A Novel Approach to Structure Generation for Texture Improvement in a Soymilk-Dairy Gel

by

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The current study attempts to improve the texture properties of a fermented product containing soymilk and milk. Preferred Attribute Elicitation (PAE) was examined as a novel sensory methodology for extracting important attributes influencing consumer liking. This method was applied on commercial yogurt products, and it was determined that texture was important to consumer acceptance, and that texture attributes such as graininess and runny texture were detrimental to consumer liking. Outcomes of the PAE method were compared to those obtained from a conventional trained panel method. It was determined that the PAE method was able to characterize the product textures in a meaningful way, resulting in a product map that closely resembled that obtained by the trained panel method. A mixed protein network was then generated, and simultaneous gelation of both soy and dairy proteins lead to an improved gel structure as compared to gelation of either soy or dairy proteins alone in the mixed system. In addition, it was determined that the presence of homogenized fat globules in the network resulted in fermented products with increased mouthcoating and thickness, particularly when cream was homogenized with dairy milk, with or without soymilk in the mix. It was noted that the order of homogenization (cream with either soymilk, milk alone or with the mix) affected the size and number of aggregates as well as number of interconnecting strands. Additionally, aggregation of milk proteins before soy proteins generated gels with higher slipperiness and fattiness perceptions than gels made from simultaneous milk and soy protein aggregation. This study suggested that it is possible to generate desirable texture properties from a mixture of soy protein and milk proteins, and that it is of fundamental importance to fine tune the structure within the matrix to obtain optimal texture perception.
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LIST OF ABBREVIATIONS

DWS .......................................................................................................................... diffusing wave spectroscopy

GDL .......................................................................................................................... glucono-δ-lactone

GPA ......................................................................................................................... generalized procrustes analysis

MF ............................................................................................................................ milk fat

MFGM ...................................................................................................................... milk fat globular membrane

MFA ........................................................................................................................ multiple factor analysis

MSD ........................................................................................................................ mean square displacement

PAE ........................................................................................................................ preferred attribute elicitation

PCA ........................................................................................................................ principal components analysis

SDS-PAGE ...................................... Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

SM ............................................................................................................................. skim milk
CHAPTER 1
GENERAL INTRODUCTION

At the start of industrialization, food manufacturing was driven by a need to increase food production to avoid shortages (Cengage, 2003). Manufacturers were concerned with food preservation and the products obtained depended mainly on ingredient availability. Products were introduced without any prior market research and their success depended mainly on similarities among consumer preferences (Linnemann et al., 2006). Today’s food manufacturers have to face fierce competition and to remain competitive they need well-informed, continuous new food product development that generates products that meet consumer needs and sensory preferences (Linnemann et al., 2006). As a result, food product development has undergone a chain-reversal, meaning that food development is now a demand-driven process rather than supply-driven: by using a demand-driven food product development process, relying on consumer insights, food companies are more likely to maintain their competitive edge and to waste less time and funds developing products that do not meet consumer needs.

The present research evaluated this approach for the development of a challenging food matrix, a mixed soy and dairy protein gel. Sensory testing was employed in the early stages of the research project, to better identify the most important textural attributes from a consumer standpoint. This approach should accelerate the development of a novel product, and it is of relevance to the food industry. When faced with a new food matrix, there are many aspects of the food that can be examined such as the impact of composition, various ingredients, processing methods and processing conditions on the food structure. However, not all of these factors will have an effect on a product’s sensory properties, for example, because of limits in human detection. It has been
shown that soft particles that are < 80 µm in size are hardly detectable on the human tongue (Tyle, 1993). Such a study implies that although processing techniques that decrease the size of soft particles to below 80 µm may have an impact on microstructure, this change may not be reflected in the product’s texture properties. This example shows the importance of carrying out sensory testing in the initial stages of a project.

The current project will utilize consumer insights to help direct the improvement of a challenging system. There is great demand for protein products, especially fermented high protein products, as evidenced by the recent dramatic increase in popularity of Greek yogurts (Thompson, 2011). There is potential for a novel, high protein fermented gel to be made from a mixture of soymilk and cow’s milk. However, soy products are often associated with poor sensory qualities such as beany flavor and grainy texture (Ankenman Granata & Morr, 1996; Yazici et al., 1997). Thus, making a fermented product from a mixture of cow’s milk and soymilk presents a great challenge to consumer acceptability. Additionally, little research exists on parameters that affect the structure and texture of mixed soy-dairy protein gels (Chronakis & Kasapis, 1993; Comfort & Howell, 2002; Drake & Gerard, 2006; Roesch et al., 2004; Roesch & Corredig, 2005; Roesch & Corredig, 2006). Understanding of these systems is still in the early stages and more work is required before the details of the mixed aggregation of these protein matrices are understood sufficiently well to result in optimized processes and formulations. Modern consumers are quite selective and they expect to have products which are both healthy and have good sensory properties. Thus, although a mixed protein system containing milk proteins and soymilk would have great potential for development because of the health benefits deriving from both components, some important questions are still unanswered. Although mixed soymilk-dairy milk
yogurts would be healthy products, is it possible to generate consumer-acceptable textures with these systems? What processing steps can be used to modify the textures of soymilk-dairy milk systems? How will such texture modifications impact acceptability? These are some of the questions that this thesis will focus on.

The challenges to product acceptability presented by the soy component of this system as well as the lack of information regarding structuring of soymilk-dairy milk mixtures, make it a very challenging system to work with. This presents an opportunity to employ this matrix as a proof of concept model system to validate the usefulness of the sensory-first approach in texture improvement.

The first step in this project was to select a method to elicit consumer preferences in commercially available yogurts. This allowed the establishment of sensory goals for this product category. The traditional means for doing this involves the use of a trained panel for descriptive analysis of a product set, followed by coupling of this data with consumer liking scores for the same products to produce an external preference map. External preference mapping generates a two- or three-dimensional map of the products, mapped according to their attributes. Thus, the location of each product on the map corresponds to certain sensory properties (ex.: thick, thin, grainy). Consumer preferences are then regressed onto the product map and the direction of the vector indicates preference for particular sensory properties (Guinard et al., 2001). Although this method is well accepted and it can provide a detailed description of the products, it is also very time-consuming, costly and requires a knowledgeable panel leader. An alternative method, which we have named Preferred Attribute Elicitation (PAE), employs a panel of consumers to
characterize a set of products in a single session according to attributes that stand out most to the consumers.

The PAE method has experienced only limited usage in the food industry, and it is not yet understood how the results of this technique compare to those of conventional profiling. In this research, we improved our understanding of the PAE method by comparison of this technique with conventional profiling, as shown in Chapter 3. The results reported in Chapter 3, collected using commercial yogurt products, also allowed establishing a texture goal for a fermented or acidified protein gel. The texture attributes derived from the PAE will then allow setting of texture goals for the study of a food system with yogurt-like textures but composed of milk proteins and soymilk.

A mixed soymilk-dairy milk product presents major challenges to consumer-acceptability. While the texture of a dairy product is often well received by western consumers, the texture of soymilk derived gels such as tofu, is less liked (Drake & Gerard, 2006). Thus, some texture manipulation will be required to generate consumer-acceptable textures from a fermented mixture of soymilk and cow’s milk.

Using a mixed protein system represents an opportunity, as different gelation mechanisms can be employed to fine tune the gelation of the two protein species. In particular, in the case of soy and milk proteins, these proteins aggregate under different conditions; hence, selective conditions may be used to manipulate the incorporation of each protein type into the gel matrix. A combined gelation induced by the enzyme rennet, which specifically destabilizes caseins,
together with acidification using pH (which at pH >5.4 destabilizes only soy protein) was studied in detail, to better understand if by modulating these gelation mechanisms it may be possible to generate a range of microstructures and textures. The results are summarized in Chapter 4. It was clearly shown that it is possible, by fine-tuning the point of gelation between soymilk particles and renneted casein micelles, to obtain mixed gels. Hence it was important to determine if with these mixed gelation processes it is possible to obtain textures exhibiting attributes among those that were identified as being preferred by consumers.

For the last part of the work, lactic acid cultures were employed for the acidification experiments on the mixture, to obtain a product more acceptable to consumers. In Chapter 5 we examined the similarities in the gelation behaviour of such systems induced by bacterial culture or addition of GDL, which was employed in earlier chapters.

After acquiring understanding of the conditions required for gelation of the mixed soymilk-dairy milk system, gels with different structures were obtained using different methods, as reported in Chapter 6. In particular, fat globules were included in the mixture, and the order of processing was varied, namely, homogenizing the fat globules with soymilk or with milk first or all together, before preparing the mixtures. These variations in processing, together with variation of different gelation mechanisms (renneting and acid induced gelation) should have an impact on texture. This hypothesis was then tested using the Napping® technique combined with ultra-flash profiling. In this technique, panelists were required to taste the products and to place them on a piece of paper, at a distance that depended on the degree of perceived difference in their sensory properties. Chapter 6, provides information on some of the attributes used to characterize
the mixed soymilk-dairy milk gels and the extent of the difference in texture perceived by the consumers for the various products. To better understand the role played by the physico-chemical properties and microstructure on the texture differences perceived by the panelists, the samples were characterized in Chapter 7. It was important to determine if an improved understanding of the underlying structures that generate desirable textures can facilitate some predictions of other processing steps or ingredients that could be used to further improve mixed soymilk-dairy fermented gels.

Overall, this work allowed determining structural features affecting the perception of texture in a protein matrix, using a very challenging mixture of proteins as the model system. The work showed very clearly that texture in protein gel matrices is an important sensory property and identified challenges when texture development is approached using only chemical and microstructural analysis.
CHAPTER 2
LITERATURE REVIEW

2.1. Texture as a sensory property

Texture is a sensory property of food which is derived from the material properties of the food matrix. It is defined as “the sensory and functional manifestation of the structural, mechanical and surface properties of foods detected through the senses of vision, hearing, touch and kinesthetics” (Surmacka-Szcześniak, 2002). As described in the definition, texture attributes can be classified into three categories: structural, mechanical and attributes related to surface properties. Attributes related to structure are associated with the size, shape and orientation of structures within a food matrix. Examples of these include gritty, coarse, and fibrous attributes. Mechanical attributes are instead related to the reaction of food to stress and are measured by exerting pressure on the food with the teeth, tongue and roof of the mouth. Hardness, brittleness, cohesiveness and chewiness are examples of mechanical texture attributes. Finally attributes specific to surface properties relate to the ability of the food matrix to interact with the tongue, and are usually correlated to the fat content, moisture content and the ability of the matrix’s components to interact with salivary components (Surmacka-Szcześniak, 1963).

Texture can be detected by several senses. Attributes such as thickness and chunkiness can be perceived visually by manipulating a food in its container or with a utensil (Rosenthal, 1999). Auditory senses also contribute to detection of food texture such as in the case of crispiness and crunchiness where sounds of different pitches are produced when the food is crushed (Duizer, 2001). However, the majority of food texture is perceived by the sense of touch by manipulating a food with the hands or in the mouth (Chen, 2009).
2.1.1. The process of texture perception

Before a food is placed into the oral cavity, texture perception has already begun. Visual cues such as colour, shape, size and structure (e.g. how porous a sample appears) provide a basis for our texture expectations. Manipulation of the food either within a container (e.g. how quickly the product pours down the side of a container) or with a utensil provide more clues about the texture and shape (Rosenthal, 1999). Expectations about how a food will feel in the mouth can change how the food is perceived (Deliza & MacFie, 1996).

Before the first bite, the first touch of food in the mouth occurs at a relatively low shear rate and provides information regarding the shape, size and presence of particles or surface indentations in the food. In the case of solid foods, food structure is broken within the first few bites and from this, information is gathered regarding how brittle, fibrous or chewy a food is. These chewing cycles achieve a high degree of shearing and chews may be irregularly spaced. During chewing, the tongue moves the food around in the oral cavity, contributing to shearing and gathering information about how the food flows within the mouth. After the first few bites, a food is mixed with saliva and broken down; in the next phase of chewing, chewing cycles become more regular and the food is made into a bolus (Chen, 2009; Prinz et al., 2007; Rosenthal, 1999). When the bolus achieves a consistency and level of lubrication that is judged appropriate for swallowing, it is finally swallowed (Coster & Schwarz, 1987). After swallowing, residual particles or mouthcoating may be perceived and additional swallowing is usually performed to clear the mouth (Chen, 2009).
Semi-solid foods, such as yogurts, are processed somewhat differently because they are already in a form that is comparable to a bolus. Although such foods require very little mastication before swallowing, they are often kept in the mouth for a longer period of time than that required to transfer the food from the front of the mouth to the oropharynx (de Wijk et al., 2011). During the time that the food is kept in the oral cavity, the temperature of the food is brought up to match the temperature of the mouth, the foods are diluted with saliva, and they are spread in the mouth via various complex movements to enhance sensory perception. In fact, it has been shown that when panelists are instructed to follow specific movements during oral processing of semi-solid foods, such as smearing in a figure-eight or moving the tongue up and down, perception of attributes such as thickness or creaminess is reduced compared to when panelists are given no instructions (de Wijk et al., 2011). Thus it appears as though individuals optimize movements inside the mouth to achieve maximum intensity of sensory perceptions.

Not all attributes benefit equally from complex intra-oral manipulations and the degree of complexity involved in perceiving attributes has been found to be linked to the timing of their evaluation. For example, thickness is generally evaluated early in the mastication process and thickness evaluation has been shown to benefit less from complex oral movements. On the other hand, creaminess requires more complex oral manipulations for detection and it is evaluated later during mastication. Overall, although texture perception of semi-solid foods requires very little mastication compared to solid foods, the foods continue to undergo complex manipulations in the oral cavity and as with solid foods, texture perception of semi-solids continues while the food is in the mouth as well as after swallowing (e.g. residual particles, mouthcoating) (de Wijk et al., 2011).
Food texture perception is a complex process involving many steps, whereby information is gathered simultaneously by different senses and different areas of the mouth. The information is interpreted by the brain and can be influenced by the psychological state of the subject. Because of the inherent variability of texture perception from individual to individual, as well as from day to day in a single subject, attempts have been made to correlate texture with instrumental measurements.

2.1.2. Relating sensory and instrumental measurements of texture

From the definition of texture, it is clear that it is a food material property that is based on perception. As humans are the only living beings able to verbalize their perceptions, texture can only truly be assessed by humans. Instrumental measurements occur under highly controlled conditions, and most of them only measure one dimension in a multidimensional experience. Texture is a collective of many texture attributes perceived simultaneously under highly variable conditions. Because texture is such a complex experience, it is difficult to know for certain when an instrumental measurement will have a relation to the texture perceived by humans.

There are many factors that can cause texture to be perceived differently by humans compared to what is expected from instrumental measurements. Some factors may be psychological but many are related to environmental conditions. Instrumental measurements are carried out under specified conditions of temperature, flow and shear rate, among others, and the conditions are not subjected to any feedback mechanism, unlike in reality, where conditions inside the oral
cavity are dynamic, and change greatly from first contact, to chew, to swallow during consumption of food (van Vliet, 2002; van Vliet et al., 2009).

When food is placed into the oral cavity, the temperature will immediately begin to change to body temperature. This can induce melting in foods such as ice cream or cooling such as in the case of hot soup. Temperature changes will vary the consistency of food products and hence their perception in the mouth. Instrumental measurements of texture are usually carried out at one selected temperature.

The food material properties change quickly in the mouth as food is mixed with saliva. Saliva acts as a lubricant and can reduce the perception of roughness or dryness. In other cases, interactions between food components (i.e. tannins or proteins) and salivary mucin proteins can lead to particle sedimentation on the tongue and generate a sensation of roughness, such as with astringency (Chen, 2009). Salivary enzymes may also act on foods to change their consistency. For example, in starch containing foods, salivary α-amylases quickly break down starch and can lead to a decrease in viscosity within seconds (Evans et al., 1986).

In general, the effect of salivary components on foods is omitted during rheological assessment (Rosenthal, 1999). Rheological measurements also cannot precisely mimic food behaviour inside the mouth because they require laminar flow. However in the mouth, semi-liquids and liquids experience extensional flow and turbulence (van Vliet, 2002). Additionally, shear rates in the oral cavity vary greatly throughout chewing and from individual to individual. Shear rates in the mouth can vary between 0.1-1000 s⁻¹. The shear rate used also depends on the viscosity of the
food. High shear rates are applied in the mouth when consuming low viscosity foods while higher viscosity foods experience low shear rates. Since most foods are non-Newtonian, their viscosity will change depending on the shear rate and temperature, thus the shear rate within the mouth will be adjusted throughout chewing (Shama & Sherman, 1973).

Although food texture perception is too complex to be reproduced instrumentally, there are some approaches for using instruments to measure certain material properties that can be related to texture perception. For example, as a screening tool to evaluate how the addition of a particular ingredient may impact the viscosity of a food product, a viscometer could provide some correlation to sensory thickness. Earlier work (Skriver et al., 1999) measured viscosity of stirred yogurts using a rheometer at shear rates varying between 1 and 230 s\(^{-1}\). The researchers found that, for a particular set of stirred yogurt products, viscosity measurements correlated best with sensory thickness when measured at a shear rate of 100 s\(^{-1}\) (Skriver et al., 1999). However, other reports, also employing a rheometer, have demonstrated a positive correlation between oral thickness of stirred yogurts and instrumental viscosity measured at a lower shear rate, around 10 s\(^{-1}\) (Lee & Lucey, 2006). Thus, it can be concluded that it is possible to estimate some texture attributes using instrumental methods, however the conditions that make the measurement relevant to texture are highly dependent on the particular food product. In addition, separate instrumental techniques will be required to assess multiple attributes (Rosenthal, 1999).

Some authors have also reported a positive correlation between oral thickness and other types of rheological measurements, namely small deformation measurements of storage modulus, G' (Lee & Lucey, 2006; Tarrega & Costell, 2007). However, such statements must be regarded with
some reservation as the levels of strain exerted on a sample during these measurements are below the sensory perception threshold (Dickinson, 2005). The correlation between the storage modulus and thickness has more to do with the correlation between the storage modulus and the yield stress of a sample than the actual rheological properties during in mouth processing.

Some attempts have been made to measure more than one dimension of texture by combining various instrumental methods. This is particularly interesting for approximating multi-component sensory attributes. Creaminess is an important attribute of yogurt and it can be approximated by the evaluation of thickness and smoothness (Cayot et al., 2008). While thickness is known to be commonly associated with instrumental viscosity, smoothness is an attribute related to frictional properties, and it can be estimated using tribological measurements (Chen & Stokes, 2011). It has been recently reported that using a tribology accessory attached to a conventional rheometer it is possible to detect sample rheological properties as well as friction parameters simultaneously, to generate an improved instrumental assessment of creaminess (Krzeminski et al., 2012).

Although the human experience of texture cannot be fully replicated instrumentally, some headway is being made in instrumental measurements to help better estimate individual texture parameters. Instrumental measurements are less costly and more reproducible than sensory evaluation methods; however they are very limited to certain dimensions of texture perception. They can be used for purposes of quality control or as screening tools, as they can provide a basis for estimating how food texture might be impacted by some parameters. However, for an accurate assessment of texture, sensory panels are indispensable.
2.1.3. *Texture in semi-solid fermented products*

Yogurts exhibit a number of textural attributes including creaminess, thickness, smoothness, slipperiness and mouthcoating, to name a few. Creaminess appears to be a particularly important attribute in yogurt products (Cayot *et al.*, 2008). However, as mentioned above, unlike thickness or smoothness, creaminess is a multi-dimensional attribute. Studies have shown that creaminess consists of both flavour and texture attributes. Dairy flavour in particular has been shown to contribute to enhancement of creaminess perception (Janhoj *et al.*, 2008). Products exhibiting texture properties including thickness, smoothness and slipperiness are generally rated as creamy. However, it has been shown that in the specific case of dairy products, the combination of thickness and smoothness (absence of chalkiness) alone can usually be used to predict creaminess sufficiently well (van Vliet *et al.*, 2009). These attributes have been extensively studied and will be discussed in detail in the ensuing sections. As already mentioned, yogurt may also exhibit a number of other texture attributes. However, many of these are far less commonly studied and thus little is known about how they are perceived or how they may be generated in semi-solid gels such as yogurt. A brief description of some the less understood attributes (wateriness, slipperiness, mouthcoating) will be included at the end of this section.

2.1.3.1. *Thickness*

Sensory thickness is based on the perception of viscous consistency of a product. Products are processed differently in the mouth depending on their thickness. A low viscosity sample will freely flow in the mouth, and it is thought that for these types of samples, the in-mouth assessment of texture is carried out at a constant shear rate, based on how easily the food flows between the upper surface of the tongue and the palate (van Vliet *et al.*, 2009). The transit time
in the mouth is very short. Foods with higher viscosity or a yield stress will be processed differently. Foods with higher viscosities or those with a yield stress (such as acid gels like yogurt) will first be compressed between the tongue and the palate to separate the structure of the food and induce flow (van Vliet, 2002). The pressure required to cause flow of the material will contribute to the sensory assessment of sample thickness (van Vliet et al., 2009).

There are many factors that can influence yogurt thickness and some of these include protein fortification, milk heat treatment conditions, fat content, homogenization conditions, stabilizers, selection of a bacterial culture, incubation temperature and the final pH (Lucey, 2004).

Yogurt type products are made by acidification of milk using bacterial cultures. As the pH of the substrate is decreased, proteins approach their isoelectric point and begin to interact to form a gel network. The strength and type of bonds formed will ultimately determine the final thickness of the acid milk gel (Lucey, 2004). Milk is often fortified by addition of skim milk powder (SMP), milk concentrates, caseinates or whey proteins. A higher protein concentration will lead to an increased thickness. Caseins are the building blocks of the gel structure in acid milk gels. Thus, increasing the casein content will naturally lead to more interconnections in the gel network and a firmer product. Whey proteins also impart a significant increase in gel thickness when milk is extensively heat treated. With heating, whey proteins are denatured and form complexes with the caseins, and with acidification, these complexes actively participate in the network, forming additional bonds and contributing to product thickness (Alexander & Dalgleish, 2004; Lucey, 2002; Donato & Guyomarc’h, 2009).
Increasing the fat content of the milk base will also increase the thickness of the fermented gel, especially if the milk is homogenized with fat (Sodini et al., 2004). During homogenization, fat globules are disrupted to smaller sizes leading to an increase in surface area. While the native milk fat globule membrane does not have significant amounts of adsorbed casein or whey proteins, after homogenization, the newly formed surface area of fat globules is coated with caseins and whey proteins (Cano-Ruiz & Richter, 1997). Because caseins are now present on the surface of the milk fat globule membrane, the fat globules become an integral part of the gel network. These interacting fat globules in the gel network contribute significantly to sample thickness (Sodini et al., 2004).

Polysaccharides may also be used to alter product thickness (Azim et al., 2010; El-Sayed et al., 2002), usually by increasing the viscosity of the serum phase. These high molecular weight molecules help increase the water holding capacity of the gel, decreasing yogurt syneresis (van Marle et al., 1999). Amongst the polysaccharides employed are pectin, locust bean gum, carrageenan, carboxymethyl cellulose and guar gum (Everett & McLeod, 2005). In a number of formulations, modified starch granules are also added to yogurt mixes, as they increase the thickness and decrease syneresis of the acid gel (Azim et al., 2010).

Although the primary function of bacterial cultures in yogurt is to acidify, some cultures also produce exopolysaccharides. These are polysaccharide molecules secreted from the lactic acid bacteria during fermentation, and they impart viscosity as well as increase water holding capacity of the acid milk gels. This result is similar to other polysaccharides, however no stabilizer is added which has important implications in labeling (Duboc & Mollet, 2001).
Low incubation temperatures (for example, 40ºC instead if 45ºC) lead to slower acidification than higher temperatures, but can result in yogurt products with higher viscosities and therefore increased sample thickness. The final pH of yogurts is another important factor determining yogurt thickness. As caseins approach their isoelectric point, there is a corresponding increase in sample thickness between pH 5.1 and 4.6. However, if the pH continues to drop below pH 4.6, the isoelectric point of caseins, there is once again an increase in repulsive charges and a corresponding decrease in product thickness (Lucey, 2004).

2.1.3.2. Chalkiness

Chalkiness, sometimes also referred to as roughness or grittiness (Josephson, 1954; Schmidt & Bates, 1976), can be defined as the sensation of very fine particles on the tongue (Lee & Lucey, 2006). It is considered to be a part of the geometrical class of texture attributes as it relates to the size and shape of particles in a food product (Surmacka-Szcześniak, 2002).

Chalkiness is likely perceived by mechanoreceptors in the mouth when food is moved over the mouth surface or when the tongue is rubbed against the palate. The sensation of roughness may be enhanced by rubbing the food between a soft tissue in the mouth (tongue or lip) and a hard surface (teeth). If particles are as soft, or softer, than the oral mucosa, then the particle will deform under compression and will not contribute much to a sensation of chalkiness. On the other hand, if a particle is harder than the oral mucosa, compression will cause deformation of the oral mucosa and trigger detection by the mechanoreceptors (Engelen et al., 2005).
Various factors affect the perception of chalkiness including particle size, shape, hardness, concentration and lubrication. Hardness and roughness of particle surfaces increases the perception of chalkiness and decreases the detection limit for particle size. For example, hard and sharp particles of silica as small as 2 µm in diameter have been found to contribute to grittiness (Engelen et al., 2005). However, soft and round particles of polyethylene are not perceived as gritty up to a size of 80 µm in diameter (Tyle, 1993). Increases in particle size will increase the perception of roughness only to a certain point. After a critical size is reached, panelists may perceive the further increase in particle size as an increase in a different attribute, which was not examined by the authors (Engelen et al., 2005).

Increases in particle concentration often correspond with increased perception of roughness. Additionally, particles that were too small to be detected at low concentrations, may become detectable as their concentration is increased (Imai et al., 1995).

Lubrication can have an important impact on reducing the perception of roughness, as it reduces the friction between particles and oral surfaces (Engelen et al., 2005). Fat is known to have lubricating properties and it has been shown that, as fat content is increased, the perception of roughness tends to decrease (de Wijk & Prinz, 2005). It is interesting to note that lubrication has a more prominent effect on small particles. This is likely because the lubricating layer can more easily cover the sharp edges of small particles. When particles are larger, the shape of the particles becomes more important because the lubricant, usually fat, cannot coat the rough edges entirely and the shape will then contribute more to a sensation of roughness compared to small particles (Engelen et al., 2005).
Although fat can contribute to lubrication and therefore reduce perceptions of roughness, its ability to lubricate also changes with size and number of fat globules. Smaller fat globules result in reduced friction. It is thought that this may be because smaller globules are less deformable, due to their higher surface area to volume ratio, and the globules therefore have a smaller surface contact area resulting in reduced friction (de Wijk & Prinz, 2005).

When there is a need to predict chalkiness in soft materials without the use of a sensory panel, both particle size analysis and friction measurements can be used. Both types of measurements have been used successfully to correlate strongly with sensory perception of grittiness (Cayot et al., 2008).

Chalkiness is an important attribute in yogurt. A good quality yogurt gel is smooth, relatively thick and free of clumps. Chalkiness is considered a defect in yogurt and it is known to detract from perceptions of creaminess (Cayot et al., 2008). Addition of an excessive amount of whey protein (Lucey, 2004) or addition of soy protein concentrate to dairy yogurts can lead to chalkiness (Drake & Gerard, 2006). Chalkiness has also been reported after excessive heating of milk before yogurt making (Lucey & Singh, 2003), as well as after slow acid development and low incubation temperatures (Labropoulos et al., 1984) as these conditions can lead to formation of larger protein aggregates.

In the case of fermented soy products, it has been shown that addition of 0.25% caseinate or casein hydrolyzate can reduce grittiness (Ankenman Granata & Morr, 1996) while a high total
solids content (about 11%) results in an increase in grittiness (Estevez et al., 2010). In this case, the chalkiness may be controlled in the soymilk base by increasing homogenization temperature and pressure, by thorough bean grinding and by filtration through a finer mesh (Kuntz et al., 1978). However very little is reported on how to control chalkiness in fermented soymilk based products.

2.1.3.3 Wateriness and fat-related attributes

As mentioned earlier, texture attributes are classified into 3 categories: mechanical, geometrical and other attributes, mostly related to surface properties (Surmacka-Szcześniak, 2002). Thickness is an important mechanical texture property of yogurts while chalkiness is a key geometrical property of yogurts. However, there are a number of other attributes in yogurts which are classified under the “other” category of texture attributes. These include wateriness and some fat-related attributes such as slipperiness and mouthcoating.

Wateriness is the sensation perceived when a sample “melts unusually quickly to an uncharacteristically thin water-like fluid” (Soukoulis et al., 2010). The watery sensation arises when serum exudes upon compression of a sample. Thus, it is a property related to the microstructure of samples (van den Berg et al., 2008). It has been shown that samples which have higher porosity release more serum during deformation and would therefore be rated as higher in wateriness (van den Berg et al., 2007).

Slipperiness and mouthcoating are attributes which are typically associated with the fat content of a sample, particularly in dairy products (Richardson et al., 1993; Tepper & Kuang, 1996).
Slipperiness, or oral lubricity (Malone et al., 2003), is the amount of lubrication provided by a product in the oral cavity (Richardson-Harman et al., 2000). The perception of slipperiness has been shown to be inversely proportional to the average of viscous and frictional forces (Kokini et al., 1977). Thus the perception of a low viscosity, combined with a low coefficient of friction results in the perception of high slipperiness.

While slipperiness is an attribute that is perceived due to the lubricating effects of a sample, mouthcoating is another fat-related attribute based on a different property of fat; its ability to coat oral surfaces. Thus, mouthcoating is the amount of film perceived on the oral surfaces and it is usually judged as an afterfeel, after swallowing the food sample (Tepper & Kuang, 1996). Mouthcoating is usually associated with the fat content of samples. As the fat content of products such as milk increases, the mouthcoating perception also increases (Phillips et al., 1995). Additionally, mouthcoating has been found to be an important parameter in panelists’ judgment of the fat content of samples (Tepper & Kuang, 1996). It has been shown that oral movements may influence how mouthcoating properties are perceived by panelists. More complex tongue movements result in perception of a more creamy and less fatty mouthcoating (de Wijk et al., 2011). Perception of fat-related attributes such as slipperiness and fattiness has been shown to be enhanced in samples in which there is more fat globule coalescence during mastication (Dresselhuis et al., 2008).

2.2 Distinguishing between applications for trained panels and consumer panels

Panelist training is often employed in the field of sensory evaluation during profiling of food products. Training procedures generally involve training panelists in sessions of 30-60 min for 5-
15 days. During the sessions, panelists are trained on a particular food and taught to detect a set of attributes within that food and to evaluate those attributes on intensity scales. The attributes are well defined and panelists are sometimes tested to ensure that all panelists are interpreting the attribute in the same way. Generally, the longest part of panelist training involves developing a frame of reference. At this stage, panelists practice using intensity scales according to predetermined standards, such as a sugar solutions to denote the upper and lower ends of the sweetness scale (Meilgaard et al., 2007a).

A well trained panel will behave much like a well calibrated instrument and when presented with a sample, all panelists should provide a similar intensity rating for each attribute in that sample. Trained panelists are taught to evaluate product attributes objectively and in a predictable manner using scientific language. They are an effective tool for characterizing complex products in great detail (Lawless & Heymann, 2010; Meilgaard et al., 2007a; 2007b). Trained panels are often applied for purposes of quality control (Meilgaard et al., 2007a), market research (Sinesio et al., 2010; Thompson et al., 2007) or for sensory research requiring highly controlled conditions (de Wijk et al., 2011; Lovely & Meullenet, 2009). However, depending on the purpose of the study, this objective and scientific approach to product evaluation may be quite the opposite of what is required. The ensuing section explains a number of instances where use of a consumer panel may be more appropriate.

2.2.1. Collection of affective data

Collection of affective (liking) data is a case where only consumer panels may be used. Trained panelists are taught to think about products more scientifically, and are sometimes trained to
detect subtle defects or differences in food products. Therefore, they may have a different approach and attitude towards foods. Trained panelists are not representative of the general population and they cannot be considered typical, “naive” consumers and should not participate in liking tests (Meilgaard et al., 2007a).

2.2.2. Uncovering consumer language

Consumer panels may be used when there is a need to gather knowledge of the consumer language used to describe a product (Santosa, et al., 2010). Trained panels use agreed-upon vocabulary that is often more scientific than vocabulary used by consumers (Moskowitz, et al., 2004), therefore, once again, this is an area where trained panels are not preferred. Thus, to characterize products in consumer language, techniques such as free-choice profiling may be employed, wherein each consumer selects their own set of attributes to describe the products and rates the products according to their own scale (Santosa et al., 2010).

There are instances where the use of a consumer panel and a trained panel should be used together. One of the disadvantages of using consumer panels is the difficulty in interpreting some of the terminology used by participants. Since there is no prior agreement about the meaning of the vocabulary used to describe the products, consumers tend to use terminology according to their own interpretation. In this instance, the trained panel can be employed to explain consumer language. If a set of products is characterized by both a consumer panel and a trained panel, the product maps of both panels can be overlaid. Trained panel attributes which overlap attributes on the consumer map, may then explain the terms used by consumers (Richardson-Harman et al., 2000).
### 2.2.3. Generating a holistic product description

Sometimes the intention of the researcher is to acquire a “holistic” description of the product where the attributes of the product as a whole are described (Pagès et al., 2010). Indeed, flavour, texture and appearance can all influence each other. For example, it was demonstrated that adding fatty-type flavors to mashed potatoes can enhance the perception of a fatty mouthfeel in the product (Yackinous & Guinard, 2000). Trained panelists are taught to distinguish between appearance, flavour and texture, and to give an objective assessment of each attribute. However, this assessment may not necessarily reflect the perception of the product by consumers who are not trained to be objective about each sensory parameter separately.

Some researchers are turning to methods such as Napping® where consumers can be used to describe their overall perception of a product. In the Napping® procedure, panelists are asked to place a set of products on a sheet of paper according to how similar or different their attributes are. The more similar two products are, the closer together they should be placed on the sheet and the more different they are, the further apart they should be on the sheet. Napping® is often coupled with descriptive tasks such as ultra-flash profiling, where after placement of the products on the paper, panelists are requested to write a short description of each product. Thus, the technique offers an overall assessment of the degree of difference between products as well as a short description, in consumer language, explaining the differences (Pagès et al., 2010).
2.2.4. Rapid-profiling

During the course of product development, sometimes a rough description of a product is desired to obtain some more specific indication of how well the development of the product is progressing. In such cases, rapid-profiling techniques employing consumers such as Napping®, free-choice profiling or sorting tasks may be used. The techniques allow generation of a product description in less than a day, they are cost-effective and usually provide sufficient information to guide product developers (Perrin et al., 2008; Williams & Arnold, 1985). However, whenever reliable data is required, such as when the data will influence the decision to spend large sums of money on a new product launch, a trained panel is a safer choice for product characterization.

2.3. The model system

2.3.1. Soymilk

Soymilk is a beverage made from ground soybeans and water. The soymilk making process involves soaking the soybeans in water for approximately 10-14 hours followed by washing the soybeans and grinding with water. This slurry is centrifuged or filtered to remove okara, the insoluble fiber. The soymilk must be heat treated, to ensure its safety and nutritional quality (Liu, 1997). Heating inactivates antinutritional factors such as trypsin inhibitors, denatures soy proteins and inactivates enzymes such as lipoxygenases, which contribute to the beany flavour, and overall increases shelf-life, physical appearance and microbial quality of the soymilk (Kwok & Niranjan, 1995). Heating may be initiated either before or after grinding and temperatures around the boiling point are generally held for 15-20 minutes. The resulting product is a smooth, beige, colloidal suspension (Liu, 1997). While in domestic settings soymilk heating is
accomplished by boiling the soymilk for 15-20 min, industrial manufacture of soymilk typically involves ultra-high temperature processing of soymilk (Prabhakaran & Perera, 2006).

The composition of soymilk is dependent on the soybean variety used and soymilk processing conditions (Kwok & Niranjan, 1995; Malaki Nik et al., 2009). However, typical soymilk composition is approximately 3.6% protein, 2% fat, 2.9% carbohydrates and 0.5% ash (Liu, 1997).

Soy proteins are classified according to their sedimentation coefficients, when separated by ultracentrifugation in pH 7.6 phosphate buffer with an ionic strength of 0.5. The four major fractions include 2S (15%), 7S (34%), 11S (42%) and 15S (9%) (Tay et al., 2005).

The 2S fraction consists of Bowman-Birk and Kunitz trypsin inhibitors, cytochrome C and α-conglycinin. The 7S is a heterogeneous fraction containing β-conglycinin, γ-conglycinin, lipoygenases, α-amylases, and hemagglutinins (lectins). The 11S and 15S are nearly pure fractions of glycinin and polymers of glycinin, respectively. The combination of the two main storage soybean proteins, glycinin and β-conglycinin, accounts for over 70% of the total soy protein (Catsimpoolas & Ekenstam, 1969; Wolf, 1970). For this reason, these proteins have been the most studied, in relation to their processing functionality in food products such as soymilk and soy curd (tofu).

Glycinin is a heterogeneous hexamer with a molecular weight ranging from 320 to 360 kDa (Liu, 1997). Each monomer of glycinin consists of one acidic and one basic polypeptide linked by a
disulphide bond. To date, six different acidic and five basic polypeptides have been isolated. The quarternary structure of glycinin is formed by two trimers stacked one on top of the other, held together by hydrogen bonds and electrostatic interactions (Adachi, et al., 2003).

β-conglycinin is a trimeric glycoprotein with a molecular weight around 150-200 kDa and it is approximately 4-5% glycosylated (Liu, 1997). The protein is composed of three types of subunits: α’, α and β. These subunits are non-covalently linked through hydrophobic and hydrogen bonding to form a trimer (Maruyama et al., 2001).

During soymilk making, heat treatment reduces the average particle size by disrupting large aggregates and generating more medium sized particles. While the average particle size in unheated soymilk is around 150-400 nm, the average particle size of heated soymilk is around 100-200 nm (Malaki Nik et al., 2009). During heating, soy proteins modify their structure, exposing reactive amino acid side groups such as nonpolar groups and sulfhydryl groups, making the proteins more prone to aggregation (Fukushima, 1980). The large particles present in raw soymilk are not formed with addition of 2-mercaptoethanol, indicating that the particles are held together by disulfide bonds between proteins. During heating, the disulfide bonds holding large protein particles together are disrupted resulting in smaller particles held together mainly by non-covalent bonds (Ono et al., 1991; Ren et al., 2009).

Soy fat mainly consists of triglycerides composed largely (86%) of unsaturated hydrocarbon chains (Liu, 1997). In cooked soymilk, only 3% of total lipids are found inside protein particles (Shun-Tang et al., 1997). The remainder is found as oil droplets in suspension having a diameter
of 200-400 nm (Ono, 2000). The oil droplet interface is coated mostly with small proteins, such as oleosins, and also some glycinin and β-conglycinin and some triglycerides (Guo et al., 2002).

2.3.2. Cow’s milk

Milk is a highly complex colloidal suspension, containing around 12 % solids consisting of 3.7% fat, 3.3% protein, 1.9% non-protein nitrogen, 4.8% lactose and 0.7% ash (Muir, 1998). There are two main classes of milk proteins: casein and whey proteins. Whey is a heterogeneous mixture of proteins, soluble under conditions where caseins sediment out (pH 4.6). The mixture is composed of α-lactalbumin (19%), β-lactoglobulin (49%), immunoglobulins (11%), serum albumin (5%), trace amounts of lactoferrin, lysozyme and approximately 16% miscellaneous proteins. α-lactalbumin (molecular weight ~14 kDa (Barber et al., 1987)) is a small monomeric globular molecule with four disulfide bonds, and a calcium binding site (Romero et al., 2010). With the calcium ion bound to its structure, α-lactalbumin has a denaturation temperature around 65°C at pH 6.7 and exhibits 80-90% renaturation upon cooling. However, at low pH values the protein is unable to bind calcium and at a pH of 3.5, α-lactalbumin is easily denatured around 40°C and it exhibits less than 50% renaturation upon cooling (Bernal & Jelen, 1984).

The main whey protein, β-lactoglobulin is a globular protein found as a dimer with a molecular weight around 36 kDa (de Wit, 2009). It has two disulfide bonds and one free thiol group, which becomes highly reactive during heating (Hoffman & van Mil, 1997). Up to a temperature of 60°C, the conformational changes occurring to β-lactoglobulin are reversible. Above 60°C irreversible conformational changes begin to take place, and around 65-75°C, the free thiol groups, normally buried in the interior of the protein, become exposed. Above 75°C, disulfide
exchange reactions dominate, and non-covalent interactions contribute to building larger aggregates (de Wit, 2009).

When milk is heated, β-lactoglobulin interacts not only with itself, but also with α-lactalbumin, and with κ-casein. These complexes form with a mass ratio of 1-5 whey proteins for every κ-casein in serum complexes and 0.5-3.5 whey proteins for every κ-casein in micelle-bound complexes and the formation of these complexes has important implications in milk functionality, as will be discussed in the gelation section (Donato & Guyomarc'h, 2009).

The caseins are phosphoproteins (Horne, 2006), classically defined as the milk proteins which precipitate at pH 4.6. They account for nearly 80% of milk proteins and include: 54% α-caseins (αs1 and αs2), 33% β-casein and 13% κ-casein (Hambraeus & Lonnerdal, 2003). While whey proteins are soluble in the serum phase, casein proteins are arranged in polydisperse colloidal particles referred to as casein “micelles”. These micelles range in size from 80-400 nm with an average size of approximately 200 nm. A typical casein micelle contains over 20 000 protein molecules. Caseins do not possess any significant amounts of secondary structures and their tertiary structure adapts to changes in the environment (Dalgleish, 2011). The four caseins are differently distributed between the inside and the outside of the micelles: while α- and β-caseins are generally found in the interior, κ–casein is usually found in the outer region of the casein micelle. α- and β-caseins are both highly phosphorylated and therefore can interact with calcium phosphate nanoclusters to stabilize the micelle structure. Unlike α- and β-caseins, κ-caseins only contain one phosphate group and they are therefore less prone to interacting with calcium phosphate nanoclusters. In addition, κ-caseins have a glycosylated C-terminus fraction, which
makes the protein highly amphiphilic. This amphiphilic nature leads to the presence of κ-casein mostly on the surface of casein micelles, creating what is often referred to as the casein micelle’s “hairy layer”. This polyelectrolyte layer of κ-casein around the casein micelles acts to stabilize these protein particles in solution (Dalgleish, 2011).

Bovine milk naturally contains approximately 3.7% fat. The fat globules range in size from less than 0.2 to greater than 15 µm. Because of the large size of milk fat globules, they have a tendency towards creaming if they are not homogenized. The globules are stabilized in solution via the milk fat globular membrane (MFGM). This membrane is largely composed of lipids and protein. The lipid portion consists mainly of triacylglycerols (62%) and phospholipids (26-31%). The MFGM contains a wide variety of proteins including enzymes, mucins and mucin-like glycoproteins. However it is important to note that the native membrane does not contain significant amounts of casein or whey proteins (Huppertz & Kelly, 2003).

### 2.3.3. Gelation

#### 2.3.3.1. Soy gels

Gelation is an important functional property of soy proteins, particularly in the manufacture of some soy-based foods such as soy yogurt and tofu (Donkor et al., 2007; Kohyama et al., 1995). Above a critical concentration, soy protein isolates can form heat-induced gels (Utsumi & Kinsella, 1985), but after heating even lower concentrations of soy proteins can be induced to gel using acid (for example, glucono-δ-lactone (GDL)) or salts (Kohyama et al., 1995). Depending on the processing conditions, the type of gel obtained will be different. The protein composition in the isolate also affects the type of protein network. Glycinin generates stiffer gels with larger
deformability before fracture than gels made from β-conglycinin. Because glycinin has better gel-forming abilities than β-conglycinin, the ratio of glycinin to β-conglycinin is an important parameter in determining gelling ability of soy protein mixtures (Renkema et al., 2001).

Just as in gelation of soy protein solutions, in the preparation of soymilk curd, before GDL or salts are added, soymilk must be heat treated, to cause protein rearrangements (Renkema et al., 2002). Unlike in soy protein isolate solutions, when soymilk particles come together to form a gel network, they do so while entrapping other non-protein components including lipids and carbohydrates (Ono et al., 1996; Shun-Tang et al., 1999; Yazici et al., 1997). However, because soy proteins are the building blocks of the gel network of soymilk curd, it has been proposed that the mechanism of gel network formation in soymilk gels is the same as in gel formation in soy protein solutions (Ono et al., 1996).

Soy proteins at pH near neutral have a net negative charge. When enough charges are neutralized, either using acid or ions (for example, MgCl₂), the protein particles can approach each other and allow for short-range interactions to take place and induce soy protein aggregation (Kohyama & Nishinari, 1993). Although disulphide interchange plays an important role in heat-induced interactions, acid-induced gelation or salt-induced gelation may occur once heating has been applied, and in this case non-covalent forces such as hydrogen bonding, van der Waals interactions and electrostatic bridging play a major role in bond formation in the network (Kohyama et al., 1995; Ringgenberg et al., 2012a). It has recently been shown that, during acidification, A₃ and basic subunits of glycinin and β subunits of β-conglycinin are incorporated
in the network first while α and α’ subunits of β-conglycinin and acidic subunits of glycinin are incorporated into the gel network at lower pH values (Ringgenberg et al., 2012a).

There are many factors that influence the gel structure. Some of these include the final pH of the gel, protein concentration, protein composition, and the type and amount of coagulant. The overall charge of soy proteins is lowest around pH 6, with many soy polypeptides having an isoelectric point around that value. It has been demonstrated that gels made below pH 6 are stiffer than gels above pH 6 due to increased incorporation of proteins in the gel network (Renkema et al., 2002). Higher protein concentrations lead to increased gel stiffness because of the increased number of bonds participating in the network (Wang & Damodaran, 1991). Protein composition of the soybeans used to make soymilk also has an important impact on the rheological properties of the gel (Renkema et al., 2001).

2.3.3.2. Milk gels

Caseins are very heat stable and can withstand heating above 100°C (Fox & Morrissey, 1977). However, the micelles can form gels using acidification or enzymatic hydrolysis, particularly chymosin (Alexander & Dalgleish, 2004; Lucey et al., 1998).

Acid coagulation of milk is used to prepare products such as yogurt and kefir. The acidification is usually achieved by means of lactic acid bacteria, or chemically with glucono-delta-lactone or organic acids (Lucey et al., 1998). The surface of the casein micelle has a net negative charge. During acidification, the charged hairy layer which normally extends out into the serum phase, interacting with water to keep caseins suspended in solution, is slowly neutralized, reducing both
steric stabilization and electrostatic repulsion (Dalgleish, 2011). When milk is acidified from 6.7
to around 5.6, there is a small decrease in apparent radius (~10 nm), most probably caused by the
collapse of the hairy layer. The colloidal calcium phosphate (CCP) present in the micelles
becomes solubilized during acidification. Around pH 5.0 the casein micelles aggregate
(Alexander & Dalgleish, 2004). Yogurt fermentation is typically continued down to a pH of 4.6,
the isoelectric point of caseins. This pH generates the maximum gel strength. Below pH 4.6,
proteins begin to acquire a positive charge, once again inducing electrostatic repulsion and
reducing the gel strength (Lucey, 2004).

Yogurts are often made with heat-treated milk as this increases the gel strength. When milk is
heated, β-lactoglobulin undergoes thiol/disulfide interchange with α-lactalbumin and κ-caseins,
resulting in whey protein-κ-casein complexes in the serum phase as well as on the surface of the
casein micelle. When the heat-treated milk is acidified, the gel point is increased from around pH
5.0 to pH 5.3 (closer to the isoelectric point of β-lactoglobulin) and the whey-κ-casein complexes
become an integral part of the gel network (Donato & Guyomarc’h, 2009; Lucey, 2004). Compared to acid gels made from unheated milk, heated milk acid gels result in gels with higher
viscosity and firmness and more homogeneous microstructures with higher interconnectivity and
lower porosity (Donato & Guyomarc’h, 2009).

Another widespread method for destabilizing casein micelles involves the addition of a protease
enzyme, chymosin. This process is most commonly used in cheese making, as it results in a
firmer gel than simple acidification. Chymosin is known to specifically hydrolyze κ-caseins in
two parts: para-κ-casein and caseinomacropeptide (CMP). The para-κ-casein portion remains
anchored to the casein micelle, while CMP diffuses away from the micelle and into the serum phase. The action of chymosin results in a decrease in the steric repulsion (Sandra et al., 2007), and when > 80% of CMP has been removed from the casein surface, caseins begin to aggregate and form a gel network (Dalgleish, 2011). In dairy processing, lactic acid bacteria fermentation and acidification is often used in combination with renneting as it increases the rate of gelation, gel strength and contributes to the flavour of the final product (Li & Dalgleish, 2006). Acidification reduces the repulsive charges between the micelles, facilitating rennet induced aggregation at a lower level of CMP release (Li & Dalgleish, 2006).

In instances where renneting is used as the primary mode of gelation, heating of milk is detrimental to gel formation. In such cases, the deposited β-lactoglobulin on the surface of the casein micelle obstructs the access of chymosin to the cleavage site on κ-casein, resulting in slower gelation and lower final gel stiffness (Vasbinder et al., 2003).

2.3.4. Homogenization

2.3.4.1. Effect of homogenization on soymilk

Soymilk homogenization is commonly employed in soymilk manufacturing to improve stability of soymilk particles. Homogenization of heated soymilk has been shown to cause a reduction in particle size and to generate a narrower particle size distribution than that of heat treated soymilk alone. It has also been suggested that homogenization may induce further protein rearrangements (Malaki Nik et al., 2008). It has not been demonstrated whether homogenization has an effect on the gelation properties of soymilk. However, it is known that ultra high pressure homogenization (UHPH) does impact gelation properties. Cruz et al. showed that following UHPH treatment, the
onset of gelation was delayed and the aggregation rate and gel network density were reduced. Despite the slower gel development and lower density, the final gels made from UHPH treated soymilk had higher gel firmness, and higher deformability and water holding capacity than gels made from heated, unhomogenized soymilk (Cruz et al., 2009).

2.3.4.2. Effect of homogenization on milk

Homogenization results in a decrease in size of milk fat globules. The newly formed surfaces are too large to be fully coated by the original MFGM material and the coating is completed with caseins and their fragments. If the milk was heat-treated prior to homogenization, some whey proteins will also adhere to the surface of the fat globule (Lee and Sherbon, 2002). This change in MFGM material has an important influence on the type of gel networks obtained. When homogenized milk is coagulated by either acid coagulation or renneting, the fat globules become an integral part of the gel network (Huppertz & Kelly, 2003). When caseins aggregate to form a gel network, the casein-coated fat globules are incorporated in the gel network via the caseins on their surface and may be referred to as “interacting fillers”. When unhomogenized milk is coagulated, the fat globules are not interacting with the gel network and are simply loosely held in the pores of the gel (van Vliet, 1988). In the case of yogurt, interacting fat globules are highly desirable as they increase the gel firmness and body of the yogurt (Cho et al., 1999; Pannell & Schoenfuss, 2007).

2.3.5. Studies of mixed soymilk-cow’s milk systems

As described above, milk and soy proteins can be induced to gel under varying conditions. Several studies have followed the gelling behaviour of a mixture of soy and milk proteins
(Comfort & Howell, 2002; Roesch et al., 2004; Roesch & Corredig, 2005; 2006). It has been shown that when a system containing a combination of milk protein and soy protein is acidified, aggregation begins earlier in the mixture (~pH 5.8) than in skim milk (~pH 5.3). This behaviour can be explained by the instability of soy proteins around pH 6.0. Confocal studies revealed that gelled soy-milk mixtures have less branching and a more particulate structure than pure milk samples (Roesch et al., 2004).

When mixtures of soy and dairy proteins are heated before acidification, the initial size of protein aggregates increases (Roesch et al., 2004) and the samples show higher viscous and elastic moduli and less frequency dependence than unheated samples (Roesch & Corredig, 2006). Additionally, heated samples containing whey, casein and soy proteins have a faster onset of gelation and higher $G'$ values than samples containing only casein and soy proteins, suggesting that whey proteins play a role in the interactions (Roesch et al., 2006). Heating studies of soy-dairy mixtures have demonstrated that complexes form between β-conglycinin and caseins as well as between β-conglycinin, glycinin and β-lactoglobulin (Roesch & Corredig, 2005; 2006). Thus it is possible to expect that in a mixture of soymilk and cow’s milk, all of these reactions will occur to some extent.

It was recently reported that a dual gelation mechanism employing both rennet and GDL allows formation of a protein network with soymilk protein particles and casein micelles (Lin et al., 2012). This system was further studied during this work.
CHAPTER 3
EXTRACTION OF CONSUMER TEXTURE PREFERENCES FOR YOGURT: COMPARISON OF THE PREFERRED ATTRIBUTE ELICITATION METHOD TO CONVENTIONAL PROFILING

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3.1. Abstract

During development of a new product, it is important to understand the key attributes of importance to consumers. From a sensory perspective, it is not possible to determine this via conventional profiling where consumer information is not sought. The method examined in this study, which we will refer to as preferred attribute elicitation (PAE), attempts to derive this information from an untrained panel of consumers by asking the group of panelists to agree on a set of attributes which they consider important. This approach also aids in reducing the amount of ambiguity in interpreting terms selected by consumers as all panelists must agree to the terms and feel capable of rating them. The employment of the PAE method in the food industry has so far been limited, therefore, there exists a need to study the strengths and weaknesses of the PAE method in comparison to descriptive analysis to better understand its place among sensory analysis methodologies. The method was effective for extracting key sensory properties of yogurts and panelists were capable of characterizing yogurts in a meaningful way. There is a need to continue assessment of the technique, particularly with more complex food systems.
3.2. Introduction

Gaining knowledge of the sensory properties affecting consumer preference for food products is an important part of successful product development. Conventional profiling methods, employing trained panelists, are commonly used to extract a descriptive profile of a food product. The methods are ideally suited for generating a detailed description of products (Meilgaard et al., 2007b). However, they do not provide any indication of the importance of attributes generated (Perrin et al., 2008). Thus, it may be difficult for product developers to know which attributes to focus their attention to. Recently there has been an increasing interest in methods such as Napping®, which employs an untrained panel of consumers to generate a more “holistic” description of products and can also provide insight into the importance of attributes. The method is a rapid approach capable of showing the perceived differences among products and, in combination with ultra-flash profiling, it is also capable of extracting the attributes responsible for those perceived differences (Pagès et al., 2010; Perrin et al., 2008). However, because the method is carried out on an individual basis, it generates a large set of attributes, some of which may be difficult to interpret.

The current study will examine the use of a method which we will refer to as Preferred Attribute Elicitation (PAE). In this method, a group of consumers is asked to agree on a set of attributes to describe a sample of products and to rank the attributes according to how important they believe the attributes are in influencing their liking of the products. It is expected that this approach will generate a smaller number of terms which are of importance to all the panelists and thus provide product developers with a clear idea of the attributes which should be targeted during product improvement. Additionally, it is expected that there will be reduced ambiguity in interpretation
of attributes as all panelists must agree to the terms and feel capable of rating the products based on the attributes they have selected. The method has experienced only limited usage in food industry settings; hence, there exists a lack of information regarding application of the method and its strengths and limitations. The ensuing study will attempt to improve understanding of the method by comparison with conventional profiling, which will be regarded as the reference method.

The sensory properties affecting yogurt liking have traditionally been examined using conventional profiling (Allgeyer et al., 2010; Folkenberg & Martens, 2003; Lovely & Meullenet, 2009). Yogurt texture can be characterized by many attributes. These include both non-oral textural attributes such as spoon impression, clumpiness, thickness, smoothness, and oral texture attributes including thickness, stickiness, chalkiness and dairy film, meltdown rate, and fatty after mouthfeel (Janhøg et al., 2006; Lovely & Meullenet, 2009). Although most studies of yogurt texture tend to focus on thickness and smoothness of yogurt, there is some disagreement over whether or not these texture attributes affect overall liking. Lovely and Meullenet (2009) found that when consumers judged a yogurt to be either too thick, too thin or lacking in smoothness, the overall liking score of the yogurt decreased significantly. On the other hand, Jaworska et al. (2005) found that both thickness and smoothness were not significantly correlated with consumer acceptability. The difference in findings can be explained by the many differences between the studies including differing study objectives and methodologies used to collect and analyze the data. Cross-cultural differences may also have contributed to the differences in the findings.
Given that the importance of texture may change with different sets of yogurt products or different populations, the secondary objective of this study was to examine the role of texture in consumer liking of commercial yogurts found in the Canadian market.

3.3. Materials

Seven vanilla yogurts were selected, by bench-top tasting, from a pool of 19 commercial yogurts. Yogurts were selected to represent a range of textures. The yogurts were purchased from local grocery stores in Guelph, Ontario, Canada. The products chosen were from a variety of national brands and store brands and the products varied in fat content, stabilizer composition, and sugar content. For each yogurt, the product from different containers was poured into a large bowl and gently mixed before dispensing into sample cups. This ensured that there was no variability among yogurts from the same batch, packaged in different containers. To equalize the time between tasting and the last time each yogurt was stirred, panelists were instructed to stir the yogurts three times immediately before tasting. Samples were dispensed into plastic sample cups in 25 g portions one day before testing and stored in a refrigerator at 3.5 °C. Sample cups were labeled with 3-digit blinding codes.

3.4. Methods

In the procedures below, in addition to texture attributes, which are the interest of this study, attributes associated with taste, flavour and appearance were also evaluated by consumers and trained panelists to avoid dumping effects.
3.4.1. Preferred Attribute Elicitation

The PAE method has been previously described (Nash, 2003). The steps involved in the PAE method as applied in the current study are summarized in Figure 3.1 and they were carried out in 6 steps as follows:

(1) Texture liking, flavour liking and overall liking of each yogurt was evaluated individually by panelists on a 9-point hedonic scale. Since texture was the focus of the study, texture liking was evaluated first to avoid halo effect on texture liking assessment. After each evaluation of liking, space was provided for panelists to write down in words what attributes they liked or disliked about the yogurt to encourage panelists to start thinking about yogurt attributes.

Steps (2) to (5) were then accomplished through round-table discussions moderated by the researcher.

(2) In this step, the researcher asked panelists to name attributes that differed among yogurt products. As panelists named attributes, the attributes were written down on sticky notes and posted on a white board.

(3) In the third step, when panelists could not think of any more attributes, they were asked to group the generated attributes into groups in any way they considered appropriate.

(4) Next, 7-point scales were generated for the attribute groups and panelists assigned anchor descriptor terms for intensity for each attribute. Panelists were asked to group synonymous and antonymous attributes into a single scale.
Figure 3.1 Flow chart summarizing the various steps in the Preferred Attribute Elicitation method.
Additionally, panelists were informed that if the scale did not sufficiently encompass all of the important attributes within a group, they could make an additional scale. At this stage, attributes were also narrowed down further by asking questions such as, “Do any more of these terms mean the same thing to everyone?” and ”Are there any terms here that you feel you won’t be able to easily evaluate in the product?”

(5) Panelists were then asked to rank the attributes governing each scale according to importance in driving liking. The group was informed that if they feel that certain attributes are equally important, those attributes can hold the same rank in order of importance.

(6) After a short break, panelists were provided with evaluation sheets and were presented with fresh yogurt samples to evaluate each attribute for each yogurt on a 7-point scale with the anchor terms they had selected.

All sensory evaluations were computed individually by panelists, using paper ballots. Panelists were provided with plain soda crackers and water for palate cleansing between samples. The PAE sessions lasted 70-90 min each.

The above procedure was carried out with four groups of consumers; two groups of men and two groups of women. Approximately 10 panelists were recruited for each session. In total, PAE was carried out using 42 consumers (22 women and 20 men) recruited by posting advertisements in coffee shops, grocery stores, around campus and through online classified advertisements. All participants consumed dairy yogurt at least once per week and did not work for a food company.
nor study food-related subjects. The age range of participants was 19 to over 66 with the majority being university students between the ages of 19 and 25. Research ethics approval was obtained from the university’s ethics board. Samples were stored in a refrigerator at 3.5 °C until testing and then distributed two at a time to minimize wait times in between samples and to avoid panelist boredom.

Preliminary triangle tests showed that yogurts with different expiry dates had significantly different sensory properties. For this reason, the first two sessions of PAE, one of men and one of women, were carried out one day apart and in the same week as the conventional profiling method to ensure that the yogurts used for both methods had the same expiry date and were as close as possible in storage age. The second set of two sessions was carried out one month later using a different set of the seven yogurts, thus these sessions cannot be directly compared to the first sessions as they employed products with noticeably different sensory properties. However, the second set of PAE sessions can be used to judge if the method consistently extracts the same information when it is repeated.

3.4.2. Conventional profiling

Ten panelists were selected based on tasting ability, ability to verbalize their perceptions and availability to take part in the panel. The panel consisted of 7 women and 3 men. Panelists were in the age range of 19-45 with the majority being in the range 26-35. Half of the panel members were experienced in descriptive analysis while the remaining members were new to descriptive analysis.
Table 3.1 Attributes used in trained panel and their corresponding references.

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Definition</th>
<th>Reference</th>
<th>Location of reference on 15-point scale</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stringiness</strong></td>
<td>Formation of string-like strands of yogurt when sample is slowly poured off spoon</td>
<td>Sour cream (14% fat, Gay Lea Foods Co-operative, Guelph, Ontario)</td>
<td>1</td>
</tr>
<tr>
<td><strong>Vanilla Aroma</strong></td>
<td>Aroma of vanillin. A clean, white, sweet smell of vanilla icing.</td>
<td>Vanilla flavour 13032 (NovoTaste Corporation Inc., Brossard, QC)</td>
<td>7</td>
</tr>
<tr>
<td><strong>Sour Aroma</strong></td>
<td>Aromatic produced by lactic acid bacteria.</td>
<td></td>
<td>None</td>
</tr>
<tr>
<td><strong>Sweet</strong></td>
<td>Taste associated with table sugar.</td>
<td>Sucrose in water solution (50g/L)</td>
<td>14</td>
</tr>
<tr>
<td><strong>Sour</strong></td>
<td>Taste associated with acids.</td>
<td>Tartaric acid (2g/L)</td>
<td>14</td>
</tr>
<tr>
<td><strong>Dairy</strong></td>
<td>A flavour reminiscent of raw milk or cream. Described as “cow-y” or “farm-y”.</td>
<td>Evaporated partly skimmed milk (2% fat, No Name® brand, No Frills, Guelph, Ontario)</td>
<td>14</td>
</tr>
<tr>
<td><strong>Thickness</strong></td>
<td>Amount of force required to slurp yogurt into mouth while making “O” shape with lips. High thickness associated with higher amount of force.</td>
<td>Sour cream (14% fat, Gay Lea Foods Co-operative)</td>
<td>14</td>
</tr>
<tr>
<td><strong>Chalkiness</strong></td>
<td>Perception of chalk-like particles when tongue is rubbed against the roof of the mouth immediately after swallowing sample.</td>
<td>No Name® vanilla instant pudding made with double the recommended amount of milk</td>
<td>1</td>
</tr>
<tr>
<td><strong>Aftertaste Intensity</strong></td>
<td>Intensity of aftertaste perceived 2 seconds after swallowing.</td>
<td></td>
<td>None</td>
</tr>
<tr>
<td><strong>Colour</strong></td>
<td>Colour of yogurt as perceived after mixing 3 times</td>
<td>Paint chip (WD720) (Behr, Santa Ana, California, USA)</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Paint chip (WD700) (Behr, Santa Ana, California, USA)</td>
<td>1</td>
</tr>
</tbody>
</table>
Panelists were trained using a generic descriptive analysis technique for one hour per day for ten
days followed by six days of testing to obtain three replicates of testing for each of the seven yogurts. Table 3.1 lists the attributes panelists were trained for and their corresponding
references. Testing took place in sensory booths under red light at the Human Nutraceutical
Research Unit of the University of Guelph. Descriptive data were collected using 15 cm scales
labeled with appropriate anchors for each attribute. Each booth was equipped with a computer
and product evaluations were recorded by panelists in Compusense® Five (Compusense, Guelph, Ontario, Canada). Colour evaluation was carried out in a separate room under white
light, using paper ballots.

Yogurts were distributed in randomized order, one at a time, in portions of 25 g in 30 mL plastic
cups labeled with three digit codes. Samples were stored in the refrigerator (3.5 °C) until
distribution. Panelists were provided with filtered water and plain soda crackers for palate
cleansing between samples. Research ethics approval was obtained from the ethics board of the
University of Guelph.

3.4.3. Consumer liking scores
A total of 90 consumers (58 women and 32 men) were recruited from the University of Guelph
and surrounding area. The age range of the consumers was 19 to 55 with the majority in the age
range of 19-25. Consumers were screened for dairy allergies and frequency of yogurt
consumption. Only those who consumed yogurt at least once per month and did not have dairy
allergies were invited to take part in sensory tests. Testing was completed in the sensory booths
and consumers were asked to rate appearance liking, texture liking, flavour liking and overall
liking of each of the seven yogurts on a 9-point hedonic scale under white light using Compusense® Five. Yogurts were stored in the refrigerator until testing. Samples were presented monadically to panelists to maintain the temperature. Standard sensory practices were applied, including random sample presentation, plain soda crackers and filtered water for palate cleansing and sample labeling with three digit codes. Research ethics approval was also obtained for this part of the study.

3.4.4. Statistical analysis

3.4.4.1 Preferred attribute elicitation

The ensuing statistical analyses were accomplished using XLStat 2010 (Addinsoft SARL, New York, NY) in Microsoft Excel™ 2007. Analysis of PAE data collected from each group of individuals was accomplished in a multi-stage process. First, the mean and standard deviations were calculated for the liking data from part (1) of the PAE method. The liking data were also subjected to a one-way ANOVA followed by a Tukey’s HSD to determine if there were significant differences in liking between the yogurts. The liking data were then centered and summarized using Agglomerative Hierarchal Clustering (XLStat, 2010).

Next, the descriptive data acquired from part (6) of the method were collected from the different PAE groups and combined and normalized using Generalized Procrustes Analysis (XLStat, 2010). After normalization by GPA, liking data was regressed onto product coordinates to generate an external preference map (XLStat 2010).
3.4.4.2 Preference testing

Consumer liking scores were subjected to a 2-way random factor ANOVA and a Tukey’s HSD test (SAS 9.2 © SAS Institute Inc., Cary, NC, USA) to establish significant differences among the yogurts for liking. Responses were centered and clustered using Agglomerative Hierarchal Clustering (XLStat, 2010).

3.4.4.3 Conventional profiling

Trained panel data were evaluated by a 3-way fixed factor ANOVA with interactions and a Tukey’s HSD test to assess panel performance and significant differences between products (SAS 9.2 © SAS Institute Inc., Cary, NC, USA). XLStat 2010 was used for the ensuing analyses. Means and standard deviations were calculated for descriptive data and consumer liking scores.

Descriptive data were analyzed using Principal Components Analysis (PCA) to generate a correlation matrix (XLStat) and the resulting coordinates were then used to build a preference map by regressing the liking scores onto the product coordinates.

3.4.4.4 Comparison of conventional profiling and preferred attribute elicitation

Multiple Factor Analysis (MFA) was used to obtain a visualization of how closely the texture descriptions obtained from the PAE method were aligned with those obtained from the conventional profiling method. PCA coordinates of data from PAE and conventional profiling were used to generate the multiple factor analysis in XLStat 2010. RV coefficients and their significance were determined using the coeffRV function in FactoMineR 1.14 package (Husson,
3.5. Results and discussion

Desirable sensory attributes for commercial vanilla yogurts were elicited using two methods: Preferred Attribute Elicitation (PAE) and conventional profiling paired with consumer liking scores. Selection of yogurts for testing was limited to vanilla flavours to reduce the effects of flavour on consumer perception of the yogurts, as texture was the focus of this study. For the same reason, note that in the ensuing discussions although all attributes were elicited, the discussion will focus mainly on results related to texture perception followed by a brief discussion on the use of PAE for non-texture terms.

3.5.1. Preferred attribute elicitation

The PAE method was carried out four times. Men and women were placed into separate sessions to avoid any potential response biases due to the presence of the opposite gender (specifically with regard to desirability of high fat content). Two sessions (one for men and one for women) were carried out one day apart using yogurts from the same batch and the following two sessions were carried out on the same day one month later using a different batch of yogurts. For purposes of clarity, the first pair of sessions will be referred to as T1 sessions and the sessions carried out one month later will be referred to as T2 sessions. Table 3.2 lists the attributes rated by the trained panel as well as the four sessions of PAE. It was found that all sessions generated texture attributes related to thickness and smoothness (in some sessions the opposite of smoothness, graininess, was generated instead).
Table 3.2 Attributes used by the trained panel and in PAE sessions to characterize yogurt products.

<table>
<thead>
<tr>
<th></th>
<th>Trained Panel</th>
<th>PAE (T1 Men)</th>
<th>PAE (T1 Women)</th>
<th>PAE (T2 Men)</th>
<th>PAE (T2 Women)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Appearance</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colour</td>
<td>Colour</td>
<td>Visual Thickness</td>
<td>Colour</td>
<td>Colour</td>
<td>Colour</td>
</tr>
<tr>
<td>Stringiness</td>
<td>Visual Graininess</td>
<td>Visual Separation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Aroma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sour aroma</td>
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</tr>
<tr>
<td>Vanilla aroma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Texture</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thickness</td>
<td>Thickness</td>
<td>Thickness</td>
<td>Thickness</td>
<td>Thickness</td>
<td>Thickness</td>
</tr>
<tr>
<td>Chalkiness</td>
<td>Smoothness</td>
<td>Graininess</td>
<td>Smoothness</td>
<td>Smoothness</td>
<td>Smoothness</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Chalkiness</td>
</tr>
<tr>
<td><strong>Taste</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dairy flavour</td>
<td>Sweetness</td>
<td>Sweetness</td>
<td>Sweetness</td>
<td>Sweetness</td>
<td>Sweetness</td>
</tr>
<tr>
<td>Sweet</td>
<td>Tanginess</td>
<td>Sourness</td>
<td>Sourness</td>
<td>Sourness</td>
<td>Sourness</td>
</tr>
<tr>
<td>Sour</td>
<td>Flavour intensity</td>
<td>Flavour intensity</td>
<td>Chemical taste</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Aftertaste</strong></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Aftertaste</td>
<td>Aftertaste intensity</td>
<td>Aftertaste intensity</td>
<td>Aftertaste intensity</td>
<td>Aftertaste intensity</td>
<td>Aftertaste intensity</td>
</tr>
</tbody>
</table>
The round-table discussion was very helpful for generating vocabulary during the PAE session. The discussion allowed panelists to brainstorm terms with each other and find words to describe sensory perceptions they might not all have otherwise come up with had they been participating in a method where product descriptions are generated individually.

It has been previously shown (Cayot et al., 2008) that when a yogurt is smooth and thick, it is perceived as creamy. Knowing that “creamy” is a multidimensional term, when consumers elicited the term “creamy”, the panel leader initiated a discussion to probe the panelists’ definition of the term. The consumers came to an agreement that creamy texture in yogurt is a combination of smoothness and thickness. Thus, their opinions reflected what was already reported in the literature above.

In all PAE sessions, consumers consistently ranked both texture and flavour as very important for acceptability. This is in agreement with previous results (Lovely & Meullenet, 2009). The products used by Jaworska et al. (2005) possessed attributes such as bitterness and off-flavours which negatively impacted consumer liking. It is known that, for many people, flavour has the ability to push texture into the background; however if a product’s flavour lacks in distinctiveness, texture importance increases (Szcześniak & Kahn, 1971). It may be possible that in the previously cited study (Jaworska et al., 2005), some of the negative flavour attributes may have caused flavour perception to become more prominent and to overshadow the impact of texture on consumers, resulting in no correlation between texture and yogurt liking. In the results of the study by Lovely and Meullenet (2009), as well as in the current study, sweetened yogurts were used, and sweetness is known to decrease perception of bitterness (Burns & Noble, 2007).
This could have caused the yogurts to have flavours which were less outstanding thus increasing the importance of texture and resulting in positive correlations between texture and consumer liking. Overall, it seems that the importance of texture in yogurts may vary depending on the flavour profile of the products. It is also important to note that texture has a larger impact on consumer liking when it does not meet expectations (Szczeniak & Khan, 1971). Hence, if the texture in a yogurt is problematic, its importance in determining liking may also increase.

In all the PAE sessions consumers ranked aftertaste as lower in importance than texture and flavour. Appearance attributes were ranked as the least important. This suggests that improving appearance and aftertaste is unlikely to have a significant impact on consumer liking if texture or flavour is unappealing. Although it is known that product appearance can influence panelists’ perception of the sensory attributes of a product (Phillips et al., 1995), when flavour and texture of a product are highly desirable, consumers may be more willing to sacrifice appearance as it is less important to them. Similarly, if a product has a pleasant appearance but has unpleasant texture or flavour, it would not be judged favourably by consumers. This was demonstrated by yogurt D which had a relatively high score for appearance liking (6.5 on a 9 point hedonic scale) but a very low score for overall liking (3.6 on a nine point hedonic scale) due to its low texture and flavour scores.

Attribute ratings obtained from part (6) of the PAE method were subjected to Generalized Procrustes Analysis (GPA) to combine each pair of sessions and to account for different use of scales by the panelists and different use of vocabulary by the different sessions. The coordinates
of the products and attributes following normalization by GPA were then combined with the liking data to build an external preference map.

Figure 3.2 shows the preference map produced from the T1 PAE sessions. The map indicated that yogurt graininess and chunkiness are negatively correlated with consumer liking. This was in agreement with previous reports (Folkenberg & Martens, 2003; Lovely & Meullenet, 2009). Additionally, although consumers appear to like a range of thicknesses, none of the consumer clusters were negatively correlated with thickness suggesting that consumers may dislike yogurt products with very low thickness. This statement was confirmed by panelists verbally and in writing during the PAE session, wherein consumers expressed that runniness was unappealing in a yogurt. These consumer texture preferences were confirmed by the results of the T2 PAE sessions and conventional profiling combined with consumer liking data. Consumer clusters 2 and 3 from the T1 PAE session are quite close together thus consumers in both clusters might have similar yogurt preferences. Consumer cluster 1 was quite a bit more positive in the first dimension. Since the first dimension was dominated by thickness (on the negative end), this cluster may represent consumers that preferred less thick yogurts than those in clusters 2 and 3.

3.5.2. Comparison of PAE and conventional profiling

Preliminary triangle tests indicated that all yogurts tested were noticeably different from batch to batch (based on expiry date of the yogurts). Due to these batch-to-batch differences among yogurt products, the T1 PAE provides the truest comparison to the trained panel approach; these data were collected using the same batch of yogurt products and were carried out within one week of each other, thus minimizing the impact of storage age on yogurt sensory properties.
Figure 3.2 Preference map generated from the results of the T1 PAE. Letters A-G represent the yogurt products tested and consumer clusters represent the direction of liking of each cluster of consumers.
RV coefficients were used to provide a numerical value for the degree of similarity between PCA plots of descriptive data obtained by different methods (PAE and trained panel). The RV coefficient applies to multivariate data and it is interpreted in much the same way as the Pearson correlation coefficient (Josse et al., 2008; Schlich, 1996; Smilde et al., 2009). Table 3.3 lists the RV coefficients and \( p \)-values of interest. The RV coefficient relating the PCA plots from the T1 PAE and the trained panel was \( 0.877 \) \( (p = 0.0011) \), indicating a highly significant correlation. Figure 3.3 shows a plot of the projected points of each yogurt as described by the T1 PAE, by the trained panel and by the average of the two points following multiple factor analysis (MFA). All the yogurts were described very similarly by the panelists of T1 PAE and conventional profiling. It is possible that this high correlation was due in part to the simple sensory space involved and also to the use of unidimensional attributes, which consumers already have a clear concept for. It is likely for these reasons that consumers interpreted the attributes similarly to each other and similarly to the trained panelists.

Liking scores from the traditional consumer liking panel and from the PAE method were clustered using agglomerative hierarchal cluster analysis, to summarize liking of each yogurt by individuals within those groups. Following MFA, the RV coefficient relating the clustered scores for each yogurt from the T1 PAE \( (n = 23) \) and the 90 consumers (in booths) was found to be \( 0.816 \) \( (p = 0.0085) \). Thus it was found that the scores were highly correlated even though the yogurt samples were assessed in different conditions (more natural round-table setting compared with within booths) and by a small number of consumers. It is possible that, for more complex products that have many consumer clusters, using such a small number of consumers to determine consumer clusters would not be representative of the population.
Table 3.3 Comparison of PCA plots of 7 commercial yogurt products mapped according to their texture attributes. PCA plots were generated using data from 2 different methods: preferred attribute elicitation and a generic descriptive analysis technique.

<table>
<thead>
<tr>
<th>PCA plots being compared</th>
<th>Significant Correlation?</th>
<th>RV coefficient</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 Men PAE vs. T1 Women PAE</td>
<td>Yes</td>
<td>0.642</td>
<td>0.025</td>
</tr>
<tr>
<td>T2 Men PAE vs. T2 Women PAE</td>
<td>Yes</td>
<td>0.683</td>
<td>0.023</td>
</tr>
<tr>
<td>T1 PAE vs. Conventional profiling</td>
<td>Yes</td>
<td>0.877</td>
<td>0.001</td>
</tr>
</tbody>
</table>
Figure 3.3 Projected points of each yogurt as mapped according to texture attributes following multiple factor analysis of the T1 PAE and conventional profiling. Both methods used the same batch of yogurts.
Nevertheless, it can be argued that even if the first step of the PAE method (liking assessment and noting what attributes were and were not liked) cannot be used to generate consumer clusters, the step is useful as it propels consumers to think about which attributes stood out and contributed to their liking. Developing these individual opinions is quite important for the group discussion in the later steps of the PAE method and consequently for product characterization by PAE.

3.5.3. Comparison of different PAE sessions

Comparison of the mens’ and womens’ sessions can provide some insight into the consistency of the PAE method. The intention in comparing the mens’ and womens’ sessions is not to examine gender differences but rather to treat the panelists in each session as a new set of consumers and to examine if the result is the same when a new set of consumers is used on the same product set. Comparison of the men’s PAE session with the women’s PAE session from T1 and T2 resulted in RV coefficients of 0.642 ($p = 0.0246$) and 0.683 ($p = 0.0231$), respectively. Because only two attributes are often sufficient to describe the most important traits of yogurt textures, it may not be surprising that those two terms were generated in all four PAE sessions and by the trained panel. However, it is interesting to note that product characterizations were not found to be significantly different when repeated by different consumers, in separate PAE sessions. This demonstrated that consumers were able to provide consistent product descriptions of yogurt textures.

Unfortunately, because of the batch-to-batch differences in yogurts, it was not possible to compare the results of the T1 PAE sessions with the results of the T2 PAE sessions. Further
examination of the repeatability of the PAE method would have to be carried out with products that can be made to have consistent sensory attributes.

3.5.4. Application of the PAE method to non-texture attributes

Although texture was the focus of this study, it may also be possible to use PAE to extract important appearance or flavour attributes and differences in these attributes among products. In this study, when yogurts were visualized in a PCA plot according to only their flavour attributes, the PCA plots generated from the T1 PAE session and from the trained panel were not significantly correlated (RV coefficient = 0.508, \( p = 0.4841 \)). However, this by no means indicates that the data obtained from the PAE session were meaningless. In fact, of the five flavour attributes generated by the panelists of the PAE session, four of the attributes were highly correlated (>0.77) with either the positive or negative side of the first dimension and only one (overall flavour intensity) could not be explained by the three dimensions used in this study. This indicated that those four attributes were used similarly by panelists and could be used to provide a description of the flavour profile of the yogurt products. It is likely that overall flavour intensity could not be explained by the first three dimensions because flavour intensity is a multidimensional term and the intensity can differ at different times during mastication (Sprunt et al., 2001). Thus, the term was likely interpreted differently by the panelists and may have been rated at different times during mastication. This brings to attention that in a consumer focused method such as this where panelists rate products on a set of attributes selected by the group, better quality data is more likely when consumers characterize the products according to unidimensional attributes that they already have a clear concept for and would not require training to be able to identify correctly.
The lack of correlation between the PCA plots of the trained panel and the PAE panel can be explained by the fact that the trained panel used a different set of attributes to describe the yogurts than the PAE panel. While the PAE panel used the terms tanginess, sweetness, aftertaste intensity, chemical taste and overall flavour intensity, the trained panel used the terms sweet, sour, dairy, vanilla, sour aroma and aftertaste intensity. In the cases where similar terminology was used, those attributes were highly correlated with the same products by both the trained panel and PAE panel. For example, according to the trained panel, the yogurt most highly correlated with the terms sour and aftertaste intensity was yogurt D and for the PAE panel, the yogurt most highly correlated with the terms tanginess and aftertaste intensity was also yogurt D. Similarly, the attribute sweetness was highly correlated with yogurts C and G in both panels. The inability of the PAE panel to extract terms such as dairy and vanilla likely has to do with consumers’ less developed sensory vocabulary. It is likely that most consumers would not be able to express and rate terms such as “dairy flavour”. Although the yogurts were all vanilla flavoured, this was not clearly stated to the consumers and they did not appear to recognize all of the yogurts as vanilla flavoured. This is not surprising as the yogurt products varied greatly in terms of their “vanilla” flavour, thus consumers did not view this as an attribute that was common to all products and were not able to express any particular flavour as ranging in intensity in the yogurts. On the other hand, the panelists in the conventional profiling method were trained to detect only the intensity of vanillin-like vanilla flavours thus creating a reasonable basis for assessment of “vanilla” flavour.

The above results show that the PAE method may be used to characterize the flavour profile of products, however, due to the limited sensory vocabulary of typical consumers only terms which
are familiar to the general public are likely to be used by panelists on a PAE panel. It was also noted that terms which are unidimensional, such as sweet or sour, are used more consistently among consumers and provide more meaningful data than multidimensional terms.

3.5.5. General comments

Overall, it was found that PAE and conventional profiling yielded highly similar results when extracting texture attributes affecting liking of yogurt products. It is likely that the simplicity of the sensory space contributed to these findings. Training is important for panelists to use sensory descriptors more exactly (Lawless & Heymann, 2010); it helps to specify attributes for which consumers have no clear concept. Thus, in order for consumer-directed methods such as PAE to be successful, one must expect to characterize products using attributes that consumers generally have a clear concept for already. Additionally, if panelists generate multidimensional terms such as “fresh” or “creamy”, panel leaders should encourage consumers to explain these terms, as multidimensional terms are unlikely to generate meaningful descriptors and will instead generate confusion. This is by no means a suggestion that panel leaders should limit the descriptors that panelists are “allowed” to use, but rather encourage panelists to break down vocabulary to singular terms when multi-component descriptors arise. For these reasons it is important for the moderator to be a trained sensory scientist who would be able to pick out multi-component terms and also to probe panelist responses in order to attach more meaningful descriptions for the attributes that the consumer panel selects.

It is expected that the method would be most useful in contexts of product development where investigators are searching for key attributes to focus their attention to during the product
development or improvement process. The method is capable of extracting attributes that stand out to consumers and which appear to have a significant impact on consumer liking of the product. Additionally, consumers were able to use most of the terms they had generated in a meaningful way. Thus the method can also be used to track changes in the key sensory properties of a product throughout the product development process. Although unlike conventional profiling, the method does not provide a detailed description of products, the method would be useful for providing direction to product developers.

It has been mentioned several times that yogurt texture is an unusually simple sensory space as far as most food products are concerned. Thus there remains a need to assess the applicability of the PAE method with more complex systems. As with any new method, it is important to start with a simple system and work upwards in complexity to determine a method’s limits.

3.6. Conclusions

Although more research is needed to fully understand the capabilities of this method, the current data suggest that the method may find some applicability to product developers searching for a handful of key attributes to focus their efforts on. The method does not extract a detailed or holistic description of products but rather extracts attributes which stand out most to consumers. Thus the method would likely be most applicable earlier on in the product development process and would not be helpful for any work requiring “fine-tuning” of product attributes. The method is most useful if panelists use unidimensional attributes that they already have a clear concept for. Therefore, PAE sessions should be mediated by a trained sensory scientist to guide panelists away from multidimensional terms. Due to the lack of extensive research on this method, more
work is required to improve understanding of the capabilities of the PAE method particularly with regard to evaluation of more complex sensory spaces.

With regard to our secondary objective, the study revealed that yogurt texture was an important factor affecting consumer liking. Consumers consistently expressed the importance of texture suggesting that the lack of agreement in literature regarding the importance of texture in yogurts may be the result of the dynamic relationship of the relative importance of flavor and texture. The work also confirmed previous reports that runny texture and gritty mouthfeel negatively impacted yogurt liking.
CHAPTER 4

COMBINED ACID AND RENNET INDUCED GELATION OF A MIXED SOYMILK-DAIRY MILK SYSTEM

4.1. Abstract

Although there is great potential for generating new high protein foods using combinations of protein particles, very little fundamental understanding is available on gels obtained with mixed proteins. The objective of this study was to better understand the gelation behaviour of a mixed soymilk-dairy milk system, by forming different reactive protein particles using combinations of rennet and acid gelation. By modulating the reactivity of the building blocks, it is possible to fine tune structure formation. The results demonstrated that by using both rennet and acid, both soy proteins and caseins become involved in the final gel network. It was clearly demonstrated, following structure formation using diffusing wave spectroscopy and rheology, that when only one protein source was induced to gel, protein aggregation was hindered. Confocal microscopy analysis of the gel networks suggested that gels had a unique structure compared to those of gels obtained with either soymilk particles or milk proteins alone. It was concluded that by careful control of the gelation of soymilk and milk particles using a combination of destabilization mechanisms, it is possible to obtain novel gels with unique microstructure.

4.2. Introduction

The production of mixed protein gels is an area of great potential for future development; however, optimization of the structures in such products is not trivial as aggregation of the different protein particles needs to be fine-tuned to obtain desired sensory and texture attributes. The present work focuses on mixed soymilk and skim milk gels, as there are numerous health benefits associated with the consumption of a high protein product containing both proteins.
Such gels would deliver the health benefits of both dairy products (Park et al., 2009; Schaafsma, 2005) and soy products (Pham & Shak, 2009; Schaafsma, 2005). In addition to the individual benefits of dairy and soy, it has been reported that addition of skim milk powder to soy yogurts enhances isoflavone glycoside transformation to a biologically active form during yogurt storage (Pham & Shak, 2009).

The protein particles present in soymilk and skim milk can be easily gelled by heating, as well as by salt, acid, or enzymatic destabilization (Campbell et al., 2009; Lucey et al., 1996; Renkema & van Vliet, 2002; Sandra et al., 2007; Speroni et al., 2010). Soy proteins must be heated in order for gelation to take place (Renkema & van Vliet, 2002). During heating of soymilk, the protein particles dissociate, rearrange and aggregate. The result is particles composed of acidic and basic polypeptides of glycinin as well as minor amounts of α and α’ subunits of β-conglycinin. The polypeptides within these protein particles may be covalently linked via disulfide bonds, whereas aggregates of such particles interact by non-covalent forces including hydrophobic interactions and hydrogen bonding (Ren et al., 2009). When glucono-δ-lactone (GDL) is added to soymilk, its hydrolysis causes a gradual reduction in pH. The protons neutralize repulsive charges on soy protein particles, allowing them to approach one another and to interact by short range interactions such as hydrogen bonding and van der Waals forces (Ringgenberg et al., 2012a). It has been reported that rennet addition to soy protein isolates results in very little protein degradation (Kim et al., 1990; Park, & Rhee, 1990) and does not result in soymilk clotting (Stanley & deMan, 1978).
Caseins are the major protein fraction in cow’s milk, accounting for 80% of milk proteins (Hambraeus & Lonnerdal, 2003). Caseins are found as colloidal particles referred to as casein micelles with α- and β-caseins found in the interior of the micelles and κ-casein found mostly on the exterior, creating a polyelectrolyte layer around the particles. κ-caseins are highly amphiphilic polypeptides and their charged hydrophilic end protrudes into solution, offering colloidal stability to the casein micelle (Horne, 1998). When GDL is added, to slowly acidify milk, protons neutralize the repulsive negative charges on the κ-casein surface, leading to a collapse of the hairy layer and subsequent aggregation of caseins. The casein micelle contains large amounts of colloidal calcium phosphate and when milk is gradually acidified, colloidal calcium phosphate becomes solubilized and is released from the interior of the casein micelle (Lucey et al., 1996). Renneting is also commonly applied to induce aggregation of caseins, as it specifically cleaves κ-caseins, decreasing the steric and electrostatic stabilization of casein micelles and resulting in casein aggregation (Lucey, 2002). When acidification and renneting are used simultaneously, the decrease in pH results in increased rennet activity and a reduction in repulsive charges leading to more rapid aggregation (Lucey, 2002).

It has previously been reported that using GDL acidification as well as combined acidification and renneting, it is possible to induce interactions between soy proteins and caseins to form a mixed protein gel network (Lin et al., 2012; Roesch & Corredig, 2006). Thus it may be possible to generate novel fermented products from these mixed protein systems. Several studies have employed GDL acidification to examine gelation of soy-dairy mixtures (Chronakis & Kasapis, 1993; Comfort & Howell, 2002; Lin et al., 2012; Roesch & Corredig, 2005; 2006; Roesch et al., 2004). When the pH is slowly decreased, by addition of glucono-δ-lactone, soy proteins
aggregate to form a particulate gel network around pH 5.8, close to their isoelectric point (Malaki Nik et al., 2011). Milk proteins, on the other hand, usually show acid induced gelation at lower pH values. Heated milk shows a gelation pH around 5.4, due to the interactions between casein micelles and heat induced whey protein complexes (Donato & Guyomarc’h, 2009; Lucey, 2002). However, in unheated milk, the gelation pH is around 5.0, closer to the isoelectric point of caseins (Alexander & Dalgleish, 2004; Lucey, 2002). Thus, it is expected that when only acidification is used to acidify soy-dairy protein mixes, the soy proteins will aggregate well before milk proteins. Addition of rennet during GDL acidification, may be used to induce simultaneous aggregation of milk and soy proteins and therefore generate a more homogeneous arrangement of milk and soy proteins within the mixed gel network. It has been shown that combined rennet and acid-induced gelation of mixtures of soy and dairy proteins leads to the formation of a mixed protein gel (Lin et al., 2012).

Although some information is known about gelation of soy-dairy protein mixtures, much more work is needed before a consumer-acceptable product can be developed. The ensuing chapter will examine the details of structure formation in a mixture of fresh soymilk and fresh skim milk with rennet and GDL added. It is expected that this dual gelation method will allow for selective gelation of the proteins, and the results will provide a better understanding of the role played by soy and milk proteins during mixed gelation.
4.3. Materials and Methods

4.3.1. Soymilk preparation

Soymilk (5% soy protein) was prepared as previously reported (Malaki Nik et al., 2011), with slight modifications. In brief, Harovinton soybeans were soaked overnight in milliQ water for hydration. The hydrated soybeans were blended (Osterizer BLSTMG-WOO-033, Oster®, Brampton, ON, Canada) with a measured amount of water at room temperature (calculated to obtain the desired protein content) before being passed through a kitchen juicer (Professional Series 211, The Juiceman®, Korea) to further liquefy the sample. The soymilk was then passed through a cheesecloth to remove the okara (mainly composed of insoluble fiber material) and heated at 95°C for 7 min before cooling it in ice and storing in a refrigerator at 4 °C until use. Soy serum was prepared by first removing large particles by centrifugation (Optima™ LE-80K, Beckman Coulter, Mississauga, ON, Canada) of the soymilk for 30 min at 20°C and at 8,000 g. Next, the soymilk was transferred to Macrosep Centrifugal Devices (10 kDa molecular weight cut-off) from Pall Corporation (Mississauga, ON) and centrifuged for two hours at 5000 g and 10°C. Following centrifugation, soy serum was poured out from the filtrate receivers and kept in the refrigerator (4 °C) until further use, always within 5 days.

4.3.2. Skim milk preparation

Fresh milk was collected from the Elora dairy research station of the University of Guelph. Sodium azide was added at a concentration of 0.02% (w/v) to prevent bacterial growth. Milk was centrifuged at 6000 g for 20 min at 4 °C using a Beckman J2-21 centrifuge and JA-10 rotor (Beckman Coulter). Milk was then filtered four times through Whatman glass fiber filters (Fisher Scientific, Whitby, ON, Canada) before being subjected to ultrafiltration (PLGC 10k
regenerated cellulose cartridge, Millipore Corp., Bedford, MA). During ultrafiltration, both the milk permeate (free of protein) and the retentate (concentrated milk) were collected. Ultrafiltration was continued until a protein concentration of 4% was reached, estimated by volume reduction. The skim milk was stored in the refrigerator at 4 °C until use.

4.3.3. Protein determination

Protein contents of skim milk and soymilk were analyzed using the DC protein assay kit (BIO RAD, Mississauga, ON, Canada).

4.3.4. Soymilk characterization

Mineral content of soymilk serum was determined using inductively coupled plasma optical emission spectroscopy by the advanced analytical laboratories at the University of Guelph (Guelph, Ontario, Canada). The serum was found to contain the following amounts of minerals per kg of soy serum: 80 mg calcium, 190 mg magnesium, 200 mg phosphorus, 1400 mg potassium, <95 mg sodium and 83 mg sulfur.

4.3.5. Gel preparation

Samples were generated by mixing equal volumes of unheated concentrated skim milk (SM, 4% protein) and soymilk (SOY, 5% protein) resulting in a total protein concentration of 4.5%. Calcium chloride (Fisher Scientific) was added to all samples at a concentration of 1 mM as in previous reports (Li & Dalgleish, 2006). GDL (Sigma-Aldrich Co., St. Louis, MO, USA) was added at 0.6% at 30°C to slowly reduce the pH from ~6.6 to ~5.5 over the course of the 3 hour experiments. Rennet (Chymax Ultra Rennet (790 IMCU/mL), Chr. Hansen, Milwaukee, WI,
USA) was added in concentrations of 0.1074 IMCU/mL or 0.0537 IMCU/mL (referred to as high and low rennet, respectively) at 30°C. It was anticipated that the interpretation of binary gelation of a mixed system would be challenging considering the lack of information available and the possible synergistic/competitive effects. Thus, a number of controls were deemed essential to provide a better understanding of the gelation behaviour of the mixed system, as reported in Table 4.1. Controls included the mixed system with each of the gelling conditions alone (high rennet alone, low rennet alone or GDL alone). Additionally, every combination of the gelling agents (Table 4.1) was applied to 4% skim milk, 2% skim milk (diluted 1:1 with soy serum), 5% soymilk and 2.5% soymilk (diluted 1:1 with milk permeate). Gelation experiments were repeated in triplicate if they showed a sol gel transition and in duplicate if they did not show it.

4.3.6. Gelation studies

Each sample for rheology and DWS was prepared in volumes of 35 mL and distributed as follows: 1.5 mL for light scattering experiment, 20 mL for rheology and the remaining amount was used for pH measurement. Rheology, light scattering and pH measurement were carried out simultaneously. Final gels were observed using confocal microscopy.

4.3.6.1. Diffusing Wave Spectroscopy

Diffusing wave spectroscopy (DWS) was employed to measure the diffusion coefficient in undiluted samples, which can then be used to calculate the hydrodynamic particle radius with the Stokes-Einstein relation. A more detailed description of DWS theory is found elsewhere (Weitz et al., 1993). The light source was a solid diode pumped Nd:YAG laser emitting light with a
Table 4.1 Treatment combinations for soymilk (2.5 and 5% protein, SOY), skim milk (2% and 4% protein, SM) and mixture (4.5% protein, Mix) examined in this study. Examined treatments X; not tested -. X_G indicates treatments that resulted in a gel.

<table>
<thead>
<tr>
<th></th>
<th>GDL</th>
<th>High Rennet</th>
<th>Low Rennet</th>
<th>GDL High Rennet</th>
<th>GDL Low Rennet</th>
</tr>
</thead>
<tbody>
<tr>
<td>2% protein SM</td>
<td>-</td>
<td>X</td>
<td>-</td>
<td>X_G</td>
<td>X_G</td>
</tr>
<tr>
<td>4% protein SM</td>
<td>X</td>
<td>X_G</td>
<td>X_G</td>
<td>X_G</td>
<td>X_G</td>
</tr>
<tr>
<td>2.5% protein SOY</td>
<td>X_G</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5% protein SOY</td>
<td>X_G</td>
<td>X</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>4.5% protein Mix</td>
<td>X_G</td>
<td>X</td>
<td>X</td>
<td>X_G</td>
<td>X_G</td>
</tr>
</tbody>
</table>
wavelength of 532 nm and a power of 350 mW, as detailed in earlier publications (Alexander & Dalgleish, 2004; Malaki Nik et al., 2011).

All samples except the 2% skim milk were placed into a 5 mm, 1.5 mL glass cuvette and measured at 30°C controlled by an external water bath. The 2% skim milk samples were analyzed in a 10 mm (3 mL) glass cuvette to ensure sufficient multiple scattering. In all cases, the light scattering measurements were collected for 2 minutes with intervals of 1 second and analysis was carried out until gelation. Data was analyzed using DWS-Fit software to calculate apparent radius and 1/1* (Mediavention Engineering, Guelph, ON, Canada) and then plotted using Sigma Plot 10.0 (SPSS Inc., Chicago, IL, USA). The gel point was extrapolated from the plot of increase in radius as a function of pH/time.

4.3.6.2. Rheology

Experiments were carried out using a controlled stress rheometer with a cup and bob set-up at a constant strain of 0.01, a frequency of 1 Hz and an initial stress of 6 mPa. The temperature was controlled with an external water bath and kept at 30 °C. Rheological measurements were carried out in triplicate and each experiment was continued for 3 hours. Rheology was not performed on 2% skim milk samples due to the difficulty in obtaining large volumes of soy serum. The gel point was taken as the pH/time at tan δ=1.
4.2.6.3 pH Measurement

Samples were kept at 30°C in a circulating waterbath during pH measurements. pH measurements were automatically recorded every 11 s into an excel spreadsheet by AR15 pH recorder software (Mediavention Engineering) for 3 h.

4.3.7. Confocal microscopy of final gels

20 μL of Rhodamine B (0.2% w/v in milliQ water) was added to 5 mL of sample. Two drops of sample were placed into grooves of a concave microscope slide and a cover slip was placed overtop and sealed. Before analysis, slides were incubated at 30°C for three hours in sealed petri dishes placed in a circulating water bath. Images were taken using an inverted confocal laser scanning microscope (Leica TCS SP2, model Leica DM IRE2, Leica Microsystems CMS GmbH, Mannheim, Germany) with an Ar/Kr visible light laser, 63x (oil) objective. Experiments were repeated in triplicate.

4.3.8. Statistical Analysis.

Point of interest from DWS and rheology studies were collected and analyzed for significant differences using ANOVA and Tukey’s HSD.

4.4. Results and Discussion

Mixtures of soymilk (5% protein) and concentrated dairy milk (4% protein) were prepared in a 1:1 ratio, and the formation of structure was followed using DWS and rheology. The combination of the two techniques allowed investigation of the beginning stages of the sol-gel transitions and the formation of the gel network. The microstructure of the gels was also analyzed using confocal microscopy.
All samples were supplemented with 1 mM CaCl$_2$, as it is known that soluble calcium is necessary for rennet aggregation of casein micelles (Horne, 1998). As mentioned earlier, soy proteins have a higher isoelectric point than milk proteins. Thus, if only acidification is used, soy proteins will aggregate far earlier than milk proteins. Rennet was added to induce aggregation of casein micelles in skim milk. The relatively low concentration of GDL (0.6%) resulted in a gradual decrease in the pH of the sample allowing for more careful observation of the early stages of gelation, and the pH could be maintained higher than that reported for acid induced milk protein gelation (Lucey, 2002). Two concentrations of rennet were used (high and low) to evaluate the effect of varying the kinetics of casein destabilization.

Table 4.1 summarizes the treatments examined in this study. To distinguish between the activity of rennet and acid, control experiments were also conducted wherein GDL and rennet (at both concentrations) were added to the system in isolation. In addition to observing the activity of each gelation mechanism in isolation, it was also important to observe the behaviour of each protein source in isolation. Thus additional control experiments were conducted on the gelation behaviour of soymilk and skim milk alone, under the conditions used in this work. The actual protein concentrations in the mixed system were 2.5 and 2% for soymilk and skim milk, respectively. However, these control experiments with low protein concentrations do not accurately represent the actual conditions of the more crowded mixed system, thus control experiments were also carried out at high protein concentrations (4% protein skim milk and 5% protein soymilk). Only some of the treatments resulted in a visible gel, and they are indicated with a G subscript in Table 4.1. In the cases where the sample with the high protein content did
Figure 4.1 DWS (A,B) and rheology (C,D) parameters (radius and G', respectively) measured during rennet induced gelation for 4% (w/w) protein skim milk (A,C) and 5% (w/w) protein soymilk (B,D). For skim milk gelation, two concentrations of rennet are shown (triangles, high; diamonds, low). Representative curves are shown, for average values and statistical significance see Table 4.2.
not gel, the low protein content sample was not tested. Similarly, samples which did not gel with high rennet concentrations, were not tested with low rennet concentrations as it could safely be assumed the system would also not gel.

4.4.1. Rheological behaviour of control samples

The effect of rennet on the gelation of control soymilk (5% protein) and skim milk (4% protein) is shown in Figure 4.1. The apparent radius measured by DWS for skim milk (Figure 4.1A) was approximately 120 nm, consistent with previous results (Dalgleish et al., 2004).

Under the conditions used in this study (1 mM CaCl$_2$ added), aggregation of skim milk occurred within 30 min (see Table 4.2 for average gelation parameters), and more specifically, 18 and 29 min for high and low rennet concentrations, respectively. Aggregation of the casein micelles was observed with a drastic increase in the radius measured by DWS (Figure 4.1A). On the other hand, as expected, in the case of soymilk, the protein particles did not show destabilization induced by rennet, within the experimental time (Figure 4.1B). It is important to note that the apparent size of the soymilk particles was much larger (about 800 nm) (Ringgenberg et al., 2012b) than that measured for the casein micelles.

Similarly, when the gelation behaviour was followed by rheology, the elastic modulus ($G'$) in skim milk remained low and constant during the initial stages and then increased rapidly at the time of gelation (Figure 4.1C). The $G'$ did not demonstrate a plateau during the course of the experiment and the gradual increase in $G'$ may be due to the further addition of protein particles to the network or it may be due to continuing structural rearrangements resulting in an increase in gel stiffness (Mellema, 2002; Roefs, 1990). There was a noticeable difference in coagulation
Table 4.2 Gelling parameters measured using DWS and rheology. SM= Skim Milk; SOY= Soymilk; Mix= mixture of skim milk and soymilk; HR=high rennet; LR= low rennet; G= GDL. Values of pH and time of aggregation measured by DWS, pH and time of gelation measured by rheology (as tanδ=1), and values of G’ measured at pH 5.6 or 180 min. Means in a column followed by the same letter are not significantly different at p>0.05.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Aggregation pH (DWS)</th>
<th>Time of aggregation (DWS) (min)</th>
<th>Gelation pH (rheology)</th>
<th>Gelation pH (min)</th>
<th>G’ at pH 5.6 (Pa)</th>
<th>G’ at 180min (Pa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4% SM+ HR</td>
<td></td>
<td>18.2 ± 0.9a</td>
<td>15.9 ± 0.6a</td>
<td></td>
<td>194 ± 6.0a</td>
<td></td>
</tr>
<tr>
<td>4% SM+ LR</td>
<td></td>
<td>29.5 ± 1.3b</td>
<td>26.2 ± 0.3b</td>
<td></td>
<td>181 ± 4.9a</td>
<td></td>
</tr>
<tr>
<td>4% SM+HR+G</td>
<td>6.33 ± 0.04a</td>
<td>6.35 ± 0.04a</td>
<td></td>
<td>199 ± 28a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4%SM+LR+G</td>
<td>6.35 ± 0.01a</td>
<td>6.29 ± 0.03a</td>
<td></td>
<td>192 ± 28a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2%SM+HR+G</td>
<td>6.1 ± 0.06b,d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2%SM+LR+G</td>
<td>6.12 ± 0.07b,d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5% SOY+G</td>
<td>5.93 ± 0.01d</td>
<td>5.93 ± 0.05d</td>
<td></td>
<td>134 ± 44b,c</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5% SOY+G</td>
<td>6.00 ± 0.01c</td>
<td>6.04 ± 0.05c</td>
<td></td>
<td>27 ± 6d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mix+G</td>
<td>6.16 ± 0.02b,c</td>
<td>6.10 ± 0.03b,c</td>
<td></td>
<td>32 ± 6.5d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mix+HR+G</td>
<td>5.97 ± 0.06b,c</td>
<td>6.13 ± 0.04b,c</td>
<td></td>
<td>86 ± 8c,d</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mix+LR+G</td>
<td>5.95 ± 0.01b</td>
<td>6.17 ± 0.01b</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
point for the skim milk gelled with the low rennet concentration (~29 min) and high rennet concentration (~18 min) as more enzyme resulted in a higher rate of cleavage taking place (Sandra et al., 2007). The value of $G'$ measured 180 min after rennet addition was not significantly different between the samples treated with low and high rennet (approximately 185 Pa) (Figure 4.1C and Table 4.2). Experiments with skim milk at 2% protein showed only limited aggregation during the experiment time, possibly because of the very low level of gelling protein present in the samples.

In agreement with the DWS data, the value of $G'$ did not change, in the case of the 5% protein soymilk with a high amount of rennet added (Figure 4.1D). Overall, the results shown in Figure 4.1 and Table 4.2 suggest that if rennet were added to a mixed soymilk-dairy milk sample, only the dairy component would be affected by the action of rennet.

Control experiments were also performed to evaluate the effect of acidification with no rennet added, as illustrated in Figure 4.2. At the pH levels employed in this study, skim milk showed no aggregation, namely, no increase in the apparent radius measured by DWS (Figure 4.2A) and no change in the elastic modulus measured by rheology (Figure 4.2C). On the other hand, soymilk particles showed aggregation at a pH near 6, regardless of the concentration of the soy protein particles present (Table 4.2). Figure 4.2B shows the change in the apparent radius of soymilk particles as a function of pH. The apparent radius of the 5% protein sample was higher than that of the 2.5% protein soymilk because of crowding effects (Alexander et al., 2002), which dictate that the higher the volume fraction, the more dominant the hydrodynamic effects will be, resulting in a seemingly larger apparent radius. After an initial period with invariant size
Figure 4.2 DWS (A,B) and rheology (C,D) parameters (radius and G', respectively) measured during GDL induced gelation for 4% (w/w) protein skim milk (A,C) and 5% or 2.5% (w/w) protein soymilk (B,D). High protein concentration, filled symbols; low protein concentration, empty symbols. Representative curves are shown. For statistical significance see Table 4.2.
and G' (Figure 4.2B and D, respectively), there was an increase in these parameters at a pH near the isoelectric point of soy proteins (pH 6, see Table 4.2). The values of pH of gelation measured with DWS and rheology were in good agreement (Table 4.2). In addition, the pH of gelation was in good agreement with the literature (Ringgenberg et al., 2012a). The G' measured at pH 5.6 was quite different between 5% protein soymilk (~130 Pa) and 2.5% protein soymilk (~25 Pa). The high value of G' may reflect the presence of a higher number of bonds, thicker strands or a change in the structural elements in samples with more protein. The final tan δ values for the 4% soymilk and 2% soymilk samples with added GDL were 0.23 ± 0.012 and 0.21 ± 0.005, respectively. These values were not significantly different from each other, indicating that both gels were composed of similar types of bonds. Under the conditions used in this study, with 0.6% GDL, the pH of the 4% skim milk control reached a plateau at pH 5.6, a pH value insufficient to induce destabilization of the casein micelles. On the other hand, in the presence of both rennet and acid, both caseins and soy proteins should undergo destabilization in a mixed system.

The gelation behaviour of skim milk with both GDL and rennet added is depicted in Figure 4.3. Previous literature has shown that when rennet is added to skim milk in combination with acid, the activity of rennet on the casein micelles is enhanced because of the increased activity of rennet at lower pH values, as well as the charge neutralization occurring on the surface of the casein micelles (Lucey et al., 1996). Measurements were carried out for both 2 and 4% protein skim milk, as shown in Figure 4.3, using DWS and rheology. When GDL and either high or low rennet were added to 4% skim milk, there was a short period of constant radius, lasting
Figure 4.3 DWS (A) and rheology (B) parameters (radius and $G'$, respectively) measured during gelation of skim milk with added rennet and GDL. High rennet, squares; low rennet, inverted triangles; filled symbols, 4% protein; empty symbols, 2% protein. Representative curves are shown. For statistical significance see Table 4.2.
approximately 6 min, followed by a rapid increase in radius around pH 6.3 (Figure 4.3A). No difference in gel point between the two rennet concentrations was noted under these conditions (see Table 4.2), probably because of the short time needed to destabilize the micelles. When the experiment was repeated with 2% skim milk (Figure 4.3A), although there was no difference in gelation point between the samples containing low or high rennet, skim milk at lower protein concentration gelled at a lower pH than the skim milk containing 4% protein. The lower gelation pH can be attributed to the lower concentration of milk proteins as well as the lower ionic strength of the system (samples were diluted in soymilk serum). The reason for the pH difference was not further explored, as it was outside the scope of this work.

In agreement with previous literature (Lucey et al., 2000) gels prepared with different concentrations of rennet showed a similar value of $G'$ at around pH 5.6 (Table 4.2). It is important to note that the structure formation was studied only to pH 5.6. A further decrease in the pH would result in a downward slope of the elastic and viscous modulus, as increasing amounts of calcium are released from the casein micelles resulting in a loosening of the casein structure (Lucey et al., 1996).

The gelation of milk at 2% protein was not measured by rheology, as there was not enough protein to obtain a clear change in the $G'$ values. In addition, soymilk with added rennet and GDL was not studied, as the addition of rennet did not have an effect on soymilk and the plots would therefore be identical to those of soymilk with GDL only (shown in Figure 4.2B and D).

As a result of the control experiments it was concluded that while in the case of soymilk, the decrease in the overall charge of the protein particles would lead to gelation of the proteins at a
**Figure 4.4** DWS (A, B) and rheology (C,D) parameters measured during gelation of skim milk (2% protein) and soymilk (2.5% protein) mixtures with added high rennet (A,C) or with added GDL (B,D). In samples with added GDL, G’ and tan δ are shown as circles and crosses, respectively. Representative curves are shown. For statistical significance see Table 4.2. Lines have been drawn in Figure B to better demonstrate the changes in slope during acidification. Insert in B shows the beginning stages of aggregation.
pH around 6, in the case of skim milk, the combination of rennet and GDL would lead to a gelation pH between 6.3 and 6.1 depending on the casein micelles concentration in the system. It was therefore possible to predict that under the conditions used in this study, both proteins (from skim milk and soymilk) would contribute to the formation of a network.

4.4.2. Rheological behaviour of mixed soymilk-skim milk systems

Figure 4.4 depicts the gelation behaviour of the mixture of soymilk and skim milk with only rennet or only GDL. When rennet was added to the mixture, no aggregation was observed (Figure 4.4A). In addition, no changes were noted in the elastic modulus up to 3 h (Figure 4.4C). On the other hand, in the presence of GDL, there was an increase in the apparent radius measured by DWS (Figure 4.4B) as well as an increase in the value of G' (Figure 4.4D) at a pH of about 6.1, indicating a sol gel transition. The pH of gelation measured by rheology was statistically equivalent to the gelation pH of the soymilk control containing 2.5% protein (Table 4.2). Interestingly, while DWS measurements of soymilk alone (Figure 4.2B, Table 4.2) showed the rapid increase in apparent radius immediately after the gel point around pH 5.9, the DWS measurements of the mixed skim milk and soymilk system with only GDL added, showed only a slow increase in radius immediately after the gel point (see Figure 4.4B, inset) with rapid aggregation beginning only at a lower pH, around pH 5.7.

It may be speculated that in the mixed system, destabilization of soy protein particles occurred as for the soymilk in isolation, but rapid aggregation was hindered by the presence of casein micelles. In the mixture, the soy protein particles are the main contributor of the scattering intensity (Ringgenberg et al., 2012b), leaving the stable casein micelles mostly undetected in DWS experiments. Thus in the present system, the apparent delay in the increase in apparent
radius was due to a delay in soy protein aggregation and not the result of the detection of freely diffusing casein micelles.

Results from gelation experiments of the mixture with GDL only (Figure 4.4B and C) clearly suggested that without rennet added, the sol gel transition was due only to soymilk particles. It is important to note that the value of $G'$ measured at pH 5.6 (this pH was arbitrarily chosen for comparison) was not significantly different from that of the 2.5% protein soymilk alone with GDL (Table 4.2). When only GDL was added to the mix, the gel formed was a soy protein network with inclusions of non-interacting casein micelles in the gel pores. Figure 4.4B also shows the value of tan $\delta$ as a function of pH, after sol gel transition (tan $\delta$=1). The tan $\delta$ steeply decreased reaching a plateau value of 0.25 ± 0.01. This value was significantly higher than the values measured for acid soymilk gels (see above).

It was hypothesized that by combining rennet and GDL it was possible to destabilize casein micelles together with soymilk protein particles. Figure 4.5 shows the initial stages of structure formation as measured using DWS and rheology. Regardless of whether the high or low rennet concentration was used in combination with GDL, the mixed soy-milk systems showed a drastic increase in radius and gelled at pH 6 (Figure 4.5A). Under the conditions used in this experiment, acidified 2.5% protein soymilk gelled at a of pH 6.0 and 2% protein milk at a pH of 6.1 (Table 4.2). Hence, it was possible to conclude that in the mixed system, the pH of gelation was not significantly different from the control treatment (Table 4.2). It was concluded that the sol gel transition pH shown in Figure 4.5 was the result of the destabilization of both soymilk
Figure 4.5 DWS (A,B) and rheology (C, D) parameters measured during gelation of skim milk and soymilk mixtures containing both rennet and GDL. High rennet, squares; low rennet, inverted triangles; tan δ, crosses; G', squares. Representative curves are shown. For statistical significance see Table 4.2.
and milk protein particles, and that both contributed in the mixed gel network. It is also important to point out that under the mixed gelation, the combined system did not show the discrepancy in the radius increase discussed for Figure 4.4B. Thus, it could be speculated that the presence of rennet was effective in making casein micelles prone to aggregation and enabled them to participate in network formation rather than, as shown in Figure 4.4B, interfere with network formation.

Although the pH of gelation of the mixed system was statistically equivalent with high and low rennet added, there were differences in the gelation behaviour as measured by rheology. Indeed, in the case of low rennet, the values of $G'$ at pH 5.6 were highly variable, while the elastic modulus $G'$ with high rennet and acid was around 86 Pa (Table 4.2). This suggested that the extent of the $\kappa$-casein cleavage affected the bond formation of the mixed protein network. This is well known in acid milk gels (Li & Dalgleish, 2006). The discrepancy in $G'$ values between the high and low rennet in the mixed systems would indicate that also in mixed systems the destabilization of casein micelles needs to be fine tuned to obtain optimal network structures.

When the elastic modulus of the mixed system was compared to that of the control samples, containing either skim milk or soymilk alone (4 and 5% protein, respectively) the modulus at pH 5.6 for the mixed gel (~86 Pa) was still far lower than that of the control treatments (Table 4.2). This might be further indication that there was less efficient reorganization of the protein linkages in the mixed gels, and suggests differences in the final gels texture. As with the mixed system with only GDL added, when both rennet and GDL were added, the tan $\delta$ showed a rapid
**Figure 4.6** Confocal microscopy images of acidified soymilk gel at 5% (w/w) protein (A), skim milk with both GDL and high rennet at 4% (w/w) protein (B), mixed soymilk-skim milk gels (2+2.5% (w/w)) made with: high rennet and GDL (C), low rennet and GDL (D) and GDL alone (E). Bar size is 50 µm.
decrease reaching a plateau at $0.27 \pm 0.01$ (Figures 4.5B). The higher values of tan $\delta$ compared to the mix with GDL or soymilk alone suggested a mixed milk and soymilk particles gel.

### 4.4.3. Confocal microscopy

The microstructure of the gels at pH 5.6 was observed using confocal microscopy (Figure 4.6). The top images are representative of an acidified soymilk gel at 5% protein (w/w) (Figure 4.6A) and of a skim milk gel with both GDL and high rennet at 4% protein (w/w) (Figure 4.6B). The bottom three images show mixed soymilk-skim milk gels (2+2.5% protein (w/w)) made with high rennet and GDL (Figure 4.6C), low rennet and GDL (Figure 4.6D) and GDL alone (Figure 4.6E). The bright areas in the images represent the protein aggregates, stained with rhodamine B. The soymilk gel, produced with GDL, appeared as densely packed aggregates of soy proteins. The milk gel, produced with GDL and high rennet, exhibited a very different structure: the network showed large pores and interconnecting strands. The mixed soymilk-skim milk gels had appearances which were in between, exhibiting a network of strands of aggregated protein. These images indicate that the mixed systems may have microstructures different from those of pure soy gels and pure milk gels. When confocal images of the mixed system with the different gelling agents were compared, no obvious differences could be observed. In general, the structures were more open than those of soymilk alone (Figure 4.6A) but more closely packed than those of skim milk alone (Figure 4.6B).
4.5. Conclusions

Mixed soymilk and milk gels have great potential to be used to obtain products with added health benefits. This study provided insight into the gelation behaviour of these systems. Overall, the mixtures with a 4.5% total protein produced gels that had lower gel stiffness than those produced from either 4% skim milk or 5% soymilk alone. Confocal microscopy also revealed that the microstructure of the mixed system differed from that of either soymilk or dairy milk alone, suggesting the combined system generates a different gel structure. It was concluded that by fine tuning the composition and the gelling behaviour of the proteins in the mixed gels it is possible to form novel platforms for delivery of high protein foods.
CHAPTER 5

GELATION OF SOYMILK BY LACTIC ACID BACTERIA: DIFFERENCES IN MICROSTRUCTURE AND RHEOLOGY COMPARED TO ACIDIFICATION WITH GLUCONO-DELTA-LACTONE

5.1. Abstract

Because of the increase in consumer demand for soy protein, there has been a growth in the variety of soy based products developed in recent years. Although a number of different acid soymilk curd products are currently available in the market, the mechanisms associated to the formation of a gel in soymilk still need to be studied in detail. The objective of this work was to compare the gelation of soymilk induced by acid producing cultures to that resulting from chemical acidification by addition of glucono-δ-lactone (GDL). GDL is commonly employed to induce gelation and to obtain a homogeneous soybean curd. Soymilk was prepared with procedures comparable to those employed in a domestic environment, and bacterial culture or GDL were added to obtain a bean curd. The formation of the structure was followed using rheology, and the microstructure of the gels was analyzed with confocal microscopy. Acidification of lactic acid bacteria resulted in a higher gelation pH (pH 6.29 ± 0.05) compared to that of a gel induced by GDL (pH 5.9 ± 0.04). In spite of the earlier gelation pH, there were no observed differences in the final gel stiffness measured at pH 5.1, the value of tan δ (where δ is the phase angle of the oscillatory rheological measurements) and the frequency dependence of the gels measured at the final pH. In addition, stress sweep tests did not reveal differences in the linear viscoelastic range, and microstructural observations also showed a similar protein network structure between the two acid gels. It was concluded that although GDL could be a suitable substitute for bacterial cultures when examining the final structure of soymilk acid-gels, lactic
acid bacteria fermentation caused a faster protein destabilization, probably because of the slower acidification rate and longer time available for rearrangements of the soymilk protein particles.

5.2. Introduction

In addition to the use of soymilk as a base for traditional soy based products such as soy beverages and tofu, soymilk is increasingly employed as a protein matrix to design novel food products. For this reason, a better understanding of the details of its colloidal stability and gelation behaviour are needed. Soymilk is a beverage produced by grinding soaked soybeans with water, followed by cooking around boiling temperatures for around 15 min and removal of insoluble fiber (okara) by filtration or centrifugation (Canabady-Rochelle et al., 2009; Prabhakaran et al., 2006). Heating of soymilk is an essential step to denature anti-nutritional compounds, and to modify the structure of soymilk particles to improve their colloidal stability and decrease their size (Malaki Nik et al., 2009). In addition, soy protein denaturation induced by heating is a necessary step for gel formation (Renkema & van Vliet, 2002). During heating, soluble protein aggregates form. These aggregates are mostly composed of acidic and basic polypeptides of glycinin linked via disulphide bonds as well as a small amount of α and α’ subunits of β-conglycinin (Ren et al., 2009). Previous work has shown that the protein aggregates interact by hydrophobic interactions and hydrogen bonding to make protein particles with basic subunits of glycinin in the interior and the acidic glycinin subunits and α and α’ subunits of β-conglycinin on the exterior (Ren et al., 2009). When acid curds are prepared, acidification is usually carried out using glucono-δ-lactone (GDL), as the pH decreases gradually, creating a homogeneous gel (Malaki Nik et al., 2011). Unlike in heat-induced gelation of soy protein isolates (Renkema & van Vliet, 2002), except for the bonds that develop during
heating, covalent bonds do not play a major role in the network formation of acid induced gels. The driving forces behind acid gelation of soy proteins are non-covalent in nature (Kohyama et al., 1995), and include salt bridging (Zhang et al., 2012) and short range interactions such as hydrogen bonding and van der Waals forces (Ringgenberg et al., 2012a).

As the pH of soy protein suspensions decreases to values around 6, it has been observed that the basic subunits of glycinin and the β subunit of β-conglycinin are the first to destabilize. As the system approaches a net neutral charge, the acidic subunits of glycinin and the α and α’ subunits of β-conglycinin then also begin to participate in gel network formation (Ringgenberg et al., 2012a).

The use of lactic acid bacteria to produce soymilk curds has been evaluated in the past (Liu et al., 2009; Mital & Steinkraus, 1975). Lactic acid bacteria are known to primarily ferment sucrose in soy products. However, some lactic acid cultures are also capable of fermenting other low molecular weight carbohydrates found in soybeans such as raffinose and stachyose (Mital & Steinkraus, 1975). Although several studies have focused on lactic acid bacteria metabolism of soy products (Mital et al., 1974; Mital & Steinkraus, 1975), very little data is available on the beginning stages of structure formation of soymilk acid-gels produced using lactic acid fermentation.

Several studies exist on the acid-induced aggregation of soymilk particles acidified with GDL (Kohyama et al., 1995; Malaki Nik et al., 2011; Tay & Perera, 2006). It has been reported that acidification of soymilk using GDL results in a gel point in the range of 5.6-5.8 depending on the
variety of soybean used, and different concentrations of GDL do not cause differences in the gelation pH (Malaki Nik et al., 2011). However, acidification using lactic acid bacteria is known to be far slower than GDL (Lucey et al., 1998).

In consideration of the complex composition of soymilk particles, it may be hypothesized that allowing more time for protein rearrangements to occur may influence the gelation behaviour of soymilk. Thus the purpose of this study was to evaluate the difference in the gelation behaviour of soymilk particles during acidification induced with GDL or bacterial culture. Soymilk was prepared using a commonly used soymilk maker, with a food grade process. Structure formation was followed using rheology, and microstructural differences were analyzed using confocal scanning microscopy.

5.3. Materials and methods

5.3.1. Soymilk preparation

Soymilk was prepared using food grade materials with a procedure that would resemble that of a household process. Soybeans were obtained from a local grocery store and characterized as described in the following section. A portion of 175 g of beans was washed with filtered water (Brita® Faucet Filtration System (Model FF-100), Brita Canada Corp., Brampton, ON, Canada) and soaked overnight in water. The hydrated soybeans were rinsed once again with water and placed into a household soymilk maker (Soyquick™ Premier Milk Maker Model SQ930P, Kitchen’s Best Manufacturing Group Ltd., Nanaimo, BC, Canada) with 933 mL of filtered water to produce 4% protein soymilk. The soymilk maker cycles involved the following steps: soybeans and water were heated to 80°C (in approximately 5 min) and once the temperature was
reached, the soybeans were ground for 5 s. After this short grinding cycle, the soymilk temperature was brought up to just below boiling temperature (1 min) and then four grinding cycles were performed, each cycle lasting 40 s with a 5 s pause between cycles. After grinding, the soymilk maker continued to hold the soymilk just below boiling temperature for 10 min. The hot soymilk was then immediately poured through a strainer (Kitchen’s Best Manufacturing Group Ltd., Vancouver, BC, Canada) to remove the okara, and then passed twice through a cheese cloth (Kitchen’s Best Manufacturing Group Ltd, Vancouver, BC, Canada). After filtration the soymilk was cooled to refrigeration temperatures.

5.3.2. Soybean and soymilk characterization

To determine the amount of protein as well as the polypeptide distribution of the soybeans, the beans were milled with a grain mill (IKA® Works Inc., Wilmington, NC, USA) and the ground flour was analyzed using the Dumas combustion method in a LECO FP-528 (Leco Corp., St. Joseph, MI, USA) with 6.25 as a conversion factor for % protein from % N (AACC method 46-30.01, 1999). The protein concentration in soymilk was also measured with Dumas, while solids were determined by weighing 1 mL soymilk in dry aluminum pans containing dry sand (Fisher Sci., Mississauga, ON, Canada) as a dispersing agent. The pans were placed into an IsoTemp forced air oven (Fisher Sci., Mississauga, ON, Canada) at 105°C for 24 h. Particle size distribution of the soymilk was determined using laser light scattering (Mastersizer S, Malvern Southborough, MA, USA), using 1.46 as the refractive index of the soymilk particles and 1.333 as the refractive index of the dispersant (water) as previously reported (Malaki Nik et al., 2009). The ratio of 11S:7S protein in the soy flour and soymilk was determined using SDS-Poly Acrylamide Gel Electrophoresis, using conditions published in the literature (Keerati-u-rai &
Corredig, 2009a). Gel analysis was carried out using a Gel Doc™ EZ Imager (Bio-Rad, Mississauga, ON, Canada). Crude oil content of the soybeans was determined using the soxhlet oil extraction method with petroleum ether (AOAC method 945.16) with a multi-unit extraction heater (Labline Thermo-Scientific, Asheville, NC, USA). The fat content of soymilk was determined using the Babcock method (AOAC method 989.04, 2000) with a Babcock System for Fat Analysis (Cole-Parmer, Montreal, QC, Canada) as previously reported in the literature (Buono, Erickson, Fung, & Jeon, 1990). Mineral analysis was determined by using inductively coupled plasma optical emission spectroscopy by the advanced analytical laboratories at the University of Guelph (Guelph, Ontario, Canada).

5.3.3. Gelation experiments

Soymilk was acidified at 40°C with either 0.6% Glucono-δ-lactone (GDL) (Sigma-Aldrich Co., St. Louis, MO, USA) or YO-MIX™ 511 LYO 375 DCU (Danisco Canada Inc., Scarborough, ON, Canada). The starter culture was pre-diluted by adding 0.2 g of the freeze-dried starter culture to 30 mL of warm soymilk (40°C) and stirring for 30 s before adding 157 µL of the diluted culture to 30 mL of soymilk sample for a final concentration of 0.00349% starter culture.

Yo-mix is a starter culture commonly employed in the fermentation of milk for yogurt production, containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *Bulgaricus*. Soymilk was pre-warmed at 40°C for 5 min. After addition of GDL or bacterial culture, the soymilk was mixed for 30 s and then immediately placed in the rheometer. Aliquots of the same samples were kept at 40°C in a circulating waterbath to measure pH in parallel to the rheology experiments. The pH was recorded on line using an Accumet AR15 pH meter (Fisher
Sci., Mississauga, ON, Canada) connected to a computer, using AR15 pH recorder software (Mediavention Engineering Inc., Guelph, ON, Canada). Gel formation was followed using an Advanced Rheometer AR 1000 with Rheology Advantage Instrument Control AR software v5.4.0 (TA Instruments Ltd., New Castle, DW, USA) at a constant strain of 0.01, and a frequency of 1 Hz. The temperature was controlled with an external water bath and kept at 40 °C. Once the samples reached a pH of 5.1, a frequency sweep was performed, using a constant strain of 0.01. Finally, a strain sweep was carried out at a frequency of 1 Hz, to determine a yield strain, defined as the value of strain at which the elastic modulus deviated by 10% from its value in the linear viscoelastic range.

5.3.4. SDS-PAGE of fermented soymilk

Samples of fermented soymilk were collected at pH 5.8 and 5.5 and analysed by SDS-PAGE to determine if there was any protein hydrolysis occurring during fermentation. The method employed has been previously described by others (Keerati-u-rai & Corredig, 2009a). The gels were scanned using a Bio-Rad Gel Doc™ EZ Imager (Bio-Rad, Mississauga, ON, Canada). The protein composition of fermented soymilk was compared to that of unfermented soymilk and of unfermented soymilk incubated at 40°C for 2.5 h.

5.3.5. Gel Microstructure

Images of the gel microstructure were taken using an inverted confocal scanning laser microscope (Leica TCS SP2, model Leica DM IRE2, Leica Microsystems CMS GmbH, Mannheim, Germany) with an Ar/Kr visible light laser and 63x (oil) objective. Resolution of the acquired digital images was 1024 x 1024 pixels. A fluorescence dye (rhodamine B) (Fisher Sci.,
Fairlawn, NJ, USA) (excitation and emission wavelengths 543 and 625 nm, respectively) was used for staining the protein. 20 μL of rhodamine B (0.2% w/v in milli-Q water) was added to 5 mL of sample for staining. Two drops of sample were placed into grooves of a concave microscope slide and a cover slip was placed overtop and sealed. Slides were incubated at 40°C until pH 5.1 was reached, and then cooled in a refrigerator for at least 30 min at 4°C before viewing under the microscope.

5.3.6. Statistical Analysis

Measurements were carried out in triplicate and treatments were subjected to a two sample F-test to test for equal variances, then analyzed using an unpaired student’s t-test assuming equal variances using the data analysis function in Microsoft Office Excel 2007.

5.4. Results and Discussion

5.4.1. Characterization of soybeans and soymilk

In the present study the soymilk was prepared using common household practices, using only food grade ingredients. The soybeans, purchased from a local grocery store contained 36.8±0.2% (n=3) protein and 15.32±0.01% fat (n=3) (dry basis), and had a ratio between glycinin and β-conglycinin polypeptides of approximately 1.7, as determined by SDS-PAGE and densitometry. The soymilk obtained had a total solids content of 8.55±0.09%, 4.00±0.04% protein and 1.0±0.3% fat. Mineral analysis revealed that the soymilk contained the following amounts of minerals per kilogram of soymilk: 250 mg calcium, 330 mg magnesium, 630 mg phosphorus, 2100 mg potassium, 87 mg sodium and 360 mg sulfur.
The particle size distribution of the soymilk (Figure 5.1) was measured using integrated light scattering. The soymilk particles were characterized by a bimodal distribution, with two populations at approximately 0.2 and 10 µm. These two populations represented mostly soymilk protein particles and fat globules. Incubation of the samples with 1% SDS did not affect the size distribution (data not shown). It has been previously reported (Malaki Nik et al., 2008) that after centrifugation at 8000 g, soymilk exhibited a monomodal distribution with an average particle size of 0.2 µm. The distribution was mostly due to soymilk protein particles. In the present work centrifugation was not carried out, hence fat globules were present, together with larger protein particles and some cell wall debris. It was indeed demonstrated that the centrifugation step decreases the amount of total solids and causes creaming of the fat globules in soymilk without decreasing significantly the amount of protein (Malaki Nik et al., 2008).

5.4.2. Gelation experiments

Acidification was conducted with addition of 0.6 % GDL or the addition of lactic acid bacteria. The differences in the pH profile during acidification are clearly shown in Figure 5.2. The differences in the decrease in the value of pH for bacterial and GDL acidification have been previously reported, for example, in milk matrices (Amice-Quemeneur et al., 1995; Lucey et al., 1998). The lactic acid bacteria culture was able to acidify soymilk to pH 5.1 in about 240 min. The decrease in pH caused by bacterial acidification began slowly, most probably due to an adapting lag phase of the bacteria, which lasted about 100 min. After this initial phase, where the pH was constant at about 6.6, the pH started to decrease. After approximately 4 h, a pH of 5.1 was reached.
Figure 5.1 Particle size distribution, measured by integrated light scattering, of soymilk prepared using a household soymilk maker. Distribution is representative of at least three independent experiments.
Figure 5.2 Acidification profile of soymilk with GDL (triangles) or bacterial culture (circles). The results are the average of 3 replicates and bars represent the standard deviation.
In contrast with the slow acidification by lactic cultures, when 0.6% GDL was added, the pH drop was very rapid due to the fast hydrolysis of GDL. After about 50 min, acidification slowed down as GDL began to approach equilibrium and the soymilk reached pH 5.1 after approximately 2 h. This value of pH was therefore used to compare the final gels. A comparison between the two modes of acidification allows determining possible differences in the acid induced gelation behavior of the soymilk particles with varying rates of acidification.

The development of the gel structure during acidification was followed using rheology and a summary of the measured rheological parameters is shown in Table 5.1. Figure 5.3 illustrates the changes in the elastic modulus (G') and tan δ (where δ is the phase angle) as a function of pH for soymilk acidified with GDL or with bacterial cultures. The environmental pH is critical to soy protein stability; during acidification, protons gradually neutralize surface charges. When the net charge approaches zero, soymilk particles destabilize, aggregate and form a particulate gel network (Kohyama et al., 1995). The gelation pH was defined as the point where tan δ =1. In both cases, the initial value of the elastic modulus (G’) remained very low and constant until the point of gelation, which was earlier in the case of lactic acid bacteria fermentation. The sudden increase in G’, which occurs around the isoelectric point of soy proteins is in full agreement with earlier reports, indicating that the gelation of the soymilk particles is a result of short range interactions, as gelation occurs when protein particles have reduced repulsive charges and can be in close proximity to each other (Malaki Nik et al., 2008; Ringgenberg et al., 2012a). However, there was a significant difference in the pH of gelation of the soymilk particles depending on the mode of acidification (Table 5.1).
Table 5.1 Rheological parameters determined for soymilk acidified with GDL and soymilk acidified with lactic acid bacteria. Means in a column followed by the same letter are not significantly different at p>0.05.

<table>
<thead>
<tr>
<th>Rheological Parameter</th>
<th>soymilk + GDL</th>
<th>soymilk + lactic acid bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gelation pH</td>
<td>5.90±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.29±0.05&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>tan δ at plateau</td>
<td>0.138±0.003&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.142±0.002&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>G' at pH 5.1</td>
<td>412±72&lt;sup&gt;a&lt;/sup&gt;</td>
<td>467±62&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>log/log slope</td>
<td>0.073±0.004&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.072±0.002&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Strain at yield</td>
<td>0.128±0.046&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.113±0.063&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
**Figure 5.3** Development of the elastic modulus ($G'$) (filled symbols) and tan $\delta$ (empty symbols) during acidification of soymilk with bacterial cultures (circles) or GDL (triangles). The results are representative of three replicates. For statistical analysis see Table 5.1.
The gelation pH for soymilk acidified with lactic acid bacteria was 6.29±0.05. After this pH, the G' began to increase and continued to increase in a smooth upward curve until the end of the gelation experiment (which was arbitrarily set at pH 5.1). The average final G' at pH 5.1 was 467±62 Pa. On the other hand, when soymilk was acidified using GDL the gel point was pH 5.9 ±0.04, in agreement with previous results (Malaki Nik et al., 2008; 2011; Ringgenberg et al., 2012a). The average final G' of soymilk acidified with GDL was 412±72 Pa. This was not significantly different from the final G' when bacterial acidification was used. Both gels also showed a similar final tan δ value. Although the values of G' and tan δ for the two gels at pH 5.1 were similar, there seemed to be a faster gel development with pH for the gels prepared with GDL. The values of tan δ also reached a similar final value, regardless of the mode of acidification. The slower fermentation time of soymilk by bacteria allowed for more protein rearrangements during acidification and may have been the cause of the higher pH of destabilization in those gels. However, the molecular details of this early gelation are not known, as this effect has never been reported before.

Although soymilk contains fermentable carbohydrates (Mital & Steinkraus, 1975), previous literature has shown that some strains of lactic acid bacteria may produce proteolytic enzymes (Aguirre et al., 2008). To test the occurrence of proteolysis during fermentation, the composition of the polypeptides of soymilk incubated with or without lactic acid bacteria, at pH near neutral, as well as pH 5.8 and 5.5 was analyzed by SDS-PAGE (Figure 5.4). No apparent proteolysis was observed in the samples: all the subunits were still present below the gelation pH. It was then concluded that the difference in gel point with bacterial acidification compared to GDL acidification may be the difference in rate of pH reduction.
Figure 5.4 SDS-PAGE gel of unfermented and fermented soymilk. Lanes are labelled at the top and represent the following samples: (1) Unfermented soymilk, (2) Unfermented soymilk incubated at 40°C for 2.5 hours, (3) Soymilk fermented to pH 5.8, (4) Soymilk fermented to pH 5.5.
When bacterial acidification is used, the soymilk approaches the isoelectric point of soymilk very slowly, allowing more time for proteins to rearrange and interact with each other, resulting in a higher gelation point. When GDL is used, the isoelectric point of soy proteins is surpassed very rapidly so it is possible that the pH must drop further before proteins have time to interact sufficiently to generate a gel structure. Indeed, the complex composition of the soymilk protein may cause inhomogeneities in the structure of the soymilk particles, and with more time, particles may be able to orient themselves more optimally for short range attractions. It has been recently shown that the basic subunits of glycinin and β subunits of β-conglycinin begin to aggregate above the gel point (Ringgenberg et al., 2012a), probably because of their elevated isoelectric points (pH 6.8-8.5 and 5.7-6.0, respectively) (Staswick et al., 1981; Thanh & Shibasaki, 1977). As these protein subunits approach a net neutral charge and aggregate, the other subunits of soy proteins, which are covalently linked to basic glycinin subunits and β subunits of β-conglycinin, may also approach each other. When these subunits are in closer proximity to each other, short range interactions may begin to take place and contribute to the development of a gel network. However, until the pH is reduced, some repulsion still exists as these subunits have lower isoelectric points (Staswick et al., 1981; Thanh & Shibasaki, 1977).

With the ample time provided by bacterial acidification for proteins to rearrange into optimal conformations for interaction, the initial aggregation of basic subunits of glycinin and β-subunits of β-conglycinin may be sufficient to make a network before the remaining protein subunits are incorporated as well. Figure 5.3 also shows that during structure development, there was a statistically significant difference in the slope of the increase of G' as a function of pH. Although
bacterial acidification results in a higher gelation pH, the presence of still a number of proteins with repulsive charges would prevent a rapid development of the gel network.

As previously mentioned, although the gelation occurred earlier for soymilk acidified with bacteria, and the structure development also appeared earlier, the small deformation properties of the gel at pH 5.1 did not differ. Figure 5.5 illustrates the frequency dependence of soymilk acid gels produced using a bacterial culture and using GDL. The curves using both modes of acidification are parallel to each other and rise with increasing frequency. The log/log slopes of frequency dependence on $G'$ for the GDL-induced soymilk gel and the bacteria-induced soymilk gel (0.073 and 0.072, respectively) were not statistically different (Table 5.1). The positive slope suggested that the gels are non-covalently linked and possibly particulate in nature, as has been previously reported for soymilk acid gels (Malaki Nik et al., 2011). The average yield strain of soymilk gels formed using a bacterial culture was $0.113 \pm 0.063$ and the same value for the gel made by GDL acidification was $0.128 \pm 0.046$. These values were also not found to be significantly different, again suggesting that the final gel formed by either GDL or bacterial acidification did not differ.

The confocal microscopy images are also in full agreement with the conclusion that at the final pH of 5.1, the gels were very similar. Figure 5.6 shows confocal microscopy images of soymilk samples gelled using either lactic acid bacteria or GDL. The microstructure of the protein gel appears densely packed and composed of many small structures assembled into a network of protein aggregates.
**Figure 5.5** Frequency dependence of soymilk gels prepared with bacterial culture and GDL. Results are the average of three replicates and bars represent standard deviations.
Figure 5.6 Confocal microscopy of the soymilk gels prepared with bacterial culture (A) or GDL (B). Bar size 50 μm.
5.5. Conclusions

Comparison of gelation of soymilk induced by acidification using commercial lactic cultures or GDL hydrolysis revealed that bacterial fermentation resulted in a significantly earlier gel point. The slower rate of acidification was responsible for a higher gelation pH. This slower rate of acidification compared to that of samples chemically acidified may be allowing enough time for the protein subunits with higher isoelectric points to rearrange for more optimal interaction with each other, resulting in a change in the elastic modulus at an earlier pH. In the case of chemical acidification, the rate of acidification is much higher, and all the subunits will be incorporated much faster as their lower isoelectric points will be reached faster. It was hypothesized that because of the range of isoelectric points of soy protein subunits, at the pH where some subunits become destabilized and start to aggregate, other subunits still exhibit repulsive charges, hindering the ability of destabilizing proteins to form a network and when there is rapid acidification a gel network will appear only after more protein species have reached their isoelectric point.

Although the pH of gelation is significantly different with the two modes of acidification, the final gel structure of the gels did not appear to be different based on small oscillatory measurements, yield strain and visual microstructure. These results may suggest that in studies where the final gel structure is investigated, GDL may be a suitable substitute for bacterial acidification.
CHAPTER 6

IMPACT OF STRUCTURE MODIFICATION ON TEXTURE OF A SOYMILK AND COW’S MILK GEL ASSESSED USING THE NAPPING® PROCEDURE

6.1. Abstract

It was hypothesized that with a careful control of the structure of a mixed protein matrix it is possible to obtain different texture properties without changing ingredients or their concentrations. A model system containing soymilk, cow’s milk and cream was employed for this study. The structure of the gel was modified by varying the organization of the components, using different processes to make the gels, while maintaining a constant composition. To modify the texture of the final matrix, the mode of gelation of the protein as well as the order of homogenization of the cream (with soymilk, with skim milk or together in the final mixture) were investigated. Using a partial Napping® and the ultra-flash profiling procedure, it was demonstrated that as long as milk was homogenized with cream, with or without the presence of soymilk during homogenization, the mixed protein gels had high thickness and mouthcoating. When the samples were not homogenized or soymilk was homogenized with cream before addition of dairy milk, the resulting gels were thin and watery and did not show mouthcoating properties. It was found that aggregation of milk proteins before that of soy proteins, using renneting in addition to acid gelation, resulted in more prominent fat-related attributes such as slipperiness and fattiness. On the other hand, simultaneous aggregation of casein micelles and soymilk particles generated mixed protein gels with less oily/fatty characteristics.
6.2. Introduction

Soy proteins are commonly used as dairy substitutes, for example, in fermented gel matrices such as soy yogurt. However, soy yogurts have not been nearly as successful as their dairy counterparts, due in large part to their less desirable sensory properties which often include beany and grassy off-flavours and poor texture attributes such as chalkiness, lack of body and wateriness (Ankenman Granata & Morr, 1996; Yazici et al., 1997; Schmidt & Bates, 1976). Mixed protein gels made with a combination of soymilk and cow’s milk could not only provide consumers with the health benefits derived from the consumption of these products (Ankenman Granata & Morr, 1996; Pham & Shak, 2009; Zemel, 2004), but they may also act as a new source of soy proteins with consumer-acceptable textures.

A previous study identified the challenges related to fortifying dairy yogurts (1.9% milk fat) with 1-5% soy protein concentrate (Drake et al., 2000). The study kept the total solids content of the samples constant by replacing non-fat dried milk with soy protein concentrate. It was clearly shown that at low concentrations of soy protein (1-2.5%), the yogurt textures are similar to those of unfortified dairy yogurts, but at higher protein levels, the final yogurt products are more chalky, thicker and darker in colour than control yogurts (Drake et al., 2000). Although a few studies (Drake et al., 2006; Roesch and Corredig, 2006) have demonstrated that addition of soy proteins to dairy products can have a negative impact on texture, to date there have been no studies examining approaches to minimize this effect in mixed soy and dairy gels.

There are many approaches to improving the texture of fermented gels. Many of these involve changing the ingredients including the starter culture or the protein base (Sodini et al., 2004).
The present study will determine the potential for improving the texture of soymilk and cow’s milk protein gels by arranging the constituents within the matrix. Hence, the effect of homogenization order, which will cause changes in the mode of incorporation of the fat constituents within the matrix, as well as different types of protein gelation will be evaluated in this research.

It is known that homogenization creates different protein structures at the newly created interface and in such a way can change the structure and texture of a gel (van Vliet & Dentener-Kikkert, 1982; Sala et al., 2007a). In the case of mixed protein gels containing soymilk, cow’s milk and cream, there are three options for homogenizing the fat globules: fat globules can be homogenized either with soymilk or cow’s milk first, before the addition of the other component, or the fat globules can be homogenized with the mixture of soymilk and cow’s milk. In each case, the fat globule membranes will have a different composition and will be interacting differently with the protein network. This should result in varying textures.

It has also been previously demonstrated that by fine tuning the gelation of the protein components in the mixed matrix it is possible to affect the structure of the final gels (Chapter 4). Therefore to further affect the texture of the final mixed protein matrix, it was chosen to induce aggregation of one protein type (soy or milk) before aggregation of the other or to induce simultaneous aggregation.

As previously mentioned, a mixed gel containing soymilk, cow’s milk and cream is a very good model system for the present study, as the protein gelation and the order of fat globules
homogenization may modify the structure of the matrix without modifying its composition. By preparing different gels it will be then possible to evaluate the effect of structure on the texture properties of the gels.

Due to the labour-intensive preparation of the samples, it would not be possible to produce samples quickly enough to supply a trained panel of tasters. Napping® is a novel rapid-profiling technique which allows for a holistic characterization of a set of products using a small number of panelists and fewer samples than conventional profiling. Additionally, the method is also capable of providing some insight into the importance of attributes to the overall perception of the products (Perrin et al., 2008). Thus to test the hypothesis, the texture of the final protein gels was evaluated using the Napping® procedure combined with ultra-flash profiling, to verify if these different preparation methods are in fact having an impact on texture and what the sensorial changes may be.

6.3. Materials and methods

To modify the structure and cause different arrangements in the matrix of the mixed gels, two sets of treatments were employed: the order of homogenization and the type of gelation. Table 6.1 lists the 8 treatments used in the study. The letters indicate a different homogenization treatment, namely, unhomogenized (A), homogenized with soymilk (B) or cow’s milk (C) first, or homogenized together (D). It was previously shown (see Chapter 4) that to achieve improved gel structure, it was important to induce gelation of both soy and cow’s milk proteins in the gel network. Therefore, acid gelation alone was not included in the treatments, as this would induce
Table 6.1 Treatments applied in this study. SOY = soymilk; SM = skim milk; MF = milk fat from cream.

<table>
<thead>
<tr>
<th>Treatment identifier</th>
<th>Homogenization Treatment</th>
<th>Gelation type</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non homogenized</td>
<td>SOY+MF</td>
</tr>
<tr>
<td>A1</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>A2</td>
<td>X</td>
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gelation of soymilk particles without gelation of the milk proteins at the pH values used in this study. The use of both rennet and acid was an attractive means for controlling the timing of dairy and soy protein aggregation. Whereas in previous work (see Chapter 4) acidification was induced by GDL, bacterial acidification was employed in this research. This allowed for better tasting samples and it also resulted in a slow decline in pH during fermentation (see Chapter 5). It was then possible to add rennet at two carefully selected pH values to either induce rapid aggregation of casein micelles (pH 6.6) before soymilk particles, or simultaneous aggregation of both protein species (pH 6.4), (treatments indicated with 1 and 2, respectively).

6.3.1. Skim milk preparation

Low-heat skim milk powder (Gay Lea Foods Co-operative, Guelph, ON, Canada) was added to pasteurized skim milk (Crown Dairy Ltd., Guelph, ON, Canada) to reach a milk protein content of 6%.

Protein content was analysed using the Dumas combustion method in a LECO FP-528 (Leco Corp., St. Joseph, MI, USA). The solution was stirred for 30 min before placing the milk in the refrigerator overnight to ensure skim milk powder was fully hydrated before use.

6.3.2. Soymilk preparation

Soybeans were purchased from a local grocery store, and soymilk was prepared as previously described (see 5.3.1). Based on preliminary tasting, the concentration of proteins was increased to 4.8% (as determined by the Dumas combustion method) by using 240g of dry beans and mixing with 855 mL of filtered water to produce soymilk.
6.3.3. Homogenization

Cream (~40% fat content) was obtained from Gay Lea Foods Co-operative, Guelph, ON, Canada). Samples were pre-heated to 40°C then homogenized using a two-stage homogenizer (31MR Laboratory Homogenizer, APV Gaulin Inc., Wilmington, MA, USA). The first stage was set at 170 bar and the second stage at 35 bar. Four homogenization treatments were examined: A) unhomogenized mixture of milk, soy milk and cream (control), B) soymilk homogenized with cream before combination with skim milk (skim milk was homogenized alone), C) skim milk homogenized with cream before combination with soymilk (soymilk was homogenized alone), and D) soymilk, skim milk and cream mixed together before homogenization (Table 6.1). Skim milk and soymilk were mixed in a 1:1 ratio for a final protein content of 3.0% milk proteins and 2.4% soy proteins in the mixture. Cream was added to achieve a final milk fat content of 1.5%, contributing to an additional 0.03% skim milk protein.

6.3.4. Gelation

Calcium chloride (33% solution, Glengarry Cheesemaking and Dairy Supply Ltd., Massena, NY, USA) was added to all samples at a concentration of 1 mM to ensure there was sufficient calcium for a firm skim milk gel. Two gelation mechanisms were investigated: 1) casein protein aggregation before soy protein aggregation (rennet addition at pH 6.6), 2) simultaneous casein and soy protein aggregation (rennet addition at pH 6.4). Samples A-D were pre-heated to 40°C before addition of the bacterial culture (YO-MIX™ 511 LYO 375 DCU, Danisco Canada Inc., Scarborough, ON, Canada) at a usage level of 0.2 DCU/L. Samples were then split into two in preparation for initiation of the two different gelation mechanisms. Samples in which the first gelation mechanism (casein protein aggregation before soy aggregation) was initiated will be
referred to as samples A1-D1. Samples in which the second gelation mechanism (simultaneous casein and soy protein aggregation) was initiated will be referred to as samples A2-D2. Immediately after addition of the bacterial culture, rennet (Chymax Ultra Rennet (790 IMCU/mL) from CHR Hansen, Milwaukee, WI, USA) was added at a concentration of 0.2212 IMCU/mL to samples A1-D1. Samples were incubated until pH 6.4 was reached (approximately 2 hours after addition of the bacterial culture). At this point, 0.2212 IMCU/mL rennet was added to samples A2-D2. Preliminary work was done to determine the gel point of the soymilk used in the present study (see Chapter 5). It was determined that the soymilk gelled around pH 6.3, thus rennet was added slightly before the gel point to minimize any damage that may be done to the gel structure by mixing the solution at the gel point.

After rennet addition, all samples were dispensed into 4 oz. styrofoam serving cups in portions of 50 mL. Samples were fermented at 40°C in a gravity convection incubator Model 4L (GCA Precision, Winchester, VA, USA). One cup of each of the sample treatments was used to track acidification using a Denver Instrument UltraBasic pH meter (Bohemia, NY, USA). When samples reached pH 5.2, they were removed from the incubator and placed into a refrigerator to halt fermentation. Samples were stored in the refrigerator for approximately 24 hours before sensory testing.

6.3.5. Sensory testing: Napping® procedure

A partial Napping® procedure combined with ultra flash-profiling was carried out to determine differences only in texture properties of the samples. The panel consisted of 7 women and 5 men with the majority being students in the age range of 25-35. All of the panelists were experienced
in trained panel descriptive analysis and 8 of the 12 panelists had previously participated in a trained yogurt panel. Trained panelists were selected to take part in the Napping® procedure because they were experienced in focusing on individual sensory properties (in this case texture) whereas a naive consumer would have more difficulty separating assessment of texture from other sensory modalities. To aid in focusing on texture properties, panelists were asked to wear nose plugs to minimize effects of flavour on their judgment of the samples. Research ethics approval was obtained from the university’s ethics board.

Panelists were given 40 cm x 60 cm paper sheets and provided with instructions similarly to what has been previously published (Pagès, 2005). Panelists were instructed to place the products on the sheet of paper according to texture similarities and differences. The more similar two samples were, the closer they were to be placed to each other. Conversely, the more different two samples were, the further their locations were to be on the sheet. Additionally, panelists were instructed that once they had finished placing the products they should write the sample code underneath the cup and 2-5 words to describe the sample. Panelists were requested to avoid using comparisons of samples (ex.: sample X was whiter than sample Y) and instead to use words such as low, medium, high if they wish to express intensity. Participants were informed that there was no right or wrong answer and that they may differentiate the product according to their own criteria.

A second replicate of the Napping® procedure was carried out two months later to confirm the results of the first session. This replicate used the same trained panelists as the first session,
Table 6.2 Organization of data table for analysis of Napping® data by multiple factor analysis.

<table>
<thead>
<tr>
<th>Product</th>
<th>Product Coordinates of Panelist 1</th>
<th>Product Coordinates of Panelist 12</th>
<th>Frequency of use of each attribute (Supplementary Variables)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Attribute 1</td>
</tr>
<tr>
<td>A1</td>
<td>X1 Y1</td>
<td>X12 Y12</td>
<td>Frequency</td>
</tr>
<tr>
<td>B1</td>
<td>X1 Y1</td>
<td>X12 X12</td>
<td>Frequency</td>
</tr>
<tr>
<td>...</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>D2</td>
<td>X1 Y1</td>
<td>X12 X12</td>
<td>Frequency</td>
</tr>
</tbody>
</table>
except for 2 panelists that could not attend the session and had to be substituted with 2 other trained panelists.

6.3.6. Data Analysis

The bottom, left corner of each 40 cm x 60 cm paper sheet was set as the origin, and the coordinates of each product (in centimetres) were collected. The data table was organized in 24+52 columns and eight rows (one for each product) as shown in Table 6.2. The first 24 columns represented the X- and Y-coordinates of the 12 panelists. The following 52 columns represented one of each of the attributes that was cited by panelists. For each attribute, the number of times the attribute was cited for a particular product was recorded in the table. In cases where an intensity of attribute was expressed, each intensity was considered as a separate attribute (ex.: thin, medium thickness, medium to high thickness, high thickness were all entered as separate attributes). Data was subjected to a multiple factor analysis with the attributes as supplementary variables using the FactoMineR 1.14 package (Husson, Josse, Lê & Mazet) in R version 2.13.0 (R Development Core Team, The R Foundation for Statistical Computing, Vienna, Austria).

6.4. Results and discussion

To evaluate texture perceptions, a rapid technique that does not require panelist training was used. This approach is particularly useful in situations where due to the time demands in sample preparation, to perform a trained panel assessment, tasting on a daily basis would be resource intensive. For example, in the present study, due to the time sensitivity of the samples, the products would have to be prepared fresh every day.
The main goal of the study was to determine if the texture of the products is different between treatments and if so, to be able to have an overall evaluation of what these differences might be. Napping® was selected as a means to evaluate the sensory properties of the samples. The basic principle of Napping® involves asking panelists to evaluate the degree of difference between samples.

This technique was also coupled with ultra-flash profiling, to provide an indication of the attributes that differentiated the products. With the information derived from these two techniques, it was then possible to determine if the homogenization order and/or the gelation mechanism could indeed be used to manipulate the texture of mixed soymilk-cow’s milk mixed protein gels or if the differences between samples would be too subtle for sensory detection.

A combination of rennet and acid gelation was employed to fine-tune the aggregation of soymilk particles and casein micelles. It has previously been shown that rennet is not capable of inducing soy protein gelation, and would thus only contribute to milk protein gelation (Section 4.4.1). Acidification can cause both milk and soy protein gelation (Alexander & Dalgleish, 2004; Lucey et al., 1996; Malaki Nik et al., 2011). However, the gel point of soy proteins is far higher (pH 6.3) than that of low-heat milk proteins (pH 5.0) (Alexander & Dalgleish, 2004). Thus, at pH values above 5.0, acidification may only induce aggregation of soy proteins. Acidification only was not employed in this study, as it has been previously shown that the incorporation of casein micelles within the network formed a stiffer gel (Chapter 4).
Following fermentation, samples were stored in a refrigerator over-night. The resulting samples were opaque, off white in colour, and with a consistency similar to that of yogurts. On the morning of the Napping® session, one sample of each permutation was taken for pH measurement and the final pH of the samples was found to range from 5.0 to 5.15. This narrow pH range ensured that differences in texture were not caused by differences in final pH.

Only the results from the first Napping® session will be discussed herein. The results obtained from the second session only confirmed the results of the first session and did not provide any new information. By analysis of the data with MFA, a visual representation of the products was generated in graphical form, wherein products that were perceived as being more similar in texture by the panelists were closer together and products that were perceived as very different were depicted graphically as being farther apart.

Table 6.3 lists the eigenvalues and inertia for the first four axes of the MFA. These four axes accounted for just over 85% of the variance and they will be kept for data interpretation. The representations of the mixed protein gels following MFA are presented in Figure 6.1, where the top panel (Figure 6.1A) depicts the dimensions 1 and 2, and Figure 6.1B dimensions 3 and 4.

The results shown in Figure 6.1A suggest that samples that were homogenized the same way (as represented by the same letter) are similarly described by the first dimension (similar x-coordinate).
Table 6.3 Eigenvalues and percent of variance accounted for by each dimension of the multiple factor analysis of Napping® data.

<table>
<thead>
<tr>
<th></th>
<th>Factor 1</th>
<th>Factor 2</th>
<th>Factor 3</th>
<th>Factor 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eigenvalue</td>
<td>5.09</td>
<td>3.29</td>
<td>2.40</td>
<td>1.38</td>
</tr>
<tr>
<td>Percent of variance</td>
<td>35.62</td>
<td>23.04</td>
<td>16.76</td>
<td>9.62</td>
</tr>
<tr>
<td>Cumulative percent of variance</td>
<td>35.62</td>
<td>58.66</td>
<td>75.42</td>
<td>85.04</td>
</tr>
</tbody>
</table>
Figure 6.1 Product map generated following multiple factor analysis of Napping® data. Dimensions 1 and 2 (A); dimensions 3 and 4 (B). A1-D2 refer to treatments described in Table 6.1.
On the other hand, it appears that for the most part, the second dimension separated samples according to their gelation mechanism with most of the samples in which milk proteins were aggregated before soy proteins, on the negative side of the second dimension (apart from sample D1) and all of the samples with simultaneous milk and soy protein aggregation, belonging to the positive side of the second dimension. Dimensions 3 and 4 did not generate such distinctions between homogenization method or gelation mechanism.

The frequency with which each attribute was mentioned and correlations of the attributes with dimensions are listed in Table 6.4. Only attributes which had a significant correlation with one of the first four axes are shown. Correlations of >0.6 were considered as having loaded high on that dimension. Within the table, these are highlighted in bold. Correlations which were just below this cut-off (correlation >5.9) are highlighted.

The first dimension appears to be governed by mechanical texture properties (Surmacka-Szcześniak, 1963) as well as some surface-related properties. The positive side of the dimension is associated with terms expressing a thick consistency (high thickness, dense), high mouthcoating and some roughness (less smooth, mouthdrying, grainy). The negative side of the first dimension is correlated with terms expressing a thin consistency (low firmness, thin, high runny), smoothness, no mouthcoating and high wateriness (very liquidy). The term “low residue” was also associated with the negative side of the first dimension. This term is more difficult to interpret as it may refer to lack of mouthcoating (Claassen & Lawless, 1992) or to the mouthdrying sensation (Porubcan & Vickers, 2005).
Table 6.4 Correlations of attributes with the first four dimensions of multiple factor analysis of Napping® data and frequency of usage of the terms. Correlations >0.6 are highlighted in bold. Correlations just under 0.6 are in bold and italicized.

<table>
<thead>
<tr>
<th>Attribute</th>
<th>Usage Frequency</th>
<th>Correlation with dimensions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Usage Frequency</td>
<td>Dimension 1</td>
</tr>
<tr>
<td>High Runny</td>
<td>2</td>
<td>-0.657</td>
</tr>
<tr>
<td>Thin</td>
<td>14</td>
<td>-0.764</td>
</tr>
<tr>
<td>Medium Thickness</td>
<td>12</td>
<td>-0.449</td>
</tr>
<tr>
<td>Medium-High Thickness</td>
<td>8</td>
<td>0.692</td>
</tr>
<tr>
<td>High Thickness</td>
<td>18</td>
<td>0.914</td>
</tr>
<tr>
<td>Medium Watery</td>
<td>3</td>
<td>0.221</td>
</tr>
<tr>
<td>High Watery</td>
<td>8</td>
<td>-0.844</td>
</tr>
<tr>
<td>No Mouthcoating</td>
<td>5</td>
<td>-0.614</td>
</tr>
<tr>
<td>High Mouthcoating</td>
<td>11</td>
<td>0.858</td>
</tr>
<tr>
<td>Not Oily/Fatty</td>
<td>5</td>
<td>0.345</td>
</tr>
<tr>
<td>High Oily/Fatty</td>
<td>7</td>
<td>0.147</td>
</tr>
<tr>
<td>Not Chalky</td>
<td>1</td>
<td>0.245</td>
</tr>
<tr>
<td>Low Creamy</td>
<td>2</td>
<td>-0.006</td>
</tr>
<tr>
<td>Medium Slipperiness</td>
<td>6</td>
<td>-0.097</td>
</tr>
<tr>
<td>High Slipperiness</td>
<td>8</td>
<td>-0.526</td>
</tr>
<tr>
<td>Pasty</td>
<td>1</td>
<td>0.245</td>
</tr>
<tr>
<td>Airy</td>
<td>1</td>
<td>0.039</td>
</tr>
<tr>
<td>Medium Astringent</td>
<td>1</td>
<td>-0.595</td>
</tr>
<tr>
<td>Whipped-Like</td>
<td>1</td>
<td>0.245</td>
</tr>
<tr>
<td>Less Smooth</td>
<td>4</td>
<td>0.727</td>
</tr>
<tr>
<td>Smooth</td>
<td>8</td>
<td>-0.612</td>
</tr>
<tr>
<td>Low Firmness</td>
<td>2</td>
<td>-0.834</td>
</tr>
<tr>
<td>Medium Firmness</td>
<td>5</td>
<td>-0.249</td>
</tr>
<tr>
<td>High Firmness</td>
<td>7</td>
<td>0.848</td>
</tr>
<tr>
<td>Low Residue</td>
<td>1</td>
<td>0.258</td>
</tr>
<tr>
<td>Dense</td>
<td>6</td>
<td>0.768</td>
</tr>
<tr>
<td>Grainy</td>
<td>4</td>
<td>0.727</td>
</tr>
<tr>
<td>Mouth-Drying</td>
<td>7</td>
<td>0.697</td>
</tr>
<tr>
<td>Slightly Clumpy</td>
<td>2</td>
<td>0.450</td>
</tr>
<tr>
<td>High Clumpy</td>
<td>2</td>
<td>0.484</td>
</tr>
<tr>
<td>Very Liquidy</td>
<td>4</td>
<td>-0.725</td>
</tr>
</tbody>
</table>
Gels C1 and C2 were prepared with skim milk homogenized with cream followed by addition of soymilk. Gels D1 and D2 were prepared by homogenization of skim milk, soymilk and milk cream together. After analysis, these four mixed protein gels all fell on the positive end of the first dimension. The results would then indicate that as long as cow’s milk is homogenized with cream, whether or not soymilk is present in the mixture during homogenization, the resulting soy-dairy milk gels exhibit high thickness, mouthcoating and roughness. Gels B1 and B2 were prepared by homogenization of soymilk with cream followed by addition of skim milk. Gel B2 was very close to the Y-axis, suggesting that this treatment resulted in products with an intermediate consistency, mouthcoating and smoothness. Gel B1 was slightly lower on the first axes indicating it may have been perceived as thinner and more watery than gel B2. Mixed protein gels that were unhomogenized (A1 and A2) were perceived as the most thin, watery and having no mouthcoating.

The second dimension shown in Figure 6.1A appears to be dominated mostly by fat-related texture properties. The attribute “not oily/fatty” loads high on the positive side of the axis and the attributes “high slipperiness”, a property of fats related to lubrication (Richardson-Harman et al., 2000), “medium thickness” and “low creamy” load high on the negative end of the axis. Additionally, the term “high oily/fatty” also loaded relatively high on the negative side of the second dimension (correlation of 0.592), opposing the term “not oily/fatty”. When yogurt samples exhibit an oily coating this has been shown to detract from the perception of creaminess (de Wijk et al., 2003). Hence, it seems logical that the term “low creamy” was found on the negative end of the second dimension alongside fat-related terms such as “high slipperiness” and “high oily/fatty”. Overall, the negative end of the second dimension was governed by more.
fatty/oily attributes and the positive side by less fatty/oily attributes. This dimension is particularly interesting because the fat content of all the samples was the same. These results therefore clearly demonstrated that the configuration of the fat within the gel matrix (even between homogenized samples) changed the perception of fat attributes.

The second dimension shown in Figure 6.1A clearly demonstrated that samples A1-C1 were different from A2-D2 and also D1. Therefore, the majority of samples in which milk proteins were aggregated before soy proteins during mixed protein gel production (A1-C1) were associated with more prominent fat-related attributes (slipperiness, low chalky); whereas samples in which milk and soy proteins were aggregated simultaneously (A2-D2) were described as lower in fat-related attributes (not oily/fatty). This effect was confirmed by the second Napping® session where it was once again shown that A1, B1, C1 and D1 were more closely related to fat-related attributes than samples A2, B2, C2 and D2, respectively.

The dimensions 3 and 4, shown in Figure 6.1B, were more difficult to interpret. These dimensions did not distinguish the products according to their treatments and thus did not contribute any clear information regarding the effects of the treatments on sensory properties of the mixed protein gels. Additionally, although some terms did correlate with the dimensions, their characterizations of the products were not repeatable in the second Napping® session. Thus it was concluded that the third and fourth dimensions were not helpful with any further characterization of the mixed protein gels.
It has previously been shown that consumers prefer yogurts that are smooth, have a medium to high thickness, and that are creamy (Lovely & Meullenet, 2009; van Vliet et al., 2009). Based on this it is expected that the consistency of mixed protein gels generated using homogenization orders C and D would be preferred by consumers as these samples exhibited higher thickness. It was then concluded that as long as cow’s milk was homogenized with the fat component, the product would be more likely preferred by consumers. However, roughness of these same samples may detract from the liking scores of these products and further work is required to evaluate the intensity of the rough mouthfeel and how it may be reduced if it is in fact problematic.

Although a fatty after mouthfeel contributes to creaminess perception (Janhøg et al., 2006), it is known that excessive perception of fattiness or oiliness detracts from creaminess (de Wijk et al., 2003). Because it was not possible to determine the level of fattiness of the samples, nor their relation to consumer liking, more work is needed before it can be determined which mode of gelation would result in products that would be preferred by consumers.

6.5. Conclusions

Homogenization order was found to have a clear effect on sample thickness, mouth coating, mouthdrying and wateriness. As long as dairy cream was homogenized with milk proteins, whether or not soymilk was present in the mix, the samples exhibited high thickness, roughness and high mouthcoating. The timing of protein gelation also appeared to have an effect on the mixed protein gels’ sensory properties. Gels prepared by aggregation of milk proteins before soy proteins appeared to be associated with more fat-related attributes such as high slipperiness and
oily/fattiness. Mixed protein gels in which milk and soy proteins were aggregated simultaneously, were described as not oily/fatty.

Although some effects of homogenization order and mechanism were observed, it has not yet been possible to explain what causes these sensory differences. A study on the chemical structure of these mixed protein gels will be needed to better understand the structures that generate a more desirable texture in mixed soymilk-dairy milk gels.
CHAPTER 7

GELATION OF RECOMBINED SOYMILK AND COW’S MILK GELS: EFFECT OF HOMOGENIZATION ORDER AND MODE OF GELATION ON MICROSTRUCTURE AND TEXTURE OF THE FINAL MATRIX.

7.1. Abstract

The present study examined the development of the gel network as well as the final structure obtained from mixed soymilk-skim milk gels recombined with cream. An understanding of the structural changes occurring as a result of different process manipulations may aid with understanding the reported texture differences in these matrices (see Chapter 6). Results showed that upon changing the mode of homogenization, the composition of the fat globule membrane changed. When both milk and soymilk were homogenized with the cream, the resulting gel had the highest gel stiffness at the end of fermentation. Confocal microscopy demonstrated that as long as milk was homogenized with cream (regardless of the presence of soymilk during homogenization), the resulting gel was composed of finer protein aggregates. On the other hand, without homogenization or when soymilk was homogenized with cream before milk addition, the result was a more coarse gel structure. The composite gels where both casein micelles and soy proteins were aggregated simultaneously appeared as more homogeneous structures compared to gelation of caseins before soy proteins. This work, in combination with the results shown in Chapter 6 clearly demonstrated that by modulating the timing of casein and soy protein aggregation, as well as the composition of the fat interface, it was possible to modify the gel structure and affect texture properties.
7.2. Introduction

Previous studies have demonstrated that it is possible to obtain composite gels containing soy proteins and casein micelles (Lin et al., 2012; Roesch & Corredig, 2005). Protein gels made from a mixture of protein particles from soymilk and milk show great potential as platforms for a new category of food products providing additional health benefits. Indeed, there are demonstrated synergies in consuming milk and soy products. For example, it has been shown that addition of skim milk powder to soy yogurts can increase conversion of soy isoflavone glycosides to a more biologically active form (Pham & Shak, 2009). However, the presence of a significant amount of soy in mixed soymilk-dairy milk products presents a challenge to consumer acceptance in the western world as soybeans tend to impart beany and grassy flavors (Ankenman Granata & Morr, 1996; Yazici et al., 1997). In addition to flavor, texture is also a challenge, due to the different types of gels obtained with soymilk protein particles compared to casein micelles (Malaki Nik et al., 2011; Roefs et al., 1990).

Soymilk gels are typically prepared by heating soymilk to denature the proteins, followed by addition of ionic salts, such as MgCl₂, or by acidification using glucono-δ-lactone or bacterial cultures (Kohyama et al., 1995; Wang et al., 2002). Heating is a necessary step in soy protein gelation. Heating denatures the soy proteins and leads to formation of soluble aggregates of protein subunits linked by disulfide bonds. These covalently linked aggregates may also interact with each other via hydrophobic interaction and hydrogen bonding (Ren et al., 2009). Addition of ionic salts or acidification induces charge shielding or charge neutralization on these heat-induced protein aggregates allowing for short-range interactions such as hydrogen bridging and van der Waals forces to take place and induce gel formation (Kohyama et al., 1995). Soymilk
gels are not typically network gels but rather tend to be particulate in nature (Malaki Nik et al., 2011). Casein gels, on the other hand, are made up of a network of strands of aggregated caseins (Roefs et al., 1990). Such gels can be generated by destabilization of the κ-casein hairy layer and subsequent aggregation of casein micelles by non-covalent interactions. This can be accomplished either by enzymatic cleavage of κ-caseins using rennet or by causing the hairy layer to collapse by charge neutralization via acidification (Lucey, 2002; Alexander & Dalgleish, 2004). However, unlike the high gel point of acidified soymilk (around pH 6) (Malaki Nik et al., 2011), unheated dairy milk must be acidified further, to around pH 5.0 to produce an acid-gel (Alexander & Dalgleish, 2004). Such differences in protein properties can be capitalized upon when making mixed soymilk-skim milk gels.

It has previously been demonstrated that by simultaneous use of rennet and acidification, it is possible to make mixed soymilk and dairy milk gels in which both soy proteins and caseins contribute to the formation of the gel network (Lin et al., 2012). It is hypothesized that by careful manipulation of the gelation mechanism (i.e. aggregation of milk proteins before soy proteins or simultaneous aggregation) it may be possible to control the distribution of caseins and soy proteins within the gel network. If caseins are aggregated first, it is possible that when soy proteins begin to aggregate, they are incorporated in an already pre-formed network of casein micelles. Thus the resulting gel network might be less homogeneous. However, if caseins and soy proteins are aggregated simultaneously, they may be more evenly dispersed throughout the network. The previous chapter demonstrated that the gelation mechanism may have an impact on the perception of fattiness in mixed soymilk-dairy milk gels with added cream. However, the underlying causes of this effect have yet to be examined.
In addition to changing how proteins come together to form a gel network, the sensory properties of protein gels may also be modified by addition of fat globules. Recently, some strides have been made in understanding perception of fatty attributes and creaminess in semi-solid gels through studies of oil droplet release and oil droplet-matrix interactions (Dresselhuis et al., 2008; Sala et al., 2007b; Sala et al., 2008). It has been shown that it is possible to alter perception of fat-related attributes by modifying the level of interaction between oil droplets and the gel matrix or by selecting gel matrices with differing melting properties to control release of oil droplets (Sala et al., 2007; Sala et al., 2008). In the case of dairy yogurt, addition of fat globules is known to increase gel thickness and perception of creaminess (de Wijk et al., 2006; Lucey, 2004). While simple addition of fat globules to a gel is sufficient to make a large impact on sensory properties, the interaction of fat globules with the gel network structure can also be modified, for example, by homogenization to modify sensory properties of the gel (Lucey, 2004). When fat globules are added to a protein network in unhomogenized dairy milk, few casein proteins are present on the milk fat globule membrane. When this milk is gelled, for example in the case of yogurt-making, the gel network forms around the fat globules and these large globules become entrapped in the pores of the gel network (van Vliet, 1988). However, when cow’s milk is homogenized with fat, caseins are the main component present on the fat globule membrane (Cano-Ruiz & Richter, 1997). During gelation of homogenized milk, the fat globules become an integral part of the network, and contribute to the gel modulus (van Vliet, 1988). In such gels, the fat globules are referred to as “interacting fillers”. The majority of the work on interacting and non-interacting fillers has been carried out on the platform of dairy proteins and dairy fat. To date, there is no such published work carried out on mixed soymilk-casein gels.
It has been previously demonstrated (Chapter 6) that modifying the homogenization order and gelation mechanism had a significant impact on the thickness, mouthcoating and fattiness of the samples. The present study will examine in further detail the physico-chemical details underlying these changes in texture of mixed protein networks. The purpose of this study will be to examine the differences in gel network development and microstructure between soymilk-dairy milk gels produced using different homogenization orders and gelation mechanisms. The results will provide increased understanding of the structure-texture relationships, and will provide principles for the design of functional structures in protein matrices.

7.3. Materials and methods

7.3.1. Preparation of materials and gelation

See sections 6.3.1-6.3.4 for preparation of materials and sample gelation. See Table 6.1 for treatment list.

7.3.2. SDS-PAGE

Milk and soymilk mixtures that were homogenized via the three homogenization orders as well as an unhomogenized sample were analyzed by electrophoresis. Additionally, soymilk in this study contained 0.9\% ± 0.8 lipid (as determined by the Babcock method described in chapter 5), as it was not subjected to centrifugation. Thus, soy fat was also analyzed to examine the type of protein present at the interface. Samples were centrifuged at 12 486 g at 25°C for 15 minutes in an Eppendorf 5415 D Centrifuge (Brinkmann Instruments, Mississauga, ON, Canada). The cream layer was carefully removed and placed on a filter paper. The cream was then gently rinsed with Milli-Q water to remove any serum proteins then allowed to dry before being
resuspended in Milli-Q water to the original volume fraction. This same procedure was used to remove soy fat from soymilk and to prepare it for electrophoresis. Electrophoresis was carried out using two methods, with different acrylamide concentrations and with sample preparations with or without 6M Urea, to optimize separation of the different proteins, as previously detailed in the literature (Acero-Lopez et al., 2010; Keerati-urai & Corredig, 2009b). The gels were scanned using a Bio-Rad Gel Doc™ EZ Imager (Bio-Rad, Mississauga, ON, Canada).

7.3.3. Particle size analysis

Particle sizes of the differently homogenized samples were determined using a Mastersizer 2000S (Malvern Instruments Inc., Southborough, MA, USA). To determine if oil droplets were coalesced or if oil droplets were bridged by protein-based aggregates, samples were treated with urea (Sigma-Aldrich Ltd., St. Louis, MO, USA) and sodium dodecyl sulfate (Fisher Sci., Fairlawn, NJ, USA) and analyzed again for particle size. Samples were prepared by adding 2 mL of sample to 8 mL solution of either 6 M urea (pH 7.0) or 10% sodium dodecyl sulfate (SDS). Samples were allowed to stand at room temperature for one hour before analysis.

7.3.4. Gelation experiments

Samples were prepared in volumes of 30 mL and after bacteria and rennet addition, 1.5 mL of the sample was injected into a glass cuvette for diffusing wave spectroscopy, 20 mL was dispensed into a rheometer and the remainder of the sample was used for pH measurement. The pH was recorded every 11 s using an Accumet AR15 pH meter (Fisher Sci., Mississauga, ON, Canada) connected to a computer, using AR15 pH recorder software (Mediavention Engineering Inc., Guelph, ON, Canada). Each treatment was measured in triplicate.
The light source for DWS was a solid diode pumped Nd:YAG laser emitting light with a wavelength of 532 nm and a power of 350 mW. Samples were placed in 5 mm, 1.5 mL glass cuvettes and measured at 40 °C, controlled by an external water bath. Measurements were collected for 2 minutes with intervals of 1 second until pH 5.1. The mean square displacement (MSD) was analyzed using DWS Linear Fit software (Mediavention Engineering Inc., Guelph, ON, Canada) and Sigma Plot 10.0 (SPSS Inc., Chicago, IL, USA). Unhomogenized samples were not analyzed by DWS due to their large oil droplets.

Rheology was performed using an Advanced Rheometer AR 1000 with Rheology Advantage Instrument Control AR software v5.4.0 (TA Instruments Ltd., New Castle, DW, USA) at a constant strain of 0.01 and a frequency of 1 Hz. The temperature was kept steady at 40°C using an external water bath. Experiments were continued until a pH of 5.1, at which point a frequency sweep was initiated using a frequency of 0.01-10 Hz with a strain of 0.01. A strain sweep was performed with a constant frequency of 1 Hz. The yield strain was considered to be the point at which the storage modulus deviated by 10% from the value in the linear viscoelastic range. Significant differences for G' at pH 5.1, the gel point and the frequency slope for the different permutations were analysed by 2-way ANOVA with interactions followed by Tukey’s HSD in R version 2.13.0 (R Development Core Team, The R Foundation for Statistical Computing, Vienna, Austria).
7.3.5. Confocal microscopy

Samples were prepared in volumes of 10 mL and gelling agents were added as described above. 20 μL of 0.2% w/v (in milli-Q water) Rhodamine B fluorescent dye (Fisher Sci., Fairlawn, NJ, USA) was added to 5 mL of each sample for staining. Three drops of dyed sample were placed into grooves of a concave microscope slide and a cover slip was placed overtop and sealed. The remaining 5 mL of sample was placed into a tube and used for pH measurement. Slides and samples for pH measurement were incubated at 40°C until pH 5.1 at which point the slides were placed into a refrigerator at 4°C for cooling to halt fermentation. Images of the gel microstructure were taken using an inverted confocal scanning laser microscope (Leica TCS SP2, model Leica DM IRE2, Leica Microsystems CMS GmbH, Mannheim, Germany) with an Ar/Kr visible light laser and 63x (oil) objective. Resolution of the digital images was 1024 x 1024 pixels. Experiments were repeated in triplicate.

7.4. Results and discussion

Chapter 6 examined the effect of homogenization order and gelation mechanism on the sensory properties of mixed soymilk-dairy milk gels. Both treatments were found to have an impact on the texture of these mixed protein networks. Thus, the present study was initiated to attempt to examine the structural differences which generate the textures created by varying homogenization order and gelation mechanism.

The first treatment applied to the samples was that of varying homogenization order. The homogenization orders examined included: 1) homogenization of soymilk with dairy cream followed by addition of skim milk (homogenized separately), 2) homogenization of dairy milk
with dairy cream followed by addition of soymilk (homogenized separately), and 3) homogenization of mixed soymilk, skim milk and dairy cream. A sample of unhomogenized mixed soymilk, dairy milk and dairy cream was included as a control.

7.4.1. SDS-PAGE

During homogenization, the size of lipid droplets decreases and more protein adsorbs onto the newly formed interface. In milk fat, as there is not sufficient membrane around the fat globules to cover the entire surface area after homogenization, skim milk proteins will adsorb at the interface (Cano-Ruiz & Richter, 1997). Although both whey proteins and caseins are present on the surface of fat globule after homogenization, casein micelles are preferentially adsorbed because of their size (Walstra, 1995). Soy protein particles in soymilk may also adsorb onto the oil droplet during homogenization, as soy proteins have also been shown to be good emulsifiers (Keerati-u-rai & Corredig, 2009b).

Although it was expected that either soy or dairy milk proteins may be present on the surface of the oil droplets after homogenization of the cream with either soymilk or skim milk, the preferential adsorption of soy proteins or casein micelles in a mixed system when soymilk and skim milk are homogenized with cream has never been reported. Figure 7.1 illustrates the polypeptide composition in the oil phase after homogenization as analyzed by SDS-PAGE.
Figure 7.1 SDS-PAGE electrophoresis of protein adsorbed on the fat globules after homogenization. Lane 1 = skim milk; lane 2 = soymilk; lane 3 = sample D; lanes 4-7 protein present in the fat fraction separated by centrifugation: lane 4: unhomogenized mix (A); lane 5 cream homogenized with soymilk and then mixed with skim milk (B); lane 6: cream homogenized with skim milk then mixed with soymilk (C); lane 7: and skim milk, soymilk and cream homogenized together (D).
The first three lanes in Figure 7.1 are reference lanes containing skim milk, soymilk and the homogenized mix (sample D). Lanes 4-8 show the migration of the polypeptides present on the surface of the oil droplets. Bands were identified according to previous literature (Malaki Nik et al., 2009; Titapiccolo et al., 2010). By comparing the bands in the reference lanes (Lanes 1-2, Figure 7.1) to the cream phase of samples A-D (Lanes 4-7, Figure 7.1) it was possible to evaluate which type of protein was present at the interface after homogenization, and if any protein was preferentially adsorbed.

In the case of non-homogenized milk, caseins as well as some β-lactoglobulin were adsorbed at the interface together with the original components of the milk fat globule membrane. Although some of the soy proteins in the high molecular weight range would migrate similarly to those of the milk fat globule membrane (Titapiccolo et al., 2010) it is clear that even in the unhomogenized milk some soy protein seemed to adsorb on the fat globules, and in particular, the bands of α and β-conglycinin as well as the acidic and basic subunits of glycinin migrated in this sample. Similar results were shown in the gels run using urea buffer as a solubilizing buffer (data not shown). However, it is important to point out that some of the soy proteins appearing in the SDS-PAGE analysis of the fat fraction may actually be those found at the soy oil fat interface, as a small amount of soy oil droplets would also be present in the mix. It has been reported that the cream layer of centrifuged soymilk contains some soy proteins (Shun-Tang et al., 1997), and this was confirmed by testing the cream layer of soymilk in this study (Figure 7.2). In particular, the cream layer of soymilk was found to contain some α, α’ and β subunits of β-conglycinin.
Figure 7.2 SDS-PAGE electrophoresis of protein found on the fat globule interface of soy oil. Lane 1= soymilk; lane 2= protein present in the soy fat fraction separated by centrifugation.
While in sample A some protein was present, a higher amount of protein was isolated from homogenized milk fat droplets (Lanes 5,6,7, for droplets of treatment B, C and D, respectively). When soymilk and dairy cream were homogenized and skim milk added post-homogenization, all of the main soy storage proteins were present on the fat globules. There seemed to be a higher amount of $\alpha$-conglycinin, as well as the acidic and basic subunits of glycinin. Caseins were also present in these samples, as well as traces of $\beta$-lactoglobulin, perhaps the result of some competitive displacement during storage. Although the preferential adsorption of the different proteins present in the mixture has not been fully elucidated and has never been reported in the literature, a more in depth study of this system was outside the scope of this research.

It was clear that amongst the treatments applied in this study, treatment B was the only treatment where soy protein was clearly present at the interface. In treatment C and D, most of the proteins migrated in the gel were derived from skim milk, and in particular, caseins and $\beta$-lactoglobulin. The $\alpha$-lactalbumin was absent from all the samples. When both milk and soymilk were homogenized together with cream, there appeared to be mostly milk proteins, with traces of acidic and basic polypeptides of glycinin at the interface (Lane 7, Figure 7.1). Overall, SDS-PAGE analysis confirmed that it was possible to selectively adsorb more milk or soy proteins to the fat interface by varying homogenization order. The results suggested that there are differences in composition at the interface, and further work should be carried out to elucidate the dynamics of adsorption and displacement occurring at the interface in these mixed systems, during homogenization and storage.
Figure 7.3 Particle size distribution of a mixture of soymilk, cow’s milk and cream following various homogenization treatments. (A) unhomogenized (●); (B) cream homogenized with soymilk first (Δ); cream homogenized first with skim milk dairy milk homogenized with cream (◊); cream homogenized with the mixture (□).
7.4.2. Particle size analysis

To determine possible differences in the particle size distribution of the fat globules, the various mixes were analyzed using integrated light scattering. Figure 7.3 shows the particle size distribution of the samples following the four different homogenization treatments. The mixture containing unhomogenized cream showed a bimodal distribution of sizes, with a population around 0.5 μm and a second population of larger size <10 μm, consistent with the presence of unhomogenized milk fat globules (Walstra, 1995).

Following homogenization the particle size significantly decreased, with a reduction of the large population, although some large particles (>10 μm) were present. There were no obvious differences in the particle size distribution of the three homogenized samples, namely, treatments B, C and D. Thus, it may be concluded that possible differences in the gelation kinetics would not be due to particle size distribution differences.

To determine the extent of bridging between the particles, the homogenized samples were treated with urea and with SDS (see methods). There were no differences in the size distribution after disruption with urea and SDS, hence it was concluded that the large droplets were not in a bridged state, but the large population was most likely composed of a mix of large fat globules and soymilk protein aggregates.

7.4.2. Gelation behaviour

To evaluate possible changes in the gelation behaviour of the mixtures, rheology and DWS were employed to observe the development of the gel. Figure 7.4 illustrates the changes in the gel
Figure 7.4 Development of the elastic modulus ($G'$) (A) and $\tan \delta$ (B) during acidification of mixed soymilk-dairy milk gels prepared by various homogenization orders: (A) unhomogenized (●); (B) soymilk homogenized with cream (▲); dairy milk homogenized with cream (♦); soymilk, dairy milk and cream homogenized together (■). Gelation mechanisms: casein aggregation before soy (filled symbols); simultaneous casein and soy aggregation (empty symbols).
modulus as well as the values of \( \tan \delta \) (where \( \delta \) is the phase angle) as a function of pH of acidification. In all cases, the \( G' \) remained very low until a certain pH, when the \( G' \) showed a steep increase. The experiment was terminated at pH 5.1, to be able to compare the gel properties between the different mixtures and to be consistent with the experiments reported in Chapter 6.

The increase in \( G' \) for treatments A, B and C progressed very similarly and their \( G' \) values at pH 5.1 were not significantly different from each other (see Table 7.1, Figure 7.4A). On the other hand, the development of \( G' \) with pH was significantly different for treatment D, where the mixture was homogenized with cream. In this case, the value of \( G' \) was higher at pH 5.1 (Table 7.1). Surprisingly, unhomogenized samples were similar to homogenized samples in this study, although this is not usually the case for recombined milk samples (Ion Titapiccolo et al., 2010). This is possibly due to the similar protein composition and the larger droplets still present in the mixture after homogenization as shown in Figure 7.3.

It was concluded that homogenization of soymilk, cow’s skim milk and cream together (sample D) generates a stiffer gel compared to all the other treatments, regardless of the similarity in size distribution to treatments B and C, and a high amount of skim milk derived protein adsorbed at the interface (see Figure 7.1), as in treatment C. As shown in Table 7.1, treatments A, B and C resulted in an average \( G' \) of 172 Pa measured at pH 5.1, while treatment D resulted in a final \( G' \) of 282 Pa. Homogenization of cream together with the soymilk and skim milk mixture resulted in both skim milk and some soy proteins at the interface, however it is not possible to estimate at this stage how much the fat globule interface composition may have impacted the interactions with the gel network and the final gel stiffness.
Table 7.1 Elastic modulus, gel point, tan δ plateau and slope of the frequency sweep measured for each of the permutations of soymilk-dairy milk gel. Means in a column followed by the same letter are not significantly different at p>0.05.

<table>
<thead>
<tr>
<th>Permutation</th>
<th>G' at pH 5.1</th>
<th>Gel point (pH)</th>
<th>tan δ plateau</th>
<th>Slope of frequency sweep</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>151.8\textsuperscript{a} ± 22.9</td>
<td>6.16\textsuperscript{a} ± 0.04</td>
<td>0.222\textsuperscript{a} ± 0.027</td>
<td>0.119\textsuperscript{a} ± 0.022</td>
</tr>
<tr>
<td>A2</td>
<td>139.0\textsuperscript{a} ± 31.3</td>
<td>5.93\textsuperscript{b} ± 0.07</td>
<td>0.216\textsuperscript{a} ± 0.018</td>
<td>0.133\textsuperscript{a} ± 0.017</td>
</tr>
<tr>
<td>B1</td>
<td>199.5\textsuperscript{a} ± 19.5</td>
<td>6.26\textsuperscript{a} ± 0.13</td>
<td>0.236\textsuperscript{a} ± 0.012</td>
<td>0.135\textsuperscript{a} ± 0.011</td>
</tr>
<tr>
<td>B2</td>
<td>178.3\textsuperscript{a} ± 19.9</td>
<td>5.97\textsuperscript{b} ± 0.12</td>
<td>0.223\textsuperscript{a} ± 0.007</td>
<td>0.124\textsuperscript{a} ± 0.005</td>
</tr>
<tr>
<td>C1</td>
<td>171.5\textsuperscript{a} ± 29.2</td>
<td>6.03\textsuperscript{a} ± 0.07</td>
<td>0.221\textsuperscript{a} ± 0.006</td>
<td>0.128\textsuperscript{a} ± 0.006</td>
</tr>
<tr>
<td>C2</td>
<td>188.6\textsuperscript{a} ± 60.1</td>
<td>5.99\textsuperscript{b} ± 0.05</td>
<td>0.231\textsuperscript{a} ± 0.009</td>
<td>0.134\textsuperscript{a} ± 0.004</td>
</tr>
<tr>
<td>D1</td>
<td>285.5\textsuperscript{b} ± 25.6</td>
<td>6.09\textsuperscript{a} ± 0.07</td>
<td>0.221\textsuperscript{a} ± 0.005</td>
<td>0.118\textsuperscript{a} ± 0.015</td>
</tr>
<tr>
<td>D2</td>
<td>278.6\textsuperscript{b} ± 41.0</td>
<td>5.97\textsuperscript{b} ± 0.06</td>
<td>0.224\textsuperscript{a} ± 0.002</td>
<td>0.110\textsuperscript{a} ± 0.008</td>
</tr>
</tbody>
</table>
Since the gelation mechanism appeared to have an effect on sensory properties (see Chapter 6), the development of the gel matrix was followed using rheology. The development of the gel was not different between treatments (Table 7.1), although, it was confirmed that by addition of rennet at a higher pH (to induce earlier gelation of casein micelles) there was an earlier gelation pH (Table 7.1 and Figure 7.4B). The tan δ change during the course of acidification clearly showed an earlier pH of gelation, as taken as the pH at which tan δ = 1 (Donato et al., 2010). Samples A1, B1, C1 and D1 all gelled at pH of about 6.1, while when milk and soy proteins were aggregated simultaneously (treatment 2), the gel point was reached at a later pH (pH 5.9). However, it is important to point out that it was previously shown (Chapter 4) that when mixed with soy, rennet induced aggregation of the casein micelles may be hindered, hence, it may be possible that the later pH (6.1) than expected may be caused by such hindrance. Indeed rennet was added at pH 6.6 (treatment 1), much earlier than for treatment 2, thus it is surprising that the gel point is only at pH 6.1 (approximately 90 minutes after rennet addition). Previous work (Chapter 4) demonstrated that when rennet was added to a mixture of milk and soy proteins, without acidification, aggregation of milk protein alone was not sufficient to generate a gel network.

To better observe the changes in dynamic mobility of the fat globules during gelation, for each homogenized mixture observed by rheology, a sample was observed concurrently using DWS. Because homogenized milk fat globules are far larger than the protein aggregates and have a higher refractive index contrast (Corredig et al., 2011; Ion Titapiccolo et al., 2010), it is possible to approximate the light scattering signal to that of homogenized fat globules. Hence, an important event that can be observed with DWS which cannot be observed by rheology is the pH
at which the homogenized fat globules decrease their mobility. If fat globules are indeed interacting with the gel network, their gelation behaviour will resemble that of the protein. On the other hand, if the fat globules are not interacting with the gel network, the oil droplets may just be “held down” in the protein gel network once a sufficient hindrance to their movement has been created by the network surrounding them.

The mean square displacement (MSD) obtained using DWS can provide insight into the degree of particle attenuation in a concentrated system (Romer et al., 2000). The slope of the MSD curve depends on the frequency of collisions between scatterers. Thus if motion of scatterers in a system is constrained, the frequency of collisions will be very low. If scatterers are freely diffusing, they will collide frequently. The log-log plot of the MSD over correlation time generates a linear relationship (slope= 1) if scatterers are freely diffusing. If the motion of scatterers is attenuated, the slope will be less than 1 (Corredig et al., 2011; Romer et al., 2000; Weitz, 1993).

Figure 7.5 shows a representative plot of the log/log MSD slope plotted as a function of pH for all homogenized samples. The log/log MSD slope remained constant around a value of 1 until pH of about 6. This was expected as before the gel point, the globules in the soymilk-dairy milk mixture should be freely diffusing. When the gel point (~pH 6.1) was reached, the slope showed a lower value, indicating the decrease in the motion of the fat globules. The same trend was followed by all the homogenized treatments (B-D) and independently of the mode of gelation confirming that in all the homogenized samples, the fat globules were behaving as interacting fillers.
Figure 7.5 Change of the attenuation of fat globules during gelation of mixed soymilk-dairy milk gels prepared by various homogenization orders: (B) soymilk homogenized with cream (▲); dairy milk homogenized with cream (♦); soymilk, dairy milk and cream homogenized together (■). Gelation mechanisms: casein aggregation before soy (filled symbols); simultaneous casein and soy aggregation (empty symbols).
A frequency sweep was performed to examine the frequency dependence of the gels generated after the different modes of homogenization and different gelation mechanisms. It is known that true cross-linked network gels exhibit little frequency dependence whereas entanglement networks and physical gels show frequency dependence (Stading & Hermansson, 1990, Tunick, 2011). The log/log slope of frequency dependence on $G'$ was determined for each permutation and no significant differences were found. This suggested that the nature of the interactions is quite similar in all the gels. The average log/log slope of all the permutations was $0.125 \pm 0.013$, thus the gels exhibited some frequency dependence, as is expected with milk and with soy gels (Hussain et al., 2011; Malaki Nik et al., 2011). A strain sweep was also performed on the gel once pH 5.1 was reached, to determine the linear viscoelastic range of the mixed protein gels. The maximum strain in the linear range is a property of the gel, which can be related to how easily the strands of a gel may be disrupted and it is often taken as an indication of the gel brittleness (van Vliet, 2002). The linear viscoelastic range was not significantly different among the various treatments. The average maximum strain was $0.065 \pm 0.022$. The low yield strain indicates that the gels were quite brittle.

While parameters which are within the viscoelastic range are below the sensory threshold (Dickinson, 2005), parameters which are beyond the viscoelastic range may be detected by consumers. The yield strain of a fermented protein has been correlated to oral viscosity (Lee & Lucey, 2006). In the present study this was not found to be the case. While all the mixed soymilk-dairy milk gels tested did not have significantly different yield strains, panelists rated the samples as having a range of thicknesses. However, it is important to note that rheological measurements were carried out at 40°C immediately after the gels reached pH 5.1. On the other
hand, the texture perception of the soy-casein gels was tested after storage in the refrigerator (4°C) overnight. The differences may be related to the structural rearrangements occurring during cooling, especially for soy protein gels, because of the increase in hydrogen bridging (Renkema et al., 2001).

7.4.4. Confocal laser scanning microscopy

Whereas rheology and DWS provided insight into the development of the gel over the course of gelation, confocal microscopy images allowed for a visualization of the final gel structure after cooling. Since these are the same structures present in the soy-casein gels when they are eventually consumed by panelists, these images provide important information regarding the structure-texture relationships in mixed soymilk-dairy milk fermented gels.

Figure 7.6 shows confocal microscopy images of each of the treatments. The bright signal in the images derives from the rhodamine B fluorescence signal, indicating the location and structure of the protein network. To observe the effect of homogenization order, the images should be compared horizontally. Unhomogenized samples (samples A1 and A2) appear as many large aggregates with few interconnecting strands. Due to their larger sizes, the fat globules are clearly identifiable in the system. Some branching is evident in the oil droplets as shown by the arrows in Figure 7.6A2. Overall the protein strands could be very well identified and the images also showed a porous network, both for A1 and A2. It is possible that the large fat globules somehow hindered the aggregation of the soy and milk proteins resulting in a low amount of interconnecting branches. These gels were perceived by panelists as having a thin consistency.
Figure 7.6 Confocal laser scanning microscopy images of recombined mixtures. A: non homogenized mixture. B; cream homogenized with soymilk first and then mixed with skim milk; C: cream homogenized with skim milk first and then with soymilk; C: cream homogenized with the mixture. Treatment 1 rennet added earlier; Treatment 2, rennet added later. Bar is 50 μm.
(Chapter 6). Since few strands need to be broken for the gel structure to be fractured, flow may be easily induced. It is known that when a sample’s structure is easily broken and when only a little pressure is required to significantly induce flow, then the sample is perceived as having a thin consistency (Van Vliet, 2002; van Vliet et al., 2009). It is also known from the literature that the perception of “wateriness” arises when a serum is exuded from a sample upon compression. It is a property related to the microstructure of the sample. When samples with high porosity are compressed, they tend to release more serum and therefore to be perceived as more watery (van den Berg et al., 2007; 2008).

Samples B1 and B2 were obtained by homogenizing cream with soymilk, followed by addition of dairy milk (homogenized separately). The images showed larger clusters of protein aggregates with a number of interconnecting strands between the aggregates. The more dense structure may explain the increase in perceived sample thickness compared to the unhomogenized samples. More strands are visible in treatment C, while treatment D showed a much more defined structure, although with high porosity (D1).

The presence of finer structures in samples C1, C2, D1 and D2 may be the cause of the roughness sensation reported by the panelists (Chapter 6). The sensation of roughness is known to be caused by the presence of many small particles (Lee & Lucey, 2006). When soymilk, milk and cream were homogenized together (samples D1 and D2), a densely packed gel network formed composed of very fine protein aggregates. It could be concluded that as long as milk is homogenized with cream (either first or in the mixture) there is a change in density and
interconnectivity within protein aggregates compared with previous samples. This increase in the
density of aggregates might be responsible for the increase in perceived sample thickness.

Surprisingly, although samples D1 and D2 appeared to have more dense aggregates than samples
C1 and C2, there were no obvious differences in perceived thickness. One explanation may be
that fat globules may associate weakly with soy proteins and more strongly with dairy proteins.
Thus strands which are composed of interacting fat globules may be stronger if those fat globules
are interacting with the gel network via dairy proteins rather than soy proteins. If fat globules are
weakly associated with soy protein aggregates, then the point of interaction between fat globules
and soy proteins could be easily broken and may not contribute greatly to the strength of
interconnecting strands. Since samples C1 and C2 are made by homogenization of milk fat with
skim milk, the fat is likely interacting mainly via milk proteins. On the other hand, samples D1
and D2 are prepared by homogenization of milk, soymilk and cream. Thus the milk fat is
interacting partly via milk proteins and partly via soy proteins. Thus, if strands with inclusions of
fat globules interacting via milk proteins are in fact stronger than those interacting via soy
proteins, this may explain the similarity in the perceived thickness between samples C and D
even though samples D appear to have a denser structure than samples C.

Overall, homogenization order had a marked effect on the size of protein aggregates and the
density of aggregates. Networks constructed from finer protein aggregates with higher
interconnectivity within aggregates were associated with increased mouthcoating, roughness and
sensory thickness (Chapter 6). Homogenization of milk proteins with cream was particularly
important for increasing sensory thickness and mouthcoating, which are considered as desirable
attributes in other fermented protein gels such as yogurts (Lovely & Meullenet, 2009; van Vliet et al., 2009). This presents a potential novel application of milk proteins as texture improvers in food systems containing high amounts of non-milk proteins with less desirable texture properties. When milk proteins are appropriately incorporated in the gel structure, they appear to be capable of “carrying forward” their desirable texture properties.

To examine the effect of gelation mechanism on the microstructure of the samples, the images should be compared vertically. From microstructural studies it was clear that samples in which both milk and soymilk proteins were aggregated simultaneously (A2-D2) show a more homogeneous structure, less porous and with more interconnecting strands than when milk proteins are aggregated before soy proteins (A1-D1). When milk proteins are aggregated before soy proteins, by the time soy proteins begin to aggregate, the casein micelles likely already formed well defined clusters, and soy proteins would then interact with these clusters. The aggregates in treatment 1 appear to be larger with fewer interconnecting strands between aggregates compared to samples in which milk and soy proteins were aggregated simultaneously, as in treatment 2.

It is important to note that the particle size distribution of the homogenized samples was not different between samples. Hence, fat globule size cannot be responsible for the increase in perception of fat-related attributes following simultaneous casein and soy aggregation, as reported in the previous chapter. It has been shown that an increase in in-mouth coalescence leads to increased perception of fat-related attributes such as oiliness, creaminess and fatty-mouthfeel (Dresselhuis et al., 2008). It may be possible that an uneven distribution of fat
globules within the gel network may lead to increased in-mouth coalescence due to the proximity of the fat globules to each other during in-mouth shearing compared to when the fat globules are more evenly distributed throughout the network and better separated by strands of proteins. However, at this stage, no work has been completed to examine this possibility and more research will be necessary to better understand the relationship between milk fat globule distribution in a gel network and sensory perception of fat-related attributes.

7.5 Conclusions

Homogenization order and gelation mechanism were found to have significant effects on the final structure of mixed soymilk-dairy milk gels. Sample permutations that were reported as having thicker textures had structures made of finer strands and a more dense network. Samples characterized as having more prominent fat-related attributes had less homogeneous gel networks, and showed some porosity.

Overall, it was determined that rearrangement of soy and milk components in the mixed gel matrix can have a profound impact on the texture and structure of the resulting samples. Additionally, it was found that when milk proteins were appropriately incorporated in the gel network, they were capable of imparting desirable texture attributes to a system containing high amounts of a protein typically exhibiting less desirable texture attributes.
CHAPTER 8
GENERAL CONCLUSIONS

The current project aimed at improving texture of mixed protein gels, composed of soymilk particles and milk proteins. This challenging system was employed as a model for high protein dairy gels containing composite systems, which may require a new way to approach product development. In the present project, a “sensory-first” approach was employed. In order to improve the texture it was necessary to define a texture benchmark. The preferred attribute elicitation method was employed to extract important product attributes that contribute to consumer liking. The method was found to be successful in extracting key attributes and consumers were capable of characterizing products in a meaningful way. The use of this method on commercial yogurt products revealed that texture was important for consumer liking and, in particular, a runny consistency and grainy texture detracted from consumer liking. This work, for the first time, investigated the use of attribute elicitation and compared the outcomes of this methodology with a more established technique.

The model system chosen for this work demonstrated the importance of understanding how to manipulate the gelation behaviour of the individual proteins. Rennet and acidification were selected to gel the mixture of soymilk and cow’s milk as the combined mechanism could be used to selectively induce aggregation of the soy and dairy proteins. However, before any attempts at texture improvement could be undertaken, there was a need to better understand the gelation behaviour of mixed soymilk-dairy milk systems, gelled using both rennet and acidification. With the use of this dual gelation mechanism, it was possible to generate a mixed protein gel network,
with unique rheological and microstructural properties compared to those of the systems in isolation. To better understand the gelation behaviour of the mixed protein gels, glucono-δ-lactone (GDL) was initially employed, because of its ease to use. In subsequent studies, bacterial cultures were employed, as they result in slower acidification than GDL and the cultures also produce desirable flavour compounds. Although the similarities between GDL and bacterial acidification of dairy milk are well documented, no data was available for soymilk. Although the two modes of acidification seemed to slightly differ in the gelation kinetics, the final gels did not show significant differences in their gel stiffness, frequency dependence, yield point and microstructure.

With better understanding of the gelation behaviour of the mixed soymilk–dairy milk systems, it was possible to select some conditions to generate a mixed protein gel to use as a model system for examining methods for improving the system’s texture. It was hypothesized that it would be possible to modify the texture of mixed soy-dairy gels by changing the structure of the mixed protein gel without changing any ingredients or their concentrations. To examine this hypothesis, homogenization order was varied to modify the incorporation of fat globules in the gel matrix and two different gelation mechanisms were used to modify the type of protein aggregates formed to build the gel network. Homogenization order was found to have a profound effect on thickness and mouthcoating of the mixed soymilk-dairy milk gels. When milk proteins were homogenized with cream, with or without the presence of soy proteins during homogenization, the resulting samples had high mouthcoating and high thickness. However when samples were either unhomogenized or when the cream was homogenized with only soymilk, the resulting gels had low mouthcoating and a watery texture. When milk proteins were aggregated before soy
proteins, the mixed protein gels exhibited more apparent fat-related attributes, such as slipperiness and fattiness, than when both soy and milk proteins were aggregated simultaneously.

Physico-chemical characterization of the systems tested by panelists demonstrated that, by varying homogenization order and gelation mechanism, it was possible to change the microstructure of the gel matrix. Varying the homogenization order enabled selective embedding of either milk or soy proteins or a combination of both. Homogenization resulted in a reduction of fat globule size and behaviour of the fat globules as active fillers in the gel matrix. The highest final gel stiffness was achieved when both milk and soymilk were homogenized together with cream. However, all the treatments resulted in gels with similar frequency dependence and yield strain. Confocal microscopy showed that, as long as milk was homogenized with cream (regardless of the presence of soymilk during homogenization), the resulting gel was composed of finer aggregates. Simultaneous gelation of milk and soy proteins resulted in more homogeneous structures compared to gelation of caseins before soy proteins.

By relying on sensory data to guide the research, the present study was able to pinpoint some techniques which lead to an improvement in texture of a challenging system such as a mixed soymilk and cow’s milk gel. This thesis represents the evolution of the research, where the system continued to evolve, led by sensory data. This approach clearly demonstrates how sensory studies can accelerate the development of a more consumer-acceptable texture for a mixed protein network.
It was determined that it was possible to make significant changes in the textural properties of mixed soymilk-dairy gels without changing the ingredients, but rather by rearranging the components within the structure. It was demonstrated that it is possible to generate desirable textures in a protein-based matrix by careful control of the mechanisms of gelation of the proteins and rearrangement of the recombined oil droplets. Additionally, milk proteins were found to be able to improve the texture of systems containing high levels of proteins which are normally associated with poor sensory properties. This highlighted the possibility of the use of milk proteins as texture improvers in mixed protein systems.


the mechanical properties of gelatinised starch granules. *Journal of the Science of Food and Agriculture, 37*(6), 573-590.


All experiments utilizing human subjects were carried out with approval from the University of Guelph ethics board (REB# 09JA028, REB# 09MY020, REB# 11JL022).