QUALITY OF YOGURT SUPPLEMENTED WITH WHEY PROTEIN CONCENTRATE AND EFFECTS OF WHEY PROTEIN DENATURATION

by

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Abstract

Yogurt is a good source of whey proteins, which have been reported to provide positive health benefits. During yogurt manufacture, the yogurt mix receives a heat treatment which pasteurizes the product, denatures the whey proteins affecting their availability, and enhances quality attributes. Thus the objective of this research was to improve the undenatured whey protein content in yogurt. The study was divided in two parts. The first part focused on the effect of pasteurization treatments of yogurt mixes (65 °C for 30 min vs. 90 °C for 10 min) on the yogurt firmness, G', L*, syneresis and water holding capacity (WHC), and how these properties change as a function of storage. Nonfat dry milk (NFDM) was reconstituted (~11% w/v) pasteurized, cooled, inoculated with yogurt culture, incubated to pH 4.5, stored at 5 °C ±1 and evaluated for various physical and chemical properties on days 1, 15 and 29. The experiment was replicated 3 times and data were analyzed by SAS®. Yogurt samples had a 5-fold difference in whey protein denaturation (WPD) and the greater the WPD the greater the firmness, G', L* and WHC but lesser the syneresis. During yogurt storage, L*, G', syneresis and WHC increased. The second part of this research focused on whey protein concentrate (WPC) addition (3%) in yogurt mix combined with two pasteurization treatments (70 °C for 30 min vs. 90 °C for 10 min) to determine their effects on the yogurt quality. Yogurt mixes were formulated using 12.5% NFDM or 9.5% NFDM and 3% WPC and a procedure similar to the previous study was followed. The WPC addition resulted in a yogurt with decreased firmness, G', WHC but increased syneresis. Yogurt made from mixes pasteurized at 90 °C for 10 min had ~60% WPD and comparable quality attributes regardless of WPC addition. Thus, additional WPC and less WPD in this study resulted in a yogurt with slightly lesser quality attributes but more undenatured whey proteins in the final yogurt.

Table of Contents

List of Figures

List of Tables

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Dedication

I would like to dedicate this work to my parents Laxman and Urmila and my elder brother Dr. Vikrant for their support, understanding and help to reach this point.

CHAPTER 1 - INTRODUCTION

The relationship between food and health is established and studies have shown that food can reduce some risk factors that affect health. Prevention of disease in the future will be just as important as treatment of diseases today and many consumers are highly aware of the health-properties of foods. The market for health-promoting food is showing promising growth with an annual increase of 7%, which is projected to last through 2012 (Haug *et al.* 2007, O'Donnell 2009).

Bovine milk is intended for the nutrition, growth, stimulation and immunological protection of the young calf, but it has been consumed by humans in different forms since prehistoric times (de Wit 1998). Bovine milk contains the nutrients needed for growth and development of the calf, and is a resource of lipids, proteins, amino acids, lactose, vitamins and minerals. It contains immunoglobulins, hormones, growth factors, cytokines, nucleotides, peptides, polyamines, enzymes and other bioactive peptides (Haug *et al.* 2007).

Bovine milk contains about 32 g protein/l. Milk proteins (casein and whey) have high biological value, and milk is therefore a good source for essential amino acids (Haug *et al.* 2007). In milk, caseins predominate, approximately 80% of total protein. Caseins carry calcium and phosphate and enhance efficient digestion. The whey proteins are globular-shaped and are more water soluble than caseins, with the principle fractions being β-lactoglobulin, α-lactalbumin, bovine serum albumin and immunoglobulins (Shah 2000).

The rates at which the amino acids are released during digestion and are absorbed into circulation differ among the milk proteins. Whey proteins are considered as rapidly digested proteins that provide high concentrations of amino acids in the body (Nielson *et al.* 2007). The benefit of drinking whey (a cheese manufacturing byproduct) has been known for centuries, and two ancient proverbs from the Italian city of Florence say, "If you want to live a healthy and active life, drink whey" and, "If everyone was raised on whey, doctors would be bankrupt" (Brink 2008).

Yogurt–a milk based mix fermented by lactic acid bacteria–is a valuable health food for both young and old. Yogurt proteins are hydrolyzed increasing their availability. In comparison

with cheeses, whey proteins (mainly α-lactalbumin and β-lactoglobulin) remain in yogurt. All of these factors contribute to the increased nutritional value of yogurt (Ayar *et al.* 2006).

Milk is pasteurized to kill pathogens at 72 °C for 15 s-a process known as High Temperature/Short Time (HTST) pasteurization. Traditionally the yogurt manufacturing process includes a mix pasteurization step at ≥80 °C for 10 to 30 min prior to inoculation with lactic acid bacteria. Milk pasteurization at temperatures that exceed 70 °C results in the thermal denaturation of the globular whey proteins in which the native conformation is disrupted. Denaturation of the whey proteins can result in the exposure of reactive amino acid side groups that are normally buried within the native conformation (Anema and Li 2003). In yogurt, denaturation of whey proteins contributes to overall quality of the final product, but that final quality is contingent on not only the whey proteins denaturing but also their subsequent interactions with casein in the yogurt mix (Lucey and Singh 1998). Yogurt mixes pasteurized at ≥ 80 °C for 10 to 30 min yield yogurt with greater firmness and rheological properties but less syneresis (Parnell-Clunies *et al.* 1986, Lucey and Singh 1998).

Whey proteins contain all amino acids the human body requires for muscle protein synthesis (Tipton *et al.* 2004). But excessive heating of yogurt mixes denatures whey proteins which could further negatively affect overall protein quality in yogurt (Cribb *et al.* 2007, Haug *et al.* 2007, Hoffman and Flavo 2004, de Wit *et al.* 1998). Thus there is a need to improve yogurt protein quality by minimizing whey protein denaturation in yogurt.

This research will evaluate the approach to supplement yogurt mix with whey protein concentrate combined with minimum pasteurization (65 °C or 70 °C for 30 min) to produce a yogurt mix with less whey protein denaturation. Increased whey protein content with less whey protein denaturation should enhance the nutritional value of yogurt and may affect quality attributes negatively. Mixes will be fermented and yogurt gels will be evaluated for quality during storage. Little information is available on a yogurt quality as a function of pasteurization with a goal to maximize undenatured whey protein. Thus, the objective of this research is to investigate the quality of yogurt made with a mix containing whey protein concentrate and minimal whey protein denaturation.

CHAPTER 2 - LITERATURE REVIEW

Yogurt History

Yogurt is a popular food (Sodini *et al.* 2005). Although no records exist regarding yogurt origin, yogurt is believed to be one of the oldest fermentation products known to humans, originating in the Middle East and Asia (Chandan 2006, Tamime and Robison 1999). Yogurt making dates back thousands of years, possibly to the domestication of cows, sheep or goats. During the last few decades the manufacturing process has become more controlled because of discoveries and advances in fermentation science. However yogurt making is a complex process combining art and science (Tamime and Robison 1999, Varnam and Sutherland 2001).

Overview of Yogurt in United States

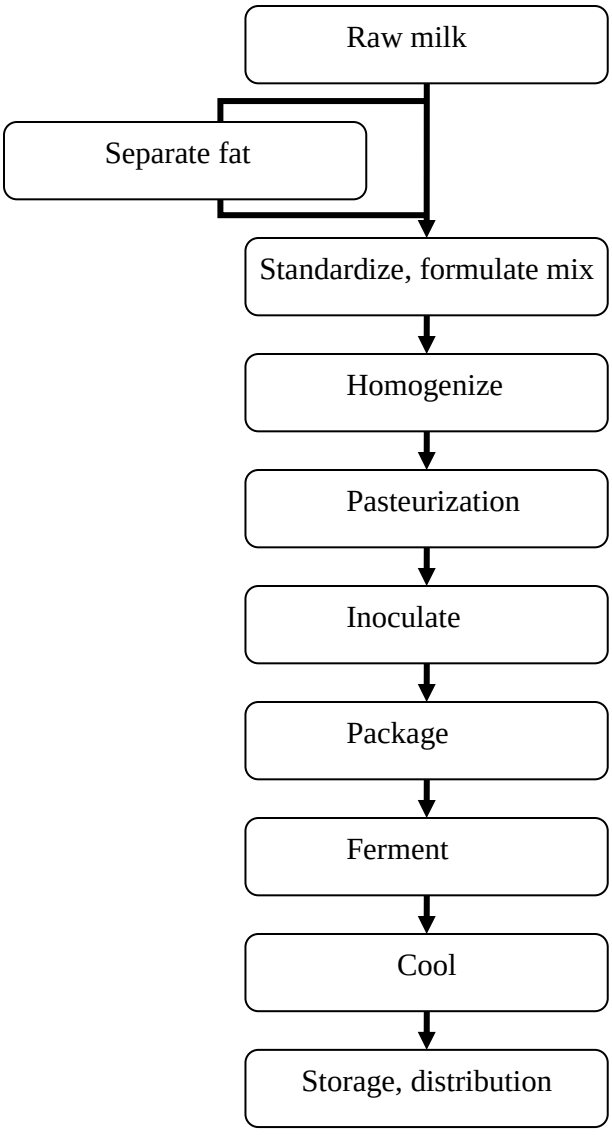
The U.S. Food and Drug Administration (2009) defines yogurt as, "the food produced by culturing one or more of the optional dairy ingredients cream, milk, partially skimmed milk or skimmed milk, used alone or in combination with a characterizing bacterial culture that contains the lactic acid-producing bacteria, *Lactobacillus bulgaricus* and *Streptococcus thermophilus*" (21CFR 131.200|a). Other approved optional ingredients include, "vitamins, concentrated skimmed milk, nonfat dry milk, buttermilk, lactose, lactalbumins, lactoglobulins, or whey (modified by partial or complete removal of lactose and/or minerals, to increase the nonfat solids content of the food), sugar (sucrose), beet or cane; invert sugar (in paste or syrup form); brown sugar; refiner's syrup; molasses (other than blackstrap); high fructose corn syrup; fructose; fructose syrup; maltose; maltose syrup, dried maltose syrup; malt extract, dried malt extract; malt syrup, dried malt syrup; honey; maple sugar; flavoring ingredients, color additives and stabilizers" (21CFR 131.200 b| c| d).

In the U.S., yogurt production increased from 19.95 million kg in 1960 to 1577.46 million kg in 2007 with an annual per capita consumption of 4.99 kg in 2007 (IDFA 2008). Increased yogurt production and consumption are attributed to yogurt's perceived health benefits and wide consumer appeal (Chandan 2006, Lucey and Singh 1998).

General Manufacturing

Yogurt can be classified based on physical state - set or stirred. Set yogurt is recognized by a gel-like structure because it is fermented in the container (Chandan 2006, Tamime and Robinson 1999). Once a desired acidity is attained, a rapid cooling is done to arrest additional acid production (Tamime and Robinson 1999). However in the U.S., the stirred-style, flavored yogurt is more popular. The yogurt manufacturing process has changed little over the years, although there are some refinements in relation to yogurt cultures. Figure 2-1 shows a typical flow diagram for yogurt manufacturing.

Figure 2-1: Set yogurt manufacturing schematics



Source: Varnam and Sutherland 2001

Ingredients for Yogurt Base

Milk is the main ingredient of yogurt. However, most yogurts contain additional solids such as milk solids non fat (MSNF), to boost the non fat milk solids from 8.25% to 16% (CFR131.200a, Tamime and Robinson 1999). The range of total solids in commercial yogurt is 9 to 30% (Tamime *et al.* 1987). The non-milk solids consist of sweeteners, stabilizers, fruits and colorants. Sweeteners such as sucrose, invert sugar, fructose, glucose or galactose syrup are added mainly for taste preferences (Tamime and Robinson 1999). Stabilizers such as natural gums, modified natural gums or synthetic gums are added to improve and maintain gel firmness and consistency, while also (to many people) improving appearance and mouthfeel. Typical yogurt stabilizers include carboxy methyl cellulose (CMC), guar gum, xanthan gum, κ-carrageenan or pectins (Soukoulis *et al.* 2007, Tamime and Robison 1999). Hydrocolloids specifically stabilize gel structures, increase viscosity and either form networks with milk constituents (e.g. pectin) or establish a separate gel structure (e.g. xanthan gum) (Teles and Flores 2007, Keogh and O'Kennedy 1998).

Once the formulation has been set, these dry and liquid ingredients are mixed to form a homogeneous mixture.

Homogenization

In general, yogurt mix is homogenized at 15 MPa and can be done before or after pasteurization. Homogenization reduces the size of the milk fat globules (≤ 2 μm) (which in their native state range from 1 to 10 μm), which prevents cluster formation and surface aggregation (Chandan 2006). Homogenization induces interactions between milk proteins (predominately casein) and fat, due to increased surface area of the fat globules (Cano-Ruiz and Richter 1997).

Pasteurization

Yogurt mix is pasteurized (80 to 85 °C for 30 min or 90 to 95 °C for 10 min) to destroy pathogens, but as temperature|time exceeds pasteurization minimums (63 °C for 30 min or 72 °C for 15 s) (CFR 1240.61), other desirable outcomes occur–for instance, inactivation of some nonpathogenic microorganisms, production of stimulatory/inhibitory factors for starter cultures, inactivation of enzymes and alterations to the physicochemical properties of milk constituents (Tamime and Robison 1999). Almost immediately, the mix is cooled to 42 to 44 °C.

Inoculation, Fermentation and Gel Formation

S. thermophilus is a Gram-positive, facultative anaerobic homofermentative bacterium with a spherical or ovoid shape, a diameter <1 µm and commonly associates into chains or pairs. These microorganisms exhibit optimum growth at 39 to 46 °C (Chandan 2006). Some strains produce exopolysaccharides (EPS) (Mediedo *et al.* 2002).

L. delbrueckii spp. *bulgaricus* is a Gram-positive, nonsporing homofermentative bacterium rod with rounded ends that forms chains which vary in length from 0.8 to 6 µm. These organisms can grow below 10 °C but optimum growth is between 40 to 47 °C. Some strains are capable of producing exopolysaccharides (EPS). These two bacteria are added to the cooled pasteurized mixes (40 to 44 °C) (Chandan 2006).

In the initial stage of fermentation *S. thermophilus* grows faster than *L. bulgaricus*, fermenting the lactose and producing lactic acid. When the pH reaches ~5, growth of *S. thermophilus* slows and *L. bulgaricus* grows at a faster rate than *S. thermophilus* (Chandan 2006). In yogurt, gel formation is a result of biological, chemical and physical actions. The microbial growth causes the mix pH to decrease from 6.8 to ~5, which at that point solubilizes colloidal calcium ions (Lee and Lucey 2004a). A further pH decrease from ~5 to 4.6 induces the physical aggregation of the casein micelles. At pH 4.6, the isoelectric point of casein, the charges on the casein micelles are neutralized. At pH < 4.5, rearrangement and aggregation of casein micelles lead to protein gel formation and thus a particle gel structure (Tamime and Robison 1999).

Acid casein gels such as yogurt are defined as particle gels formed by the aggregation of casein micelles (Ozer 2004, Dickinson 1994). The 3-dimensional structure of the particle gel is stabilized by covalent (thiol and disulfide exchange reactions) and non-covalent protein interactions (hydrophobic effect, steric effect, Van der Waals attraction/repulsion forces and electrostatic and ionic interactions) (Rohm and Kovacs 1994, Mitchel 1980). The properties of an acid casein gel are closely related to the casein concentration, enthalpic/entrophic nature of the gel, the extent of repulsion/attraction forces between casein particles and the gelation mechanism (Ozer 2004, Dickinson 1994). The size and distribution of casein micelles and the number of protein contact points also influence the structure of a gel. To summarize, the balance between the strong and permanent protein bonds to the weak and non-permanent bonds, determines the characteristics of an acid casein gel (Ozer 2004).

Increasing inoculation rates (0.5 to 4%) and incubation temperatures (40 to 45 °C) yield yogurt gels with compact structures due to increased acidification rates which in turn decrease the time to reach the isoelectric point of casein. Colloidal calcium phosphate in the form of Ca^{2+} ions increases electrostatic repulsions, causing gel formation at a decreased pH (~5.2 to 5), as gelling pH is defined as the pH of gel formation (e.g. storage modulus (G') equals 1 Pa) (Lucey *et al.* 1997). When incubation temperature increases (40 to 44 °C), increased hydrophobic interactions occur, which contribute to a more compact conformation within the gel as well as more contractions of the casein particles (Lee and Lucey 2004b).

Cool and Package

At pH 4.5 the yogurt is cooled to $\leq$ 5 ºC primarily to restrict microbial activity. The packaging of yogurt ensures safety of the product until consumption (Tamime and Robinson 1999).

Distribution

Distribution of yogurt involves the storage, transport and marketing (retail/wholesale/food service), leading towards the consumer. Typical expected shelf life is 28 to 49 days at 0 to 7 °C (Chandan 2006). However NDC (2009) recommended a shelf life of 7 days at the consumer side.

Nutritional Aspects of Yogurt

For humans, yogurt provides nutrients such as proteins (caseins and whey), minerals and vitamins. The starter cultures degrade the lactose, the main sugar in milk which may be beneficial for lactose-intolerant people (Chandan 2006). Yogurt contains bacteria, which are acid tolerant, survive stomach acidity and secrete lactic acid in the human intestine (Shah 2007, Tamime and Robison 1999). *Lactobacillus delbrueckii* spp. *bulgaricus* and *Streptococcus thermophilus* establish themselves as the gut dominant bacteria, decreasing the number of putrefactive organisms and producing a variety of therapeutic compounds such as bioactive peptides (casein phosphopeptides, lactoferroxins and casooxins) which provide attributes such as antistress, anticarcinogenic, antihypertensive, immunostimulants, antithrombotic and mineral transportation (Chandan 2006, Zsivkovits *et al.* 2003, Wollowski *et al.* 2001, Kawase *et al* 2000, Wollowski *et al.* 1999). Bioactive peptides are active only after the peptides are derived from the native form of the protein. Peptides such as casein phosphopeptides, lactoferroxins and casooxins contain 3 to 64 amino acids, display hydrophobic characteristics and resist hydrolysis in the gastrointestinal tract. Lactoferroxins and casooxins act as opoid antagonists, which have analgesic properties similar to aspirin. Casein phosphopeptides enhance bioavailability of calcium, phosphorus and magnesium which contribute to optimum bone health and prevent dental caries (Chandan 2006, Tamime and Robison 1999). Although yogurt cultures are not natural inhabitants of the human intestine, current theories suggest that they offer health benefits such as improved protein digestibility, alleviated lactose-intolerance, enhanced mineral absorption, controlled intestinal health and enhanced immunity (Shah 2007).

Milk Proteins

Protein availability is defined as the ratio of the amount of the protein available to be absorbed and utilized in the human body to the protein intake and differs with protein type (Shane and Neil 2006). Two major groups of proteins–casein and whey–exist in milk. Table 2-1 shows the distribution and some properties of casein and whey proteins in bovine milk.

Table 2-1: Protein distribution in bovine milk

Type	Characteristics of type	Fraction	Total protein g/Kg milk	Number of amino acids
Casein	Hydrophobic; MW[1]-20 to 25 KDa; except κ-casein all Ca^{+2} insensitive; β-casein heat sensitive; form micelles and exist as colloids in milk; micelles heat stable < 140 °C; average micelle diameter 100 to 300 nm; 10^{14}-10^{16} micelles ml^{-1} of milk	α_{s1}-Casein	12 to 15	199
		α_{s2}-Casein	3 to 4	207
		β-Casein	9 to 11	209
		κ-Casein	3 to 4	169
Whey	Hydrophilic; globular proteins; MW[1]-14 to 69 KDa (except immunoglobulin and proteose/peptone fraction); diameter 1.8 to 6 nm; heat sensitive; contain no phosphate residues	α-Lactalbumin	1 to 1.7	123
		β-Lactoglobulin	3 to 4	162
		Immunoglobulin	0.5 to 1.8	-
		Serum albumin	0.2 to 0.4	582
		Proteose/peptones	0.6 to 1.7	-
Other	Miscellaneous proteins contain enzymes; lactoferrins- bind iron; membrane proteins bound fat moieties	Glycomacropeptide	1.2	-
		Miscellaneous	0.8	-
		-Enzymes		
		-Lactoferrins		
		-Membrane protein		
Total			35.5	

Sources: Chandan (2006), Shah (2000), Tamime and Robison (1999), Fox and McSweeney (1998), Walstra and Jenness (1984).
[1]MW-molecular weight of individual fraction

Caseins exist in micelle forms, which are colloids of the different caseins viz. - α_{s1}-, α_{s2}-, β- and κ-casein and have an average diameter of ~150 nm in the native form (Haug *et al.* 2007, Fox and McSweeney 1998). The α-$_{s1}$-casein, α_{s2}-casein, β-casein and κ-casein vary in the sequence and number of amino acids and contain 199, 207, 209 and 169 amino acids, respectively (Swaisgood 1993). The four casein proteins differ in charge distribution and aggregation sensitivity (Tamime and Robison 1999). In the human diet, casein is one of the greatest sources of calcium. The α_{s1}-, α_{s2}- and β-casein are sensitive to calcium whereas κ-casein is insensitive to calcium as the calcium is a structural component of the micelle. Kappa-casein, which has a hydrophilic tail covers the casein micelle; stabilizing the other caseins against precipitation (Swaisgood 1993, Horne 2006).

On the other hand, whey proteins are globular, water-soluble and categorized into 5 fractions: β-lactoglobulin, α-lactalbumin, bovine serum albumin, immunoglobulins and proteose peptone fractions (de Wit 1998). Whey protein diameters vary from as small as 1.6 nm for α-lactalbumin to 6 nm for immunoglobulins (de Wit 1998, Haug e*t al.* 2007). Small proteins (such as α-lactalbumin) form a single globular domain, which provides stability and overall rigidity (de Wit 1998). In general whey proteins do not react with calcium and casein in the native states; however once denatured, whey proteins react with the casein micelle. Hence in milk, the role of whey proteins is a function of the heat treatment (Tamime and Robison 1999). Whey proteins have high (>90%) protein availability, because they contain significant amounts of sulpher-containing amino acids (such as cysteine). Whey proteins are a good source of branched-chain amino acids (more importantly leucine), which are important in skeletal muscle protein synthesis (Ha and Zemel 2003). Overall whey proteins digest rapidly compared to casein and thus provide greater quantities of the essential amino acids such as cysteine (2.4 vs. 0.3%), leucine (12.4 vs. 10.4%) and lysine (8 vs. 7.5%) (Hoffman and Flavo 2004, Haug *et al.* 2007) (Table 2-2).

Evidence suggests that whey proteins, found naturally in milk, increase muscle protein synthesis which in combination with resistance exercise can improve skeletal muscle composition (Tipton *et al.* 2004). Beta-lactoglobulin is a rich source of cysteine (151 mg acyl/g of true protein nitrogen: an essential amino acid) and stimulates synthesis of glutathione, which is an anticarcinogenic tripeptide (Mehmoud 1994, Jost *et al.* 1999). Alpha-lactalbumin supports biosynthesis of lactose which is an important energy source for newborn babies. Serum albumin binds free fatty acids in blood exhibiting immunoenhancement (de Wit 1998). Serum albumin,

lactoferrins and immunoglobins are whey proteins that could act as potential modulators of various regulatory processes (predominantly immunity) in the human body (de Wit 1998).

Table 2-2: Amino acid distribution of casein and whey proteins in the bovine milk

Essential amino acid	α_{s1}-Casein	α_{s2}-Casein	κ-Casein	β-Casein	α-Lactoglobulin	β-Lactalbumin
1/2Cysteine	0	2	2	0	6	5
Alanine	9	8	15	5	3	14
Arginine	6	6	5	4	1	3
Aspargine	8	14	7	5	12	5
Aspartic acid	7	4	4	4	9	11
Glutamic acid	24	25	12	18	8	16
Glutamine	15	15	14	21	5	9
Glycine	9	2	2	5	6	3
Histidine	5	3	3	5	3	2
Isoleucine$^{\delta}$	11	11	13	10	10	8
Leucine$^{\delta}$	17	13	8	22	22	13
Lysine	14	24	9	11	15	12
Methionine	5	4	2	6	1	4
Phenylalanine	8	6	4	9	4	4
Proline	17	10	20	35	8	2
Ser phosphate	8	11	1	5	0	0
Serine	8	6	12	11	7	7
Threonine	5	15	14	9	7	8
Tryptophan	2	2	1	1	2	4
Tyrosine	10	12	9	4	4	4
Valine$^{\delta}$	11	14	11	19	10	6

Source: Swaisgood 1993, Fox and McSweeney (1998).
$^{\delta}$Branched chain amino acids; underlined amino acids are essential amino acids

WHEY PROTEIN CONCENTRATE

Whey is a major by-product of the cheese industry and whey is further processed by separation or fractionation techniques to remove water, lactose, lipids and minerals. Typically these products are then dried to produce food ingredients such as whey powder, whey protein concentrates (WPC) (protein content 30 to 90%) and whey protein isolates (WPI) (protein content >90%) (Lopes *et al.* 2006, Delaney 1976). In the food industries, WPC and WPI are used extensively due to their nutritional and functional properties, especially their gel-forming ability which produces viscoelastic gels after denaturation. Gelation of globular proteins has been extensively investigated and can be induced via chemical, enzymatic or thermal actions (Kinsella and Whitehead 1989, Lopes *et al.* 2006).

Many yogurt formulations contain plant hydrocolloids or animal proteins to impart desired thickening, stabilizing or gelling properties. Inclusion of these compounds can lead to flavor problems even at the recommended low levels such as 0.3 to 0.5%. Due to the flavor problems, combined with a trend to produce "all natural" yogurt, hydrocolloids have been replaced with milk-based proteins and milk solids (Modler *et al.* 1983). A variety of ingredients are listed as potential stabilization and fortification agents in yogurt formulations; such as 3 to 4% nonfat dry milk (NFDM), whey powder concentrates (1 to 2%), sodium caseinate (1 to 2%), and milk concentrated by ultrafiltration (UF) or reverse osmosis (RO) (18 to 20% total solids) (Modler *et al.* 1983, de Wit 1998). In the past, NFDM was used to enrich the milk before fermentation; however, increased quality and availability of other and generally cheaper dairy ingredients, such as WPC, have provided a cost-effective alternative to NFDM. In addition, whey proteins offer functional properties different from the whole milk proteins in NFDM such as gelation, foam formation, solubility and emulsification (Sodini *et al.* 2005, Schmidt *et al.* 1984). Whey protein concentrate has been added as an ingredient to yogurt mix to reduce whey separation, increase firmness and enhance viscosity (Lucey *et al.* 1999).

CHEMISTRY OF YOGURT MIX

Whey-casein interactions

While mix pasteurization at 65 °C for 30 min inactivates pathogens and most enzymes, whey proteins remain intact in the mix (whey protein denaturation < 10%) (Chandan 2006). However yogurt mix pasteurization at 85 °C for 30 min or 95 °C for 5 min, denatures greater than 80% of whey proteins, where temperature and time govern the amount of whey protein denaturation (Lucey *et al.* 1998, Parnell-Clunies *at al.* 1986). When whey proteins are denatured, they complex with casein micelles present in a yogurt mix (Anema and Li 2003, Lucey and Singh 1998). Thus if milk is exposed to ≥ 80 ºC, whey proteins unfold and the reactive sulpher groups become available (thiols in cysteine) (Lee and Lucey 2004a). Beta-lactoglobulin, the predominant whey protein in whey can form disulfide bonds with other cysteine containing proteins such as β-lactoglobulin and bovine serum albumin (BSA) or disulphide bridges with non-cysteine containing proteins such as α-lactalbumin, κ-casein and α_{s2}-casein. The denatured β-lactoglobulin forms hair-like structures, which subsequently reacts with κ-casein increasing the effective micelle size (Anema and Li 2003, Vasbinder *et al.* 2003).

Alpha-lactalbumin also complexes with κ-casein; however, different factors affect these interactions (Vasbinder *et al.* 2003, Mottar *et al.* 1989). Hollar *et al.* (1995) reported that β-lactoglobulin denatured to a greater extent as compared to α-lactalbumin when simulated whey dispersions were pasteurized to 66 or 71 °C and held for 30 to 120 min. Although, α-lactalbumin was not needed for β-lactoglobulin to react with κ-casein; β-lactoglobulin was needed for α-lactalbumin to react with κ-casein (Corredig and Dalgleish 1999). Anema and Li (2003) showed that the association rate of denatured whey proteins with casein micelles was slower than the denaturation rate of whey proteins; thus, unbound denatured whey proteins existed in milk.

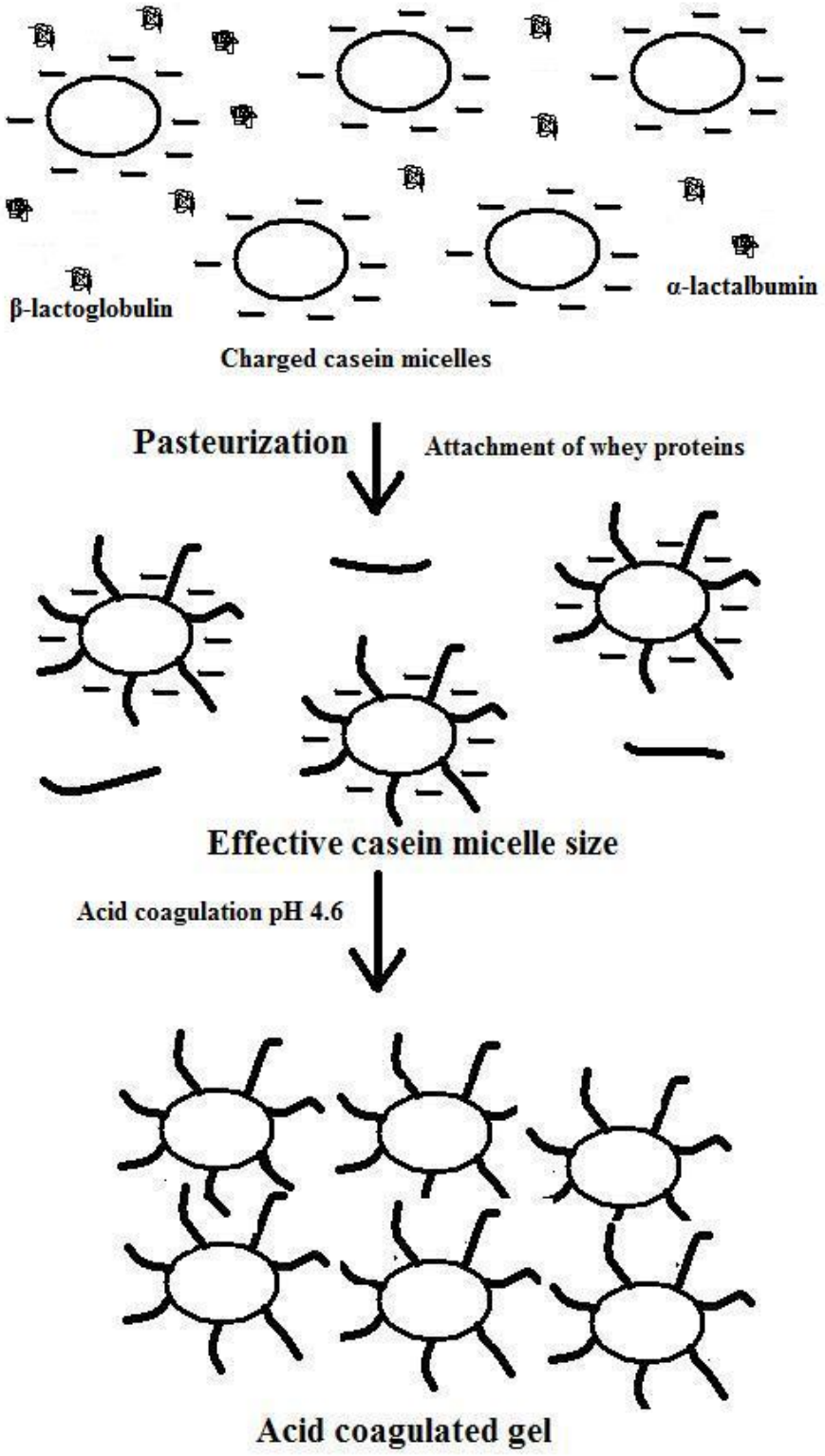
In a yogurt mix, whey proteins can attach directly to casein micelles or the casein-denatured whey protein complexes via cross-linkings. Reports have been made that casein micelle sizes increase from 100 to ~400 nm as a function of the heat treatment due to the whey protein attachment to the casein micelles (de Wit 1998, Needs *et al.* 2000, Anema and Li 2003).

Aziznia *et al.* (2008) reported that microstructure of nonfat yogurt containing WPC (0.75, 1.5 or 2%) had a more compact structure consisting of fused casein particles and large aggregates as compared to nonfat yogurt made from NFDM. The WPC-added yogurt gel structure was

characterized as extensively large, fused protein clusters close to each other, but protein chains were not observed. Excessive addition (2%) of WPC in the yogurt mix led to the formation of extremely large whey protein aggregates which were entrapped within the casein micelle network saturating the binding capacity of κ-casein. Puvanenthiran *et al*. (2002) reported casein micelle size increased up to ~700 nm. These attachments altered gel formation during acidification by increasing the distances among the casein clusters (increasing voids) and loosening the associations of casein-casein. Thus in the yogurt, whey proteins in excess of ≥ 1% lead to whey protein self-aggregating and forming a separate gel structure embedded among the casein-whey protein gel structure (Aziznia *et al.* 2008).

Figure 2-2 gives the schematics of yogurt manufacturing where, whey proteins attach to caseins on heating and gel is formed due to charge neutralization of casein micelles.

Figure 2-2: Yogurt gel formation due to milk acidification



Adopted from Mellema 2000

YOGURT QUALITY

The gel quality of yogurt is defined by parameters such as color, firmness, rheology (e.g. G', loss tangent), syneresis and water holding capacity (Lee and Lucey 2006, Lee and Lucey 2004a, Lee and Lucey 2004b, Anema and Li 2003, Scorsch *et al.* 2001, Vasbinder *et al.* 2003, Parnell-Clunies *et al.* 1986).

Color

Food acceptance and preference are functions of product quality. Often color is the first sensory characteristic perceived by the consumer and color tends to modify other perceptions such as flavor and aroma. Food color is generally expressed in terms of the CIELAB color space with the color coordinates: lightness (L*) (estimation of food lightness), red/greenness (a*, ±red-green), and yellow/blueness (b*, ±yellow-blue) (Garcia-Perez *et al.* 2005).

Whiteness in fluid milk is a result of the larger particles, such as milk fat globules (2 to 10 μm) and casein micelles (~100 nm) which scatter light in the visible spectrum. Consumers show the highest appeal for fluid milks with visual properties characteristic of whole milk; thus, milk whiteness has a positive influence on increasing consumer appeal (Philips *et al.* 1995, Garcia-Perez 2005).

Harte *et al.* (2003) reported that the L* increase in milk was a function of milk pasteurization and further a function of the casein micelle size. Mix pasteurized at 85 °C for 30 min induced whey protein denaturation, allowing for whey proteins to attach to the casein micelles and increasing the effective casein micelle size. Needs *et al.* (2000) reported that if the casein micelle size increased in yogurt mix, L* and a* increased (81.6 to 84.73 and -4.2 to -3.85, respectively) and b* decreased (7.7 to 6.10), but yogurt color was not affected probably because the casein micelles aggregated into the gel. Aziznia *et al.* (2009) reported that incorporation of gum tragacanth and WPC in yogurt mixes did not affect the L*, a* and b* significantly, but the presence of WPC in yogurt, which has inherent yellow color, affected the yellowness (b*) in nonfat yogurt as opposed to full fat yogurt (10.53 vs. 10.72, respectively).

Rheology

Milk gels are visco-elastic, thus milk's rheological properties can be characterized using both the viscous and elastic components. Dynamic rheology testing, which involves an oscillatory applied (shear) strain or stress, provides information on gels and the gel formation.

The main parameters used to characterize gels are: 1) the elastic or storage modulus (G'), which is a measure of the energy stored per oscillation cycle, 2) the viscous or loss modulus (G"), which is a measure of the energy dissipated as heat per oscillation cycle and 3) the loss tangent (LT) (also known as tan δ), which is the ratio of the viscous to elastic properties. These parameters are defined as follows:

$$G' = \frac{\tau 0}{\gamma 0}\cos\delta$$

$$G'' = \frac{\tau 0}{\gamma 0}\sin\delta$$

$$LT = \frac{G''}{G'}$$

where τ0 is the amplitude of the shear stress, γ0 is the amplitude of the strain and δ is a phase angle (Lucey 2002). Thus, the rheological properties of a viscoelastic gel can be determined by measuring the resistance of permanent protein bonds against the force applied.

Storage modulus (G'), loss modulus (G") and viscosity are often used to characterize yogurt (Lee and Lucey 2006, Hassan *et al.* 2003). In simple terms G' represents the elasticity whereas G" indicates the viscous or liquid character of the gel network. Elasticity is the ability of a material to return to the original state after shape deformation due to applied stress, whereas the flow property-viscosity (liquid character) is unable to regain its shape after deformation (Bohlin *et al.* 1984). From a sensory standpoint, yogurt rheology contributes to mouth feel and flavor release (Chandan 2006) and most U.S. consumers perceive yogurt as a "solid gel".

Storage Modulii

Comparing yogurts made from different mixes, Lee and Lucey (2006) observed lower G' (134 vs. 232 Pa) if the mixes were pasteurized at 75 °C for 30 min as opposed to 85 °C for 30 min. Yogurts made from mix pasteurized at 75 °C for 30 min had a greater G' (134 vs. 62 Pa), when incubated at 32 vs. 44 °C. These researchers observed that yogurt made from mixes pasteurized at >80 °C, exhibited greater G' compared to yogurts made from mixes pasteurized at 68 to 75 °C (Lee and Lucey 2006, Lee and Lucey 2004b, Anema and Li 2003, Vasbinder *et al.* 2003, Corredig and Dalgleish1999). The greater G' for yogurt gels was attributed to the increased casein and whey proteins interactions as a function of increased pasteurization temperatures and greater overall protein concentrations (Megenis *et al.* 2006, Lee and Lucey

2004, Lucey and Singh 1998, Parnell-Clunies *et al.* 1986). On the other hand, high pressure (>400 MPa) has been reported to decrease G' (546 vs. 445 Pa) as evidenced by a weaker network with less cross-linkings or protein-protein interactions (Grady *et al.* 2001).

Puvanenthiran *et al.* (2002) observed that increased whey protein content (while maintaining constant solids content) decreased the gelation pH of yogurt (from 5.5 to 5.3 for yogurts with whey protein contents 1.08 and 2.07 g, respectively). Increased gelation pH was attributed to the increased participation of denatured whey proteins in the gel structure as their isoelectric pH are greater than casein (5.6 for whey proteins vs. 4.6 for casein micelles) (Lucey *et al.* 1997). During acidification, the denatured whey proteins aggregate at the isoelectric pH (Lucey *et al*, 1997), resulting in increased cross-linking or bridging within the gels. Lucey *et al.* (1999) reported that the G' of yogurt increased 100X if made from pasteurized vs. unpasteurized mix as with added WPC (1%) reiterating the importance of the pasteurization step to yogurt quality.

Loss tangent (LT)

Loss tangent is the tangent of the phase displacement angle between stress and strain; LT indicates the proportion of viscous (G") to elastic (G') moduli. The system information is as follows,

- pure viscous liquid, $\delta = 90°$
- pure elastic solid, $\delta = 0°$
- viscoelastic solid, $0° < \delta < 90°$ (Endress *et al.* 2006).

Loss tangent in acid milk gels tend to increase to a maximum followed by a decrease, thus the maximum LT has been reported to occur at ~5.0 pH- the gelation pH of milk (Lee and Lucey 2004a, Lucey *et. al.* 1998). This pH induces the solubilization of colloidal calcium phosphate (which acts as a bridge in the casein micelle, holding the micelle together), resulting in disintegrated micelles and hence decreasing elasticity in the system. However at maximum LT, the decreasing repulsion in response to decreasing pH allows the aggregates of the casein micelles to form, which strengthens the gel structure. Increased LT is an indication of increased relaxation of the bonds in the gel which also is interpreted as increased rearrangement of the gel structure (van Vliet *et al.* 1991). Thus LT is a function of the different types of protein bonds present in network (Lee *et. al.* 2004a). Lee and Lucey (2004a) reported for set-style yogurts made from mixes pasteurized at 93 °C, 82.5°C and 72 °C (30 min each) LT of 0.507, 0.521 and

0.635, respectively; thus yogurts tended to be more elastic as the pasteurization temperature increases.

Lee and Lucey (2004b) reported a faster acidification rate (i.e. increasing inoculation rate from 0.5% to 4%) reduced the pH at the maximum LT (from 5.14 to 5.04), because calcium phosphate solubilizes slower in the shorter time. When colloidal calcium is dissolved within the casein micelles, the electrostatic repulsion between the exposed phosphoserine residues tends to increase (Lucey, 2002). Van Vliet and Walstra (1994) reported that LT values change over storage due to bond rearrangements.

Syneresis

Syneresis is defined as gel shrinkage that occurs concomitantly with liquid/whey expulsion and relates to the inability of the gel network to entrap all of the liquid phase. Most consumers consider syneresis to be a defect (Lucey *et al.* 1998, Megenis *et al.* 2006). When the casein particles rearrange in the gel network, whey expulsion is spontaneous, as the gel shrinks without the application of any external force (Lucey 2002). The exact causes of whey separation in yogurt are unknown. Commercial manufacturers try to prevent syneresis by increasing total solids contents (14 to 16%) or by adding stabilizers like pectin and gelatin (Lucey *et al.* 1998, Amatayakul *et al.* 2006). Another alternative includes the use of exopolysaccharide (EPS)-producing starter cultures which can minimize syneresis (Amatayakul *et al.* 2006). Due to consumer awareness of natural products, the extensive use of stabilizers is minimized (Amatayakul *et al.* 2006, Lucey 2002).

Yogurt made from mix pasteurized at 93 °C yielded yogurt with low syneresis as compared to the yogurt made from mix pasteurized at 72 °C (Lee and Lucey 2004a). Spontaneous whey separation was related to an unstable network, which could be due to an increase in rearrangements within the gel network losing whey (Lee and Lucey 2004a, Lee and Lucey 2004b). In yogurt made from mixes pasteurized at >80 °C, the casein network and denatured whey proteins form stronger bonds in the protein network, which reduced the likelihood of casein rearrangements.

Researchers using SEM reported that the yogurt gel microstructure had larger pores ($(1.52(x10^{-13}\ m^2)^3$ vs. $1.43\ (x10^{-13}\ m^2)^3$) and less cross-links if the mixes were pasteurized at 82 °C vs. 93 °C. These microstructural observations were related to syneresis, as yogurts had less

syneresis if the mix was pasteurized at 93 °C vs. 82 °C (Lee and Lucey 2004). The weaker gel structure (evidenced as less cross-linkings) was related to greater syneresis susceptibility, which was associated with increased breakage of protein strands in the network junctions, resulting in the formation of weak spots and a less stable gel network (Lee and Lucey 2006).

Amatayakul *et al.* (2006) compared three methods of syneresis in yogurt as – 1. siphoning surface whey 2.draining inverted a yogurt gel on a specified size mesh and 3.centrifuging yogurt at specified G force, time and temperature. The same yogurt (9% total solids) exhibited 7.5% syneresis by siphon, 40% syneresis by drainage and 75% syneresis by centrifugation. They concluded that measuring syneresis via siphon was more relevant as gravity and external forces were avoided, which could destroy the gel matrix.

Puvanenthiran *et al.* (2002) reported that the increased whey protein contents in the yogurt affected syneresis as yogurts with 0.75 vs. 2.07 g whey protein contents had 44 and 16% syneresis, respectively. However the syneresis was relatively constant for yogurts with similar whey protein content but varying total solids (yogurts with 12 vs. 9.5% solids had syneresis of 11 vs. 10%).

Texture

Textural characteristics of yogurts have been defined by firmness, adhesiveness, cohesiveness and springiness (Megenis *et al.* 2006). Yogurt with increased solids (10 to 20%) had increased protein content (value not reported) and hence increased the number of interactions which in turn increased firmness (Gastaldi *et al.* 1997). Substitution of casein (100% milk retentate) with whey (80% milk retentate with added 20% whey retentate) yielded yogurt that was less firm (14.14 vs. 9.60 g). Researchers further found that decreased protein content (3.52 to 3.31%) in yogurt mix decreased casein-casein and casein-whey interactions and decreased firmness of yogurt (Megenis *et al.* 2006). Antunes *et al.* (2003) reported that the greater mix protein contents (6 to 12%) combined with increased pasteurization (81 vs. 89 °C) yielded firmer yogurts (>205 g). Parnell-Clunies *et al.* (1986) reported a correlation of 0.8 between whey protein denaturation and firmness.

For yogurts containing added WPC and different total solids, the increased whey protein in the yogurt mix increased the firmness (yogurt with whey protein contents of 0.75 vs. 2.07 g had firmness 15.10 vs. 32.44 g, respectively (total solids maintained at 12%)). However if whey

contents were constant (1:1) and total solids were varied (9.5 to 12%), the firmness remained somewhat constant (25.91 vs. 30.30 g) (Puvanenthiran *et al.* 2002). Decreased whey protein content yielded casein micelles with greater sizes in the gel network (191 vs. 711 nm); thus the bond types, particle sizes and protein interactions contributed to yogurt texture (Puvanenthiran *et al.* 2002).

Cohesiveness indicates structural integrity and is often discussed in terms of the bond strength; adhesiveness indicates adherence of yogurt; whereas springiness reflects the structural integrity of yogurt (Megenis *et al.* 2006, Gastaldi *et al.* 1997). Greater cohesiveness and springiness may be related to stronger gel structures, whereas greater magnitudes of adhesiveness reflects greater association with the probe surface, indicating greater structural integrity; perhaps due to increased charged groups on the amino acid groups-a function of whey protein denaturation (Megenis *et al.* 2006).

Water holding capacity (WHC)

Water holding capacity measures the amount of water absorbed in the protein structure (Parnell-Clunies *et al.* 1986). Parnell-Clunies *et al.* (1986) reported an increased WHC in a yogurt gel (27.51 vs. 28.39%) if the yogurt mix was pasteurized at 85 vs. 98 °C. Increased WHC was attributed to increased hydration of the protein network (2.44 vs. 2.47 g water/g solids) and the increased amount of denatured whey proteins (72.7 vs. 88.4%) as their subsequent incorporation with caseins increased the total water hydration capacity of the gel network. Whey protein denaturation exposes charged amino acids and increases the surface area which in turn allowed increased water retention (72.7 vs. 88.4%) in the yogurt matrix (Parnell-Clunies *et al.* 1986). However Parnell-Clunies *et al.* (1986) hypothesized that as the β-lactoglobulin interacted with κ-casein, more covalent bonds were formed and larger micelle sizes might cause steric hindrances; all resulting in lower WHC (covalent bonds decrease the number of charged groups present in the gel network). In yogurt, increased micelle size and increased whey-casein and casein-casein interactions lead to a more porous gel, which could retain more water (Sodini *et al.* 2005, Lee and Lucey 2004a).

Sodini *et al.* (2005) compared stirred yogurts made from mixes with added WPC (protein content increased to 4.5%) and reported that added WPC resulted in greater WHC (50 for only

NFDM yogurt vs. 63.8% for NFDM and added WPC yogurt), and was attributed to increased interactions between whey and casein leading to increased water retention of the gel network.

Yogurt storage

Yogurt storage has been studied to understand the effects of time on sensory attributes, quality and bacterial viability. Damin *et al.* (2008) reported that counts of *Lactobacillus bulgaricus* decreased 2 logs (8.52 to 6.50 log CFU/ml) while *Streptococcus thermophilus* remained constant (9.30 to 9.00 log CFU/ml) for yogurt stored for 35 days (Salvador and Fiszman 2004). Salvador and Fiszman (2004) reported that yogurt firmness and TA remained constant, while syneresis increased when yogurts were stored at 10, 20 and 30 °C for 91, 21 and 3 days, respectively. Parnell-Clunies *et al.* (1986) reported that WHC increased (26.32 to 28.90%) when yogurt was stored for 42 days. Serra *et al.* (2009) reported increased G' of 1481 to 1552 Pa in yogurts stored for 28 days; while Weidendorfer *et al.* (2008) reported increased G' of 200 to 250 Pa in yogurts stored for 21 days.

These increases of G', syneresis and WHC have been attributed to rearrangements in the casein gel network. Over time, casein particles rearrange into a more compact structure which increases bond numbers and decreases the total free energy of the system, moving the system to a more thermodynamically stable state. When rearrangements occur, the casein particles which are part of the gel network deform and form new junctions as evidenced by syneresis and changes in dynamic moduli (Serra *et al.* 2009, Lucey 2002). Thus this allows changes in yogurt quality attributes during the storage period.

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CHAPTER 3 - RESEARCH OBJECTIVES

1. To determine the impact of minimum whey protein denaturation on yogurt quality
2. To improve yogurt quality with whey protein supplementation, while minimizing whey protein denaturation
3. To determine the effect of storage on the quality of yogurt made with different supplements and different amounts of whey protein denaturation

CHAPTER 4 - MATERIALS AND METHODS

Mother culture

Mother culture was prepared by mixing 4.5 g of low heat-nonfat dry milk (NFDM) (Dairy America, Fresno, CA) to 50 ml of de-ionized, distilled water in a 250 ml volumetric flask (Fischer Scientific, Pittsburgh, PA) using a magnetic stirrer (Fisher stirring hotplate, Fischer Scientific) for 5 min at 24 °C ±1. The rehydrated mix was covered with cheesecloth and aluminum foil, autoclaved at 121 °C at 15 psi for 15 min (AMSCO Eagle Series 2021 Gravity, American Sterilizer Co., Erie, PA) and cooled to 24 °C ±1. Approximately 1% w/w yogurt culture ("Yo-Mix" 651 DPL yogurt 500GL, Danisco Inc., USA, New Century, KS) was transferred aseptically to the milk and incubated (Equitherm, Environmental Incubator, Curtis Matheson Sciences, Houston, TX) for 18 hrs at 35 °C ±1 until a pH of 4.1 to 4.4. Mother culture was transferred and maintained at 5 °C ±1 until yogurt manufacture (approximately 6 hr).

Yogurt mix processing

Table 4-1 provides the formulations used for the two experiments, which includes the codes for yogurt, as well as the ingredient amounts for each formulation. Nonfat dry milk was reconstituted by adding NFDM (experiment 1) or the combination of NFDM and WPC (Avonlac 134, Glanbia Nutritionals Inc., Monroe, WI – specifications sheet can be found in APPENDIX A) (experiment 2) to de-ionized, distilled water in a Erlenmeyer flask and stirred for 10 min at 24 °C ±1 (Corning stirrer PC310), after which heat was applied until the yogurt mix attained 65 (experiment 1), 70 (experiment 1) or 90 °C (experiment 1 and 2). Mixes were pasteurized by placing the flasks in a pre-heated water bath (Isotemp 220, Fischer Scientific) set at 65, 70 or 90 °C and maintained for 30, 30 or 10 min, respectively. Yogurt mixes were cooled to 43 °C for 15 min in ice water with periodic shaking.

Mother culture was aseptically transferred (~3 % (w/v)) to the pasteurized, cooled yogurt mixes and the yogurt mixes were shaken for ~1 min to ensure uniform distribution of microorganisms. Inoculated, pasteurized mixes were incubated (Isotemp Incubator, Fischer Scientific) in 120 ml capacity sterile cups (Fisher Scientific) or 50 ml centrifuge tubes (Nalgene, Rochester, NY) at 43 °C ±1, to pH 4.5 to 4.6 (4.5 to 6 hrs). Following fermentation, yogurts were stored at 5 °C ±1 (Roper, Whirlpool Corporation, Benton Harbor, MI) for up to 1, 15, 29 days.

The procedure was repeated on 3 and 6 different days indicating three replications for experiment 1 and 2, respectively.

Table 3-1: Codes and formulations for the yogurt mixes

Experiment	Code	Pasteurization Temperature (°C)	Pasteurization time (min)	Ingredients		
				Water (ml)	NFDM‡ (g)	WPC‡ (g)
1	65-Y	65	30	500	55	0
	90-Y	90	10	500	55	0
2	N-70 β	70	30	437.5	62.5	0
	N-90 β	90	10	437.5	62.5	0
	W-70β	70	30	437.5	47.5	15
	W-90 β	90	10	437.5	47.5	15

β N= Yougrt containing 12.5% NFDM only; W= yogurt containing 9.5% NFDM and 3% of WPC

‡ NFDM is nonfat dry milk; WPC is whey protein concentrate

Chemical Methods

pH

pH was measured using a Fischer universal glass pH electrode (Acumet Portable AP61, Fisher Scientific) after pH calibration with standardized solutions to pH 4 and 7 at 24 °C ±1.

Total solids (TS)

Total solids were measured using a forced–air oven method as described by Hooi *et al.* (2004). Yogurt was mixed with a spoon for 30 sec and then approximately 3 g was added in a previously heated, weighed, desiccated aluminum dish (Fisher Scientific), covered with another previously heated, weighed, desiccated aluminum dish. Samples were placed in a forced draft oven (Isotemp Oven, Fisher Scientific) for drying at 103 °C ±1, for 24 hrs. Samples were desiccated and weighed.

Total solids were calculated as follows:

$$\%\ Total\ Solids = \frac{Dried\ sample\ with\ dish\ weight - Dish\ weight}{Initial\ yogurt\ weight} \times 100$$

Titratable acidity (TA)

Titratable acidity was measured as described by Hooi e*t al.* (2004). Ten g of yogurt was placed in a beaker and titrated with 0.1 N sodium hydroxide (Fisher Scientific) solution using phenolphthalein as an indicator. End point of titration was the transition from colorless to pink.

TA was calculated as follows:

$$\%\ \mathrm{TA} = \frac{9 \times 0.1 \times ml\ of\ NaOH}{yogurt\ weight}$$

Whey protein denaturation analysis

Whey protein denaturation (WPD) was measured by modification of the method given by Grady *et al.* (2001). Protein contents of the various filtrates were determined using a Leco Analyzer (LECO analyzer, LECO, St Joseph, MI), using the nitrogen conversion factor of 6.38 for dairy proteins (IDF 1964).

Unpasteurized mix whey protein content

Ten ml of unheated, yogurt mix was transferred to a 100 ml volumetric flask and 50 ml of de-ionized, distilled water (50 °C ±1) was added. Three ml of 10% (v/v) acetic acid (Fisher Scientific) was added, and the mixture set quiescently for 10 min (24 °C ±1). Three ml of 1 N sodium acetate (Fisher Scientific) was added followed by water to 100 ml (pH of mixture was 4.6 after sodium acetate addition). The solution was filtered through a Whatman filter paper no. 42 of diameter 10 cm (Fisher Scientific) using a funnel. The filtrate was analyzed for protein content.

Pasteurized mix whey protein content

Ten ml of pasteurized yogurt mix was processed as described in the previous procedure.

Total protein content of mix

The unheated yogurt mix was diluted as 1:10 times and 3 ml sample was used to ascertain protein content.

Whey protein denaturation (WPD):

Calculated using formula:

$$\%Denaturation = \frac{(Unpasteurized\ mix\ whey\ protein - Pasteruized\ mix\ whey\ protein\ )}{Unpasteurized\ mix\ whey\ protein} \times 100$$

Casein content of mix:

Casein was determined as:

$$\text{Casein content} = Total\ protein - Unpasteurized\ mix\ whey\ protein$$

Gel analyses

Color

The color of yogurt was measured as described by Schmidt *et al.* (2001), using a Hunter D-54 Reflectance Lab Ultra Scan Sphere Spectrophotometer (Hunter Associate Laboratories, Reston, VA). The colorimeter used L*, a* (redness) and b* (yellowness) scales and illuminant C (the average daylight) at an observed angle of 10º to measure the color of the yogurt surface. Two readings were taken and averaged.

Rheology – G' and Loss tangent

Rheological measurements were done as described by Hassan *et al*. (2003). The sample cup contained a concentric cylinder device consisting of a cup (28 mm diameter) and a bob (25 mm diameter, 42 mm length).Yogurt samples were gently stirred 10 times by spoon prior to rheological analysis. Flow curves were obtained using a Bohlin VOR Rheometer System (Bohlin Instruments Inc., Cranbury, NJ). About 17 to 20 ml of yogurt sample was transferred into the cup and the bob was lowered until its whole surface was covered. A strain oscillation frequency sweep of 0.1 to 10 Hz was applied and readings were taken at 1 Hz at an interval of 5 sec. The

fundamental dynamic parameters-storage modulus (G') and loss modulus (G") were determined for the yogurt gels using shear rate of 0.1 to 10 Hz and values obtained at 1 Hz were compared. The temperature was maintained at 4 °C ±1. Loss tangent (LT) was measured using the equation (Lucey and Singh, 1998):

$$LT = \frac{G"}{G'}$$

Syneresis

Syneresis was determined as described by Amatayakul *et al.* (2006). A 120 ml cup of yogurt of known weight was maintained at a 45° angle for 2 hrs at 5 °C ±1 (Equitherm, Environmental Incubator, Curtis Matheson Sciences). Free whey was siphoned from the surface using a syringe (10 s) and weighed. The syneresis was calculated as a percent weight of whey over initial weight of yogurt.

$$\%\ Syneresis = \frac{siphoned\ whey\ weight}{initial\ yogurt\ weight} \times 100$$

Texture

Yogurt texture was determined using a modified method by Megenis *et al*. (2006). Textural analysis was done with TA.XT2, Texture Analyzer (Stable Micro System, Scarsdale, NY) using a 25 mm (P25/L) acrylic probe in a 120 ml yogurt cup at 5 °C ±1. Test velocity, time and distance were 2 mm/s, 5 sec and 5 mm, respectively. Firmness, cohesiveness, adhesiveness and springiness values were obtained using texture analyzer software (Stable Micro System, Scarsdale, NY).

Water holding capacity(WHC)

Water holding capacity was measured as described by Parnell-Clunies *et al.* (1986). Yogurt incubated in the sterile centrifuge tubes were centrifuged at 10 °C at 13500 × g for 30 min (Marathon 21000R, Fischer Scientific). The supernatant fluid was drained for 20 min by inverting tubes at 24 °C ±1. Water holding capacity was expressed as percent pellet weight over original yogurt weight.

$$\%WHC = \frac{drained\ tube\ weight\ -tube\ weight}{initial\ yogurt\ weight} \times 100$$

Experimental design and Statistical analysis

Statistical analyses were done using SAS® software (SAS Institute Inc., v 9.1, Cary, NC). For the first experiment, the experiment was designed using a randomized block design with three replications, where each block represented 2 pasteurization treatments/day (65 °C for 30 min or 90 °C for 10 min). Compositional data were analyzed using GLM procedure, while day 1 data were analyzed using MIXED procedure. Storage data were analyzed using the MIXED procedure with a split plot design, where pasteurization treatment (65 °C for 30 min or 90 °C for 10 min) was whole plot factor and storage time (day 1, 15 or 29) was the split plot factor. Significant means of main effects were differentiated using the P-diff procedure.

In the second experiment, an incomplete randomized block design was used with 3 replications, where a block was a yogurt mix formulations/day (NFDM or NFDM-WPC). Compositional data were analyzed using MIXED procedure. Day 1 data were analyzed using a split plot, where formulation (NFDM or NFDM-WPC) was whole plot factor and pasteurization treatment (70 °C for 30 min or 90 °C for 10 min) was split plot factor. Storage data were analyzed using split-split plot, where formulation (NFDM or NFDM-WPC) was the whole plot factor, pasteurization treatment (70 °C for 30 min or 90 °C for 10 min) was the split plot factor and storage time (day 1, 15 or 29) was the split-split plot factor. Data were analyzed using MIXED procedure, where significant means of main effects were differentiated using the P-diff procedure.

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CHAPTER 5 - HEAT TREATMENT EFFECTS ON PHYSICAL AND CHEMICAL PROPERTIES AND STORAGE OF SET-STYLE NONFAT YOGURT

INTRODUCTION

A yogurt mix is formulated and subsequently pasteurized to inactivate pathogens and to induce desirable physicochemical properties (Tamime and Robinson 1999). Pasteurization that exceeds 80 °C for 30 min denatures whey proteins, which are then available to complex with caseins (Anema and Li 2003; Lucey and Singh 1998).

In milk, pasteurization (>80 °C) denatures whey proteins, exposing the reactive thiol in the most abundant whey protein-β-lactoglobulin, which then can react and form disulfide bonds with cysteine-containing proteins or disulphide bridges with non-cysteine-containing proteins. Although initially denatured β-lactoglobulin forms hair-like structures, it subsequently reacts with κ-casein resembling a polymerization process, with increased sizes of casein micelles as the end result (Anema *et al.* 2003, Vasbinder *et al.* 2003). Anema *et al.* (2003) showed that the association rate of denatured whey proteins with casein micelles was slower than the denaturation rate of whey proteins in milk; thus, unbound denatured whey proteins existed in milk. In yogurt, whey proteins contribute to the overall quality of the final product, but that final quality is contingent on whey protein denaturation (WPD) in the yogurt mix and the subsequent interactions of denatured whey proteins with casein in the yogurt mix (Lucey and Singh 1998).

When WPD increased from 25 to 75% in yogurt mixes, the mixes exhibited greater viscosity and the yogurt had increased firmness and WHC and less syneresis (Parnell-Clunies *et al.* 1986, Mottar *et al.* 1989, Zbikowskia *et al.* 1998, Lee and Lucey 2004). In addition, yogurt firmness has been positively correlated to total solids and protein contents as well as protein type in the yogurt mix (Megenis *et al.* 2006; Salvador and Fiszman 2004). Yogurt mixes pasteurized at 85 °C for 20 min had increased micelle size (3 X) and L* (1.009 X) compared to the unpasteurized mixes, but the resultant yogurts did not differ in L*. The researchers explained these results as the heat–induced whey protein attachment to the casein micelles, which in turn increased their effective sizes and light scattering ability in the mixes, but the protein aggregation

during gel formation negated the overall effect on the color properties of the yogurt (Harte *et al.* 2003, Needs *et al.* 2000).

Whey proteins contain all the essential amino acids required for muscle synthesis and are rapidly digestible proteins (Tipton *et al.* 2004). But, WPD in the yogurt mix could decrease the whey proteins availability in the yogurt (Cribb *et al.* 2007, Haug *et al.* 2007, Hoffman and Flavo 2004, de Wit 1998). Most yogurt research is focused on maximizing the WPD, not minimizing it, so as to induce desirable quality and thus to establish a baseline of the relationship of the WPD to yogurt to yogurt quality this study was undertaken.

The objectives of this experiment were 1) to quantify the gel quality as a function of whey protein denaturation, and 2) to study the effects of storage on the gel quality of these yogurts. To fully understand the cause and effect system, a yogurt mix of minimum solids (~9%), protein contents (~3%) and pasteurization conditions (for milk) were used for comparison purposes.

MATERIALS AND METHODS

Yogurt Manufacture

Mother culture was prepared by rehydrating 4.5 g of NFDM (low-heat nonfat dry milk, Dairy America) in 50 ml of de-ionized, distilled water at 24 °C ±1. The milk was autoclaved at 121 °C at 15 psi for 15 min (AMSCO Eagle Series 2021 Gravity; American Sterilizer Co.), cooled at 24 °C ±1, inoculated with approximately 1% w/w yogurt culture ("Yo-Mix" 651 DPL yogurt 500GL (Danisco) and incubated (Equitherm, Environmental Incubator, Curtis Matheson Scientific) for 18 hr at 35 °C until a pH of 4.1 to 4.4 and stored at 5 °C ±1 thereafter.

For yogurt, 55 g of NFDM was rehydrated in 500 ml of de-ionized, distilled water, heated to 65 or 90 °C and then pasteurized in a preheated water bath (Isotemp 220, Fischer Scientific) at 65 or 90 °C for 30 or 10 min, respectively, and cooled to 43 °C ±1. Mother culture was aseptically transferred (approximately 3% (w/v)) to the pasteurized yogurt mix, and samples were mixed thoroughly. Yogurt mix was poured in to 120 ml sterile cups (Fisher Scientific) or 50 ml capacity centrifuge tubes (Nalgene) and incubated (Isotemp, Fischer Scientific) at 43 °C ±1 for 4 to 6 hrs to pH 4.5 to 4.6. Then samples were placed in a cold storage at 5 °C ±1 (Roper, Whirlpool Corporation, Benton Harbor, MI). The yogurts were made on three different days as

mentioned for a total of three replications. These yogurts were referred to as 65-Y or 90-Y to reflect their mix pasteurization conditions of 65 °C for 30 min or 90 °C for 10 min, respectively.

Chemical Methods

Acidity Measurements

Prior to pH measurement (Fischer Universal pH meter (Fisher Scientific)) the instrument was calibrated with standardized solutions (Fisher Scientific) to pH 4 and 7. Titratable acidity (TA) was measured as described by Hooi *et al.* (2004). Measurements were done in duplicate and the average was reported.

Total Solids Content

Total solids were measured using a forced air oven method (Isotemp Oven, Fisher Scientific) as described by Hooi *et al.* (2004).

Total Protein and Whey Protein Denaturation (WPD)

Total protein and WPD were measured as described by Grady *et al.* (2001). A LECO analyzer was used for nitrogen measurement and the conversion factor of 6.38 was used (IDF 25, 1964).

Physical Methods

Color Properties

Yogurt color was measured as described by Schmidt *et al.* (2001). A Hunter D-54 Reflectance Lab Ultra Scan Sphere Spectrophotometer (Hunter Associate Laboratories) was standardized using a white tile and light trap, and single readings were obtained. The yogurt was placed in a sample cup (diameter 6.5 cm and depth 4.5 cm). White color reflectance standards were X = 83.4, Y = 88, Z = 93.9 and D 65/10°. Two readings were taken and averaged.

Rheological Properties

Rheological measurements were done as described by Hassan *et al.* (2003) using a Bohlin VOR computer-controlled rheometer system (Bohlin Instruments Inc). The sample cup contained a concentric cylinder device consisting of a cup (28 mm diameter) and a bob (25 mm diameter, 42.5 mm length). About 17 to 20 ml of yogurt sample was transferred into the cup and the bob

was lowered until its whole surface was covered. A strain oscillation frequency shear sweep of 0.1 to 10 Hz was applied and readings were taken at 0.1 Hz at an interval of 5 s. The fundamental dynamic parameters-storage modulus (G') and loss modulus (G") were determined at 4 °C ±1. The G' was expressed at 1 Hz and loss tangent (LT) was calculated as the ratio of G" to G' at 1 Hz.

Syneresis Analysis

Syneresis was determined as described by Amatayakul *et al.* 2006. A 120 ml cup of yogurt of known weight was maintained at a 45° angle for 2 hrs at 5 °C ±1. The free whey was siphoned using a syringe and weighed. Syneresis was expressed as a percentage of the whey weight over initial yogurt weight. Greater values indicate greater amounts of syneresis.

Texture Profile Analysis

Yogurt texture was determined using a method described by Megenis *et al*. (2006), using TA.XT2, Texture Analyzer (Stable Micro System) equipped with a 25 mm (P25/L) acrylic probe on a 120 ml cup of yogurt maintained at 5 °C ±1. Test velocity, time and distance were 2 mm/s, 5 s and 5 mm, respectively. Computer generated results included cohesiveness, adhesiveness (g.s), firmness (g) and springiness.

Water Holding Capacity

Water holding capacity (WHC) was measured as described by Parnell-Clunies *et al.* (1986). Yogurt samples were centrifuged at 10 °C at 13,500 x g for 30 min (Marathon 21000R, Fisher Scientific). The supernatant was drained for 10 min and the pellet weight was determined. Water holding capacity was expressed as percentage of the pellet weight over the original yogurt weight.

Shelf life/Storage study

Immediately post fermentation, yogurt samples were cooled overnight at 5 °C ±1. Yogurt samples were stored (Roper, Whirlpool Incorporation) at 5 °C ±1 and then on days 1, 15 and 29, evaluated for chemical and physical properties.

Experimental design

The experiment was designed using a randomized block design with three replications, where each block represented 2 pasteurization treatments/day (65 °C for 30 min or 90 °C for 10 min) (Kuehl 2000). For storage studies a split plot design was used, where pasteurization treatment (65 °C for 30 min or 90 °C for 10 min) was whole plot factor and storage time (day 1, 15 and 29) was the split plot factor.

Statistical analysis

Protein analyses and day 1 yogurt quality: Compositional data were analyzed by GLM procedure, while day 1 data were analyzed by MIXED procedure of SAS® (SAS v 9.1) using a significance of α=0.05.

Storage study: Data were analyzed by the MIXED procedure of SAS® (SAS v 9.1) using a significance of α=0.05. Significant main effects and interactions were further analyzed to determine the differences. Significant means of main effects were differentiated by P-diff procedure.

Appendix B provides average raw data for the experiment; Appendix C provides the SAS® program used to analyze data; and Appendix D provides P values; from the ANOVA results.

RESULTS

Yogurt characterization

Table 5-1 shows the compositional analyses results as well as pH and TA for the yogurt samples at 24 hrs post fermentation. Yogurts did not differ in pH, TA, total protein or total solids indicating that yogurt samples were similar in many factors such as acidity, protein type, protein content and total solids that are known to influence quality parameters, such as texture, syneresis and rheology (Megenis *et al*. 2006). However the 65-Y had statistically less WPD (5 X) than did the 90-Y; thus, the yogurts samples were similar in compositions but differed in WPD.

Table 5-1: Initial yogurt[‡Δ] means and standard errors for composition and chemical quality as a function of pasteurization treatments

Attribute	**Pasteurization**	
	65 °C[‡]	**90 °C[‡]**
Composition of yogurt mixes		
WPD[α] (%)	8.76[b] ±2.19	47.93[a] ±7.33
TP[α] (g)	3.36 ±0.12	3.36 ±0.09
Whey protein (g)	0.67 ±0.03	0.74 ±0.05
Initial yogurt quality		
TS[α] (%)	9.36 ±0.02	9.37 ±0.02
pH	4.45 ±0.03	4.47 ±0.01
TA[α] (%)	0.90 ±0.01	0.90 ±0.00

[a-b] means in a row with a different superscript differ ($P < 0.05$)
[Δ]n=3
[‡]65 °C: Yogurt mixes pasteurized at 65 °C for 30 min; 90 °C: yogurt mixes pasteurized at 90 °C for 10 min.
[α]TA= titratable acidity; TP=total proteins; TS=total solids; WPD=whey protein denaturation

Day 1 yogurt

Both 65-Y and 90-Y formed visible gels and Figure 5-1 confirms this observation. From Figure 5-1, the day1 yogurt samples exhibited gel structures as evidenced by G' being greater than G" at any shear rate. Despite the minimal solids (9%) and total protein (3.36%) contents in both 65-Y and 90-Y and the 5 fold difference in WPD both mixes formed gel structures.

Table 5-2 provides yogurt means for different quality attributes on day 1. The 90-Y had greater b* (1.05 X), cohesiveness (1.11 X), firmness (2 X), G' (4.34 X), L* (1.012 X), springiness (1.02 X) and WHC (1.28 X) but greater adhesiveness (4.22 X) and syneresis (2.19 X) than did the 65-Y. Yogurts did not differ in a* or LT as a function of pasteurization (Appendix E).

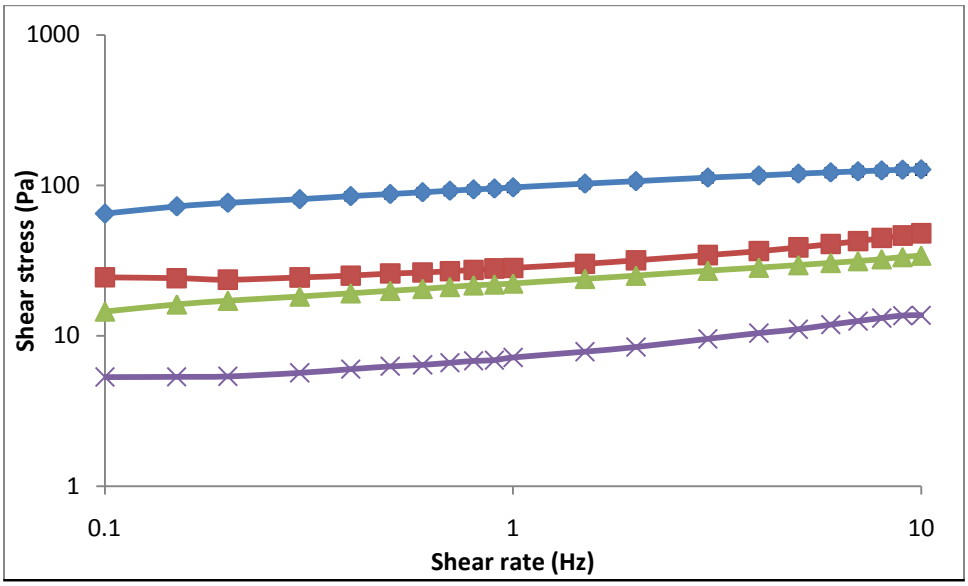
Thus yogurt gels were made at two different WPD; with yogurt having greater WPD exhibiting greater firmness, G' and water binding properties.

Table 5-2: Initial (day 1) yogurt^{‡Δ} means and standard errors for color, textural, rheological and water binding properties as a function of pasteurization treatments

Attribute	Pasteurization	
	65-Y‡	**90-Y‡**
L*	84.67^{b} ±0.16	85.71^{a} ±0.06
b*	6.44^{b} ±0.05	6.78^{a} ±0.10
Adhesiveness (g.s)	-5.10^{a} ±0.18	-21.57^{b} ±5.51
Cohesiveness	0.55^{b} ±0.00	0.61^{a} ±0.01
Firmness (g)	40.32^{b} ±0.66	82.84^{a} ±1.18
Springiness	0.96^{b}±0.00	0.98^{a} ±0.00
G' (Pa)	22.27^{b} ±2.10	96.70^{a} ±3.86
Syneresis (%)	12.21^{a} ±0.31	5.58^{b} ±0.57
WHC^{α} (%)	13.22^{b} ±0.42	16.95^{a} ±0.27

$^{a-b}$ means in a row with a different superscript differ as a function of pasteurization ($P < 0.05$)
^{Δ}n = 3
‡65-Y: Yogurts made from mixes pasteurized at 65 °C for 30 min; 90-Y: yogurts made from mixes pasteurized at 90 °C for 10 min.
^{α}WHC=water holding capacity

Figure 5-1: Rheological profile of yogurt made from mixes pasteurized at 65 °C for 30 min or 90 °C for 10 min and stored at 5 °C ±1 (▲-G' of yogurt made from mix pasteurized at 65 °C for 30 min, ♦-G' of yogurt made from mix pasteurized at 90 °C for 10 min, X–G" of yogurt made from mix pasteurized at 65 °C for 30 min, ■–G" of yogurt made from mix pasteurized at 90 °C for 10 min) for day 1; n=3



Storage study

Yogurts had been formulated to be similar in composition, but were pasteurized differently to induce a 5 fold difference in WPD (See Table 5-1). All yogurts formed gels (See Figure 5-1); thus, the yogurts were studied during storage to determine quality attributes which were affected by the WPD. Statistically, significant differences were observed for both pasteurization (Table 5-3) and storage time (Table 5-4). Three pasteurization*storage time interactions were observed for cohesiveness, G' and syneresis. The means in Table 5-3 show that the 90-Y had greater adhesiveness (9 X), b* (1.05 X), cohesiveness (1.12 X), firmness (2 X), G' (4 X), L* (1.015 X) and WHC (1.21 X), but LT (1.12 X) and syneresis (2.37 X) than did the 65-Y. These trends are similar to day 1 yogurt quality trends (Table 5-2); except for LT.

Table 5-3: Yogurt[‡Δ] means and standard errors for color, textural, rheological and water binding properties as a function of pasteurization treatments averaged for storage days

	Pasteurization	
Attribute	**65-Y[‡]**	**90-Y[‡]**
L*	84.12[b] ±0.17	85.38[a] ±0.10
b*	6.57[b] ±0.04	6.88[a] ±0.03
Adhesiveness (g.s)	-2.84[a] ±0.68	-25.32[b] ±0.76
Cohesiveness	0.55[b] ±0.01	0.62[a] ±0.00
Firmness (g)	39.56[b] ±0.25	83.35[a] ±0.43
Springiness	0.97[b] ±0.00	0.98[a] ±0.00
G' (Pa)	31.39[b] ±2.48	126.12[a] ±9.32
LT[α]	0.3116[a] ±0.00	0.2782[b] ±0.00
Syneresis (%)	12.51[a] ±0.15	5.27[b] ±0.18
WHC[α] (%)	16.69[b] ±0.52	20.17[a] ±0.98

[a-b] means in a row with a different superscript differ as a function of pasteurization ($P < 0.05$)
[Δ]n = 9 (collapsed for storage day 1, 15 and 29)
[‡]65-Y: Yogurts made from mixes pasteurized at 65 °C for 30 min; 90-Y: yogurts made from mixes pasteurized at 90 °C for 10 min.
[α]LT=loss tangent; WHC=water holding capacity

The yogurt quality parameters of a*, G', L*, LT, pH, TA and WHC were significantly affected by the storage time (Table 5-4); whereas adhesiveness, b*, cohesiveness, firmness and syneresis were not influenced by the storage time (Appendix E). From day 1 to 15, G' (1.53 X), pH (1.05 X), TA (1.06 X) and WHC (1.24 X) increased, while L* (1.006 X) decreased and then stabilized. Yogurt a* (1.009 X) decreased from day 15 to 29. Loss tangent decreased from day 1 to 29, indicating very gradual decrease in LT.

Table 5-4: Yogurt[‡Δ] means and standard errors for chemical, color, rheological and water binding properties as a function of storage day averaged for pasteurization treatments

	Storage Day		
Attribute	**1**	**15**	**29**
pH	4.46[a] ±0.02	4.31[b] ±0.00	4.34[b] ±0.01
TA[α] (%)	0.90[b] ±0.00	0.95[a] ±0.00	0.95[a] ±0.00
L*	85.16[a] ±0.06	84.66[b] ±0.09	84.42[b] ±0.03
a*	-2.50[a] ±0.02	-2.52[a] ±0.01	-2.60[b] ±0.02
G' (Pa)	57.35[b] ±1.80	88.08[a] ±1.10	87.82[a] ±3.40
LT[α]	0.3093[a] ±0.00	0.2922[ab] ±0.00	0.2841[b] ±0.00
WHC[α] (%)	15.85[b] ±0.47	19.64[a] ±0.10	19.76[a] ±0.36

[a-b] means with a different superscript within a row differ as a function of storage ($P<0.05$);
[Δ] n = 6
[‡] 65-Y: Yogurts made from mixes pasteurized at 65 °C for 30 min; 90-Y: yogurts made from mixes pasteurized at 90 °C for 10 min.
[α] LT=loss tangent; TA=titratable acidity; WHC=water holding capacity

Significant interactions were observed in case of the cohesiveness, G' and syneresis. Figure 5-2 to 5-4, represent the graphical interpretation of the interactions; whereas Table 5-5 contains the means and standard errors for cohesiveness, G' and syneresis.

The G' increased from day 1 to 15 by 1.52 X for the 90-Y as compared to 1.61 X for the 65-Y. But the G' of the 65-Y and 90-Y remained basically unchanged from day 15 to 29. When comparing the relative impacts, the G' was greater (4 X) for 90-Y compared to the 65-Y (Table 5-5). Cohesiveness and syneresis increased slightly from day 1 to 29 for 65-Y; whereas cohesiveness and syneresis increased slightly from day 15 to 29 for 90-Y.

Figure 5-2: Yogurt means and standard errors for G' as a function of pasteurization treatments (65 °C represents mixes pasteurized at 65 °C for 30 min and 90 °C represents mixes pasteurized at 90 °C for 10 min) and storage days (blue bars are day 1, green bars are day 15 and red bars are day 29); n=3

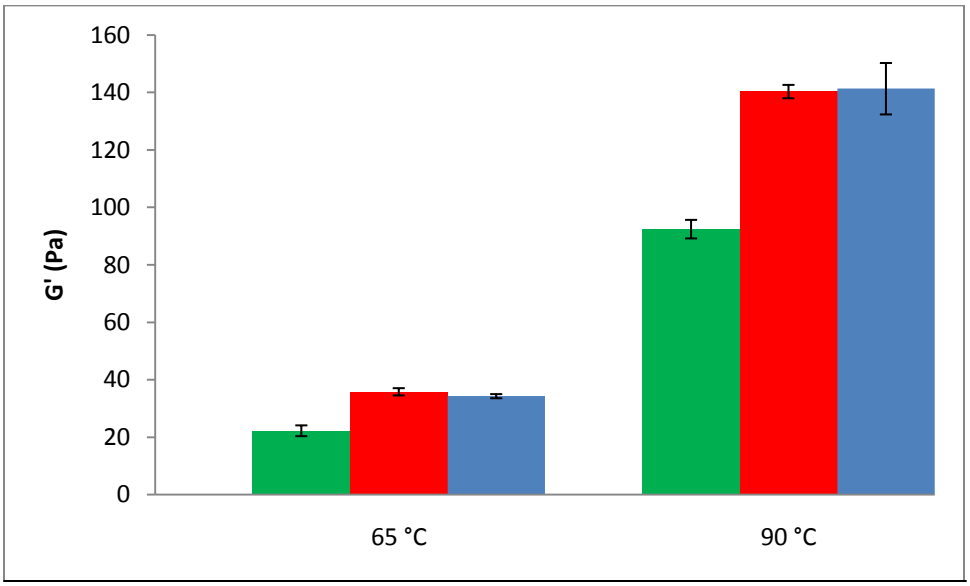


Figure 5-3: Yogurt means and standard errors for syneresis as a function of pasteurization treatments (65 °C represents mixes pasteurized at 65 °C for 30 min and 90 °C represents mixes pasteurized at 90 °C for 10 min) and storage days (blue bars are day 1, green bars are day 15 and red bars are day 29); n=3

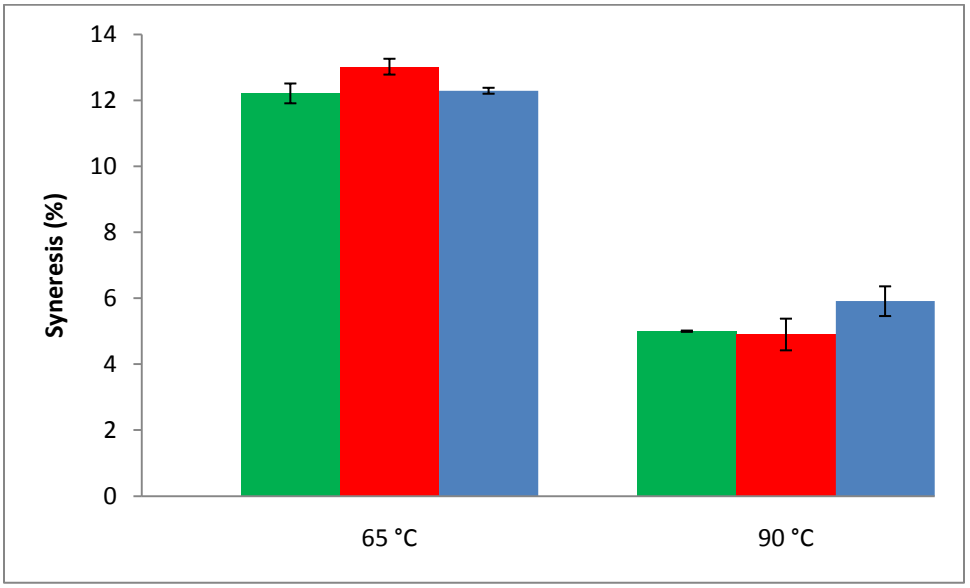


Figure 5-4: Yogurt means and standard errors for cohesiveness as a function of pasteurization treatments (65 °C represents mixes pasteurized at 65 °C for 30 min and 90 °C represents mixes pasteurized at 90 °C for 10 min) and storage days (blue bars are day 1, green bars are day 15 and red bars are day 29); n=3

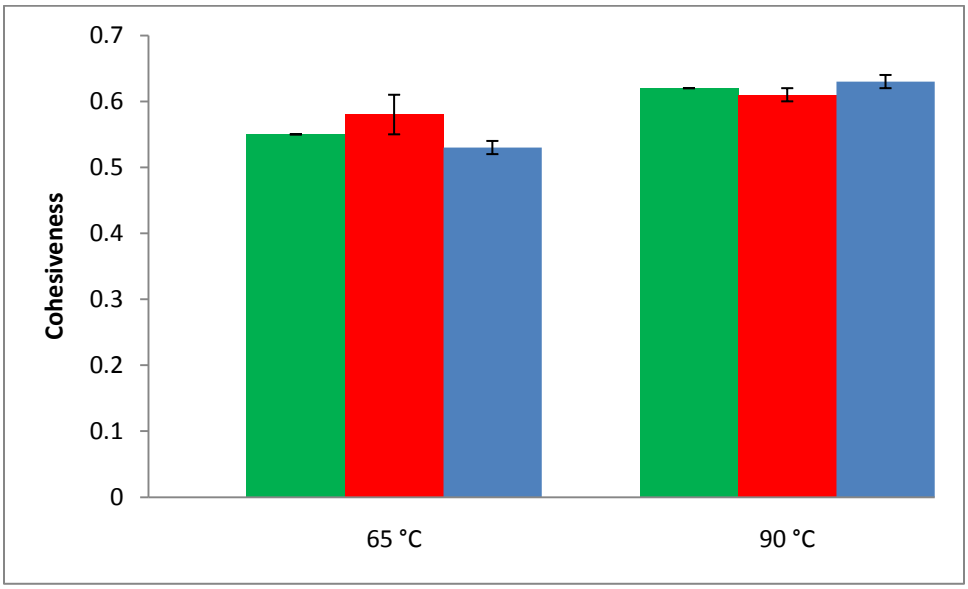


Table 5-5: Yogurt[‡Δ] means and standard errors for cohesiveness, G' and syneresis as a function of pasteurization treatments and storage days

Yogurts[‡]	Day	Cohesiveness	G' (Pa)	Syneresis (%)
65-Y	1	0.55 ±0.00	22.27 ± 1.88	12.21±0.30
	15	0.58 ±0.03	35.83 ± 1.26	13.02 ±0.24
	29	0.53 ±0.01	34.30 ± 0.72	12.29 ±0.09
90-Y	1	0.62 ±0.00	92.43 ±3.23	5.00 ±0.02
	15	0.61 ±0.01	140.3 ±2.33	4.90 ±0.48
	29	0.63 ±0.01	141.3 ±8.95	5.91 ±0.45

[Δ]n=3

[‡]65-Y: Yogurts made from mixes pasteurized at 65 °C for 30 min; 90-Y: yogurts made from mixes pasteurized at 90 °C for 10 min.

Figures 5-1, 5-5 and 5-6 show typical shear stress and shear rate plots for yogurt stored for day 1, 15 and 29, respectively and at any given point G' > G" which indicated stability of yogurt gels during the storage period.

Figure 5-5: Rheological profile of yogurt made from mixes pasteurized at 65 °C for 30 min or 90 °C for 10 min and stored at 5 °C ±1 (▲-G' of yogurt made from mix pasteurized at 65 °C for 30 min, ♦-G' of yogurt made from mix pasteurized at 90 °C for 10 min, X–G" of yogurt made from mix pasteurized at 65 °C for 30 min, ■–G" of yogurt made from mix pasteurized at 90 °C for 10 min) for day 15; n=3

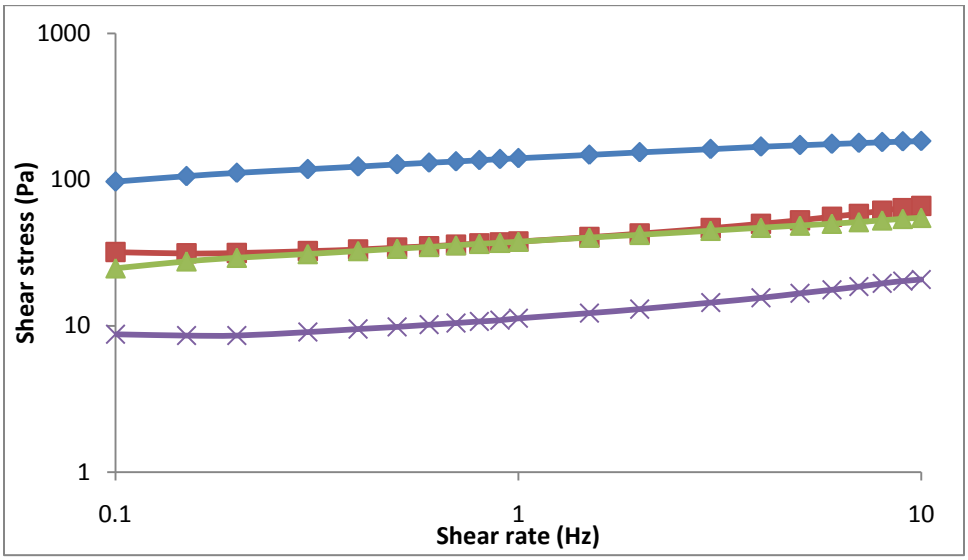
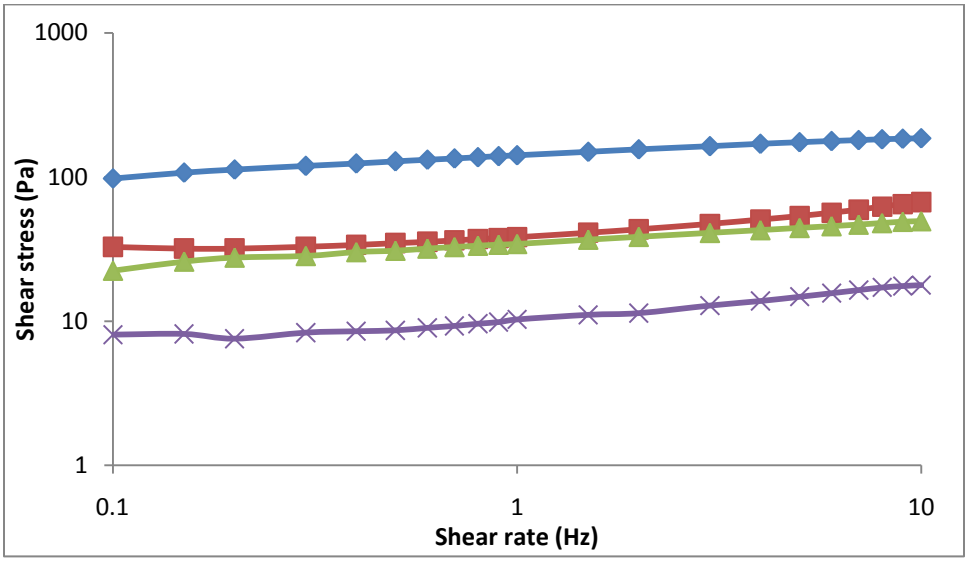


Figure 5-6: Rheological profile of yogurt made from mixes pasteurized at 65 °C for 30 min or 90 °C for 10 min and stored at 5 °C ±1 (▲-G' of yogurt made from mix pasteurized at 65 °C for 30 min, ♦-G' of yogurt made from mix pasteurized at 90 °C for 10 min, X–G" of yogurt made from mix pasteurized at 65 °C for 30 min, ■–G" of yogurt made from mix pasteurized at 90 °C for 10 min) for day 29; n=3



DISCUSSION

The yogurt mixes pasteurized at 65 °C for 30 min and 90 °C for 10 min had ~9 and 48% WPD, respectively. Although these were significantly different in this study, both WPD were relatively minimal compared to other research results. Lucey *et al.* (1997) reported 95 and 81% WPD for β-lactoglobulin and α-lactalbumin, respectively in the milk heated at 85 °C for 30 min; Parnell-Clunies *et al.* (1986) reported WPD of 88.4 or 98.4% for yogurt mixes heated at 85 °C for 10 and 15 min, respectively and Hollar *et al.* (1995) reported that when whey protein solutions (whey proteins content ranged from 16 to 35%) pasteurized at 66 °C, produced < 20% WPD in the heated mix. Thus 90-Y as well as 65-Y had less WPD than most of previously reported heat treatments.

Researchers reported that yogurts made from the mixes pasteurized at <70 °C had minimal whey–κ-casein complexes and thus a "minimum" gel structure (Anema and Li 2003). These yogurt gels had smaller voluminosities of casein micelles and fewer as well as less casein-whey protein junctions (observed through electron microscopy).

Over time, casein particles rearrange into a more compact structure, which increases bond numbers and decreases the total free energy of the system, moving the system to a more thermodynamically stable state (van Vliet and Walstra 1994). Loss tangent reflects bond rearrangements in a gel and if measured over time, may relate to quality changes. In the present study, LT did not differ for either yogurt on day 1; however in storage, the 90-Y had less LT and greater G' than did the 65-Y, which agrees with other reports on the relationship between WPD, yogurt gel strength and bond rearrangements (Lee and Lucey 2004, Boye *et al.* 1997, van Vliet *et al.* 1991, van Vliet and Walstra 1994).

Denatured whey protein complexes lose solubility as pH decreases resulting in enhanced interactions with casein micelles (Lee and Lucey 2004). These interactions lead to a highly branched and cross-linked structure that allowed increased bond strength and a greater G' (Lee and Lucey 2006, 2004; Mottar *et al.* 2001, Lucey *et al.* 1997). These previous reports support current findings for 90-Y and 65-Y. When gel rearrangements occur, the casein particles which are part of the gel network, deform and form new junctions as evidenced by the changes in G' (Serra *et al.* 2009, Lucey 2002, van Vliet and Walstra 1994). Thus the G' changes in the 65-Y and 90-Y over time indicated that the gels were dynamic and particles shifting occurred, predominantly from day 1 to 15.

Syneresis can be related to the number of whey-casein interactions. The greater the number, the more junctions in the network-which then retain increased amounts of water (Lee and Lucey 2004, Parnell-Clunies *et al.* 1986). In this experiments, the 90-Y had greater WPD and less syneresis than did the 65-Y.

Boye *et al.* (1997) reported increased turbidity of WPC gels (1.5 g suspended in water) with increased WPD. Needs *et al.* (2000) reported similar results, where increased WPD (>90 %) increased the casein micelle diameter to ~300 to 400 nm, which scattered more light as observed by increased L* in the yogurts made from pasteurized mixes as opposed to yogurts made from unpasteurized mixes. Similar results were obtained in this study, where 90-Y exhibited greater L* than 65-Y. The increased b* observed in the 90-Y could reflect a greater quantity of 'associated' whey proteins in the gel and the decreased whey release due to gel shrinkage (Harte *et al.* 2003, Needs *et al.* 2000, Lee and Lucey 2004).

Parnell-Clunies *et al.* (1986) reported that yogurt made from mix pasteurized at 98 °C for 1.87 min (WPD 72.7%) had 76.5 g ±3.5 firmness, while yogurt made from unpasteurized mix (effective WPD ~0%) had 32.9 g ±3.4 firmness, a doubling of firmness due to pasteurization. Firmness was attributed to the increased whey-casein interactions that occurred as a result of the pasteurization. In this study, 90-Y had comparable firmness (83.35 g) but comparatively less WPD (~48%) than reported by Parnell-Clunies *et al.* (1986). Other research groups have reported that yogurt firmness increased from 56.12 to 74.49 g, if stored for 91, 21 and 3 days at 10, 20 and 30 °C, respectively (Salvador and Fiszman 2004). In the present study, the yogurts did not show this trend, perhaps the differences in storage temperatures, storage times or yogurt composition may contribute to these different results. The 90-Y in this study exhibited greater cohesiveness than the 65-Y, indicating more structural integrity and stronger bonds (Megenis *et al.* 2006).

In this study, yogurts were made from the 'same unpasteurized mix' so the mix contained minimal protein (3.3%) and total solids (9.4%). Denatured whey proteins in a gel network contribute charged groups and increased surface area which allows for increased water-protein interactions and increased water retention in gels (Parnell-Clunies *et al.* 1986). This supports these WHC results in this study.

The 90-Y and 65-Y did not receive a heat treatment post-fermentation, thus it can be assumed that the starter cultures were alive during the storage and contributed to the increase in

TA and decease in pH as a function of storage time. Salvador and Fiszman (2004) reported similar trends of decreased pH and increased TA but their yogurts were stored at 10 °C for 42 days.

In summary, the 90-Y exhibited greater cohesiveness, firmness, springiness, G', L*, WHC and WPD but less adhesiveness, LT and syneresis than did the 65-Y. The 5 fold difference in WPD in these mixes influenced the intra- and inter-connections of whey proteins and casein micelles prior to gel formation and also during gel formation. Yogurt stability was influenced by the dynamics of these interactions.

But most importantly, gels were formed and maintained during 29 days of refrigerated storage. These results showed that despite only ~48% WPD in the 90-Y, these yogurt gels had similar quality attributes as other yogurt gels which had been reported to have greater WPD (72 to 90%) (Lee and Lucey 2004, Needs *et al.* 2000, Lucey *et al.* 1997, Parnell-Clunies *et al.* 1986).

More work is needed to increase whey protein availability in yogurt and possible approaches could be 1) increasing total solids of yogurt mixes, 2) use of stabilizers in the yogurt mixes and 3) use of exopolysaccharides producing dairy cultures. Because whey proteins are some of the best proteins for human consumption, supplementation of yogurt mix with WPC might be the best possible approach to improve final yogurt quality.

CONCLUSIONS

WPD exhibited a positive relationship with almost all of the yogurt quality parameters. Although yogurt gel structures changed over time as evidenced by changes in LT, the gel structure was maintained and these gel rearrangements tended to stabilize during storage. Yogurts with less WPD (8 and 48%) were made and at least the 90-Y (~48%) had similar quality to previously reported yogurts, suggesting that yogurt can be made with more undenatured whey proteins and maintain comparable quality. But more studies are necessary to understand how to improve yogurt quality made from mixes with low levels of WPD. Addition of whey protein concentrate due to its high nutritional quality and wide availability, in the yogurt mixes pasteurized at lower temperature could be plausible measure to improve yogurt gel quality.

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CHAPTER 6 - ADDITION EFFECT OF WHEY PROTEIN CONCENTRATE (WPC) ON PHYSICAL AND CHEMICAL PROPERTIES OF SET YOGURT

INTRODUCTION

The functional role of a protein as a food ingredient depends on complex interactions of various factors. Whey proteins are known to have excellent gelation properties. In the solution, they are capable of forming viscoelastic gels after heat denaturation (Kinsella and Whitehead 1989). The texture of whey protein gels is affected by heating and cooling rates, protein concentration, pH, ionic strength and interactions (Boye *et al.* 1997).

The amount of total solids in the yogurt mix, to a large extent, determines the physical and textural properties of the final yogurt (Aziznia *et al*. 2008). Subsequently lower total solids in the yogurt mixes result in a yogurt with a weak body, poor texture, and whey separation (syneresis), unless stabilizer blends or ropy strains of starter cultures are used (Trachoo and Mistry 1998, Mistry and Hassan 1992). Sandoval-Castilla *et al.* (2004) reported that in yogurt supplemented with WPC, the casein micelles were predominantly linked by particle-to-particle attachments in large chains with comparatively small interspatial voids; however yogurt without WPC had casein micelles that were fused into aggregates which were closely packed. Decreased interspatial voids increase gel strength. Thus, addition of WPC in yogurt mixes affected the microstructure of the yogurt gel. Exceeding optimal amounts of WPC (~1.5%) saturates the binding capacity of κ-casein, which leads to the formation of whey protein aggregates as opposed to κ-casein-whey aggregates. These whey protein aggregates may interfere with the gel formation and consequently microstructural and rheological properties of the yogurt are altered. Some researchers have suggested that these aggregates might 1) act as fillers in the gel network; 2) might form another network; or 3) simply hinder the present gel network (Aziznia *et al.* 2008, Guyomarch *et al*. 2003, Puvanenthiran *et al*. 2002).

Over the years WPC production techniques to make “low heat” and “high heat” powders have been developed. Undenatured whey proteins have greater biological values and they are desired for their health benefits (Ha and Zemel 2003). Adding WPC to yogurt mix contributes peptides and enhances the amino acids content and profile in the yogurt mix (Dave and Shah

1998). The nutrient increase may enhance the starter culture growth and possibly increase yogurt TA (Amatayakul *et al*., 2006). Modler and Kalab (1983) reported that yogurts fortified with 0.5, 1 and 1.5% WPC had increased firmness (56.0, 55.7 and 78.9 g, respectively) and decreased syneresis (41.1, 34.0 and 23.1%, respectively). Increased WPC in yogurt mix increased bound water and firmness (Puvanenthiran *et al*. 2002, Trachoo and Mistry 1998).

Puvanenthiran *et al.* (2002) reported that increasing whey protein content in yogurt mixes (from 0.75 to 2.07 g) (total proteins kept constant), increased the yogurt fermentation time (4.30 to 5.75 hrs, respectively), because additional proteins increased the buffering capacity of the mix. They further reported that the increased whey proteins in the yogurt mixes increased firmness (13.57 vs. 32.44 g) and decreased syneresis (44 vs. 16%) in the yogurt.

Researchers reported that increased denatured whey proteins increased cross-linking or bridging within the gels, and was responsible for the increase in G' (~10 vs. ~1000 Pa for unheated vs. heated yogurt mixes containing 1% WPC) (Lucey *et al.* 1999). They interpreted that the increased firmness and decreased syneresis were the result of the increased number of whey-whey and whey-casein bonds in the gel (mostly covalent in nature). Sodini *et al.* (2005) reported increased WHC in the yogurts if whey protein addition was done, which was attributed to increased interactions of whey and casein leading to increased water retention in the gel network.

Addition of WPC to a yogurt mix combined with pasteurization conditions that minimize WPD, may produce a yogurt gel with enhanced nutritional quality. Thus the objectives of this research were- 1) to investigate the effects of adding a greater amount of WPC (3%) in yogurt mixes pasteurized at two different temperatures 2) to assess the yogurt quality made from mixes with different formulations and different WPD and 3) to determine the effects of WPC addition on the quality of yogurt stored for 29 days.

MATERIALS AND METHODS

Yogurt Manufacture

Yogurt culture

A mother culture was prepared by mixing 4.5 g of NFDM (low-heat nonfat dry milk, Dairy America) to 50 ml of distilled, de-ionized water in a 250 ml volumetric flask (Fischer Scientific) using a magnetic stirrer (Fisher stirring hotplate, Fischer Scientific) for 5 min at 24 °C ±1 in a 250 ml Erlenmeyer flask. Milk was then covered with cheesecloth and aluminum foil, autoclaved at 121 °C at 15 psi for 15 min (AMSCO Eagle Series 2021 Gravity) and cooled to 23 °C. Approximately 1% w/w yogurt culture ("Yo-Mix" 651 DPL yogurt 500GL (freeze dried culture, Danisco) was transferred aseptically to the milk and incubated (Equitherm, Environmental Incubator, Curtis Matheson Sci) for 18 hrs at 35 °C until a pH of 4.1 to 4.4. Mother culture was transferred to 4 °C storage until usage (approximately 6 hrs).

Yogurt mix processing

Nonfat dry milk was reconstituted by adding 62.5 g of NFDM or 47.5 g NFDM and 15 g of WPC (Avonlac 134, Glanbia Nutritionals Inc., Monroe, WI) to 437.5 ml of de-ionized, distilled water in a flask. For convenience, the mixes were referred as N and W to reflect the milk base with N as the NFDM and W as the NFDM-WPC blend. The formulated mix was magnetically stirred at 24 °C ±1 for 10 min (Corning stirrer PC310) then heat was applied until the yogurt mix attained 70 or 90 °C (~5 min). The flask was transferred to a pre-heated water bath (Isotemp 220, Fischer Scientific) at 70 or 90 °C and maintained for 30 or 10 min, respectively. Yogurt mix was cooled to 43 °C within 15 min by placing the flask in ice water with periodic shaking.

Mother culture was aseptically transferred (approximately 3% (w/v)) to the pasteurized yogurt mix and the yogurt mix was shaken for about 1 min to ensure adequate distribution of microorganisms. Yogurt mix was incubated (Isotemp incubator, Fischer Scientific) in 120 ml capacity sterile cups (Fisher Scientific) at 43 °C for 2.5 to 4 hrs, to pH 4.5 to 4.6. Additionally 50 ml of yogurt mix was poured in centrifuge tubes (50 ml, Nalgene) and incubated with the yogurt cups. Yogurt was stored at 5 ºC ±1 (Roper, Whirlpool Corporation, Benton Harbor, MI) until test

day. For convenience yogurts are referred as W-70, W-90, N-70 or N-90 and their descriptions are given in Table 6-1.

Table 6-1: Codes and formulations for the yogurt mixes and resultant yogurts

Code	Pasteurization Temperature (°C)	Pasteurization Time (min)	Ingredients		
			Water (ml)	NFDM (g)	WPC (g)
N-70 [β]	70	30	437.5	62.5	0
N-90 [β]	90	10	437.5	62.5	0
W-70[β]	70	30	437.5	47.5	15
W-90 [β]	90	10	437.5	47.5	15

[β] N= Yougrt containing 12.5% NFDM only; W= yogurt containing 9.5% NFDM and 3% of WPC

Chemical Methods

Acidity Measurements

Prior to pH measurement (Fischer Universal pH meter (Fisher Scientific)) the instrument was calibrated with standardized solutions (Fisher Scientific) to pH 4 and 7. Titratable acidity (TA) was measured as described by Hooi *et al.* (2004). Measurements were done in duplicate and the average was reported.

Total Solids Content

Total solids were measured using a forced air oven method (Isotemp Oven, Fisher Scientific) as described by Hooi *et al.* (2004).

Total Protein, Casein, Whey and Whey Protein Denaturation (WPD)

Total protein, casein, whey and WPD were measured as described by Grady *et al.* (2001). A LECO analyzer was used for nitrogen measurement using a conversion factor of 6.38 (IDF 25, 1964).

Physical Methods

Color Properties

Yogurt color was measured as described by Schmidt *et al.* (2001). A Hunter D-54 Reflectance Lab Ultra Scan Sphere Spectrophotometer (Hunter Associate Laboratories) was standardized using a white tile and light trap, and single readings were obtained. The yogurt was placed in a sample cup (diameter 6.5 cm and depth 4.5 cm). White color reflectance standards were X = 83.4, Y = 88, Z = 93.9 and D 65/10°. Two readings were taken and averaged.

Rheological Properties

Rheological measurements were done as described by Hassan *et al.* (2003) using a Bohlin VOR computer-controlled rheometer system (Bohlin Instruments Inc). The sample cup contained a concentric cylinder device consisting of a cup (28 mm diameter) and a bob (25 mm diameter, 42.5 mm length). About 17 to 20 ml of yogurt sample was transferred into the cup and the bob was lowered until its whole surface was covered. A strain oscillation frequency shear sweep of 0.1 to 10 Hz was applied and readings were taken at 1 Hz at an interval of 5 s. The fundamental dynamic parameters-storage modulus (G') and loss modulus (G") were determined at 4 °C ±1. The G’ was expressed at 1 Hz and loss tangent (LT) was calculated as the ratio of G” to G’ at 1 Hz.

Syneresis Analysis

Syneresis was determined as described by Amatayakul *et al.* 2006. A 120 ml cup of yogurt of known weight was maintained at a 45° angle for 2 hrs at 5 °C ±1. The free whey was siphoned using a syringe and weighed. Syneresis was expressed as a percentage of whey weight over initial yogurt weight. Greater values indicate greater amounts of syneresis.

Texture Profile Analysis

Yogurt texture was determined using a method described by Megenis *et al*. (2006), using TA.XT2, Texture Analyzer (Stable Micro System) equipped with a 25 mm (P25/L) acrylic probe on a 120 ml cup of yogurt maintained at 5 °C ±1. Test velocity, time and distance were 2 mm/s, 5 sec and 5 mm, respectively and firmness (g) was reported.

Water Holding Capacity

Water holding capacity (WHC) was measured as described by Parnell-Clunies *et al.* (1986). Yogurt samples were centrifuged at 10 °C at 13,500 x g for 30 min (Marathon 21000R, Fisher Scientific). The supernatant was drained for 10 min and pellet weight was determined. Water holding capacity was expressed as percent pellet weight over original yogurt weight.

Shelf life/Storage study

Immediately post fermentation yogurt samples were cooled overnight at 5 °C ±1 (Roper, Whirlpool Corporation). Yogurt samples were stored at 5 °C ±1 and evaluated on days 1, 15 and 29 for chemical and physical properties.

Experimental design

The experiment was designed using a randomized incomplete block with 3 replications, where each block was a yogurt formulation/day (NFDM or NFDM-WPC) (Kuehl 2000). Day 1 data were analyzed by split plot design while storage data were analyzed using split-split plot design.

Statistical analysis

Protein analyses and day 1 yogurt quality: Compositional data were analyzed using MIXED procedure. Day 1 data were analyzed using a split plot, where formulation (N and W) was whole plot factor and pasteurization treatment (70 °C for 30 min or 90 °C for 10 min) was split plot factor by the MIXED procedure of SAS® (SAS v9.1, SAS Institute Inc, Cary, NC) using a significance of $\alpha = 0.05$. Means of the significant main effects were differentiated by P-diff procedure, while interactions were graphically reported and interpreted.

Storage study: Storage data were analyzed using split-split plot, where formulation (N and W) was the whole plot factor, pasteurization treatment (70 °C for 30 min or 90 °C for 10 min) was the split plot factor and storage time (day 1, 15 or 29) was the split-split plot factor. Data were analyzed by the MIXED procedure of SAS® (SAS v9.1, SAS Institute Inc, Cary, NC) using a significance of $\alpha=0.05$. Means of the significant main effects were differentiated by P-diff procedure, while interactions were graphically reported and interpreted.

Appendix B provides average raw data for the experiment; Appendix C provides SAS® program used to analyze data; Appendix D provides P values and Appendix E provides data that were determined to be non-significant.

RESULTS

Yogurt Characterization

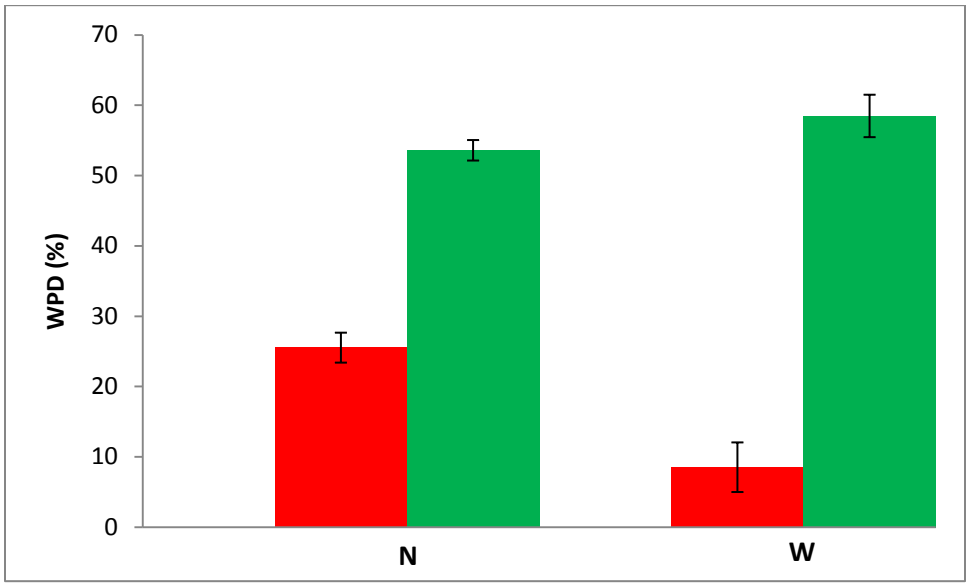
Table 6-2 provides the initial yogurt mix composition, fermentation time and yogurt pH and TA, while figure 6-1 provides the formula*pasteurization interaction for WPD. Yogurts did not differ in casein contents, fermentation time, pH, TA, total protein and total solids indicating yogurt samples were similar in some of the factors that affect yogurt quality. But yogurts did differ in whey protein contents as a function of formulation (Table 6-2) and pasteurization (Figure 6-1). The W-70 had less WPD (3 X) than N-70; while N-90 and W-90 had similar WPD (56.02% ±5.74).

Table 6-2: Yogurt[‡Δ] means and standard errors for fermentation time (FT), protein compositions and chemical quality as a function of formulation

Attribute	Formulation[‡]	
	N	W
FT[β] (hrs)	4.99[a] ±0.21	4.97[a] ±0.19
Initial yogurt quality		
TS[α] (%)	12.11[a] ±0.02	12.10[a] ±0.02
pH (%)	4.40[a] ±0.01	4.35[a] ±0.03
TA[α] (%)	1.05[a] ±0.02	1.02[a] ±0.02
Composition of yogurt mixes		
TP[α] (g)	3.73[a] ±0.25	4.24[a] ±0.02
Whey protein (g)	1.24[b] ±0.03	1.63 [a] ±0.07

[β]Fermentation time- measured as time (hrs) for yogurt mix pH to achieve 4.6 during incubation
[Δ]Means (n=3) in a row with a different superscript differ ($P < 0.05$)
[‡]N: yogurt containing 12.5% NFDM; W: yogurt containing 9.5% NFDM and 3% WPC
[α]TA=titratable acidity; TP=total protein; TS=total solids

Figure 6-1: Means and standard error for whey protein denaturation (%WPD) as a function of formulation (N consists of yogurt containing 12.5% NFDM, and W consists of yogurt containing 9.5% NFDM and 3% WPC) and pasteurization treatments (red bars represent 70 °C for 30 min, whereas green bars represent 90 °C for 10 min); n = 3.



Figures 6-2 and 6-3 show the shear stress vs. shear rate graphs for the yogurt samples at day 1. Yogurt samples exhibited gel structures as evidenced by G' being greater than G" at any specified shear rate, despite the different WPD of the mixes.

Figure 6-2: Rheological profile of N yogurts made from 12.5% NFDM mixes pasteurized at 70 °C for 30 min or 90 °C for 10 min and stored at 5 °C ±1 (♦-G' of yogurt made from mix pasteurized at 70 °C for 30 min, ▲-G' of yogurt made from mix pasteurized at 90 °C for 10 min, X–G" of yogurt made from mix pasteurized at 70 °C for 30 min, ■–G" of yogurt made from mix pasteurized at 90 °C for 10 min) for day 1; n=3

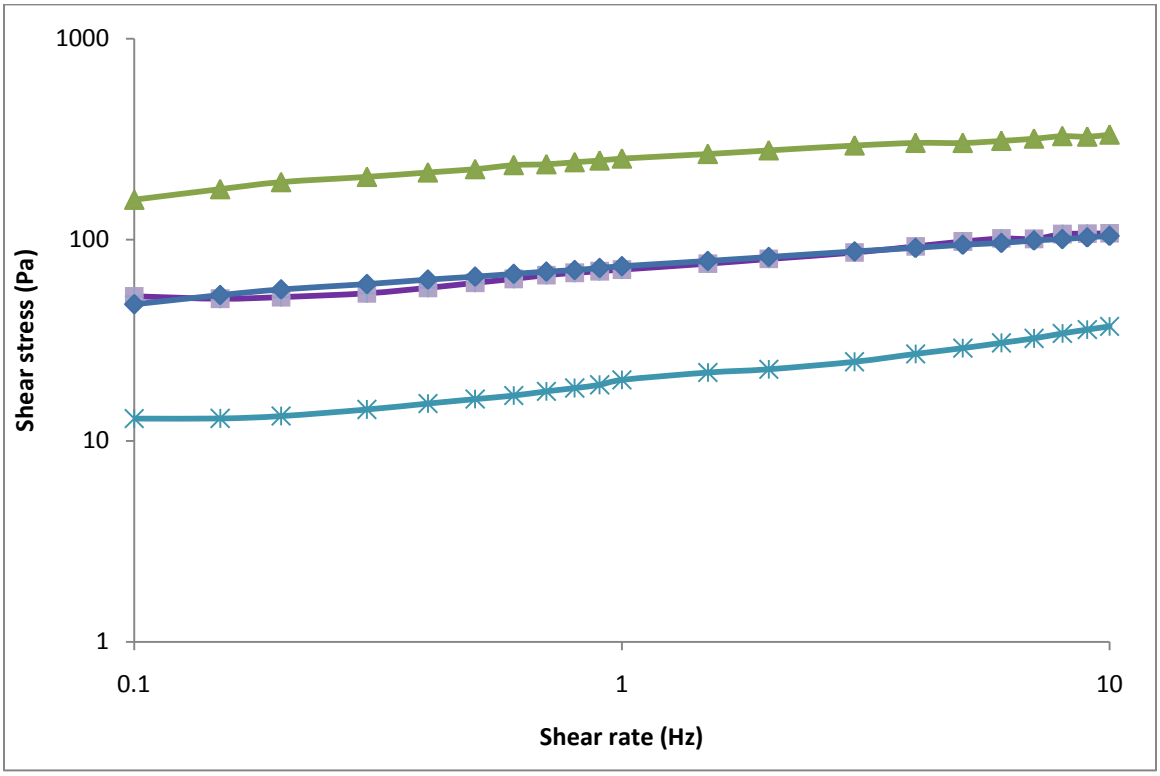
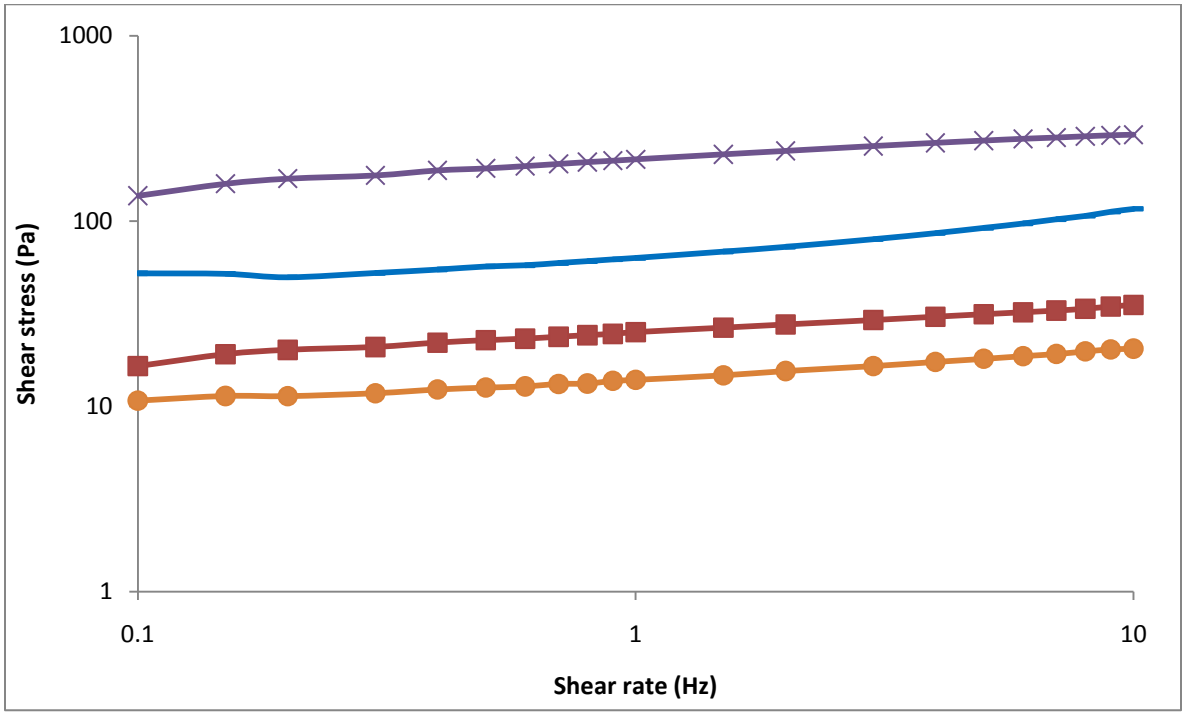


Figure 6-3: Rheological profile of W yogurts made from 9.5% NFDM and 3% WPC mixes pasteurized at 70 °C for 30 min or 90 °C for 10 min and stored at 5 °C ±1 (■-G' of yogurt made from mix pasteurized at 70 °C for 30 min, X-G' of yogurt made from mix pasteurized at 90 °C for 10 min, ●–G" of yogurt made from mix pasteurized at 70 °C for 30 min, ♦–G" of yogurt made from mix pasteurized at 90 °C for 10 min) for day 1; n=3



Day 1 yogurt quality

Day 1 yogurts differed as a function of formulation (Table 6-3), where N yogurts had greater firmness (1.33 X) and G' (1.34 X) but lower pH (1.01 X) than did the W yogurts.

Table 6-3: Initial (day 1) yogurt[‡Δ¥] means and standard errors for firmness, G' and pH as a function of formulation averaged for pasteurization treatments

	Formulation[‡]	
Attribute	**N**	**W**
Firmness (g)	102.88 [a] ±20.12	76.95[b] ±21.08
G' (Pa)	161.61 [a] ±41.45	120.23 [b] ±41.45
pH	4.35 [b] ±0.03	4.40 [a] ±0.01

[Δ]Means (n=6) in a row with a different superscript differ ($P < 0.05$)
[‡]N: yogurt containing 12.5% NFDM; W: yogurt containing 9.5% NFDM and 3% WPC
[¥]Yogurt mixes pasteurized at 70 °C for 30 min or 90 °C for 10 min

Day 1 yogurts differed as a function of pasteurization (Table 6-4), where yogurts made from mixes pasteurized at 90 °C for 10 min had greater firmness (2.90 X), G' (4.75 X), L* (1.02 X), WHC (1.34) but less syneresis (3 X) than did the yogurts made from mixes pasteurized at 70 °C for 30 min. Appendix E provides the non significant dataset of this experiment.

Table 6-4: Initial (day 1) yogurt[‡Δ¥] means and standard errors for color, rheological, textural and water binding properties as a function of pasteurization treatments averaged for formulation

Attribute	Pasteurization[¥]	
	70 °C	**90 °C**
Firmness (g)	46.37 [b] ±5.95	134.46 [a] ±9.34
G' (Pa)	49.34 [b] ±12.00	234.50 [a] ±13.14
L*	85.01 [b] ±0.23	86.51 [a] ±0.08
Syneresis (%)	9.20 [a] ±0.61	3.19 [b] ±0.33
WHC (%)	17.88 [b] ±0.53	24.01 [a] ±0.57

[Δ]Means (n=6) in a row with a different superscript differ ($P < 0.05$)
[‡]N: yogurt containing 12.5% NFDM; W: yogurt containing 9.5% NFDM and 3% WPC
[¥]70 °C – yogurt mix pasteurized at 70 °C for 30 min; 90 °C – yogurt mix pasteurized at 90 °C for 10 min

At day 1, yogurts differed in WPD ranged from 8.9% to 58% and all these factors are known to affect the yogurt gel quality attributes.

Storage study

Statistical analyses indicated that yogurt quality differed as a function formula, where N yogurt had increased firmness (1.21 X), G' (1.64 X), WHC (1.09 X) but less LT (1.11 X) and pH (1.01 X) compared to the W yogurts (Table 6-5). Formulation did not affect color properties, syneresis and TA (Appendix E).

Table 6-5: Yogurt[‡Δ¥ δ] means and standard errors for chemical, rheological, textural and water binding properties as a function of formulation averaged for storage days and pasteurization treatments

	Formulation[‡]	
Formula	**N**	**W**
Firmness (g)	105.42 [a] ±1.18	86.55 [b] ±13.15
G' (Pa)	199.31 [a] ±28.54	148.20 [b] ±29.44
LT	0.2713 [b] ±0.00	0.3006 [a] ±0.00
pH	4.30 [b] ±0.01	4.35 [a] ±0.01
WHC (%)	23.02 [a] ±0.93	21.07 [b] ±0.81

[Δ]Means (n=18) in a row with a different superscript differ ($P < 0.05$)
[‡]N: yogurt containing 12.5% NFDM; W: yogurt containing 9.5% NFDM and 3% WPC
[¥]Yogurt mixes pasteurized at 70 °C for 30 min or 90 °C for 10 min
[δ] Yogurts analyzed at day 1, 15 and 29

Pasteurization affected yogurt firmness, G', L*, syneresis and WHC (Table 6-6); but not a*, b*, LT, pH and TA (see Appendix E for non significant dataset). Yogurts made from mixes pasteurized at 90 °C for 10 min had greater firmness (3X), G' (4.86X), L* (1.02 X) and WHC (1.34X) but less syneresis (2.76X) than did the yogurts made from mixes pasteurized at 70 °C for 30 min.

Table 6-6: Yogurt[‡Δ¥δ] means and standard errors for color, rheological, textural and water binding properties as a function of pasteurization treatments averaged for formulation and storage days

	Pasteurization[¥]	
Attribute	**70 °C**	**90 °C**
Firmness (g)	47.26 [b] ±3.41	144.71 [a] ±4.14
G' (Pa)	59.23 [b] ±7.00	288.28 [a] ±12.87
L*	85.07 [b] ±0.15	86.42 [a] ±0.08
Syneresis (%)	9.41 [a] ±0.49	3.40 [b] ±0.19
WHC (%)	18.68 [b] ±0.33	25.41 [a] ±0.45

[Δ]Means (n=18) in a row with a different superscript differ ($P < 0.05$)
[‡]N: yogurt containing 12.5% NFDM; W: yogurt containing 9.5% NFDM and 3% WPC
[¥]70 °C – yogurt mix pasteurized at 70 °C for 30 min; 90 °C – yogurt mix pasteurized at 90 °C for 10 min
[δ] Yogurts analyzed at day 1, 15 and 29

Storage significantly affected yogurt firmness, G', pH, TA and WHC (Table 6-7); but did not affect a*, b*, syneresis, L* and LT (see Appendix E for non significant dataset). Overall yogurts stored for day 1 to 29 had increasing G' but decreasing pH; whereas yogurt firmness, TA and WHC increased from day 1 to 15 and remained constant thereafter. Results obtained were similar to chapter 5, where G', TA and WHC increased from day 1 to 15 and then stabilized, while pH decreased from day 1 to 29.

Table 6-7: Yogurt[Δ‡¥] means and standard errors for chemical, rheological, textural and water binding properties as a function of storage days averaged for formulation and pasteurization treatments

	Storage day		
Attribute	**1**	**15**	**29**
Firmness (g)	89.91 [b] ±14.43	100.95 [a] ±15.85	97.09 [ab] ±15.78
G' (Pa)	141.92 [c] ±29.10	177.64 [b] ±36.77	201.70 [a] ±41.37
pH	4.37 [a] ±0.01	4.32 [b] ±0.01	4.28 [c] ±0.01
TA (%)	1.04 [b] ±0.01	1.11 [a] ±0.01	1.11 [a] ±0.00
WHC (%)	20.95 [b] ±1.00	22.64 [a] ±1.28	22.54 [a] ±1.00

[Δ]Means (n=12) in a row with a different superscript differ ($P < 0.05$)
[‡]N: yogurt containing 12.5% NFDM; W: yogurt containing 9.5% NFDM and 3% WPC
[¥]70 °C – yogurt mix pasteurized at 70 °C for 30 min; 90 °C – yogurt mix pasteurized at 90 °C for 10 min

Interactions

During storage, 4 significant interactions were observed-formula*pasteurization interactions for L* and syneresis and pasteurization*day interactions for G' and syneresis.

*Formula*pasteurization interactions*

Interaction results for L* and syneresis are shown as means and standard deviations in Table 6-8 and graphically in figure 6-4 and 6-5, respectively.

Yogurts made from mixes pasteurized at 90 °C for 10 min were lighter (L*) and expressed less syneresis than did the yogurts made from mixes pasteurized at 70 °C for 30 min. N-90 and W-90 had comparable L* and syneresis; while N-70 had greater L* and less syneresis than the W-70.

Table 6-8: Yogurt[‡Δ] means and standard errors for L* and syneresis as a function of formulation and pasteurization treatments averaged for storage days

Attribute	Formulation*pasteurization[‡]			
	N-70	N-90	W-70	W-90
L*	85.59±0.14	86.49±0.08	84.56±0.07	86.35±0.13
Syneresis (%)	8.23±0.70	3.59±0.22	10.59±0.41	3.21±0.31

[Δ]n=9 (collapsed for storage days 1, 15 and 29)
[‡]N-70: yogurts made from mixes containing 12.5% NFDM, pasteurized at 70 °C for 30 min;
N-90: yogurts made from mixes containing 12.5% NFDM, pasteurized at 90 °C for 10 min;
W-70: yogurts made from mixes containing 9.5% NFDM and 3% WPC, pasteurized at 70 °C for 30 min
W-90: yogurts made from mixes containing 9.5% NFDM and 3% WPC, pasteurized at 90 °C for 10 min

Figure 6-4: Yogurt means and standard errors for L* as a function of formulation (N consists of yogurt containing 12.5% NFDM, and W consists of yogurt containing 9.5% NFDM and 3% WPC) and pasteurization treatments (red bars represent 70 °C for 30 min, whereas green bars represent 90 °C for 10 min) averaged for storage days (1, 15 and 29); n= 9

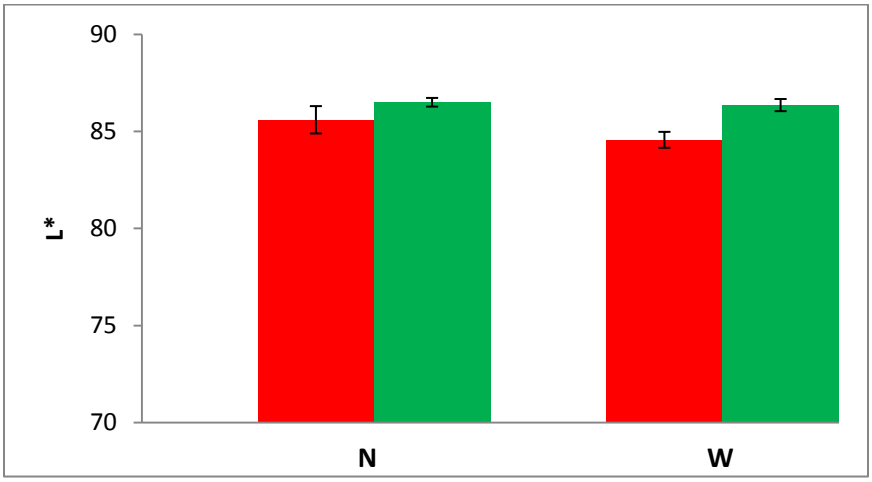
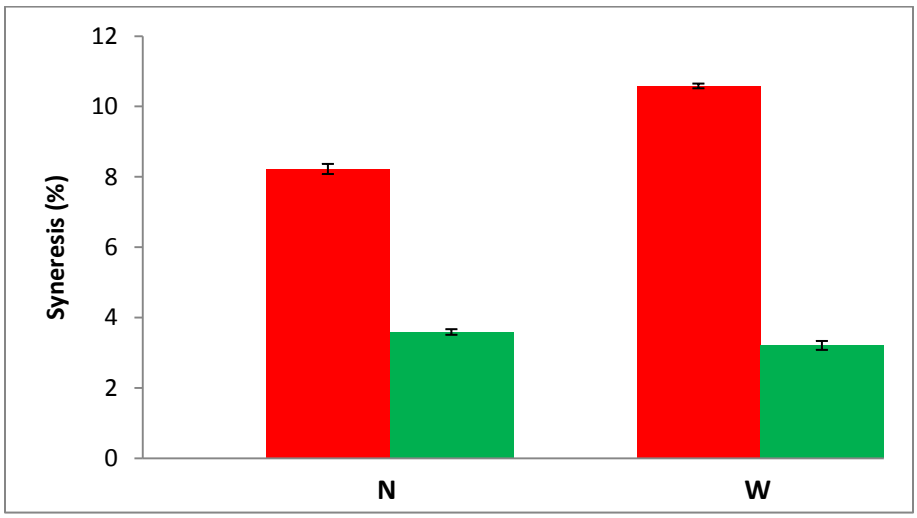


Figure 6-5: Yogurt means and standard errors for syneresis as a function of formulation (N consists of yogurt containing 12.5% NFDM, and W consists of yogurt containing 9.5% NFDM and 3% WPC) and pasteurization treatments (red bars represent 70 °C for 30 min, whereas green bars represent 90 °C for 10 min) averaged for days (1, 15 and 29); n= 9



Pasteurization*day interactions

Table 6-9 shows the means of the pasteurization*day interactions for G' and syneresis; whereas figures 6-6 and 6-7 shows the graphical representation of the G' and syneresis interactions.

The 90-N and 90-W had G' ~5 X greater than the 70-N and 70-W (Figure 6-4). However the G' increase from day 1 to 15 was equivalent (~1.24 X) regardless of pasteurization treatments; but from day 15 to 29, G' increased ~1.14 X vs. ~1.08X for yogurts made from mixes pasteurized at 90 °C for 10 min as compared to yogurts made from mixes pasteurized at 70 °C for 30 min.

Syneresis means for 90-N and 90-W were comparable and were ~3 X less than that for 70-N and 70-W. The 70-N had less syneresis than did the 70-W. Syneresis for the yogurts made from mixes pasteurized at 70 °C for 30 min increased over 29 days, while syneresis decreased for yogurts made from mixes pasteurized at 90 °C for 10 min. As shown in figure 6-5, the syneresis slightly decreased for yogurts made from mixes pasteurized at 70 °C for 30 min from day 1 to 15 and then increased afterwards as opposed to yogurts made from mixes pasteurized at 90 °C for 10 min where syneresis increased from day 1 to 15 and decreased afterwards.

Table 6-9: Yogurt[‡Δ¥] means and standard errors for G' and syneresis as a function of pasteurization treatments and storage days averaged for formulation[¥]

	Pasteurization[‡]	70 °C			90 °C		
Attribute	**Day**	**1**	**15**	**29**	**1**	**15**	**29**
G' (Pa)		49.34±4.28	61.62±13.43	66.73±12.75	234.5±13.15	293.67±19.57	336.67±9.09
Syneresis (%)		9.20±0.61	8.75±1.25	10.27±0.50	3.19±0.33	4.15±0.24	2.87±0.17

[Δ]n=6 (collapsed for formula)
[¥]N: yogurt containing 12.5% NFDM; W: yogurt containing 9.5% NFDM and 3% WPC
[‡]70 °C – yogurt mix pasteurized at 70 °C for 30 min; 90 °C – yogurt mix pasteurized at 90 °C for 10 min

Figure 6-6: Yogurt means and standard errors for G' as a function of pasteurization treatments (70 °C represents mixes pasteurized at 70 °C for 30 min and 90 °C represents mixes pasteurized at 90 °C for 10 min) and storage days (blue bars are day 1, green bars are day 15 and red bars are day 29) averaged for formulation (N consists of yogurt containing 12.5% NFDM, and W consists of yogurt containing 9.5% NFDM and 3% WPC); n=6

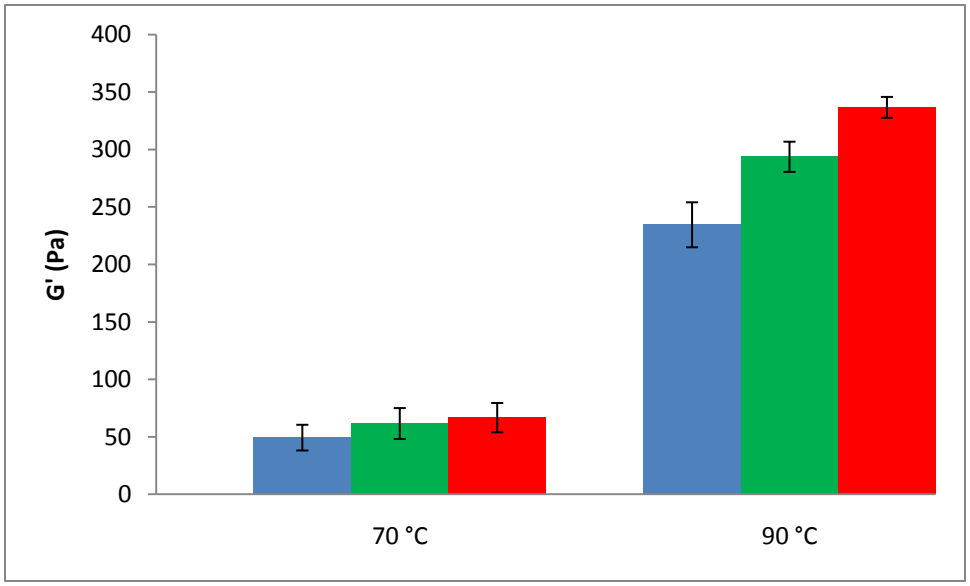
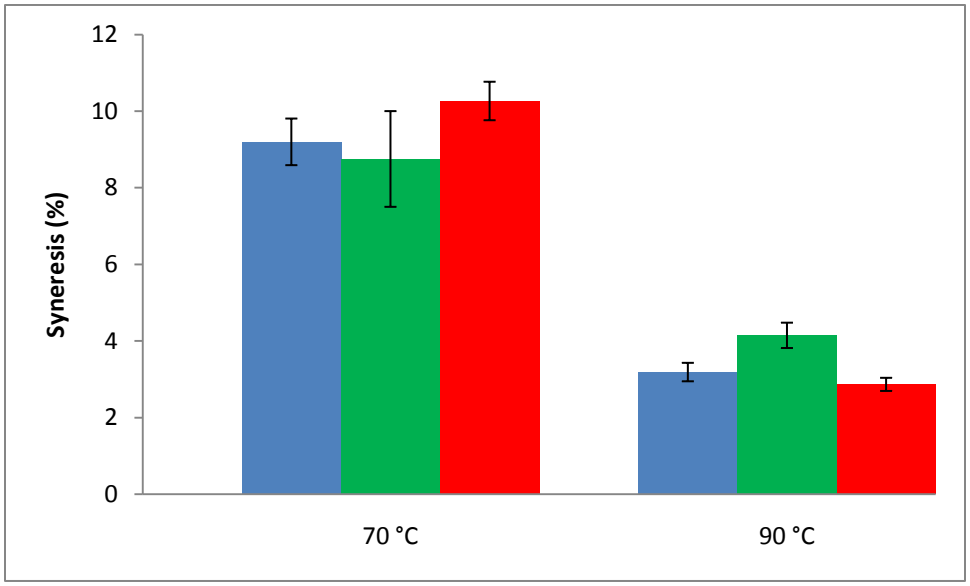


Figure 6-7: Yogurt means and standard errors for syneresis as a function of pasteurization treatments (70 °C represents mixes pasteurized at 70 °C for 30 min and 90 °C represents mixes pasteurized at 90 °C for 10 min) and storage days (blue bars are day 1, green bars are day 15 and red bars are day 29) averaged for formulation (N consists of yogurt containing 12.5% NFDM, and W consists of yogurt containing 9.5% NFDM and 3% WPC); n=6



DISCUSSION

Yogurts were successfully formulated from mixes containing 3% WPC and pasteurized at 70 °C for 30 min as evidenced by their gel structures. The physical and chemical properties of yogurts on day 1 were functions of the changes in whey protein contents as well as WPD.

Vasbinder and de Kruif (2003) reported that if yogurt mix pH was > 6.55, soluble denatured whey protein aggregates were formed during heating; however, if yogurt mix pH <6.55, insoluble denatured whey proteins aggregates were formed when heated. Denatured whey proteins subsequently associate with casein micelles increasing the effective casein micelle size; which further affect firmness, G', L*, WHC and decrease syneresis (Anema and Li 2003, Vasbinder *et al.* 2003, Lucey 2002, Mottar *et al.*1989, Parnell-Clunies *et al.* 1986). Lee *et al.* (1999), reported that in the acid gels made from mixes that were heated prior to the WPC addition, the native whey proteins in the yogurt mix were almost completely denatured and contributed to the gel structure; however, the WPC added after heating remained undenatured and probably acted as a filler in the gel matrix.

In this work, the pH of the mixes were similar (6.60 for N yogurts and 6.56 for W), but the W mix pH was close to the 6.55-a cutoff point and thus during pasteurization, more insoluble whey proteins aggregates might have formed (due to lower pH and greater WPC concentration). Also WPC was added prior to pasteurization at 3% level, which was 1.5 X more than what previous researchers have added (maximum 2%). All of these combined differences might have saturated the binding capacity of casein micelles (mainly κ-casein in mix) (Aziznia *et al.* 2008), so that large saturated casein micelles might have formed with decreased charges, which caused a loose interspatial packing in the final gel network. Saturation phenomenon could prevent further coalescence (due to increased covalent bonds and lower energy) which might have affected gel properties as exhibited by decreased firmness, G' and increased LT in the W yogurts compared to N yogurts (Amatayakul 2006b, Guyomarch *et al.* 2003, Lee and Lucey 2004a, Sandoval-Castilla *et al.* 2004, van Vliet and Walstra 1994).

van Vliet and Walstra (1994) reported that yogurt bonds rearrange over time to decrease energy thermodynamically and WPD is known to affect the LT in yogurt (Lee and Lucey 2004). In this study LT was affected as a function of formulation where W yogurts had greater LT than N yogurts. Loss tangent did not change as a function of storage, but G' increased during storage. Serra *et al.* (2009) reported similar results-increased yogurt G' during 28 days of storage.

Furthermore initial changes in stored yogurt G' from day 1 to 15, indicated that yogurts were rearranging at similar rates irrespective of pasteurization; however their rates differed in later part of storage which could be a function of the saturated binding capacity of caseins due to additional whey proteins in the gel network (Aziznia *et al.* 2008, Lucey *et al.* 1999).

Puvanenthiran *et al.* (2002) reported firmness increased with supplementation of WPC which contradicts present results; however the other researchers adjusted yogurt mixes to pH 7 prior to pasteurization, to maximize the soluble denatured whey protein aggregates, which might have accounted for difference. However the present results were in accordance with Amatayakul *et al.* (2006b), who reported decreasing firmness for yogurts made from mixes containing added WPC. Salvador and Fiszman (2004) reported that commercial yogurt samples increased in firmness from 56.12 to 74.49 g, if stored for 91, 21 and 3 days at 10, 20 and 30 °C, respectively; whereas in this study the firmness increased and then decreased.

Also researchers reported that supplementing yogurt mixes with WPC reduced syneresis in the resultant gels due to a more compact structure and free water immobilization (Puvanenthiran *et al.* 2002, Bhullar *et al.* 2002, Guzman-Gonzalez *et al.* 1999), but present results did not show this relationship, providing additional evidence that a loose structure may have formed in the W yogurts due to saturating the binding capacity of the casein in the mix, prior to the gel formation.

Sodini *et al.* (2005) reported greater WHC for yogurts fortified with WPC which contradicts present results. However, they measured WHC at a lower G-force and less time than the conditions used in the present study. It is important to note that WPD differed significantly for N-70 and W-70 and other researchers have reported that WPD and WHC are positively correlated in yogurt (Parnell-Clunies *et al.* 1986).

Needs *et al.* (2000) reported that pasteurization increased L* in yogurts, where Aziznia *et al.* (2009) reported that WPC addition to the yogurt could affect the b* of yogurt which in turn affects overall color perceptions of yogurt. However in the present experiment L* were affected slightly as a function of pasteurization but WPC had little to no effect on yogurt color. This suggests that the level of WPC addition and the WPD did not alter the light scattering abilities in the present yogurt.

Kailashpathy *et al.* (1996) reported that WPC increased buffering capacity of yogurt mixes which was confirmed in present studies. O'Neil *et al.* (1979) reported increased yogurt

acidity with storage time due to the acidity of the starter culture during storage, as they continued to generate more lactic acid and thus increased TA and lowered pH in this study confirmed these findings.

Overall yogurts made from W mixes had lower quality parameters such as firmness, G', pH and WHC than did the yogurts made from mixes containing NFDM. Pasteurization of yogurt mixes at 90 °C for 10 min resulted in greater WPD (2 to 6 fold) compared to the mixes pasteurized at 70 °C for 30 min. Yogurts demonstrated greater G', firmness, L* and WHC if pasteurized at 90 °C for 10 min and the firmness, G', pH, TA and WHC increased during storage. Yogurts made from mixes containing only NFDM had greater WPD as compared to yogurt made from mixes containing NFDM and WPC when pasteurized at 70 °C for 30 min.

In this set of experiment, W-70 had lower quality attributes compared to N-70; however WPD was much lower (8.52% vs. 25.52%). While W yogurts had greater whey protein content as compared to N yogurts (0.68 g vs. 0.58 g), mixes pasteurized at 90 °C for 10 min had comparable quality attributes and WPD. But most importantly, W-90 had less WPD but similar quality to other previously reported results indicating that the objective of manufacturing a yogurt with more undenatured whey proteins but similar can be achieved.

CONCLUSIONS

Adding WPC to yogurt mix increased the whey protein content and if yogurt mixes were pasteurized at 70 °C for 30 min, whey protein denaturation was less compared to the yogurt mixes made from only NFDM and pasteurized at 90 °C for 10 min.

Addition of WPC at 3% level had mixed effects on the quality (initial and storage); however WPC added yogurt showed lower WPD when pasteurized at 70 °C for 30 min indicating increased amounts of undenatured whey protein. It is likely that 3% WPC could have saturated binding capacity of casein micelles which affected the gel network. Yogurts containing added WPC and pasteurized at 70 °C for 30 min showed potential for the manufacture of a quality yogurt with a promise of added nutritional benefit. Further studies are needed to optimize amount of WPC to add to yogurt as well as the pasteurization conditions to improve quality attributes further.

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CHAPTER 7 - SUMMARY

Experiment 1

Yogurt quality is dependent on the presence and amount of denatured whey proteins. Yogurts made from mixes pasteurized at 90 °C for 10 min had significantly greater L*, firmness, G' water holding capacity (WHC) and whey protein denaturation (WPD) but less loss tangent (LT) and syneresis than did the yogurts made from mixes pasteurized at 65 °C for 30 min. Yogurt properties such as titratable acidity, G' and WHC increased while LT decreased over 29 days. Whey protein denaturation was almost 5 X greater in yogurts made from mixes pasteurized at 90 °C (~48%) than in yogurts made from mixes pasteurized at 65 °C (~9%). But a yogurt made from mixes pasteurized at 90 °C for 10 min had less WPD compared to previous reports. Increasing total solids and boosting whey proteins may be an alternative to improve quality attributes of yogurts as well as nutritional quality.

Experiment 2

Added whey protein concentrate (WPC) in the yogurt mix increased the whey protein content in the formula, which in turn increased whey protein content. In yogurts, WPC addition decreased firmness, G' and WHC but increased pH. Mix pasteurization at 90 °C for 10 min, increased firmness, G' and WHC while decreased syneresis confirming the positive effects of WPD. When stored, yogurt firmness, G' and WHC increased during 29 days, confirming that the gel networks rearrange over time. Results showed that the WPC supplemented yogurt mixes pasteurized at 70 °C for 30 min had less WPD than the NFDM mixes, which suggest that quality in the WPC-containing yogurt may be improved, if similar WPD contents are achieved. This may be the next research study.

Table 7-1 provides a direct comparison of chapter 5 and 6 followed by conclusions on the same.

Table 7-1: Initial (Day 1) Yogurt[Δ‡] chemical, color, rheological, textural and water binding properties means and standard errors as a function of pasteurization or formula*pasteurization in experiment 1 and 2, respectively

	Experiment 1[Λ]		Experiment 2[‡]			
Attribute	**65-Y[‡]**	**90-Y[‡]**	**N-70**	**N-90**	**W-70**	**W-90**
Firmness (g)	40.32 [b] ±0.66	82.84 [a] ±1.18	58.39 ±1.89	147.37 ±6.37	32.35 ±1.94	121.55 ±15.15
G' (Pa)	22.27[b] ±2.10	96.70[a] ±3.86	73.55 ±5.92	253.67 ±21.07	25.13 ±1.91	215.33 ±7.26
L*	84.67[b] ±0.16	85.71[a] ±0.06	85.44 ±0.31	86.42 ±0.14	84.64 ±0.04	86.60 ±0.02
LT[δ]	0.3222 ±0.01	0.2947 ±0.00	0.2642 ±0.01	0.2790 ±0.00	0.2943 ±0.02	0.2927 ±0.00
pH	4.45[a] ±0.03	4.47[a] ±0.01	4.36 ±0.03	4.33 ±0.03	4.40 ±0.02	4.40 ±0.02
Syneresis (%)	12.21 [a] ±0.31	5.58 [b] ±0.57	8.46 ±0.41	3.52 ±0.32	9.94 ±1.07	2.86 ±0.58
WHC[δ] (%)	13.22 [b] ±0.42	16.95 [a] ±0.27	18.43 ±0.79	24.41 ±1.09	17.35 ±0.72	23.62 ±0.54
WPD[δ] (%)	8.76 ±2.19	47.93 ±7.33	25.52 ±3.02	53.58 ±3.53	8.52 ±1.45	58.46 ±2.13
TS [δ] (%)	9.36 ±0.02	9.37 ±0.02	12.09 ±0.01	12.13 ±0.03	12.09 ±0.03	12.10 ±0.01
TP [δ] (%)	3.36 ±0.12	3.36 ±0.09	3.73 ±0.25	3.73 ±0.25	4.24 ±0.02	4.24 ±0.02

[‡]N-70: yogurts made from mixes containing 12.5% NFDM, pasteurized at 70 °C for 30 min;
W-70: yogurts made from mixes containing 9.5% NFDM and 3% WPC, pasteurized at 70 °C for 30 min
N-90: yogurts made from mixes containing 12.5% NFDM, pasteurized at 90 °C for 10 min;
W-90: yogurts made from mixes containing 9.5% NFDM and 3% WPC, pasteurized at 90 °C for 10 min
[Δ]65-Y: yogurts made from mixes containing 9.5% NFDM pasteurized at 65 °C for 30 min
90-Y: yogurts made from mixes containing 9.5% NFDM pasteurized at 90 °C for 10 min.
[‡Δ]n = 3
[δ]LT=loss tangent; WHC= water holding capacity; WPD=whey protein denaturation; TS=total solids; TP=total proteins

RESEARCH CONCLUSIONS

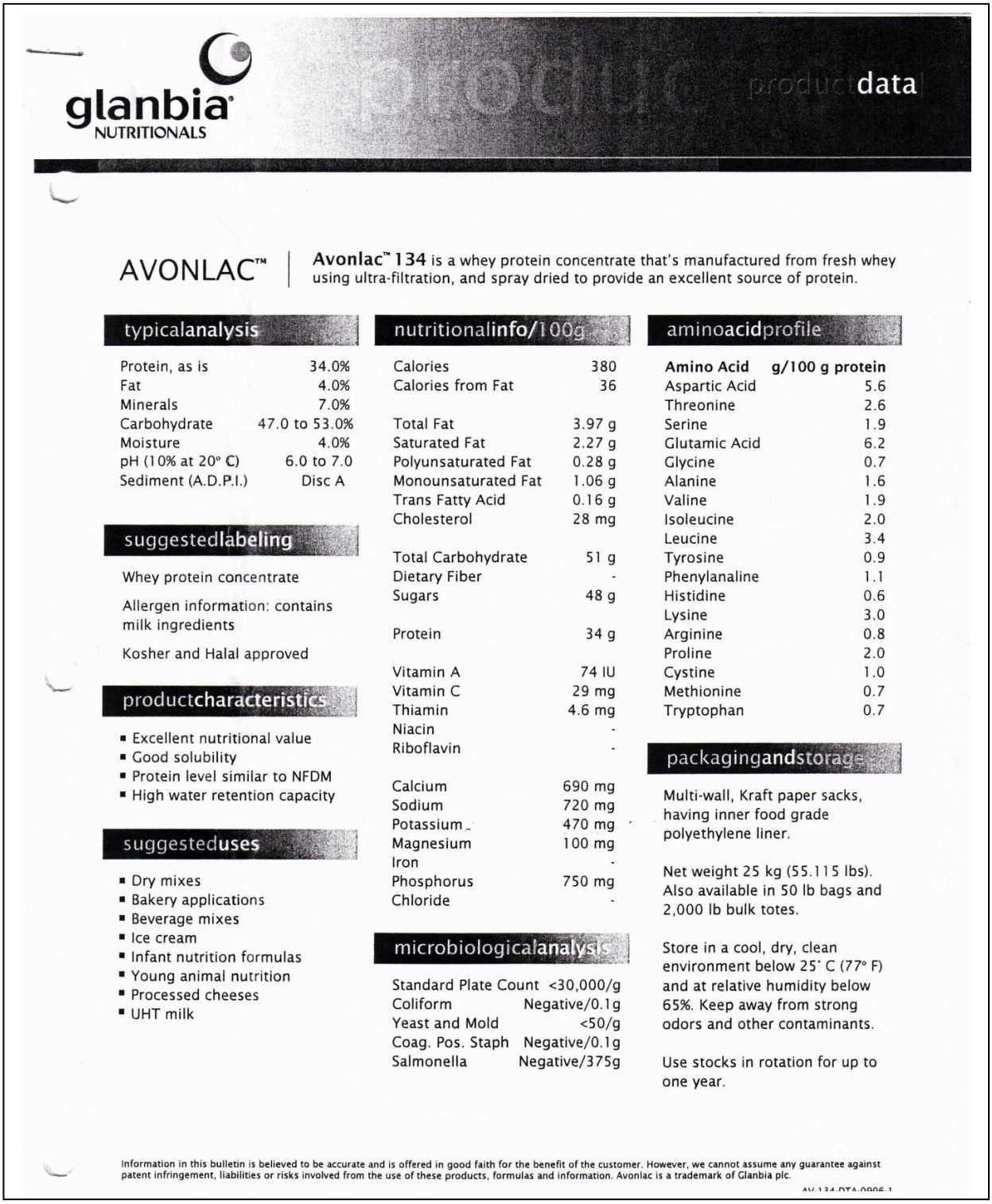
From Table 7-1, whey protein concentrate addition increased protein content and total solids in W-70 and W-90 as compared to 65-Y and 90-Y. The W-70 and 65-Y mixes had similar WPD and thus they produced yogurts with comparable G', L*, LT and pH as shown; however W-70 had less syneresis and greater water holding capacity compared to N-65, suggesting positive effects of WPC addition on physical quality of yogurt, which confirms previous results. Experiments indicated that 3% WPC addition might interfere with the gel structure in W-70 and W-90, but W-90 had greater quality attributes than 90-Y. The W-90 had less WPD than previous reported literature and greater whey protein content (0.68 g) compared to the 90-Y and N-90 (0.35 and 0.58 g, respectively). Results support the fact that the yogurt gels tended to rearrange over time, evidenced by changes in G', LT, syneresis and WHC.

Overall yogurts with supplemented whey protein concentrate were successfully formulated with lower whey protein denaturation and thus increased nutritional quality. Future studies may focus on slightly lower levels of whey proteins addition and slightly increased pasteurization treatments.

APPENDIX INDEX

Appendix A - WPC certificate of analysis

Figure 7-1: Typical composition of Glanbia whey protein concentrate Avonlac



glanbia NUTRITIONALS

product data

AVONLAC™

Avonlac™ 134 is a whey protein concentrate that's manufactured from fresh whey using ultra-filtration, and spray dried to provide an excellent source of protein.

typical analysis

Protein, as is	34.0%
Fat	4.0%
Minerals	7.0%
Carbohydrate	47.0 to 53.0%
Moisture	4.0%
pH (10% at 20° C)	6.0 to 7.0
Sediment (A.D.P.I.)	Disc A

suggested labeling

Whey protein concentrate

Allergen information: contains milk ingredients

Kosher and Halal approved

product characteristics

- Excellent nutritional value
- Good solubility
- Protein level similar to NFDM
- High water retention capacity

suggested uses

- Dry mixes
- Bakery applications
- Beverage mixes
- Ice cream
- Infant nutrition formulas
- Young animal nutrition
- Processed cheeses
- UHT milk

nutritional info/100g

Calories	380
Calories from Fat	36
Total Fat	3.97 g
Saturated Fat	2.27 g
Polyunsaturated Fat	0.28 g
Monounsaturated Fat	1.06 g
Trans Fatty Acid	0.16 g
Cholesterol	28 mg
Total Carbohydrate	51 g
Dietary Fiber	-
Sugars	48 g
Protein	34 g
Vitamin A	74 IU
Vitamin C	29 mg
Thiamin	4.6 mg
Niacin	-
Riboflavin	-
Calcium	690 mg
Sodium	720 mg
Potassium	470 mg
Magnesium	100 mg
Iron	-
Phosphorus	750 mg
Chloride	-

microbiological analysis

Standard Plate Count	<30,000/g
Coliform	Negative/0.1g
Yeast and Mold	<50/g
Coag. Pos. Staph	Negative/0.1g
Salmonella	Negative/375g

amino acid profile

Amino Acid	g/100 g protein
Aspartic Acid	5.6
Threonine	2.6
Serine	1.9
Glutamic Acid	6.2
Glycine	0.7
Alanine	1.6
Valine	1.9
Isoleucine	2.0
Leucine	3.4
Tyrosine	0.9
Phenylanaline	1.1
Histidine	0.6
Lysine	3.0
Arginine	0.8
Proline	2.0
Cystine	1.0
Methionine	0.7
Tryptophan	0.7

packaging and storage

Multi-wall, Kraft paper sacks, having inner food grade polyethylene liner.

Net weight 25 kg (55.115 lbs). Also available in 50 lb bags and 2,000 lb bulk totes.

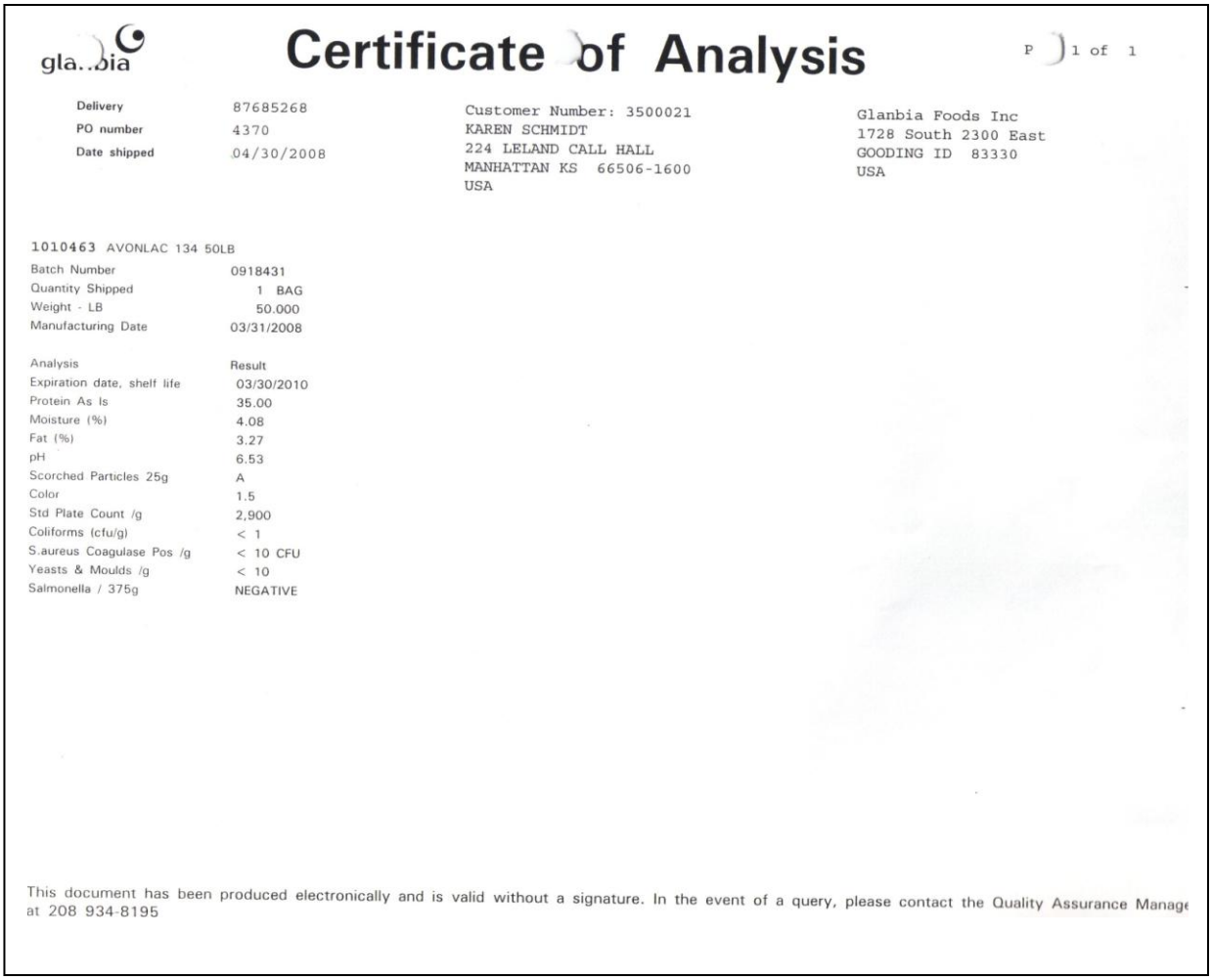
Store in a cool, dry, clean environment below 25° C (77° F) and at relative humidity below 65%. Keep away from strong odors and other contaminants.

Use stocks in rotation for up to one year.

Information in this bulletin is believed to be accurate and is offered in good faith for the benefit of the customer. However, we cannot assume any guarantee against patent infringement, liabilities or risks involved from the use of these products, formulas and information. Avonlac is a trademark of Glanbia plc.

AV-134-DTA-0906-1

Figure 7-2: Certificate of analysis of whey protein concentrate Avonlac used in study



gla..bia

Certificate of Analysis

P 1 of 1

Delivery	87685268
PO number	4370
Date shipped	04/30/2008

Customer Number: 3500021
KAREN SCHMIDT
224 LELAND CALL HALL
MANHATTAN KS 66506-1600
USA

Glanbia Foods Inc
1728 South 2300 East
GOODING ID 83330
USA

1010463 AVONLAC 134 50LB

Batch Number	0918431
Quantity Shipped	1 BAG
Weight - LB	50.000
Manufacturing Date	03/31/2008

Analysis	Result
Expiration date, shelf life	03/30/2010
Protein As Is	35.00
Moisture (%)	4.08
Fat (%)	3.27
pH	6.53
Scorched Particles 25g	A
Color	1.5
Std Plate Count /g	2,900
Coliforms (cfu/g)	< 1
S.aureus Coagulase Pos /g	< 10 CFU
Yeasts & Moulds /g	< 10
Salmonella / 375g	NEGATIVE

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Appendix B - Dataset of experiment

Experiment 1

Table 7-2: Yogurt properties measured as chemical and physical properties of yogurts on day 1, 15 and 29

Rep[β]	Past[δ]	Day	Firmness (g)	Adhesiveness (g.s)	Springiness	Cohesiveness	Siphon (%)	LT
1	65	1	40.8	-5.03	0.956	0.549	12.36	0.3019
1	65	15	40.73	-2.9	0.964	0.54	12.55	0.3333
1	65	29	39.15	-1.8	0.974	0.546	12.11	0.2826
2	65	1	39.02	-5.45	0.952	0.552	12.65	0.3418
2	65	15	40.8	-1.98	0.974	0.557	13.3	0.2932
2	65	29	40.3	-0.19	0.976	0.501	12.38	0.2896
3	65	1	41.15	-4.83	0.974	0.555	11.63	0.323
3	65	15	37.15	-2.06	0.974	0.642	13.21	0.3192
3	65	29	36.94	-1.36	0.966	0.532	12.37	0.3202
1	90	1	80.89	-16.49	0.988	0.616	5.03	0.2922
1	90	15	89.17	-20.78	0.974	0.604	4.76	0.2603
1	90	29	77.88	-10.33	0.984	0.651	5.03	0.2746
2	90	1	82.67	-32.6	0.976	0.616	4.98	0.296
2	90	15	78.95	-26.2	0.976	0.596	5.79	0.2787
2	90	29	84.03	-26.05	0.974	0.607	6.17	0.2702
3	90	1	84.96	-15.62	0.988	0.586	6.72	0.3019
3	90	15	85.82	-30.69	0.974	0.616	4.64	0.3333
3	90	29	88.03	-32.08	0.97	0.642	6.54	0.2826

[δ]Past=pasteurization 5=65 °C for 30 min; 90=90 °C for 10 min; [β]Rep= replication

Rep[β]	Past[δ]	Day	L*	a*	b*	WHC (%)	G' (Pa)	G" (Pa)	TA (%)	pH
1	65	1	84.87	-2.63	6.53	13.65	26	7.85	0.89	4.48
1	65	15	83.55	-2.58	6.9	18.15	39.3	11.3	0.96	4.28
1	65	29	83.88	-2.64	7.15	17.85	33.1	9.52	0.93	4.32
2	65	1	84.79	-2.39	6.35	13.63	20.8	7.11	0.92	4.38
2	65	15	84.23	-2.48	6.39	17.25	38.2	11.2	0.94	4.32
2	65	29	83.74	-2.65	6.35	18.68	34.1	9.9	0.95	4.35
3	65	1	84.36	-2.57	6.43	12.37	20	6.56	0.9	4.48
3	65	15	84.14	-2.52	6.48	17.53	35.4	11.3	0.93	4.32
3	65	29	83.5	-2.62	6.54	16.36	35.6	11.4	0.95	4.38
1	90	1	85.69	-2.43	6.96	16.47	98.9	28.9	0.89	4.5
1	90	15	85.27	-2.51	7.03	21.73	136	35.4	0.93	4.38
1	90	29	84.99	-2.57	6.72	21.62	126	34.6	0.95	4.36
2	90	1	85.62	-2.5	6.74	16.97	89.2	26.4	0.91	4.46
2	90	15	85.48	-2.51	6.84	21.67	141	39.3	0.96	4.28
2	90	29	85.22	-2.63	7.12	22.77	141	38.1	0.97	4.28
3	90	1	85.81	-2.39	6.63	17.4	102	29.4	0.94	4.32
3	90	15	85.31	-2.51	7.02	21.78	144	38.7	0.96	4.28
3	90	29	85.19	-2.5	6.73	21.57	157	42	0.94	4.35

[α]Past=pasteurization 65=65 °C for 30 min; 90=90 °C for 10 min;; [β]Rep= replication
LT=loss tangent; TA=titratable acidity; WHC=water holding capacity

Table 7-3: Yogurt$^{\Delta\delta}$ chemical properties‡, total solids (TS) and whey protein denaturation (WPD)

Pasteurization$^{\delta}$	TA$^{\alpha}$ (%)	pH	TS (%)	WPD (%)
65	0.89	4.48	9.34	11.67
65	0.92	4.38	9.34	10.14
65	0.90	4.48	9.40	4.48
90	0.89	4.5	9.33	54.02
90	0.91	4.46	9.38	56.45
90	0.91	4.46	9.39	33.33

$^{\delta}$65=65 °C for 30 min; 90=90 °C for 10 min;
$^{\Delta}$n=3
‡Expressed on day 1
$^{\alpha}$TA=titratable acidity

Experiment 2

Table 7-4: Yogurt properties measured as chemical and physical properties of yogurts on day 1, 15 and 29

Rep	Past[δ]	form[Δ]	Day	G' (Pa)	LT	pH	WHC (%)	Syneresis (%)	Firmness (g)	TA (%)	L	a*	b*
1	70	N	1	84	0.2857	4.31	17.24	9.22	62.17	1.10	85.56	-2.41	7.48
1	90	N	1	290	0.2876	4.29	24.2	3.96	137.28	1.05	86.59	-2.31	7.49
1	70	W	1	24.5	0.2567	4.37	15.94	10.39	31.37	1.10	84.71	-2.34	7.60
1	90	W	1	217	0.2618	4.39	24.61	1.8	94.26	1.03	86.64	-2.47	7.58
1	70	N	15	91.6	0.2502	4.27	18.79	8.32	64.61	1.13	85.88	-2.29	7.38
1	90	N	15	322	0.2878	4.22	28.51	4.39	158.39	1.15	86.88	-2.29	7.50
1	70	W	15	38.1	0.2714	4.29	18.03	10.93	34.81	1.10	84.82	-2.56	7.79
1	90	W	15	256	0.2853	4.3	24.17	3.24	141.73	1.10	86.77	-2.64	7.89
1	70	N	29	95.3	0.2874	4.25	19.84	9	63.46	1.15	85.71	-2.39	7.42
1	90	N	29	341	0.2907	4.23	26.83	3.64	160.50	1.15	86.82	-2.25	7.54
1	70	W	29	34.2	0.3240	4.27	18.12	11.3	38.74	1.11	84.67	-2.57	7.84
1	90	W	29	313	0.3020	4.33	25.13	2.56	135.80	1.09	86.67	-2.72	7.91
2	70	N	1	63.5	0.3100	4.43	18.14	7.82	56.60	1.04	85.91	-2.27	7.59
2	90	N	1	217	0.2571	4.3	26.39	2.9	159.17	0.99	86.54	-2.45	8.04
2	70	W	1	22.2	0.2397	4.42	17.8	11.53	29.59	1.00	84.64	-2.31	7.97
2	90	W	1	227	0.2584	4.4	22.76	3.8	146.59	1.01	86.60	-2.27	7.65
2	70	N	15	91.8	0.3068	4.33	19.36	9.57	73.76	1.09	86.03	-2.34	7.59
2	90	N	15	358	0.2808	4.28	27.02	3.59	163.60	1.10	86.43	-2.30	8.00
2	70	W	15	22.2	0.3517	4.42	17.45	11.01	33.74	1.19	84.51	-2.28	7.72
2	90	W	15	227	0.3141	4.4	25.01	4.68	168.54	1.16	85.86	-2.13	7.31
2	70	N	29	93.2	0.2874	4.3	1044.12	9.44	57.60	1.15	86.00	-2.32	7.61
2	90	N	29	351	0.2907	4.25	0.00	3.03	158.01	1.15	86.38	-2.33	8.04

2	70	W	29	36.6	0.3585	4.3	145.51	12.18	33.30	1.10	84.58	-2.22	7.70
2	90	W	29	305	0.2908	4.3	-379.17	2.8	138.28	1.05	85.81	-2.23	7.39
3	70	N	1	73.14	0.2455	4.35	19.91	8.33	56.39	1.02	84.86	2.09	8.38
3	90	N	1	254	0.2792	4.39	22.63	3.71	145.66	1.10	86.14	-2.17	8.10
3	70	W	1	28.7	0.2382	4.42	18.3	7.91	36.09	1.00	84.57	-2.37	8.01
3	90	W	1	202	0.2741	4.41	23.48	2.98	123.79	1.00	86.57	-2.36	7.66
3	70	N	15	90.3	0.2906	4.32	20.27	2.84	61.83	1.10	85.33	-2.19	8.56
3	90	N	15	317	0.2732	4.35	29.3	4.56	131.23	1.05	86.37	-2.18	8.14
3	70	W	15	35.7	0.2789	4.31	17.99	9.85	34.67	1.10	84.14	-2.32	7.70
3	90	W	15	282	0.2821	4.31	25.75	4.44	144.52	1.02	86.11	-2.41	7.70
3	70	N	29	96.7	0.3361	4.23	21.85	9.5	46.24	1.08	85.02	-2.21	8.43
3	90	N	29	358	0.2810	4.24	27.08	2.57	141.07	1.08	86.27	-2.10	8.20
3	70	W	29	44.4	0.3131	4.33	18.02	10.18	35.66	1.08	84.37	-2.39	7.73
3	90	W	29	352	0.2648	4.3	24.22	2.62	156.44	1.10	86.09	-2.48	7.76

[δ]Past=pasteurization; 70=70 °C for 30 min; 90=90 °C for 10 min
N: yogurt containing 12.5% NFDM only and W: yogurt containing 9.5% NFDM and 3% WPC
Form=formula; LT=loss tangent; TA=titratable acidity; WHC=water holding capacity

Table 7-5: Yogurt[‡Δ] mixes as a function of casein, total proteins and whey proteins

formula[‡]	Total proteins (g)	Whey protein before (g)	Casein present (g)	W/C ratio
N	3.65	1.24	2.41	0.51
W	4.27	1.67	2.60	0.64
N	4.09	1.29	2.80	0.46
W	4.19	1.72	2.47	0.7
N	3.44	1.19	2.25	0.53
W	4.25	1.49	2.76	0.54

[Δ] n=3

[‡]N: yogurt containing 12.5% NFDM pasteurized at 70 °C for 30 min or 90 °C for 10 min and W: yogurt containing 9.5% NFDM and 3% WPC pasteurized at 70 °C for 30 min or 90 °C for 10 min

Table 7-6: Yogurt mix as a function of pasteurization affecting whey protein denaturation

Formula	Pasteurization temperature (°C)	whey protein content (g)		% denaturation
		before pasteurization (g)	after pasteurization (g)	
N	70	1.24	0.94	24.29
	90		0.66	47.15
W	70	1.67	1.54	7.77
	90		0.76	54.45
N	70	1.29	1.00	22.60
	90		0.59	54.28
W	70	1.72	1.61	6.45
	90		0.75	56.56
N	70	1.19	0.83	29.66
	90		0.48	59.30
W	70	1.49	1.32	11.33
	90		0.53	64.37

Appendix C - SAS® program

Experiment 1

Overall dataset was put in and SAS® was run.

```
Data yogurt;
Input rep temp day firm adhes spring cohes syn L a b WHC G1 G2 LT acid pH ;
Cards
;
*Proc print data=yogurt;
*title2 ‘print of data’;
*ods rtf file='I:\Split-Plot Complete with PDIFFS.rtf';

%macro run1(y, ytitle);
proc mixed covtest data=yogurt cl;
 title &ytitle;
 Class Rep Temp Day;

model &y=Temp|Day/ddfm=satterth;
 random Rep Rep*Temp;
 lsmeans Temp|Day;
lsmeans day/pdiff;
ods output lsmeans=lsm;
data lsm; set lsm;
  dayvalue=Day;
  if day='15' then dayvalue=2;
  if day='29' then dayvalue=3;

 proc plot;
 where effect='Temp*Day';
 plot estimate*Temp=dayvalue;

 %mend run1;

%run1 (G1, 'G prime');
%run1 (LT, 'LT');
%run1 (pH, 'pH');
%run1 (WHC, '%WHC');
%run1 (syn, '%syneresis');
%run1 (firm, 'firmness');
%run1 (adhes, 'adhesiveness');
%run1 (cohes, 'cohesiveness');
%run1 (spring, 'springiness');
%run1 (acid, 'TA');
%run1 (L, 'L');
%run1 (a, 'a*');
%run1 (b, 'b*');
*ods rtf file='I:\Split Plot Complete with PDIFFS.rtf' close;
  quit;
```

Experiment 2

Following program was used to get SAS® output. The data file was imported from XL in order to run this program.

```
proc sort data=yogurt; by Day;
*ods rtf file='I:\Split-Split-Plot Complete with PDIFFS.rtf';
proc print;

%macro run1(y, ytitle);
proc mixed covtest data=yogurt cl;
  title &ytitle;
  class Rep Formula Temp Day;
  model &y=Formula|Temp|Day/ddfm=satterth;
  random Rep Rep*Formula Rep*Formula*Temp;
  lsmeans Formula|Temp|Day/cl pdiff;

  ods output lsmeans=lsm;
  data lsm; set lsm;
  if Formula='W' then plotvalue=7;
  if Formula='N' then plotvalue=8;
  dayvalue=Day;
  if day='15' then dayvalue=2;
  if day='29' then dayvalue=3;
  proc sort data=lsm; by Temp;
  proc plot;
  where effect ='Formula*Temp*Day'; by Temp;
  plot estimate*Day=plotvalue;
  proc plot;
  where effect='Formula*Temp';
  plot estimate*Formula=Temp;
  proc plot;
  where effect='Formula*Day';
  plot estimate*Formula=dayvalue;
  proc plot;
  where effect='Temp*Day';
  plot estimate*Temp=dayvalue;

  %mend run1;

%run1 (G1, 'G prime');
%run1 (G2, 'G double prime');
%run1 (pH, 'pH');
%run1 (WHC, '%WHC');
%run1 (syn, '%syneresis');
%run1 (firm, 'firmness');
%run1 (TA, 'TA');
%run1 (L, 'L');
%run1 (a, 'a*');
%run1 (b, 'b*');
*ods rtf file='I:\Split-Split-Plot Complete with PDIFFS.rtf' close;
  quit;
```

Appendix D - P values

Experiment 1

Day 1

Class	Values
Replication	1 2 3
Day	1
Pasteurization[δ]	65 90

[δ] Pasteurization; 65=65 °C for 30 min; 90=90 °C for 10 min

Table 7-7: P values for main effects and interactions

Effect	G'	pH	WHC	Syneresis	Firmness	LT	TA
Formula	<.0001	0.7701	0.0017	0.0005	<.0001	0.0023	0.5879

Effect	L*	a*	b*	Adhesiveness	Cohesiveness	Springiness
Formula	0.0035	0.3179	0.0366	0.0407	0.006	0.0412

WHC=water holding capacity; TA=titratable acidity; LT=loss tangent

Storage study

Description

Class	Values
Replication	1 2 3
Day	1, 15, 29
Pasteurization[δ]	65 90

[δ] Pasteurization; 65=65 °C for 30 min; 90=90 °C for 10 min

Table 7-8: P values for main effects and interactions

Effect	G'	pH	WHC	Syneresis	Firmness	LT	TA
Past	<.0001	0.8758	<.0001	<.0001	<0.0001	0.0003	0.3558
Day	<.0001	0.0002	<.0001	0.2612	0.8337	0.0355	0.0002
Past*day	0.0012	0.5298	0.1251	0.0396	0.6455	0.5134	0.7984

Effect	L*	a*	b*	Adhesiveness	Cohesiveness	Springiness
Past	<.0001	0.0334	0.1645	0.0097	0.0020	0.0182
Day	0.0002	0.2467	0.3141	0.0686	0.6615	0.6705
Past*day	0.1725	0.7087	0.5689	0.8172	0.0439	0.1375

WHC=water holding capacity; TA=titratable acidity; LT=loss tangent

Experiment 2

Day 1

Description

Class	Values
Replication	1 2 3
Formula	N W
Day	1
Pasteurization[δ]	70 90

[δ]70=70 °C for 30 min; 90=90 °C for 10 min
N: yogurt containing 12.5% NFDM only and W: yogurt containing 9.5% NFDM and 3% WPC

Table 7-9: P values for main effects and interactions

Attribute	Firmness	G'	pH	WHC	Syneresis
Effect	**Pr > F**	**Pr > F**	**Pr > F**	**Pr > F**	**Pr > F**
Formula	0.0162	0.0335	0.0418	0.2796	0.6117
Pasteurization	<.0001	<.0001	0.3976	<.0001	0.0004
Formula*pasteurization	0.9896	0.6014	0.4767	0.8604	0.1274

Attribute	TA	LT	L*	a*	b*
Effect	**Pr > F**	**Pr > F**	**Pr > F**	**Pr > F**	**Pr > F**
Formula	0.2828	0.1686	0.2591	0.3780	0.5792
Pasteurization	0.5769	0.4431	0.0001	0.3551	0.5127
Formula*pasteurization	0.7780	0.3508	0.0067	0.3709	0.2881

LT=loss tangent; TA=titratable acidity; WHC=Water holding capacity

Storage study

Description

Class	Values
Replication	1 2 3
Formula	N W
Day	1, 15, 29
Pasteurization[δ]	70 90

[δ]70=70 °C for 30 min; 90=90 °C for 10 min
N: yogurt containing 12.5% NFDM only and W: yogurt containing 9.5% NFDM and 3% WPC

Table 7-10: P values for main effects and interactions

Attribute	Firmness	G'	pH	WHC	Syneresis
Effect	**Pr > F**	**Pr > F**	**Pr > F**	**Pr > F**	**Pr > F**
Formula	0.0290	<.0001	0.0121	0.0013	0.0816
Pasteurization	<.0001	<.0001	0.4142	<.0001	<.0001
Formula*pasteurization	0.2040	0.5353	0.3772	0.4991	0.0275
Day	0.0360	<.0001	<.0001	0.001 0	0.7330
Formula*Day	0.3164	0.1360	0.8763	0.1258	0.3355
Pasteurization*Day	0.2057	<.0001	0.8683	0.0515	0.0330
Formula*Pasteurization*Day	0.1329	0.2586	0.9701	0.4702	0.6655

Attribute	TA	LT	L*	a*	b*
Effect	**Pr > F**	**Pr > F**	**Pr > F**	**Pr > F**	**Pr > F**
Formula	0.1661	0.0006	0.0627	0.3627	0.6164
Pasteurization	0.2852	0.2120	0.0005	0.3182	0.7973
Formula*pasteurization	0.4563	0.0797	0.0271	0.3996	0.4519
Day	0.0002	0.1992	0.4745	0.3634	0.8770
Formula*Day	0.3409	0.5771	0.0066	0.4844	0.7707
Pasteurization*Day	0.9170	0.1969	0.2856	0.3644	0.6246
Formula*Pasteurization*Day	0.9635	0.5311	0.6142	0.3451	0.7612

LT=loss tangent; TA=titratable acidity; WHC=Water holding capacity

Appendix E - Non significant dataset

Experiment 1

Day 1

Table 7-11: Yogurt as a function of pasteurization conditions[β]

Pasteurization	a*	TA (%)	pH	LT
65	-2.53	0.9	4.45	0.3222
90	-2.44	0.91	4.43	0.2947

[β] n=3; [δ]65=65 °C for 30 min; 90=90 °C for 10 min; TA= titratable acidity

Storage study

Table 7-12: Yogurt as a function of pasteurization conditions[β]

Pasteurization	TA (%)	a*
65	0.93	-2.56
90	0.94	-2.51

[β] n=9; [δ]65=65 °C for 30 min; 90=90 °C for 10 min; TA= titratable acidity

Table 7-13: Yogurt as a function of storage days[β]

Day	Adhesiveness (g.s)	b*	Cohesiveness	Springiness	Syneresis (%)
1	-13.34	6.61	0.58	0.97	8.90
15	-14.10	6.78	0.59	0.97	9.04
29	-11.97	6.77	0.58	0.97	9.10

[β] n= 12

Table 7-14: Pasteurization*days interactions observed for yogurts stored for 29 days[β]

Pasteurization[δ]	Day	pH	WHC (%)	Firmness (g)	TA (%)	L*
65	**1**	4.45	14.89	40.32	0.90	84.67
65	**15**	4.31	17.64	39.56	0.94	83.97
65	**29**	4.35	17.54	38.80	0.94	83.71
90	**1**	4.47	16.8	82.08	0.90	85.64
90	**15**	4.31	21.73	84.65	0.95	85.35
90	**29**	4.33	21.99	83.31	0.95	85.13

[β] n =6; [δ]65=65 °C for 30 min; 90=90 °C for 10 min; WHC=water holding capacity; TA=titratable acidity

Pasteurization[δ]	Day	a*	b*	LT	Adhesiveness (g.s)	Springiness
65	**1**	-2.53	6.44	0.3222	-5.1	0.96
65	**15**	-2.53	6.59	0.3152	-2.31	0.97
65	**29**	-2.64	6.68	0.2975	-1.12	0.97
90	**1**	-2.47	6.81	0.2947	-27.25	0.98
90	**15**	-2.51	6.96	0.2693	-25.89	0.97
90	**29**	-2.57	6.86	0.2708	-22.82	0.98

[β] n =6; [δ]65=65 °C for 30 min; 90=90 °C for 10 min; LT=loss tangent; WHC=water holding capacity; TA=titratable acidity

Experiment 2

Day 1

Table 7-15: Yogurt quality as a function of formulation[β]

Formula	Syneresis (%)	TA (%)	L*	a*	b*	LT	WHC (%)
N	5.99	1.05	85.93	-2.59	7.85	0.2716	21.42
W	6.40	1.02	85.62	-2.35	7.75	0.2935	20.48

[β]n=18, N: yogurt containing 12.5% NFDM only and W: yogurt containing 9.5% NFDM and 3% WPC
LT=loss tangent; TA=titratable acidity; WHC=water holding capacity

Table 7-16: Yogurt quality as a function of pasteurization conditions[β]

Pasteurization[δ]	pH	TA (%)	a*	b*	LT
70	4.38	1.04	-2.60	7.84	0.2792
90	4.36	1.03	-2.34	7.75	0.2859

[β]n=18; [δ]70=70 °C for 30 min; 90=90 °C for 10 min; LT=loss tangent; TA=Titratable acidity

Table 7-17: Formulas*pasteurization interactions means for yogurts[β]

Type	Past[δ]	G' (Pa)	pH	LT	WHC (%)	Syneresis
N	**70**	73.55	4.36	0.26	18.43	8.46
N	**90**	253.67	4.33	0.28	24.41	3.52
W	**70**	25.13	4.40	0.29	17.35	9.94
W	**90**	215.33	4.40	0.29	23.62	2.86

[β]n=9;
[δ]Past=pasteurization; 70=70 °C for 30 min; 90=90 °C for 10 min
N: yogurt containing 12.5% NFDM only and W: yogurt containing 9.5% NFDM and 3% WPC
LT=loss tangent; WHC=water holding capacity

Type	Past[δ]	Firmness (g)	TA (%)	L*	a*	b*
N	**70**	58.38	1.05	85.44	-2.86	7.82
N	**90**	147.37	1.05	86.42	-2.31	7.88
W	**70**	32.35	1.03	84.64	-2.34	7.86
W	**90**	121.55	1.01	86.60	-2.37	7.63

[β]n=9;
[δ]Past=pasteurization; 70=70 °C for 30 min; 90=90 °C for 10 min
N: yogurt containing 12.5% NFDM only and W: yogurt containing 9.5% NFDM and 3% WPC
TA=titratable acidity

Storage study

Table 7-18: Yogurt quality as a function of formulation[β]

Formula	Syneresis (%)	TA (%)	L*	a*	b*
N	5.91	1.09	86.04	-2.04	7.86
W	6.90	1.07	85.45	-2.39	7.72

[β]n=18, N: yogurt containing 12.5% NFDM only and W: yogurt containing 9.5% NFDM and 3% WPC
TA=titratable acidity

Table 7-19: Yogurt quality as a function of pasteurization conditions[β]

Pasteurization[δ]	pH	TA (%)	a*	b*	LT
70	4.33	1.09	-2.09	7.81	0.2907
90	4.32	1.08	-2.34	7.77	0.2812

[β]n=18; [δ]70=70 °C for 30 min; 90=90 °C for 10 min; LT=loss tangent; TA=Titratable acidity

Table 7-20: Yogurt quality as a function of storage dayβ

Day	Syneresis (%)	L*	a*	b*	LT
1	6.2	85.78	-1.97	7.8	0.2955
15	6.45	85.76	-2.33	7.77	0.2797
29	6.57	85.7	-2.35	7.8	0.2825

[β]n=12; LT=loss tangent

Table 7-21: Formulas*pasteurization interactions means for yogurts stored for 29 days[β]

Type	Past[δ]	G' (Pa)	pH	LT	WHC (%)	Firmness (g)	TA (%)	a*	b*
N	**70**	86.62	4.31	0.2693	19.53	60.3	1.1	-1.81	7.83
N	**90**	312	4.28	0.2733	26.51	150.55	1.09	-2.26	7.89
W	**70**	31.84	4.35	0.3121	17.83	34.22	1.09	-2.37	7.78
W	**90**	264.56	4.35	0.2891	24.3	138.88	1.06	-2.41	7.65

[β]n=9;
[δ]Past=pasteurization; 70=70 °C for 30 min; 90=90 °C for 10 min
N: yogurt containing 12.5% NFDM only and W: yogurt containing 9.5% NFDM and 3% WPC
LT=loss tangent; TA=titratable acidity; WHC=water holding capacity

Table 7-22: Pasteurization*day interactions for yogurts stored for 29 days[β]

Past[δ]	Day	pH	WHC (%)	Firmness (g)	TA (%)	LT	L*	a*	b*
70	1	4.38	17.89	45.37	1.04	0.2792	85.04	-1.60	7.84
70	15	4.32	18.65	50.57	1.12	0.2859	85.12	-2.33	7.79
70	29	4.28	19.51	45.83	1.11	0.3090	85.06	-2.35	7.79
90	1	4.36	24.01	134.46	1.03	0.2820	86.51	-2.34	7.75
90	15	4.31	26.63	151.34	1.10	0.2837	86.40	-2.33	7.76
90	29	4.28	25.58	148.35	1.10	0.2757	86.34	-2.35	7.81

[β]n=6; [δ]Past=pasteurization; 70=70 °C for 30 min; 90=90 °C for 10 min;
LT=loss tangent; TA=titratable acidity; WHC=water holding capacity

Table 7-23: Formula*day interactions for yogurts stored for 29 days[β]

Formula	Day	G' (Pa)	LT	pH	WHC (%)	Syneresis (%)
N	**1**	163.61	0.2716	4.35	21.42	5.99
N	**15**	211.78	0.2755	4.30	23.88	5.55
N	**29**	222.53	0.2668	4.25	23.77	6.20
W	**1**	120.23	0.2935	4.40	20.48	6.40
W	**15**	143.50	0.3155	4.34	21.40	7.36
W	**29**	180.87	0.2927	4.31	21.32	6.94

Formula	Day	Firmness (g)	TA (%)	L*	a*	b*
N	**1**	102.88	1.05	85.93	-1.59	7.85
N	**15**	108.90	1.10	86.15	-2.27	7.86
N	**29**	104.48	1.13	86.03	-2.27	7.87
W	**1**	76.95	1.02	85.62	-2.35	7.75
W	**15**	93.00	1.11	85.37	-2.39	7.69
W	**29**	89.70	1.09	85.37	-2.44	7.72

[β]n=6

N: yogurt containing 12.5% NFDM only and W: yogurt containing 9.5% NFDM and 3% WPC

LT=loss tangent; TA=titratable acidity; WHC=water holding capacity