0.516**		0.411*	0.440*	0.470*

**p*-value ≤ 0.05; ***p*-value ≤ 0.01; ****p*-value ≤ 0.001;
[1]μ: friction coefficient; γ_c: critical strain; G*: complex modulus

Table 4.12. Correlations between viscoelastic results and friction coefficients of acid milk gels with addition of HWS (n=24). [1]

Attribute	μ at 10 mm/s at 25°C	μ at 15 mm/s at 25°C	μ at 20 mm/s at 25°C	μ at 25 mm/s at 25°C	μ at 30 mm/s at 25°C
γc (%) at 8°C	-0.429*	-0.430*	-0.471*	-0.483*	-0.429*
γc (%) at 25°C	-0.466*	-0.476*	-0.510*	-0.522*	-0.466*
G* (Pa) at 8°C				0.442*	
G* (Pa) at 25°C			0.407*	0.489*	

**p*-value ≤ 0.05; ***p*-value ≤ 0.01; ****p*-value ≤ 0.001;
[1]μ: friction coefficient; γ_c: critical strain; G*: complex modulus;

Based on the correlation results, γ_c showed the best correlation to friction coefficients measured at sliding speeds of 10-30 mm/s. Overall, viscoelastic parameters better correlated to tribological results compared to viscosity parameters, and correlations differed based on the sliding speeds and HWS application.

4.5 Conclusions

The presence of CMC in acid milk gel formulations produced samples with positive texture attributes based on descriptive sensory analysis results. Both rheological and tribological results showed significant correlations with sensory results. Correlating sensory evaluation results with the parameters from viscosity models showed a strong correlation between flow index and zero viscosity. Friction coefficients at 25 and 30 mm/s had the most correlations with sensory attributes. The correlations were mostly with attributes e.g. graininess, chalkiness afterfeel, astringency, smoothness, and mouth viscosity at 5 different sliding speeds (10-30 mm/s). The correlations of mechanical, frictional and sensory properties showed a meaningful relationship among these behaviors in texture perception of acid milk gels. Addition of HWS increased the number of correlations found among these parameters.

These results showed that saliva application during instrumental testing (rheometry and tribometry) can result in better prediction of semisolid food texture perception from instrumental data. In general, the results of this study can help in designing new reduced or non-fat semisolid products with desirable texture properties.

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CHAPTER 5: USING HUMAN WHOLE SALIVA TO BETTER UNDERSTAND HOW YOGURT RHEOLOGICAL AND TRIBOLOGICAL BEHAVIORS INFLUENCE THEIR SENSORY TEXTURE

5.1 Abstract

Saliva plays a critical role in texture perception of semisolid foods. Therefore, human whole saliva (HWS) application during rheometry and tribometry may help determining how texture attributes are perceived during oral processing. The formulation of these products can significantly impact their textural properties as well as their extent of breakdown after incorporation with HWS. Hydrocolloids are used in reduced or non-fat semisolid foods as texture enhancers. One popular reduced-fat semisolid food is yogurt which is considered as a healthy food due to its probiotic bacteria. Understanding the effect of HWS and hydrocolloids on texture perception of yogurts can help manufacturers to design reduced or non-fat products with similar texture to their full-fat counterparts. Thus, the objective of this study was to determine the effects of HWS on yogurt structure, rheological, tribological, and texture relationships. Twelve formulations of yogurts were prepared using hydrocolloids (carboxymethyl cellulose, locust bean gum, potato starch, corn starch, whey protein isolate, and skim milk powder), skim milk, and cream. Viscosity, viscoelastic behaviors, and confocal microscopy evaluations were performed with and without HWS. Descriptive sensory analysis was also performed to evaluate yogurt textural attributes. Overall, microstructural images showed that hydrocolloids and HWS addition resulted in a denser protein network with thicker chains and fat coalescence for the formulations with milkfat compared to the control.

Viscosity flow curves were fit to four shear thinning model; Cross-Williams (R^2>0.998), Cross (R^2>0.961), Herschel Bulkley (R^2>0.74), and power law (R^2>0.985). In general, yogurt viscosity, viscoelastic, and Stribeck curve profiles were significantly affected by applying hydrocolloids and HWS. Additionally, yogurt formulation significantly impacted sensory textural attributes. Texture attributes were significantly correlated to both rheological and tribological behaviors, and these correlations were affected by HWS application during instrumental testing. The results of this study not only showed that rheology and tribology can be useful for indicating sensory texture but also showed that addition of HWS during instrumental testing provided a better approximation of how semisolid food texture is perceived during oral processing. This information can be used in optimizing fat-free or reduced fat semisolid products when hydrocolloids are used as fat enhancers.

Key words: Yogurt, rheology, tribology, sensory, saliva, hydrocolloids, texture perception

5.2 Introduction

Yogurt, a popular semisolid food in many countries including the USA, is produced by fermentation of milk using lactic acid bacteria, *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. bulgaricus*. The demand for reduced or non-fat yogurts has been increased during recent years due to health concerns. However, reduction or removal of fat from yogurts can compromise their texture attributes, since fat play a major role in creating a smooth, creamy texture in dairy products (De Wijk et al., 2006b, Chojnicka-Paszun et al., 2012). Application of hydrocolloids has been an effective solution to improve the textural properties of reduced-fat yogurts. There are a wide range of hydrocolloids used in dairy products as fat replacers (Ognean et al., 2006, Peng and Yao, 2017), including carboxymethyl cellulose (CMC), locust bean gum (LBG), and starch (Cho and Prosky, 1999, Peng and Yao, 2017). CMC is an anionic hydrocolloid that is used widely in dairy products as a fat replacer to enhance their textures (Cho and Prosky, 1999). This gum is not only an effective stabilizer in dairy systems but also a dietary fiber with health benefits such as reduction of blood cholesterol and improvement of digestion and absorption (Cho and Prosky, 1999). LBG is a galactomannan with a 1:4 ratio of galactose:mannose, and its mannan part has been made soluble by side chains of single galactoses. LBG is a neutral (non-ionic) hydrocolloid that is stable at a pH range of 3.5 to 11 (Cho and Prosky, 1999). Corn starch and potato starch with modified structures are usually used as fat mimetics in dairy products (Cho and Prosky, 1999, Peng and Yao, 2017).

When evaluating the use of hydrocolloids in dairy products, rheometry and tribometry are typically applied in conjunction with sensory analysis to evaluate the impact of the hydrocolloids on food texture attributes. (Janssen et al., 2007, Sonne et al., 2014, Morell et al., 2016). However, the type of hydrocolloid selected as a fat replacer in dairy products may affect their texture not only through hydrocolloid functional properties, but also through hydrocolloid–saliva interactions, which may differ from lipid–saliva interactions. Thus, human whole saliva (HWS) has been incorporated during rheological and tribological measurements because of its important role in food texture perception (Guinard et al., 1997). During the initial stages of oral processing, rheological properties are the dominant influence on oral behaviors because they are related to the deformation and change in particle size of foods due to the mastication. After the food is mixed with HWS, broken into small pieces, and formed into a bolus (a mix of food and HWS), food tribological behaviors become more important than rheological behaviors. The importance of food tribological behaviors continues with swallowing the food and sensing the remaining food residue on the tongue and palate (Stokes et al., 2013). Different textural attributes may be perceived during different stages of oral processing through sensory measurement. Correlating rheological, tribological, and sensory behaviors along with incorporation of HWS during instrumental evaluation of food products can open a better way for predicting semisolid food texture attributes for targeted design of nutrient-dense foods that have textures as close as possible to their full-fat counterparts. Thus, the objective of this study was to determine the effects of HWS on yogurt structure, function, and texture relationships.

5.3 Materials and Methods

5.3.1 Materials

Skim milk was purchased from a local supermarket (WinCo Foods, Moscow, ID., U.S.A.). Whey protein isolate (WPI) (Provon 190, 89.4% protein) was donated by Glanbia Nutritionals (Fitchburg, Wis., U.S.A.). Low heat skim milk powder (SMP) and heavy cream (Darigold, 40% fat) were provided by the WSU Creamery (Pullman, WA., U.S.A.). Corn starch (CS) and modified potato starch were donated by Ingredion (Bridgewater, N.J., U.S.A.). Locust bean gum (LBG) and carboxymethyl cellulose (CMC) (pre-hydrated Ticalose CMC 2500 powder) were donated by TIC Gums (TIC Gums, Inc., Belcamp, Md., U.S.A.). Glucono-delta-lactone (GDL) was donated by Jungbunzlauer (Jungbunzlauer, Inc., MA., U.S.A.). The protein assay kit (Quick Start Bradford) used for measuring the protein concentration of HWS was obtained from Bio-Rad laboratories (Bio-Rad laboratories, Inc. CA., U.S.A.). Teflon balls (6 mm) for tribometry were purchased from McMaster-Carr (Atlanta, Ga., U.S.A.). GluconoFluorescein Isothiocyanate (FITC) dye and cavity slides for confocal imaging were purchased from Sigma (Sigma-Aldrich, St. Louis, MO., U.S.A.), and Nile red dye was purchased from TCI America (Portland, OR., U.S.A.).

5.3.2 Yogurt preparation

Twelve yogurts were prepared using skim milk (89.15-97.2% w/w), SMP (0-2.8% w/w), cream (0-3.5% w/w), WPI (0-2.8% w/w), and hydrocolloids, including corn starch (0-1% w/w), potato starch (0-0.7% w/w), LBG (0-1.8% w/w), and CMC (0-1% w/w) (Table 5.1). These yogurts were selected from 24 previously-studied formulations of acid milk gels based on their significant differences in rheological and tribological properties (Chapters 3 and 4). Dry powders and cream were added to the skim milk at room temperature (22 ± 2°C). To disperse the powders, the mixture was stirred with a spatula for 3 min in a water bath (Precision, Thermo Fisher Scientific, Waltham, MA, U.S.A.) at 85°C. Samples were held at 85°C for 30 min to both ensure pasteurization and complete hydrocolloid dissolution. Samples were then homogenized at 5,000 rpm for 1 min using a stand homogenizer (Polytron, Kinematica AG, NY, U.S.A.). GDL (1.1%-1.55% w/w, see Table 5.1) was added to samples after cooling to 42.2°C on the benchtop. Samples were then incubated at 42.2°C for 4 hr to reach a pH of 4.55-4.6. The gel was broken with a metal laboratory spatula, then the samples were stored in a refrigerator at 4°C overnight. Yogurts were blended at 350 rpm for 10 s before testing. Each sample was made in duplicate, and samples were tested the day after preparation.

Table 5.1. Experimental design for yogurts

Formula number	SMP (w/w)	Sweet WPI (w/w)	LBG (w/w)	CMC (w/w)	Potato starch (w/w)	Corn starch (w/w)	Skim milk (w/w)	Cream (w/w)	Starter culture (w/w)
1	2.8	0	0	0	0	0	97.2	0	0.04
2	2.83	0	0	0	0	0	95.96	1.21	0.04
3	2.89	0	0	0	0	0	92.26	4.85	0.04
4	2.95	0	0	0	0	0	89.15	7.9	0.04
5	1.8	1	0	0	0	0	97.2	0	0.04
6	1.8	0	1	0	0	0	97.2	0	0.04
7	1.8	0	0	1	0	0	97.2	0	0.04
8	2.1	0	0	0	0.7	0	97.2	0	0.04
9	2.1	0	0	0	0	0.7	97.2	0	0.04
10	0	2.8	0	0	0	0	97.2	0	0.04
11	0	0	1.8	0	0	1	97.2	0	0.04
12	0.2	0.8	0.45	0.45	0.45	0.45	97.2	0	0.04

5.3.3 Proximate analyses

All proximate analyses were performed in duplicate. Protein contents were determined with a Leco FP-528 nitrogen analyzer (Leco Corp., St. Joseph, MI, USA) according to the manufacturer's instructions (Kjeldahl conversion factor = 6.38). Fat contents were determined only for samples with added cream using Mojonnier method 989.05 (AOAC, 1995). Moisture contents were determined with a DKN 400 oven (Yamato Scientific America, INC., Santa Clara, CA., U.S.A.), according to the method of the AOAC (1999). Ash contents were determined by using the method from AOAC (1995) based on dry basis weight. Carbohydrate contents were determined by difference.

5.3.4 HWS collection

HWS collection procedure were approved by the University of Idaho Institutional Review Board (protocol 17-196). HWS was collected from 5 healthy people (3 females and 2 males, ages 20-35) with normal saliva flow according to the method of (Bongaerts et al., 2007b). Panelists were asked to refrain from eating and drinking anything except water for 2 hr prior to collection. At the beginning of collection, they were required to rinse their mouth twice with deionized water and expectorate into a waste cup. They were then asked to chew on the bulb-shaped end of a disposable plastic pipette to stimulate saliva flow and expectorate into a 2-oz. cup. Fresh HWS was collected every two hr and used for both rheological and tribological testing within two hr of collection for the testing.

5.3.5 Rheometry

Yogurt rheological behaviors were measured with an Anton Paar MCR 302 rheometer (Anton Paar, Graz., Austria) using a 50 mm diameter parallel plate with a gap height of 1 mm. All tests were carried out at 25°C and 8°C with and without addition of HWS (Section, 5.3.4). Samples were equilibrated at the testing temperature for 60 s prior to the test, and all samples were evaluated in triplicate.

Shear rate sweeps (0.01 to 100 s^{-1}) were carried out to measure yogurt viscosity profiles. Oscillatory tests including strain sweeps (0.01-100%, 1 Hz) and frequency sweeps (0.1-100 rad/s and 0.75% strain) were performed to measure yogurt viscoelastic behaviors. Frequency sweeps were performed at 75% of the lowest critical strain to ensure samples remained in the linear viscoelastic region (LVR). Critical strain was calculated by determining the strain at which G* deviated by >1% for this study.

5.3.6 PDMS plate production

Polydimethylsiloxane (PDMS) plates were manufactured for tribometry using the method reported by (Bongaerts et al., 2007a). A curing agent and a base (Dow Corning Corporation, Midland, MI, U.S.A.) were used to prepare the plates. The mixture was poured into an aluminum mold (4 mm height, 60 mm diameter). Air bubbles were removed by a cabinet vacuum desiccator (Bel-Art Products, Wayne, N.J., U.S.A) under a pressure of -90 kPag. Vacuum was applied cyclically up to 10 times until all bubbles were removed. PDMS plates were cured in the mold at 55°C for 2 hr in a DKN 400 oven (Yamato Scientific America, INC., Santa Clara, CA., U.S.A.), then stored overnight at room temperature (22 ± 2°C) to complete curing. The plates were removed and stored at room temperature (22 ± 2°C) until used for testing.

5.3.7 Tribometry

Tribometry was performed using an Anton Paar MCR 302 (Anton Paar, Graz., Austria) with a three-ball (Teflon, 6 mm diameter) geometry on a 60-mm diameter PDMS plate. The materials of the plate and balls were selected to mimic the oral surfaces (tongue–palate) (Johnson et al., 1993, Prakash et al., 2013). A 1 N normal force used was used to mimic the in-mouth force during swallowing, which is between 0.01 and 10 N (Miller and Watkin, 1996). The PDMS plate was placed on top of the rheometer base plate and pressed firmly to adhere the two surfaces. A line was marked on both the PDMS plate and rheometer plate using a laboratory pen to provide a visual indicator that the PDMS plate did not move during testing. Friction coefficient was measured at sliding speeds of 0.01-1000 mm/s. Samples were tested at 25°C with and without addition of HWS. For samples tested with HWS, 0.5 mL of HWS was added to 3 g of sample and held at room temperature (22 ± 2°C) for 5 min for complete digestion (Joyner (Melito) et al., 2014). At least three replicates for each sample duplicate were performed with and without HWS. The PDMS plate was cleaned after each run with 70% ethanol and laboratory wipes for non-fat samples; 70% ethyl ether was used for the samples with fat to prevent fat film build-up on the surface of PDMS plates and balls and then rinsed with 70% ethanol. Plates and balls were changed after every 6 runs to prevent the wear effect to impact the results.

5.3.8 Textural evaluation of yogurts

Sensory evaluation of yogurts was performed with the approval of the University of Idaho’s IRB (protocol 17-195). Panelists (n=10) were recruited from Washington State University and University of Idaho by email and social media. Participants (100% female; ages 25-55 yr, mean age of 34 yr) were trained for 11 hr before evaluating all samples in two sessions. Total training and evaluation of samples was completed over 2 mo.

Textural attributes (n=13) were introduced to the participants for describing the texture of the yogurts (Table 5.2); texture attributes and reference samples were selected from previous related studies (Saint-Eve et al., 2004, Pascua et al., 2013). During training, panelists profiled each yogurt individually using a 15-cm line scale to indicate the intensity of each attribute present in the samples. Hard copies of descriptions of the 13 attributes along with a 15-cm line scale for each attribute were provided for each training session.

Table 5.2. Texture attributes and reference products used for sensory evaluation of yogurts
(Saint-Eve et al., 2004, Pascua et al., 2013).

Attribute	Definition	Reference (scale 0 to 15)
	Visual terms	
Lumpiness	The presence of lumps observed in yogurts after being stirred	Yoplait vanilla yogurt=1 Jell-O tapioca pudding=15
Spoon viscosity	The thickness of food after being stirred back and forth for 10 times	Water=1 Jell-O pudding=10.5
	Mouthfeel terms	
Grainy	The feeling of food granules (small particles) on tongue after expectorating	Reddi wip whipped cream=1 Baby rice cereal (Gerber)=12
Mouthcoating	The force required to clear sample adhered to the mouth/with the tongue during eating	Cream cheese=10 Reddi wip whipped cream =1
Mouth Viscosity	The force needed to draw food from a spoon over the tongue	Water=1 Chocolate Jell-O pudding=12
Firmness	The firmness of food in the mouth when food is compressed up and down via tongue motions	Reddi wip whipped cream =1 Cream cheese (Philadelphia)=14
Lumpiness in-mouth	The feeling of lumps in the mouth during eating	Yoplait yogurt=1 Jell-O tapioca pudding=15
Smooth	The lack of individual food particles, opposite of grainy and lumpy attributes	Yoplait yogurt=13 Baby rice cereal (Gerber)=15
Melting	Food spreads out in the mouth at different rates.	Reddi wip whipped cream =1 Jell-O pudding=1
Grittiness in-mouth	The feeling of gritty/chalky particles in the oral cavity during eating	Walmart non-fat Greek yogurt =10 Reddi wip whipped cream =1
	After-feel mouth terms	
Astringent	The astringent/dry sensation in the mouth after food is swallowed or expectorated	Atkins strawberry protein drink=10 Reddi wip whipped cream =1
Chalky/Gritty after-feel	The feeling of chalk-like particles in the mouth after food is swallowed or expectorated	Walmart non-fat Greek yogurt =10 Reddi wip whipped cream =1
Slimy	Difficulty to clear the mouth from food in the mouth after food is swallowed or expectorated associated with gruel	Banana baby food Gerber=7 Reddi wip whipped cream=1

Panelists were allowed to practice with the sensory data collection software (Compusense Cloud, Guelph, Ontario, Canada) for the last 2 training sessions to familiarize themselves with the software for the formal evaluations.

Formal sensory evaluation of the twelve yogurt samples was performed in duplicate in separated sensory booths under white light. Samples were coded with 3-digit numbers and evaluated at 8°C within 48 hr of preparation. Six samples were evaluated in duplicate per session. 4 oz. plastic soufflé cups were used for serving the samples. Panelists were asked to rinse their mouth with filtered water, expectorate the samples after each evaluation, and clean their palate with unsalted crackers after evaluation of each sample to prevent fatigue. After evaluation of six samples, a 5 min break was required to minimize fatigue and errors. Attribute intensity was marked using a 15-cm line scale with anchors at 1.5 cm for low intensity and 13.5 cm for high intensity. Attribute data were collected from Compusense software for further analysis.

5.3.9 Confocal imaging

Confocal laser scanning microscopy (CLSM) was used to image yogurt microstructures. GluconoFluorescein isothiocyanate (FITC) and Nile red dyes were applied to stain yogurt proteins and fat globules, respectively. 8 mg of FITC was added to 500 μL of ethanol in a 1 mL vial and vortexed for 10 s. 500 μL of deionized water was then applied to the FITC solution and vortexed for another 10 s. Nile red solution was prepared similarly, except 5 mg of Nile red was used. FITC and Nile red were used for samples with fat, but only FITC was used for non-fat samples. Concentrations were adjusted for 120 g yogurt samples.

Dyes were added to the yogurt mix before incubation. Samples were incubated, stirred, and stored as described in Section 5.3.2; microscopy analysis was done the next day. For testing, 500 µL of each sample was transferred to a cavity slide and covered with a glass coverslip. Samples were imaged at 20X and 4-8°C. The wavelengths of Nile red and FITC were excited at 488 nm and 559 nm, respectively.

5.3.10 Data analyses

Statistical analyses were performed using SAS version 9.1 (SAS, Cary, NC) and XLSTAT (version 16.11; Addinsoft, Boston, U.S.A.). Rheology and tribological graphs were plotted with Origin 8 software (OriginLab, Northampton, MA, USA). Error bars on graphs represent standard deviations of duplicate samples (6 data points total). Viscosity profiles were fitted to four models: Cross-Williams (Equation 1), Cross (Equation 2), Herschel Bulkley (Equation 3), and power law (Equation 4) using TRIOS software version 4.4.0 (TA Instruments; New Castle, Delaware, USA).

$$\eta = \frac{\eta o}{[1+(c\gamma\cdot)^{1-n}]} \qquad (1)$$

$$\eta = \eta\infty + \frac{\eta o - \eta\infty}{1+(k\gamma\cdot)^{n}} k\gamma\,^{\cdot n-1} \qquad (2)$$

$$\sigma = \sigma_o + k\dot{\gamma}^{n} \quad (3)$$

$$\sigma = k\dot{\gamma}^{n-1} \qquad (4)$$

In Equation 1, η_o is the zero-shear rate viscosity (Pa s), *c* is the time constant (s), and *n* is the flow behavior index (unitless). In Equation 2 η_o is the zero-shear rate viscosity (Pa s), η_∞ is infinite viscosity (Pa s), *c* is the time constant (s), and *n* is flow behavior index (unitless). In Equation 3, σ_0 is the yield stress (Pa), *k* is the consistency coefficient (Pa s^{1-n}) and *n* is the flow behavior index (unitless). The power law equation is shown in equation 4 and is also a general model for materials that are weak gels with shear-dependent behavior. In Equation 4, *k* is the consistency coefficient (Pa s^{1-n}) and *n* (unitless) is the flow behavior index.

Friction coefficients between 10–100 mm/s sliding speeds were selected for correlation analysis to mimic oral sliding speed (Malone et al., 2003). These values were used for correlation analysis between tribological-sensory and tribological-rheological results.

ANOVA was used to determine significant differences in sensory results considering three main variables (panelists, replicates, and samples), as well as significant differences among yogurt proximate analysis results and rheological and tribological parameters. Tukey's HSD (Honest Significant Difference) test was used for mean separations. Principle Component Analysis (PCA) was used to determine drivers behind variation of yogurt sensory attributes. Partial Least Square (PLS) analysis was performed to correlate rheological–tribological, rheological–sensory and tribological–sensory results.

5.4 Results and Discussion

Significant differences were observed for yogurt protein contents (Table 5.2**)**. Differences in protein content were attributed to the reduction of SMP for adjustment of other ingredients in the formulation. Sample 11 (added LBG and CS) had the lowest amount of protein, which was expected since no SMP powder was added to this formulation. Sample 10 (high WPI level used) had the highest protein concentration due to the use of WPI as the only hydrocolloid.

Table 5.3. Yogurt proximate compositions

Formula number	Protein (%)	Moisture (%)	Fat (%)	Ash (%)	Carbohydrate (%)[2]
1	5.61 ± 0.075[bc1]	85.12 ± 0.092[abc]	0	0.78 ± 0.028[a]	8.8[abc]
2	5.36 ± 0.249[dc]	83.54 ± 0.922[c]	0.50 ± 0.022[a]	0.65 ± 0.006[a]	9.9[ab]
3	4.64 ± 0.129[efg]	86.00 ± 0.406[a]	1.98 ± 0.008[b]	0.70 ± 0.048[a]	7.26[de]
4	4.62 ± 0.006[fg]	85.01 ± 0.496[abc]	3.52 ± 0.142[c]	0.62 ± 0.008[a]	6.99[e]
5	6.07 ± 0.094[b]	85.86 ± 0.132[abc]	0	0.79 ± 0.017[a]	7.90[dc]
6	4.65 ± 0.015[efg]	86.61 ± 0.207[a]	0	0.69 ± 0.003[a]	8.77[bc]
7	4.56 ± 0.075[fg]	85.21 ± 0.084[abc]	0	0.74 ± 0.09[a]	9.73[ab]
8	5.04 ± 0.053[de]	85.68 ± 0.394[ab]	0	0.70 ± 0.024[a]	9.1[abc]
9	4.82 ± 0.035[efg]	85.06 ± 0.612[bc]	0	0.76 ± 0.069[a]	9.45[a]
10	6.79 ± 0.153[a]	86.07 ± 0.363[abc]	0	0.73 ± 0.088[a]	7.06[de]
11	4.48 ± 0.034[g]	86.62 ± 0.061[a]	0	0.67 ± 0.082[a]	8.83[abc]
12	4.96 ± 0.024[def]	84.43 ± 0.162[bc]	0	0.65 ± 0.025[a]	10.03[a]

Different letters in a given column indicate significant differences among yogurts for different components at *p*-value ≤0.05.[1]
Carbohydrates were calculated by difference.[2]

There were significant differences in moisture content among the samples. Yogurts with higher amounts of hydrocolloids may have retained more water in their structure and reduce the availability of the water on the surface for more evaporation. The amount of carbohydrate increased by increasing CS, PS, CMC, and LBG, and decreased with addition of fat and protein, mainly WPI, which was expected. This effect was observed in samples 12 (all hydrocolloids added) and 9 (CS added), which had the highest carbohydrate content, and samples 4 (full-fat) and 10 (high WPI level used), which had the lowest carbohydrate content. There were no significant differences in the ash content of yogurts.

5.4.1 The impact of hydrocolloids and HWS on yogurt microstructures

Overall, CLSM results showed the conformation of protein network structure (green) was dependent on yogurt formulation, and the size of serum pores increased with addition of hydrocolloids (black space in Figures 5.1, 5.2, and 5.3). Pores size in samples with gums (samples 6, 7, and 12) were significantly bigger than those in the control sample (sample 1). network with more protein cross-linking and aggregation. LBG, used in sample 6, is a neutral hydrocolloid, so there would be minimal electrostatic interaction between LBG and caseins at the pH of casein (approximately the pH of all samples), causing a weaker network. However, LBG can increase viscosity by increasing the continuous phase viscosity, which would likely result in the smaller, more weakly aggregated protein network in these samples (Perrechil et al., 2009).

The higher amount of serum observed in the microscopy images for sample 6 (added LBG) was in line with its higher moisture content (Table 5.3). Also, the moisture content of the sample containing all hydrocolloids (sample 12) was significantly less than that of samples (added LBG) and 7 (added CMC), which may be related to differences in their pores sizes. The protein network in the sample with added CMC (sample 7) was shown to be thicker and more compact than that of the sample with added LBG (sample 6). This was attributed to the casein–CMC interactions due to their opposite charge, resulting in a stronger protein network. The effect of additional protein to the formulation was observed for the sample with added WPI (sample 5).

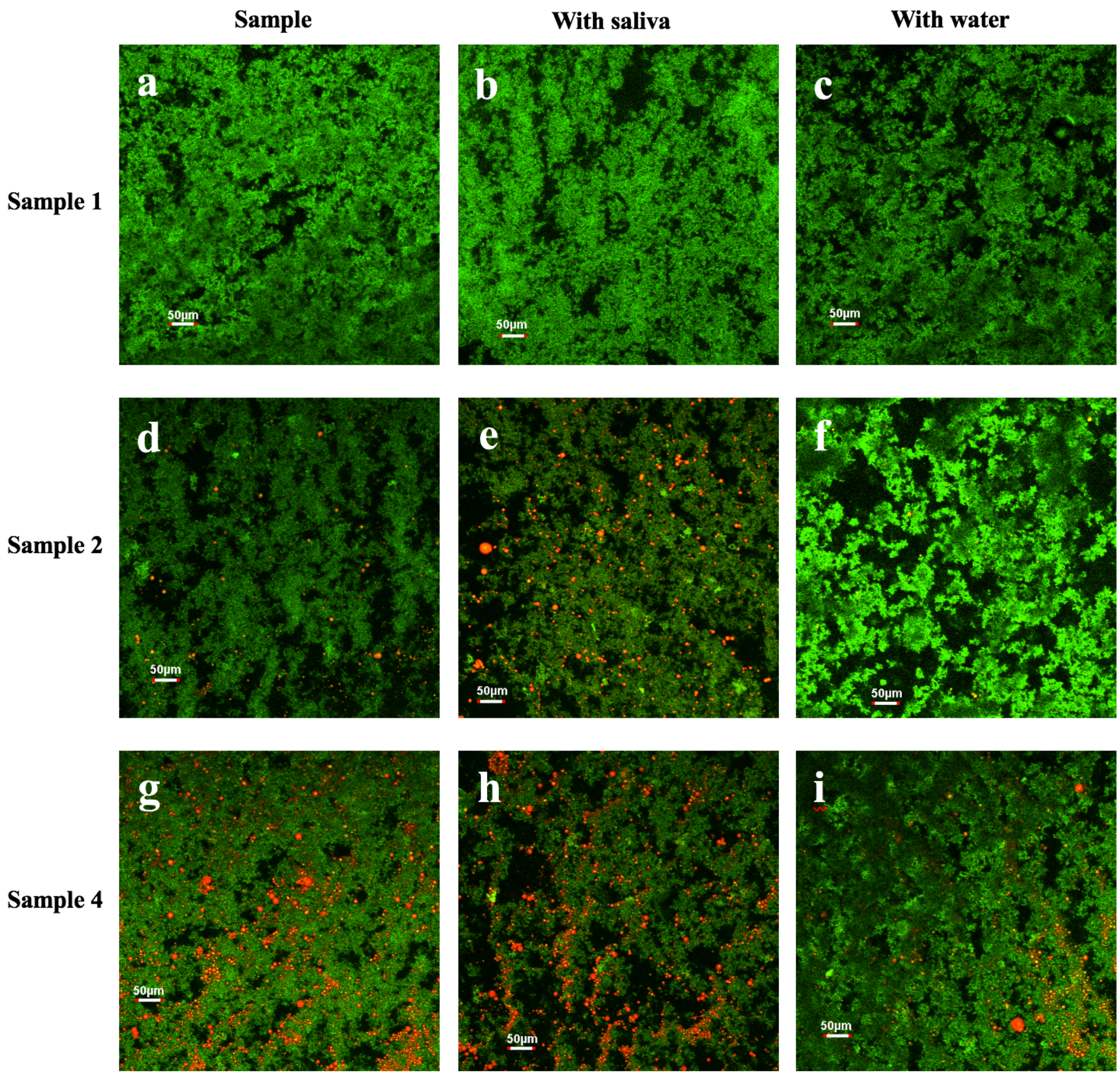

Figure 5.1. CLSM results of yogurts;
a) sample 1; b) sample 1 with HWS; c) sample 1 with water; d) sample 2; e) sample 2 with HWS; f) sample 2 with water; g) sample 4; h) sample 4 with HWS; i) sample 4 with water. The protein network, fat globules, and serum pores are shown in green, red, and black, respectively.

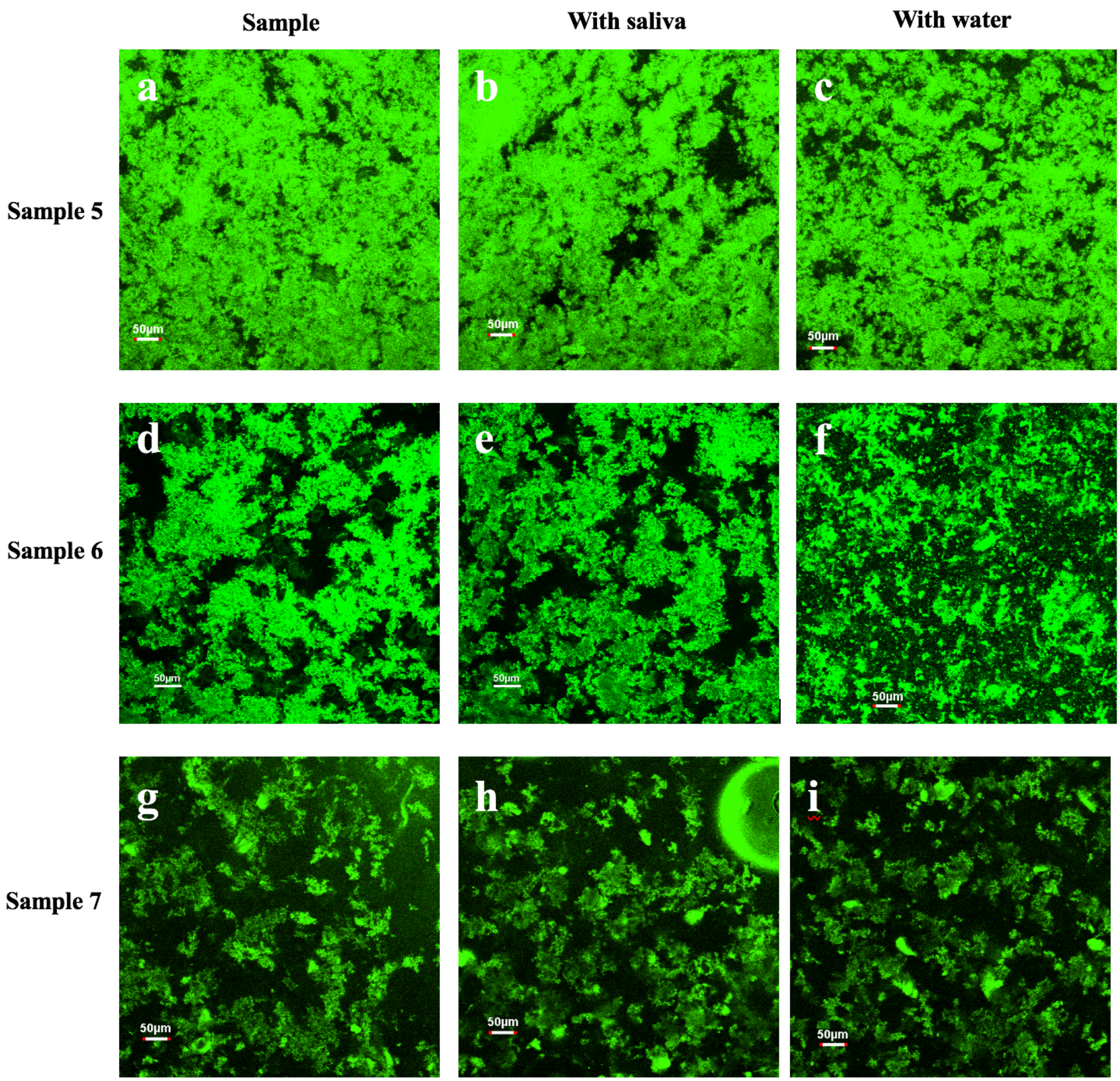

Figure 5.2. CLSM results of yogurts;
a) sample 5; b) sample 5 with HWS; c) sample 5 with water; d) sample 6; e) sample 6 with HWS; f) sample 6 with water; g) sample 7; h) sample 7 with HWS; i) sample 7 with water. The protein network and serum pores are shown in green and black, respectively.

The denser protein matrix of sample 5 compared to the control sample (sample 1) was attributed to higher protein content (Table 5.3). Addition of WPI can increase the level of casein-casein and casein-whey interactions and result in greater cross- linking and a denser conformation with more protein chains. Addition of fat in samples 2 and 4 impacted the protein structure compared to the control sample (sample 1). The interaction of fat and caseins can result in a more condensed protein matrix in yogurts produced by homogenized milk (Serra et al., 2007).

In general, addition of HWS caused greater protein aggregation regardless of formulation. This effect was most clearly illustrated for the control sample (sample 1), and samples with added PS (sample 8), and CS (sample 9) when HWS was added. The greater effect on the starch-containing samples was likely because amylase breaks down amylose in starch to smaller sugars (Janssen et al., 2007). This would cause disruption of starch embedded in the casein network and result in a larger serum phase. However, the effect of HWS was not notable on the structure of the samples with gums (samples 6, 7, and 12). On the other hand, HWS caused fat coalescence in samples 2 and 4. This observation was attributed to depletion flocculation created by the osmotic pressure from salivary proteins (Chen, 2015).

Overall, addition of HWS versus water affected the protein network differently in terms of aggregation and conformation. Addition of water in the sample notably increased the porosity in all yogurts due to the dilution effect of water and its integration into the serum pores. The dilution effect of water was shown most clearly for the sample containing LBG (sample 6).

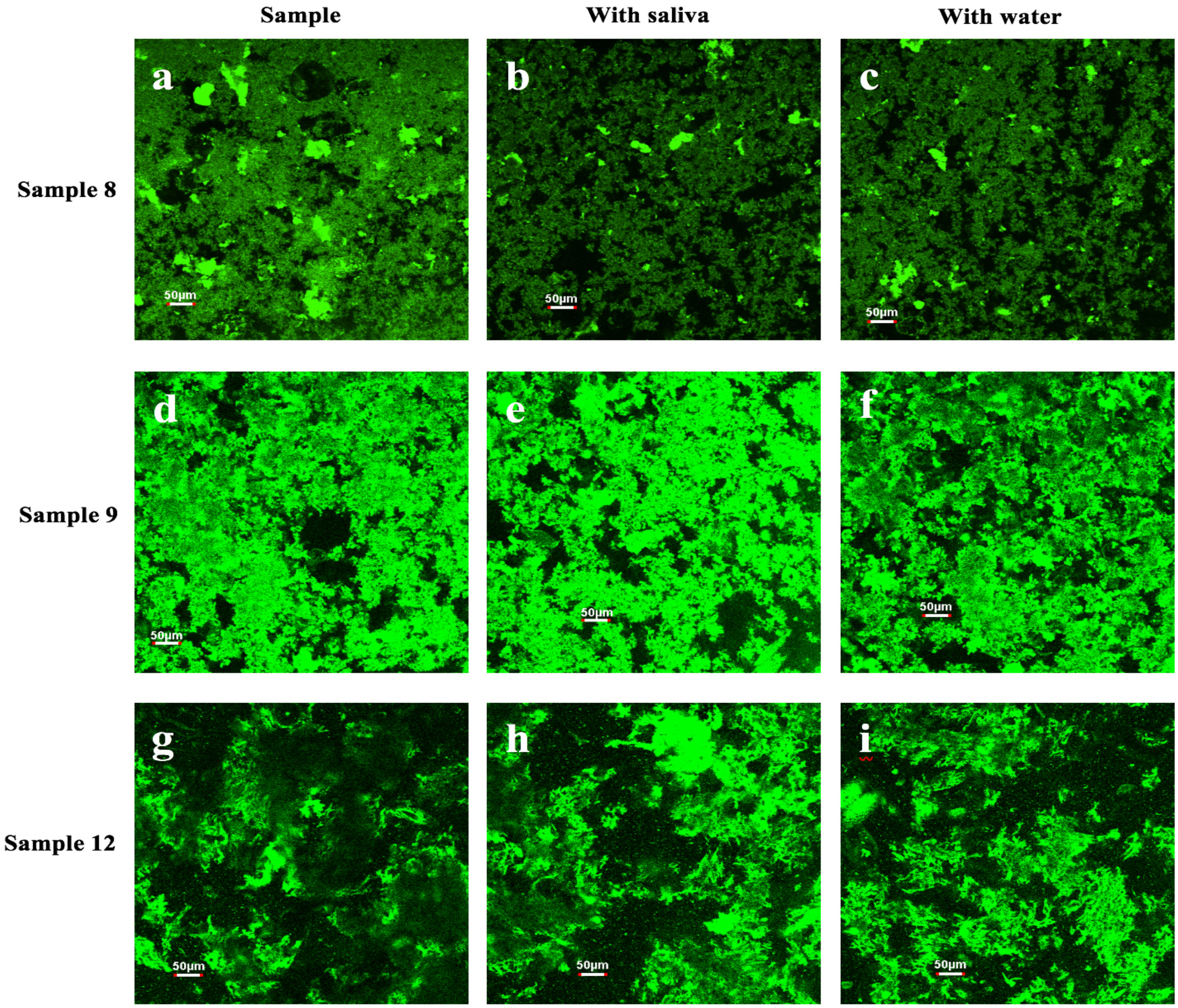

Figure 5.3. CLSM results of yogurts;
a) sample 8; b) sample 8 with HWS; c) sample 8 with water; d) sample 9; e) sample 9 with HWS; f) sample 9 with water; g) sample 12; h) sample 12 with HWS; i) sample 12 with water. The protein network and serum pores are shown in green and black, respectively.

This results was attributed to the weaker structure of LBG in the continuous phase due to disrupted weak interactions, e.g. hydrogen and non-covalent bonds, upon addition of water

(Murray and Phisarnchananan, 2014) compared to the samples with stronger interactions, such as covalent bonds and hydrophobic interactions with casein micelles.

5.4.2 The impact of hydrocolloids and HWS on yogurt rheological behaviors

5.4.2.1 Viscosity profiles

Overall, all yogurts showed shear thinning behavior (Table 5.4, 5.5, 5.6, and 5.7). Shear thinning behavior in a yogurt system is typically due to alignment of entangled protein molecules with the shear field. The viscosity curves of samples were fit to Cross- Williams, (R^2>0.935) Cross (R^2>0.748), Herschel Bulkley (R^2>0.74), and power law (R^2>0.985).

Table 5.4. Viscosity profiles for yogurts (n=12) at 8°C without added HWS

Formula	Model	ηo (Pa s)	η∞ (Pa s)	n	c (s)	k (Pa s1-n)	σy (Pa)	R2
1	Herschel-Bulkley	N/A	N/A	0.955	N/A	0.177	14.9	0.740
2	Cross	2190	0.212	0.935	31.2	N/A	N/A	0.891
3	Cross	693	0.222	0.930	19.8	N/A	N/A	0.895
4	Cross	627	0.214	0.926	19.7	N/A	N/A	0.919
5	Cross	532	0.020	0.966	65.5	N/A	N/A	0.820
6	Power law	56.1	n/A	0.286	N/A	N/A	N/A	0.985
7	Cross-Williamson	1358	N/A	0.928	13.0	N/A	N/A	0.935
8	Herschel-Bulkley	N/A	N/A	0.902	N/A	0.157	26.0	0.806
9	Herschel-Bulkley	N/A	N/A	0.772	N/A	0.682	19.5	0.856
10	Cross	13476	0.02	0.971	19.5	N/A	N/A	0.748
11	Cross-Williamson	370	N/A	0.697	8.80	N/A	N/A	0.999
12	Cross-Williamson	416	n/a	0.834	10.33	N/A	N/A	1.000

Table 5.5. Viscosity profiles for yogurts (n=12) at 8°C with added HWS

Formula	Model	η_o (Pa s)	η_∞ (Pa s)	n	c (s)	k (Pa s^{1-n})	σ_y (Pa)	R^2
1	Cross-Williamson	254	N/A	0.891512	64	N/A	N/A	0.999
2	Cross	637	0.101	0.914	25.5	N/A	N/A	0.938
3	Cross	418	0.122	0.908	28.2	N/A	N/A	0.965
4	Cross	531	0.174	0.911	37.8	N/A	N/A	0.922
5	Cross	461	0.010	0.949	52.1	N/A	N/A	0.917
6	Power law	29.3	n/A	0.324	N/A	N/A	N/A	0.992
7	Cross-Williamson	232	N/A	0.805	11.3	N/A	N/A	1.000
8	Herschel-Bulkley	N/A	N/A	0.746	N/A	0.176	6.61	0.975
9	Herschel-Bulkley	N/A	N/A	0.387	N/A	3.05	5.71	0.962
10	Cross	3976	0.09	0.961	34.5	N/A	N/A	0.961
11	Cross-Williamson	184	N/A	0.699	5.13	N/A	N/A	0.999
12	Cross-Williamson	141	n/a	0.755	11.3	N/A	N/A	1.000

Most samples showed good fit to the Cross model. The Cross model is suitable for shear-thinning materials, and its parameters can be related to food texture attributes.

Table 5.6. Viscosity profiles for yogurts (n=12) at 25°C without added HWS

Formula	Model	η_o (Pa s)	η_∞ (Pa s)	n	c (s)	k (Pa s^{1-n})	σ_y (Pa)	R^2
1	Herschel-Bulkley	N/A	N/A	0.856	N/A	0.199	7.39	0.871
2	Cross	1177	0.054	0.928	19.9	N/A	N/A	0.881
3	Cross	364	0.109	0.920	22.6	N/A	N/A	0.924
4	Cross	344	0.086	11.6	0.916	N/A	N/A	0.948
5	Cross	471	0.013	0.957	55.8	N/A	N/A	0.952
6	Power law	29.5	n/A	0.358	n/A	N/A	N/A	0.993
7	Cross-Williamson	454	N/A	0.825	12.6	N/A	N/A	0.998
8	Herschel-Bulkley	N/A	N/A	0.776	n/a	0.289	15.3	0.924
9	Herschel-Bulkley	N/A	N/A	0.841	n/a	0.322	11.0	0.880
10	Cross	4712	0.01	0.957	79.691	N/A	N/A	0.970
11	Cross-Williamson	223	N/A	0.649	7.49	N/A	N/A	0.999
12	Cross-Williamson	219	n/a	0.776	10.6	N/A	N/A	1.000

The only difference between the Cross model and the Cross-Williams model is the presence of η_∞ (Pa s) in the Cross model; η_∞ is indicative of shear-independent flow behavior under high shear conditions. The difference between the power law and Herschel-Bulkley models

Table 5.7. Viscosity profiles for yogurts (n=12) at 25°C with added HWS

Formula	Model	η_o (Pa s)	η_∞ (Pa s)	n	*c* (s)	*k* (Pa s^{1-n})	σ_y (Pa)	R^2
1	Cross-Williamson	84.4	N/A	1	19.2	N/A	N/A	0.999
2	Cross	305	0.047	0.910	23.7	N/A	N/A	0.964
3	Cross	226	0.061	0.907	26.5	N/A	N/A	0.975
4	Cross	345	0.083	0.909	33.2	N/A	N/A	0.943
5	Cross-Williamson	214	n/a	0.946	33.1	N/A	N/A	0.900
6	Power law	15.5	N/A	0.391	N/A	N/A	N/A	0.996
7	Cross-Williamson	106	N/A	0.755	10.6	N/A	N/A	1.000
8	Herschel-Bulkley	N/A	N/A	0.601	n/a	0.272	4.89	0.954
9	Herschel-Bulkley	N/A	N/A	0.532	n/a	0.830	3.69	0.961
10	Cross	648	0.01	0.908	11.5	N/A	N/A	0.760
11	Cross-Williamson	106	N/A	0.624	6.96	N/A	N/A	0.999
12	Cross-Williamson	79.9	n/a	0.693	13.7	N/A	N/A	1.000

is the yield stress in Herschel-Bulkley materials. However, they both show shear-dependent viscosity (Steffe, 1996).

Three-way ANOVA was used to determine the effects of formulation (hydrocolloids), HWS, and temperature on the flow parameters from non-Newtonian models of yogurts including η_o, n, and c (Table 5.8.). For η_o, formulation, HWS, and temperature showed significant effects at $p \leq 0.001$ and $p \leq 0.01$ for interaction of formulations with each of the other two parameters. Interaction of HWS with temperature for η_o was not significant. Surprisingly, no significant effects were observed on n or k for any combination of parameters. These results might have been caused by the dominant role of η_o in the non-Newtonian viscosity models compared to n and k. The significant impact of hydrocolloids can be mainly explained by the electrostatic interactions between counterions of anionic hydrocolloids with casein micelles, swelling starch granules in the presence of water and heat in the yogurt system, dispersion of large particles of neutral hydrocolloids in the continuous phase and their depletion flocculation effect in the system. These factors can also significantly change the structure of protein network and overall conformation of yogurts (Figure 5.1, 5.2, and 5.3). The significant effects from HWS can be explained by digestive, dissolving, and coalescence properties of HWS resulted mostly by enzymes, salivary proteins and electrolyte presented in the HWS. Temperature can weaken the intermolecular bonds in a semisolid food system, decrease resistance to flow and lower the viscosity (Berk, 2018). Based on these results, the importance of hydrocolloids and HWS on yogurt flow properties as a model system for the semisolid foods can be helpful in designing semisolid foods with better rheological properties related to texture quality.

Overall, η_o, η_∞, n, and σ_y decreased with increasing temperature and addition of HWS. Addition of hydrocolloids increased the viscosity, except for the sample with LBG (sample 6).

Table 5.8. Effect of main sources of variations on flow properties of yogurts (n=12) determined by F-values obtained from three-way ANOVA[1].

Source of variation	η_o	n	k
Formulations	16.4***	1.2	1.8
HWS	12.5***	1.3	0.4
Temperature	9.7***	1	1.2
HWS*Temperature	1.9	1	0.1
Formulation*HWS	6.1**	1	1.2
Formulation*Temperature	4.8**	1	0.8

[1] *, **, and *** indicate significant differences at p-value ≤ 0.05, p-value ≤ 0.01, and p-value ≤ 0.001, respectively.

This sample was also the only one fit to a power law model, which is mostly applicable to weak gels, although the protein structure was shown highly entangled (Figure 5.2) (Murray and Phisarnchananan, 2014) compared to the control sample (sample 1, Figure 5.1). LBG is a neutral hydrocolloid that increases the viscosity of the system by increasing the continuous phase, not through interactions with protein network (Hansen, 1993). Another mechanism for viscosity increase observed for neutral hydrocolloids is depletion flocculation. The large molecules of LBG created an osmotic pressure space between the casein micelles, which would push the caseins together and cause flocculation (Thaiudom and Goff, 2003). Samples with LBG (sample 6) and LBG and CS (sample 11) also showed the least decrease in their viscosity upon addition of HWS, which was expected based on the confocal images (Figure 5.2). Similar results was shown with LBG solutions and HWS (Zinoviadou et al., 2008).

The viscosity of sample 11 slightly increased compared to the control due to inclusion of LBG and CS, which was expected because addition of CS and LBG together in a system can make a stronger gel than when LBG is used individually (Murray and Phisarnchananan, 2014). The viscosity of sample 7 increased notably from the control due to addition of CMC. CMC is an anionic gum that interacts with positively charged casein micelles through aggregative phase separation to create a strong matrix (van de Velde et al., 2015). The yield stress of sample 8, which contained PS, was higher than that of sample 9, which contained CS. PS can increase viscosity due to the large size of it swollen starch granules in the dispersed phase. CS is a neutral polysaccharide that can increase viscosity through weak interactions in the continuous phase (Dang et al., 2009). The network formed by CS and milk protein is not as strong as the PS–milk protein network; hence, the force (σ_y) that is needed for sample 8 to flow is greater than sample 9 due to the increased size of the starch granule.

Sample 10, which incorporated WPI, had the highest viscosity of all samples, likely because of its high protein content (Table 5.2.). Denaturation of whey proteins occurs due to heat treatment above 70°C. High concentration of denatured whey proteins results not only in increased interactions with casein micelles but also in interactions among non-associated whey proteins. These interactions yield a stronger protein gel network with more cross-linking and aggregate structure (Lucey and Singh, 1997). Sample 5, which also contained WPI but at a lower concentration, showed similar results, although its viscosity was lower than sample 10 due to its lower protein content (Table 5.2.).

Interestingly, the viscosity of sample 12 was not highly impacted by containing all hydrocolloids. This result was attributed to the conflicting contributions of hydrocolloids in this sample. Addition of fat notably increased viscosity (samples 2, 3, and 4), likely due to the embedded fat globules throughout the protein matrix creating a resistance to flow (Chojnicka-Paszun et al., 2012, Nguyen et al., 2017). Full-fat yogurt (sample 4) showed higher viscosity than samples 2 and 3, supporting this hypothesis.

Yogurt viscosity and *n* decreased with addition of HWS and increased temperature (Table 5.4., 5.6.). Samples evaluated with added HWS at 25°C showed the lowest viscosity (Table 5.4.), which was expected. Increasing temperature weakens the intermolecular bonds in a yogurt system, decreasing resistance to flow and lowering the viscosity (Berk, 2018). The effect of HWS varied based on type of hydrocolloids used in the formulations. Salivary proteins, enzymes, and other HWS components can disrupt semisolid food microstructures

(Janssen et al., 2007). For examples, HWS has been shown to cause protein flocculation when mixed with yogurt (Vingerhoeds et al., 2009) (Sarkar and Singh, 2012). These effects can be observed in the microstructural images (Figure 5.1, 5.2, and 5.3). Samples containing PS (sample 8) and CS (sample 9) showed the greatest decrease in yield stress upon addition of HWS. Similar results were reported for starch-based custards (Janssen et al., 2007). Amylase in the HWS would break down the amylose in the starches into simple sugars like maltose and glucose (Humphrey and Williamson, 2001). Although this effect was not clearly observed in confocal images of sample 8 in this study, the digestion of starch by amylase was visually shown by another study (Janssen et al., 2007).

5.4.2.2 Viscoelastic behaviors

The effects of formulation (hydrocolloids), HWS, and temperature on yogurt viscoelastic properties including critical strain (γ_c), G* (complex modulus), and tan δ, were determined using F-values from three-way ANOVA (Table 5.9.). Formulation and temperature showed significant effects at $p \leq 0.001$ for tan δ and γ_c.; HWS also showed significant effects on tan δ ($p < 0.001$) and γ_c ($p < 0.01$). Additionally, significance at $p < 0.01$ was observed for the interaction of formulation with temperature on tan δ and γ_c. HWS was the only parameter that had significant effect on G* ($p \leq 0.05$). However, temperature was borderline for significance ($p \leq 0.07$). This finding might explain the significant differences of G* values from Tukey's HSD with different temperatures (Table 5.10.). The significant changes in G* may have been due to the increased stability and resistance to the permanent deformation of yogurts when hydrocolloids were added to the samples compared to the control sample. Starches (PS and

CS) and gums (CMC and LBG) can improve gel stability by increasing the number of internal molecular interactions as well as stronger bonds through different mechanisms. On the other hand, HWS can disrupt the structure of semisolid foods through digestion, osmotic pressure, dilution or altering their net charges. Increasing temperature changes the thermodynamic condition of materials. Internal molecules can move more easily and faster with the heat energy and the strength of molecular bonds either electrostatic or neutral may decrease. As a result, the structure of acid milk gels can lose their original structure and become more susceptible to external shear when mechanical force is applied.

Overall, there were significant differences in γ_c (critical strain, %), G^* (complex modulus at γ_c, Pa), and tan δ (phase angle at γ_c, rad) among the 12 yogurt formulations (Table 5.10.). γ_c increased or remained constant by increasing temperature except sample 4 (full-fat yogurt).

Table 5.9. Effect of main sources of variations on viscoelastic properties of yogurts (n=12) determined by F-values obtained from three-way ANOVA[1].

Source of variation	γ_c	G*	Tan δ
Formulations	45***	2.5	418***
HWS	10.8**	4.6*	121***
Temperature	75.1***	4	72.4***
HWS*Temperature	1.5	1.8	0.4
Formulation*HWS	2.3	1.2	38.7***
Formulation*Temperature	5.1**	1.2	4.4**

[1] *, **, and *** indicate significant differences at p-value ≤ 0.05, p-value ≤ 0.01, and p-value ≤ 0.001, respectively.

These results were not expected but may have been due to increased thermal energy of the oil-in-water emulsion in sample 4 at increased temperature, which would decrease the viscosity of the fat globules, resulting in fat coalescence. The molecules can then deform more easily when shear stress is applied and result in a smaller γ_c. The increasing γ_c values for other samples can be explained by thermodynamics. At higher temperatures, molecular mobility increases, resulting in more fluid-like behavior and requiring a greater force to overcome the resistance for permanent deformation. γ_c values of fat-containing samples (samples 2, 3, and 4) increased with addition of HWS. This could be due to fat flocculation resulting from the osmotic pressure of salivary proteins throughout the sample (Huc et al., 2016) and resulting in more resistance to permanent deformation from applied strain. HWS had a different impact on critical strain values compared to that of temperature. γ_c decreased for most of samples with added hydrocolloids.

Table 5.10. Viscoelastic parameters of 12 yogurts measured by strain sweep at 1 Hz[1]

Formula number	γ_c (%) 8°C NS	γ_c (%) 8°C S	γ_c (%) 25°C NS	γ_c (%) 25°C S	G* (Pa) 8°C NS	G* (Pa) 8°C S	G* (Pa) 25°C NS	G* (Pa) 25°C S	tan δ (rad) 8°C NS	tan δ (rad) 8°C S	tan δ (rad) 25°C NS	tan δ (rad) 25°C S
1	1.74^{a}	1.30^{a}	3.12^{a}	2.33^{a}	66.2^{e}	43.6^{h}	56.4^{e}	33.2^{f}	0.313^{cd}	0.345^{e}	0.3475^{c}	0.355^{d}
2	0.731^{d}	0.976^{b}	1.74^{b}	1.30^{b}	448^{b}	249^{c}	178^{c}	171^{c}	0.298^{cde}	0.317^{fg}	0.336^{cd}	0.334^{g}
3	0.411^{f}	0.547^{e}	0.731^{e}	0.730^{e}	230^{d}	103^{gf}	133^{d}	63.8^{e}	0.296^{cdef}	0.320^{f}	0.320^{de}	0.336^{fg}
4	0.731^{d}	0.411^{f}	0.547^{f}	0.730^{e}	323^{bcd}	133^{e}	168^{c}	67.1^{e}	0.314^{cd}	0.329^{ef}	0.327^{cd}	0.349^{ef}
5	0.548^{e}	0.731^{c}	0.976^{d}	0.976^{c}	415^{b}	278^{b}	245^{b}	205^{b}	0.319^{c}	0.333^{ef}	0.341^{cd}	0.332^{g}
6	0.548^{e}	0.411^{f}	0.547^{f}	0.411^{g}	380^{bc}	206^{d}	187^{c}	102^{d}	0.571^{a}	0.591^{b}	0.665^{a}	0.639^{b}
7	1.30^{b}	0.976^{b}	1.74^{b}	1.30^{b}	257^{cd}	125^{ef}	128^{d}	63.1^{e}	0.502^{b}	0.555^{c}	0.588^{b}	0.626^{bc}
8	0.548^{e}	0.411^{f}	0.976^{d}	0.547^{f}	360^{bcd}	90.0^{g}	231^{b}	39.1^{f}	0.287^{def}	0.340^{e}	0.295^{f}	0.352^{e}
9	0.411^{f}	0.411^{f}	0.411^{g}	0.547^{f}	253^{cd}	122^{ef}	134^{d}	65.8^{e}	0.280^{ef}	0.300^{g}	0.287^{f}	0.3137^{h}
10	0.547^{e}	0.548^{d}	0.976^{d}	0.976^{c}	3410^{a}	516^{a}	499^{a}	329^{a}	0.268^{f}	0.300^{g}	0.298^{ef}	0.301^{h}
11	0.961^{c}	0.731^{c}	1.29^{c}	0.975^{d}	327^{bcd}	198^{d}	245^{b}	115^{d}	0.553^{a}	0.861^{a}	0.595^{b}	0.984^{a}
12	0.411^{f}	0.411^{f}	0.731^{e}	0.730^{e}	347^{bcd}	136^{e}	184^{c}	67.3^{e}	0.502^{b}	0.532^{d}	0.595^{b}	0.621^{c}

[1]γc: critical strain, G*: complex modulus at γc, tan δ: phase angle at γc.
Letters in each column that are different indicate significant differences.

This may have been due to the destabilization of the protein structure after incorporation of HWS. The largest effect of HWS on all of the yogurt samples was on the sample with PS (sample 8). This result was attributed to enzymatic breakdown of the starch granules by the amylase in the HWS. However, the changes in the structure of semisolid food can also be explained by other mechanisms, e.g. depletion flocculation. The non-adsorbing molecules in the HWS can create an osmotic pressure that forces the aggregation of the emulsion droplets and result in the disruption of the formulations (Chen, 2015).

Overall, G* decrease with increasing temperature and HWS addition to the samples. G* values of samples with added PS (sample 8) drastically decreased with the addition of HWS, but this effect was not shown for samples with CS (sample 9). This result was probably due to the high degree of PS-salivary amylase interactions. A similar effect for samples 8 and 9 was also seen in the shear rate sweep results (Section 5.4.2.1). This can be explained by the higher amount of amylose in CS and larger size of amylopectin (Singh et al., 2003). PS has more highly branched amylopectin and lower amylose content compared to high content of linear amylose in the CS. Saliva can pass through the freely large granules from than highly compacted structure of CS (Bird et al., 2000). Amylose is more difficult to digest compared to highly branched amylopectin because linear amyloses can pack tightly because of their shape. This results in less accessible area on the starch granules for digestion. Additionally, the structure of CS results in an increase in gelatinization temperature; when gelatinized, a high proportion of amyloses cause rapid retrogradation (Bird et al., 2000, Singh et al., 2003). Retrogradation is the molecular interaction of starch chains via hydrogen bonds when a gelatinized starch paste is cooled (Hoover, 2001). This phenomenon results in the formation of small, insoluble amylose crystallites that move to the available spaces between the amylopectin branches and increase resistance to enzymatic digestion (Bird et al., 2000).

Tan δ increased with added HWS and increased temperature, indicating increased viscous-type behavior. Sample 10, containing WPI, showed the lowest tan δ values, and samples containing LBG (samples 6 and 11) had the highest. Sample 11, containing LBG and CS, showed tan δ=0.99, indicating approximately equal viscous and elastic moduli with added HWS at 25°C. Addition of CS, PS, or high levels of WPI (samples 9, 8, and 10, respectively) resulted in a smaller tan δ compared to those of the samples containing fat (samples 2, 3, and 4). As expected, the addition of HWS resulted in greater tan δ values for starch-containing samples (samples 8 and 9) due to starch breakdown by salivary α-amylase. Tan δ values for the sample with CMC (sample 7) and the sample with all hydrocolloids (sample 12) were similar. These results may have been indicative of the similar matrix conformation of CMC and milk proteins with the protein network formed by all hydrocolloids and milk proteins. This effect is clearly shown in the confocal images of these two samples (Figure 5.2., 5.3.). Frequency sweep results (Figure 5.4) showed that samples with high levels of WPI (sample 10) had low dependence of frequency as indicated by the small slope of the viscoelastic moduli. These results were attributed to covalent bonds in the protein matrix for this sample (Laverse et al., 2011).

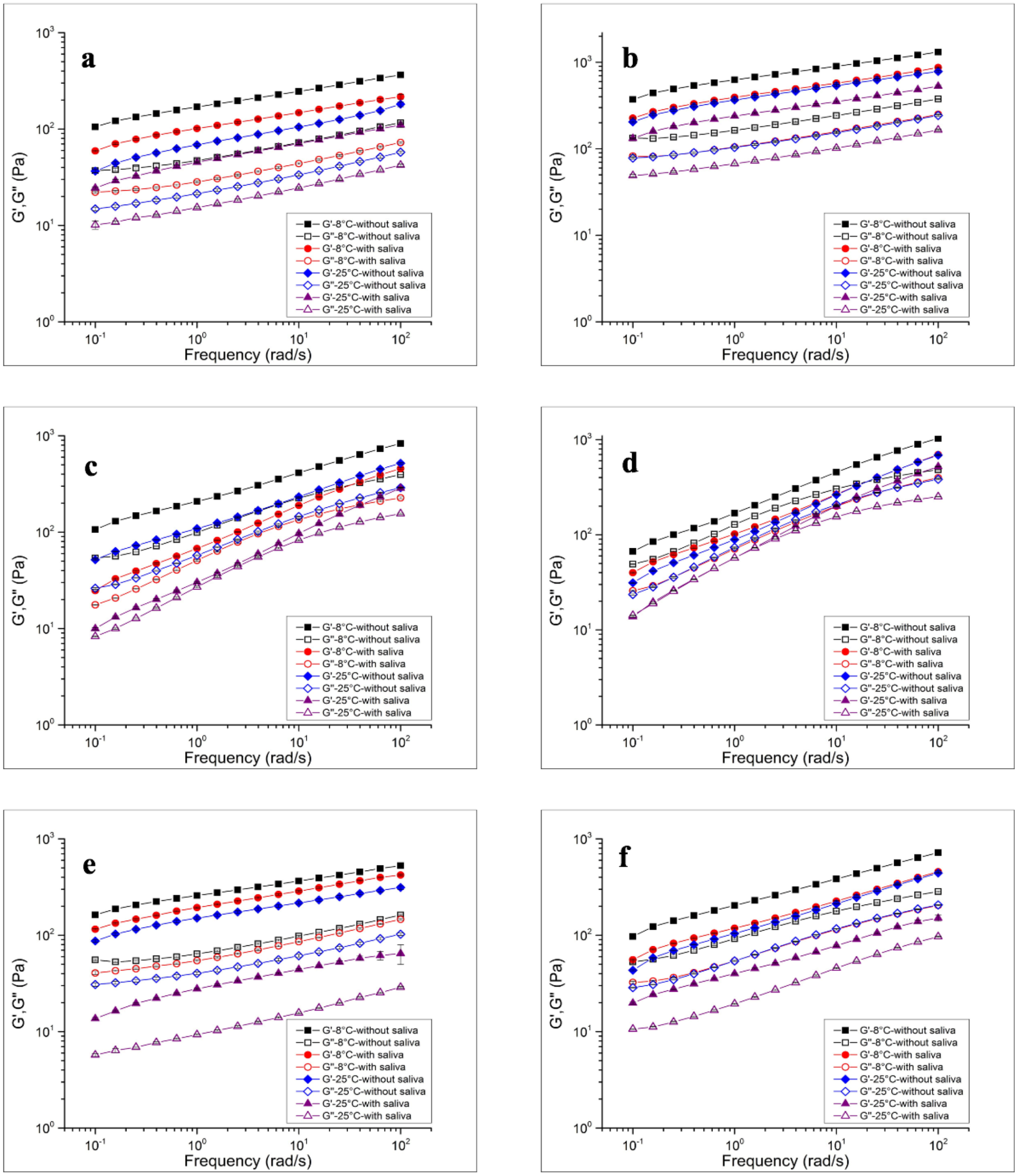

Figure 5.4. Frequency sweep results of yogurts;
a) sample 4; b) sample 10; c) sample 6; d) sample 11; e) sample 8; f) sample 12.

G' values for samples with LBG (sample 6) and LBG and CS (sample 11) showed high frequency dependence. These samples were weak gels with non-covalent linkages such as hydrogen bonds rather than electrostatic bonds (Laverse et al., 2011). This structure can be explained by the neutral charges of LBG and high numbers of amyloses in CS. Furthermore, it has been shown that addition of LBG and milk proteins can result in a weak gel due to the thermodynamic incompatibility of LBG (Thaiudom and Goff, 2003).

5.4.3 Friction profiles

Three-way ANOVA was performed to determine the impact of formulation (hydrocolloids), HWS, and different rates of sliding speeds on yogurt friction coefficients (Table 5.11.). Five sliding speeds were selected in the range of sliding speed experienced during oral processing (Malone et al., 2003). The effects of formulation, sliding speed, and the interaction of formulation with the other two parameters were significant at $p \leq 0.001$. HWS was significant at $p \leq 0.05$ and the interaction of sliding speed with HWS was significant at $p \leq 0.01$. Salivary proteins, mainly high molecular weight and proline-rich proteins, e.g. mucins, are the main source for the high lubricity of HWS (Bongaerts et al., 2007b). HWS has been shown to have friction coefficients that were two orders of magnitude less than those of water in its boundary regime. The significant impact of hydrocolloids is due to the addition of additives with significantly different functionalities to the yogurt system due to their different electrostatic charges, molecular size, and adhesive properties which can result in significantly different network structures, number of intermolecular interactions, bond strength, and aggregate size that can dramatically alter frictional properties of the formulations. For instance, addition of WPI can lead to a larger particle size that can increase friction coefficients. Additionally, sliding speed can change the position of food between the two surfaces (balls and PDMS plate) and impact the friction coefficient.

Table 5.11. Effect of main sources of variations on frictional properties of yogurts (n=12) determined by F-values obtained from three-way ANOVA[1].

Source of variations	Friction coefficient
Formulation	377***
Sliding speed	26.6***
HWS	710*
Sliding speed*HWS	6.6**
Formulation*HWS	131***
Formulation*Sliding speed	2.8***

[1] *, **, and *** indicate significant differences at *p*-value ≤ 0.05, *p*-value ≤ 0.01, and *p*-value ≤ 0.001, respectively.

Sample Stribeck curves showed an increase in friction coefficient at the beginning of the curve up to approximately 0.1 mm/s of sliding speed (Figure 5.5.). This increase was not the hydrodynamic regime, but was due to elastic deformation of the PDMS plate because the

rotational speed of the double-ball attachment was not high enough to promote slip (Zinoviadou et al., 2008). This start-up behavior typically disappeared at a sliding speed of ~0.1 mm/s and was minimized in full-fat yogurt (sample 4). During testing, fat globules form an interfacial film between the sliding surfaces, acting as a lubricant and resulting in a notable decrease in friction coefficient, which would promote sliding rather than stretching of the PDMS plate (Prakash et al., 2013, Huc et al., 2016).

The profile shape of Stribeck curves significantly changed for various hydrocolloids (Figure 5.5.). The length of boundary regime for samples with PS (sample 8), CS (sample 9), and high WPI levels (sample 10) was similar to the that of the control sample (sample 1). This result was likely due to the larger molecule size of the WPI, SMP, and starches. These molecules would not be able to fit between the PDMS surface and balls when the gap between the surfaces was small due to low siding speed, resulting in similar friction behavior to that of the control sample at those speeds. Sample 4 (full fat sample) showed the distinct friction curve shape, likely due to its milkfat content. Part of hydrodynamic region can be seen at the end of the mixed regime for this sample when HWS was not added to the sample. Samples containing CMC (sample 7) and all hydrocolloids (sample 12) had similar friction curves, as did the control sample (sample 1) and samples containing PS (sample 8), CS (sample 9), and high levels of WPI (sample 10). Sample 7 and 12 had also very similar phase angles (Table 5.6.) and microstructures (Figure 5.3). The curves for (samples 6 and 7) were notably different in their shape. Addition of LBG (sample 6) caused higher friction coefficients than addition of CMC (sample 7). This result was likely due to the type of structures that LBG and CMC form in the yogurt systems. The small, aggregated LBG molecules dispersed throughout the protein network would have been easier to deform and made a more particulate structure compared to the cohesive structure formed with addition of CMC. Samples with added LBG (sample 6), CMC (sample 7), and all hydrocolloids (sample 12) transitioned to the mixed regime at lower sliding speeds compared to the control sample (sample 1) and the samples with added PS (sample 8) or CS (sample 9). The full-fat sample (sample 4) showed a transition to the mixed regime at the lowest sliding speed. The use of hydrocolloids, particularly WPI, LBG, and SMP (used in samples 6, 10, 7, 12, and 1, respectively) resulted in increased friction coefficients in the boundary regime.

Samples with added LBG (sample 6), CS (sample 9), and all hydrocolloids (sample 12) had the least changes in their friction profiles after addition of HWS, which was in

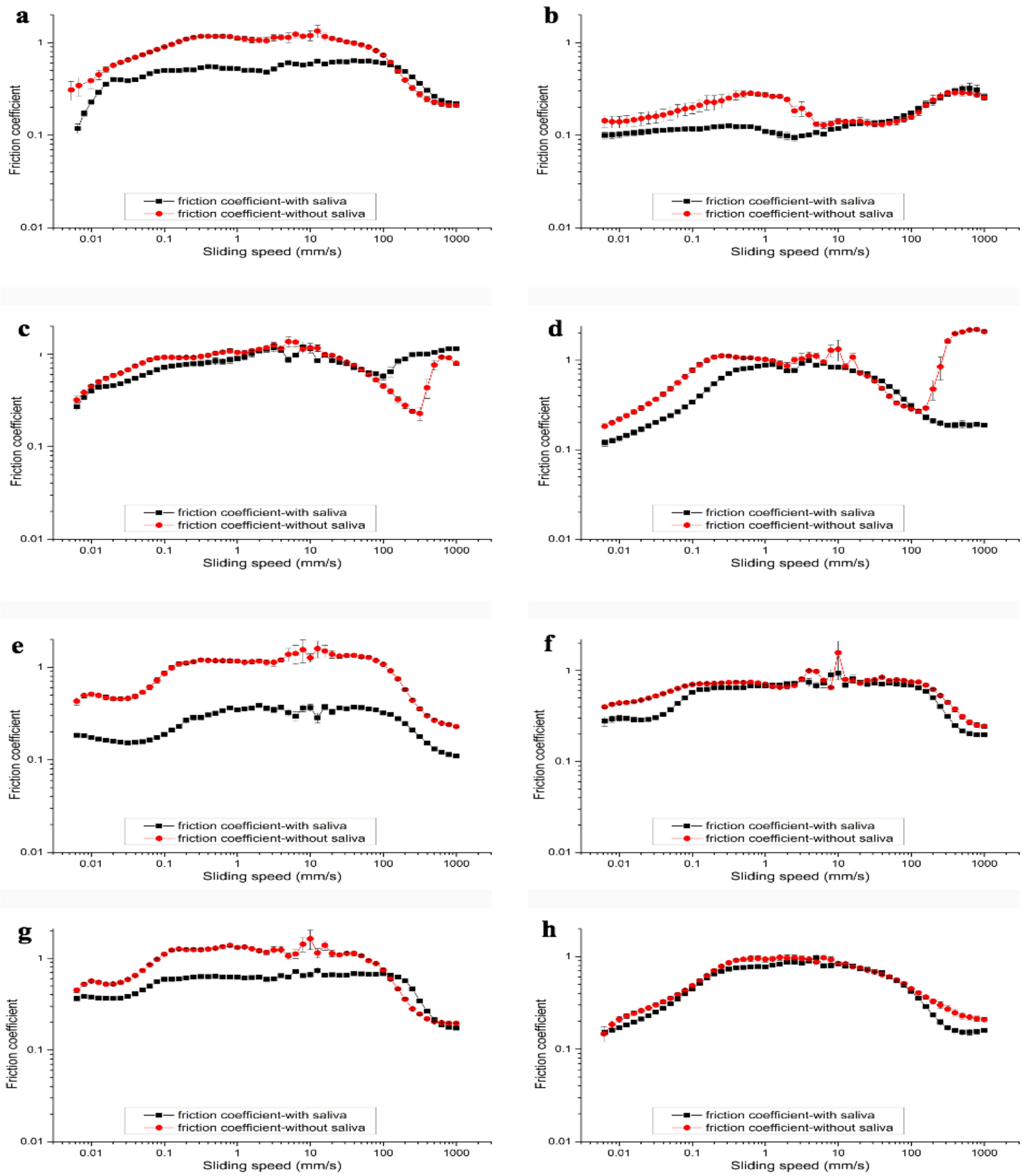

Figure 5.5. Tribological results of yogurts;
a) sample 1 (control); b) sample 4; c) sample 6; d) sample 7; e) sample 8; f) sample 9; g) sample 10; h) sample 12;

agreement with previous results. LBG, used in sample 6, is a neutral polysaccharide that has been shown to be less affected by HWS (Zinoviadou et al., 2008). The effect of HWS on the friction of CMC was greater than that for LBG. Addition of HWS to samples containing PS (sample 8) resulted in a drastic decrease in friction coefficient compared to samples made with CS (sample 9). This effect was attributed to the larger granule size and branched structure of PS compared to CS and lower amylose content of PS, as discussed in the viscoelastic section (5.7.). However, these samples showed similar Stribeck curve profiles. Overall, the frictional properties of yogurts changed with addition of HWS and hydrocolloids. Correlation of these observations with sensory results can be more helpful to decide whether using HWS is necessary in tribometry to determine relationships between friction behaviors and sensory texture.

5.4.4 Texture attributes

Formulations and panelists showed significant influence on textural attributes of yogurts at $p \leq 0.001$ (Table 5.12). Three-way ANOVA also showed significant differences at ($p < 0.05$) among panelists and samples for all attributes tested (Table 5.7.). There were no significant differences between replicates.

Table 5.12. Effect of different formulations (hydrocolloids) on textural attributes of yogurt (n=12) determined by F-values obtained from three-way ANOVA[1].

Source	panelist	sample	replicate	Sample*replicate
Spoon viscosity	14.4***	57.6***	1.3	0.7
Graininess	15.4***	32.9***	1.2	0.3
Mouthcoat	10***	17.6***	1.3	0.4
Firmness	10.4***	26.7***	0.9	1.1
Mouth viscosity	7.4***	32.6***	0.1	0.2
Lumpiness	16***	65.3***	1.6	1.1
Lumpiness-in-mouth	17.9***	57.1***	0.1	0.9
Smoothness	15.8***	44.3***	0.1	0.7
Melting	14.5***	11.3***	0.8	0.7
Grittiness-in-mouth	13.3***	12.8***	0.3	1.3
Astringency	34.5***	9.6***	0.9	0.7
Chalkiness-Afterfeel	10.1***	9.6***	0.1	0.6
Sliminess	2.6**	30.1***	0.9	0.7

[1] *, **, and *** indicate significant differences at p-value ≤ 0.05, p-value ≤ 0.01, and p-value ≤ 0.001, respectively.

Samples without gums and starches (samples 1-5) showed the highest spoon lumpiness. These samples contained SMP (sample 1), low levels of WPI (samples 5) and different fat ratios (samples 2, 3, 4). This intensity of this attribute significantly decreased when WPI was used in a higher ratio and without addition of SMP (sample 10), as well as LBG in sample 6, CS in sample 9, and the combination of all hydrocolloids in sample 12. Addition of milk-base additives can cause unpleasant texture attributes in yogurts (Morell et al., 2015). This has been attributed to protein aggregation and a possibility of two different protein matrices (Morell et al., 2015). Additionally, an increase in particle size may occur upon addition of whey powders (Beaulieu et al., 1999). The next group of samples with high spoon lumpiness were those with PS and CS (sample 8, 9, 10, and 6 respectively). Samples with CMC (sample 7), a combination of CS and LBG (sample 11), and the sample with all hydrocolloids (sample 12) had the lowest spoon lumpiness. Lumpiness in mouth followed similar trends as spoon lumpiness, which was not surprising.

Samples with added LBG (sample 6), CMC (sample 7), and all hydrocolloids (sample 12) had the highest degree of mouthcoat. Mouthcoating was significantly lower in the sample with high levels of WPI (sample 10), the low-fat sample (sample 2) and the samples with PS (sample 8). Samples 1, 3, 4, and 5 were not significantly different from samples 2 and 8 for intensity of mouthcoat. The lack of mouthcoating in samples containing starches was likely due to the role of amylase in starch breakdown. This effect was noted by De Wijk et al. (2009). The low mouthcoating for the sample containing high levels of WPI may be due to its high melting attribute, which would remove the feeling of a coating on the oral surfaces due to rapid meltaway.

Attributes scores for samples 1-5 (control sample, samples containing fat, and sample with low levels of WPI), showed that the effects of SMP were dominant to those of the milkfat content in perceived sensory texture. SMP is a popular additive that is used to alter yogurt texture (Karam et al., 2013) through short chains of proteins in the system. They can easily break once the product is in the mouth, resulting in a low mouthcoat; longer protein chains are needed to provide a mouthcoat. Increased astringency, grittiness, and graininess due to addition of SMP and WPI in these samples may have been due to increased particle size when these proteins were added to a yogurt system, resulting in a higher sensation of astringency, grittiness, and graininess (Sano et al., 2005), (Andrewes et al., 2011). Another reason for increased astringency, grittiness, and graininess could be aggregation of milk.
Sliminess/ropiness is an attribute that can be caused by exopolysaccharide (EPS) producing bacteria. These EPS from these bacteria can make long chains with milk proteins, resulting in a long, stringy texture. The EPS's can have negative or neutral charges based on the strains of bacteria (van de Velde et al., 2015). The mechanism of EPS interaction with milk proteins has been linked to the similar mechanism of hydrocolloid interactions with milk proteins. Samples containing CMC (sample 7) and all hydrocolloids (sample 12) showed the highest intensity of sliminess, probably due the presence of CMC, which has an opposite charge to that of milk proteins. These electrostatic interactions, as well as hydrophobic interactions, can form longer chains of proteins and cause a slimier texture. It seemed that the presence of strong interactions was required for this attribute in the yogurt samples since samples with LBG (sample 6), a neutral hydrocolloid with weak bonds to protein, had significantly lower sliminess than sample 7 or 12.

Table 5.13. Yogurt sensory attributes as evaluated by trained panelists

Formula Number	Spoon lumpiness	Spoon viscosity	Graininess	Mouthcoat	Mouth viscosity	Firmness	Lumpiness in mouth	Smoothness	Melting	Grittiness in mouth	Astringency	Chalkiness afterfeel	Sliminess
1	10.0^{a}	4.05^{d}	7.28^{b}	3.79^{c}	3.16^{cd}	3.81^{bc}	8.53^{a}	3.49^{d}	7.00^{bc}	5.09^{a}	5.94^{a}	4.16^{ab}	1.78^{c}
2	10.6^{a}	3.82^{d}	6.96^{b}	3.53^{cd}	2.94^{cd}	3.48^{bc}	8.49^{a}	3.45^{d}	7.12^{bc}	4.69^{ab}	5.85^{a}	3.98^{ab}	1.88^{c}
3	10.5^{a}	3.68^{d}	6.18^{b}	3.84^{c}	3.02^{cd}	3.47^{bc}	8.38^{a}	3.77^{d}	7.58^{b}	4.46^{ab}	5.76^{a}	4.13^{ab}	1.91^{c}
4	9.82^{a}	4.07^{d}	5.91^{bc}	3.98^{c}	3.33^{cd}	3.45^{bc}	7.85^{a}	4.00^{d}	7.61^{ab}	4.52^{ab}	5.32^{ab}	4.40^{ab}	1.97^{c}
5	10.4^{a}	3.86^{d}	6.48^{b}	3.75^{c}	3.06^{cd}	3.49^{bc}	8.15^{a}	3.71^{d}	7.51^{bc}	4.7^{ab}	5.80^{a}	4.21^{ab}	1.98^{c}
6	4.67^{c}	7.30^{b}	9.64^{a}	6.28^{a}	6.67^{a}	5.76^{a}	4.54^{b}	3.31^{d}	4.40^{e}	4.31^{ab}	4.32^{bcd}	3.68^{bc}	3.39^{b}
7	2.53^{d}	7.63^{ab}	2.51^{d}	5.38^{ab}	6.70^{a}	5.52^{a}	1.93^{d}	10.1^{a}	5.23^{cde}	2.19^{c}	3.77^{cd}	2.38^{c}	4.58^{a}
8	6.97^{b}	3.37^{de}	3.79^{d}	3.29^{cd}	2.92^{cd}	2.8^{dc}	4.07^{bc}	6.94^{c}	9.00^{ab}	3.57^{bc}	5.69^{a}	3.60^{bc}	1.82^{c}
9	$5.5b^{c}$	5.69^{c}	$4.15d^{c}$	$4.45b^{c}$	4.92^{b}	4.16^{b}	2.95^{bcd}	7.66^{bc}	6.80^{bcd}	4.76^{ab}	5.60^{ab}	4.51^{ab}	2.38^{c}
10	4.58^{c}	2.44^{e}	2.66^{d}	2.39^{d}	2.13^{d}	2.03^{d}	2.21^{d}	8.99^{ab}	9.93^{a}	2.75^{c}	5.56^{ab}	2.40^{c}	1.65^{c}
11	2.53^{d}	3.86^{d}	9.40a	4.03^{c}	3.61^{bc}	2.94^{dc}	2.73^{cd}	3.31^{d}	8.21^{ab}	5.60^{a}	4.96^{abc}	5.36^{a}	3.30^{b}
12	3.77cd	8.46^{a}	3.95d	5.84^{a}	6.91^{a}	6.15^{a}	2.36^{cd}	9.07^{ab}	4.56^{de}	2.51^{c}	3.49^{d}	2.56^{c}	4.44^{a}

Letters in each column that are significantly different indicate significant differences

Overall, sample 7 (CMC) and 12 (all hydrocolloids) showed similar trends in increasing or decreasing attributes. For instance, spoon viscosity, firmness, viscosity in mouth had the greatest and graininess, chalkiness, and grittiness intensity had the lowest intensity. Sample 11 (combination of LBG and CS) was very similar to these two sample for some of attributes. Improving ideal attributes in yogurts in these samples can introduce CMC, combination of LBG and CS, and combination of all hydrocolloids the best combination of hydrocolloids as semisolid food enhancers. The suitability of these combinations were also shown in other studies (Alakali et al., 2008, Murray and Phisarnchananan, 2014).

5.4.5 Principal component analysis of sensory results

Principal component analysis (PCA) was performed to visualize the relationships between the samples and textural attributes (Figure 5.6.). The first two principle components accounted for 59.9% and 26.3% of the variance, respectively, in the thirteen-variable system. The most positively correlated attributes with component 1 were mouth viscosity, spoon viscosity, sliminess, and firmness. Negative correlations were with astringency and low-melting. component 2 was positively correlated with graininess and negatively related with smoothness and astringency. This results from this plot were in accordance with the results for the sensory attributes (Table 5.13.).

Thirteen textural attributes of 12 yogurt were clustered in three groups (purple circles) in the PCA plot based on cluster analysis (Figure 5.7.). The first cluster (green) was positively described by smoothness, sliminess, both viscosity related attributes, firmness, and mouthcoating for samples 7, 12, 9 and negatively related to samples 8 and 10. Samples 8 and

10 were positively related to astringency and low-melting; these attributes had negative relations with samples 7, 12, and 9. As explained in the previous section (Section 5.4.4.), the CMC in sample 7 and all hydrocolloids in sample 12 contributed to these attributes due to a possible higher number of electrostatic and hydrophobic interactions, as well as covalent bonds formed by negatively charged hydrocolloids (CMC and PS) (Alakali et al., 2008). Additionally, samples 7 and 12 showed the highest intensities of ideal texture attributes in Section 5.4.4, which was attributed to the addition of CMC. The palatability of the yogurt produced by CS was likely produced by its structural features (Alakali et al., 2008). CS granules are very small compared to PS granules, and they can reduce the sensation of dry attributes in the mouth. Alakali et al. (2008) suggested that the residual corn oil in CS may be partially responsible for the palatability of CS-containing yogurts.

The second cluster (red) included samples 11 and 6. Sample 6 was positively related to most attributes related to the first cluster (green) and negatively to the lumpiness-related attributes, graininess, low-melting, and astringency. In the LBG-containing samples (sample 6), the neutral hydrocolloid would increase the viscosity of the continuous phase, promoting these attributes (Thaiudom and Goff, 2003).

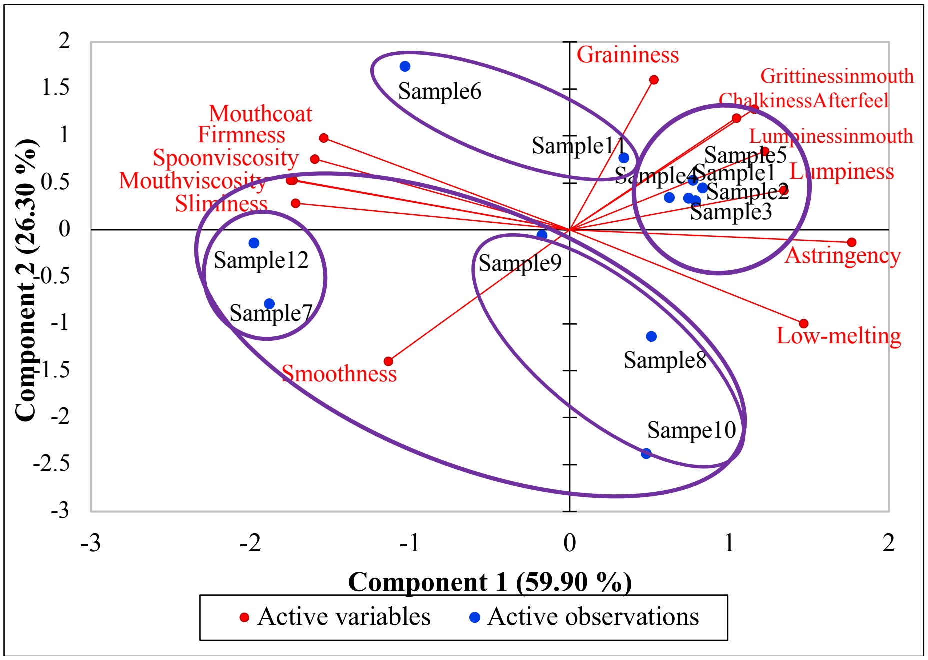

Figure 5.6. Principal Component Analysis (PCA) for texture attributes of yogurts (n=12) analyzed by descriptive sensory panelists (n=10)
Clusters have been circled based on cluster analysis (Figure 5.7.)

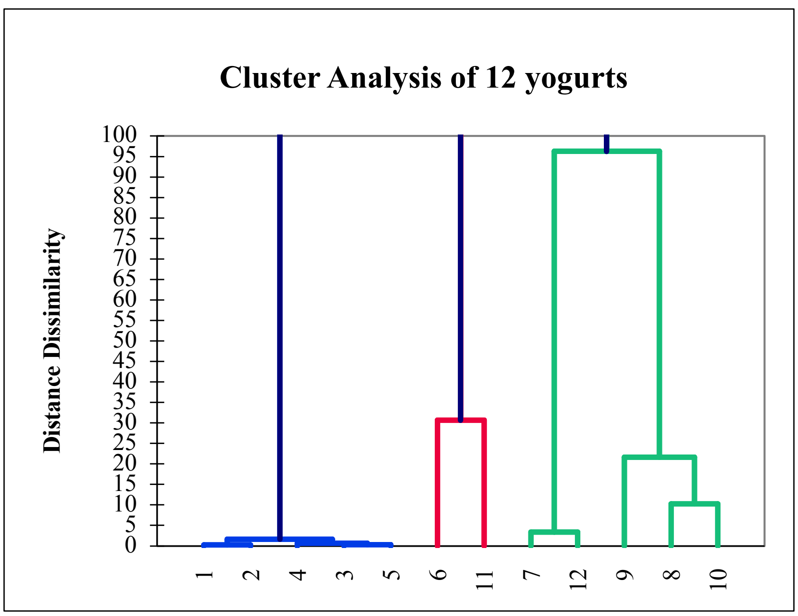

Figure 5.7. Cluster Analysis for yogurts from descriptive analysis data
(n=12 formulations composition in table 5.1.)

Sample 11 from the same cluster was positively related to umpiness-related attributes, graininess, low-melting, and astringency. Howeber, the intensity of these undesirable defects was significantly lower than the first cluster (green). The presence of CMC was hypothesized to be the reason for this observation. The third cluster (blue) consisted of samples 1, 2, 3, 4, and 5. These samples were mostly related to grittiness in mouth, chalkiness afterfeel, both related lumpiness attribtes, graininess, and astringency. SMP was the common additive in these samples. Addition of milk powders e.g., SMP and WPI is known to increase these attributes (Isleten and Karagul-Yuceer, 2006); Overall, PCA was helpful for illustrating significant attributes as well as categorizing samples.

5.4.6 Correlations among yogurt textural attributes

Overall, textural attributes showed significant correlations (Table 5.14.). There were two major groups of attributes in this matrix. The first group included lumpiness, lumpiness in mouth, low-melting, grittiness in mouth, astringency, and chalkiness afterfeel. These attributes showed negative correlations with the second group including the palatable attributes: spoon viscosity, mouthcoat, mouth viscosity, firmness, smoothness, and sliminess. Drivers behind these correlations included particle size and the extent of susceptibility of the hydrocolloid to salivary components, specifically, salivary enzymes or proteins.

Table 5.14. Correlation matrix for yogurt textural attributes[1,2]

Attribute	Spoon lumpiness	Spoon viscosity	Graininess	Mouthcoating	Mouth viscosity	Firmness	Lumpiness in mouth	Smoothness	Melting	Grittiness in mouth	Sliminess
Spoon viscosity	-0.534*	1.00			0.991***				-0.942***		
Mouthcoat		0.953***		1.00					-0.961***		
Mouth viscosity	-0.609*	0.991***		0.958***	1.00				-0.920***		
Firmness		0.975***		0.958***	0.953***	1			-0.986***		
Lumpiness in mouth	0.955***										
Smoothness	-0.602*		-0.893***				-0.761**			-0.878**	
Low-melting		-0.942***		-0.961***	-0.920***	-0.986***					
Grittiness in mouth			0.814**				0.548*	-0.878**	1.00	1.00	
Astringency	0.740**	-0.884***		-0.822**	-0.896**	-0.809**	0.628*		0.750**	0.562*	-0.964***
Chalkiness afterfeel			0.727**					-0.785**		0.960***	
Sliminess	-0.745**	0.884***		0.829**	0.900***	0.808**	-0.614*		0.761**		1.00

[1] Coefficients that were not statistically significant (p>0.05) and repetitive correlations were removed from cells and columns

[2] **p*-value ≤ 0.01***p*-value ≤ 0.001; ***p*-value ≤ 0.0001

As expected, the highest correlations were for spoon lumpiness and lumpiness in mouth, as well as spoon viscosity and mouth viscosity. This result was likely due to the fact that appearance may highly affect other senses, or the intensity of these attributes remained similar to their appearance. Viscosity-related attributes had the highest number of correlations among other attributes. In terms of the undesirable attributes, astringency and lumpiness had highest correlation. These results emphasize that yogurt textural attributes may be related to each other, potentially due to structural features that have a variety of effects. Therefore, care must be taken in formation of new yogurt products so that changing one textural attribute does not cause undesirable changes in a second.

5.4.7 Correlations among yogurt viscoelastic and flow behaviors

Correlations were found among yogurt viscosity and viscoelastic behaviors for samples with and without added HWS (Table 5.15.). G* (complex modulus) was significantly correlated with η_o and c at 8°C and 25°C and phase angle was only correlated to η_o at 8°C without HWS application. These results showed with increasing G*, η_o also increases. These correlations are shown in viscosity and strain sweep results. By increasing the phase angle, η_o decreases. Meaning, the greater the η_o is, the more solid-like behavior is the material, since phase angle is G'/G". The results from when samples were tested with addition of saliva, G* at both temperatures was correlated to η_o, but phase angle were not significant in this correlation. Critical strain was related to n and c from viscosity parameters. The only difference in the explanation of samples with HWS is by increasing critical strain, c and n would also increase. The type of correlations changed when HWS was considered in the test, but it was not very notable.

Table 5.15. Viscosity profiles for yogurts (n=12) at 25°C

Formula	η_o at 8°C no HWS	η_0 at 25 °C no HWS	c at 25°C no HWS	n at 8°C no HWS	η_o at 8°C HWS	η_0 at 25 °C HWS	c at 8°C HWS	n at 25°C HWS
G*, 8°C, no HWS	***0.985	***0.980	*0.817					
G*, 25°C, no HWS	*0.892	**0.900	*0.855					
tan δ, 8°C, no HWS				*-0.676				
tan δ, 25°C, no HWS				*-0.650				
G*, 8°C, HWS					*0.864	*0.780		
G*, 25°C, HWS					*0.844	*0.791		
γ_c, 25°C, HWS							*0.712	*0.628

[1]Only significant correlations at *p-value ≤ 0.05; **p-value ≤ 0.01; ***p-value ≤ 0.001, are shown.

Sensory results were correlated to viscosity parameters of yogurts including η_o (Pa s), c (s), and flow behavior (n). n was correlated with fiction coefficients at all tested friction coefficients excluding 1 mm/s at 8°C when HWS was not added. This result can be interpreted as when n increases, the friction coefficient become greater. The lower the n is, the yogurt can show the weaker gels and in other words the shear thinning behavior of material, the less material is shear thinning. In addition to the correlations with n, c was also significantly correlated with the friction coefficient at 25°C when HWS was not added. The negative correlations of time constant with tribological results might suggest that addition of HWS to the samples for testing can be useful.

No significant correlations among yogurt viscoelastic and friction behaviors. This result was not expected as some viscoelastic properties e.g. viscoelastic moduli or loss factor, have been found to be related to friction behavior in other studies (Chen and Engelen, 2012).

The lack of correlation implies that structural features that control viscoelastic properties do not have a significant on friction behaviors and vice versa.

5.4.8 Correlations among yogurt flow parameters and textural behaviors

Correlation of viscosity parameters with sensory results showed few correlations. However, η_o was positively correlated with firmness at 25°C (R^2=0.87) and *n* was negatively correlated with sliminess at 8°C (R^2=0.88) when HWS was added. Firmer yogurts showed higher instrumental viscosity, since there a greater force was needed to induce flow. Firmer materials would have stronger bonds and interactions, making their structure more resistant to flow. As previously discussed, sliminess is the result of strong interactions between milk proteins and hydrocolloids. Slimy materials can show shear thinning behavior, and this behavior was intensified by the long chain proline rich mucin ad other salivary proteins as the molecules of the sample and HWS are highly intact. *c* was negatively correlated with low-melting (R^2=0.930), grittiness in mouth (R^2=0.822), and astringency (R^2=0.844) at 8°C when HWS was not added. The parameter *c* is the time frame needed for a material to flow and is known to be the result of protein aggregations or larger particle size. Therefore, more time is needed to disrupt more aggregated structures or shear larger molecules.

5.4.9 Correlations among yogurt viscoelastic and textural behaviors

Viscoelastic parameters that correlated well to yogurt texture attributes included only tan δ (phase angle) at γ_c obtained from strain sweeps; γ_c (critical strain) and G* (complex modulus) at γ_c, did not show significant correlations with sensory terms (Table 5.16). tan δ was positively correlated with viscosity-related attributes, mouthcoat, firmness, and sliminess. It was also negatively correlated with low-melting, lumpiness, and astringency.

Table 5.16. Correlations between yogurt viscosity and sensory results[1]

Viscosity parameters	Spoon lumpiness	Spoon viscosity	Mouthcoat	Mouth viscosity	Firmness	Low-melting	Astringency	Sliminess
Tan δ (rad) at 8°C, no HWS	-0.695*	0.702*	0.783*	0.750*	0.656*	-0.663*	-0.832**	0.860**
Tan δ (rad) at 8°C, with HWS	-0.698*						-0.626*	0.706*
Tan δ (rad) at 25°C, no HWS	-0.690*	0.748*	0.807*	0.787*	0.710*	-0.710*	-0.870**	0.886**
Tan δ (rad) at 25°C, with HWS	-0.708*						-0.654*	0.734*

[1]Only significant correlations at *p-value ≤ 0.05; **p-value ≤ 0.01; ***p-value ≤ 0.001, are shown.

As tan δ increases, the samples would show more viscous-type behavior, i.e. the sample would flow more readily over its own weight. A potential explanation for the correlation of tan δ with firmness and viscosity is that panelists may have interpreted the increased flowability of the yogurts as increased viscosity, sliminess, and mouthcoat. Correlations at different temperatures were not significantly different. This can be explained by the short time of the product holding in the mouth for semisolid foods before swallowing it.

5.4.10 Correlations among yogurt frictional and textural behaviors

Friction coefficients of yogurts at 1 mm/s, 5, 10, 15, 20, 25, 30, 40, 50, 60, 80, and 100 mm/s of sliding speeds were correlated with sensory results at 25°C (Table 5.17). These sliding speeds were selected since oral sliding speeds have been reported to be in the range of 10 to 100 mm/s (Malone et al., 2003).

Spoon lumpiness was positively correlated to friction coefficients at all sliding speeds, excluding 1 and 5mm/s for the measurements without HWS, and excluding 1 mm/s for friction coefficients measured for sample with added HWS. However, friction coefficients with and without saliva at 1 mm/s were correlated with mouth viscosity and smoothness. Negative correlation of smoothness with friction coefficient were expected: smoother yogurts would have lower friction coefficients. Mouth viscosity was positively correlated with friction coefficients at 60, 80, and 100 mm/s when no HWS was applied during tribological testing. This result was opposed to findings for model hydrocolloid solutions (De Vicente et al., 2006). However, the positive correlation between viscosity and friction has been found in a more recent study on semisolid dairy products (Sonne et al., 2014). The conflicting results may be due to the larger particle size in semisolid foods with higher protein content, particularly if WPI is used as the protein source (Krzeminski et al., 2011). The protein molecules might be trapped between or adhere to the two surfaces, increasing the friction.

Table 5.17. Correlations between sensory results and friction coefficients of yogurts with and without HWS addition at 25°C (n=12).[1]

Sensory attributes	μ at 1mm/s	μ at 5 mm/s	μ at 10 mm/s	μ at 15 mm/s	μ at 20 mm/s	μ at 25 mm/s	μ at 30 mm/s	μ at 40 mm/s	μ at 50 mm/s	μ at 60 mm/s	μ at 80 mm/s	μ at 100 mm/s
Lumpiness		0.615*	0.990***	0.997***	0.999***	0.999***	0.999***	0.999***	0.999***	0.999***	0.999***	1.00***
Spoon viscosity	-0.840**	-0.692*										
Smoothness	-0.902***	-0.684*										
Low-melting	0.617*											
mouth viscosity												
Correlations without HWS												
Lumpiness			0.914***	0.967***	0.992***	0.996***	0.998***	0.998***	0.999***	0.999***	0.999***	0.999***
Spoon viscosity	-0.840**											
Smoothness	-0.902***											
Low-melting	0.616*											
Mouth viscosity										0.577*	0.579*	0.581*

*p-value ≤ 0.05; **p-value ≤ 0.01; ***p-value ≤ 0.001;

[1]μ: friction coefficient

5.5 Conclusions

Overall, the combination of rheology, tribology, sensory, and confocal imaging were found to be useful techniques to determine different texture-related properties in yogurts. Addition of different hydrocolloids to the yogurt formulations significantly changed flow, viscoelastic, friction, and textural behaviors in yogurts. Microstructural images were a beneficial tool for determining protein network conformations, which showed relationships with multiple instrumental parameters and texture attributes. For instance, addition of CMC in the formulation changed the protein matrix of that formulation to a more aggregated structure with longer chains. This structure appeared to be related to the increased viscosity of this sample as well as a decrease in its phase angle compared to the control sample. This sample also showed higher values of pleasant texture attributes compared to samples containing other individual hydrocolloids. These changes were due to the strong interaction of negatively charged CMC with milk protein along with hydrophobic bonds in the system. HWS has significant influence on all instrumental parameters and can be used to determine some of the mechanisms of food disruption when used during instrumental testing. However, correlations among yogurt rheological, tribological, and sensory behaviors did not significantly change for samples tested with or without HWS. More work is needed to illustrate the optimization of semisolid food textures by hydrocolloids when fat is completely or partially removed, with consideration of HWS effects during oral processing.

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CHAPTER 6: CONCLUSIONS

Rheological, tribological, sensory, and confocal imaging of acid milk gels and yogurts showed significant differences upon addition of hydrocolloids (corn starch (CS), potato starch (PS), locust bean gum (LBG), and caboxymethylcellulose (CMC)) and human whole saliva (HWS). Confocal images showed that the protein network had notable differences when hydrocolloids and HWS were added. Hydrocolloids produced thicker chains and larger aggregates. HWS application resulted in fat coalescence in samples with fat and a higher number of aggregates with larger serum pores.

Five non-Newtonian models, including Cross, Cross-Williamson, Herschel Bulkley, and power law, were fit to the viscosity profiles of the samples. All samples showed shear-thinning behaviors regardless of formulation or addition of HWS. However, n (flow index) decreased with HWS application and increasing temperature. Acid milk gels and yogurt samples with added WPI had the greatest viscosity, and samples with LBG had the lowest. Samples containing CMC, or all hydrocolloids showed similar viscosity profiles. This similarity in behavior carried over to the results for tribology, confocal imaging, and sensory evaluation. Stribeck curve profiles from tribometry mostly showed boundary and mixed regimes for all samples, but their shapes changed notably for different formulations. Yogurt and acid milk gel samples with added WPI (sample 16 from acid milk gels and sample 10 from yogurts) had the greatest friction coefficients, while full-fat acid milk gels and yogurts showed the lowest. Friction coefficient decreased with addition of HWS for most samples due to enzymatic breakdown and lubrication effects of HWS. The most notable decrease in friction coefficient among the samples occurred when HWS was applied to the sample with PS (sample 8) due to the enzymatic breakdown of PS with salivary α-amylase. However, this effect was not observed for the samples with CS due to differences between CS and PS amylose content, as well as differences in microstructural conformation. Similar results to viscosity and friction behaviors occurred for the shear rate sweep results. tan δ increased upon addition of HWS and increased temperature and decreased with greater number of hydrocolloids used in the formulation. In other words, the resistance to deformation increased with addition of hydrocolloids in the formulations and decreased with HWS and increased temperature.

Acid milk gel and yogurt samples made with CMC, or more than two hydrocolloids showed the greatest number of desirable textural attributes, i.e. smoothness, firmness, mouth-viscosity. These samples were also least related to negative attributes, i.e. grittiness, graininess, astringency, and chalkiness afterfeel. The opposite results were found for samples containing SMP, WPI, and starches either individually or in combinations (acid milk gel and yogurt samples 1-5, 8 and 9, and acid milk gel sample 13). These differences were attributed to differences in net charges, hydrophobic interactions, and particle size.

Rheological and tribological results were well-correlated with sensory results. Sensory attributes of grittiness, graininess, and astringency were positively correlated with acid milk gel and yogurt friction coefficients. Not surprisingly, viscosity in mouth, firmness, smoothness, and melting were negatively correlated with acid milk gel and yogurt friction coefficients. Friction coefficients were correlated with sensory results at different sliding speeds in yogurts compared to acid milk gels. Friction coefficients were also correlated with viscosity and viscoelastic parameters for acid milk gels, but this correlation was only with viscosity for yogurts. Overall, there were a greater number of correlations among different properties of acid milk gels than for yogurt. One possible reason is the greater number of formulations for acid milk gels, which increases the probability of significant correlations in statistical analysis.

The results from this dissertation are beneficial in formulating new semisolid products with a desirable texture similar to their full-fat counterparts. There are several areas of future work for this project. Based on the results from this project, significant instrumental tests and sensory attributes can be selected for future study to determine what changes are caused by HWS in the samples from individual panelists rather than a combined group of panelists. Ideally, the HWS donators would also perform the descriptive sensory evaluation. Differences in panelist HWS components would also be determined. This study would help connect the variation in rheological, tribological, and confocal images with the sensory results from each individual, providing a clearer picture on how individual variation in HWS composition impacts food instrumental and sensory behaviors.

The application of hydrocolloids can cause adverse effects on the flavor of semisolid foods. Expanding sensory evaluation to determine hydrocolloid impact on flavor can help evaluate these effects. Additionally, combining flavor impact with texture perception may be provide a better understanding of food sensory profiles than when only texture is being evaluated.

A third area of possible future work is generalizing the results of this project to other semisolid foods that have high fat content, e.g. mayonnaise or sour cream, which may not be acceptable for more health-conscious people. For instance, mayonnaise may not show similar results to those from this work due to the differences in formulation. The combination or type of hydrocolloids may be adjusted in semisolid food systems to evaluate their effect of structural, rheological, tribological, and sensory behaviors.

Another area of future work is the application of exopolysaccharide (EPS)-producing bacteria for yogurt preparation. These EPS are able to produce ropy textures and are a popular method to improve texture and syneresis in yogurts. Both EPS and non-EPS producing bacteria can be used in yogurt preparation with and without hydrocolloids. Rheological and tribological measurements along with sensory evaluation will be helpful to evaluate those yogurts, understand the impact of EPS- producing bacteria versus hydrocolloids impact yogurt behaviors, and determine what would be the best fat replacer; EPS-producing bacteria, hydrocolloids, or the combination of both; in texture improvement. The same methods are also applicable for the optimization of reduced or non-fat yogurts that have been flavored with fruits and are sweetened by either sucrose or any artificial sugar. Fruits and a sweetening source can significantly change rheological properties of yogurts as well as their tribological and sensory behaviors. Some yogurt defects can be improved by addition of the right type and concentration of hydrocolloids. Finally, consumer preference tests can be added to sensory evaluation to find out how rheological and tribological results can be related to product liking. Overall, the experimental design and measurement design of this project can be used in the optimization of textures of any semisolid food.

APPENDIX A: SUPPLEMENTAL FIGURES

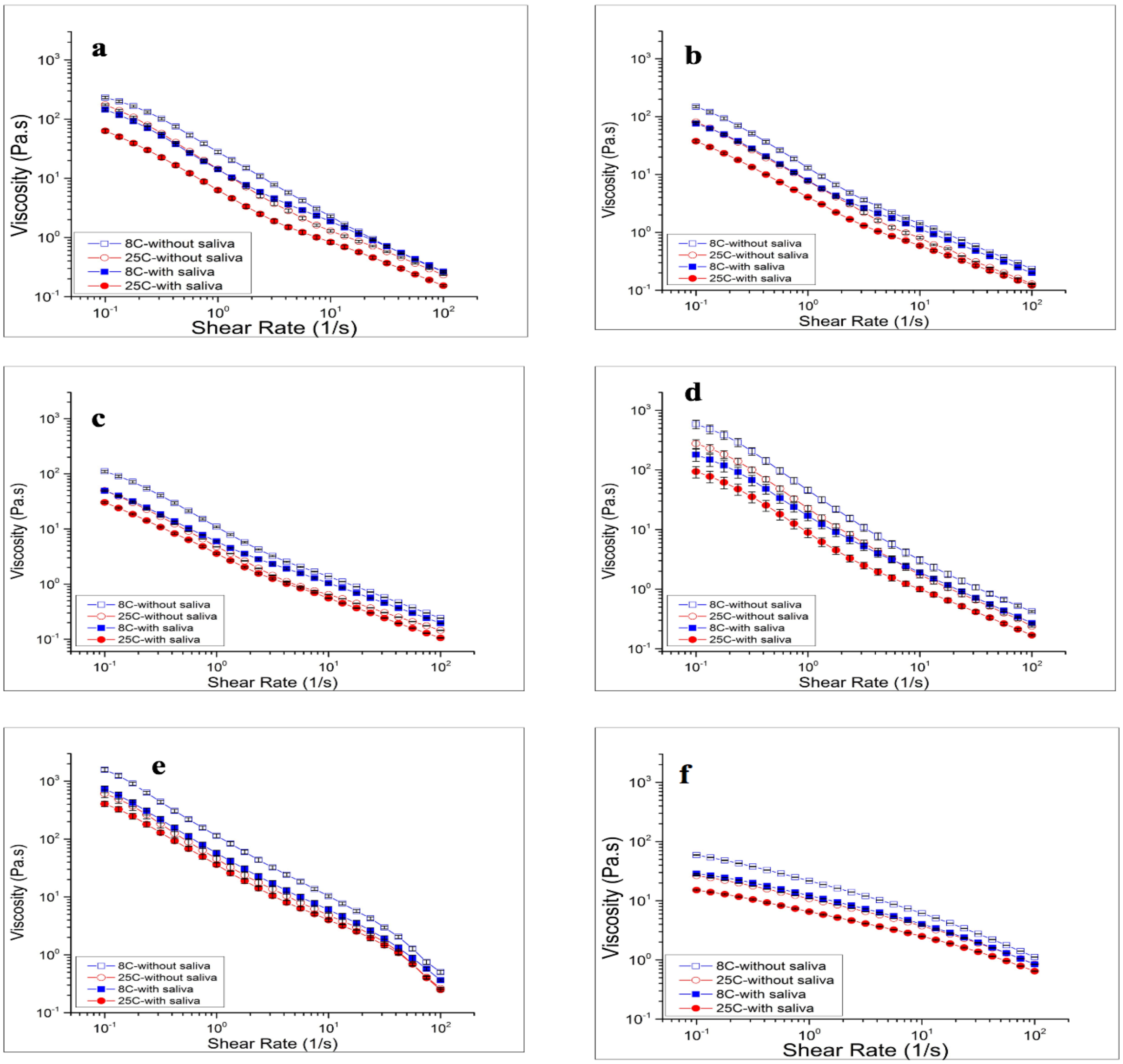
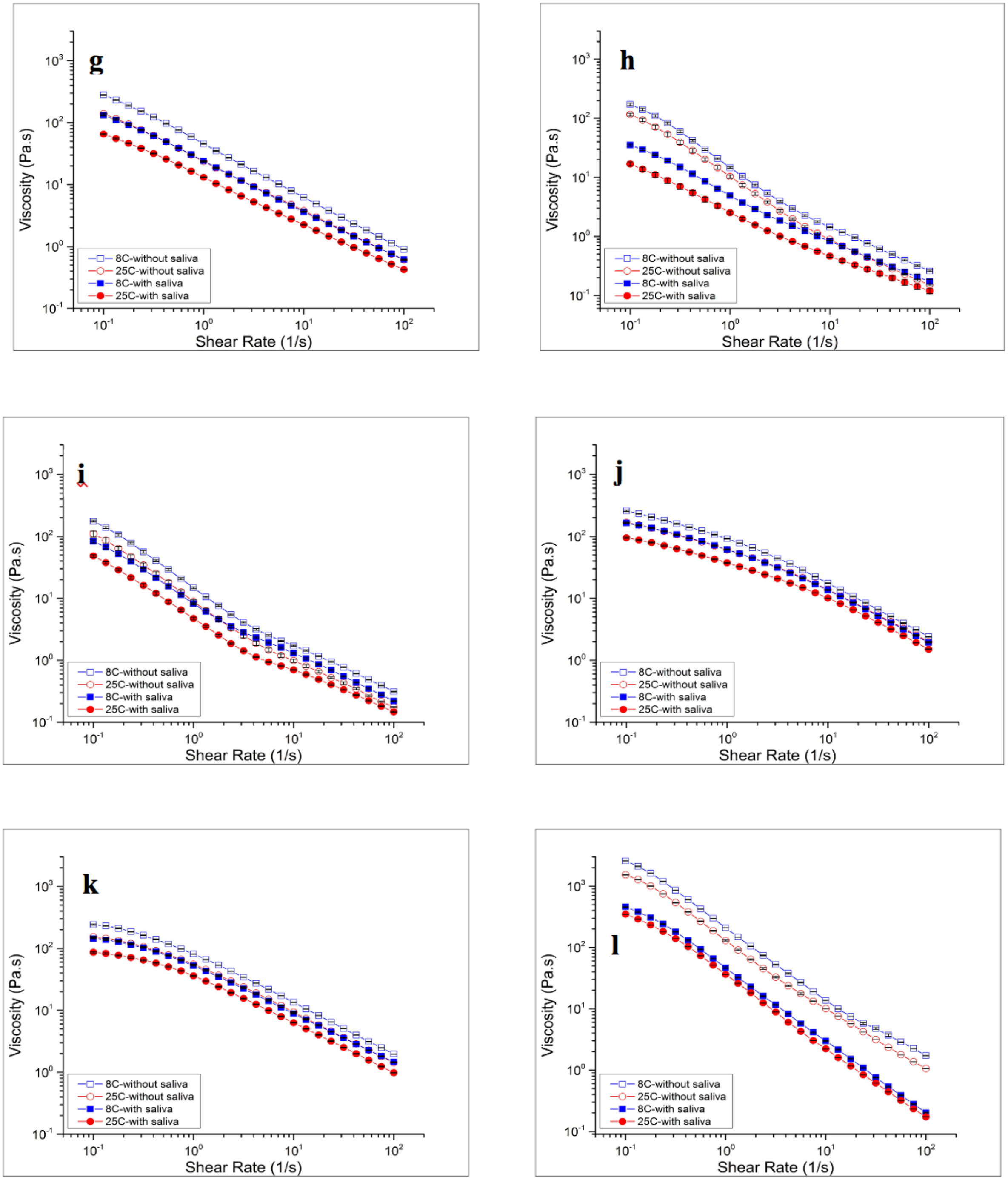


Figure A.3.1. Shear rate sweep results of acid milk gels;
a) sample 1; b) sample 2; c) sample 3; d) sample 4; e) sample 5; f) sample 6; g) sample 7; h) sample 8; i) sample 9; j) sample10; k) sample 11; l) sample 12; m) sample 13; n) sample 14; o) sample 15; p) sample 16; q) sample 17; r) sample 18; s) sample 19; t) sample 20; u) sample 21; v) sample 22; w) sample 23; x) sample 24.



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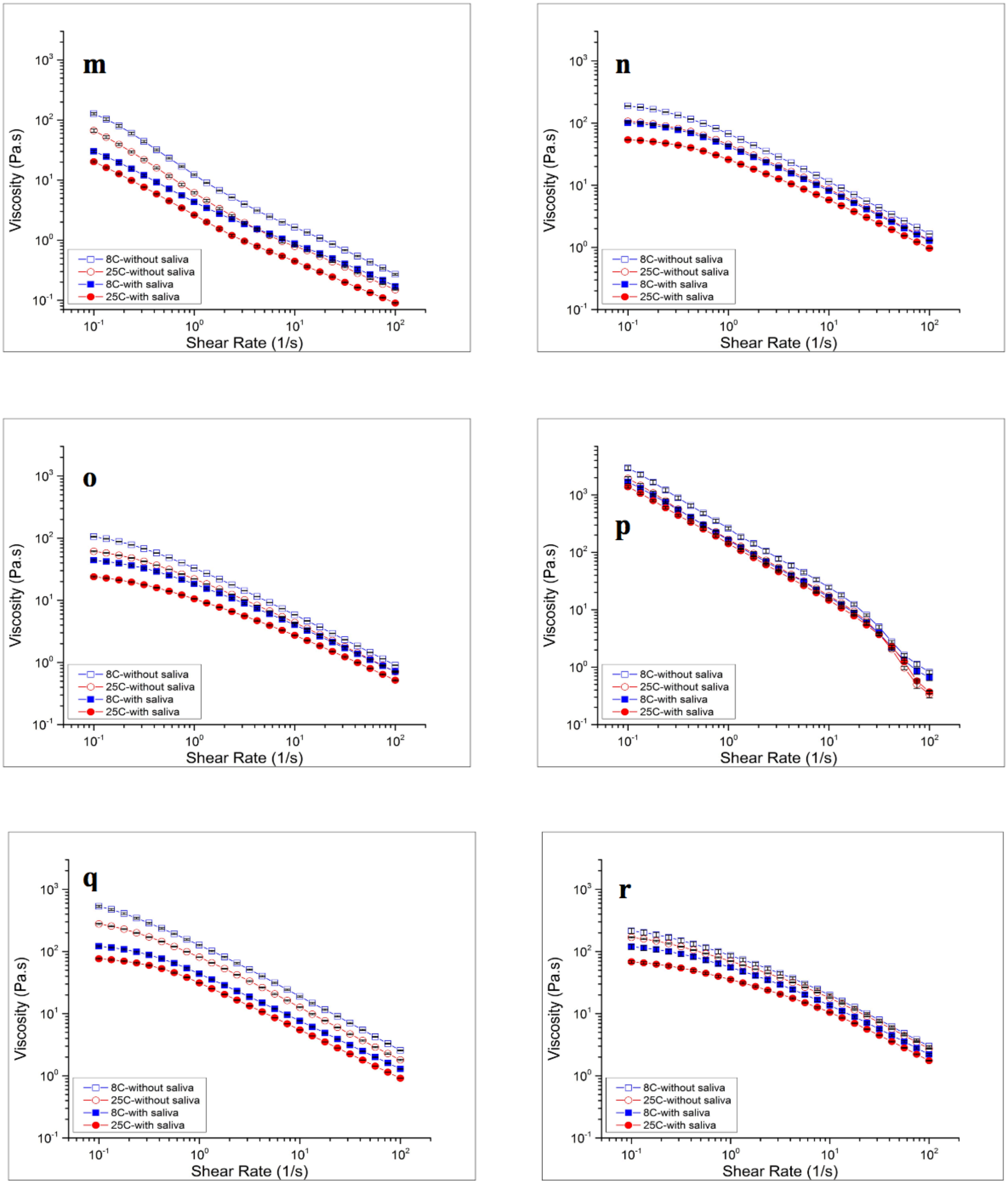
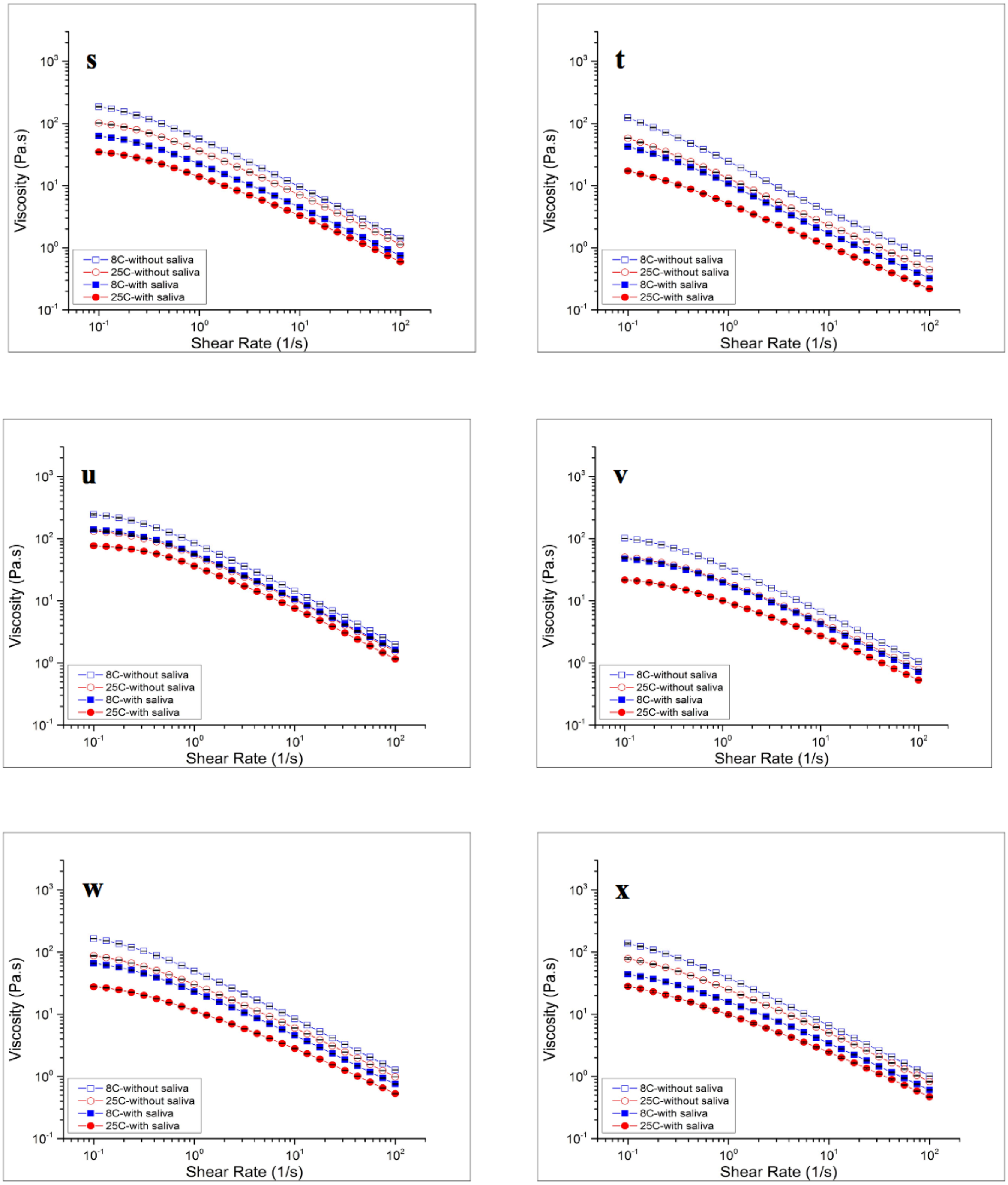


Continued from Figure A.3.1. shear rate results of acid milk gels.



Continued from Figure A.3.1. shear rate results of acid milk gels

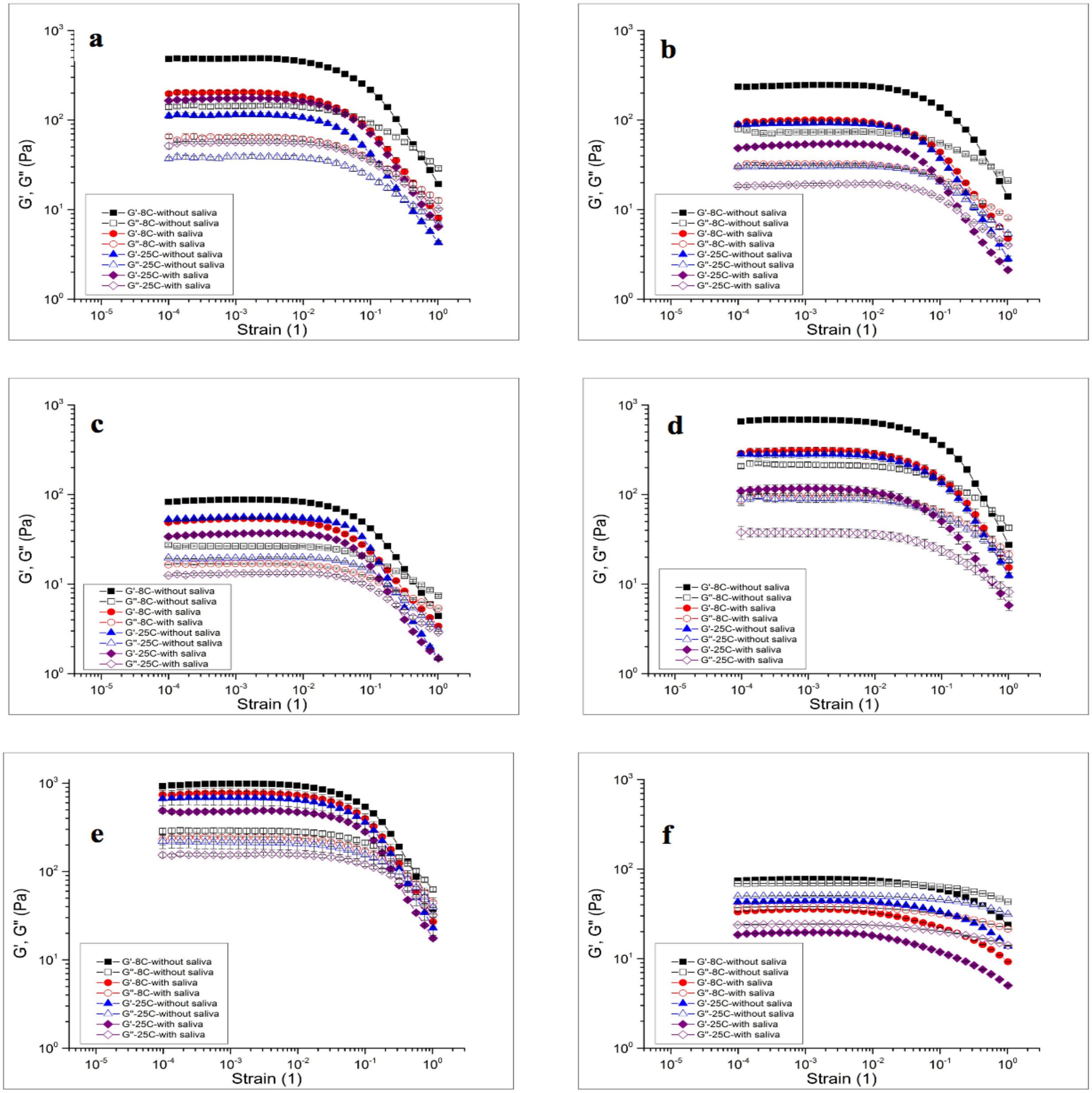
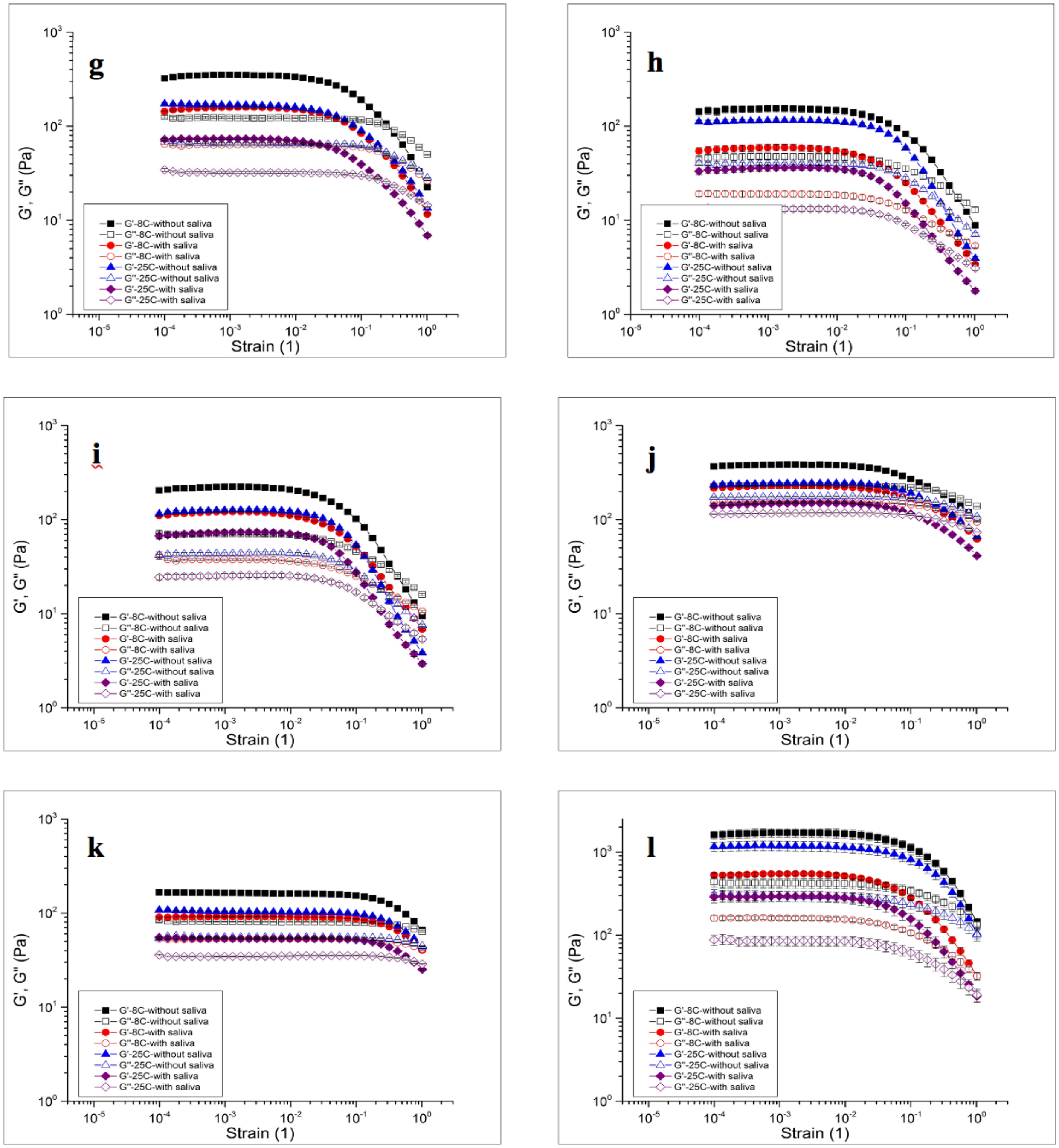


Figure A.3.2. Strain sweep results of acid milk gels;
a) sample 1; b) sample 2; c) sample 3; d) sample 4; e) sample 5; f) sample 6; g) sample 7; h) sample 8; i) sample 9; j) sample10; k) sample 11; l) sample 12; m) sample 13; n) sample 14; o) sample 15; p) sample 16; q) sample 17; r) sample 18; s) sample 19; t) sample 20; u) sample 21; v) sample 22; w) sample 23; x) sample 24.



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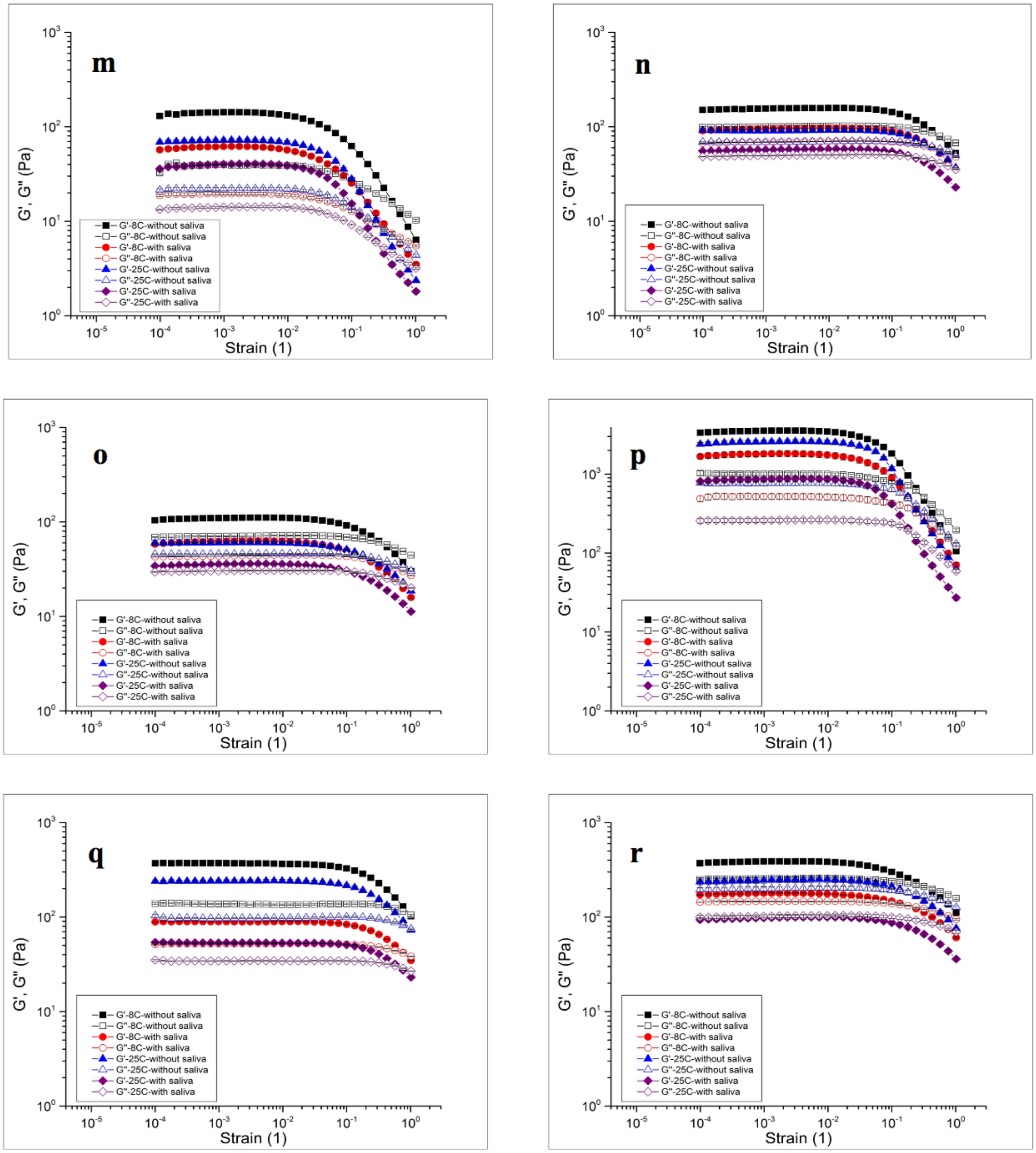

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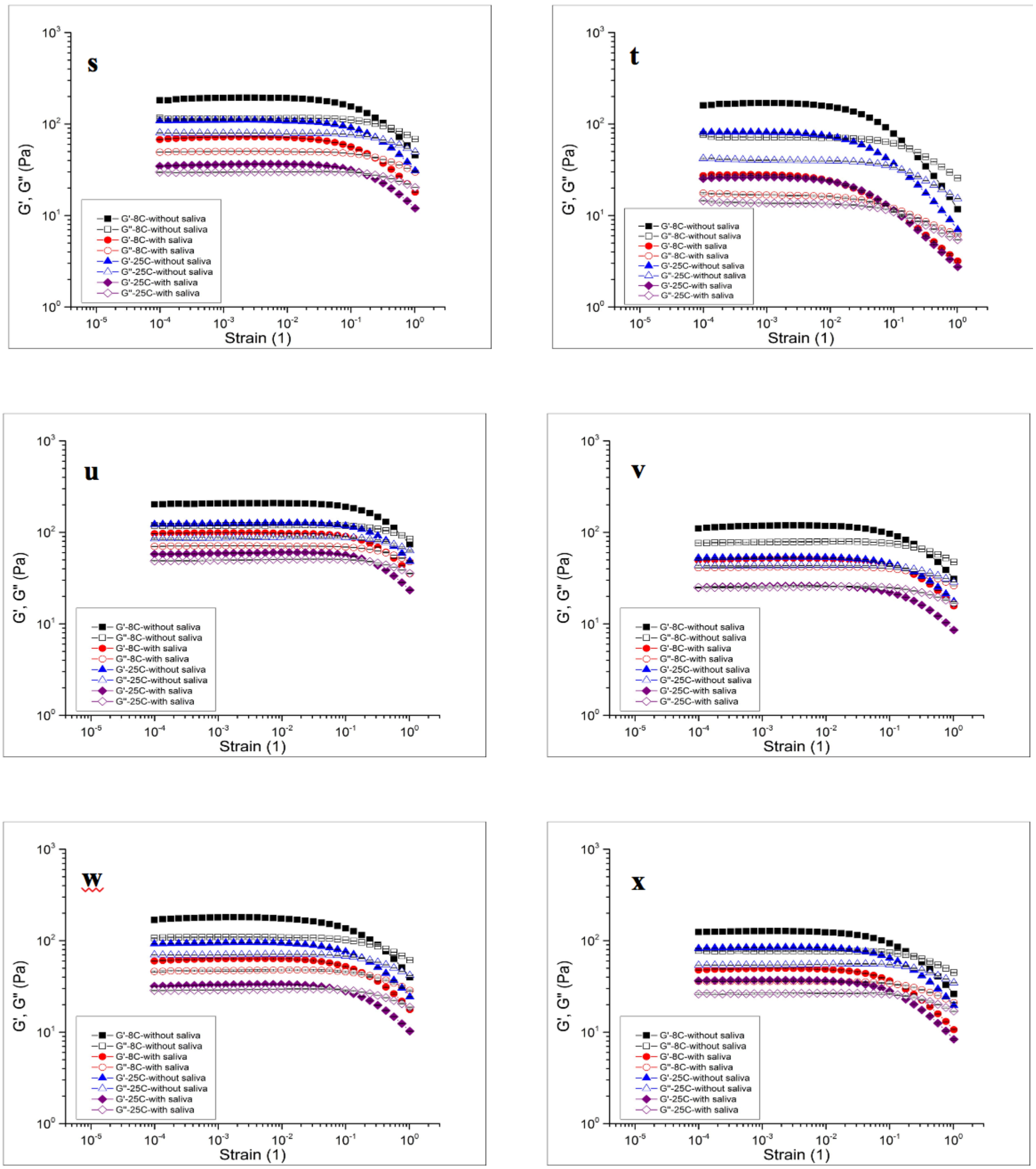


Continued from Figure A.3.2. Strain sweep results of acid milk gels.

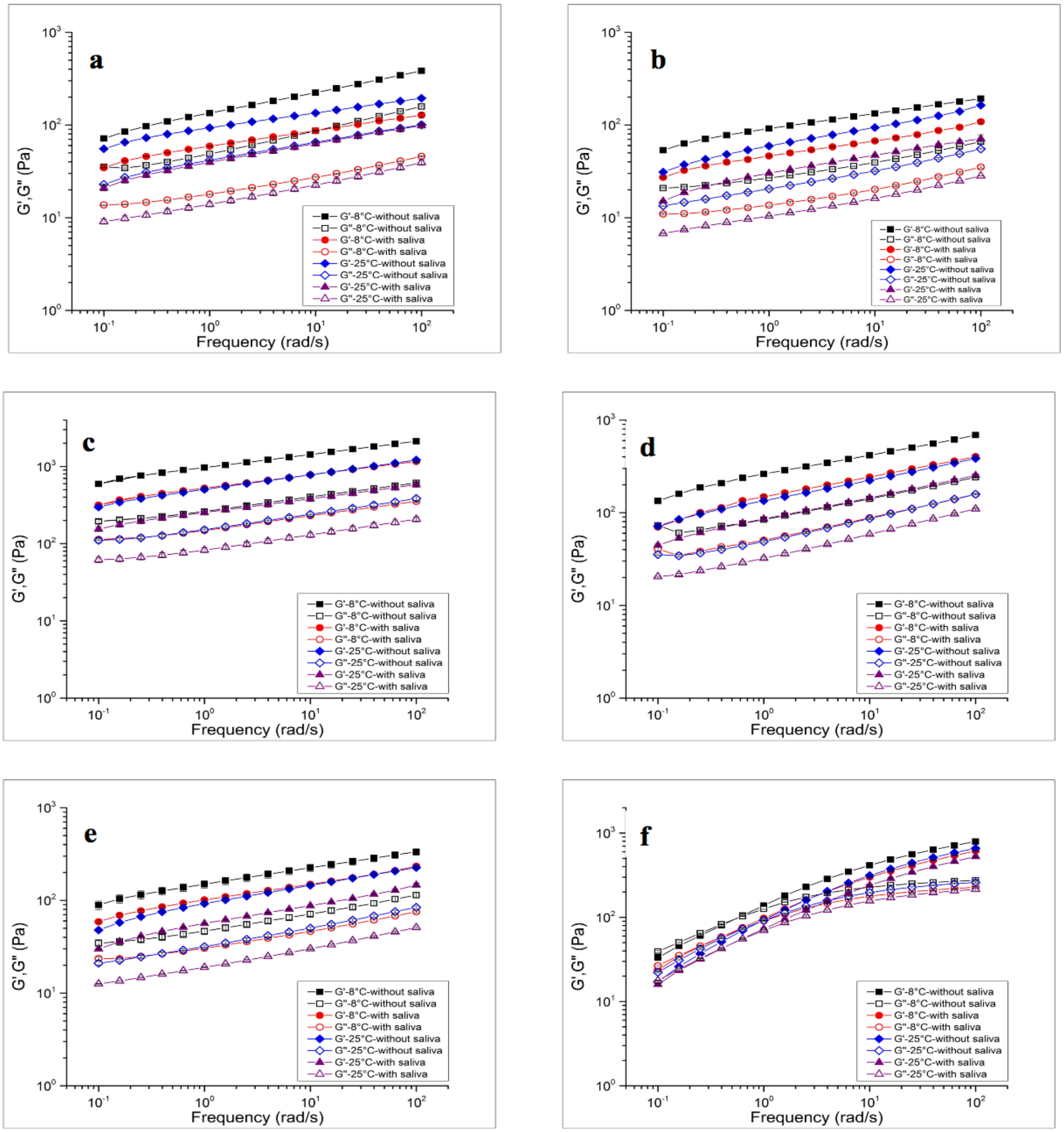
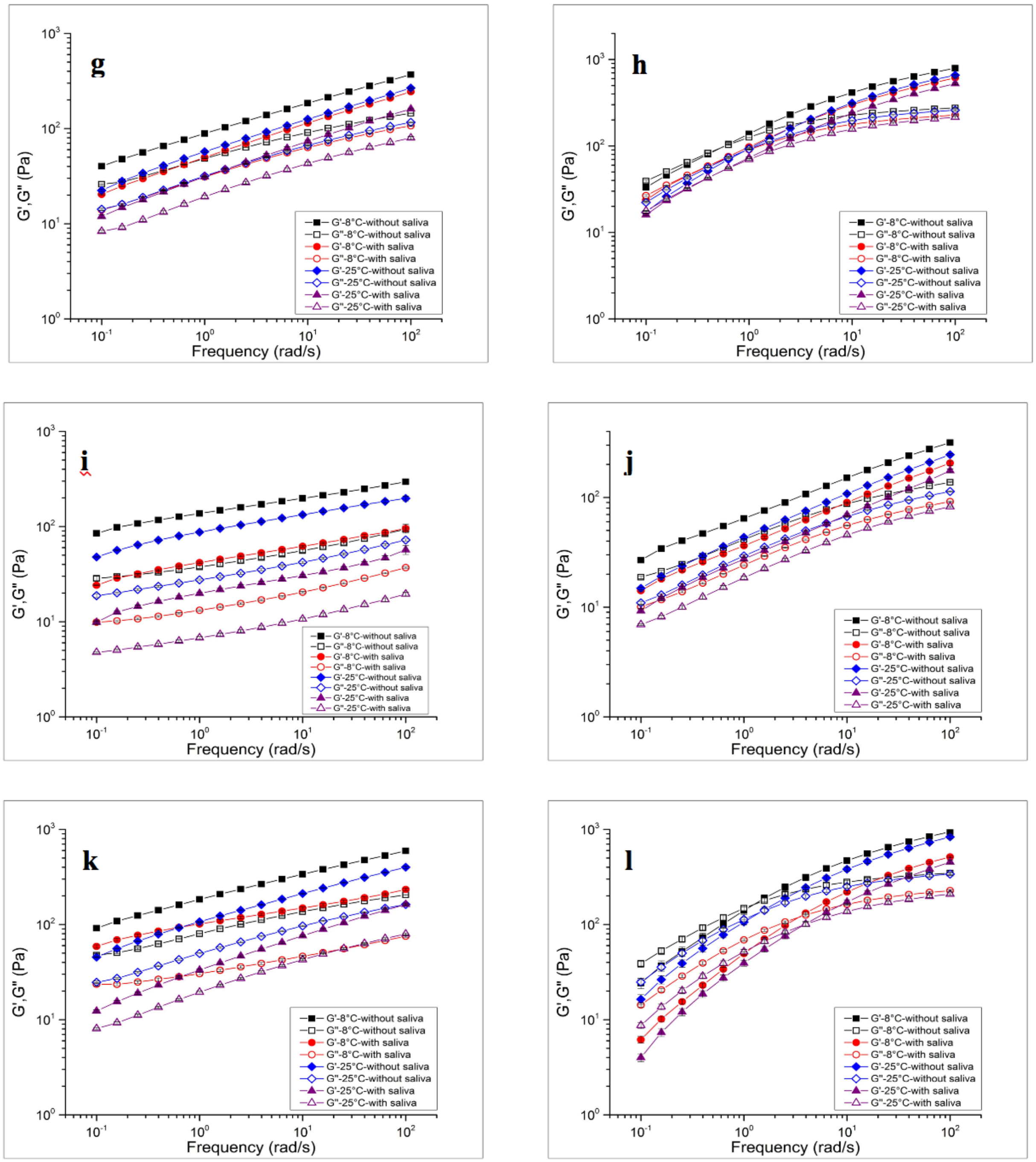


Figure A.3.3. Frequency sweep results of acid milk gels;
a) sample 2; b) sample 3; c) sample 5; d) sample 7; e) sample 9; f) sample 10; g) sample 11; h) sample 12; i) sample 13; j) sample15; k) sample 17; l) sample 18; m) sample 19; n) sample 21; o) sample 22; p) sample 24.



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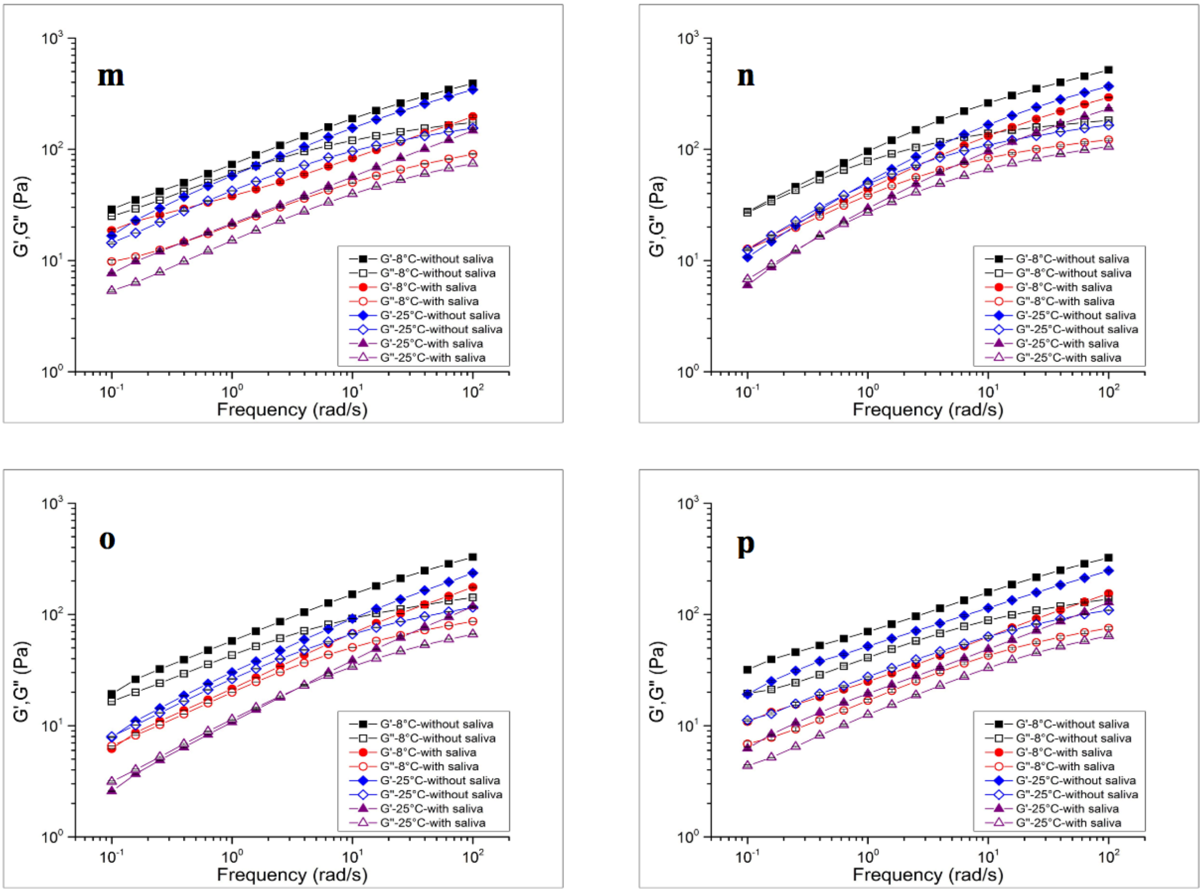


Continued from Figure A.3.3. Frequency sweep results of acid milk gels.

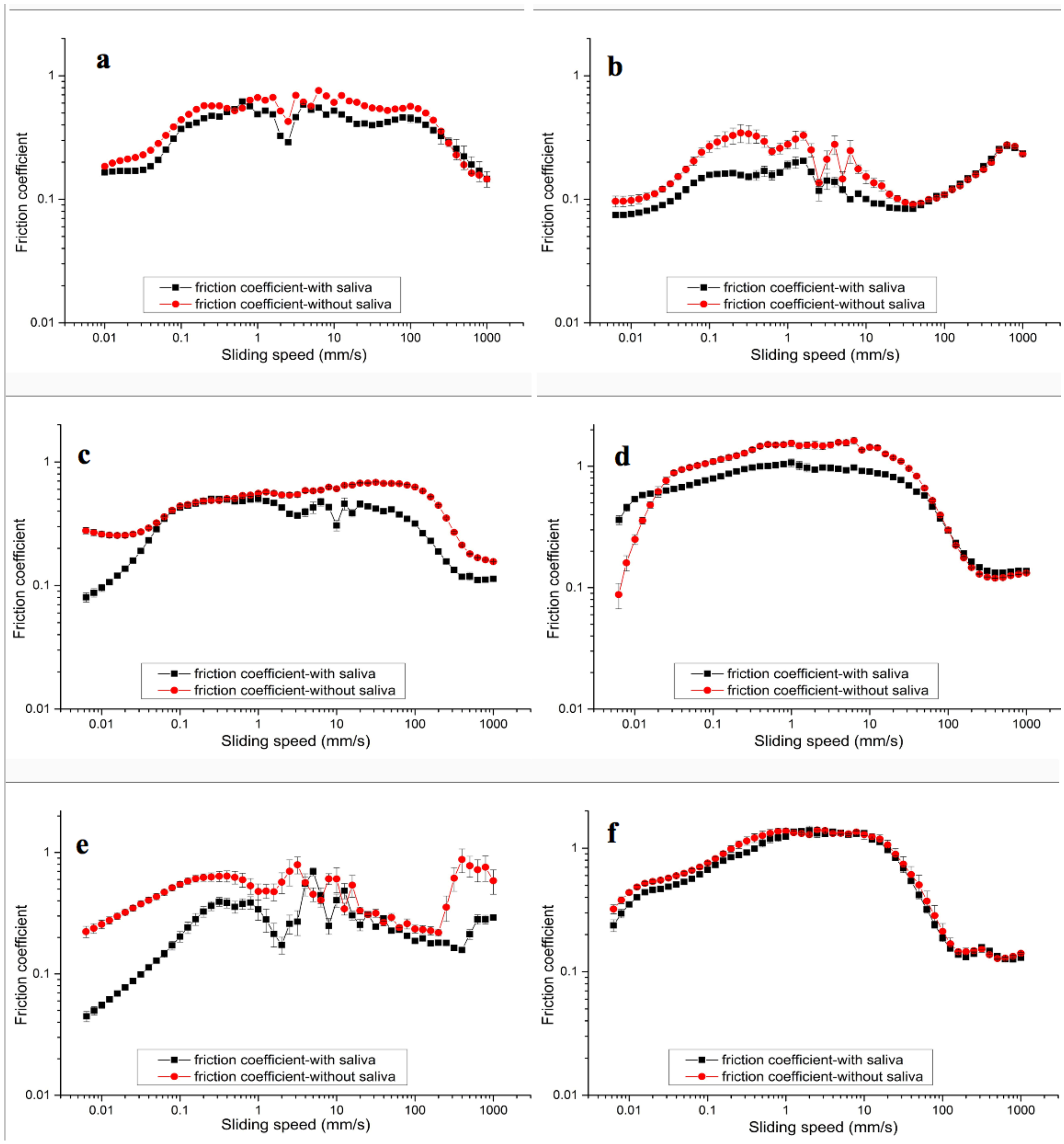
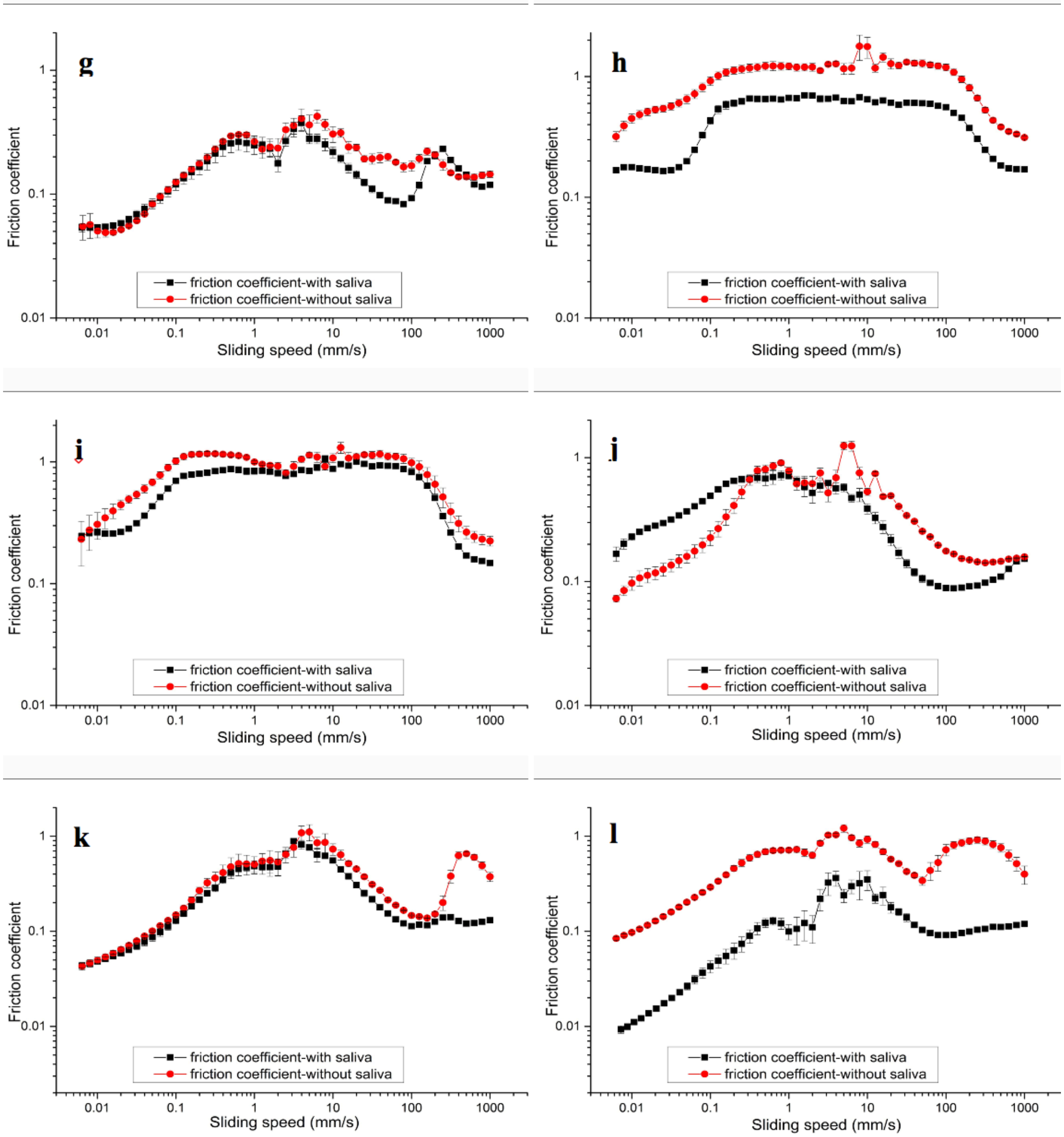


Figure A.3.4. Tribological results of acid milk gels;
a) sample 2; b) sample 3; c) sample 5; d) sample 6; e) sample 9; f) sample 10; g) sample 11; h) sample 12; i) sample 13; j) sample14; k) sample 15; l) sample 17; m) sample 18; n) sample 19; o) sample 20; p) sample 24.



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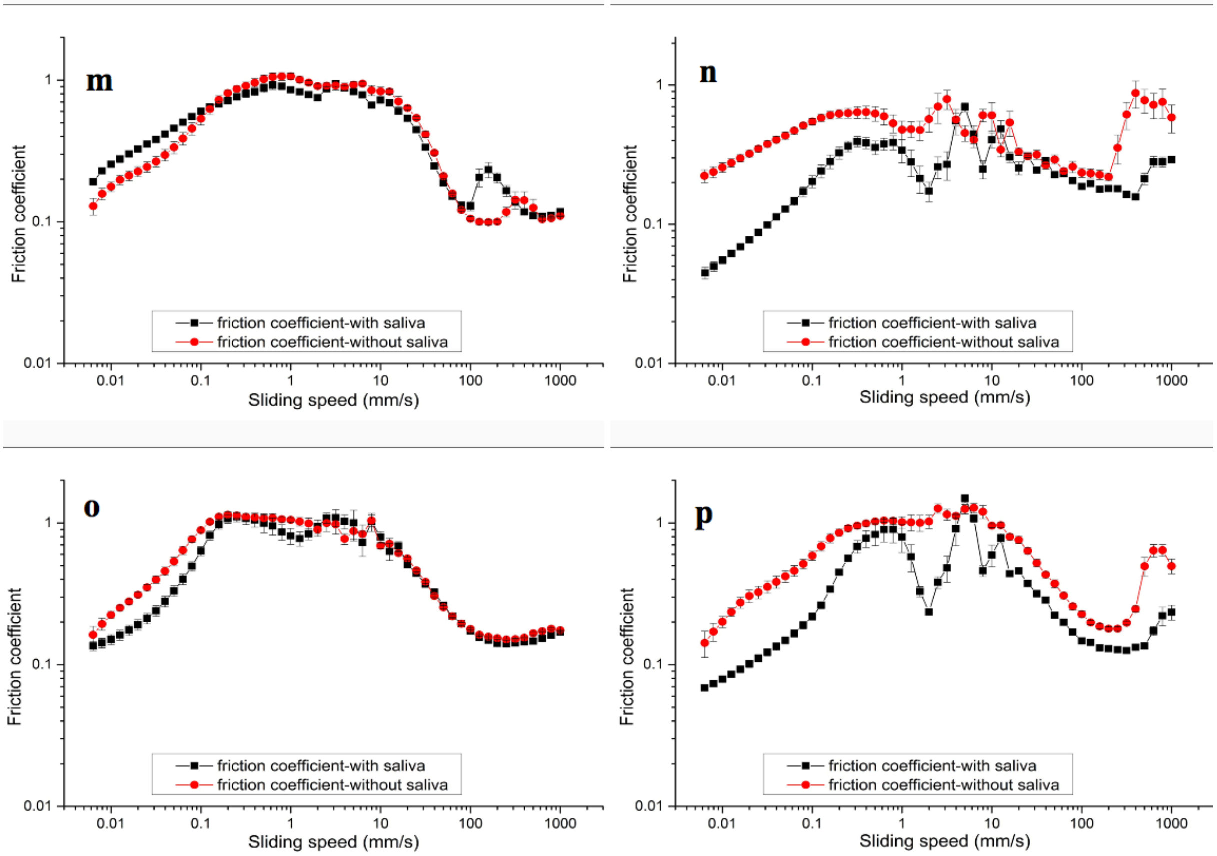

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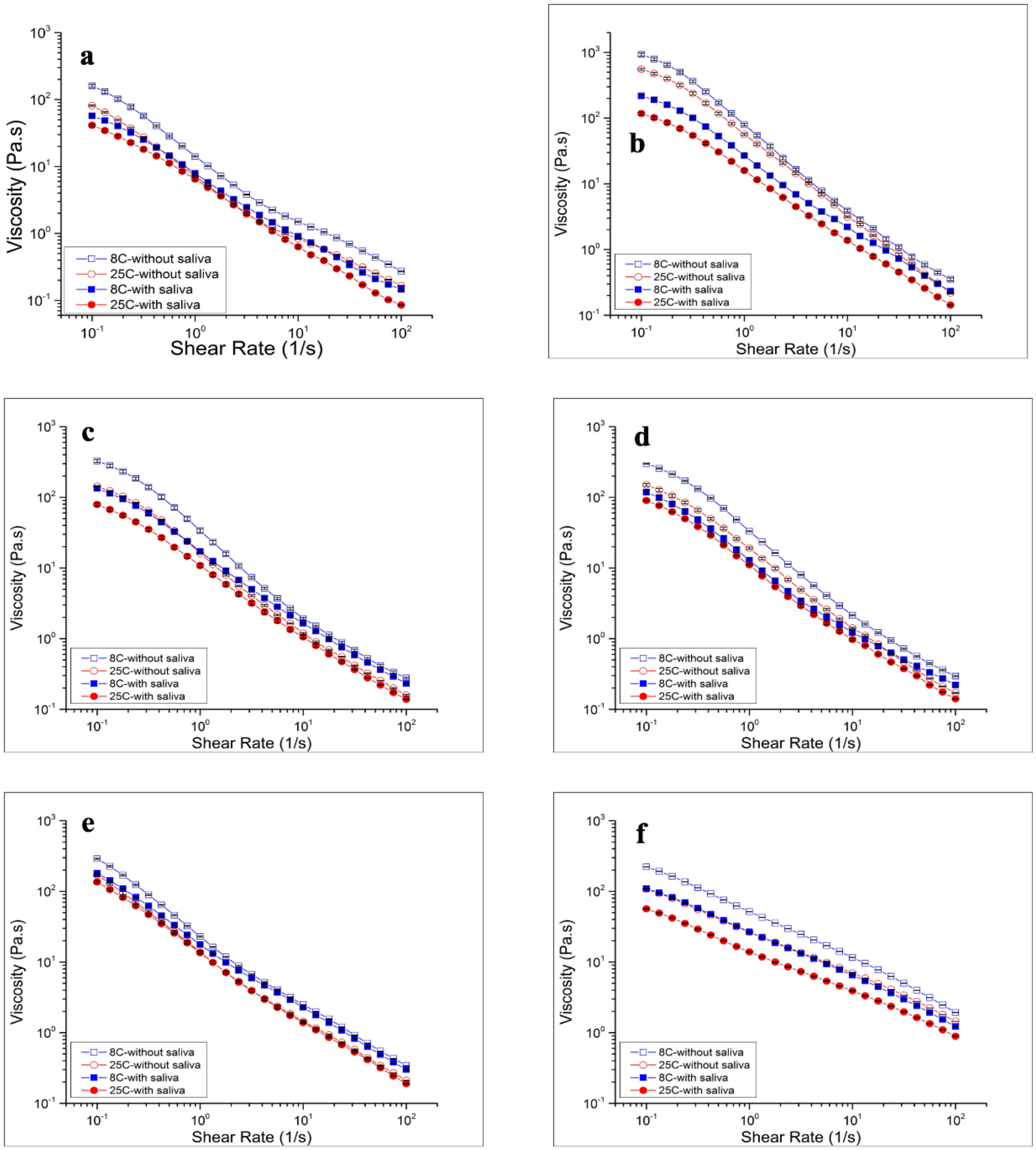
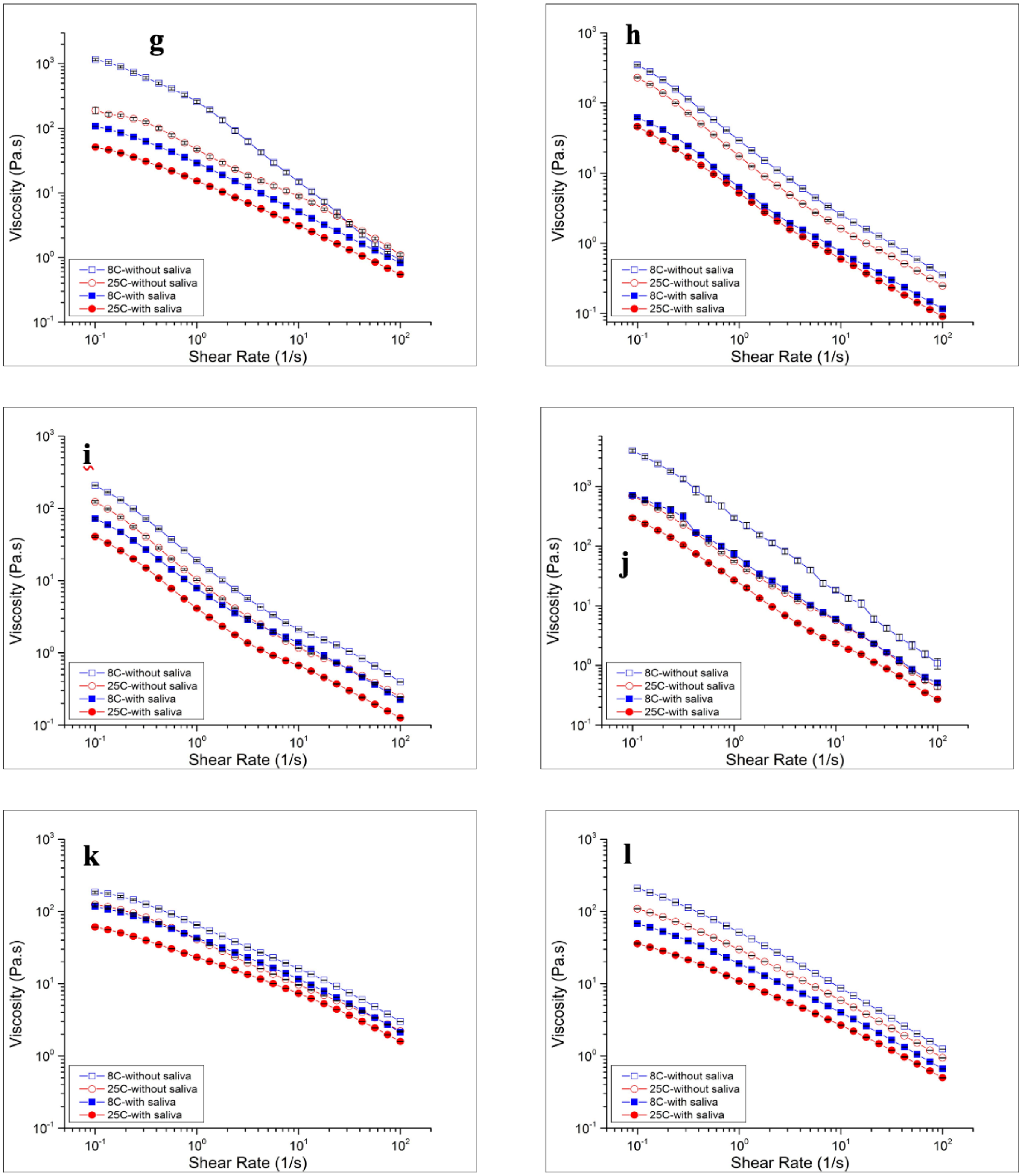


Figure A.5.1. Shear rate sweep results of yogurts;
a) sample 1; b) sample 2; c) sample 3; d) sample 4; e) sample 5; f) sample 6; g) sample 7; h) sample 8; i) sample 9; j) sample10; k) sample 11; l) sample 12.



Continued from Figure A.5.1. Shear rate sweep results of yogurts.

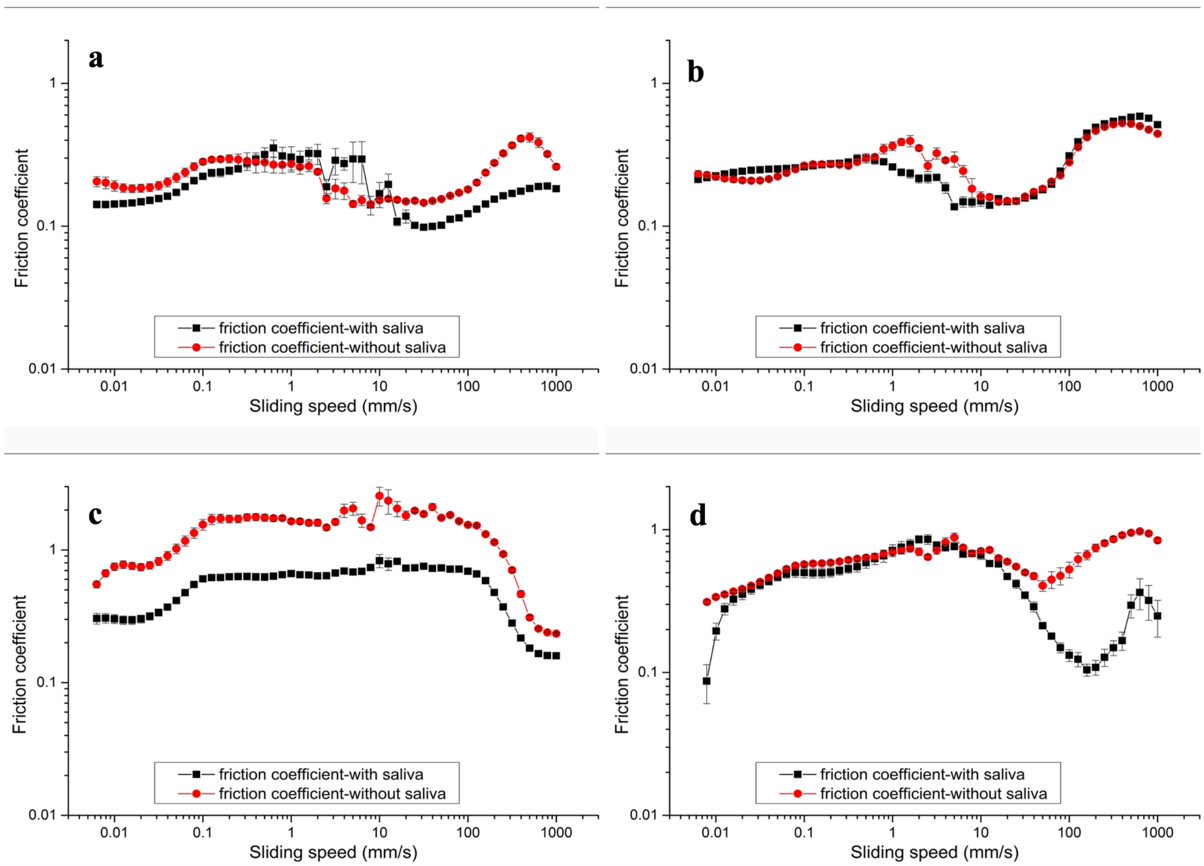


Figure A.5.2. Tribological results of yogurts;
a) sample 3; b) sample 2; c) sample 5; d) sample 11.

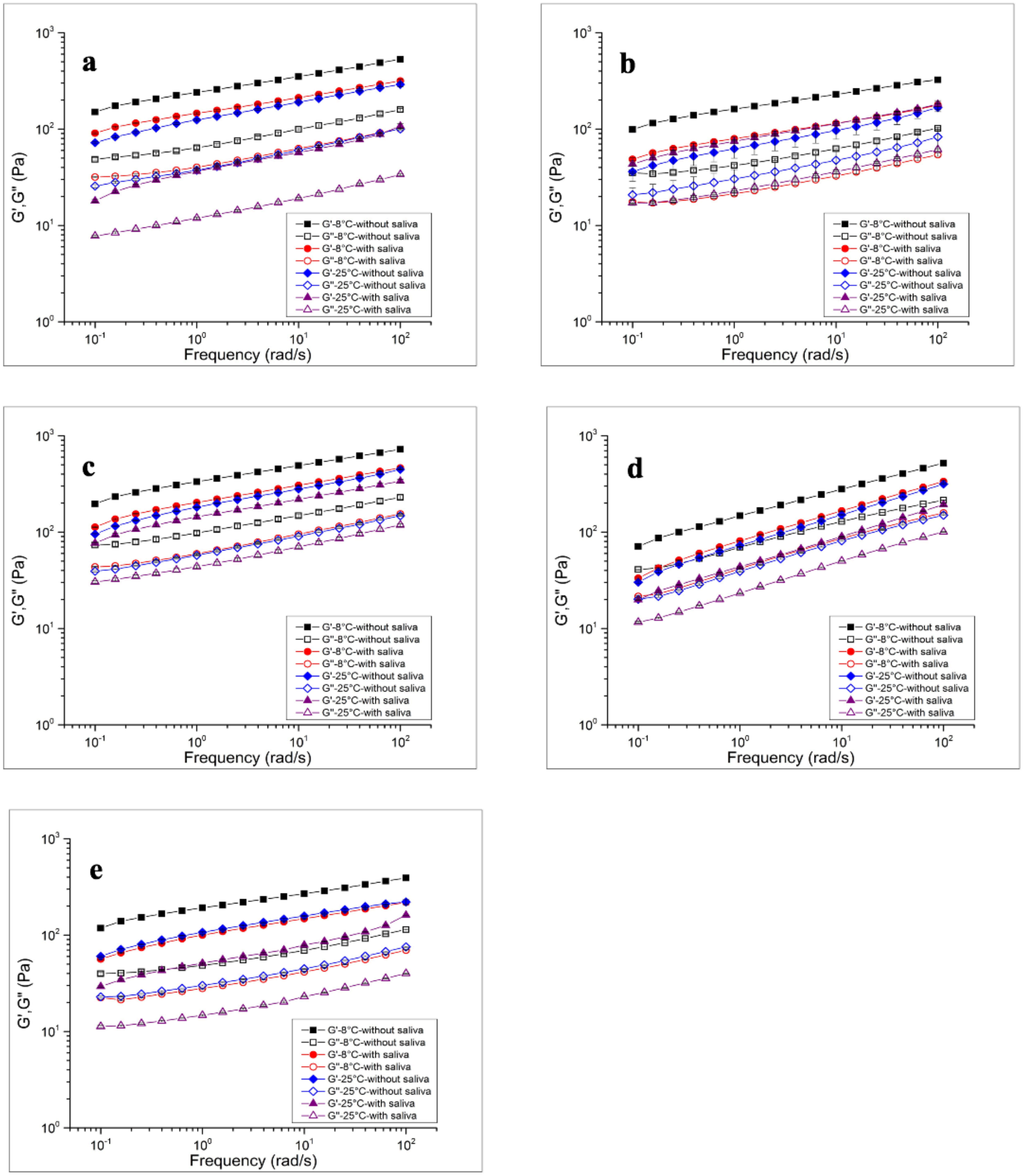


Figure A.5.3. Frequency sweep results of yogurts;
a) sample 2; b) sample 3; c) sample 5; d) sample 7; e) 9.

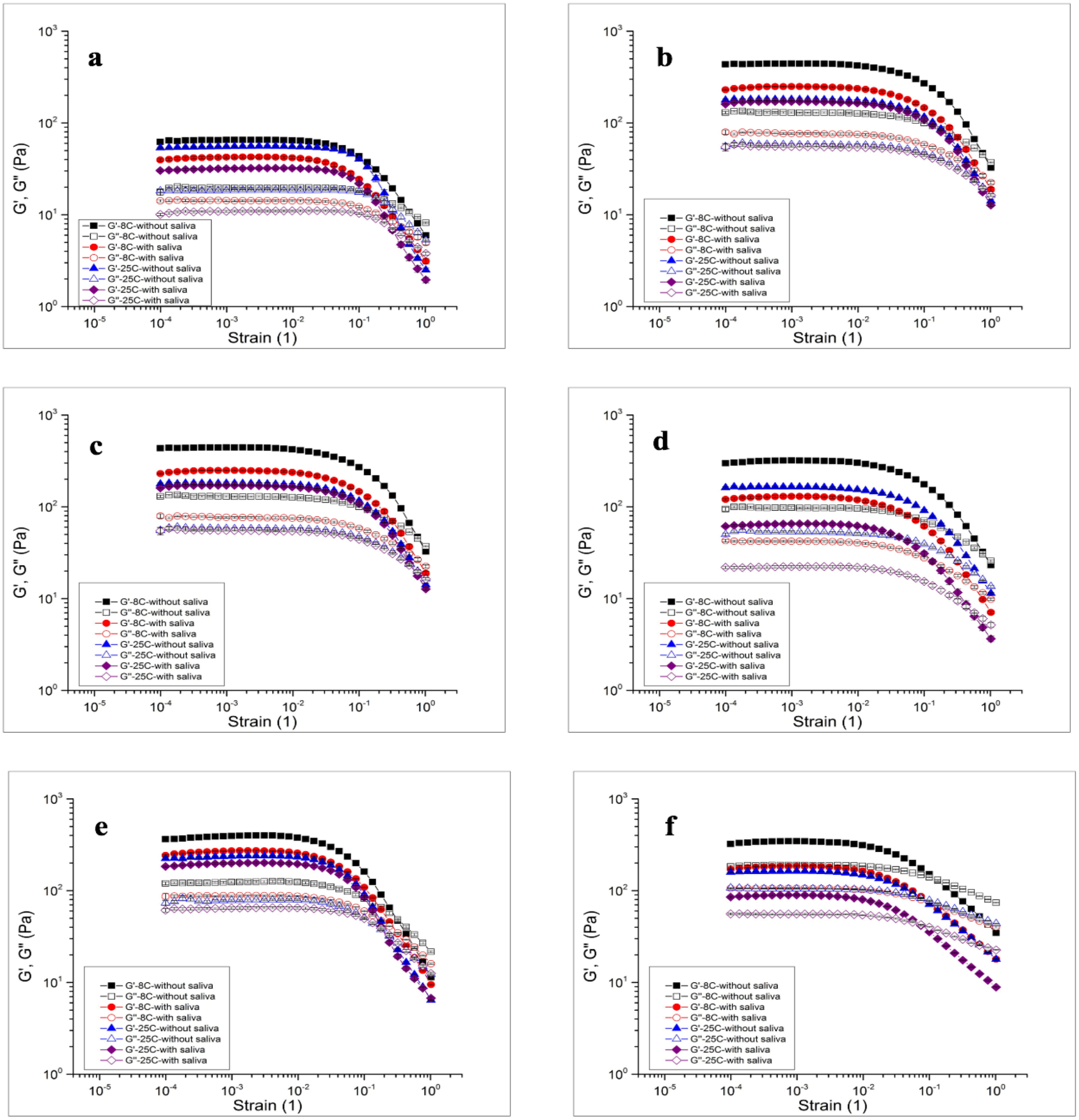
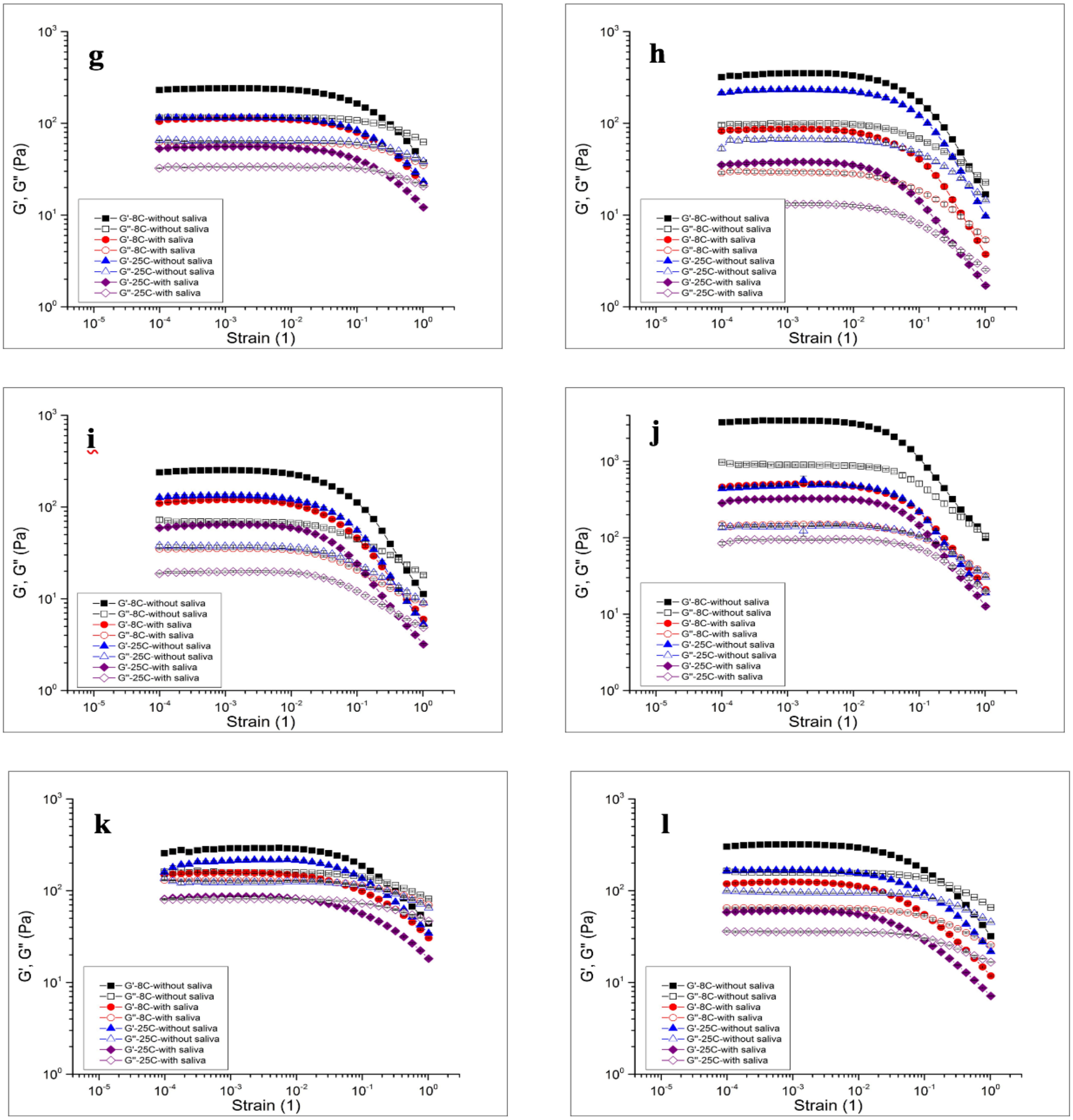


Figure A.5.4. Strain sweep results of yogurts;
a) sample 1; b) sample 2; c) sample 3; d) sample 4; e) sample 5; f) sample 6; g) sample 7; h) sample 8; i) sample 9; j) sample10; k) sample 11; l) sample 12.



Continued from Figure A.5.4. Strain sweep results of yogurts.

APPENDIX B: SUPPLEMENTAL FIGURES

Table B.3.1. HWS composition[1]

HWS samples	α-amylase	protein concentration
1-morning	32.1[a]	1.26[a]
2-afternoon	31.8[a]	1.24[a]
3-morning	31.7[a]	1.18[ab]
4-afternoon	31[a]	1.15[ab]
5-morning	30.8[a]	1.06[abc]
6-afternoon	30.4[a]	0.991[bcd]
7-morning	29.4[a]	0.905[dc]
8-afternoon	27.4[a]	0.816[d]

[1]Different letters in a given column indicate significant differences among HWS for protein concentration and α-amylase at *p*-value ≤ 0.05.