

The Impact of Formulation on the Rheological, Tribological, and Microstructural Properties of Acid Milk Gels



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1 Introduction

Rheometry and tribometry are techniques that have been used to indicate food texture attributes. Rheology is the study of mechanical properties of foods, e.g. flow and deformation, which are often important to functionality and texture attributes. For example, viscosity has been correlated with several textural attributes of semi-solid foods, such as mouth viscosity, sliminess, (creaminess), stickiness, and thickness (Stanley and Taylor 1993; Malone et al. 2003; Chojnicka-Paszun et al. 2014; Sonne et al. 2014). 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 generally not sufficient to completely predict the perception of friction-related textural attributes such as graininess, smoothness, and chalkiness. Tribology, the science of friction, lubrication, and wear, has become popular in food texture and oral processing studies due to its ability to simulate the rubbing of two oral surfaces, producing a friction-related sensation that is important for perceiving certain textural attributes, e.g., astringency and 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 three different regimes. First is the *boundary regime*, in which the 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

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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. 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 has been shown that astringency and slipperiness are mostly related to boundary regime (Prakash et al. 2013). Chapter 5, “[Semisolid Food Tribology](#)”, provides a more detailed explanation of Stribeck curves and how they may be used for developing relationships among food behaviors.

Besides mechanical and frictional properties, microstructural imaging can help describe the textural differences in semisolid foods with different formulations. For instance, addition of starch to semisolid foods 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 that showed swollen potato starch granules embedded in the acid milk gel protein network; the density of the protein matrix increased with increased potato starch concentration (Oh et al. 2007). Indeed, viewing microstructural changes among samples can help explain why textural attributes are altered with changes in formulation or processing.

Different types (e.g. gums, proteins, starches) 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 mechanisms of hydrocolloid behaviors in a food system depend on the physicochemical properties of the hydrocolloid as well as its origin. The effect of hydrocolloids on semisolid food has received significant attention in the literature (Chojnicka-Paszun et al. (2009), Janssen et al. 2007, Oh et al. 2007, Milani and Maleki 2012, Chojnicka-Paszun et al. 2014). 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, promoting astringency, chalkiness, and grittiness (Lucey and Singh 1997; Lee and Lucey 2010; Morell et al. 2016). Cellulose gum can 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). Other food ingredients can show similar sensitivity to components of HWS. Thus, considering the effect of HWS in predicting texture perception of semisolid foods is key for better interpretation of sensory data.

HWS is secreted during all the stages of oral processing. 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 (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 as well as the oral surfaces and food bolus during mastication (Chen and Engelen 2012). HWS can disrupt food structure by dilution, enzymatic hydrolysis,

and protein complexation. Salivary proteins, mainly mucin, can alter food structures by altering the net charge, potentially resulting in particle precipitation (Chen and Engelen 2012). Overall, α -amylase and protein concentration have been shown to have the greatest effect on texture perception of semisolid foods, particularly those that are starched-based (Engelen et al. 2007).

Temperature change is another factor that can affect texture perception of semisolid foods and needs to be considered during *in vitro* testing. Accordingly, controlling temperature, adding HWS, and using materials that mimic the oral surfaces during instrumental testing can provide a more accurate 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 formulation and HWS impact semisolid food microstructure, rheological, and tribological behaviors of acid milk gels at 25 °C and 8 °C. This study was the first step in understanding the microstructure–rheology–texture relationships in acid milk gels with different formulations. In a followup study (Chap. 11, “Among Acid Milk Gel Sensory, Rheological, and Tribological Behaviors”), selected textural attributes of the acid milk gels used in this study were measured and correlated to their rheological and tribological behaviors to create a better understanding of these relationships. The results of these two studies can be applied to targeted development of a wide variety of reduced or non-fat semisolid foods with desirable textural properties.

2 Materials and Methods

2.1 Materials

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

2.2 *Sample Preparation*

Twenty-four different formulations of acid milk gels were prepared with skim milk, SMP, cream, WPI, and hydrocolloids, including LBG, corn starch, potato starch, and CMC. Specific formulations are given in Table 1. Formulations were designed to maintain a total solids content of 13% w/w in the finished acid milk gels. After adding all the hydrocolloids 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 a water bath (Precision, Thermo Fisher Scientific, Waltham, MA, USA) at pasteurization temperature (85 °C) for complete dissolution. The mixture was then held at 85 °C for an additional 30 min without stirring. Subsequently, the mixture was homogenized at 5000 rpm for 1 min using a stand homogenizer (Polytron, Kinematica AG, NY, USA), and cooled to 42.2 °C for addition of GDL (1.1–1.55% w/w, Table 1). The mixture was incubated at 42.2 °C for 4 hr. 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 s before testing to remove lumps; preliminary testing showed that this preshear procedure did not impact sample rheology and improved data repeatability. Each sample was made in duplicate on different days and tested within 24 hr of production.

2.3 *Proximate Analysis*

All proximate analysis was conducted 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, USA), according to the method of AOAC (1999). Fat contents were determined only for samples with added cream using the Mojonnier method 989.05 (AOAC 1995a). Skim milk with negligible fat content was used for preparation of other samples. Hydrocolloids also had negligible fat content according to their specification sheets that were provided by their manufacturers. Therefore, fat content was considered zero for samples made without cream. Ash contents were determined by the method of AOAC (1995b), dry basis. Carbohydrate contents were calculated by difference.

Table 1 Experimental design of acid milk gel formulations

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

2.4 HWS Collection

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. Panelists were asked to refrain from eating and drinking anything except water 2 hr. prior to each collection. At the start of collection, panelists 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 HWS stimulation and expectorated their saliva into a 2-oz. cup. The first two expectorations discarded were into a discard cup. Fresh HWS was collected every 2 hr to minimize aging effects during testing. HWS was used for both rheological and tribological testing within 2 hr of collection for the testing, and any excess was discarded.

For testing samples with HWS, all HWS was pooled in a single container and mixed thoroughly. 0.5 ml of the pooled 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). Care was taken to minimize bubble formation in the HWS.

2.5 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. All tests were carried out at 25 °C and 8 °C with and without addition of human whole saliva (HWS) collected per Sect. 2.4. Samples were equilibrated to the test temperature for 60 s prior to testing. Each sample was tested in triplicate and results were averaged for data analysis. Samples were tested with and without addition of HWS.

Shear rate sweeps (0.01 – 100 s⁻¹) were carried out to measure acid milk gel viscosity profiles. Oscillatory tests were performed to measure acid milk gel viscoelastic properties. Strain sweeps were performed at 0.01–100% and a frequency of 1 Hz. Frequency sweep tests were performed at 0.1–100 rad s⁻¹ and 75% of the lowest critical strain calculated from strain sweeps (1.0%) so that tests would be performed in the linear viscoelastic region (LVR), using and 0.75% strain. Critical strain was calculated by determining the strain at which G^* deviated by >1% from the previous value within the LVR (Steffe 1996; Tunick 2010).

2.6 PDMS Plate Production

Polydimethylsiloxane (PDMS) gel plates were generated for tribometry using the method of Bongaerts et al. (2007a). Briefly, plates were made by mixing a curing agent and a base (Dow Corning Corporation, Midland, MI, USA) 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 placing them in a cabinet vacuum desiccator (Bel-Art Products, Wayne, NJ, USA) under a pressure of -90 kPag 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, USA), 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.

2.7 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 palate–tongue contact (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 an indelible laboratory pen to provide visual confirmation that the PDMS plate did not move during testing. Friction coefficient was measured at sliding speeds of 0.01–1000 mm s⁻¹. Samples were tested at 25 °C. At least three replicates for each sample duplicate were performed with and without HWS. The contact surfaces were cleaned after each run with 70% ethanol and laboratory wipes for non-fat samples. 70% ethyl ether followed by a 70% ethanol rinse was used to clean the contact surfaces when testing fat-containing samples to prevent fat film buildup on the contact surfaces. Both plates and balls were changed after every 6 runs to prevent wear from affecting the results (determined by preliminary testing).

2.8 HWS Analyses

The composition of HWS can significantly affect the texture perception of semi-solid foods. Protein concentration and α -amylase activity were reported to have the greatest impact among other components of HWS (Engelen et al. 2007). Accordingly, collected HWS (Sect. 2.4) was centrifuged at 10,000 rpm (14,087 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).

A Bradford protein kit was used to determine the protein concentration in HWS (Quick Start™ Bradford Protein Assay, Bio-Rad). Eight samples were collected from 5 healthy panelists within 2 wk. and tested in triplicate. The assay was performed according to the manufacturer's guidelines (Bio-Rad Laboratories, Inc. CA., USA). A microplate standard assay using bovine serum albumin (BSA) was used as a protein standard.

A modified Somogyi-Nelson assay was performed to determine salivary α -amylase activity (Shao and Lin 2018). Different concentrations of maltose, a source of reducing sugar, in a series of 0–0.6 mM was used to create the standard curve for the assay; maltose is one of the sugars produced by α -amylase from amylose and amylopectin from cleaving the starch chain. The curve was plotted based on absorbance as a function of sugar concentration with a linear relationship of

$R^2 = 0.97$. The procedure and mechanisms of this test are 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 a 1.7 mL capacity (Sorenson, BioScience, Inc., Salt Lake City, UT, USA). A dilution of 1:250 of HWS:DI water was made due to high α -amylase activity in HWS samples. 50 μL of the diluted HWS was added to each of 15 microtubes containing the starch solution. These microtubes were incubated at 37 °C for 0, 3, 5, 7, and 9 min. Samples were further diluted with DI water at a ratio of 1:5 sample:DI water for the reaction times of 3 and 5 min, and 1:7 sample:DI water for the reaction times of 7 and 9 min. Blank samples of HWS and no soluble starch were created as a control (zero reaction time). After incubation, 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 silicone 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 read at 600 nm using a microplate reader (Spectra Max 190 Microplate Reader, Molecular Devices, CA, USA). Considering the amounts of released reducing sugar from HWS samples and their protein concentration from Bradford assay, 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^{-1}).

2.9 Confocal Imaging

Microstructural properties of the acid milk gels were imaged 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 each acid milk gel mixture before addition of GDL. Samples were then stirred with a spatula to mix the dyes evenly. Samples were incubated for 4 hr. to reach a pH of 4.55–4.6 and refrigerated overnight; microscopy analysis was performed the next day. 500 μL of each acid milk gel sample was transferred to a cavity slide and covered with a glass coverslip. Samples were imaged at 20X and 4–8 °C with an excitation wavelength of 559 nm for FITC and 488 nm for Nile red.

2.10 Data Analyses

Rheological and tribological data 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 (Eq. 1), Cross (Eq. 2), and Herschel-Bulkley (Eq. 3) using TRIOS software (TA Instruments; New Castle, Delaware, USA).

$$\eta = \frac{\eta_o}{\left[1 + (c\dot{\gamma})^{1-n}\right]} \quad (1)$$

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

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

In the Cross-Williams model (Eq. 1), η_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 unity for Newtonian materials. In the Cross model (Eq. 2) η_o is the zero-shear rate viscosity (Pa.s), η_∞ is the infinite viscosity plateau (Pa.s); k is the time constant (s), and n is flow behavior index (unitless). In the Herschel-Bulkley model (Eq. 3), σ_o is the yield stress (Pa), the minimum force needed to induce flow. k is the consistency coefficient (Pa.s¹⁻ⁿ) and n is the flow behavior index (unitless). In shear-thinning materials ($0 < n < 1$), pseudoplastic behavior increases as n approaches zero. These model 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 $\tan \delta$ (phase angle, rad) were selected for statistical analysis. Friction coefficients at 10, 15, 20, 25, and 30 mm s⁻¹ sliding speeds were used for data analysis. This selection of speeds was to mimic the oral speed, reported to be 10–30 mm s⁻¹ for semi-solid foods (De Wijk and Prinz 2006). Data analysis included three-way ANOVA for determining the impact of formulation, HWS, and temperature on selected rheological and tribological, as well as ANOVA ($\alpha = 0.05$) followed by Tukey's HSD (Honest Significant Difference) test, which was used to determine significant differences among acid milk gel rheological, tribological, and proximate analysis results.

3 Results and Discussion

3.1 Acid Milk Gel Proximate Compositions

Acid milk gel proximate compositions showed significant differences for moisture, protein, ash, fat, and carbohydrate contents (Table 2). Total solids content was kept constant for all formulations at 13% w/w. The 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 the higher level of WPI addition. The moisture contents of the acid milk gels prepared with LBG (sample 6, 18) or all hydrocolloids except CS (sample 19) were the greatest amount among all acid milk gels. These results were

Table 2 Acid milk gel proximate composition^a

Samples	Protein (%)	Moisture (%)	Fat (%)	Ash (%)	Carbohydrate (%) ^b
1	4.51 ± 0.045 ^{de}	86.22 ± 0.043 ^{abcd}	0	0.63 ± 0.009 ^{bcd}	8.64 ^{efgh}
2	4.4 ± 0.054 ^{defgh}	85.87 ± 0.206 ^{abcde}	0.49 ± 0.008 ^a	0.58 ± 0.001 ^{def}	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	812.17 ± 0.015 ^{hg}	0	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 ^{bcd}	8.15 ^{hij}
20	4.46 ± 0.06 ^{defg}	85.47 ± 0.269 ^{cde}	0	0.62 ± 0.008 ^{cde}	9.45 ^{cdef}
21	12.19 ± 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	12.12 ± 0.014 ^{ijkl}	85.67 ± 0.481 ^{bcd}	0	0.62 ± 0.002 ^{bcd}	9.59 ^{cde}

^aDifferent letters in a given column indicate significant differences in that column at $p \leq 0.05$. Fat contents of non-fat samples were considered zero based on ingredient compositions rather than fat analysis

^bCarbohydrates were calculated by difference

attributed to the weak gel structure formed between LBG and 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. The sample prepared with CMC (sample 7) had the lowest moisture content. Differences in moisture content may have been 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 formulation. 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 inverted 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 may explain the differences in ash content.

3.2 Saliva Composition

The composition analysis of 8 HWS samples showed no significant differences ($p > 0.05$) among α -amylase activity ($U\ mg^{-1}$). However, significant differences ($p \leq 0.05$) were found for protein concentration (Appendix A). It was difficult to relate these differences to any variations in rheological, tribological, and microstructural imaging, as the time needed to test the HWS samples (over 1 hr) resulted in the samples aging past the point where they could be used for rheological and tribological testing. Thus, the samples used for compositional analysis were not the same samples used for rheological and tribological testing. On the other hand, the samples tested with added HWS were held for 5 min after mixing with saliva and before testing. This hold time would compensate for any differences in reaction rates due to HWS compositional differences, rendering any effects from compositional difference negligible.

3.3 Acid Milk Gel Microstructural Properties

Overall, all acid milk gel microstructures comprised a particulate protein network containing serum; the specific conformation of the protein network structure was dependent on acid milk gel formulation (Figs. 1, 2, and 3). The control sample (sample 1) showed a more homogenous protein network with smaller pores sizes (Fig. 1a). The branches of protein network became thicker and the size of the open-

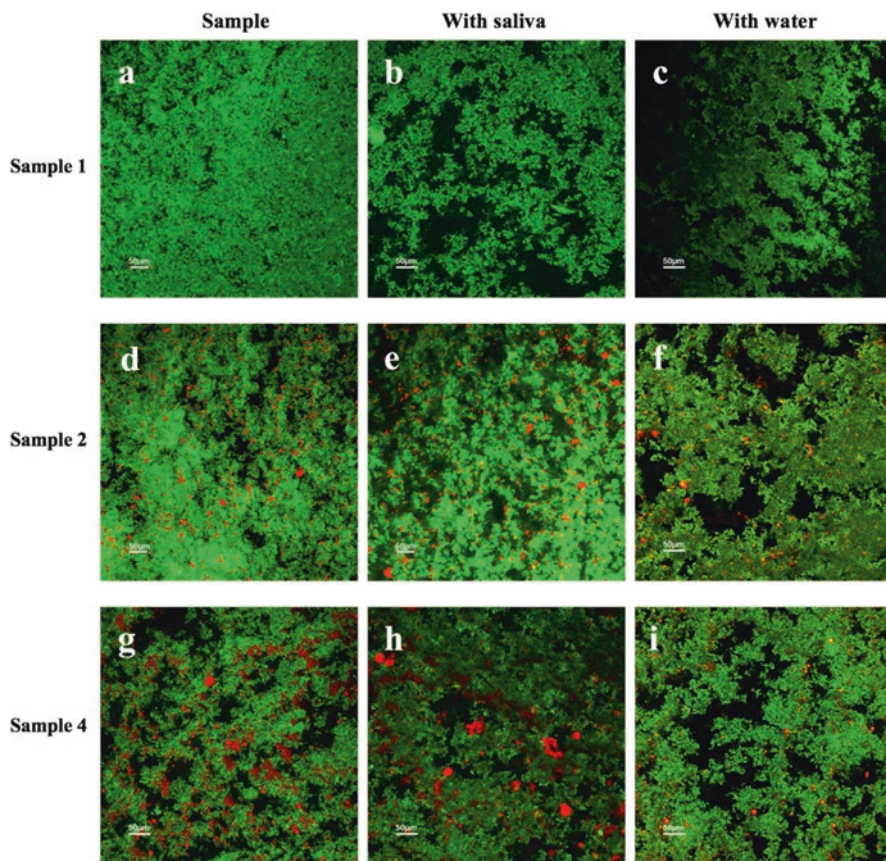


Fig. 1 CLSM results of acid milk gels; (a) sample 1; (b) sample 1: with HWS; (c) sample 1: with water; (d) sample 2; (e) sample 2: with HWS; (f) sample 2: with water; (g) sample 4; (h) sample 4: with HWS; (i) sample 4: with water. The protein network, fat globules, and serum pores are shown in green,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

ings (aqueous phase) increased with addition of hydrocolloids. However, the micro-structural features differed with the specific hydrocolloid used in the formulation. The density of the protein structure for samples with PS (sample 8, Fig. 3a) decreased and the aqueous phase increased compared to the control. However, the sample with CS (sample 9, Fig. 3d) had smaller pores and was more homogenous and similar to the control sample. Although the branches of the protein network for the sample with CMC (sample 7, Fig. 2g) were 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 (Table 2). The thickest and largest clusters in the protein network were shown in the sample with all hydrocolloids (sample 24, Fig. 3g), indicating that addition of all hydrocolloids caused the most notable differ-

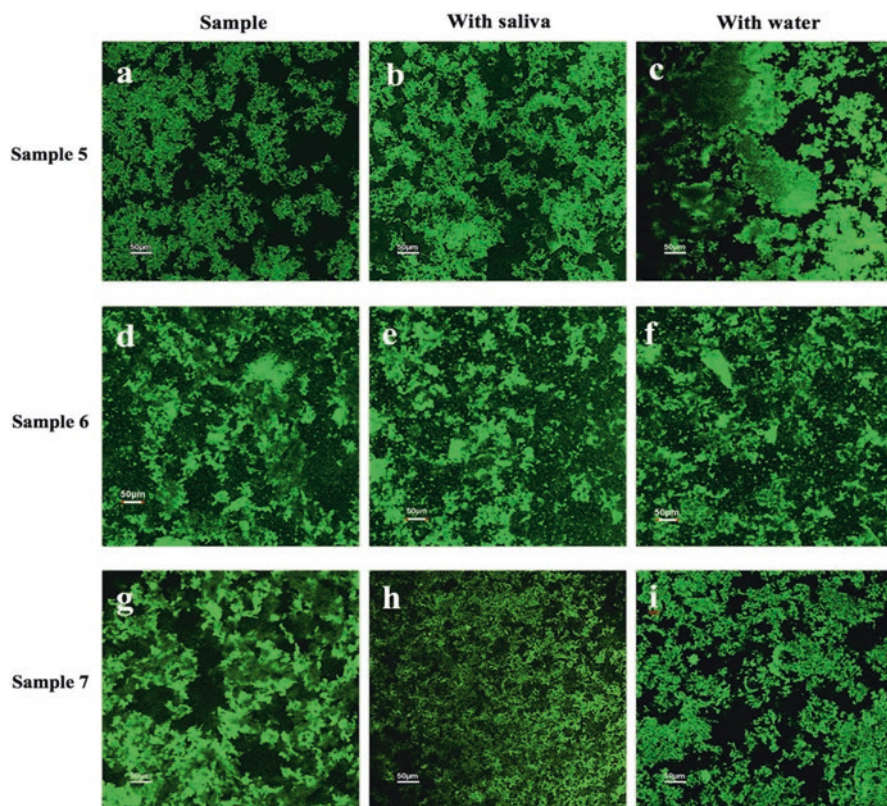


Fig. 2 CLSM results of acid milk gels; (a) sample 5; (b) sample 5: with HWS; (c) sample 5: with water; (d) sample 6; (e) sample 6: with HWS; (f) sample 6: with water; (g) sample 7; (h) sample 7: with HWS; (i) sample 7: with water. The protein network and serum pores are shown in green and black, respectively

ences in the protein matrix compared to the control due to different interactions of hydrocolloids with protein network.

In general, addition of water did not have as notable an effect on the microstructure as addition of HWS. More dark areas appeared for several of the samples upon addition of water, which was expected since this area corresponded to the serum phase. However, samples with added LBG (sample 6), CMC (sample 7), and PS (sample 8) did not show notable differences in the amount of serum present (Figs. 2 and 3). This result may have been due to the water-holding ability of these hydrocolloids, which would prevent larger serum pools from forming upon addition of water.

Addition of HWS generally increased protein aggregation and amount of free serum, regardless of formulation. Samples with added HWS showed more distinct protein clusters compared to samples with added water. This result was attributed to the digestive, dissolving, and coalescing effects of HWS caused by enzymes,

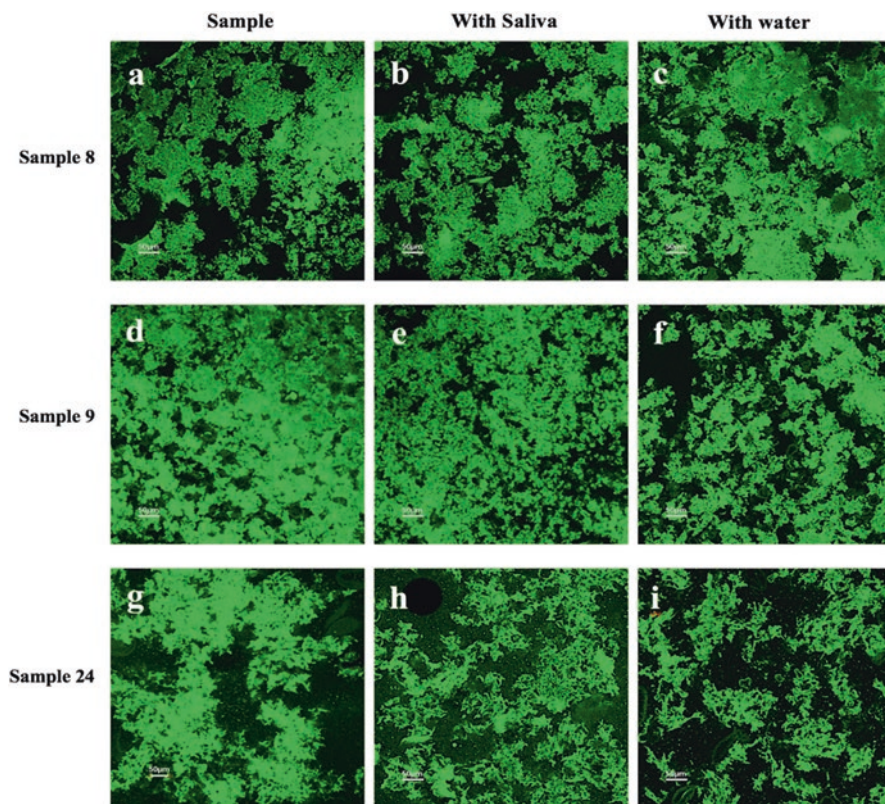


Fig. 3 CLSM results of acid milk gels; (a) sample 8; (b) sample 8: with HWS; (c) sample 8: with water; (d) sample 9; (e) sample 9: with HWS; (f) sample 9: with water; (g) sample 24; (h) sample 24: with HWS; (i) sample 24: with water. The protein network and serum pores are shown in green and black, respectively

salivary proteins (e.g. mucins) and electrolyte presented in HWS but not water. However, samples with added CS (sample 9, Fig. 3e) did not show many structural changes upon addition of HWS. This result was interesting considering that adding of HWS significantly impacted the structure of samples containing PS (sample 8, Fig. 3b). Amylose content can decrease the enzymatic digestion 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 reason for the reduced effects of HWS on samples with CS compared to those containing PS since the amylose content in CS is higher than that of PS. HWS showed the least effect on the sample with LBG (sample 6, Fig. 2e). This minimal effect can also be observed in rheological and tribological results in agreement with Zinoviadou et al. (2008).

HWS also had a significant impact on fat globule size: addition of HWS caused coalescence of fat globules. Fat coalescence was most visible in the full-fat acid milk gel with added HWS (sample 4, Fig. 1h). 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 in the fat-containing samples (samples 2 and 4, Fig. 1). Overall, the changes to acid milk gel microstructures caused by HWS were distinctly different from those caused by addition of water, indicating that salivary changes to food products were more than simple dilution and incorporation of saliva during instrumental testing may give a better understanding of how saliva changes food rheological behaviors during oral processing.

3.4 Acid Milk Gel Flow Behaviors

All samples showed pseudoplastic behavior regardless of formulation or addition of HWS (Tables 3, 4, 5, and 6). Pseudoplastic behavior occurs when the rate of structural breakdown from external forces exceeds the rate of formation of internal entanglements, reducing the number of internal molecular interactions and resulting in decreased viscosity (Morris et al. 1981). Pseudoplastic behavior is typical in yogurt systems, particularly in yogurts prepared with hydrocolloids, which are known for their shear-thinning behavior.

The averaged viscosity profiles from 6 replicates of each formulation were individually fitted to Cross-Williams ($R^2 > 0.813$), Cross ($R^2 > 0.720$), and Herschel-Bulkley ($R^2 > 0.692$) models. The Cross model is a popular model for pseudoplastic materials that is extensively used in food dispersions and polymers. In this model, zero-shear viscosity (η_o) is the viscosity at low shear rates, which shows a Newtonian plateau. η_o can be measured at low shear rates and indicates the resistance of a material to flow at rest (Mezger 2011). Infinite viscosity (η_∞) can be used to determine food flow behaviors under high shear conditions, such as those experienced during industrial processing. This parameter is considered zero in Cross-Williams model.

For the formulations fitted to the Cross model, η_o and η_∞ decreased with increased temperature and addition of HWS. Interestingly, the decrease in η_o due to application of HWS was greater than that caused by increased temperature. This may indicate that addition of HWS caused a greater reduction of protein entanglements in acid milk gels compared to the reduction promoted by greater molecular mobility at increased temperatures. Samples with WPI (samples 5, 12, and 16) had the highest η_o values. This was attributed to the greater number of casein–whey protein interactions 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, which was expected based on the previously discussed microstructural changes. c has been attributed to the extent of entanglement density in a system (Bourbon et al. 2010). Acid milk gel structures were denser with more entanglements in the protein net-

Table 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_o (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

work before applying HWS and/or increasing temperature (Figs. 1, 2, and 3). Because the freedom of movement for individual protein strands becomes more restricted by increasing time, the strands would require longer time to form new entanglements to replace the ones depleted by the external force (Bourbon et al. 2010). This was reflected in the higher c values for samples tested at lower temperature and without HWS.

Similar trends for the viscosity parameters were observed for 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. 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. 1997). Full-fat samples (sample 4) also had a notably high value for η_o . Fat globules interact with the protein network, resulting in a stronger gel. For samples with no added HWS, η_o increased in full-fat acid milk gels (sample 4)

Table 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_o (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

compared to the control acid milk gel (sample 1) in the Cross model. For samples with added HWS, σ_o in the Herschel-Bulkley models was noticeably higher in the full-fat acid milk gel compared to the control sample. The bigger size and greater number of fat globules in the full-fat sample compared to the control sample would cause increased resistance to flow (Chojnicka-Paszun et al. (2009) 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 (Figs. 1, 2, and 3).

Samples containing CMC (sample 7), PS (sample 8), and both WPI and PS (sample 12) were fitted to the Cross model and had $\eta_\infty > 0$; these results were attributed to greater gel strength under high shear compared to the other samples. 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 ability of CMC to prevent casein precipitation and maintains a higher viscosity at the isoelectric point of casein (pH = 4.6) (Alakali et al. 2008; Andiç et al. 2013).

Table 5 Viscosity profiles for acid milk gels ($n = 24$) at 25 °C without added HWS

Formula	Model	η_0 (Pa.s)	η_∞ (Pa.s)	n	c (s)	k (Pa.s ¹⁻ⁿ)	σ_0 (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

The greatest effect of HWS was seen in samples with PS (sample 8) and both WPI and PS (sample 12). The η_0 value in sample 12 decreased by >60% of the original value when HWS was added at both 8 and 25 °C. In sample 8 with added HWS, this decrease was >33% at 8 °C and ~50% at 25 °C. The key driver for this result was PS. α -amylase in HWS breaks down starch to smaller monosaccharides (Humphrey and Williamson 2001). The greater impact of PS on viscosity parameters compared to CS (sample 9) after addition of HWS may have been due to the larger granule size of PS and its higher swelling power, higher solubility, and 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-Bulkley model. As expected, k decreased with both addition of HWS and increased temperature for all samples. A smaller stress was needed to deform the acid milk gels under these conditions since the higher temperature and/or HWS addition disrupted their structure. While the values of k are related to viscosity, using this parameter alone to determine sample viscosity is not advised because it neglects shear thinning behavior. Still, it serves as a criterion for comparison when the degree of shear thinning among samples is similar.

Table 6 Viscosity profiles for acid milk gels ($n = 24$) at 25 °C with added HWS

Formula	Model	η_0 (Pa.s)	η_∞ (Pa.s)	n	c (s)	k (Pa.s ¹⁻ⁿ)	σ_0 (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

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, with the exception of temperature not significantly affecting n values (data not shown). Interaction of HWS and temperature showed significant influence on values of c ($p < 0.05$), n ($p < 0.05$), and η_0 ($p < 0.01$). The significant effect of formulation was attributed to a combination of electrostatic bonds between oppositely charged molecules of anionic hydrocolloids with casein micelles, swollen starch granules throughout the system, and dispersion of large, neutral hydrocolloid molecules in the continuous phase, as well as depletion flocculation. These factors can significantly change the protein network structure and the overall conformation of acid milk gels (Figs. 1, 2, and 3). The significant effects from HWS can be explained by microstructural disruption and breakdown caused by salivary enzymes, proteins and electrolytes. Temperature can weaken the intermolecular bonds in a semisolid food system, decreasing resistance to flow (Berk 2018).

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 speed and extent of oral movements would result in decreased oral viscosity of the acid milk gels. Addition of hydrocolloids and HWS significantly affected acid milk gel viscosity parameters. These parameter changes can provide useful information about how flow behaviors change under different conditions. Because these rheological properties can be related to oral processing actions as well as correlated with sensory attributes such as thickness, tuning acid milk gel flow behaviors by adjusting their structures is a feasible way to create products with desirable oral and industrial processing behaviors.

3.5 Acid Milk Gel Viscoelastic Behaviors

Formulation, HWS, temperature, and all their interactions showed significant differences ($p \leq 0.001$) for G^* (Table 7). Formulation and HWS had significant effects on $\tan \delta$ ($p \leq 0.001$). Temperature and formulation significantly impacted γ_c ($p \leq 0.001$); HWS impacted γ_c ($p \leq 0.05$). These results were attributed to increased microstructural stability and rigidity when hydrocolloids were added to the samples. Gums (CMC and LBG) and starches (PS and CS) improve gel stability and rigidity by increasing the number of internal molecular interactions as well as promoting stronger bonds through different mechanisms. HWS can disrupt the structure of semi-solid foods through digestion, alteration of osmotic pressure, dilution, or alteration of polymer net charges. Increasing temperature alters the thermodynamic condition of materials. Internal molecules can move faster with increased heat energy, potentially decreasing the strength of molecular bonds. As a result, microstructures become more susceptible to deformation when mechanical force is applied.

Strain sweep results showed γ_c values varied significantly with formulation, temperature, and HWS. The significance level of HWS was higher than formulation and temperature, indicating that formulation and temperature had a greater impact on γ_c values compared to HWS. In other words, HWS had less effect on disrupting the stability of the acid milk gels compared to temperature and formulation.

Addition of hydrocolloids significantly increased γ_c values (Table 8). Samples 11 (containing WPI and CMC), 21 (containing LBG, CMC, CS, and PS), and 17 (containing PS and CMC) showed the highest γ_c values, and samples 1 (control), 20 (containing WPI, CMC, PS, and CS), 13 (containing WPI and CS), and 4 (full-fat sample) had the lowest γ_c values. Oppositely charged bonds between the surface of casein micelles and hydrophilic ends of CMC (Everett and McLeod 2005) along with increased bonds between denatured–denatured, and denatured–undenatured whey proteins due to presence of additional whey proteins from WPI would result in a more stable gel in sample 11 (Lukey et al. 1998). Thus, there was a larger force required to cause permanent structural deformation. In sample 22, the strong bonds between the caseins and CMC, along with the contribution of starches and LBG that increased the viscosity of the continuous phase, resulted in a high γ_c compared to that of the control sample. The combination of PS and CMC appeared to build a

Table 7 Main sources of variation of viscosity parameters and viscoelastic properties of acid milk gels ($n = 24$)^a

Source of variation	η_0 (Pa.s)	n	c (s)	γ_c	G^* (Pa)	$\tan \delta$ (rad)
Formulations	79.2***	97.5***	70.6***	65.4***	589.9***	19.4***
HWS	156.7***	339.1***	41.9***	4.7*	798.5***	19.8***
Temperature	60.7***	172.5***	3.5	27***	302.5***	3
HWS \times temperature	11.5**	5.3*	4.7*	0.1	16.2***	1.5
Formulation \times HWS	18.3***	18.3***	7.1***	1.9	100.6***	1.8
Formulation \times temperature	7.3***	6.9***	3.9***	1.1	27.4***	1

^a *, **, and *** indicate significant differences at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively

strong, stable gel in sample 17 whereas in sample 20, their combination with WPI and CS significantly decreased the resistance to permanent deformation.

Addition of fat had low effect on γ_c values in comparison to hydrocolloids. Increasing temperature generally increased γ_c values. This can be explained by thermodynamics occurring in acid milk gels. Heat can increase internal energy and molecular mobility, which increases fluid-like behavior. Therefore, greater instrumental stress was required to cause permanent and irreversible deformation. Addition of HWS showed different results on γ_c values depending on formulation. γ_c decreased upon addition of HWS for samples 6 (containing LBG), 8 (containing PS), 9 (containing CS), 14 (containing WPI, CMC, and PS), and 19 (containing WPI, CMC, LBG, and PS). HWS can easily disrupt the dispersion of neutral LBG molecules in the continuous phase, as there are no electrostatic bonds between LBG molecules and caseins. This disruption results in depletion flocculation and changes the osmotic pressure in the system, subsequently decreasing viscosity. The decrease in γ_c values for samples containing starches may have been due to starch digestion by amylase, which can disrupt the gel microstructure and decrease their stability and strength. This effect was stronger for PS (used in sample 8) due to its larger granules compared to CS (used in sample 9). γ_c showed no notable change after addition of HWS for several formulations. These samples were mainly those with strong bonds such as CMC and caseins, WPI, or a combination of these interactions with other gums. Saliva would not have a major impact on the number and strength of these bonds, so the structure and therefore critical strain would remain unchanged.

G^* values at critical strain for all samples decreased with addition of HWS, increased temperature, or both. $G' < G''$ for most formulations with and without added HWS for all strains tested (0.01–100%) (Table 8). Thus, these acid milk gels were viscoelastic solids in this strain range. Accordingly, $\tan \delta < 1$ for all samples without added HWS except for samples 6 (containing LBG) and 23 (containing all hydrocolloids but WPI). Additionally, $\tan \delta > 1$ for samples 6 (containing LBG) and 22 (containing all hydrocolloids but CS) when HWS was added. This viscous-dominant behavior of LBG has been also reported by (Perrechil et al. 2009). Additionally, these results were expected since these samples had relatively low G^* values even at 8 °C; the additional energy at increased temperatures would alter 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). Similarly, addition of HWS would weaken the microstructure, promoting viscous flow.

In general, increasing temperature and addition of HWS to the formulations increased $\tan \delta$ values, but most acid milk gels still showed viscoelastic solid behavior. These results indicated that the mechanical forces applied at strains below γ_c were insufficient to overcome the intermolecular forces among the molecules within the acid milk gel microstructures, so samples stored more energy than they dissipated at these strains.

G^* values decreased with increasing temperature, HWS addition, or both. Applying both higher temperature and HWS resulted in the lowest G^* values. Nevertheless, samples generally maintained viscoelastic solid behavior upon addition of HWS, suggesting that the elasticity of the protein network was not strongly disrupted by HWS. The decrease in viscoelastic moduli with increased temperature was attributed to increased energy in the acid milk gel molecular structures. At higher temperatures, molecules have increased kinetic energy that can overcome the intermolecular bonding forces, allowing the samples to flow more easily. Addition of HWS can also disrupt food structures due to dilution, mucin interactions, and enzymatic breakdown of amylose by amylase (Janssen et al. 2007; Vingerhoeds et al. 2009).

Several acid milk gel samples showed a crossover between G' and G'' at $>10\%$ strain during the strain sweeps. 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. The strain range under which a formulation remained elastic-dominant (see Appendix A for data) also differed with formulation due to changes in gel stability.

Frequency sweep results were dependent on formulation (selected results shown in Fig. 4). Overall, G' and G'' decreased with increasing temperature, HWS addition, or both. Increasing temperature and application of HWS decreased G' and G'' . Overall, addition of WPI (sample 16), CMC (sample 7), and fat (samples 2, 3, and 4) had the greatest impact on the moduli values compared to the control sample (sample 1) via the same mechanism explained earlier for the strain sweep results. The significant increase of viscoelastic moduli for sample 16 (high levels of WPI) 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. Furthermore, non-associated, denatured whey proteins can interact with the bound, denatured whey proteins, forming a stronger gel (Lucey et al., 1998). Viscoelastic moduli values decreased when WPI was used in combination with SMP (sample 5), likely because the protein content of WPI was higher than SMP (approximate ratio of 3:1). Because the total solids content of both formulations was equal, there would be less protein in sample 5 compared to sample 16, which was shown in the proximate results (Table 2). WPI also resulted in increased viscoelastic moduli values of samples prepared with additional hydrocol-

Table 8 Viscoelastic parameters of acid milk gels^a

Formula number	γ_c (%)	γ_c (%)	γ_c (%)	γ_c (%)	G^* (Pa)	G^* (Pa)	G^* (Pa)	G^* (Pa)	G^* (Pa)	$\tan \delta$ (rad)	$\tan \delta$ (rad)	$\tan \delta$ (rad)	$\tan \delta$ (rad)
	8 °C	25 °C	25 °C	25 °C	8 °C	8 °C	25 °C	25 °C	25 °C	8 °C	8 °C	25 °C	25 °C
	NS	S	NS	S	NS	S	NS	S	NS	S	NS	NS	S
1	0.411 ^j	0.417 ^k	0.411 ^k	0.555 ^m	486.62 ^{de}	208.64 ^{def}	179.2 ^d	118.9 ^{def}	0.300 ^{nm}	0.325 ^h	0.329 ^{nop}	0.346 ⁿ	
2	0.976 ^f	0.73 ^b	0.975 ^h	0.975 ⁱ	249.6 ^{efgh}	101.63 ^{ef}	94.5 ^d	56.6 ^{ef}	0.307 ^{lm}	0.333 ^h	0.339 ^{mmo}	0.365 ^l	
3	0.730 ⁱ	0.411 ^l	0.975 ⁱ	1.30 ^b	88.8 ^b	56.0 ^f	58.12 ^d	38.12 ^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	700.67 ^{cd}	318 ^d	284 ^d	119.45 ^d	0.319 ^{kl}	0.314 ^h	0.334 ^{mmo}	0.329 ^{no}	
5	0.731 ^h	0.731 ^g	0.731 ^g	0.731 ^k	998 ^c	782.5 ^b	697.7 ^c	503.42 ^b	0.299 ^{mmo}	0.320 ^h	0.321 ^{op}	0.326 ^{no}	
6	1.74 ^e	0.73 ^b	1.74 ^h	0.73 ^l	100 ^b	50.2 ^f	64.4 ^d	30.4 ^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}	166.42 ^{def}	174.24 ^d	78.29 ^{def}	0.36 ^{lj}	0.413 ^g	0.403 ^l	0.450 ^k	
8	0.731 ^h	0.547 ⁱ	0.975 ⁱ	0.975 ⁱ	156 ^{gh}	66 ^f	118 ^d	37.66 ^f	0.316 ^{klm}	0.331 ^h	0.355 ^{mm}	0.375 ^l	
9	0.731 ^h	0.411 ^l	0.975 ⁱ	0.73 ^l	224 ^{efgh}	124 ^{def}	130 ^d	76.61 ^{def}	0.326 ^k	0.314 ^h	0.355 ^{mm}	0.354 ^{lmm}	
10	1.74 ^e	1.74 ^e	2.33 ^e	2.33 ^f	431 ^{defg}	265.5 ^{de}	287.6 ^d	185.8 ^{de}	0.618 ^e	0.71 ^c	0.745 ^g	0.815 ^g	
11	10.0 ^b	10.0 ^b	10.0 ^b	13.4 ^a	172.7 ^{gh}	101.4 ^{ef}	112 ^d	61.7 ^{ef}	0.518 ^g	0.606 ^f	0.561 ^l	0.692 ^l	
12	0.975 ^g	0.548 ⁱ	0.975 ⁱ	0.975 ⁱ	1715.3 ^b	560.2 ^c	1177.7 ^b	295.1 ^c	0.248 ^p	0.293 ⁱ	0.250 ^h	0.301 ^o	
13	0.411 ^j	0.411 ^l	0.73 ^l	0.73 ^l	145 ^{gh}	63.8 ^{ef}	73.2 ^d	42.2 ^{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	182.5 ^{gh}	115.9 ^{def}	111.6 ^d	75.5 ^{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}	75.5 ^{ef}	73.0 ^d	45.7 ^{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	3600.6 ^a	1823.8 ^a	2640 ^a	879.3 ^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	379.2 ^{efgh}	100.3 ^{ef}	250 ^d	61 ^{ef}	0.386 ⁱ	0.606 ^f	0.436 ^k	0.677 ^h	
18	1.74 ^e	2.33 ^d	3.12 ^d	4.18 ^d	450.8 ^{def}	224.24 ^{def}	315.2 ^d	140.9 ^{def}	0.681 ^{bc}	0.859 ^b	0.843 ^{bc}	1.08 ^b	
19	2.33 ^d	1.74 ^e	3.12 ^d	4.18 ^d	219.8 ^{efgh}	85.64 ^{ef}	131.42 ^d	46 ^{ef}	0.621 ^{de}	0.713 ^c	0.758 ^g	0.866 ^f	
20	0.411 ^j	0.232 ^m	0.411 ^m	0.547 ⁿ	180.1 ^{gh}	32 ^f	87.9 ^d	28.34 ^f	0.433 ^h	0.609 ^f	0.506 ^l	0.541 ^j	
21	5.59 ^b	7.48 ^b	7.48 ^b	7.48 ^c	233.44 ^{efgh}	116.31 ^{def}	150 ^d	77.11 ^{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	139.5 ^{gh}	63.64 ^{ef}	64.6 ^d	35.17 ^{ef}	0.692 ^b	0.852 ^b	0.864 ^b	1.04 ^c	

(continued)

Table 8 (continued)

Formula number	γ_c (%) 8 °C NS	γ_c (%) 8 °C S	γ_c (%) 25 °C NS	γ_c (%) 25 °C S	γ_c (%) 25 °C NS	G^* (Pa) 8 °C S	G^* (Pa) 8 °C S	G^* (Pa) 25 °C NS	G^* (Pa) 25 °C S	$\tan \delta$ (rad) 8 °C S	$\tan \delta$ (rad) 8 °C NS	$\tan \delta$ (rad) 25 °C NS	$\tan \delta$ (rad) 25 °C S
23	1.74 ^e	2.33 ^d	2.33 ^d	3.12 ^e	201 ^{e,figh}	78.12 ^{ef}	115.0 ^d	43.6 ^{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	143.6 ^{gh}	59.5 ^f	97.9 ^d	44.21 ^f	0.638 ^d	0.750 ^d	0.692 ^h	0.759 ^h	

^aNS: no HWS added; S: HWS added

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

loids compared to when they were used individually in the formulations (samples 10, 11, 12, and 13). The presence of additional whey protein would increase the number of interactions with both other polysaccharides and caseins in the system, resulting in a stronger gel (Laneuville et al. 2000).

The effect of HWS was significant for several samples prepared with PS due to enzymatic digestion of large granules of PS with α -amylase. This effect was particularly notable when PS was used alone (sample 8) or in combination with one other hydrocolloids (samples 12 and 17). However, when PS was used with more than 2 hydrocolloids (samples 14, 19, 20, 21, 22, 23, and 24), this effect was not observed. This may have been because the quantity of PS used in these formulations

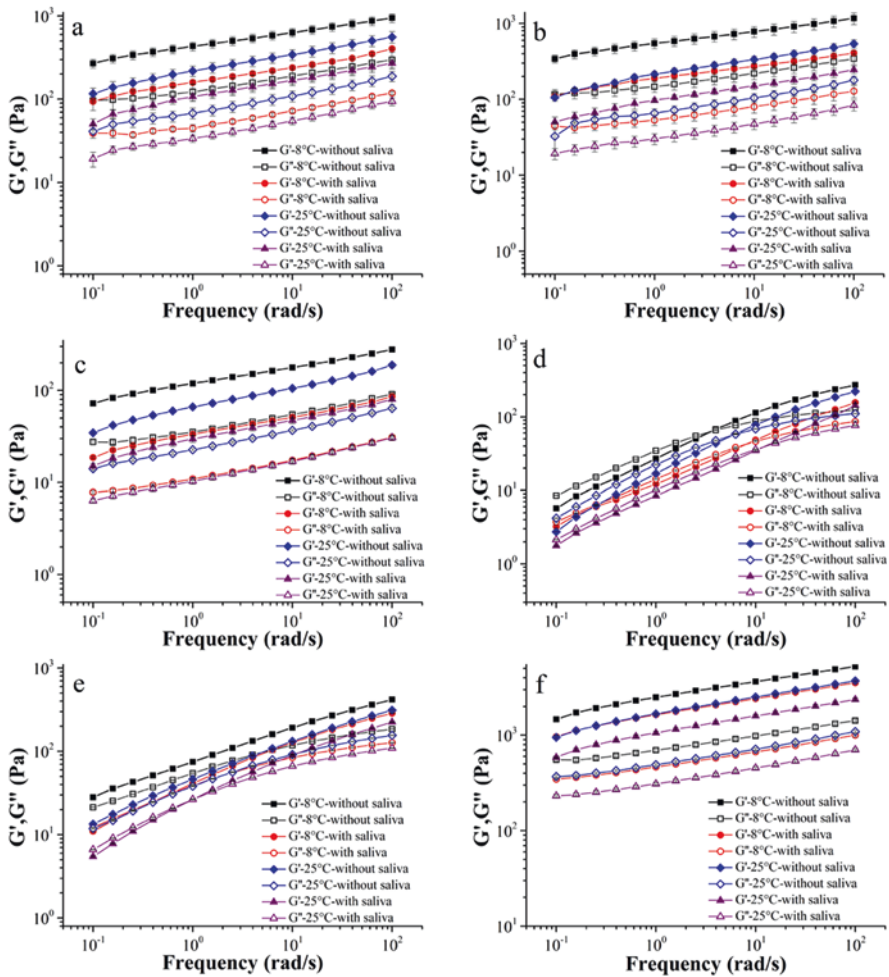


Fig. 4 Selected frequency sweep results of acid milk gels; (a) sample 1; (b) sample 4; (c) sample 8; (d) sample 6; (e) sample 14; (f) sample 16

would decrease to balance the total solids content (13% w/w in all samples), resulting in less PS available for digestion. An example of the significant impact of PS was observed for sample 14 with added HWS, for which a crossover of the moduli values occurred regardless of temperature. Digestion of the sample by HWS was sufficient to disrupt the sample and promote viscous-dominant behavior at low frequencies.

Similarly, several acid milk gel formulations showed a crossover between G' and G'' within the frequency range of 0.1–100 rad s^{-1} , and saliva was not always necessary to promote this crossover (Table 9). These samples showed viscous-dominant behavior ($G'' > G'$) at low frequencies but solid-like behavior ($G' > G''$) at higher frequencies. Crossovers were attributed to the different responses of the microstructures to forces applied 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.

Overall, HWS, formulation, and temperature had significant effects on acid milk gel viscoelastic parameters. Formulation appeared to have a greater impact than the other parameters. The rigidity, stability, and viscoelastic moduli of the samples were highly affected when WPI, fat, CMC, or a combination of more than two hydrocolloids were added to the formulations. Among the hydrocolloids used, LBG decreased viscoelastic moduli in comparison to the control, which can be explained by the neutral charge on LBG. Although LBG would not notably interfere with interactions in the protein network, it can destabilize the network by depletion flocculation due to altered osmotic pressure. The general explanation of this significant difference among formulations with added WPI and CMC was most likely the difference in strength and quantity of the electrostatic interactions as well as differences in hydrophobicity of the internal molecules. PS and CS changed the viscoelastic properties of the acid milk gels by swelling in and increasing the viscosity of the continuous phase.

Both HWS and temperature generally resulted in lower moduli values and higher phase angles. HWS had the highest effects on the viscoelastic properties of formulations that included starch due to the enzymatic breakdown, especially for samples

Table 9 Crossover frequencies for acid milk gels^a

Formula number	8 °C-S (rad s^{-1})	8 °C-NS (rad s^{-1})	25 °C-S (rad s^{-1})	25 °C-NS (rad s^{-1})
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

^aNS: no HWS added; S: HWS added

with PS rather than CS due to the larger PS granule size. HWS had also a greater impact on the sample with LBG since there were no strong bonds or interaction between milk proteins and LBG molecules. The overall impact of HWS was attributed to changes in osmotic pressure and protein net charges and disruption of acid milk gel structures by digestion and dilution. Increased temperatures increased the internal mobility and weakened the internal bonds, resulting a decreased in stability, rigidity, and a resistance to permanent deformation due to the fluid-like properties of more viscous-dominant materials.

The impact of hydrocolloids, and HWS on viscoelastic properties of acid milk gels was in accordance with the notable differences in microstructural conformation in various formulations with and without addition of HWS (Figs. 1, 2, and 3). Overall, this information about viscoelastic properties of acid milk gels provides an understanding of how their formulation and testing parameters can result in different degrees of microstructural stability and a subsequent variety in viscoelastic behaviors. The information can be used to assist in proper selection of hydrocolloids during product development to generate microstructures that create desirable processing behaviors. It can also be used to indicate sensory textures, as will be discussed in Chap. 11, “[Relationships Among Acid Milk Gel Sensory, Rheological, and Tribological Behaviors](#)”.

3.6 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 on acid milk gel friction coefficients were significant ($p \leq 0.001$, Table 10). The interaction of sliding speed and HWS 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 and molecular size. For instance, addition of WPI may result in a larger particle size that can increase friction coefficients. The significant impact of HWS can be explained by disruption of acid milk gel structures by digestion, altered osmotic pressure, dilution, or changes to milk protein net charges, mainly caused by salivary enzymes, proteins, electrolytes, and additional water. The significant impact of sliding speed was most likely due to the thickness of the lubricant (food) between the two surfaces during tribometry, which increases with increased sliding speed and can profoundly impact the measured friction due to increased surface separation and greater dependence on the bulk rheology of the fluid at higher sliding speeds.

Stribeck curves showed that the boundary and mixed regime were the dominant regimes for all samples (Fig. 5). These regimes have been shown for semisolid food with added hydrocolloids during tribological testing (De Vicente et al. 2006; Dresselhuis et al. 2007; Chojnicka et al. 2008; Chojnicka-Paszun et al. 2012;

Table 10 Main sources of variation of frictional properties of acid milk gels ($n = 24$)^a

Source of variation	Friction coefficient
Formulations	2612.1 ^{***}
Sliding speed	92.8 ^{***}
HWS	596.1 ^{***}
Sliding speed*HWS	3.5 [*]
Formulation*HWS	39.2 ^{***}
Formulation * Sliding speed	3.7 ^{***}

^a *, **, and *** indicate significant differences at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively

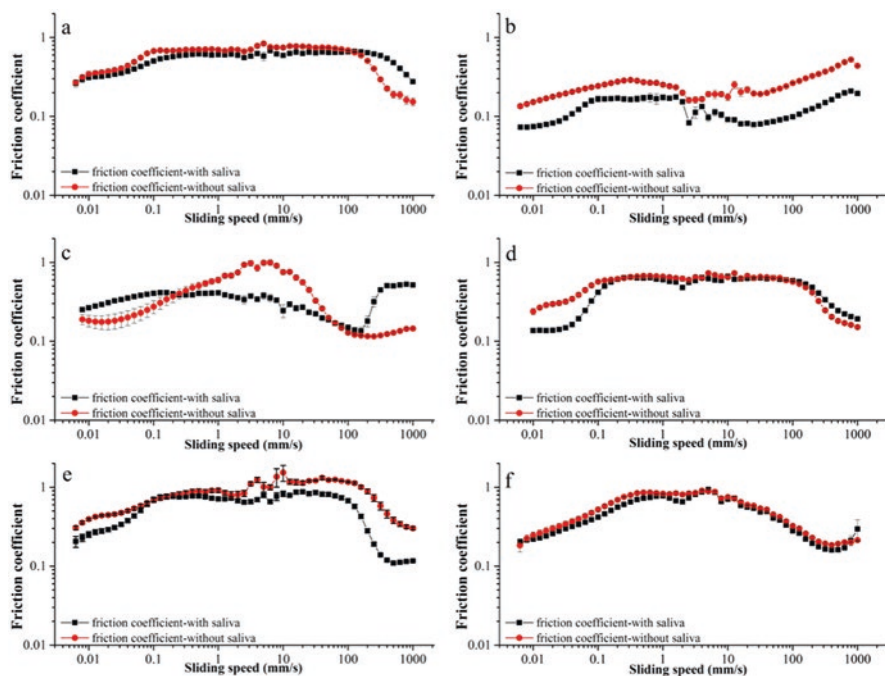


Fig. 5 Tribology results of acid milk gels; (a) sample 1; (b) sample 4; (c) sample 7; (d) sample 8; (e) sample 16; (f) sample 22

Morell et al. 2016). Most acid milk gels showed startup behaviors from 0.01 mm s^{-1} to 1 mm s^{-1} (Fig. 5). The increase in friction coefficients at these speeds was due to deformation of the PDMS plate, rather than actual sliding of the contact surfaces against each other at low sliding speeds (Zinoviadou et al. 2008).

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. Fat globules can decrease the friction coefficient, as they can become trapped in the contact area and form a thin film of fat due to local fat coalescence or flocculation, acting as a lubricant (Huc et al. 2016). It should be noted that this behavior is typically local to the contact zone, not in the bulk material, so it is not necessary to see fat separation during or after testing for this phenomenon to occur.

Samples with formulations that included LBG or high levels of WPI (samples 6, 18, and 16) had notably increased friction coefficient within the boundary regime. Samples with high levels of 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. (2009), 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 friction behaviors contributed by WPI. Stribeck curves for samples with high levels of 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). Samples containing LBG at a lower level (sample 6) had small changes in friction coefficient within the boundary regime. This effect was also shown by (Zinoviadou et al. 2008).

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) showed notably lower friction coefficients upon addition of HWS, but addition of HWS to samples with PS (sample 8) showed little impact on friction behaviors, which was unexpected based on the rheological results. In contrast, samples with CS (sample 9) showed a notable decrease in friction coefficient when mixed with HWS. This result was not in accordance with the viscosity and viscoelastic results for samples 8 and 9; in those results, sample 8 showed greater changes upon addition of saliva than sample 9. The differences between the rheological and tribological results for these samples support differences in sample interaction with α -amylase in HWS, as previously discussed (Sect. 3.3). These results highlight the importance of testing foods with and without HWS using a range of tests: saliva may have significantly different effects on sample behaviors under different conditions. The main reason for the decrease in friction coefficient in 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 >99% water (Humphrey and Williamson 2001).

4 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, structures comprising thicker clusters and bigger chains after addition of hydrocolloids were associated with greater mechanical viscosity. This result can be attributed to the greater resistance of those larger clusters to deformation by mechanical force, causing increased viscosity. Addition of HWS to the acid milk gels resulted in decreased viscosity, viscoelastic moduli, and frictional coefficients. A decrease in the void area after addition of saliva, observed in confocal images, was in agreement with this finding. The most notable differences were for samples with either PS individually or in combination with other hydrocolloids. However, these results were not found for CS-containing 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 an improved ability to designing ideal textures for the human palate.

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Appendix A: Supplemental Figures

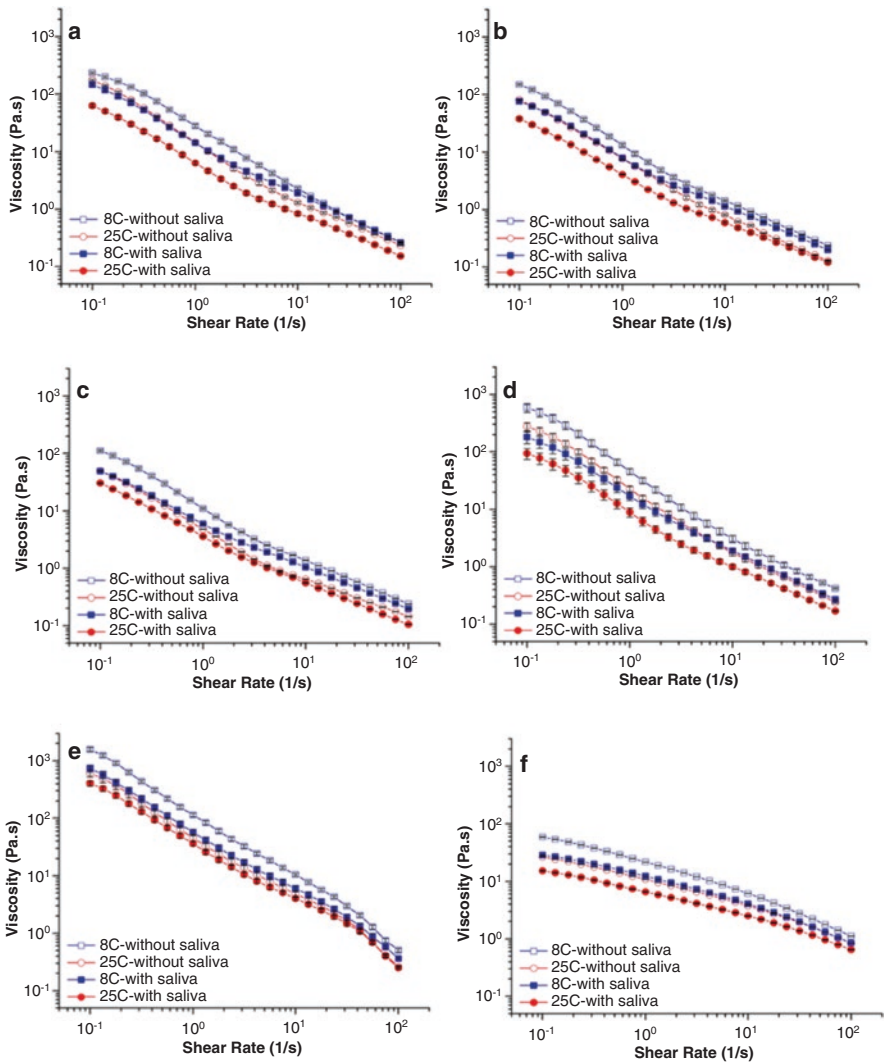


Fig. 6 Acid milk gel shear rate sweep results; (a) sample 1; (b) sample 2; (c) sample 3; (d) sample 4; (e) sample 5; (f) sample 6; (g) sample 7; (h) sample 8; (i) sample 9; (j) sample 10; (k) sample 11; (l) sample 12; (m) sample 13; (n) sample 14; (o) sample 15; (p) sample 16; (q) sample 17; (r) sample 18; (s) sample 19; (t) sample 20; (u) sample 21; (v) sample 22; (w) sample 23; (x) sample 24

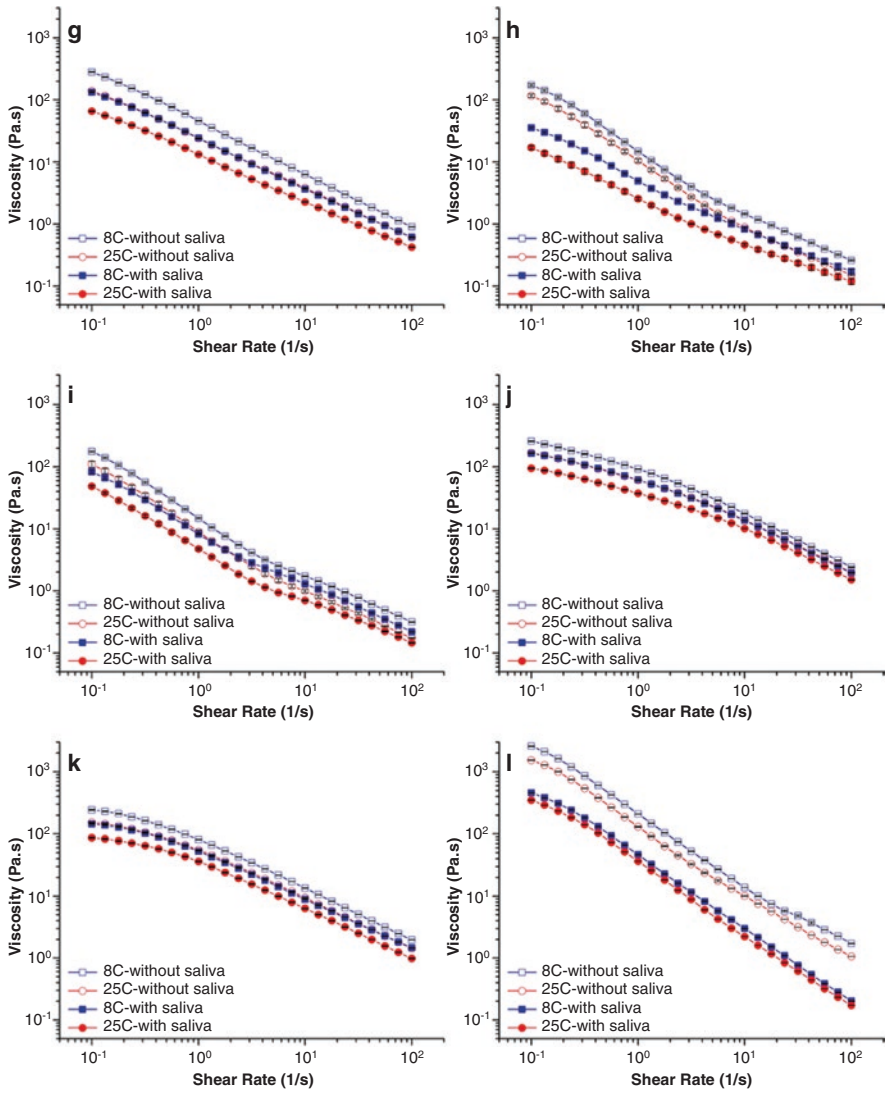


Fig. 6 (continued)

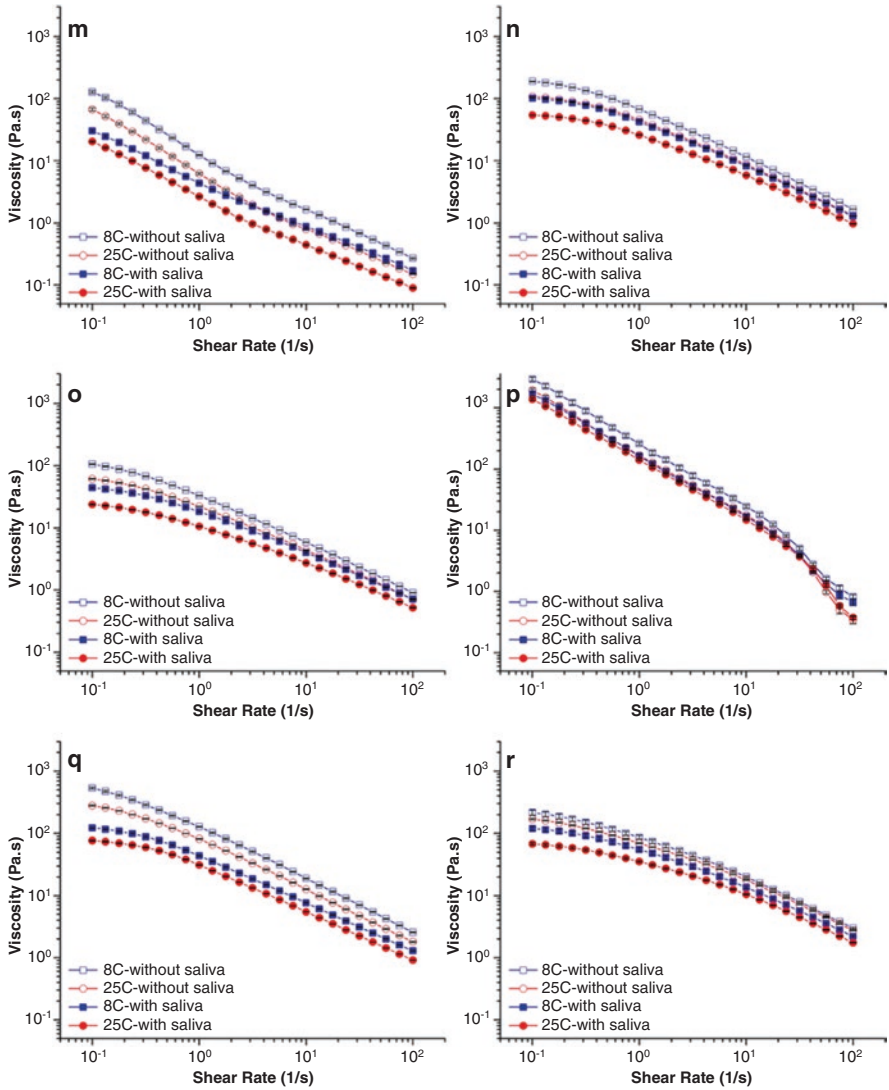


Fig. 6 (continued)

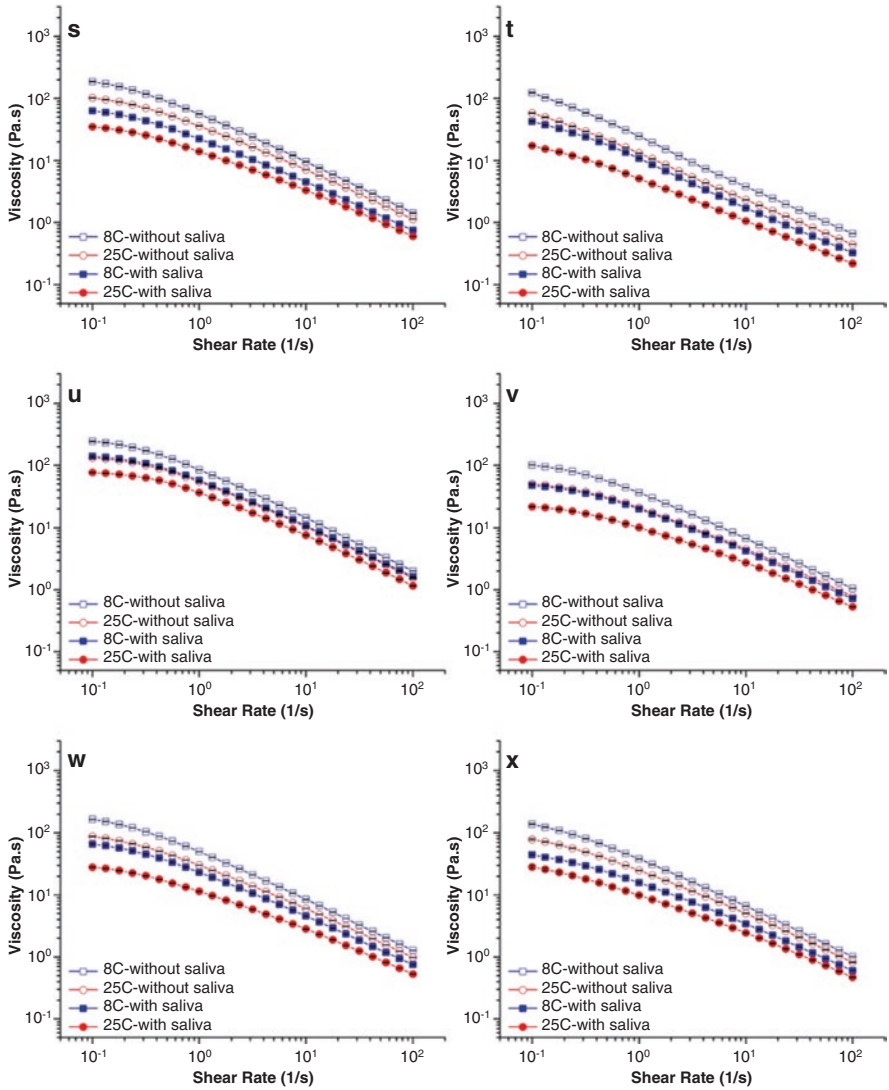


Fig. 6 (continued)

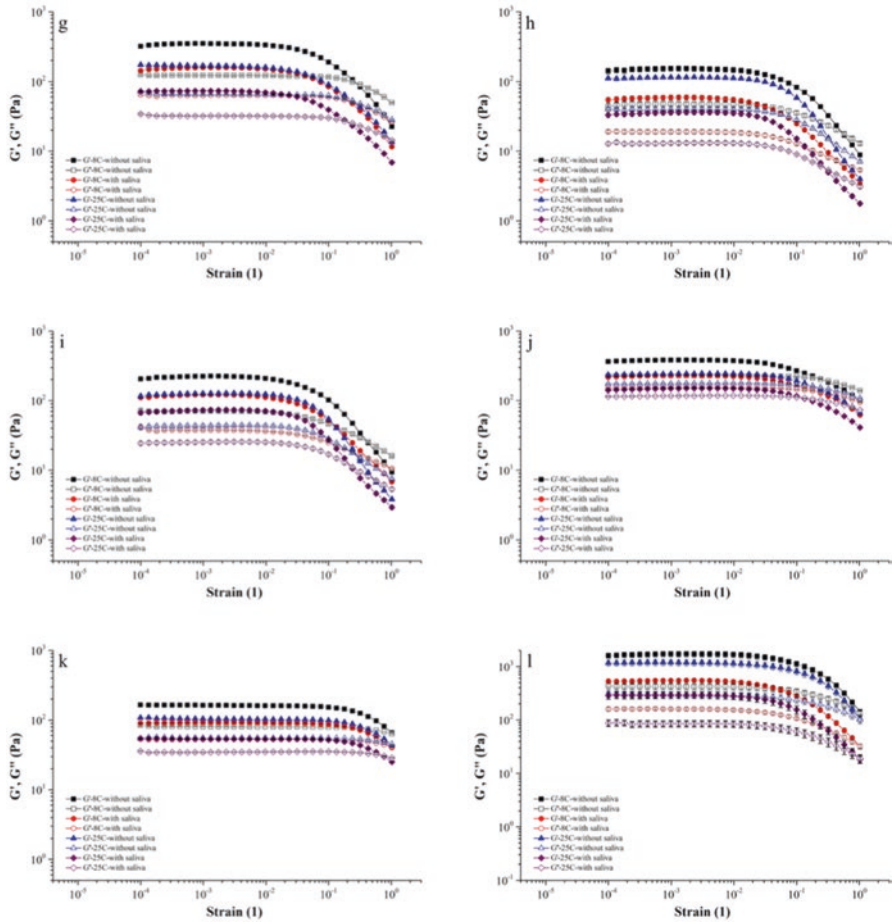


Fig. 7 (continued)

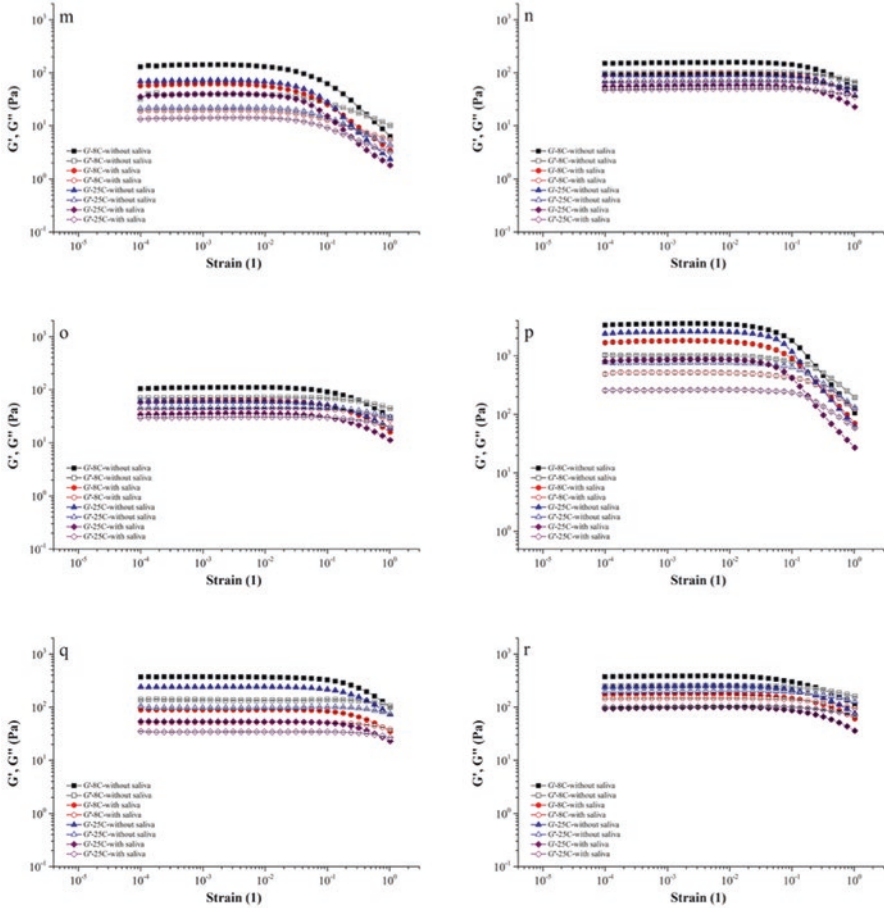


Fig. 7 (continued)

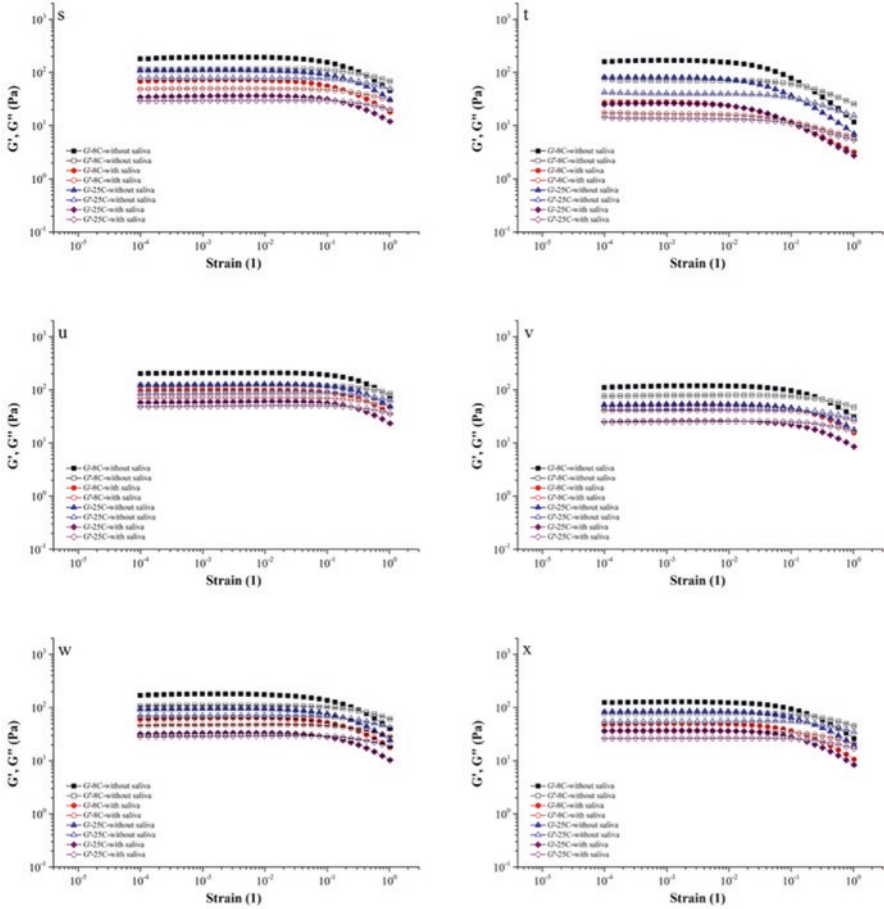


Fig. 7 (continued)

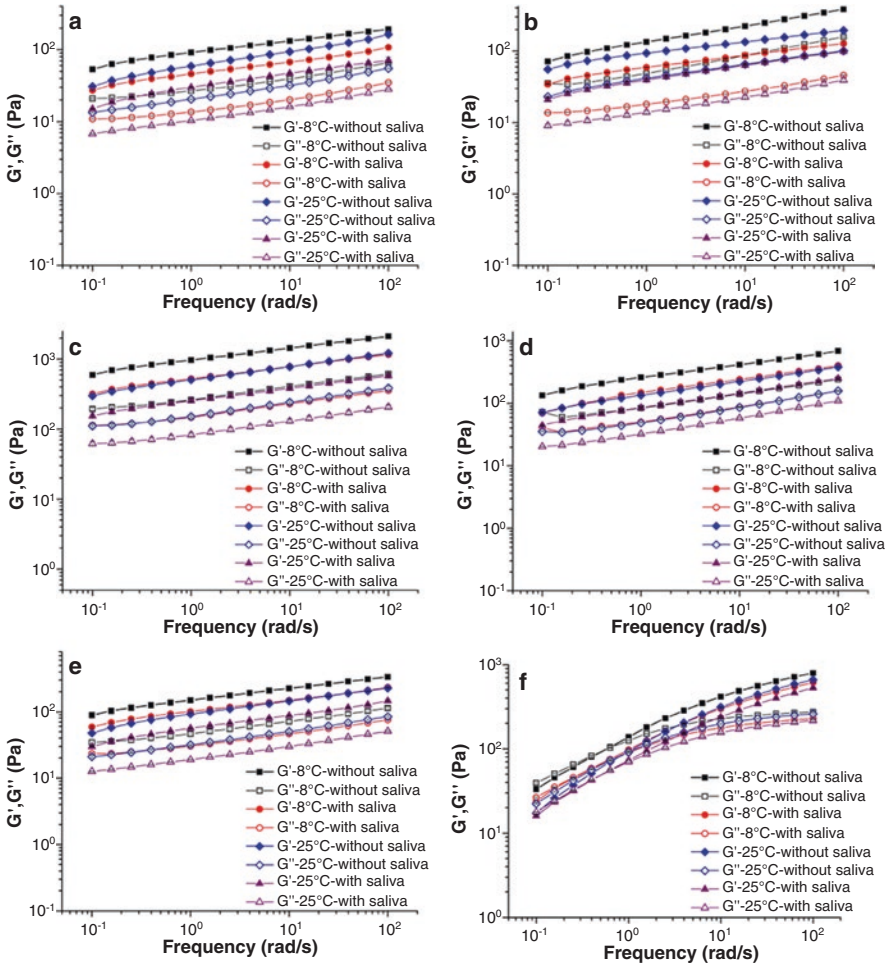


Fig. 8 Acid milk gel frequency sweep results; (a) sample 2; (b) sample 3; (c) sample 5; (d) sample 7; (e) sample 9; (f) sample 10; (g) sample 18; (h) sample 11; (i) sample 12; (j) sample 13; (k) sample 15; (l) sample 17; (m) sample 23; (n) sample 19; (o) sample 20; (p) sample 21; (q) sample 22; (r) sample 24

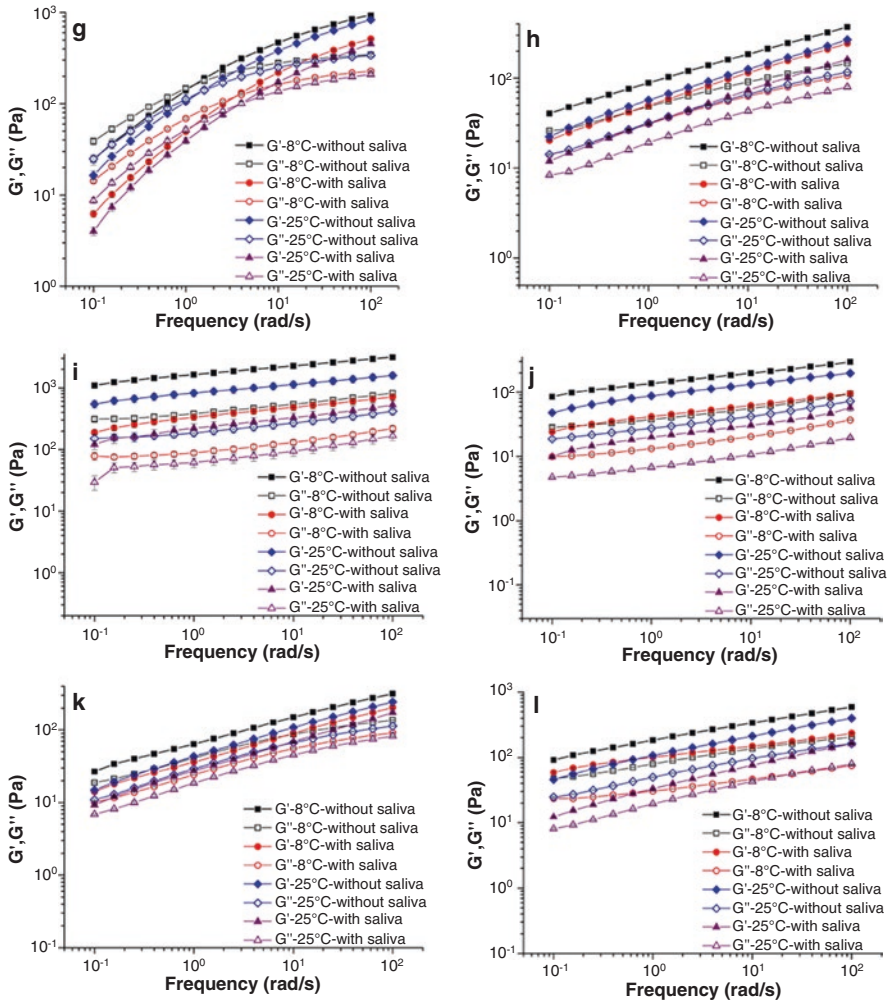


Fig. 8 (continued)

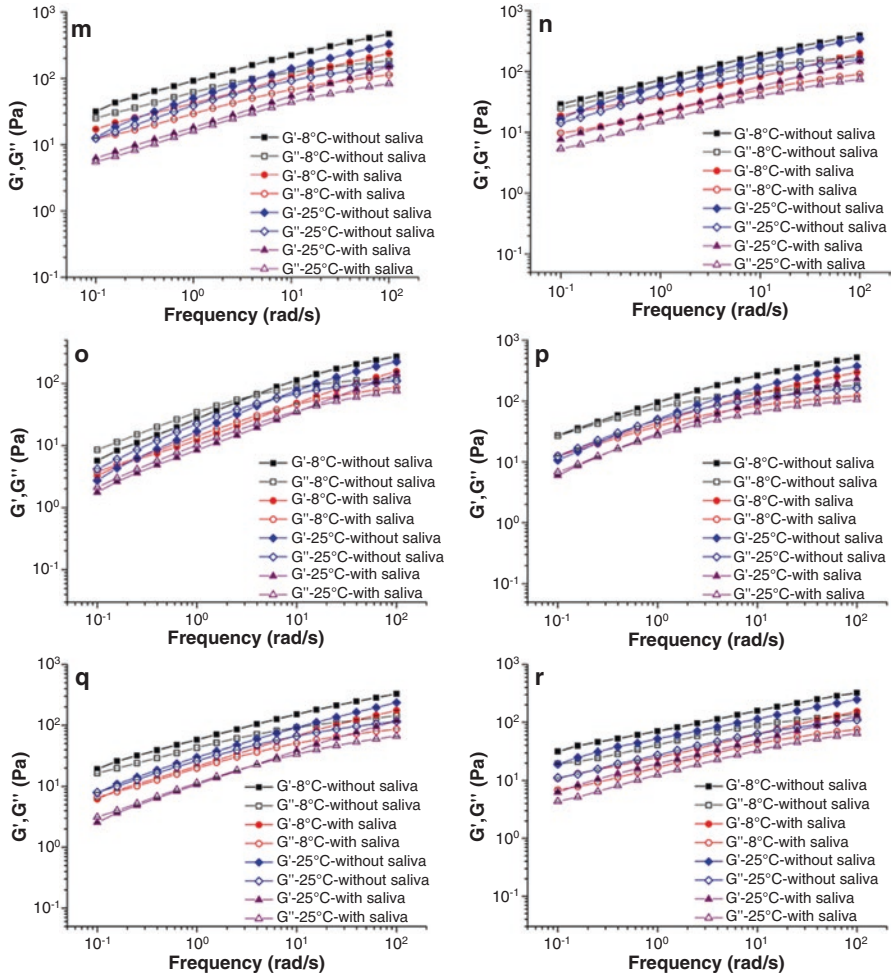


Fig. 8 (continued)

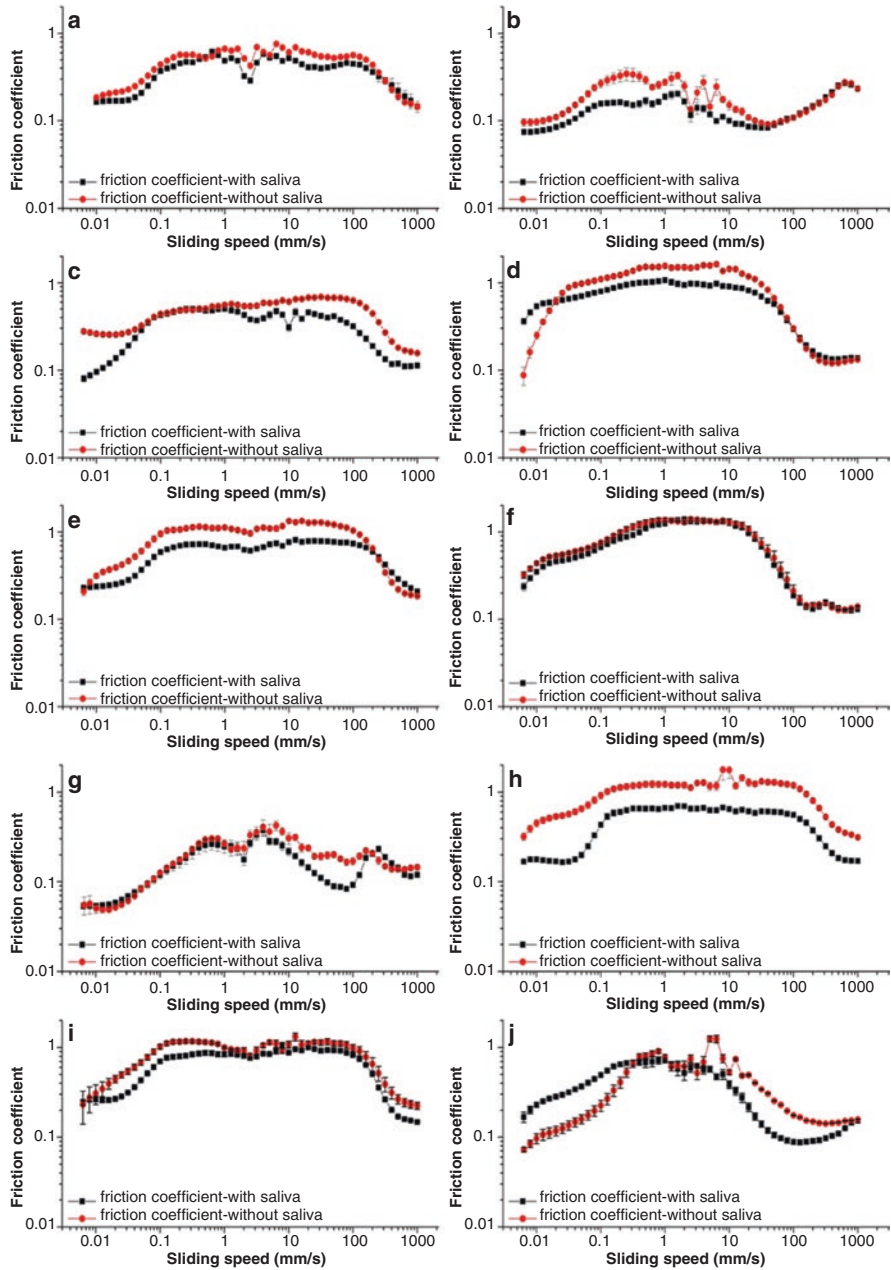


Fig. 9 Acid milk gel tribological results; (a) sample 2; (b) sample 3; (c) sample 5; (d) sample 6; (e) sample 9; (f) sample 10; (g) sample 11; (h) sample 12; (i) sample 13; (j) sample 14; (k) sample 15; (l) sample 17; (m) sample 18; (n) sample 19; (o) sample 20; (p) sample 21; (q) sample 24; (r) sample 23

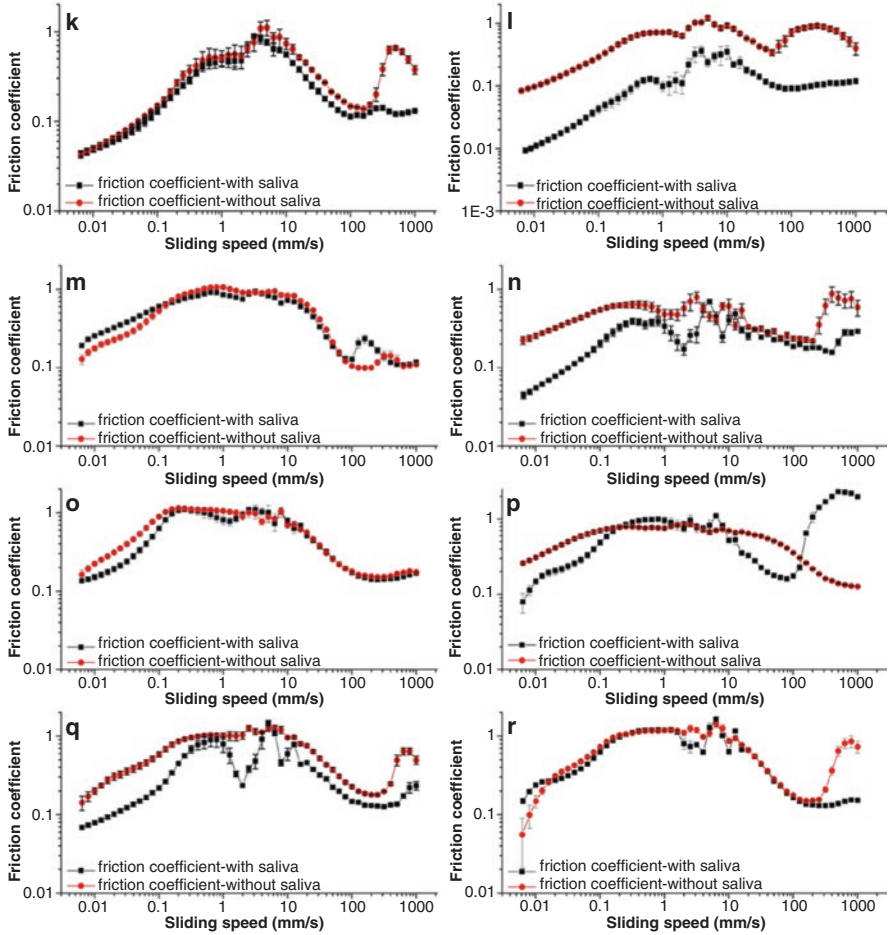


Fig. 9 (continued)

Appendix B: Supplemental Tables

Table 11 HWS composition^a

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

^aDifferent letters in a given column indicate significant differences among HWS for protein concentration and α -amylase at $p \leq 0.05$

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