A DAIRY-BASED BEVERAGE DEVELOPMENT BY ALPHA-LACTALBUMIN/BETA-LACTOGLOBULIN RATIO ADJUSTMENT FOR DYSPHAGIA PATIENTS

by

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Abstract

People who suffer from swallowing disorders are diagnosed with dyphasgia. The beverage for the dyphagia patients should have the apparent viscosity in the range of nectar-like (51 to 350 mPa·s) or honey-like (351 to 1750 mPa·s). Due to the swallowing problems, dysphagia patients usually consume beverages slowly. Thus, the apparent viscosity of beverage for such patients should be high enough to be in the suitable range during the entire time of consumption.

Three ratios of α-lactalbumin (α-la)/β-lactoglobulin (β-lg) (3:8, 1:1 and 8:3) were used to prepare the milk systems. These ratio adjusted milk systems were either processed at 70, 80, and 90ºC for 30 min or at 25ºC, and cooled to 25 ± 1ºC. After the process was completed, the milk systems were set quiescently 120 min at 25 ±1ºC. Physical and chemical properties were assessed at various time. For the milk systems at 0 min, the apparent viscosity increased in all 90°C processed-samples, and the increase was in the order of 8:3 (15.96%), 1:1 (6.38%) and 3:8 (2.11%) compared with the 25ºC samples at each ratio. When the milk systems set for 120 min, apparent viscosity increased slightly by 3.7%.

The maximum apparent viscosity was 2.18 mPa·s, which was less than nectar-like. Therefore, xanthan gum was added at 0.15 w/w % to enhance rheological properties of the milk systems. α-La/β-lg ratio adjusted milk systems either with or without xanthan gum were prepared, and processed at 90ºC or 25ºC, and cooled to 25

± 1ºC. Apparent viscosity increased by 48.61 and 89.61% in 3:8 and 8:3 milk systems, respectively for those at 0.15% xanthan gum concentration and processed at 90ºC compared with at 25ºC. Apparent viscosity of 8:3 milk systems at xanthan gum concentration of 0.15% processed at 90°C was 58.7 ± 2.12 mPa·s which was within the nectar-like range. When the samples were set for 120 min, no changes were found in the apparent viscosity of the milk systems. If the rheological properties of the milk systems can be controlled by ingredients interactions, this can be used to develop nutritious products with different forms for dysphagia patients.

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Chapter 1 - Literature review

1.1 Milk proteins

Milk is the secretion from mammals providing complete nutritional requirements for the infants of that species and contains numerous components such as proteins, minerals, vitamins, hormones, enzymes, and miscellaneous compounds (Fox 1989). Milk proteins are split into two major categories, casein and whey proteins depending on the solubility at pH 4.6 (Ellsworth 2003). Milk proteins are frequently used as an ingredient in foods because of their physiochemical properties such as foam stability, emulsion stability, and gelation (Liu and others 2013). Table 1.1 depicts the typical components of bovine milk proteins.

Table 1.1 Typical components of bovine milk protein[1]

Protein	g/L	% of total protein	Phosphate amount	Cysteine residues
Total protein	33	100		
Total casein	26	79.5		
α_{s1}	10	30.6	8-9	0
α_{s2}	2.6	8.0	12-13	2
β	9.3	28.4	5	0
κ	3.3	10.1	1	2
Total whey protein	6.3	19.3		
α-Lactalbumin	1.2	3.7	0	8
β-Latoglobulin	3.2	9.8	0	5
Bovine serum albumin	0.4	1.2	0	35
Immunoglobulines	0.7	2.1		
Proteose peptone	0.8	2.4		

[1] Adapted from Walstra and Jennes 1984.

1.1.1 Casein micelles

Casein is a spherical-shaped protein, provides 76-86% of the total nitrogen content in bovine milk, possesses an isoelectric point (pI) of 4.6, and has an average diameter of 150 nm, with an average molecular weight of about 10^8 Da (Fox 2003). Its biological function is to help protein digestion by forming a clot in the stomach and carrying calcium and phosphate (Haug and others 2007). As shown in Table 1.1, α_{s1}- and β-casein contain no cysteine or cystine while α_{s2}- and κ-casein contain two cysteine residues per mole, which can naturally form disulfide bonds (Dalgleish 1997). Among the many models that have been used to describe the structure of casein micelles, the nanocluster model proposed by Holt seems to capture the main features (de Kruif and others 2012). In the nanocluster model, casein micelles associate mainly through colloidal calcium phosphate nanoclusters. The attachment of the nanocluster is the essential part of the phosphorylation.

Casein protein fractions contain high phosphate amounts (Table 1.1), which possess negative charges; thus, they strongly bind polyvalent cations, principally Ca^{2+} as well as Zn^{2+}. Therefore, α_{s1}-, α_{s2}-, and β-caseins, which contain high amounts of phosphate groups are Ca-sensitive caseins while κ-casein which possesses fewer phosphate groups is regarded to be Ca-nonsensitive (Fox 1989). Some weak interactions (hydrophobic, hydrogen bond, ion bond, Van der Waal's force) induce tails of caseins that associate with each other. Proline, which is highly concentrated and uniformly distributed, imparts a random coil secondary structure to all casein

subunits, which increases the interactions via hydrophobic and ionic bonds. α_{s1}-Casein could self-aggregate through hydrophobic bonds; α_{s2}-casein contains 2 or 3 phosphoserine clusters and 2 hydrophobic regions; β-casein offers one phosphoserine cluster within the hydrophilic region and one hydrophobic C-terminal tail (Walstra and Jenness 1984; de Kruif and others 2012). κ-Casein cannot offer nanoclusters due to low phosphate content, and it only has one hydrophobic N-terminal tail; thus, the expansion of casein micelles is prohibited when κ-casein is attached (Horne 2009; de Kruif and others 2012).

1.1.2 Whey proteins

Whey proteins are highly functional, and used more frequently in either animal feed or human protein sources (Cayot and Lorient 1997; Bryant and McClements 2000). Industrially, two kinds of whey are produced: sweet and acid. Sweet whey is generated from rennet-coagulated cheese production like cheddar, whereas acid whey is produced from acid-coagulation such as cottage cheese (Panesar and others 2007). Categories of whey proteins are listed in Table 1.1; α-lactalbumin and β-lactoglobulin, the major whey protein fractions in whey are introduced further.

1.1.2.1 α-Lactalbumin

α-Lactalbumin (α-la) is only found in mammal's milk and binds two atoms of Ca which is considered to be the unique structure of α-la (Edwards and others 2009; Walstra and Jennes 1984). α-La is a globular protein with a molecular weight of

14,200 Da with a pI of ~4.2 (Bramaud and others 1997). It is widely found in all mammals' milk, acting as a specifier protein for lactose synthetase in the formation of lactose from galactose and glucose. Functionally, it possesses good emulsifying and foaming functions although, the native molecule reveals low surface hydrophobicity (Edwards and others 2009; Fox 1989).

1.1.2.2 β-Lactoglobulin

β-Lactoglobulin (β-lg) is the major whey protein in bovine milk; but is absent in human and rodent milks (Fox 1989). It is a globular protein with molecular weight of 18,000 Da, and a pI of 5.2 (Hegg 1982). β-Lg is known to self-associate and the conformation of β-lg depends on the pH (Table 1.2).

Table 1.2 Conformation of β-lactoglobulin as a function of pH[1]

pH	Conformation of β-lg
<3.5	Monomer
3.5-5.2	Octamer
5.2-8.0	Dimer
>8.0	Monomer

[1]Adapted from Fox 1989

The electrostatic interactions between Asp^{130} and Glu^{134} of one monomer with the corresponding lysine residues of another monomer is the reason for the dimer presence (Koffi 2003). Native β-lg has five cysteines: two disulfide bonds, Cys^{66}-Cys^{160} and Cys^{106}-Cys^{119}/Cys^{121}; and one free buried thiol group shifting between residues 119 and 121. The secondary structure of β-lg holds 15% α-helix, 50% β-sheet, and 15-20% reverse turn (Edwards and others 2009). The native

conformation is sensitive to heat and pH, as the free sulfhydryl (also known as thiol) groups are exposed in thermal denaturation. The mechanism for heat denaturation is that the dimer dissociates, followed by the monomer unfolding to permit more rapid sulfhydryl reactivity, which leads to disulfide interchange and whey protein aggregation.

1.1.3 Casein micelles and whey proteins interactions

Environmental factors can influence the interactions of casein and whey proteins such as pH, temperature, heating time, enzyme, and water activity in dry ingredients, as well as the ratio of casein and whey protein (Corredig and Dalgleish 1996; Gu and others 2011; Gulzar and others 2011). Denaturation of a protein occurs when the tertiary and secondary structures are ruptured, while the peptide bonds of the primary structure are maintained; side chain groups which are buried in the core of the protein are exposed due to the unfolding of the native proteins (Walstra and Jenness 1984). Association between denatured β-lg and κ-casein occurs via thiol-disulphide bond interchange (Figure 1.1) resulting in an intermolecular disulfide bond, which shifts the location of the disulphide bond from R'-R" to R-R' (Hae and Swaisgood 1990).

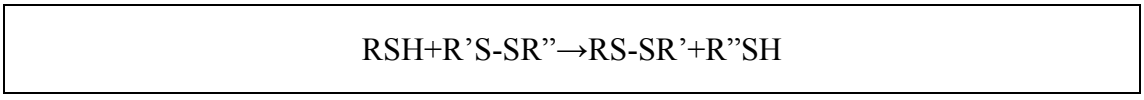

Figure 1.1 The mechanism of sulfhydryl/disulfide bond exchange

The association between whey protein and casein is also sensitive to pH as a higher rate of interaction was reported in a liquid system at pH 6.5 compared with 6.7 (Anema and Li 2003b). Most denatured whey proteins associate with casein micelles at pH of 6.5 during heating; however, the association between whey protein and casein micelles decreases when pH continues to increase after heating. Thirty percent of whey proteins associated with casein micelles at pH 6.7 whereas 50% of whey proteins associated with casein micelles at pH 6.5 when both were processed at 80ºC for 30 min (Anema and others 2004a). At a pH near the pI of a protein, the charge on the protein is balanced and hydrophobic forces predominant; this noncovalent bonding of hydrophobic forces leads to the binding of two molecules in the solution resulting in aggregation.

Change of pH can also affect electrostatic interactions (which is defined as "the attraction or repulsion between (partially) charged parts of the protein molecules") (Koning and Visser 1992). In normal milk pH (6.6-6.8), β-lg is present as a dimer, while monomers or octamers are formed when pH is below 3.5 and 5.2, respectively (Bonnaillie and Tomasula 2008). Change of conformation leads to exposure of disulfide bonds and cysteine residues, allowing for association between whey proteins and casein micelles.

Temperature is another major factor impacting association of whey protein and casein. Corredig and Dalgleish (1996) reported that β-lg was more sensitive to temperature than α-la; but the interaction kinetics between α-la/κ-casein and

β-lg/κ-casein were similar at 75 and 90ºC. On the other hand, the rate of interaction between β-lg/κ-casein was faster than α-la/κ-casein when > 90ºC. The results suggested that the interaction mechanism between proteins might be different if temperatures exceed 90ºC (Corredig and Dalgleish 1996). Greater association (80%) between denatured whey proteins and casein micelles occurs when milk is heated slowly (e.g. lab water bath) versus heated rapidly (e.g. direct heating) which has been reported to cause 50% of the association (Anema and Li 2003a). Sulfhydryl/disulfide bond exchange, which is highly dependent on temperature, is responsible for whey protein and casein micelles association. Disulfide bonds and cysteine residues are exposed at elevated temperatures (Gezimati and others 1997). Moreover, the increasing temperature promotes the mobility of molecules (Schuck 2013). The hydrophobic association is positive enthalpy, and higher temperature will induce hydrophobic associations to occur. On the other hand, hydrogen bonds break when temperature rises, which induces protein denaturation as both intermolecular or intramolecular bonds are altered. Interaction of pH and temperature can lead to the shift of gelation pH of the milk systems. Heat treatment of milk results in the shift of gelation pH towards a higher pH due to the coating of whey proteins on the casein micelles causing the increase of the pI from 4.6 (pI of casein) to 5.2 (pI of β-lg), leading to a decrease in electrostatic repulsions at higher pH and gels within a shorter time (Vasbinder and others 2003).

The ratio of α-la/β-lg in the milk system also influences the association

between whey protein and casein micelles. α-La associates with casein micelles through β-lg because no sulfhydryl groups are present in α-la (Corredig and Dalgleish 1999). However, the number of binding sites for β-lg on casein micelles seems limited. A previous study conducted by Corredig and Dalgleish (1996) reported that the binding sites on casein micelles for β-lg were already saturated as addition of β-lg into reconstituted skim milk did not increase associations of β-lg and casein micelles. However, the addition of α-la increased the associations between whey proteins and casein micelles when the milk systems were processed at 80ºC for 30 min. Specifically, the associations between β-lg/κ-casein (~0.7 mg/mg) did not change when an extra 2 g/L of β-lg was added while associations between α-la/κ-casein increased from ~0.3 to ~0.7 mg/mg when an extra 2 g/L α-la was added adjusting the ratio of α-la/β-lg to 1.

Moreover, Dalgleish and others (1997) studied the associations between whey proteins and casein micelles in a casein-resuspended model system that had different ratios of α-la/β-lg (3:8, 1:1, and 8:3). The results showed that associations were the greatest in the 8:3 α-la/β-lg and the least in the 3:8 α-la/β-lg system. Therefore, a conclusion was made that the association between α-la and casein micelles was through the formation of an intermediate that consisted of α-la and β-lg. Furthermore, the amount of α-la associated with the casein micelles was affected by the concentration of α-la in the milk system. However, the physical properties were not monitored; so the impacts to viscosity or turbidity were uncertain if the α-la/β-lg ratio

increased in a milk system.

1.2 Sulfhydryl groups measurement

Free sulfhydyrl groups buried in the core of β-lg are considered to be responsible for the interactions between whey proteins and casein micelles when whey proteins are thermally denatured in the milk system (Shimada and Cheftel 1989; Clare and others 2005). It is known that the free sulfhydryl group contributes to the oxidation of sulfhydryl groups into disulfide bonds and sulfhydryl/disulfide bonds exchange (Shimada and Cheftel 1988; Hoffmann and Van Mil 1997). Whey proteins and casein micelles do not interact with each other at 25 ± 1ºC; however, when the temperature exceeds 70°C, whey proteins start to denaturate, exposing the buried free sulfhydryl groups; therefore, allowing them to associate with casein micelles (Corredig and Dalgleish 1996). Whey proteins as a group have been found to completely denaturate when processed at 80ºC for 30 min (Anema and Li 2003a).

Several methods for testing of the sulfhydryl group in milk systems have been unsed: fluorescence method (Fahey and others 1981), titration with aqueous Ag+ (Zweig and Block 1953), and Ellman's reagent (5,5'-dithiobis-(2-nitrobenzoic acid or DTNB) (de Wit and Nieuwenhuijse 2008). Ellman's reagent has been used more frequently in recent years (Stancluc and others 2012; Gulzar and others 2011). The principle of the interaction between DTNB and a sulfhydryl group is shown in Figure 1.2.

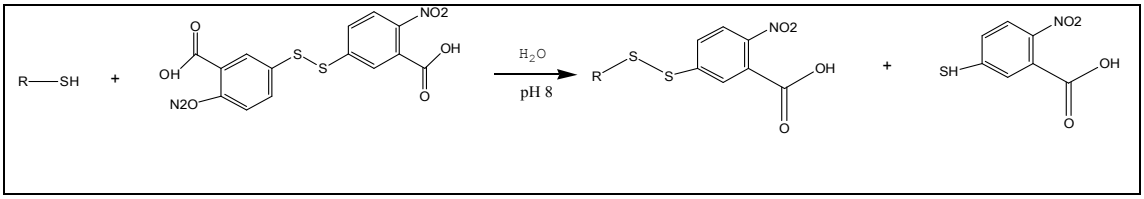

Figure 1.2 The reaction between sulfhydryl group and DTNB reagent (adapted from Sedlak and Lindsay 1968).

One mole of sulfhydryl group interacts with 1 mole of DTNB reagent, forming 1 mole of 2-nitro-5-thiobenzoic acid (TNB) which has a yellow color and high molar extinction coefficient of $13{,}600 M^{-1}cm^{-1}$ at 412 nm and pH 8.0 (Ellman 1959). Heating causes the whey protein to unfold, exposing the buried free sulfhydryl group; thus, higher concentrations of sulfhydryl groups should be formed after the heating.

1.2 Xanthan gum

1.2.1 Structure of xanthan gum

Xanthan gum is an exocellular heteropolysaccharide produced by the *Xanthomonas campestric pv. campestris* (Becker and others 1998). The molecular weight of the xanthan gum ranges from $2*10^6$ to $20*10^6$ Da (Braun and Rosen 2001). The primary structure (Figure 1.3) consists of a helical trisaccharide side chain including D-glucose, D-mannose and D-gluconate attached to acetate and pyruvate aligned onto a backbone of D-glucose (Bertrand and Turgeon 2007). The secondary structure of xanthan gum in aqueous media is an "order-disorder" transition from

helix to coil structure depending on temperature, ionic strength, acetyl, and pyruvate concentration (Khouryieh and others 2007). Most of the researchers agree with the double-helix model (Pelletier and others 2001). Because of its nontoxic properties, xanthan gum is designated by the United States Food and Drug Administration (FDA) as a safe food additive (Garcia-Ochoa and others 2000).

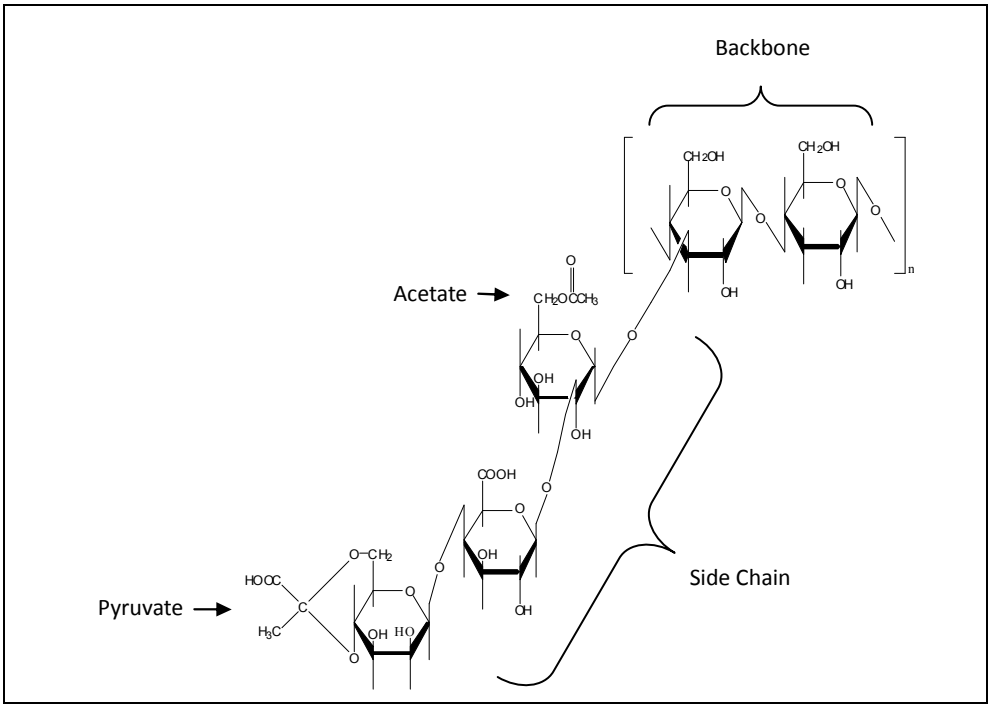

Figure 1.3 Typical structure of xanthan gum (Adapted from Becker and others 1998).

1.2.2 Rheological properties of the xanthan gum in aqueous media

Xanthan gum is highly soluble in both cold and hot water (Khouryieh 2006). The rheology of the aqueous xanthan gum at low concentrations presents a pseudoplastic behavior, and the rheological properties are largely influenced by the concentration as opposed to temperature and pH (Ahmed and Ramaswamy 2005).

Song and others (2006) reported that storage modulus (G′) increased from $2*10^2$ to 10^3 dyne/cm^2 and loss modulus (G″) increased from 60 to 200 dyne/cm^2 as the xanthan gum concentration increased from 1 to 4% when tested at frequency of 1.0 rad/s and strain of 1%.

Additionally, Whitcomb (1978) tested the apparent viscosity of different concentrations of xanthan gum solution, finding that the apparent viscosity of xanthan gum solution at 5,000 ppm was almost twice that at 3,000 ppm. The backbone of xanthan gum remains disordered in aqueous solution at 25°C, and this disordered state can be highly extended because of the electrostatic repulsion between the charged groups on the side chains; hence, a weak structure can be formed through hydrogen bonds between the xanthan gum molecules (Song and others 2006).

The viscosity of the xanthan gum solution depends on the measurement temperature. As temperature increased from 10 to 80ºC, apparent viscosity decreased from 0.07 to 0.015 Pa·s; however, decrease in apparent viscosity was fully reversible when temperature declined from 80 to 10ºC (Garcia-Ochoa and others 2000). The secondary structure of xanthan gum is that side chain reversely twists the backbone through hydrogen bonds to maintain the double helix structure; tertiary structure is a helical complex formed network structure maintained by weakly non-polar covalent bonds (Han and others 2012). Therefore, these structures play an important role in the rheologial properties of xanthan gum (Lai and others 2006).

Pyruvate and acetyl are located on the mannose units of xanthan gum in the

side chain (Callet and others 1987). Previous studies conducted on the native xanthan, only acetyl-free xanthan, only pyruvic-free xanthan, and both acetyl- and pyruvic-free xanthan in aqueous media, concluded that the pyruvate groups, due to the electrostatic repulsion, disabled the ordered conformation of xanthan gum while the acetyl groups stabilized the ordered conformation of xanthan gum (Dentimi and others 1984). Bradshaw and others (1983) tested the influence of pyruvic acid and acetate in xanthan gum solutions on viscosity. Results revealed that viscosities of pyruvate-free, pyruvate/acetate-free and native xanthan were similar at shear rates ranging from 8.8 to 88.3 s^{-1}. However, Tako and Nakamura (1984) showed that dynamic viscosity and dynamic modulus of deacetylated xanthan gum solution had a greater increase compared to native xanthan gum solution when concentration increased at angular velocity of 3.768 rad/s at 25°C. Dynamic viscosity increased by ~16 (0.7 to 16.7) and 7.1 (0.9 to 8) poise, and dynamic modulus increased by 198 and 56 dyne/cm^2 when concentration increased from 0.1 to 1% w/v for deacetylated and native xanthan solutions, respectively. Results suggested that acetyl groups contributed to stabilization of the structure of xanthan gum in a water-based solution through intermolecular associations of the xanthan gum backbone.

1.2.3 Protein-polysaccharide interaction

Among the food ingredients (lipids, sugars, protein, minerals, etc.), proteins and polysaccharides are important to the final structure and stabilization of many food systems through gelling, thickening, and other functional properties (Sanchez and

others 1997). The conjugates of protein-polysaccharide are expected to have potential for use in the food and health industries due to its non-toxic nature (Kasran 2013). Protein-polysaccharide interactions have been detected and studied due to their unique functions on texture, stability, and development of new structures in food (Schmitt and others 1998). Various types of intermolecular forces are responsible for the interactions between protein and polysaccharide, including covalent bonds, electrostatic interactions, excluded volume, hydrogen bonds, hydrophobic bonds, ion-bridges, and Van de Waals forces (Dickinson 1998).

1.2.3.1 Electrostatic interactions

The interactions between polymers based on electrostatic interactions can be classified into three types: interactions between a) charged macromolecules, b) oppositely charged side groups, and c) other available side groups of macro-ions (Tolstoguzov 1997). Electrostatic interactions predominantly influence the interactions between protein and polysaccharide when a pH below the pI of proteins and at low ionic strengths (<20 mM) (Kasran 2013). Generally, food proteins have pIs of ~5, and polysaccharides have pIs of ~3 (Dickinson 1998). Both proteins and polysaccharides possess negative charge when the pH is above pI; however, protein-polysaccharide may be formed between positively charged residues (mostly $–NH_3^+$) on the protein and negatively charged polysaccharide (Schmitt and others 1998). Soluble complexes form when opposite charges carried by the two macro-ions

are not equal. The unequal charged complexes interact with solvent molecules, which makes the complexes soluble (Kasran 2013).

1.2.2.2 Non electrostatic interactions

Non electrostatic interactions involve hydrogen bonds, hydrophobic bonds, and covalent bonds. Covalent bonds form when the chemical reaction of amino groups from the proteins and carboxylic groups from the polysaccharides occur to generate an amide covalent bound. It is a strong linkage which endues the protein-polysaccharide complexes to be stable and irreversible (Dickinson 1998).

1.2.2.3 Environmental influences

Environmental factors such as pH, ionic strength, process temperature, and ratio of protein and polysaccharide have been studied to detect their influence on protein-polysaccharide interactions. pH has a significant effect on the rheological properties of protein-polysaccharide system. A system of xanthan (0.1%) and whey protein isolate (3%) were processed at 85°C for 30 min and the highest apparent viscosity was found at pH of 7 (1000 mPa·s), followed by pH 5 (300 mPa·s), but the apparent viscosity was lowest at pH 10 (160 mPa·s) (Gustaw and others, 2003). Another experiment detected the effect of pH (ranged from 5.5 to 6.5) on the elastic modulus of whey protein isolate (12.5% v/v) and low concentrations of xanthan gum (0.01% to 0.06% v/v). Results demonstrated a synergistic effect at pH 6.5 and 6

whereas, an antagonist effect was observed at pH 5.5 (Bertrand and Turgeon 2007). These two experiments suggested that pH plays a key role on the electrostatic interactions, and further influences the rheological behaviors of the liquid systems (Dickinson 1998; Bertrand and Turgeon 2007). Process temperature is another aspect that impact protein-polysaccharide interaction.

The hydrophobic bonds are enhanced when temperature exceed 60ºC (Skrovanek and others 1985). The hydrogen bonds are diminished when the temperature exceeds 30ºC (Skrovanek and others 1985). Moreover, the whey protein denaturates at 70°C; thus, the exposed active groups due to the denaturation are attributed to form covalent bonds with polysaccharides (Schmitt and others 1998).

The concentration of xanthan gum also contributes to the interaction of proteins and polysaccharides. Sanchez and others (1997) tested the apparent viscosity of whey protein isolate (14% w/w) and various concentrations of xanthan gum solution (0.05, 0.1, 0.2 and 0.5%) at pH 7. The apparent viscosity of WPI-xanthan gum mixture increased from 20 mPa·s to 120 mPa·s at shear stress of 10 Pa when the concentration increased from 0.05 to 0.5%.

1.3 Rheological properties

1.3.1 Apparent viscosity

The viscosity and the flow behavior of skim milk is largely influenced by increased total solids, decrease of pH, salt addition such as Na^+ and K^+, increased temperature and pressure which impact the aggregation of casein micelles, leading to the increase in particle size, and thus contribute to a viscosity increase (Anema and others 2004a and b; Karlsson and others 2005; Oldfield and others 2000). When the total solids is increased, the removal of water increases the interaction between casein micelles while decreasing the distance between them so that the viscosity can be increased. The flow behavior of reconsitituted whole milk was changed from a Newtonian to a shear-thinning behavior when the total solids exceed 30% (Trinh and others 2007).

The flow behavior index (n) of a fluid may influence the mouthfeel of liquids. Liquids with low n values (which is also regarded as high degree of shear-thinning behavior) has been reported to have less slimy mouthfeel than those liquids with high n values (Cho and others 2012).

The relationship between shear stress, viscosity and shear rate can be presented in the following equation:

$$\tau = -\mu \frac{dv}{dy}$$

where τ is the shear stress, $-\frac{dv}{dy}$ (usually represented by the symbol $\dot{\gamma}$) is the shear rate, μ is the viscosity. The apparent viscosity of a non-Newtonian fluid

sample is the viscosity at a specific shear stress or shear rate (Steffe 1996).

The apparent viscosity can be split into time-independent or time-dependent based on whether the apparent viscosity changes as a function of time in one measurement. For a time-independent material, a general relationship to describe the flow behavior of non-Newtonian fluids is the Herschel-Bulkley model:

$$\tau=K(\gamma)^n+\tau_0$$

Where τ=shear stress (Pa), γ=shear rate (1/s), τ_0=intercept or yield stress, K=consistency coefficient (Pas^n), and n=flow behavior index (-).

Table 1.3 compares the different flow behaviors based on the Herschel-Bulkely model.

Table 1.3 Newtonian, shear-thinning, shear-thickening, and Bingham plastic flow behaviors expressed in the Herschel-Bulkley model[1].

Fluid behavior	K[2]	n[3]	τ_0[4]
Herschel-Bulkley	>0	$0<n<\infty$	>0
Newtonian	>0	1	0
Shear-thinning	>0	$0<n<1$	0
Shear-thickening	>0	1	0
Bingham plastic	>0	1	>0

[1]Adapted from Steffe 1996; [2]Consistency coefficient; [3]Flow behavior index; [4]Yield stress.

Other models also are used to describe the relationship between shear stress and shear

rate of time-independent fluid (Hassan and others 2003; Sopade and others 2007), and are shown in Table 1.4.

Table 1.4 Other models for time-independent fluid

Flow behavior	Model
Casson	$(\tau)^{1/2}= K(\gamma)^{1/2}+(\tau_0)^{1/2}$
Generalized Herschel-Bulkley	$(\tau)^{m}= K(\gamma)^{n}+(\tau_0)^{m}$
Heinz-Casson	$(\tau)^{n}= K(\gamma)^{n}+\tau_0$
Mizrahi-Berk	$(\tau)^{1/2}= K(\gamma)^{n}+(\tau_0)^{1/2}$
Vocadlo	$(\tau)^{1/m}= K\gamma+(\tau_0)^{1/m}$

Where τ=shear stress (Pa), γ=shear rate (1/s), τ_0=intercept or yield stress, K=consistency coefficient (Pas^n), m=a form of flow behavior index (index of yield stress), n=flow behavior index (index of shear rate).

The associations between whey proteins and casein micelles influence apparent viscosity in skim milk (Jeurnink and de Kruif 1993). Environmental factors such as pH, total solids, true protein content, fat content, and temperature can cause the apparent viscosity of a milk systems to change (Anema and Li 2003b; Anema and others 2004b; Bakshi and Smith 1984; Quinones and others 1997; Trinh and others 2007; Vasbinder and de Kruif 2003). In the experiment of Anema and others (2004b), the pH of reconstitutued skim milk were adjusted to 6.5, 6.55, 6.6, 6.65 and 6.7, and were held at 90ºC for 30 min. The relative viscosity increased from 1.04 to 1.13 as pH decreased from 6.7 to 6.5. Langely and Temple (1985) used a model to describe the relationship between relative viscosity and total solids in skim milk:

$$\eta_r=e^{KC}$$

where η_r is the relative viscosity, C is the total solids (w/v %) and K is a constant. K increased as the temperature increased from 80 to 140ºC. Quinones and others (1997) measured the relative viscosity (taking water as the reference) of milks with different true protein contents (1.0 to 4.8%) in skim milk and 1% milk. The relative viscosity increased by 35.14 and 39.10% when true protein content increased from 2.0 to 4.8% in skim milk and 1% milk, respectively. In Jeurnink and de Kruif's (1993) experiment, relative viscosity of skim milk were 1.099 if held at 90ºC for 600 s; however, relative viscosity were 0.988 when skim milk was held at 60ºC for 600 s.

1.3.2 Dynamic oscillation testing

Dynamic oscillation testing is applied to understand the gelation mechanism in food systems (Tang and others 1995). To study the microstructure of the material system, a small deformation based on rheological measurement (small oscillatory shear test) is applied (Song and others 2006). Typically, this dynamic test starts with amplitude of strain or stress at a constant frequency (large amplitude oscillatory shear test) to ensure the strain is small enough to be within the linear viscoelastic region of the material. Once the linear viscoelastic region is defined, further experiments such as a frequency-sweep test can be conducted to detect the gel properties of the systems. The small amplitude oscillatory shear (SAOS) test allows for testing systems without damage; hence, the microstructure of the material can be described (Macosko 1994).

The relationship between storage modulus and loss modulus can be described by the following equation:

$$G' = (\sigma_0/\Upsilon_0)\cos(\delta)$$

$$G'' = (\sigma_0/\Upsilon_0)\sin(\delta)$$

$$\tan(\delta) = G''/G'$$

Where, G′=storage modulus, G″=loss modulus, σ_0=the amplitude of the shear stress, Υ_0=the amplitude of the strain, δ=the phase shift. Table 1.5 shows the gel properties of a material as a function of δ.

Table 1.5 The relationship between phase shift and gel properties in a material (Bryant and McClements 2000; Tang and others 1995).

Gel properties	Phase shift
Hookean solid	δ=0
Viscoelastic	0<δ<90
Newtonian fluid	δ=90

Tang and others (1995) tested the rheological properties of the heat-induced gelation of whey protein concentrate solutions as a function of pH. Even though pH varied in whey protein concentrate solutions, gels were formed when heated at 80ºC after 44 min. Cho and others (2012) used frequency sweeps to detect gel structures of thickened beverages (apple juice, orange juice and whole milk) prepared with xanthan or guar gum based commercial thickeners. The results suggested that G′ and G″ increased with frequency increases and the tan(δ) at 6.28 rad/s (1Hz) was < 1, which meant that a weak gel-like behavior was exhibited. The structural product types are different if tan(δ) differs, even though the apparent viscosities are the same for

various beverages (Payne 2011).

1.4 Turbidity

1.4.1 Principle of absorbance

White light can be separated into different single colors by various wavelengths (r) (Table 1.6) with higher wavelengths having lower energy. When light shines on a substance, reflection, absorption, or transmission occurs. Different chemical substances have different absorbances, transmittances, and reflection properties. Absorption occurs when a photon of electromagnetic energy is transferred to an atom or molecule. The atom or molecule with certain functional groups absorbs the energy from the photon and shifts from group state to excited state. The absorption peak is found at a wavelength which depends on the difference between the energy levels of the electronic transitions in the chromophores (McClements 1999; Tinker 1975).

Table 1.6 The wavelength of ultraviolet, visible light, and infrared[1].

Color/Radiation	Wavelength range (nm)
Ultraviolet	1-385
Violet	385-425
Blue	425-491
Green	491-575
Yellow	575-585
Orange	585-647
Red	647-730
Infrared	730-100,000

[1] Adapted from Tinker 1975

Absorbance of light measures the total amount of light scattered as it passes through a cuvette containing liquid systems, and larger particle sizes have more light scattering. The three regions of the light scattering are: long-wavelength regime ($r<<\lambda$), intermediate-wavelength regime ($r\approx\lambda$), and short-wavelength regime ($r>>\lambda$), and the turbidity reaches the highest when the size of the droplets is similar to that of the wavelength (Figure 1.4).

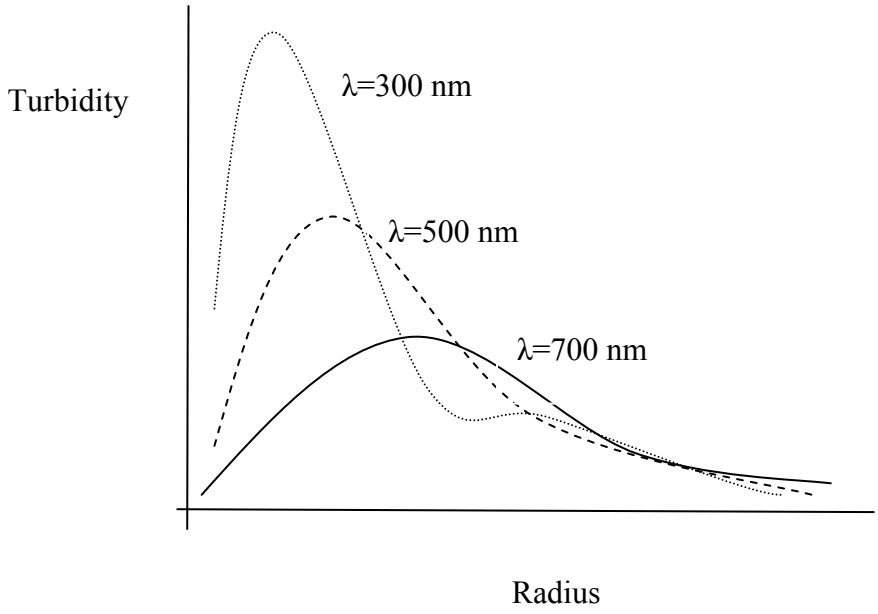

Figure 1.4 Turbidity as function of radius of particle size at different wavelengths (adapted from McClements 1999).

However, this rapid method can only provide an average of the particle size instead of the distribution, and assumptions must be made that the distribution of the particles in the liquid system is known in advance (Dalgleish 2004). The type and concentration of chromophores present in the solution decide the absorption of light, while the size, concentration, and relative refractive index of any particulate matter relate to the light scattering. The Lambert-Beer law fits a wide range of wavelength from UV to infrared (Zijlstra and others 2000). According to the Lambert-Beer law, turbidity has a positive relationship to the absorbance:

$$T=I_S/I_R=\exp(-\tau l)$$

$$A=\log(I_R/I_S)=\alpha lc$$

$$\tau = 2.303A/l$$

Where T is the transmittance, τ is the turbidity, l is the sample path length, A is the absorbance, I_S is the intensity of the light that travels directly through the sample, I_R is the intensity of the light which has traveled directly through the reference cell, α is the molar absorbtivity (L mol^{-1} cm^{-1}), and c is the concentration of the compound in solution (mol L^{-1}) (Pearce and Kinsella 1978; McClements 1999).

1.4.2 Application of absorbance in milk systems

Absorbance has been applied to determine concentrations of milk compounds such as protein and lactose (Nakai and Le 1970; Yoshida and Ye 1991; Teles and others 1978). Nakai and Le (1970) tested milk protein and milk fat contents simultaneously: using acetic acid to dissociate and dissolve both milk protein and fat. The milk protein was tested at a wavelength of 280 nm; urea solution containing imidazole was added further into the solution, and the fat content was tested at a wavelength of 400 nm.

For a complex milk system, the association between denatured whey protein and casein micelles can be detected through absorbance indirectly. Anema and Klostermeyer (1997) tested the turbidity of pH adjusted and heat processed reconstituted skim milk at 900 nm using a UV/VIS spectrophotometer (Kontron Uvikon 941 spectrophotometer), finding that the absorbance of the milk systems decreased by ~2% if processed at 70ºC for 15 min at pH 6.7 while the absorbance

increased by ~7 and 12% when the samples were processed at 80 and 90ºC for 15 min at pH 6.7, respectively. Therefore, a conclusion was drawn that the association of denatured whey protein and κ-casein was responsible for the increase of absorbance.

Particle sizes of the casein in the skim milk processed at various temperatures for 15 min at pH 6.55 have been measured with a Malvern Zetasizer 4 instrument and the associated ZET5110 particle sizing cell by Anema and Li (2003a). The average particle size increased by 4.85% and 10.98% when processed at 80 and 90ºC, respectively. Hence, the absorbance and the particle size seemed to have a positive relationship. Le and others (2013) tested the turbidity of acid-induced gelation of a xanthan gum and β-lg system by measuring absorbance of the system at 800 nm using a UV/VIS spectrophotometer. The absorbance of the β-lg/xanthan gum at ratio=5 (total solid=0.3 w/w%) increased from 0.009 to 0.13 (arbitrary unit) when pH decreased from 6.0 to 5.2. The conclusion was drawn that the association between β-lg and xanthan gum occurs when pH is decreased.

1.5 Dysphagia

Patients who have difficulty in food mastication and swallowing are diagnosed to have dysphasia (Vivanti and others 2009). It is caused by the damage of nerve tissue, cavity structure, or upper digestive tract, which may be the sequela of disease such as stroke, brain injury, spinal cord injury, Parkinson's, etc. Dysphagia mostly occurs in the early post-operative period (Lotong and others 2003; Zeng and others

2013). A survey conducted in a clinic waiting room revealed that ~23% of the patients reported to have symptoms of dysphagia, further it would be more likely to occurr amongst older people (> 65 years) as 53 to 57% of patients who had a stroke experienced dysphagia (Garcia and Chambers 2010).

Older adults suffer from dysphagia more frequently because elderly are more likely to have esophageal disorders such as reflux and hiatus hernia (Davis and Spicer 2007). The normal swallow is a four-step action: (1) oral preparatory phase, the food is held in the mouth by closing lips and cheeks; (2) oral transport phase, the bolus is pushed back into the pharynx by tongue, the nasal cavity is sealed by the soft palate, and the bolus is transported downwards by peristaltic waves; (3) pharyngeal phase, reflexes in laryngo-pharynx occur to prevent the bolus moves towards the airway; and lastly (4) the bolus passes through the crico-pharyn-geal sphincter and downward to the stomach. Dysphagia may occur during any one of the four steps of swallowing (Germain and others 2006).

Dysphagia patients may suffer from aspiration, dehydration, pneumonia, weight loss, malnutrition, and even mortality (Vivanti and others 2009). Aspiration occurs when the foreign material is inhaled into the lower airway (Tutor and Gosa 2012). It may occur during any phases of swallowing, resulting in cough, chest pain, lung abscess, and death. Infants, whose swallowing mechanisms are immature, have a significant amount of suffering from aspiration along with dysphagia. Thin liquids which are difficult to control in the mouth for swallowing-disordered patients may

increase the risk of aspiration. To avoid aspiration, texture-modified and more viscous liquids are prepared for dysphagia patients (Vivanti and others 2009). But at the same time, dysphagia is also associated with dehydration. Such symptom pervades among older adults in all kinds of long-term care agencies including hospitals and living communities (Bratlund and others 2010). A previous report showed that over half of hospitalized elderly adults with dehydration suffer risks of death if no additional care was applied (Allison and Lobo 2004). Due to the negative effects brought by aspiration and dehydration, diets that can be both tolerated by patients and supply adequate moisture contents are needed.

Speech-language pathologists are often involved in recommending food modifications for dysphagia patients to ensure swallowing safety; however, people who take care of the patients such as a nursing staff are often the ones directly preparing and serving the recommended food (Garcia and others 2010). A recommended labeling for diets based on various "consistency" have been developed and established by the American Dietetic Association (ADA) for the National Dysphagia Diet (NDD) (American Speech-Language-Hearing Association 2003). The categories are aimed to guide the liquid food preparation based on thickness and has an instrument measurement of apparent viscosity using centipoises (mPa·s) at a shear rate of 50 s^{-1} at 25ºC (Lotong and others 2003) (Table 1.7).

Table 1.7 Standard consistency classes for foods targeted for dysphagia based on the National Dysphagia Diet (Lotong and others 2003).

Consistency class	Apparent viscosity[1] (mPa·s)
Thin	1-50
Nectar-like	51-350
Honey-like	351-1750
Pudding-like	>1750

[1]The apparent viscosity is measured at shear rate of 50 s^{-1} at 25 ± 1ºC.

Leder and Judson (2012) conducted the safe swallowing tests on 84 patients with dysphagia resulting from different diseases (e.g. cancer, cardiothoracic surgery, dementia, etc.) and reported that the liquid foods of nectar-like or honey-like consistency are safe for these patients.

1.6 Beverages

The consumption of beverages in human history dates back to 1500 BC with fairly limited variability (Grivetti and Wilson 2004). Nowadays, beverage categories have expanded and classified as: water, tea and coffee, low fat (1.5% or 1%) and skim milk, and soy beverages, noncalorically sweetened beverages, caloric beverages with some nutrients, and calorically sweetened beverages (Popkin and others 2006; Sharkey and others 2012). Whey, as an ingredient, has been involved in the beverage development for several decades (Jelen 1987). Whey is a nutritious source of vitamins, minerals, and high quality proteins. The sulfur containing amino acids in β-lg are of

great value because of the anticancer activities; and insoluble free fatty acids are transported by bovine serum albumin. Lactoferrin and lactoperoxidase are known as bioactive proteins with functions of antibacterial, antioxidant, and antiviral properties (Djuric and others 2004; Tunick 2008). Generally, beverages in the market are considered to be "thin" liquids. Typical apparent viscosity of the beverages are listed in Table 1.8.

Table 1.8 Typical flow behavior of common beverages in the market

Beverage	Measured temperature (ºC)	Apparent viscosity (mPa·s)	Fluid behavior	References
Water	25	0.89	Newtonian	Rao 2007
Skim milk	25	1.26	Newtonian	Bakshi and Smith 1984
1% milk	25	1.26	Newtonian	Bakshi and Smith 1984
2% milk	25	1.26	Newtonian	Bakshi and Smith 1984
Whole milk	25	1.26	Newtonian	Bakshi and Smith 1984
Depectinized apple juice (50º Brix)	25	2.7	Newtonian (all concentrations)	Saravaco 1970
Grape juice (30º Brix)	25	3.7	Newtonian (< 50º Brix)	Saravaco 1970
Oragen juice (16º Brix)	25	3	Newtonian (< 20º Brix)	Saravaco 1970
Coffee	-	2.12-2.38	Newtonian	Nemtanu and others 2005
Beer	-	~1.6	Newtonian	Bamforth 2004

1.6.1 Beverage for dysphagia

To treat dysphagia, consistency-adjusted fluids are recommended to support patients, either as ready-to-serve or by adding instant thickener (Claes 2012). Thickened beverages have slowed flow, which allow for muscle control to match the accurate time of swallowing; hence ensuring the safety of swallowing (Atherton and others 2007).

Thickening agents are available commercially and intended specifically as dysphagia foods (Matta and others 2006). Most of the instant thickening agents are starch- or gum-based such as guar gum or xanthan gum (Payne and others 2012). For liquid systems thickened by these agents, viscosity varies according to the diversity of the thickener that is used. Starch-based, instant thickeners have a greater ability to increase the apparent viscosity compared with the gum-based commercial ones (Garcia and others 2005; Hamlet 1996). However, viscosity increases when the thickened liquids set if certain kinds of thickeners are used (Garcia and others 2005; Hamlet 1996).

In Garcia's experiment (2005), commercial thickening agents (Thick & Easy®, Hormel HealthLab, Austin, MN; Thicken Up®, Novartis Nutrition Corporation, Minneaplois, MN; Thick-it®, Precision Foods Inc., St. Louis, Mo; Simply Thick® Phagia-Gel Technologies, St, Louis, MO; and Thick & Clear®, Nutritional Focus, Indianapolis, IN) were used to prepare nectar- and honey-like beverages using water, fruit juice, 2% milk, and coffee. It was observed that the apparent viscosity at 50 s^{-1}

increased after setting for 30 min for all starch-based thickening agents (Thick & Easy®, Thicken Up®, and Thick-it®) while the gum-based thickening agents (Simply Thick® and Thick & Clear®) (xanthan gum and cellulose gum standardized with maltodextrin) did not significantly change during the setting time of 30 min. For instance, magnitude of increase ranged from 105.8 to 312.9% for a 2% milk thickened by different starch-based thickeners (Garcia and others 2005).

Another experiment using carrageenan as the hydrocolloid illustrated its interaction with κ-casein since no κ-casein band was observed through polyacrylamide gel electrophoresis after a 30 min reaction between the gum and protein (Grindrod and Nickerson 1968). Because of the interaction of κ-casein and carrageenan, the apparent viscosity of milk system with carrageenan as a thickener would probably be too inconsistent if the milk set for 30 min. When the gum-based thickeners are used across different beverages (2% milk, apple juice, orange juice, coffee), viscosity seems to be more consistent and less variable compared with beverages thickend by starch-based agents (Matta and others 2006). Sensory evaluation of the starch-based and gum-based commercial thickeners showed that starch-based thickened products generated a grainy texture while gum-based thickened products yielded more slicker textures (Matta and others 2006).

Ready-to-serve beverages or commercially packaged prethickened beverages are another form of beverages that are designed for dysphagia patients. Prethickened fruit juices (including orange and apple juice), milk, and flavored water can be found

in the market (Adeleye and Rachal 2007; Payne and others 2011). However, there are still some parameters that need to be considered about the ready-to-serve beverages in the market. A study conducted by Adeleye and Rachal (2007) compared the rheological properties of ready-to-serve beverages with instant food thickened prepared beverages. Preparation of beverages with instant food thickener strictly followed the instructions for usage amount. The results suggested that the ready-to-serve beverages had much greater apparent viscosities compared with the instant food thickener prepared beverages (303.13 ± 3.56 cPs for nectar-like apple juice of commercially prethickened beverage while 89.07 ± 0.46 cP for nectar-like apple juice of instant food thickened beverage). For some ready-to-serve beverages, apparent viscosities were obviously out of the range of nectar-like or honey-like as presented in Table 1.6 (commercial prethickened beverage of nectar-like orange juice was 447.98 ± 6.32 cP at 20ºC). In another experiment testing the consistency of prethickened beverages in the UK market, the results indicated that the consistencies of the starch-based prethickened beverages were not constant among different batches (Payne and others 2011). Due to the disadvantages of the current ready-to-serve beverages in the market, development of a beverage with suitable apparent viscosity as well as stable consistency is needed.

Xanthan gum has been largely used in the industry to modify the rheology of the products. The association between whey protein and casein micelles can be enhanced by adding α-la. An economic and nutritious ready-to-serve beverage with

suitable and stable apparent viscosity may be developed based on the enhanced protein interactions by α-la/β-lg ratio adjustment, or with the addition of a polysaccharide.

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Chapter 2 - Research objectives

The overall objective of this research was to develop a dairy-based beverage with the rheological properties that are suitable for dysphagia patients by adjusting the ratio of α-lactalbumin (α-la) and β-lactoglobulin (β-lg) and controlling the interactions between milk proteins and xanthan gum.

In the first study, the objective was to determine the effects of temperatures and ratios of α-la/β-lg and setting time on the apparent viscosity of milk systems. The objective of the second study was to determine the effects of temperature and ratio of α-la/β-lg and setting time on the rheological properties of the milk systems either with or without xanthan gum.

Chapter 3 - Material and methods

3.1 Milk systems preparation

3.1.1 α-la/β-lg ratio adjusted milk system

Low heat nonfat-dry milk (NDM) (Dairy America, Fresno, CA, USA) and LACPRODAN® ALPHA-20 (Arla Foods Inc., Basking Ridge, NJ, USA; Appendix A) were obtained from commercial suppliers. The milk systems with total solids of 10% w/w at α-la/β-lg ratio of 3:8 (control), 1:1, and 8:3 were achieved by rehydrating specific amounts of NDM and commercial α-la into dionized, distilled water. The powder mixtures were rehydrated in approximately 80 mL of dionized, distilled water by magnetically-stirring (Isotemp Stirring Hotplate model # 11-600-495SH, Fisher Scientific) for 40 min at 25 ± 1°C. The dispersion was transferred into a 100 mL volumetric flask and made to volume. Milk suspensions achieved the desired process temperature (70, 80, or 90ºC) on the heat/stir plate. The milk systems were either not processed (25ºC) or processed at 70ºC for 30 min, 80ºC for 30 min, and 90ºC for 30 min by heating in a water bath (Isotemp 220, Fisher Scientific) set to the designated temperature. The processed milk systems were immediately cooled to 25 ± 1ºC within 5 min by placing in ice bath.

3.1.2 Xanthan gum added α-la/β-lg ratio adjusted milk system

Dispersions consisting of 0.5% w/w xanthan gum (Ticaxan® Xanthan 200,

TIC Gum, White Marsh, MD; Appendix A) were prepared by mixing 5.5078 g of xanthan gum powder (moisture content of 8.732 ± 0.148%) with 600 mL water, in a an Oster blender (Model: 6870, Jarden Consumer Solutions, Boca Raton, Florida, USA) on speed "blend" for 10 min at 25 ± 1°C. The solution was made to 1,000 mL after centrifuging (Marathon 21000R, Thermo IEC, Needham Heights, MA) at 1,000 g for 5 min to remove the incorporated air (Ahmed and Ramaswamy 2004). The xanthan gum dispension set at 4 ± 1°C for 12 h. Milk systems were prepared by adding 0 or 33.5 mL of xanthan gum solution into NDM and commercial α-lactalbumin dispersions to achieve the desired xanthan gum concentration of 0 or 0.15%. The mixture was stirred on a magnetic stir (Isotemp Stirring Hotplate model # 11-600-495SH, Fisher Scientific) for 2 h at 25 ± 1°C. The milk systems were made up to volume in a 100 mL volumetric flask. Milk systems were either processed at 90°C for 30 min or remained at 25 ± 1°C.

For the 90°C samples, milk systems were transferred to a 100 mL beaker, and were quickly heated to 85 ± 1°C on a hot plate (Isotemp Stirring Hotplate model # 11-600-495SH, Fisher Scientific, Pittsburgh, PA, USA) within 3 min, then samples were processed at 90°C in a water bath (Isotemp 220, Fisher Scientific) for 30 min. Processed dispersions were cooled to 25 ± 1°C in ice bath covered with aluminium foil within 10 min.

3.2 Assessments

3.2.1 Rheological properties

3.2.1.1 Apparent viscosity of the α-la/β-lg ratio adjusted milk systems

The apparent viscosity for α-la/β-lg ratio adjusted milk systems were obtained on a speed controlled viscometer (Brookfield Programmable LVDV-II Viscometer, Brookfield Engineering. Middleboro, MA, USA) fitted with ULA spindle and UL adaptor. Approximately 25 mL sample was measured at shear rates of 14.7, 24.5, 36.7, 61.2, 73.4 and 122 s^{-1} at 25°C. Since the milk system is a Newtonion fluid (Karlsson and others 2005), the apparent viscosity at a shear rate of 50 s^{-1} was calculated by forming a linear regression model of shear stress and shear rate as:

$$\tau = \mu * \dot{\Upsilon}$$

where τ is the shear stress, $\dot{\Upsilon}$ is the shear rate, μ is the apparent viscosity (Glassburn and Deem 1998); all R^2 were > 0.999.

3.2.1.2 Apparent viscosity of xanthan gum and α-la/β-lg ratio adjusted milk systems

Apparent viscosity of xanthan gum milk systems was measured at a shear rate range from 0.1 to 100 s^{-1} using a shear rate control rheometer (VISCOANALYSER DSR, ATS RheoSystems, 231 Crosswicks Road, Bordentown, NJ) with the accompanying Rheoexpolorer software (RheoExplorere Version 5) at 25 ± 1°C. Ten points in the logarithm scale were taken every 20 s (Pollen 2002). Apparent viscosity at 50 s^{-1}, consistency coefficient, and flow behavior index were recorded for further

statistical analyses. Apparent viscosity at 50 s^{-1} was calculated by power law (Steffe 1996):

$$\tau=K(\gamma)^n$$

$$\mu= \tau/ \dot{Y}$$

Where τ=shear stress (Pa), γ=shear rate (1/s), K=consistency coefficient, n=flow behavior index, $\dot{Y}$ is the shear rate, and μ is the apparent viscosity at shear rate of 50 s^{-1}; R^2 were between 0.992-0.999.

3.2.1.3 Phase shift, storage modulus (G'), and loss modulus (G") of xanthan gum added α-la/β-lg ratio adjusted milk systems

Phase shift, G′, and G″ were obtained using a frequency sweep ranging from 0.1 to 100 Hz at a constant strain of 1% using a shear rate control rheometer (VISCOANALYSER DSR, ATS RheoSystems, 231 Crosswicks Road, Bordentown, NJ) with the accompanying Rheoexpolorer software (RheoExplorere Version5) at 25 ± 1°C. A strain sweep was conducted (strain ranged from 0.1 to 100% at a constant frequency of 1 Hz) to ensure the strain of 1% was within the linear elasticity range. Ten measurements were taken every 20 s. The phase shift, G′, and G″ at 1 Hz were taken for statistical analyses (Keshtkaran and others 2013).

3.2.2 Turbidity

The turbidity of xanthan gum added α-la/β-lg ratio adjusted milk systems were measured following the method given be Le and Turgeon (2013). Milk systems were diluted into 1:10 with dionized, distilled water; turbidity was detected by measuring absorbance of approximately 3 mL diluted sample at 800 nm with 1 cm quartz cells (Fisher Scientific) using a UV/VIS-light spectrophotometer and each sample was measured twice. Approximately 3 mL dionized, distilled water was used as the blank.

3.2.3 pH

pH (post-process) was measured by a pH meter at 25°C (Accumet AP63 portable pH meter, Fisher Scientific) calibrated with standard pH 4 and pH 7 buffer solutions (Fisher Scientific) at 25 ± 1ºC.

3.2.4 Total solids (TS)

Total solids were measured using the forced-draft oven method (Hooi and others 2004). Approximately 3 mL of sample was added into a pre-heated and pre-dessicated aluminum, disposable dish with the diameter of 56 mL (Fisher Scientific), and covered with another aluminum, disposable dish. Samples were placed in the oven to dry at 100 ± 2ºC for 12 h. Samples were cooled and dessicated in a dessicator for 15 min. Total solids were calculated as follows:

$$\% \text{ Total Solids} = \frac{\text{Dry sample weight}}{\text{Initial sample weight}} \text{ x100}$$

3.2.5 Free and total sulfhydryl groups

Free sulfhydryl groups were measured following the method by Hashizume and Sato (1988). Approximately 3 mL of the sample for the first study and 1 mL for the second study were dissolved in 5 mL of sodium phosphate buffer (pH 8.0; prepared by Na_2HPO_4 and NaH_2PO_4; Fisher Scientific) and 0.1 mL of 10^{-2} M 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB; Sigma-Aldrich, St. Louis, MO, USA). After holding for 5 min, 2 g of ammonium sulfate (Fisher Scientific) was added to coagulate the milk proteins, and held for 2 min more at 25°C. Milk samples were filtered through Whatman No. 1 filter paper (Fisher Scientific). The absorbance of 3 mL of the supernatant was measured at 412 nm using a UV-Visible wavelength spectrophotometer (GENESYS 5, Thermo Electron Corporation, Madison, WI, USA) in a 1 cm disposable quartz cell (Fisher Scientific) at 25ºC. The blank consisted of the same amount of reagents treated equally as samples.

Total sulfhydryl groups were measured following Shimada and Cheftel (1989). Approximately 0.265 mL of milk sample at ratio of 3:8 and 1:1, and approximately 0.225 mL of milk samples at the ratio of 8:3 were diluted in 10 mL of 8 M urea (Sigma-Aldrich) and 0.5% sodium dodecyl sulfate (SDS; Sigma-Aldrich) dissolved in pH 8 sodium phosphate buffer (Fisher Scientific) to achieve protein concentrations of approximately 0.1%. Diluted samples were votexed for 1 min, followed by centrifugation (Marathon 21000R, Thermo IEC, Needham Heights, MA) at 15,000*g for 15 min at 25°C. The supernantant was carefully removed by filtration through

Whatman No.1 filter paper, and 3 mL of the filtrated sample was transferred to a 1 cm disposable quartz cell (Fisher Scientific). Then, 0.03 mL of DTNB reagent was added into each cell and the mixture was shaken 10 times thoroughly. Absorbance was recorded at 412 nm after 10 min using the same amount of reagent without sample as the blank at 25°C, and the average of the two measurements was calculated as the absorbance for each sample. The concentrations of free and total sulfhydryl groups were calculated by the following equation (Shimada and Cheftel 1989):

$$C_0 = \frac{A}{\epsilon} * D$$

Where C_0 = original concentration (mol/L), A = absorbance at 412 nm, Є = extinction coefficient = 13,600/M/cm, and D = dilution factor (Appendix B, Table B.13 for the first study; Appendix C, Table C.10 for the second study).

Chapter 4 - Heat-induced Interactions between Casein Micelles and Whey Proteins at Varying α-Lactalbumin (α-la) and β-Lactoglobulin (β-lg) Ratios

4.1 Abstract

Heat-induced interactions between casein micelles and whey proteins are known to alter physical properties of milk and milk-based products by increasing viscosity, particle sizes, and changing opacity. The ratio of the two major whey protein fractions (α-lactalbumin **(**α-la) and β-lactoglobulin (β-lg)) also may have a role in altering the physical properties of the final liquid systems, which may be useful for functional beverages. Liquid systems consisting of reconstituted nonfat dry milk and a commercial source of α-la were prepared to targeted α-la/β-lg ratios of 3:8, 1:1, and 8:3 then processed at 70, 80, and 90°C (30 min) or not. After processing, samples were cooled to 25°C and evaluated at 0, 30, 60, and 120 min.

An incompete block design was used and three replications were done; statistical analyses were done by ANOVA and Tukey's HSD. Apparent viscosity was the least for the samples processed at 70°C. An increase in apparent viscosity was observed in the 80°C processed samples at the ratio of 8:3 while no changes in apparent viscosity were found in the other two ratios compared with the 25ºC samples. Apparent viscosity increased by 15.96, 6.38, and 2.11% for 8:3, 1:1, and 3:8 milk systems at 90°C samples compared with at 25ºC, respectively. When milk systems had set for 120 min, apparent viscosity increased by 3.74%. All milk systems had similar pH (6.53) and total solids (10.74%). Sulfhydryl/disulphide bond exchange as well as the formation of disulphide bonds explain the association between casein micelles and whey proteins; decreased total sulfhydryl group decreased and free

sulfhydryl group increased when comparing 80 and 90ºC processed samples with the 25ºC samples.

Results suggested that the apparent viscosity had increased due to the associations between whey proteins and casein micelles. The milk system at α-la/β-lg ratio of 8:3 processed at 90°C had the greatest apparent viscosity of all milk systems.

4.2 Introduction

As a group, whey proteins begin to thermally denature at 70°C; as the free sulfhydryl groups on β-lactoglobulin (β-lg) are exposed, leading to the association between whey proteins and casein micelles (Anema and Li 2003a; Corredig and Dalgleish 1996). Elevated temperatures (> 60ºC) promote molecule mobility and induce hydrophobic associations (Scheraga and others 1962), while at the same time, the strength of hydrogen bonds decrease when temperature exceeds 30ºC (Skrovanek and others 1985).

The ratios of α-lactalbumin (α-la)/β-lg, α-la/κ-casein, and β-lg/κ-casein affect milk protein associations. Increased concentrations of α-la promote the association between α-la and κ-casein while increased concentration of β-lg does not increase the associations between β-lg and κ-casein (Corredig and Dalgleish 1996; 1999); however, intermediates might form between α-la/β-lg, which could later attach to the κ-casein (Corredig and Dalgleish 1999). α-La does not associate with κ-casein if β-lg is not present; but, β-lg associates with κ-casein without α-la (Corredig and Dalgleish 1999). Furthermore, the amount of β-lg that can associate with κ-casein seems to be restricted by the number of available sites on the casein micelles. When an extra 2 mg/mL of β-lg was added to skim milk by no change in the band of the SDS-PAGE (final amounts of α-la and β-lg were 1.2 mg/mL and 5.2 mg/mL, respectively). The maximum amount of β-lg that could associate to κ-casein was ~0.72 mg/mg as additional β-lg did not increase the association. On the other hand, adding 2 mg/mL of

α-la in the skim milk, increased the association between α-la and κ-casein from 0.22 to 0.65 mg/mg (the amount of α-la and β-lg for the milk systems were 3.2 mg/mL and 3.2 mg/mL, respectively) (Corredig and Dalgleish 1999).

Dalgleish and others (1997) studied the protein associations that occurred when different concentrations of α-la and β-lg (α-la/β-lg=0.8/0.8, 1.2/1.2, 1.2/3.2, and 3.2/1.2 g/L) were added to resuspended casein micelles. The results showed that the maximum α-la/κ-casein and β-lg/κ-casein associations occurred in the milk systems that contained the ratio of α-la/β-lg=3.2/1.2 g/L. The associations between α-la/κ-casein and β-lg/κ-casein in the milk systems at ratio of 0.8/0.8 g/L and 1.2/1.2 g/L were greater than those at ratio of 1.2/3.2 g/L (which is the normal ratio of α-la/β-lg in skim milk). Thus, a conclusion was made that the amount of α-la associated with the micelles depended on the concentration of α-la present whereas the association between β-lg and casein micelles were limited by the number of binding sites on the casein micelles.

Denaturation and association of milk proteins can be classified by many ways: insoluble/soluble, covalent/noncovalent, reversible/ irreversible, and native/denatured according to the mechanisms of association (Cromwell and others 2006; Gulzar and others 2011). Denaturation and aggregation can be explained by the following equations:

$$(P_N)_n \leftrightharpoons nP_N$$

$$P_N \leftrightharpoons P_U$$

$$P_U + A \rightarrow (P - A)$$

where $(P_N)_n$ is the native conformation of the whey protein fraction (such as dimer or octamer of β-lg), P_U is the unfolded structure, and A is any other compound that associated with the whey protein fraction (Anema 2009).

The reactions are reversible when non-covalent bonds are disrupted in the secondary and tertiary structures of the protein, whereas irreversible reactions occur when further associations with other compounds occur via covalent bonds. The associations between β-lg and casein micelles in milk are mainly through the active monomer (denatured β-lg monomer) with the sulfhydryl group, and the association reactions end by the formation of disulfide bridges (Roefs and deKruif 1994). Hence, protein associations occur between β-lg and β-lg, β-lg and κ-casein, as well as β-lg and α-la (Roefs and deKruif 1994; de Jong and Van der Linden 1998). The sulfhdryl/disulfide bond exchange between β-lg and κ-casein is considered to be the predominant reaction for association. Free sulfhydryl groups are buried in the core of β-lg, and thus they are barely detected in native β-lg (Shimada and Cheftel 1989).

Once a specific temperature is achieved, the unfolding of β-lg exposes the free sulfhydryl groups so that they can be detected by various methods (Fahey and others 1981; Zweig and Block 1953; de Wit and Nieuwenhuijse 2008). Previous experiments tested for free sulfhydryl groups in milk after heating at 90ºC for 30 min using Ellman's reagent and reported that free sulfhydryl groups increased by approximately $2.1*10^{-6}$ mol/g of protein (Hashizume and Sato 1988); whereas the

total sulfhydryl groups decreased as the heat was applied (Shimada and Cheftel 1989; de Wit and Nieuwenhuijse 2008). In the experiment by Taylor and Richardson (1979), total sulfhydryl groups decreased by 5.48% when skim milk was processed at 90ºC for 1 min compared with raw skim milk.

Denaturation and association of milk proteins have been reported to increase the particle size in a liquid system, viscosity, and turbidity of liquid systems (Anema and others 2004b; Anema and Li 2003a). The relative viscosity (milk system/water) of reconstituted skim milk (pH 6.55) processed at 90°C for 30 min was increased by 0.2 Nsm^{-2} compared with skim milk at 25ºC (Anema and Li 2003a). Previous researchers suggested that increased viscosity in skim milk was mainly due to the β-lg and κ-casein associations as aggregates of β-lg did not increase viscosity (Jeurnink and deKruif 1993). Heated milk samples (> 80ºC) have been reported to have increased particle sizes compared to the unheated milk; for example, particles increased by 40 nm when milk was processed at 90ºC for 30 min at pH 6.5 compared with 25ºC (Anema and others 2004a).

It is known that the heat-induced associations of whey proteins and casein micelles can be increased with increased concentrations of α-la (Corredig and Dalgleish 1996); but the magnitude of apparent viscosity increases due to the associations between whey proteins and casein micelles enhanced by addition of α-la is unknown. Hence, this experiment was conducted to determine the influence of ratio of α-la/β-lg on the apparent viscosity of milk.

4.3 Materials and Methods

4.3.1 Products

Low-heat nonfat dry milk (NDM) (Dairy America, Fresno, CA, USA) and LACPRODAN® ALPHA-20 (Arla Foods Inc., Basking Ridge, NJ, USA) were obtained and analyzed for composition by the following standardized methods. Moisture and ash contents were determined using the forced air oven and gravimetric methods, respectively (Hooi and others 2004). Total protein was calculated from the nitrogen data obtained from the combustion method, using a conversion factor of 6.38 as described by ISO8968-5/IDF20-5 (2001). True protein and protein fractions were determined using ISO 8968-4/IDF20-4 (2001), ISO8968-5/IDF20-5 (2001), and ISO/TC 34/SC 5 N (2011), respectively. True protein, casein and whey protein contents were calculated by the following formulas:

True protein = (Total protein nitrogen – non protein nitrogen) * 6.38

Casein = True protein – non casein

Whey protein = Non casein – non protein nitrogen

The percentage of α-la and β-lg were calculated (α-la/whey protein: 18.75% and β-lg/whey protein: 50%) based on Goff (2012). Results are displayed in Table 4.1.

Table 4.1 Composition of NDM powder and commercial α-lactalbumin (α-la)[1]

Component	Commercial α-la	Low-heat nonfat dry milk	
	% of Weight	% of Weight	% of Total protein
Moisture	5.409 ± 0.021	2.880 ± 0.0133	
Ash		7.986 ± 0.0055	
Total protein (wet basis)	90.44 ± 0.020	31.54 ± 0.1307	100.00
True protein	90.41 ± 0.27	31.15 ± 0.0823	98.89
Casein		25.21 ± 0.2240	79.94
Whey		5.941 ± 0.2754	18.84
α-la[2]			3.588
β-lg[2]			9.568

[1]Values are means ± SE (n=2). [2]Percent of total protein of α-la and β-lg were calculated based on the normal percentage in the skim milk from Table 1.1.

4.3.2 Milk systems

NDM and commercial α-la were mixed in dionized, distilled water to prepare 10% (w/w) milk systems with adjusted ratios of α-la and β-lg. Table 4.2 depicts the weights of the ingredients to obtain the desired ratios.

Table 4.2 Amounts of NDM and LACPRODAN® ALPHA-20 in 100 mL milk systems

Ratio[1] (α-la/β-lg)	NDM[2] (g)	Commercial α-la[3] (g)	dH_2O[4] (g)	Casein[5] (g)	α-la[5] (g)	β-lg[5] (g)
3:8	11.4784	0	100	2.8939	0.1299	0.3464
1:1	11.1032	0.3860	100	2.7993	0.3350	0.335
8:3	10.2128	1.3019	100	2.5748	0.8218	0.3082

[1]Target ratio of α-la/β-lg in the milk system; [2]Nonfat dry milk; [3]LACPRODAN® ALPHA-20; [4]Dionized, distilled water; [5]Values were calculated from Table 2.1.

Powders were rehydrated in approximately 80 mL of dionized, distilled water and magnetically stirred (Isotemp Stirring Hotplate model # 11-600-495SH, Fisher Scientific, Pittsburgh, PA, USA) for 40 min at 25 ± 1°C. The mixture was transferred

to a 100 mL volumetric flask and made to volume. Samples were then transferred to a 100 mL beaker and processed: 25°C (non-heated), 70°C for 30 min, 80°C for 30 min, and 90°C for 30 min, by holding in a water bath (Isotemp 220, Fisher Scientific) set at the designated temperature after each milk system had achieved the desired temperature (70ºC, 80ºC and 90ºC for < 3 min) on the heat/stir plate. When the process was completed, samples were immediately cooled to 25 ± 1°C within 5 min by placing in an ice bath.

4.3.3 Assessments

The apparent viscosity was evaluated on a speed-controlled viscometer (Brookfield Programmable LVDV-II Viscometer, Brookfield Engineering, Middleboro, MA, USA) fitted with ULA spindle and UL adaptor. Approximately 25 mL sample were analyzed at shear rates of 14.7, 24.5, 36.7, 61.2, 73.4 and 122 s^{-1} at 25°C. Since milk is a Newtonion fluid (Karlsson and others 2005), apparent viscosity at a shear rate of 50 s^{-1} was calculated by forming a linear regression model of shear stress and shear rate as:

$$\tau = \mu * \dot{\Upsilon}$$

where τ is the shear stress, $\dot{\Upsilon}$ is the shear rate, μ is the viscosity (Glassburn and Deem 1998). All R^2 in the linear regression models were > 0.999.

pH (post-process) was measured by a pH meter at 25°C (Accumet AP63 Portable pH meter, Fisher Scientific) that had been calibrated with pH 4 and pH 7 standarized buffer solution (Fisher Scientific). Total solids for the milk systems were

measured using the forced air method (Hooi and others 2004).

Free sulfhydryl groups were measured following the method by Hashizume and Sato (1988). Approximately 3 mL of milk sample was dissolved in 5 mL of sodium phosphate buffer (pH 8.0; prepared by Na_2HPO_4 and NaH_2PO_4; Fisher Scientific) and 0.1 mL of 10^{-2} M 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB; Sigma-Aldrich, St. Louis, MO, USA). After holding for 5 min, 2 g of ammonium sulfate (Fisher Scientific) were added to coagulate the milk proteins, and then held for another 2 min at 25°C. Milk samples were filtered through Whatman No. 1 filter paper (Fisher Scientific). The absorbance of 3 mL of the supernatant was measured at 412 nm using a UV/VIS wavelength spectrophotometer (GENESYS 5, Thermo Electron Corporation, Madison, WI, USA) in a 1 cm disposable quartz cell (Fisher Scientific).

Total sulfhydryl groups were measured following Shimada and Cheftel (1989). Approximately 0.265 mL of milk sample at ratio of 3:8 and 1:1, and approximately 0.225 mL of milk samples at the ratio of 8:3 was diluted in 10 mL of 8 M urea (Sigma-Aldrich) and 0.5% sodium dodecyl sulfate (SDS; Sigma-Aldrich) dissolved in pH 8 sodium phosphate buffer (Fisher Scientific) to achieve a protein concentration of approximately 0.1%. The diluted samples were votexed for 1 min, followed by centrifugation (Marathon 21000R, Thermo IEC, Needham Heights, MA) at 15000*g for 15 min at 25°C. The supernant was carefully removed by filtration through Whatman No.1 filter paper and 3 mL of the filtrated sample were transferred to a 1

cm disposable quartz cell. Then, 0.03 mL of DTNB were added into each cell and the mixture was shaken 10 times thoroughly. Absorbance of the samples with DTNB was recorded at 412 nm after 10 min at 25°C, and the average of the two measurements was calculated as the absorbance for each sample (Shimada and Cheftel 1989). The free and total sulfhydryl groups were calculated using the following equation (Ellman 1959):

$$C_0 = \frac{A}{\epsilon} * D$$

Where C_0 = original concentration (mol/L), A = absorbance at 412 nm, Є = extinction coefficient = 13,600/M/cm, and D = dilution factor (Appendix B, Table B.13).

Apparent viscosity and turbidity of the milk systems were tested at 0, 30, 60 and 120 min after the cooling of milk systems to 25ºC. Free and total sulfhydryl groups were assessed at 0, 30 and 120 min. Total solids and pH of the milk systems were assessed only at 0 min.

4.4 Experimental Design and Statistical Analyses

A connected, incomplete randomized block design was used to explore the effects of temperatures (25, 70, 80, and 90ºC), ratios of α-la/β-lg (3:8, 1:1, and 8:3) and time (0, 30, 60, and 120 min) taking “half day” as a block (Appendix B, Table B.1). From Table 4.3, the milk system at α-la/β-lg ratio of 3:8 at 25ºC was given number “0”. Samples with a specific ratio, temperature and time were given a number

(0-47) (Appendix B, Table B.2). Because of the limitations of the Brookfield viscometer, only 2 samples could be assessed during 30 min. Therefore, to compare the effects among all ratios, temperatures, and times, the "0" sample was prepared and assessed during each half day (Appendix B, Table B.2). Three replications of the experiment were done; however, for "0" sample was 33 replications. Apparent viscosity was measured at 0, 30, 60, and 120 min; pH and TS were recorded at 0 min only. Three-way ANOVA for apparent viscosity, two-way ANOVA for pH and total solids and Tukey's HSD were used to detect the significant effects and means of ratio, temperature and time using SAS (SAS institute Inc., V 9.2. Cary, NC).

Because of time constrants, free and total sulfhydryl groups in milk systems were done in a different design. A split-plot design with the whole plots arranged in a randomized complete block design of temperature, ratio of α-la/β-lg and time (0, 30 and 120 min). Time with 3 levels was the split plot. Three replications were used. Split-plot ANOVA and Tukey's HSD were used to detect the significant effects of ratio, temperature and time as well as the mean differences between each ratio, temperature and time by SAS (SAS institute Inc., V 9.2. Cary, NC).

4.5 Results

4.5.1 Apparent viscosity

The interaction of temperature and ratio as well as the main effect of time significantly affected the apparent viscosity of the milk systems ($P \leq 0.05$) (Appendix B, Table B.4). The flow behaviors of all milk systems were Newtonian with $R^2 >$ 0.999. Comparing the 3:8 milk systems at 25 ºC, the apparent viscosity decreased by 5.79 and 2.11% at 70 and 80ºC, respectively, but increased by 2.11% at 90ºC. Similar observations have been observed by other researchers. In the experiment of Jeurnink and de Kruif (1993), relative viscosity of the skim milk was either processed at 60ºC for 600 s or 90ºC for 600 s were tested. The relative viscosity increased by 9.9% when skim milk was processed at 90ºC for 600 s, but decreased by 1% if processed at 60ºC for 600 s.

When the ratio increased from 3:8 to 1:1 and 8:3, apparent viscosity increased by 0.53 and 4.76% (Appendix B, Table B.7). Compared with 25ºC milk systems, apparent viscosity decreased by 4.76% at 70ºC, but the increased by 2.65 and 7.94% at 80 and 90ºC (Appendix B, Table B.8). Figure 4.1 shows the effect of ratio and temperature on apparent viscosity (actual values were shown in Apprendix B, Table B.4). At 25ºC, apparent viscosities were equivalent with a mean of 1.89 mPa·s. As temperature increased to 70ºC, decreased apparent viscosity was observed at each ratio. Lower apparent viscosity was observed in 3:8 milk systems but greater apparent viscosity was observed in 1:1 and 8:3 milk systems at 80ºC compared with those at

25ºC. A 10.21% increase was observed when the ratio increased from 3:8 to 8:3. When samples were processed at 90ºC, the apparent viscosity of the 1:1 and 8:3 systems increased by 3.09 and 12.37% compared with the 3:8 system, respectively. Apparent viscosity increased by 2.11, 6.38, and 15.96% in the milk systems processed at 90ºC compared with the 25ºC samples at ratio of 3:8, 1:1, and 8:3, respectively.

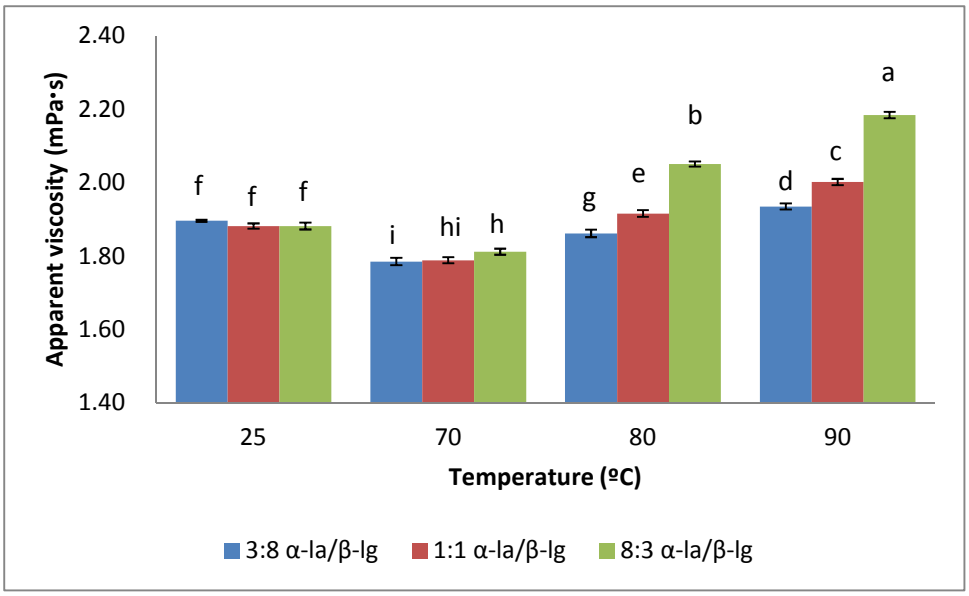

Figure 4.1 The apparent viscosity of milk systems containing different α-lactalbumin (α-la)/β-lactoglobulin (β-lg) ratios and heated at different temperatures. Data are means ± SE (n=132 for control at ratio of 3:8 and 25ºC; n=12 for other ratios and temperatures). [a,b,c]Means on the bars with different superscript letters differ ($P \leq 0.05$).

Apparent viscosity increased in all milk systems as time increased. Figure 4.2 depicted apparent viscosity as a function of time (actual means are depicted in Appendix B, Table B.6). Apparent viscosity increased by 3.74% at 120 min compared

with at 0 min.

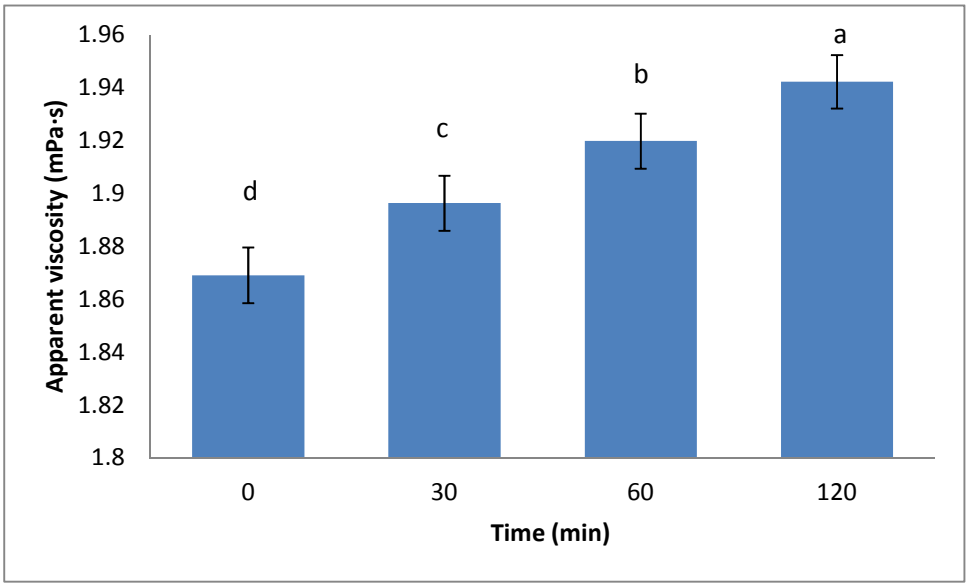

Figure 4.2 The effect of time on the apparent viscosity of milk systems. Data are means ± SE (n=66). [a,b,c]Means on the bar with different superscript letters differ (*P ≤ 0.05*).

4.5.2 pH and Total solids

No significant differences ($p > 0.05$) were observed in pH. According to the fixed effect table (Appendix B, Table B.12), neither interactions nor main effects significantly affected pH. The pH ranged from 6.51 to 6.55 with an overall average of 6.53 (Appendix B, Table B.9).

Total solids (TS) did not show a significant difference among all samples ($p \leq 0.05$) (Appendix B, Table B.11 and B.11). The TS ranged from 10.62 to 10.79% with an overall average of 10.74% (Appendix B, Table B.9).

4.5.3 Free sulfhydryl groups

A three-way interaction of ratio, temperature, and time was significant for free sulfhydryl groups (Appendix B, Table B.15). Figure 4.3 depicts the effects of ratio and time at the four temperatures. Free sulfhydryl groups at 25ºC at 0 min were 0.4632, 0.9926, and 1.4559 μM for the α-la/β-lg ratio of 3:8, 1:1 and 8:3, respectively (Appendix B, Table B.14). Free sulfhydryl groups in the rehydrated NDM systems were fairly small. In several other reports, free sulfhydryl groups in pasteurized skim milk could not be detected (Hutton and Patton 1952; de Wit and Nieuwenhuijse 2008). However, at all three times, free sulfhydryl groups significantly increased for all three ratios when the milk systems were processed at 70, 80 and 90ºC. The 80 and 90ºC samples were equivalent and had significantly greater ($P \leq 0.05$) than the 70ºC samples. The ratio effect on free sulfhydryl groups was significant when the samples were processed at 80 and 90ºC ($P \leq 0.05$). The α-la/β-lg ratio of 8:3 had the greatest free sulfhydryl groups, followed by ratio of 1:1, and ratio of 3:8 had the least. Free sulfhydryl groups increased by 46.59, 66.18, and 112.2 μM when comparing milk systems processed at 90ºC with 25ºC at the ratios of 3:8, 1:1, and 8:3, respectively.

Time did not affect free sulfhydryl groups at 25 and 70ºC. However, decreased free sulfhydryl groups were observed in the milk systems at 80 and 90ºC. Free sulfhydryl groups decreased by 37.44 and 39.58% at 80 and 90ºC, respectively. The The decease in free sulfhydrl groups might because that the exposed sulfhydryl groups had interacted with water as sulfhydryl groups may bind water (Hutton and

Campbell 1981).

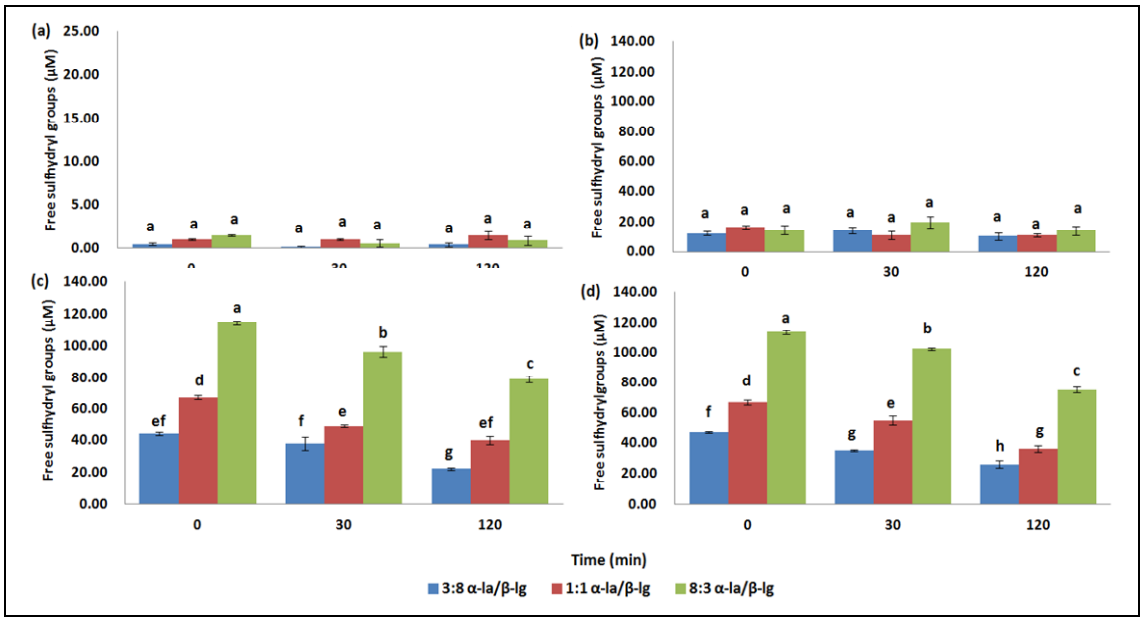

Figure 4.3 Free sulfhydryl groups of milk systems consisting of different ratios of α-lactalbumin (α-la)/β-lactoglobulin (β-lg) and temperatures at (a) 25ºC, (b) 70ºC, (c) 80ºC and (d) 90ºC. Data are means ± SE (n=3). [a,b,c]Means on the bars within each temperature with different superscript letters differ ($P \leq 0.05$).

4.5.4 Total sulfhydryl groups

The interaction of temperature and ratio had a significant effect on the total sulfhydryl groups while the time did not affect the total sulfhydryl groups ($P \leq 0.05$) (Appendix B, Table B.17). Total sulfhydryl groups in the milk systems at ratio of 3:8 and 25ºC of this study was 175.5 μM, which was within the range of the accepted value of 100 to 200 μM (Taylor and Richardson 1980). Total sulfhydryl groups in the 1:1 and 8:3 milk systems compared with the 3:8 milk systems decreased as the temperature increased from 25 to 90ºC; total sulfhydryl groups decreased by 50.8, 71.3 and 134.2 μM when comparing the 90ºC samples with the 25ºC samples at 3:8,

1:1, and 8:3, respectively (Figure 4.4). The results suggested that the association between casein micelles and whey proteins increased due to the addition of α-la.

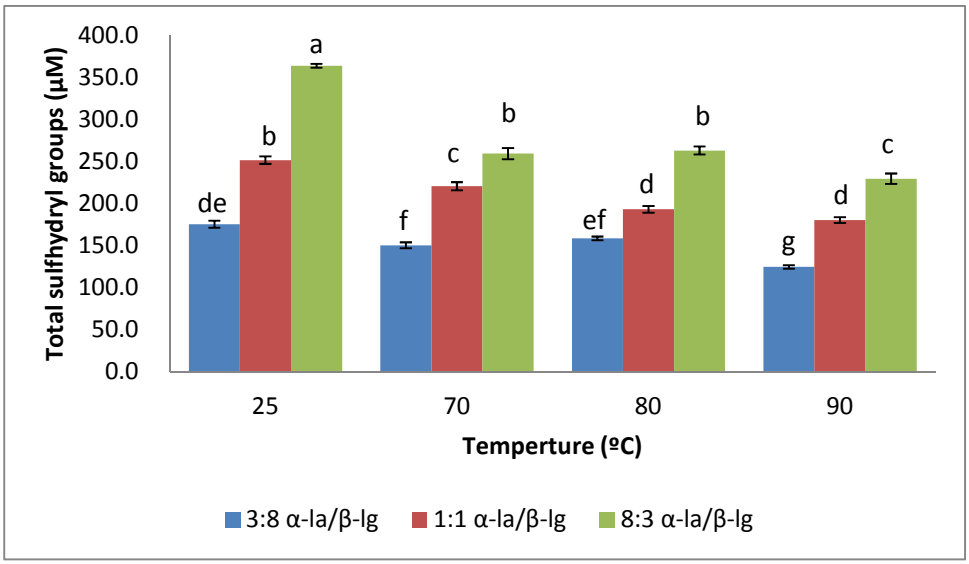

Figure 4.4 Total sulfhydryl group (µM) of milk systems containing different α-lactalbumin (α-la)/β-lactoglobulin (β-lg) ratios and processed at different temperatures. Data are means ± SE (n=9). [a,b,c]Means on bars with different superscript letters differ ($P \leq 0.05$)

4.6 Discussion

4.6.1 Effect of free sulfhydryl groups and total sulfhydryl groups in the proteins association

Almost all whey proteins are denaturated at ≥ 80ºC for 30 min (Anema and Li 2003b), and the denaturated whey proteins mainly associate with κ-casein through sulfhydryl groups (de Wit and Nieuwenhuijse 2008). Figure 4.5 decipts the reactions related to sulphur groups in milk systems. When temperatures exceed 70ºC, tertiary and secondary structures were broken, leading to the exposure of sulfhydryl groups,

which is considered as reversible. The oxidation of exposed sulfhydryl bonds form disulfide bonds or H_2S which is released from the milk systems (de Wit and Nieuwenhuijse 2008). Sulfhydryl/disulfide exchange is also involved in the reaction, resulting in the positional shift of sulfhydryl bonds (Vasbinder and de Kruif 2003). Therefore, whey proteins and casein micelles associate and form a structure via intramolecular and intermolecular disulfide bonds.

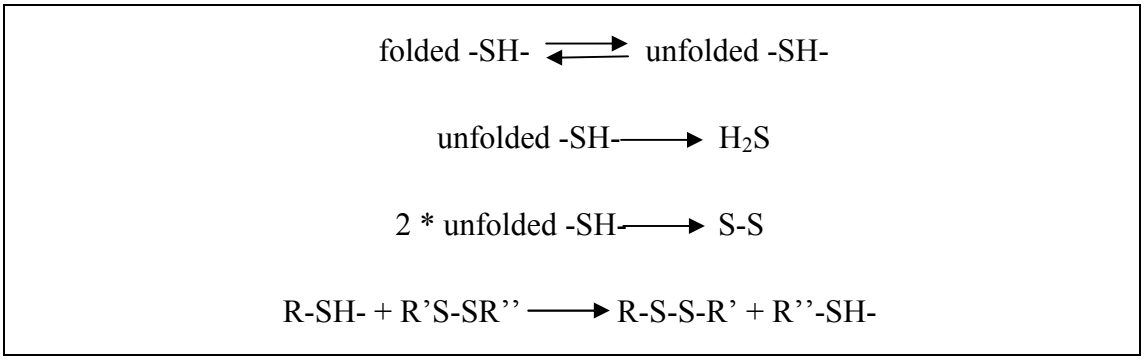

Figure 4.5 Partial reactions related to sulphur components in milk systems (adapted from de Wit and Nieuwenhuijse 2008).

In this experiment, total sulfhydryl groups in the milk systems decreased when comparing 25ºC milk systems to those processed at 90ºC, confirming that intermolecular and intramolecular reactions occurred through the oxidation of sulfhydryl bonds and sulfhydryl/disulfide bond exchange (between β-lg and κ-casein, β-lg and α-la, or β-lg and β-lg). The disulfide bonds and H_2S were formed which were responsible for the loss in total sulfhydryl groups. Free sulfhydryl groups (unfolded sulfhydryl groups) in the milk systems were constant at ≥ 80ºC. Within a ratio, the addition of α-la increased free sulfhydryl groups in the milk systems as expected.

4.6.2 The proteins association structures

Figure 4.6 depicts the structure of a casein micelles with or without the addition of the commercial α-la and processed at 90ºC or 25ºC. β-Lg is attached directly to the surface of casein micelles with κ-casein mainly through sulfhydryl/disulfide bond exchange. α-La associated to the casein micelles by attaching to β-lg, since α-la fails to attach to κ-casein without the presense of β-lg at ≥ 70ºC (Corredig and Dalgleish 1996; Gezimati and others 1997). Extented hairy layers may be formed when denatured whey proteins associate to casein micelles, expanding the effective particle size of the complex (Bienvenue and others 2003).

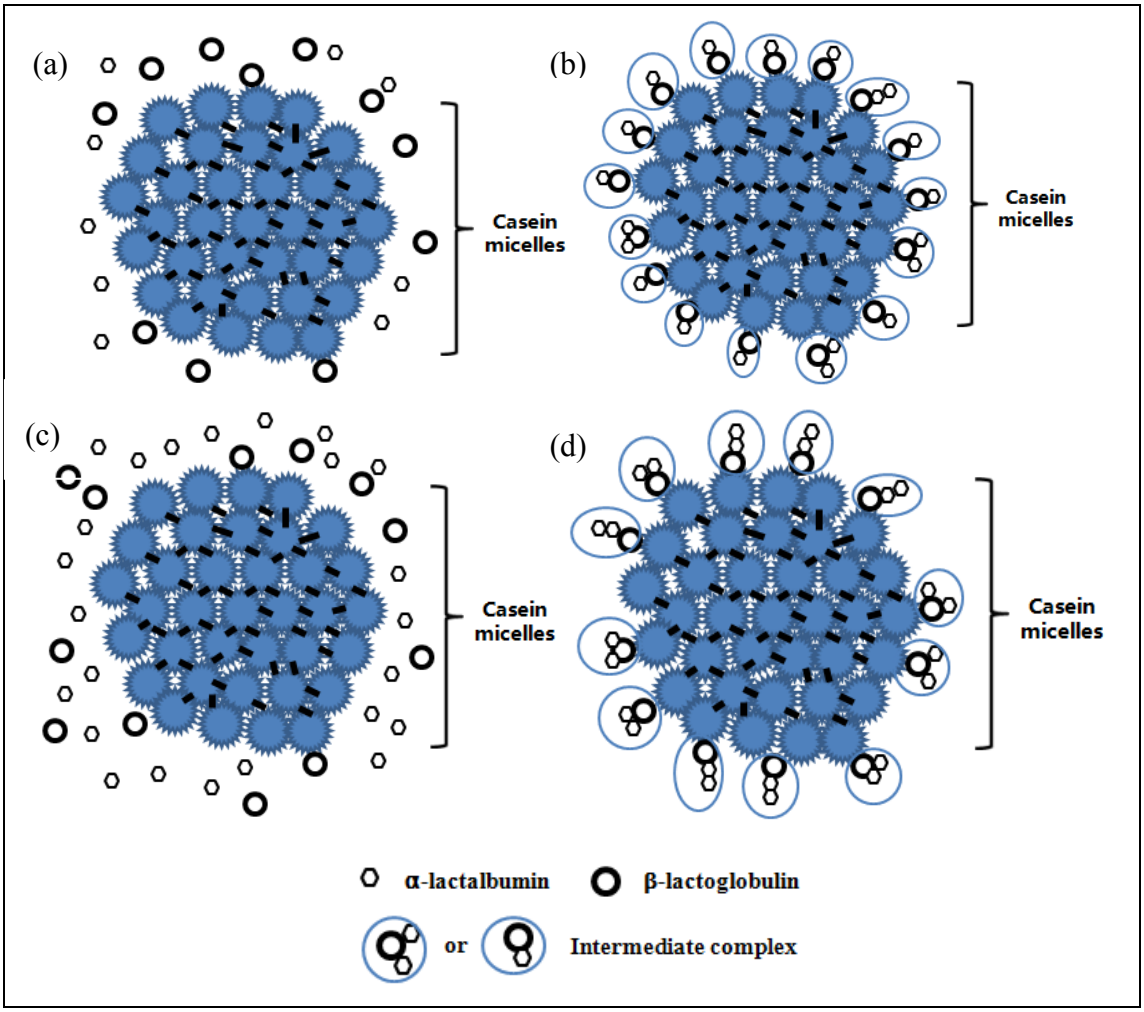

Figure 4.6 The structure of casein micelles associated with α-la and β-lg (a) at

α-lactalbumin (α-la)/β-lactoglobuln (β-lg) ratio of 3:8 processed at 25ºC; (b) at α-la/β-lg ratio of 3:8 processed at 90ºC; (c) at α-la/β-lg of 8:3 at 25ºC; (d) at α-la/β-lg of 8:3 at 90ºC (adapted from de Kruif and others 2012)

Casein micelles provide a large number of positions for attachment of β-lg, which α-la can attach indirectly to κ-casein by their attachment to β-lg. The association between α-la and β-lg occurs before β-lg attaches to κ-casein, forming intermediates (Corredig and Dalgleish 1999). Different intermediates have been formed at various α-la/β-lg ratios such as two moles of α-la with one mole of β-lg, one mole of α-la with one mole of β-lg, or one mole of α-la with two moles of β-lg (Livney and others 2003). A previous experiment studied the rheological properties of a model system without casein but with various ratios of α-la/β-lg (0%/10%, 2%/8%, 5%/5%, 8%/2%, and 10%/0% w/v), and reported the maximum G′ occured at a ratio of 2%/8% (α-la/β-lg=0.25) (Gezimati and others 1997). However in my study which contains commercial sourse of α-la, the maximum apparent viscosity occurred in the milk system at ratio of 8:3 (0.8218% and 0.3081% for α-la and β-lg, respectively). Perhaps, the presence of casein micelles can increase the number of intermediates formed by α-la and β-lg, which increases the effective size of the final complexes.

4.6.3 Effect of ratio and temperature on apparent viscosity

Whey protein-casein compexes are predominately responsible for the increased viscosity (Jeurnink and de Kruif 1993; Anema and Li 2003b). Increased micelle size cause the increase in volume fraction in skim milk (Jeurnink and de Kruif 1993). Viscosity well demonstrates the protein associations in skim milk as the relationship between viscosity and volume fraction has been decribed by the Einstein equation (Anema and others 2004b). pH and total solids have been proven to affect the apparent viscosity of milk systems (Vasbinder and de Kruif 2003; Fernandez-Martin 1972). When milk is at pH < 6.4, whey protein-casein micelles interactions increase, resulting in a viscosity increase (Vasbinder and de Kruif 2003; Anema and others 2004b). It has been reported that apparent viscosity of reconstituted whole milk at 45ºC increased by ~0.3 mPa·s when total solids increased from 10 to 20% (Trinh and others 2007). A model was also formed by Langely and Temple (1985) to describe the relationship between relative viscosity and total solids in skim milk showing that the relative viscosity increases as total solids increases. The consistency of total solids and pH which was above 6.4 in those milk systems indicated that these parameters were not additional influences on the increase of apparent viscosity.

At 25°C, whey proteins remained in their native form, and have little ability to associate with casein; therefore, in my study, the apparent viscosities were equivalent despite any alteration in the α-la/β-lg ratios. As the whey proteins started to denaturate ~70°C, decreased apparent viscosities were observed in the skim milk systems

(despite the ratio), which had been explained as a possible precipitation of calcium phosphate onto the casein micelles which leads to the shrinkage of the particle size; thus, decreased the viscosity (Jeurnink and deKruif 1993). The additional α-la in the 1:1 and 8:3 milk systems may have increased the number of intermediates, which in term can increase the associations among whey proteins and casein micelles.

Significant differences in apparent viscosities were observed between each ratio when processed at 80 and 90ºC (Figure 4.1). At 80ºC, no significant differences in apparent viscosity were observed in the 3:8 and 1:1 milk systems while significant increases in apparent viscosity were observed in 8:3 milk system. Even though the whey proteins had been fully denatured (Anema and Li 2003b), association between whey proteins and casein micelles were limited in the milk systems at 80ºC. At 90ºC, association between whey proteins and casein micelles increased, leading to the greater increase in apparent viscosity compared with at 80ºC. The addition of α-la increased the association between whey proteins and casein micelles as the association between α-la and κ-casein was mainly influenced by the concentration of α-la (Corredig and Dalgleish 1999). As a consequence, particle size of the complexes expanded due to the increased association between α-la and κ-casein, and further increased apparent viscosity.

4.6.4 Effect of the time

Both covalent and noncovalent bonds are responsible for associations and aggregations between whey proteins and casein micelles (Hae and Swaisgood 1990). In a previous study, formation of β-lg octomers is via disulphide bonds while larger aggregates are formed through noncovalent bonds (de la Fuente and others 2002). Hae and Swaisgood (1990) suggested that complexes formed at low temperatures (< 70ºC) would primarily involve noncovalent bonds. In this experiment, total sulfhydryl groups remained constant after 120 min (Appendix B, Table B.15), supporting that the increased apparent viscosity with time resulted from the noncovelent bonds between whey proteins and casein micelles. Also, free sulfhydryl groups decreased when the milk systems were processed at 90ºC. Polar groups such as carbonyl, hydroxyl, amino, , and sulfhydryl groups are responsible for the water-protein interactions; also, unfolding of proteins allows amino acids to bind more water (Hutton and Campbell 1981). Perhaps, the observed decreased free sulfhydryl groups in milk systems at 120 min were due to interactions of proteins and water as sulfhydryl groups bonded to polar groups in the water (Figure 4.3).

4.7 Conclusions

The adjustment of α-la/β-lg ratio in the milk system and processed at > 80ºC changed the physical and chemical properties of the milk systems. Apparent viscosity at shear rate of 50 s^{-1} and 25ºC can be increased if the ratio of α-la/β-lg in the milk systems is increased and processed at > 80ºC. The increase in apparent viscosity was mainly attributed to the association between whey proteins and casein micelles through sulfhydryl/disulfide bond exchange. The milk system at ratio of 8:3 processed at 90ºC was the most stable during the 120 min. If those changes in the physical properties of the milk systems are applied, a beverage with suitable and stable rheological properties for dysphagia patient may be obtained.

However, the maximum apparent viscosity was 2.18 mPa·s in the milk systems at α-la/β-lg ratio of 8:3 and processed at 90ºC, which is far less than the consistency recommended by the National Dysphagia Diet. Further adjustments are needed to achieve the consistency that is suitable for dysphagia patients.

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Chapter 5 - Milk-based Beverage Prepared by Interactions between Dairy Proteins and Xanthan Gum

5.1 Abstract

Viscosity recommendations for suitable beverages for these dysphagia patients ranges from 51 to 1750 mPa·s. The objective of this study was to develop a dairy-based beverage with rheological properties that are suitable for dysphagia patients. Apparent viscosity of milk systems increased from 1.94 to 2.18 mPa·s when α-lactalbumin (α-la)/β-lactoglobulin (β-lg) ratio increased from 3:8 to 8:3 at 90ºC in the previous study; however, the increased apparent viscosity was far less than the recommendation. For this study, physical and chemical properties of the milk systems at α-la/β-lg ratio of 3:8 and 8:3 with xanthan gum concentrations of 0 and 0.15% processed at 25 or 90°C for 30 min were assessed.

A split polt ANOVA and Tukey's HSD with three replication and SAS were used. Apparent viscosity increased by 48.61 and 89.61% in 3:8 and 8:3 milk systems, respectively for milk systems with a 0.15% xanthan gum concentration and processed at 90ºC compared with 25ºC. Apparent viscosity of the 8:3 milk systems at xanthan gum concentration of 0.15% processed at 90°C was within the nectar-like range. Furthermore, the apparent viscosity did not change after setting for 120 min. Turbidity increased by by 65.7, 91.5, and 96.1% for milk systems at 3:8 ratio with xanthan gum, 8:3 ratio without xanthan gum, and 8:3 ratio with xanthan gum, respectively when processed at 90ºC compared with 25ºC. No significant differences were found in the total solids (10.82%) of the milk systems; but, a significant lower pH in the 3:8 milk systems (6.46) was observed compared with the 8:3 milk systems (6.49) at xanthan

gum concentration of 0.15% processed at 90ºC. Phase shift, G′, and G″ increased if suspensions were processed at 90ºC. When comparing samples processed at 90ºC with 25ºC, free sulfhydryl groups increased by 44.0 and 96.8 μM while total sulfhydryl groups decreased by 48.6 and 85.3μM in 3:8 and 8:3 milk systems, respectively. When the milk systems were set for 120 min, the free sulfhydryl groups decreased by 11.3 μM if processed at 90ºC.

A dairy-based product with suitable rheological properties can be developed based on the interaction between milk protein and xanthan gum. If the rheological properties of a product can be controlled by interaction of ingredients, nutritious products with different forms could be developed.

5.2 Introduction

Dysphagia patients experience problems in swallowing typically due to a medical or physical malady (Matta and others 2006). Approximately 20% of the adult primary care population and 50% of the elderly in nursing homes exhibit dysphagia as a symptom (Cho and others 2012). Many of these patients rely on beverages for their nutritional needs; hence, recommendations for beverage viscosity have been distributed to dieticians and clinics. Suitable beverages should range from 51 to 1750 mPa·s at 25ºC at a shear rate of 50 s^{-1} (Garcia and others 2005); however, sub-categories have been developed to address specific swallowing needs.

To control the viscosity of beverage systems, hydrocolloids are frequently used (Hemar and others 2001; Laneuville and others 2000). Pectin, xanthan, carrageenan, arabic, guar, and tragacanth gums are some of the most commonly used hydrocolloids in food emulsions and foams (Rodriguez-Patino and Pilosof 2011). Xanthan gum, a polysaccharide produced by *Xanthomonas camplestris* pv. *campestris*, is a safe, suitable food additive approved by the U.S. Food and Drug Administration (Becker and others 1998; Garcia-Ochoa and others 2000). Xanthan gum concentrations of 0.1 to 0.2% (w/w) have been used for dairy foods and beverages for industrial applications (Garcia-Ochoa and others 2000). Some commercial thickeners are available that incorporate xanthan gum (e.g. QuikThik™, Simply Thick®) into their base for food applications specific for dysphagia patients (Sopade and others 2008; Garcia and others 2005).

Previous research illustrated that interactions between milk proteins and xanthan gum were observed by altered rheological properties of the liquid system (Schmitt and others 1998). Rheological properties of different milk proteins and xanthan gum differed from each other dependent on the milk protein base, as greater apparent viscosities were reported if whey protein concentrate (WPC) was used versus nonfat dry milk (NDM) (Schmidt and Smith 1992). Sanchez and others (1997) reported that apparent viscosity increased in mixtures of whey protein isolate (14 w/w %) and xanthan gum by 37.5 and 125% when the concentration of xanthan gum changed from 0.1 to 0.2 and 0.5%, respectively (tested at shear stress of 10 $N \cdot m^{-2}$ at 20 ± 0.1ºC).

Both electrostatic interactions and non-electrostatic interactions including hydrogen, hydrophobic and covalent bonds are responsible for protein-polysaccharide interactions (Dickinson 1998; Schmitt and others 1998). In my previous experiment (Chapter 4), the apparent viscosity increased by 5.41% when the ratio of α-la/β-lg changed from 3:8 to 8:3 in a milk system. The maximum apparent viscosity was 2.18 mPa·s at ratio of 8:3 when processed at 90ºC, and the milk system at such conditions was the most stable when it was held for 120 min. Therefore, the milk system at ratio of 8:3 and processed at 90ºC was used in this study. Because thermal denaturation induced associations between whey proteins and casein micelles as well as interactions between milk proteins and xanthan gum (Corredig and Dalgleish 1996; Laneuville and others 2000), a beverage with a stable, controllable viscosity suitable

for dysphagia patients may be possible. The objective of this study was to develop a beverage with apparent viscosity within the nectar-like (51-350 mPa·s) or honey-like (351-1750 mPa·s) categories by controlling the interactions of milk proteins and xanthan gum through heat processing and ratio adjustment of α-lactalbumin (α-la)/β-lactoglobulin (β-lg).

5.3 Materials and Methods

5.3.1 System preparation

To prepare milk systems at with xanthan gum concentration of 0.15%, first a master solution of 0.5% w/w xanthan gum (Ticaxan® Xanthan 200, TIC Gum, White Marsh, MD, USA) were prepared using 5.5078 g of xanthan gum (moisture content of 8.732 ± 0.1483%) to 600 mL deionized water. The master solution was blended in an Oster blender (Model: 6870, Jarden Consumer Solutions, Boca Raton, Florida, USA) on speed "blend" for 10 min at 25 ± 1°C. The suspension was made to 1,000 mL after centrifuging (Marathon 21000R, Thermo IEC, Needham Heights, MA) at 1,000*g for 5 min to remove the incorporated air (Ahmed and Ramaswamy 2004). The xanthan gum solution remained at 4 ± 1°C for 12 hours to allow for full rehydration. Milk systems were prepared by adding 0 or 33.5 mL of xanthan gum solution into nonfat dry milk (NDM) and commercial α-lactalbumin (α-la; LACPRODAN® ALPHA-20, Arla Foods Inc., Basking Ridge, NJ, USA) dispersions to achieve the desired xanthan

gum concentration (Table 5.1). The mixture was stirred on a magnetic stir plate (Isotemp Stirring Hotplate model # 11-600-495SH, Fisher Scientific, Pittsburgh, PA, USA) for 120 min at 25 ± 1°C. The milk systems were made to volume in a 100 mL volumetric flask. Milk systems were either processed at 90°C for 30 min or set at 25 ± 1°C. For the 90°C samples, milk systems were transferred to a 100 mL beaker, quickly heated to 85 ± 1°C on a hot plate (Isotemp Stirring Hotplate model # 11-600-495SH, Fisher Scientific) within 3 min, then placed in a 90°C water bath (Isotemp 220, Fisher Scientific) for 30 min. Processed dispersions were cooled to 25 ± 1°C in an ice bath within 10 min. Non-processed samples set at 25ºC for 40 min before evaluation to maintain equality in time.

Table 5.1 Formulations of milk systems varying in commercial α-lactalbumin (α-la) and xanthan gum contents

Ratio[1] (α-la/β-lg)	Xanthan gum concentration[2] (w/w%)	NDM[3] (g)	Commercial α-la[4] (g)	Xanthan gum solution[5] (mL)	dH_2O[6] (mL)
3:8	0	11.4784	0	0	100
8:3	0	10.2128	1.3019	0	100
3:8	0.15	11.4784	0	33.5	66.5
8:3	0.15	10.2128	1.3019	33.5	66.5

[1]Target ratio of α-latalbumin (α-la)/β-lactoglobulin (β-lg); [2]Target xanthan gum concentration in the milk systems; [3]Low-heat non-fat dry milk; [4]LACPRODAN® ALPHA-20; [5]The volume of xanthan gum master solution (0.5%) used to achieve target xanthan gum concentration; [6]Dionized, distilled water.

5.3.2 Assesments

Apparent viscosity of the milk systems was measured at shear rate range from 0.1 to 100 s^{-1} using a shear rate control rheometer (VISCOANALYSER DSR, ATS RheoSystems, 231 Crosswicks Road, Bordentown, NJ) with Rheoexpolorer software (RheoExplorer version5) using a cup and bob system (concentric cylinder CC25) at 25 ± 1°C. Ten points in the logarithm scale were taken every 20 s (Pollen 2002). The apparent viscosity at 50 s^{-1}, consistency coefficient and flow behavior index were recorded for further statistical analyses. Apparent viscosity at 50 s^{-1} was calculated by the power law (Steffe 1996):

$$\tau = K(\dot{Y})^n$$

$$\mu = \tau/\dot{Y}$$

Where τ=shear stress (Pa), $\dot{Y}$=shear rate (1/s), K=consistency coefficient, n=flow behavior index, and μ is the apparent viscosity at shear rate of 50 s^{-1}.

Turbidity was measured following the method given by Le and Turgeon (2013). Milk systems were diluted into 1:10 with dionized, distilled water, and absorbance was recorded at 800 nm with 1 cm quartz cells (Fisher Scientific) using a UV/VIS-light spectrophotometer (GENESYS 5, Thermo Electron Corporation, Madison, WI, USA). Each sample was measured twice and values averaged.

pH of samples (post-process) was measured on a pH meter (Accumet AP63 portable pH meter, Fisher Scientific) after calibrating with standardized pH 4 and pH 7 buffer solutions (Fisher Scientific) at 25 ± 1ºC.

Total solids (TS) of dispersions were tested by the forced air oven method given by Hooi and others (2004).

Phase shift, storage modulus (G′) and loss modulus (G″) for 0.15% xanthan gum milk systems were obtained using a frequency sweep ranging from 0.1 to 100 Hz at a constant strain of 1% at 25 ± 1°C. A shear rate control rheometer with a cup and bob system (concentric cylinder CC25) was used for the dynamic oscillation testing; data was recoded by Rheoexpolorer software (RheoExplorer version5). Initially, a strain sweep was conducted (strain ranged from 0.1 to 100% at a constant frequency of 1 Hz) to ensure the strain of 1% was within the linear elasticity range. Ten points were taken every 20 s (Keshtkaran and others 2013).

Free sulfhydryl groups were measured following the method by Hashizume and Sato (1988). Approximately 1 mL of milk system was dissolved in 5 mL of sodium phosphate buffer (pH 8.0; prepared by disodium phosphate and monosodium phosphate; Fisher Scientific) and 0.1 mL of 10^{-2} M 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB; Sigma-Aldrich, St. Louis, MO, USA). After holding for 5 min, 2 g of ammonium sulfate (Fisher Scientific) was added to coagulate the milk proteins, and held for 2 min more at 25°C. Milk samples were filtered through Whatman No. 1 filter paper (Fisher Scientific). The absorbance of 3 mL of the supernatant was measured at 412 nm using a UV-Visible wavelength spectrophotometer (GENESYS 5, Thermo Electron Corporation, Madison, WI, USA) in a 1 cm disposable quartz cell (Fisher Scientific).

Total sulfhydryl groups were measured following Shimada and Cheftel (1989). Approximately 0.265 mL of milk sample at ratio of 3:8, and approximately 0.225 mL of milk samples at the ratio of 8:3 was diluted in 10 mL of 8 M urea (Sigma-Aldrich) and 0.5% sodium dodecyl sulfate (SDS; Sigma-Aldrich) dissolved in pH 8 sodium phosphate buffer (Fisher Scientific) to achieve a protein concentration of approximately 0.1%. The diluted samples were votexed for 1 min, followed by centrifugation (Marathon 21000R, Thermo IEC, Needham Heights, MA) at 15000*g for 15 min at 25°C. The supernatant was carefully removed by filtration through Whatman No.1 filter paper, and 3 mL of the filtrated sample was transferred to a 1 cm disposable quartz cell. Then, 0.03 mL of DTNB was added into each cell and the mixture was shaken 10 times thoroughly. Absorbance of the samples with DTNB was recorded at 412 nm after 10 min at 25°C, and the average of the two measurements was calculated as the absorbance for each sample (Shimada and Cheftel 1989). Free and total sulfhydryl groups was calculated use the following equation (Ellman 1959):

$$C_0 = \frac{A}{\epsilon} * D$$

Where C_0 = original concentration (mol/L), A = absorbance at 412 nm, Є = extinction coefficient = 13600/M/cm, and D = dilution factor (Appendix C, Table C.10).

Apparent viscosity, turbidity, free and total sulfhydryl groups of all samples, phase shift, G′, and G″of 0.15% xanthan gum samples were evaluated at 0 min and 120 min thereafter to determine the physical and chemical properties stability. pH and TS were only measured at 0 min.

5.4 Experimental Design and Statistical Analyses

A split-plot design with the whole plot arranged in a randomized complete block design was used to explore the effects of temperature (25 or 90ºC), ratio of α-la/β-lg (3:8 or 8:3), xanthan gum concentration (0 and 0.15%) and time (0 and 120 min) on pH, total solids, turbidity, apparent viscosity as well as free and total sulfhydryl groups. The time with 2 levels was the split plot. Three replications (3) were done and was used as the block. For analyses, three-way ANOVA was used for the total solids and pH, whereas a split plot was used for apparent viscosity, turbidity, free and total sulfhydryl groups. A split plot design was also used on phase shift, G′ and G″ to determin the effects of temperature (25 or 90ºC), ratio of α-la/β-lg (3:8 or 8:3), and time (0 and 120 min).To determine differences between significant ($P \leq 0.05$) means or interactions, Tukey's HSD were applied by SAS® (V 9.2; SAS Institute Inc., Cary, NC) ($P \leq 0.05$).

5.5 Results

5.5.1 Apparent viscosity

All milk systems fitted the models well with R^2 ranging from 0.991 to 0.999 (Appendix C, Table C.1). A Newtonian flow behavior was observed in the milk systems at 0% xanthan gum concentration whereas a shear-thinning flow behavior was seen in the milk systems at 0.15% xanthan gum concentrations. Because the values of the apparent viscosities were not normally distributed, raw data was

transformed into the natural logarithm and analyzed. Main effect of ratio and two way interaction of temperature and xanthan gum concentration were significant ($P \leq 0.05$) (Appendix C, Table C.2). However, time did not affect the apparent viscosity (Appendix C, Table C.2), which means that the apparent viscosity of milk systems were stable during this period of time. The apparent viscosity increased by 68.64% in 0.15% xanthan gum milk systems whereas no changes were observed in 0% xanthan gum milk systems if processed at 90ºC compared with 25ºC (Table 5.2). The main effect of xanthan gum concentration significantly increased the apparent viscosity. The apparent viscosity was almost 25 fold greater in the 0.15% xanthan gum concentration milk systems compared with the 0% xanthan gum milk systems. Temperature significantly influenced the apparent viscosity. In milk systems processed at 90ºC, apparent viscosity was 65.76% greater than those at 25ºC. Moreover, apparent viscosity increased by 24.58% comparing 3:8 milk systems with 8:3 milk systems (Appendix C, Table C.3, C.4 and C.5).

Table 5.2 The apparent viscosity as functions of xanthan gum concentration and temperature[1].

Xanthan gum concentration (%)	Temperature (ºC) 25	90
0	$1.53^{c} \pm 0.054$	$1.68^{c} \pm 0.037$
0.15	$29.97^{b} \pm 0.45$	$50.54^{a} \pm 2.68$

[1]Data are means ± SE (n=12). [a,b,c]Means with different superscript letters differ ($P \leq 0.05$).

5.5.2 Turbidity

Two-way interactions of ratio × temperature and temperature × concentration, as well as time had significant effects on turbidity (Appendix C, Table C.6). When temperature increased from 25 to 90ºC, turbidity increased by 43.07 and 93.61% in 3:8 and 8:3 milk systems, respectively (Table 5.3). Turbidity increased by 54.74 and 79.62% in 0 and 0.15% xanthan gum concentration milk compared 90ºC with 25ºC (Table 5.4). The associations between whey proteins and casein micelles occur when the whey proteins thermally denature at > 70ºC (Corredig and Dalgleish 1996). Particle sizes of casein micelles which are represented by turbidity measurement increased due to the attachment of thermal-denatured whey proteins (Anema and Klostermeyer 1997). When the milk systems were held for 120 min, turbidity slightly increased from 1.101 to 1.140.

Table 5.3 The turbidity of milk systems as functions of α-lactalbumin (α-la)/β-lactoglobulin (β-lg) ratio and temperature[1]

Ratio	Temperature (ºC)	
(α-la/β-lg)	25	90
3:8	$0.880^{c} \pm 0.017$	$1.259^{b} \pm 0.062$
8:3	$0.798^{c} \pm 0.020$	$1.545^{a} \pm 0.043$

[1]Data are means ± SE (n=12). [a,b,c]Means with different superscript letters differ ($P \leq 0.05$).

Table 5.4 The turbidity of milk systems as functions of temperature and xanthan gum concentration[1]

Xanthan[2] (%)	Temperature (ºC)	
	25	90
0	$0.844^{c} \pm 0.018$	$1.306^{b} \pm 0.070$
0.15	$0.834^{c} \pm 0.026$	$1.498^{a} \pm 0.053$

[1]Data are means ± SE (n=12). [a,b,c]Means with different superscript letters differ ($P \leq 0.05$); [2]Xanthan gum concentration (%).

5.5.3 Total solids (TS)

No statistical differences were observed in the TS in any milk systems ($P > 0.05$) (Appendix C, Table C.8). The mean total solids were 10.82 ± 0.022%, ranging from 10.71 ± 0.031% (ratio of 3:8 at 25ºC without xanthan gum) to 10.90 ± 0.099% (ratio of 8:3 at 25ºC and xanthan gum concentration of 0.15%) (Appendix C, table C.3). Total solids are known to impact viscosity and absorbance (Hemar and others 2001) because polymers interact with each other due to the swept-out spheres (McClements 1999); thus, as the total solids increase, apparent viscosity and absorbance increase.

5.5.4 pH

Two-way interaction between temperature and ratio as well as the main effect of xanthan gum concentration had significant effects on the pH ($P \leq 0.05$) (Appendix C, Table C.9). Table 5.5 depicts the significant mean differentiations for the pH of the milk systems.

Table 5.5 pH of the milk systems as a function of the temperature and α-lactalbumin (α-la)/β-lactoglobulin (β-lg) ratio[1]

Ratio (α-la/β-lg)	Temperature (ºC)	
	25	90
3:8	$6.53^{a} \pm 0.05$	$6.47^{c} \pm 0.03$
8:3	$6.54^{a} \pm 0.03$	$6.51^{b} \pm 0.05$

[1]Data are means ± SE (n=6). [a,b,c]Means with different superscript letters differ ($P \leq 0.05$).

pH decreased by 0.06 and 0.03 for ratio of 3:8 and 8:3, respectively when processed at 90ºC compared with 25ºC. The pH increased from 6.51 to 6.52 when the xanthan gum concentration changed from 0 to 0.15%. The decreased pH in the milk systems after heating might be due to the thermal oxidation of lactose to organic acids, hydrolysis of organic phosphate, or precipitation of tertiary calcium phosphate, which release H^{+} (Singh 2004). When xanthan gum was added into the milk system and processed at 90ºC, calcium connected with xanthan gum through a cross-linking interaction (Bergmann and others 2008); removal of calcium increased the release of H^{+}; and thus, a significant decrease of pH in the xanthan gum added milk system was observed (Chandrapala and others 2010).

5.5.5 Free and total sulfhydryl groups

Two-way interactions of temperature and ratio, as well as time and temperautre affected the free sulfhydryl groups (Appendix C, Table C.11). Free sulfhydryl groups increased when the process was applied. A greater increase in free

sulfhydryl groups was observed in the 8:3 milk systems compared with the 3:8 milk systems (Table 5.5). Free sulfhydryl groups increased by 44.0 and 96.8 μM for the milk systems at ratio of 3:8 and 8:3, respectively if processed at 90ºC compared with 25ºC. When the milk systems were processed at 90ºC, increased free sulfhydryl groups were observed in both ratios of milk system (Table 5.6). No sulfhdyrl groups are present in xanthan gum according to its structure (Becker and others 1998). Thus, the detected free sulfhydryl groups should be from the milk powder. At 25ºC, β-lg has not thermally denaturated (Anema and Li 2003b). The free sulfhydryl groups decreased by 11.3 μM when the milk systems were processed at 90ºC and set for 120 min, while no changes were found in 25ºC samples after 120 min (Table 5.7).

Table 5.6 Free and total sulfhydryl groups of milk systems as functions of α-lactalbumin (α-la)/β-lactoglobulin (β-lg) ratio and temperature[1]

Ratio (α-la/β-lg)	Temperature (ºC)	Total sulfhydryl group (μM)	Free sulfhydryl group (μM)
3:8	25	$181.83^{c} \pm 5.45$	$3.70^{c} \pm 1.27$
3:8	90	$133.16^{d} \pm 5.20$	$47.69^{b} \pm 1.98$
8:3	25	$323.86^{a} \pm 6.72$	$4.56^{c} \pm 1.64$
8:3	90	$238.64^{b} \pm 4.17$	$101.41^{a} \pm 2.60$

[1]Data are mean ± SE (n=12). [a,b,c]Means within a column with different superscript letters differ ($P \leq 0.05$)

Table 5.7 Free sulfhydryl groups as functions of temperature and time[1]

Temperature	Time (min)	
(ºC)	0	120
25	$4.49^{c} \pm 1.60$	$3.78^{c} \pm 1.31$
90	$80.25^{a} \pm 8.14$	$68.85^{b} \pm 8.35$

[1]Data are means ± SE (n=12). [a,b,c]Means within the same column with different superscript letters differ ($P \leq 0.05$).

The interaction of ratio and temperature had a significant effect on total sulfhydryl groups; time did not affect total sulfhydryl groups (Appendix C, Table C.12 and C.13). Total sulfhydryl groups decreased if systems were processed at 90ºC compared with 25ºC. The decrease was 48.6 and 85.3μM for ratios of 3:8 and 8:3, respectively (Table 5.6). Total sulfhydryl groups should decrease if whey proteins associate with casein micelles due to the release of H_2S as well as the formation of disulfide bonds (de Wit and Nieuwenhuijse 2008). As described by previous researchers, whey proteins associate with casein micelles mainly through the oxidation of sulfhydryl groups and sulfhydryl/disulphide bond exchange (Anema and Li 2003); the concentration of xanthan gum did not affect total sulfhydryl groups. Therefore, associations between whey proteins and casein micelles did not change even though the xanthan gum was present in the milk systems.

5.5.6 Dynamic oscillation

Dynamic oscillation testing was conducted in the milk systems at 0.15% xanthan gum concentration to detect the gel properties which would further influence the mouthfeel. A frequency sweep was used to determine phase shif, G′ and G″.

Temperature had significant effects ($P \leq 0.05$); however, ratio and time did not affect the phase shift (Appendix C, Table C.14). The phase shift increased by 17.86% when the temperature increased from 25 to 90ºC (Table 5.8). Bryant and McClemnets (2000) characterized system by the phase shift (δ). Systems with δ=90º were true liquids; systems with δ ranging from 0 to 90 were viscoelastic; systems with δ=0º were true solids. Materials with δ < 45° are defined as gels (Bryant and McClements 2000). G′ illustrates the strength (e.g. disulphide and hydrophobic bonds) of the structure of individual particles and between particles (Gezimati and others 1997). A more liquid-like system seems to have been formed if the systems were processed at 90ºC.

Table 5.8 Phase shift as a function of temperature[1]

Temperature (ºC)	Phase shift (degree)
25	$38.7^{b} \pm 0.836$
90	$45.6^{a} \pm 0.562$

[1] Values are means ± SE (n=12). [a,b,c]Means with different superscript letters differ ($P \leq 0.05$)

Table 5.9 G′ and G″ as a function of α-lactalbumin (α-la)/β-lactoglobulin (β-lg) ratio and temperature[1]

Ratio (α-la/β-lg)	Temperature (ºC)	G′ (Pa)	G″ (Pa)
3:8	25	$0.413^{c} \pm 0.0206$	$0.329^{c} \pm 0.0126$
3:8	90	$0.645^{b} \pm 0.0277$	$0.637^{b} \pm 0.0222$
8:3	25	$0.472^{c} \pm 0.0191$	$0.380^{c} \pm 0.0222$
8:3	90	$0.952^{a} \pm 0.0350$	$1.004^{a} \pm 0.0427$

[1] Values are means ± SE (n=6). [a,b,c]Means with different superscript letters differ ($P \leq 0.05$)

The main effects of ratio and temperature significantly influenced G′ ($P \leq 0.05$) (Appendix C, Table C.15). Table 5.9 depicts G′ and G″ as functions of raito and temperature. G′ increased by 56.17% when the samples at ratio of 8:3 were compared to 3:8 whereas the G′ increased by 101.69% when the samples were processed at 90ºC compared with 25ºC. The solid-like properties were formed when the temperature and ratio increased. The addition of α-la had a function of creating a more solid-like structure in the milk protein/xanthan gum systems. The interaction of temperatures and ratios had significant influence on G″ (Appendix C, Table C.16). G″ increased as the temperature and ratio increased.

5.6 Discussion

5.6.1 The protein-polysaccharide complex structure

The interactions of milk proteins and xanthan gum after thermal processing can result in a complex structure which in turn can alter physicochemical properties of the systems. At 25ºC, the interaction between milk proteins and xanthan gum is mainly through electrostatic interactions and hydrogen bonds (Tostoguzov 1991). When thermal treatment is applied to milk, casein micelles and whey proteins associate with each other forming larger protein complexes through sulfhydryl-disulfide bond exchange (Anema and others 2003). Thus, increased apparent viscosity, turbidity, free sulfhydryl groups as well as decreased total sulfhydryl groups were observed as a function of temperature in this experiment.

Proteins further associate with the xanthan gum, which is seen as the core of the protein-polysaccharide complexes (Sanchez and others 1997). The increased ratio of α-la/β-lg effectively increased the association between whey proteins and casein micelles, further attaching to the xanthan gum which forms the larger complex, and thus increases the apparent viscosity. The association between whey proteins and casein micelles are more favored than milk proteins and xanthan gum association (Laneuville and others 2000). This may be explained as the less energy is required for the association between the same biopolymers than between different biopolymers X

5.6.2 The protein-polyssacharide interaction on apparent viscosity of the milk systems

Xanthan gum at low concentrations present pseudoplastic behavior while the milk exhibits a Newtonian fluid behavior (Ahmed and Ramaswamy 2005; Karlsson and others 2005). Therefore, flow behaviors of the milk protein/xanthan gum mixtures were predominantly influenced by xanthan gum. An explanation for the increase in apparent viscosity when milk systems were processed at 90ºC was that the association between whey protein and casein micelles through the formation of disulfide bonds and hydrophobic interactions (Gustaw and others 2003). Another experiment tested the rheological properties of 1% xanthan gum solutions heated at 110 and 130ºC for 30 min or not heated (at 20ºC); the results showed that the temperature did not affect yield stress, consistency index, and flow behavior index (Ahmed and Ramaswamy 2005). Bergmann and others (2008) proposed that two xanthan gum molecules could

associate with each other through bivalent ions such as Ca^{2+}. For the 3:8 milk systems, significant increased apparent viscosity was observed in 0.15% xanthan gum milk systems while no differences were observed in the 0% milk systems as a function of temperature changed. Thus, in the milk system, interactions between protein and protein and polysaccharide and polysaccharide were responsible for the increase in apparent viscosity. Some of xanthan gum might associate with proteins to form a larger network structure instead of becoming the original disordered structure (Sanchez and others 1997). The presence of Ca^{2+} in the milk protein might also have enhanced the interaction between xanthan gum molecules which may responsible for polysaccharide-polysaccharide interaction increased and leading to the increase in viscosity (Bergmann and others 2008). This may also explain the greater decrease of pH in 0.15% xanthan gum milk systems. If Ca^{2+} was removed due the interaction of xanthan gum molecules, H^{+} would be released (Chandrapala and others 2010).

5.7 Conclusions

The addition of xanthan gum significantly increased the apparent viscosity as well as altering the flow behavior and gel properties of the milk systems at both α-la/β-lg ratio of 3:8 and 8:3 whether processed at 90ºC or 25ºC. When the milk system at ratio of α-la/β-lg of 8:3 with xanthan gum concentration of 0.15% processed at 90ºC, apparent viscosity started to reach the range of nectar-like. The increased

particles size of the casein micelles as well as the formation of the net structure due to the interaction between milk proteins and xanthan gum were responsible for the increased apparent viscosity. A dairy-based beverage with the apparent viscosity within the consistency recommended by National Dysphagia Diet (NDD) and stable during 120 min can be obtained. If the rheological properties of the milk systems can be controlled by ingredients interactions, nutritious products with different forms for dysphagia patients can be developed.

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Chapter 6 - Research Summary

Patients who have difficulty in food mastication and swallowing are diagnosed to have dysphasia. Liquid systems in the range of nectar-like (51 to 350 mPa·s) and honey-like (351 to 1750 mPa·s) are known to be suitable for dysphagia patients. Whey proteins start to denaturate at 70ºC. The associations between whey proteins and casein micelles change the rheological properties of the milk system. The ratio of α-lactalbumin (α-la)/β-lactoglobulin (β-lg) can affect the associations between whey proteins and casein micelles, and thus influence the rheological properties. Apparent viscosity increased if whey protein adjusted milk systems were heated to 80ºC or higher temperatures for 30 min; in particular, apparent viscosity increased as the ratio increased from 3:8 to 8:3. Apparent viscosity increased in all 90°C samples, and the magnitude of increase was in the order of 8:3 (15.96%), 1:1 (6.38%) and 3:8 (2.11%) compared with the 25ºC samples at each ratio. When the milk systems were held for 120 min, apparent viscosity increased (3.74%). All milk systems had similar pH (6.53) and total solids (10.74%). When the temperature increased to > 70ºC, free sulfhydryl groups increased whereas total sulfhydryl groups decreased for milk systems at all ratios. Decreased free sulfhydryl groups were observed when processed at 80ºC and 90ºC; while no changes were seen in the total sulfhydryl groups after 120 min. The maximum apparent viscosity found in my first study was 2.18 mPa·s, which was not in the range of recommended consistencies.

To develop a dairy-based beverage with suitable rheological properties for dysphagia pateints, a concentration of 0.15% xanthan gum solution was used in the ratio-adjusted milk systems. Apparent viscosity increased by 48.61 and 89.61% in 3:8 and 8:3 milk systems at 0.15% xanthan gum concentration when temperature changed from 25ºC to 90ºC. The apparent viscosity of 8:3 milk systems at xanthan gum concentration of 0.15% processed at 90°C was 58.7 ± 2.12 mPa·s which was within the nectar-like range. No changes in apparent viscosity were found after 120 min. pH decreased (post-process pH for 3:8 and 8:3 milk systems were 6.46 and 6.49 respectively) as the temperature changed from 25ºC to 90ºC. Total solids were similar for all milk systems. In 0.15% xanthan gum milk systems, phas shift, G′ and G″ increased as temperature increased; greater increase in G′ and G″ were observed in 8:3 milk systesm compared with 3:8 milk systems.

A ready-to-drink dairy-based beverage with suitable rheological properties can be developed by controlling the ingreadients interaction in the milk system.This product can be further turned into the powder form for the convenience of distribution and serving. The appropriate drying methods are needed to be detected in the future. On the other hand, heat process may be applied on NDM and commercial α-la directly to induce the whey protein and casein micells interaction. Such powder product can be rehydrated in water and to detect its effect on rheological properties of the liquid system.

Appendix

Appendix A Information of ingredients

Arla Foods Ingredients
Product Information
Nutrition

LACPRODAN® ALPHA-20
α-LACTALBUMIN ENHANCED
WHEY PROTEIN ISOLATE

Description

LACPRODAN® ALPHA-20 is a highly nutritious native whey protein isolate with a high content of α-lactalbumin obtained by careful fractionation. The high α-lactalbumin content makes it ideally suited as a protein source in infant formula.

Chemical Specifications

Protein (Nx6.38) as is		88-94 %
Lactose	max.	3.0 %
Fat	max.	2.0 %
Ash	max.	3.5 %
Moisture	max.	5.5 %
α-lactalbumin content of protein	approx.	60.0 %

Minerals (approx.)

Sodium	Na	0.5 %
Magnesium	Mg	0.03 %
Phosphorus	P	0.3 %
Chloride	Cl	0.08 %
Potassium	K	0.8 %
Calcium	Ca	0.5 %

Physical Specifications

pH		6.5 – 7.0
Solubility index	max.	1.0 ml

Microbiological Specifications

Total plate count		< 10,000/g
Bacillus cereus		< 100/g
Coliforms		< 10/g
Staphylococcus aureus coagulase +	neg. in	1 g
Mould/yeast	max.	10/g
Salmonella	neg. in	100 g

Packaging

Paper bags with a polyethylene inner bag containing 15 kg net.

Storage

Store in closed bags under cool, dry conditions away from strong odours.

Shelf Life

Minimum two years if kept under the prescribed storage conditions.

Amino Acids (AA)
Typical amino acid composition
g AA/100 g protein

Alanine		3.1
Arginine		2.2
Aspartic acid(asparagine)		15.1
Cysteine (cystine)		3.8
Glutamic acid (glutamine)		17.6
Glycine		2.4
Histidine	*	2.9
Isoleucine	*	6.7
Leucine	*	11.5
Lysine	*	11.1
Methionine	*	1.6
Phenylalanine	*	4.2
Proline		4.3
Serine		5.5
Threonine	*	5.4
Tryptophan	*	3.1
Tyrosine		3.7
Valine	*	5.3
Total BCAA/TAA:		21.5

* Essential amino acids

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TIC GUMS
We're your Gum Guru

Ticaxan® Xanthan 200 Powder

PRODUCTDATA

A fine mesh Xanthan Gum, suitable for dry mix applications where rapid hydration is required.

Typical Usage Level	0.05% to 0.35%			
Solubility	Cold Water Soluble			
Suggested Uses	Salad Dressings, Sauces, Marinades, Gravies, Low pH Solutions, High pH Solutions, Dry Mix Beverages, Relish, Functional Foods			
Label Declaration	Xanthan Gum			
Country of Origin	Product of Austria and/or USA			
CFR #	21 CFR 172.695	Kosher Ⓤ	Y	(Y/N)
CAS #	11138-66-2	Kosher for Passover Ⓤ	N	(Y/N)
EU #	415	Halal	Y	(Y/N)
HS Tariff #	3913.90.2000	Allergen	N	(Y/N)
Minimum Qty				
Standard Packing	50# Cartons, 1,200# per pallet	All Natural	Y*	(Y/N)
Lead Time	Stock Product	Shelf-Life	3 years	
Storage & Handling	Each container is identified with the product name and lot number. Store in cool dry place for maximum shelf life.			

*TIC Gums Natural Definition Disclaimer: When deciding which of its product offerings to classify as "natural" or "all natural," TIC Gums applies the following *internal* definition to either term interchangeably: "A finished product derived from naturally occurring raw materials that were processed without modifying the native chemical structure of any of the materials."
Our processes may involve more than minimal processing of the raw materials to maximize functionality with processing aids that may be synthetic or that may be derived from ingredients developed through the use of recombinant DNA technology. TIC Gums will consider an ingredient "natural" or "all natural" when there has been no modification to its native chemical structure. TIC Gums makes no representations regarding the consistency of its definition of "natural" or "all natural" with any legal or regulatory lexicon, or dictionary. TIC Gums makes no representations in particular as to whether this definition is superior to or in conformance with definitions of "natural" or "all natural" promulgated by the USDA, Health Canada, or the FDA. Each customer must make its own decision regarding what definition of "natural" or "all natural" the customer will utilize in describing its own products. As part of its decision-making process, each customer should obtain independent regulatory and legal advice regarding the definition of "natural" or "all natural" most appropriate for that customer. Each customer is solely responsible for determining the nature and content of any claims that may or should appear on its own products. Each customer is also solely responsible for compliance with all pertinent legal requirements worldwide.

NUTRITIONAL INFORMATION

Calories (Total)	324 Kcal	Sodium	3180 mg	Insoluble Dietary Fiber	2.0 g
%Calories from Fat	0.00 %	Potassium	363 mg	Simple Carbohydrates	0 g
Calories from fat	0.00 Kcal	Calcium	28 mg	Complex Carbohydrates	0 g
Total Fat	0.00 g	Total Carbohydrates	78.00 g	Protein	5 g
Trans Fat	0.00 g	Soluble Dietary Fiber	76 g	Vitamins, Other Minerals	*ND
Cholesterol	0 mg				

(per 100 grams). This data is from analysis and calculation and should be considered "typical" and not a specification. Data is reported on an "as is" basis. Total fat and protein values are rounded to the nearest whole number.
* Calculated based on typical assay of component(s)
* N.D.; Not determined
**Total Calories are calculated in compliance with FDA Regulations requiring the inclusion of 4 Kcal/g for all soluble dietary fiber. However dietary fiber by definition consists of plant material that is resistant to hydrolysis by endogenous enzymes of the mammalian digestive tract. TIC GUMS has participated in discussions with the FDA as part of the Calorie Control Council to amend the FDA regulations to 2Kcal/g.
If all nutritional information is listed as "0" then these findings have yet to be evaluated.

SPECIFICATIONS

	Minimum	Maximum	
Bacteriological			
Aerobic Plate Count (Typical)	< 1000 cfu/g		-
E. coli (Typical)	Negative		-
S. aureus (BAM Typical)	Negative /10g		-
Salmonella (Typical)	Negative		-
Total Coliforms (Typical)	< 30 /g		-
Yeast and Mold (Typical)	< 200/g		-
Mesh	**Minimum**	**Maximum**	
USS#200 Mesh Through	70	100	%
Physical and Chemical	**Minimum**	**Maximum**	
Flavor (Typical)	Typical Bland		-
Moisture (Infrared)	0	15	%
Odor (Typical)	Characteristic		-
pH (viscosity solution)	5.5	8	pH
Powder Color (Visual)	Off White-Tan		-
Texture (Qualitative)	Free Flowing Powder		-
Viscosity (1.0%,KCL,LV@60rpm,25C)	1000	1600	cps

The information provided is based upon tests and observations made under laboratory conditions and is believed to be accurate. Test results may, however, vary depending upon testing conditions. In furnishing samples and product data and specifications, TIC Gums, Inc. makes no Warranty, either express or implied, including any warranty of merchantability or fitness for a particular purpose. It is expressly understood and agreed that it is the buyer's responsibility to determine suitability of the product for a particular purpose, product or process. To obtain a description of our testing methodologies, please contact TIC Gums, Inc. at (800) 899-3953 or (410) 273-7300.
This product, or ingredients used to make this product, has/have been demonstrated to conform with current Food Chemical Codex requirements

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Appendix B Tables and Analyses for Chapter 4

Table B.1 Experimental design of connected incomplete randomized block

Half day[1]	No.											
	0	1	2	3	4	5	6	7	8	9	10	11
	12	13	14	15	16	17	18	19	20	21	22	23
	24	25	26	27	28	29	30	31	32	33	34	35
	36	37	38	39	40	41	42	43	44	45	46	47
1	√	√										
2	√		√									
3	√			√								
4	√				√							
5	√					√						
6	√						√					
7	√							√				
8	√								√			
9	√									√		
10	√										√	
11	√											√
12	√	√										
13	√		√									
14	√			√								
15	√				√							
16	√					√						
17	√						√					
18	√							√				
19	√								√			
20	√									√		
21	√										√	
22	√											√
23	√	√										
24	√		√									
25	√			√								
26	√				√							
27	√					√						
28	√						√					
29	√							√				
30	√								√			
31	√									√		
32	√										√	
33	√											√

[1]Half day was taken as block

Table B.2 Number given for each combination of ratio, temperature and time

Temp[1] (ºC)	Ratio[2] (α-la/β-lg)	Time[3] (min)	Number	Rep[4]
25	3:8	0	0	33
25	1:1	0	1	3
25	8:3	0	2	3
70	3:8	0	3	3
70	1:1	0	4	3
70	8:3	0	5	3
80	3:8	0	6	3
80	1:1	0	7	3
80	8:3	0	8	3
90	3:8	0	9	3
90	1:1	0	10	3
90	8:3	0	11	3
25	3:8	30	12	33
25	1:1	30	13	3
25	8:3	30	14	3
70	3:8	30	15	3
70	1:1	30	16	3
70	8:3	30	17	3
80	3:8	30	18	3
80	1:1	30	19	3
80	8:3	30	20	3
90	3:8	30	21	3
90	1:1	30	22	3
90	8:3	30	23	3
25	3:8	60	24	33
25	1:1	60	25	3
25	8:3	60	26	3
70	3:8	60	27	3
70	1:1	60	28	3
70	8:3	60	29	3
80	3:8	60	30	3
80	1:1	60	31	3
80	8:3	60	32	3
90	3:8	60	33	3
90	1:1	60	34	3
90	8:3	60	35	3
25	3:8	120	36	33
25	1:1	120	37	3
25	8:3	120	38	3

70	3:8	120	39	3
70	1:1	120	40	3
70	8:3	120	41	3
80	3:8	120	42	3
80	1:1	120	43	3
80	8:3	120	44	3
90	3:8	120	45	3
90	1:1	120	46	3
90	8:3	120	47	3

[1]Target temperature (ºC); [2]Target ratio of α-la/β-lg; [3]Assessment time since the samples were cooled to 25 ± 1ºC; [4]Replications for each milk system.

Apparent viscosity

Table B.3 Mean apparent viscosity of the milk systems as a function of the temperatures, α-lactalbumin (α-la)/β-lactoglobulin (β-lg) ratios and times[1]

Ratio	Temp[2]	Apparent viscosity (mPa·s)			
(α-la/β-lg)	(ºC)	0 min	30 min	60 min	120 min
3:8	25	1.86 ± 0.00	1.88 ± 0.00	1.91 ± 0.00	1.93 ± 0.00
1:1	25	1.85 ± 0.01	1.87 ± 0.01	1.89 ± 0.00	1.91 ± 0.01
8:3	25	1.84 ± 0.00	1.87 ± 0.01	1.90 ± 0.01	1.92 ± 0.00
3:8	70	1.75 ± 0.01	1.77 ± 0.01	1.79 ± 0.01	1.83 ± 0.01
1:1	70	1.75 ± 0.01	1.78 ± 0.00	1.80 ± 0.00	1.82 ± 0.01
8:3	70	1.78 ± 0.01	1.80 ± 0.01	1.82 ± 0.01	1.85 ± 0.01
3:8	80	1.82 ± 0.02	1.86 ± 0.01	1.88 ± 0.02	1.89 ± 0.02
1:1	80	1.88 ± 0.02	1.90 ± 0.01	1.93 ± 0.01	1.95 ± 0.01
8:3	80	2.02 ± 0.00	2.05 ± 0.00	2.06 ± 0.00	2.08 ± 0.01
3:8	90	1.91 ± 0.01	1.92 ± 0.01	1.94 ± 0.01	1.97 ± 0.02
1:1	90	1.97 ± 0.01	1.99 ± 0.01	2.02 ± 0.01	2.03 ± 0.01
8:3	90	2.15 ± 0.01	2.17 ± 0.02	2.20 ± 0.00	2.21 ± 0.01

[1]Data are means ± SE (n=33 for samples at 3:8 α-la/β-lg ratio at 25ºC at 0, 30, 60 and 120 min; n=3 for other combinations of temperatures, ratios and the times).
[2]Temperature.

Table B.4 P and F values from ANOVA of the temperature, α-lactalbumin (α-la)/β-lactoglobulin (β-lg) ratio and time on apparent viscosity of the milk systems

Type 3 Tests of Fixed Effects				
Effect	Num DF[4]	Den DF[5]	F Value	Pr > F
ratio[1]	2	44.1	536.03	<.0001
temp[2]	3	44	1128.17	<.0001
ratio*temp	6	43.9	182.62	<.0001
time[3]	3	162	374.07	<.0001
temp*time	9	162	1.19	0.3063
ratio*time	6	162	0.55	0.7691
ratio*temp*time	18	162	0.86	0.6216

[1]α-la/β-lg ratio; [2]temperature; [3]set time; [4]numerator degree of freedom; [5]denominator degree of freedom.

Table B.5 Mean apparent viscosity of the milk systems as a function of the temperature and α-lactalbumin (α-la)/β-lactoglobulin (β-lg) ratio[1]

Ratio	Temperature (ºC)			
(α-la/β-lg)	25	70	80	90
3:8	$1.90^{f} \pm 0.0027$	$1.79^{i} \pm 0.010$	$1.86^{g} \pm 0.010$	$1.94^{d} \pm 0.0083$
1:1	$1.88^{f} \pm 0.0072$	$1.79^{hi} \pm 0.0083$	$1.92^{e} \pm 0.0093$	$2.00^{c} \pm 0.0084$
8:3	$1.88^{f} \pm 0.0093$	$1.81^{h} \pm 0.0082$	$2.05^{b} \pm 0.0070$	$2.18^{a} \pm 0.0085$

[1]Data are means ± SE (n=132 for the samples at 3:8 α-la/β-lg ratios at 25ºC; n=12 for other temperatures and ratios). [a,b,c]Means with different superscript letters differ ($P \leq 0.05$).

Table B.6 Mean apparent viscosity of the milk systems as a function of the times[1]

Time (min)	Apparent viscosity (mPa·s)
0	$1.87^{d} \pm 0.0105$
30	$1.90^{c} \pm 0.0104$
60	$1.92^{b} \pm 0.0104$
120	$1.94^{a} \pm 0.0101$

[1]Data are means ± SE (n=66). [a,b,c]Means with different superscript letters differ ($P \leq 0.05$).

Table B.7 Mean apparent viscosity of the milk systems as a function of ratios[1]

Ratio (α-la/β-lg)	Apparent viscosity (mPa·s)
3:8	$1.89^{c} \pm 0.00$
1:1	$1.90^{b} \pm 0.01$
8:3	$1.98^{a} \pm 0.02$

[1]Data are means ± SE (n=168 for 3:8; n=48 for other temperatures). [a,b,c]Means with

different superscript letters differ ($P \leq 0.05$).

Table B.8 Mean apparent viscosity of the milk systems as a function of temperatures[1]

Temperautre (ºC)	Apparent viscosity (mPa·s)
25	$1.89^{c} \pm 0.00$
70	$1.80^{d} \pm 0.01$
80	$1.94^{b} \pm 0.01$
90	$2.04^{a} \pm 0.01$

[1]Data are means ± SE (n=156 for 25ºC; n=36 for other temperatures). [a,b,c]Means with different superscript letters differ ($P \leq 0.05$).

pH and total solids

Table B.9 Mean pH and total solids of the milk systems as a function of the temperatures and α-lactalbumin (α-la)/β-lactoglobulin (β-lg) ratios [1]

Ratio (α-la/β-lg)	Temperature (ºC)	pH	Total solids (%)
3:8	25	6.51 ± 0.03	10.73 ± 0.01
1:1	25	6.55 ± 0.01	10.73 ± 0.03
8:3	25	6.56 ± 0.00	10.62 ± 0.03
3:8	70	6.54 ± 0.01	10.76 ± 0.06
1:1	70	6.54 ± 0.01	10.72 ± 0.02
8:3	70	6.55 ± 0.01	10.75 ± 0.06
3:8	80	6.53 ± 0.00	10.73 ± 0.02
1:1	80	6.53 ± 0.00	10.78 ± 0.06
8:3	80	6.55 ± 0.01	10.75 ± 0.02
3:8	90	6.50 ± 0.01	10.77 ± 0.03
1:1	90	6.52 ± 0.01	10.70 ± 0.04
8:3	90	6.53 ± 0.00	10.79 ± 0.07

[1]Data are means ± SE (n=33 for samples at 3:8 α-la/β-lg ratio at 25ºC; n=3 for other temperatures and ratios).

Table B.10 P and F values from ANOVA of the temperature and α-lactalbumin (α-la)/β-lactoglobulin (β-lg) ratio on pH of the milk systems

Effect	Num DF[3]	Den DF[4]	F Value	Pr > F
ratio[1]	2	53.2	0.13	0.881
temp[2]	3	53.2	0.04	0.9905
ratio*temp	6	52.9	0.03	0.9998

[1]α-la/β-lg ratio; [2]temperature; [3]numerator degree of freedom; [4]denominator degree of freedom.

Table B.11 P and F values from ANOVA of the temperature and α-lactalbumin (α-la)/β-lactoglobulin (β-lg) ratio on total solids of the milk systems

Effect	Num DF[3]	Den DF[4]	F Value	Pr > F
ratio[1]	2	51.5	0.69	0.5056
temp[2]	3	51.4	3.05	0.0367
ratio*temp	6	51.2	2.13	0.0656

[1]α-la/β-lg ratio; [2]temperature; [3]numerator degree of freedom; [4]denominator degree of freedom.

Table B.12 Mean total solids of the milk systems as a function of the temperatures[1]

Temperature (ºC)	TS (%)
25	$10.72^{a} \pm 0.01$
70	$10.74^{a} \pm 0.02$
80	$10.75^{a} \pm 0.01$
90	$10.76^{a} \pm 0.03$

[1]Data are means ± SE (n=39 for the milk systems at 25ºC; n=9 for the milk systems processed at 70, 80, and 90ºC). [a]Means with different superscript letters differ ($P \leq 0.05$).

Free sulfhydryl groups

Table B.13 Dilution factors (D) for free and total sulfhydryl groups measurement

Ratio (α-la/β-lg)	D (Free sulfhydrylgroup)	D (Total sulfhydrylgroup)
3:8	2700000	39123208
1:1	2700000	39123208
8:3	2700000	45898889

Table B.14 Free sulfhydryl groups (μM) of milk systems consisting of α-lactalbumin (α-la)/β-lactoglobulin (β-lg) ratio and heated at different temperature at 0, 30, and 120 min[1]

Ratio	Temperature	Time (min)		
(α-la/β-lg)	(ºC)	0	30	120
3:8	25	0.46 ± 0.17	0.13 ± 0.07	0.40 ± 0.23
3:8	70	12.24 ± 1.24	14.10 ± 2.33	10.13 ± 2.60
3:8	80	44.27 ± 1.00	37.92 ± 4.19	22.50 ± 0.81
3:8	90	47.05 ± 0.41	35.34 ± 0.40	26.21 ± 2.43
1:1	25	0.99 ± 0.11	0.99 ± 0.11	1.4559 ± 0.48
1:1	70	16.41 ± 1.33	10.99 ± 2.84	10.92 ± 3.47
1:1	80	67.57 ± 1.33	49.50 ± 0.87	40.04 ± 2.52
1:1	90	67.17 ± 1.63	55.39 ± 2.84	36.26 ± 2.04
8:3	25	1.46 ± 0.07	0.5294 ± 0.43	0.86 ± 0.52
8:3	70	14.29 ± 3.00	19.52 ± 3.84	14.10 ± 2.97
8:3	80	114.42 ± 1.50	96.29 ± 3.34	79.01 ± 2.02
8:3	90	113.69 ± 1.34	102.31 ± 0.59	75.24 ± 2.01

[1]Data are means ± SE (n=3).

Table B.15 P and F values from ANOVA of the temperature, α-lactalbumin (α-la)/β-lactoglobulin (β-lg) ratio, and time on free sulfhydryl groups in the milk systems.

Type III Tests of Fixed Effects				
Effect	Num DF[5]	Den DF[6]	F Value	Pr > F
rep[1]	2	22	1.02	0.3785
ratio[2]	2	22	616.25	<.0001
temp[3]	3	22	1751.25	<.0001
ratio*temp	6	22	178.41	<.0001
time[4]	2	48	249.68	<.0001
ratio*time	4	48	6.88	0.0002
time*temp	6	48	70.27	<.0001
ratio*time*temp	12	48	3.88	0.0004

[1]Replication; [2]α-la/β-lg ratio; [3]temperature; [4]set time (min); [5]numerator degree of freedom; [6]denominator degree of freedom.

Total sulfhydryl groups

Table B.16 Total sulfhydryl groups (μM) as a function of the time[1]

Ratio (α-la/β-lg)	Temp[2] (ºC)	Time (min) 0	30	120
3:8	25	175.5 ± 8.4	178.4 ± 9.0	173.1 ± 7.3
3:8	70	147.2 ± 7.2	152.5 ± 9.4	152.0 ± 0.5
3:8	80	156.3 ± 6.7	161.1 ± 3.0	158.7 ± 0.5
3:8	90	122.3 ± 1.4	128.5 ± 4.3	123.7 ± 4.6
1:1	25	248.8 ± 9.8	255.1 ± 8.5	251.7 ± 7.9
1:1	70	215.8 ± 9.8	219.1 ± 5.7	227.7 ± 10.7
1:1	80	193.7 ± 11.8	199.0 ± 3.4	187.5 ± 3.5
1:1	90	175.0 ± 5.5	186.5 ± 6.3	180.3 ± 6.0
8:3	25	362.2 ± 2.5	365.1 ± 5.4	364.5 ± 5.1
8:3	70	250.3 ± 7.6	256.5 ± 17.7	272.2 ± 5.9
8:3	80	253.7 ± 10.7	268.3 ± 8.0	267.7 ± 5.0
8:3	90	229.5 ± 6.1	240.2 ± 13.7	219.4 ± 11.7

[1]Data are means ± SE (n=3). [2]Temperature.

Table B.17 P and F values from ANOVA of the temperatures, α-la/β-lg ratios and times on total sulfhydryl groups in the milk systems

Type III Tests of Fixed Effects				
Effect	Num DF[5]	Den DF[6]	F Value	Pr > F
rep[1]	2	22	1.44	0.259
ratio[2]	2	22	795.63	<.0001
temp[3]	3	22	190.14	<.0001
ratio*temp	6	22	25.41	<.0001
time[4]	2	48	2.22	0.1193
ratio*time	4	48	0.13	0.969
time*temp	6	48	0.87	0.5218
ratio*time*temp	12	48	0.39	0.9588

[1]Replication; [2]α-la/β-lg ratio; [3]temperature; [4]set time (min); [5]numerator degree of freedom; [6]denominator degree of freedom.

Appendix C Tables and Analyses for Chapter 5

Apparent viscosity

Table C.1 The apparent viscosity, consistency coefficient (K), and flow behavior index (n) of milk systems at different α-lactalbumin (α-la)/β-lactoglobulin (β-lg), temperature, xanthan gum concentration and time[1]

Ratio[2]	Xanthan[3]	Temp[4]	Time[5]	App[6]	K (Pas^n)	n (-)	R^2
3:8	0	25	0	1.40 ± 0.00	0.0014 ± 0.00	1.00 ± 0.00	0.997-0.998
3:8	0	90	0	1.50 ± 0.00	0.0016 ± 0.0001	1.00 ± 0.00	0.998
3:8	0.15	25	0	28.6 ± 0.164	0.1866 ± 0.0036	0.52 ± 0.0058	0.993-0.995
3:8	0.15	90	0	42.45 ± 1.45	0.3562 ± 0.0249	0.45 ± 0.0030	0.992-0.997
8:3	0	25	0	1.50 ± 0.58	0.0015 ± 0.0001	1.00 ± 0.00	0.992-0.996
8:3	0	90	0	1.77 ± 0.033	0.0018 ± 0.00	1.00 ± 0.00	0.998-0.999
8:3	0.15	25	0	30.79 ± 1.05	0.2260 ± 0.0110	0.49 ± 0.0040	0.994-0.996
8:3	0.15	90	0	58.37 ± 2.93	0.5602 ± 0.0220	0.42 ± 0.0056	0.995-0.997
3:8	0	25	120	1.73 ± 0.17	0.0017 ± 0.0002	1.00 ± 0.00	0.996-0.998
3:8	0	90	120	1.63 ± 0.33	0.0016 ± 0.00	1.00 ± 0.00	0.996-0.999
3:8	0.15	25	120	29.39 ± 0.47	0.1831 ± 0.0050	0.53 ± 0.0039	0.995-0.996
3:8	0.15	90	120	42.37 ± 1.23	0.3367 ± 0.0300	0.46 ± 0.0076	0.991-0.995
8:3	0	25	120	1.50 ± 0.058	0.0015 ± 0.0001	1.00 ± 0.00	0.995-0.998
8:3	0	90	120	1.80 ± 0.00	0.6012 ± 0.5994	1.00 ± 0.00	0.997-0.999
8:3	0.15	25	120	31.06 ± 1.04	0.2183 ± 0.0144	0.50 ± 0.0076	0.994-0.995
8:3	0.15	90	120	58.99 ± 3.73	0.5604 ± 0.0390	0.42 ± 0.0040	0.993-0.994

[1]Data are means ± SE (n=3). [a,b,c]Means with different superscript letters differ ($P \leq 0.05$); [2]α-lactalbumin (α-la)/β-lactoglobulin (β-lg) ratio; [3]xanthan gum concentration (w/w%); [4]temperature (ºC); [5]set time (min); [6]Apparent viscosity (mPa·s) was measured at shear rate of 50 s^{-1} at 25 ± 1ºC.

Table C.2 P and F values from ANOVA of temperature, α-lactalbumin (α-la)/β-lactoglobulin (β-lg) ratio, xanthan gum concentration and time on the apparent viscosity in the milk system.

Type III Tests of Fixed Effects				
Effect	Num DF[6]	Den DF[7]	F Value	Pr > F
rep[1]	2	14	1.21	0.3267
ratio[2]	1	14	5.2	0.0388
temp[3]	1	14	32.51	<.0001
ratio*temp	1	14	4.18	0.0602
conc[4]	1	14	3695.72	<.0001
ratio*conc	1	14	1.96	0.1833
conc*temp	1	14	16.08	0.0013
ratio*conc*temp	1	14	0.19	0.6707
time[5]	1	16	0.95	0.3441
ratio*time	1	16	0.58	0.4565
temp*time	1	16	0.13	0.7196
ratio*temp*time	1	16	0.23	0.6363
conc*time	1	16	0.54	0.4727
ratio*conc*time	1	16	0.56	0.4669
conc*temp*time	1	16	0.05	0.8204
ratio*conc*temp*time	1	16	0.1	0.7589

[1]Replication; [2]α-la/β-lg ratio; [2]temperature; [3]xanthan gum concentration; [4]set time; [5]numerator degree of freedom; [6]denominator degree of freedom.

Table C.3 Mean apparent visocisty of the milk systems as a function of xanthan gum concentration[1]

Xanthan gum (%)	Apparent viscosity (mPa·s)
0	$1.60^{b} \pm 0.06$
0.15	$40.26^{a} \pm 6.82$

[1]Data are means ± SE (n=12). [a,b]Means with different superscript letters differ ($P \leq 0.05$).

Table C.4 Mean apparent viscosity of the milk systems as a function of temperature[1]

Temperature (ºC)	Apparent viscosity (mPa·s)
25	$15.75^{b} \pm 8.22$
90	$26.11^{a} \pm 14.49$

[1]Data are means ± SE (n=12). [a,b]Means with different superscript letters differ ($P \leq 0.05$).

Table C.5 Mean apparent viscosity of the milk systems as a function of α-la/β-lg ratio[1]

Ratio (α-la/β-lg)	Apparent viscosity (mPa·s)
3:8	$18.64^{b} \pm 10.23$
8:3	$23.22^{a} \pm 13.69$

[1]Data are means ± SE (n=12). [a,b]Means with different superscript letters differ ($P \leq 0.05$).

Turbidity

Table C.6 P and F values from ANOVA of temperature, α-lactalbumin (α-la)/β-lactoglobulin (β-lg) ratio and time on the turbidity in the milk system

Type III Tests of Fixed Effects				
Effect	Num DF[6]	Den DF[7]	F Value	Pr > F
rep[1]	2	14	0.53	0.5974
ratio[2]	1	14	5.88	0.0294
temp[3]	1	14	179.14	<.0001
conc[4]	1	14	4.67	0.0486
ratio*temp	1	14	19.26	0.0006
ratio*conc	1	14	4.2	0.0597
temp*conc	1	14	5.8	0.0304
ratio*temp*conc	1	14	4.31	0.0569
time[5]	1	16	4.72	0.0452
ratio*time	1	16	1.76	0.2031
time*temp	1	16	0.35	0.5606
ratio*time*temp	1	16	0.09	0.7623
time*conc	1	16	0.88	0.3626
ratio*time*conc	1	16	0.05	0.8226
time*temp*conc	1	16	0.01	0.9171
ratio*time*temp*conc	1	16	0.94	0.3464

[1]Replication; [2]α-la/β-lg ratio; [3]temperature; [4]xanthan gum concentration; [5]set time; [6]numerator degree of freedom; [7]denominator degree of freedom.

pH and Total solids

Table C.7 Mean pH and total solids (TS) of the milk systems as a function of the temperatures, xanthan gum concentration and α-lactalbumin (α-la)/β-lactoglobulin (β-lg) ratio[1]

Ratio (α-la/β-lg)	Xanthan[2] (%)	Temp[3] (ºC)	pH	Total solids
3:8	0	25	6.54 ± 0.0058	10.71 ± 0.031
3:8	0	90	6.48 ± 0.010	10.85 ± 0.040
3:8	0.15	25	6.53 ± 0.0033	10.80 ± 0.091
3:8	0.15	90	6.46 ± 0.0033	10.78 ± 0.042
8:3	0	25	6.54 ± 0.0088	10.81 ± 0.070
8:3	0	90	6.52 ± 0.0058	10.91 ± 0.063
8:3	0.15	25	6.54 ± 0.0088	10.81 ± 0.031
8:3	0.15	90	6.49 ± 0.0033	10.90 ± 0.099

[1]Data are means ± SE (n=3); [2]xanthan gum concentration (w/w%); [3]temperature (ºC)

Table C.8 P and F values from ANOVA of the temperature, xanthan gum concentration and α-lactalbumin (α-la)/β-lactoglobulin (β-lg) ratio on total solids of milk systems

Type III Tests of Fixed Effects				
Effect	Num DF[5]	Den DF[6]	F Value	Pr > F
rep1	2	14	0.31	0.7402
ratio[2]	1	14	2.1	0.1695
temp[3]	1	14	2.65	0.1259
conc[4]	1	14	0.03	0.8744
ratio*temp	1	14	0.12	0.7391
ratio*conc	1	14	0.02	0.9022
temp*conc	1	14	0.77	0.3957
ratio*temp*conc	1	14	0.59	0.4547

[1]Replication; [2]α-la/β-lg ratio; [3]temperature; [4]xanthan gum concentration; [5]numerator degree of freedom; [6]denominator degree of freedom.

Table C.9 P and F values from ANOVA of the temperature, xanthan gum concentration and α-lactalbumin (α-la)/β-lactoglobulin (β-lg) ratio on pH of milk systems

Type III Tests of Fixed Effects				
Effect	Num DF[5]	Den DF[6]	F Value	Pr > F
rep[1]	2	14	0.26	0.7784
ratio[2]	1	14	20.66	0.0005
temp[3]	1	14	98.65	<.0001
conc[4]	1	14	8.19	0.0126
ratio*temp	1	14	6.38	0.0243
ratio*conc	1	14	0.03	0.8687
temp*conc	1	14	2.3	0.152
ratio*temp*conc	1	14	1.39	0.2583

[1]Replication; [2]α-la/β-lg ratio; [3]temperature; [4]xanthan gum concentration; [5]numerator degree of freedom; [6]denominator degree of freedom.

Total and free sulfhydryl groups

Table C.10 Dilution factors (D) for free and total thiol groups test

Ratio (α-la/β-lg)	D (Free thiol group)	D (Total thiol group)
3:8	6100000	39123208
1:1	6100000	39123208
8:3	6100000	45898889

Table C.11 P and F values from ANOVA of temperature, α-lactalbumin (α-la)/β-lactoglobulin (β-lg) ratio, xanthan gum concentration and time on free sulfhydryl groups in the milk system

Type III Tests of Fixed Effects				
Effect	Num DF[6]	Den DF[7]	F Value	Pr > F
rep[1]	2	14	0.7	0.5133
ratio[2]	1	14	290.91	<.0001
temp[3]	1	14	1937.55	<.0001
conc[4]	1	14	2.03	0.1759
ratio*temp	1	14	272.85	<.0001
ratio*conc	1	14	0.05	0.8188
temp*conc	1	14	11	0.0051
ratio*temp*conc	1	14	0.29	0.5994
time[5]	1	16	47.65	<.0001
ratio*time	1	16	1.53	0.2343
time*temp	1	16	37.13	<.0001
ratio*time*temp	1	16	0.01	0.9336
time*conc	1	16	1.86	0.191
ratio*time*conc	1	16	7.2	0.0163
time*temp*conc	1	16	3.69	0.0729
ratio*time*temp*conc	1	16	1.23	0.2845

[1]Replication; [2]α-la/β-lg ratio; [3]temperature; [4]xanthan gum concentration; [5]set time; [6]numerator degree of freedom; [7]denominator degree of freedom.

Table C.12 P and F values from ANOVA of temperature and α-lactalbumin (α-la)/β-lactoglobulin (β-lg) ratio, xanthan gum concentration and time on the total sulfhydryl groups in the milk system

Type III Tests of Fixed Effects				
Effect	Num DF[6]	Den DF[7]	F Value	Pr > F
rep[1]	2	14	1	0.3936
ratio[2]	1	14	306.1	<.0001
temp[3]	1	14	89.57	<.0001
conc[4]	1	14	0.01	0.9189
ratio*temp	1	14	6.67	0.0217
ratio*conc	1	14	0.02	0.8942
temp*conc	1	14	0.2	0.6612
ratio*temp*conc	1	14	0.32	0.5799
time[5]	1	16	1.61	0.2231
ratio*time	1	16	1.45	0.2464
time*temp	1	16	3.1	0.0973
ratio*time*temp	1	16	1.56	0.2301
time*conc	1	16	1.65	0.2177
ratio*time*conc	1	16	6.54	0.0211
time*temp*conc	1	16	2.47	0.1354
ratio*time*temp*conc	1	16	0.05	0.8234

[1]Replication; [2]α-la/β-lg ratio; [3]temperature; [4]xanthan gum concentration; [5]set time; [6]numerator degree of freedom; [7]denominator degree of freedom.

Table C.13 Total sulfhydryl groups as functions of α-la/β-lg ratio, xanthan gum concentration and time[1]

Ratio	Xanthan[2]	Time (min)	
(α-la/β-lg)	(%)	0	120
3:8	0	$164.70^{b} \pm 12.30$	$150.07^{b} \pm 10.44$
3:8	0.15	$150.53^{b} \pm 12.94$	$164.68^{b} \pm 15.94$
8:3	0	$284.33^{a} \pm 24.65$	$279.85^{a} \pm 16.08$
8:3	0.15	$287.42^{a} \pm 22.00$	$273.38^{a} \pm 18.84$

[1]Data are means ± SE (n=6); [2]xanthan gum concentration (w/w%).

Dynamic oscillation testing

Table C.14 P and F values from ANOVA of temperature, α-lactalbumin (α-la)/β-lactoglobulin (β-lg) ratio and time on the phase shift in 0.15% xanthan gum concentration milk system

Type III Tests of Fixed Effects				
Effect	Num DF[5]	Den DF[6]	F Value	Pr > F
rep[1]	2	6	0.45	0.6552
ratio[2]	1	6	0.87	0.386
temp[3]	1	6	50.54	0.0004
ratio*time	1	8	2.57	0.1475
time[4]	1	8	0.39	0.5488
time*temp	1	8	2.74	0.1365
ratio*time*temp	1	8	0.15	0.7096

[1]Replication; [2]α-la/β-lg ratio; [3]temperature; [4]set time; [5]numerator degree of freedom; [6]denominator degree of freedom.

Table C.15 P and F values from ANOVA of temperature, α-lactalbumin (α-la)/β-lactoglobulin (β-lg) ratio and time on the storage modulus (G′) in 0.15% xanthan gum concentration milk systems

Type III Tests of Fixed Effects				
Effect	Num DF[5]	Den DF[6]	F Value	Pr > F
rep[1]	2	6	0.26	0.7819
ratio[2]	1	6	26.15	0.0022
temp[3]	1	6	98.29	<.0001
ratio*temp	1	6	11.95	0.0135
time[4]	1	8	0.22	0.6504
ratio*time	1	8	0.11	0.7529
time*temp	1	8	0.01	0.9162
ratio*time*temp	1	8	5.59	0.0457

[1]Replication; [2]α-la/β-lg ratio; [3]temperature; [4]set time; [5]numerator degree of freedom; [6]denominator degree of freedom.

Table C.16 P and F values from ANOVA of temperature, α-lactalbumin (α-la)/β-lactoglobulin (β-lg) ratio and time on the loss modulus (G″) index in 0.15% xanthan gum concentration milk systems

Type III Tests of Fixed Effects				
Effect	Num DF[5]	Den DF[6]	F Value	Pr > F
rep[1]	2	6	0.64	0.5591
ratio[2]	1	6	29.9	0.0016
temp[3]	1	6	148.81	<.0001
ratio*temp	1	6	17.3	0.0059
time[4]	1	8	0.86	0.3815
ratio*time	1	8	2.76	0.1355
time*temp	1	8	2.24	0.1728
ratio*time*temp	1	8	1.45	0.2624

[1]Replication; [2]α-la/β-lg ratio; [3]temperature; [4]set time; [5]numerator degree of freedom; [6]denominator degree of freedom.

Appendix D Raw Data

Chapter 4

Table D.1 Raw data of pH, total solids, apparent viscosity and turbidity at 0 min, 30 min, 60 min and 120 min

Hd[1]	Ratio (α-la/β-lg)	Temp[2] (ºC)	Viscosity (mPa·s)				pH	TS (%)
			0	30	60	120		
1	3/8	25	1.86	1.89	1.9	1.93	6.53	10.63
1	1/1	25	1.85	1.88	1.89	1.9	6.54	10.72
2	3/8	25	1.84	1.87	1.89	1.94	6.55	10.76
2	1/1	25	1.84	1.88	1.89	1.92	6.57	10.76
3	3/8	25	1.86	1.89	1.92	1.92	6.55	10.78
3	8/3	25	1.84	1.89	1.91	1.92	6.56	10.68
4	3/8	25	1.85	1.87	1.92	1.92	6.54	10.74
4	8/3	25	1.84	1.86	1.91	1.92	6.56	10.55
5	3/8	25	1.84	1.86	1.89	1.92	6.54	10.73
5	3/8	70	1.76	1.77	1.77	1.82	6.53	10.76
6	3/8	25	1.84	1.89	1.92	1.95	6.56	10.75
6	3/8	70	1.76	1.79	1.81	1.84	6.55	10.82
7	3/8	25	1.84	1.87	1.9	1.94	6.54	10.78
7	1/1	70	1.75	1.78	1.8	1.83	6.53	10.74
8	3/8	25	1.86	1.89	1.92	1.93	6.55	10.76
8	1/1	70	1.74	1.78	1.8	1.83	6.54	10.75
9	3/8	25	1.85	1.88	1.91	1.93	6.53	10.70
9	8/3	70	1.78	1.81	1.82	1.84	6.54	10.76
10	3/8	25	1.87	1.91	1.93	1.94	6.56	10.76
10	8/3	70	1.79	1.81	1.83	1.86	6.54	10.72
11	3/8	25	1.86	1.89	1.9	1.95	6.53	10.77
11	3/8	80	1.85	1.88	1.91	1.91	6.53	10.76
12	3/8	25	1.85	1.89	1.9	1.92	6.55	10.70
12	3/8	80	1.8	1.85	1.87	1.9	6.53	10.69
13	3/8	25	1.87	1.88	1.92	1.95	6.56	10.73
13	1/1	80	1.9	1.92	1.94	1.96	6.54	10.80
14	3/8	25	1.87	1.89	1.92	1.94	6.53	10.71
14	1/1	80	1.89	1.91	1.94	1.95	6.53	10.75
15	3/8	25	1.85	1.88	1.91	1.94	6.53	10.75
15	8/3	80	2.02	2.05	2.06	2.08	6.54	10.76
16	3/8	25	1.85	1.88	1.91	1.94	6.53	10.77
16	8/3	80	2.02	2.05	2.06	2.09	6.56	10.75
17	3/8	25	1.87	1.89	1.91	1.93	6.53	10.63
17	3/8	90	1.92	1.94	1.93	1.99	6.49	10.72

18	3/8	25	1.86	1.9	1.9	1.95	6.54	10.69
18	3/8	90	1.91	1.92	1.95	1.98	6.50	10.72
19	3/8	25	1.86	1.9	1.92	1.94	5.54	10.64
19	1/1	90	1.98	2.0	2.02	2.04	6.50	10.72
20	3/8	25	1.86	1.88	1.9	1.94	6.54	10.55
20	1/1	90	1.97	1.99	2.03	2.05	6.52	10.76
21	3/8	25	1.85	1.89	1.91	1.94	6.53	10.66
21	8/3	90	2.14	2.15	2.2	2.23	6.53	10.75
22	3/8	25	1.87	1.91	1.92	1.94	6.53	10.74
22	8/3	90	2.17	2.20	2.20	2.2	6.53	10.79
23	3/8	25	1.86	1.89	1.90	1.92	6.55	10.70
23	1/1	25	1.86	1.86	1.90	1.91	6.54	10.71
24	3/8	25	1.85	1.86	1.89	1.92	6.54	10.89
24	8/3	25	1.85	1.85	1.88	1.91	6.56	10.64
25	3/8	25	1.85	1.89	1.92	1.94	6.54	10.70
25	3/8	70	1.72	1.76	1.79	1.83	6.54	10.71
26	3/8	25	1.86	1.87	1.90	1.92	6.53	10.67
26	1/1	70	1.76	1.78	1.80	1.81	6.55	10.66
27	3/8	25	1.85	1.89	1.92	1.93	6.54	10.84
27	8/3	70	1.76	1.79	1.81	1.84	6.56	10.76
28	3/8	25	1.86	1.89	1.92	1.93	6.54	10.68
28	3/8	80	1.81	1.84	1.86	1.86	6.54	10.74
29	3/8	25	1.85	1.89	1.92	1.94	6.53	10.70
29	1/1	80	1.85	1.88	1.91	1.94	6.53	10.79
30	3/8	25	1.85	1.89	1.91	1.93	6.54	10.73
30	8/3	80	2.01	2.05	2.05	2.07	6.55	10.75
31	3/8	25	1.86	1.87	1.9	1.92	6.53	10.75
31	3/8	90	1.89	1.91	1.94	1.94	6.52	10.88
32	3/8	25	1.86	1.88	1.91	1.93	6.54	10.84
32	1/1	90	1.96	1.97	2.00	2.01	6.53	10.62
33	3/8	25	1.85	1.87	1.92	1.92	6.55	10.80
33	8/3	90	2.14	2.17	2.20	2.21	6.53	10.84

[1]Half days; [2] temperatures.

Table D.2 Total and free sulfhydryl groups at 0 min, 30 min, and 60 min

Rep[1]	Ratio (α-la/β-lg)	Temp[2] (ºC)	Total sulfhydryl groups (μM)			Free sulfhydryl groups (μM)		
			0	30	120	0	30	120
1	3:8	25	188.4	191.3	162.5	0.1985	0.1985	0.0000
2	3:8	25	178.4	161.1	169.7	0.3971	0.0000	0.7941
3	3:8	25	159.7	182.7	187.0	0.7941	0.1985	0.3971
1	3:8	70	133.8	171.2	152.5	11.32	9.728	5.360
2	3:8	70	149.6	142.4	151.0	14.69	14.89	10.72
3	3:8	70	158.2	143.8	152.5	10.72	17.67	14.29
1	3:8	80	143.8	159.7	158.2	42.29	35.54	21.04
2	3:8	80	158.2	166.8	159.7	45.46	46.06	23.82
3	3:8	80	166.8	156.8	158.2	45.07	32.16	22.63
1	3:8	90	123.7	136.6	116.5	46.85	35.74	30.97
2	3:8	90	119.4	122.3	132.3	46.46	35.74	24.62
3	3:8	90	123.7	126.6	122.3	47.85	34.54	23.03
1	1:1	25	263.2	267.5	267.5	1.191	0.9926	0.7941
2	1:1	25	253.2	238.8	244.5	0.9926	1.191	1.191
3	1:1	25	230.1	258.9	243.1	0.7941	0.7941	2.382
1	1:1	70	211.4	230.1	248.8	18.86	14.29	10.92
2	1:1	70	234.5	211.4	220.1	16.08	13.30	12.51
3	1:1	70	201.4	215.8	214.3	14.29	5.360	9.331
1	1:1	80	179.8	192.7	182.7	67.90	51.22	37.32
2	1:1	80	217.2	199.9	185.5	69.68	48.84	37.72
3	1:1	80	184.1	204.2	194.2	65.12	48.44	45.07
1	1:1	90	164.0	174.0	185.5	68.49	60.95	40.30
2	1:1	90	179.8	194.2	168.3	69.09	53.60	33.75
3	1:1	90	181.2	191.3	187.0	63.93	51.62	34.74
1	8:3	25	362.8	374.6	374.6	1.390	1.390	0.0000
2	8:3	25	366.2	364.5	359.4	1.390	0.1985	1.787
3	8:3	25	357.7	356.1	359.4	1.588	0.0000	0.7941
1	8:3	70	246.4	273.4	273.4	8.537	26.01	18.66
2	8:3	70	239.6	275.1	281.8	15.68	12.71	8.537
3	8:3	70	264.9	221.1	261.6	18.66	19.85	15.09
1	8:3	80	275.1	253.1	275.1	117.3	100.7	82.99
2	8:3	80	243.0	271.7	270.0	113.6	98.47	76.43
3	8:3	80	243.0	280.1	258.2	112.4	89.74	77.63
1	8:3	90	241.3	266.6	199.1	114.2	102.0	78.82
2	8:3	90	226.1	232.9	219.4	115.7	101.4	71.87
3	8:3	90	221.1	221.1	239.6	111.2	103.4	75.04

[1]Replication; [2]temperature

Table D.3 pH and TS measured at different ratios, xanthan gum concentrations and temperatures.

Rep[1]	Ratio (α-la/β-lg)	Xanthan[2] (%)	Temp[3] (ºC)	pH	TS
1	3:8	0	25	6.55	10.66
2	3:8	0	25	6.54	10.77
3	3:8	0	25	6.53	10.71
1	8:3	0	25	6.54	10.92
2	8:3	0	25	6.53	10.82
3	8:3	0	25	6.56	10.68
1	3:8	0.15	25	6.53	10.63
2	3:8	0.15	25	6.53	10.85
3	3:8	0.15	25	6.52	10.93
1	8:3	0.15	25	6.54	10.76
2	8:3	0.15	25	6.56	10.81
3	8:3	0.15	25	6.53	10.87
1	3:8	0	90	6.47	10.80
2	3:8	0	90	6.47	10.82
3	3:8	0	90	6.5	10.93
1	8:3	0	90	6.52	10.97
2	8:3	0	90	6.51	10.96
3	8:3	0	90	6.53	10.78
1	3:8	0.15	90	6.47	10.87
2	3:8	0.15	90	6.46	10.75
3	3:8	0.15	90	6.46	10.74
1	8:3	0.15	90	6.5	11.07
2	8:3	0.15	90	6.49	10.90
3	8:3	0.15	90	6.49	10.73

[1]Replications; [2]xanthan gum concentration (w/w%); [3]temperatures (ºC)

Table D.4 Total and free sulfhydryl groups, turbidity, apparent viscosity, consistency coefficient, and flow behavior index measured at different ratios, xanthan gum concentration, temperatures and times

Rep[1]	Ratio (α-la/β-lg)	Xanthan[2] (%)	Temp[3] (ºC)	Time[4] (min)	Total -SH-[5] (μM)	Free -SH-[5] (μM)	Apparent Viscosity[6] (mPa·s)	K[7] (Pas^n)	n[8] (-)	R^2	Turbidity (Arbitrary unit)
1	3:8	0	25	0	178.4	0.4485	1.40	0.0014	1.0000	0.997	0.856
2	3:8	0	25	0	198.5	0.00	1.40	0.0014	1.0000	0.997	0.830
3	3:8	0	25	0	189.9	0.00	1.40	0.0014	1.0000	0.998	0.862
1	8:3	0	25	0	364.5	1.346	1.40	0.0014	1.0000	0.996	0.770
2	8:3	0	25	0	339.2	0.8971	1.50	0.0015	1.0000	0.995	0.808
3	8:3	0	25	0	305.4	0.00	1.60	0.0016	1.0000	0.992	0.837
1	3:8	0.15	25	0	191.3	6.728	28.53	0.1819	0.5265	0.995	0.857
2	3:8	0.15	25	0	185.5	9.419	28.96	0.1842	0.5271	0.994	0.851
3	3:8	0.15	25	0	153.9	11.21	28.42	0.1937	0.5094	0.993	0.825
1	8:3	0.15	25	0	362.8	16.15	32.59	0.2465	0.4828	0.994	0.691
2	8:3	0.15	25	0	330.7	0.00	30.81	0.2227	0.4944	0.996	0.790
3	8:3	0.15	25	0	302.1	7.625	28.97	0.2088	0.4950	0.994	0.798
1	3:8	0	90	0	117.9	54.72	1.50	0.0015	1.0000	0.998	1.071
2	3:8	0	90	0	156.8	55.62	1.50	0.0015	1.0000	0.998	1.043
3	3:8	0	90	0	146.7	53.38	1.50	0.0019	1.0000	0.998	1.076
1	8:3	0	90	0	226.1	101.37	1.80	0.0018	1.0000	0.998	1.613
2	8:3	0	90	0	226.1	112.13	1.70	0.0017	1.0000	0.999	1.508
3	8:3	0	90	0	244.7	122.00	1.80	0.0018	1.0000	0.998	1.497
1	3:8	0.15	90	0	119.4	56.51	41.00	0.3168	0.4450	0.997	1.339
2	3:8	0.15	90	0	125.1	49.34	45.35	0.4022	0.4421	0.992	1.232
3	3:8	0.15	90	0	128.0	53.82	41.00	0.3496	0.4522	0.994	1.621
1	8:3	0.15	90	0	243.0	102.71	64.22	0.5995	0.4290	0.995	1.444
2	8:3	0.15	90	0	263.2	100.92	55.33	0.5236	0.4255	0.997	1.773
3	8:3	0.15	90	0	222.7	100.47	55.56	0.5574	0.4106	0.995	1.441
1	3:8	0	25	120	145.3	0.00	1.90	0.0019	1.0000	0.998	1.007
2	3:8	0	25	120	169.7	0.4485	1.40	0.0014	1.0000	0.996	0.902
3	3:8	0	25	120	187.0	0.00	1.90	0.0019	1.0000	0.998	0.860
1	8:3	0	25	120	324.0	0.90	1.60	0.0016	1.0000	0.998	0.806
2	8:3	0	25	120	317.2	0.00	1.40	0.0014	1.0000	0.998	0.787
3	8:3	0	25	120	300.4	0.00	1.50	0.0015	1.0000	0.995	0.807
1	3:8	0.15	25	120	211.4	4.934	28.47	0.1736	0.5379	0.995	0.870
2	3:8	0.15	25	120	197.1	1.794	29.97	0.1849	0.5349	0.995	0.993
3	3:8	0.15	25	120	174.0	9.419	29.73	0.1908	0.5249	0.996	0.852
1	8:3	0.15	25	120	335.8	9.419	32.77	0.2390	0.4921	0.995	0.752
2	8:3	0.15	25	120	303.7	12.56	31.23	0.2254	0.4948	0.995	0.978

3	8:3	0.15	25	120	300.4	5.831	29.18	0.1906	0.5160	0.994	0.750
1	3:8	0	90	120	116.5	41.26	1.60	0.0016	1.0000	0.998	1.080
2	3:8	0	90	120	130.9	44.85	1.70	0.0017	1.0000	0.999	1.081
3	3:8	0	90	120	151.0	41.71	1.60	0.0016	1.0000	0.996	1.105
1	8:3	0	90	120	261.6	85.67	1.80	0.0018	1.0000	0.999	1.596
2	8:3	0	90	120	246.4	95.54	1.80	1.8000	1.0000	0.999	1.513
3	8:3	0	90	120	229.5	104.5	1.80	0.0018	1.0000	0.997	1.490
1	3:8	0.15	90	120	102.1	38.13	41.14	0.2812	0.4761	0.994	1.447
2	3:8	0.15	90	120	156.8	40.82	44.83	0.3841	0.4509	0.991	1.468
3	3:8	0.15	90	120	146.7	42.16	41.14	0.3449	0.4565	0.995	1.543
1	8:3	0.15	90	120	221.1	95.54	66.42	0.6378	0.4218	0.994	1.436
2	8:3	0.15	90	120	246.4	99.13	55.83	0.5137	0.4327	0.994	1.878
3	8:3	0.15	90	120	232.9	96.88	54.71	0.5296	0.4197	0.993	1.357

[1]Replications; [2]xanthan gum concentration (w/w%); [3]temperatures (ºC); [4]the time of the milk systems set after cooled to 25 ± 1ºC (min); [5]total and free sulfhydryl groups; [6]the apparent viscosity at shear rate of $50s^{-1}$; [7]consistency coefficient; [8]flow behavior index.

Table D.5 Dynamic oscillation testing for the 0.15% xanthan gum concentration milk systems at different ratios, temperatures and time

Rep[1]	Ratio(α-la/β-lg)	Temp[2](ºC)	Time[3](min)	Phase(degree)	G′(Pa)	G″(Pa)
1	3:8	25	0	38.2	0.4155	0.3267
2	3:8	25	0	40.1	0.4253	0.3587
3	3:8	25	0	39.3	0.3570	0.2927
1	8:3	25	0	40.4	0.5585	0.4753
2	8:3	25	0	38.9	0.4831	0.3895
3	8:3	25	0	41.8	0.4332	0.3874
1	3:8	90	0	45.2	0.5954	0.6002
2	3:8	90	0	42.4	0.7708	0.7046
3	3:8	90	0	42.0	0.6465	0.5827
1	8:3	90	0	48.0	1.0310	1.1460
2	8:3	90	0	47.4	0.8536	0.9281
3	8:3	90	0	45.5	0.9206	0.9366
1	3:8	25	120	41.9	0.3592	0.3221
2	3:8	25	120	31.6	0.4927	0.3037
3	3:8	25	120	41.0	0.4273	0.3720
1	8:3	25	120	35.9	0.4543	0.3294
2	8:3	25	120	38.0	0.4721	0.3686
3	8:3	25	120	37.0	0.4334	0.3270
1	3:8	90	120	45.1	0.5941	0.5967
2	3:8	90	120	46.8	0.6596	0.7019
3	3:8	90	120	46.5	0.6013	0.6333
1	8:3	90	120	47.0	1.0570	1.1320
2	8:3	90	120	47.1	0.8648	0.9321
3	8:3	90	120	44.0	0.9856	0.9519

[1]Replications; [2]temperatures (ºC); [3]set time (min)

Appendix E SAS® Program

Chapter 4

pH and total solids

```
data one;
input hd trtno pH TS ratio $ temp $;
datalines;
run;
%macro mix(response, rtitle);
proc mixed data=one;
class ratio temp hd;
model &response=ratio temp ratio*temp/ddfm=satterth;
random hd;
lsmeans ratio temp ratio*temp;
lsmeans ratio/pdiff=control('3/8') adjust=dunnett;
lsmeans ratio/pdiff adjust=tukey;
lsmeans temp/pdiff=control('25') adjust=dunnett;
lsmeans temp/pdiff adjust=tukey;
%mend mix;
%mix(pH,'pH');
%mix(TS,'TS');
run;
quit;
```

Apparent viscosity

```
data all;
input hd trtno app ratio$ temp$ time @@;
cards;
proc mixed data=all;
class ratio temp time hd;
model app=ratio temp ratio*temp time time*temp ratio*time
ratio*time*temp/ddfm=satterth;
random hd hd*ratio*temp;
lsmeans time/pdiff=control('0') adjust=dunnett;
lsmeans time/pdiff adjust=tukey;
lsmeans ratio*temp/pdiff adjust=tukey;
title 'apparent viscosity';
run;
```

Free and total sulfhydryl groups

```
data freeSH;
input ratio$ temp rep free time @@;
```

```
cards;
proc glimmix data=freeSH;
class rep ratio time temp;
model free=rep ratio|time|temp;
random rep*ratio*temp;
title'freeSH';
run;

data free25;
input ratio $ temp rep free time @@;
cards;
proc glimmix data=free25;
class rep ratio time;
model free=ratio|time;
random rep rep*ratio;
lsmeans ratio time time*ratio/adjust=tukey lines;
title'free25';
run;
data free70;
input ratio $ temp rep free time @@;
cards;
proc glimmix data=free70;
class rep ratio time;
model free=ratio|time;
random rep rep*ratio;
lsmeans ratio time time*ratio/adjust=tukey lines;
title'free70';
run;

data free80;
input ratio $ temp rep free time @@;
cards;
proc glimmix data=free80;
class rep ratio time;
model free=ratio|time;
random rep rep*ratio;
lsmeans ratio time time*ratio/adjust=tukey lines;
title'free80';
run;

data free90;
input ratio $ temp rep free time @@;
```

```
cards;
proc glimmix data=free90;
class rep ratio time;
model free=ratio|time;
random rep rep*ratio;
lsmeans ratio time time*ratio/adjust=tukey lines;
title'free90';
run;

data free0;
input ratio $ temp rep free time@@;
cards;
proc glimmix data=free0;
    class ratio temp rep;
    Model free = rep ratio temp ratio*temp;
    lsmeans ratio*temp/pdiff adjust=tukey lines;
    title '0 min';
run;

data free30;
input ratio $ temp rep free time@@;
cards;
proc glimmix data=free30;
    class ratio temp rep;
    Model free = rep ratio temp ratio*temp;
    lsmeans ratio*temp/pdiff adjust=tukey lines;
    title '30 min';
run;

data free120;
input ratio $ temp rep free time@@;
cards;
proc glimmix data=free120;
    class ratio temp rep;
    Model free = rep ratio temp ratio*temp;
    lsmeans ratio*temp/pdiff adjust=tukey lines;
    title '120 min';
run;

data totalSH;
input ratio$ temp rep total time @@;
cards;
```

```
proc glimmix data=totalSH;
class rep ratio time temp;
model total=rep ratio|temp|time;
random rep*ratio*temp;
lsmeans time/pdiff adjust=tukey;
title'totalSH';
run;
```

Chapter 5

Apparent viscosity

```
data study_2;
input rep ratio$ conc temp time app @@;
cards;
proc glimmix data=study_2;
class rep ratio conc temp time;
model app=ratio|temp|conc|time;
random rep rep*ratio*temp*conc;
run;

proc glimmix data=study_2;
class rep ratio conc temp time;
model app=ratio|temp|conc|time/link=log;
random rep rep*ratio*temp*conc;
run;

data study_2;
input rep ratio$ temp conc time app@@;
logapp=log(app);
cards;
proc glimmix data=study_2;
class rep ratio conc temp time;
model logapp=ratio|temp|conc;
random rep rep*ratio*temp*conc;
lsmeans ratio*temp*conc/pdiff adjust=tukey lines;
run;
```

Turbidity

```
data Turbidity;
input rep ratio$ conc temp time T @@;
cards;
proc glimmix data=Turbidity;
class rep ratio time temp conc;
```

```
model T=rep ratio|temp|conc|time;
random rep*ratio*temp*conc;
lsmeans ratio*temp temp*conc/pdiff adjust=tukey lines;
title'Turbidity';
run;
```

pH and total solids

```
data study_2;
input rep ratio$ conc temp pH TS;
cards;
run;
%macro study2 (y);
title &y;
proc glimmix data=study_2;
class ratio temp conc rep;
model &y=ratio temp conc ratio*temp ratio*conc temp*conc temp*ratio*conc
rep;
lsmeans ratio conc temp ratio*conc ratio*temp conc*temp
conc*temp*ratio/pdiff adjust=tukey lines;
run;
%mend;
%study2(pH)
%study2(TS)
run;
```

Phase shift, G′, and G″

```
data study_2;
input rep ratio$ conc temp time phase G1 G2 @@;
cards;
%macro study2 (y);
title &y;
proc glimmix data=study_2;
class rep ratio time temp;
model &y=rep ratio|temp|time;
random rep*ratio*temp;
lsmeans temp/pdiff adjust=tukey;
lsmeans ratio*temp/pdiff adjust=tukey lines;
run;
%mend;
%study2(phase)
%study2(G1)
```

```
%study2(G2)
run;
```

Free and total sulfhydryl groups

```
data SH;
input rep ratio$ conc temp time total free @@;
cards;
proc glimmix data=SH;
class rep ratio time temp conc;
model free= rep ratio|temp|conc|time;
random rep*ratio*temp*conc;
lsmeans ratio*temp time*temp/pdiff adjust=tukey lines;
title'freeSH';
run;
proc glimmix data=SH;
class rep ratio time temp conc;
model total= rep ratio|temp|conc|time;
random rep*ratio*temp*conc;
lsmeans ratio*temp ratio*conc*time/pdiff adjust=tukey lines;
title'totalSH';
run;
```