EVALUATION OF AN ALTERNATE PROCESSING METHOD FOR GREEK STYLE YOGURT USING MICELLAR CASEIN CONCENTRATE:
TECHNICAL AND BUSINESS ASPECTS

A Thesis
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ABSTRACT

The rising popularity of Greek Style Yogurt (GSY) is one of the most remarkable events in food production and sales in recent years. However, the generation of large quantities of acid whey during the centrifugation step of production is an important factor that may limit the growth of GSY. Acid whey presents both economic and environmental challenges in GSY manufacturing. The focus of the work this work was to develop an alternative processing method for GSY, which can help address the challenges posed by acid whey in GSY production. The alternate process uses micellar casein concentrate (MCC), which represents a by-product in serum protein concentrate production by microfiltration. The optimization of the alternate processing method for GSY using MCC was based on reaching a fermentation time similar to the current industry practice for strained GSY and obtaining a final product with similar chemical and physical characteristics to those of GSY made using the traditional make process.

MCC preparations with two different protein levels (58 % and 88 %) were added to milk to bring the protein content of the milk base to the level desired in the final product. The yogurt milk bases were inoculated with starter culture and fermented until pH 4.5 was reached for both products. The fermentation time of MCC fortified GSY was shorter compared to GSY made from traditional straining process. Rheological analyses indicated a similar, weak gel structure for both the control and the MCC fortified GSY but a difference in the susceptibility of the two types of GSY to syneresis was found. A key finding in this work was that the physical and chemical properties of MCC fortified GSY were similar to those of a commercially available GSY manufactured using the traditional whey removal process. Despite some differences in the physico-chemical properties between the MCC fortified GSY and strained GSY, the make process developed in this study is a feasible alternative to the traditional GSY manufacturing process because it does not generate acid whey and therefore has the potential to reduce the environmental impact of acid whey currently generated by the commercial production process of GSY, and possibly bring financial benefits to the dairy industry.
The commercialization potential of the alternative GSY method using MCC was also examined from the standpoint of business strategy of GSY manufacturing companies. GSY is currently one of the trendiest foods in the United States and it comes in various brands, format and flavor. GSY manufacturing companies have to compete aggressively for a valuable share of this lucrative market. A brief business strategy analysis was conducted with the aid of a business model framework to evaluate the potential of adoption of the alternate make process by the major players in the GSY market. This analysis suggests that despite the potential cost saving advantages conferred by the alternative production method, not all GSY manufacturers will be attracted to adopt it. Tradition oriented GSY manufacturers are expected to be less responsive towards the alternative make process compared to their non-tradition oriented competitors. The factors involved in the decision making process to adopt and implement an alternative make process for GSY is not a simple question of economic viability, and ultimately hinges on the compatibility of the alternative production method to the business strategy of individual GSY manufacturing company as an independent business entity.
BIOGRAPHICAL SKETCH

Davin Bong was born in Jakarta, Indonesia. He started his education in Jakarta but he later moved to Singapore to continue his education. Davin showed great interest in the chemical and life sciences early on in his academic career. He earned a B.S degree in Food Science with Honors in research from the University of Wisconsin-Madison (UW-Madison), during which time he conducted a research on foaming properties of whey proteins under the supervision of Dr John Lucey. Throughout his undergraduate years, Davin received several academic and research awards from the food science department and competed in the Institute of Food Technologist (IFT) product development (PD) team in 2009. Davin also conducted market research and recommended market entry strategies for Roundy’s supermarket as part of his internship program with the company.

After completion of his B.S degree, Davin worked as a Quality Assurance Microbiologist at a beverage company in Jakarta, Indonesia. He came to Cornell University in 2011 to pursue a M.S degree in the field of dairy foods processing technology under the mentorship of Dr Carmen I. Moraru. He participated in the IFT DSDC product development team and successfully led a team of students to the final round of Idaho Milk Processor Association (IMPA) Dairy PD competition with an innovative yogurt product. He also attended and presented his research at the American Dairy Science Association (ADSA) annual meeting in 2013 and was a finalist in the graduate student poster competition at that meeting. Davin has always been inspired by his father’s entrepreneurial spirit and decides to marry his passion in food product development with his interest in entrepreneurship. During his time at Cornell, Davin developed a formulation for an avocado ice cream recipe and wrote a business plan for a dairy free ice cream business. He was awarded the Hussey Family Business Plan Award by Entrepreneurship @Cornell for his effort.

Upon completion of his M.S degree, Davin intends to pursue a career as a product development scientist in the food industry, with the goal of gaining practical experience to become a successful food entrepreneur.
I dedicate this work to my family especially my beloved parents for loving me for who I am and supporting me no matter what I choose to do in life.

**Personal Quotes**

In graduate school, I learned that I know so little and yet I learned so much about the things that I know so little about.

*Buy some humility for your success but never sell your pride to your failure*

*If life were a lecture let discipline be the lecturer, integrity be the student and love be the classroom*
My heartfelt appreciation goes to everyone who has played a role in making this thesis possible.

I would like to express my deepest gratitude to my major advisor, Dr Carmen I. Moraru, for giving me the opportunity to be involved in such an interesting research project and for being a wonderful mentor, teacher and friend. I appreciate very much her advice, guidance and feedback during my time at Cornell, which helped me to be a better inquisitor and presenter of scientific research. I am also sincerely grateful to Dr Deborah Streeter, my Applied Economics and Management advisor, for kindling the fire of entrepreneurship in me and making a committed investment in helping me understand the connection between business and technological innovation.

I would like to thank past and present members of the Moraru lab: Yifan Cheng, Lillian Hsu, Jade Proulx Teng Ju Tan, Anne Sauer, Brittany Miller, Guoping Feng, Wanyu Li, Deepti Ananth, Dongjun Zhao, Markus Walkling-Ribeiro and Iuliana Aprodu for their companionship, technical help, insights and some fun that we share in the lab. My time in the lab would have not been the same without them. I am thankful to the Food Science department faculty and students who have assisted me in one way or another. I would also like to acknowledge the administrative staff of the food science department for their help with navigating the paperwork required for projects and assignments.

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LIST OF ABBREVIATIONS

ARES = Advanced Rheometric Expansion System
ANOVA= Analysis of Variance
BMG = Business Model Generation
CCP = Colloidal Calcium Phosphate
CM = Casein Micelle
CN = Casein
CN:SP = Casein to Serum Protein
CPF = Consumer Packaged Food
DVS = Direct Vat Set
EPS = Exopolysaccharide
FDA = Food and Drug Administration
GSY = Greek Style Yogurt
HSD = Honest Significant Difference
LAB = Lactic Acid Bacteria
*L. bulgaricus = Lactobacillus delbrueckii ssp bulgaricus*
LVR = Linear Viscoelastic Range
MCC= Micellar Casein Concentrate
MPC = Milk Protein Concentrate
NPN = Non Protein Nitrogen
RO= Reverse Osmosis
SD= Standard Deviation
SE= Standard Error
SP= Serum Protein
SNF = Solids Non Fat
SPC = Serum Protein Concentrate

*S.thermophilus* = *Streptococcus thermophilus*

TA = Titratable Acidity

TNP = Total Nitrogen Protein

TP = True Protein

TS = Total Solid

UF = Ultrafiltration

UHT = Ultra Heat Temperature

U.S = United States

WHC = Water Holding Capacity
LIST OF SYMBOLS

\(G'\) = elastic modulus

\(G''\) = viscous modulus

\(\tan \delta\) = ratio of viscous to elastic modulus

\(\eta_{\text{app}}\) = apparent viscosity (\(\text{Pa} \times \text{s}\))

\(\eta_{100}\) = apparent viscosity at shear rate = 100 \(\text{s}^{-1}\)

\(n\) = flow behavior index

\(K\) = consistency coefficient (\(\text{Pa} \times \text{s}^n\))

\(\sigma\) = shear stress (\(\text{Pa}\))

\(\dot{\gamma}\) = shear rate (\(\text{s}^{-1}\))
CHAPTER 1

INTRODUCTION: AN OVERVIEW OF PROCESSING METHODS AND PHYSICO-CHEMICAL PROPERTIES OF YOGURT

Yogurt is a cultured dairy product made from milk that is fermented using *Lactobacillus delbrueckii ssp bulgaricus* (*L.bulgaricus*) and *Streptococcus thermophilus* (*S.thermophilus*) as starter cultures. Fermentation is one of the oldest methods used by human to transform milk into products with extended shelf-life (Tamime and Robinson, 2007). The exact origin of yogurt is unclear, but many believe it originated from the Middle East. Today, yogurt is a general name for a range of fermented milk products that are produced in many countries; approximately 400 generic names are used for traditional and industrialized fermented milk products manufactured around the world (Tamime and Robinson, 2007). Although these products are recognized by different names, they are similar for practical purposes and a more accurate list might include fewer varieties. According to the classification proposed by Robinson et al (2002), yogurt can be defined as thermophilic lactic acid bacteria (LAB) fermented milk product. In certain countries, the term “yogurt” is restricted to a product made exclusively using the two types of LAB in the starter culture, whereas in other countries a product can also be labeled as yogurt when it uses adjunct probiotic cultures such as *Bifidobacterium* spp, *Lactobacillus acidophilus* and *Propionibacterium* spp in addition to the starter cultures (Chandan, 2006).

Yogurt can also be classified according to its physical, chemical and flavor properties (Figure 1.1). The physical states of yogurt products range from liquid in drinkable yogurt to solid in frozen yogurt. Tamime and Deeth (1980) proposed a scheme of classification that categorizes yogurt into four different categories based on their physical characteristics (Table 1.1). The starter culture ferments lactose to lactic acid during the incubation step of yogurt processing.
Lactose is a disaccharide that is naturally found in milk. The gradual accumulation of lactic acid during fermentation results in milk pH reduction. Acidification of milk leads to disruption of the structure of milk proteins. Casein, the major protein present in milk, undergoes structural rearrangement during acidification of milk and coagulates to yield a cohesive gel that is composed of strands of casein micelles with serum proteins entrapped within the matrix mainly by means of hydrogen bond (Damin et al, 2009). The orderly secondary structure of serum proteins, especially whey proteins, is lost as they unfold and become denatured during heat treatment of the milk prior to fermentation. The yogurt gel structure is primarily the result of aggregation of casein micelles. The structure of individual aggregates is stabilized by means of hydrophobic interactions and electrostatic attraction between casein micelles. However, the strength of the casein aggregates gel network is further reinforced by means of formation of covalent disulfide bonds between κ-casein on the surface of casein micelles and denatured whey proteins during the heat treatment step (Damin et al, 2009; Lee and Lucey, 2010; Haque et al, 2010).

<table>
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*Table 1.1. Proposed scheme of classification of yogurt products.*

Figure 1.1 Generalized scheme for classification of yogurt.
Source: Tamime and Robinson (2007)

**Yogurt processing**

*Standardization* of milk with milk fat (cream) is the first processing step in commercial production of yogurt and is conducted to achieve the desired fat and Solids Non Fat (SNF) content of the yogurt milk base (Lucey, 2004). Standardization of milk fat for yogurt production is typically carried out to meet legal compositional standards for yogurt and this ranges from as low as 0.1 grams to 10 grams of milk fat per 100 grams of yogurt in different parts of the world (Tamime and Robinson, 2007). Standardization of fat content of yogurt milk base is conducted by partially removing the fat content of milk, mixing full fat milk with skimmed milk or addition of cream to skim milk. The required SNF (mainly lactose, protein and mineral) content of yogurt ranges from 8.2 g to 8.6 g/100 g; the lower limit serves to protect customers from adulterated products (Tamime and Robinson, 2007). Besides achieving the legal minimum standard of SNF content for yogurt, standardization of the fat and SNF content of yogurt milk is also done to tailor the sensory properties and shelf-life of the product in accordance to the manufacturer’s
preference (Tamime and Robinson, 2007). The SNF content of milk can be increased by several methods, which include the following:

1. Addition of milk protein (e.g. milk protein concentrate)
2. Concentration techniques (e.g. membrane filtration, spray drying and evaporation)
3. Addition of non-protein solids (e.g. lactose, vitamins, minerals)

*Homogenization* of the yogurt milk base is the next important step in the industrial manufacture of yogurt. This step is important for reducing the size of fat globules and prevents the clustering of fat globules which may otherwise give rise to a separate fat layer in the final product. Homogenization of yogurt milk base typically occurs in two stages. In the first stage, fat globules in milk are reduced to an average of less than 2 μm in diameter using high pressures of 10-17 MPa at a temperature of 55-71 °C (Lucey, 2004). The purpose of the second stage of homogenization is to prevent the clumping of fat globules following the first and to improve the efficiency of the first by supplying a constant and controlled back pressure (Chandan, 2006). The second stage of homogenization is typically carried out at a lower pressure than the first (~ 3.4 MPa) (Lucey, 2004). Homogenization also helps to minimize the occurrence of graininess and lumpiness in yogurt due to aggregation of fat globules (Chandan, 2006). Additionally, homogenization helps promote the interaction between milk components, most notably the binding of casein and whey proteins at the interface of the newly formed fat globules, which is helpful for the stabilization of fat globules and increasing the number of structure building components (Tamime and Robinson, 2007). Homogenization of yogurt milk is also advantageous for the quality of yogurt because it can help to reduce whey separation and increases the intensity of the product whiteness (Tamime and Robinson, 2007). It has been shown that a higher homogenization pressure generally leads to increased firmness and viscosity.
of yogurt because of an increase in the availability of surface area caused by the formation of larger number of smaller fat particles (Lucey, 2004).

**Heat treatment** is the next critical processing step in the manufacture of yogurt. Heat treatment of milk is the manufacture of yogurt has a great influence on the physical properties and microstructure of the final product (Remeuf et al, 2003; Lucey et al, 1998; 1999). The time-temperature combination that is most commonly used for heat treatment of the milk base for yogurt production is in the upper range of pasteurization (85°C for 30 minutes or 90-95°C for 5 minutes) (Tamime and Robinson, 2007). The primary purpose of heat treatment of yogurt milk is to destroy unwanted microorganisms in the milk, which helps reduce the competition for the starter culture (Lee and Lucey, 2010). Milk pasteurization is sufficient to destroy the majority of vegetative cells of both pathogenic and spoilage microorganisms in milk. However, spores and heat resistant enzymes in milk will not be inactivated (Tamime and Robinson, 2007). In the case of UHT treatment, both vegetative cells and spores present in yogurt milk base are destroyed. Heat treatment of yogurt milk base is also important for imparting a good textural attribute to the final product. In addition, heat treatment is also helpful for removing dissolved oxygen in milk, which is helpful for the growth of microaerophilic LAB in the starter culture (Lee and Lucey, 2010; Chandan, 2006). One of the most important factors to consider in the selection of time-temperature combination for heat treatment is its effect on the physicochemical properties of milk, as this can greatly influence the physical properties of the final yogurt product.

**Inoculation** of the yogurt milk base with starter culture is conducted after cooling it to yogurt incubation temperature. Yogurt fermentation is a biological process and cooling of yogurt milk base to a temperature that is optimum for the growth of the starter culture is critical for a successful fermentation. The fermentation of yogurt is typically carried out using LAB starter
culture consisting of L. bulgaricus and S. thermophilus. The incubation temperature of yogurt ranges from 40-45°C and the process typically takes about 3-6 hours (Lucey, 2004; Lee and Lucey, 2010). This temperature range includes the optimum temperature for the growth of both L. bulgaricus and S. thermophilus (Lee and Lucey, 2010). The optimum growth temperature for L. bulgaricus is 45°C and a temperature of 42-43°C may be used to accommodate the lower optimum growth temperature of S. thermophilus (Chandan, 2006). It is well established that S. thermophilus is active during the first stage of fermentation (pH range ~ 6.6 to 5.4). L. bulgaricus and S. thermophilus display associative growth in yogurt fermentation because they are able to utilize each other’s metabolites to increase their fermentation activity (Tamime and Robinson, 2007). L. bulgaricus possesses significantly more proteolytic enzymes compared to S. thermophilus and these enzymes hydrolyze milk proteins to produce peptides and amino acids which have stimulatory effect on the growth of the starter culture (Tamime and Robinson, 2007; Chandan, 2006). During the second stage of fermentation (pH range~5.4 to 4.6), L. bulgaricus starts to grow rapidly and this growth is stimulated by the production of formic acid and CO₂ by S. thermophilus (Chandan, 2006). The production of CO₂ also helps to remove oxygen, which in turn stimulates the activity of the microaerophilic starter culture (Chandan, 2006). Depending on the type of yogurt produced, the end point of yogurt fermentation ranges from pH 4.5 to 4.7 (Lucey, 2004). The primary objective of cooling yogurt after fermentation to <10°C in the shortest time possible is to minimize the fermentation activity of L. bulgaricus and S. thermophilus and control acidity of the final product (Tamime and Robinson, 2007). The cooling process of yogurt may occur in one or two phases (Tamime and Robinson, 2007). In one phase cooling, the yogurt is cooled directly from the incubation temperature to <10°C. For fruit-on-the-bottom and stirred style yogurt, flavor ingredients and stabilizers (e.g. fruit pieces and pectin) are
typically added after the cooling process, before packaging (Tamime and Robinson, 2007). In two-phase cooling, the first phase reduces the temperature of yogurt from the incubation temperature to about 20°C, followed by addition of non-dairy ingredients for fruit-on-the-bottom and stirred style yogurt and packaging of yogurt into retail containers. The yogurt gel is less viscous at 20°C and the product can be disturbed (e.g. pumping) with minimal structural damage (Tamime and Robinson, 2007). Therefore, two-phase cooling of yogurt is usually the preferred cooling method for yogurt in the dairy industry. The second phase cools the packaged yogurt to <10°C where the product is left undisturbed before storage (Tamime and Robinson, 2007).

**Refrigerated storage** at 4-7°C for at least 24 hours is the final step in yogurt processing. This is done to give the final product a desirable textural attribute and, more importantly, to extend its shelf-life by slowing down the rate of physicochemical and microbiological degradation in the product (Chandan, 2006). A summary of all the main processing steps involved in the industrial manufacture of various types of yogurt is shown in Figure 1.2.
Figure 1.2. Flow chart of the main processing steps in the manufacture of plain set style, fruit on the bottom style and stirred style yogurt

Source: Lucey, 2004
An overview of production technology for concentrated yogurt

Concentrated yogurt is defined as a semisolid yogurt obtained by draining away part of the yogurt’s serum phase and its water soluble components (Nsabimana et al, 2005). Like regular yogurt, concentrated yogurt is typically fermented using *L. bulgaricus* and *S. thermophilus* as the starter culture. Figure 1.3 summarizes the various methods that can be used in production of concentrated yogurt. Concentrated yogurt is traditionally made by straining yogurt after fermentation to reach the desired solids content. This simple method consists of hanging yogurt in a cloth bag overnight to remove some of the whey by gravity and achieve the semi-solid consistency expected in concentrated yogurt (Ozer et al, 1998; Nsabimana et al, 2005). Pressure may be applied using a vertical press to shorten the whey drainage process (Nsabimana et al, 2005). Exopolysaccharide (EPS) producing starter culture strains are not suitable for this process because they reduce whey separation in yogurt gels and consequently the whey removal process will require a longer time (Tamime and Robinson, 2007). Concentrated yogurt is typically manufactured by mechanically separating the serum phase which in the dairy industry is typically achieved using a centrifugal separator (Nsabimana et al, 2005). The whey separation process helps concentrate the milk proteins (mainly casein) in the yogurt gel and remove lactose from the product. An alternative method involves elevating the solids and protein content of concentrated yogurt by means of Ultrafiltration (UF). UF can be applied to fermented yogurt immediately at the end of fermentation or it can be used to concentrate proteins in yogurt milk prior to fermentation (Ozer et al, 1998). In the latter, the UF retentate may be fermented in retail containers as in the case of set-style yogurt (Nsabimana et al, 2005). The firmness of UF retentate concentrated yogurt product is much greater in comparison to similar product made using the traditional straining method or UF of warm, fermented yogurt (Tamime et al, 1989).
Most of the whey proteins, lactose and minerals in milk prior to fermentation are removed from the retentate during UF. Consequently, the concentration of casein in the UF retentate increases. The UF retentate is then heat-treated, cooled to incubation temperature (40-45 °C), fermented at the incubation temperature until the desired level of acidity and cooled overnight at refrigeration temperature (5-7°C) before it is ready for consumption (Tamime et al, 1989). In the case of UF treatment of fermented yogurt, pasteurized milk standardized for its solids and fat content is preheated to about 60°C, homogenized and similar to processing of regular yogurt, heat treated and then cooled to the incubation temperature. After fermentation, the warm yogurt is cooled to 40°C and concentrated in a two-to-four stages UF process, cooled to about 20°C before it is packaged for retail or wholesale (Nsabimana et al, 2005).
Figure 1.3. Flow Chart of the main processing steps in concentrated yogurt production using quark separator, UF and traditional straining in a cloth bag method.

This figure has been reproduced by courtesy of Chr Hansen, Milwaukee, WI
The role of milk proteins in yogurt fermentation

Caseins are phosphoproteins with no proper tertiary protein structures, which precipitate at an isoelectric pH of 4.6. They make up about 80 % of the proteins in bovine milk and are further subdivided into four distinct protein molecules: α\textsubscript{S1}-casein, α\textsubscript{S2}-casein, β-casein and κ-casein. More than 95% of casein molecules in milk are organized into casein micelles (Haque et al, 2001). Casein micelles (CM) are polydisperse, roughly spherical particles ranging from 50 to 600 nm, with a weighted average diameter of 200 nm (de Kruif, 1998). Approximately 94 % of the volume of a casein micelle is composed of the four types of casein molecules and the rest is mostly made up of calcium and phosphate salts that are organized into insoluble threads of calcium phosphate network called colloidal calcium phosphate (CCP). CCP stabilizes internal structure of CM by suppressing the electrostatic repulsion between the phosphoserine residues of casein (Haque et al, 2001; Lee and Lucey, 2010). In addition, hydrophobic interactions between individual caseins in CM also help to maintain their structural integrity. Various CM models have been developed in the past and some were dismissed more quickly than others because they are unable to elucidate some functions or properties of CM. Despite the fundamental differences among these models, there is a general agreement related to some aspects of the structure of the CM, particularly about the location of the κ-casein on the surface of casein micelles and its role in preventing the aggregation of micellar casein through steric and electrostatic repulsion (Walstra, 1990; Holt and Horne, 1996). The sub-micelle model is one of the longest lasting models for CM. In this model, each individual CM was thought to be composed of 15-25 sub-micelles with a size of about 10-15 nm which are linked together by CCP (Walstra et al, 1984). However, there is yet to be a consensus on the validity of any single model. A model developed by Horne (1998) based on the sub-micelle model, describes the CM as a roughly spherical
structure with the α and β caseins clustered in the center of the micelle while κ-casein molecules lie at the surface of the micelle with its glycosylated C-terminal amino acids protruding to form a “hairy layer” which acts as both steric and electrostatic barrier to aggregation of casein micelles. Horne (1998) proposed that hydrophobic interactions and CCP are the two main forces that stabilize the internal structure of CM.

Figure 1.4. Schematic diagram of the Horne dual-binding model for the casein micelle. Rectangular bars and black lines represent hydrophobic and hydrophilic segments, respectively of the protein sequence.

Source: Horne (1998)
The structure of the casein micelle is essential in the processing of milk into fermented milk products such as yogurt (Horne, 1998). In commercial yogurt production, the extended heat treatment step of yogurt milk causes denaturation of whey proteins. Denaturation of β-lactoglobulin, a globular whey protein that constitutes 7-12% of total whey protein in milk, is of great importance in yogurt making (Chandan, 2006). The unfolding of β-lactoglobulin exposes sulfhydryl containing cysteine residues that are buried in the hydrophobic core of the native protein and causes association of denatured β-lactoglobulin to the surface of casein micelles by means of disulfide bonds between the cysteine residues located on the N-terminal region of the κ-casein molecules and their counterparts on the denatured β-lactoglobulin molecules (Haque et al, 2001). This cross-linking of whey proteins and casein introduces another steric barrier against aggregation of CM (Horne & Davidson, 1993). Acidification of milk during lactic fermentation decreases the net negative charge on CM and correspondingly there is an increasing electrostatic attraction between casein molecules as the pH of milk decreases to the isoelectric pH of casein (Lucey, 2004). The sequence of events leading to the aggregation of casein micelles during yogurt fermentation can be discussed for three distinct pH regions (Lee and Lucey, 2010):

1. pH 6.5 to 6.0: Initial acidification decreases the net negative charge of casein micelles, which leads to the weakening of electrostatic repulsion between CM. Solubilization of CCP is minimal above pH 6.0 and the integrity of CM is maintained.

2. pH 6.0 to 5.0: Further acidification from continued fermentation results in solubilization of CCP within casein micelles, which leads to partial arrangement of the internal structure of casein micelles and electrostatic repulsion between exposed phosphoserine residues of individual α and β casein molecules.
The net result of these two events is the weakening of the internal structure of CM. The net negative charge on CM is greatly reduced at this pH region partly because of shrinking of negatively charged κ-casein “hairs”. The dissociation of phosphate groups from casein micelles as a result of solubilization of CCP may also be partly responsible for the decrease in net negative charge of casein micelles. This causes further weakening in electrostatic repulsion between casein micelles. The size of individual casein micelles also shrinks because of the disruption of its internal structure due to CCP solubilization during acidification.

3. pH 5.0 to 4.5: As pH of milk approaches the isoelectric point of casein, further solubilization of CCP causes a further decline in the electrostatic repulsion between CM, which increases attraction between individual casein molecules within the micelle and promotes aggregations of CM. Hydrophobic interactions also help to cement the attraction between casein molecules. The end result of acidification of milk is the formation of a three dimensional network of caseins stabilized by a localized balance of electrostatic forces and hydrophobic interactions with disulfide crosslinks between denatured whey proteins and κ-casein on the surface of casein micelles.

**Physical properties of yogurt**

**A. Rheological properties of yogurt**

Dynamic (small deformation) and steady shear (large deformation) rheological studies can provide much useful information on characteristics of food gels such as yogurt (Figure 1.5). Knowledge about rheological properties of yogurt is useful for gaining an understanding of the textural properties of the product and for optimization of the process and equipment design in yogurt manufacturing. The gel network of yogurt involves specific interactions between whey proteins and aggregated casein (Rao, 2007). Rheological properties of yogurt can give important
information about microstructure of the product. Past studies suggest that yogurt can be considered as a material that has a “weak gel” system with soft viscoelastic solid behavior (Rao, 2007; Kasapis and Boskou, 2001). Characterization of rheological properties of yogurt can be conducted during the gel formation process (fermentation) and on the final product.

Figure 1.5. Rheological tests used in food characterization.

Source: Munizaga and Barbosa-Canovas (2005)
**Dynamic rheological properties of yogurt**

The objective of characterizing the dynamic rheological properties of yogurt is to provide information on intermolecular interactions and bonding characteristics of the gel structure (Tunick, 2011). Dynamic rheology typically uses small deformation and is not expected to destroy the weak gel structure typically found in yogurt. Dynamic rheological parameters (G’, G”, G* and tan δ) are typically used to characterize the viscoelastic behavior of yogurt in the linear viscoelastic range (LVR), a region in which the dynamic rheological parameters of a material are independent of the applied strain (or stress) (Steffe, 1996). The elastic modulus (G’) measures energy stored per deformation cycle of the material and is indicative of solid like behavior, while the viscous modulus (G”) expresses the amount of energy lost as viscous dissipation per deformation cycle for the material and reflects liquid like behavior. The complex modulus (G*) is the combined magnitude of the elastic and viscous moduli while tan δ represents the ratio of G” to G’. High tan δ values indicate that the material has a pronounced liquid like behavior, while low tan δ values mean that the material behaves more like a solid (Rao, 2007).

The first step used in the characterization of these parameters is a strain (or stress) sweep which is conducted to establish the linear viscoelastic range (LVR) of the viscoelastic material being tested. Yogurt is a particulate weak gel system that exhibits a high frequency dependence of the dynamic modulus which suggests the occurrence of relaxation processes even at short time scales, and a low difference between G’ and G” values is typically observed for yogurt, indicating that a low proportion of the stored energy in the gel is recovered (Rao, 2007). A frequency, time or temperature sweep can be subsequently carried out to monitor changes in G’ and G” as a function of experimental timescale, time or temperature respectively at a strain value.
in LVR of the sample (Lee and Lucey, 2010). Frequency sweeps can be used to elucidate the type of gel network present in the yogurt and linkages that bond the proteins together in the network (Tunick, 2011). Previous studies have established that yogurt exhibits characteristics typical of a weak viscoelastic gel, with $G'$ greater than $G''$ at the range of frequency being investigated (Hassan et al, 2003; Haque et al, 2001). Yogurt milk has a low magnitude of $G'$ and $G''$, with $G'' > G'$, prior to fermentation. The development of $G'$ and $G''$ during yogurt fermentation can be monitored using a time sweep. The magnitude of $G'$ and $G''$ increases steadily until the onset of gel network formation during which both $G'$ and $G''$ increase sharply by several orders of magnitude over a short period of time (Haque et al, 2001). $G'$ eventually surpasses $G''$ at the end of fermentation, indicating a pronounced solid-like behavior (Tunick, 2011). Characterization of the dynamic rheological properties of yogurt during fermentation can be useful in determining the gelation point of yogurt and the pH at which this occur. Gelation point is correlated to fermentation time for yogurt, which is an important decision factor in starter culture selection for yogurt manufacturers. The gelation point of yogurt can be determined using the cross-over point, defined as the time when $G'$ becomes greater than $G''$ ($G'$-$G''$ crossover).

The dynamic rheological parameters of yogurt are expected to change during storage, since structural changes in yogurt continue to occur during storage as bonds continue to be built and broken down. Some of the important factors that influence the extent of this change include solids & protein content (Marafon et al, 2011), amount of starter culture for inoculation (Lee and Lucey, 2004) and incubation temperature (Lee and Lucey, 2004; Haque et al, 2001). Marafon et al (2011) found that yogurt fortified with whey protein concentrate showed a significant increase in $G'$ value during storage at 4°C for 14 days, whereas yogurts made from unfortified milk
exhibited a steady decrease in $G'$ value for the same storage condition and period. Haque et al (2001) reported that increased incubation temperature led to an increase in $G'$ values of the yogurt samples after stored at 5°C for 2 days. Lee and Lucey (2004) found that a lower amount of starter culture used for inoculation could significantly lower $G'$ for yogurt. In addition, they reported that an increase in incubation temperature led to a decrease in $G$ of the yogurt samples, which was attributed to decreased contact area for interactions among casein particles (Lucey, 2002).

**Flow behavior of yogurt**

The flow behavior of yogurt can be determined using a steady shear rate sweep (Ramaswamy and Basak, 1991; Lee and Lucey, 2006; Lee and Lucey, 2010). Yogurt exhibits non-Newtonian behavior, with viscosity changing as a function of shear rate. The apparent viscosity of yogurt decreases with an increase in shear rate and this is called a shear thinning behavior (Lee and Lucey, 2006).

The flow curves (viscosity vs. shear rate) of yogurt have been fitted to several rheological models, most commonly to the power law model (Keogh and O’Kennedy, 1998; Geraghty and Butler, 1999). It is meaningful to note that the power law model does not take into account yield stress of the yogurt gels. Yield stress is defined as the minimum shear stress required to initiate flow (Steffe, 1996). Stirred yogurt has a yield stress, unless no time for recovery of the structure is allowed after stirring (Lee and Lucey, 2006). The absence of yield stress from the power law model may be a potential drawback when modeling the flow curves of stirred yogurts. Flow behavior models that include yield stress as a parameter were chosen to describe the flow properties of stirred yogurts in several studies (Ramaswamy and Basak, 1991; van Marle et al,
These include the Casson (Skriver et al, 1993; Lee and Lucey, 2006) and Herschel-Buckley (Ramaswamy and Basak, 1991) models. Although these models can adequately describe the flow behavior of stirred yogurts at low shear rate (0.01 to 0.1 s\(^{-1}\)), they have been shown to be not satisfactory at high shear rate (10 to 600 s\(^{-1}\)) (Lee and Lucey, 2006). All the models discussed so far have a parameter called flow behavior index, which measures the deviation of a material from Newtonian flow and offers an indication of the rate of structure change with a change in shear rate (Beliciu and Moraru, 2011).

**Rheological properties of concentrated yogurt**

The rheological properties of concentrated yogurt have been studied extensively (Ozer et al, 1998; Mohameed et al, 2004; Kasapis and Boskou, 2001; Ozer et al, 1997; Abu-Jdayil et al, 2000; Abu-Jdayil et al, 2002). Some of these studies primarily looked at the effect of processing methods on the rheological properties of concentrated yogurt (Ozer et al, 1998; Abu-Jdayil et al, 2000; Abu-Jdayil et al, 2002, Tamime et al, 1989). Ozer et al (1997) have investigated the rheological properties of labneh (a type of full fat concentrated yogurt widely consumed in the Middle East) made using different methods of production. They found that labneh, much like regular yogurt, is a viscoelastic gel in which G’ > G”, which indicates a predominantly solid-like behavior. Differences in the rheological properties of labneh were attributed to differences in the concentration methods (straining, UF & RO) and the severity of mechanical agitation during processing. Differences in concentration method led to differences in the protein content of the final product, which is the principal factor affecting the rheological properties of yogurt (Ozer et al, 1999; Prentice, 1992). Among the concentration methods investigated in these studies, UF was found to be the most promising in producing labneh that closely resembles the traditional strained product as evaluated by its firmness and visual appearance (Tamime et al, 1989). Similar
Several studies have described the flow behavior of labneh as shear thinning, regardless of the experimental variables investigated (Abu-Jdayil and Mohameed, 2002; Mohameed et al, 2004; Abu-Jdayil et al, 2000). Labneh was shown to exhibit thixotropy, a time dependent thinning behavior at a constant shear rate (Abu-Jdayil and Mohameed, 2002; Mohameed et al, 2004; Abu-Jdayil et al, 2000). The thixotropic behavior of labneh was also found to be correlated with solids content of the product (Mohameed, et al, 2004). Abu Jdayil et al (2000) suggested that the shear thinning behavior of concentrated yogurt was attributed to the destruction of weak non-covalent forces such as electrostatic and hydrophobic interactions at the energy level of the flow shear input, which in turn could affect the texture of the product (Kinsella, 1984). Abu-Jdayil and Mohameed (2002) observed that for the same range of shear rate, the apparent viscosity of labneh increased with storage time. This was attributed to the protein interactions that occurred during storage, which led to a rearrangement of the protein network (Ross-Murphy, 1990). The increase in apparent viscosity during storage is advantageous when a firm product is desired. Another important rheological behavior of labneh is the frequency dependence of $G'$. Labneh displayed characteristics of “weak gels” with a strong dependence of $G'$ on frequency (Ozer et al, 1997; Kasapis and Boskou, 2001; Tunick, 2011). Differences in the extent of labneh’s viscoelastic behavior have been attributed to a dissimilarity in processing methods. According to Ozer et al (1998), high $G'$ values indicate that strained labneh had the firmest gel structure among the labneh samples investigated. Labneh produced by UF had the next greatest $G'$ value, while labneh prepared by RO had the lowest $G'$ value, indicating a loose gel structure (Ozer et al, 1998). The microstructure of RO and UF processed labneh showed some
discontinuity between strands of casein aggregates because of the shearing of casein aggregates during membrane processing (Ozer et al, 1998). Ozer et al (1998) concluded that the structural damage of labneh was a function of shearing intensity of the production method. The higher pressure associated with RO treatment might have caused a greater structural damage of labneh, due to a greater disruption of casein aggregates, while the lower transmembrane pressure of UF might have led to a smaller extent of structural damage because casein aggregates were stretched rather than disrupted (Ozer et al, 1998). Structural damage in labneh was correlated to poor textural attributes of the product, and the authors concluded that membrane processing with a low shearing intensity would be the most suitable method to produce labneh.

**B. Whey separation in yogurt**

Whey separation or syneresis in yogurt represents the expulsion of whey from the yogurt gel network which then becomes visible as surface whey (Lucey, 2010). Whey separation is considered as a common defect in yogurt, and a high level of whey separation may negatively affect consumers’ perception of the product (Amatayakul et al, 2006; Lucey, 2010). The type of syneresis that occurs naturally in yogurt is spontaneous syneresis, which is the contraction of intact yogurt gel network without the application of any external forces (Lucey, 2010). Non-spontaneous syneresis, on the other hand, is induced by the application of an external force (e.g. high shear, centrifugation or pumping), which causes expulsion of whey from yogurt gel network under pressure. The latter is often taken advantage of in tests that measure the susceptibility of yogurt to syneresis during distribution and storage. Whey separation due to spontaneous syneresis is relevant to set style yogurt, while non-spontaneous syneresis is more relevant to stirred style yogurt (Lucey, 2010).
Evaluation of whey separation in yogurt is typically done by quantifying the amount of surface whey that is expelled from the gel. The drainage method measures the level of whey separation from yogurt gels under the influence of gravity (Amatayakul, Sherkat and Shah, 2006; Lee and Lucey, 2010). This method is useful for quantifying the level of whey separation in products that have a serum separation step such as concentrated yogurt and cottage cheese (Lucey and Singh, 1998; Lee and Lucey, 2010). The centrifugation method is more suitable to measure the level of whey separation in yogurt gels that have been subjected to shear after fermentation, such as stirred yogurt. The centrifugation method measures the level of whey separated from collapsed gel network as a result of centrifugal force (Amatayakul, Sherkat and Shah, 2006; Lee and Lucey, 2010). Amatayakul, Sherkat and Shah (2006) investigated the level of whey separation in set yogurt with different solids content and different characteristic of EPS produced by the starter cultures. They observed significant differences in the level of whey separation for the same yogurt sample when different evaluation methods were used. This suggest that these methods do not give similar information about whey separation in yogurt gels (Amatayakul, Sherkat and Shah, 2006)

The protein composition of yogurt is an important factor that determines the extent to which yogurt is able to retain whey in the gel network (Puvanenthiran et al, 2002; Amatayakul et al, 2006). In addition, solids content of the yogurt milk base (Amatayakul, Sherkat and Shah, 2006; Jaros et al, 2002; Hassan et al, 1996), inoculation level (Lee and Lucey, 2004), EPS characteristics of the starter culture (Amatayakul et al, 2006; Amatayakul, Sherkat and Shah, 2006) and yogurt incubation temperature (Lee and Lucey, 2004) are also important factors that influence the extent of whey separation in yogurt gels.
One of the indicators of the protein composition of yogurt is the casein to serum protein ratio (CN:SP ratio), which is influenced by various factors including addition of dried dairy ingredients, membrane separation techniques and seasonal variation of milk. Amatayakul et al (2006) observed that syneresis in yogurt generally decreased when the CN:SP ratio was reduced, regardless of the type of starter culture used. This was attributed to the microstructure of yogurt gels network becoming finer and the protein matrix becoming denser as the CN:SP ratio of yogurt decreased (Lucey, 2001; Puvanenthiran et al, 2002; Amatayakul et al, 2006). The compactness of the gel structure of yogurt with low CN:SP ratio also led to a firmer yogurt (Lucey, 2004). The increase in compactness of yogurt microstructure decreased the size of pores in the gel network, which in turn led to immobilization of a high amount of free water (Puvanenthiran et al, 2002; Amatayakul et al, 2006). In addition, whey proteins are known to have a high water binding capacity, which helps to retain water in the gel structure (Cerning, 1990; DeVuyyst and Degeest, 1999). The addition of dried milk protein ingredients to yogurt milk base can also help reduce whey separation in yogurt (Fox, 2009).

Certain strains of *L. bulgaricus* and *S. thermophilus* in yogurt starter culture are able to produce EPS, a group of heteropolysacharides that give yogurt a slimy texture (Amatayakul et al, 2006). EPS produced by the yogurt starter culture exists in two forms: capsular EPS, which is attached to bacterial cell surface, and ropy EPS, which is secreted into the protein matrix (Cerning, 1990). Some bacteria can produce both forms of EPS. EPS is also known to possess a high water binding capacity (Cerning, 1990; De Vuyst and Degeest, 1999). While it is not entirely clear how EPS reduces syneresis in yogurt, de Kruif (1998) suggests that EPS and casein have similar charges at pH values above the isoelectric point of casein, and thus are incompatible with casein above pH 4.60. This incompatibility may be due to depletion-induced repulsion of
casein micelles with EPS, in which EPS was assumed to be excluded from the surface of casein micelles, resulting in a depletion layer (Hassan et al, 2003). Therefore, the presence of EPS in the yogurt gel microstructure may trigger a different gelation mechanism compared to cases in which non-EPS producing cultures are used. The result is a more compact protein network structure characterized by EPS filled gel network and smaller pores which potentially help to reduce whey separation in yogurt.

REFERENCES


CHAPTER 2
RESEARCH OBJECTIVES AND JUSTIFICATION

The rising popularity of Greek style yogurt (GSY) in recent years makes it one of the most desirable yogurt products in the dairy aisle of supermarkets. However, the generation of large amounts of acid whey from traditional GSY manufacturing can potentially be a limiting factor in the growth of this trendy yogurt product. The use of micellar casein preparations obtained by microfiltration has been receiving increased interest from the dairy and food industry. The casein micelle in these preparations remains close to its native state during the microfiltration process which gives them unique functionality as a dairy ingredient. Micellar casein concentrate (MCC) is a novel, emerging dairy food ingredient that has a variety of potential commercial applications in foods, including increasing the protein content of yogurt. The use of MCC as a protein fortification ingredient in GSY production can help elevate the protein content of the product to the desired level without whey removal. If this method were to become available for commercial manufacture of GSY, it has the potential to mitigate the economic and environmental challenges posed by acid whey in traditional GSY manufacturing.

The objectives of this thesis were:

1. Develop and optimize an alternative make process for GSY in which the desired composition of the product is reached by fortification with MCC instead of whey removal.
2. Evaluate the physical and chemical properties of GSY fortified with MCC and compare them with those of GSY made from traditional straining process.
3. Investigate the feasibility of adoption of the alternate manufacturing approach for GSY using MCC by GSY producers from the standpoint of business strategy with the aid of a business model framework.
CHAPTER 3

EFFECT OF MICELLAR CASEIN CONCENTRATE FORTIFICATION ON THE CHEMICAL AND PHYSICAL PROPERTIES OF GREEK STYLE YOGURT

ABSTRACT

The objective of this work was to develop and optimize an alternative make process for GSY, in which the desired level of protein is reached by fortification with Micellar Casein Concentrate (MCC) obtained by microfiltration. MCC preparations with 2 levels of total protein: MCC-58 and MCC-88 were used to fortify yogurt milk to 9.80 % (w/w) protein. Strained GSY of similar protein content was used as control. Yogurt milk bases were inoculated with 0.02 % (w/w) or 0.04 % (w/w) Direct Vat Set starter culture and fermented until pH 4.5. The acidification rate was faster for the MCC fortified GSY than the control, regardless of the inoculation level, which was attributed to a higher level of non-protein nitrogen content in the MCC fortified milk. Steady shear rate rheological analysis indicated a shear-thinning behavior for all GSY samples, which fitted well with the power law model. Dynamic rheological analysis at 5°C showed a weak frequency dependency of the elastic modulus (G’) and viscous modulus (G”) for all GSY samples, with G’>G”, which indicated a weak gel structure. Differences in the magnitude of viscoelastic parameters between the two types of GSY were found, with G’(MCC fortified GSY) < G’(control), indicating a different extent of protein interactions in the two types of yogurt. Differences were also noticed in water holding capacity, which was lower for the MCC fortified GSY compared to the control, which was attributed to a lower serum protein content in the former. Despite some differences in the physico-chemical of
the final product, the developed alternate process is a feasible alternative to the traditional GSY manufacturing process, with environmental and possibly financial benefits to the dairy industry.

**INTRODUCTION**

The rising popularity of Greek Style Yogurt (GSY) is one of the most remarkable events in food production and sales in recent history. GSY is traditionally made by straining fermented yogurt curd in a cloth bag to reach the desired solids level by removing acid whey. This step is achieved by mechanically separating the whey from the curd using either a centrifugal separator or membrane filtration (Nsabimana et al., 2005). The production of large quantities of acid whey presents both economic and environmental challenges (Dairyreporter, 2013; Foodnavigator USA, 2013). An alternate make process for GSY that eliminates the acid whey removal might therefore be attractive to GSY producers. The alternate processes that are currently available involve fortification of milk with milk protein concentrates to enhance the protein content of the final product. Micellar casein concentrate (MCC) obtained by microfiltration is an emerging protein ingredient, which is garnering increased interest from the dairy industry (Mulvihill and Ennis, 2003; Nelson and Barbano, 2005; Afferstsholt, 2009; Sauer and Moraru, 2012), and is a good candidate for protein fortification of yogurt milk base.

The change in chemical composition of yogurt milk base as a result of protein fortification has been shown to influence the rheological and physical properties of yogurt (Prentice, 1992; Skriver et al, 1999; Peng et al, 2009; Lee and Lucey, 2010). Several studies investigated the effects of fortification of milk with whey protein concentrates and caseinates on the physical properties of yogurt (Peng et al, 2009; Isleten and Karagul-Yuceer, 2006; Remeuf et al, 2003; Akalin et al, 2012; Marafon et al, 2011), but comparatively little work has been done to
investigate the effect of micellar casein fortification on the make process and properties of yogurt (Peng et al, 2009).

The objectives of this study were: 1) develop and optimize an alternate make process for GSY, in which the desired level of protein is reached by fortification with MCC; and 2) evaluate the effect of MCC fortification on the chemical and physical properties of GSY as compared to GSY made using a traditional straining process.

MATERIALS AND METHODS

Materials

Commercial pasteurized skim milk (Crowley Foods, Binghamton, NY) was obtained from a local grocery store and stored at 4°C until use. The chemical composition of skim milk was tested at the Dairy One Laboratory (Ithaca, NY), and the following parameters were determined: total nitrogen protein (TNP), true protein (TP), total solids (TS), casein (CN), fat, lactose and ash. The average composition of the milk is shown in Table 3.1. Commercial spray dried micellar casein concentrate with 88 % TNP (w/w) (MCC-88) obtained from American Casein Company (Burlington, NJ) and dried micellar casein concentrate with 58% TNP (w/w) (MCC-58) produced by Dr. Barbano’s research group at Cornell University, obtained using the methodology described in Hurt et al (2010), were used for fortification of skim milk. The chemical composition of MCC-58 and MCC-88 was tested at the Dairy One Forage Analysis Laboratory (Ithaca, NY), using the methodology described by Beliciu and Moraru (2012). A commercial sample of plain GSY (Chobani Inc., Norwich, NY) sourced from a local grocery store was used for comparison purposes. The chemical composition of the commercial GSY sample is shown in Table 3.2
Table 3.1. Chemical composition of skim milk, MCC-58 and MCC-88 on dry solids basis (w/w).
Values represent means of triplicate measurements (n=3).

<table>
<thead>
<tr>
<th>Composition</th>
<th>TS (%)</th>
<th>TNP¹ (%)</th>
<th>TP² (%)</th>
<th>NPN³ (%)</th>
<th>CN (%)</th>
<th>Fat (%)</th>
<th>Lactose (%)</th>
<th>Ash (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skim milk</td>
<td>9.35</td>
<td>3.38</td>
<td>3.19</td>
<td>0.19</td>
<td>2.67</td>
<td>0.13</td>
<td>4.97</td>
<td>0.68</td>
</tr>
<tr>
<td>MCC-88</td>
<td>92.90</td>
<td>87.93</td>
<td>87.51</td>
<td>0.42</td>
<td>74.74</td>
<td>2.11</td>
<td>0.53</td>
<td>9.47</td>
</tr>
<tr>
<td>MCC-58</td>
<td>96.99</td>
<td>57.64</td>
<td>56.35</td>
<td>1.29</td>
<td>50.81</td>
<td>2.16</td>
<td>30.54</td>
<td>9.63</td>
</tr>
</tbody>
</table>

¹TNP = total nitrogen × 6.38

²TP = true protein × 6.38

³ NPN = TNP – TP

Starter culture
A freeze dried Direct Vat Set (DVS) yogurt culture (FD-DVS YC-380) containing a mixed strain culture of non EPS producing strains of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* was obtained from Chr. Hansen, Inc. (Milwaukee, WI, USA). The culture was stored at -80°C until use.

Preparation of GSY milk bases
Skim milk was heated to 40 °C on a bench top stirring hot plate (Thermoscientific, Waltham, MA) prior to preparation of MCC fortified yogurt milk bases. MCC fortified yogurt milk bases were prepared by adding the appropriate amount of MCC-58 and MCC-88 respectively, to obtain mixtures with a target protein content of 9.80 %. This protein content was chosen to match the average protein content of the commercial GSY sample (Table 3.2). To allow a good dispersion and reconstitution of the MCC in milk, the MCC-88 GSY milk base was subjected to high shear agitation at 6500 rpm for 20 min using Ultra Turrax Model T25 fitted with S 25 N-18 G dispersing tool (IKA, Wilmington, NC, USA). Since MCC-58 could be
dispersed easily in milk, high shear agitation was not used and the milk base was stirred for 20 min on a stirring plate. The MCC fortified GSY milk bases were stored at 4 °C for 18 hours before use to allow sufficient time for hydration of the MCC powder.

**Preparation of GSY**

To prepare the control GSY, skim milk was heated to 90 °C for 5 min on a stirring hot plate (Thermoscientific, Waltham, MA), and subsequently cooled to 43 °C in an ice bath. The sample was then inoculated with 0.02 % (w/w) or 0.04 % (w/w) of starter culture suspension. A 10% (w/w) starter culture suspension was prepared by dispersing 3 g of the freeze dried DVS culture in 27 g of skim milk at 43 °C. The culture was mixed thoroughly in the skim milk and placed in an incubator at 43 °C (Fisher Scientific, Pittsburgh, PA, USA) for 10 minutes to acclimatize the starter culture to the incubation temperature prior to inoculation. After inoculation, the GSY milk bases were placed in an incubator (Fisher Scientific, Pittsburgh, PA), at 43 °C. The pH of the GSY samples was measured hourly at 43°C using a Fisher Scientific Accumet Excel XL20 pH meter (Fisher Scientific, Pittsburgh, PA), calibrated at the measurement temperature before use. When the pH of the GSY samples reached 4.5, the fermentation was arrested by cooling the samples to ≤ 10 °C in an ice bath. The GSY samples were subsequently subjected to a low shear stirring for 10 s using a hand mixer (Heritage Series model 2551, Sunbeam Inc.). After that, 300 g of the yogurt sample was strained using cheesecloth for 18-21 h at 4 °C in a refrigerator (Fisher Scientific, Pittsburgh, PA, USA), to obtain strained GSY with a target protein content of 9.80 % (w/w). To reach the target protein content, the amount of whey that had to be drained from the yogurt was established using a mass balance. The strained GSY control samples were subsequently evaluated for their chemical, physical and rheological properties.
To prepare the MCC fortified GSY, the MCC-58 and MCC-88 GSY milk bases were subjected to a similar thermal treatment, inoculation and incubation conditions as the control sample. Similar to the control sample, incubation was stopped at pH 4.5 and the yogurt was subjected to low shear mixing for 10 s using a hand mixer. MCC-58 and MCC-88 GSY samples were stored at 4 °C for 24 h before conducting chemical, physical and rheological analyses.

**Rheological characterization of GSY**

The rheological properties of the GSY samples were characterized using a dual transducer Advanced Rheometric Expansion System (ARES) strain-controlled rheometer, in conjunction with the Orschtrator data collection and analysis software (TA instruments, New Castle, DE). A parallel plate geometry (25 mm diameter Teflon plates) with a 1 mm gap was used for testing, and temperature control was ensured using a Peltier heating system. For each of the rheological analysis described in the subsequent section, approximately 1 g of sample was gently loaded onto the bottom plate of the rheometer. A 1 min temperature equilibration and relaxation step was allowed before proceeding with a test. Testing was conducted at 5°C, which is within the range of temperature at which yogurt is usually consumed (Farinade et al, 2009).

Steady rate sweeps were used to characterize the flow behavior of GSY. Strain-controlled, steady shear rate sweeps were conducted at 5 °C, with shear rates between 0.01-100 s⁻¹, at a frequency of 1 rad/s, in a clockwise direction. Since all GSY samples had a shear dependent, non-Newtonian behavior across the investigated shear rate range, the term apparent viscosity (\( \eta_{\text{app}} \)) was used to describe their resistance to flow.

Dynamic strain sweeps were conducted at a frequency of 1 rad/s, and a temperature of 5°C. A strain range between 0.01 % - 5 %, which is typical for rheological testing of yogurt (Peng et al, 2009; Lee and Lucey, 2006; Haque et al, 2001; van Marle and Zoon, 1995), was used
for all GSY samples. Strain sweeps were used to determine the linear viscoelastic region (LVR) of the GSY samples, which is the region where the elastic (G’) and viscous (G”) moduli are independent of the applied strain. Dynamic frequency sweeps were conducted between 1-100 rad/s, at a strain level in the upper limit of the LVR. The strain values for each of the GSY samples were as follows: 0.7 % for control and commercial GSY, 0.6 % for MCC-58 GSY and 0.3 % for MCC-88 GSY. All rheological analyses were performed in triplicate.

Water Holding Capacity

The water holding capacity (WHC) of GSY samples was determined using a modified version of the procedure reported by Sodini et al (2004). Ten grams of GSY was centrifuged at 1250 g for 10 min in a refrigerated high speed centrifuge (Sorvall RC-5B, Thermoscientific, Asheville, NC), at 5 °C. The amount of whey expelled during centrifugation (W, g) was weighed, and WHC calculated as:

\[
WHC = \frac{10 - W}{10} \times 100 \% \quad \text{Eq 3.1}
\]

The measurement was carried out in duplicates for each set of experimental replicate.

Titratable Acidity

Titratable Acidity (TA) of the GSY samples was determined using the standard International Dairy Federation procedure (Chandan and Rell, 2006). TA was expressed as g lactic acid/100 g of GSY sample, and was obtained by titrating 9 grams of GSY sample, diluting sample with 18 grams of deionized water and then titrating with 0.1 N NaOH. Approximately 0.5 ml phenolphthalein was used as an indicator, and titration was conducted until the first permanent shade of pink that lasted for longer than 30 s was achieved. The volume of 0.1 N NaOH required in the titration was used to determine TA, as follows (Chandan and Rell, 2006):

\[
\text{TA} \left( \frac{g \text{ lactic acid}}{100 \text{ g sample}} \right) = \frac{ml \text{ of } 0.1 \text{ NaOH}}{10} \quad \text{Eq 3.2}
\]
**Statistical Analysis**

The statistical software package JMP 9.0 (SAS Institute Inc., Cary, NC) was used for statistical analysis of the experimental data. One way analysis of variance (ANOVA) was used to determine significant differences between the experimental GSY samples (P<0.05). Significant differences (P<0.05) between means of dependent variables or measured parameters for the samples were compared using the Tukey-Kramer Honest Significant Difference (HSD) test.
Figure 3.1. Acidification profiles for GSY samples at: (A) 0.02 % inoculation level; (B) 0.04 % inoculation level. Plotted are means of triplicates (n=3), with the exception of strained GSY at 0.02 % inoculation level (n=6), error bars represent standard errors.
RESULTS AND DISCUSSION

*Effect of chemical composition on the acidification profile of GSY*

The chemical composition of the MCC-58 and MCC-88 is shown in Table 3.1. The main difference between these two types of MCC was the degree of removal of the serum phase during diafiltration step of the microfiltration process (Sauer and Moraru, 2012). It is important to note after adjusting the GSY milk bases to a similar protein content, MCC-58 GSY had higher TS, lactose and casein to serum protein ratio (CN:SP ratio) than MCC-88 GSY (Table 3.2). The lactose and casein content of the yogurt milk base and its CN:SP ratio are important variables that influence the fermentation time of yogurt (Puvanenthiran et al, 2002; Amatayakul et al, 2006). The CN:SP ratio of yogurt also changes during fermentation because the bacteria in the starter culture release proteases capable of hydrolyzing casein molecules (Beskhova et al, 1998; Tamime and Robinson, 2007). The peptides and free amino acids present in the soluble nitrogen portion of milk are important for the growth of the starter culture bacteria during yogurt fermentation (Carminati et al., 1995; Courtin and Rul, 2004; Tamime and Robinson, 2007).
Table 3.2. Chemical composition of GSY samples on dry solids basis (w/w). Values represent means of duplicate measurements (n=2).

<table>
<thead>
<tr>
<th>Sample</th>
<th>TS (%)</th>
<th>TNP(^1) (%)</th>
<th>TP(^2) (%)</th>
<th>NPN(^3) (%)</th>
<th>CN(^4) (%)</th>
<th>CN/SP(^5)</th>
<th>Fat (%)</th>
<th>Lactose (%)</th>
<th>Ash (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strained GSY (control)</td>
<td>14.89</td>
<td>9.61</td>
<td>9.42</td>
<td>0.19</td>
<td>7.91</td>
<td>5.25:1</td>
<td>0.20</td>
<td>4.33</td>
<td>0.75</td>
</tr>
<tr>
<td>MCC-88 fortified GSY</td>
<td>16.06</td>
<td>9.65</td>
<td>9.44</td>
<td>0.21</td>
<td>7.70</td>
<td>5.53:1</td>
<td>0.16</td>
<td>4.21</td>
<td>1.18</td>
</tr>
<tr>
<td>MCC-58 fortified GSY</td>
<td>19.40</td>
<td>9.90</td>
<td>9.58</td>
<td>0.32</td>
<td>7.99</td>
<td>6.30:1</td>
<td>0.32</td>
<td>9.90</td>
<td>1.75</td>
</tr>
<tr>
<td>Commercial strained GSY</td>
<td>15.06</td>
<td>9.81</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.24</td>
<td>4.03</td>
<td>0.88</td>
</tr>
</tbody>
</table>

\(^1\)TNP = total nitrogen \times 6.38

\(^2\)TP = true protein \times 6.38

\(^3\)Calculated based on the NPN content of skim milk and MCC

\(^4\)Calculated based on the casein content of skim milk and MCC

\(^5\)SP = TP-CN. CN/SP values represent means of triplicate measurement

A consequence of fortification of skim milk with MCC was an increase in the NPN content of the GSY milk base. The higher NPN content of MCC fortified GSY samples relative to the unfortified control sample resulted in a slightly shorter fermentation time for the fortified samples compared to the control (Figure 3.1). The difference in fermentation time was statistically significant (P<0.05) only at 0.04% inoculation level; there was no significant difference in fermentation time between MCC-58 and MCC-88 GSY, at either 0.02 % or 0.04 % inoculation level (Table 3.3). Furthermore, it is noteworthy to mention that despite a significant difference (P<0.05) in the lactose content between the MCC-58 and MCC-88 fortified GSY
samples (Table 3.2), there was no significant difference in the fermentation time between the two samples. This also supports the conclusion that the main reason for the shorter fermentation time in the MCC fortified GSY was the higher level of NPN.

The acidification parameters (pH and TA) for MCC-58 and MCC-88 GSY were measured after 24 h of storage at 4 °C. The pH of the GSY samples at the end of fermentation prior to refrigeration storage was 4.50. The pH of the control sample was measured after straining such that its protein content similar to the MCC fortified GSY (Table 3.2). The final pH of the control and MCC fortified GSY samples were not significantly different (P>0.05) at either inoculation level (Table 3.3). Damin et al (2009) reported a small decrease in the pH of nonfat yogurt after storing the samples at 4 °C for 24 h, a phenomenon known as post-acidification. According to Tamime and Robinson (2007), post acidification during cold storage occurs mainly as a result of the continued conversion of lactose to lactic acid by L. bulgaricus. Damin et al (2009) found that post acidification occurred independently of the level of fortification or the ingredients used in the yogurt milk base. This was also observed in the current study, where differences in composition and solids content of the GSY samples had little impact on the degree of post-acidification. There was, however, a significant difference (P<0.05) in TA values among the experimental GSY samples, both at the end of fermentation and after refrigerated storage. The TA value for the MCC fortified GSY was significantly higher (P<0.05) than that of the control, at both inoculation levels (Table 3.3). The sample fortified with MCC-58 had the highest TA value. This was expected because of both the higher NPN level and the higher lactose level in the MCC-58 fortified GSY (Table 3.2), which probably resulted in a higher amount of lactic acid in this particular sample. The peptides in the NPN fraction of milk were reported to have a
stimulatory effect on the growth of *S. thermophilus* and consequently increased the extent of conversion of lactose to lactic acid during the first few hours of fermentation (Shah, 2003).

**Table 3.3.** pH and TA profiles for GSY samples. Shown are mean values (n=3) ± 1SE, with the exception of strained GSY with 0.02 % inoculation (n=6).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Time to reach pH 4.5 (min)</th>
<th>TA at the end of fermentation (g lactic acid/100 g sample)</th>
<th>Final pH&lt;sup&gt;1,2,3&lt;/sup&gt;</th>
<th>Final TA&lt;sup&gt;1,2,3&lt;/sup&gt; (g lactic acid/100 g sample)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.02 % (w/w) inoculation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strained GSY&lt;sup&gt;1&lt;/sup&gt;</td>
<td>305±16&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>0.66 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.26 ± 0.01&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.50 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>MCC-88 GSY&lt;sup&gt;2&lt;/sup&gt;</td>
<td>255±0&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.52 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.32 ± 0.02&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.72 ± 0.05&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>MCC-58 GSY&lt;sup&gt;2&lt;/sup&gt;</td>
<td>320±10&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.11 ± 0.03&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.35 ± 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.46 ± 0.05&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>0.04 % (w/w) inoculation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strained GSY&lt;sup&gt;1&lt;/sup&gt;</td>
<td>330±0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.73 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.26 ± 0.02&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.68 ± 0.05&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>MCC-88 GSY&lt;sup&gt;2&lt;/sup&gt;</td>
<td>250±10&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.60 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.25 ± 0.02&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.62 ± 0.05&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>MCC-58 GSY&lt;sup&gt;2&lt;/sup&gt;</td>
<td>255±9&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.92 ± 0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.33 ± 0.02&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>2.07 ± 0.05&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Commercial GSY&lt;sup&gt;3&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
<td>4.23 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.60 ± 0.05&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
Means within a column not sharing a common superscript are significantly different (P<0.05)

1 pH and % TA measured on strained yogurt with a protein content of ≈ 9.80%

2 pH and % TA measured after storage at 4°C for 24 hours

3 pH and % TA measured immediately after retail purchase

**Water Holding Capacity of GSY**

The water holding capacity (WHC) of yogurt is an indicator of its ability to retain serum in the gel structure. The ability of a yogurt product to exhibit minimal whey separation is an important factor for its retail success, since whey separation negatively affects consumer perception (Lee and Lucey, 2010).

Previous studies on yogurt have shown that when non-EPS producing strains are used as the starter culture, CN:SP ratio of yogurt milk base and TS are important factors that influence the ability of the yogurt gel network to retain water (Lee and Lucey, 2010; Amatayakul et al, 2006; Harwalkar and Kalab, 1986). According to Puvanenthiran et al (2002), decreasing CN:SP ratio in yogurt milk base increases firmness of the fermented yogurt gels. In this study, WHC of strained GSY sample was significantly higher (P<0.05) than that of the MCC fortified samples (Table 3.4), which could be attributed to the lower CN:SP ratio of the control relative to the MCC fortified samples. As the CN:SP ratio of the yogurt decreases, the density of the cross-linked protein network in the gel structure increases. This helps reduce the size of pores in the gel network structure, which consequently hinders the flow of liquid from the gel structure (Puvanenthiran et al, 2002). The combination of high TS and low CN:SP ratio (Table 3.2) for the control GSY may have resulted in a denser microstructure for the yogurt gel network, which helped immobilize more free water in the gel structure. Further, whey proteins are known to possess high water binding capacity (Cerning, 1990; Degeest and DeVuyyst, 1999). The
synergistic effect of a compact yogurt microstructure and whey proteins helped to enhance the water holding capacity of the yogurt gels. The significantly higher (P<0.05) WHC of MCC-58 GSY relative to the MCC-88 sample (Table 3.4) was mainly attributed to its higher solids content. The WHC of the MCC-58 GSY was similar (P>0.05) to that of the commercial GSY sample, which showed minimal whey separation even after 1 month of refrigeration storage.
**Table 3.4.** Rheological parameters and water holding capacity (WHC) for the GSY samples.  
Shown are mean values (n =3) ± 1SD.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Rheological parameters</th>
<th>Water holding capacity (WHC), %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Flow index ( (n) )</td>
<td>Consistency coefficient ( (K) ), Pa s(^n)</td>
</tr>
<tr>
<td>Strained GSY (^1)</td>
<td>0.03 ± 0.02(^a)</td>
<td>546 ± 90(^a)</td>
</tr>
<tr>
<td>MCC-88 GSY (^2)</td>
<td>0.21 ± 0.02(^c)</td>
<td>35 ± 5(^b)</td>
</tr>
<tr>
<td>MCC-58 GSY (^2)</td>
<td>0.09 ± 0.02(^{ab})</td>
<td>182 ± 49(^c)</td>
</tr>
<tr>
<td>Strained GSY (^1)</td>
<td>0.04 ± 0.02(^a)</td>
<td>571 ± 146(^a)</td>
</tr>
<tr>
<td>MCC-88 GSY (^2)</td>
<td>0.27 ± 0.03(^c)</td>
<td>45 ± 16(^b)</td>
</tr>
<tr>
<td>MCC-58 GSY (^2)</td>
<td>0.09 ± 0.03(^{ab})</td>
<td>176 ± 41(^c)</td>
</tr>
<tr>
<td>Commercial GSY (^3)</td>
<td>0.12 ± 0.03(^b)</td>
<td>196 ± 87(^c)</td>
</tr>
</tbody>
</table>

\(^{a-c}\) Means within a column not sharing a common superscript are significantly different (P<0.05)

\(^1\) Measurements conducted on strained yogurt with a protein content of \( \approx 9.80\% \)

\(^2\) Measurements conducted after storage at 4\(^\circ\)C for 24 hours

\(^3\) Measurements conducted immediately after retail purchase
**Rheological properties of GSY**

The rheological properties of all GSY samples were evaluated at a similar protein content, since the protein content is known to greatly influence the rheological properties of yogurt gels (Lee and Lucey, 2010). Before discussing the data, it is also important to note that MCC-58 and MCC-88 were dried using freeze drying and spray drying, respectively. Nonetheless, Beliciu and Moraru (2009, unpublished data) found that the rheological properties of MCC-58 and MCC-88 dispersions were not affected by the drying method (freeze drying vs. spray drying). Therefore, it is expected that the drying method of the two MCC preparations did not affect the rheological properties of the MCC fortified GSY samples.

**Viscosity and flow behavior of GSY.** The MCC-58 GSY had a higher apparent viscosity ($\eta_{app}$) than MCC-88 GSY over the investigated shear rate range (Figure 3.2). This is partly attributed to higher casein content in the former, and also because of a stronger gel structure due to a high degree of acidification. The higher density of casein molecules in MCC-58 GSY probably allowed for an extensive formation of weak hydrophobic interactions between and within casein aggregates in the gel network. The apparent viscosity data at shear rate of 100 s$^{-1}$ ($\eta_{100}$) was used to make direct comparison of viscosity among the GSY samples. This shear rate was chosen because it is associated with processing operations such as pumping and stirring (Steffe, 1996). Strained GSY had a significantly higher $\eta_{100}$ (P<0.05) than MCC fortified GSY. There was however, no significant difference (P>0.05) between the $\eta_{100}$ of MCC-58 and MCC-88 GSY (Table 3.4). The $\eta_{100}$ value for MCC-58 GSY was statistically similar (P>0.05) to that of the commercial GSY sample, at both inoculation levels (Table 3.4).

All GSY samples exhibited shear thinning behavior, with viscosity decreasing as a function of shear rate (Figure 3.2). Concentrated yogurt was characterized as a pseudoplastic
material in other studies and this behavior was attributed to weak electrostatic and hydrophobic interactions within the yogurt matrix, which are easily disrupted by shear (Abu-Jdayil et al, 2000; Abu-Jyadil and Mohameed, 2002; Mohameed et al, 2004). The power law model was found to be a good fit for the flow behavior of both strained GSY and MCC fortified GSY, in agreement with other studies on concentrated yogurt (Abu-Jyadil and Mohameed, 2002; Mohameed et al, 2004).

The power law model has the following mathematical form:

$$\sigma = K(\dot{\gamma})^n$$

Eq 3.3

where $\sigma$ is the shear stress (Pa), $K$ is the consistency coefficient (Pa s$^n$), $\dot{\gamma}$ is the shear rate (s$^{-1}$) and $n$ is the flow behavior index. The flow behavior index ($n$) measures deviation from Newtonian flow ($n=1$), and offers an indication of the rate of structure change with a change in shear rate. The fit for all data sets was very good, with the coefficient of determination $>98\%$ in all cases. Values for the consistency coefficient ($K$) and flow behavior indices ($n$) for all samples are presented in Table 3.4. The range of $n$ values for the MCC fortified GSY observed in the study was below the range reported by Sauer and Moraru (2012) for MCC dispersions at a similar protein content. The observed difference was attributed to a difference in structure between the two systems, with the MCC fortified GSY being a weak gel network consisting of casein aggregates linked by weak, non-covalent bonds including hydrogen bonds, electrostatic and hydrophobic interactions. In addition, solubilization of colloidal calcium phosphate (CCP) during yogurt fermentation weakens the internal structure of casein micelles and increases the likelihood of dissociation of casein from the micelles, causing a partial loosening of bonds within and between casein molecules (Lee and Lucey, 2010). Furthermore, at the low temperature (5 °C)
at which the flow behavior of the GSY samples was characterized, the hydrophobic interactions involved in casein association are very weak (Lee and Lucey, 2010).

The flow behavior index for the MCC-88 GSY was significantly higher than that of MCC-58 GSY and the strained control GSY, at both inoculation levels (Table 3.4). It is likely that the higher acidity developed in the MCC-58 GSY led to more aggregation of casein micelles and a more gel-like structure in comparison to MCC-88 GSY, which probably resembled more a concentrated suspension of casein micelles. There was little to no significant effect of inoculation level on the flow behavior parameters of GSY (Table 3.4). The magnitude of consistency coefficient (K) of the GSY samples was affected by solids content and chemical composition of GSY, with K values increasing as total solids content increased (P<0.05) (Table 3.2). This is in agreement with other studies on flow behavior of stirred yogurts (Penna et al, 2006; Keogh and O’Kennedy, 1998; Ramirez-Sucre and Velez-Ruiz, 2013; Celik et al, 2006). Keogh and O’Kennedy (1998) found that K values of stirred yogurt were mostly influenced by its protein content. Overall, the flow behavior parameters (K, n and η100) of the MCC-58 GSY were not statistically different (P>0.05) compared to the commercial strained GSY sample (Table 3.4).
Figure 3.2. Apparent viscosity as a function of shear rate (flow curves) for GSY samples at (A) 0.02 % inoculation level; (B) 0.04 % inoculation level. Plotted are means of triplicate measurements (n=3), error bars represent one standard deviation.
**Dynamic rheological properties of GSY.** Dynamic rheological parameters (G’, G” and tan δ) were used to characterize the viscoelastic behavior of GSY samples. In the context of gel systems like yogurt, dynamic strain sweeps can be used to differentiate weak gels and strong gels. Strong gels remain in the linear viscoelastic region (LVR) over a greater strain range compared to weak gels (Steffe, 1996). Figure 3.3 shows an example of frequency sweep for the strained GSY (control), at 0.04 % inoculation level. Dynamic rheological testing revealed that G’ was greater than G” for all GSY samples over the tested frequency range at both inoculation levels which is indicative of a solid-like behavior. Another important viscoelastic parameter evaluated was tan δ, which can be used to indicate the extent of viscoelastic behavior for yogurt. A higher tan δ denotes a more viscous, liquid-like behavior, while lower tan δ values suggest a more solid-like behavior. The tan δ for all experimental GSY samples ranged from 0.23-0.30, which is typical of a weak gel with a predominantly elastic behavior. No meaningful difference in viscoelastic behavior was observed between the commercial, strained and MCC fortified GSY samples at both inoculation levels which suggest that all samples had a similar gel structure.
Figure 3.3. Frequency sweep for strained GSY (control), at 0.7 % strain, 5°C and 0.04 % inoculation level. Plotted are means of triplicate measurements (n=3), error bars represent one standard deviation.

The frequency dependence of the prevailing dynamic modulus, G’ as expressed by the slope of the modulus vs. frequency curve (m), was used as an indication of the type of gel structure and degree of interactions found within the yogurt matrices (Figure 3.4). All GSY samples exhibited a weak dependence of G’ on frequency, with m ranging from 0.12 to 0.14 (Table 3.4), which indicates a soft solid with gel like properties (Kasapis and Boskou, 2001). The amount of starter culture used for inoculation had no significant impact on the frequency dependence of G’ which implies that the same can be said for the type of gel network found in the GSY samples.

The value of the elastic modulus (G’), which is an indication of gel firmness, was significantly higher (P<0.05) for the experimental strained GSY (control) as compared to the MCC fortified GSY (Figure 3.4). This dissimilarity could be attributed to differences in the degree of both covalent and non-covalent interactions in the two types of GSY arising from a difference in chemical composition. Additionally, G’ values of the experimental control sample
were significantly higher \((P<0.05)\) than \(G'\) of the commercial GSY sample. This may be attributed to a difference in the method used for whey removal. The experimental control sample was strained, while whey was removed by centrifugation for the commercial GSY sample. Abu Jdayil et al (2002) also observed significant differences in rheological properties between concentrated yogurt samples that were subjected to whey removal method by straining in cloth bags and centrifugal separation. Another reason for the observed difference could be due to a difference in the heat treatment condition applied to yogurt milk base prior to fermentation, which could have influenced the extent of denaturation of whey proteins and thus the extent of disulfide crosslink formation between \(\kappa\)-casein and \(\beta\)-lactoglobulin (Lucey et al, 1998; 1999).

\(G'\) values of MCC-58 GSY were significantly higher \((P<0.05)\) compared to those of MCC-88 GSY. As discussed previously, this could be attributed to a difference in the solids and casein content of the two types of MCC fortified GSY, which was likely to have an impact on their microstructure. Marafon et al (2011) reported that the microstructure of unfortified yogurt showed numerous large sized pores that are evenly distributed in the protein matrix whereas the microstructure of sodium caseinate fortified yogurt at a similar protein content showed a more compact protein matrix, with fewer and smaller pores. The presence of a high numbers of pores in the microstructure of yogurt gels indicates a weaker gel (Lee and Lucey, 2004). In the current study, it is expected that the structure of the MCC-58 GSY was more compact as compared to MCC-88 GSY, which contributed to a stronger, firmer gel network in the former.

\(G'\) of the MCC-58 GSY was similar \((P>0.05)\) to the commercial GSY sample. This, combined with the observation that the two types of yogurt have virtually overlapping flow curves (Figure 3.1), suggest that the two types of yogurt have similar textural properties. This is very significant since texture is a very important sensory attribute for consumers’ liking and
acceptance of a yogurt product. Furthermore, a correlation between the G’ of GSY gels and their WHC was observed. Lucey (2001) stated that gel networks with high modulus are able to prevent excessive syneresis. In this study, the MCC fortified GSY had a significantly lower WHC (P<0.05) compared to its strained control counterpart. This suggests that MCC fortified GSY may be prone to more syneresis than the experimental strained GSY. Among the two types of MCC fortified GSY, MCC-58 GSY had a higher WHC compared to MCC-88 GSY (Table 3.4) which suggests that the former is likely to have a lower propensity for whey separation during storage.
Figure 3.4. Frequency dependence of $G'$ for GSY samples at 5 °C: (A) 0.02 % inoculation level; (B) 0.04 % inoculation level. Plotted are means of triplicate measurements (n=3), and error bars represent one standard deviation.
CONCLUSIONS

This study demonstrates that fortification of milk with MCC obtained by microfiltration is a promising alternative to the conventional make process of GSY by whey removal. MCC-58 is potentially a suitable source of micellar casein for milk fortification because the physical properties of the GSY fortified with this protein preparation resemble GSY manufactured by the conventional whey removal process. These findings can help GSY processors design appropriate conditions for the alternative production method of GSY by MCC fortification. Future work in this area will focus on the evaluation of the sensory properties and shelf life of the GSY made by the alternate process as compared to the traditional whey removal process.

ACKNOWLEDGMENTS

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REFERENCES


CHAPTER 4
A BUSINESS ANALYSIS FOR AN ALTERNATIVE GREEK STYLE YOGURT MANUFACTURING TECHNOLOGY

Key business issues

The success of traditional GSY product in the U.S market is not without its share of woes. Acid whey, the by-product of traditional GSY manufacturing, poses considerable economic challenge to producers with limited financial resources. In addition, the high cost of procuring a centrifugal separator, an essential piece of equipment needed for traditional GSY production process, is potentially a barrier to entry to the lucrative GSY market, especially for companies with limited financial resources. Development of an alternative production method for GSY using protein ingredient, which is the focus of this thesis, can potentially be a cheaper production method for GSY compared to the traditional process (Minneapolis Business Journal, 2012) and thus could become a more affordable option for small producers to enter the GSY market.

The adoption of this method by a GSY producer is expected to have an impact on the various aspects of the business. It is expected that a GSY producer who wishes to adopt this alternate production technology will not need any new processing equipment. However, a strategic partnership with milk protein ingredient suppliers and distributors has to be forged by GSY producers in order to ensure a reliable and cheap supply of the ingredient. Some marketing effort will be expected to familiarize consumers with the ingredient and promote it as a nutritious ingredient for GSY. The expertise of dairy ingredient specialists is likely needed to help with the production of the “formulated” GSY product. If the alternative GSY production method using protein ingredients were to become widely used in the industry, the method is expected to be strategically advantageous for GSY manufacturers who desire to increase the price...
competitiveness of their product offerings. Price competitiveness is an important strategic tool for these companies in light of the recent market trend in which major players in the industry aggressively compete in a zero sum game for valuable share of the GSY market (Food Navigator USA, 2013).

**An Overview of Greek style yogurt business in the United States**

Greek style yogurt (GSY) started out as imported niche yogurt product in the United States (U.S) (Ad Age, 2013). Historically, not many niche food products have successfully traversed into the mainstream market. GSY is essentially a derivative of traditional concentrated yogurt product that resonates heavily with the culture of Middle Eastern and Mediterranean countries. GSY is currently one of the trendiest foods in the U.S, dominating dairy aisles in grocery stores and even spawning stand-alone yogurt cafes (NPR, 2012). There are many reasons why consumers like this product. According to Mintel (2012), the desirable taste profile of GSY products coupled with its high protein, low fat content, along with consumers’ perception that it is a healthy product with all natural ingredients were among the top reasons why consumers crave for this product. GSY is a type of concentrated yogurt product that has existed in Mediterranean countries for many years. GSY is named and marketed as Greek yogurt because it is believed to be first introduced to the U.S by a Greek immigrant, Costas Mastoras, who claimed to have imported the product from Fage, a Greek-based GSY manufacturing company (Forbes, 2011). GSY has transformed the competitive landscape of the U.S dairy industry by rapidly evolving itself from what was once known as a specialty product for a niche market to the single most desirable dairy product in mainstream retail stores. Chobani, a U.S based manufacturing company founded by a Turkish immigrant in 2005, along with Fage, were the key early players of GSY in the U.S market. The initial marketing strategy of Fage was, centered on a “niche”
approach because it did not want to alert potential competitors of the market opportunity for a thick, creamy yogurt in the U.S (Ad Age, 2012). According to Osterwalder and Pigneur, this strategy indicates that the company practices the closed innovation business model. This business model relies on controlling a company’s innovation process so that competitors cannot profit from the innovation. The closed innovation business model is still very much embraced by successful companies competing in this market space because proprietary information regarding processing technique is a critical element of the booming GSY business (NPR, 2012). An open innovation business model is not likely to be practiced by these companies in the near future unless there is a major incentive for collaboration among these companies such as through merger or acquisition.

The market share of GSY has grown tremendously since it was first introduced to the U.S market. GSY accounted for only 0.7% of the U.S yogurt market in 2006 but it has since experienced a phenomenal growth and accounted for 25% and 35% of the US yogurt market in 2011 and 2013, respectively (Food Navigator USA, 2013). Today, the U.S GSY market is an impressive $2 billion business (Food Navigator USA, 2013). Chobani and Fage, the early players in the GSY market, champion the traditional approach of GSY manufacturing as part of their “go-to-market” strategy, because they believe it is what makes the product authentic and desirable. Tradition inspired GSY manufacturers like Chobani hope to deliver the value of simplicity and healthfulness through their product offering which is aimed at feeding consumers’ appetite for a thick, creamy yogurt product made from natural food ingredients. It is not until much later that huge multinational food companies with a diverse product portfolio such as Dannon, General Mills and Kraft begin to compete in this market. Dannon did not introduce its brand “Oikos” Greek until 2007 while General Mills did not roll out its Yoplait Greek yogurt
until early 2010 (Ad Age, 2012). These companies are not inherently traditional GSY manufacturers and producing an “authentic” product made by the traditional straining process is not necessarily part of the value that they intend to deliver to customers. For example, General Mills uses milk protein concentrate (MPC) in its GSY product (Food Navigator USA, 2012). Despite the early momentum gained by traditional companies like Chobani and Fage in the form of capturing the early market segment(s) for this product, gaining key partners and establishing important relations with both the former and the latter, not all these companies are able to carve out a sustainable competitive advantage over their competitors. According to Ad Age (2012) and Food Navigator USA (2012), non-traditional manufacturers like Dannon are gaining market share at the expense of some of their traditional competitors (Figure 4.1). In fact, Fage has lost a substantial portion of the market share since 2007 and is trailing Dannon as of 2012 (Figure 4.1). Nielsen (2012) predicted that Dannon’s market share is going to increase even further in the next few years. This clearly illustrates that the traditional approach of GSY manufacturing does not necessarily confer a sustainable competitive advantage for all the early players in the market. A sustainable competitive advantage is crafted for a company who is able to implement an holistic business strategy that is effective against their competitors and “authentic”, strained GSY is just a part of a marketing strategy that traditional GSY producers have in common. The other components of the business strategy are different for each company, and they play an important role in the long term viability of a traditional GSY manufacturer in this competitive market. Consumers’ expectation of GSY changes with time and the company that is best able to tailor their products to meet those changes will be successful in this lucrative business.
Is acid whey a significant problem for traditional GSY manufacturers?

GSY is traditionally made by straining fermented yogurt in a cloth bag to reach the desired protein content by removing the water soluble portion called acid whey. In the dairy industry, this whey removal step is achieved by means of a centrifugal separator (Nsabimana et al., 2005). Acid whey, the by-product in this traditional approach of GSY production has recently received some media spotlight. The media has recently portrayed that America’s favorite high protein snack is better for consumers’ health than that of the environment, which has sparked some controversy in the dairy industry. GSY is not the only food product that generates organic waste product during processing. However, the media claims that acid whey, at the level at which it is generated in GSY production, can be toxic to aquatic ecosystems when disposed into rivers and lakes because it releases aerobic sugar eating bacteria that removes enough oxygen from the water to harm fishes and other aquatic species (Modern Farmer, 2013; Dairy reporter, 2013; Foodnavigator USA, 2013).
To put matters into perspective, a traditional GSY manufacturing company procures about 21 million pounds from local farms for its weekly production activity in its New York state facility in 2012 and produces about one pound of Greek yogurt from every four pounds of milk, and the remainder becomes acid whey (U.S Small business administration, 2012). Consequently, the company produces about 16 million pounds of acid whey weekly in its upstate New York facility alone. The most common uses for acid whey is currently as an ingredient in cattle feed and fertilizer (ModernFarmer, 2013). However, there are limits as to how much acid whey each farmer can take before they run the risk of creating runoff into streams and rivers that can potentially be toxic to aquatic life. A more promising, but less economical option is using an anaerobic digester, which is able to convert the acid whey to biofuel (e.g. methane) that in turn can be fed into generators that supply electrical power (NPR, 2012). Some GSY manufacturers currently have access to an anaerobic digester in the vicinity of their production facilities (NPR, 2012). However, this solution is by no means a panacea because anaerobic digesters require substantial financial investment and have limited capacity. A functional unit of anaerobic digester costs approximately $ 4.5 million (Modern Farmer, 2013). According to Modern Farmer (2013), the northeast region in the U.S alone generated around 150 million gallons of acid whey in 2012, and yet there is currently no industry wide statistics available on where all the acid whey is going.

These sources seem to have overlooked two important facts when evaluating the matter. First, production facilities of GSY manufacturers are subjected to strict regulations and testing from government regulatory agencies regarding acid whey disposal. Acid whey leakage from a dairy production facility into the environment, accidental or otherwise, is illegal and could result in loss of license to operate the facility (CNN, 2013). Second, acid whey from GSY
manufacturing is not the only organic by-product from dairy foods processing that can adversely affect the environment. For example, sweet whey, the by-product of cheese production, which has a much higher economic value compared to acid whey because of its higher protein content and a more desirable taste profile, can also become an environmental issue if not handled properly (CNN, 2013). By the same token, acid whey may not be an environmental concern if appropriate measures are taken (Dairy reporter, 2013). Nonetheless, GSY manufacturers are constantly seeking more cost-effective ways to remove acid whey from their manufacturing facilities and their focus is on improving acid whey utilization from nutritional and environmental perspectives (Dairy reporter, 2013). Overall, acid whey may be quite a significant problem in traditional GSY production, but not urgent enough to warrant a complete overhaul of the production method for all manufacturers (Euromonitor International, 2013).

**Cost analysis of traditional and alternative GSY manufacturing approaches**

The rising popularity of GSY in recent years is a phenomenal trend in the food industry business today. The traditional straining process that produces “authentic” GSY is still the most popular approach among producers. However, there are two major cost issues associated with this approach, which may interfere with the creation of a sustainable competitive advantage for traditional GSY manufacturers over their competitors:

a. The variable costs of acid whey disposal

b. The high fixed cost of procurement of a centrifugal separator. A unit of centrifugal separator with a production capacity of 700 kilograms/hour costs $650,000 (GEA Westfalia separator division)

These are important financial issues to consider for the long term profitability of the traditional GSY business. Larger producers may be able to leverage on the economies of scale
associated with large volume production in order to offset these costs and make the business viable in the long run. However, smaller producers may not have this advantage. The more significant issue behind the traditional approach of GSY production is the sustainability of the production process from the standpoint of resource utilization. Acid whey is a by-product that constitutes about 75% of the milk input in the production process (Dairy reporter, 2013). A reduction of the amount of fluid milk required in GSY production can help to make the product more environmentally friendly. Advancement in dairy processing research has led to the development of an alternative GSY processing approach that can improve the utilization of milk in GSY production, and has the potential to trim the production cost of GSY. The idea behind the alternative approach is the incorporation of food additives in yogurt in order to obtain a product with thick, creamy texture and a taste profile similar to that of GSY made by whey removal. One can make Greek yogurt that resembles the original strained version in terms of taste and texture profile by using milk protein ingredient (e.g. MPC) or stabilizers (e.g. pectin and corn starch) in the product formulation (NPR, 2012). The potential sources of cost saving for this alternative GSY production approach in comparison to the traditional production method are:

a. The alternative avoids high capital investment in an expensive centrifugal separator
b. Companies do not incur additional costs for acid whey disposal
c. Lower storage and transportation costs due to lower amount of fluid milk needed for production as dry additives have a comparatively lower volume for the same weight compared to fluid milk (Euromonitor International, 2013)

The major source of additional cost for alternative GSY production is using MPC obtained by membrane filtration. Despite the extra cost of membrane filtration, MPC is expected to be the cheaper ingredient in GSY production compared to fluid milk, due to lower storage and
transportation costs (Minneapolis Business Journal, 2012). A summary of the major differences in manufacturing cost components of tradition inspired GSY manufacturers and their non-traditional competitors is shown in Table 4.1. The alternative GSY production method using additives has the potential to be financially more attractive than the traditional straining method and may be especially appealing to companies with limited financial resources that wish to compete in this market space. Such companies are more likely to see an advantage in the price competitiveness of their product offering relative to bigger competitors. The alternative GSY production approach is currently also a legal way for these companies to enter the GSY market because the U.S Food and Drugs Administration (FDA) currently has not yet developed a “standard of identity” for GSY and there is no clear indication from FDA that additives like MPC are prohibited in commercial GSY formulation (Star tribune, 2012). Therefore, this alternative approach for manufacturing GSY may serve as a viable way for small businesses to enter the lucrative GSY market. The alternative GSY production approach can potentially boost price competitiveness and quality of product offerings which in turn benefit the consumers.

**Table 4.1.** Key differential manufacturing cost components for traditional and alternative GSY production methods.

<table>
<thead>
<tr>
<th>Traditional</th>
<th>Alternative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Centrifugal separator</td>
<td>Procurement/production cost of additive ingredients</td>
</tr>
<tr>
<td>Acid whey disposal</td>
<td></td>
</tr>
</tbody>
</table>
Tradition or Technology? A case study of a traditional GSY manufacturer

The decision making factors involved that lead to the embrace and potentially adoption a new, alternative method or to keep the current method ultimately differs from one company to another. Different companies conceive different business strategies in order to compete in the given market space and therefore are likely to have different priorities and approaches when addressing the various components of the business at any given time. Chobani is currently the leader in the GSY market, owning nearly 50% of the available market share in 2012 (Figure 4.1) (Business insider, 2013). It was discussed previously that Chobani is unlikely to incorporate additives into its GSY formulation because this approach interferes with the value proposition of delivering traditional strained GSY to customers. More importantly, Chobani has proven that its concept of introducing a simple, natural GSY product to the U.S market worked well to fill the gap for a thick, creamy yogurt offering in the market. As a result, the gastronomic experience of Chobani’s product was a big hit with consumers. Chobani firmly believes that its product will be successful in the market and it uses a risky marketing strategy of pricing its product based on what it would cost in the future if sales were to improve, instead of factoring in the high input costs that the company had to face at the onset of production (Business insider, 2013). This was in line with Chobani’s business strategy to make their products affordable for everyone. This strategy helps to turn Chobani’s GSY into a sensation in the U.S yogurt market and it did not take long before Chobani became the leader in the GSY market (Figure 4.1), generating more than $1 billion in sales in a $2 billion industry (Business insider, 2013). This is a proof that Chobani’s value proposition of offering only traditional strained GSY resonates with customers’ expectation of the product. The success of Chobani’s business model is a testament that its overall business strategy works well with the U.S market. Unless there is a severe change in
market conditions that work against traditional GSY, it very difficult for the company to consider an alternative production method regardless of changes in competitors’ strategy. This case study highlights the complexity of the decision for a GSY manufacturer to adopt a new, alternative production approach despite the attractive advantages that it offers. Cost saving may not be the top priority of every company in the market and the reason(s) for (or for not) adopting the alternative production method are likely to be different for different companies in accordance to their business strategy at a particular point in time and therefore, adoption should be considered on a case-to-case basis.

**Business strategy considerations for alternative GSY manufacturing approach**

The decision to adopt and implement a new production method is ultimately dependent on how the decision fits with the value and related branding message that a company upholds as an individual business entity. It is not a simple question of which method is economically more viable for these companies. The Business Model Generation (BMG) canvas developed by Osterwalder and Pigneur provides a useful framework to help explain the strategic reasoning behind a company’s decision whether or not to adopt a new production technology modify the existing technology or change nothing (Figure 4.2). There are nine elements of the business that together constitute the building blocks of the BMG canvas. These components make up the two upper halves of the canvas. The right upper half of the canvas focuses on value creation while the left half focuses on production processes, resources and partnerships. The components that make up the right upper half of the canvas are customer segments, customer relationship, distribution channel and revenue streams for the business. These components identify a market segment, pinpoint the most important value proposition of the business, describe the customer-company relationship, and describe the “go-to market” channels. The left upper half of the canvas
describes the activities that have to be in place for the value proposition to be created and the necessary resources needed for the business to function, and the partnerships required for a successful business strategy. The lower part of the BMG tells the financial story of the company, the costs associated with the needed resources and the revenues that the company gains in exchange for the value delivered to the customer segment(s). Taken as a whole, the components help the company analyze the right business model that will produce a sufficient level of profitability.

Figure 4.2. Business Model Generation Canvas.

Source: Entrepreneur Magazine (2011)

The value creation components of the canvas do not only complement but are essential for the proper functioning of their BMG counterparts and vice versa. For example, procurement of key resources needed for the key activities of the business operations would not have been possible without a steady, reliable stream of revenue for the business. At the heart of the BMG
canvas is the value proposition that the business offers to customers. Together, the building blocks of the BMG canvas constitute a foundation that is crucial for showing how value is delivered in a profitable way to a company’s customer base for a sustainable business model.

The value proposition that consumer packaged foods (CPF) manufacturers offer to their customers is inherently based on their belief that customers will make repeat purchase of their products because they enjoy the experience of consuming their products. The taste profile of the food product plays an important role in giving customers an enjoyable eating experience. GSY is no different. GSY manufacturers tailor their products according to what they believe about consumers’ expectations of the product. Companies that rely on the traditional whey removal method to manufacture GSY believe that consumers want natural product made using the simple traditional approach when the product first was first conceived. (NPR, 2012; Huffington Post, 2012). Chobani is an example of a GSY manufacturer that champions the traditional approach of making the product. The company believes that its “authentic” product offering as a result of adhering to the traditional approach of GSY manufacturing is the reason why it is the largest and one of the most successful producers of GSY in the U.S (Huffington Post, 2012). The production method of using protein ingredients or stabilizers is not likely to be attractive to GSY manufacturers like Chobani because it threatens the core value proposition of the business regardless of the value of cost saving that is creates. A close examination of the various business components that make up the BMG canvas of tradition-inspired GSY manufacturers like Chobani is useful to illuminate the reasoning behind why adoption of the alternative technology is unlikely for these companies (Figure 4.3). These companies will find little incentive to substitute the traditional approach of manufacturing GSY for a cheaper manufacturing technology regardless of the economic benefits it confers because they believe that their
customer segment(s) desire “authentic “strained GSY. The major assumption is that these companies have sufficient financial resources to sustain the costly business operations of traditional GSY manufacturing. In contrast, other companies that are cost conscious and do not regard the alternative method as a threat to their value proposition are more likely to be attracted to adopt the alternative manufacturing approach because they see an incentive in the cost savings aspect of this approach (Figure 4.4). With regards to the BMG Canvas, it is expected that for a GSY manufacturer who wish to adopt this alternative technology for making the product will not need new production equipment (key resources) beyond what is needed for regular yogurt production. However, the company will need to source suppliers of the additives (key business partners) that they intend to use and most likely enlist the expertise of dairy product developers (key resources) to help them formulate the product (Figure 4.4).

The alternative manufacturing approach has the potential to fit the business model of GSY manufacturing companies that desire to improve price competitiveness of their products and deliver quality products to customers at the best economic value possible without compromising the value proposition that they offer to customers. In summary, one of the key decision factors that GSY companies face is whether adoption of the alternative method poses a threat to the brand (value proposition) even though it can potentially streamline the manufacturing cost of these companies in the long term. Besides tangible cost-benefit analysis from a financial perspective, the more subtle, less obvious impact of the alternate method such as the effect of the additive on the taste and/or texture profile on the final product should be considered. Replacing an existing manufacturing method with a new method that has yet to be proven is a critical decision that companies will not take lightly. Different companies will react
differently according to what they perceive to be important to the business and to reiterate, this is why adoption is not a simple question of economic viability.

Figure 4.3. BMG Canvas for a traditional GSY manufacturing company.
The focus of the previous chapter of this thesis is on an alternative method for GSY production using micellar casein concentrate (MCC) as a protein fortification ingredient. MCC is a new dairy ingredient, which has the potential to be used in a wide range of food applications including protein fortification of dairy foods (Mulvihill and Ennis. 2003). The alternative production method using MCC provides an acid whey free way to manufacture GSY without addition of thickeners (e.g. yogurt stabilizers) and achieve the textural properties and high protein content desired in GSY. MCC has all the cost saving potential of the alternative method summarized in Table 4.2. However, MCC possesses an additional source for cost saving because MCC is a functional high protein by-product of serum protein concentrate (SPC) production by
membrane filtration (Table 4.2). The cost saving is derived from the partial if not full coverage of the cost of membrane filtration by the revenue stream from sale of the SPC product. As a result, if this alternative production method using MCC were to become commercially available to producers, MCC is expected to be a more cost-effective ingredient in GSY production compared to yogurt stabilizers (e.g. corn starch) and MPC. In addition, MCC offers added macro nutritional benefit to consumers because it is a rich source of milk protein. An important assumption that needs to be addressed in the discussion is that the sensory profile of MCC fortified GSY is desirable if not, acceptable to consumers and does negatively affect the quality of customers’ eating experience. Further consumers’ testing for MCC fortified GSY is required to verify this. Membrane filtration is an established technique for commercial production of milk components. However, membrane filtration is a relatively new method for the commercial production of SPC. Potential commercial applications exist for MCC (Hurt and Barbano, 2010) but MCC is currently an underutilized dairy ingredient possibly because there are very few vendors that supply this dairy ingredient - only two known suppliers of MCC could be identified in the U.S in 2012. However, MCC is an emerging dairy ingredient that is garnering increased interest from the food and dairy industry since the number of new products containing casein or caseinates has grown by 22% from the year of 2000-2008 (Affertsholt, 2009). An improvement in the supply of MCC along with enhancement cost effectiveness of MCC as a protein fortification ingredient in GSY production is expected as SPC production by membrane filtration is likely to become more common in the dairy industry in the near future.

If GSY production method using MCC were to become widely adopted by producers in the dairy industry, the method is expected to be strategically advantageous for GSY manufacturers who want to increase both the price competitiveness and nutritional quality of
their product offering. The adoption of this method by a GSY producer is expected to have an impact on the various components of the BMG canvas for the business. New strategic partnerships with MCC suppliers and distributors will need to be forged by GSY manufacturers in order to ensure a reliable and cheap supply of MCC. Some marketing effort will be expected to familiarize consumers with MCC and promote it as a natural, nutritious dairy ingredient in GSY. The expertise of dairy ingredient specialists is likely needed to help with formulation of MCC fortified GSY as well as verification that MCC is a safe, nutritious ingredient in the GSY product. General Mills has adopted an iteration of this approach using MPC to increase the protein content of its Greek yogurt product (Foodnavigator USA, 2012). The type of processor that is likely to adopt this method will be small businesses and new entrants in the market that desire to distance themselves from the environmental concern of acid whey in GSY manufacturing (Dairy reporter, 2013). Examples include small processors that manufacture for private labels and new players in the GSY market such as Muller Quaker Dairy, a subsidiary of PepsiCo and Ultima Foods (Dairy reporter, 2013).

Table 4.2. Key differential sources of manufacturing cost savings for traditional and alternative GSY production methods.

<table>
<thead>
<tr>
<th>Traditional</th>
<th>Alternative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lesser amount of food additives needed in production</td>
<td>Centrifugal separator</td>
</tr>
<tr>
<td></td>
<td>Acid whey disposal</td>
</tr>
<tr>
<td></td>
<td>Transportation and storage costs of fluid milk</td>
</tr>
<tr>
<td></td>
<td>Coverage of by-product cost from main product sales revenue (e.g MCC)</td>
</tr>
</tbody>
</table>
Conclusions

GSY is one of the most successful dairy products in the U.S. market and sales of the product show no sign of slowing down despite the media portrayal that acid whey has a negative impact on the environment (Euromonitor International, 2013). The strategic analysis for adoption of an alternative GSY manufacturing method using dairy protein ingredients shows that the developed alternate processing technology can potentially avoid the acid whey generation in addition to conferring cost reduction benefit to manufacturers which can help in enhancing the price competitiveness of their product offering. The alternative manufacturing method is expected to benefit any GSY manufacturers who desire to leverage the economic benefit of the technology while not compromising the quality and nutritional value of their products. The alternative technology threatens the value proposition of traditional GSY manufacturing companies and therefore is not expected to be an attractive option for these companies. The technology is expected to be more attractive to companies with more limited financial resources that wish to compete in this market space as well as those who wish to distance themselves away from the consequences of acid whey production in traditional GSY manufacturing. The strategic analysis of technology adoption for GSY manufacturing presented in this chapter can be extended to other technology to help innovators evaluate the commercial potential of their technological innovation and identify possible commercial oriented users of the innovation. Expectations about adoption of the innovation should be informed by an understanding of the marketplace dynamics and the business models of the key users of the innovation.
REFERENCES


CHAPTER 5
CONCLUSIONS

The demand of Greek style yogurt continues to grow despite the challenges associated with the generation of acid whey in traditional Greek style yogurt (GSY) manufacturing. Nonetheless, the alternative processing method presented in this thesis has the potential to make GSY a more environmentally friendly and economical product. The technical part of this work demonstrated that the manufacture of GSY using MCC as a protein fortification ingredient is a promising alternative to the traditional straining make process for the product. The knowledge generated from this work has the potential to aid GSY manufacturers design appropriate conditions for an alternate processing method for GSY using dried or concentrated dairy ingredients. Future work in this area could involve an investigation of sensory properties and shelf life of the GSY made by the alternative processing method using MCC. The impact of the alternative method to the value proposition and related marketing message for a company’s GSY product is particularly of great importance when companies the business decision to adopt the alternative method. The analysis for business part of this work indicated that adoption of an alternative production method for GSY using dairy protein ingredients by GSY manufacturing companies is not a simple question of profitability only and ultimately depends on the fit of the alternative method to the business strategy of the company.
FIGURES

A

B
C

Figure A.1 Frequency sweep for (A) Strained GSY (control) (0.7 % strain; 5°C), (B) MCC-58 GSY (0.6 % strain; 5°C), (C) MCC-88 GSY (0.3 % strain; 5°C) and (D) Commercial GSY at 0.02 % inoculation level. Plotted are means of triplicate measurements (n=3), error bars represent one standard deviation.
Figure A.2. Frequency sweep for (A) Strained GSY (control), (B) MCC-58 GSY (0.6 %), (C) MCC-88 GSY at 0.04 % inoculation level (0.7 % strain; 5°C) Plotted are means of triplicate measurements (n=3), error bars represent one standard deviation.

Figure A.3 Examples of \( \log G' = m(\log \omega) + c \) curves, illustrating the frequency dependence of \( G' \) for strained GSY (control), MCC-58 GSY and MCC-88 GSY at 0.04 % inoculation level.
**TABLES**

Table A.1 Composition of MCC-58 and MCC-88 fortified GSY milk bases, on dry solids basis (w/w). Values represent means of duplicate measurements. (n=2)

<table>
<thead>
<tr>
<th>Sample</th>
<th>TS</th>
<th>TNP(^1)</th>
<th>CN(^2)</th>
<th>Fat</th>
<th>Lactose</th>
<th>Ash</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCC-88</td>
<td>16.06</td>
<td>9.92</td>
<td>8.17</td>
<td>0.16</td>
<td>4.74</td>
<td>1.25</td>
</tr>
<tr>
<td>MCC-58</td>
<td>19.97</td>
<td>9.83</td>
<td>8.54</td>
<td>0.36</td>
<td>8.14</td>
<td>1.64</td>
</tr>
</tbody>
</table>

\(^1\) TNP = total nitrogen × 6.38

\(^2\) Calculated based on the casein content of skim milk and MCC