Graduate Theses and Dissertations
Graduate College

2012

Modification of milk protein concentrate and applicability in high-protein nutrition bars

Justin Charles Banach
Iowa State University

Follow this and additional works at: http://lib.dr.iastate.edu/etd

Part of the Food Science Commons

Recommended Citation

Banach, Justin Charles, "Modification of milk protein concentrate and applicability in high-protein nutrition bars" (2012). *Graduate Theses and Dissertations*. Paper 12786.

Modification of milk protein concentrate and applicability in high-protein nutrition bars

By

Justin Charles Banach

A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Food Science and Technology

Program of Study Committee:
Buddhi P. Lamsal, Major Professor
Stephanie Clark
James Hollis

Iowa State University

Ames, Iowa

2012

TABLE OF CONTENTS

LIST OF FIGURES

v

LIST OF TABLES

CHAPTER 1. GENERAL INTRODUCTION

1.1 Research Premise

Milk protein concentrates (MPCs) are dairy protein powders with protein content in the range of 42–85% that maintain the ratio of proteins, namely, casein to whey proteins in skim milk. They are manufactured by removing the lactose and minerals from skim milk using membrane technology. The retentate obtained from this process is further concentrated by evaporation, and spray dried. MPCs are mostly used for process cheese milk fortification, low-fat yogurt production, and nutritional applications.

Currently, commercial high-protein nutrition (HPN) bar formulations rely on casein, caseinates, whey proteins and their hydrolysates, and soy proteins to make HPN bars with 20% to 50% protein by weight. Each of these protein ingredients has some setbacks. Despite good performance in HPN bars, casein and caseinates are heavily imported into the United States. The whey protein market is volatile and demand for specialty whey products, such as those found in HPN bars, has increased steadily since its time of disposal (Smithers 2008). Although nutritionally complete, soy proteins are avoided by some male consumers due to their phytoestrogen effect that may decrease energy and muscle mass (Hamilton-Reeves and others 2010). Replacing, supplementing, or blending these commonly used proteins with MPC in HPN bar formulations allows for an economic alternative to produce a widely accepted HPN bar.

Although MPCs contain casein and whey proteins, two protein fractions commonly employed in HPN bar applications, bars formulated with high concentrations of MPC harden quickly, become unpalatable, and have reduced product shelf-life. Another drawback of using MPC in HPN bar formulations is that the finished product tends to lack cohesiveness

and is too crumbly. This research study seeks to tackle the shelf-life problem of MPC-incorporated HPN bars by exploring ways to reduce hardening.

Modification of protein structure is the key to bring out targeted functionality in protein-rich food applications. Depending on the degree of denaturation, protein molecules unfold, opening up the tertiary and quaternary structures, enabling interactions with other protein molecules and food constituents. Application of heat and shear to protein through extrusion has been one technique to partially unfold and denature proteins to bring about desired functionalities. Low temperature toasting has also been used to impart changes to protein powders for better performance in food systems.

Extrusion and toasting modification of MPC with protein content at 80% (MPC80) were compared for potential incorporation of the resulting ingredient in HPN bars. Extrusion can modify the macromolecular state of proteins in MPC80, and generate an inert source of protein with limited interaction between constituents to prevent or greatly reduce the onset of quality degrading mechanisms. MPC80 was also toasted and compared with extruded MPC80 for changes in functional properties brought about by heat. It was hypothesized that macro- and micro-molecular changes due to extrusion, and toasting, would result in functional changes leading to reduced HPN bar hardening. HPN bar hardening reactions are well studied for whey proteins, but research is lacking on why HPN bars suffer rapid quality decline when formulated with MPCs and what modifications could eliminate or reduce such decline, the subject of this research.

1.2 Thesis Organization

This thesis is presented in five chapters. Chapter 2 contains a review of MPC, the mechanisms of HPN bar hardening, and protein modification techniques that can be applied

to MPC to potentially slow quality change in HPN bars. Chapter 3 looks at the molecular and functional property changes in MPC80 that are brought about by extrusion and toasting; the two modification techniques that are yet to be applied to MPC. Chapter 4 focuses on utilizing extruded or toasted MPC80s in a model HPN bar system and monitoring of quality parameters over an accelerated shelf-life study. Chapter 5 provides general conclusions, recommendations, and future research possibilities, and is followed by acknowledgement of those who have helped in the completion of this study. Formatting of this thesis follows the guidelines specified by the *Journal of Food Science,* the flagship publication of the Institute of Food Technologists (IFT).

1.3 References

Hamilton-Reeves JM, Vazquez G, Duval SJ, Phipps WR, Kurzer MS, Messina MJ. 2010. Clinical studies show no effects of soy protein or isoflavones on reproductive hormones in men: results of a meta-analysis. Fertil Steril 94(3):997-1007.

Smithers GW. 2008. Whey and whey proteins-From 'gutter-to-gold'. Int Dairy J 18(7):695-704.

CHAPTER 2. MILK PROTEIN CONCENTRATES IN HIGH-PROTEIN BARS: HARDENING MECHANISMS AND PROTEIN MODIFICATIONS WITH POTENTIAL IMPLICATIONS

Justin C. Banach[1,2], Stephanie Clark[3], and Buddhi P. Lamsal[4,5]

2.1 Abstract

Milk protein concentrates are powdered milk ingredients produced with membrane filtration to contain a range of protein contents maintaining the casein to whey ratio found in fluid milk. If used properly, the nutritional and functional properties of milk protein concentrates can be used to enhance food products without cluttering ingredient labels. However, whey protein concentrates, whey protein hydrolysates, and imported caseinates, are more commonly used to make high-protein nutrition bars. In this category, the use of milk protein concentrates has not fared well when compared with whey and casein ingredients. Protein ingredients are often subjected to modifications like enzyme hydrolysis and pH precipitations during production for an application at hand. Milk protein concentrates are relatively new powdered milk ingredients, with a better flavor profile than purified casein or caseinates, yet less published work is available about modification of milk protein concentrates and resulting effect on protein functionality. This review intends to present and analyze protein modification techniques, with emphasis on dairy proteins, and resulting functionality. Major modification techniques reviewed include enzyme hydrolysis,

---

[1] Graduate Student, Department of Food Science and Human Nutrition, Iowa State University.
[2] Primary researcher and author.
[3] Associate Professor, Department of Food Science and Human Nutrition, Iowa State University.
[4] Assistant Professor, Department of Food Science and Human Nutrition, Iowa State University.
[5] Author for correspondence, lamsal@iastate.edu, p: 515-294-8681, f: 515-294-8181.

extrusion, toasting, and high-power ultrasonication of dairy proteins, mainly whey, with intent to look at their applicability for milk protein concentrates to improve performance in high-protein nutrition bars.

2.2 Milk and Powdered Dairy Products

Fluid milk derived from the bovine species is approximately 87.3% water and 12.7% solids, including fat (3.7%), protein (3.4%), lactose (4.8%), and ash (0.7%) (Fox and McSweeney 1998). About 80% of the protein is classified as casein and the remaining 20% is whey (Oldfield and Singh 2005). Fluid milk is prone to spoilage due to its high moisture content. Milk powders, obtained by drying fluid milk to less than 5% moisture (Codex Standard 207-1999), provide a means to extend milk's shelf-life and reduce shipping as well as refrigeration costs (Heldman and Hartel 1998). According to Codex Standard 207-1999, the ratio of whey to casein must be preserved after drying in order to be called a milk powder. The United States Department of Agriculture (USDA) defines the following as dry milk powder: dry whole milk, nonfat dry milk (NFDM), skim milk powder, dry skim milk, dry buttermilk, and milk protein concentrate (MPC).

MPCs are powdered milk products with protein content ranging from 42% to 85%, generally with casein and whey in the same proportion found in skim milk. Moisture in MPCs ranges from 3.5% to 5% and lactose content is inversely related to protein content. Lactose, a reducing disaccharide consisting of β-D-galactose and α-D-glucose, impacts the stability of dried milk products in storage (Oldfield and Singh 2005). MPC powders are divided into three categories: low-protein (≤ 55% protein), intermediate-protein (55% < protein < 80%), and high-protein (> 80% protein) (De Castro-Morel and Harper 2002). Milk

protein powders with very high protein content ($\geq$ 90%), contain very low concentrations of lactose and other minerals and are called milk protein isolates (Kelly 2011).

MPCs are relatively new powdered milk ingredients; consequently, the US has no standard of identity (Mistry 2002; Gerdes 2008). The USDA Dairy Products Summary first reported MPC production numbers in the 2009 report, which defined MPC as a dry milk powder containing 40.0% to 89.9% protein (USDA 2010). Under Chapter 4 in the harmonized tariff schedule of the US, the phrase "milk protein concentrate" refers to "any complete protein (casein plus lactalbumin) concentrate that is 40 percent or more protein by weight". In contrast, Chapter 35 of the harmonized tariff schedule of the US defines "milk protein concentrate" as "any complete milk protein (casein plus lactalbumin) concentrate". Using the Chapter 35 definition, MPC must contain at least 90% casein protein (Bailey 2011).

Lacking identity standards allows for variations in what is produced and labeled as MPC in the US. MPCs are produced by one of three main methods: dry blending, precipitation, and membrane filtration (Kelly 2011). Dry blending takes prepared dairy protein powders (e.g., casein, caseinates, WPC, WPI, skim milk powder) and simply mixes them together in the powder state to obtain MPC. The precipitation method starts with skim milk and can then proceed down two slightly different paths. The skim milk can be acidified to produce acid casein, which is then re-dissolved and combined with whey protein followed by spray drying to manufacture MPC (Kelly 2011). Alternatively, skim milk can be treated with calcium chloride plus heat to create a co-precipitate of casein and whey proteins that is followed by dissolution and spray drying to make MPC (Kelly 2011). The preferred method of domestic MPC production relies heavily on membrane technology and the retention of

casein and whey proteins in skim milk by micro- and ultrafiltration. To increase the protein content greater than or equal to 70%, lactose and minerals are washed from the retentate via diafiltration. The liquid MPC is further concentrated with evaporation, and spray dried at inlet temperatures of 120 to 125°C and outlet between 75 and 80°C (Baldwin and Pearce 2005). MPC manufactured with ultrafiltration eliminates both extrinsic pH adjustments and the addition of calcium chloride which allows the casein micelle to remain intact and offers different functionality than MPCs produced by dry blending or precipitation (Henning and others 2006; Kelly 2011).

Domestic MPCs manufactured with ultrafiltration differ from conventional casein-based protein powders, including lactic acid casein, mineral acid casein, rennet casein, and caseinates, because casein remains in the micellar state, and whey proteins are present (Henning and others 2006; Kelly 2011). Lactic acid casein curd is precipitated from fluid skim milk by acidification through inoculation with lactic acid bacteria, causing slow conversion of lactose to lactic acid, or by direct addition of dilute lactic acid. Mineral acid casein is produced by adding dilute sulfuric or hydrochloric acid to skim milk to precipitate the casein protein at its isoelectric point (Southward 2012). Rennet casein curd is isolated from skim milk without pH adjustment; rather the addition of chymosin or synthetic rennet produced by enzyme fermentation cleaves κ-casein, which disrupts micellar casein, causing it to clot in the presence of released calcium. After casein precipitation, the curd is then heated to expel entrapped whey and washed several times with water before mechanical pressing or centrifugation (Southward 2012). The washed casein curd is then dried on a fluidized bed dryer to around 10% moisture, cooled, milled, sieved, and bagged (Southward 2012). Washed or washed and dried acid casein curd can be neutralized with sodium, potassium, or

calcium hydroxides or other alkaline chemicals such as ammonia, to produce caseinates. Neutralization to pH 6.6 causes dissolution of the acid casein curd and the resultant solution is spray dried to produce sodium, potassium, or calcium caseinates. Caseinates retain better solubility, and hence functional properties, when compared with purified casein powders. In some instances the functional properties of MPCs are similar to caseinates and thus substitution of caseinates with MPC in food applications may be possible (Kelly 2011).

MPCs contribute to water binding, gelation, whipping, emulsification, browning, flavor enhancement, thickening, and nutrition when used in food applications (Baldwin and Pearce 2005). Such properties generally depend on quality and protein content in MPC, however, in a study of 37 internationally produced MPCs, properties such as solubility, viscosity, foaming, and emulsification did not correlate with protein content (De Castro-Morel and Harper 2002). The functional properties of MPCs are not uniform and vary with both processing and the final product composition. If used properly, functional properties of MPCs can enhance foods without cluttering ingredient labels (Chandan 1997). Despite being a nutritionally complete protein, a potential drawback of using MPC in food applications is limited reconstitution and dissolution, a much needed prerequisite for many other functional properties. MPC flavor varies with protein content, with a better flavor profile than purified casein or caseinates (Baldwin and Pearce 2005). Low-protein MPCs have cooked, sweet, and milky flavors that increase in intensity at higher protein levels (Gerdes 2008).

MPCs offer different functional properties than other powdered milk products, e.g., NFDM (Sparrow 2009). Domestic production of MPCs is limited, and in order to obtain the desired functionality, companies import MPCs into the US (Mistry 2002). Domestic production of MPC and casein-based powders are limited because it is more profitable to

produce NFDM than to invest in the capital needed (e.g., membrane filtration units) to prepare high-quality MPCs (Bailey 2001). Little is known about domestic production costs of these high-protein concentrates whereas production of NFDM is well established and most certainly cheaper to produce (Jesse 2003). There is some concern that low MPC tariffs allow economical imports that discourage domestic milk production (Mistry 2002), which is somewhat justified by an inverse relation between MPC imports and the use of domestic NFDM (Bailey 2001). However, imported MPCs might not affect domestic milk prices as more NFDM is purchased by the government compared to the amount of MPC that, if functionality is compatible, is substituted for NFDM (Jesse 2003). The International Dairy Foods Association has opposed increased tariffs on MPC because the growing demand and use of MPC in food applications exceeds domestic supply (Sparrow 2009; Kruse 2009). US producers are starting to enjoy the benefits of the expanding MPC markets, but have yet to focus on casein-based powders. As imported MPC continues to increase due to its functionality and ability to supply concentrated protein, actions will be taken to correct marketplace signals making domestic MPC a competitive choice. Seeking additional ways to increase the functional properties of MPC in high-protein nutrition bars offers a way to utilize domestic produced MPC as well as create a competitive protein concentrate that is utilized as a replacement for imported caseinates in these applications.

2.3 Applications of Milk Protein Concentrates in Food Products

MPCs are used as ingredients in nutritional products, cheese analogs, cultured dairy products, ice creams, spreads, baked goods, soups, coffees, and whipping creams (Kelly 2011; Baldwin and Pearce 2005). Nutritional products using MPC in their formulation include beverages, enteral products, instant powders, sports products, weight management

products, infant formulas, medical nutritionals, and other protein-fortified foods (Baldwin and Pearce 2005). Some applications use MPCs to standardize milk protein content for greater consistency during processing (Anema and others 2006).

The processed cheese industry is a heavy user of MPCs, but they are prohibited in natural cheeses with a federal standard of identity, except for use in the starter cultures of some cheddars and mozzarellas (Mistry 2002). For pizza cheese, a non-standardized cheese, producers add MPC to increase overall yield and protein content (Rehman and others 2003; Singh 2007). MPCs are preferred over NFDM and condensed milk for pizza cheese standardization because these products contain excess lactose, which causes unwanted fermentation and excessive browning during baking (Rehman and others 2003).

Cultured dairy products, including low-fat and Greek yogurts, are other applications for MPC (Havea 2006). Grade A MPC is approved for use in yogurt production in the US (Gerdes 2008). MPC fortified low-fat yogurt with up to 5.6% protein had acceptable flavor, texture, and minimal whey separation without any additional stabilizers (Mistry and Hassan 1992). Yogurt milk fortified with caseinates produced a firm, grainy texture; when fortified with NFDM, excessive fermentation was problematic (Mistry and Hassan 1992). In contrast, MPC is advantageous in full-fat yogurt milk fortification as it can improve overall texture (Singh 2007).

The specialty nutrition industry, including, but not limited to liquid nutritional beverages, enteral products, instant powders, sports products, weight management products, infant formulas, adult nutritionals, and protein-fortified foods, is another large consumer of MPCs (UBIC Consulting 2010; Baldwin and Pearce 2005). According to a report by Beverage Management Corporation, beverages based for wellness and nutrition are a

growing trend and sales are predicted to surpass carbonated soft drinks becoming the largest

market of nonalcoholic beverages (Dairy Foods 2005). Consequently, the incorporation of

high nutritional quality MPCs into this beverage category is also increasing (Singh 2007).

High-protein WPCs or WPIs (≥90% protein) are traditionally used in beverages containing

up to 10% protein, but undesirable tartness permits for more MPC application (Bastian

2004). MPCs are not common in high-protein nutrition bars, but their neutral flavor and

nutritional quality make incorporation favorable (Baldwin and Pearce 2005). Some milk

protein isolates and milk protein hydrolysates have been used in HPN bars (Hutchinson

2009), however, in some cases, crumbly texture and little cohesiveness in bars were reported

(Li and others 2008).

2.4 Milk Protein Concentrates and High-Protein Nutrition Bars

2.4.1 Formulation of High-Protein Nutrition Bars

High-protein nutrition bars (HPN) were once marketed to athletes and competitors,

but today are formulated, marketed, and sold to the everyday consumer (Hutchinson 2009).

HPN bars often have a particular marketing target such as for meal replacement, athletic

supplements, body building aids, and balanced nutrition. Some HPN bars tap into dietary

trends like low carbohydrate consumption. Meal replacement HPN bars offer more balanced

nutrition than snack or candy bars and provide satiety between meals (Book 2008).

HPN bars are formulated primarily for stability, their ability to maintain palatable

texture, and limited texture changes during storage (Gallo-Torres 2003). Lesser regard is

placed on taste and nutrition; nonetheless, these are important attributes for a successful HPN

bar (Gallo-Torres 2003). Without careful ingredient selection and identification of potential

interactions, the formulated HPN bar will lack stability, harden quickly, and will lose flavor

and nutritional value (Zhu and Labuza 2010). HPN bars should maintain shelf stability for a minimum of six months if stored at room temperature (McMahon and others 2009). Although stability is imperative for a successful HPN bar formulation, they contain 30% protein, 40% carbohydrate, and 30% fat based on approximate caloric contribution (Gallo-Torres 2003).

Protein is an important component in HPN bars, and although there is no official standard, they usually contain 20-40% protein by weight (Zhu and Labuza 2010). HPN bar packaging usually displays how much protein is packed into the bar on the front of the product label. Common protein sources include: whey, soy, caseinates, egg, and gelatin (Liu and others 2009). The protein source will impact bar texture, flavor, consumer acceptance, and stability (Childs and others 2007; Adams 2008). Protein blends and hydrolysates can be employed to improve flavor, texture, and stability (Adams 2008).

Some attempts have been made to modify the protein functionality and thus improve overall performance of MPCs for HPN bar products. PowerProtein™ 4857 and PowerProtein™ 4861 are specialty MPCs produced by Fonterra™; they are said to perform similar to calcium caseinates and milk protein isolates, respectively, in HPN bars (Hutchinson 2009). These functionally-enhanced MPCs and an improved, bar-specific WPC were used to create model HPN bars that were compared instrumentally and by a trained sensory panel for changes in firmness and cohesiveness during storage (Imtiaz and others 2012). The bar-specific WPC was non-hydrolyzed, but was able to improve softening and cohesiveness when used as the sole protein and in combination with the functionally-enhanced MPCs (Imtiaz and others 2012). HPN bars formulated entirely with 4861 and blends of 4861, and the two other modified proteins, had minimal change in hardness, but

had increased crumbliness during 12 months storage at 20°C (Imtiaz and others 2012). The ingredient 4857 showed increased firmness during storage over 12 months at 20°C, but had lower influence on HPN bar cohesion (Imtiaz and others 2012). Essentially MPC can be modified, although the exact techniques are unknown, to improve its performance in HPN bar systems. Improvements over unmodified MPC include decreased bar hardening and improved HPN bar cohesion, two important qualities in these products (Imtiaz and others 2012).

Carbohydrates make up 10% to 50% of the protein bar matrix by weight (Zhu and Labuza 2010). Carbohydrates are supplied as a mixture of crystalline sugars such as dextrose, sucrose, fructose, and lactose, and sugar syrups such as high-fructose corn syrup (HFCS), corn syrup, brown rice syrup, and glucose syrup. Sugar alcohols are not technically carbohydrates, rather are used as low calorie sweeteners and for their humectant properties in HPN bars. Sorbitol and maltitol are two common sugar alcohols used in HPN bars (Adams 2008). Glycerol, a polyol with a water activity-lowering effect, may also be incorporated into the HPN bar matrix (Liu and others 2009; Loveday and others 2009; Loveday and others 2010).

Fats make up about 10% to 15% of the HPN bar matrix (Zhu and Labuza 2010), although higher percentages in commercially produced bars, especially low carbohydrate varieties, are available. Fats are incorporated into the HPN bar with vegetable shortening (McMahon and others 2009; Adams 2008) or cocoa butter (Loveday and others 2009; Loveday and others 2010). Other oils, such as canola or soy, can also be added to the bar matrix (Adams 2008). Any type of food grade oil can be used in the production of HPN bars (Gautam and others 2006).

In addition to protein, carbohydrate, and fat, HPN bars contain water that acts as a plasticizer in maintaining stability. HPN bars are intermediate moisture foods with low moisture content and water activity ($a_w \leq 0.65$) (Loveday and others 2009). Moisture content may be so low that water may be excluded from a bar formulation (McMahon and others 2009; Adams 2008) or added at 15% of the formulation (Loveday and others 2009; Loveday and others 2010). Low water activity is needed to prevent microbial growth and ensure consumer safety since many HPN bars are not subjected to heat treatment (Liu and others 2009).

Besides the high-protein matrix, other components in HPN bars add value, consumer appeal, and increase eating quality. Components include flavor layers (e.g., chocolate, peanut butter, strawberry), textural components (e.g. crisps, nuts, wafers), and nutritional bonuses (e.g. fiber, vitamins, minerals) (Loveday and others 2009). Protein is commonly blended with the additional components and is necessary to obtain the higher protein contents in some HPN bars.

2.4.2 Hardening Mechanisms in High-Protein Nutrition Bars

A number of mechanisms exist explaining why some HPN bar formulations are unstable and become unpalatable with the development of rapidly increasing hardness. Model HPN bars stored over a couple weeks can generally be used to determine how well a particular formulation will fare (Gallo-Torres 2003). The main mechanisms proposed for HPN bar hardening include moisture migration, protein aggregation, and macro constituent phase separation within the HPN bar matrix. Proposed minor mechanisms include Maillard reactions between protein and reducing sugars, crystallization of sugars, and shifts in the glass transition temperature (Hutchinson 2009). Additional interactions between proteins

and minor components, such as Na^+, K^+, Ca^{2+}, or Mg^{2+}, may lead to altered protein conformation, inducing moisture migration and HPN bar hardening (Book 2008).

Moisture content, water activity, and osmotic pressure influence the migration of moisture in the HPN bar matrix (Hazen 2010; Loveday and others 2010). After HPN bar components are mixed together, water may migrate, not necessarily from components of high moisture to low moisture, but from those with high water activity to low water activity (Li and others 2008). Water may migrate from sugar syrups into protein powders that were only partially hydrated during bar manufacture, meaning that the syrups will lose their ability to act as plasticizers and the bars will harden as a function of the moisture migration (Li and others 2008; Hazen 2010). Proteins draw water molecules away from sugar syrups through non-covalent forces, which leave the sugars in a state of low hydration, prone to crystallization and increased bar hardness (Gautam and others 2006).

NMR spectra of model HPN bars made with WPI, sodium caseinate, or MPC80 showed that water may not migrate to partially hydrated protein (Loveday and others 2009; Loveday and others 2010). Increased mobility of water and polyhydroxy compounds (i.e., glucose and glycerol) was characteristic of softer bar texture (Loveday and others 2009; Loveday and others 2010), but also a physiological state susceptible to change with high component mobility (Li and others 2008). Model HPN bars made with WPI maintained low and fairly constant fast relaxation rate constants for water and polyhydroxy compounds when compared to model bars formulated with sodium caseinate (Loveday and others 2010). Such WPI HPN bars remained too soft for textural analysis throughout the accelerated storage study and were hence prone to little textural change. Low and consistent fast-relaxing proton mobility for water and polyhydroxy compounds indicated that the molecular association of

water and polyhydroxy compounds with protein in the HPN bar matrix did not change with time and therefore water did not migrate to the protein (Loveday and others 2010). Model HPN bars prepared with sodium caseinate (Loveday and others 2010) and MPC80 (Loveday and others 2009) lack water-protein interactions and become firmer as lower molecular weight compounds with higher osmotic pressure pull water away from and dehydrate the protein.

The addition of vitamins and minerals to the HPN bar matrix, especially calcium, magnesium, or other mineral complexes, may influence the rate of hardening (Coleman and others 2005). Mineral and vitamin fortified layers, external coatings, and/or sandwiches between the HPN bar matrix increase bar stability when compared with a consistent amount of minerals and vitamins mixed directly into the HPN bar matrix (Coleman and others 2005). This theory assumes that partially hydrated proteins slowly attract water from other HPN bar constituents and the incorporation of minerals, specifically calcium, is hypothesized to interact with individual amino acids, increasing the protein's affinity for water molecules, thus exacerbating moisture migration and its contribution to HPN bar hardening (Coleman and others 2005). Proteins with high surface hydrophobicity may prevent the migration of water molecules to protein, keeping all components adequately hydrated to slow changes in HPN bar stability (Gallo-Torres 2003).

In addition to movement of water molecules within the HPN bar matrix, the physical state of water also impacts the rate of hardening. Water within a soft-textured HPN bar is categorized into one of three physical states: State A – bulk water that freezes at 0°C, State B – intermediate water loosely associated with surrounding components that freezes completely at -40 °C and melts between -40 °C and 0 °C, and State C – bound water that does not freeze

because of hydrogen bonds and dipole interactions (Zhou and others 2008b). State B water in the HPN bar matrix is critical for maintaining bar flexibility (Li and others 2008). Water that migrates to partially hydrated protein becomes bound and reduces HPN bar flexibility (Gallo-Torres 2002). In a model HPN bar formulated with WPI/buffer, storage at 34°C and 45°C caused water in State B to decrease and water in State C to increase after storage, as estimated by differential scanning calorimetry (Zhou and others 2008b). Model HPN bars formulated with WPI and high-fructose corn syrup had increased water activity (0.63 to 0.69) after storage at 32°C that was attributed to a shift of water molecules from State B to State A and thus loss of its plasticizing ability (McMahon and others 2009). When HPN bars were formulated with hydrolyzed WPI, the initial water activity was lower than those bars prepared with native WPI (McMahon and others 2009). The water activity level of the hydrolyzed WPI HPN bars remained constant during storage and these same HPN bars hardened at a slower rate than bars prepared with native WPI (McMahon and others 2009). A decrease in State B water due to movement to State C or State A is a possible mechanism of HPN bar hardening.

Insoluble protein aggregation may also be associated with HPN bar hardening. In a model intermediate moisture food system of WPI/buffer, protein aggregation and reduced protein solubility was determined to be caused predominantly by intermolecular disulfide bonds with participation from β-lactoglobulin (β-lg), α-lactalbumin (α-la), and bovine serum albumin (BSA) (Zhou and others 2008a). Hardening occurred when primary disulfide-bonded proteins interacted with others, forming an aggregated network (Zhou and others 2008a). Plasticizing humectants including glycerol, sorbitol, and maltitol in the same WPI/buffer matrix decreased protein aggregation and slowed hardening (Liu and others

2009). Propylene glycol, a humectant with low water activity, induced protein aggregation and caused rapid intermediate moisture food hardening (Liu and others 2009). Cysteine (Cys) monohydrate or N-ethylmaleimide (NEM) was added to the same WPI/buffer matrix to reduce disulfide bonds and to block free thiols from linking together (Zhu and Labuza 2010). The bar hardening rate was not significantly reduced when Cys was added in the molar ratio of 0.05 Cys:WPI compared with the no Cys control. The addition of Cys in the molar ratio of 0.25 Cys:WPI increased the rate of intermediate moisture food hardening. NEM, added to the intermediate moisture food matrix at a molar ratio of 2 NEM:WPI, significantly reduced the rate of hardening (Zhu and Labuza 2010). The shelf-life for each intermediate moisture food was 26, 41, 15, and 161 days for the control, 0.05 Cys:WPI, 0.25 Cys:WPI, and 2 NEM:WPI, respectively, based on time to reach a predetermined textural hardness (Zhu and Labuza 2010). Too much Cys in the intermediate moisture food model led to accelerated disulfide bond formation, but the correct addition served to extend shelf-life. The addition of NEM extended the intermediate moisture food model shelf-life because only non-covalent protein interactions contributed to texture change (Zhu and Labuza 2010). MPC modifications that seek to block free sulfhydryl groups from disulfide bond formation within the HPN bar matrix may be useful in limiting textural changes during storage.

Macro constituent phase separation and increased water activity were observed with model HPN bars that hardened under accelerated storage conditions (McMahon and others 2009). Confocal laser scanning microscopy was used to evaluate regions of lipid, protein, and carbohydrate within model protein bars prepared with WPI and substituted with partially hydrolyzed WPI. The separation of carbohydrate syrup (HFCS) or polyol syrup (sorbitol) from the bar matrix, as determined by the appearance of black regions on the micrographs,

was common in HPN bars with increased hardness. HPN bars without carbohydrate or polyol separation were stable and more pliable. The model HPN bars prepared with blends of WPI and partially hydrolyzed WPI maintained stability compared to bars formulated strictly with WPI because solvent (i.e., water) and co-solvent (i.e., HFCS or sorbitol) interaction with protein domains was greater due to increased hydrophilicity and decreased hydrophobicity of the protein from hydrolysis (McMahon and others 2009). The solvent and co-solvent are less excluded from the local protein domain; they interact more with the protein stabilizing its native structure preventing moisture induced aggregations and disulfide bond formations (McMahon and others 2009). However, too much co-solvent interaction will also contribute rapid hardening, as is the case when propylene glycol was used in model HPN bar formulations (Liu and others 2009). Partial exclusion of the solvent and co-solvent from local protein domain due to a decrease of free energy greater than the amount of free energy required to increase the concentration of HFCS or sorbitol in the aqueous phase makes the gradual phase separation energetically favorable (McMahon and others 2009).

Maillard browning occurred in model HPN bars prepared with partially hydrolyzed WPI and HFCS, but was limited in model bars consisting of WPI and sorbitol (McMahon and others 2009). The bars formulated with HFCS remained softer but became darker, whereas the bars formulated with sorbitol had less color change and became firmer. Maillard browning was determined to occur during HPN bar storage, but was not identified as major mechanism for hardening (McMahon and others 2009). Although, Maillard reactions are signs of quality decline in HPN bars, they are not a strong mechanism of HPN bar hardening. Although, the Maillard reaction in itself does not really contribute to HPN bar stability, the

generation of Maillard browning products may lead to increased disulfide bond formation and thus some attention should be given to these reactions (Le and others 2011).

2.5 Modifications of Milk Protein Concentrate to Potentially Alleviate High-Protein Nutrition Bar Hardening

2.5.1 Enzyme Hydrolysis of Dairy Proteins and Resulting Functional Properties

MPC hydrolysis has been limited by difficulties in controlling degree of hydrolysis (DH), reproducibility, and the development of potential bitterness (Urista and others 2011). DH will influence the functionality of the final hydrolysate, whereas reproducibility is necessary to ensure that ingredients perform consistently. Reproducibility in milk protein hydrolysis is hindered two-fold: (1) the starting material may differ with season, breed, etc. and (2) the enzymatic reaction system utilized may offer inconsistencies. For example, cheese whey differs with make and thus WPC could have varied functionality, influencing industry to select a more consistent protein source to use in hydrolysate production (Sinha and others 2007). A large amount of published work exists on enzyme hydrolysis of whey, casein, and purified dairy proteins and has been compiled into literature reviews (Urista and others 2011; Kilara and Panyam 2003). Research has increased in the area of peptide separation with membrane technology and continuous flow systems using continuous stirred tank membrane reactors (Bouhallab and others 1993; Kilara and Panyam 2003). Enzymatic modification of MPC is rare, but to increase applicability in HPN bars and other food applications, it is necessary to study resulting functionality after processing (Urista and others 2011).

Trypsin and Chymotrypsin were used to modify reconstituted MPC produced from reconstituted skim milk powder, ultrafiltration, diafiltration, and freeze drying (Amiot and

others 2004). The enzymatic hydrolysis of MPC was employed to obtain bioactive peptides for growing human skin cells in culture. DH values, as determined with pH-Stat, between 1.5% and 6.0% were obtained for each enzyme. More than 40% of the peptides had mass greater than 3000 Da in the Trypsin hydrolysate (6% DH). The Chymotrypsin hydrolysate (6% DH) produced many peptides (80%) with molecular weight between 300 and 1200 Da. Proteolysis of β-lg was likely limited when compared to the casein protein because of its compact globular structure that is typically resistant to hydrolysis, whereas the casein is designed for easy digestion to nurture a young calf (Guo and others 1995). MPC hydrolysis is a possibility to modify functionality while maintaining a low degree of hydrolysis to negate the development of potential bitterness.

Hydrolysates of whey protein and plant proteins are commonly used in HPN bars to increase stability. Peptides from protein hydrolysates maintain high water activity levels upon hydration through hydrogen bonding, which eliminates a water activity gradient within the HPN bar matrix, preventing water molecule migration to the protein during storage (Gautam and others 2006). Milk protein hydrolyzed with Alcalase and Neutrase to less than 10% DH as determined by pH-Stat had decreased water holding capacity, but had a remarkable increase in protein solubility (Mietsch and others 1989). WPC hydrolysates produced by hydrolysis with Papain and a fungal protease had increased water holding capacity with lengthened hydrolysis time (Sinha and others 2007). This increase in water holding capacity may be due to differences in methodology since the volume of water added may not be enough to completely dissolve the WPC, but may indicate that these WPC hydrolysates hydrate rapidly when exposed to water and are better able to hydrate completely during HPN bar manufacture. Rapid protein hydration and increased protein solubility,

allows for adequate hydration during HPN bar manufacture, which prevents subsequent moisture migration in the HPN bar and may slow bar hardening.

Hydrolyzed proteins introduce more charged peptide ends than the intact protein, but the extra charges cancel each other out and the protein powder incorporated into the HPN bar maintains charge neutrality. The development of charge neutrality also inhibits the protein and peptides from pulling water molecules away from other HPN bar constituents during storage to stop moisture migration and resultant HPN bar hardening (Gautam and others 2006). Hydrated peptides that maintain an ideal water activity level ($0.75 > a_w > 0.55$) form HPN bars that pack less densely and disrupt any potential gradients within the HPN bar matrix, and can prevent unwanted moisture migration to limit the progression of HPN bar hardening (Gautam and others 2006).

Amino acid profiling of the Papain- and a fungal protease-hydrolyzed WPC revealed that the available lysine deceased (Sinha and others 2007). A decrease in available lysine could potentially limit the amount of Maillard browning that occurs within the HPN bar matrix during storage. Maillard browning products (e.g., furosine and hydroxymethylfurfural) increase the formation of crosslinks between adjacent particles of native MPC during storage (Le and others 2011). Native MPC solubility decreased as the number of crosslinks increased, but only a few crosslinks were needed for significant solubility reduction (Anema and others 2006). Insoluble MPC contained disulfide linked β-lg and κ-casein complexes, but these same complexes were also present in soluble MPC suggesting that formation takes place during MPC production and that these β-lg and κ-casein complexes likely do not contribute to insolubility (Havea 2006). If all MPC enzyme hydrolysates limit available lysine, the Maillard browning reactions and the rate of internal

protein cross linking within the HPN bar could potentially be slowed during storage. Inhibiting internal disulfide bonds by adding N-ethylmaleimide to a model intermediate moisture food matrix slowed the rate of hardening (Zhu and Labuza 2010). Maillard products speed up the disulfide exchange reaction between adjacent proteins; the inhibition of the reaction by decreasing available lysine may help maintain HPN bar stability.

2.5.2 Extrusion Processing of Dairy Proteins and Resulting Functional Properties

Extrusion processing has been used by the food industry to manufacture pastas, ready-to-eat cereals, puffed snacks, pet foods, candies, and meat analogs (Heldman and Hartel 1998). Feed properties and extruder settings influence the final extruded product. Feed properties include moisture content, macro nutrients (i.e., carbohydrate, protein, and fat), minor constituents (e.g., minerals), pH, particle size distribution, and resulting interactions (Heldman and Hartel 1998). Extruder settings include extruder type (e.g., single- or twin-screw), barrel length and diameter, temperature, feed rate, screw geometry and RPM, die geometry, and applied specific mechanical energy (Heldman and Hartel 1998). An experimental approach is necessary to achieve optimum parameters to obtain the desired extrudate.

Extrusion processing has been utilized to modify the functionality of powdered dairy proteins. Information about extruding MPC is limited, however, extrusion processing of whey protein and to lesser extent casein/caseinates is well established. Extruded dairy proteins are often texturized, that is they are subjected to high pressure, temperature, and shearing forces that cause the protein to melt and partially denature with strand realignment occurring at the die (Heldman and Hartel 1998). If dairy protein concentrates rather than

isolates are extruded, lactose will contribute to Maillard browning, decreasing protein availability and nutritional quality (Heldman and Hartel 1998).

Extruding high protein concentrates, such as WPC80, is difficult, so starch, water, acid, and/or base may be added to ease processing, but will manipulate the extrudates' functional properties (Manoi and Rizvi 2008). Co-extruded dairy proteins, such as MPC40 with corn starch in Mexican-based third-generation snacks, add nutritional value to snack foods (Limón-valenzuela and others 2010). MPC40 (0% to 10%) was added to a blend of quality protein maize and corn starch (80:20) at adjusted moisture (20% and 30%), extruded, and then extrudate pellets were cooked in a microwave oven; the preparation method for third-generation snacks. High feed moisture (30%) and high MPC addition (10%) led to greater expansion after microwaving. Increasing MPC content in the low moisture range (20%) decreased product expansion and increased bulk density. Penetration force was minimized at intermediate moisture (25%) and MPC (5%) addition, but provided good product expansion. MPC was used to improve overall nutritional quality of extruded third-generation snacks.

WPC (80%, 85.1%, and 85.7% protein) was blended with corn starch (0%, 10%, 20%, and 30%) with different amylose levels (0, 25%, 50%, and 70%) to determine interaction between protein and carbohydrates after extrusion (Matthey and Hanna, 1997). A co-rotating extruder with a 3 mm die processed WPC/corn starch blends at constant moisture (22% dry-basis), die temperature (140°C), and screw speed (140 RPM). Increasing WPC did not affect the shear strength, but increasing amylose content of the feed increased the final extrudate shear strength. High amylose and high WPC content decreased radial expansion and so amylose-free starch may be desired for protein crisp production. Increasing amylose

increased extruder specific mechanical energy whereas color difference was attributed to increased WPC. WPC addition did not affect water holding capacity or the water solubility index. Co-extrusion of dairy proteins adds more variables that may or may not influence the functional properties of the extrudate.

WPC and sweet whey solids (SWS) were each mixed with corn meal, potato flour, or rice flour and were extruded to assess whey protein addition in puffed snacks (Onwulata and others 1998). Feed moisture was increased with a dosing pump without accounting for starting material moisture prior to extrusion. Whey protein and flour were fed through a twin-screw extruder at constant die temperature (125°C), constant speed (300 RPM), and processed with either low- or high-shear screws. Radial extrudate expansion and breaking strength differ with carbohydrate, screw profile, and protein source and concentration. Radial expansion generally decreased with increased whey protein incorporation whereas breaking strength increased. Processing with high-shear screws decreased solubility and increased water holding capacity. Due to the complex interaction between processing parameters, there was no set trend, but the recognized main effects were reduced water holding and increased water solubility. The effect of incorporating WPC or SWS into corn, potato, and rice flour for extrusion was characterized again (Onwulata and others 2001b). Some results differed between studies demonstrating that although co-extrusion of whey proteins with corn meal, potato flour, and rice flour is possible, variability in extrusion is common and must be taken into consideration when modifying protein functionality.

Dairy protein powders (casein, WPC, or WPI at 25%) plus wheat fiber (5% or 12.5%) in combination with corn meal flour (70% or 62.5%) were processed with high-shear screws through a twin-screw extruder at constant die temperature (125°C) and screw speed (300

RPM) (Onwulata and others 2001a). Corn meal flour was extruded by itself, with wheat fiber (5% and 12.5%), and then only with each dairy protein (25%). Corn meal (70% and 62.5%) plus wheat fiber (5% and 12.5%) plus each dairy protein (25%) were also extruded. Specific mechanical energy decreased when WPC, WPI, and casein are extruded with corn meal. Corn meal/WPC retained the most moisture and had high water holding capacity when compared with corn meal, WPI/corn meal, and casein/corn meal extrudates. Adding wheat fiber (5%) to corn meal decreased retained moisture, but higher addition (12.5%) increased retained moisture. Corn meal water holding capacity was not influenced by wheat fiber addition, but it reduced solubility when compared with the corn meal extrudate.

WPC80 (94%), pre-gelatinized corn starch (6%), $CaCl_2$ (0.6%), and NaCl (0.6%) was extruded with super critical CO_2 injection on a twin-screw machine at constant screw speed (180 RPM) and temperature (90°C) (Manoi and Rizvi 2008; Manoi and Rizvi 2009a; Manoi and Rizvi 2009b). Extrudates were dried and ground into powders. Solutions of texturized WPC80, with and without supercritical CO_2 injection, had increased apparent viscosity with shear thinning properties (Manoi and Rizvi 2008). Increasing texturized WPC in solution increased apparent viscosity, but the same increase of unprocessed WPC in solution had no change (Manoi and Rizvi 2008). Water holding capacity was greatest for WPC extruded at acidic conditions, followed by the basic extrudates, and then the control WPC extrudate (Manoi and Rizvi 2008). Super critical CO_2 injection into extruder allows for product expansion at lower temperatures and may result in unique structural properties as CO_2 flashes off when the extrudate reaches atmospheric conditions (Manoi and Rizvi 2008).

WPC extruded at basic and acidic conditions had decreased solubility from increased sulfhydryl reactivity with disulfide bond formations and increased hydrophobic aggregations

respectively (Manoi and Rizvi 2009a). Native, acidic, and basic WPC extrudates had

decreased free sulfhydryl content with the greatest decrease at native pH. Protein

aggregation occurred via hydrophobic and non-covalent interaction with acidic extrusion

(Manoi and Rizvi 2009a). WPC texturized at acidic pH formed stable emulsions and

exhibited improved gelling ability (Manoi and Rizvi 2009b). pH is a feed property that

affects the resultant functional properties of extruded WPC and thus should be taken into

consideration in applications that look to extrude MPC.

WPC, WPI, and whey lactalbumin were extruded to make functional ingredients with

limited interaction with other constituents, being more inert to limit potential quality

degradation reactions in snack foods (Onwulata and others 2003). Each dairy protein was

processed through a twin-screw extruder with nine temperature-controlled zones (zones 1-3 =

35°C; zones 4-5 = 75°C; zones 6-9 = 35°C, 50°C, 75°C, or 100°C) with low-shear imparting

screws at constant speed (300 RPM) and moisture content (38%). At 75°C, WPC

denaturation was minimal; solubility decreased 19% compared with native WPC. Whey

lactalbumin and WPI solubility extruded at the same conditions, decreased 25.7% and 66.8%

compared to their respective non-extruded controls and thus underwent more protein

denaturation. WPI solubility decreased with increased melt temperature and level of protein

denaturation, but protein digestibility was unaffected; 89.6% digestible at 35°C and 84.5%

digestible at 100°C. Insoluble protein aggregates formed disulfide bonds as corroborated

with SDS-PAGE. Gel strength increased and foaming properties were maintained for WPI

extruded at or below 50°C, but at 75°C and 100°C extrudates lost gelling ability and foam

formation declined. It is possible to modify the functionality of whey protein without co-

extrusion such that solubility is decreased through formation of disulfide aggregates that may

be less reactive if incorporated into HPN bars while still maintaining good protein digestibility.

NFDM, WPC, and WPI were also extruded to evaluate applicability for inclusion in puffed snacks (Onwulata and others 2010). Dairy powder moisture contents were adjusted by varying a dosing pump output and then were processed through a twin-screw extruder at 50°C, 75°C, and 100°C melts. NFDM proteins were not texturized because solubility and water holding capacity remained the same when compared with native NFDM (Onwulata and others 2010). WPC80 solubility (52% to 64%) and WPI solubility (72% to 93%) remained high after extrusion 50°C and tended to decrease with increased feed moisture. Extrusion processing of WPC80 and WPI at 75°C and 100°C decreased water holding capacity and protein solubility (Onwulata and others 2010). Extrusion puffing NFDM to create textural crisps might serve as good use for NFDM due to its abundant supply and minimal effect on the protein functionality (Tremaine and Schoenfuss 2012). However, fine grinding extruded NFDM, unlike WPC and WPI, and utilizing in a food system would tend to behave like the unmodified version.

The effect of melt temperature at constant moisture (Qi and Onwulata 2011a) and the effect of moisture content (Qi and Onwulata 2011b) were studied by processing WPI through a twin-screw extruder. WPI was adjusted to 50% moisture and extruded at melt temperatures from 5°C to 100°C (Qi and Onwulata 2011a). WPI was extruded at 20%, 30%, 50%, and 75% moisture at 50°C, 75°C, and 100°C (Qi and Onwulata 2011b). Low temperature extrusion (< 35°C) did not decrease WPI solubility (Qi and Onwulata 2011a). WPI solubility decreased slightly after extrusion at 50°C, even though β-lg denatures around 70°C (Fox and

McSweeney 1998), because additional shear, friction, and pressure exerted by the extruder increased WPI denaturation which was exemplified at greater melt temperatures (Qi and Onwulata 2011a; Qi and Onwulata 2011b). WPI extruded with higher feed moisture had better solubility, especially at 50°C, but extrusion at 75°C and 100°C caused significant solubility reductions (Qi and Onwulata 2011a; Qi and Onwulata 2011b). Soluble β-lg content remained constant with low temperature (< 50°C) and moisture extrusion with increased degradation or insolubility with increased moisture (20% to 50%), and when extruded at 100°C, β-lg was undetectable by RP-HPLC (Qi and Onwulata 2011a; Qi and Onwulata 2011b). The α-la content was stable when extruded at 75°C or less, but extrusion at 100°C significantly reduced its soluble portion as detected by RP-HPLC. Feed moisture had no effect on the free primary amine, free secondary amine, or free sulfhydryl content of extruded WPI, but temperatures greater than 50°C reduced each moiety (Qi and Onwulata 2011a; Qi and Onwulata 2011b). Decreased free primary amine, free secondary amine, and free sulfhydryl content indicates increased hydrophobic and disulfide interactions. WPI extruded at high temperature and moisture reduced tryptophan reflectance and depleted protein tertiary structure (Qi and Onwulata 2011a; Qi and Onwulata 2011b). Temperature and feed moisture content influence the resultant functional properties of extruded WPI. This highlights the importance of extruder settings and feed properties when extruding dairy proteins. These same parameters might influence whey protein in MPC the same way during extrusion, but experimentation is necessary to see how the casein and whey protein interact together.

It is well established that extrusion can be used to modify the structure and hence functionalities of concentrated dairy proteins. Little research has focused on extruded MPC because, unlike whey protein, MPC is not a by-product of another process (e.g., cheese making), but is instead a high-quality, complete protein concentrate. Some characteristics of dairy protein concentrate extrudates, more specifically the effect extrusion could have on protein solubility, water holding capacity, and gelling ability, may improve HPN bar texture and limit quality decline.

Extruding dairy proteins at higher temperatures and with high-shear imparting screws led to reduced solubility (Onwulata and others 1998; Manoi and Rizvi 2008; Qi and Onwulata 2011a; Qi and Onwulata 2011b; Onwulata and others 2003; Onwulata and others 2010). Decreased protein solubility post-extrusion was attributed to the formation of disulfide bonds, increased sulfhydryl reactivity, associated hydrophobic interactions, and hence increased protein denaturation (Manoi and Rizvi 2009a; Onwulata and others 2003; Qi and Onwulata 2011a; Qi and Onwulata 2011b). As long as reactive sulfhydryl groups react to form disulfide bonds prior to incorporation to HPN bars, extrusion processing may be a good way to generate an inert ingredient that prevents HPN bar instability by limiting ingredient interactions within the HPN bar matrix. The disulfide bonds formed during extrusion might prevent the aggregation of primary disulfide bonded proteins and thus should eliminate one of the mechanisms of HPN bar hardening (Zhou and others 2008a).

Water holding capacity (e.g., water absorption, water-imbibing, and water-binding) is another relevant property of dairy proteins, which could be described as the amount of retained water under defined, but varying procedures (Kneifel and others 1991). A number of water holding capacity methods with specific regard to dairy proteins have been reviewed:

baumann apparatus, viscometric techniques, farinographic techniques, rehydration analysis, cryoscopic osmometry, equilibration at relative humidity, the net test, centrifugation techniques, differential scanning calorimetry, filtration procedures, and NMR (Kneifel and others 1991). Water absorption describes the ability of a dairy protein to swell taking in water whereas retention is the ability to retain associated water molecules after exposure to an expelling force (Hardy and others 2002).

Extruded dairy proteins have decreased (Onwulata and others 1998; Onwulata and others 2010), increased (Onwulata and others 1998), or unaffected (Matthey and Hanna, 1997) water holding capacities depending on processing and testing methods. Although water holding capacity depends heavily on the employed methodology, reduced water holding capacity of dairy protein powders may indicate possible prevention of moisture migration within the HPN bar matrix. However, testing methodology often looks at water hydration of a specific dairy protein within a short period of time incomparable to desired storage time, 6 to 9 months, for HPN bars. Proteins with low water holding capacity over a shortened testing procedure may just absorb water at a slower rate and thus may not be able to prevent moisture migration in the HPN bar matrix. Protein powders with slow hydration properties were hypothesized to be responsible for partial protein hydration during HPN bar manufacture that serves as the driving force of water migration as the partially hydrated proteins slowly pull water away from other constituents (Li and others 2008; Hazen 2010).

The effect of dairy protein gelation in HPN bars is minimal because these products are not exposed to heat treatment, acidification, or enzymes (e.g., rennet) used to initiate dairy protein gelation. Without any chemical or physical force to initiate gelation, the colloidal calcium phosphate associated with the casein micelle remains intact and κ-casein is

unperturbed, preventing aggregation and gelation (Lucey 2002). Whey protein denaturation, hydrolysis, and tertiary unfolding through high pressure or heat treatment induce gelation (van Vliet and others 2004). In order to achieve whey protein gelation in a mixture of casein and whey (e.g., MPC) a preheat step is necessary to cause whey protein denaturation, followed by the initiation of casein gelation (van Vliet and others 2004). Other constituents in HPN bars are likely to disrupt and prevent formation of particle gels. Some thought, although not tested in the realm of model HPN bar studies, should be given to a protein that has low gel strength and gel forming ability for incorporation into HPN bars. Dairy proteins extruded at high temperatures produced diminished gelling ability whereas low temperature and acidic dairy protein extrudates exhibited improved gelling (Onwulata and others 2003; Manoi and Rizvi 2009b). Dairy proteins that do not form gels may not be able to entrap or attract water from other components, but remain inert and have no effect on HPN bar stability. Lower gel strength may be suggestive of a dairy protein that is capable of forming a softer, more palatable HPN bar versus a protein with higher gel strength.

2.5.3 Glycation, Phosphorylation, and Toasting of Dairy Proteins and Resulting Functional Properties

The functional properties of WPI (Li and others 2005), β-lg (Enomoto and others 2007), and BSA (Enomoto and others 2008) were modified by phosphorylating with sodium pyrophosphate, glycating with maltopentaose, or a combination of glycation plus phosphorylation. Glycated dairy proteins were dissolved with maltopentaose, lyophilized, toasted, dialyzed, and lyophilized. Dairy protein phosphorylation after or without glycation followed the same procedure, except after toasting the protein or sugar-conjugated protein was dissolved in a pyrophosphate buffer, lyophilized, toasted, dialyzed, and lyophilized.

Sugar content of the modified dairy proteins, including WPI, β-lg, and BSA, increased when subjected to glycation; when the same dairy proteins were subjected to phosphorylation, the phosphorous content increased, indicating successful glycation and phosphorylation when used in combination. Native PAGE showed decreased band migration for glycated proteins because of increased molecular weight and increased migration due to increased negative charge from phosphorous bound to each protein. SDS-PAGE revealed that phosphorylated plus glycated WPI formed a β-lg/α-la complex that was not completely reducible, suggesting aggregation formation by sulfhydryl and chemical bonds (Li and others 2005). β-lg aggregation also occurred through sulfhydryl linkages and covalent bonds (Enomoto and others 2007). However, the β-lg/α-la complex and β-lg aggregation might be the result of low temperature toasting rather than a result of glycation, phosphorylation, or the combination of the two.

WPI, β-lg, and BSA are known for being very soluble, but whey proteins display lower heat stability. Glycation and subsequent phosphorylation increased heat stability of these proteins. The heat stability of the glycated WPI increased with degree of phosphorylation (Li and others 2005). Glycated plus phosphorylated β-lg was 79.2% soluble and glycated β-lg was 57.0% soluble after heating at 90°C for 10 min, whereas unmodified β-lg was less than 5% soluble. Phosphorylation and glycation in combination greatly improved the heat stability of BSA, being almost completely soluble after heating at 80°C for 10 min (Enomoto and others 2008). Glucose glycation of β-casein improved protein solubility and overall thermal stability (Darewicz and others 1998; Lee and others 1979). The emulsion activity of both glycated, and glycated and phosphorylated WPI was retained; however, glycation plus phosphorylation without adding the maltopentaose and dissolving in the

pyrophosphate buffer, resulted in decreased emulsion stability. Glycated and glycated plus phosphorylated WPI produced firmer gels than native WPI (Li and others 2005). MPC does not suffer from heat instability, but is often criticized for lacking solubility upon rehydration; especially those in the high-protein category (Singh 2007; Fang and others 2011; Mimouni and others 2009). Glycation, phosphorylation, and/or the combination of the two may be useful in improving the overall solubility of MPC making it more appealing for some food applications.

Glycation and glycation plus phosphorylation had minimal change on the secondary structures of β-lg and BSA, respectively (Enomoto and others 2007; Enomoto and others 2008). Glycation and glycation plus phosphorylation decreased retinol-binding and tryptophan reflectance of β-lg indicating change in tertiary structure (Li and others 2005). Tryptophan reflectance of BSA also decreased with glycation, toasting, and phosphorylation, but overall structure changes were minor (Enomoto and others 2008).

Toasting protein powders under a variety of environmental conditions (e.g., temperatures, relative humidity, pH) can modify and even improve protein functionality, depending on desired attributes. Toasting or heating protein powders in hydrocarbon solutions to limit oxidation was reported, but contact with the organic solution could potentially influence protein structure during heating (Mecham and Olcott 1947). Soybean meal subjected to low degree of toasting (85°C, 20 min) had increased non-covalent interactions whereas extruded counterparts were modified more prevalently by increased formation of covalent disulfide bonds (Marsman and others 1998). Improving the functional properties of dried egg white proteins is possible, with results depending on pH (Mine 1997). Increased alkalinity (pH 7 to 9.5) in egg white powder toasted for up to 15 d at 75°C showed

little to no detrimental effect on protein solubility and improved gel strength (Mine 1997). Toasted egg white powder at the same temperature-time combination with pH adjusted to 10.4 had reduced protein solubility, free sulfhydryl content, and gelling ability, but had increased deamidation and surface hydrophobicity (Mine 1997).

Toasting powder dairy proteins, especially without intended glycation or phosphorylation, is limited (Gulzar and others 2011). WPI was toasted for up to 24 h at 100°C at pH 6.5, 4.5, and 2.5 such that net charge on the WPI was negative, neutral, and positive (Gulzar and others 2011). Toasted WPI powder was more resistant to disulfide bond formation and non-covalent interactions at acidic pH, whereas increasing pH led to larger soluble protein aggregates, the appearance of insoluble aggregates, and a more turbid solution with increased toasting time (Gulzar and others 2011). Dairy proteins are somewhat resistant to denaturation due to limited mobility, elevated denaturation temperature, and lower pH conditions (Ibrahim and others 1993; Zhou and Labuza, 2007; Gulzar and others 2011). β-lg and α-la were toasted separately from each other at 80°C for up to 10 days for surface functional property improvement (Ibrahim and others 1993). The solubility of each toasted protein declined slightly with increased time, but foaming properties and emulsion activity improved (Ibrahim and others 1993). Emulsion stability and surface hydrophobicity decreased with toasting time for β-lg, but both were increased for α-la (Ibrahim and others 1993). β-lg formed soluble aggregates whereas α-la remained predominantly in the monomeric form (Ibrahim and others 1993).

Toasting alone can also be used to modify the structure and functionality of dairy proteins for potential incorporation into HPN bars and alleviation of hardening. Toasting of β-lg decreased surface hydrophobicity, whereas that of α-la increased, the latter of which

could be indication that toasting may prevent the moisture migration within a HPN matrix (Ibrahim and others 1993). Toasting exposed additional free sulfhydryl groups in WPI, but oxidized enough to cause a total free sulfhydryl reduction (Gulzar and others 2011). By decreasing the overall free sulfhydryl content it might be possible to lessen internal disulfide bond formation and protein aggregation within HPN bar matrix, consequently, weakening one of the proposed mechanisms of hardening. Phosphorylation may increase moisture migration within the HPN bar matrix due to increased negative charges resulting in increased protein hydrophilicity, leading to more rapid HPN bar hardening.

Dairy protein glycation, or the non-enzymatic Maillard browning, consists of linking a reducing sugar onto the ε-amine of lysine or, to a lesser extent, the N-terminus of the protein. Maillard reactions can have detrimental effects on intermediate moisture foods, such as HPN bars; however, it was shown that the rate of HPN bar hardening did not correlate well with decrease in free amine content, hence did not depend heavily on Maillard browning (Loveday and others 2009; Loveday and others 2010). Others have negated the effects of Maillard browning as a mechanism of HPN bar hardening with addition of sulfites, through nucleophilic interactions can prevent Maillard browning, yet still experienced hardening (Baier and others 2007). Since Maillard browning reactions are not directly related to HPN bar hardening, it is unlikely that glycation would provide enough modification to prevent associated hardening. Phosphorylation and glycation of dairy proteins by themselves may not bode well in a HPN bar matrix if the goal is to alleviate the HPN bar hardening mechanisms, although improvements of other functional properties are possible.

2.5.4 High-power Ultrasonication Processing of Dairy Proteins and Resulting Functional Properties

Sonication, the application of mechanical wave energy at a frequency above the audible range (>20 kHz), can be used to probe (low-intensity) and modify (high-intensity) food matrices (Torley and Bhandari 2007; Mulet and others 2002; McCarthy and others 2005). High-power ultrasound (HPU) utilizes low frequency (20 kHz to 300 kHz) sound waves and high power levels (10 W cm^{-2} to 1000 W cm^{-2}) to produce functional changes in a food matrix (Mulet and others 2002). Wave propagation through solution fabricates internal pressure gradients, leading to the generation of air cavities. Cavities grow with gas diffusion until cavitation, or rupture of the pulsating air bubble, that impart localized temperature (>5000 K) and pressure (>100 atm) extremes (Bhaskaracharya and others 2003; Torley and Bhandari 2007; Zisu and others 2010).

In addition to high temperature and pressure, cavitation produces shear force exploitable to modify structure of proteins, among other potential uses, such as to kill bacteria, inactivate enzymes, and degas and homogenize solutions in theoretical dairy processing systems (Zisu and others 2010). The functional properties of dairy proteins can be improved with altered secondary and tertiary conformational changes often associated with free and oxidized sulfhydryls, thermal property changes, and internal protein aggregation (Chandrapala and others 2011). With respect to other applications, the effect of HPU on dairy proteins, especially on the pilot plant scale, and resulting functional property analysis is under-studied (Gülseren and others 2007; Zisu and others 2010; Chandrapala and others 2011)

Reconstituted WPC processed with HPU after an initial pre-heat treatment had increased heat stability (Ashokkumar and others 2009; Zisu and others 2010). Heating alone increases WPC solution viscosity, but WPC solutions processed with intermediate sonication retained viscosity similar to the native WPC solutions when subjected to post-heating. The pre-heat step formed aggregates that are demolished upon sonication leading to increased heat stability that is preserved during spray drying (Ashokkumar and others 2009; Zisu and others 2010).

Reconstituted WPC, evaporated whey protein retentate (54% solids, 27% protein), UF-concentrated whey protein retentate (33% solids, 27% protein), milk protein retentate concentrated to (18% solids 27% protein), and calcium caseinate at 24% solids were processed on a pilot-scale sonicator (20 kHz, 4 kW max) (Zisu and others 2010). Sonication reduced solution viscosity and particle size at the conditions tested for all preparations. Intense power delivery for times exceeding ten minutes caused protein aggregation leading to increased particle size, but this far exceeds exposure levels seen with normal operation of the pilot-scale HPU system (Zisu and others 2010). Sonicated whey protein retentate had improved gel strength over the unprocessed control. Milk protein retentate processed at 60% amplitude, 20 kHz, and 1 kW decreased viscosity by 0.02 Pa-s through disruption of internal protein interactions (Zisu and others 2010).

BSA (3×10^{-4} M), maintained at 2°C, was sonicated at 20 W cm^{-2} for up to 90 min (Gülseren and others 2007). Increasing sonication time increased overall surface activity, decreased denaturation enthalpy, and did not affect denaturation temperature. PRODAN, a charge neutral surface hydrophobicity probe, revealed increased BSA surface hydrophobicity with longer sonication time. The magnitude of surface potential and particle size also

increased with increased sonication time. Sonication resulted in increased alpha helical content and decreased free sulfhydryl content with increased exposure time.

Casein solutions were sonicated to explore effect on casein micelles (Madadlou and others 2009) and acid gel texture (Madadlou and others 2010). 3% casein solutions were processed in a sonication water bath (35 kHz) for 6 h at three power levels (2.0, 4.1, and 6.6 W) and three alkaline pH (8.0, 9.7, and 11.4). Turbidity decreased at lower pH and with increased power level due to particle size reduction from ultrasonic treatment (Madadlou and others 2009). The sonication water bath did not affect primary amine content and was not powerful enough to break peptide bonds (Madadlou and others 2009). Again 3% casein solutions were sonicated, but this time dual ultrasound was applied to the solution using a sonication water bath (130 kHz) and an ultrasonic homogenizer (24 kHz) for 0, 60, or 120 min (Madadlou and others 2010). Gelation initiated with glucono delta-lactone (GDL) was delayed after casein was processed with HPU (Madadlou and others 2010). Casein reactive groups are partially exposed with HPU producing gels with higher complex modulus and overall firmness (Madadlou and others 2010). Micrographs revealed greater connectivity in structure of gels formed from casein first subjected to HPU, which correlates well with the observed texture (Madadlou and others 2010).

While very little work is reported on HPU processing of concentrated MPC streams, e.g., Zisu et al. (2010), more focus is being placed on sonication of fluid milk, possibly to serve as a means of pasteurization and enzyme inactivation to extend shelf-life. Several other purified dairy protein streams including WPC, BSA, and casein have been subjected to HPU processing and it is possible that the resultant effects will carry over to HPU applied to all the proteins in one solution, e.g., concentrated MPC streams. Increased protein heat

stability that results from sonication of some dairy proteins may also lead to increased HPN bar stability. Protein aggregates formed during the preheat step, which may need to be more severe to aggregate casein, followed by sonication and drying may produce a more inert protein for HPN bars. Similarly increased surface hydrophobicity and decreased free sulfhydryl content, as obtained by processing BSA with HPU (Gülseren and others 2007), may prove to limit moisture migration and internal disulfide bond formation within the HPN bar. However, increased surface potential of BSA may be counterproductive in HPN bar stability. Firmer casein gels may indicate a more compact protein network that may allow for the formation of a tighter protein matrix in HPN bars. Water holding capacity and viscosity in yogurts produced from sonicated milks was higher than conventionally set type yogurts (Riener and others 2009). If dairy proteins incorporated into HPN bars contain a high water holding capacity, they may pull water away from other constituents increasing the rate of moisture migration. Conversely, these proteins may be better able to hydrate during HPN bar manufacture leading to increased HPN bar stability during storage.

2.6 Conclusion

Modifying MPCs via physical and chemical methods can lead to changes in protein structures, which may result in desired protein functionality, as has been done with other dairy proteins, namely whey proteins. The modifications imparted by enzyme hydrolysis, extrusion, toasting, and sonication to proteins in MPC could promote greater HPN bar stability, and allow for an extended shelf-life of HPN bars. Care must be taken when extrapolating dairy protein functional property analysis to performance within a HPN bar matrix because conflicting results may potentially eliminate a promising protein modification.

Additionally, modification of milk proteins is one among many possible ways to increase HPN bar stability. Careful ingredient selection, including the incorporation of protein blends, plasticizers, and sugar alcohols, are also known to add stability and extend shelf-life. Discussion on such topics is beyond the scope of this review, which was focused primarily on comparing results from protein processing methods for other dairy proteins ingredients to MPC. Protein functional properties, post-modification, and their potential for performance in HPN bars were discussed. However, model protein bars need to be produced with modified MPC, and studied for their instrumental texture and sensory panel evaluation to gain a full understanding of the effect of a proposed modification on eating quality and quality decline during storage.

2.7 References

Adams SL. 2008. Mechanisms of Nutrition Bar Hardening: Effect of Hydrolyzed Whey Protein and Carbohydrate Source. [MSc thesis]. Utah: Utah State Univ. 79 p.

Amiot J, Germain L, Turgeon S, Lemay M, Ory-Salam C, A. Auger F. 2004. Peptides from milk protein hydrolysates to improve the growth of human keratinocytes in culture. Int Dairy J 14(7):619-26.

Anema SG, Pinder DN, Hunter RJ, Hemar Y. 2006. Effects of storage temperature on the solubility of milk protein concentrate (MPC85). Food Hydrocoll 20(2-3):386-93.

Ashokkumar M, Lee J, Zisu B, Bhaskarcharya R, Palmer M, Kentish S. 2009. Hot topic: Sonication increases the heat stability of whey proteins. J Dairy Sci 92(11):5353-6.

Baier SK, Guthrie BD, Smith SA, Lendon CA, Muroski, AR. 2007. Influence of extrusion on protein conformation and shelf-life extension of nutritional bars. Paper presented at International Symposium of the Properties of Water X, 2-7 September, Bangkok, Thailand.

Bailey KW. 2001. Imports of Milk Protein Concentrates: Assessing the Consequences. Penn State - Department of Agricultural Economics and Rural Sociology Staff Paper #343

Baldwin A, Pearce D. 2005. Chapter 15 - Milk Powder. In: C. Onwulata, editor. Encapsulated and Powdered Foods. 1st ed. New York: Taylor & Francis. p 387-433.

Bhaskaracharya RK, Kentish S, Ashokkumar M. 2009. Selected Applications of Ultrasonics in Food Processing. Food Eng Rev 1(1):31-49.

Book S. 2008. Protein ingredients for health and texture -- February 2008. Prepared Foods 177(2):61-65. [serial online] 2012 (January 18): Available from http://www.preparedfoods.com/articles/print/105676.

Bouhallab S, Mollé D, Léonil J. 1993. Continuous hydrolysis of β-casein in a membrane reactor: Preparation of a bioactive peptide. Biotechnology Letters 15(7):697-702.

Chandan R. 1997. Production and Specifications of Milk Concentrates. In: Anonymous Dairy-Based Ingredients. 1st ed. St. Paul: American Association of Cereal Chemists, Inc. p 23-40.

Chandrapala J, Zisu B, Palmer M, Kentish S, Ashokkumar M. 2011. Effects of ultrasound on the thermal and structural characteristics of proteins in reconstituted whey protein concentrate. Ultrason Sonochem 18(5):951-7.

Childs JL, Yates MD, Drake MA. 2007. Sensory Properties of Meal Replacement Bars and Beverages Made from Whey and Soy Proteins. J Food Sci 72(6):S425-34.

Codex Alimentarius, FAO/WHO Food Standards. Codex standard for milk powders and cream powders. Codex Stan 207-1999. 2012.

Coleman EC, Akashe A, Tancredi M, Smith BK, inventors; Inc Kraft Food Holdings, assignee. 2005 March 10. Reducing Bar Hardness via Repositioning of Micronutrients within a Matrix U.S. patent US 2005/0053695 A1.

Dairy Foods. (2005). Where's Milk in the Functional and New-age Beverage Businesses? Dairy Foods. 106 : July 12, 2012.

Darewicz M, Dziuba J, Mioduszewska H. 1998. Some physico-chemical properties and structural changes of bovine beta-casein upon glycation. Nahrung 42(3-4):213-4.

De Castro-Morel M, Harper WJ. 2002. Basic functionality of commercial milk protein concentrates. Milchwissenschaft 57(7):367-70.

Enomoto H, Li C, Morizane K, Ibrahim H, Sugimoto Y, Ohki S, Ohtomo H, Aoki T. 2008. Improvement of functional properties of bovine serum albumin through phosphorylation by dry-heating in the presence of pyrophosphate. J Food Sci 73(2):C84-91.

Enomoto H, Li C, Morizane K, Ibrahim HR, Sugimoto Y, Ohki S, Ohtomo H, Aoki T. 2007. Glycation and phosphorylation of beta-lactoglobulin by dry-heating: effect on protein structure and some properties. J Agric Food Chem 55(6):2392-8.

Fang Y, Selomulya C, Ainsworth S, Palmer M, Chen XD. 2011. On quantifying the dissolution behaviour of milk protein concentrate. Food Hydrocolloid 25(3):503-10.

Fox PE, McSweeney PLH. 1998. Dairy Chemistry and Biochemistry. 1st ed. United Kingdom: Blackie Academic & Professional. 478 p.

Gallo-Torres JM. 2002. Raising the Bar. Prepared Foods 171(9):65. [serial online] 2012 (July 12): Available from http://www.preparedfoods.com/articles/raising-the-bar.

Gallo-Torres JM. 2003. Made to Last. Prepared Foods 172(9):96. [serial online] 2012(January 18): Available from http://www.preparedfoods.com/articles/made-to-last.

Gautam A, Garcia AC, Hander RJ, inventors; Slim-Fast Foods Company, division of Conopco, Inc., assignee. 2006 June 1. Nutrition Bar. U.S. patent US 2006/0115554 A1.

Gerdes S. 2008. Are All Milk Protein Concentrates Alike? Dairy Foods 109(1):90. [serial online] 2012 (July 12): Available from http://www.innovatewithdairy.com/SiteCollectionDocuments/Are%20all%20Milk%20Protein%20concentrates%20alike.pdf.

Gülseren İ, Güzey D, Bruce BD, Weiss J. 2007. Structural and functional changes in ultrasonicated bovine serum albumin solutions. Ultrason Sonochem 14(2):173-83.

Gulzar M, Bouhallab S, Jeantet R, Schuck P, Croguennec T. 2011. Influence of pH on the dry heat-induced denaturation/aggregation of whey proteins. Food Chem 129(1):110-6.

Guo MR, Fox PF, Flynn A, Kindstedt PS. 1995. Susceptibility of beta-lactoglobulin and sodium caseinate to proteolysis by pepsin and trypsin. J Dairy Sci 78(11):2336.

Hardy J, Scher J, Banon S. 2002. Water activity and hydration of dairy powders. Le Lait 82(4):441452-452.

Havea P. 2006. Protein interactions in milk protein concentrate powders. Int Dairy J 16(5):415-22.

Heldman DR, Hartel RW. 1997. Chapter 10 - Food Extrusion In: D. R. Heldman, editor. Principles of Food Processing 1st ed. New York: Chapman & Hall. p 253-83.

Heldman DR, Hartel RW. 1997. Chapter 8 - Dehydration In: D. R. Heldman, editor. Principles of Food Processing. 1st ed. New York: Chapman & Hall. p 177-218.

Henning DR, Baer RJ, Hassan AN, Dave R. 2006. Major Advances in Concentrated and Dry Milk Products, Cheese, and Milk Fat-Based Spreads. J Dairy Sci 89(4):1179-88.

Hutchinson M. 2009. R&D: Meeting Snack Bar Challenges -- June 2009. Prepared Foods 178(6):60. [serial online] 2012(January 18): Available from http://www.preparedfoods.com/articles/print/107894.

Ibrahim HR, Kobayashi K, Kato A. 1993. Improvement of the Surface Functional Properties of Beta-Lactoglobulin and Alpha-Lactalbumin by Heating in a Dry State. Biosci Biotech Bioch. 57(9):1549-52.

Imtiaz SR, Kuhn-sherlock B, Campbell M. 2012. Effect of Dairy Protein Blends on Texture of High Protein Bars. J Texture Stud 43(4):275-86.

Jesse E. 2003. U.S. Imports of Concentrated Milk Proteins: What We Know and Don't Know?. University of Wisconsin-Madison - Department of Agricultural and Applied Economics. Staff Paper #80.

Kelly P. 2011. MILK PROTEIN PRODUCTS | Milk Protein Concentrate. In: Editor-in-Chief: John W. Fuquay, editor. Encyclopedia of Dairy Sciences (Second Edition). 2nd ed. San Diego: Academic Press. p 848-54.

Kilara A, Panyam D. 2003. Peptides from milk proteins and their properties. Crit Rev Food Sci Nutr 43(6):607-33.

Kneifel W, Paquin P, Abert T, Richard JP. 1991. Dairy Foods: Water-Holding Capacity of Proteins with Special Regard to Milk Proteins and Methodological Aspects - A Review. J Dairy Sci 74(7):2027-41.

Kruse P. 2009. Statement of Paul Kruse CEO and President Blue Bell Creameries, L.P. on behalf of International Dairy Foods Association. International Dairy Foods Association. [serial online] 2012 (July 12): Available from http://www.idfa.org/files/news/4453_kruse_testimony_0713.pdf.

Le TT, Bhandari B, Deeth HC. 2011. Chemical and physical changes in milk protein concentrate (MPC80) powder during storage. J Agric Food Chem 59(10):5465.

Lee HS, Sen LC, Clifford AJ, Whitaker JR, Feeney RE. 1979. Preparation and nutritional properties of caseins covalently modified with sugars. Reductive alkylation of lysines with glucose, fructose, or lactose. J Agric Food Chem 27(5):1094.

Li CP, Enomoto H, Ohki S, Ohtomo H, Aoki T. 2005. Improvement of Functional Properties of Whey Protein Isolate Through Glycation and Phosphorylation by Dry Heating. J. Dairy Sci. 88(12):4137-45.

Li Y, Szlachetka K, Chen P, Lin X, Ruan R. 2008. Ingredient Characterization and Hardening of High-Protein Food Bars: an NMR State Diagram Approach. Cereal Chem 85(6):780-6.

Limón-valenzuela V, Martínez-bustos F, Aguilar-palazuelos E, Caro-corrales JJ, Zazueta-morales JJ. 2010. Physicochemical Evaluation and Optimization of Enriched Expanded Pellets with Milk Protein Concentrate. Cereal Chem 87(6):612-8.

Liu X, Zhou P, Tran A, Labuza TP. 2009. Effects of Polyols on the Stability of Whey Proteins in Intermediate-Moisture Food Model Systems. J Agric Food Chem 57(6):2339-45.

Loveday SM, Hindmarsh JP, Creamer LK, Singh H. 2009. Physicochemical changes in a model protein bar during storage. Food Res Int 42(7):798-806.

Loveday SM, Hindmarsh JP, Creamer LK, Singh H. 2010. Physicochemical changes in intermediate-moisture protein bars made with whey protein or calcium caseinate. Food Res Int 43(5):1321-8.

Lucey JA. 2002. Formation and physical properties of milk protein gels. J Dairy Sci 85(2):281.

Madadlou A, Emam-Djomeh Z, Mousavi ME, Mohamadifar M, Ehsani M. 2010. Acid-induced gelation behavior of sonicated casein solutions. Ultrason Sonochem 17(1):153-58.

Madadlou A, Mousavi ME, Emam-djomeh Z, Ehsani M, Sheehan D. 2009. Sonodisruption of re-assembled casein micelles at different pH values. Ultrason Sonochem 16(5):644-8.

Manoi K, Rizvi SSH. 2008. Rheological characterizations of texturized whey protein concentrate-based powders produced by reactive supercritical fluid extrusion. Food Res Int 41(8):786-96.

Manoi K, Rizvi SSH. 2009a. Physicochemical changes in whey protein concentrate texturized by reactive supercritical fluid extrusion. J Food Eng 95(4):627.

Manoi K, Rizvi SSH. 2009b. Emulsification mechanisms and characterizations of cold, gel-like emulsions produced from texturized whey protein concentrate. Food Hydrocoll 23(7):1837-47.

Marsman GJP, Gruppen H, de Groot J, Voragen AGJ. 1998. Effect of toasting and extrusion at different shear levels on soy protein interactions. J Agric Food Chem 46(7):2770-7.

Matthey FP, Hanna MA. 1997. Physical and Functional Properties of Twin-screw Extruded Whey Protein Concentrate - Corn Starch Blends. Lebensm-Wiss Technol 30(4):359-66.

McCarthy K, Wang L, McCarthy M. 2005. Ultrasound Properties. In: M. A. Rao , A. K. Datta, S. S. H. Rizvi, editors. Engineering Properties of Foods. 3rd ed. CRC Press.

McMahon DJ, Adams SL, McManus WR. 2009. Hardening of High-Protein Nutrition Bars and Sugar/Polyol-Protein Phase Separation. J Food Sci 74(6):E312-21.

Mecham DK, Olcott HS. 1947. Effect of Dry Heat on Proteins. Industrial engineering chemistry 39(8):1023-7.

Mietsch F, Fehér J, Halász A. 1989. Investigation of functional properties of partially hydrolyzed proteins. Nahrung 33(1):9-15.

Mimouni A, Deeth HC, Whittaker AK, Gidley MJ, Bhandari BR. 2009. Rehydration process of milk protein concentrate powder monitored by static light scattering. Food Hydrocoll 23(7):1958-65.

Mine Y. 1997. Effect of dry heat and mild alkaline treatment on functional properties of egg white proteins. J Agric Food Chem 45(8):2924-8.

Mistry VV, Hassan HN. 1992. Manufacture of nonfat yogurt from a high milk protein powder. J Dairy Sci 75(4):947-57.

Mistry VV. 2002. Manufacture and application of high milk protein powder. Le Lait 82(4):515-22.

Mulet A, Carcel JA, Benedito J, Sanjuan N. 2002. Applications of Low- Intensity Ultrasonics In The Dairy Industry. In: J. Welti-Chanes, G. V. Barbosa-Canovas, J. M. Aguilera, editors. Engineering and Food for the 21st Century. 1st ed. CRC Press.

Oldfield D, Singh H. 2005. Chapter 14 - Functional Properties of Milk Powders. In: C. Onwulata, editor. Encapsulated and Powdered Foods. 1st ed. New York: Taylor & Francis. p 365-86.

Onwulata CI, Konstance RP, Cooke PH, Farrell HMJ. 2003. Functionality of Extrusion - Texturized Whey Proteins. J Dairy Sci 86(11):3775-82.

Onwulata CI, Konstance RP, Smith PW, Holsinger VH. 1998. Physical Properties of Extruded Products as Affected by Cheese Whey. J Food Sci 63(5):1-5.

Onwulata CI, Konstance RP, Smith PW, Holsinger VH. 2001a. Co-extrusion of Dietary Fiber and Milk Proteins in Expanded Corn Products. LWT-Food Sci Technol 34(7):424-9.

Onwulata CI, Phillips JG, Tunick MH, Qi PX, Cooke PH. 2010. Texturized Dairy Proteins. J Food Sci 75(2):E100-9.

Onwulata CI, Smith PW, Konstance RP, Holsinger VH. 2001b. Incorporation of whey products in extruded corn, potato or rice snacks. Food Res Int 34(8):679-87.

Qi PX, Onwulata CI. 2011a. Physical Properties, Molecular Structures, and Protein Quality of Texturized Whey Protein Isolate: Effect of Extrusion Temperature. J Agric Food Chem (59):4668-75.

Qi PX, Onwulata CI. 2011b. Physical properties, molecular structures, and protein quality of texturized whey protein isolate: Effect of extrusion moisture content. J Dairy Sci 94(5):2231-44.

Rehman S, Farkye NY, Yim B. 2003. Use of dry milk protein concentrate in pizza cheese manufactured by culture or direct acidification. J Dairy Sci 86(12):3841-48.

Riener J, Noci F, Cronin DA, Morgan DJ, Lyng JG. 2009. The effect of thermosonication of milk on selected physicochemical and microstructural properties of yoghurt gels during fermentation. Food Chem. 114(3):905-11.

Singh H. 2007. Interactions of milk proteins during the manufacture of milk powders. Le Lait 87(4-5):413-23.

Sinha R, Radha C, Prakash J, Kaul P. 2007. Whey protein hydrolysate: Functional properties, nutritional quality and utilization in beverage formulation. Food Chem. 101(4):1484-91.

Southward CR. 2012. Casein Products. New Zealand Dairy Research Institute. [serial online] 2012 (August 28): Available from http://nzic.org.nz/ChemProcesses/dairy/3E.pdf.

Sparrow K. 2009. Schumer Introduces Bill to Raise Tariffs on MPCs, Caseins and Caseinates. International Dairy Foods Association. [serial online] 2012 (January 18): Available from http://www.idfa.org/key-issues/category/global-markets/tariffs/details/4333/

Torley PJ, Bhandari BR. 2007. Chapter 29 - Ultrasound in Food Processing and Preservation. In: M. S. Rahman, editor. Handbook of Food Preservation, Second Edition. 2nd ed. CRC Press. p 713-39.

Tremaine A, Schoenfuss TC. 2012. Twin-Screw Extrusion Puffing of Nonfat Dry Milk and Starch – The Effects of Acid Addition, Fluid Addition Rate and Nonfat Dry Milk Concentration on Extruded Products. J Food Process Preserv doi:10.1111/j.1745-4549.2012.00769.x.

UBIC Consulting. 2012. Milk Protein Concentrate Ingredient Market. [serial online] 2012 (July 12): Available from http://www.ubic-consulting.com/template/fs/documents/Dairy-Ingredients/milkproteinconcentrate-tmp-ingredient-market.pdf.

Urista CM, Fernández RA, Rodriguez FR, Cuenca AA, Jurado AT. 2011. Review: Production and functionality of active peptides from milk. Food Sci Technol Int 17(4):293-317.

USDA. 2010. Dairy Products 2009 Summary National Agricultural Statistics Service Da 2-1 (10)

van Vliet T, Lakemond CMM, Visschers RW. 2004. Rheology and structure of milk protein gels. Curr Opin Colloid In 9(5):298-304.

Zhou P, Labuza TP. 2007. Effect of Water Content on Glass Transition and Protein Aggregation of Whey Protein Powders During Short-Term Storage. Food Biophys 2(2-3):108-16.

Zhou P, Liu X, Labuza TP. 2008a. Effects of Moisture-Induced Whey Protein Aggregation on Protein Conformation, the State of Water Molecules, and the Microstructure and Texture of High-Protein-Containing Matrix. J Agric Food Chem 56(12):4534-40.

Zhou P, Liu X, Labuza TP. 2008b. Moisture-Induced Aggregation of Whey Proteins in a Protein/Buffer Model System. J Agric Food Chem 56(6):2048-54.

Zhu D, Labuza TP. 2010. Effect of Cysteine on Lowering Protein Aggregation and Subsequent Hardening of Whey Protein Isolate (WPI) Protein Bars in WPI/Buffer Model Systems. J Agric Food Chem 58(13):7970-9.

Zisu B, Bhaskaracharya R, Kentish S, Ashokkumar M. 2010. Ultrasonic processing of dairy systems in large scale reactors. Ultrason Sonochem 17(6):1075-81.

CHAPTER 3. MODIFICATION OF MILK PROTEIN CONCENTRATE WITH EXTRUSION, AND TOASTING, AND CHARACTERIZATION FOR POTENTIAL HIGH-PROTEIN BAR APPLICATIONS

Modified from a paper to be submitted to the *Journal of Food Science*

Justin C. Banach[1,2], Stephanie Clark[3], and Buddhi P. Lamsal[4,5]

3.1 Abstract

Important functional properties of milk protein concentrate with 80% protein, modified with low- and high-shear extrusion, or low temperature toasting were compared. High- and low-shear screw profiles in a co-rotating extruder, and four different ramped temperature profiles with die temperatures of 65°C, 75°C, 90°C, and 120°C were compared for extrusion of moisture adjusted milk protein concentrate. Extrudates were pelletized, dried, and ground to a fine powder. Toasting was done at 75°C and 110°C for 4 h for milk protein modification. The resulting functional properties of modified milk protein concentrate, including protein solubility profiles, water holding capacity, gel strength, and soluble protein surface hydrophobicity were evaluated. Reduced and non-reduced sodium dodecyl sulfate polyacrylamide gel electrophoresis revealed peptide structural changes that occurred due to processing, especially the effect on whey protein subunits. Results are discussed in terms of applicability of extruded or toasted milk protein concentrates in potential high-protein nutrition bar applications.

---

[1] Graduate Student, Department of Food Science and Human Nutrition, Iowa State University.
[2] Primary researcher and author.
[3] Associate Professor, Department of Food Science and Human Nutrition, Iowa State University.
[4] Assistant Professor, Department of Food Science and Human Nutrition, Iowa State University.
[5] Author for correspondence, lamsal@iastate.edu, p: 515-294-8681, f: 515-294-8181.

3.2 Introduction

Milk protein concentrates (MPC) are powdered milk products with protein content ranging from 42% to 85%. High-quality MPCs are produced with micro- and ultrafiltration of skim milk to concentrate casein and whey proteins in the retentate with lactose permeating. Following ultrafiltration, diafiltration may be employed to increase protein content higher than 70%; the retentate is concentrated with evaporation and spray dried (Baldwin and Pearce 2005). Ultrafiltration is the preferred method of MPC production because it allows the casein micelle to remain intact offering emulsion stability, heat stability, opacity, flavor, and protein fortification in food applications (Baldwin and Pearce 2005).

MPCs are mostly used in processed cheese products to standardize protein content and increase yield, but are prohibited in cheeses with standard of identity (Mistry 2002). MPCs from Grade A milk are also used in low-fat and Greek yogurts and have been shown to improve the texture of the former as well as full-fat varieties (Mistry and Hassan 1992; Singh 2007). Specialty nutrition products, including beverage, enteral, powder, and sport products may use MPCs (Baldwin and Pearce 2005). Despite having good flavor, nutritional quality, and being produced domestically, MPCs are rarely employed in high-protein nutrition (HPN) bar formulations (Baldwin and Pearce 2005). Instead, whey protein concentrates (WPC), whey protein isolates (WPI), whey protein hydrolysates, and soy proteins are used for better functionality (Imtiaz and others 2012).

MPCs produce HPN bars that lack cohesiveness and have crumbly texture (Li and others 2008). Another important textural parameter in HPN bars is hardness, which during storage tends to increase to undesirable levels in HPN bars formulated with unmodified

MPCs (Imtiaz and others 2012). The exact mechanism of instability in HPN bars formulated with unmodified MPCs is not clear, but likely a combination of moisture migration, limited free water availability, phase separation, and internal disulfide bond formation due to conformational changes occurring for lack of water in the local protein domain (Loveday and others 2009; Loveday and others 2010; Li and others 2008; McMahon and others 2009, Zhou and others 2008a; Zhou and others 2008b). Low moisture content limits free water available to plasticize, but also ensures water activity less than 0.65 to stop microbial growth (Loveday and others 2009).

Modification of MPCs via extrusion or toasting could result in an inert ingredient that is less susceptible to moisture migration, while still providing quality protein. Extrusion of WPC, WPI, whey lactalbumin, and nonfat dry milk (NFDM) was done to create inert protein ingredients for use in snack foods (Onwulata and others 2003; Onwulata and others 2010). Extruding dairy proteins, especially at temperatures greater than 75°C, reduced protein solubility (Onwulata and others 1998; Manoi and Rizvi 2008; Qi and Onwulata 2011a; Qi and Onwulata 2011b; Onwulata and others 2003; Onwulata and others 2010). Extruded dairy proteins have had decreased (Onwulata and others 1998; Onwulata and others 2010), increased (Onwulata and others 1998), or unchanged (Matthey and Hanna 1997) water holding capacity.

Reduced MPC solubility and altered water holding capacity are some potential results from modification that may indicate promise for modified MPCs in HPN bars. Enhanced disulfide bond formation with extrusion was seen in other dairy proteins and MPC85 stored at 40°C (Onwulata and others 2010; Havea 2006). Preformed disulfide bonds due to

extrusion may limit internal aggregations within the HPN bar matrix, preventing one suggested mechanism of HPN bar hardening.

In this study, the functionality of milk protein concentrate with 80% protein (MPC80) was modified through twin-screw extrusion and dry toasting. To the best of our knowledge, neither modification technique has been applied to MPC80 without addition of starchy materials during extrusion or adding phosphorous and/or sugars during toasting. Specific objective of the study was to modify MPC80 with extrusion or toasting, and elucidate the structural and functional property changes brought upon by these modifications for potential application in HPN bars.

3.3 Materials and Methods

3.3.1 Materials and Reagents

MPC80 was purchased from Idaho Milk Products (Jerome, ID). Bovine serum albumin (BSA), sodium pyrophosphate tetrabasic (TSPP), and 8-Anilino-1-napthalene-sulfonic acid (ANS) were purchased for Sigma-Aldrich Inc. (St. Louis, MO). Sodium dodecyl sulfate (SDS) and tris were purchased from Biorad (Hercules, CA). β-mercaptoethanol and the Pierce BCA protein assay kit where obtained from VWR International (Radnor, PA). All other chemicals were obtained from Thermo Fisher Scientific (Waltham, MA).

3.3.2 MPC80 Extrusion and Toasting

Based on information from preliminary operation of the extruder, the moisture content of the feed MPC80 was adjusted to about 38% by misting dry MPC80 with distilled water with continual mixing in a mechanical mixer (Kitchen Aid, model# KS55, St. Joseph, MI). After moisture adjustment, the MPC80 was placed in a large plastic bag, sealed in a 5

gal pail, and stored at 4°C overnight for adequate moisture equilibration. Prior to extrusion,

the sample was hand-mixed to break up large clumps and equilibrated to room temperature.

Prior to further processing, moisture content was measured to assure that adjustment was

±1% of 38%.

Moisture-adjusted MPC80 was fed via a hopper into a co-rotating twin-screw

extruder (Micro 18, American Leistritz Extruder, Somerville, NJ) with length (540 mm) to

diameter (18 mm) ratio equal to 30. The feeding zone (Z0) of the barrel was water cooled,

followed by five temperature controlled zones (Z1 - Z5), and zone 6 (Z6), the die

temperature. Screw speed was kept constant at 60 RPM, but two screw profiles, high- and

low-shear, were utilized. The low-shear screws had more feeding sections, whereas the high-

shear screws had more kneading blocks. Four-temperature profiles in combination with each

screw type produced eight extruded samples (Table 3-1). Extrusion of MPC80 was

replicated a minimum of two times; LS65 and LS120 extrusions (Table 3-1) were replicated

three times.

Table 3-1 Temperature and Screw Profile during Extrusion of MPC80

Code	Screw Profile	Zone Temperature (°C)					
		Z1	Z2	Z3	Z4	Z5	Z6
LS65	Low-shear	25	35	45	55	65	65
LS75	Low-shear	35	45	55	65	75	75
LS90	Low-shear	50	60	70	80	90	90
LS120	Low-shear	35	45	55	65	120	120
HS65	High-shear	25	35	45	55	65	65
HS75	High-shear	35	45	55	65	75	75
HS90	High-shear	50	60	70	80	90	90
HS120	High-shear	35	45	55	65	120	120

Following extrusion, the extrudates were pelletized with a laboratory cutter (model#

BT25, Scheer Bay Co., Bay City, MI) to increase surface area for drying and to ease fine

grinding. The pellets were spread on a metal pan and dried for 72 h in a forced draft oven at

50°C. After drying samples were ground with a centrifugal mill (model# ZM1, Retsch, Newtown, PA) equipped with a 0.5 mm mesh.

MPC80 was toasted in a laboratory oven to mimic extrusion cooking without applying shearing force (Murphy and others 2002). MPC80 was spread into a thin layer (2 to 5 mm) on an aluminum foil lined oven rack and toasted for 4 h at 75°C (T75) or 110°C (T110) in a laboratory oven. The toasted MPC80 was cooled at room temperature for 45 min. Toasting experiments were replicated three times.

All ground samples, including unmodified MPC80, were sieved through 250 μm to ensure particle size uniformity. Samples were stored under refrigerated conditions in double zipper-seal bags after drying, grinding, sieving, and prior to any functional property analysis.

3.3.3 Functional Properties Characterization

Solubility profiles were determined following methods used by Rickert and others (2004). One percent (w/w) sample dispersions were prepared in distilled water and the pH was adjusted to 2.0, 3.0, 4.6, 5.5, 6.0, 7.0, 9.0, or 11.0 using 2 *N* HCl or 2 *N* NaOH. The dispersions were mixed for 1 h and the pH was readjusted if needed at 15, 30, and 45 min after the initial pH adjustment. A portion of the dispersion was centrifuged at $10,000 \times g$ for 10 min and protein in supernatant was determined with Biuret assay with a BSA standard. Solubility was expressed as a percent by dividing protein content in the supernatant by total protein in the dispersion. Duplicate measurements were made for each sample preparation; only the second and third preparations of toasted MPC80 were analyzed for solubility.

Water holding capacity (WHC) was determined following a procedure similar to that used by Onwulata and others (2010). Two g of sample was weighed into a 50-mL centrifuge tube and 20 mL (±0.4 mL) of distilled water was added. Tubes were agitated for two 1 min

intervals during a 15 min hold period. Each centrifuge tube was spun at 10,000 × *g* for 15 min. Supernatant was decanted into aluminum weigh dishes and tubes were inverted above each dish at a 45° angle for 5 min. WHC is the grams of water occluded per gram of dry sample. Triplicate measurements were made for each sample preparation.

Modified and unmodified MPC80s were gelled following procedure by Mizuno and Lucey (2007) with slight deviations. Samples were reconstituted at 10% (w/w) protein in distilled water and allowed to hydrate with continual mixing for 1 h. pH was checked and if needed was readjusted to 6.8, the pH of unmodified MPC80 in solution, with 2 *N* NaOH or 2 *N* HCl. Eighteen g of sample dispersion was transferred to glass vials (height = 85 mm, diameter = 23 mm). Gelation was initiated by adding 2 mL of TSPP solution containing 5% (w/v) TSPP and 0.2% (w/v) sodium azide. The ratio of protein to TSPP in each vial was kept constant at 18. The vials were capped, inverted three times, vortexed at low speed for 3 s, left to gel at room temperature for 24 h, and then chilled for 24 h at 4°C.

Gel strength was evaluated without temperature equilibration by puncturing each gel in the vial at 1 mm/s with an 11 mm diameter, blunted stainless steel cylindrical probe (TA-212) (TA-XT2, Texture Technologies, Scarsdale, NY). Peak force exerted during 20 mm compression was taken as the gel strength. Triplicate measurements were made for each sample preparation.

Surface hydrophobicity of control and modified MPC80 was determined following a procedure modified from Hayakawa and Nakai (1985). Based on protein solubility at pH 7 (Figures 3-4 and 3-5) and percent protein, samples were weighed into beakers, and were mixed for 1 h after addition of 50 mL 10 mM sodium phosphate buffer (pH 7). The dispersion was centrifuged for 10 min at 10,000 × *g* and supernatant was filtered through

0.22 µm low protein binding syringe filter (Millex-GV, EMD Millipore, Billerica, MA). The protein in supernatant was determined with the with bicinchoninic acid (BCA) assay (Mao and others 2012). The soluble protein was diluted to 1000 µg/mL with 10 mM sodium phosphate buffer (pH 7). The standardized protein solution was diluted to seven concentrations between 0.008% and 0.03% protein and a total volume of 3 mL. A Biorad VersaFluor™ fluorometer (Hercules, CA) with 390 nm excitation and 460 nm emission wavelengths was used to measure relative fluorescence intensity (RFI) of each protein solution after the addition of 40 µL of 8 mM ANS in 10 mM sodium phosphate buffer (pH 7). Prior to RFI measurement, gain was set to medium, the instrument was zeroed with 10 mM sodium phosphate buffer, and the range was set to 30% full scale for 15 µL 8 mM ANS in 3 mL of methanol. RFI was corrected for each sample by subtracting the RFI for blank sodium phosphate buffer plus 40 µL of 8 mM ANS. Corrected RFI was plotted versus protein concentration (%), and the slope of a linear regression line was taken as surface hydrophobicity.

3.3.4 Analytical Procedures

Samples were prepped for reduced and non-reduced sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) by mixing 50 mg of protein for 1 h at room temperature with 25 mL of reduced (5% w/v SDS and 5% v/v β-mercaptoethanol) or non-reduced (5% w/v SDS) protein extraction buffer prepared in 10 mM sodium phosphate buffer (pH 7). Dispersions were centrifuged at 10,000 × *g* for 10 min and supernatants were diluted 1:1 with reduced or non-reduced (without β-mercaptoethanol) 2x sample buffer for discontinuous gel systems (Shi and Jackowski 1998). Samples were loaded at equal volume (12 µL) into 4% stacking gels and separated on 13% gels at 200V along with a low-range

molecular weight marker (Catalog#M3913, Sigma-Aldrich). Gels were stained with

Coomassie blue, and destained with methanol (50%), water (40%), and acetic acid (10%).

Crude protein content was determined by Dumas nitrogen combustion method 992.23

(AOAC 1998) in an Elementar Vario MAX CN analyzer (Elementar Americas, Inc., Mt.

Lauerel, NJ). Protein content was taken as the average of two measurements for each sample

preparation.

Sample moisture content was determined by drying approximately 4 g of sample at

102°C (±2°C) for 3 h in a laboratory oven with three measurements for each sample

preparation (IDF/ISO 2004).

3.3.5 Statistical Analyses

Data were analyzed using SAS® software (version 9.3, SAS Institute Inc., Cary, NC).

Gel strength, surface hydrophobicity, and WHC were modeled as a function of screw-profile,

temperature category, and the interaction for the extruded MPC80 samples after correcting

for the random error of each preparation and applying Satterthwaite's correction to estimate

denominator degrees of freedom. The mean gel strength, surface hydrophobicity, and WHC

of all modified and unmodified MPC80 samples were compared pair-wise at a significance

level of $p<0.05$. Protein solubility at each level of pH was compared after correcting for the

random preparation error using Tukey's adjusted p-value.

3.4 Results and Discussion

3.4.1 Changes in Protein Subunits

Reduced and non-reduced SDS-PAGE was used to evaluate the effect extrusion and

toasting had on structural changes such as denaturation, and possible disulfide bond

formation. Reduced and non-reduced SDS-PAGE of extruded MPC80 (Figures 3-1a and 3-

1b) indicated that α-lactalbumin (α-la), which was present in unmodified MPC80 under reduced conditions (Figure 3-1a) became insoluble after extrusion. The α-la subunits of extruded MPC80s disappeared from non-reduced gel including unmodified MPC80. We observed smearing of β-lactoglobulin (β-lg) bands for extruded samples under reduced conditions (Figure 3-1a), which suggested partial peptide denaturation. The β-lg remained soluble in non-reduced SDS-PAGE in unmodified MPC80 whereas extrusion caused insolubility of β-lg as indicated by the disappearance of the bands (Figure 3-1b).
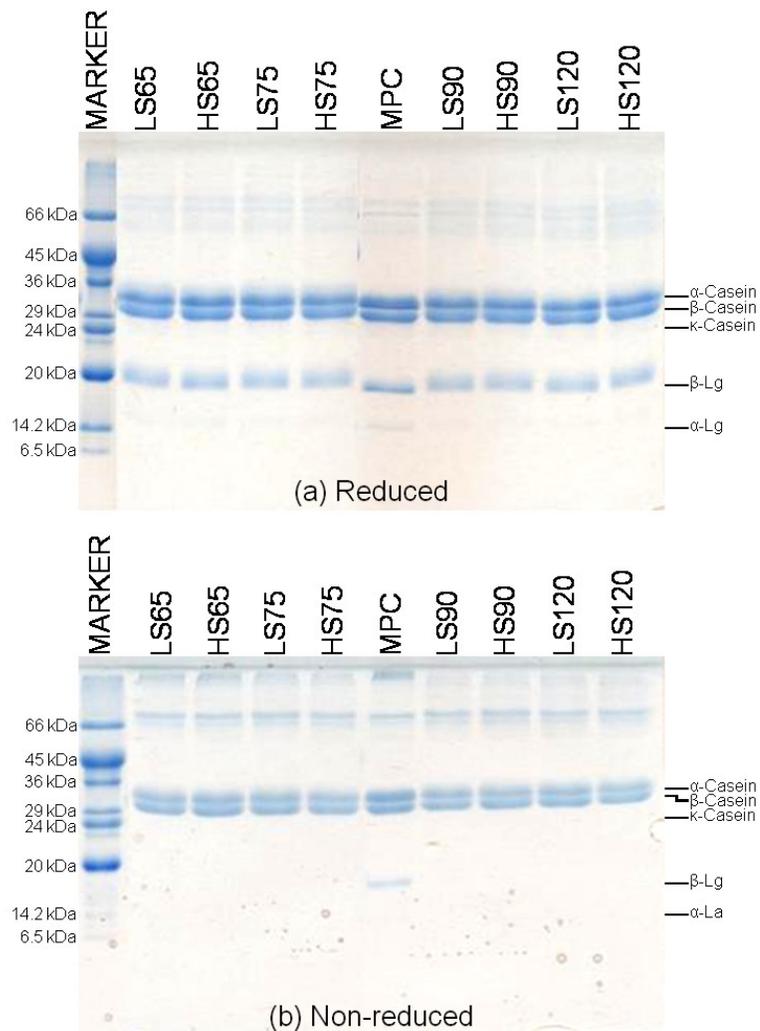
Figure 3-1 Reduced (a) and Non-Reduced (b) SDS-PAGE of Unmodified and Extruded MPC80
LS indicates 'low-shear' and HS indicates 'high-shear' screw profile. Number following distinguishes die temperature. Samples defined in Table 3-1. MPC is unmodified MPC80.

SDS-PAGE of MPC80 toasted at 110 °C (T110) showed aggregation as indicated by smeared bands near the top of both the reduced and non-reduced SDS-PAGE gels (Figures 3-2a and 3-2b). In addition to non-reduced protein aggregation, T110 had decreased casein solubility in the both extraction buffers. Havea (2006) reported that decreased casein solubility, including α-, β-, and κ-casein, was more prominent than decreased whey solubility under non-reduced conditions during storage; visual differences in peptide bands were apparent after storing MPC85 3 to 7 days at 40°C. Casein solubility in modified MPC80 remained similar to unmodified MPC80 when toasted at 75°C (T75) suggesting heat treatment was too mild to affect casein.
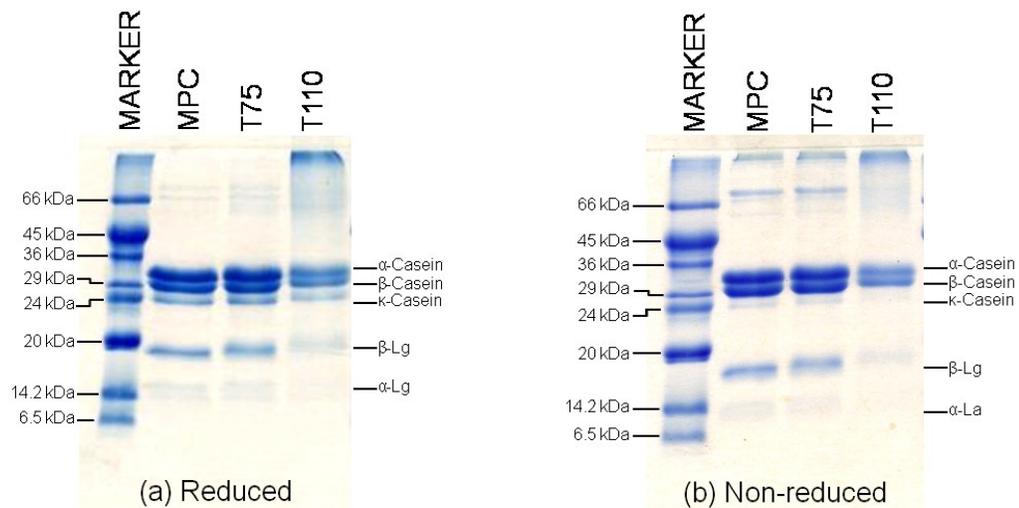
Figure 3-2 Reduced (a) and Non-Reduced (b) SDS-PAGE of Unmodified and Toasted MPC80
MPC is unmodified MPC80, T75 is MPC80 toasted 4 h at 75°C, and T110 is MPC80 toasted 4 h at 110°C.

Disappearance or fading of peptide bands on non-reduced gels compared to a reduced gels of extruded WPC80, WPI, and NFDM, suggested peptide insolubility from sulfhydryl-disulfide cross linking (Onwulata and others 2010). The disappearance of β-lg from non-reduced extruded MPC80 indicated disulfide bond formation. Subunit β-lg, with 1 mole of cysteine, can form a disulfide crosslink with κ-casein during heat denaturation (Fox and

McSweeney 1998). Although susceptible to heat, α-la may also interact through disulfide interchange, with α_{s2}-casein (Fox and McSweeney 1998). Disulfide bond formation, observed to be more prominent in extruded MPC80 than toasted MPC80, may possess decreased reactivity when incorporated into food. Inhibition of disulfide bond formation in a model intermediate moisture food system was shown to extend shelf-life (Zhu and Labuza 2010).

3.4.2 Surface Hydrophobicity.

The surface hydrophobicity of extruded MPC80s (Figure 3-3) was not influenced significantly by screw-profile or die temperature, but pair-wise comparisons between all sample means revealed that surface hydrophobicity of the soluble protein in each extruded sample was lesser than unmodified MPC80. T110 and T75 also had decreased surface hydrophobicity when compared with unmodified MPC80 ($p < 0.05$). Among toasted samples, higher temperature led to a larger reduction in surface hydrophobicity ($p < 0.05$).

Surface hydrophobicity is a measure of hydrophobic amino acid exposure, the tendency of protein molecules to aggregate, and lose solubility (Wagner and others 2000). ANS-based surface hydrophobicity increased with increasing solubility in MPCs produced by adding dilute sodium chloride during diafiltration (Mao and others 2012). Extruded and toasted MPC80 had reduced solubility (Figures 3-4 and 3-5) and a reduced ANS surface hydrophobicity response. ANS surface hydrophobicity of WPC textrurized by supercritical CO_2 extrusion increased due to exposure of hydrophobic residues (Mustapha and others 2012). Natural tryptophan reflectance decreased in extruded WPI indicating increased exposure and mobility of this residue (Qi and Onwulata 2011a).
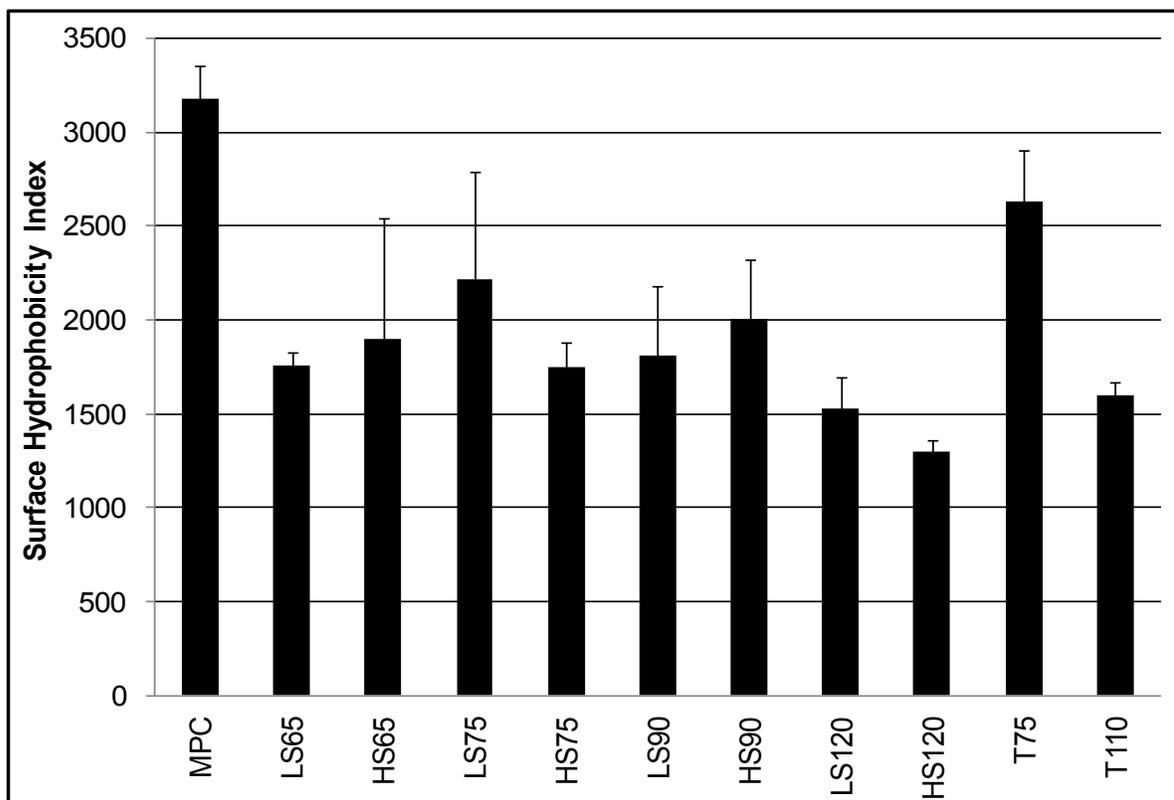
Figure 3-3 Surface Hydrophobicity of Extruded, Toasted, and Unmodified MPC80
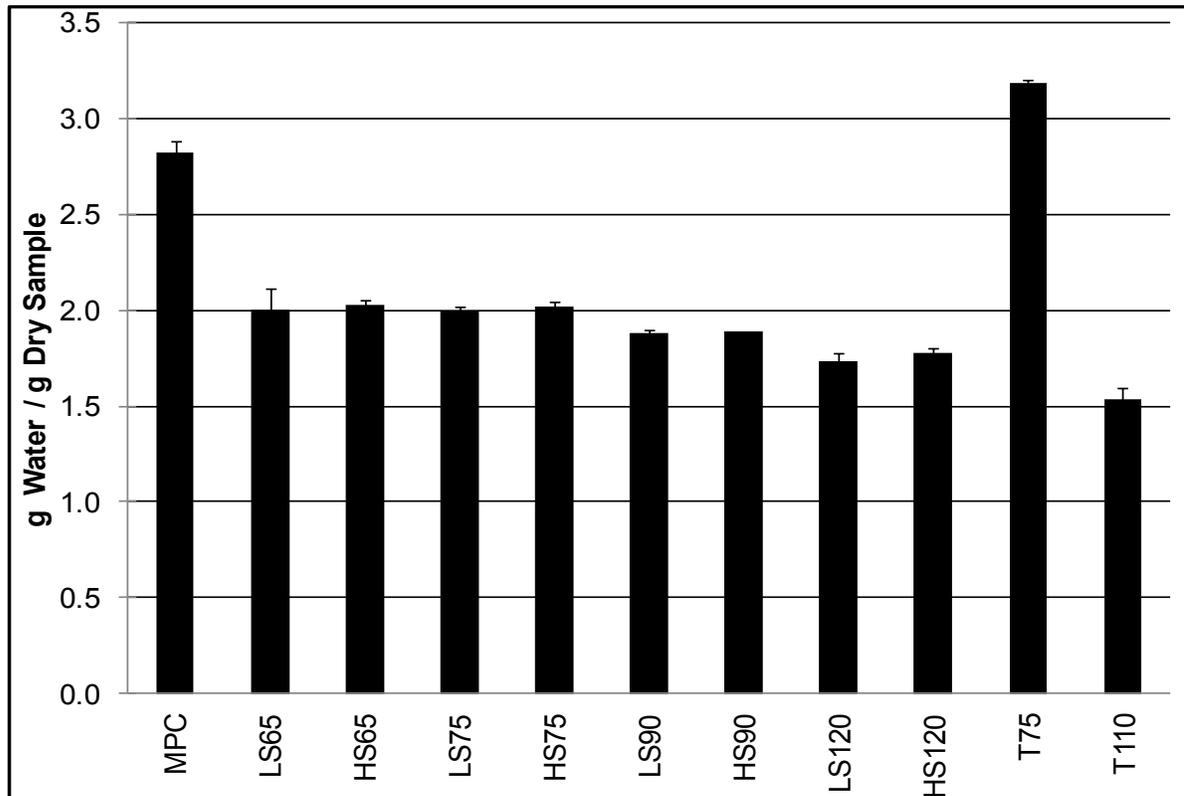*LS indicates 'low-shear' and HS indicates 'high-shear' screw profile. Number following distinguishes die temperature. Samples defined in Table 3-1. MPC is unmodified MPC80, T75 is MPC80 toasted 4 h at 75°C, and T110 is MPC80 toasted 4 h at 110°C. Bars are one standard deviation.*

Processing reduced soluble protein surface hydrophobicity, but the soluble protein (Figures 3-4 and 3-5) at pH 7 ranged from 9% to 12% for extruded MPC80, 16% to 33% for toasted MPC80, and was 45% for unmodified MPC80. Proteins with more hydrophobic exposure may be in the insoluble portion, and it is not uncommon for ANS surface hydrophobicity to correlate positively with protein insolubility (Hayakawa and Nakai 1985). Increased protein surface hydrophobicity may help prevent moisture migration within food matrices including HPN bars (Gallo-Torres 2003).

3.4.3 MPC80 Functional Properties

Protein solubility profiles for extruded and toasted MPC80 are shown in Figure 3-4 and Figure 3-5 respectively. At each pH, pair-wise comparisons were made between the

solubility of each sample with significance based on the Tukey's adjusted p-value ($p < 0.05$). The extruded MPC80 samples amongst themselves were not significantly different in solubility, except at pH 9 where practicality in food applications is limited. At pH 2, only the samples extruded at 120°C (LS120 and HS120) have significantly lower solubility than unmodified MPC80 ($p < 0.05$). At pH 3, LS65 also became significantly less soluble than unmodified MPC80. All the extruded samples have significantly reduced solubility when compared with unmodified MPC80 at pH 5.5 through pH 9. Toasting MPC at 110°C also significantly reduced protein solubility at each pH tested ($p < 0.05$), except for pH 4.6 where all samples where statistically equivalent. Toasting at 75°C was not sufficient to bring about difference in MPC80 solubility.
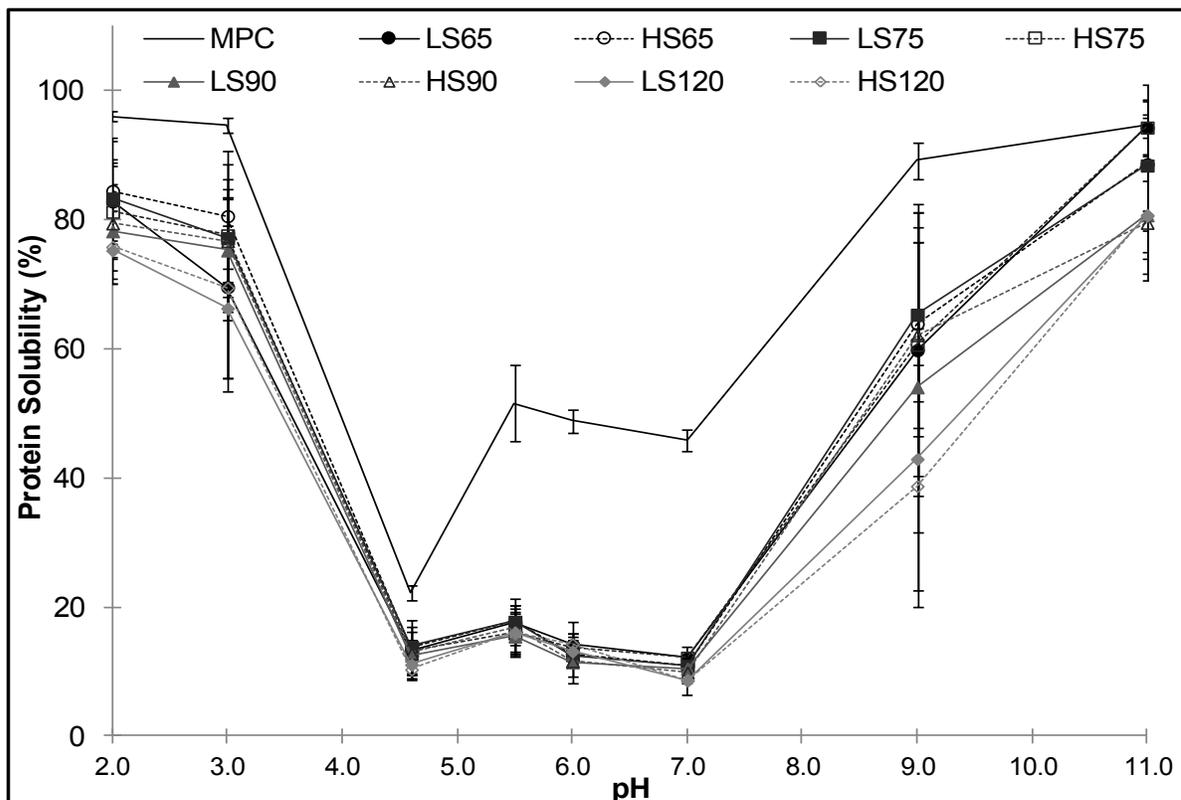
Figure 3-4 Protein Solubility Profiles of Extruded and Unmodified MPC80
LS indicates 'low-shear' and HS indicates 'high-shear' screw profile. Number following distinguishes die temperature. Samples defined in Table 3-1. MPC is unmodified MPC80. Bars are one standard deviation.
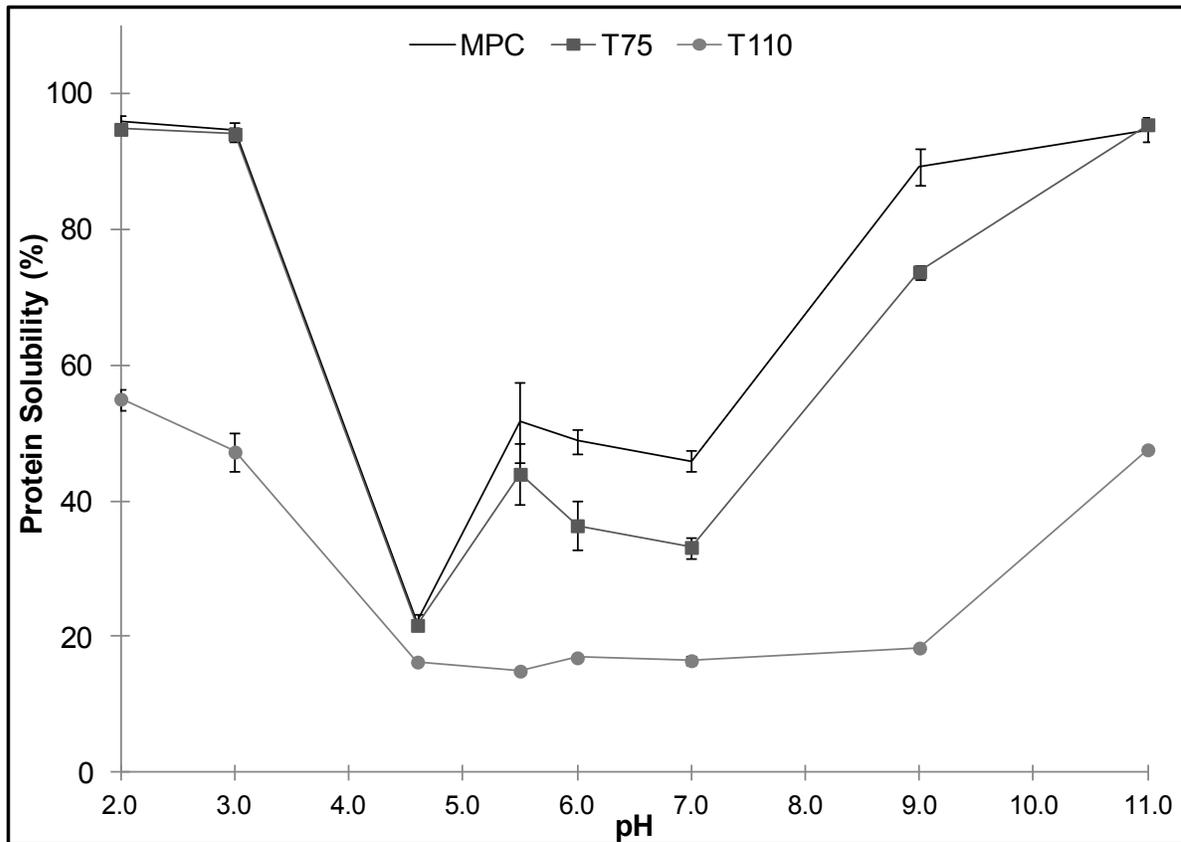
Figure 3-5 Protein Solubility Profiles of Toasted and Unmodified MPC80
MPC is unmodified MPC80, T75 is MPC80 toasted 4 h at 75°C, and T110 is MPC80 toasted 4 h at 110°C.
Bars are one standard deviation.

Reduced protein solubility may be indicative that the modified MPC80 will be more

inert with limited interactions with other ingredients when incorporated into food matrices.

Onwulata and others (2003) also reported decreased solubility of extruded dairy proteins,

including WPC, WPI, and whey lactalbumin. Extruded WPI and WPC80 for inclusion in

puffed snacks also had reduced solubility at higher extrusion temperatures, indicating that

more proteins are textrurized (Onwulata and others 2010). We observed that toasting,

especially at 110°C, reduced protein solubility without being textrurized. Decreased

solubility in toasted MPC80 was attributed to hydrophobic folding, hydrogen bonds, and

increased Maillard products that increase the formation of protein-protein crosslinks that

form a barrier to rehydration (Anema and others 2006; Le and others 2011). Maillard

browning was more apparent in T110 and consequently it was more insoluble, which may

inhibit functionality in some beverage applications, but still may be applicable in HPN bars.

Water holding capacity (WHC) (Figure 3-6) of the extruded MPC80 was not affected

significantly by the screw-profile used during extrusion, but was significantly influenced by

die temperature ($p < 0.05$). Extruding at a die temperature of 120°C with both high- and low-

shear screw profiles resulted in significantly lower WHC when compared with all the lower

extrusion temperatures ($p < 0.05$).

Figure 3-6 Water Holding Capacity of Extruded, Toasted, and Unmodified MPC80
LS indicates 'low-shear' and HS indicates 'high-shear' screw profile. Number following distinguishes die temperature. Samples defined in Table 3-1. MPC is unmodified MPC80, T75 is MPC80 toasted 4 h at 75°C, and T110 is MPC80 toasted 4 h at 110°C. Bars are one standard deviation.

The WHC difference between samples extruded at 65°C and 75°C was not significant,

whereas it was significantly different ($p < 0.05$) between 65°C, and 75°C compared with

90°C. Higher die temperature produced samples with lower WHC when averaged over both screw-profiles. Pair-wise WHC comparisons revealed that T75 had increased WHC when compared with all other samples ($p < 0.05$). Each extruded MPC80 had reduced WHC when compared with unmodified MPC80, but higher WHC when compared to T110 ($p < 0.05$). Toasting MPC80 for 4 h at110°C reduced WHC when compared with the unmodified control ($p < 0.05$).

Water migration, to or away from proteins in HPN bars, influences the rate of bar hardening (Li and others 2008; Hazen 2010). Proteins during HPN bar manufacture may hydrate slowly, thus are subject to only partial hydration during production. They could then pull moisture away from other constituents within the protein matrix leaving solutes in higher concentration, a state prone to crystallization, and loss of plasticizing effect (Li and others 2008). NMR analysis of model HPN bars formulated with sodium caseinate (Loveday and others 2010) and MPC80 (Loveday and others 2009) revealed that water molecules lacked interaction with the local protein domains and hence were expelled to the bulk phase through osmotic pull of lower molecular weight compounds. Whey protein hydrolysates function well in HPN bars because high WHC allows for rapid hydration during manufacture and the resultant high water activity maintained during storage prevents gradient driven moisture migration (Sinha and others 2007; McMahon and others 2009; Gautam and others 2006).

WHC analysis is rapid because the hold period is only 15 min. Thus, T75 with high WHC may function well in HPN bar applications with the ability to hydrate more readily than unmodified MPC80. However, HPN bar quality will decline if T75 pulls moisture away from other constituents during storage. If T75 functions in this manner in HPN bars, then the

extruded MPC80 samples may be a better option in these products because they had lower initial and possibly lower sustained WHC.

Although there would be interference from other ingredients and a strong mechanism to initiate milk protein gelation in the HPN bar matrix would be lacking, gel strength may yield some information about potential performance. Denaturation from modification might produce non-gelling proteins that may be inert when incorporated into food matrices. Extrusion or toasting had limited effect on gel strength (Figure 3-7), especially compared with unmodified MPC80.
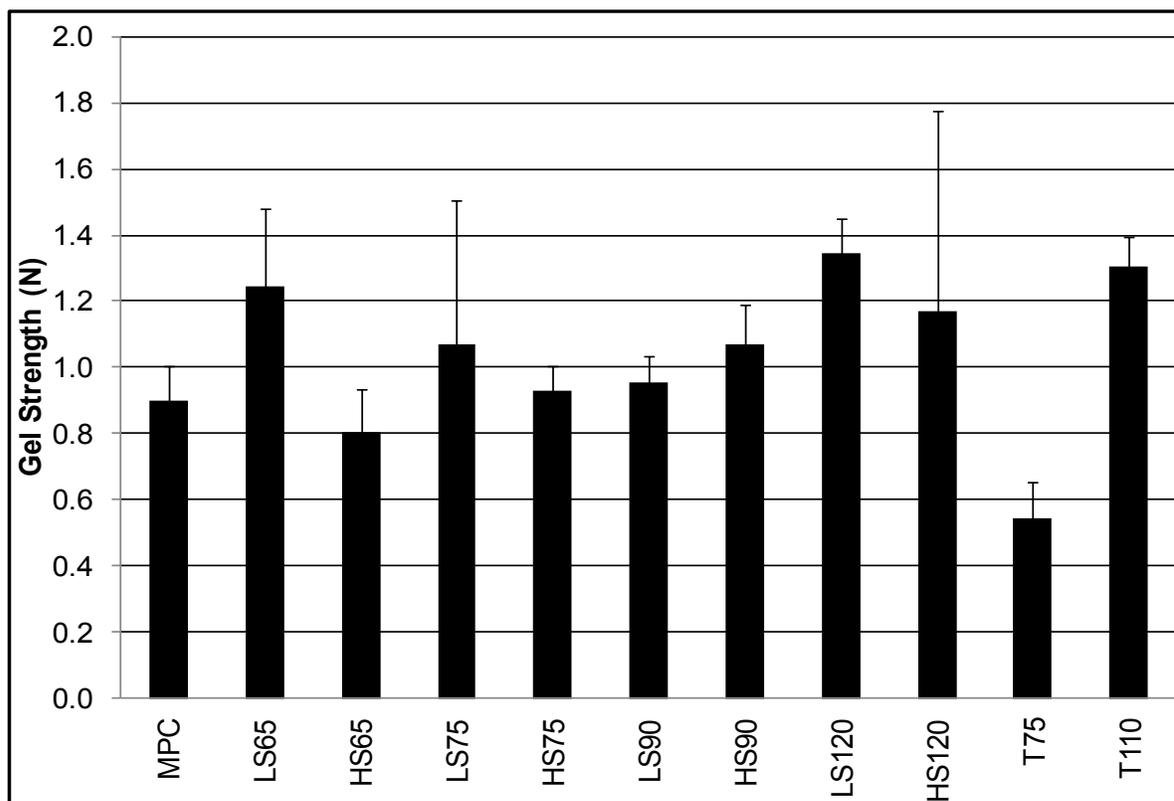
Figure 3-7 Gel Strength of Extruded, Toasted, and Unmodified MPC80
LS indicates 'low-shear' and HS indicates 'high-shear' screw profile. Number following distinguishes die temperature. Samples defined in Table 3-1. MPC is unmodified MPC80, T75 is MPC80 toasted 4 h at 75°C, and T110 is MPC80 toasted 4 h at 110°C. Bars are one standard deviation.

The screw-profile and the die temperature had no significant effect on the resultant gel strength. When compared pair-wise, only the gels of MPC80 modified at higher

temperatures (LS120and T110) were significantly stronger than of unmodified MPC80 ($p <$ 0.05). T110 gels were stronger than T75 gels ($p < 0.05$), and increased toasting temperature seemed to correspond to increased gel strength.

All samples gelled in the presence of TSPP without the addition of heat, were uniform, and had no syneresis. TSPP induces gelation through destruction of the casein micelle, re-aggregation of hydrophobic residues, and formation of calcium-pyrophosphate complexes to balance repulsive forces for gel stabilization (Mizuno and Lucey 2007). Differences in gel strength with unmodified MPC80 may have suggested that casein micelles were sheared or partially denatured.

3.5 Conclusions

Both extrusion and toasting can modify the functionality of MPC80. Extrusion processing and toasting at 110°C have the ability to induce disulfide bond formation and may create non-reducible protein associations which lead to modified functionality such as reduced protein solubility in the pH range common to foods. Low solubility may indicate less reactivity in food matrices, and along with altered WHC, may provide a good way to modify the functionality of MPCs prior to use in HPN bars. Although functional properties can be used to predict performance of modified MPCs in HPN bars, a model should be used to determine applicability of modifications in a realistic matrix.

3.6 References

Anema SG, Pinder DN, Hunter RJ, Hemar Y. 2006. Effects of storage temperature on the solubility of milk protein concentrate (MPC85). Food Hydrocoll. 20(2-3):386-93.

[AOAC] Assn. of Official Analytical Chemists. 1998. Method 992.23. In: Official methods of analysis of Assn. of Analytical Chemists. 16th ed. Arlington, VA.: AOAC.

Baldwin A, Pearce D. 2005. Chapter 15 - Milk Powder. In: C. Onwulata, editor. Encapsulated and Powdered Foods. 1st ed. New York: Taylor & Francis. p 387-433.

Fox PE, McSweeney PLH. 1998. Dairy Chemistry and Biochemistry. 1st ed. United Kingdom: Blackie Academic & Professional. 478 p.

Gallo-Torres JM. 2003. Made to Last. Prepared Foods 172(9):96. [serial online] 2012(January 18): Available from http://www.preparedfoods.com/articles/made-to-last.

Gautam A, Garcia AC, Hander RJ, inventors; Slim-Fast Foods Company, division of Conopco, Inc., assignee. 2006 June 1. Nutrition Bar. U.S. patent US 2006/0115554 A1.

Havea P. 2006. Protein interactions in milk protein concentrate powders. Int Dairy J 16(5):415-22.

Hayakawa S, Nakai S. 1985. Relationships of Hydrophobicity and Net Charge to the Solubility of Milk and Soy Proteins. J Food Sci 50(2):486-91.

Hazen C. 2010. Texture Solutions for Snack Bars. Food Product Design June:40-58.

[IDF/ISO] International Dairy Federation/International Organization for Standardization. 2004. Method IDF 26/ISO 5537. Dried milk - determination of moisture content.

Imtiaz SR, Kuhn-sherlock B, Campbell M. 2012. Effect of Dairy Protein Blends on Texture of High Protein Bars. J Texture Stud 43(4):275-86.

Le TT, Bhandari B, Deeth HC. 2011. Chemical and physical changes in milk protein concentrate (MPC80) powder during storage. J Agric Food Chem 59(10):5465.

Li Y, Szlachetka K, Chen P, Lin X, Ruan R. 2008. Ingredient Characterization and Hardening of High-Protein Food Bars: an NMR State Diagram Approach. Cereal Chem 85(6):780-6.

Loveday SM, Hindmarsh JP, Creamer LK, Singh H. 2009. Physicochemical changes in a model protein bar during storage. Food Res Int 42(7):798-806.

Loveday SM, Hindmarsh JP, Creamer LK, Singh H. 2010. Physicochemical changes in intermediate-moisture protein bars made with whey protein or calcium caseinate. Food Res Int 43(5):1321-8.

Manoi K, Rizvi SSH. 2008. Rheological characterizations of texturized whey protein concentrate-based powders produced by reactive supercritical fluid extrusion. Food Res Int 41(8):786-96.

Mao XY, Tong PS, Gualco S, Vink S. 2012. Effect of NaCl addition during diafiltration on the solubility, hydrophobicity, and disulfide bonds of 80% milk protein concentrate powder. J Dairy Sci 95(7):3481-8.

Matthey FP, Hanna MA. 1997. Physical and Functional Properties of Twin-screw Extruded Whey Protein Concentrate - Corn Starch Blends. Lebensm-Wiss Technol 30(4):359-66.

McMahon DJ, Adams SL, McManus WR. 2009. Hardening of High-Protein Nutrition Bars and Sugar/Polyol-Protein Phase Separation. J Food Sci 74(6):E312-21.

Mistry VV, Hassan HN. 1992. Manufacture of nonfat yogurt from a high milk protein powder. J Dairy Sci 75(4):947.

Mistry VV. 2002. Manufacture and application of high milk protein powder. Le Lait 82(4):515-22.

Mizuno R, Lucey JA. 2007. Properties of Milk Protein Gels Formed by Phosphates. J Dairy Sci 90(10):4524-31.

Murphy PA, Barua K, Hauck CC. 2002. Solvent extraction selection in the determination of isoflavones in soy foods. J of Chromatogr B 777(1-2):129-38.

Mustapha NA, Ruttarattanamongkol K, Rizvi SSH. 2012. The effects of supercritical fluid extrusion process on surface hydrophobicity of whey protein concentrate and its relation to storage and heat stability of concentrated emulsions. Food Res Int 48(2):470-7.

Onwulata CI, Konstance RP, Cooke PH, Farrell HMJ. 2003. Functionality of Extrusion - Texturized Whey Proteins. J Dairy Sci 86(11):3775-82.

Onwulata CI, Konstance RP, Smith PW, Holsinger VH. 1998. Physical Properties of Extruded Products as Affected by Cheese Whey. J Food Sci 63(5):1-5.

Onwulata CI, Phillips JG, Tunick MH, Qi PX, Cooke PH. 2010. Texturized Dairy Proteins. J Food Sci 75(2):E100-9.

Qi PX, Onwulata CI. 2011a. Physical Properties, Molecular Structures, and Protein Quality of Texturized Whey Protein Isolate: Effect of Extrusion Temperature. J Agric Food Chem 59(9):4668-75.

Qi PX, Onwulata CI. 2011b. Physical properties, molecular structures, and protein quality of texturized whey protein isolate: Effect of extrusion moisture content. J Dairy Sci 94(5):2231-44.

Rickert DA, Johnson LA, Murphy PA. 2004. Functional Properties of Improved Glycinin and β-Conglycinin Fractions. J Food Sci 69(4):FCT303:11

Shi Q, Jackowski G. 1998. One-dimensional polyacrylamide gel electrophoresis. In: B. D. Hames, editor. Gel Electrophoresis of Proteins: A Practical Approach. 3rd ed. Oxford University Press. p 23.

Singh H. 2007. Interactions of milk proteins during the manufacture of milk powders. Le Lait 87(4-5):413-23.

Sinha R, Radha C, Prakash J, Kaul P. 2007. Whey protein hydrolysate: Functional properties, nutritional quality and utilization in beverage formulation. Food Chem. 101(4):1484-91.

Wagner JR, Sorgentini DA, Añón MC. 2000. Relation between solubility and surface hydrophobicity as an indicator of modifications during preparation processes of commercial and laboratory-prepared soy protein isolates. J Agric Food Chem 48(8):3159-65.

Zhou P, Liu X, Labuza TP. 2008a. Moisture-Induced Aggregation of Whey Proteins in a Protein/Buffer Model System J Agric Food Chem 56(6):2048-54.

Zhou P, Liu X, Labuza TP. 2008b. Effects of Moisture-Induced Whey Protein Aggregation on Protein Conformation, the State of Water Molecules, and the Microstructure and Texture of High-Protein-Containing Matrix. J Agric Food Chem 56(12):4534-40.

Zhu D, Labuza TP. 2010. Effect of Cysteine on Lowering Protein Aggregation and Subsequent Hardening of Whey Protein Isolate (WPI) Protein Bars in WPI/Buffer Model Systems. J Agric Food Chem 58(13):7970-9.

CHAPTER 4. MODIFIED MILK PROTEIN CONCENTRATES IN HIGH-PROTEIN NUTRITION BARS: EFFECT ON TEXTURAL CHARACTERISTICS

Modified from a paper to be submitted to the *Journal of Dairy Science*

Justin C. Banach[1,2], Stephanie Clark[3], and Buddhi P. Lamsal[4,5]

4.1 Abstract

Milk protein concentrate with 80% protein was extruded or toasted prior to incorporation into model high-protein nutrition bars. Two temperature profiles, with die temperatures of 65°C and 120°C, were used to extrude the milk protein concentrate on a twin-screw co-rotating extruder with low-shear screw profile. Milk protein concentrate was also heat-modified without shear, by toasting at 75°C or 110°C for 4 h in a laboratory oven. Model high-protein nutrition bars with control and modified milk protein concentrates were formulated to contain 30% protein by weight in combination with other constituents to mimic commercial high-protein nutrition bars. The model high-protein nutrition bars were stored at room temperature (22°C), 32°C, or 42°C for accelerated storage study. Texture, water activity, and color were measured periodically over a 42 d storage period. Reduced and non-reduced sodium dodecyl sulfate polyacrylamide gel electrophoresis suggested formation of internal disulfide bonds and protein aggregations that possibly contributed to increased hardness in the model high-protein nutrition bars.

[1] Graduate Student, Department of Food Science and Human Nutrition, Iowa State University.
[2] Primary researcher and author.
[3] Associate Professor, Department of Food Science and Human Nutrition, Iowa State University.
[4] Assistant Professor, Department of Food Science and Human Nutrition, Iowa State University.
[5] Author for correspondence, lamsal@iastate.edu, p: 515-294-8681, f: 515-294-8181.

4.2 Introduction

Milk protein concentrates (MPCs), the spray-dried membrane concentrate of skim milk, are commonly used in processed cheeses and yogurt applications to improve texture (Mistry 2002; Mistry and Hassan 1992; Singh 2007a). Whey, caseinate, and soy proteins are typically used in high-protein nutrition (HPN) bars with 20% to 50% protein by weight, but the nutritional value and flavor of MPC make it suitable for these applications too (Imtiaz and others 2012). Whey protein prices have risen continually with the development of functional and protein fortified foods (Smithers 2008). Soy proteins, although nutritionally complete, contain phytoestrogens that may contribute to decreased energy and muscle mass in males and thus might be avoided by some consumers (Hamilton-Reeves and others 2010; Hughes and others 2011). Whey protein and soy protein are nutritionally comparable though the caseins in MPC are digested slower allowing for nitrogen retention and muscle growth post-exercise (Tang and others 2009).

Despite these advantages, MPCs perform poorly when incorporated into HPN bars without prior modification. Rapid bar hardening of MPC-incorporated high-protein matrices results in loss of consumer acceptability and limited product shelf-life. The minimum shelf-life for HPN bar or other nutritional bar products on the market is 6 months, but stability for greater than 12 months is desired (McMahon and others 2009; Imtiaz and others 2012). HPN bars manufactured with unmodified MPCs will not allow for this, and also will have a crumbly texture lacking cohesiveness needed to hold the HPN bar together (Li and others 2008). The exact mechanism of instability in HPN bars formulated with MPC has not been determined. Model protein bars formulated with other dairy proteins indicate that quality decline is most likely due to a combination of moisture migration, limited free water, phase

separation, and internal disulfide bond formation and protein aggregation (Loveday and others 2009; Loveday and others 2010; Li and others 2008; McMahon and others 2009, Zhou and others 2008a; Zhou and others 2008b).

MPC80, incorporated into model HPN bars at 20% by weight, saw water migration from the protein to lower molecular weight constituents that left the proteins in a state prone to aggregation and texture change (Loveday and others 2009; Loveday and others 2010). The model HPN bars formulated with MPC80 hardened substantially during 1 2/3 months storage at room temperature, well short of the 6 month minimum requirement, suggesting limited functionality and applicability of MPCs in HPN bars.

Macronutrient phase separation in HPN bars has also been suggested as another bar-hardening mechanism. It can occur due to preferential exclusion of the solvent (e.g., water) and cosolvent (e.g., sugar alcohol, sugar syrup) from the localized protein domain (McMahon and others 2009). Good interaction between protein and sugar alcohol (e.g., sorbitol, maltitol, xylitol) helps stabilize the protein, preventing phase separation and the formation of internal disulfide bonds (McMahon and others 2009). However, too much interaction between the protein and some cosolvents such as propylene glycol can also lead to rapid aggregations and subsequent hardening (Liu and others 2009). Without the addition of sugar alcohols, the local protein domains can freely interact with other amino acid residues to form disulfide bonds and aggregated proteins. HPN bar hardening can also occur as the disulfide bonded aggregates come together to form more complete networks (Zhou and others 2008b).

In this study, we report instrumental texture evaluation of HPN bars formulated with modified milk protein concentrates with 80% protein (MPC80) during accelerated storage at

different temperatures. Extrusion and toasting were utilized for physical modification of protein for possible inclusion in HPN bars.

4.3 Materials and Methods

4.3.1 Materials and Reagents

MPC80 was purchased from Idaho Milk Products (Jerome, ID). The high-fructose corn syrup (HFCS), CornSweet® 55, and the palm kernel stearin were donated by Archer Daniels Midland (Decatur, IL). The maltitol/sorbitol syrup (LYCASIN® 80-55) was donated by Roquette America (Keokuk, IA). Reagents were of analytical grade, and were purchased from Thermo Fisher Scientific (Waltham, MA), Sigma-Aldrich Inc. (St. Louis, MO), Biorad (Hercules, CA), and VWR International (Radnor, PA).

4.3.2 MPC80 Extrusion

The moisture content of the MPC80 was adjusted to about 38% (±1%) by misting with distilled water during constant mixing by a mechanical mixer equipped with a wire whip attachment (Kitchen Aid, model# KS55, St. Joseph, MI). Following moisture content adjustment, the MPC80 was placed into a large plastic bag, sealed in a 5 gal pail, and stored at 4°C overnight for adequate moisture equilibration. Prior to extrusion, moisture content was measured.

Moisture-adjusted MPC80 was fed by hopper into a co-rotating twin-screw extruder (Micro 18 American Leistritz Extruder, Somerville, NJ) with length (540 mm) to diameter (18 mm) ratio of 30. The feeding zone (Z0) of the barrel was water cooled, followed by five temperature controlled zones (Z1 - Z5), and zone 6 (Z6) the die temperature. Screw speed was constant at 60 RPM and screw profile was designed to impart low-shear. Low-shear screws were characterized by more feeding sections than kneading blocks that impart higher

shear force on extruded material. Extrusion conditions are summarized and sample codes are

defined in Table 4-1. Following extrusion the extrudate was pelletized with a laboratory

cutter (model# BT25, Scheer Bay Co., Bay City, MI). The pellets were spread into a metal

pan and dried at 50°C for 72 h in a force-draft oven with periodic hand mixing. Dry pellets

were finely ground using a centrifugal mill (model ZM1, Retsch, Newtown, PA) fitted with a

0.5 mm mesh.

Table 4-1 Extruded MPC80 Temperature and Screw Profile

Sample	*Screw Profile*	*Zone Temperature (°C)*					
		Z1	*Z2*	*Z3*	*Z4*	*Z5*	*Z6*
E65	Low shear	25	35	45	55	65	65
E120	Low shear	35	45	55	65	120	120

4.3.3 MPC80 Toasting

MPC80 was toasted in a laboratory oven to modify MPC80 with heat (Murphy and

others 2002). MPC80 was spread into a thin layer (2 to 5 mm) on an aluminum foil lined

oven rack and toasted for 4 h at 75°C (T75) or 110°C (T110). The toasted MPC80 was

cooled at room temperature for 45 min.

Each extruded MPC80 and toasted MPC80 were prepared in duplicate and were

sieved through a 250 μm mesh to remove any coarse material. Samples were stored at 4°C in

double zipper-seal bags prior to HPN bar manufacture and at any intermediate steps during

MPC80 modification.

4.3.4 Model High-Protein Nutrition Bar Manufacture

HPN bars were prepared from two duplicates of modified MPC80s (n = 2). Model

HPN bars were formulated to contain 30% protein by weight (Table 4-2). Three 5-qt

mechanical mixers (Kitchen Aid, model# K5SS, St. Joseph, MI) were used simultaneously to

prepare three 840 g batches of HPN bar dough with modified (E65, E120, T75, T110) or

unmodified MPC80s. The protein ingredient, glycerol, maltitol/sorbitol syrup, and water

were combined with a wire whip on 'stir' for 60 s and were then mixed 2 min on speed #4.

Palm kernel stearin and HFCS were heated together until fat liquefaction, cooled to 55°C,

and the solution was transferred into the protein mixture where it was combined with mixing

on speed #4 for an additional 2 min. All mixing times are discontinuous as the mixers were

paused every 30 s to scrap the edges of the mixing bowl.

Table 4-2 High-Protein Nutrition Bar Formulations

	High-Protein Nutrition Bar Formulations (w/w %)				
Ingredient	*MPC*	*E65*	*E120*	*T75*	*T110*
MPC	37.39	-	-	-	-
E65	-	38.11	-	-	-
E120	-	-	37.41	-	-
T75	-	-	-	36.89	-
T110	-	-	-	-	36.30
Glycerol	21.50	21.48	21.50	21.50	21.50
Palm Kernel Stearin	18.46	18.43	18.45	18.45	18.45
Maltitol/Sorbitol Syrup	12.00	11.99	12.00	12.00	12.00
High-Fructose Corn Syrup	10.00	9.99	10.00	10.00	10.00
Water	0.65	0.00	0.63	1.16	1.75

The dough from three mixing bowls was mixed together into a single batch. HPN bar

dough was uniformly packed into PVC cylindrical molds with 21 mm internal diameter and

13 mm (shorter), and 107 mm (longer) length. The HPN dough in each mold was leveled

with a spatula and the mold was sealed at both ends with parafilm. Care was taken to fill

molds uniformly without inclusion of air bubbles. Water activity sample cups (Aqua Lab,

Decagon Devices Inc., Pullman, WA) were filled halfway with HPN bar dough, covered with

a lid, and wrapped with parafilm.

Six short molds, one long mold, and three water activity cups packed with HPN bar

samples were placed into separate zipper-seal bags. After all molds were filled, sealed, and

bagged, about 2.5 h from the start of HPN bar manufacture, the model HPN bars rested for 1

h at room temperature. Samples were randomly transferred to incubators set to 42°C, 32°C, or were left at room temperature (22°C).

4.3.5 Instrumental Texture Evaluation of Model HPN Bars

Texture profile analysis (TPA) and shearing, was initially performed when samples were moved to their respective incubators and was defined as time zero. Samples for texture analysis were taken out from incubators after 1, 2, 4, 6, 13, 22, 32, and 42 d. Prior to all texture testing, samples were allowed to equilibrate at least 1 h a room temperature.

Samples from the small cylindrical molds were plunged from the mold with a wooden dowel and were used in TPA analysis. Each HPN bar specimen was compressed with a flat circular plate (TA-30) moving at 2 mm/s to 60% strain with trigger force set to 0.05 N using a texture analyzer (TA-XT2, Texture Technologies, Scarsdale, NY). A second compression was performed after a 2 s pause from when the first compression ended. Three TPA measurements were made for each storage time, temperature, and type of MPC80 modification. Hardness was defined as the peak force attained at a given deformation (i.e., 60% strain) and fracturability was the force where a significant break occurred during the first compression (Gunasekaran and Ak 2003).

Another set of small cylindrical HPN bar sample were plunged from PVC molds and were used for stress-relaxation analysis. A HPN bar sample was compressed at 3.3 mm/s with a flat plate (TA-30) to 10% strain and resultant force was recorded using a texture analyzer (TA-XT2, Texture Technologies, Scarsdale, NY). For data analysis, time zero was adjusted for sample loading and was set to the maximum force during the 10% compression. Force data were converted to stress (kPa) by dividing by area of applied force (1.33×10^{-4}

m^2). After adjustments, 300 s worth of data was analyzed using linearization technique

(Peleg 1979; Lamsal and others 2007).

In the following equations, σ_0 was stress in the sample at time zero, t was time, σ_t was

stress in the sample at time t, and k_1 and k_2 were constants characteristic of viscoelastic

properties (Lamsal and others 2007). First stress-time data were normalized with the left-

hand side of equation 4-1.

$$\textit{[Equation 4-1]} \quad (\sigma_0 \times t)/(\sigma_0 - \sigma_t) = k_1 + k_2 \times t$$

Normalized stress was plotted against time and a liner regression line was fit.

Average slope from the linearized plot was k_2 and the y-intercept of the regression line was

k_1, the inverse of which corresponds to the initial decay rate of the stress ratio. The

equilibrium stress (σ_e), the internal stress in the sample at infinite time, was estimated using

equation 4-2.

$$\textit{[Equation 4-2]} \quad \sigma_e = \sigma_0 \times (1 - 1/k_2)$$

Samples were plunged from the longer cylindrical molds and were used in shearing

analysis adapted from McMahon and others (2009). The cylindrical HPN bar was sheared

along the circular cross-section with a 45° chisel blade (TA-42, Texture Technologies,

Scarsdale, NY) at 1 mm/s to 85% of the sample height based on the initial point of contact

and a 0.05 N trigger force. Three cuts were made per long HPN bar sample and average

maximum force during shear was taken as shear strength. Three measurements were

acquired for each preparation, batch, storage temperature, and time combination for TPA,

stress-relaxation, and shear strength tests.

4.3.6 Color and Water Activity Measurement

Color and water activity measurements were made on Day 0, 1, 4, 13, 22, and 42, after equilibrating at room temperature for at least 1 h prior to measurement. L^*, a^*, and b^* values were acquired with a LabScan XE (Hunter Laboratory Associates, Inc., Reston, VA) bench-top colorimeter operating with D65 northern daylight light and a 10° standard angle observer for the samples in each water activity cup and were used to calculate total color change (ΔE) using equation 4-3.

$$[Equation\ 4\text{-}3] \qquad \Delta E = [(a - a_0)^2 + (b - b_0)^2 + (L - L_0)^2]^{\frac{1}{2}}$$

Three successive color measurements were made on each water activity cup, averaged to determine the color values of that particular sample; the color values for the three samples from each batch, storage temperature, and time combination were averaged. The ΔE value was determined for each preparation with the reference color values (a_0, b_0, and L_0) set to the initial values for each preparation × batch combination on Day 0. ΔE values are reported as the average of both preparations.

Water activity was measured with a water activity analyzer (Aqua Lab 4TE Duo, Decagon Devices Inc., Pullman, WA, USA) following color analysis. Prior to measurement, the water activity analyzer was standardized using 6 m sodium chloride and 13.41 m lithium chloride. Three separate water activity measurements were made for each batch, temperature, and time combination. Water activity values are reported as the average of both preparations.

4.3.7 Analytical Procedures

Following texture analysis, samples were frozen in liquid nitrogen, ground to a fine powder with a laboratory blender, and were stored at -80°C (Loveday and others 2009). The

second preparation of HPN bars was used for SDS-PAGE analysis after protein content determination.

Reduced and non-reduced protein extractions from the frozen HPN bars were performed following the procedures of Loveday and others (2009) with slight modifications. A 100 mM borate buffer was prepared, adjusted to pH 9.0, and filtered through a 0.22 μm membrane filter (Cold Spring Harbor Protoc 2009). The borate buffer was diluted to 50 mM with Millipore water for non-reduced protein extraction and with addition of 5% (v/v) β-mercaptoethanol for reduced protein extraction. If needed, the pH of each extraction buffer was adjusted back to 9.0 with 4 N sodium hydroxide. Based on the average protein content, 100 mg of protein (315.8 mg to 320.9 mg HPN bar) was weighed directly into a centrifuge tube and 25 mL of reduced or non-reduced extraction buffer was added. A small stir bar was used to mix the samples for 16 h at 4°C.

Samples were spun at 10,000 × g for 10 min and supernatant was filtered through a 0.22 μm low protein binding syringe filter (Millex-GV, EMD Millipore, Billerica, MA). Filtered supernatants were diluted 1:1 with reducing (with β-mercaptoethanol) or non-reducing 2x Laemmli buffer. Samples were heated at 90°C for 5 min.

Equal sample volume (10 μL) of was loaded into precast 4% to 20% gradient gels. Peptides were separated at 150 V for 50 min. Gels were stained overnight in Coomassie blue stain solution and were destained until a clear background was obtained.

Crude protein content of samples was determined by using Dumas nitrogen combustion method 992.23(AOAC 1998) in an Elementar Vario MAX CN analyzer (Elementar Americas, Inc., Mt. Lauerel, NJ). Protein content was taken as the average of two measurements for each sample.

Sample moisture content was determined by drying approximately 4 g of sample at 102°C (±2°C) for 3 h in a laboratory oven with three measurements per sample (IDF/ISO 2004).

4.3.8 Statistical Analyses

Statistical analyses were conducted using SAS® software (version 9.3, SAS Institute Inc., Cary, NC). Tukey's adjusted p-value was used to determine significance ($p < 0.05$) after applying Satterthwaite's correction to estimate denominator degrees of freedom and correcting for the random errors due to batch preparation and the division of samples into their storage groups. Texture response variables, including TPA hardness, TPA fracturability, shear strength, initial sample stress (σ_0), average slope from linearized relationship (k_2), initial decay rate of stress ratio ($1/k_1$), equilibrium stress (σ_e), and mean ratio (σ_{300s}/σ_e) were modeled as the function of batch, storage temperature, storage time, and all possible interactions. At each batch × temperature × time combination a temperature × time slicing factor was applied to analyze batch differences at each level of time at each temperature separately. It was not of interest to look at changes that occur between temperature levels here because the mechanism of change does not differ; only the rate increases at higher temperature. Total color change and water activity were modeled separately as a function of batch, storage temperature, storage time, and all possible interactions. At each batch × temperature × time combination a batch × temperature slicing factor was applied to analyze total color and water activity change within each HPN bar batch over time at constant temperature. The differences in color change and water activity at different temperatures was not of interest because change was occurring via the same mechanism only at faster rates. Color and water activity changes over time within a

particular HPN bar batch were of more interest than differences between each batch at temperature × time slices because these variables can be related to internal changes in HPN bar texture.

4.4 Results and Discussion

4.4.1 Texture Analysis

Significant ($p < 0.05$) differences in hardness, fracturability, and shear strength between the HPN bars are shown in Tables 4-3 through 4-5, respectively. Comparisons in these Tables are made between all batches at constant temperature and time. On the day of manufacture (Day 0), there was no statistical difference in hardness, fracturability, and shear strength between the five types of HPN bars.

HPN bars produced with MPC80 toasted at 75°C were not significantly different in terms of hardness, fracturability, or shear strength when compared to unmodified MPC80 at each temperature × time slice. HPN bars produced with MPC80 toasted at 110°C closely followed unmodified MPC80 HPN bars in terms of hardness, fracturability, or shear strength at each temperature × time slice over the storage period; some significant differences between them were undesirable. For example, the shear strength of T110 on day 42 at 42°C was significantly greater than unmodified MPC80 ($p < 0.05$). Toasting at the temperatures studied was not useful for slowing textural changes in model HPN bars.

Table 4-3 Model High-Protein Nutrition Bar Hardness (N)

	Storage Temperature														
	22°C					32°C					42°C				
Day	MPC	E65	E120	T75	T110	MPC	E65	E120	T75	T110	MPC	E65	E120	T75	T110
0	92.5^a	39.1^a	65.6^a	74.1^a	102.2^a	-	-	-	-	-	-	-	-	-	-
1	110.3^{ab}	49.0^b	67.3^{ab}	119.7^{ab}	135.1^a	152.8^a	55.8^b	81.5^{ab}	135.7^{ab}	151.9^a	171.7^a	62.4^b	83.9^b	191.0^a	130.3^{ab}
2	125.9^{abc}	54.0^c	82.1^{bc}	130.2^{ab}	131.3^a	164.8^{ab}	54.0^c	82.1^{bc}	170.6^a	144.1^{ab}	191.9^a	69.3^b	103.1^b	196.3^a	140.0^b
4	133.3^a	65.2^a	71.0^a	148.8^a	115.3^a	163.9^{ab}	68.7^c	91.3^{bc}	180.9^a	141.6^{abc}	207.1^a	80.5^b	110.6^b	218.9^a	122.2^b
6	149.8^{ab}	70.0^{bc}	64.0^c	154.6^a	123.2^{abc}	175.9^a	82.4^b	110.2^{ab}	164.6^{ab}	126.3^{ab}	213.9^{ab}	113.0^c	119.3^c	246.0^a	155.4^{bc}
13	160.7^a	74.5^b	111.8^{ab}	174.0^a	119.8^{ab}	186.8^a	90.7^b	128.0^{ab}	198.1^a	126.9^{ab}	223.3^{ab}	164.7^b	186.7^{ab}	254.4^a	200.6^{ab}
22	178.5^{ab}	96.3^b	112.4^{ab}	186.1^a	119.4^{ab}	217.9^a	112.0^b	144.1^{ab}	179.1^{ab}	138.4^{ab}	323.3^a	174.0^b	177.6^b	304.5^a	274.6^a
32	225.3^a	81.1^c	120.4^{bc}	198.8^{ab}	139.1^{bc}	207.7^{ab}	127.4^b	142.4^{ab}	223.9^a	135.7^b	350.0^a	151.5^b	153.1^b	323.4^a	350^a
42	196.6^{ab}	76.6^c	117.2^{bc}	201.8^a	170.1^{ab}	229.6^a	111.4^b	155.1^{ab}	208.8^a	174.9^{ab}	350.0^a	112.6^c	207.4^b	291.3^a	350.0^a

Sample means for each batch of high-protein nutrition bars are presented at each combination of day × temperature. Sample means are significantly different ($p < 0.05$) if they do not share a common alphabetical superscript for the same day (row) at constant temperature.

Table 4-4 Model High-Protein Nutrition Bar Fracturability (N)

	Storage Temperature														
	22°C					*32°C*					*42°C*				
Day	*MPC*	*E65*	*E120*	*T75*	*T110*	*MPC*	*E65*	*E120*	*T75*	*T110*	*MPC*	*E65*	*E120*	*T75*	*T110*
0	7.2^a	4.4^a	0.0^a	11.6^a	18.2^a	-	-	-	-	-	-	-	-	-	-
1	19.5^a	13.2^a	6.3^a	31.9^a	56.0^a	89.8^a	32.3^a	30.2^a	82.5^a	95.4^a	128.6^{ab}	28.6^c	55.7^{bc}	120.8^{ab}	182.0^a
2	64.2^{ab}	0.0^b	15.6^{ab}	56.9^{ab}	75.5^a	112.0^{ab}	27.3^c	42.0^{bc}	98.0^{abc}	118.4^a	132.5^a	34.6^b	50.6^b	126.6^a	185.6^a
4	96.0^{ab}	43.0^b	29.5^b	82.7^{ab}	128.4^a	139.1^a	41.0^c	48.3^{bc}	120.0^{ab}	171.5^a	186.0^a	84.2^b	107.2^b	153.0^{ab}	223.5^a
6	121.9^a	33.4^b	9.6^b	112.3^a	154.4^a	140.1^{ab}	54.2^c	83.6^{bc}	125.1^{abc}	184.3^a	194.2^{ab}	148.8^{bc}	108.8^c	186.4^{ab}	243.7^a
13	127.5^{ab}	46.8^c	81.8^{bc}	123.8^{ab}	193.3^a	153.5^{ab}	70.1^c	88.8^{bc}	161.4^{ab}	218.1^a	243.3^a	144.5^b	154.0^b	253.2^a	309.7^a
22	146.2^{ab}	60.7^c	90.6^{bc}	145.0^{ab}	218.7^a	192.7^{ab}	87.6^c	132.4^{bc}	162.8^{ab}	233.0^a	308.3^a	194.5^b	209.7^b	299.4^a	341.5^a
32	174.5^a	73.3^b	68.7^b	163.0^a	210.7^a	204.6^{ab}	102.9^c	149.3^{bc}	180.0^{ab}	229.0^a	350.0^a	228.7^b	233.2^b	301.5^{ab}	350.0^a
42	189.0^a	70.5^b	82.9^b	166.1^a	230.0^a	228.4^{ab}	136.8^c	136.3^c	195.0^{bc}	279.6^a	350.0^a	270.3^b	332.1^{ab}	341.2^{ab}	350.0^a

Sample means for each batch of high-protein nutrition bars are presented at each combination of day × temperature. Sample means are significantly different ($p < 0.05$) if they do not share a common alphabetical superscript for the same day (row) at constant temperature.

84

Table 4-5 Model High-Protein Nutrition Bar Shear Strength (N)

						Storage Temperature									
			22°C					32°C					42°C		
Day	MPC	E65	E120	T75	T110	MPC	E65	E120	T75	T110	MPC	E65	E120	T75	T110
0	7.2[a]	3.3[a]	7.4[a]	5.1[a]	7.9[a]	-	-	-	-	-	-	-	-	-	-
1	7.4[a]	6.0[a]	7.9[a]	7.4[a]	13.2[a]	23.0[a]	7.0[a]	8.2[a]	16.4[a]	18.2[a]	25.7[ab]	9.2[b]	15.2[b]	25.5[ab]	40.0[a]
2	8.8[a]	5.4[a]	8.3[a]	11.5[a]	18.6[a]	21.4[ab]	4.9[b]	9.0[ab]	19.1[ab]	25.1[a]	31.1[ab]	6.3[c]	15.2[bc]	27.9[ab]	37.8[a]
4	22.2[a]	7.4[a]	10.1[a]	19.4[a]	25.6[a]	34.0[a]	8.6[c]	13.0[bc]	27.1[abc]	32.1[ab]	52.9[a]	20.0[b]	29.2[b]	37.0[ab]	51.0[a]
6	23.7[ab]	6.2[b]	14.4[ab]	23.2[ab]	28.7[a]	36.4[a]	10.5[b]	17.4[ab]	28.9[ab]	35.8[a]	48.6[a]	20.3[b]	32.3[ab]	36.1[ab]	46.8[a]
13	28.0[ab]	5.1[c]	13.6[bc]	33.2[a]	37.7[a]	39.6[ab]	16.1[c]	24.7[bc]	33.8[abc]	49.1[a]	66.6[ab]	46.4[c]	47.8[bc]	48.4[bc]	67.8[a]
22	34.6[ab]	10.8[c]	24.3[bc]	33.3[ab]	44.8[a]	46.7[a]	23.0[b]	37.3[ab]	36.4[ab]	44.7[a]	71.4[ab]	56.1[b]	68.3[ab]	53.5[b]	77.8[a]
32	45.9[ab]	14.3[c]	27.3[bc]	37.0[ab]	49.4[a]	44.5[a]	32.0[a]	38.5[a]	36.4[a]	48.9[a]	65.2[ab]	70.2[ab]	81.8[a]	61.1[b]	77.5[ab]
42	48.0[a]	16.7[c]	27.2[bc]	45.8[ab]	54.1[a]	48.7[ab]	33.1[b]	38.8[ab]	43.0[ab]	58.0[a]	72.0[b]	66.1[b]	79.8[ab]	63.9[b]	93.8[a]

Sample means for each batch of high-protein nutrition bars are presented at each combination of day × temperature. Sample means are significantly different ($p < 0.05$) if they do not share a common alphabetical superscript for the same day (row) at constant temperature.

HPN bars prepared with MPC80 extruded at 65°C had significantly lower hardness, fracturability, and shear strength compared with HPN bars formulated with unmodified MPC80. This was especially true for hardness and fracturability at most temperature × time combinations throughout storage. The hardness and fracturability behavior of HPN bars formulated with MPC80 extruded at 65°C were similar to HPN bars prepared with MPC80 extruded at 120°C when stored at 42°C. However, at lower storage temperatures, significant difference in hardness and fracturability between the HPN bars formulated with MPC80 extruded at 120°C and those prepared with unmodified MPC80 was intermittent. Shear strength of HPN bars formulated with MPC80 extruded at 120°C did not differ significantly with HPN bars formulated with unmodified MPC80, except on day 42 with storage at room temperature ($p < 0.05$).

HPN bars prepared with MPC80 extruded at 65°C and 120°C maintained lower hardness and fracturability towards the end of storage as well as at most instances in the beginning. Textural change at 42°C may not be via the same mechanism as change at room temperature or 32°C, because the HPN bar matrix was more fluid at 42°C which was greater than the melting point of palm kernel stearin.

Hardness and fracturability data should be compared side-by-side because in some instances the force needed to cause initial fracture during the first bite was greater than the hardness value obtained at 60% compression due to the crumbly nature of HPN bars formulated with unmodified and toasted MPC80s. In general, HPN bars prepared with MPC80 extruded at 65°C and 120°C maintained lower hardness and fracturability during storage at each temperature × time combination and may prove to be applicable in HPN bars.

Stress-relaxation analysis parameters, including initial sample stress (σ_0), the average slope from linearized relationship (k_2), the initial decay rate of the stress ratio ($1/k_1$), the predicted sample stress at equilibrium (σ_e), the mean ratio or stress after 300 s divided by calculated equilibrium stress (σ_{300s}/σ_e), are shown in Tables 4-6 through 4-10 respectively. Sample means were compared at each temperature $\times$ time combination to look at significant differences between the HPN bars formulated with unmodified and modified MPC80s. The initial sample stress (σ_0) (Table 4-6) increased numerically with storage time. The HPN bars formulated with MPC80 extruded at 65°C and 120°C tended to have lower loading stress which corresponds to decreased firmness. Average slopes from the linearized relationships (k_2) (Table 4-7) suggested that HPN bars exhibited solid-like behavior with $k_2 > 1$ (Lamsal and others 2007). Initial decay rate of the stress ratio ($1/k_1$) remained relatively the same amongst samples for each temperature $\times$ time slice, meaning stress decayed internally at about the same rate (Table 4-8). Calculated equilibrium stress (σ_e) was typically larger in the HPN bars formulated with MPC80 toasted at 110°C, but significant difference between the other samples at each temperature $\times$ time combination was intermittent. Table 4-10 shows that the stress remaining in the sample after 300 s of fixed strain compression was only slightly greater than the calculated stress at equilibrium, since the ratio was only slightly greater than 1 and added validity to this model. No stress-relaxation analysis has been reported in literature before for HPN bars made with modified MPC80s.

Table 4-6 High-Protein Nutrition Bar Stress Relaxation Analysis: Initial Sample Stress (σ_0, kPa)

	Storage Temperature														
	22°C					32°C					42°C				
Day	MPC	E65	E120	T75	T110	MPC	E65	E120	T75	T110	MPC	E65	E120	T75	T110
0	39[a]	25[a]	33[a]	58[a]	116[a]	-	-	-	-	-	-	-	-	-	-
1	41[a]	39[a]	51[a]	115[a]	221[a]	507[a]	62[c]	83[bc]	468[ab]	566[a]	683[ab]	202[c]	352[bc]	584[bc]	1017[a]
2	269[a]	30[a]	51[a]	235[a]	413[a]	588[a]	64[b]	140[b]	605[a]	730[a]	777[ab]	237[d]	370[cd]	696[bc]	1104[a]
4	596[a]	46[b]	128[b]	531[a]	703[a]	775[a]	162[c]	356[bc]	656[ab]	1008[a]	1000[ab]	457[c]	666[bc]	833[bc]	1270[a]
6	609[a]	77[b]	94[b]	558[a]	897[a]	637[b]	176[c]	449[bc]	697[b]	1132[a]	1063[ab]	726[b]	774[b]	844[b]	1332[a]
13	750[ab]	164[c]	472[bc]	784[ab]	1067[a]	750[ab]	164[c]	472[bc]	784[ab]	1110[a]	1066[b]	863[b]	1194[ab]	1227[ab]	1462[a]
22	948[ab]	274[d]	514[cd]	821[bc]	1292[a]	1125[ab]	549[c]	820[bc]	1012[ab]	1294[a]	1348[a]	1320[a]	1356[a]	1372[a]	1437[a]
32	909[a]	433[b]	482[b]	945[a]	1122[a]	1141[a]	747[b]	957[ab]	972[ab]	1145[a]	1451[a]	1483[a]	1517[a]	1224[a]	1513[a]
42	1006[b]	469[c]	492[c]	765[bc]	1464[a]	867[b]	1041[b]	1009[b]	1000[b]	1555[a]	1846[a]	1570[ab]	1578[ab]	1321[b]	1746[a]

Sample means for each batch of high-protein nutrition bars are presented at each combination of day × temperature. Sample means are significantly different ($p < 0.05$) if they do not share a common alphabetical superscript for the same day (row) at constant temperature.

Table 4-7 High-Protein Nutrition Bar Stress Relaxation Analysis: Average Slope from Linearized Relationship (Equation 4-1) (k_2)

Storage Temperature

Day	*22°C*					*32°C*					*42°C*				
	MPC	*E65*	*E120*	*T75*	*T110*	*MPC*	*E65*	*E120*	*T75*	*T110*	*MPC*	*E65*	*E120*	*T75*	*T110*
0	1.12[b]	1.17[ab]	1.15[ab]	1.17[ab]	1.27[a]	-	-	-	-	-	-	-	-	-	-
1	1.09[a]	1.19[a]	1.20[a]	1.17[a]	1.23[a]	1.27[a]	1.19[a]	1.22[a]	1.24[a]	1.31[a]	1.26[a]	1.24[a]	1.26[a]	1.24[a]	1.36[a]
2	1.19[a]	1.16[a]	1.16[a]	1.21[a]	1.24[a]	1.24[a]	1.17[a]	1.22[a]	1.25[a]	1.31[a]	1.22[a]	1.19[a]	1.21[a]	1.24[a]	1.33[a]
4	1.31[ab]	1.20[b]	1.31[ab]	1.30[ab]	1.36[a]	1.33[a]	1.29[a]	1.39[a]	1.30[a]	1.40[a]	1.30[ab]	1.27[b]	1.31[ab]	1.28[ab]	1.43[a]
6	1.29[a]	1.27[a]	1.31[a]	1.27[a]	1.37[a]	1.30[a]	1.29[a]	1.38[a]	1.29[a]	1.43[a]	1.31[b]	1.34[b]	1.34[ab]	1.34[ab]	1.49[a]
13	1.34[a]	1.29[a]	1.36[a]	1.35[a]	1.42[a]	1.31[b]	1.35[b]	1.39[ab]	1.34[b]	1.52[a]	1.45[ab]	1.32[b]	1.33[b]	1.44[ab]	1.56[a]
22	1.41[ab]	1.33[b]	1.41[ab]	1.35[ab]	1.48[a]	1.38[a]	1.38[a]	1.40[a]	1.35[a]	1.49[a]	1.56[b]	1.51[b]	1.48[b]	1.54[b]	1.73[a]
32	1.40[a]	1.38[a]	1.42[a]	1.35[a]	1.47[a]	1.40[ab]	1.39[ab]	1.45[ab]	1.34[b]	1.54[a]	1.78[ab]	1.57[c]	1.64[abc]	1.63[bc]	1.79[a]
42	1.40[a]	1.40[a]	1.40[a]	1.41[a]	1.47[a]	1.43[a]	1.43[a]	1.43[a]	1.44[a]	1.55[a]	1.84[b]	1.81[bc]	1.79[bc]	1.66[c]	2.01[a]

Sample means for each batch of high-protein nutrition bars are presented at each combination of day × temperature. Sample means are significantly different (p < 0.05) if they do not share a common alphabetical superscript for the same day (row) at constant temperature.

Table 4-8 High-Protein Nutrition Bar Stress Relaxation Analysis: Initial Decay Rate of Stress Ratio (Equation 4-1) ($1/k_1$, s^{-1})

Storage Temperature

Day	22°C					32°C					42°C				
	MPC	*E65*	*E120*	*T75*	*T110*	*MPC*	*E65*	*E120*	*T75*	*T110*	*MPC*	*E65*	*E120*	*T75*	*T110*
0	0.43[a]	0.29[b]	0.24[bc]	0.27[c]	0.17[c]	-	-	-	-	-	-	-	-	-	-
1	0.26[a]	0.22[ab]	0.18[ab]	0.17[ab]	0.13[b]	0.11[a]	0.20[a]	0.16[a]	0.12[a]	0.11[a]	0.11[a]	0.15[a]	0.12[a]	0.12[a]	0.08[a]
2	0.14[a]	0.21[a]	0.20[a]	0.13[a]	0.12[a]	0.11[a]	0.19[a]	0.15[a]	0.11[a]	0.09[a]	0.12[a]	0.17[a]	0.14[a]	0.11[a]	0.08[a]
4	0.10[a]	0.20[a]	0.14[a]	0.11[a]	0.10[a]	0.10[a]	0.13[a]	0.11[a]	0.10[a]	0.08[a]	0.10[a]	0.12[a]	0.12[a]	0.10[a]	0.07[a]
6	0.11[a]	0.17[a]	0.14[a]	0.11[a]	0.09[a]	0.11[a]	0.14[a]	0.11[a]	0.11[a]	0.08[a]	0.10[a]	0.11[a]	0.11[a]	0.09[a]	0.07[a]
13	0.11[a]	0.14[a]	0.12[a]	0.10[a]	0.08[a]	0.11[a]	0.12[a]	0.10[a]	0.10[a]	0.07[a]	0.07[a]	0.13[a]	0.10[a]	0.07[a]	0.06[a]
22	0.11[a]	0.12[a]	0.11[a]	0.11[a]	0.08[a]	0.10[a]	0.10[a]	0.11[a]	0.11[a]	0.08[a]	0.06[a]	0.07[a]	0.07[a]	0.06[a]	0.05[a]
32	0.10[a]	0.12[a]	0.11[a]	0.10[a]	0.08[a]	0.10[a]	0.11[a]	0.10[a]	0.10[a]	0.07[a]	0.05[a]	0.08[a]	0.06[a]	0.05[a]	0.04[a]
42	0.10[a]	0.11[a]	0.10[a]	0.10[a]	0.14[a]	0.09[a]	0.09[a]	0.09[a]	0.08[a]	0.06[a]	0.04[a]	0.05[a]	0.05[a]	0.05[a]	0.03[a]

Sample means for each batch of high-protein nutrition bars are presented at each combination of day × temperature. Sample means are significantly different ($p < 0.05$) if they do not share a common alphabetical superscript for the same day (row) at constant temperature.

Table 4-9 High-Protein Nutrition Bar Stress Relaxation Analysis: Equilibrium Stress (Equation 4-2) (σ_e, kPa)

| | Storage Temperature | | | | | | | | | | | | | | |
| | 22°C | | | | | 32°C | | | | | 42°C | | | | |
Day	*MPC*	*E65*	*E120*	*T75*	*T110*	*MPC*	*E65*	*E120*	*T75*	*T110*	*MPC*	*E65*	*E120*	*T75*	*T110*
0	7^a	4^a	4^a	12^a	29^a	-	-	-	-	-	-	-	-	-	-
1	4^a	7^a	9^a	19^a	42^a	107^a	11^a	16^a	89^a	135^a	143^{ab}	40^b	72^b	112^{ab}	273^a
2	44^a	4^a	7^a	40^a	79^a	112^a	9^a	25^a	120^a	173^a	141^{ab}	40^b	65^b	136^{ab}	275^a
4	141^a	8^a	30^a	123^a	185^a	192^{ab}	36^b	99^b	154^{ab}	292^a	229^{ab}	97^b	154^b	183^b	379^a
6	138^{ab}	17^b	23^b	120^{ab}	240^a	147b	39^b	125^b	155^b	339^a	255^b	184^b	194^b	215^b	439^a
13	189^{ab}	38^b	128^b	205^{ab}	317^a	190^b	119^b	182^b	237^{ab}	375^a	327^b	211^b	297^b	374^{ab}	523^a
22	276^{ab}	67^c	150^{bc}	213^{bc}	422^a	309^{ab}	150^b	233^b	262^{ab}	427^a	484^a	450^a	442^a	485^a	602^a
32	269^{ab}	120^b	143^b	246^{ab}	358^a	327^{ab}	207^b	297^{ab}	246^{ab}	396^a	638^{ab}	552^{ab}	588^{ab}	482^b	669^a
42	290^{ab}	132^b	139^b	223^b	433^a	254^b	314^b	304^b	305^b	553^a	842^{ab}	695^{bc}	689^{bc}	525^c	878^a

Sample means for each batch of high-protein nutrition bars are presented at each combination of day × temperature. Sample means are significantly different (p < 0.05) if they do not share a common alphabetical superscript for the same day (row) at constant temperature.

Table 4-10 High-Protein Nutrition Bar Stress Relaxation Analysis: Mean Ratio (σ_{300s}/σ_e)

	Storage Temperature														
	22°C					*32°C*					*42°C*				
Day	*MPC*	*E65*	*E120*	*T75*	*T110*	*MPC*	*E65*	*E120*	*T75*	*T110*	*MPC*	*E65*	*E120*	*T75*	*T110*
0	1.10[a]	1.10[a]	1.06[b]	1.04[b]	1.05[b]	-	-	-	-	-	-	-	-	-	-
1	1.08[a]	1.07[a]	1.06[a]	1.06[a]	1.05[a]	1.06[a]	1.06[a]	1.04[a]	1.06[a]	1.05[a]	1.06[a]	1.05[a]	1.05[a]	1.06[a]	1.05[a]
2	1.07[a]	1.04[a]	1.06[a]	1.07[a]	1.06[a]	1.06[a]	1.04[a]	1.05[a]	1.06[a]	1.06[a]	1.07[a]	1.05[a]	1.06[a]	1.06[a]	1.06[a]
4	1.05[a]	1.03[a]	1.03[a]	1.05[a]	1.05[a]	1.05[a]	1.04[a]	1.03[a]	1.05[a]	1.05[a]	1.06[a]	1.05[a]	1.05[a]	1.06[a]	1.05[a]
6	1.05[a]	1.03[a]	1.03[a]	1.05[a]	1.05[a]	1.05[a]	1.04[a]	1.04[a]	1.05[a]	1.04[a]	1.06[a]	1.05[a]	1.04[a]	1.05[a]	1.05[a]
13	1.04[a]	1.04[a]	1.04[a]	1.04[a]	1.04[a]	1.05[a]	1.04[a]	1.04[a]	1.05[a]	1.04[a]	1.05[a]	1.04[a]	1.05[a]	1.05[a]	1.04[a]
22	1.03[a]	1.05[a]	1.03[a]	1.04[a]	1.04[a]	1.04[a]	1.04[a]	1.04[a]	1.04[a]	1.04[a]	1.04[a]	1.04[a]	1.04[a]	1.04[a]	1.04[a]
32	1.04[a]	1.03[a]	1.03[a]	1.04[a]	1.04[a]	1.04[a]	1.04[a]	1.03[a]	1.05[a]	1.04[a]	1.03[a]	1.03[a]	1.03[a]	1.04[a]	1.04[a]
42	1.04[a]	1.03[a]	1.04[a]	1.04[a]	1.04[a]	1.04[a]	1.04[a]	1.04[a]	1.04[a]	1.04[a]	1.03[a]	1.03[a]	1.03[a]	1.04[a]	1.03[a]

Sample means for each batch of high-protein nutrition bars are presented at each combination of day × temperature. Sample means are significantly different ($p < 0.05$) if they do not share a common alphabetical superscript for the same day (row) at constant temperature.

4.4.2 Water Activity

Water activity (Table 4-11) of the HPN bars increased slightly during storage, but remained less than 0.65 in all samples ensuring shelf stability and preventing microbial growth (Loveday and others 2009). All model HPN bars had significantly increased water activity after 42 days of storage at room temperature except for those prepared with MPC80 extruded at 65°C, which only increased by 0.03 ($p < 0.05$). At room temperature, the water activity of HPN bars formulated with unmodified MPC80 increased by 0.07. However, the water activity at 32°C was more stable for HPN bars formulated with unmodified MPC80 than those formulated with MPC80 extruded at 65°C. Increased water activity may indicate that water molecules have moved from the intermediate phase, where they act as a plasticizer, to the bulk phase (Li and others 2008). Small magnitude increases in water activity in model HPN bars in this study are consistent with other HPN bar studies that used 32°C as the accelerated storage temperature; such increase corresponded with HPN bars that harden quicker compared with those HPN bars with stable water activity (Li and others 2008; McMahon and others 2009). Lack of water molecules associated with the local protein domain allows neighboring amino acids to form disulfide bonds that cause subsequent protein aggregation, one of the suggested mechanisms of HPN bar hardening when formulated with whey protein (Zhou and others 2008a; Zhou and others 2008b). Although some increases in water activity within each batch at constant temperature over time were significant ($p < 0.05$), the relative increase was small and may have no influence on HPN bar hardening. Water activity measurement is not a sensitive technique and differential scanning calorimetry may be a better option to more thoroughly describe the state of water molecules in HPN bar matrices (Li and others 2008; Zhou and others 2008b).

Table 4-11 Water Activity of High-Protein Nutrition Bars during Storage

			Storage Temperature													
			22°C						*32°C*						*42°C*			
Day	*MPC*	*E65*	*E120*	*T75*	*T110*	*MPC*	*E65*	*E120*	*T75*	*T110*	*MPC*	*E65*	*E120*	*T75*	*T110*
0	0.39^c	0.40^{ab}	0.40^{cd}	0.40^c	0.40^c	-	-	-	-	-	-	-	-	-	-
1	0.41^{bc}	0.39^b	0.39^d	0.42^{bc}	0.41^{bc}	0.44^a	0.40^c	0.40^c	0.45^a	0.43^a	0.48^a	0.43^b	0.43^b	0.47^a	0.46^a
4	0.44^{ab}	0.40^b	0.41^{bcd}	0.44^{ab}	0.43^{ab}	0.46^a	0.41^{bc}	0.43^{cb}	0.46^a	0.45^a	0.46^a	0.46^a	0.46^a	0.48^a	0.46^a
13	0.44^a	0.40^{ab}	0.42^{abc}	0.45^a	0.45^a	0.45^a	0.43^{abc}	0.45^{ab}	0.45^a	0.46^a	0.47^a	0.46^a	0.46^a	0.47^a	0.46^a
22	0.45^a	0.41^{ab}	0.44^{ab}	0.45^a	0.45^a	0.46^a	0.43^{ab}	0.46^a	0.46^a	0.45^a	0.45^a	0.47^a	0.47^a	0.46^a	0.45^a
42	0.46^a	0.43^a	0.45^a	0.45^a	0.46^a	0.46^a	0.45^a	0.46^a	0.46^a	0.45^a	0.47^a	0.46^a	0.44^{ab}	0.46^a	0.44^a

Sample means for each batch of high-protein nutrition bars are presented at each combination of batch × temperature. Sample means are significantly different ($p < 0.05$) if they do not share a common alphabetical superscript for the same batch (column) at constant temperature.

MPC80 dissolved partially during protein bar dough preparation and was followed by dehydration during storage as polyhydroxy compounds (i.e., glucose and glycerol) pulled water away through osmotic force (Loveday and others 2009). Fast proton relaxation rates, as determined through NMR, decreased for water and the polyhydroxy compounds suggesting lower mobility of these constituents as a result of increased glucose precipitation (Loveday and others 2009).

4.4.3 Total Color Change

Total color change (ΔE) in HPN bars is shown in Table 4-12. Although HPN bar hardening is typically the main reason for unacceptable quality, color change can also be an indicator of quality decline. Magnitude of ΔE was numerically greater at higher storage temperature. HPN bars prepared with unmodified MPC80 underwent extensive color change during storage, whereas Maillard browning occurred extensively in MPC80 toasted at110°C during processing, and thus total color change during storage was more reserved. Inhibition of Maillard browning may help slow HPN bar hardening because Maillard browning products have increased the rate of disulfide bond and hydrophobic network formation, that expels water currently associated with the local protein domain (Anema and others 2006; Le and others 2011). However, when Maillard browning was inhibited in HPN bars by sulfite addition, they continued to harden, prompting some researchers (Baier and others 2007) to refute this suggested mechanism of HPN bar hardening.

**Table 4-12 Total Color Change (ΔE) of High-Protein Nutrition Bars during Storage**

	22°C					*32°C*					*42°C*				
Day	*MPC*	*E65*	*E120*	*T75*	*T110*	*MPC*	*E65*	*E120*	*T75*	*T110*	*MPC*	*E65*	*E120*	*T75*	*T110*
0	0[a]	0[a]	0[c]	0[a]	0[a]	-	-	-	-	-	-	-	-	-	-
1	1.4[a]	1.5[a]	1.2[c]	4.1[a]	2.7[a]	2.6[c]	2.2[b]	1.6[a]	3.8[c]	3.3[a]	4.3[c]	4.2[b]	3.1[cd]	4.3[c]	3.6[c]
4	1.9[a]	1.4[a]	2.7[bc]	3.4[a]	3.5[a]	1.9[c]	3.1[ab]	2.9[a]	5.4[c]	3.9[a]	11.0[c]	6.1[b]	2.5[d]	10.3[c]	10.5[abc]
13	1.7[a]	3.6[a]	5.1[abc]	3.4[a]	6.2[a]	7.9[bc]	8.8[ab]	3.2[a]	19.0[ab]	5.8[a]	26.8[b]	19.8[a]	10.6[bc]	26.3[b]	9.2[bc]
22	2.6[a]	3.2[a]	10.1[ab]	3.4[a]	7.5[a]	14.6[ab]	6.5[ab]	7.0[a]	14.2[b]	6.4[a]	32.8[ab]	24.0[a]	15.7[ab]	32.2[ab]	12.9[ab]
42	4.7[a]	7.4[a]	12.5[a]	6.6[a]	6.2[a]	22.1[a]	11.1[a]	8.3[a]	23.3[a]	6.4[a]	38.7[a]	27.5[a]	19.8[a]	37.5[a]	18.2[a]

Storage Temperature

Sample means for each batch of high-protein nutrition bars are presented at each combination of batch × temperature. Sample means are significantly different ($p < 0.05$) if they do not share a common alphabetical superscript for the same batch (column) at constant temperature.
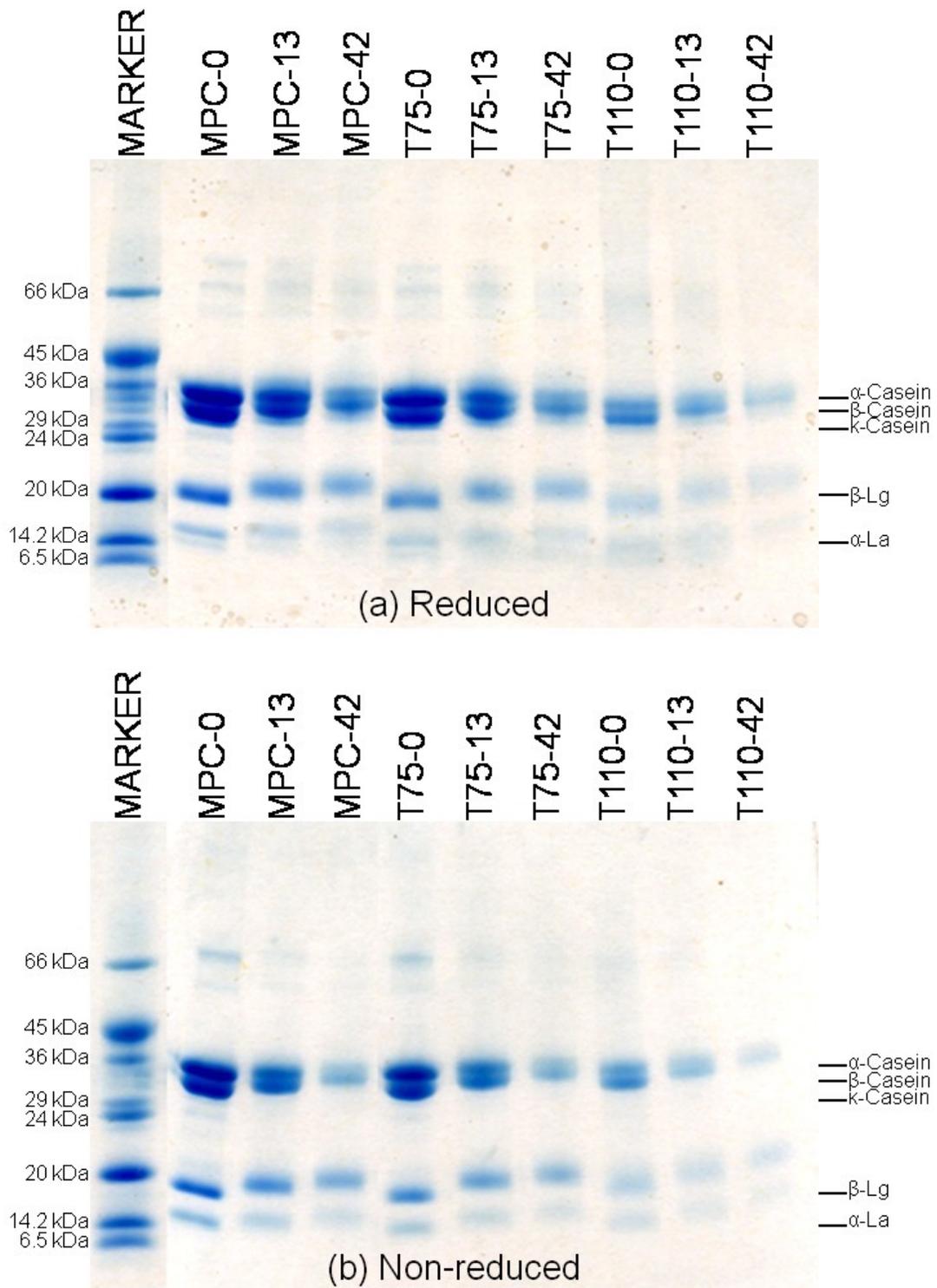
The HPN bars formulated with MPC80 extruded at 65°C and 120°C had lower magnitude of color change, which may be from the limited lysine available to participate in the Maillard browning reaction since lysine was suggested to have low levels of retention when milk protein isolate was extruded in combination with reducing sugars (Singh and others 2007b). Therefore the extruded MPC80 samples will potentially have limited ability to participate in further Maillard reactions because of lysine destruction. Since internal production of advanced Maillard products would be slowed, the HPN bars produced with extruded MPC80 may have slowed internal disulfide bond formation resulting in a soft and stable HPN bar. HPN bars formulated with MPC80 toasted at 110°C, a sample that was already visually brown, likely contained advanced Maillard browning products that may have contributed to more rapid disulfide bond formation and faster textural changes.

4.4.4 Reduced and Non-Reduced SDS-PAGE

Reduced and non-reduced SDS-PAGE profiles of the protein subunits in HPN bars prepared with extruded MPC80 on the day of manufacture (Day 0), Day 13, and Day 42 of storage at 32°C are presented in Figure 4-1. The reduced and non-reduced SDS-PAGE profiles of the HPN bars prepared with toasted MPC80 at the same storage conditions are shown in Figure 4-2. HPN bars formulated with unmodified MPC80 are shown on each reduced and non-reduced gel.
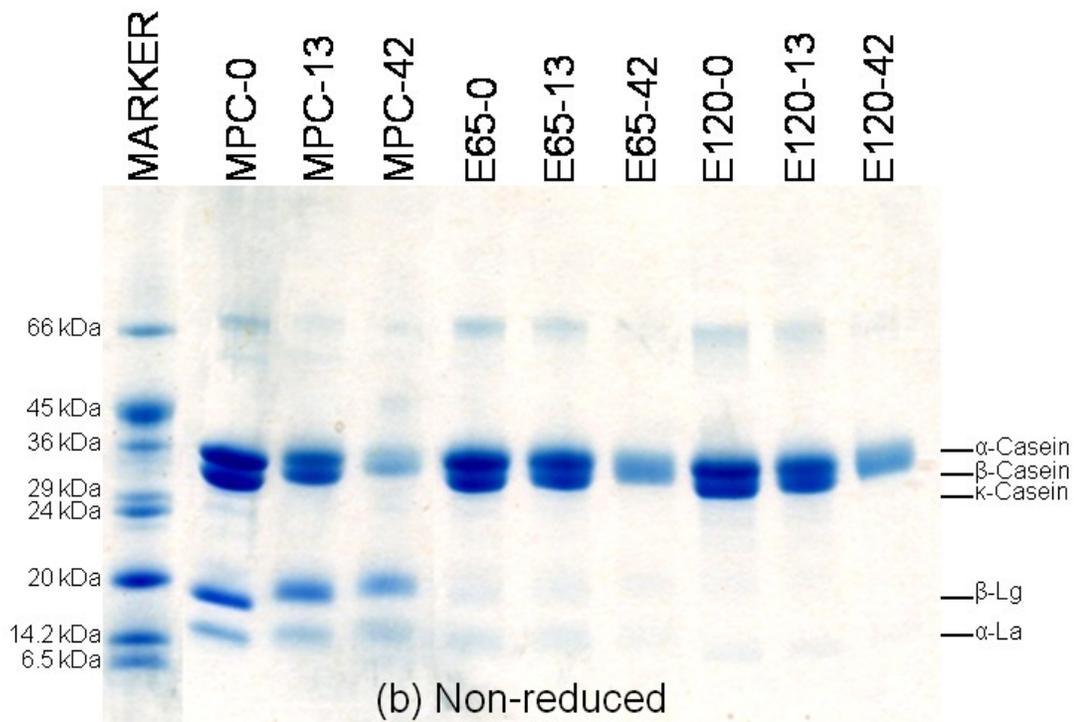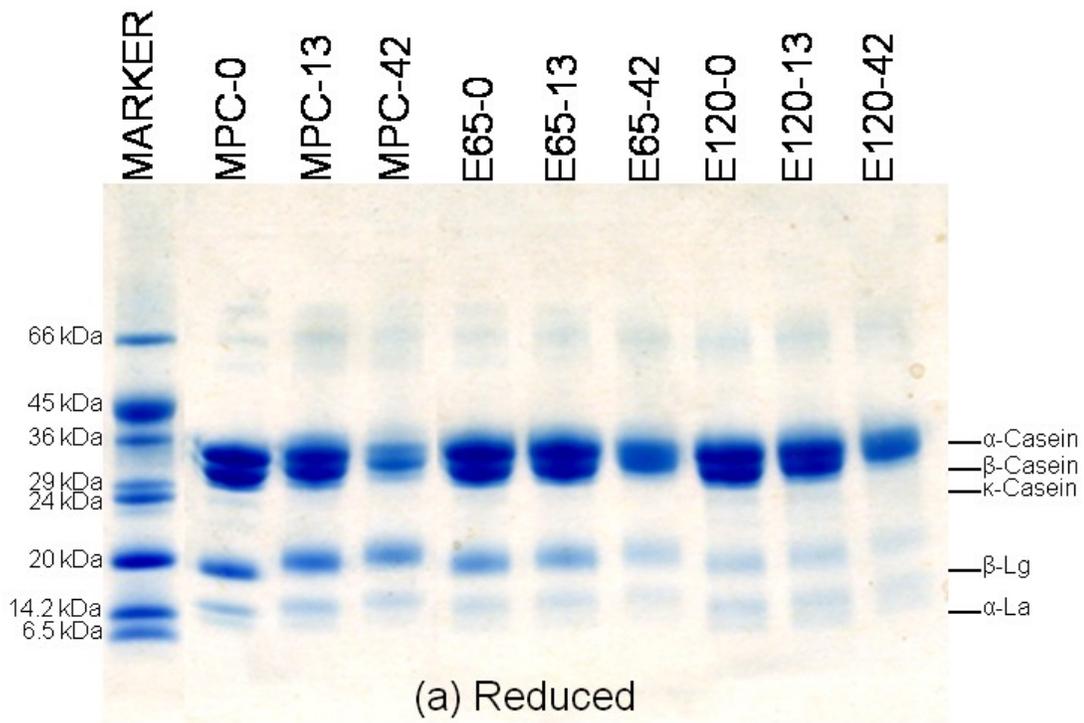
Figure 4-1 Reduced (a) and Non-Reduced (b) SDS-PAGE for HPN Bars with Extruded MPC80
MPC indicates HPN bars that were formulated with unmodified MPC80. E65 and E120 designate HPN bars formulated with MPC80 extruded at 65°C and 120°C. Days of storage at 32°C are 0, 13, and 42 for each batch as indicated above each lane.

Figure 4-2 Reduced (a) and Non-Reduced (b) SDS-PAGE for HPN Bars with Toasted MPC80
MPC indicates HPN bars that were formulated with unmodified MPC80. T75 and T110 designate HPN bars formulated with MPC80 toasted at 75°C and 110°C. Days of storage at 32°C are 0, 13, and 42 for each batch as indicated above each lane.

HPN bars prepared with MPC80 extruded at 65°C and 120°C retained reduced peptide profiles (Figure 4-1a) that closely resembled the control HPN bars. With increased storage time, the casein subunits showed decreased intensity, and in the extruded samples, the casein subunits merged into one band by Day 42. The unmodified MPC80 still retained casein subunit distinction between α-, β-, and κ-casein under reduced conditions. The β-lactoglobulin (β-lg) and α-lactalbumin (α-la) bands remained visible in the HPN bars formulated with extruded MPC80 under reduced conditions, but had decreased intensity when compared with the MPC80 control corresponding to the same length of storage. On Day 0, the extruded samples had whey protein bands that were slightly smeared, suggesting some denaturation as a result of extrusion of MPC80 prior to incorporation to the HPN bars.

Peptide solubility, as corroborated by decreased band intensity between the reduced and non-reduced gel, was lesser when β-mercaptoethanol was absent during extraction (Figure 4-1b). The relative extent of subunit disappearance with increased storage time was greater for non-reduced protein extraction in unmodified MPC80 HPN bars. The β-lg and α-la protein bands in HPN bars formulated with MPC80 extruded at 65°C were faintly present on Day 0 and disappeared completely by Day 42. The same was true for the whey protein bands in HPN bars formulated with MPC80 extruded at 120°C, except they were even more difficult to see on Day 0. Also, casein subunits in the extruded samples were less pronounced compared with the reduced extractions and became more insoluble without a reducing agent with increased storage time. Under non-reduced conditions, α-, β-, and κ-casein became indistinguishable by Day 42 in the HPN bars prepared with MPC80 extruded at 65°C and 120°C.

The protein subunits in HPN bars formulated with toasted MPC80 showed decreased intensity under both reduced and non-reduced conditions with longer storage at 32°C (Figure 4-2). Under reduced conditions, HPN bars formulated with MPC80 toasted at 75°C behaved similar to unmodified MPC80, but toasting at 110°C prior to incorporation into the HPN bar reduced initial peptide band intensity and became fainter, especially when compared with the control MPC80 HPN bars with increased storage time. Non-reduced gels in Figures 4-1 and 4-2 showed all subunits at reduced intensity compared with their respective reduced gels, which indicated reduced solubility in the non-reduced extraction buffer. The β-lg and α-la bands on the non-reduced gels shifted upward when compared with Day 0 for each respective HPN bar sample.

The disappearance or fading of dairy protein subunits on non-reduced compared to reduced extractions suggested insolubility from disulfide bond formation (Onwulata and others 2010). The decreased intensity in whey protein bands with longer HPN bar storage with non-reduced extraction could be attributed to possible increases in disulfide bond formation within the HPN bar matrix. The subunit β-lg contains 1 mole of cysteine that can form a disulfide crosslink with κ-casein, and possibly with α_{s2}-casein or α-la (Fox and McSweeney 1998). MPC85 stored as a powder at 40°C without any modification was reported to have decreased casein solubility that was more prominent than decreased β-lg and α-la solubility (Havea 2006). β-lg and α-la had decreased mobility on the reduced gels, and on the non-reduced gel for toasted MPC80 (Figures 4-1a, 4-2a, and 4-2b). Loveday and others (2009) attributed decreased protein subunit mobility to Maillard reactions and increased peptide molecular weight. The non-reduced SDS-PAGE profile for model HPN bars formulated with unmodified MPC80 revealed fainter whey protein bands with increased

storage time at 20°C (Loveday and others 2009). In the same study, β- and α- casein were reported to retain similar band intensity after 48 days storage compared with band intensity on Day 1 for the reduced and non-reduced gels (Loveday and others 2009). κ-casein became progressively fainter with time on the non-reduced gel, but maintained intensity on the reduced gel (Loveday and others 2009). A model intermediate food containing whey protein experienced similar protein insolubility due to primary disulfide bond formation and hardened with subsequent aggregations (Zhou and others 2008a, Zhou and others 2008b).

Since HPN bars formulated with extruded MPC80, especially at 65°C, retained a softer texture over storage period, discussion will be focused on the proposed mechanism for reduced internal hardening. The whey proteins, β-lg and α-la, in this particular HPN bar, will not be able to contribute to internal disulfide bond formation because they were already disulfide bonded, as demonstrated with their reappearance in the presence of a reducing agent in reduced SDS-PAGE (Figure 4-1a). Extrusion enhanced disulfide bond formation prior to incorporation into the HPN bar that prevented internal aggregations and decreased peptide solubility with storage time. Inhibition of disulfide bond formation by low levels of cysteine or N-ethylmaleimide addition to a model intermediate moisture food system improved stability and extended shelf-life based on time to reach a predetermined hardness level (Zhu and Labuza 2010). N-ethylmaleimide, which prevents disulfide bond formation by thioether linkage with free cysteine residues, extended the shelf-life of the model intermediate moisture food up to 6-times the control (Zhu and Labuza 2010).

4.5 Conclusion

Unmodified, extruded, and toasted MPC80s were used to formulate model HPN bars and study textural, color, and water activity change during storage. MPC80 toasted at 75°C

and 110°C behaved similarly to unmodified MPC80 when incorporated into the HPN bars.

Toasting MPC80 at these conditions did not improve performance in model HPN bars and so

it probably will not enhance commercial viability. However, MPC80 extruded at 65°C and

120°C lessened textural change when compared with HPN bars formulated with unmodified

MPC80. The MPC80 extruded at 65°C outperformed the MPC80 extruded at 120°C because

the textural parameters measured were often lower and statistically less than those in the

HPN bars formulated with unmodified MPC80. This suggested that modification of MPC80

with low temperature extrusion may be suitable to improve performance in HPN bars,

especially if final formulation is similar to that used in this study.

4.6 References

Anema SG, Pinder DN, Hunter RJ, Hemar Y. 2006. Effects of storage temperature on the solubility of milk protein concentrate (MPC85). Food Hydrocoll 20(2-3):386-93.

[AOAC] Assn. of Official Analytical Chemists. 1998. Method 992.23. In: Official methods of analysis of Assn. of Analytical Chemists. 16th ed. Arlington, VA.: AOAC.

Baier SK, Guthrie BD, Smith SA, Lendon CA, Muroski, AR. 2007. Influence of extrusion on protein conformation and shelf-life extension of nutritional bars. Paper presented at International Symposium of the Properties of Water X, 2-7 September, Bangkok, Thailand.

Cold Spring Harbor Protoc. 2009. Borate buffer. Cold Spring Harbor Protocols 2009(7).

Fox PE, McSweeney PLH. 1998. Dairy Chemistry and Biochemistry. 1st ed. United Kingdom: Blackie Academic & Professional. 478 p.

Gunasekaran S, Ak MM. 2003. Chapter 7 - Cheese Texture. In: Cheese Rheology and Texture. 1st ed. United States of America: CRC Press.

Hamilton-Reeves JM, Vazquez G, Duval SJ, Phipps WR, Kurzer MS, Messina MJ. 2010. Clinical studies show no effects of soy protein or isoflavones on reproductive hormones in men: results of a meta-analysis. Fertil Steril 94(3):997-1007.

Havea P. 2006. Protein interactions in milk protein concentrate powders. Int Dairy J 16(5):415-22.

Hughes GJ, Ryan DJ, Mukherjea R, Schasteen CS. 2011. Protein Digestibility-Corrected Amino Acid Scores (PDCAAS) for Soy Protein Isolates and Concentrate: Criteria for Evaluation J Agric Food Chem 59(23):12707-12.

[IDF/ISO] International Dairy Federation/International Organization for Standardization. 2004. Method IDF 26/ISO 5537. Dried milk - determination of moisture content.

Imtiaz SR, Kuhn-sherlock B, Campbell M. 2012. Effect of Dairy Protein Blends on Texture of High Protein Bars. J Texture Stud 43(4):275-86.

Lamsal BP, Koegel RG, Gunasekaran S. 2007. Some physiochemical and functional properties of alfalfa soluble leaf proteins. LWT Food Sci Technol 40(9):1520-6.

Le TT, Bhandari B, Deeth HC. 2011. Chemical and physical changes in milk protein concentrate (MPC80) powder during storage. J Agric Food Chem 59(10):5465.

Li Y, Szlachetka K, Chen P, Lin X, Ruan R. 2008. Ingredient Characterization and Hardening of High-Protein Food Bars: an NMR State Diagram Approach. Cereal Chem 85(6):780-6.

Liu X, Zhou P, Tran A, Labuza TP. 2009. Effects of Polyols on the Stability of Whey Proteins in Intermediate-Moisture Food Model Systems. J Agric Food Chem 57(6):2339-45.

Loveday SM, Hindmarsh JP, Creamer LK, Singh H. 2009. Physicochemical changes in a model protein bar during storage. Food Res Int 42(7):798-806.

Loveday SM, Hindmarsh JP, Creamer LK, Singh H. 2010. Physicochemical changes in intermediate-moisture protein bars made with whey protein or calcium caseinate. Food Res Int 43(5):1321-8.

McMahon DJ, Adams SL, McManus WR. 2009. Hardening of High-Protein Nutrition Bars and Sugar/Polyol-Protein Phase Separation. J Food Sci 74(6):E312-21.

Mistry VV, Hassan HN. 1992. Manufacture of nonfat yogurt from a high milk protein powder. J Dairy Sci 75(4):947.

Mistry VV. 2002. Manufacture and application of high milk protein powder. Le Lait 82(4):515-22.

Murphy PA, Barua K, Hauck CC. 2002. Solvent extraction selection in the determination of isoflavones in soy foods. J of Chromatogr B 777(1-2):129-38.

Onwulata CI, Phillips JG, Tunick MH, Qi PX, Cooke PH. 2010. Texturized Dairy Proteins. J Food Sci 75(2):E100-9.

Peleg M. 1979. Characterization of the Stress Relaxation Curves of Solid Foods. J Food Sci 44(1):277-81.

Singh H. 2007a. Interactions of milk proteins during the manufacture of milk powders. Le Lait 87(4-5):413-23.

Singh S, Wakeling L, Gamlath S. 2007b. Retention of essential amino acids during extrusion of protein and reducing sugars. J Agric Food Chem 55(21):8779-86.

Smithers GW. 2008. Whey and whey proteins-From 'gutter-to-gold'. Int Dairy J 18(7):695-704.

Tang JE, Moore DR, Kujbida GW, Tarnopolsky MA, Phillips SM. 2009. Ingestion of whey hydrolysate, casein, or soy protein isolate: effects on mixed muscle protein synthesis at rest and following resistance exercise in young men. J Appl Physiol 107(3):987-92.

Zhou P, Liu X, Labuza TP. 2008a. Effects of Moisture-Induced Whey Protein Aggregation on Protein Conformation, the State of Water Molecules, and the Microstructure and Texture of High-Protein-Containing Matrix. J Agric Food Chem 56(12):4534-40.

Zhou P, Liu X, Labuza TP. 2008b. Moisture-Induced Aggregation of Whey Proteins in a Protein/Buffer Model System J Agric Food Chem 56(6):2048-54.

Zhu D, Labuza TP. 2010. Effect of Cysteine on Lowering Protein Aggregation and Subsequent Hardening of Whey Protein Isolate (WPI) Protein Bars in WPI/Buffer Model Systems J Agric Food Chem 58(13):7970-9.

CHAPTER 5. GENERAL CONCLUSIONS

5.1 Summary

The overall goal of this study was to modify MPC80 in an effort to alleviate the hardening effect it has when incorporated into high-protein nutrition (HPN) bars unmodified. After a thorough review of processing techniques that were available for protein modification, it was decided to modify the proteins in MPC80, mainly casein and whey, with extrusion and low-temperature toasting. Both methodologies have been used to modify other proteins, but neither has been applied to MPC80 without additional processing aids. Extrusion modification looked at the effect of two different screw profiles, low- and high-shear, and four different ramped temperature profiles with four different die temperatures. MPC80 was toasted at 75°C or 110°C for 4 h to impart modification without the shearing forces and high pressures imparted during extrusion.

The functional properties of modified MPC80 were analyzed, including solubility profiles, water holding capacity, gelation and gel strength, surface hydrophobicity, and SDS-PAGE. In General, extruded MPC80 had reduced protein solubility, reduced surface hydrophobicity, reduced water holding capacity, and no strong effect on gel strength. Toasted MPC80 also had reduced protein solubility, decreased surface hydrophobicity, and no strong effect on gel strength. Toasting MPC80 at the lower temperature (75°C) increased water holding capacity, but at the higher temperature it led to slightly increased gel strength. Reduced protein solubility was common as a result of heat treatments; either through extrusion or toasting. Reduced protein solubility indicated that proteins would likely be less reactive in HPN bars.

Both extruded and toasted MPC80 were compared for their performance in a model HPN bar study. MPC80 extruded at 65°C and 120°C, MPC80 toasted at 75°C and 110°C, and control unmodified MPC80 were used for formulating model HPN bars with similar ingredients and in realistic concentrations as commercial HPN bars. The low-shear screws were selected for extrusion because processing was easier and screw-profile never had a significant effect on the functionalities tested. The formulated model remained shelf-stable in terms of water since water activity remained lower than 0.65 to prevent microbial growth at all time points during storage. MPC80 toasted at 110°C behaved very similar to control MPC80 used in the model HPN bar formulations, and produced the hardest product with accelerated storage. Extruded MPC80s used in HPN bars lowered hardness, fracturability, and shear strength towards the end of the 42 day storage period at each storage temperature; in many instances each parameter tested was significantly ($p < 0.05$) lower than in HPN bars prepared with unmodified MPC80.

5.2 Recommendations

The functionality of MPC80 can be modified with low-temperature toasting and extrusion processing. The modification technique should be selected based on desired functionality in the food product being formulated. In terms of applicability in HPN bars, extruded MPC80s, specifically the MPC80 extruded at 65°C die temperature, tended to lessen TPA hardness, TPA fracturability, and shear strength while still forming a cohesive model HPN bar with the formulation tested. Of the modifications tested, extrusion processing MPC80 at 65°C is the recommended processing technique to improve performance in HPN bars. Feasibility of using an extruded MPC80 on bar forming equipment was not carried out as part of this study and so it is unknown how HPN bar dough

will perform on processing equipment. Although extrusion processing improved performance in HPN bars, commercial production of an extruded MPC product will depend on economical feasibility of the process. However, if the new HPN bar texture imparted by pre-extruding MPC is preferred by the consumer, then extruded MPCs will gain a competitive advantage. Ideally, these modified MPCs will be able to displace or supplement imported caseinates in HPN bar applications and may work well in combination with domestically produced whey protein concentrates, isolates, and hydrolysates.

5.3 Future Work.

Based on research findings and observations, the following work is suggested for further research in relation to MPC80 utilization in high-protein nutrition bars:

1. Moisture migration and phase separation in HPN bars formulated with extruded MPC80

Macronutrient phase separation and moisture migration are two HPN bar hardening mechanisms that were not looked at during this study. Moisture may migrate to the protein or away from the protein in HPN bars. Increased water activity in HPN bars may indicate increased water in the bulk phase that is less associated with protein and hence less able to plasticize. However, water activity measurement is not as sensitive as differential scanning calorimetry which can be used to assess the state of water molecules as bound, loosely associated, or free within the HPN bar. Further elucidation of the state of water in HPN bars formulated with extruded MPC80 might help explain why extrusion processing softened the HPN bars when subjected to accelerated storage. Confocal laser scanning microscopy (CLSM) can be used to look a macronutrient phase separation within a HPN bar matrix. HPN bars formulated with extruded MPC80 might be able maintain uniform phase as opposed to HPN bars formulated with unmodified MPC80. Limited macronutrient phase

separation has been linked to slower hardening reactions in HPN bars formulated with partially hydrolyzed whey protein isolate.

2. *Evaluation of other MPC ingredients in HPN bars*

There are some other concentrated milk protein ingredients produced on a research scale that are relatively new, understudied, and may have good performance in HPN bars. Transglutaminase cross-linked micellar casein concentrate (MCC), transglutaminase cross-linked MPC, and mineral-reduced MPC are modified concentrated dairy protein ingredients that have not been evaluated for their applicability in HPN bars. Transglutaminase crosslinks in MCC and MPC may slow aggregations that occur within the HPN bar matrix. Reduced-calcium MPCs may also be favorable in HPN bars because calcium and other minerals in HPN bars can affect the rate of hardening by altering protein conformations and affinity for water. The use of transglutaminase cross-linked MCC and MPC, and mineral-reduced MPC might result in HPN bars with favorable textural properties and slowed hardening reactions.

3. *Influence of α-lactalbumin bound calcium versus free calcium in HPN bars*

Calcium may influence the functionality of α-lactalbumin (α-la), either for better or worse, in HPN bars. α-La is a metallo-protein that can bind up to one mole of calcium. If zinc or aluminum is bound to α-la, the molecular conformation changes, the calcium atom is released from the binding loop, and the peptide converts to its apo form. By altering the molecular conformation, the functionality of α-la in food systems will also be altered. The addition of minerals, especially free calcium, may increase the rate of quality decline in HPN bars by increasing the rate of moisture migration to the protein and subsequently increasing the rate of HPN bar hardening. However, the addition of calcium chloride buffer to a dough system roughly modeling a HPN bar formulated with α-la slowed the rate of protein

aggregate formation, a mechanism associated with protein bar hardening. It is proposed to study how the functionality of apo and holo α-la differ in terms of solubility, water hydration capacity, surface hydrophobicity, gel strength, and textural properties when incorporated into a HPN bar matrix. If decalcifying α-la improves functionality, removing the calcium from other dairy proteins, namely the casein in MPC, may help increase demand for these dairy proteins.

ACKNOWLEDGEMENTS