Evaluating the Effect of Milk Protein Concentrates (MPC) Fortification on Rheological Properties of Nonfat Set Yogurt Using Vane Rheometry

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Abstract

The purpose of the study was to evaluate and compare the effects of fortification of yogurt with skim milk powder (SMP) and milk protein concentrate (MPC) on the rheological properties and viscosity of the yogurt. The SMP and MPC bases were formulated by adding different levels of SMP and MPC to common milk base of 8.8% total solids to an equivalent protein content (4.6%) in the final mixes. A control sample was prepared without any protein fortification. The yogurts were analyzed for gel strength (yield value) and viscosity after one and five days of storage. MPC fortified yogurt had a higher gel strength and viscosity compared to SMP fortified yogurt. There was slight increase in the strength of yogurt gels in all samples on Day 5 compared to Day 1. The control sample was found to have the lowest gel strength and viscosity. Fortification with SMP or MPC did not affect rates of fermentation.

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Chapter I: Introduction

Fermentation is one of the oldest methods of preserving the milk and dates back thousands of years (Tamime & Robinson, 1999). Yogurt is a fermented product that can be obtained by the lactic acid fermentation of milk through addition of a starter culture typically comprised of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus.* In rare cases, unconventional microorganisms, such as *Lactobacillus helveticus* and *Lactobacillus delbrueckii* subsp. *lactis* are also used for culturing purpose (McKinley, 2005). In addition to the added benefit of a longer shelf life, these starter cultures can produce products with different varieties of flavors, consistencies, and textures. In fermented food markets, yogurt is produced and sold with a variety of textures, such as liquid, set and stirred. In terms of fat content, the product is categorized as regular fat, low fat, and non-fat yogurt. The industry has also developed natural and fruit flavored yogurt to suit the demands of the market. All these value additions to yogurt have tremendously increased its popularity across all consumer subgroups (McKinley, 2005).

In the United States, yogurt was launched in 1940. At that time, it was considered a product for dieters (Katz, 2001). But over the last decade, the trend in the yogurt consumption in the United States has shifted dramatically. In 1996, the estimated annual yogurt consumption in the United States was 1,588 million pounds, which increased to 3295 million pounds in 2006 (*Total fluid milk sales*, n. d.). An increasing demand for yogurt may be due to increased awareness of the product's neutraceutical properties (Katz, 2001). Yogurt is considered a very good source of protein, calcium, phosphorus, riboflavin (vitamin B2), thiamin (vitamin B1), and Vitamin B12. Yogurt also contains considerable amounts of niacin, magnesium, and zinc. The proteins, vitamins and minerals present in yogurt are bioavailable. Low fat yogurt is believed to contribute to a healthy and nutrient-rich diet (McKinley, 2005). Low fat plain yogurt contains 5.0 g of protein/100 g, 1.0 g of fat/100 g, 5.0 g of lactose/100 g, 0.18 g of calcium/100 g, 0.14 g of phosphorous/100 g, 0.08 g of sodium/100 g and 0.24 g of potassium/100 g. Type of fruit added for fruit yogurt manufacturing can also alter nutrient levels (Deeth & Tamime, 1981).

Yogurt gels are produced by fermentation of milk with thermophilic starter cultures. Milk is pasteurized at 85°C for 30 minutes, which denatures whey proteins. Interaction and cross-linking of the denatured whey proteins with κ-casein occur on the surface of casein micelles. The decrease in the pH from ~6.6 to ~4.6, the isoelectric point of casein, during fermentation increases the casein micelle interaction and forms a gel (Lee & Lucey, 2004).

The quality of yogurt products and acceptance by consumers are largely determined by the rheological and physical properties of yogurt gels. These properties are dependent on processing conditions (Cobos, Hone, & Muir, 1995) and fortification while manufacturing (Rohm, 1993).

Low total solids in yogurt without any protein fortification can result in whey expulsion, weak body, poor texture, and inconsistent product over time. In order to resolve these problems, yogurts are generally fortified with different types of stabilizers to improve stability, thickness, and gelling properties. However, adding stabilizers is not a common practice in many countries where yogurt is manufactured (Tamime, Kalab, & Davies, 1984). Low fat yogurt faces these quality concerns most because of lower total solids in the yogurt mixes. Therefore, in commercial yogurts, it is very important to increase the total solids of the low fat mixes by fortification with milk proteins (Tamime & Robinson, 1999).

Skim milk powder (SMP) is used commonly to enrich yogurt at a rate of 3 to 4% to increase the total solids (Tamime & Robinson, 1985). Addition of milk protein helps form a firmer body and reduces whey expulsion in the final product (Mistry & Hassan, 1992). There is a limit to the quantity of the skim milk powder that can be used to get firmer body because large amounts of SMP can produce powdery mouthfeel, and excess lactose from the SMP can lead to extreme acidity during storage of the final product (Tamime & Robinson, 1985).

Casein (82%) and whey proteins (18%) are the primary proteins found in milk. Many milk protein ingredients, such as nonfat dry milk, whey powder, whey protein concentrate, whey protein isolates, caseins and caseinates and recent ones, such as milk protein concentrates, milk casein concentrates, milk soluble protein concentrates are used to fortify yogurt mixes (Barbano, 2009).

Milk Protein Concentrates (MPC)

As a possible source of protein fortification to the product, milk protein concentrate (MPC) is an alternative to SMP. In the United States, the definition of MPC is "any complete milk protein (casein plus lactalbumin) concentrate that is 40% or more protein by weight" (*Harmonized tariff schedule*, 2009). Milk protein concentrate is highly soluble with no off flavor and is stable at typical pasteurization temperature. The protein concentration of MPC can vary from 40% to 85% depending upon the purpose of final use. As the level of protein increases in MPC, lactose level decreases. Milk protein concentrate can be used as a protein source for protein-enriched beverages and low-carbohydrate foods (*Milk protein concentrate*, n.d.). Milk protein concentrate production begins with ultra filtration of skim milk to make a lactose-reduced, skim milk concentrate, which is further concentrated in an evaporator by using two-stage drying process and, finally, spray dried into powder form. Milk protein concentrate contains high levels of protein and low lactose. Milk protein concentrate preserves the unique casein to whey ratio in milk (Novak, 1992).

Labeling of Milk Protein Concentrates (MPC)

Food products fortified with milk protein concentrates must be appropriately labeled to specify the use of milk derived ingredients in product to alert consumers who are allergic to milk proteins (*Milk protein concentrate,* n.d.).

Vane Rheometry

In the last two decades the use of rotating vane geometries for the measurement of the flow behaviors and characteristics of non-Newtonian fluids has increased in popularity. In the past, vane rheometry was utilized to determine the apparent yield stress of inorganic dispersions. Now it is used for measuring rheological properties, such as steady-state flow-curves of structured fluids. Vane geometries are simple to fabricate and clean, and the geometry also minimizes wall-slip effects. Vane rheometry is currently used to characterize foods, inorganic colloidal dispersions, and bioengineering fermentation broths (Barnes & Nguyen, 2001).

Statement of the Problem

With the increase in the popularity of healthy and low calorie products in the world today, low fat and other types of yogurt are seen as healthy alternatives for customers. There are three sorts of yogurts generally seen in the market: set, stirred, and drinking yogurt. In spite of its widespread appeal, yogurt with weak body and poor texture is considered to have quality defects. The common solution to these defects has been addition of skim milk powder (SMP) to the yogurt mix to increase the total solids content of the final yogurt. The disadvantage of using SMP in yogurt manufacturing is the potential development of excess acidity during storage of the product and texture defects (Tamime & Robinson, 1985). Researchers have tested several dairy ingredients to improve rheological properties, of which whey protein concentrates, whey powder, caseinates, ultra-filtered milk, reverse osmosis concentrated skim milk have shown enhanced viscosity and firmness as well as reduction in syneresis in yogurts (Jaros & Rohm, 2003; Chandan, White, Kilara, & Hui, 2006). Milk protein concentrate (MPC) can be added to yogurt to serve this purpose. In this study yogurt was fortified with SMP or MPC, and fermentation rates and rheological characteristics for each were compared.

Objectives

The objective of the study was to determine the effects of fortification of yogurt with skim milk powder (SMP) and milk protein concentrates (MPC) on rheological properties such as gel strength and viscosity of the yogurt gels using vane rheometry.

Chapter II: Literature Review

Introduction

This chapter will discuss background of yogurt, the textural properties and factors affecting yogurt. The chapter will also address the textural characteristics of yogurt prepared by fortification of with MPC and SMP.

Background of Yogurt

The history of yogurt goes back over six thousand years. It is believed that the word "yogurt" evolved from the Turkish word *jugurt* (Rasic & Kurmann, 1978). Today, yogurt is known by different names in different regions in the world. In Iraq, it is known as *roba* and Finland it is called *fiili* (Tamime & Deeth, 1980; Tamime & Robinson, 1985). It is assumed that limited availability of milk due to dry desert surroundings in Middle East led to development of a yogurt like product. In Turkey, it was thought to be consumed as a preserved milk product (Akin & Rice, 1994; Rasic & Kurmann, 1978; Tamime & Robinson, 1985).

Traditionally, Greek yogurt is prepared from ewe's milk, yet cow milk is used commercially. In South Asia the yogurt is called *dahi*, and it exhibits soft coagulum, lumpy texture and mild acidic flavor. In India, *raita* is made from *dahi* with addition of grated cucumber or grated bottle gourd, black pepper, cumin seeds and coriander. Bulgarian yogurt has a unique flavor and taste due to different microbial strains in the yogurt preparation. In Indonesia different varieties of yogurt called *dadiah* are prepared by fermenting milk in a bamboo container surrounded with banana leaves. *Taratur* is a variety of yogurt made in Albania and Republic of Macedonia by mixing yogurt with vegetables, walnuts, garlic, oil, and water. *Rahmjoghurt*, yogurt with higher milk fat content (10%), is produced in Germany and other European countries. *Matsoni* is another variety of yogurt product made by using

Lactococcus lactis which gives it a distinctive viscous texture. In Middle Eastern countries, such as Jordan and Palestine, yogurt named *Jameed* is combined with salt and dried for preservation. (*Food production and patisserie*, 2009).

The health and nutritional properties of yogurt have inspired numerous research and product development studies. Nutrition awareness and health education has resulted in the widespread promotion of low fat yogurt in recent decades. The Food and Drug Administration defines yogurt as follows:

> [Yogurt consists of] food produced by culturing one or more of the optional dairy ingredients (cream, milk, partially skimmed milk, and skim milk) with a characterizing bacteria culture that contains the lactic acid-producing bacteria, *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* (GPO No. 21 CFR 131.200).

In the Code of Federal Regulation 131.200, the FDA states that yogurt may include a number of optional ingredients to increase the non-fat solids, including "concentrated skim milk, non-fat dry milk, buttermilk, whey, lactose, lactalbumins, lactoglobulins and modified whey" (No. 21). The FDA regulations also list the following specifications for yogurt:

> Yogurt, before the addition of bulky flavors, contains not less than 3.25% milkfat and not less than 8.25% milk solids not fat, and has a titratable acidity of not less than 0.9%, expressed as lactic acid. Yogurt may be heat treated after fermenting to destroy viable microorganisms for a longer shelf life of the food (GPO No. 21 CFR 131.200).

According to the FDA's Code of Federal Regulations, non-fat yogurt and low-fat yogurt may contain less than 0.5% and 0.5 to 2.0% fat respectively (GPO No. 21 CFR 131.206; GPO No. 21 CFR 131.206).

Yogurt Texture and Factors Affecting Texture

Jaros and Rohm (2003) stated that the texture of a food can be characterized as a flow, deformation, or disintegration of the food material under an applied force. Technically rheology of food is the study of flow and deformation of materials. Texture is an important parameter contributing to sensory properties of food.

Texture affects the mouthfeel of a product. Mouthfeel is described as perception of sensations on the tongue and throughout the mouth after intake of a food. The movement of food in the mouth and the sensations perceived contribute to overall acceptance and likeness of the product. Texture is comprised of physical properties like density, viscosity, and surface tension. Yogurt is considered a non-Newtonian food, and the textural properties of yogurt can be generalized as viscosity, strength of the gel, and syneresis (Jaros & Rohm, 2003).

Analysis of the rheological properties of dairy foods is helpful in determining the structure of the product (Jaros & Rohm, 2003). The textual properties of yogurt gels are important factors in establishing consumer preference and manufacturing strategies for yogurt (Charm, 1971; Tunick, 2000). The main factors affecting the rheological and textural properties of yogurt are composition and preparation of milk base; dry matter fortification, homogenization, pre-heat treatments, starter culture, incubation time-temperature, and post-incubation treatments (Jaros & Rohm, 2003).

Dry Matter Fortification. The amount of total solids affects the physical and rheological properties of yogurt. The typical solids level for skim milk yogurt production is the MSNF content for milk, but many types of concentrated yogurt milk bases may contain more than 20% of dry matter. The dry matter content of typical commercial yogurts ranges from 13-17% because of enrichment of the base milk (Kulkarni, Huss, Kessler, & Plock, 1990). Enrichment can be accomplished by concentrating the milk base or by adding non fat dry milk or other ingredients, such as whey or casein-based protein powders to the base. The choice of method is determined by process restrictions and final product quality. Irrespective of the type of protein added, the total level of protein content in yogurt base is critical to the rheological and physical properties of yogurt gels. As the protein level increases, the fermented gel strength increases, and water mobility is restricted (Snoeren, Damman, & Klok, 1982). However, high levels of whey protein can affect flavor by contributing to bitterness. Therefore, high whey to casein ratios should be avoided (de Boer, 1996).

Homogenization. Homogenization of whole milk prevents creaming during fermentation. The process is accomplished at pressures between 10 and 20 MPa at temperatures of 55-65°C. High shear during homogenization reduces the size of milk fat globules, which are stabilized by milk fat globule membrane. Fractions of milk proteins coat the enlarged fat globule surface and form a secondary fat globule membrane which is of great significance for the unique properties in fermented dairy foods (Schkoda, 1999).

In unhomogenized milk, the large fat globules reduce the firmness of the product by interrupting the protein gel network (Aguilera & Kessler, 1988). Full fat yogurt made from whey protein fortified homogenized milk resulted in increased viscosity and firmness when homogenization followed heat treatment (Kulkarni, Huss, Kessler, & Plock, 1990). Plock, Huss, Kennel, and Kessler (1992) found that as the diameter of the fat globule decreases, the gel firmness increases.

Pre-heat Treatments. Proper heat treatment of the milk is important for optimal texture of yogurt. Heat denatures whey proteins after which they associate with the casein micelles. The association of whey proteins with caseins takes place through disulphide linkages and hydrophobic interactions (Law, 1996). In commercial yogurt production, time-temperature profiles ranging from 80-85 °C for 30 min to 90-95 °C for five minutes is sufficient to manufacture good quality yogurt (Lucey & Singh, 1997). Extensive heating resulted in maximal denaturation of whey proteins, mainly β-lactoglobulin, which increased the storage modulus of the final yogurt gel (Lucey, Teo, Munro, & Singh, 1997). Furthermore, gelation occurred earlier in severely heat-treated milks resulting in a decrease in coagulation time of directly acidified milks (Lucey, Munro, & Singh, 1999). Yogurt made from unheated or less heated milk was found to have a weaker gel, poorer texture and firmness, and higher levels of syneresis (Tamime & Robinson, 1999).

Starter Culture. The type of culture used determines the physical properties of stirred type yogurts. Lactic acid bacteria can produce extracellular polysaccharides (EPS), which can increase apparent viscosity and form more stable yogurt. A correlation was found between the amount of EPS, the type of EPS and viscosity of the product (Sebastiani & Zelger, 1998). In addition to the amount, the charge, type, and molecular mass of EPS may also be important (Bouzar, Cerning, & Desmazeaud, 1997; Laws & Marshall, 2001; Marshall & Rawson, 1999; Pleijsier, De Bont, Vreeker, & Ledeboer, 2000; Ruas-Madiedo, Hugenholtz, & Zoon, 2002). Some researchers assumed that the effect of EPS may relate to its association with the casein matrix (Skriver et al., 1995). Uncharged EPS did not affect the storage modulus in set yogurt at

12% solids. However, increased stiffness with lower fortification was observed (Jaros, Rohm, Haque, Bonaparte, & Kneifel, 2002; Pleijsier, De Bont, Vreeker, & Ledeboer, 2000).

Time-Temperature During Fermentation. Rate of fermentation as a function of time and temperature can influence the rheological properties of yogurt. The optimum temperature for thermophilic lactic acid bacteria is 40 to 43°C. Lower fermentation temperatures resulted in longer time required to reach final pH of 4.6. However, the final product was found to be much firmer (Walstra, Geurts, Noomen, Jellema, & Van Boekel, 1999). When comparing the results of yogurt fermented at 30 or 42°C, Lankes, Ozer and Robinson (1998) found that yogurt produced at 42°C had higher viscosity and gel firmness.

Post-incubation Treatments. In the manufacture of set style yogurt, yogurt is fermented in the container. Upon reaching a desired pH, it is necessary to avoid vibrations while transferring containers to a cooling unit, to prevent breakage of the gel which can cause subsequent syneresis. Rapid cooling is essential to retard further growth of lactic acid bacteria. Such bacteria would otherwise result in undesired acid production. Excessive acidification below a pH 4 could also encourage whey separation and gel defects in the final product (Jaros & Rohm, 2003).

Textural Characteristics of Yogurt and Vane Method

Some of the common descriptors of textual properties of yogurt-like products are firmness, creaminess, viscosity, sliminess, curdness, chalkiness, and syneresis (Muir & Hunter, 1992; Tamime & Robinson, 1999). The difficulties with sensory analysis include cost associated with training of panelists, highly variable data, and uncertain terminology and scaling. This has forced researchers to search for instrumental techniques to objectively measure textural properties of foods (Kuehl, 1994). As a non-Newtonian material, yogurt's rheological properties have been characterized by empirical or imitative methods (Benezech & Maingonnat, 1994; Fiszman & Salvador, 1999; Hellinga, Somsen, & Koenraads, 1986; Skriver, Holstborg, & Qvist, 1999). With the exception of drinkable yogurts, all intact yogurts exhibit viscoelastic properties (Keogh & O'Kennedy, 1998).

Measurement of apparent viscosity is more common in stirred-type yogurt. The firmness of set style yogurt gels is mainly measured by its yield point value on stress-strain plots. Yield point is obtained by measuring the force required to break the gel structure expressed in yield stress, at the strain where material actual starts flowing (Chandan, White, Kilara, & Hui, 2006). Yield stress is the minimum stress required for the material to begin to flow (Steffe, 1996). A low-yield value indicates yogurt with a weak gel network (Lucey, 2001). Forces responsible for yield properties of gel network are strength and relaxation times of protein-protein bonds, arrangement of number of bonds in each cross-section of the strand, and direction of strands in the network (van Vliet, van Dijk, Zoon, & Walstra, 1991). The apparent viscosity of yogurt is a function of shear rate and as such yogurt is considered a thixotrophic material. In case of thixotrophic fluids with the increase in shear rate, the apparent viscosity decreases (Chandan, White, Kilara, & Hui, 2006).

The vane method is a common way to study yield stress in foods (Yoo, Rao, & Steffe, 1995). In the vane method, a vane with different number of blades, generally four to eight is immersed in a food test sample and rotated at defined rate. Resulting torque applied on the blades is computed as a function of time. Stress and deformation values at the rupture can be calculated by using torque-time data to characterize the textural properties of foods (Troung & Daubert, 2001). The advantages of the vane method are as follows:

a. The vane is placed in the material limits slip on the wall,

b. There is less damage to the tested material compared to other methods,

c. There are no slight annular gaps generally experienced with other geometries, and

d. Yield is measured under quasi-static conditions. (Barnes & Nguyen, 2001; James, Williams, & Williams, 1987; Nguyen & Boger, 1983)

Effect of Fortifications on Textural Properties of Yogurt

Researchers have reported numerous fortification methods to improve the physical and textural properties of yogurt. Trachoo and Mistry (1998) stated that non-fat and low-fat set yogurt prepared by enrichment with buttermilk powder (BP) (3.7% protein level) was smoother than those prepared with addition of skim milk powder (SMP) (4% protein level). Low-fat yogurt with BP was also found to be slightly smoother than yogurt with SMP. Guzman-Gonzalez, Morais, Ramos and Amigo (1999) reported no difference in quality between yogurts prepared with SMP and condensed skim milk. Replacement of SMP with whey powder at 4.2% protein produced more firm, viscous, and better flow properties in yogurt product than did the control group with same level of protein content (González-Martínez, Cháfer, Albors, Carot, & Chiralt, 2003). When skim milk was replaced by whey protein concentrates, yogurt showed an increase in water-holding capacity and a reduction in syneresis (Augustin, Cheng, Glagovskaia, Clarke, & Lawrence, 2003; Cheng, Augustin, & Clarke, 2000; Remeuf, Mohammed, Sodini, & Tissier, 2003).

Additionally, a low-fat yogurt made with the addition of 1% microparticulated whey protein resulted in a sensory profile similar to full-fat yogurt (Sandoval-Castilla, Lobato-Calleros, Aguirre-Mandujano, & Vernon-Carter, 2004). Guirguis, Versteeg and Hickey (1987) found an increase in the firmness and a decrease in the whey expulsion in yogurt prepared by reverse osmosis concentrated skim milk in contrast to yogurt prepared by addition of milk powder. Yogurt with added caseinate had higher gel firmness and viscosity (Guinée, Mullins, Reville, & Cotter, 1995; Remeuf, Mohammed, Sodini, & Tissier, 2003; Rohm, 1993; Tamime, Kalab, & Davies, 1984) but reduced smoothness and rougher texture compared to yogurt made by addition of SMP (Modler, Larmond, Lin, Froelich, & Emmons, 1983; Remeuf, Mohammed, Sodini, & Tissier, 2003).

Non-Fat Dry Milk (NFDM). Non-Fat Dry Milk (NFDM) is manufactured from condensed skim milk (Chandan, White, Kilara, & Hui, 2006). In the spray drying process, condensed milk is atomized and exposed to an air stream at 180-200°C. Different designs of atomizers, such as a pressure nozzle or a centrifugal disc may be used. The moisture removal is dictated by size of the droplets, air temperature, and air flow rate. Spray drying produces dried milk with excellent solubility, flavor, and color. The heat treatment prior to drying determines the functional properties of NFDM. No preheating prior to pasteurization results in "low-heat" powder. In production of "high-heat" powder, milk is heated to 85-88 °C for 15 to 30 min. For "medium-heat" powder, a heat treatment between simple pasteurization and "high-heat" treatment is employed. The amount of undenatured whey protein is quantified by the whey protein nitrogen (WPN) index. For yogurt making only low-heat (WPN≥6.0 mg/g), NFDM can be used (Chandan, White, Kilara, & Hui, 2006).

Various studies have established a relationship between enrichment of yogurt with milk powder and its impact on rheological and physical properties of yogurt (Becker & Puhan, 1989; Harwalkar & Kalab, 1986; Rohm, 1993). Rohm (1993) compared the viscosity of yogurt made without fortification and viscosity of yogurts made with 1%, 2% and 3% addition of SMP. Skim milk powder at 1%, 2% and 3% increased viscosity by 22%, 43%, and 70% respectively. In a similar study, the addition of 1% SMP increased the gel strength by 25% and the viscosity by

15% in comparison to yogurt prepared with no enrichment (Becker & Puhan, 1989). There was no difference in syneresis between yogurts with 10%, 12.5%, 15% and 20% total solids (Harwalkar & Kalab, 1986).

Milk Protein Concentrates (MPC) (OR Ultrafiltered Milk). Milk protein concentrate (MPC) is fairly a recent dairy ingredient employed in dairy formulations. Milk protein concentrate is manufactured from skim milk by ultrafiltration, microfiltration, diafiltration, and evaporation (Mistry & Hassan, 1991) followed by spray drying. Milk protein concentrate is classified by protein content. Milk protein concentrate contains high protein and low lactose. Milk protein concentrate also possesses unique casein to whey ratio (Novak, 1992). Some of these qualities make MPC an alternative to NFDM in yogurt (Alvarez, Wolters, Vodovotz, & Ji, 2005).

According to Chandan, White, Kilara and Hui (2006), MPC can be used to raise the protein level of yogurt without raising the lactose content of the mix resulting in a "low-carbohydrate" yogurt. Milk protein concentrate can be labeled as "ultrafiltered skim milk" (Chandan, White, Kilara, & Hui, 2006). At the same protein level, replacing SMP with MPC does not alter firmness (Mistry & Hassan, 1990; Modler, Larmond, Lin, Froelich, & Emmons, 1983), texture (Mistry & Hassan, 1992), syneresis (Modler, Larmond, Lin, Froelich, & Emmons, 1983), viscosity (Guzman-Gonzalez, Morais, Ramos, & Amigo, 1999; Rohm, 1993) or flavor of yogurt (Mistry & Hassan, 1992; Modler, Larmond, Lin, Froelich, & Emmons, 1983).

The high protein content in MPC (50-85%) requires much less powder for fortification than SMP (34-36%). Alternatively, MPC could be used directly as the yogurt milk. At the same level of fortification, yogurt made from ultra-filtered milk demonstrated higher viscosity and firmness in contrast to yogurts made from SMP because of the higher protein content in the ultrafiltered milk base (Becker & Puhan, 1989; Biliaderis, Khan, & Blank, 1992; Lankes, Ozer, & Robinson, 1998). In a comparison of yogurt prepared from ultra-filtered milk and yogurt fortified with SMP to 5% protein, Savello and Dargan (1995) observed a higher viscosity (100%) and higher gel strength (50%) in the yogurts made from ultra-filtered milk.

The purpose of this study is to quantify the effect of MPC fortification on nonfat, set yogurt texture as measured by yield stress and apparent viscosity.

Chapter III: Methodology

In this experiment, four main ingredients were used: (a) low-heat skim milk powder (SMP) from Dairy America Inc., Fresno, California, USA, (b) milk protein concentrate (MPC) from Main Street Ingredients, La Crosse, Wisconsin, USA, (c) DVS lactic acid culture from Dairy Connection Inc., Wisconsin, USA (d) distilled water.

Milk Base Preparation

Three different milk bases—SMP base, MPC base, and Control base—were prepared by mixing commercial low-heat, non-fat dry milk powder and distilled water with and without addition of milk protein concentrate (Table 1). Separate yogurt bases were prepared from SMP. The first base containing 13.0% total solids was prepared by gradually adding 195 g of skim milk powder to 1305 ml of distilled water in a 2 liter beaker with continuous agitation with a magnetic stirrer at 800 rpm (Figure 1). The beaker was covered and the base was mixed for 60 minutes to insure complete hydration of the skim milk powder. The second, a control yogurt base, containing 8.8% total solids was prepared in the same way using 132 g SMP in 1368 ml of distilled water. The final yogurt base containing MPC was made by adding 132 g of SMP and 28.5 g of MPC to 1339.5 ml of distilled water as described above. The resulting base had total solids of 10.7%. The bases with 13.0% total solids and 10.7% total solids were formulated to have identical protein contents (4.6%) in the final mix.

Table 1

Level of Ingredients and Approximate Composition of Milk Bases used in Yogurt Manufacturing

Ingredients	**Experimental Milk bases**		
	Control	**SMP**	**MPC**
Distilled water (ml)	1368.0	1305.0	1339.5
SMP (g)	132.0	195.0	132.0
MPC (g)			28.5
Solids (%)	8.8	13.0	10.7
Protein (%)	3.1	4.6	4.6

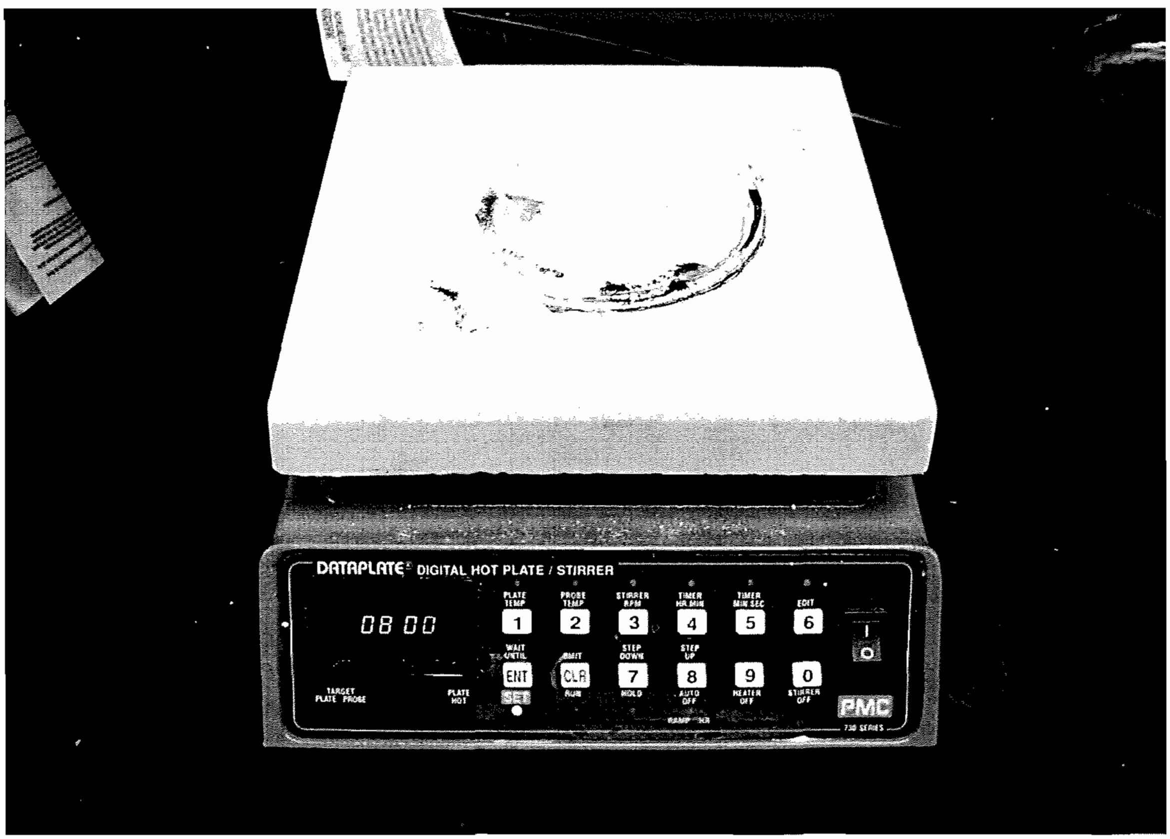

Figure 1. Hot Plate Magnetic Stirrer used for mixing the Ingredients

Preparation of Yogurt Culture

A Direct Vat Set (DVS) culture DCI612 (Dairy Connection Inc., Madison, Wisconsin) was used in the experiment to acidify the milk during yogurt manufacture. This culture consisted of two commercial strains of *Streptococcus thermophillus* and *Lactobacillus bulgaricus,* which are commonly used in the yogurt industry. The culture was stored at temperature of -15°C in a dried flaky form before use.

To insure consistent inoculation of the yogurt bases, a standard culture was prepared. A milk base was prepared by adding 70 g of SMP to 430 ml of distilled water and mixing on a magnetic stirrer for 45 min at 800 rpm. This base was heated at 95°C for 3 minutes by immersion in a circulating in a hot water bath. 130 mg of DCI612 was added to 500 ml of prepared milk base at 35°C in 750 ml jar. The jar was sealed and incubated at 35°C for 9 hours. The fermentation was stopped by refrigerating to 5°C when the pH reached 4.5. The resulting culture was used as an inoculum for the fermentation step.

Pasteurization of Yogurt Mix

Pasteurization to deactivate the enzymes and destroy microorganisms in the milk and to denature whey proteins was accomplished in a Stephan Universal Machine-5 (Stephanplatz 2-31789, Hamein, Germany) heated by a circulating water bath (Julabo, Pennsylvania, USA) to 80°C. All bases were held at 80°C for 25 minutes (Figure 2). The Stephan machine is a jacketed kettle with agitation (300 rpm) to facilitate rapid uniform heating of the milk bases. After pasteurization, the heated milk was poured into a glass beaker, covered with a plastic foil, and immediately refrigerated to 5°C. The pasteurized bases were stored overnight at 5°C to insure complete hydration of proteins and to minimize any foam generated during the heating/cooling process.

Figure 2. Stephan Cooker Assembly used for Pasteurization of Yogurt Mixes

Sample Preparation, Fermentation and Storage of Yogurt

The pasteurized milk mix was warmed up to 45°C in a hot water bath at 75°C. As soon as the temperature of the milk reached 45°C, mother culture was added to it at an inoculation rate of 2%, and the mixture was agitated. 200 ml replicates were transferred to 250 ml plastic cups. Lids were placed on the cups, and the cups were immediately transferred to an air incubator (Model 12-140, Quincy Lab Inc., Illinois, USA), which was maintained at 42°C. Seven replicates were made for each of the three bases —SMP base, MPC base, and Control base— in the experiment; two cups for gel strength analysis on Day 1, two cups for gel strength analysis on

Day 5, one cup for viscosity, one cup for pH and one cup for extra sample. Time was recorded when incubation began, and the pH was measured at 1 hour intervals. Upon reaching pH 4.6, the cups were immediately cooled to 5°C. After 24 hours at 5C, the samples were evaluated for gel strength and pH.

Analyses

Determination of pH. Measurement of pH occurred throughout the entire experiment for each of the different milk base variables. The pH of all the yogurt samples was measured by inserting the pH probe in the sample and lightly stirring for some moments and taking the reading. The pH of each of the samples was determined and recorded at the start of incubation and at one hour intervals until pH reached 4.6 when the fermentation was terminated. The pH values of all the samples stored at 10 °C were also measured after 24 hours and on Day 5.

Brookfield Viscometer. Viscosity and yield values of prepared yogurt samples were determined using a Brookfield DV-III Ultra Programmable Rheometer (Brookfield Engineering Lab Inc., Massachusetts, USA) (Figure 3). The operating principle of the DV-III is to drive a spindle, which is immersed in the sample through a calibrated spring. A deflection in the spring measures the viscous drag of the food sample against the spindle. A rotary transducer measures the spring deflection. Viscosity is calculated from size and shape of spindle, speed of the spindle, container of the sample in which the spindle is rotating, and full scale torque of the calibrated spring. All units of measurement for viscosity, shear stress, shear rate, and torque are calulated in accordance with the CGS or the SI system (*Brookfield DV-III ultra*, n. d.).

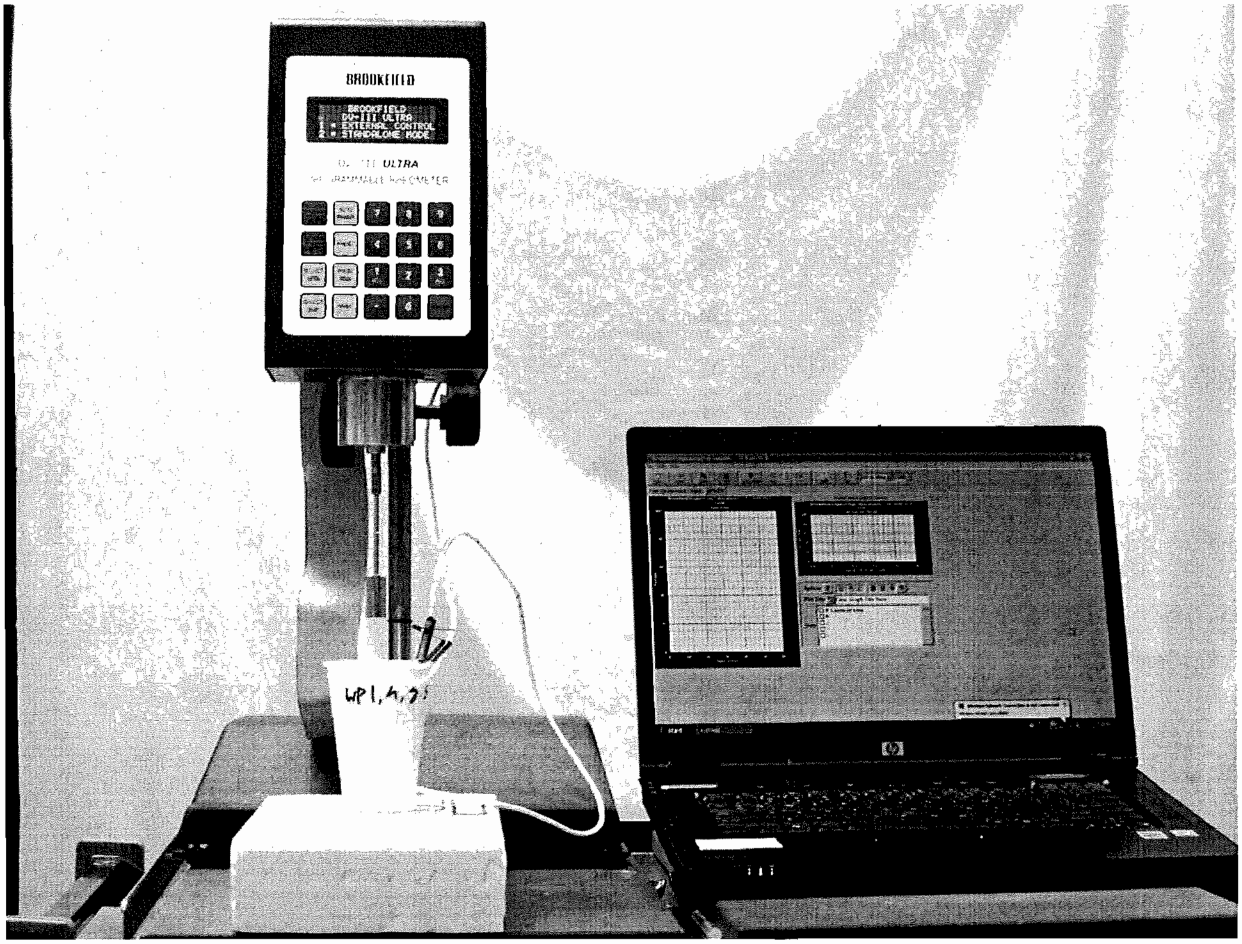

Figure 3. Brookfield DV-III Ultra Programmable Rheometer with Software

Measurement of Yield. The static shear yield stress and yield strain of test samples was determined using the Brookfield DV-III Ultra Rheometer. A sample was placed under the vane assembly and the four-bladed vane spindle, V-73 (1.267 cm diameter, 2.535 cm length), was immersed to the primary immersion mark into the sample and rotated at 0.1 RPM. Two readings of yield value were taken from each of the samples. The temperature of the sample was also recorded during the analysis. As a function of time, the torque required to maintain a fixed rotation was also recorded. Data for yield stress, yield strain and torque were calculated and recorded. When the test was complete, the data were saved and converted to MS Excel format.

After every sample test, the spindle was carefully rinsed and dried. Yield values were determined for each sample after 24 hours and on the fifth day of storage.

Measurement of Viscosity. The viscosity of the sample was also measured using the Brookfield DV-III Ultra Rheometer on the fifth day of sample storage. The V-73 spindle was immersed to the primary immersion mark into the sample and rotated at constant shear rate of 100 RPM and viscosity readings were recorded at increasing time intervals over 4 minutes. The viscosity measurements were carried out at sample temperature of 25°C. After every sample test, the spindle was carefully rinsed with water and wiped out gently before next use.

Chapter IV: Results and Discussion

The results of pH development during fermentation, gel strength of the final product based on yield stress determination and time dependent viscosity are discussed as follows.

Yield Stress

The results of yield stress analysis are shown in Figure 4. The average yield stress value of all three trials for Control sample on Day 1 was 128.12 (SD=7.22) and on Day 5 it was 144.31 (SD=7.69). There was an increase of 12% in yield stress value of Control between Day 1 and Day 5. For the SMP sample, average yield stress value of Day 1 for all the trials was 255.02 (SD=12.21) and for Day 5 the value was (M=279.55, SD=10.30). The yield value of SMP sample increased 9% from Day 1 to Day 5. The average yield value of all trials for MPC sample recorded on Day 1 was (M=309.36, SD=15.57) and the value for Day 5 determined was (M=332.56, SD=10.24). During storage of MPC samples, the yield value increased about 7% from Day 1 to Day 5. In previous reports, replacement of skim milk powder by MPC at the same protein level did not alter gel strength and texture (Mistry & Hassan, 1990; Mistry & Hassan, 1992; Modler et al., 1983b). In this experiment, however, yogurt fortified with MPC showed higher gel strength than SMP fortified yogurt. This could be attributed to minor differences in the composition of SMP and MPC. Generally MPC contains higher amount of calcium, magnesium and denatured whey protein which may add to the firmness of the yogurt. Similarly Savello and Dargan (1995) reported an increase in gel strength by 50% in ultrafiltered milk fortified yogurt compared to SMP enriched yogurt at a same protein level of 5%.

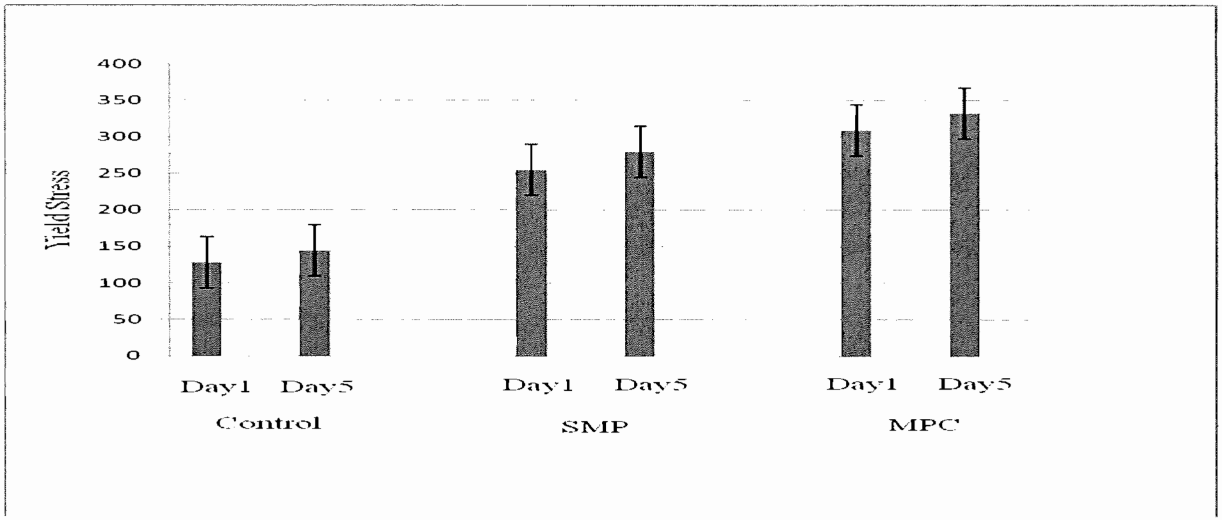

Figure 4. Average yield stress values from three trials of control, SMP and MPC yogurt on Day 1 and Day 5

Viscosity

In this study the viscosity decreases with the time for all yogurt samples at fixed shear rate. The apparent viscosity of all the varieties of yogurt is shown in (Figure 5, 6, 7). The viscosity of the yogurts which were fortified had higher viscosity than control. The yogurt fortified with NFDM+MPC had the highest initial viscosity while the control had the lowest initial viscosity. These differences might be attributed to the amount and different types of protein powders used. Looking at graphs of all three trials, the pattern of viscosity drop from initial readings occurs rapidly and is similar for all three samples. When the protein level was kept the same, no change was observed in viscosity when skim milk powder was substituted by MPC in yogurt (Guzman-Gonzalez et al., 1999; Rohm, 1993). Initial highest viscosity of MPC may be related to different composition of MPC from SMP in terms of increased protein content, decreased lactose and mineral content and thereby decrease in porosity in yogurts. In addition to this, Savello and Dargan (1995) reported increase in viscosity by 100% in ultrafiltered milk enriched yogurt compared to SMP fortified yogurt at same protein level of 5%.

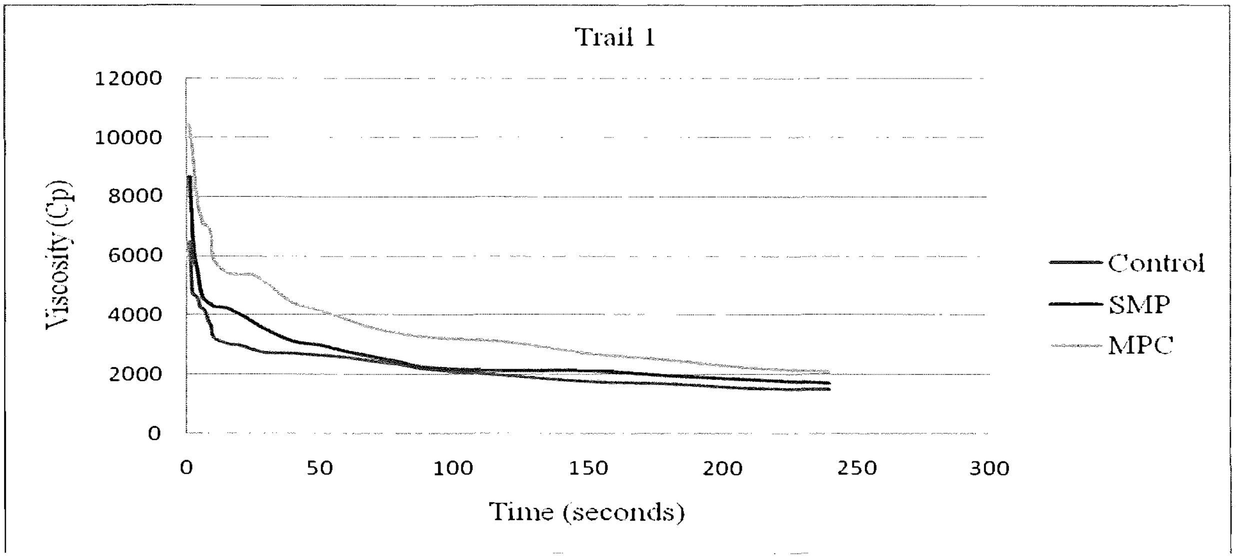

Figure 5. Time dependent viscosity measured for Control, SMP and MPC yogurts in Trial 1

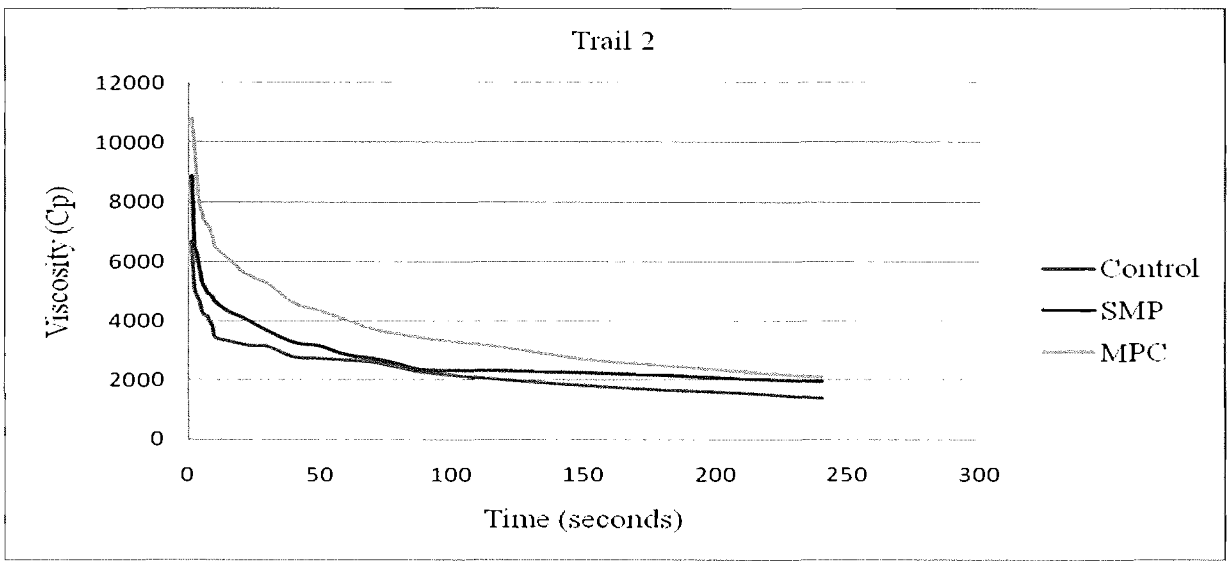

Figure 6. Time dependent viscosity measured for Control, SMP and MPC yogurts in Trial 2

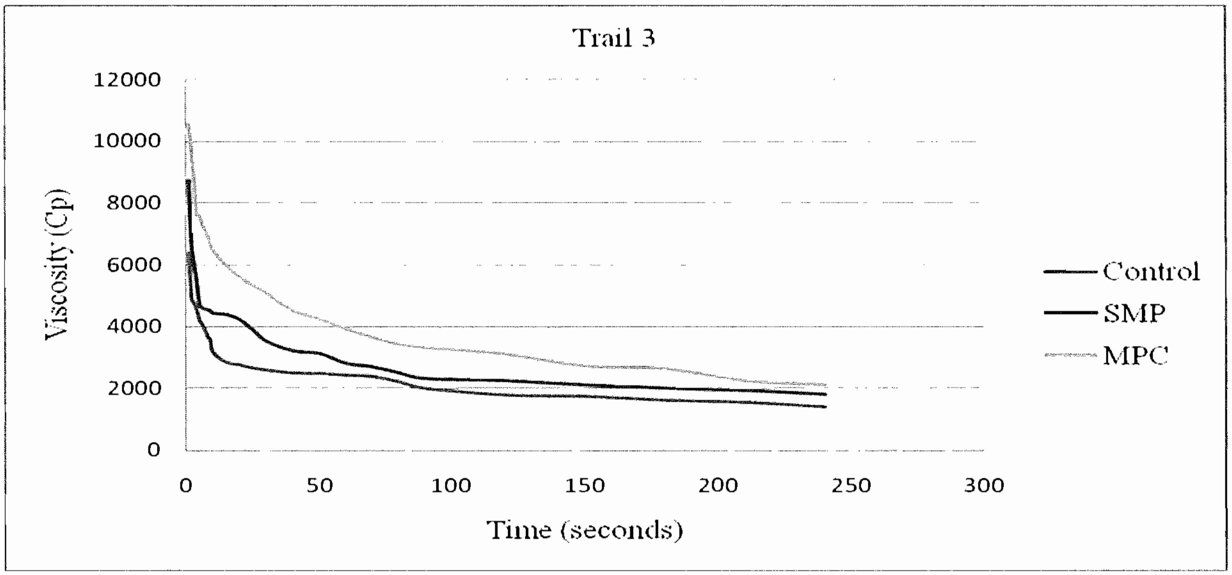

Figure 7. Time dependent viscosity measured for Control, SMP and MPC yogurts in Trial 3

pH Development

The measured pH values of Control, SMP and MPC samples at specific time interval during the entire fermentation stage until it reached pH 4.6 are shown in figure 8, 9, 10.

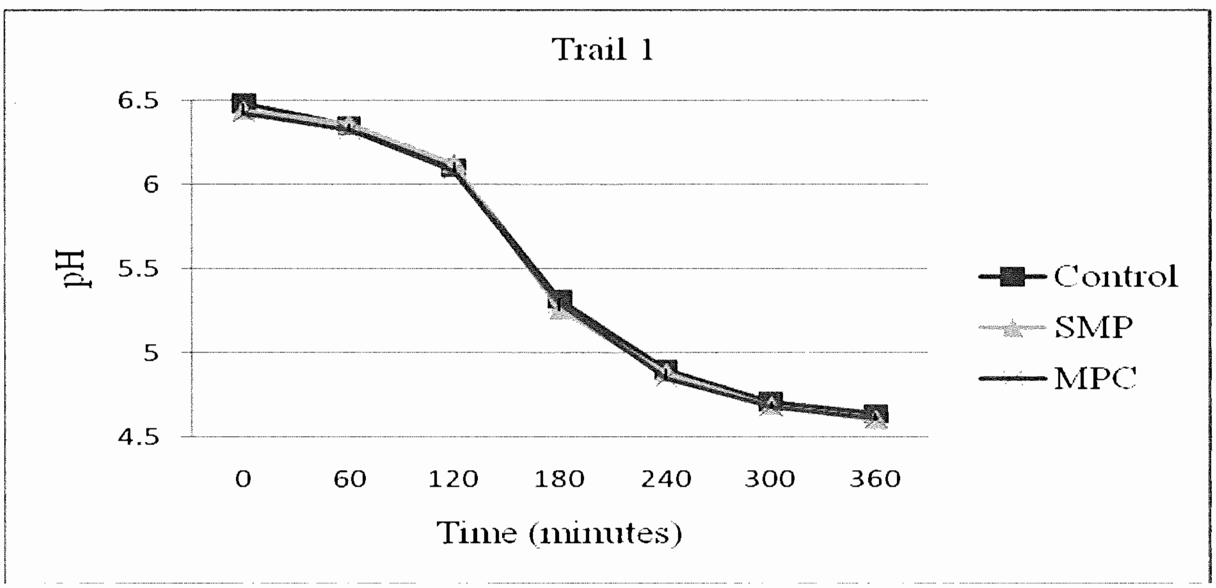

Figure 8. pH development rate during fermentation of Control, SMP and MPC samples for Trail 1

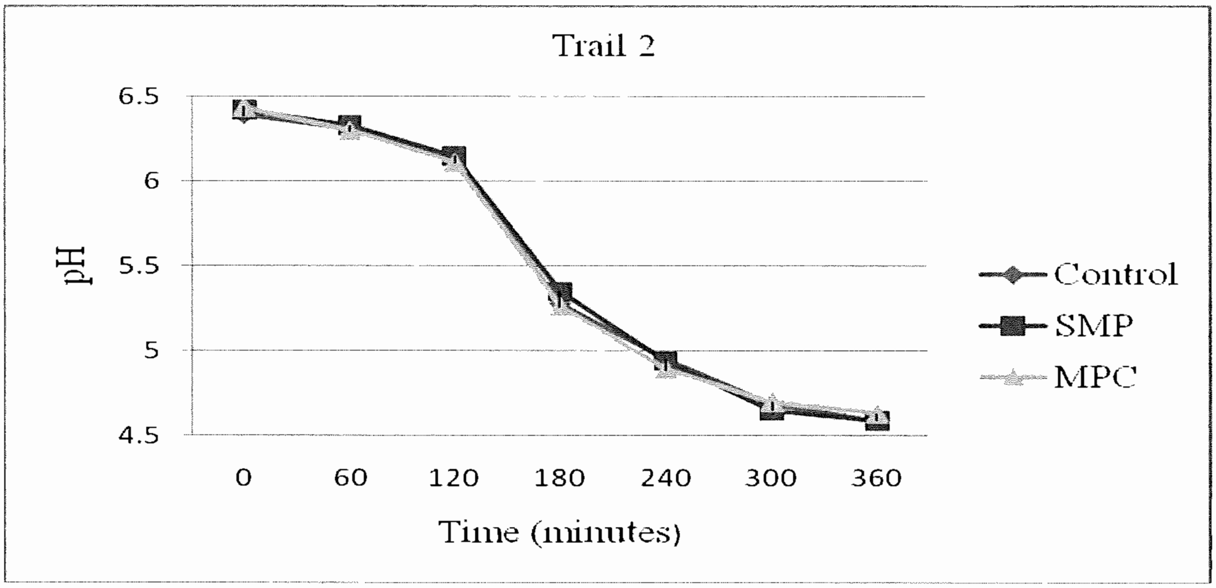

Figure 9. pH development rate during fermentation of Control, SMP and MPC samples for Trail 2

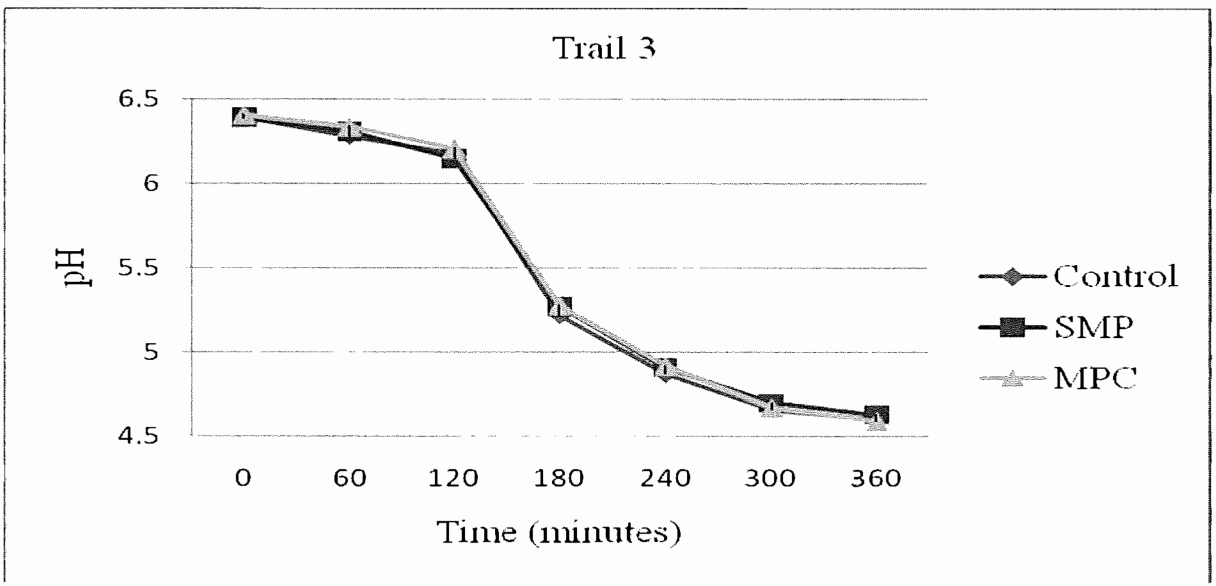

Figure 10. pH development rate during fermentation of Control, SMP and MPC samples for Trial 3

There was no difference in the gelation time for the different types of fortified yogurts. Yogurts prepared by fortifying with 8.8% SMP, 13% SMP and 10.7% combined MPC had similar pH profiles, and there was no difference in fermentation time required to reach pH 4.6 in these yogurts. This was found to be same as per literature. Fortification of yogurt with SMP did not have effect on pH development during fermentation (De Brabandere & De Baerdemaeker, 1999; Islenten & Karagul-Yuceer, 2006). Soukoulis et al (2007) reported similar incubation time for yogurts fortified with SMP and MPC when heat treated at 80°C for 30 minutes. A gradual pH drop after 24 hours (Day 1) and subsequently on Day 5 for all samples was due to slight acidity development as a result of the activity of lactic acid bacteria (Tamime & Robinson, 1999).

Chapter V: Conclusions

Set style yogurts were prepared from three milk formulations, two of which were fortified with different amounts of SMP and MPC but had the same protein concentration. One was considered a control without any addition. The yogurts were analyzed for textural properties such as gel strength and viscosity after a day and five days of storage. A rate of pH change during fermentation was also measured for each fortification. Milk protein concentrate yogurts showed higher gel strength and viscosity compared to SMP yogurts. There was slight increase in the strength of yogurt gels in all samples from Day 1 to Day 5. A control sample was found to have the lowest gel strength and viscosity. There was no difference in pH change during fermentation.

Recommendations for Further Study

Further research is recommended to evaluate and compare micro textural and sensory analysis of the yogurts prepared from SMP and MPC. In addition to this, similar studies can be conducted to compare the textural, physical and sensory properties of yogurts made from MPC and other casein based ingredients such as condensed skim milk and caseinates.

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