ENHANCING CYSTEINE CONTENT IN YOGURT WITH ADDITION OF WHEY PROTEIN ISOLATE AND ITS SENSORY EVALUATION

by

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Abstract

Milk proteins are excellent sources of sulfur-containing amino acids methionine and cysteine, in particular whey proteins. Cysteine is synthesized from methionine by γ-cystathionase. However, cysteine has to be included in the diets of certain subpopulations due to diminished γ-cystathionase activity. Cysteine, a heat- liable amino acid, may lose bioavailability during thermal processing. The objective of this research was to enhance cysteine content in yogurt while maintaining its quality. First, yogurt mixes were formulated to a total solids content of 12.5% with nonfat dry milk (**NDM**) (**N**) or a combination of NDM (10%) and whey protein isolate (**WPI**) (2.5%) (**W**), and processed at 70°C (20 min) (**70**) or 90°C (7 min) (**90**). Yogurt was prepared and maintained at 4^{o}C for 60 days. Three replications were performed and data were analyzed using SAS®. The W mixes had 65%, 32% and 190% more cysteine, true protein and whey protein contents respectively, compared to N mixes prior to processing. However in day 1 yogurt, the highest cysteine content (398.3 mg/L) was found in the W70 yogurt and its gel quality was comparable to the N90 yogurt except for firmness. During a 60 day storage period the W70 and N90 were similar in gel quality except for firmness. Secondly, a hedonic test was done on the W70 (**HC**) and N90 (**LC**) yogurts which had been reformulated to contain sugar and vanillin. One replication was performed and data were analyzed using SAS®. The LC and HC yogurts did not vary in liking of flavor (6.1), aftertaste (6.1) and overall acceptability (6.3) corresponding to the words of "like slightly" when compared. However, the appearance of the LC yogurt was liked more than the HC yogurt (6.7 *vs.* 6.1) whereas the thickness of HC yogurt was liked more than the LC yogurt (6.4 *vs.* 5.8). These results suggest that addition of WPI along with lower process treatment resulted in yogurt with enhanced cysteine; however, further studies may be needed to

optimize the WPI addition to improve the visual characteristics of the yogurt for consumer acceptance.

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Chapter 1 - Introduction

There is general agreement among nutritionists that diet is related to health, wellbeing and disease. Use of food and their constituents to prevent certain chronic diseases has been studied for years and certain dietary changes have been advocated as well. For example, to reduce cancer risk, avoid processed meat and consume foods with high anti-oxidant property (USDA, 2013a); whereas for a healthy heart, diets with lower saturated fatty acids and more polyunsaturated fatty acids should be consumed (USDA, 2013b). And to maintain a healthy gut, consumption of prebiotic and probiotic yogurt is advised (Oskar et al., 2004).

Since 2006, yogurt sales have increased by almost 40% (USDA, 2013). The increase in popularity may be attributed to its nutritional and health benefits – such as improved digestibility and lactose utilization as well as some antagonistic actions towards enteric pathogens (Chandan, 2007). In the U.S. most yogurt mixes are processed at conditions of 90 to 95 °C for 5 to 10 min to denature whey proteins, which in turn contributes to a gel with lower syneresis, greater firmness and water holding capacity (Chandan, 2008; Lucey et al. 1998). All these quality factors are functions of the number and strength of the protein interactions many initiated by thermal denaturation. Since the early 1970's researchers have studied the impact of added whey proteins to yogurt mixes (in the forms of isolates or concentrates) and as an entity reported that the resultant yogurts had less syneresis (Isleten and Yuceer, 2006; Pintro et al., 2011) and greater water holding capacity (Sodini et al., 2005; Modler et al., 1983). However, Gonzalez et al. (2002) reported that 2% whey protein concentrate addition to a yogurt mix resulted in a yogurt with increased yellowness (b*). Whereas Isleten and Yuceer (2006) reported that descriptive analysis results of yogurt containing whey protein isolate (**WPI**) indicated that these yogurts had more chalkiness, thickness, lumpiness, whey flavor but less creaminess.

In cells, cysteine is synthesized from methionine by action of γ-cystathionase (EC. 4.4.1.1) (Sastre et al., 2005). Research has shown that elderly rats (24-26 months) had less or no γ -cystathinase in their eye lenses compared to young rats (5-6 months) and as a result cysteine and glutathione contents were 50% less in the elderly rats compared to young rats which resulted in cataractogenesis (Sastre et al., 2005). For certain sub-populations such as the elderly, cysteine is considered to be a conditional essential amino acid; hence the need to rely on dietary supplements of cysteine to meet their body requirements. When considering food sources of cysteine nonfat dry milk (**NDM**) and WPI have 290 and 750 mg/100g, respectively (Glass and Hedrick, 1976). Hence, supplementation of yogurt mixes with WPI is a strategy to enhance cysteine content. Cysteine is heat-liable with losses of 20, 35, and 60% in whey protein extracts treated at 72°C for 30 sec, 142°C for 5 sec and 115°C for 30 min, respectively compared to raw milk (Carbonaro et al., 1997).

As the quality of a yogurt gel is directly related to whey protein denaturation (**WPD**) and whey protein addition in a yogurt mix can enhance gel quality; the, addition of WPI along with lower process temperature will induces less WPD may be an approach to produce a yogurt with enhanced cysteine content.

Chapter 2 - Literature Review

2.1 Milk Proteins

Milk proteins are of two distinct types- caseins and whey proteins. In bovine milk, caseins predominate with 80% of the total proteins while the whey proteins constitute the other 20% (Kailasapathy, 2008). Table 2.1 shows the total solids, total protein, casein, whey protein and non-protein nitrogen contents in milk and selected milk products. As can be seen from Table 2.1 yogurt has one of the greatest quantities of protein and whey protein per 100g. From 2005 to 2011, overall fluid milk sales in the U.S. declined by 4%; however, consumption of other dairy foods such as yogurt (40%) and cream (21%) increased (USDA, 2012).

Table 2.1 Total solids, total protein and whey protein contents in milk and milk products

Product	Total solids[a] g/100g	Total protein g/100g	Casein g/100g	Whey protein[b] g/100g	NPN[c] mg/100g
Whole milk	13.60	3.30	2.34	0.66	23-31
Skim milk	9.10	3.50	2.80	0.70	24.00
Yogurt (Whole milk)	11.00	5.70	4.56	1.14	39.00
Low fat yogurt	12-14	4.80	3.84	0.96	33
Sour cream	27	2.90	2.32	0.58	20
Ice cream	30-36	3.60	2.88	0.72	24

[a]: Based on total solids=total weight – moisture
[b]: Whey protein calculated based on 20% of total protein (Chandan, 2008)
[c]: NPN: Non protein nitrogen based on 5% of total protein nitrogen (Fox & McSweeney, 1998)

2.2 Whey Proteins

Whey, a byproduct of cheese manufacture, is a liquid product typically obtained from milk after coagulation and subsequent removal of fat and casein (Kilara, 2008). Probably one of the most valuable components in whey is the proteins as they contribute to both nutritional and physicochemical properties in foods (Kilara, 2008). As an entity, whey proteins are globular, water-soluble and categorized into fractions: β-lactoglobulin (**β-lg**), α-lactalbumin (**α-la**), bovine serum albumin (**BSA**), immunoglobulins, lactoferrin and lactoperoxidase (Bonnaillie and Tomasula, 2008). Table 2.2 depicts the protein composition in sweet whey.

Table 2.2 Whey protein composition in sweet whey

	Content (%)
Protein	
β-Lactoglobulin	48-58
α-Lactalbumin	13-19
Glycomacropeptide	12-20
Immunoglobulins	8-12
Bovine serum albumin	6
Lactoferrin	2
Lactoperoxidase	0.5

Source: (Bonnaillie and Tomasula, 2008)

As can be seen in Table 2.2, β-lg is the major component and contains 162 amino acids of which 17% are essential amino acids and 33.5% are branched chain amino acids (**BCAA**) (Etzel, 2004). For every gram β-lg of 3.3% of weight is contributed by cysteine (Chandan and Shah, 2007). The main biological function of β-lg is its nutritional value due to its amino acid composition. From a functional point of view, β-lg provides binding and gelling properties (Etzel, 2004).

α-Lactalbumin is the second predominant whey protein and contains 123 amino acids of which 17% are essential amino acids and 38.3% are BCAA (Etzel, 2004). For every gram of α-la

6.8% of weight is contributed by cysteine (Chandan and Shah, 2007). Biologically, α-la modulates the substrate specificity of galactosyltransferase which catalyzes the transfer of galactose to glucose during lactose synthesis. Functionally, α-la provides good emulsifying and foaming properties (Fox, 1989); nutritionally α- la is used in infant formulas to mimic human milk as human milk has greater α- la than casein and β-lg (de Wit, 1998).

The remaining 20 to 40% of whey proteins represents the minor constituents. BSA comprises about 6% of whey protein with about 582 amino acids of which 17% are essential amino acids and 30% are BCAA (Fox and McSweeney, 1998). For every gram of whey proteins 6.9% of weight is contributed by cysteine in BSA (Chandan and Shah, 2007). The immunoglobulins fraction constitutes 8 to 12% of the total whey proteins and range from 111 to 222 amino acids with about 17% essential amino acids and 30% BCAA. Lactoferrin is ~ 2% of the total whey protein and consists of 168 to 242 amino acids of which 17% are essential amino acids and 30% BCAA. Lactoferrin is of great interest in the food industry, as lactoferrin exhibits bactericidal properties, contains iron and has other biological functions such as immune modulation, bacteriostasis, antiviral effects, and iron absorption facilitation (Kilara, 2008; Fox, 1989). Lactoperoxidase comprises about 0.5% whey protein with 183 to 288 amino acids and has a bactericidal effect also, as it is involved in the host's natural defense system against invading microorganisms (Fox, 1989; de Wit and Hooydonk, 1996). Today, whey proteins are in demand in the food industry, as they have been associated with many health benefits, some of which are listed in Table 2.3 (Sharma and Shah, 2010).

Table 2.3 Some health benefits of whey proteins

Category
Bone and Body
Bone health and osteoporosis
Muscle strength and sports nutrition
Obesity and weight control
Immunomodulatory Properties
Cancer
Allergy
Autoimmune diseases
Antioxidant property
Growth of probiotic bacteria and gut health
Anti-bacterial activity
Organs
Cardiovascular health
Liver and lung health benefits

Source: (Sharma and Shah, 2010)

2.3 Whey Protein Isolate

Liquid whey can be further processed to increase the protein and solids contents; often this is done by fractionation techniques to remove water, lactose, lipids and minerals (Kilara, 2008). Whey powders (< 30%), whey protein concentrates (**WPC**) (protein content 30 to 90%) and whey protein isolates (**WPI**) (protein content > 90%) are the most typical products obtained after drying (Lopes et al., 2006). As described in 21 CFR § 184.1979c, WPC is produced from whey using physical separation techniques that remove non-protein constituents from whey such as lactose and water so that the finished dry product contains not less than 25% protein and is affirmed with a GRAS status. The WPI processing steps include those for WPC and a few additional steps to concentrate the proteins and can be used in food products if no new toxicants are introduced as a result of the further processing (Wilcox and Swaisgood, 2002). These dried whey products are used extensively in dairy and baked foods as ingredients (Wilcox and Swaisgood, 2002). Although, WPI is commonly used in protein supplements, it also exhibits

excellent emulsifying, gelling, thickening, foaming, and water-binding properties (Wilcox and Swaisgood, 2002). The composition of an average WPI is shown in Table 2.4.

Table 2.4 Composition of whey protein isolate

	Composition (%)
Moisture	4.5
Ash	2-3
Lactose	0.5-1.0
Fat	0.5-1.0
Protein	90-92
β-Lactoglobulin	70.2
α-Lactalbumin	14.3
Immunoglobulins	6.9
Bovine serum albumin	8.6

Source: Kilara, 2004

2.4 Amino Acids

Amino acids are the building blocks of protein. Essential amino acids are histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. On the other hand, conditional essential amino acids are these which are needed in the diet during ill health (eg; AIDS, cancer), aging, etc. (Ensminger, 1993). Arginine, cysteine, tyrosine, glycine and proline are some of the more common conditional essential amino acids (Ensminger, 1993). Table 2.5 compares amino acid composition of β-lg, α-la and a typical WPI.

Table 2.5: Amino acid compositions of β-lactoglobulin, α-lactalbumin and whey protein isolate

Amino acids	β-Lactoglobulin	α-Lactalbumin	Whey protein isolate[1]
		%wt:wt	
Essential amino acids			
Histidine	1.5	2.9	1.7
Lysine	10.5	10.9	9.5
Methionine	2.9	0.9	3.1
Phenylalanine	3.2	4.2	3.0
Theronine	4.4	5.0	4.6
Tryptophan	2.0	5.3	1.3
Branched chain amino acids			
Isoleucine	6.2	6.4	4.7
Leucine	13.6	10.4	11.8
Valine	5.4	4.2	4.7
Conditional essential amino acids			
Arginine	2.6	1.1	2.4
Cysteine	2.8	5.8	2.6
Glycine	0.9	2.4	1.7
Proline	4.2	1.4	4.2
Tyrosine	3.6	4.6	3.4
Other amino acids			
Alanine	5.4	1.5	4.9
Aspargine	3.1	9.7	3.8
Aspartic acid	6.9	7.3	10.7
Glutamine	6.3	4.5	3.4
Glutamic acid	11.3	7.3	15.4
Serine	3.3	4.3	3.9

[1]WPI: Whey protein isolate with 91% protein content (Ion exchange method)

Source: (Etzel, 2004)

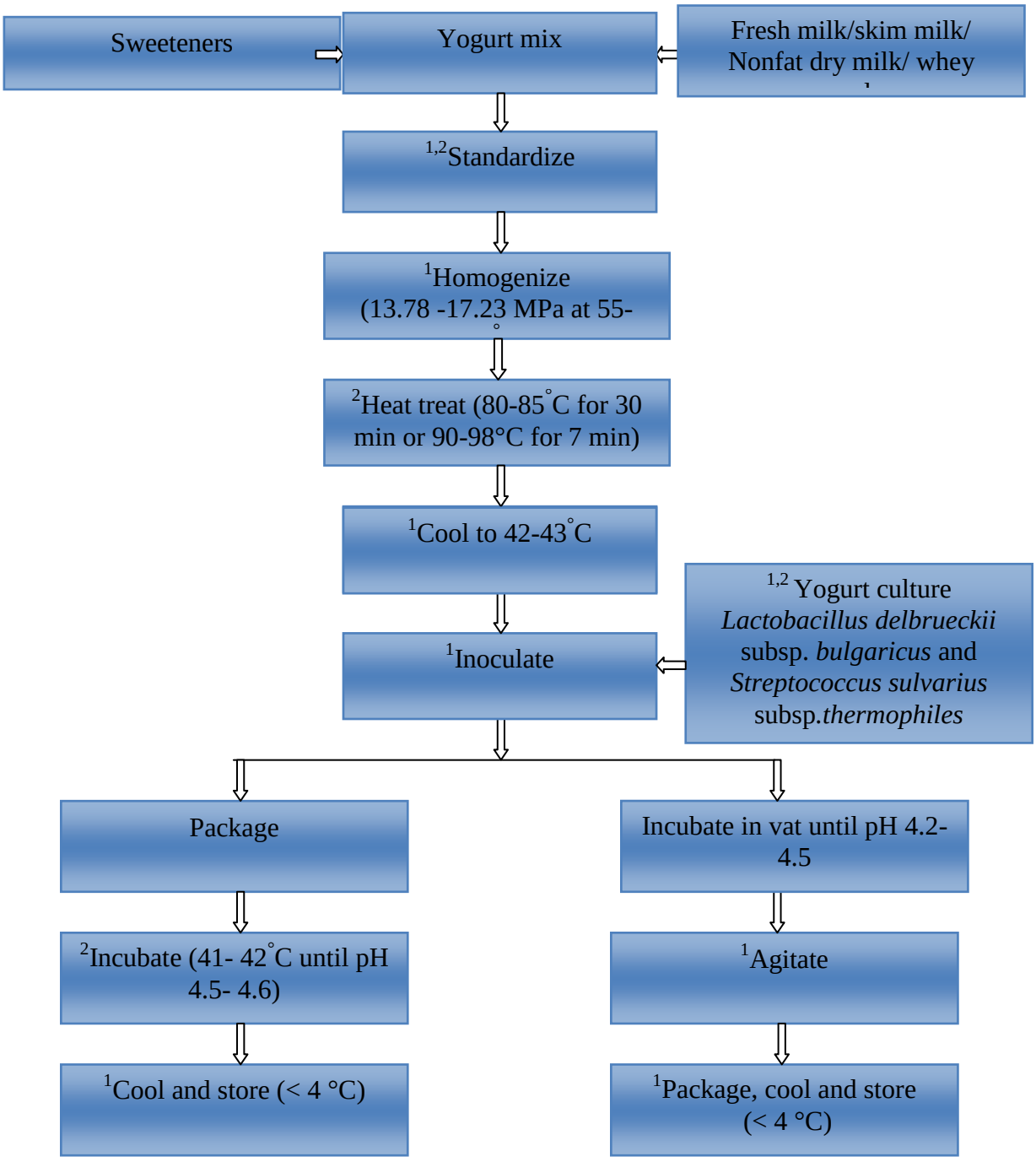
2.5 Yogurt

Yogurt popularity and sales have increased by 40% since 2006 (USDA, 2013). The increase in popularity may be attributed to its nutritional and health benefits – such as improved digestibility and lactose utilization as well as some antagonistic actions towards enteric pathogens (Chandan and Shah, 2007). Whatever the reason for spurring the growth of yogurt, it is considered to be a nutritious food product (Chandan and Shah, 2007). In the U.S., most of the yogurt consumed is made commercially and two styles of yogurt predominate in the U.S. market: set style (the yogurt is directly fermented in the package) and stirred-style, (the yogurt is fermented in a vat, curd broken, and then filled into consumer's package) (Chandan and O'Rell 2006).

2.5.1 Yogurt Processing

The contrast of the two yogurt processing methods (set *vs*. stirred style) is shown in Figure 2.1. Although yogurt can be made entirely out of whole milk, it is often formulated to contain other ingredients, predominately to meet consumer's needs and preferences. In the U.S., yogurt mix consists of milk (whole, low fat, or skim), culture, specifically *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus* and many formulations contain additional milk solids, sweetener, and stabilizer (21 CFR § 131.200a). According to FDA, yogurt is the food produced by culturing one or more of the optional dairy ingredients with a characterizing bacterial culture that contains the lactic acid-producing bacteria, *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (21 CFR § 131.200a).

Figure 2.1 An outline of yogurt processing contrasting set *vs.* stirred style



Sources: ([1]Chandan and O'Rell, 2006; [2]Tamime, 2006)

2.5.2 Yogurt Manufacturing

2.5.2.1 Yogurt Mix

Yogurt mix mainly contains milk and milk products, which are often formulated to desired milk solids contents (8.25 to 12%) (21 CFR § 131. 200a,b,c,d). The milk solids are often increased in the yogurt mix by supplementation with nonfat dry milk (**NDM**), WPC and/or condensed milk products, which increases the protein content of the mix (Chandan and O'Rell, 2006). For flavored or fruit yogurt, other ingredients may be added; generally these include: sweeteners (0 to 6%) and stabilizers (0.3 to 1.6%) (Tamime, 2006).

As far as sweetener sources in yogurt, these include sucrose, invert sugar, fructose, glucose, sorbitol, sacchrain, and cyclamate. Sucrose is the most abundant carbohydrate in the plant kingdom and is a widely used sweetener in the food industry (Tamime and Robinson, 1999). Sucrose is added during yogurt making to improve flavor; as sweetness is one of the major sensory determinants that positively affect the acceptability of yogurt (Jaworska et al., 2005). As sugar may contain vegetative contaminants, it needs to be added before heat treatment. Tamime and Robinson (1999) reported that if sugar is added after gel formation, uneven sugar distribution and reduced product acceptability results.

Stabilizers (whey proteins, gelatin, starch, pectin, xanthan gum) are usually plant and animal hydrocolloids (Tamime and Robinson, 1999). The two important reasons for adding stabilizers are to improve the consistency and viscosity while minimizing whey separation. An ideal stabilizer is one which does not impart any flavor, is effective at low pH and can easily be dispersed in normal conditions (Chandan, 2006).

Flavors are used in most yogurts sold in U.S. (Clark et al., 2009). Typically, flavors are added either at inoculation for set-style yogurts or during the agitation step for stirred-style yogurt (see Figure 2.1). Yogurt formulators typically use vanilla extracts from 1× (1-fold) to 3× (3-fold). The higher the fold or concentration of the vanilla extract, the lower the usage rate in the yogurt. For a 2× vanilla extract a typical usage rate is 0.45 to 0.60% (Chandan and O'Rell, 2006).

2.5.2.2 Standardization

Once the formula is set, the yogurt mixes are standardized to adjust the milk solids-not-fat (SNF) content ranging from 8.2 to 8.6% to 12 to 16%, Normally, standardization results in reduced fat and increased lactose, protein, vitamin and mineral contents (Chandan and O'Rell, 2006; Tamime, 2006).

2.5.2.3 Homogenization

Yogurt mix is homogenized to reduce the size of the milk fat globules. For yogurt mixes, homogenization at 13.78 to 17.23 MPa at 55 to 80°C reduces the milk fat globule diameters from 10,000 nm to < 2,000 nm (Chandan and O'Rell, 2006).

2.5.2.4 Pasteurization

Yogurt mixes are pasteurized at 65°C for 30 min, to kill vegetative spores of micro organisms; but some spores and heat-stable enzymes may be present (if raw milk is used) and the whey protein denaturation (**WPD**) is < 10% (Tamime and Robinson, 1999). To inactivate greater numbers of enzymes and microorganisms and induce greater WPD, yogurt mixes are typically processed at temperatures ranging from 85 to 95°C and times ranging from 5 to 15 min (Anema and Li, 2003; Chandan, 2006). Many researchers have reported that greater WPD (70 to 95%) in yogurt mixes is directly correlated with gel quality where the gels were firm, with lower

syneresis and greater water holding capacity (**WHC**) (Amatayakul et al., 2006; Dave and Shah, 1998a; Modler et al., 1983). Yogurt mixes are immediately cooled to 43°C, the optimum temperature for culture growth (Chandan and O'Rell, 2006).

2.5.2.5 Inoculation and Incubation

After cooling to 43°C, the yogurt culture *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus* is added to the pasteurized mix. *Str. salivarius* subsp. *thermophilus* is a gram-positive, anaerobic, non-motile, and catalase negative organism with spherical cells of 0.7 to 0.9 µ. *Str. salivarius* subsp. *thermophilus* exhibits optimum growth at 37°C and synergetic growth when *L. delbrueckii* subsp. *bulgaricus* is present (Chandan and O'Rell, 2006; Tamime and Robinson, 1999). *L. delbrueckii* subsp. *bulgaricus* is an anaerobic, homofermentative organism producing D-lactic acid, bacterium rod-shaped with rounded ends that form chains which vary in length from 0.8 to 6 µ. For yogurt production moderate acid producing strains are selected (Chandan and O'Rell, 2006; Tamime and Robinson, 1999). Although *L. delbrueckii* subsp. b*ulgaricus* grows well at 45°C, the recommendation is to use incubation temperatures between 42 to 43°C to accommodate the lower growth temperature of *Str. salivarius* subsp. *thermophilus* (Chandan and O'Rell, 2006).

Incubation is considered done when the yogurt reaches has a titratable acidity of not less than 0.9% (21 CFR § 131. 200a). At this point, the yogurt is a gel, as the biological, chemical and physical actions have occurred in the milk during this incubation period. Biologically, lactose is utilized by the culture for growth and eventually is converted to lactic acid. Chemically, lactic acid will neutralize the negative charge on the casein micelles, which allows for the initiation of the physical coagulation of milk ~pH 5.2 to 5.3. As the pH decreases to 4.6, casein micelles continue to destabilize as the calcium phosphate and citrate solubilize out of the

micelle, physically allowing the casein and whey proteins to continue to aggregate and partially coalesce at pH 4.6, the pkI of casein (O'Kennedy and Mounsey, 2006).

2.5.2.6 Cooling and Packaging

After pH 4.6 is reached, the yogurt is immediately cooled to 4°C to slow culture growth and limit further acid production (Tamime, 2006). The packaging step of yogurt depends on the type of yogurt, for set style yogurt the yogurt mixes are inoculated, packaged and then fermented whereas for stirred-style yogurts, the yogurt is fermented, stirred (flavors and fruits may be added at this point) and then packaged (Chandan and O'Rell, 2006).

2.5.2.7 Storage and Distribution

Yogurt is stored and distributed at < 4°C which maintains overall quality such as body and texture by slowing down the biological and biochemical reactions in yogurt (NDC, 2012). Biological and biochemical reactions include metabolic activity of the yogurt culture, fat oxidation in presence of oxygen, hydration of protein, and slight dehydration which may take place resulting in increased acidity, increased syneresis and also deterioration of gel structure may occur leading to gel shrinkage effecting the gel quality. Storage life of yogurt also depends on sanitation procedures used during processing and possible effects of packaging. Transportation of yogurt may also lead to increased syneresis and reduced viscosity due to shaking which affects the gel quality (Tamime, 2006).Yogurt has a shelf life of 4 to 7 weeks when stored at < 7 ºC (Chandan and O'Rell, 2006)**.**

2.5.3 Yogurt Gel Structure

Yogurt mix is a liquid that during incubation transforms to a gel; but the gel can vary in firmness, consistency, WHC and stability (Amatayakul et al., 2006; Dave and Shah, 1998; Isleten and Yuceer, 2006; Modler et al., 1983).

2.5.3.1 Whey Protein Denaturation

Undenatured whey proteins are whey proteins in their native form, i.e., the secondary and tertiary structures have not been disrupted. However, dairy proteins denature; both pH and heat are catalysts for this reaction (Monahan et al., 1995). Yogurt mixes pasteurized at 65°C for 30 min have ~ 10% WPD *vs.* mixes processed at 90 to 95°C for 5 to 10 min have 70 to 95% WPD (Tamime and Robinson, 1999; Chandan and O'Rell, 2006). Basically, heat disrupts some of the non-covalent bonds (partially responsible for stabilization of the secondary and tertiary structures), causing the protein to unfold; thus, exposing hydrophobic groups (Hoffmann and van Mil, 1997). Hydrophobic groups then can interact with each other and lead to aggregation, coagulation and precipitation. When milk is heated to 40°C, the whey proteins undergo reversible conformational changes, and on further heating the whey proteins unfold, increasing the exposure of the buried hydrophobic groups and the thiol group within the globule structure (Chandan, 2006). Thiol-disulfide interchange reactions rarely occur at acidic pH (O'Kennedy and Mounsey, 2006). Sodini et al. (2005) reported that when yogurt mixes were fortified with 45g protein per kg WPC *vs.* skim milk powder (**SMP**) greater WPD occurred in WPC (77.5%) mixes *vs.* the mixes with SMP (72.9%) although process conditions were the same and attributed the greater WPD to the increased whey protein content.

2.5.3.2 Casein – Whey Protein Interactions

When whey proteins denature they complex with casein micelles in mixes (Lucey et al., 1998). These thermal-induced associations are considered complexes of denatured whey proteins with casein; but, the denaturation rates and agglomeration rates differ and depend on several factors (Vasbinder et al., 2004, Mottar et al., 1989). In yogurt mix, the casein-denatured whey

protein complexes are cross-linked. Formation of disulfide bonds increases the molecular weight of the aggregates and aids in the gel stabilization, with a concomitant increase in gel strength (Alting et al., 2000).

2.5.3.3 Casein : Whey Ratios

Aziznia et al. (2008) reported that the microstructure of nonfat yogurt containing WPC (0.75, 1.5 or 2%) had a more compact structures consisting of fused casein particles and large aggregates as compared to nonfat yogurt made with NDM. Excessive addition (2%) of WPC in the yogurt mix led to the formation of extremely large whey protein aggregates saturating the binding capacity of κ-casein to whey proteins, which led to the formation of additional whey protein aggregates. Yogurt mixes supplemented with β-lg exhibited faster rates of aggregate formation as concentration of β-lg increased as β-lg are less heat stable than α-la (Nielsen et al., 1996). Beaulieu et al. (1999) reported that as the ratio of casein to whey decreased from 80:20 to 20:80 greater numbers of whey protein aggregates were formed based on the increase of particle size from 135 nm to 340 nm, respectively and further aggregate formation increased from 0 to 90% when mixes were heated from 70 *vs.* 90°C.

2.6 Yogurt Quality

A high-quality yogurt exhibits gel quality where the gels are firm, with lower syneresis and greater water holding capacity (**WHC**) (Amatayakul et al., 2006; Dave and Shah, 1998; Modler et al., 1983). The yogurt gel quality is affected by the WPD, as WPD induces interactions between whey protein-casein. The greater these interaction higher the gel quality as a compact yogurt gel structure will retain more water resulting in greater firmness and lower (Sodini et al., 2005).

2.6.1 Syneresis

Typically, syneresis can be seen as free liquid on the yogurt surface and is often regarded as a defect (Lucey et al. 1998). During yogurt formulation, additional milk solids and/or stabilizers are added in set-style yogurt and stirred-style yogurt respectively, to reduce syneresis (Chandan and O'Rell, 2006). Also, process conditions are selected carefully as smaller pore sizes were observed in yogurt gels made from milk heated at 82.5°C and 93°C for 30 min; further, these smaller pore sizes resulted in less rapid flow of water and whey through the gel structure (Lee and Lucey, 2004). In yogurt, syneresis can occur due to low WHC, large pore sizes, and disruptions such as mechanical shearing (Amatayakul et al., 2006; Chandan and O'Rell, 2006; Lucey et al., 1998).

Amatayakul et al. (2006) reported that when total solids in yogurt increased from 9 to 16%, yogurt syneresis decreased by 28%. Modler et al. (1983) reported that as WPC increased from 0.5% to 1.5% in a yogurt mix, yogurt syneresis decreased by 24%. More recently, Pintro et al. (2011) reported that WPI addition to a yogurt mix resulted in a 79% decrease in syneresis in the yogurt and explained this decrease due to the increased whey protein content in yogurt mix. And a 1% WPI addition directly to yogurt resulted in a 50% decrease in syneresis due to the increased whey protein content (Isleten and Yuceer, 2006).

2.6.2 Water Holding Capacity (WHC)

Water holding capacity is the amount of water trapped within the protein structure and a yogurt can contain 80-90% water (Tamime and Robinson, 1999). Parnell-Clunies et al. (1986) reported that as yogurt mix pasteurization temperatures increased from 85°C to 98°C (for 30 min) the WHC of the resultant yogurt increased from 27.5 to 28.39%. The hydrophobic-

hydrophilic properties of casein are influenced by the β-lg: α-la ratio present on the micellar surface of the casein; thus, the lower the ratio of β-lg on the micellar surface, the greater the WHC and the lower the surface hydrophobicity (Mottar et al., 1989). The WHC of WPC enriched yogurts were reported to be ~25% more compared to the control yogurt and these changes were attributed to higher cross-linkage of the network in yogurts fortified with whey protein concentrates (Sodini et al., 2005).

2.6.3 Firmness

Firmness is a textural property of yogurt and is influenced by formulation and processing (Megenis et al., 2006). Puvanenthiran et al. (2002) reported that as whey protein increased from 0.75 to 2.07 g in yogurt, the firmness increased 41%. In WPC-supplemented yogurt, casein micelles were linked by particle-to-particle attachments in long chains, whereas in yogurts made of skim milk powder (**SMP**) the yogurts had spatial distributions of the casein micelles forming a open, loose structure with more interspaced voids resulting in gel with lower firmness than WPC-supplemented yogurt (Sandoval-Castilla et al., 2004). Yogurt made from mixes supplemented with WPC had increased firmness (cone penetration 298.5 mm *vs.* 308.6 mm) compared to yogurt from mixes containing skim milk only (Dave and Shah, 1998a). Vasbinder et al. (2004) stated that the increase in gel firmness in gels (pH 4.6) was due to the additional disulfide bridges formed during gelling as the reactive thiol groups form disulfide bridges linking all particles together.

2.6.4 Color

Color of the food product is one of the factors recognized as a main attribute by consumers when selecting food products (Clark et al., 2009). Food color can be expressed in

terms of the CIELAB color space with the coordinates being L* (0-100, estimation of lightness), a* (red-green) and b* (yellow-blue) (Pagliarini et al., 1990). Whiteness index (**WI**) is determined from the L*, a* and b* coordinates using equation $100- [(100-L^*)^2+ (a^*)^2+(b^*)^2]^{½}$ (Vargas et al., 2008). Whiteness index sets the kind of color and samples with WI of 0 are black whereas, 100 are white.

Yogurt mixes treated at 85°C for 15 min, resulted in increased L* and a* values compared to untreated mixes and during incubation (43°C) further increase in L* and a* values were observed until pH 5, after which no further changes were observed (Needs et al., 2000). The lightness (L*) of yogurts did not vary with either thermal ((85°C, 30 min) or pressure treatment (300 to 676 MPa, 5 min) suggesting that the light color of yogurt does not depend on the aggregation pattern or the size of the casein micelle fraction (Harte et al., 2003).

2.6.5 Yogurt Storage

Stored yogurt is often studied to understand the effects of time and temperature on sensory, microbiological and physical qualities of the yogurt. Isleten and Yuceer, 2006 in their study observed a decrease (14%) in syneresis when yogurts were stored at 4°C for 21 days. Salvador and Fiszman (2004) compared yogurts stored at 10, 20 and 30°C for 91, 31 and 21 days and reported that although the yogurt evolved differently, negative characteristics such as syneresis, appearance defects, atypical texture / mouth feel increased during storage regardless of storage temperatures. Storage conditiond also influenced yogurt WHC and as mentioned by Parnell-Clunies et al., (1986) during heat processing of yogurt mix, WPD causes casein micelles to rearrange, but, this rearrangement deteriorates during storage resulting in release of bound water. For example, WHC decreased 10% in yogurts stored for 42 days. Sodini et al., 2005 indicated that increases in titratable acidy (**TA**) of stored yogurts (4°C) were functions of the

continual acid production by the bacterial culture. Thus, during storage yogurt chemical and functional properties change affecting the gel quality.

2.6.6 Sensory

Sensory evaluation is the measurement of a product's quality based on information received from the five senses; sight, smell, taste, touch and sound (Clark et al., 2009). Formulation and mix processing influence the body, texture and flavor of yogurt (Antunes et al. (2005); Gonzalez-Martinez et al. (2002); Isleten and Yuceer (2006); Modler et al. (1983)).

Isleten and Yuceer (2006) compared the additions of SMP, WPI, texture improver (**TI**) or sodium caseinate (**NaCn**) in yogurt mixes on the flavor and texture attributes of the yogurts throughout a 12 day storage period. Using descriptive analysis, free whey was least but lumpiness and thickness were the greatest in the WPI yogurt compared to the other yogurts. Also, the WPI yogurt had less fermented and creamier flavor scores than the other samples during storage. They attributed the lower flavor scores of the WPI yogurt to the flavor-binding properties of whey proteins. Gonzalez-Martinez et al. (2002) also used descriptive sensory analysis to evaluate yogurts formulated with whey proteins. The whey protein yogurts were reported to be lump free but have softer gel textures compared to yogurts containing SMP. However, Modler et al. (1983) used a consumer panel and reported that yogurts containing WPC had acceptable texture and appearance scores when compared to yogurts containing other protein sources like casein and NaCn. When flavored yogurts with different types of protein (NaCn, whey proteins) were compared they exhibited viscosity differences but not flavor differences (Saint-Eve et al., 2006).

In 2005, Antunes et al. reported that consumers rated yogurt containing WPC *vs.* SMP as similar in appearance, flavor, texture and overall impression which agreed with another study

where, yogurts fortified with NaCn (1%) and WPC (1%) had no significant differences in appearance aroma, taste and overall acceptability during storage (28 days) (Akalin et al., 2012).

2.7 Cysteine

Cysteine is unique among the amino acids due to its thiol group which can form a disulfide bond with another cysteine when oxidized. This dimeric compound is called cystine. Figure 2.2 shows the conversion of cysteine to cystine via disulfide linkage Table 2.5 depicts cysteine contents in β-lg, α-la and WPI.

In rats and humans, cysteine in the monomer form is absorbed 3 times more readily than cystine (Neil, 1958, Rosenberg et al., 1967). Cysteine can be synthesized from methionine by action of γ-cystathionase (EC. 4.4.1.1) (Figure 2.3) (Sastre et al., 2005). However, with age, γ-cystathionase (EC. 4.4.1.1) activity decreases; hence, the conditionally essential amino acid status for cysteine for the elderly sub-population. The effect of low γ-cystathionase (EC. 4.4.1.1) was studied by researchers and an increase in cataractogenesis was found in elderly rats (24 to 26 months) reporting 50% less γ-cystathionase (EC. 4.4.1.1) when compared to young rats (5 to 6 months) which in turn was related to the lower glutathione (**GSH**) contents (Sastre et al., 2005).

Figure 2.2 Conversion of cysteine to cystine (Nelson and Cox, 2005)

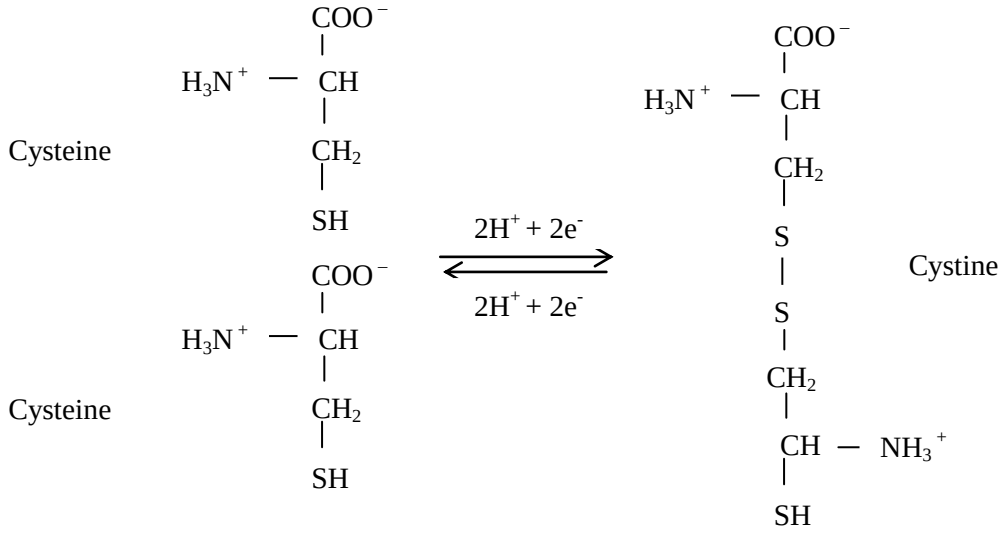
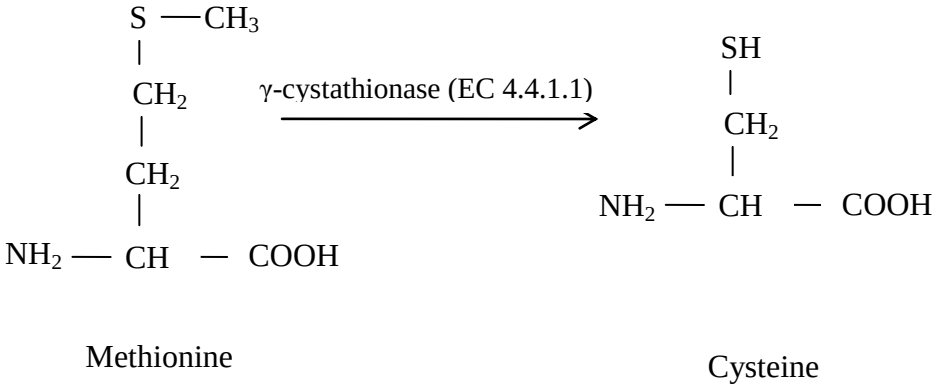


Figure 2.3 Cysteine synthesis

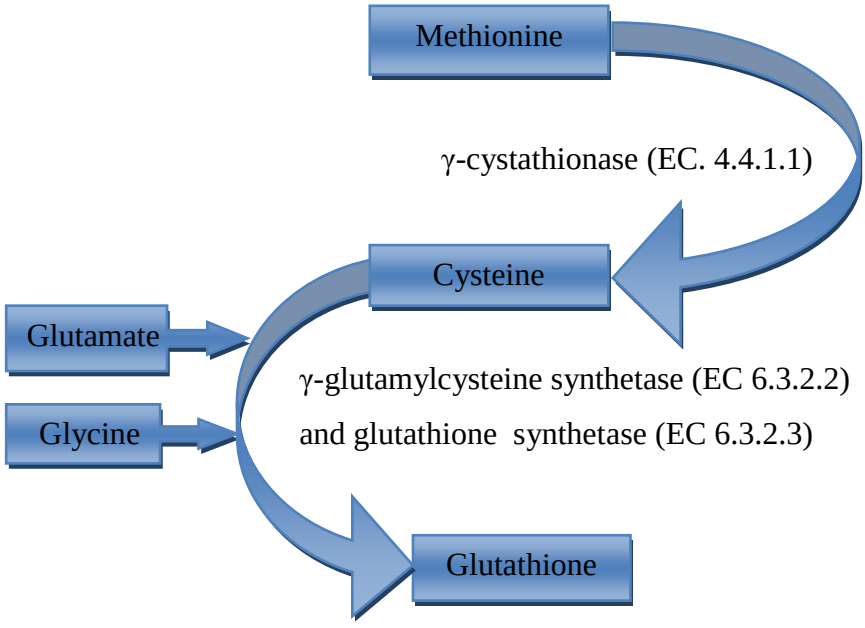


Fukagawa and Richard (2004) reported that when dietary intake of methionine increased, cysteine levels also increased; but, methionine levels increased in the blood to the extent that they were considered “adverse”, indicating hyperhomocysteinemia and endothelial dysfunction, which are considered to be promoters of cardiovascular diseases.

2.8 Glutathione

Glutathione is a tripeptide thiol synthesized from glutamate, cysteine, and glycine in the presence of enzymes γ-glutamylcysteine synthetase (EC 6.3.2.2) and GSH synthetase (EC 6.3.2.3) (Cristopher, 1998). This reaction is shown in Figure 2.4. Glutathione exists in two forms; reduced GSH and glutathione di-sulfide (GSSG). In a cell, the ratio of GSH to GSSG is an indication of cell health and viability with an optimum ratio of about 9:1 (Cristopher, 1998). There is no evidence for availability of oral GSH and transport of GSH into cells; so agreement exists that the constituent amino acids are required for intracellular GSH synthesis (Bounous et al., 1998).

Figure 2.4 Glutathione synthesis (Cristopher et al., 1998)



In 2003, Kent et al. reported that human prostate epithelial cells grown on WPI had 64% greater GSH contents compared to the cells grown on casein. HIV patients experienced an increase in blood GSH contents (from 10.22 to 17.04 n mol) when they consumed whey proteins

(Bounous et al., 1989a). When mice were fed diets containing 20 g of whey protein/100 g, GSH contents were improved by 5%; however, greatest increases were shown with the WPC diets that had 99.5% solubility (least denatured) (Bounous and Gold, 1991).Thus, the researchers suggested the effect was due to the greater availability of cysteine (Bounous and Gold, 1991).

Pau et al., 1990 reported that GSH contents in elderly eye lenses were low due to the decreased γ-cystathionase (EC. 4.4.1.1) contents. For humans, the data suggests that the diets designated for elderly populations may benefit from increased cysteine as it leads to the increase in GSH contents (Droge, 2002).

As shown in Tables 2.1 and 2.5- yogurts with WPI are excellent sources of cysteine; however, the major concern is dietary availability of cysteine in whey as the denaturation of whey proteins leads to unfolding exposing free sulphydryl group which initiates aggregation of ß-lg with other ß-lg, α-la, bovine serum albumin, casein (Tamime, 2009). These reactions decrease the amount of cysteine available for GSH synthesis (Witschi et al., 1992).

Chapter 3 - Research Objectives

The overall objective of this research is to develop a cysteine enhanced yogurt prototype by whey protein isolate (**WPI**) supplementation and to determine the consumer liking of this cysteine enhanced yogurt.

The objectives were divided into 2 parts:

1) To prepare a yogurt with enhanced cysteine content with supplementing WPI at lower processing temperatures and evaluate its storage quality.
    a. To formulate and determine cysteine contents in mixes made with different supplements (WPI *vs.* NDM).
    b. To assess changes in cysteine content in mixes and yogurt along with yogurt quality as functions of process treatments and formulations.
    c. To evaluate the impact of storage on yogurt quality.
2) To determine the liking of a high-cysteine content yogurt.

Chapter 4 - Materials and Methods

4.1 Yogurts Manufacture

4.1.1 Set-style yogurt

Low-heat nonfat dry milk (**NDM**) (Dairy America, Fresno, CA, USA), whey protein isolate (**WPI**) 895 (Glanbia Nutritionals, Fitchburg, WI, USA) and yogurt culture (Yo-Mix 495 LYO 375 DCU, DuPont, New Century, KS, USA) were obtained from commercial suppliers and maintained at -2 or -10^{o}C (culture) until usage. (Specification sheets of NDM, WPI and yogurt culture are available on request). Yogurts consisted of two formulations: N (0% WPI and 12.5% W/V NDM) and W (2.5% WPI and 10% W/V NDM) for equivalent total solids (**TS**) of 12.5%W/V. Powders were mixed in deionized, distilled water at 22 to 24^{o}C for 60 min in Erlenmeyer flask (2000 mL) (Fisher Scientific, Pittsburgh, PA, USA), on a magnetic stir plate (Fisher Scientific) then subdivided and heated to 70^{o}C or 90^{o}C on magnetic stir plate (Fisher Scientific) and placed in a pre-set water bath (Isotherm 220, Fisher Scientific) at 70^{o}C for 20 min or 90^{o}C for 7 min (referred to as 70 or 90, respectively). Mixes were cooled to 43^{o}C, inoculated with 0.4% culture, packaged into sterile 120 mL cups (Fisher Scientific) or 30 mL sterile plastic centrifuge tubes (Fisher Scientific), and incubated at 43^{o}C ± 1 (Isotemp, Fischer Scientific) until pH 4.5-4.6. Samples were removed from the incubator and placed in storage (4^{o}C ± 1) (RT18 DKXEN, Whirlpool Corporation, Benton Harbor, MI, USA) until the day of analysis. The four yogurts were designated as N70, N90, W70 and W90 and the N90 yogurt is considered as control.

4.1.2 Stirred-style yogurt

Low-heat NDM (Dairy America, Fresno, CA, USA), sugar (pure cane sugar, Domino Foods, Inc., Yonkers, NY, USA), vanillin (Midwest Country Fare, Imitation vanilla flavouring, Westown Parkway, West Des Moines, IA, USA [bought at Hyvee, Manhattan, KS, USA]), WPI 895 (Glanbia Nutritionals, Fitchburg, WI, USA) and yogurt culture (Yo-Mix 495 LYO 250 DCU, DuPont, New Century, KS, USA) were obtained from commercial suppliers. The NDM, WPI and yogurt culture were maintained at -2 or -10^{o}C (culture) and vanillin and sugar were maintained at 4°C until usage. (Specification sheets of NDM, WPI and yogurt culture are available on request). Yogurts consisted of two formulations: C (0% WPI, 12.5% W/V NDM and 5% W/V sugar) and E (2.5% W/V WPI, 10% W/V NDM and 5% W/V sugar). Both mixes were designed to deliver TS of 17.5%. Dry ingredients were mixed in deionized, distilled water at 22 to 24^{o}C for 60 min in Erlenmeyer flask (2000 mL) (Fisher Scientific) on a magnetic stir plate (Fisher Scientific). The C mix was brought to 90^{o}C and E mix was brought to 70^{o}C on magnetic stir plate (Fisher Scientific) and placed in water bath (Isotherm 220, Fisher Scientific) pre-set at the designated temperature for 7 and 20 min, respectively. Process temperature of 70^{o}C for 20 min has been used by Allgeyer et al. (2010) in their research for pasteurization of a yogurt drink and was considered safe for human consumption. Mixes were cooled to 43^{o}C and 1.6% W/V of vanillin flavor was added to both the mixes. Mixes were inoculated with 0.6% W/V culture, packaged into sterile 120 mL cups (Fisher Scientific) or 5 L stainless steel bowls (Cuisinart CTG-00-SMB, East Windsor NJ, USA) and incubated at 43^{o}C ± 1(Isotemp, Fischer Scientific) until pH 4.5 to 4.6. Samples were removed from the incubator and placed in storage (4^{o}C ± 1) (RT18 DKXEN, Whirlpool Corporation, Benton Harbor, MI, USA). On the following day the mixes were stirred with a hand mixer (Hamilton Beach 5, Hamilton Beach/Proctor-Silex, Inc.,

Washington, NC, USA) for 5 min at speed 3 and placed in the same container and stored at 4^{o}C until assessed.

4.2 Assessments

4.2.1 Consistency

Consistency was determined by method from (Penna et al., 1997). The Bostwick consistometer (Bostwick consistometer 24925-000, CSC Scientific Company, INC, Fairfax, VA) was leveled by adjusting the screws at the bottom until the leveling bubble was at the centre. The yogurt was mixed with a spoon for 30 sec before filling the sample into sample reservoir. The product gate was closed. The sample was filled up to the top of the sample reservoir. The product gate was opened and the distance (in cm) over which the material flowed at 4°C in 30 sec was measured.

4.2.2 Cysteine Content

Cysteine content was estimated as described by Shimada and Cheftel, 1989. Plastic cuvettes (1 cm length, 1.5 mL capacity) (Fisher Scientific) containing 0.1 M phosphate buffer (Fisher Scientific) (Appendix A Table A.1) at pH 8 with 8M urea (Sigma-Aldrich, St. Louis, MO, USA) and 0.5% sodium dodecyl sulfate (SDS) (Fisher Scientific) and 0.03 ml of Ellman's solution (Thermo Scientific, Rockford, IL, USA) were prepared. A sufficient size of yogurt mix or yogurt was added to cuvettes to deliver a 0.1% protein concentration and the remaining mix was made up to volume of (3.03 mL) with 0.1 M phosphate buffer. For the blank, 3 mL of the 0.1 M phosphate buffer along with 0.03 mL Ellman's solution were used. The reaction set for 5 min at 22 ± 2°C and then absorbance was measured at 412 nm on spectrophotometer (Spectronic

Genrys 5, Thermo Electron Corporation, Madison, WI, USA). The concentration of thiols was calculated from the molar absorbance.

$$Co = \frac{A}{E} \mathbf{x} D$$

Co=concentration of thiols

A=absorbance at 412nm

E=extinction coefficient=1.36×10^4 $cm^{-1}M^{-1}$

D=dilution factor

Dilution schemes for all samples are shown in Appendix A (Table A.1).

4.2.3 Firmness (Set-style yogurt)

Yogurt firmness 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, USA) using a 25 mm (P25/L) acrylic probe in a 120 ml plastic yogurt cup maintained at 4 ± 1°C. Test velocity, time and distance were 2 mm/s, 5 sec and 5 mm, respectively. Firmness reported in g was obtained using the accompanying software (Stable Micro System).

4.2.4 Firmness (Stirred-style yogurt)

Yogurt firmness was determined using method described by Saint-Eve et al. (2006) for stirred yogurts. Textural analysis was done with the TA.XT2, Texture Analyzer (Stable Micro System) using a 25 mm (P25/L) acrylic probe in a 120 ml plastic yogurt cup maintained at 4 ± 1°C. Test velocity, time and distance were 5 mm/s, 5 sec and 10 mm, respectively. Firmness reported in g was obtained using the accompanying software (Stable Micro System).

4.2.5 Syneresis (set-style yogurt)

Syneresis was determined as described by Amatayakul et al. (2006). Yogurt cups (120 mL) of known weight were maintained at approximately 45° angle for 2 hrs at 4 ± 1°C. Free whey was siphoned from the surface using a syringe (10 mL, Fisher Scientific) within 10 s and weighed. The syneresis was calculated as a percent weight of whey over initial weight of yogurt.

$$\% \text{ Syneresis} = \frac{\text{Siphoned whey weight}}{\text{Initial yogurt weight}} \text{ x } 100$$

4.2.6 Syneresis (stirred-style yogurt)

Syneresis (stirred-style) was determined as described by Damin et al. (2009) on day 1 yogurt. Forty g of stirred yogurt were transferred to 30 mL plastic, reusable centrifuge tubes (Fisher Scientific) and centrifuged (Marathon 21000 R, Fisher Scientific) at 222 x g for 10 min, at 4 ± 1°C. The supernatant was poured off and the remaining yogurt weight was weighed and expressed as percent weight relative to original weight of yogurt.

$$\% \text{ Syneresis} = \frac{(\text{Initial yogurt weight} - \text{Remaining yogurt weight})}{\text{Initial yogurt weight}} \text{ x } 100$$

4.2.7 Titratable Acidity (TA)

Titratable acidity (**TA**) was measured as described by Hooi et al. (2004). Ten g of yogurt was placed in a 100 mL beaker and titrated with 0.1N sodium hydroxide solution (Fisher Scientific) using 0.5 mL phenolphthalein (Sigma-Aldrich) as an indicator. End point of titration was when a permanent pink color (30 sec).

One mL of 0.1N sodium hydroxide neutralizes 0.009 g of lactic acid.

$$\% \text{ TA} = \frac{(9 \text{ x } 0.1 \text{ x Volume of } 0.1 \text{ N NaOH})}{\text{Weight of yogurt}}$$

4.2.8 Total Solids (TS)

Total solids were measured using a forced–draft oven method as described by Hooi et al. (2004). Yogurt was mixed for 1 min and then approximately 5 g was added into a weighed, desiccated aluminum dish (56 mL, Fisher Scientific) and covered with another 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.

$$\% \text{ TS} = \frac{\text{(Dried sample weight)}}{\text{Initial yogurt weight}} \text{ x } 100$$

4.2.9 Water Holding Capacity (WHC)

Water holding capacity (**WHC**) was measured as described by Parnell-Clunies et al. (1986). Yogurt incubated in the 30 mL sanitized, plastic, resusable centrifuge tubes (Fisher Scientific) of known weight were centrifuged (Marathon 21000 R, Fisher Scientific) at 4 ± 1°C at 13500 × g for 30 min. The supernatant fluid was drained for 10 min by inverting tubes at 24 ± 1°C. Water holding capacity was expressed as percent pellet weight to original yogurt weight.

$$\% \text{ WHC} = \frac{\text{Drained tube weight} - \text{Empty tube weight}}{\text{Initial yogurt weight}} \text{ x } 100$$

4.2.10 Whey Protein Denaturation (WPD)

Whey protein denaturation was calculated following Grady et al. (2001) and Outinen et al. (2010). Total protein, non-casein protein nitrogen and non-protein nitrogen (**NPN**) for each sample were determined (IDF 2001a; IDF, 2001b and IDF, 2004, respectively).

Total protein: Total protein content of unheated and heat treated samples were calculated by measuring the nitrogen content of rehydrated samples directly using a Leco Analyzer (LECO

TruSpecCN, LECO, St Joseph, MI). The nitrogen was converted to protein content by multiplying factor of 6.38 (IDF 1964).

Non Casein Protein: Thirty milliliters of mix was transferred to a 100 mL volumetric flask, 50 mL of distilled water at 50^{o}C and 3 mL of acetic acid (10%, v/v; Fisher Scientific) were added and the mixture was left to stand for 10 min. Three milliliters of 1 N sodium acetate (Sigma-Aldrich) was then added and the mixture cooled to 23 ± 1^{o}C before being made up to volume with distilled water (The pH was 4.6 after addition of the sodium acetate). The solution was filtered through Whatman No. 1 filter paper (Fisher Scientific). Nitrogen contents were determined using a Leco Analyzer (LECO TruSpecCN) casein content calculated by measuring the difference of nitrogen content of milk and filtrate and nitrogen was converted to protein content by multiplying factor of 6.38 (IDF 1964).

Casein protein= Total protein - Non Casein Protein

Non Protein Nitrogen: Ten milliliters of mix was transferred into a conical flask; 40 ml of trichloroacetic acid solution (Fisher Scientific) was added, mixed and allowed to stand for 5 min to allow the precipitate to settle. The solution was filtered through Whatman No. 1 (Fisher Scientific) filter paper and nitrogen content of the filtrate was estimated using a Leco Analyzer (LECO TruSpecCN), and nitrogen was converted to protein content by multiplying factor of 6.38 (IDF 1964).

True Protein: True protein is obtained from the difference of total protein and non-protein nitrogen.

$$\text{True protein} = \text{Total protein} - \text{NPN}$$

Whey protein content was obtained from difference of total protein and casein.

$$\text{Whey protein content} = \text{True protein} - \text{Casein protein}$$

Finally, whey protein denaturation was calculated from comparing the processed and non-processed mixes following Grady et al. (2001) and Outinen et al. (2010).

$$\%\ \text{WPD} = 100 - \left(\frac{\text{whey protein content in heated sample}}{\text{whey protein content in unheated sample}}\right) \times 100$$

4.2.11 Whiteness Index (WI)

Whiteness index was calculated following modified methods (Schmidt et al. 2001; Vargas et al., 2008) .A Miniscan EZ, Model 4500L, (Hunter Associates Laboratory, Reston, Virginia, USA) Hunter D-54 Reflectance Lab Ultra Scan Sphere Spectrophotometer. Standardization was done using a white and black tile (X= 80.49, Y= 85.30 and Z = 88.35). Samples were maintained at 4 ± 1°C and analyzed for color in the same cup. For each sample, surface color was measured 4 times, by rotating the sample at 90^{o} and the averaged L, a^{*} and b^{*} (Appendix B, Table B.1) values were obtained. Whiteness index was calculated and compared.). Whiteness index sets the kind of color and samples with WI of 0 are black whereas, 100 are white.

$$WI = 100 - [(100-L^{*})^{2} + (a^{*})^{2} + (b^{*})^{2}]^{½}$$

4.2.12 Sensory

The low cysteine (LC) and High cysteine (HC) stirred-style yogurts were tested for consumer liking. The stirred-style yogurt was prepared 7 days before the test date as described in 4.1.2. A consumer panel consisting of 112 subjects ranging from 18 to 70 yrs (74 females and 38

males) were recruited. All panelists were screened based on their age (≥ 18 years), interest in consuming yogurts and for any food allergies or intolerances. Demographic information on gender, background and yogurt consumption frequency was gathered (Appendix C, Table C.4). A 9-point hedonic scale (1 = dislike extremely, 5 = neither like nor dislike, 9 = like extremely) was used to evaluate the 2 products (Lawless and Heymann, 1998). Paper ballots (Appendix C) were pre-printed and contained the scales for liking of appearance, thickness, flavor, after taste and overall acceptability. A question at the end of the ballot was asked regarding willingness to buy the product (yes/no). Approximately 15 g of each sample were placed in plastic cups (60 mL) (Classic Sysco, Sysco Corporation, Houston, TX, USA), lidded and kept at 4°C ± 1. All the samples were randomly coded with a three-digit number, and the serving order for panelists was randomized. Water was provided to clean the palate between samples. See Appendix C for ballot, demographics, and consent statements. Once a sample was completed, the ballot was collected and then the second ballot and other sample were distributed. At the completion of the study, panelists were given coupons for a free ice cream cone at the Call Hall Dairy Store, Kansas State University, Manhattan, KS.

4.2.13 Experimental Design and Statistical Analysis

The objectives are divided into two parts.

Part 1: To prepare a yogurt with enhanced cysteine content with supplementing WPI at lower processing temperatures and also evaluate its storage quality. This part included 3 studies.

Study 1: In study one, the objective was to determine if cysteine content could be enhanced in a yogurt mixes.

Protein contents, initial cysteine content and pH of non-processed mixes were compared using a one way analyses. Three replications were done and statistical analyses were performed using SAS version 9.3 (SAS® Institute Inc., Cary, NC, USA) using the generalized linear mixed model which includes both fixed (formulation) and random effects (replication). ANOVA results ($P \leq 0.05$) for formulation indicated significant effects. The following model was used:

$$Y_{ijk} = \mu + F_i + \varepsilon_{ij}$$

Where, Y_{ijk} is the ijkth observation for kth replication

μ is the mean

F_i is the formulation (N or W)

ε_{ij} is the random error.

And where, i = 1, 2; j = 1, 2, 3.

Study 2: In study 2, cysteine contents of mixes and yogurt and yogurt quality was assessed.

Cysteine and pH were assessed on all the yogurt mixes; thus a 2 x 3 randomized complete block design was used. Three replication were done and statistical analyses were performed using SAS version 9.3 (SAS® Institute Inc.) using the generalized linear mixed model which includes both fixed (formulation and process treatment) and random effects (replication. ANOVA results ($P \leq 0.05$) for significant effects were further analyzed using Tukey's HSD (Kuehl, 2000). The following model was used:

$$Y_{ijk} = \mu + F_i + P_j + FP_{ij} + \varepsilon_{ijk}$$

Where, Y_{ijk} is the ijkth observation for kth replication

μ is the mean

F_i is the formulation (N or W)

P_j is the process treatment (non-heated, 70 or 90)

FP_{ij} is the interaction and

ε_{ijk} is the random error.

And where, i = 1, 2; j = 1, 2, 3 and k = 1, 2, 3.

However, for analyzing WPD, TA, TS, pH, yogurt functional characteristics (day 1) and yogurt cysteine contents (day 1) only heated mixes or yogurts were factors; thus, a 2 x 2 randomized complete block design was used. Three replications were done and statistical analyses were performed using SAS version 9.3 (SAS® Institute Inc.) using the generalized linear mixed model which includes both fixed (formulation and process treatment) and random effects (replications). ANOVA results ($P \leq 0.05$) for significant effects were further analyzed using Tukey's HSD (Kuehl, 2000). The following model was used:

$$Y'_{ijk} = \mu' + F'_i + P'_j + F'P'_{ij} + \varepsilon'_{ijk}$$

Where, Y'_{ijk} is the ijkth observation for kth replication

μ' is the mean

F'_i is the formulation (N or W)

P'_j is the process treatment (70 or 90)

$F'P'_{ij}$ is the interaction

ε'_{ijk} is the random error.

and where, i = 1, 2 ; j = 1, 2 and k = 1, 2, 3.

Study 3: The objective of this study was to determine if cysteine and gel quality could be maintained throughout the shelf life of yogurt. The 4 yogurts were designated as: N70, N90, W70 and W90 to reflect both formulation (WPI addition for the W yogurts) and the process (70

for the mixes heated at 70°C for 20 min and 90 for 90°C for 7 min). Yogurts were assessed on days 1, 15, 30, 45 and 60) for color properties, cysteine, firmness, pH, TA, syneresis, and WHC. A balanced split plot in a completely randomized design was used and three replications were completed. Whole plots were the yogurts and 5 stored days were split plot. Statistical analyses were done using SAS version 9.3 (SAS® Institute Inc.) using the generalized linear mixed model which includes both fixed (yogurts and days) and random effects (replication). ANOVA results ($P < 0.05$) for significant effects were further analyzed using Tukey's HSD (Kuehl, 2000). The following model was used:

$$Y_{ijk} = \mu + y_i + T_j + yT_{ij} + \alpha_{ik} + \varepsilon_{ijk}$$

Where, Y_{ijk} is the ijkth observation made on split-plot for lth replication

μ is the mean

y_i is the yogurt (N70, N90, W70 and W90)

α_{ik} is the random error for the whole plot experimental units

T_j is the time (day 1, 15, 30, 45 and 60)

yT_{ij}, are the interactions

ε_{ijk} is the random error for the split plot experimental units.

where, i = 1, 2,3,4; j = 1, 2,3,4,5 and k = 1, 2, 3.

Part 2: To determine the liking of a high-cysteine content yogurt.

Study 4: The objective of this study was to determine the liking of two yogurt samples: experimental and control. The design was a randomized complete block design with yogurt formulation being LC and HC treatments (2) and panelists (112) as blocks. Statistical analysis

was done using SAS version 9.3 (SAS® Institute Inc.). ANOVA results ($P < 0.05$) were obtained for significant effects. The following model was used:

$$Y_{ijk} = \mu + F_i + P_j + \varepsilon_{ij}$$

Where Y_{ijk} is the ijkth observation for kth replication

μ is the mean

F_i is the yogurt (C or E)

P_j is the panelist (main effect)

ε_{ij} is the random error.

where, i = 1, 2 ; j = 1, 2, 3,..112.

Chapter 5 - Enhancing Cysteine Content in Yogurt: Chemical and Functional Properties

5.1 Introduction

Yogurt sales have increased by 40% since 2006 (USDA 2013). The increase in popularity may be attributed to its nutritional and health benefits – such as improved digestibility and lactose utilization as well as some antagonistic actions towards enteric pathogens (Chandan, 2007). During yogurt processing the mixes are intensely processed (90 to 95°C for 5 to 10 min) to increase the whey protein denaturation (**WPD**) (75-90%) (Anema and Li, 2003). But other benefits occur as when whey proteins denaturation, contribute to a compact yogurt gel structure that retains more water and minimizes syneresis (Sodini et al., 2005).

During thermal processing loss of dietary cysteine occurs as denaturation caused by thermal treatmetns leads to unfolding of whey proteins exposing the free sulphydryl groups. These free sulphydryl groups initiate aggregation with other whey proteins decreasing the thiol content. Also, the exposed thiols form hydrogen sulfide which further adds to the losses of thiol contents (de Wit and Nieuwenhuijse, 2008). Compared to denatured whey proteins, undenatured whey proteins are rich in cysteine, a precursor of a tripeptide thiol glutathione (**GSH**) (Fig 2.5) (Lothian et al., 2000). In cells, GSH has many metabolic functions such as DNA synthesis and repair, protein synthesis, amino acid transport, toxins and carcinogens metabolism and oxidative cell damage protection (Christopher et al., 1998). Droge (2002) reported that GSH levels were related to various aging-related processes like neurological disorders and oxidative stresses; thus, GSH is often used as an indicator of overall health. Metabolically, decreased γ-cystathionase (EC. 4.4.1.1) activity (converts methionine to cysteine) resulted in decreased GSH contents in elderly human eye lenses, and decreased GSH content in eyes has been associated with cataract

formation (Reddy, 1990). As whey proteins are excellent sources of cysteine (Glass and Hedrick, 1976) researchers have studied the relationship between whey proteins and GSH. In 2003, Kent et al. reported that human prostate epithelial cells grown on whey protein isolate (**WPI**) had greater GSH (64%) contents compared to the cells grown on casein. However, the nature of the whey protein may be important as Bounous and Gold (1991) presented findings that GSH contents were greater in liver and heart tissues when undenatured *vs*. denatured whey protein concentrates (**WPC**) were fed to mice. They proposed that this effect was due to the greater availability of cysteine. These data suggest that humans may benefit from the consumption of dietary cysteine especially those subpopulations with diminished γ-cystathionase (EC. 4.4.1.1) activity, as the dietary cysteine may be used directly as a precursor for GSH.

A high-quality yogurt exhibits gel quality where the gels are firm, with lower syneresis and greater water holding capacity (**WHC**) (Amatayakul et al., 2006; Dave and Shah, 1998; Modler et al., 1983). The yogurt gel quality is affected by the WPD, as WPD induces interactions between whey proteins-casein as well as whey protein-whey protein interactions typically results in a compact yogurt gel structure which will retain more water, be mote firm and less syneresis (Sodini et al., 2005). Parnell-Clunies et al. (1986) reported that as yogurt mix pasteurization temperatures increased from 85 to 98°C (with equivalent hold periods of 30 min) the WHC of the resultant yogurt increased from 27.5 to 28.39%, while WPD increased from 72.7 to 88.4%. Larger pore sizes were observed in yogurt gels made from milk heated at 72°C compared to yogurts made from milk heated at 82.5 and 93°C (30 min). These larger pores allowed for more rapid flow of water and whey through the gel structure; hence greater syneresis (Lee and Lucey, 2004).

Addition of whey products to yogurt mixes has been studied for many years and by many researchers. Typically as whey protein concentration increased in the yogurt mix the resultant yogurts had less syneresis, increased firmness and WHC, as well as enhanced bifidobacteria viability (Amatayakul et al., 2006; Dave and Shah, 1998b; Isleten and Yuceer, 2006). When mixes were treated at 90°C for 10 min greater numbers of casein-whey protein and whey protein-whey protein interactions were observed in yogurt mixes with increased whey protein contents when there microstrucute was studied. These interactions are responsible for the yogurt gel quality (Aziznia et al., 2008). When Isleten and Yuceer (2006) formulated a non fat yogurt mix with WPI (1%), the syneresis decreased (55%) and WHC increased (125%) although processing conditions were the same (85°C for 15 min). But the addition of whey proteins can significantly alter the physical properties of yogurt. For example, Puvanenthiran et al. (2002) increased whey protein from 0.75 to 2.07 g in yogurt and subsequently the yogurt gels firmness increased from 123 to 210mN. More recently Pintro et al., (2011) showed that WPI added to yogurt (0.42 g WPI/ 4.2 g protein) resulted in 4X reduction in syneresis.

The shelf life of yogurt is often studied to understand the effects of time and temperature on overall quality - either by emphasizing changes in the sensory, chemical, functional, or microbial properties (Salvador and Fiszman, 2004; Serra et al., 2009; Isleten and Yuceer, 2008). For example, during storage the casein particles in the yogurt gel rearrange into a more compact structure which increased the bond numbers and decreased the total free energy of the system, moving the gel to a more thermodynamically stable state which in the end decreased syneresis (Serra et al., 2009). Isleten and Yuceer (2006) in their study also observed a decrease (14%) in syneresis when yogurts were stored at 4°C for 21 days. Salvador and Fiszman (2004) compared yogurts stored at 10, 20 and 30°C for 91, 31 and 21 days and reported that although the yogurt

evolved differently, negative characteristics such as syneresis, appearance defects, atypical texture / mouth feel increased during storage regardless of storage temperatures. Storage time also influenced the WHC of yogurt. During yogurt processing, WPD causes increase in casein micelles size and further these micelles coalescence, but, this rearrangement deteriorates during storage resulting in release of bound water (Parnell-Clunies et al., 1986). WHC decreased 10% in yogurts stored for 42 days. Yogurt has a shelf life of 4 to 7 weeks when stored at <7 ºC (Chandan and O'Rell, 2006). Thus, studies of yogurt quality during storage can help predict shelf life more accurately.

Compositionally, WPI has 3X the protein content as does WPC (Lopes et al., 2006) and ~2.5 X more cysteine than does nonfat dry milk (**NDM**) (Glass and Hedrick, 1976). Theoretically, a yogurt with enhanced cysteine content could be developed using WPI (to augment cysteine and whey protein contents) combined with a less severe process (to minimize cysteine loss and adjust the number of protein-protein bonds). Thus the objective of this part of research was to develop a yogurt with enhanced cysteine content while maintaining an acceptable gel quality throughout shelf life.

5.2 Materials and Methods

5.2.1 Yogurt Manufacture

Low-heat nonfat dry milk (**NDM**) (Dairy America, Fresno, CA, USA), whey protein isolate 895 (**WPI**) (Glanbia Nutritionals, Fitchburg, WI, USA) and yogurt culture (Yo-Mix 495 LYO 375 DCU, DuPont, New Century, KS, USA) were obtained from commercial suppliers and maintained at -2 or -10^{o}C (culture) until usage. Yogurts consisted of two formulations: **N** (0% WPI and 12.5% NDM) and **W** (2.5% WPI and 10% NDM) for equivalent total solids (**TS**) of

12.5%. Powders were mixed in deionized, distilled water at 22 to 24^{o}C for 60 min, then subdivided and heated to the target temperature of 70^{o}C and 90^{o}C on a magnetic stir plate (Fisher Scientific) and held at 20 and 7 min, respectively (referred to as 70 or 90, respectively) in a water bath (Isotherm 220, Fisher Scientific) that was pre-set to the target temperature. Mixes were cooled to 43^{o}C, inoculated with 0.4% culture, packaged into sterile 120 mL cups (until 120 mL mark) (Fisher Scientific) or 30 mL centrifuge tubes (about 25-30 g) (Fisher Scientific), and incubated at 43^{o}C ± 1 (Isotemp, Fisher Scientific) until pH 4.5-4.6. Yogurts were removed from the incubator and placed in storage (4^{o}C ± 1) (RT18 DKXEN, Whirlpool Corporation, Benton Harbor, MI, USA) until the day of analysis. The four yogurts were designated as N70, N90, W70 and W90 and the N90 yogurt was considered to be the most similar to a commercially produced product.

5.2.2 Chemical and Functional Properties

Cysteine was measured as described by (Shimada and Cheftel, 1989) using Ellman's reagent. Absorbance was measured at 412 nm on spectrophotometer (Thermo Electron Corporation, Spectronic Genrys 5, Madison, WI, USA). The concentration of thiols were calculated from the molar absorbance.

$$Co = \frac{A}{E} \mathbf{x} D$$

Co = Concentration of thiols, A = absorbance at 412 nm, E (extinction coefficient) = 1.36x10^{4} cm$^{-1}M^{-1}$, D = dilution factor.

pH was measured using a Fisher universal glass pH electrode (Acumet Portable AP61, Fisher Scienctific) after calibration with standardized buffer solutions (Fisher Scientific), to pH 4 and 7 at 43^{o}C±1.

Firmness was determined using a modified method by Megenis et al. (2006). Textural analysis is 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.

Syneresis was determined as described by Amatayakul et al. (2006). The syneresis was calculated as a percent weight of whey over initial weight of yogurt.

$$\%\text{Syneresis} = \frac{(\text{Siphoned whey weight})}{\text{Initial yogurt weight}} \text{ x } 100$$

Total solids (**TS**) were measured using a forced–air oven method as described by Hooi et al. (2004). Total solids were calculated as follows:

$$\%\ \text{TS} = \frac{(\text{Dried sample weight})}{\text{Initial yogurt weight}} \text{x}100$$

Titratable acidity (**TA**) was measured as described in (Hooi et al., 2004). One milliliter of 0.1 N sodium hydroxide neutralizes 0.009 g of lactic acid.

$$\%\ \text{TA} = \frac{(9 \text{ x } 0.1 \text{ x Volume of } 0.1 \text{ N NaOH})}{\text{Weight of yogurt}}$$

Water holding capacity was measured as described by Parnell-Clunies et al. (1986). Water holding capacity was expressed as percent pellet weight over original yogurt weight.

$$\%\ \text{WHC} = \frac{(\text{Drained tube weight} - \text{Tube weight})}{\text{Initial yogurt weight}} \text{ x } 100$$

Whey protein denaturation was calculated following Grady et al. (2001) and Outinen et al. (2010). Total protein, non-casein protein nitrogen and non-protein nitrogen (**NPN**) for each sample were determined (IDF, 2001a; IDF, 2001b and IDF, 2004, respectively).

Total protein: Total protein content of unheated and heat treated samples were calculated by measuring the nitrogen content of samples using a Leco Analyzer (LECO TruSpecCN, LECO, St

Joseph, MI). The nitrogen was converted to protein content by multiplying factor of 6.38 (IDF 1964).

Non Casein Protein: Thirty milliliters of mix was transferred to a 100 mL volumetric flask, 50 mL of distilled water at 50^{o}C and 3 mL of acetic acid (10%, v/v; Fisher Scientific) were added and the mixture was left to stand for 10 min. Three milliliters of 1 N sodium acetate (Sigma-Aldrich) was then added and the mixture cooled to 23 ± 1^{o}C before being made up to volume with distilled water (The pH was 4.6 after addition of the sodium acetate). The solution was filtered through Whatman No. 1 filter paper (Fisher Scientific). Nitrogen contents were determined using a Leco Analyzer (LECO TruSpecCN). Casein content calculated by measuring the difference of the nitrogen contents of milk and filtrate and nitrogen and then calculatin the in protein by multiplying factor of 6.38 (IDF 1964).

Non Protein Nitrogen: Ten milliliters of mix was transferred into a conical flask; 40 mL of trichloroacetic acid solution (Fisher Scientific) was added, mixed and allowed to stand for 5 min to allow the precipitate to settle. The solution was filtered through Whatman No. 1 filter paper and nitrogen content of the filtrate was estimated using a Leco Analyzer (LECO TruSpecCN), and nitrogen was converted to protein content by multiplying factor of 6.38 (IDF 1964).

Finally, whey protein denaturation was calculated from comparing the processed and non-processed mixes following Grady et al. (2001) and Outinen et al. (2010)..

$$\% \text{ WPD} = 100 - \left(\frac{\text{whey protein content in heated sample}}{\text{whey protein content in unheated sample}}\right) \times 100$$

Whiteness index was calculated following modified methods (Schmidt et al. 2001; Vargas et al., 2008) .A Miniscan EZ, Model 4500L, (Hunter Associates Laboratory, Reston, Virginia, USA) Hunter D-54 Reflectance Lab Ultra Scan Sphere Spectrophotometer. Standardized using a white and black tile (X= 80.49, Y= 85.30 and Z = 88.35). Four surface

readings for L, a^* and b^* were taken on each sample by rotating the sample at 90^o angles on yogurt sample with ~3 cm diameter cup at $4 \pm 1°C$ and the averaged L, a^* and b^* values were obtained. Whiteness index (**WI**) was calculated and compared (Vargas et al., 2008).

$$WI = 100 - [(100-L^*)^2 + (a^*)^2 + (b^*)^2]^{½}$$

5.2.3 Experimental Design and Statistical Analysis

For this part the overall objective was to determine if cysteine contents could be enhanced in a yogurt gel and maintained throughout and also yogurt storage quality. As this objective involved data analysis on mixes and yogurt four models were needed.

Mix: protein contents, initial cysteine content and pH of non-processed mixes were compared using a one way analyses. Three replications were done and statistical analyses were performed using SAS version 9.3 (SAS® Institute Inc., Cary, NC, USA) using the generalized linear mixed model which includes both fixed (formulation) and random effects (replication). ANOVA results ($P \leq 0.05$) for formulation indicated significant effects. The following model was used:

$$Y_{ijk} = \mu + F_i + \varepsilon_{ij}$$

Where, Y_{ijk} is the ijkth observation for kth replication

μ is the mean

F_i is the formulation (N or W)

ε_{ij} is the random error.

And where, i = 1, 2; j = 1, 2, 3.

Processed mix *vs.* non-processed mix: the second model was used for the cysteine and pH of all the yogurt mixes; using 2 x 3 randomized complete block design. Three replications

were done and statistical analyses were performed using SAS version 9.3 (SAS® Institute Inc.) using the generalized linear mixed model which includes both fixed (formulation and process treatment) and random (replication) effects. ANOVA results ($P \leq 0.05$) for significant effects were further analyzed using Tukey's HSD (Kuehl, 2000). The following model was used:

$$Y_{ijk} = \mu + F_i + P_j + FP_{ij} + \varepsilon_{ijk}$$

Where, Y_{ijk} is the ijkth observation for kth replication

μ is the mean

F_i is the formulation (N or W)

P_j is the process treatment (non-processed, 70 or 90^oC)

FP_{ij} is the interaction and

ε_{ijk} is the random error.

And where, i = 1, 2; j = 1, 2, 3 and k = 1, 2, 3.

Yogurt gel quality and WPD: For analyzing WPD, TA, TS, pH, yogurt functional characteristics (day 1) and yogurt cysteine contents (day 1) only processed mixes or yogurts were factors; thus, a 2 x 2 randomized complete block design was used. Three replications were done and statistical analyses were performed using SAS version 9.3 (SAS® Institute Inc) using the generalized linear mixed model which includes both fixed (formulation and process treatment) and random (replication) effects. ANOVA results ($P \leq 0.05$) for significant effects were further analyzed using Tukey's HSD (Kuehl, 2000). The following model was used:

$$Y'_{ijk} = \mu' + F'_i + P'_j + F'P'_{ij} + \varepsilon'_{ijk}$$

Where, Y'_{ijk} is the ijkth observation for kth replication

μ' is the mean

F'_i is the formulation (N or W)

P'_j is the process treatment (70 or 90^oC)

$F'P'_{ij}$ is the interaction

ε'_{ijk} is the random error.

and where, i = 1, 2 ; j = 1, 2 and k = 1, 2, 3.

Storage study: To evaluate the impact of different process treatments on stored yogurt quality. The 4 yogurts were designated as: N70, N90, W70 and W90 reflecting formulation (W and N) and process (70 and 90 for 70°C for 20 min and 90°C for 7 min, respectively). Yogurts were assessed on days 1, 15, 30, 45 and 60 for color properties, cysteine, firmness, pH, TA, syneresis, and WHC. A balanced split plot in a completely randomized design was used and three replications were completed, with whole plots being the 4 yogurts and the split plots were the 5 test days. Statistical analyses were done using SAS version 9.3 (SAS® Institute Inc.) using the generalized linear mixed model which includes both fixed (yogurts and days) and random (replication) effects. ANOVA results ($P \leq 0.05$) for significant effects were further analyzed using Tukey's HSD (Kuehl, 2000). The following model was used:

$$Y_{ijk} = \mu + y_i + \alpha_{ik} + T_j + yT_{ij} + \varepsilon_{ijk}$$

Where, Y_{ijk} is the ijkth observation made on split-plot for lth replication

μ is the mean

y_i is the yogurt (N70, N90, W70 and W90)

α_{ik} is the random error for the whole plot experimental units

T_j is the time (day 1, 15, 30, 45 and 60)

yT_{ij}, are the interactions

ε_{ijk} is the random error for the split plot experimental units.

where, i = 1, 2, 3 ,4; j = 1, 2, 3, 4, 5 and k = 1, 2, 3.

5.3 Results and Discussion

Study 1: To formulate and determine cysteine contents in mixes made with different supplements (WPI *vs.* NDM). (Raw data can be found Appendix B)

5.3.1 Yogurt Mixes

True protein, casein, whey protein and cysteine contents as well as pH of the non-processed mixes were determined (Table 5.1). As expected true and whey protein contents increased by 25 and 65%, respectively when comparing the N to W mixes. Cysteine contents were 39% greater in W *vs*. N mixes. These results were consistent with Glass and Hedrick (1976) who reported that WPI had 2.5 times greater cysteine content than NDM.

Table 5.1: Protein and cysteine contents in non-processed yogurt mixes (means ± standard error)

Mix	True Protein (%)	Whey (%)	Cysteine mg/L	pH
N	4.17^{b}±0.07	0.77^{b}±0.01	306.9^{b}±1.70	6.51^{a}±0.02
W	5.51^{a}±0.02	2.23^{a}±0.02	504.9^{a}±2.61	6.41^{b}±0.02

$^{a-b}$Means (n=3) within a column with different superscripts differ ($P < 0.05$).
N: Mix with 12.5% NDM, W: Mix with NDM (10%) and WPI (2.5%).

These results indicate that cysteine contents increased with the supplementation of yogurt mix with WPI significantly increased true and whey protein contents. However, pH decreased.

Mixes were then processed, assessed and the data analyzed. Significant main effects were found for: cysteine and pH (formulation) (Table 5.2); cysteine, pH and WPD (process) (Table 5.3), and significant process*formulation interactions were found for cysteine contents, pH and WPD ($P \leq 0.05$) (Table 5.4).

5.3.1.1 Formulation Effect

Table 5.2 shows the significant formulation effects on cysteine content and pH of yogurt mixes ($P \leq 0.05$). Cysteine contents were 70% greater in W mixes than N mixes. This agrees with Glass and Hedrick (1976) that WPI had 2.5 times greater cysteine content than NDM. The pH of W mixes was less than N mixes, probably a factor of the altered casein: whey protein ratios in the mixes [1.5:1 to 4.4:1 in the W *vs*. N mixes, respectively (Appendix B, Table B.2)].

Table 5.2: Cysteine contents and pH of yogurt mixes containing different formulation collapsed for process treatment (means ± standard error)

Mix	Cysteine mg/1000mL	pH
N	226.2^{b}±24.4	6.35^{a}±0.02
W	383.6^{a}±37.9	6.33^{b}±0.05

$^{a-e}$Means (n=9) within a column with different superscripts differ ($P \leq 0.05$).
N: Mix with 12.5% NDM, W: Mix with NDM (10%) and WPI (2.5%).

5.3.1.2 Process Effect

Table 5.3 shows the significant process effects on cysteine content, pH and WPD of yogurt mixes ($P \leq 0.05$). Cysteine contents decreased 22.0 and 52.7% when processed at 70°C for 20 min or 90°C for 7 min compared to non-processed mixes. Pofahl and Vakaleris (1967) reported cysteine losses ranged from 60 to 65% when skim milk was heated at 85^{o}C for 10 min, whereas Zweig and Block (1954) reported that cysteine decreased by 7 and 40% in skim milk when processed at 70^{o}C for 20 min and 90^{o}C for 7 min, respectively. Although the process conditions were the same as Zweig and Block's, but these mixes had greater total solids and protein contents which may influence the results. Mix pH decreased by 2.34 and 3.22% when processed at 70^{o}C for 20 min and 90^{o}C for 7 min, respectively compared to non-processed mixes. Researchers have reported that mineral balances can shift in milk during thermal

processing resulting in a pH decrease (Fox and McSweeney, 1998; Tamime, 2006). The WPD of mixes ranged from 8.63 to 76.10% when processed at 70°C for 20 min and 90°C for 7 min, respectively. These results were in agreement with previous studies, where yogurt mixes pasteurized at 65°C for 30 min have ≤ 10% WPD whereas mixes processed at 90 to 95°C for 5 to10 min have 70 to 95% WPD (Tamime and Robinson, 1999; Chandan and O'Rell, 2006).

Table 5.3: Cysteine contents, pH and whey protein denaturation (WPD) % of yogurt mixes when processed differently collapsed for formulation(means ± standard error)

Mix	Cysteine mg/L	pH	WPD %
Non-processed	406.0^{a}±44.3	6.46^{a}±0.03	-
70	316.6^{b}±37.7	6.31^{b}±0.02	8.63^{b}±2.37
90	192.1^{c}±24.1	6.25^{c}±0.04	76.10^{a}±2.70

$^{a-c}$Means (n=6) within a column with different superscripts differ ($P \leq 0.05$).
70: Mixes processed at 70°C for 20 min, 90: Mixes processed at 90°C for 7 min.

*5.3.1.3 Formulation*Process Effect*

Significant formulation*process interactions were found for cysteine contents, pH and WPD of the yogurt mixes ($P \leq 0.05$) (Table 5.4).

Cysteine losses were 21, 51, 23 and 55% in W and N mixes when processed at 70°C for 20 min and 90°C for 7 min, respectively compared to the non-processed mixes. Pofahl and Vakaleris (1967) and Zweig and Block (1954) have reported similar losses when researching skim milk.

The non-processed W mixes had lower pH (6.40) than did the N mixes (6.51). As mentioned earlier the altered casein: whey protein ratios in the mixes (1.5:1 to 4.4:1 in the W *vs*. N mixes, respectively (Appendix B, Table B.2) could be responsible as casein has higher buffering capacity (Kailasapathy et al., 1996) than whey proteins. The process caused the mix pH to decease, with the average pH of the 70 mixes at 6.29 *vs*. 6.27 for the 90 mixes. The N mixes exhibited greater decreases (3%) than the W mixes (1.5%). Others have reported that the

mineral balances shifts in milk during thermal processing caused pH to decrease and as the N mixes have greater mineral contents [1.025 *vs.* 0.895% in N and W mixes, respectively], this change might have also occurred also in these mixes contributing to the pH difference (Fox and McSweeney, 1998; Tamime, 2006).

Whey protein denaturation was greatest (82%) for the W90 mix and least (3%) for the W70 mix. Although literature suggested that ~90% WPD would occur in the N90 mixes (Chandan, 2006); Anema and Li (2003) reported WPD was ~ 75% when yogurt mixes were processed at 90^{o}C and 7 min which is comparable to the N90 mix results (70%). Sodini et al. (2005) reported that when yogurt mix was fortified with WPC greater WPD occurred in the mixes (72.9 to 77.5%). The W90 yogurt mixes had more WPD than those reported by Sodini et al. (2005); however both N90 and W90 mixes results followed the published trends i.e. when mixes were processed at 90^{o}C for 7 min greater WPD (70 and 82%, respectively) and cysteine losses (55 and 51%, respectively) were observed than mixes processed at 70^{o}C for 20 min.

Table 5.4: Cysteine contents, pH and whey protein denaturation (WPD) % of yogurt mixes containing different formulations and processed differently (means ± standard error)

Mix	Process	Cysteine mg/L	pH	WPD %
N	Non-processed	306.9^{c}±1.70	6.51^{a}±0.02	-
N	70	233.1^{d}±1.79	6.29^{d}±0.03	13.81^{c}±0.62
N	90	138.5^{e}±4.23	6.28^{d}±0.04	70.28^{b}±0.73
W	Non-processed	504.9^{a}±2.61	6.41^{b}±0.03	-
W	70	400.2^{b}±12.1	6.32^{c}±0.02	3.452^{d}±0.88
W	90	245.6^{d}±5.34	6.31^{c}±0.05	81.92^{a}±1.38

$^{a\text{-}e}$Means (n=3) within a column with different superscripts differ ($P \leq 0.05$).
N: Mix with 12.5% NDM and non-processed, W: Mix with NDM (10%) and WPI (2.5%) and non-processed, 70: Mixes processed at 70^{o}C for 20 min, 90: Mixes processed at 90^{o}C for 7 min.

These results indicated that addition of WPI to yogurt mix enhanced cysteine contents; however, processing of mixes at 90°C (7 min) resulted in WPD (76%) and a 54% loss in cysteine compared to the non-processed mix. The W70 mixes had minimal WPD (3%) and retained 80% of the cysteine compared to the non-processed mix. However, compared to the control N90 mix, the cysteine contents in the W70 mix was enhanced by 190%.

5.3.2 Day 1 yogurt

Yogurt gel formation and quality depend directly on the TS, protein content and WPD (Mahdian and Tehrani 2007; Pintro et al., 2011). As just shown in Tables 5.1, 5.2, 5.3 and 5.4, the W mixes had greater protein contents than N mixes, and the W70 mix had less WPD but the W90 mix had more WPD than did the N90 mix, so it was desired to see if the W mixes could be "fermented" into acceptable yogurt gels. Hence, gel quality at day 1 was assessed as well as cysteine contents in the 4 yogurts.

Statistical analysis showed that day 1 yogurts did not differ in TA (1.01%), TS (12.44%), or WI (67.92) ($P > 0.05$) (See Appendix D); however, in day 1 yogurts, cysteine contents, firmness, pH, syneresis and WHC were affected by formulation ($P \leq 0.05$); cysteine, firmness, syneresis and WHC were affected by process ($P \leq 0.05$) and cysteine, firmness and WHC were affected by the formulation*process interaction ($P \leq 0.05$).

5.3.2.1 Formulation Effects

Table 5.5 depicts the significant mean differentiations for the formulation effect. Overall, W yogurts had greater cysteine (75%), firmness (282%) and WHC (87%) *vs*. N yogurts; however, N yogurts had greater pH (0.3%) and syneresis (51%) than W yogurts. The greater pH observed in the N yogurt may be attributed to the difference in casein contents in these mixes as casein has greater buffering capacity than whey proteins (Kailasapathy et al. 1996) and the N

yogurts had about 3 times more casein than the W yogurts (Table 5.1). Isleten and Yuceer (2006) reported that nonfat yogurt made from mixes containing 1% WPI and processed at 85°C (15 min) had significant decrease in syneresis (55%) and increase in WHC (125%) compared to yogurts made without WPI. Although mixes had greater WPI and process conditions were different, still results followed the same trend.

Table 5.5: Cysteine contents, firmness, pH, syneresis and water holding capacity of yogurts at day 1 as a function of formulation collapsed for process treatment (means ± standard error)

	Formulation	
Properties	N	W
Cysteine (mg/L)	183.0^{b}±21.22	320.8^{a}±35.12
Firmness (g)	49.31^{b}±4.663	188.6^{a}±25.80
pH	4.426^{a}±0.003	4.411^{b}±0.003
Syneresis(%)	9.335^{a}±1.030	4.572^{b}±0.751
WHC(%)	19.68^{b}±1.570	37.64^{a}±6.609

^{a,b}Means (n=6) within a row with different superscripts differ ($P \leq 0.05$).
N: Nonfat dry milk (12.5%), W: Nonfat dry milk (10%) + WPI (2.5%)
WHC: Water holding capacity

5.3.2.2 Process Effects

Table 5.6 depicts the process effect on day 1 yogurt quality. Overall, cysteine contents (39.7%) and syneresis (30%) decreased, whereas firmness (43%) and WHC (47%) increased when comparing mixes processed at 70°C (20 min) *vs*. 90°C (7 min). The syneresis results agree with Lee and Lucey (2004) who reported that yogurt gels made from milk heated at 72°C had greater syneresis compared to yogurts made from milks heated to 82.5 or 93°C. Sodini et al. (2005) reported that yogurt mixes enriched with WPC (1%) had greater WPD (77.5%) compared to mixes enriched with skim milk powder (**SMP**) (1%) and suggested that although processing conditions (85-100°C) were similar, more WPD could contribute to more cross-linkages in the

gel network, which may in turn produces a firmer yogurt. Although cysteine contents were different, they reflect the cysteine losses due to the process treatment (see Table 5.4).

Table 5.6: Cysteine contents, firmness, syneresis and water holding capacity of day 1 yogurt as a function of process treatment collapsed for formulation (means ± standard error)

	Process	
Properties	70	90
Cysteine (mg/L)	314.3^{a}±37.95	189.5^{b}±24.23
Firmness (g)	86.18^{b}±21.25	151.70^{a}±41.78
Syneresis (%)	8.683^{a}±1.376	5.221^{b}±0.896
WHC (%)	19.96^{b}±1.781	37.36^{a}±6.703

^{a,b}Means (n= 6) within a row with different superscripts differ ($P \leq 0.05$).
70: Processed at 70^{o}C (20 min), 90: Processed at 90^{o}C (7 min)
WHC: Water holding capacity

*5.3.2.3 Formulation*Process Effect*

Table 5.7 shows the significant mean differentiations for cysteine contents, firmness and WHC as functions of formulation * process interaction.

Table 5.7: Cysteine contents, firmness and water-holding capacity (WHC) of day 1 yogurt as a function of formulation and process interaction (means ± standard error)

Yogurt	Cysteine mg/L	Firmness g	WHC %
N70	230.3^{b}±5.704	39.09^{d}±0.308	16.81^{b}±1.685
N 90	135.7^{c}±4.572	59.53^{c}±2.054	22.55^{b}±1.094
W70	398.3^{a}±19.83	133.3^{b}±6.414	23.11^{b}±1.748
W90	243.4^{b}±10.10	243.9^{a}±15.058	52.17^{a}±2.031

$^{a-d}$Means (n=3) within a column with different superscripts differ ($P \leq 0.05$).
N70: Nonfat dry milk(12.5%) rehydrated and processed at 70^{o}C (20 min); N90: Nonfat dry milk(12.5%) rehydrated and processed at 90^{o}C (7 min); W70: Nonfat dry milk (10%) + WPI(2.5%) rehydrated and processed at 70^{o}C (20 min); W90: Nonfat dry milk(10%) +WPI(2.5%) rehydrated and processed at 90^{o}C (7 min).

The W70 yogurt had the greatest cysteine content whereas the N90 yogurt had the least, with the W70 yogurt having 190% more than the N90 yogurt. The W90 and N70 yogurt had similar cysteine contents. Compared to the cysteine data in Table 5.4, fermentation did not affect the cysteine content in yogurt; suggesting that cysteine would be available in the final product.

The W90 yogurt had the greatest firmness and the N70 yogurt had the least with the W90 yogurt being 525% more firm than the N70 yogurt and the W70 yogurt being twice as firm as the N90 yogurt.

The WHC was the greatest in the W90 yogurt about ~ 54% greater than the N70, N90 and W70 yogurts which exhibited similar WHC. Sodini et al. (2005) reported that yogurt mixes enriched with WPC (1%) had significantly greater WPD (77.5%) which contributed to more cross-linkages of the gel network, which in turn increased the WHC (1.2X) of the yogurt. In this study, the W90 yogurt had 2.3 X the WHC when compared to the N90 yogurt which might be due to the greater protein content in WPI than WPC, and greater WPD.

The N90 yogurt system was considered to be a "control", as the resultant yogurt was formulated and processed to match recommendations for a high-quality yogurt (Chandan, 2006; Tamime, 2009). Supplementing WPI in a yogurt mix enhanced cysteine content (73%) in the yogurt gel and was retained (39.7%) if the mix was processed at 70°C for 20 min *vs*. 90°C for 7 min.

Although WPD was significantly less in the W70 (3.5%) yogurt mix, the day 1 yogurt had similar WHC and twice the firmness of the N90 yogurt (70%). A market bought sample from a local store (Plain nonfat yogurt, Dannon, The Dannon Company, Inc., White Plains, NY, USA [Bought at Walmart, Manhattan, KS, USA (June, 2012)] was analyzed for firmness. Firmness of the market bought sample (128g) and W70 yogurts were similar.

Hence, the W70 yogurt with higher cysteine content may be used as a source of dietary cysteine in the U.S. diet; however, lower WPD may affect the yogurt quality during storage. Therefore, the effect of storage on cysteine and yogurt quality needs to be assessed.

Study 3: To evaluate the impact on storage on yogurt quality.

5.3.3 Storage Study

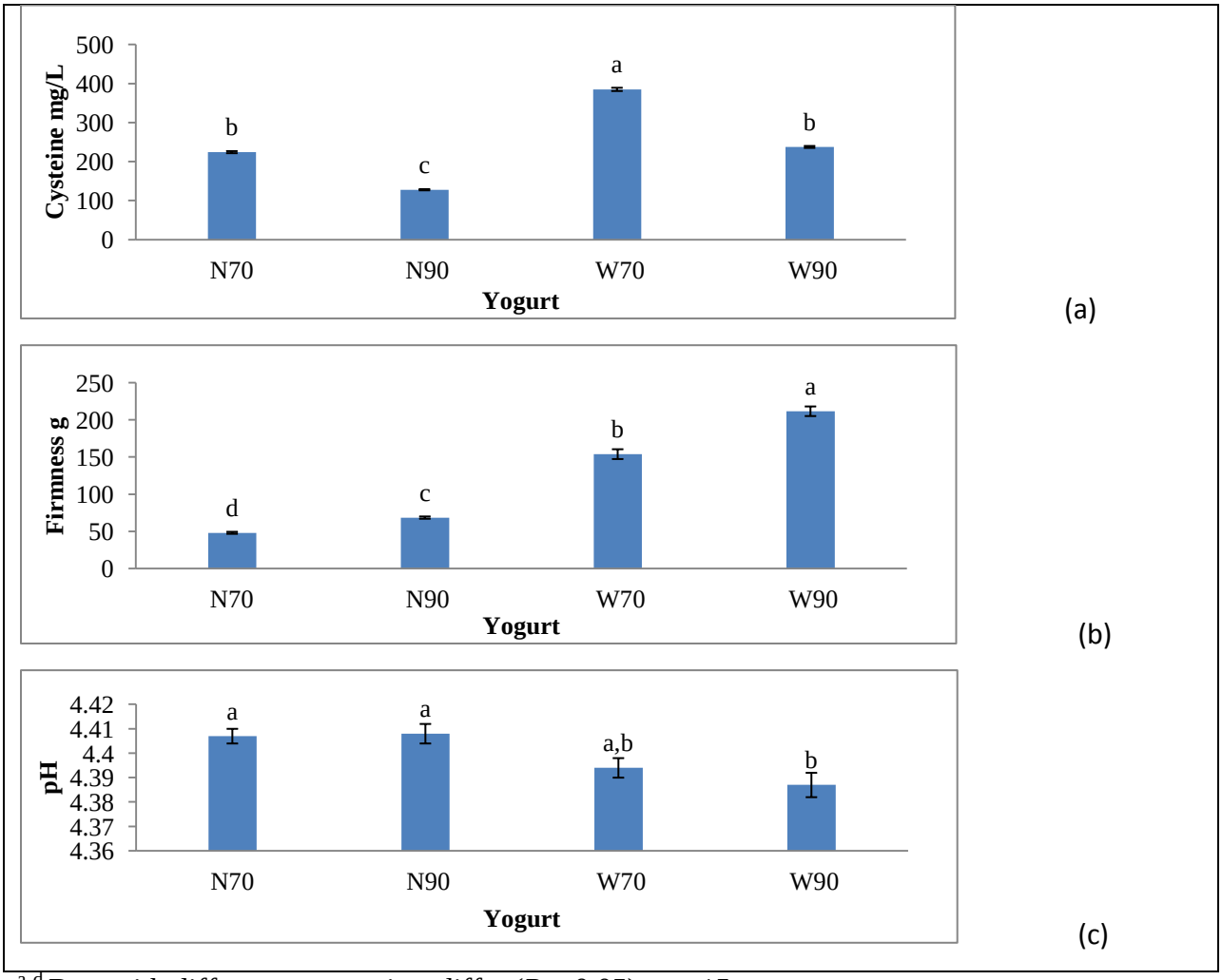
Yogurts were evaluated for functional and chemical quality during a 60 day storage period. Statistical analysis showed that yogurt type significantly affected cysteine, pH, firmness, syneresis, WHC and WI ($P \leq 0.05$); significant affect of storage day on cysteine, firmness, pH, syneresis, WHC and WI ($P \leq 0.05$) and firmness, TA, WHC and WI were affected significantly by yogurt*storage ($P \leq 0.05$).

5.3.3.1 Yogurt Effect

Cysteine content, pH, firmness, WI and WHC were functions of the yogurt product. Overall, cysteine contents were greatest in W70 yogurts and the least in the N90 yogurt, with the W70 yogurt having 3X the cysteine content as did the N90 yogurt (Figure 5.1 (a)). These results were consistent with Glass and Hedrick (1976) and Zweig and Block (1954) who reported that process conditions could decrease cysteine contents in skim milk. The W90 yogurt had the greatest firmness and the N70 yogurt had the least firmness with the W90 yogurt being 525% more firm than the N70 yogurt and the W70 yogurt being twice as firm as the N90 yogurt (Figure 5.1 (b)). Amatayakul et al. (2006) reported that as casein to whey ratio varied from 4:1 to 1:1, yogurt firmness doubled. However, when comparing W yogurts had firmness 3X that of the N yogurt. Although the pH was similar in the N70, N90 and W70 yogurts, pH was significantly less in the W90 yogurt compared to the N70 and N90 yogurts (Figure 5.1 (c)). Dave and Shah,

(1998b) in their research observed that yogurts with greater whey proteins had double the pH drop than yogurts without whey proteins. This can probably attributed to the greater viability of bacterial growth due to greater availability of amino nitrogen nutrients for microbial growth.

Figure 5.1: Cysteine (a), firmness (b) and pH (c) of various yogurts* collapsed for stored days (1, 15, 30, 45 and 60)



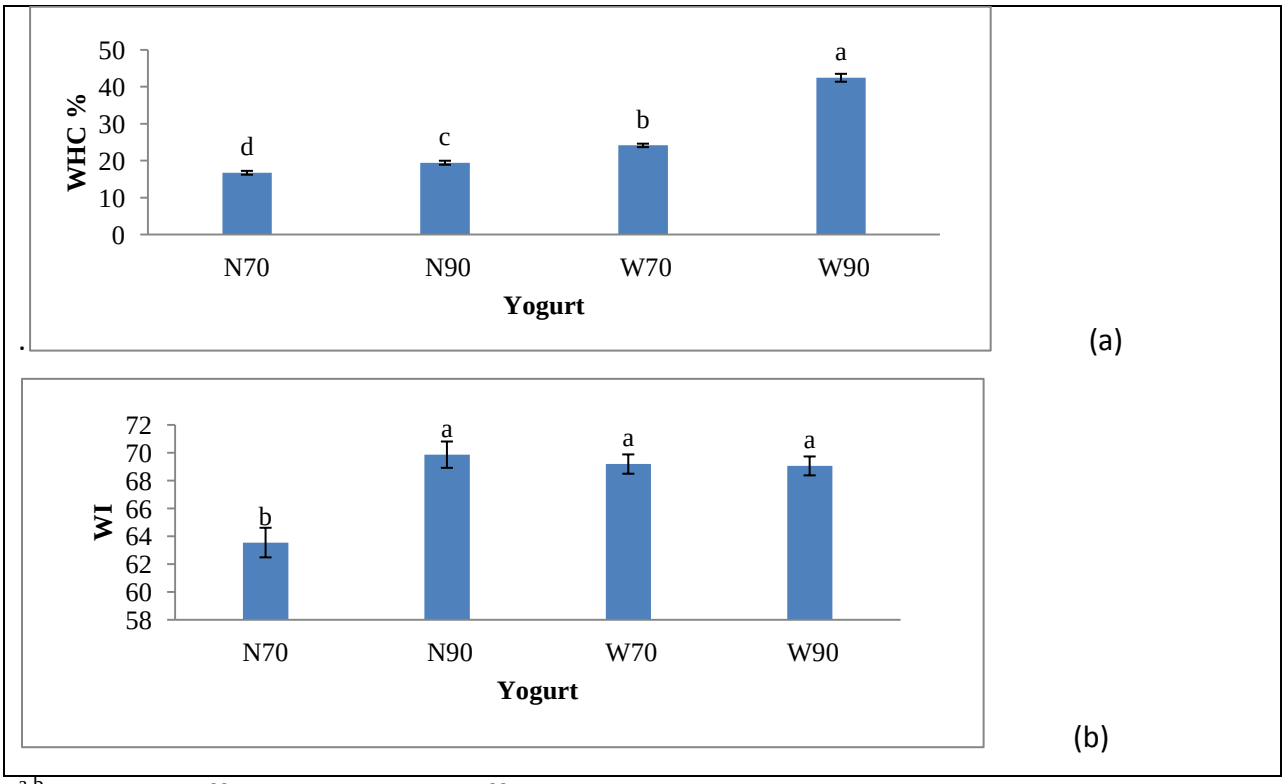
[a-d] Bars with different superscripts differ ($P \leq 0.05$), n = 15.
N70: Nonfat dry milk treated at 70°C for 20 min, N90: Nonfat dry milk treated at 90°C for 7 min
W70: Nonfat dry milk + WPI treated at 70°C for 20 min; W90: Nonfat dry milk +WPI treated at 90°C for 7 min.

Water holding capacity of N70 yogurt was least and W90 the highest (Figure 5.2(a)). Sodini et al. (2005) reported that yogurt mixes enriched with WPC (1%) had significantly greater

WPD (77.5%) and suggested that the greater WPD which contributed to more cross-linkages of the gel network, which in turn increased the WHC (1.2X) of the yogurt. Though W70 yogurt had lower denaturation than N90 yogurt it still exhibited greater WHC. In a research by Hongsprabhas and Barbut, 1997 even at lower temperatures (< 24°C) WPI solutions showed gelation and usually denaturation of whey proteins is expected at (> 65°C).

Whiteness index of N70 yogurt was lower but the other yogurts were similar in WI (Figure 5.2 (b)). Yogurt mixes treated at 85°C for 15 min, resulted in increased L* and a* values compared to untreated mixes and during incubation (43°C) further increase in L* and a* values were observed until pH 5, after which no further changes were observed (Needs et al., 2000). The WI equation comprises all the parameter of L*, a* and b*. However, the set-style plain yogurt is expected to be white in appearance. The a* and b* individually can be explained more clearly for colored food products.

Figure 5.2: Water holding capacity (a) and whiteness index (b) of various yogurts* collapsed for stored days (1, 15, 30, 45 and 60)



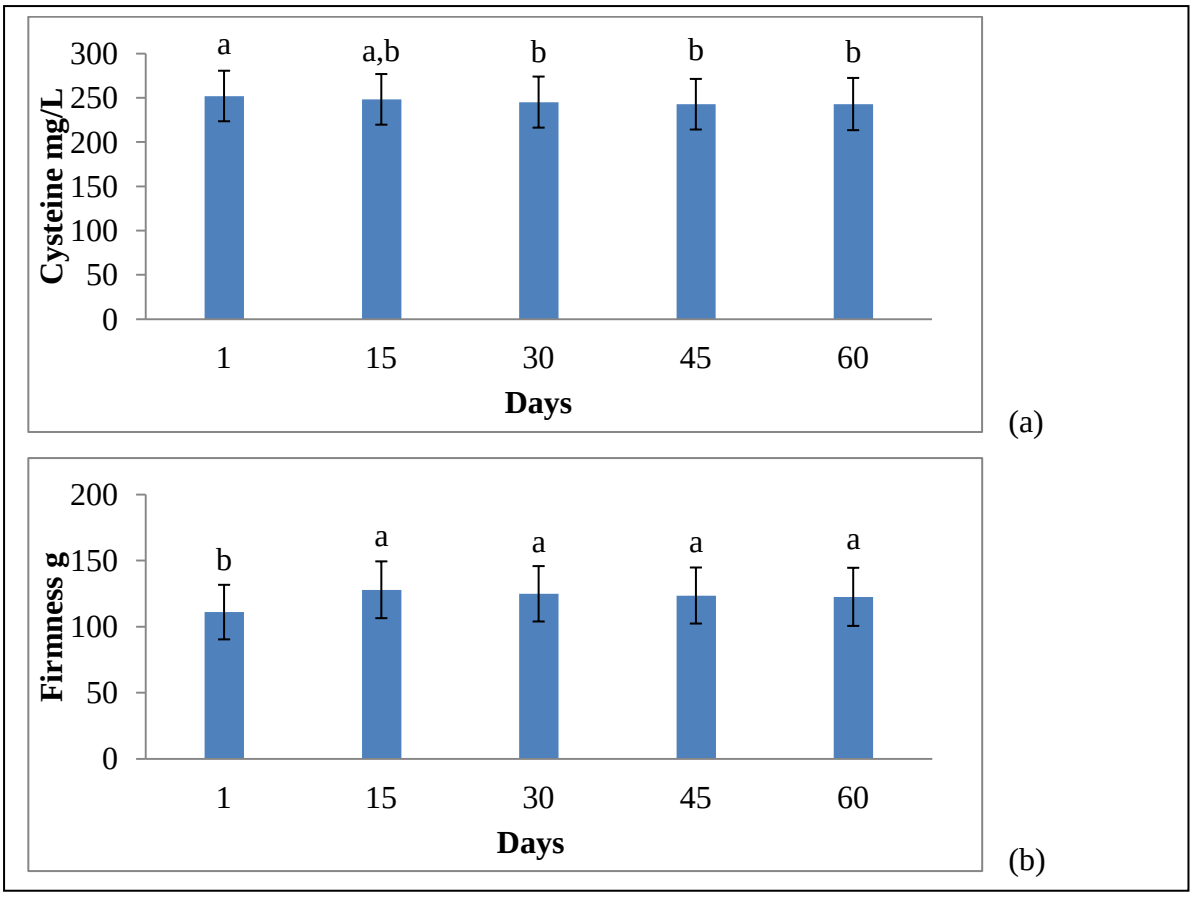
[a,b] Bars with different superscripts differ ($P \leq 0.05$), n = 15.
*N70: Nonfat dry milk treated at 70°C for 20 min, N90: Nonfat dry milk treated at 90°C for 7 min, W70: Nonfat dry milk + WPI treated at 70°C for 20 min; W90: Nonfat dry milk +WPI treated at 90°C for 7 min., WHC: Water holding capacity, WI: Whiteness index.

5.3.3.2 Day Effect

Cysteine contents, pH, firmness, syneresis, WHC and WI were affected by storage day. Overall, cysteine contents significantly decreased by 3% from day 1 to 30 and remained constant thereafter (Figure 5.3 (a)) and even though the same trend was observed for pH, the decrease was only 1% (Figure 5.4 (a)). Salvador and Fiszamna (2004) reported that yogurt pH decreased (2%) during 91 days of storage which agrees with the observations. Firmness of yogurts increased from day 1 to day 15 (111.0 and 127.9 g, respectively) (Figure 5.3 (b)) and was constant thereafter and these results agree with Salvador and Fiszamna (2004) who reported that firmness

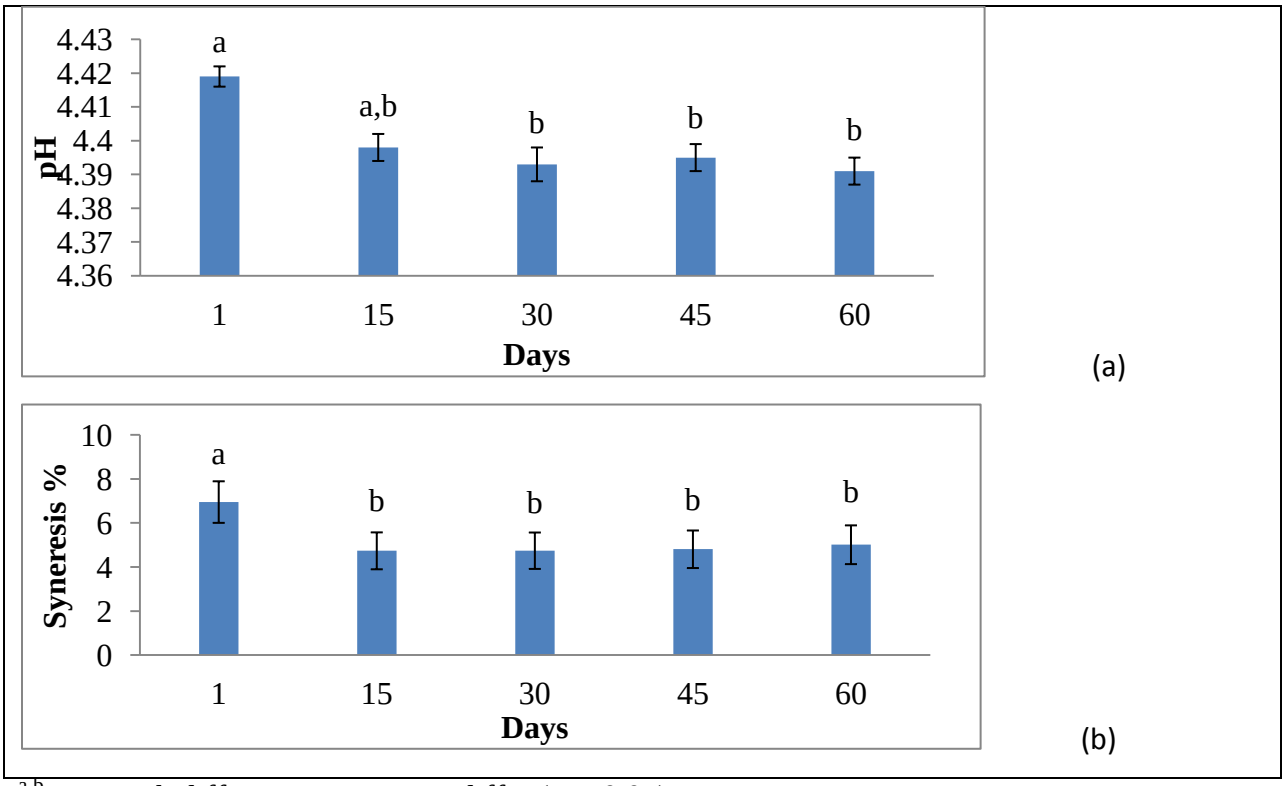
in yogurt significantly increased with storage (91 days) from 56.12 to 74.49 g. Syneresis also decreased by 32% from day 1 to 15 and then remained constant thereafter (Table 5.4 (b)). Serra et al (2009) reported that syneresis in yogurts decreased during the initial 15 day of storage and attributed the rearrangement of casein particles to a more thermodynamically stable state resulting in a more stable yogurt gel with less syneresis as the reason, which may explain the drop in syneresis from day 1 to 15.

Figure 5.3: Cysteine contents (a) and firmness (b) of yogurts stored up to 60 days collapsed for all 4 yogurt* products



[a,b] Bars with different superscripts differ ($P \leq 0.05$), n = 12.
*N70: Nonfat dry milk treated at 70°C for 20 min, N90: Nonfat dry milk treated at 90°C for 7 min W70: Nonfat dry milk + WPI treated at 70°C for 20 min; W90: Nonfat dry milk +WPI treated at 90°C for 7 min.

Figure 5.4: pH (a) and syneresis (b) of yogurts stored for up to 60 days collapsed for all 4 yogurt* products

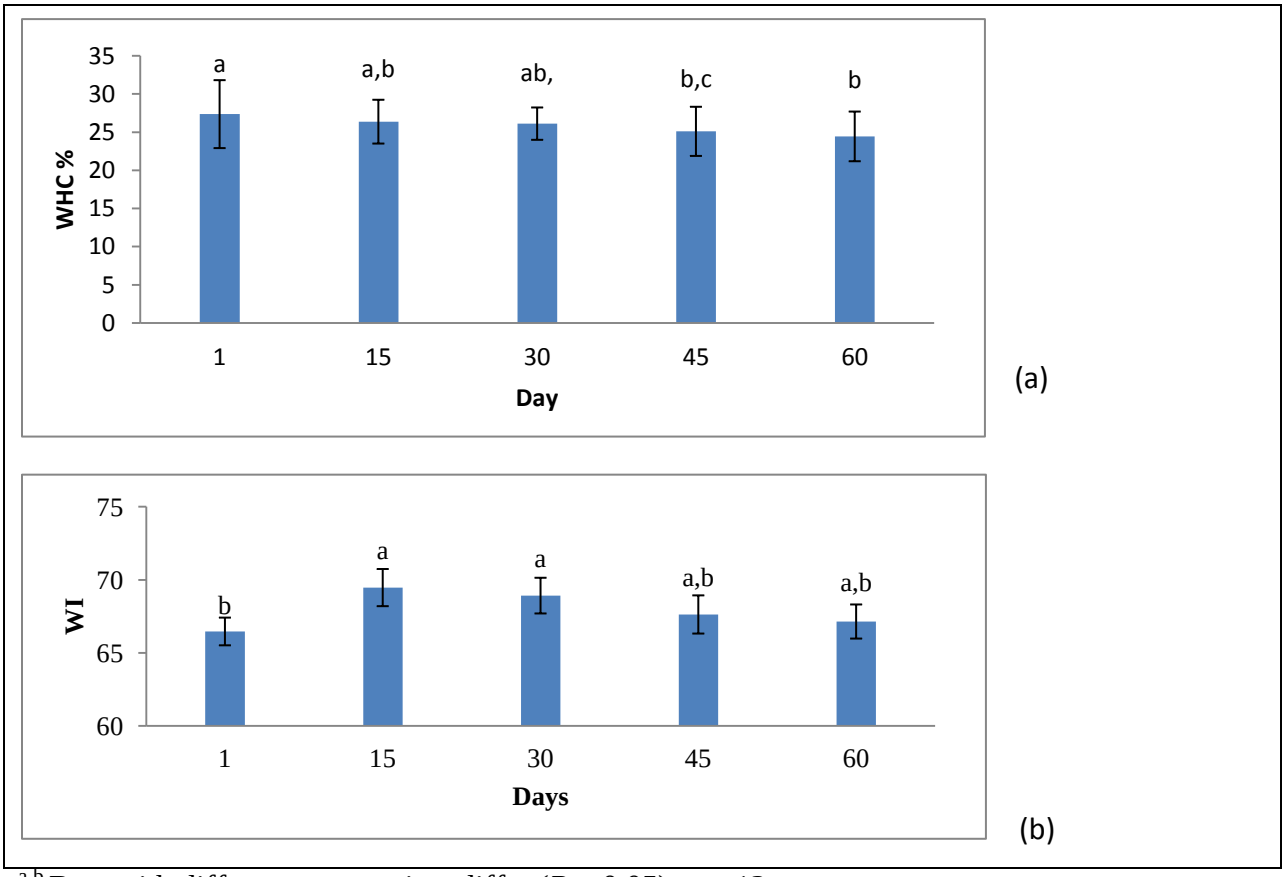


[a,b] Bars with different superscripts differ ($P \leq 0.05$), n = 12.
*N70: Nonfat dry milk treated at 70°C for 20 min, N90: Nonfat dry milk treated at 90°C for 7 min, W70: Nonfat dry milk + WPI treated at 70°C for 20 min; W90: Nonfat dry milk +WPI treated at 90°C for 7 min.

Water holding capacity was similar the first 30 days but decreased by 45/60 days on storage (Figure 5.5 (a)) and as reported by Salvador and Fiszman (2004) who explained that during WPD, casein micelles rearrange and the rearrangement deteriorates during storage resulting in release of bound water, thus, lowering the WHC. Whiteness index of yogurts increased by day 15 however, reached the original value by day 45 (Figure 5.5 (b)). Harte et al. (2003) reported that in milk, L* increased as a function of pasteurization which was caused by the increased casein micelle size. However if rearrangement of casein micelles occurred during

storage as suggested by the WHC and syneresis data – then this rearrangement may also be responsible for the changing WI of the products.

Figure 5.5: Water holding capacity (WHC) (a) and whiteness index (WI) (b) of yogurts stored for up to 60 days collapsed for all 4 yogurt* products



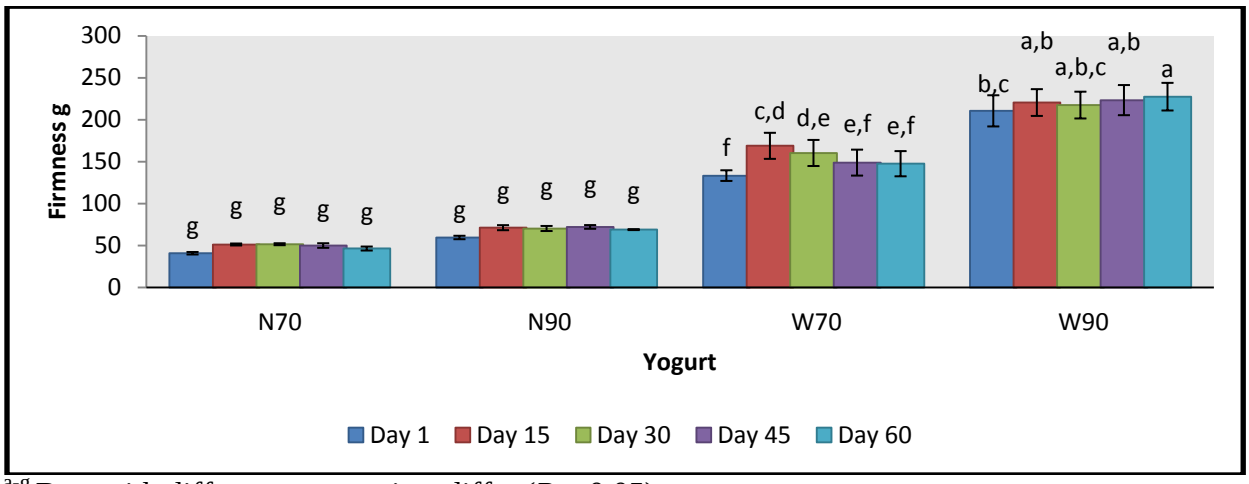
[a,b] Bars with different superscripts differ ($P \leq 0.05$), n = 12.
*N70: Nonfat dry milk treated at 70°C for 20 min, N90: Nonfat dry milk treated at 90°C for 7 min,W70: Nonfat dry milk + WPI treated at 70°C for 20 min; W90: Nonfat dry milk +WPI treated at 90°C for 7 min.

*5.3.3.3 Yogurt*Day Effect*

Significant interactions (yogurt*day) were observed for firmness, TA, WHC and WI ($P \leq 0.05$). Overall, the W70 and W90 yogurts were firmer than the N70 and N90 yogurt (Figure 5.6). The W70 yogurt increased in firmness (12%) by day 15 but decreased to the initial value by day

45; whereas the W90 yogurt increased in firmness (8%) from day 1 to 60. Firmness was consistent in N70 and N90 yogurts throughout storage. Salvador and Fiszman (2004) reported an increase in yogurt firmness (90%) during storage (91 days).

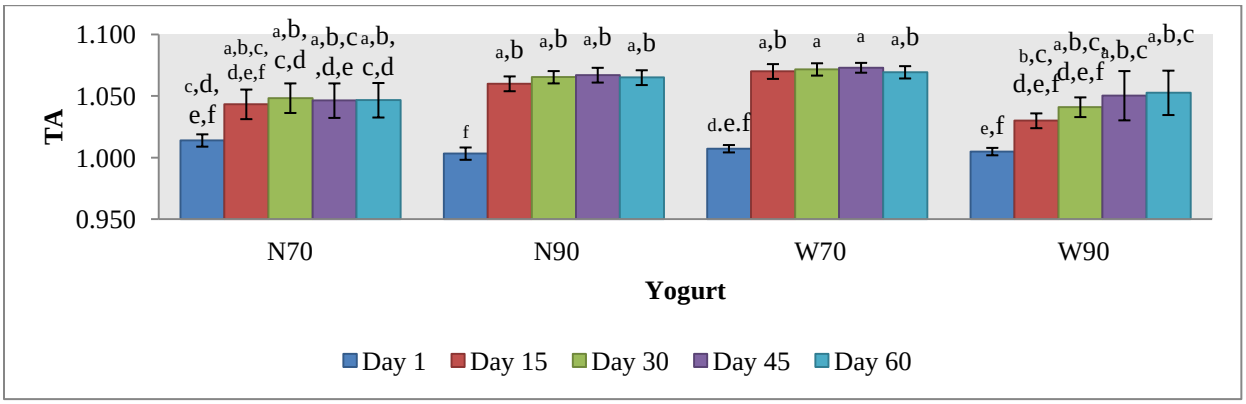
Figure 5.6: Firmness of various yogurts during a 60 days storage period



[a-g] Bars with different superscripts differ ($P \leq 0.05$).
N70: Nonfat dry milk treated at 70°C for 20 min, N90: Nonfat dry milk treated at 90°C for 7 min
W70: Nonfat dry milk + WPI treated at 70°C for 20 min; W90: Nonfat dry milk + WPI treated at 90°C for 7 min.

Titratable acidity of yogurts was the same (Figure 5.7) for all the yogurts on day 1 but significantly increased in the N90 and W70 yogurts by day 15 and in W90 yogurt by day 45, and remained constant thereafter. The N70 yogurt did not vary in TA during storage and as indicated by Sodini et al., 2005 that the increases in TA of stored yogurts were functions of continual acid production.

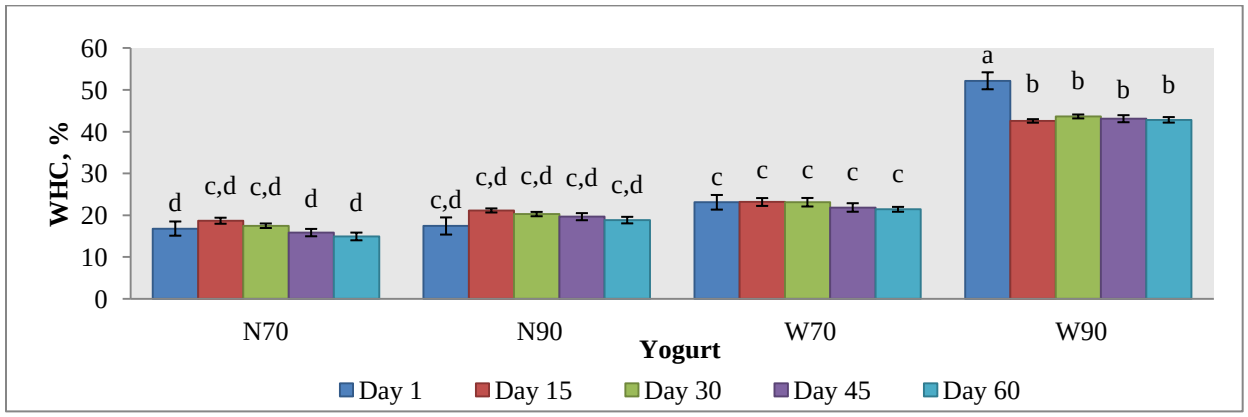
Figure 5.7: Titratable acidity (TA) of various yogurts during a 60 days storage period



[a-f] Bars with different superscripts differ ($P \leq 0.05$).
N70: Nonfat dry milk treated at 70°C for 20 min, N90: Nonfat dry milk treated at 90°C for 7 min
W70: Nonfat dry milk + WPI treated at 70°C for 20 min; W90: Nonfat dry milk + WPI treated at 90°C for 7 min.

Water holding capacity (Figure 5.8) was greatest in W90 yogurt and similar in the N90 and W70 yogurts throughout storage. The N70 yogurt had similar WHC as did the N90 yogurts throughout storage. The W90 yogurt with greatest WPD exhibited decreased (18%) WHC from day 1 to 15 and this agrees with work of Salvador and Fiszman (2004) who explained that during WPD, casein micelles rearrange and the rearrangement deteriorates during storage resulting in release of bound water, thus, lowering the WHC.

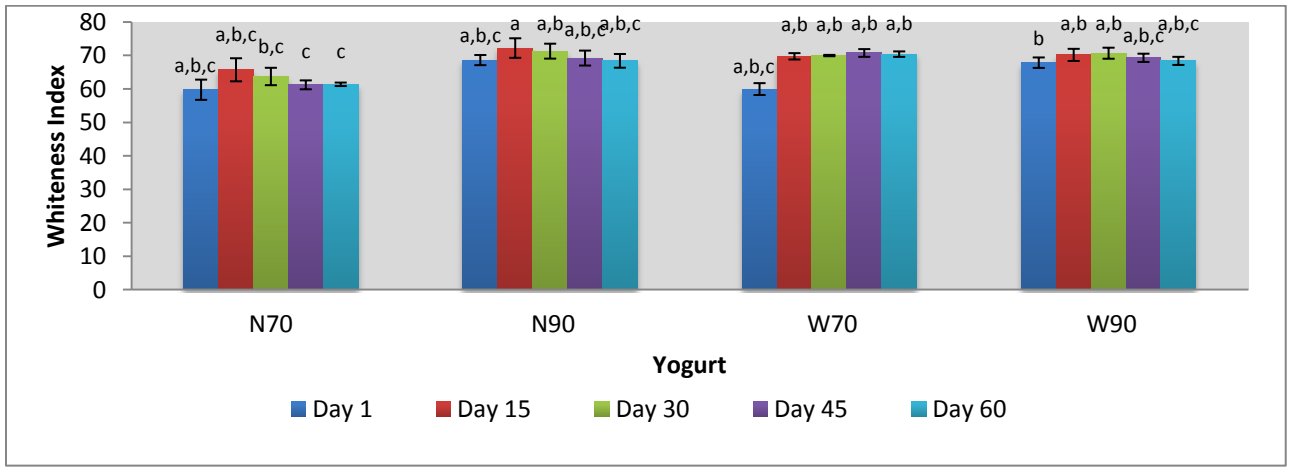
Figure 5.8: Water holding capacity (WHC) of various yogurts during a 60 days storage period



[a-f] Bars with different superscripts differ ($P \leq 0.05$).
N70: Nonfat dry milk treated at 70°C for 20 min, N90: Nonfat dry milk treated at 90°C for 7 min
W70: Nonfat dry milk + WPI treated at 70°C for 20 min; W90: Nonfat dry milk +WPI treated at 90°C for 7 min.

Whiteness index of yogurts did not vary with in the same type of yogurt but however, N70 yogurt had lower WI on day 45 and 60 when compared to yogurts day W70 and W90 yogurts. However, this difference might be due to the greater syneresis observed on N70 imparting the yellowness.

Figure 5.9: Whiteness index (WI) of various yogurts during a 60 days storage period



N70: Nonfat dry milk treated at 70°C for 20 min, N90: Nonfat dry milk treated at 90°C for 7 min
W70: Nonfat dry milk + WPI treated at 70°C for 20 min; W90: Nonfat dry milk +WPI treated at 90°C for 7 min.

Overall yogurts "deteriorated" during storage as predicted in literature – syneresis decreased, TA increased, firmness increased and WHC decreased (Salvador and Fiszman, 2004; Sodini et al., 2004). However, previous literatures showed an increase in syneresis but that was not observed in these yogurts. Also, similarity in results of the W70 yogurt (the most cysteine) and the N90 yogurt which was most similar to a commercially made yogurt which had similar processing temperature and formulations (Chandan, 2008). They exhibited similar values in WI, TA, and WHC throughout storage, but the firmness of the W70 yogurt was twice that of the N90 yogurts. When reviewing yogurt firmness that is considered as acceptable quality a market sample yogurt was purchased and evaluated with a result of 128 g which was similar with the W70 yogurt. Hence in this body of work addition of WPI in yogurt preparation have increased the cysteine content in yogurt by ~190%, combined with a less severe process treatment to control the WPD which ultimately controlled the gel structure so that the final yogurt was similar to a control yogurt. Furthermore, this enhanced cysteine product is stable during storage, as it exhibits very similar gel quality to a control. Although 3% of the cysteine is lost by 15 days it still delivered greater cysteine content than other yogurts during a 60 day shelf life; thus, this yogurt may be suitable for certain sub-populations wishing to increase their dietary cysteine contents.

5.4 Conclusions

Addition of WPI to a yogurt mix and minimum process condition affected both chemical and functional properties of final yogurt. Yogurt mix supplemented with WPI had increased protein and cysteine contents. Process treatments influenced the WPD and in turn the cysteine contents. The conventional process treatment of 90^{o}C for 7 min resulted in greater WPD and cysteine losses in yogurt mixes when compared to mixes processed at 70^{o}C for 20 min. Yogurt

containing WPI had similar textural properties when processed at lower temperature compared to yogurt with NDM and processed at 90^{o}C for 7 min. Yogurt with WPI and processed at 70^{o}C for 20 min had greater cysteine contents, firmer gels, greater water-holding capacities compared to yogurts formulated with NDM and processed at 90°C for 7 min. During storage, syneresis and cysteine contents varied during storage and decreased by day 15 but were stable thereafter. Overall, yogurt with WPI and processed at 70°C for 20 minutes had greater cysteine contents and acceptable gel quality. Further study of sensory evaluation is needed to evaluate the consumer acceptability.

Chapter 6 - Consumer Acceptance Evaluation of Cysteine Enhanced Yogurt

6.1 Introduction

Sensory evaluation is the measurement of a product's quality based on information received from the five senses; sight, smell, taste, touch and sound (Clark et al., 2009). Yogurt formulation and processing influence the body, texture and flavor of the end product ((Isleten and Yuceer, 2006; Kailasapathy and Supriadi, 1998). Research on the sensory quality of different types and flavors of yogurt has been conducted by both descriptive sensory analysis and consumer panels (Akalin et al. 2011; Antunes et al., 2005; Brown, 2010; Modler et al., 1983; Saint-Eve et al., 2006).

Descriptive sensory analysis is a method to quantify the perceived intensities of sensory characteristics by trained panelists (Lawless and Leymann, 2010). When assessing the sensory attributes of stirred strawberry-flavored yogurt with different ratios of proteins (sodium caseinate (**NaCn**) *vs*. whey proteins) the yogurts had different viscosities but flavor attributes were similar (Saint-Eve et al., 2006). Isleten and Yuceer (2006) reported that descriptive analysis results of yogurts made with additional skim milk powder (**SMP**), whey protein isolate (**WPI**), texture improver (**TI**) or NaCn varied. For instance, free whey was less but lumpiness and thickness were greatest in the WPI yogurt compared to the other yogurts. Also, the WPI yogurt had less fermented and creamier flavor scores than the other samples during a 12 day storage period. These lower flavor scores were attributed to the flavor-binding properties of whey proteins (Isleten and Yuceer, 2006). Gonzalez-Martinez et al. (2002) also used descriptive analysis to assess yogurts formulated with whey proteins and reported that yogurts with whey proteins were lump-free and had softer gel textures than yogurts prepared with SMP. More recently Brown

(2010) used descriptive analysis to evaluate 32 market-purchased, plain yogurts and reported that yogurts with ingredients such as whey protein had, significantly greater amount of off-flavors such as cardboard. Also the texture of yogurt varied with type of yogurt (set, stirred, or strained/Greek-style) as the Greek-style yogurt which was strained exhibited greater thickness and firmness. Also, the stirred yogurts had smoother consistency scores compared to others.

Consumer acceptance testing of yogurt has been used to determine and evaluate the liking of a variety of yogurt and yogurt products with panelist who are not trained. Modler et al. (1983) reported that yogurts containing whey protein concentrate (**WPC**) had acceptable texture and appearance scores when compared to yogurts containing other protein substitutes like casein and NaCN. In 2005, Antunes et al. reported that consumer results comparing the liking of set-style yogurt containing WPC (6%) and SMP were similar for appearance, flavor, texture and overall impression. Similarly, Akalin et al. (2011) reported that yogurt fortified with NaCN (1%) and WPC (1%) did not differ in appearance, taste and overall acceptability during storage (28 days); however, the texture of the yogurt with WPC was liked more than the yogurt with NaCN.

Whey proteins have been reported to influence the sensory properties of yogurt depending on the source and concentration. In particular the whey proteins have been reported to increase the chalkiness, thickness, lumpiness, whey flavor, creaminess and yellowness (Isleten and Yuceer, 2006; Kailasapathy and Supriadi, 1998). In the previous research (Chapter 5) yogurt mixes supplemented with WPI and processed to have less WPD resulted in yogurts with enhanced cysteine content, similar gel quality properties and similar storage stability as did a yogurt that was formulated and processed similar to a commercial yogurt. Although the WPI with minimal denaturation and flavor impact was chosen for the supplement, it was desired to know if consumers might like an enhanced cysteine yogurt. The characteristics of appearance,

thickness, flavor, after taste and overall acceptability were chosen to rate the yogurt as these were the factors which were influenced by the addition of the whey proteins. Thus, the objective of this research was to determine the liking of a high-cysteine yogurt.

6.2 Materials and Methods

6.2.1 Yogurt Manufacture

Low-heat nonfat dry milk (**NDM**) (Dairy America, Fresno, CA, USA), sugar (pure cane sugar, Domino Foods, Inc., Yonkers, NY, USA), vanillin (Midwest Country Fare, imitation vanilla flavoring, Westown Parkway, West Des Moines, IA, USA [Hyvee, Manhattan, KS, USA]), whey protein isolate (**WPI**) 895 (Glanbia Nutritionals, Fitchburg, WI, USA) and yogurt culture (Yo-Mix 495 LYO 250 DCU, DuPont, New Century, KS, USA) were obtained from commercial suppliers and maintained at -2 or -10^{o}C (culture) until usage. Two yogurts were formulated: the low cysteine (**LC**) consisted of 12.5% W/V NDM and 5% W/V sugar whereas the high cysteine (**HC**) consisted of 2.5% W/V WPI, 10% W/V NDM and 5% W/V sugar. These ingredients were mixed in deionized, distilled water at 22 to 24^{o}C for 30 min in Erlenmeyer flask (2 L) (Fisher Scientific, Pittsburgh, PA, USA) using a magnetic stir plate (Fisher Scientific). The LC mix was brought to 90^{o}C whereas the HC mix was brought to 70^{o}C on magnetic stir plate (Fisher Scientific) and then placed in a pre-heated water bath (Fisher Scientific) for 7 and 20 min, respectively. Process temperature of 70^{o}C for 20 min has been used by Allgeyer et al. (2010) in their research on a pasteurizaed of a yogurt drink that was considered safe for human consumption. Mixes were cooled to 43^{o}C and 1.6% W/V of vanillin flavor was added along with 0.6% W/V culture, stirred well and then packaged into sterile 120 mL cups (Fisher Scientific) or 5 L stainless steel bowls (Cuisinart CTG-00-SMB Stainless Steel Mixing Bowls, Cuisinart®, East Windsor, NJ, USA), covered and incubated at 43^{o}C ± 1 (Isotemp, Fischer Scientific) until pH

4.5- 4.6. Samples were removed from the incubator and placed in storage (RT18 DKXEN, Whirlpool Corporation, Benton Harbor, MI, USA) (4^{o}C ± 1). On the following day, the mixes were stirred with a hand mixer (Hamilton Beach, Hamilton Beach/Proctor-Silex, Inc., Washington, NC, USA) for 5 min at speed 3 and stored in the same containers and placed into storage 4^{o}C ± 1 until the day of evaluation (sensory on day 7, physical and chemical analysis on day 1).

6.2.2 Sensory Analysis

The LC and HC yogurts were evaluated for consumer acceptance. A consumer panel, consisting of 119 subjects ranging from 18 to 70 yrs (76 females and 43 males), were recruited from the general Kansas State University community. Panelists were screened on age (≥ 18 years), interest in consuming yogurt, and lack of any food allergies or intolerances. Panelists completed a consent form (see Appendix C) and once they were screened they completed a questionnaire on gender, family background and yogurt consumption frequency (Appendix C, Table C.1). After completion, a ballot consisting of a 9-point hedonic scale (1 = dislike extremely, 5 = neither like nor dislike, 9 = like extremely) for the characteristics: appearance, thickness, flavor, aftertaste and overall acceptability was given to the panelist (Lawless and Heymann, 1998). (An example of the paper ballots is shown in Appendix C, Table C.1). At the end of the ballot, panelists were asked if they were willing to buy the product (yes/no) and if they had any specific comments to share.

The stirred-style yogurts were prepared 7 days before the test (date 02/08/2013). About an hour prior to serving, approximately 15 g of each yogurt sample was placed in a (60 mL) plastic cup covered with a lid (Classic Sysco, Sysco Corporation, Houston, TX, USA) and returned to cold storage (4°C). All the samples were randomly coded with three-digit numbers,

and the serving order for panelists was randomized. Water was provided to clean the palate between samples. Once a sample was completed, the ballot was collected and then the second ballot and sample was distributed. At the completion of the study, panelists were given coupons for a free ice cream cone at Call Hall Dairy Store, Kansas State University, Manhattan, KS.

6.2.3 Chemical and Functional Properties

Consistency was determined by method from (Penna et al., 1997). The Bostwick consistometer (Bostwick consistometer 24925-000, CSC Scientific Company, INC, Fairfax, VA) was leveled by adjusting the screws at the bottom until the leveling bubble was at the centre. The yogurt was mixed with a spoon for 30 sec before filling the sample into sample reservoir. The product gate was closed before filling. The sample was filled up to the top of the sample reservoir. The product gate was opened and the distance (in cm) over which the material flowed at 4°C in 30 sec was recorded.

Cysteine was measured as described by (Shimada and Cheftel, 1989) using Ellman's reagent. Absorbance was measured at 412nm on Spectrophotometer (Thermo Electron Corporation, Spectronic Genrys 5, Madison, WI, USA). The concentration of thiols were calculated from the molar absorbance.

$$Co = \frac{A}{E} \mathbf{x} D$$

Co = Concentration of thiols, A = absorbance at 412 nm, E (extinction coefficient) = $1.36x10^4$ $cm^{-1}M^{-1}$, D = dilution factor

pH was measured using a Fisher universal glass pH electrode (Acumet Portable AP61, Fisher Scienctific) after calibration with standardized buffer solutions (Fisher Scientific), to pH 4 and 7 at $43^oC \pm 1$.

Firmness was determined using a modified method by Saint-Eve et al. (2006). Textural analysis is 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 5 mm/s, 5 sec and 10 mm, respectively.

Syneresis was determined as described by Damin et al. (2009). The syneresis was calculated as percent weight relative to original weight of yogurt.

$$\% \text{ Syneresis} = \frac{(\text{Initial yogurt weight} - \text{Remaining yogurt weight})}{\text{Initial yogurt weight}} \times 100$$

Whiteness index was calculated following modified methods (Schmidt et al. 2001; Vargas et al., 2008) .A Miniscan EZ, Model 4500L, (Hunter Associates Laboratory, Reston, Virginia, USA) Hunter D-54 Reflectance Lab Ultra Scan Sphere Spectrophotometer. Standardized using a white and black tile (X= 80.49, Y= 85.30 and Z = 88.35). Four surface readings for L, a^* and b^* were taken on each sample by rotating the sample at 90^o angles on yogurt sample with ~3 cm diameter cup at 4 ± 1°C and the averaged L, a^* and b^* values were obtained. Whiteness index (**WI**) was calculated and compared (Vargas et al., 2008).

$$WI = 100 - [(100-L^*)^2 + (a^*)^2 + (b^*)^2]^{½}$$

6.2.4 Experimental Design and Statistical Analysis

Based on the results of studies 1, 2 and 3 (Chapter 5), the objective of this study was to determine the liking of two yogurt formulations: LC and HC. Yogurt were reformulated to contain sugar and vanillin, but the HC yogurt was supplemented with 2.5% WPI and processed at 70°C for 20 min. And the LC yogurt was supplemented with NDM and processed at 90°C for 7 min.

The design was a randomized complete block design with yogurt being treatments (2) and panelists (112) as blocks. Statistical analysis was done using SAS version 9.3 (SAS®

Institute Inc., v 9.3, Cary, NC, USA). ANOVA results ($P < 0.05$) for significant effects were further analyzed. The following model was used:

$$Y_{ijk} = \mu + F_i + P_j + \varepsilon_{ij}$$

Where Y_{ijk} is the ijkth observation for kth replication

μ is the mean

F_i is the yogurt (LC or HC)

P_j is the panelist (main effects)

ε_{ij} is the random error.

where, i = 1, 2 ; j = 1, 2, 3,..112.

6.3 Results

Table 6.1 shows the chemical and functional properties of these yogurts. As only one batch was made – these scores reflect the one day's production; thus, no replications were made. As can be seen in Table 6.1, the LC and HC yogurts varied. The HC yogurt had a higher cysteine content, firmness and consistency but lower syneresis and WI than did the LC yogurt. However, the pH of yogurts was similar. The cysteine content of HC yogurt was greater (2 X) than the LC yogurt and these contents are comparable to those reported in study 1 (398 mg/L *vs*. 135 mg/L) for W70 (HC) and N90 (LC) yogurts, respectively (See Table 5.5).

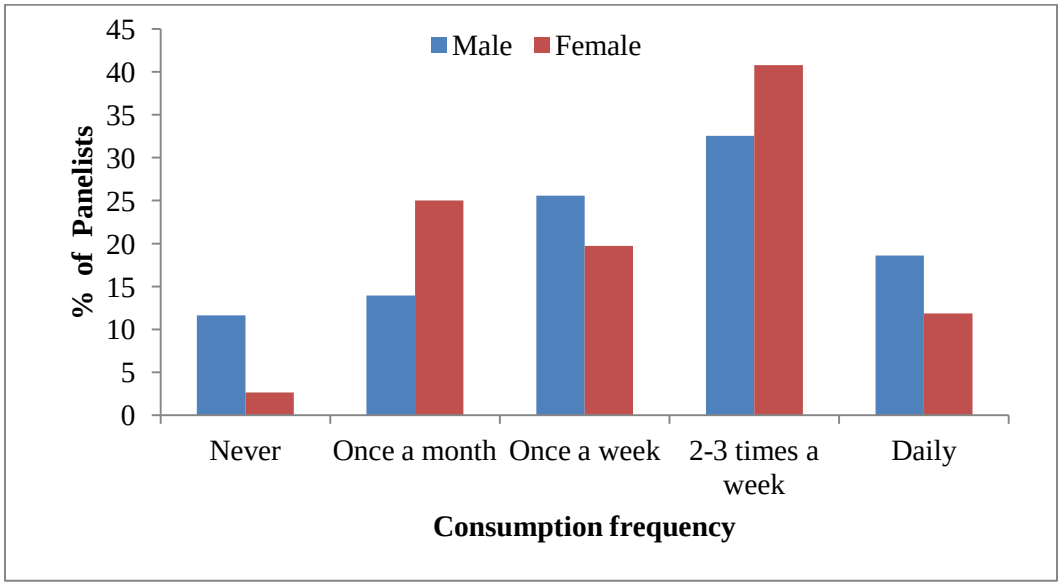
Table 6.1: Chemical and functional properties of yogurt samples

	Consistency cm	Cysteine mg/L	Firmness g	pH	Syneresis %	Whiteness Index
LC	10.1	128.5	25.63	4.49	5.065	68.22
HC	6.4	409.6	39.20	4.50	0.790	65.11

LC (Low cysteine yogurt): NDM and processed at 90°C for 7 min, HC (High cysteine yogurt): NDM + WPI and processed at 70°C for 20 min.

One hundred and nineteen panelists participated in the evaluation of which 24 were in age group of 18-20 yrs, 55 in age group 21-25 yrs, 20 in age group 26-40 yrs and 21 in age group > 40 yrs. Seventy-six females and 44 males participated. Frequency of consumption of yogurt was 17 panelists consumed daily, 45 consumed 2-3 times a week, 26 consumed once a week, 24 consumed once a month and 8 never consumed (Figure 6.1) (Appendix D, Table D.1). (Raw data and ANOVA results can be found in Appendix D).

Figure 6.1: Frequency of yogurt consumption by the panelists



Male : N=43, Female: N=76.

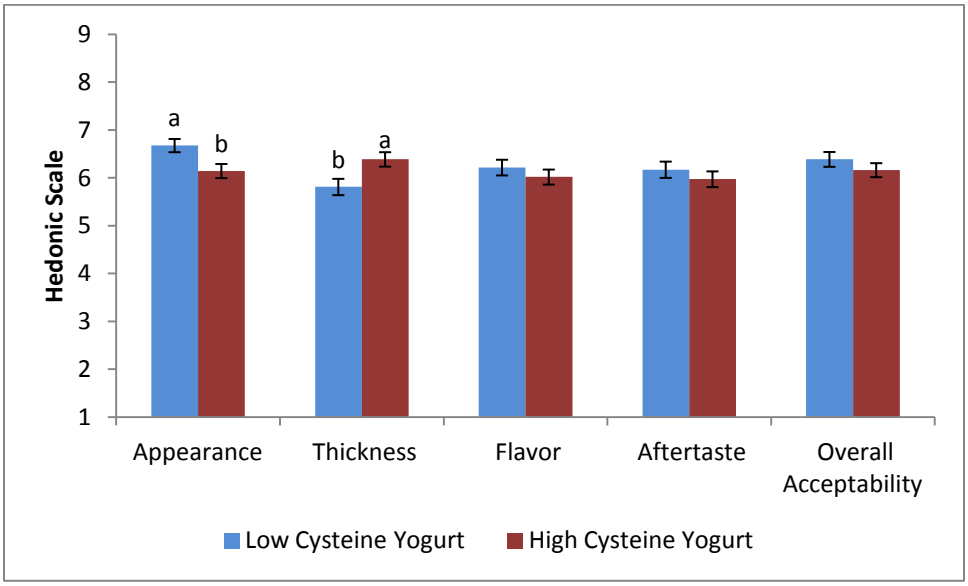
Panelists who were never consumed yogurt (n =7) were excluded for further evaluating tthe results based on Dr. Koushik Adhikari's recommendation. The mean data for 119 panelists is shown in (Table D.4, Appendix D). The consumer liking results for appearance, thickness, flavor, after taste and overall acceptance are shown in Figure 6.2. ANOVA results indicated no significant differences ($P > 0.05$) between LC and HC for the liking of overall acceptability ($P > 0.05$), flavor ($P > 0.05$) and after taste ($P > 0.05$); however, significant differences were observed in sensory scores for liking of appearance ($P < 0.05$) and thickness ($P < 0.05$) (Figure 6.2). The

appearance of LC yogurt had a mean score of 6.7 *vs*. a 6.2 for the HC yogurt. These numbers would fall between the words "like slightly" (6) to "like moderately" (7). The difference in liking of appearance may be due to the color properties of yogurt, as the HC yogurt had lower WI than did the LC yogurt (see Table 6.1 (WI: 65.11 *vs.* 68.22). Gonzalez et al. (2002) reported that yogurts containing 2% WPC instead of milk powder had lower L* values and according to WI formula the WI values depend on the L* value.

The thickness of the HC yogurt was liked more than the thickness of the LC yogurt (5.8 and 6.4, respectively). However, both scores fall better the words of "neither likes nor dislikes" (5) to "like moderately" (7). When consistency of yogurts was measured instrumentally the HC yogurt had almost double the consistency and 53% more firmness than the LC yogurt (Table 6.1).

The overall means for LC and HC yogurts for the liking of flavor, aftertaste and overall acceptability ranged from 6.1 to 6.4, which would fall between the words "like slightly" to "like moderately". The LC and HC yogurts did not vary in flavor (6.1), aftertaste (6.1) and overall acceptability (6.4) were in the range of almost "like slightly" when compared. These results were comparable to Antunes et al. (2005) who reported that consumer results of yogurt liking did not differ in appearance, flavor, texture and overall impression with addition of WPC.

Figure 6.2: Consumer liking study of yogurts



Mean bars with different letter notations are significantly different ($P \leq 0.05$) (n =112)
Low cysteine yogurt: NDM and processed at 90°C for 7 min, High cysteine yogurt: NDM + WPI and processed at 70°C for 20 min. 1-Dislike Extremely, 2-Dislike Very Much, 3-Dislike Moderately, 4-Dislike Slightly, 5-Neither Like nor Dislike, 6-Like Slightly, 7-Like Moderately, 8-Like Very Much
9-Like Extremely.

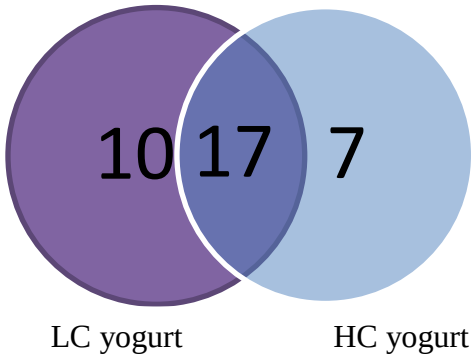
When asked for willingness to buy for LC and HC out of the 112 panelists yogurt 61% and 59% showed willingness to buy LC and HC yogurt, respectively (Table 6.2). The average overall liking scores of panelists who showed willingness to buy the yogurt are 6.8 for LC yogurt and 6.9 for HC yogurt. Table 6.3 shows the mean scores of panelists based on their willingness to buy the yogurt. In figures 6.3 and 6.4 are venn diagrams of panelists who showed willingness to buy the yogurts are shown. About 40% of the panelists showed willingness to buy both the yogurts.

Table 6.2: Number of people willing to buy the low cysteine and high cysteine yogurts.

	Low Cysteine Yogurt	High Cysteine Yogurt
Yes	68 (F:41, M:27)	67 (F:43, M:24)
No	44 (F:33, M:11)	45 (F:31, M:14)

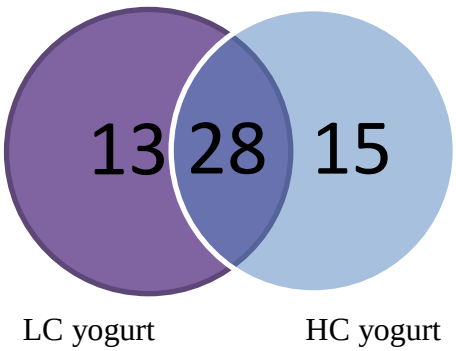
Total number of panelists =112. F: Female, M: Male. Low cysteine yogurt: NDM and processed at 90°C for 7 min, High cysteine yogurt: NDM + WPI and processed at 70°C for 20 min.

Figure 6.3: Venn diagram showing the number of male panelists who showed willingness to buy yogurts



Low cysteine yogurt: NDM and processed at 90°C for 7 min, High cysteine yogurt: NDM + WPI and processed at 70°C for 20 min

Figure 6.4: Venn diagram showing the number of female panelists who showed willingness to buy yogurts



Low cysteine yogurt: NDM and processed at 90°C for 7 min, High cysteine yogurt: NDM + WPI and processed at 70°C for 20 min

Table 6.3: Mean liking scores for low cysteine and high cysteine yogurts based on willingness to buy

Willingness to buy	Attribute	High cysteine yogurt	Low cysteine yogurt
Yes	Appearance	6.6	7.1
	Thickness	6.9	6.4
	Flavor	6.9	7.1
	Aftertaste	6.6	7.0
	Overall Acceptability	6.9	7.3
No	Appearance	5.4	6.0
	Thickness	5.6	4.8
	Flavor	4.8	4.8
	Aftertaste	5.0	4.9
	Overall Acceptability	5.0	5.0

Willingness to buy low cysteine yogurt Yes: 68, No: 44, high cysteine yogurt Yes: 67 No: 45. Low cysteine yogurt: NDM and processed at 90°C for 7 min, High cysteine yogurt: NDM + WPI and processed at 70°C for 20 min

6.4 Conclusions

A high cysteine yogurt with WPI, sweetened and vanillin flavored and processed at 70°C for 20 min affected the liking of appearance and texture. Both high cysteine and low cysteine yogurts were rated between the words of “like slightly” to “like moderately” for overall acceptability, whereas, liking of flavor and after taste of both the yogurts were rated as “liked slightly”. But yogurts varied in their liking of appearance and thickness. Liking of appearance of high cysteine yogurt was “liked slightly” whereas the low cysteine yogurt was “liked moderately” which might reflect the whiteness of yogurt. Thickness of high cysteine yogurt was “liked moderately” whereas the low cysteine yogurt was “liked slightly”. Instrumentally, the

consistency of high cysteine yogurt was greater. However, the willingness to buy high cysteine yogurt (61%) was comparable to low cysteine yogurt (59%). Further studies may be needed to improve the visual characteristics of the yogurt which doesn't impart any color to the products and also the animal studies to see the impact of a food with increased cysteine content can increase glutathione content in tissue or muscles.

Chapter 7 - Research Summary

Cysteine contents of the yogurt were dependent on the type of dairy powder added (nonfat dry milk (**NDM**) *vs.* whey protein isolate (**WPI**)) and the processing temperature. Yogurt mixes with WPI had increased cysteine contents; however, this amount decreased (22.0 and 52.7%) when processed at 70°C for 20 min and 90°C for 7 min, respectively. Yogurt supplemented with WPI and processed at 70°C for 20 min had significantly greater cysteine content (190%), firmness (123%) but similar syneresis and water holding capacity (**WHC**) than did the yogurt with nonfat dry milk (NDM) and processed at 90°C for 7 min (control). During storage the yogurt with WPI and processed at 70°C for 20 min varied in yogurt gel properties. An increase in firmness (12%) and titratable acidity was observed. Although 3% of the cysteine is lost during a 60 day shelf life – that is consistent for all yogurts; thus, this yogurt may be suitable for certain sub-populations wishing to increase their dietary cysteine contents.

The cysteine enhanced yogurt was evaluated for consumer acceptance after reformulation with sugar and flavor (vanillin). The yogurt supplemented with WPI and lower WPD but enhanced cysteine content compared to the yogurt without WPI. Both the yogurts had similar pH; however, the high cysteine yogurt (one rep) had higher firmness, consistency and less syneresis than the low cysteine yogurt. The consumer panel liked the flavor and after taste of both yogurts slightly. However the appearance of high cysteine yogurt was “liked slightly” whereas the low cysteine yogurt was “liked moderately”; and the thickness of high cysteine yogurt was “liked moderately” and the low cysteine yogurt was “liked slightly”. However, both the yogurts had similar willingness to buy.

In summary, yogurts supplemented with WPI and having less WPD had greater cysteine contents (190%) and comparable gel quality to a yogurt made without WPI and higher WPD. Though the firmness of yogurt with WPI was double that of the other yogurt, it was comparable to a market bought sample. A consumer liking test showed similar results for both yogurts for flavor, aftertaste and overall acceptability, greater liking for thickness but a lower liking for appearance of the high cysteine yogurt. Further studies may be needed to optimize the WPI addition to improve the visual characteristics of the yogurt which don't impart any color to the products and also in vivo and vitro studies are needed to understand the actual increase in glutathione by consumption of cysteine enhanced yogurt.

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Appendix A - Calculations

Table A.1: Amount of ellmen's reagent, buffer and sample for cysteine analysis

	Ellmen's Reagent mL	Buffer mL	Sample mL
NDM	0.03	2.93	0.07
NDM + WPI	0.03	2.97	0.03

NDM : Nonfat dry milk, NDM+ WPI: Nonfat dry milk and whey protein isolate

Appendix B - Raw data and ANOVA results (Experiment 1)

Table B.1: Yogurt properties measured as chemical and physical properties of yogurts on day 1, 15, 30, 45 and 60.

Day	Yogurt	Rep	Syneresis (%)	L*	a*	b*	WHC (%)	Firmness (g)	TA	pH	Cysteine (mg/L)	WI
1	N70	1	10.9	70.11	-2.12	11.87	20.13	43.69	1.010	4.42	224.5	67.77
1	N70	2	13.0	63.23	-3.15	16.04	15.72	39.71	1.009	4.43	235.8	59.76
1	N70	3	10.6	71.68	-2.06	10.85	14.57	38.87	1.023	4.42	230.5	69.60
1	N90	1	7.05	70.33	-1.95	11.09	21.48	59.08	1.005	4.44	130.5	68.26
1	N90	2	7.40	68.08	-1.70	12.76	14.82	63.29	1.011	4.43	137.2	65.58
1	N90	3	7.02	72.57	-1.82	9.78	15.98	56.23	0.994	4.42	139.5	70.82
1	W70	1	4.78	64.59	-1.57	10.92	25.89	120.6	1.008	4.40	408.7	62.91
1	W70	2	7.76	70.60	-1.81	10.82	19.88	138.5	1.012	4.41	410.7	68.62
1	W70	3	5.02	65.56	-1.73	11.04	23.55	140.7	1.002	4.41	375.5	63.79
1	W90	1	2.29	70.67	-1.44	9.58	55.55	173.7	1.000	4.41	244.9	69.11
1	W90	2	3.61	66.17	-2.22	12.64	48.52	233.1	1.010	4.42	252.6	63.82
1	W90	3	3.96	69.15	-1.81	10.19	52.45	224.7	1.005	4.42	232.5	67.46
15	N70	1	8.86	68.49	-2.66	14.21	19.97	49.28	1.050	4.40	220.6	65.33
15	N70	2	8.74	63.79	-2.76	16.85	17.50	50.98	1.020	4.42	233.6	59.96
15	N70	3	8.61	75.23	-2.62	13.05	18.59	53.34	1.060	4.40	225.9	71.88
15	N90	1	5.20	68.43	-1.82	11.09	22.12	65.26	1.070	4.42	126.9	66.49
15	N90	2	5.47	77.44	-1.66	12.58	20.73	74.94	1.060	4.40	130.5	74.11
15	N90	3	6.51	78.74	-1.92	10.83	20.59	73.49	1.050	4.40	129.7	76.06
15	W70	1	3.30	73.27	-1.91	11.92	24.92	137.9	1.080	4.38	400.5	70.67
15	W70	2	2.83	70.32	-1.85	12.22	21.69	181.1	1.070	4.40	409.0	67.85
15	W70	3	2.05	73.14	-1.88	11.49	22.89	187.3	1.060	4.40	370.6	70.73
15	W90	1	1.68	68.09	-1.51	9.49	42.60	189.1	1.020	4.38	240.6	66.67
15	W90	2	1.78	74.64	-2.01	9.76	41.82	230.5	1.030	4.37	258.6	72.75
15	W90	3	1.72	72.82	-1.78	9.69	43.29	241.5	1.040	4.40	230.9	71.09
30	N70	1	8.94	67.89	-2.89	14.21	17.01	46.55	1.052	4.40	210.8	64.77
30	N70	2	8.88	62.50	-2.73	16.85	16.89	52.89	1.026	4.41	242.2	58.79
30	N70	3	8.55	70.46	-2.51	13.05	18.56	55.06	1.067	4.40	220.5	67.61
30	N90	1	5.22	68.93	-1.00	11.09	20.76	63.87	1.075	4.42	120.6	67.00
30	N90	2	5.67	75.48	-1.77	12.58	20.83	75.15	1.063	4.39	132.6	72.39
30	N90	3	5.98	76.97	-1.85	10.83	19.24	71.80	1.058	4.41	124.5	74.48
30	W70	1	3.49	72.67	-2.00	11.92	25.13	122.2	1.082	4.37	383.2	70.12
30	W70	2	2.77	72.91	-1.90	12.22	22.39	175.2	1.068	4.39	410.8	70.22
30	W70	3	2.19	71.89	-1.79	11.49	21.84	183.4	1.065	4.41	372.5	69.58
30	W90	1	1.70	69.55	-1.58	9.49	44.39	189.7	1.025	4.38	237.8	68.06

30	W90	2	1.81	75.68	-1.98	9.76	42.78	232.4	1.045	4.36	248.9	73.72
30	W90	3	1.64	71.92	-1.82	9.69	43.74	230.0	1.053	4.39	235.4	70.24
45	N70	1	9.21	66.54	-2.16	13.46	14.46	44.49	1.051	4.40	212.5	63.87
45	N70	2	8.69	63.09	-1.89	14.83	15.59	53.12	1.020	4.41	240.8	60.18
45	N70	3	8.72	61.72	-2.89	12.50	17.49	52.25	1.068	4.41	215.7	59.63
45	N90	1	5.30	67.12	-1.27	10.99	21.31	76.51	1.078	4.41	121.7	65.31
45	N90	2	5.90	71.59	-1.46	11.48	19.17	69.71	1.067	4.40	130.7	69.32
45	N90	3	6.25	74.82	-1.69	9.48	18.49	70.14	1.056	4.41	122.4	73.05
45	W70	1	3.41	74.81	-2.27	10.45	23.85	119.5	1.081	4.39	373.3	72.64
45	W70	2	2.86	70.89	-1.89	11.50	21.24	172.0	1.072	4.37	408.2	68.65
45	W70	3	2.15	72.99	-1.83	10.48	20.49	155.0	1.066	4.41	382.8	70.97
45	W90	1	1.68	69.55	-1.69	9.89	41.76	188.5	1.026	4.38	240.5	67.94
45	W90	2	1.85	73.48	-2.08	9.48	42.87	248.4	1.036	4.38	239.7	71.76
45	W90	3	1.66	69.74	-1.97	9.60	44.66	232.9	1.089	4.40	222.5	68.19
60	N70	1	10.02	65.08	-2.46	14.89	13.79	41.86	1.055	4.40	210.3	61.96
60	N70	2	8.98	64.48	-1.87	13.79	14.24	47.34	1.020	4.40	222.5	61.85
60	N70	3	8.80	62.90	-2.78	13.70	16.76	49.91	1.065	4.40	218.4	60.35
60	N90	1	5.48	66.57	-1.29	11.42	20.24	69.29	1.075	4.40	120.5	64.65
60	N90	2	5.91	70.79	-1.48	10.76	18.75	67.79	1.065	4.39	131.6	68.83
60	N90	3	6.52	73.73	-1.87	10.32	17.54	69.71	1.055	4.40	120.3	71.71
60	W70	1	3.39	73.73	-2.30	9.45	22.39	119.5	1.078	4.39	383.5	71.99
60	W70	2	3.12	71.31	-1.87	10.77	20.39	171.0	1.070	4.38	410.6	69.29
60	W70	3	2.45	71.49	-1.95	9.37	21.50	152.0	1.060	4.40	392.1	69.92
60	W90	1	1.89	68.38	-1.89	8.84	42.93	194.9	1.035	4.37	243.0	67.11
60	W90	2	1.81	72.43	-2.07	9.38	41.37	239.0	1.034	4.37	235.2	70.80
60	W90	3	1.78	68.75	-2.71	9.49	43.59	248.2	1.089	4.40	225.2	67.23

Rep: Replication, WHC: Water holding capacity, TA: Titratable Acidity, WI: Whiteness Index. N70: Nonfat dry milk(12.5%) rehydrated and processed at 70^{o}C for 20 min, N90: Nonfat dry milk (12.5%) rehydrated and processed at 90^{o}C for 7 min W70: Nonfat dry milk (10%) + WPI (2.5%) rehydrated and processed at 70^{o}C for 20 min; W90: Nonfat dry milk (10%) +WPI (2.5%) rehydrated and processed at 90^{o}C for 7 min.

Table B.2: Protein content, Cysteine content and pH of yogurt mixes.

Treatment	Formulation	Rep	Total protein (%)	True protein (%)	Casein (Change) (%)	Whey (Change) (%)	NPN (%)	Cysteine (mg/L)	pH
UT	N	1	4.347	4.296	3.514	0.782	0.051	304.6	6.510
UT	N	2	4.160	4.121	3.361	0.760	0.039	310.1	6.540
UT	N	3	4.160	4.089	3.325	0.764	0.071	306.2	6.480
UT	W	1	5.567	5.506	3.239	2.267	0.061	508.4	6.350
UT	W	2	5.638	5.520	3.291	2.229	0.119	506.5	6.460
UT	W	3	5.638	5.520	3.334	2.186	0.119	499.9	6.410
70	N	1	4.608	4.542	3.845	0.697	0.066	230.4	6.270

70	N	2	4.487	4.450	3.798	0.652	0.037	236.4	6.360
70	N	3	4.477	4.402	3.739	0.663	0.075	232.6	6.250
90	N	1	4.393	4.348	4.056	0.292	0.045	130.9	6.190
90	N	2	4.556	4.505	4.238	0.267	0.051	139.2	6.100
90	N	3	4.418	4.348	4.124	0.224	0.070	145.5	6.240
70	W	1	5.986	5.915	3.800	2.115	0.071	412.6	6.350
70	W	2	5.783	5.664	3.467	2.197	0.119	411.9	6.330
70	W	3	5.686	5.578	3.595	1.983	0.109	376.0	6.280
90	W	1	5.451	5.398	4.994	0.404	0.053	245.7	6.240
90	W	2	5.728	5.608	5.193	0.415	0.120	254.8	6.420
90	W	3	5.715	5.602	5.226	0.376	0.113	236.5	6.310

Rep: Replication, NPN: Non protein nitrogen, UT: Untreated, 70: 70°C for 20 min, 90: 90°C for 7 min, N: Mix with 12.5% NDM and non-processed, W: Mix with NDM (10%) and WPI (2.5%) and non-processed.

Table B.3: Whey protein denaturation of yogurt mixes and total solids content of yogurt

Treatment	Formulation	Rep	WPD (%)	TS (%)
70	N	1	15.00	12.19
70	N	2	12.93	12.37
70	N	3	13.50	12.23
90	N	1	68.83	12.26
90	N	2	71.18	12.25
90	N	3	70.83	12.39
70	W	1	4.175	12.40
70	W	2	4.469	12.40
70	W	3	1.702	12.36
90	W	1	83.60	12.40
90	W	2	82.99	12.40
90	W	3	79.18	12.29

Rep: Replication, WPD: Whey protein denaturation, TS: Total solids, 70: 70°C for 20 min, 90: 90°C for 7 min, N: Mix with 12.5% NDM and non-processed, W: Mix with NDM (10%) and WPI (2.5%) and non-processed.

Table B.4: P values for protein, cysteine and pH in non-processed and processed mixes

	P (Formulation)	P (Process)	P (Formulation x Process)
Cysteine	<0.0001	<0.0001	<0.0001
pH	0.0004	0.0248	0.0113

Table B.5: P values for whey protein denaturation of non-processed yogurt mixes

	P (Formulation)	P (Process treatment)	P (Formulation x Process treatment)
Whey protein denaturation	0.5085	<0.0001	<0.0001

Table B.6: P values for chemical and functional properties of yogurt

	P (Formulation)	P(Process treatment)	P (Formulation x Process treatment)
Syneresis	<0.0001	0.0004	0.1164
WHC	<0.0001	<0.0001	0.0002
Firmness	<0.0001	0.0004	0.0027
WI	0.6393	0.3469	0.8481
TA	0.3388	0.7409	0.3388
TS	0.8846	0.0783	0.5210
pH	0.0131	0.1009	0.7119
Cysteine	<0.0001	<0.0001	<0.0001

WHC: Water holding capacity, WI: Whiteness Index, TA: Titratable Acidity, TS: Total Solids

Table B.7: P values for chemical and functional properties of yogurt during storage

	P (Yogurt)	P(day)	P (Yogurt x day)
Syneresis	<0.0001	<0.0001	0.0671
WHC	<0.0001	<0.0001	<0.0001
Firmness	<0.0001	<0.0001	0.0014
TA	0.0832	<0.0001	0.0013
pH	0.0029	<0.0001	0.2096
Cysteine	<0.0001	0.0007	0.7082
WI	0.009	0.0046	0.0249

Appendix C - Consent, Demographics and Ballot Sheets

Table C.1 Statement of informed consent, demographics sheet and ballot

Statement of informed consent

You are being asked to take part in a research project as a member of sensory panel to evaluate the characteristics of yogurt*. The purpose of this study is to evaluate two different yogurts. No discomforts or risks are anticipated. No individuals in this project will be identified in any report or publication about this study. The expected length of time of your participation is ~10 minutes.

I have read the information provided above. I have had the opportunity to ask, and have had answered, all my questions about this study. I voluntarily agree to participate in this study. I understand that I will receive a copy of this form after it has been signed if I want one.

If I have questions about the rationale or method of the study, I understand that I may contact Karen Schmidt, 224 Call Hall, or by phone at (785)532-1216.

If I have any questions about the rights of subjects in this study or about the manner in which the study is conducted, I may contact the Chair, Committee on Research Involving Human Subjects, 203 Fairchild Hall, Kansas State University, Manhattan, KS 66506, at (785)532-3224.

_____ _____

Signature of Panelist Date

_____

Name of Panelist (Print)

***CONTAINS MILK AND OTHER DAIRY PRODUCTS.**

Demographics/Consumption (Please complete by circling the correct response)

1. **Age**

   A) 18 - 20 (yrs) B) 21 - 25 (yrs)

   C) 26 - 40 (yrs) D) >40 (yrs)

2. **Gender**

   A) Female B) Male

3. **Marital Status**

   A) Married B) Single

4. **Ethnicity**

   A) African American B) Asian

   C) Caucasian D) Hispanic

   E) Others

5. **How frequently do you eat yogurt?**

   A) Daily B) 2 to 3 times/week

   C) Once a week D) Once a month

   E) Never

6. **Do you have any food allergy?**

   A) No B) Yes

Please circle the best response for the yogurt sample **615** using a 9-point hedonic scale

1-Dislike Extremely	*4-Dislike Slightly*	*7-Like Moderately*
2-Dislike Very Much	*5-Neither Like nor Dislike*	*8-Like Very Much*
3-Dislike Moderately	*6-Like Slightly*	*9-Like Extremely*

A. Appearance

1	2	3	4	5	6	7	8	9
Dislike Extremely				*Neither Like nor Dislike*				*Like Extremely*

B. Thickness

1	2	3	4	5	6	7	8	9
Dislike Extremely				*Neither Like nor Dislike*				*Like Extremely*

Extremely

C. Flavor

1	2	3	4	5	6	7	8	9
Dislike Extremely				*Neither Like nor Dislike*				*Like Extremely*

D. After Taste

1	2	3	4	5	6	7	8	9
Dislike Extremely				*Neither Like nor Dislike*				*Like Extremely*

Extremely

E. Over all Acceptance

1	2	3	4	5	6	7	8	9
Dislike Extremely				*Neither Like nor Dislike*				*Like Extremely*

F. If this product was available for sale would you buy it?

1) No 2) Yes

Any Comments:

Appendix D - Raw data and ANOVA results (Experiment 2)

Table D.1: Demographics

Age	18-20 yrs	21-25 yrs	26-40 yrs	>40yrs	
	24	55	20	21	
Gender	Female	Male			
	76	44			
Marital Status	Married	Single			
	33	87			
Ethnicity	African American	Asian	Caucasian	Hispanic	Others
	6	25	80	5	4
Frequency	Daily	2 to 3	once a week	once a month	never
	17	45	26	24	8

Table D.2: Consumer acceptance study raw data

		Appearance	Thickness	Flavor	Aftertaste	Overall Acceptability	Buy
1	C	7	3	7	7	7	Y
2	C	7	5	4	4	5	N
3	C	3	1	2	2	2	N
4	C	7	7	7	7	7	Y
6	C	6	4	7	9	7	Y
7	C	7	7	8	7	7	Y
8	C	7	6	4	4	6	N
9	C	6	6	4	5	7	N
10	C	7	6	8	7	7	Y
11	C	9	9	7	7	7	N
12	C	8	6	9	8	8	Y
13	C	6	4	6	5	6	Y
14	C	4	4	4	3	4	N
15	C	9	8	9	9	9	Y
16	C	7	6	7	7	8	Y
17	C	8	7	7	8	8	Y
18	C	7	6	8	6	7	Y
19	C	7	6	8	6	8	Y
20	C	5	4	5	6	5	N
21	C	7	4	7	5	6	N
22	C	7	6	8	6	8	Y

23	C	5	4	7	6	6	N
24	C	7	7	5	5	6	N
25	C	7	3	4	7	5	N
26	C	6	5	2	2	3	N
27	C	7	9	7	5	8	Y
28	C	9	3	6	6	6	N
29	C	7	6	4	8	6	Y
30	C	5	6	7	5	6	N
31	C	9	8	8	9	7	Y
32	C	8	8	9	8	9	Y
33	C	8	8	6	6	7	Y
34	C	6	8	5	8	8	Y
35	C	7	7	7	7	7	Y
36	C	8	8	9	6	9	Y
37	C	8	7	7	5	7	Y
38	C	8	7	8	9	8	Y
39	C	6	5	2	2	2	N
40	C	6	5	8	8	8	Y
41	C	4	4	7	6	6	Y
42	C	5	7	8	4	7	Y
43	C	6	4	4	6	6	N
44	C	5	4	4	6	4	N
45	C	5	6	6	7	6	Y
46	C	8	7	7	8	7	Y
47	C	8	8	7	7	7	Y
48	C	5	6	4	4	6	Y
49	C	8	8	6	8	8	Y
50	C	9	7	9	9	9	Y
51	C	8	9	9	9	9	Y
52	C	6	6	4	5	5	N
53	C	9	7	4	4	4	N
54	C	6	6	4	3	3	N
55	C	7	5	8	8	7	N
56	C	8	7	8	8	8	Y
57	C	5	3	5	5	4	N
58	C	8	6	8	6	8	Y
59	C	7	7	7	5	7	Y
60	C	6	4	6	7	5	N
61	C	9	9	8	7	8	Y
62	C	7	6	5	6	6	N
63	C	5	6	4	5	6	N
64	C	8	6	5	6	6	Y

65	C	7	5	5	6	6	N
66	C	8	6	6	7	7	Y
67	C	7	5	5	6	7	Y
68	C	7	7	6	5	5	N
69	C	7	3	6	6	6	Y
70	C	7	4	5	8	6	Y
71	C	8	8	7	5	6	N
72	C	6	3	7	7	6	N
73	C	8	7	7	5	6	Y
74	C	9	9	6	7	7	Y
75	C	4	6	6	6	5	N
76	C	6	6	6	6	6	N
77	C	7	7	8	8	8	Y
78	C	4	3	7	7	6	N
79	C	9	5	5	4	5	N
80	C	7	7	6	7	7	N
81	C	5	6	6	5	6	Y
82	C	5	3	7	6	6	Y
83	C	8	5	5	5	4	N
84	C	8	7	8	5	8	Y
85	C	9	8	9	9	9	Y
86	C	8	9	8	8	8	Y
87	C	6	4	6	7	6	Y
88	C	8	7	6	6	7	Y
89	C	7	8	8	8	8	Y
90	C	7	9	8	9	9	Y
91	C	7	4	6	9	8	Y
92	C	6	6	7	6	7	Y
93	C	6	6	7	7	7	Y
94	C	7	5	6	6	6	N
95	C	7	7	6	7	6	Y
96	C	5	3	5	5	5	N
97	C	4	3	3	2	2	N
98	C	4	4	7	7	7	N
99	C	8	6	7	8	8	Y
100	C	4	6	8	6	7	Y
101	C	5	3	6	3	3	N
102	C	3	2	3	2	3	N
103	C	6	7	6	6	7	Y
104	C	6	6	8	7	8	Y
105	C	9	7	8	8	8	Y
106	C	8	5	5	4	5	Y

107	C	5	3	4	7	4	N
108	C	7	4	6	8	7	Y
109	C	6	1	2	4	3	N
110	C	6	5	4	2	5	N
111	C	7	8	6	5	6	N
112	C	7	6	7	6	7	Y
113	C	5	6	4	3	3	N
114	C	7	6	7	7	7	Y
115	C	5	6	8	8	7	Y
116	C	8	3	7	7	7	Y
117	C	7	5	7	8	7	Y
118	C	7	5	6	6	5	N
119	C	6	6	7	5	6	Y
120	C	4	4	5	5	6	N
1	E	7	6	7	7	7	Y
2	E	7	6	3	3	3	N
3	E	7	7	5	6	6	N
4	E	7	7	6	6	6	Y
6	E	6	6	5	8	7	N
7	E	7	7	6	7	7	N
8	E	7	7	6	7	7	Y
9	E	5	8	7	7	8	Y
10	E	8	8	8	7	8	Y
11	E	4	6	7	7	7	N
12	E	8	8	9	7	8	Y
13	E	6	6	5	3	4	N
14	E	7	7	8	6	8	Y
15	E	8	8	5	5	6	Y
16	E	8	8	5	6	7	N
17	E	7	6	6	8	7	Y
18	E	6	7	7	7	7	Y
19	E	4	7	8	4	7	Y
20	E	4	6	6	5	5	N
21	E	5	7	5	5	5	N
22	E	7	4	5	4	6	N
23	E	5	6	7	6	7	Y
24	E	6	7	5	4	6	N
25	E	6	7	6	6	6	Y
26	E	3	7	4	4	4	Y
27	E	4	4	6	5	5	N
28	E	6	6	7	7	7	Y
29	E	8	8	8	7	6	Y

30	E	5	4	5	5	6	Y
31	E	7	8	6	8	7	Y
32	E	7	8	7	8	7	Y
33	E	6	4	8	7	6	Y
34	E	7	8	8	8	8	Y
35	E	6	4	3	4	3	N
36	E	8	8	6	7	7	Y
37	E	8	9	5	5	7	Y
38	E	4	5	6	8	5	N
39	E	4	4	3	5	4	N
40	E	7	6	7	7	7	Y
41	E	7	7	6	4	7	Y
42	E	8	7	8	4	7	Y
43	E	5	6	6	6	7	Y
44	E	5	4	4	3	3	N
45	E	5	5	4	5	5	N
46	E	8	8	8	9	8	Y
47	E	8	8	5	5	6	N
48	E	5	6	3	3	4	N
49	E	9	9	5	8	8	Y
50	E	9	8	8	9	9	Y
51	E	9	9	9	9	9	Y
52	E	5	5	5	5	5	N
53	E	9	6	4	5	5	Y
54	E	4	6	3	3	4	N
55	E	7	8	8	8	8	Y
56	E	6	7	6	5	6	Y
57	E	6	6	7	7	7	Y
58	E	7	5	7	4	7	Y
59	E	5	7	6	4	6	Y
60	E	6	4	2	2	3	N
61	E	8	9	9	9	9	Y
62	E	8	9	9	9	9	Y
63	E	7	7	8	8	8	Y
64	E	6	8	6	6	6	Y
65	E	7	7	7	8	7	Y
66	E	8	8	5	5	5	N
67	E	4	5	5	4	4	N
68	E	7	7	5	5	5	Y
69	E	7	6	6	6	6	Y
70	E	4	6	6	8	6	Y
71	E	8	8	3	4	6	N

72	E	5	5	4	5	6	N
73	E	8	6	4	4	4	N
74	E	8	8	5	5	6	Y
75	E	5	6	4	5	4	N
76	E	7	7	7	7	7	N
77	E	6	7	7	8	7	N
78	E	7	3	5	6	4	N
79	E	7	8	9	8	9	Y
80	E	5	6	9	8	8	Y
81	E	3	4	4	4	4	N
82	E	4	4	4	5	5	Y
83	E	7	5	7	8	7	Y
84	E	7	8	7	5	7	Y
85	E	9	9	8	9	9	Y
86	E	7	8	7	7	7	Y
87	E	7	7	6	7	7	Y
88	E	8	8	7	8	8	Y
89	E	7	8	6	7	6	N
90	E	6	6	7	7	6	N
91	E	5	7	8	9	8	Y
92	E	5	6	6	5	6	Y
93	E	5	6	6	6	7	N
94	E	4	5	8	8	7	Y
95	E	6	4	7	8	6	Y
96	E	5	6	3	4	2	N
97	E	4	3	5	5	5	N
98	E	7	4	4	5	5	N
99	E	6	4	7	4	5	Y
100	E	4	6	6	4	5	N
101	E	2	6	3	4	4	N
102	E	7	8	7	7	7	Y
103	E	6	4	3	2	4	N
104	E	6	6	6	6	6	Y
105	E	8	9	8	6	8	Y
106	E	4	5	6	6	6	Y
107	E	4	6	5	6	4	N
108	E	7	8	8	8	8	Y
109	E	4	1	5	6	4	N
110	E	5	6	6	5	5	Y
111	E	4	5	3	3	4	N
112	E	5	4	4	4	5	N
113	E	4	5	3	3	3	N

114	E	6	6	8	5	7	Y
115	E	5	7	8	8	8	Y
116	E	4	5	7	6	6	Y
117	E	6	8	6	7	6	Y
118	E	8	7	6	7	7	Y
119	E	4	4	6	5	5	N
120	E	6	6	7	6	7	Y

Table D.3: P values for sensory study

	Panelist	Sample
Appearance	<0.0001	0.0009
Thickness	<0.0001	0.0016
Flavor	<0.0001	0.2941
Aftertaste	<0.0001	0.2372
Overall Acceptability	<0.0001	0.1572

Table D.4: Mean liking score for 119 panelists

Attribute	High cysteine yogurt	Low cysteine yogurt
Appearance	6.1	6.7
Thickness	6.4	5.7
Flavor	6.0	6.2
Aftertaste	5.9	6.1
Overall Acceptability	6.1	6.3

Low cysteine yogurt: NDM and processed at 90°C for 7 min, High cysteine yogurt: NDM + WPI and processed at 70°C for 20 min.

Appendix E - SAS® program

For protein content, pH and cysteine content in non-processed and processed mixes.

```
data zero;
input Treatment $ Formulation  $ rep $    totalprotein      trueprotein
      casein      whey  NPN   cysteine    pH;
cards;
;

proc print data=zero;
run;

* objective 1;
%macro dayzero(y);
title &y;
proc glimmix data=zero;
class treatment formulation rep;
model &y = rep treatment|formulation;
lsmeans treatment formulation treatment*formulation/adjust=tukey lines;
run;
%mend;

%dayzero(totalprotein)
%dayzero(trueprotein)
%dayzero(casein)
%dayzero(whey)
%dayzero(NPN)
%dayzero(cysteine)
%dayzero(pH)
run;
```

For WPD

```
data three;
input Treatment $ Formulation $ rep $     wheyproteindenaturation;

cards;

title 'wheyproteindenaturation';
proc print data=three;
run;

* objective 4;
proc glimmix data=three;
class treatment formulation rep;
model wheyproteindenaturation= rep treatment|formulation;
lsmeans treatment formulation treatment*formulation/adjust=tukey lines;
run;
```

```
quit;
```

Study 1: Day 1 analysis

```
data one;
input Treatment $ Formulation  $ rep $    synersis    L     a     b     WHC
      Firmness    TA    TS    pH WI;
cards;
;

proc print data=one;
run;

* objective 1;
proc glimmix data=one;
class treatment formulation rep;
model  synersis = rep treatment|formulation;
lsmeans treatment formulation treatment*formulation/adjust=tukey lines;
title 'synersis';
run;

proc glimmix data=one;
class treatment formulation rep;
model  L = rep treatment|formulation;
lsmeans treatment formulation treatment*formulation/adjust=tukey lines;
title 'L';
run;
proc glimmix data=one;
class treatment formulation rep;
model  L = rep treatment|formulation;
lsmeans treatment formulation treatment*formulation/adjust=tukey lines;
title 'L';
run;
proc glimmix data=one;
class treatment formulation rep;
model  L = rep treatment|formulation;
lsmeans treatment formulation treatment*formulation/adjust=tukey lines;
title 'L';
run;
proc glimmix data=one;
class treatment formulation rep;
model a = rep treatment|formulation;
lsmeans treatment formulation treatment*formulation/adjust=tukey lines;
title 'a';
run;
proc glimmix data=one;
class treatment formulation rep;
model  b = rep treatment|formulation;
lsmeans treatment formulation treatment*formulation/adjust=tukey lines;
title 'b';
run;
proc glimmix data=one;
class treatment formulation rep;
model  WHC = rep treatment|formulation;
```

```
lsmeans treatment formulation treatment*formulation/adjust=tukey lines;
title 'WHC';
run;
proc glimmix data=one;
class treatment formulation rep;
model  Firmness = rep treatment|formulation;
lsmeans treatment formulation treatment*formulation/adjust=tukey lines;
title 'Firmness ';
run;
proc glimmix data=one;
class treatment formulation rep;
model  TA = rep treatment|formulation;
lsmeans treatment formulation treatment*formulation/adjust=tukey lines;
title 'TA';
run;proc glimmix data=one;
class treatment formulation rep;
model  TS = rep treatment|formulation;
lsmeans treatment formulation treatment*formulation/adjust=tukey lines;
title 'TS';
run;proc glimmix data=one;
class treatment formulation rep;
model  pH = rep treatment|formulation;
lsmeans treatment formulation treatment*formulation/adjust=tukey lines;
title 'pH';
run;
run;proc glimmix data=one;
class treatment formulation rep;
model  WI = rep treatment|formulation;
lsmeans treatment formulation treatment*formulation/adjust=tukey lines;
title 'WI';
run;
```

Study 2: Storage

```
dm"output;clear;log;clear;";
options linesize=90 pagesize=65nodate pageno=1;
data D1toD60;
input day $ yogurt       $ rep $    L     a     b     WHC   Firmness    TA
      pH cysteine h SI;
cards;
;
proc print data=D1toD60;
run;

%macro storage(y);
title &y;
proc glimmix data=D1toD60 method=laplace;
class rep yogurt day;
model &y = yogurt|day;
random day / subject=rep(yogurt) type=cs;
lsmeans yogurt day yogurt*day/adjust=tukey lines;
lsmeans yogurt/slice=day adjust=tukey lines;
run;
%mend;
```

```
%storage(L)
%storage(a)
%storage(b)
%storage(WHC)
%storage(Firmness)
%storage(TA)
%storage(pH)
%storage(cysteine)
%storage(WI)
run
quit;
```

Sensory Study

```
dm'log;clear;output;clear;';
data Sensory;
input Panelist  Sample $ Appearance Thickness Flavor Aftertaste Overall;
cards;
;
proc print data=sensory;
run;
proc glm data=sensory;
class Panelist Sample;
model Appearance Thickness Flavor Aftertaste Overall = Panelist Sample ;
lsmeans Sample /pdiff lines;
run;
```

Appendix F - Non Significant Dataset

Table F.1: Chemical and functional properties of yogurt on day 1 (n=3)

Yogurt	Syneresis (%)	TA	pH	TS (%)
N70	11.51	1.014	4.423	12.26
N90	7.155	1.003	4.430	12.30
W70	5.852	1.007	4.407	12.38
W90	3.288	1.005	4.417	12.36

TA: Titratable Acidity, TS: Total Solids. N70: Nonfat dry milk(12.5%) rehydrated and processed at 70^{o}C for 20 min, N90: Nonfat dry milk (12.5%) rehydrated and processed at 90^{o}C for 7 min W70: Nonfat dry milk (10%) + WPI (2.5%) rehydrated and processed at 70^{o}C for 20 min; W90: Nonfat dry milk (10%) +WPI (2.5%) rehydrated and processed at 90^{o}C for 7 min.

Storage study

Table F.2: Yogurt*days interactions observed for yogurts stored for 60 days (n=3)

Yogurt	Day	Syneresis %	pH	Cysteine(mg/L)
N70	1	11.5	4.4	230.3
	15	8.74	4.41	226.7
	30	8.79	4.40	224.4
	45	8.87	4.40	223.0
	60	9.27	4.40	217.0
N90	1	7.15	4.43	135.7
	15	5.73	4.41	129.0
	30	5.62	4.40	125.8
	45	5.82	4.41	124.9
	60	5.97	4.40	124.1
W70	1	4.96	4.41	359.9
	15	2.73	4.39	393.3
	30	2.81	4.39	388.8
	45	2.80	4.39	388.0
	60	2.99	4.39	395.3
W90	1	5.48	4.41	235.2
	15	1.73	4.38	243.3
	30	1.72	4.38	240.7
	45	1.73	4.39	234.2
	60	1.83	4.38	234.4

N70: Nonfat dry milk(12.5%) rehydrated and processed at 70^{o}C for 20 min, N90: Nonfat dry milk (12.5%) rehydrated and processed at 90^{o}C for 7 min W70: Nonfat dry milk (10%) + WPI (2.5%) rehydrated and processed at 70^{o}C for 20 min; W90: Nonfat dry milk (10%) +WPI (2.5%) rehydrated and processed at 90^{o}C for 7 min.