An assessment of improved functionality of radio frequency dielectric heated nonfat dry milk into model food systems

by

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Abstract

The application of a dry heat processing technology, such as radio frequency dielectric heating (RFDH) on nonfat dry milk (NFDM), has been proven to inactivate pathogens and to alter functional properties. The altered functional properties of RFDH-treated NFDM could produce a valuable, novel ingredient that may improve final product quality when used in other food systems. However, dependent upon the RFDH conditions, whey protein denaturation can occur in low-heat NFDM (LH), which in turn impacts functional properties. To understand how RFDH-treated NFDM influences the functional properties in a variety of food systems, RFDH-treated LH was the milk source in the manufacture of bread, white sauce, and caramels. Low-heat NFDM was RFDH-treated ((heated to 85°C and held for 90 min (85/90) or 180 min (85/180)) and cooled. For bread, white sauce, and caramel, the NFDM samples ((LH, 85/90, 85/180, and high heat (HH)) were directly added into formulations. Three replications were completed and all data were analyzed using SAS® statistical software to differentiate amongst the significant means. Within bread, the 85/90 and 85/180 displayed similar physical properties such as loaf volume, crumb firmness, and color properties when compared to HH. In white sauce, the 85/180 functioned similar to HH as these sauces had similar firmness, water-holding capacity, and color (darkness). When formulated within caramels, 85/90 and 85/180 displayed similar water activity, firmness, stickiness, and color properties as caramels produced with HH. Due to the nature of these particular food systems, it is possible that formulating with the RFDH-treated NFDM, will produce better quality finished products when compared to LH.

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Dedication

I would like to dedicate this to my incredibly supportive family, friends, boyfriend, and cat. Thank you for giving me unyielding love and encouraging me to always find the best version of myself.

Chapter 1 - Literature Review

1.1 Milk Proteins

Milk proteins are categorized as either caseins, which are insoluble at pH 4.6 and 20°C, or whey, which remain soluble at pH 4.6 (Smithers & Augustin, 2012). Caseins of bovine milk will compose 78% of total proteins (Table 1.1) (Varnam & Sutherland, 1994). The ratio of casein to whey protein is 4:1 (Chandan & Kilara, 2010). The quality and the functional properties of milk and dairy products are affected by their proteins more so than any other constituent (Boland, Singh, & Thompson, 2014).

Table 1.1. Characterizations of Milk Protein Fractions

Protein	Fractions	Da[1,2]	g/kg[3,5]	Total Number of Amino Acids[4]
Casein	α_{s1}-	37,000	11.5	199
	α_{s2}-	39,000	3.0	207
	β-	26,000-33,000	9.5	209
	κ-	23,000	3.4	169
Whey	α-Lactalbumin	10,000	1.2	123
	β-Lactoglobulin	15,000	3.1	162
	Serum albumin	65,000	0.4	583
	Immunogloblins	150,000	0.8	310-500

[1]Molecular weight of monomer
[2]Adapted from Varnam & Sutherland (1994); Jovanovic, Barac, Macej, Vucic, & Lacnjevac (2007).
[3]Adapted from Jensen & Newburg (1995).
[4]Adapted from Fox (2003)
[5]Assuming a density of 1.03 g/mL of milk (Fox, 2003).

1.1.1 Casein Proteins

The caseins in milk are categorized into four main classes: α_{s1}-, α_{s2}-, β-, and κ-caseins (Table 1.1) (Varnam & Sutherland, 1994). All of these caseins are mammary gland gene products that are globular proteins containing ester-bound phosphates and high concentrations of proline (Roginski, Fuquay, & Fox, 2003). Phosphoserine residues are found on all caseins and are responsible for the existing hydrophilic areas of strong negative charge. Alpha$_{s1}$-caseins have

seven to nine phosphoserine residues per mole, while α_{s2}-caseins have ten to 13, and β-caseins have five (Varnam & Sutherland, 1994). From the β-casein, the γ-casein is derived through plasmin proteolysis (Eigel, Butler, Ernstrom, Farrell, Harwalkar, Jenness, & Whitney, 1984; Varnam & Sutherland, 1994).

These caseins have a strong tendency to associate and form colloids, or casein micelles, through hydrophobic bonding due to their high concentration of phosphate groups. Due to the weak calcium binding properties of the κ-casein, the κ-casein will stabilize and protect the casein micelle (Fox, 2003).

1.1.2 Casein Micelles

Casein micelles account for around 95% of the total caseins in milk (Fox, 2003). The casein micelle vary in size ranging from 30 to 300 nm (mean diameter *ca.*150 nm, 10^8 kDa) (Varnam & Sutherland, 1994). The micelles are open, hydrated structures with an average hydration value of two to three H_2O g^{-1} protein (Roginski et al., 2003). The casein micelles contain aggregates of sub-micelles that are temperature stable due to phosphate concentrations, but coagulate at 140°C after 15 to 20 min at a pH 6.5 to 6.7. The coagulation of casein micelles is the result of dephosphorylation of the casein, cleavage of the oligosaccharide moiety of κ-casein, and denaturation of whey proteins with the precipitation of the casein micelles. The caseins will aggregate and precipitate from a solution when the pH decreases to the casein isoelectric point of 4.6. (Fox, 2003). The casein micelles can dissociate with the addition of Ca^{2+} chelating agents ((e.g., ethylenediaminetetraacetic acid (**EDTA**), phosphate, and citrate)) or by acidification (Smithers & Augustin, 2013). Casein micelles remains fixed during storage and will not change significantly with cooling (4°C) or mild heat processing, such as pasteurization (62.8°C for 30 min) (Horne, 2002).

1.1.3 Whey Proteins

The main whey protein fractions contain several proteins: α-lactalbumin (**α-LA**), β-lactoglobulin (**β-LG**), bovine serum albumin (**BSA**), and immunoglobulin (**Ig**) (Smithers & Augustin, 2012). Whey proteins are globular proteins, with uniform sequences of polar, nonpolar, and charged residues. They can undergo intramolecular folding during the formation of disulphide bonds between cysteinyl residues, which will submerge hydrophobic residues within the molecule (Varnam & Sutherland, 1994).

1.1.3.1 α-Lactalbumin

Alpha-lactalbumins are spherical and glycosylated folded Ca^{2+} metalloproteins that are composed of 162 amino acids (Table 1.1). Alpha-lactalbumin consists of four disulfide bonds, eight cysteine residues, and high concentrations of tryptophan (Chandan & Kilara, 2010). These proteins do not contain cysteines (sulfhydryl groups), phosphates, or carbohydrates (Fox, 2003). They will display greater heat stability than β-LG due to the lack of cysteine (Varnam & Sutherland, 1994). Chelating agents or low pH will result in decreased protein stability due to the loss of the tightly bound Ca^{2+} (Roginski et al., 2003).

1.1.3.2 β-Lactoglobulin

Beta-lactoglobulins are the main whey proteins in the bovine milk and constitutes ~50% of the total whey protein and ~12% of the total protein in milk (Sawyer, 2003). Beta-lactoglobulins consists of 162 residues per monomer with high concentrations of sulfur. They are comprised of one mole of cysteine and two intramolecular disulfide bonds per monomer of 18 kDa. The cysteine concentrations of β-LG react with intermolecular disulfide bonds of the κ-casein that can affect the overall heat stability of milk (Fox, 2003). Beta-lactoglobulin is more heat-labile than α-LA at

temperatures ≥ 100°C due its one free sulfhydryl group that allows the initiation of autocatalytic disulfide exchange reactions (Varnam & Sutherland, 1994).

1.1.3.3 Whey protein denaturation

During the processing of milk, whey proteins become susceptible to denaturation due to factors such as temperature, pH, and protein concentration (Dissanayake, Ramchandan, Donkor, & Vasiljevic, 2013). In comparison to caseins, whey proteins are readily denatured above 70°C, followed by aggregation and precipitation of the proteins (Wijayanti, Bansal, & Deeth, 2014). When whey proteins undergo conformational changes during thermal processing, their initially folded structure will expose sulfur-containing compounds such as hydrogen sulfide and methanethiol (Wijayanti et al., 2014). Several models have been proposed to describe the steps occurring during the denaturation of whey and the effects of denaturation on the functional properties in milk and milk products. The first proposed mechanism by Mulvihill and Donovan (1987) describes whey protein denaturation and aggregation by two-stages: (1) the unfolding of the initial folded structure of β-LG; and (2) association of the unfolded molecules to irreversibly create protein complexes. Higher temperatures (≥ 70°C) cause β-LG to unfold its native tertiary and secondary structures to expose a free sulfhydryl group that forms small and large aggregates through ionic bonding (Mulvihill & Donovan, 1987). Other models suggest the involvement of noncovalent interactions alongside with ionic interactions to form these small and large aggregates as displayed in Table 1.2. (Wijayanti et al., 2014).

Table 1.2. Theoretical reactions of whey proteins during thermal processing[1]

Theoretical reactions	Additional information	References
(1) $N2 \leftrightarrow 2N \leftrightarrow 2R \leftrightarrow 2D$ (2) $2D \rightarrow A_1 \rightarrow A_2 \rightarrow A_n$; or $2D \rightarrow A_x$	• N2 = Native dimeric β-LG • 2N = Native monomeric β-LG • R = Reversible conformation • D = Denatured monomer • A_1–A_2 = Series of small aggregates • A_n = Large aggregates • A_x = Aggregates in the presence of –SH blocking reagents	Mulvihill & Donovon (1987)
(1) $P_n \leftrightarrow D$ (2) $D + D \leftrightarrow D_2$ (3) $D + D_x \leftrightarrow D_{x+1}$	• P_n = Native protein • D = Denatured protein • D_2 = Aggregate dimer • D_x = Aggregates of x denatured protein monomers	Steventon, Gladden, & Fryer (1991)
(1) $N2 \leftrightarrow \ldots \leftrightarrow 2N \rightarrow N^*$ (2) $N + N_i^* \rightarrow N^*_{i+1}$ (3) $N_i^* + N_j^* \rightarrow N_{i+j}$	• N2 = Native dimeric β-LG • 2N = Native monomeric β-LG • N* = Reactive monomeric β-LG monomer • N^*_{i+1} = Nonnative multimers β-LG (dimer when i = 1) • N_i^*, N_j^* = Reactive multimers • N_{i+j} = Polymer	Roefs & De Kruif (1994)
(1) β-N ↔B* (2) B or $B^* + B_x^* \rightarrow B_{x+1}^* \rightarrow \ldots \rightarrow B_m^*$	• β-N = Native β-LG • B* = Unfolded state of the β-LG • B_{i+j} = Polymeric B_x^*, B_{x+1}^* and B_m^* = β-LG aggregates consisting of x, (x + 1) and m monomeric units, respectively ($x \geq 1$, $m > 2$)	Verheul, Roefs, & de Kruif (1998)
(1) $\beta\text{-N} \leftrightarrow \beta\text{-U}$ $\beta\text{-U} \leftrightarrow \beta\text{-U}^{SH}$ **or** $(\beta\text{-A})_x \rightarrow \beta\text{-U} + \beta\text{-U}^{SH}$ **or** $(\beta\text{-A})_x \rightarrow (\beta\text{-A})_{s\text{-}s} \leftrightarrow (\beta\text{-A})^*_{s\text{-}s}$ (2) $\alpha\text{-N} \leftrightarrow \alpha\text{-U}$ $\alpha\text{-U} + \beta\text{-U}^{S\text{-}H}$ **or** $(\beta\text{-A})_x \rightarrow (\alpha/\beta\text{-A})_{s\text{-}s} \rightarrow (\alpha/\beta\text{-A})^*_{s\text{-}s}$	• β-N = Native β-LG • β-U = Partially unfolded β-LG • $\beta\text{-U}^{SH}$ = Partially unfolded β-LG with exposed -SH group • $(\beta\text{-A})_x$ = Hydrophobically associated aggregates of β-Lg • $(\beta\text{-A})_{s\text{-}s}$ and $(\beta\text{-A})^*_{s\text{-}s}$ = Disulfide-linked aggregates of β-LG • α-N = Native α-LA α-U = Partially unfolded α-La A = aggregates $(\alpha/\beta\text{-A})_x$ = Hydrophobic aggregates of α-LA and β-LG $(\alpha/\beta\text{-A})_{s\text{-}s}$ and $(\alpha/\beta\text{-A})^*_{s\text{-}s}$ = disulfide linked forms of α-LA and β-LG aggregates.	Oldfield, Singh, & Taylor (1998)

[1]Adapted from Wijayanti, Bansal, & Hilton (2014)

In order to form aggregates, the high heat stability (≥ 95°C) of α-LA requires the presence of β-LG. The gelling properties of α-LA will be weak or absent due to its poor aggregation properties (Wijayanti et al., 2014). Bovine BSA denaturation is responsible for the majority of gelling properties and gelation will occur following the unfolding and aggregation of BSA (Hegg, 1982; Wijayant et al., 2014). When heated to ≥ 64°C, non-native BSA will form due to the modification and increase of β-sheet structure (Clark, Saunderson, & Suggett, 1981).

During the heating of milk (75 to 100°C), denatured whey protein (**DWP**) will associate with casein micelles through disulfide and hydrophobic reactions between the free sulfhydryl groups of the β-LG and the κ-caseins (Anema & Li, 2003). The thermally DWP will interact with κ-caseins on the surface of casein micelle during the initial stages of heating (Wijayant et al., 2014). Upon heating, casein micelles increase in size (~30 to 35 nm) due to an increased concentration of associated DWP (Anema & Li, 2003).

The process of heating milk can affect the amount of interactions between the DWP and the casein micelles. In slow heating conditions, (laboratory water bath or an indirect heat-exchanger) the denaturation of whey proteins, specifically β-LG, require longer amounts of processing time, leading to the formations of smaller aggregates and active monomers (Oldfield, Singh, & Taylor, 1998). Thus, about 80% of the denatured β-LG will be associated with casein micelles at 85 to 90°C for 30 min within thermally treated skim milk (Anema & Li, 2003; Smits & Van Brouwershaven, 1980). In comparison, rapid heating methods (direct heating methods) will denature whey proteins over a shorter amount of time and lead to the development of larger aggregates (Oldfield et al., 1998). Under rapid heating (120°C for 3 min), about 50% of the denatured β-LG and α-LA will associate with the casein micelles and the assumption that

remaining denatured whey proteins will remain as whey-whey aggregates (Singh & Creamer, 1991).

1.2 Evaluation of Milk Protein Functionalities

1.2.1 Physicochemical Properties

1.2.1.1 Nitrogen-containing fractions of milk

In 1938, Rowland reported a method that chemically fractionated different groups of nitrogen-containing components of milk. These "fractions" were then analyzed by the Kjeldahl method to obtain the nitrogen content and the different types of protein components could be estimated in each of the milk fractions. This methodology has been used by dairy scientists ever since to understand the impacts of heat and other stressors on protein associations and disassociations (Sodini, Montella, & Tong, 2005). Following Rowland's (1938) fractionation process, three fractions can be assessed directly for nitrogen content and then the protein content is obtained by multiplying the nitrogen content by the multiplication factor of 6.38 (Rowland, 1938). The three main nitrogen-containing fractions obtained within Rowland's method include: (1) total nitrogen (**TN**); (2) non-casein nitrogen (**NCN**); and (3) non-protein nitrogen (**NPN**).

Moreover, the three fractions contain:

(1) Total nitrogen (**TN**); which represents the total organic nitrogen;

(2) Non-casein nitrogen (**NCN**); which represents nitrogen-containing compounds that precipitate with an acid at 4.6 such as casein, and

(3) Non-protein nitrogen (**NPN**); which represents urea, creatinine, creatine, and other amino acids

Thus, to calculate true protein the NPN content is subtracted from TN and then multiplied by 6.38 (IDF, 2002; Sodini et al., 2005).

Rowland's methodology has been modified over the years, but the assessment still is applied in the 21st century (Sodini et al., 2005). Research conducted by Sodini et al. (2005) reported

on whey protein denaturation using this fractionation system and comparing the difference in fractions as a function of a heat treatment. They reported the amount of proteins soluble at pH 4.6 or soluble proteins (**SP**), from mixes before and after processing with the following formula:

$$\text{Protein denaturation} = \frac{SP_{\text{non-processed}} - SP_{\text{processed}}}{SP_{\text{non-processed}}} \text{ x } 100\ \%$$

More recently, gel electrophoresis has been used as an analytical procedure to qualify protein fractions following the methods proposed by Rowland (1938).

1.2.1.2 SDS-PAGE

The visual analysis of individual denatured proteins is assessed through sodium dodecyl sulfate (**SDS**) polyacrylamide gel electrophoresis (**PAGE**). Electrophoresis uses differences in electric charge, isoelectric point, hydrophobicity and mass ratio to separate proteins into bands after being fixed in an acid or alcohol precipitate, and then stained. These well-separated bands can be analytically assessed through quantitative integration measurements based upon the densitometry or molecular weight (**MW**) of the individual bands (Grappin & Ribadeau-Dumas, 1992). With the utilization of SDS-PAGE, milk proteins bind to the SDS detergent through hydrophobic interactions through a theoretical ratio of 1.4 g SDS g^{-1}. A constant charge-to-mass ratio allows for protein migration due to decreasing mass of the individual milk protein (Grappin & Ribadeau-Dumas, 1992).

With PAGE, milk proteins will separate according to their charge-to-mass ratio in alkaline conditions. Caseins are better separated in the presence of a reducing agent (2-mercaptoethanol), which dissociates the disulfide bonds between the α_{s2}-casein and κ-casein prior to their separation through the gel (Grappin & Ribadeau-Dumas, 1992; Jovanovic et al., 2007). The elution order in terms of increasing mobility is blood serum albumin (**BSA**), κ-casein and γ-casein, β-casein, β-LG, two bands of α_{s2}-casein, α_{s1}-casein, and α-LA. Whey proteins will appear diffused in

comparison to caseins that can affect quantification due to possible denaturation (Grappin & Ribadeau-Dumas, 1992; Jovanovic et al., 2007). Through SDS-PAGE analysis, the level of whey protein denaturation of the individual whey proteins can be assessed. Patel, Anema, Holroyd, Singh, & Creamer (2007) reported the SDS-PAGE pattern of thermally treated milk samples will indicate whey protein denaturation and aggregation formation.

Jovanovic et al. (2007) investigated the impact of heat processing (75, 85, and 90°C for 20 min) and demineralized whey fortification on reconstituted SMP solubility, soluble protein composition, and chemical interactions between proteins. The SP composition was determined with SDS-PAGE and densitometric analysis. Researchers found that lower thermal processing (75 to 85°C) induced greater, molecular weight complex formations and aggregates (β-LG, α-LA, and κ-casein) (14,400, 20,100, and 30,000 MW, respectively) while higher temperatures (90°C) increased aggregate degradation and solubility (Jovanovic et al., 2007).

1.2.1.3 Dynamic light scattering particle analyzer

The particle size and its distribution has the ability to affect rheological properties, reactivity, and surface area in milk systems (Beliciu & Moraru, 2009). The quantification of particle size and its distribution of food colloidal systems help identify the ability of a protein to aggregate or dissociate from one another. Dynamic light scattering (**DLS**) utilizes electromagnetic radiation of wavelength to correlate the size of particles in suspension with the average concentration of scattered light. With DLS, the quantification of apparent particle diameter (***d***) is measured by the translational diffusion coefficient (***D***) with the Stokes-Einstein equation (Belliciu & Moraru, 2009):

$$D = \frac{k_B T}{3\pi\eta(t)d'}$$

Where, k_B = the Boltzmann constant (1.38×10^{-23} m^2 kg $s^{-2}K^{-1}$)

T = the temperature (K)

$\eta(t)$ = viscosity (Pa·s)

t = time (s)

Direct light scattering was used to study heat-induced aggregation of milk proteins (Elofsson, Dejmek, and Paulsson, 1996; Horne, 1987). Dalgleish, Pouliot, and Paquin (1987) used particle sizes (90° angle at wavelengths of 360, 450, and 600 nm) to find that extensive heating (130°C for 20 to 60 min) increased casein micelles size (20%) due to aggregation of κ-casein and serum proteins. When using DLS, solutions were diluted to low concentrations (10^{-3} μg/mL) in order to prevent secondary scattering (Beliciu & Moraru, 2009). Beliciu and Moraru (2009) found that different solvents (simulated milk ultrafiltrate, ultrafiltered water, and permeate obtained by ultrafiltration of skim milk) and temperatures (6, 20, and 50°C) significantly affected the size distribution of casein micelles using DLS particle analyzer at a fixed 90° angle and a wavelength of 658 nm. Researchers found that a water solvent (ultrafiltrate water) at 6 and 50°C caused a significant dissociation of casein micelles (20%) due to low ionic strengths; thus, causing inaccurate readings (Beliciu & Moraru, 2009). Anema and Li (2003) used a calcium-imidazole buffer as solvent to measure the mean size diameter of casein due to better storage stability and ease of preparation.

1.2.2 Physical Properties

1.2.2.1 Water holding capacity

The water holding capacity (**WHC**) of foods is expressed as the ability of a food structure to prevent water from being released from the three-dimensional structure of the protein matrix (Zayas, 1997; Sodini et al., 2005). The binding of water is dependent upon the conformation and

composition of the food protein molecules. The interactions between the hydrophilic groups of protein side chains and water occurs due to hydrogen bonding. The capability of proteins to retain water is dependent on the type and concentration of polar groups in the protein polypeptide chain of the food protein (Zayas, 1997). The most common method for assessing WHC is through the application of external forces such as centrifugation. A protein-water mixture will be centrifuged in an appropriate tube and the amount of liquid released or the protein with remaining liquid will be weighed and the supernatant will be discarded (Kneifel & Seiler, 1993).

With milk proteins, intact casein micelles are capable of binding large concentrations of water due to the CCP and the hydrophilic nature of κ-casein within the submicelles (Kneifel & Seiler, 1993; Zayas, 1997). Whey proteins will display varying degrees of water binding depending on protein denaturation, aggregation, and interactions between other proteins. Thermal processing causes the unfolding of whey, which in turn increases water binding capabilities (Kneifel & Seiler, 1993).

1.2.2.2 Syneresis

Spontaneous whey separation, or syneresis, is defined as the shrinkage of a gel and occurs concurrently with expulsion of liquid and considered to be the result of protein interactions due to temperature, pH, and ionic strength rather than a physical removal of water (Lucey, Munro, & Singh, 1998). The rate of syneresis will increase with increased temperatures (Lucey et al., 1998).

Lucey et al. (1998) studied the effects of thermal processing and gelation temperature on whey separation in acid set gels using three different methods: a volumetric flask, a petri dish test, and a low speed centrifugation test. Low speed centrifugation is a separation method where the components of a sample are separated based upon their density at low centrifugal force (< 5000 rpm) (Lucey et al., 1998). Researchers found that whey separation in acid set gels was better

characterized in volumetric flasks compared to petri dishes or low speed centrifugation tests. Additionally, whey separation significantly increased with increasing heat treatment (90°C for 30 min) and gelation temperature (40 °C) (Lucey et al., 1998).

Heyman, Depypere, Delbaeare, and Dewettinick (2010) applied centrifugal force (15 min at 6000 x g at 20°C) to white sauces to monitor stability and quantify the degree of emulsion destabilization. Within sauces, centrifugation forces phase separation and is used to quantify the degree of emulsion destabilization and their stability. Heyman et al. (2010) investigated the effects of non-starch hydrocolloids (guar gum, xanthan gum, and carboxymethylcellulose) in the physicochemical properties and stability of white sauces and established that all non-starch hydrocolloids additions resulted in reduced syneresis.

1.2.2.3 Color

Color is defined as "the sensation that is experienced by an individual when radiant energy within the visible spectrum (380 to 770 nm) falls upon the retina of the eye" (Wrolstad & Smith, 2010). In food research, color-ordering systems and color spaces were developed to assess color parameters for food products. Within the food industry, color is important for food acceptance amongst consumers and measuring functional influence as seen with non-enzymatic and enzymatic reactions. By creating quality control color parameters for food products, food manufacturers can create products that appeal to consumers and influences purchase behavior. Color is one of the first attributes observed by consumers and can positively or negatively impacts their desire to consume a certain food product (Wrolstad & Smith, 2010)

One method to measure color attributes within food products is with a colorimeter. A colorimeter measures color dimensions deviated from a standard. Color differences are calculated by subtracting L^*, a^*, and b^* values for the sample from standard. L^* measures how much lighter

or darker the sample is from standard, *a** measures how much more "red" or "green" the samples is from the standard, and *b** measures how much more "blue" or "yellow" the sample is from the standard (Wrolstad & Smith, 2010). A total color difference (ΔE*) calculates the color difference from a designated standard with the following standard (Wrolstad & Smith, 2014):

$$\Delta E^{*}=[(\Delta L)^{2}+ (\Delta b)^{2}+(\Delta a)^{2}]^{1/2}$$

Chen, Michael, Phebus, Thippareddi, Subbiah, Birla, and Schmidt (2013) monitored the color effects of RFDH-treatments (27.12 MHz; 75 to 90°C for 5 to 125 min) on LH- and HH-NFDM. Researchers found that RFDH-treated LH-NFDM samples (75°C for 25 min, 85°C for 10 min, and 90°C for 0 min) significantly darker and yellower color changes suggesting Maillard reactions initiated during increased RFDH-treatments (Chen et al., 2013).

The incorporation of dairy ingredients within baked goods, such as bread, causes increased browning reactions of crust color due to the Maillard reactions between milk proteins and reducing sugars. Kenny, Wehrle, Stanton, and Arendt (2000) combined dairy ingredients ((whole milk powder (**WMP**), SMP, WPC, sodium caseinate (**SC**), and acid casein (**CH**)) within wheat bread and determined WPC incorporation produced bread with darker crust color (30%) due to possible higher concentrations of the reactive amino acid, lysine, in whey proteins. In another study, Madenci and Bilgiçli (2014) studied the influence of WPC on buttermilk powders (**BP**) on bread quality and color effect. Researchers found that WPC produced the darker bread crumb surfaces (25%) and higher ΔE* values due to the possible Maillard reactions between reducing sugars and proteins (Madenci & Bilgiçli, 2014).

Levin, Burrington, and Hartel (2016) studied the functionality and color effects of whey protein phospholipid concentrate (**WPPC**) and delactosed permeate (**DLP**), produced from the processing of cheese whey, within food systems (caramel, ice cream, and cake). The WPPC is the

co-product of whey protein isolate produced from the microfiltration of WPC, which separates native whey proteins from DWPs, fat, phospholipids, and lactose. Researchers replaced sweetened condensed skim with WPPC and DLP blends to produce caramels and found that caramels made with whey powders resulted in lighter final products when reducing sugars react with free amino groups of proteins interacted due to greater amounts of lactose in whey powders causing increased Maillard reactions (Levin et al., 2016).

1.2.2.4 Volume

The physical attributes of foods, such as size and shape, can affect the types of tests conducted to determine the volume and density of a food product (Sahin & Sumnu, 2006). These different types of volume measurements measure the density of foods. Volume and density can be in different forms such as solid, liquid, apparent, and bulk volume/density (Sahin & Sumnu, 2006).

The size and shape of a food is necessary to evaluate in cases such as fluid flow and heat to mass transfer calculations. Size is determined by using a projected area method. This method requires: (1) major diameter, (2) intermediate diameter, and (3) minor diameter. These measurements directly correlate to the length, width, and thickness of a food item (Sahin & Sumnu, 2006).

The volume of food material is defined as the amount of a three-dimensional space occupied by an object in units that are the cubes of length units (m^3) (Rahman, 2005). Volume is calculated from characteristic dimensions with regular shaped items, determined experimentally by liquid, gas, or solid displacement methods, or measured using image processing methods. In liquid displacement, food materials are placed in a calibrated pycnometer and the difference between initial and final concentration gives the volume of the sample. Solid displacement uses

rapeseeds to determine the volume by calculating the final volume of the rapeseeds with the food item and dividing by the density of the rapeseeds (Sahin & Sumnu, 2006).

Erdogdu-Arnoczky, Czuchajowska, and Pomeranz (1996) studied the effects of whey powder, dialyzed acid casein, and NFDM on physical dough properties of non-yeasted dough, yeasted dough, and fixed-style bread in an automatic bread machine. The samples were subjected to thermal processing at 80 or 95°C for 10 min. Dairy ingredients not thermally processed or dialyzed reduced overall bread volume (80 to 26%), whereas thermally processed and dialyzed dairy products had less overall dough volume loss (14 to 5.8%). Breads produced from increased concentrations of casein possessed a volume-depressing factor suggesting that different milk proteins interact with gluten in different manners and will determine the amount and size of gas cells produced in bread dough (Erdogdu-Arnoczky et al., 1996). Thermally processing dairy ingredients, such as whey protein, can cause calcium phosphate to precipitate with DWP that will improve baking characteristics due to the reduction in the concentration of ionic calcium from whey (Erdogdu-Arnoczky et al., 1996).

1.2.2.5 Water activity

Within the food industry, understanding the effects of water activity on food systems are important for food quality, stability, and for consumer safety. By controlling the amount of water activity in food products, researchers can predict potential sources of spoilage, maintain chemical stability of food over time, decrease non-enzymatic browning reactions and lipid oxidation reactions, extend the anticipated activity of enzymes and vitamins in food, and enhance physical properties of foods such as texture and shelf life (Fontana, 2000).

Water activity refers to the ratio between the vapor pressure of the food product in an undisturbed balance with surrounding air media and the vapor pressure of distilled water under identical conditions (Fontana, 2000; Fontana, Schmidt, & Labuza, 2008). The assessment of water activity depends on the total amount of water present in individual food systems. The water activity is calculated with the following ratio (Fontana, 2000):

$$a_w = \frac{p}{p_o}$$

where, a_w is derived from the laws of equilibrium thermodynamics,
p= vapor pressure of the food sample
p_o= vapor pressure of pure water at the same temperature and external pressure

Additionally, water activity is assessed as the Equilibrium Relative Humidity (**ERH**). In ERH systems, steady states of sample concentrations, system temperatures, and external pressures must be at equilibrium to drastically influence partial vapor pressure of water. The water activity is calculated by dividing the ERH by 100 (Fontana et al., 2008).

During caramel production, water activity is most affected by corn syrup to sucrose ratio and moisture content (Levin et al., 2016). Levin et al. (2016) found that WPPC and DLP displayed no significant difference on the water activity in caramels due to similar formulations; but found

the slight differences observed were attributed to possible differences in mineral contents of dairy ingredients.

1.2.2.5 Texture properties

Texture is considered the response of tactile senses to physical stimuli that results from the contact between some part of the body and the food (Bourne, 2002). The most common tactile sense, touch, is the primary method for sensing and measuring texture. In addition to tactile sense, kinesthetics, sights, and sounds are used with the assessment of texture of food products. The evaluation of texture is important for consumer attitudes with food products in the food industry. By understanding the sensory properties of foods, such as texture, food companies can develop and maintain food products with acceptable sensory profiles for consumers. Texture measurements can be conducted by using a texture analyzer that mimics the chew behavior of mouth's biting action (Bourne, 2002).

During bread production, dairy ingredients, such as WMP and SMP, impart important texture properties such as crumb firmness. Jooyandeh (2009) assessed the effect of precipitated whey protein (**PWP**) (heated at 85°C for 15 min) on the physical properties, such as firmness, of lavash flat bread. The heat treatment of PWP caused desired denaturation and coagulation for proper precipitation of whey proteins during the fermentation process. Results indicated the incorporation of PWP increased softness of bread samples due to increased concentrations of available calcium from the denatured whey protein increasing water-binding capabilities (Jooyandeh, 2008).

In caramel, firmness and stickiness, are central attributes for consumer appeal (Minifie, 2012). Stickiness, or adhesiveness, is the amount of work required to overcome the forces existing between the surfaces of a food and another material during contact (Szczesniak, 2002). The texture

of caramel can range depending upon ingredients incorporating within formulation and production (Steiner, Foegeding, & Drake, 2003). Mendenhall and Hartel (2016) studied the effects milk protein concentrations (0, 1, 2.5, 5, and 7%), caramel cooking rates (0.79°C/min, 1.21°C/min, and 2.38°C /min) and forewarming hold time (88°C water bath and held for 5, 10, and 20 min) on caramel physical properties such as hardness and stickiness. Researchers found that increased solids contents (90%) increased hardness and stickiness due to the possible formation of larger irreversible aggregates formed from greater concentrations of cross-linkages between proteins. Forewarming caramel ingredients possibly reduced aggregations in higher protein formulations due to stabilized milk proteins inhibiting aggregation allowing increased associations of whey proteins with casein micelles prior to severe heating used during caramel production (Mendenhall & Hartel, 2016).

1.3 Dairy Systems: Nonfat Dry Milk

Nonfat dry milk is produced by the separation of fat from liquid milk, followed by subsequent drying to remove water in order to obtain ≤ 5% by weight of moisture on a dry basis (**db**) and ≤ 1.5% by weight of fat (db) (21 CFR 131.125; FDA, 2018). The typical compositional ranges (%) for NFDM are displayed in Table 1.3. Nonfat dry milk can be classified into two different grades or requirements: U.S. Extra Grade (spray-drying) or U.S. Standard Grade (spray-drying). Spray-drying or roller-drying are the two main manufacturing methods to produce NFDM (Carić, 2002).

During spray-drying, evaporated milk is dispersed as fine droplets and heated to 150 to 300°C in a drying chamber at a velocity of 50 m s^{-1} (Carić, 2002; Kelly, O'Connell, & Fox, 2003). Alternatively, roller-drying removes water from pre-concentrated milk on the surface of a hot drum that will continuously be removed with a scraper (Carić, 1994). Extra Grade (spray-drying) will not exhibit any lumps within the powder. Once reconstituted, Extra Grade (spray-drying) NFDM will exhibit sensory characteristics such as cooked, feed, and fat flavor attributes. Extra Grade (roller-drying) NFDM will display scorched flavor and undesirable flavor attributes in reconstituted NFDM. In comparison to Extra Grade NFDM, Standard Grade NFDM will present undesirable flavor attributes such as light-oxidized, staleness, and bitterness (Chandan, 1997).

Table 1.3. Nonfat dry milk compositional ranges (%)[1]

Constituent	Nonfat dry milk (%)
Protein	34.0 to 37.0
Lactose	49.5 to 52.0
Fat	0.6 to 1.25
Ash	8.2 to 8.6
Moisture	3.0 to 4.0

[1]Adapted from ADPI (2009).

As seen in Figure 1.1, the production of milk powders begins with the clarification of raw milk by centrifugal separators and cooled to 4ºC by plate heat exhangers. After clarification, raw milk is standardized, which is used to adjust the ratio of milk fat to total solids (**TS**) to a recommeneded 34-37% total protein (ADPI, 2002). Following standardization, raw milk undergoes pre-thermal heat treatment for a longer holding time (≤ 71.1ºC for 2 min) than pasteurization for three reasons: (1) eliminate potential pathogenic microorganisms; (2) inactivate enzymes that could cause lipolysis during storage and cause undesirable flavor characteristics; and (3) initiate sulfhydryl (**SH**) groups exposure in ß-LG that promotes resistance of oxidation of powder during storage (Carić, 1994).

Evaporation is performed to increase the concentration of TS. The difference in the degree of concentration will be dependent on the type of drying technique performed: roller-drying (30 to 35% TS) and spray-drying (40 to 50% TS) (Varnam & Sutherland, 1994). Homogenization will be performed at a temperature of 55-85ºC and a pressure between 5 to 15 MPa in order to reduce free fat content and prevent poor powder solubility and susceptibility to light oxidation (Varnam & Sutherland, 1994; Bylund, 2003). During spray-drying, evaporated milk is dispered as fine droplets into a hot air stream ranging from 70 to 135ºC in the drying chamber, causing quick evaporation of the droplets, and milk powder will be collected from the bottom of the chamber (Kelly et al., 2003). Depending on the intensity of the heat treatment, milk powder is categorized into time/temperature combinations based upon the total temperature exposure prior to and during the evaporation and spray drying process (Bylund, 2003).

The manufacturing of NFDM and skim milk powder (**SMP**) differs from full-fat powder with lower fat standardizations (0.05 to 0.10%), no required homogenization, and intense heat treatments (Carić, 2002). Although similar, NFDM and SMP are defined by separate regulations

and authorites: U.S. Food and Drug Administration and CODEX Alimentarius, respectively. Skim milk powder will have a required minimum milk protein content of 34%, whereas NFDM will not have a standardized protein level, but will have a 34 to 37% total protein recommendation (ADPI, 2002).

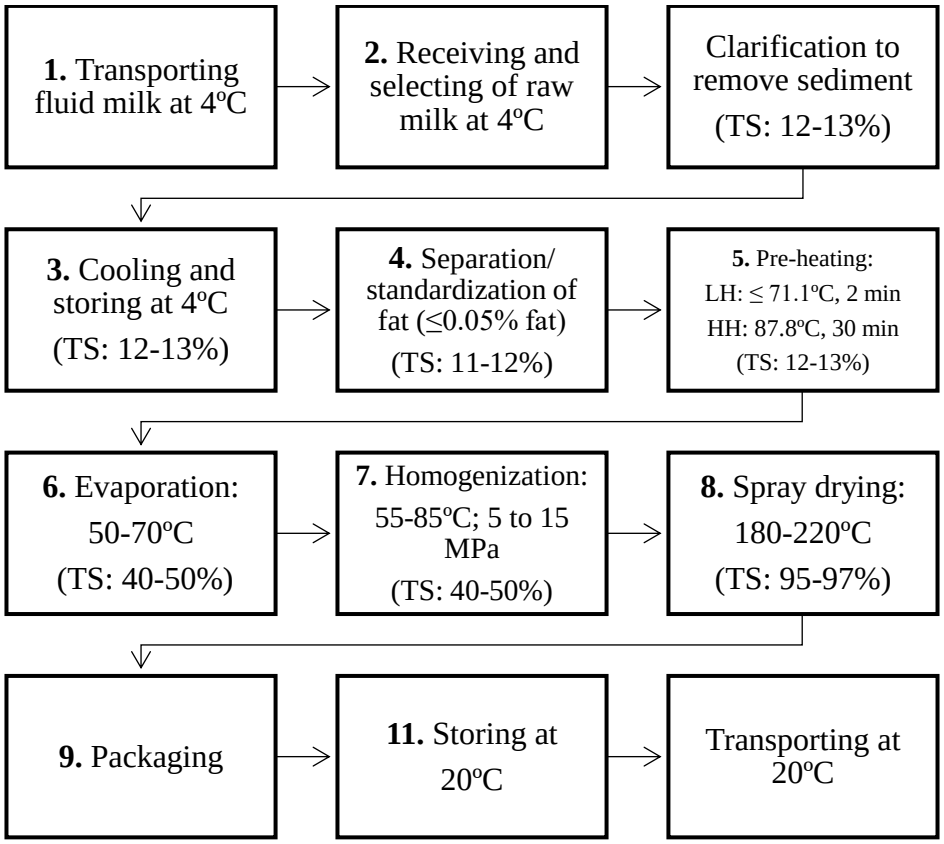

Figure 1.1. Process flow chart for nonfat dry milk (NFDM) production. Adapted from Carić, 1994.

1.3.1 Types of Nonfat Dry Milk

The concentration of protein denaturation or the whey protein nitrogen index (**WPNI**) present in NFDM solids will be defined as milligrams of WPN per gram of powder (mg g^{-1}) powder (Table 1.4) (Carić, 1994). The selection of NFDM within a variety of food systems is dependent upon the functionality and the physicochemical changes occurring during reconstitution (Parris, White, & Farrell, 1990). During the production of SMP and NFDM, varietal classification will be

dependent on the pre-heating treatment and time applied during manufacture and the amount of native WPN in the final product. The three main categories are high-heat (**HH**) (87.8ºC for 30 min; WPNI: ≤ 1.5 mg g), medium-heat (**MH**) (71.1 to 79.4ºC for 20 min; WPNI: 1.51-5.99 mg g), and low-heat (**LH**) (≤ 71.1ºC for 2 min; WPNI: ≥ 6.0 mg g) as seen in Table 1.4 (Parris et al., 1990; ADPI, 2009).

Table 1.4. Heat treatment classifications of nonfat dry milk[1]

Class	Pre-heating treatment of skim milk	WPNI[2] (mg g^{-1})	Functional properties[3]	Food applications[3]
Low heat	≤ 71.1ºC for 2 min	≥ 6.0	Solubility, lack of cooked flavoring	Recombined milk, cheese, and yogurt making
Medium heat	71.1 to 79.4ºC for 20 min	1.51 to 5.99	Emulsification, foaming, water absorption, viscosity, color, flavor	Ice cream, confectionary
High heat	87.8ºC for 30 min	≤ 1.5	Heat stability, gelation, water absorption	Recombined evaporated milk, bakery

[1]Adapted from ADPI (2009).
[2]Whey protein nitrogen index.
[3]Adapted from Kelly, O'Connell, & Fox (2003).

1.3.2 Nonfat Dry Milk in Food Systems

The different temperature and time combinations during the processing of NFDM will cause altered functional and physicochemical properties within food systems. Low heat-NFDM will be mostly incorporated within dairy products and beverages such as yogurt, cottage cheese, and other cultured skim milk products due to an increased solubility and a decreased cooked flavoring in the final product (Drake, Karagul‐Yuceer, Cadwallader, Civille, & Tong, 2003).

For MH, the most commonly used food applications will include ice cream, confectionaries, and meat products due to functional properties imparting increased emulsification, foaming, and some water absorption functional properties. With HH, improved functional properties include increased water-holding capacity (**WHC**), emulsion stability, and gelation

characteristics imperative in baked goods, confectionaries, meat products, soups, and sauces (Parris et al., 1990).

1.3.3 Trades and Exports of Milk Powders

For global and national dairy chain trade export systems, competitiveness impacts the economic sustainability for many countries' dairy sectors (Bojnec & Fertő, 2014). Based upon the USDA Dairy World Markets and Trade Reports (2019), NFDM and SMP produced in the U.S. will hold a competitive position to the European Union (**EU**) and New Zealand to market in places such as Mexico and Southeast Asia. Although U.S. exports of SMP increased at a yearly pace of 7% from 2014 to 2018, the current trade challenges are expected to cause a 4% decrease in 2019 export shipments. In 2019, the export of NFDM and SMP is expected to decrease by 1% to 686,000 tons compared to 2018 (USDA, 2019). However, according to the U.S. Dairy Export Council (**USDEC**) (2019), SMP and NFDM U.S. exports have decreased approximately 9% from 2018 due to trade disputes and the 35% prohibitive import tariff in China. United States exporters have seen export losses in China, Pakistan, and Malaysia. Due to these trade restrictions, the export value for NFDM and SMP in the U.S. has increased by 6% in 2019 compared to 2018 (USDEC, 2019).

For EU SMP exports, the 2019 export forecast has increased by 15% to 950,000 tons although SMP production is only expected to increase by 1%. Intervention stocks and decreased U.S. shipments are a major contributor to the increased exports of SMP in the EU and New Zealand (USDA, 2019).

Based upon the U.S. Export Data from the U.S. Dairy Export Council in 2018 (2019), the current top three leading import markets of NFDM and SMP, based upon an annual trend with a 12-month moving average, includes the countries of Mexico (28,862 metric tons), China (24,876

metric tons), and Indonesia (20,925 metric tons). The average U.S. export prices of NFDM and SMP in 2019 is predicted to increase by 33.2% ($2,298/metric ton) compared with 2018 export prices (USDEC, 2019).

1.4 Food Systems Incorporating Dairy Systems

1.4.1 Bread

Dairy ingredients, such as NFDM, will be incorporated into baked goods to improve the nutritional and functional properties of the final product. The nutritional benefits of dairy ingredients provide increased calcium and essential amino acid concentrations (Kenny et al., 2000). The improved functional properties of baked goods formulated with dairy ingredients include significantly increased water absorption, reduced staling, improved crumb texture, and improved crust color. In bread, dairy ingredients can cause decreased bread quality through loaf volume depression and dough slackening (Erdogdu-Arnoczky et al., 1996). The functional properties of dairy ingredients are dependent upon the inherent properties such as milk protein and fat concentrations, processing treatments, and processing temperatures (Kenny et al., 2000).

Bread ingredients will significantly affect the final quality of the baked bread. Bread formulations include flour, yeast, salt, sugar, fat, water, and other additives that are important for bread quality. In bread formulations, additives, such as NFDM, will be incorporated into bread dough to improve gas retention abilities, weaken dough resistance to deformation, and create emulsification properties. By incorporating NFDM, the overall mixing time of bread dough can increase (30 to 60 min) due to the strengthening effect of NFDM in the dough matrix from water competition between the milk proteins and wheat proteins within the bread dough (Chandan, 1997). Erdogdu-Arnoczky et al. (1996) found that bread dough containing NFDM displayed increased water absorption properties (2%) and decreased mixing times (3:00) compared with a control flour dough without dairy ingredient (3:25). However, the mixing time increases (3:15 min) whenever the NFDM is subjected to heating or dialysis (80 or 95°C) compared with untreated NFDM (3:00 min). In most bread dough formulations, a concentration of 6% NFDM is

incorporated depending upon the overall flour weight with that of a bread dough without milk products (Brouilett, Nolte, & McDuffee, 1938). Typically, a HH-NFDM should be used to avoid possible loss of dough gas retention (Cauvain & Young, 2006).

Whey and casein proteins will exhibit different functional properties during the bread-making process. Whey ingredients will exert negative effects on bread quality such as depressed loaf volumes and an increase of crumb firmness. Thermal treatments of the whey proteins could counteract negative functional properties due to protein denaturation (Kenny et al., 2000). Casein and caseinates have shown increased water absorption properties with flour systems (Gallagher, Kenny, & Arendt, 2005).

The basic bread-making procedure will include the mixture and kneading of all bread ingredients to form a dough that will then ferment, or "proof", until baked for a designated temperature and time (Figure 1.2) (Cauvain, 2007). This bread-making procedure, also referred to as the Chorleywood Bread Process (**CBP**), was modified into other bread-making procedures, such the straight-dough method and the sponge-dough method, that are commonly used in North America (Cauvain, 2007). During breadmaking, the bread dough will retain carbon dioxide gas produced from the enzymatic reactions of yeasts and will expand during proofing periods (Cauvain, 2007).

The fermentation process in bread is produced with from following steps: (1) the cell walls of starch particles destructed by cytase; (2) the starch will be converted into maltose by diastase; (3) sucrose + maltose converted into glucose + fructose by the addition of yeast; (4) alcoholic fermentation will occur from zymase in yeast; and (5) hydrolysis of proteins will cause a decrease in pH and an increase in acidity (Chandan, 1997). Milk ingredients, such as NFDM, have been suggested to cause a decrease in bread dough acidity level and an increase in pH (pH 5.94), when

compared with dough made with water with a pH 5.82. Therefore, the decreased acidity levels from an increased amounts of NFDM will cause better stabilized fermentations but increased fermentation times (10 to 30%) compared with bread made without milk solids. Additionally, bread with added NFDM can increase loaf volumes due to a possible increase of stabilized enzymatic interactions (Brouilett et al., 1938).

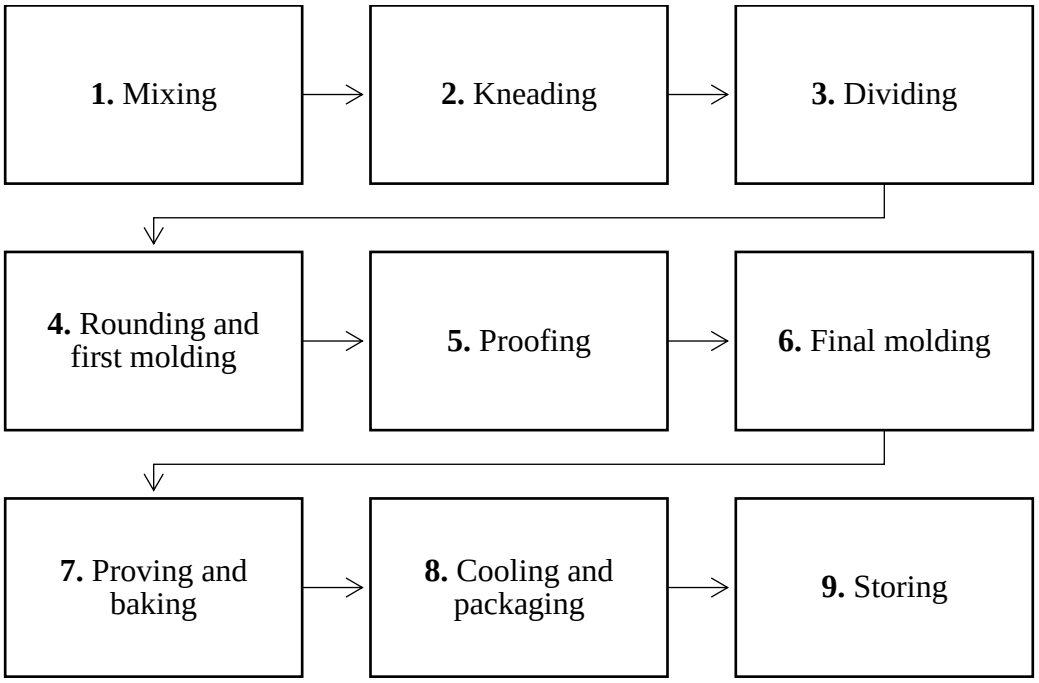

Figure 1.2. Process flow chart for basic bread-making procedure. Adapted from Cauvain, 2007.

1.4.2 Caramel

The presence of dairy within confectionaries will be used to produce specific sensory flavor and texture characteristics that affect consumer liking and acceptance (Minifie, 2012). As seen in Figure 1.3, commercial caramels are produced from a blend of protein solids, fat globules, and a supersaturated solution. The caramel mixture will be thermally processed at temperatures ranging between 120 to 130ºC to produce a browning reaction (i.e. Maillard reaction) between the milk proteins and reducing sugars. Maillard interactions will initiate at a low temperature (40ºC) and

will continue to increase with increasing processing temperatures (Figure 1.3) (Ahmed, Ramaswamy, & Pandey, 2006). During the caramelization process, increased temperatures (125 to 130ºC) will impart greater flavor properties within the finished caramel product due to Maillard reactions (Ahmed et al., 2006).

Typical caramel formulations will include sweetened condensed milk, evaporated milk, or a concentrated milk solution to impart specific milk fat flavors and textures characteristics. Milk powders can replace traditional milk ingredients, but must be properly dispersed prior to caramelizing to prevent graininess or crystallization formations within finished caramel products. During caramel formulation, a reconstituted milk powder solution with a higher moisture content will yield improved texture and sensory properties compared to standard condensed milk incorporations (Minifie, 2012).

Typical commercial caramel formulations will have a range between 2 to 2.5% protein, whereas, a higher protein caramel will have a 4 to 8% protein and considered more desirable for consumers due to the preferred texture properties imparted from the high protein concentration (Mendenhall & Hartel, 2016). The thermal processing of caramels will produce chewier and denser products depending upon the concentration and types of milk proteins used in the formula.

Milk proteins, casein and/or whey, have a significant impact on the texture, flavor, and color of caramels. Caramels produced with greater concentrations of milk proteins in the initial milk dispersion will produce firmer textures in the finished caramel product (Atapattu & Kakuda, 1998). Additionally, the type of milk protein included will affect final flavor and texture. Caramels produced with greater protein concentrations (5.0 to 6.9%) of casein derivatives can yield caramel products with increased hardness, stickiness, and viscosity (Levin et al., 2016).

Caramel graininess is considered an undesirable texture characteristic caused by the coagulation or uncontrolled aggregation of milk proteins within the caramel dispersion (Mendenhall & Hartel, 2016). Mendenhall & Hartel (2016) found that a higher protein content (7%) in caramel may require longer heat treatment times (> 10 min) during the manufacturing process of sweetened condensed milk to prevent graininess. During caramel processing, the DWPs will unfold, aggregate through disulfide bonding, and form a viscoelastic network within the sugar matrix. Caramel mixtures processed with greater initial concentrations of DWPs prior to caramel thermal processing will produce a less grainy finished caramel (Atapattu & Kakuda, 1997; Mendehall & Hartel, 2016).

Additional thermal processing of dairy ingredients (i.e. forewarming) to caramel cooking has shown to cause unwanted hardness, elasticity, and shortening of the caramel texture, which is possibly caused by increased WPD. Additionally, researchers observed that increased forewarming temperatures (88ºC) and holding times (> 10 min) of milk prior to cooking will decrease hardness (~25%) of the caramel products due to the increased concentrations of whey protein denaturation and aggregations (Mendenhall et al., 2016).

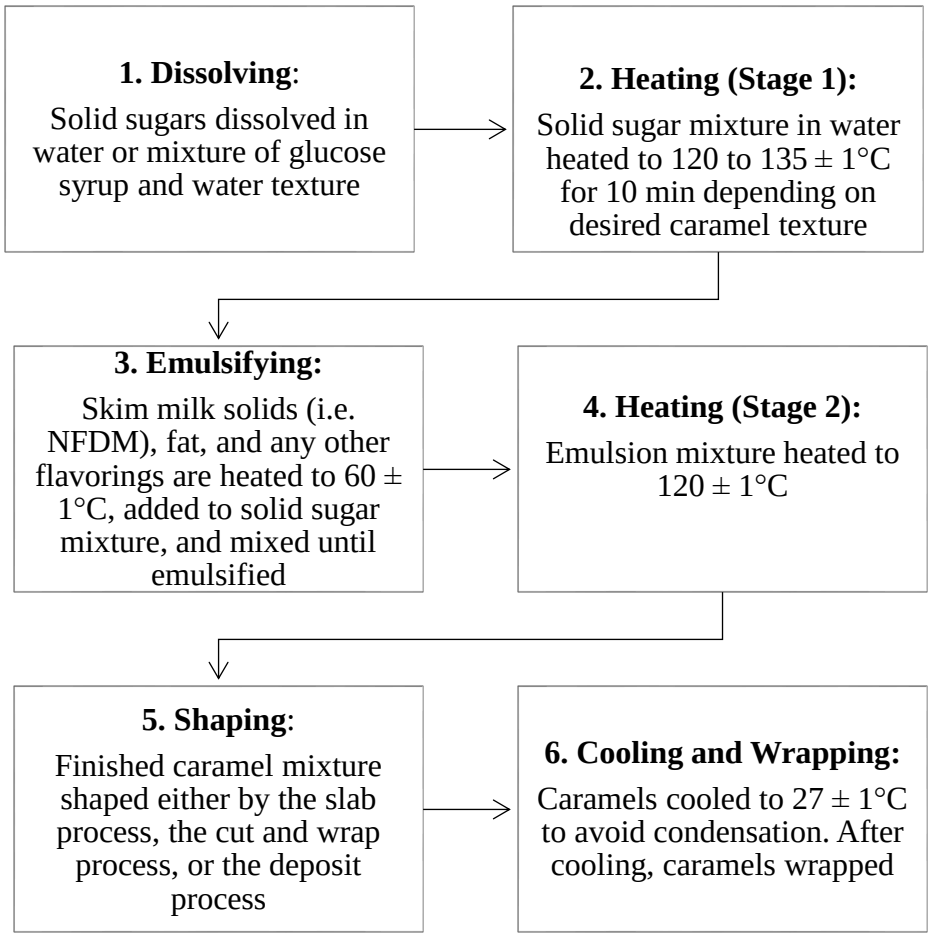

Figure 1.3. Process flow chart for caramel procedure. Adapted from Minifie, 2012.

1.4.3 White Sauce

Milk powders can be incorporated within non-starch hydrocolloids, for use in sauces, to impart protein-stabilized emulsions, gelation reactions, and desirable texture properties for consumers. Due to the expansion of the ready-to-eat (**RTE**) food sector in the consumer market, the majority of prepared food products sold will be complemented with sauces to impart certain flavor and texture characteristics (Hernández-Carrión, Guardeño, Carot, Pérez-Munuera, Quiles, & Hernando, 2011). The most common type of sauce used within RTE foods is béchamel sauce, also referred to as white sauce (Heyman et al. 2010).

A white sauce formulation will typically include milk, oil or butter, flour or starch and salt (Arocas, Sanz, & Fiszman, 2009). During white sauce production as seen in Figure 1.4, critical functional properties are developed during the starch gelatinization process. The starch gelatinization process occurs during the heating process of starch granules in an aqueous medium, such as milk, at a characterized gelatinization temperature. The continuation of starch thermal processing produces irreversible, swollen starch granules that leach amylose and amylopectin (Hernández-Carrión et al., 2011). A maximum paste viscosity is reached at a temperature range of 65 to 95°C and will possibly possess greater swollen, intact starch granules. As temperature increases to 92 to 95°C, the starch granules will dissociate and fragment, which will lead to a decrease of the final viscosity in the final product (Sanz, Tárrega, & Salvador, 2016). Following thermal processing, the reassociation, also known as retrogradation, of solubilized starch polymers and insoluble granular fragment will occur during cooling. Starch retrogradation produces undesirable sensory characteristics such as too firm or viscous sauces (Hernández-Carrión et al., 2011).

Sauce dispersions will incorporate milk ingredients to improve emulsification and facilitate uptake of essential nutrients (Bortnowska, Krudos, Schube, Krawczyńska, Krzemińska, & Mojka, 2016). During white sauce production, the addition of milk proteins, casein and whey, has shown to affect starch interactions (Arocas et al., 2009). The incorporation of certain milk ingredients within a starch solution will cause a decrease in granule swelling, which in turn, prevent gelatinization properties and affect rheological properties (Doublier, Marzin, Visdeloup, & Lefebvre, 1994). In heated sauce dispersions, casein-like ingredients tend not to undergo conformational changes due to greater resistance to heat-induced interactions (Lin, Kelly, O'Mahony, & Guinee, 2017). The protein networks formed between whey and starch dispersions

will demonstrate different observed functional properties than casein additions because of the heat sensitivity of whey (Considine, Noisuwan, Hemar, Wilkinson, Bronlund, & Kasapis, 2011). Whey protein and starch mixtures can produce greater open protein gel networks comprised of large soluble aggregates (Olsson, Langton, & Hermansson, 2002).

White sauce formulations incorporate milk powder depending on milk protein content. Muir, Hunter, and West (1991) assessed texture and viscosity properties between 34 different heat-classified NFDM samples and found that low heat powders produced from ultrafiltration improved viscosity of white sauce. The denaturation of whey protein in milk powder will decrease white sauce viscosity. Jiang, Zhang, Fang, Mujumdar, and Xu (2016) found that dielectric drying methods ((microwave drying (2450 MHz), low frequency microwave drying (915 MHz), and radio frequency (27 MHz)) influenced physicochemical properties in starch-water model systems. Higher dielectric frequency levels promoted an acceleration in drying rates in starch dispersions

that stimulated an increase in starch diameter and inhibition gelatinization (Jiang et al., 2016).

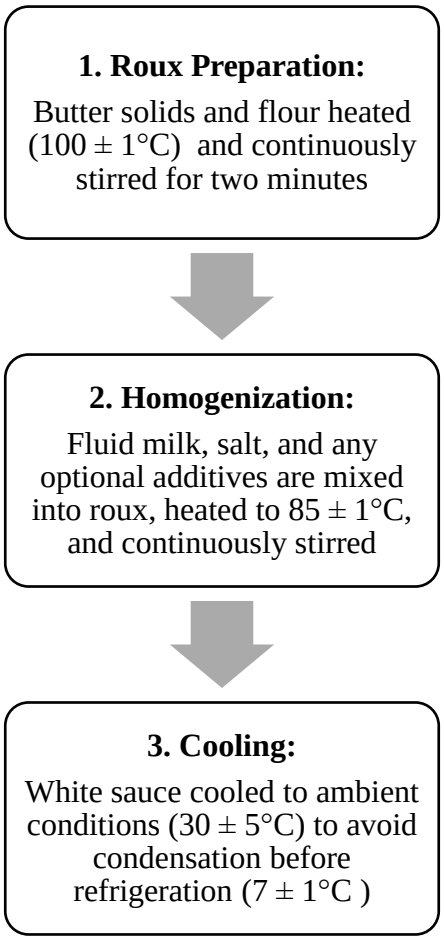

Figure 1.4. Process flow chart for white sauce procedure. Adapted from Heyman et al., 2010.

1.5 Radio Frequency Dielectric Heating

The thermal processing of foods ensures microbiological safety, eliminates or reduces enzymatic activity during storage, and can impart specific sensory characteristics necessary for consumer liking (Piyasena, Dussault, Koutchma, Ramaswamy, & Awuah, 2003). These types of thermal processing include cooking, baking, extruding, pasteurizing, sterilizing, dry heating, and electroheating, among others (Arnoldi, 2001). The process of converting electrical energy to heat, or electroheating, is categorized into two categories: direct (i.e. ohmic heating) or indirect (i.e. microwave (**MW**) or radio frequency (**RF**) heating). Direct heating refers to the immediate transfer of heat to the food material; whereas, indirect heating does not involve the contact of air, gas, or any other heating agent with the food material in order to change the final temperature of the material (De Alwis & Fryer, 1990).

Both MW- and RF-heating produce non-ionizing radiation from low energy (fewer than 10 eV) to ionize most atoms within the material (Piyasena et al., 2003). With MW-heating, magnetrons or klystrons are produced through the emission of microwaves and the transmission of resonant electromagnetic standing waves within the metal cavity holding a target material (Figure 1.4) (Marra, Zhang, & Lyng, 2009). In comparison to MW-heating, RF-heating generates heat through the application of a high voltage alternate electric fields to a target material set between two parallel electrodes (Figure 1.4) (Piyasena et al., 2003). This common RF-heating method, or the parallel plate RF-system, utilizes grounded electrodes that allows the capacitor to store the electric energy being generated when heating the food material between the pair of electrodes (Marra et al., 2009). The RF generator produces electromagnetic (**EM**) energy and will be comprised of a power supply, control circuitry, a hydraulic press, and a parallel plate system to hold food material (Piyasena et al., 2003).

Within food materials, both MW- and RF-heating represent two different types of dielectric heating. Dielectric heating (**DH**) generates heat within food products through dipolar molar friction and oscillations of dissociative ions while undergoing high frequency alternating fields (Boreddy, Thippareddi, Froning, & Subbiah, 2016). Radio frequency dielectric heating (**RFDH**) will heat a food material by forming a dielectric between two capacitor plates that conduct alternating positive and negative charges from a high frequency alternating electric field (Piyasena et al., 2003). Due to the inversely proportional nature of frequency and wavelength, the RF (lower frequency) wavelengths (11.0 m at 27.12 MHz) are longer than MW-heating (higher frequency) wavelengths (0.12 m at 2450 MHz) (Marra et al., 2009)

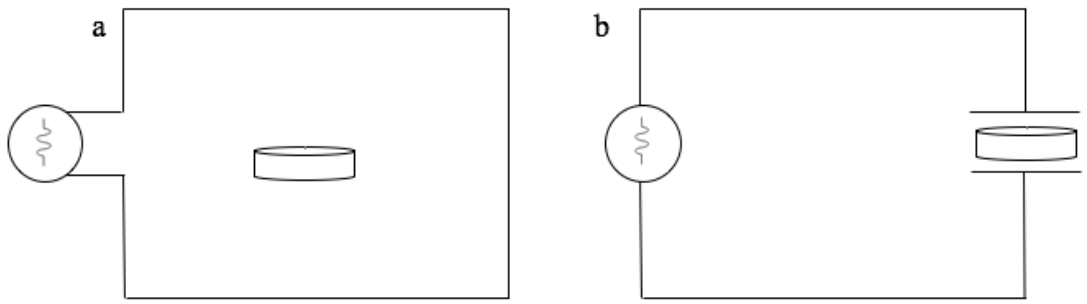

Figure 1.5. Schematic arrangement for (a) microwave heating and (b) radio frequency heating. Adapted from Marra et al. 2009

While MW- and RF-energy are classified as DH methods, RF-energy applies a lower frequency than microwave heat; RF-heat (1 to 300 MHz) vs. MW-heat (300 to 30,000 MHz) (Fu, 2004). The longer wavelengths of the RF spectrum will allow for a deeper penetration into heating food materials when compared with the MW-energy (Piyasena et al., 2003).

During the large-scale processing of low-moisture food products, RF-heating remains advantageous when compared with MW-heating due to greater uniform heating, reduced come-up times, consistent preheating temperatures, and minimized product quality degradation (Boreddy et al., 2016). Although RFDH equipment involves greater initial investment costs, food

manufacturers can purchase RF equipment at higher power levels than MW sources. Due to the higher power levels and lower prices per watt, RFDH equipment demonstrates financial advantages compared with microwave equipment (Fu, 2004; Ştefănoiu, Tănase, Mitelut, & Popa, 2016).

The dielectric properties of food systems are classified based upon the food materials' permeability, permittivity, electrical conductivity, loss tangent, and penetration depth during dielectric heating (Marra et al., 2009). The permeability represents the amount of free space not contributing to the heating process, while the permittivity indicates the dielectric constant (**ε'**) and the loss factor (**ε"**) (Piyasena et al., 2003). The ε' is a measure of a material's capacity to absorb, transmit, and reflect energy from the electric field, whereas ε" measures the correlation between the amount of energy lost from the electrical field and the amount of energy absorbed then converted to heat within the food material (Piyasena et al., 2003; Marra et al., 2009). The electrical conductivity (**σ**) assesses the ability of a material to conduct electrical currents and will directly correlate to the ionic depolarization of food materials. The loss tangent (**tan δ**) represents the dissipation factor of the food material and the penetration depth (**d_p**) measures the amount of depth in a material (Marra et al., 2009). The d_p can also be defined as the total depth of a material when the energy of a plane wave transmitting perpendicular to the surface has reduced to 1/e (1/2.72) of the surface value (Bengtsson & Risman, 1971; Piyasena et al., 2003).

1.5.1 Functions of Radio Frequency Dielectric Heating in the Food Industry

Radio frequency dielectric heat is a commonly used, conventional heat process applied in numerous food industries such as meat, grain, nut, produce, baked goods, and dairy (Fu, 2004). The first studied application of RFDH in the food industry was the blanching of vegetables by Moyer and Stotz (1945). Over the past decades, a steady increase of RFDH applications within

various food systems have been reported due to increased demands for foods with clean labeled foods with no food safety issues. The introduction of the Food Safety Modernization Act (**FSMA**) encouraging food industries to modernize current technologies to help promote a stronger food safety system in the U. S. that focuses on the prevention of food safety problems (Lau & Subbiah, 2017).

The meat industry can utilize RFDH to improve functional properties and sensory attributes of meat products. Laycock, Piyanasa, and Mittal (2003) compared time/temperature profiles (72°C in a 1.5 kW RF-heater operating at 27.12 MHz (RFDH) versus 151, 130, and 109 min in a controlled laboratory water bath (conventional), respectively) for meat quality (ground beef, comminuted meat, and muscle). The researchers reported that the meat cooked by the RFDH method had reduced cook times (5.83, 13.5, and 13.25 min for ground beef, comminuted meat, and muscle, respectively)). Further, the RFDH-treated meats displayed decreased juice losses and improved color and water holding properties compared with meats cooked through the controlled laboratory water bath system (Laycock et al., 2003; Marra, et al., 2009).

Within the grain, nut, and produce industries, manufacturers will employ RFDH as an alternative method for disinfestation and post-harvest treatments to eliminate the traditional fumigation practices that apply chemical agents such as methyl bromide (Marra et al., 2009). Alfaifi, Wang, Tang, Rasco Sablani, & Jiao (2013) studied the effects of RFDH-treatments (10 to 1800 MHz; 20 to 60°C) with raisins, dates, apricots, figs, and prunes on dielectric properties and two common insects, while relating the results to the RFDH frequency and temperature, as well as, the water content of the fruits. The authors concluded that the dielectric constant and loss factor of all five fruits decreased (~0.5 to 0.7) with increasing frequency (1800 MHz), but increased with increasing temperature at every frequency (Alfalifi et al., 2013). Additionally, the RFDH-

treatments displayed greater insect disinfection properties through an increased dielectric constant (80.2 to 99.4 at 27 MHz when temperature increased from 20 to 60°C) and an increased dielectric loss factor (307.8 to 562.7 at 27 MHz when temperature increased from 20 to 60°C) between insects. The treated dry fruit suggesting a high degree of preferential heating of insects compared to MW-heating (Alfaifi et al., 2013).

Food industries that manufacture dry food materials such as herbs, spices, bakery products, and infant formulas, will use RFDH-treatments as an alternative for cooking and sterilizing. Wang, Wig, Tang, & Hallberg (2003) conducted a pilot-scale sterilization system with RFDH (27.12 MHz; internal temperature of 135°C) to assess the effectiveness of decreased processing times of packaged macaroni cheese and whey protein gels sealed in 2.72 kg military ration polymeric trays. The packaged macaroni and cheese was selected because it is extensively used as a heat sensitive food in a parallel dielectric heating sterilization by researchers and was formulated with cooked semolina noodles and cheese sauce (Wang et al., 2003). The researchers found that the RFDH-treatments effectively achieved a process sterilizing value ($\mathbf{F_0}$) for the core, middle, and corner in the polymeric trays of macaroni and cheese (10.90, 14.00, and 29.10 min, respectively) and whey protein gels (9.40, 9.60, and 7.80 min, respectively). These results were similar to conventional retort values (macaroni and cheese: 7.30, 9.10, and 33.20 min, respectively; whey protein gels: 10.60, 12.00, and 18.00 min, respectively), but, decreased cooking time (30 min), and improved temperature uniformity were reported (Wang et al., 2003).

In conjunction with conventional hot air treatments, RFDH has shown to decrease mold growth and extend shelf-life within baked goods such as commercialized bread. When using RF-treatments with bread making, the core internal temperature of the loaf will cause severe moisture condensation on the surface, which will decrease quality and increase microbial activity. By

combining a RFDH-treatment with circulating hot air or water, bread quality and mold growth will be less prevalent due to a greater distribution of temperature causing an overall reduction of cold spots on the bread surface (Liu, Wang, Mao, Tang, and Tiwari, 2013).

Within an egg systems, RFDH-treatments have altered the physical and functional properties in egg white proteins (**EWP**). Important functional properties of EWP are gelling, foaming, and emulsifying (Ahmed, Ramaswamy, Alli, & Raghavan, 2007). Egg white proteins are incorporated within cakes, sausages, and meringues, due to their functional properties (Boreddy et al., 2016). Ahmed et al. (2007) observed that RFDH-treated (27.12 MHz for 60 to 180 s) EWP produced gels with an increased elastic modulus (**G'**) especially as EWP concentrations increased (2.5 to 12.5%) and temperature (20 to 90°C). Additionally, egg white dispersions exposed to a lower pH treatment (pH 3 to 5) and RFDH displayed a stronger gel (Ahmed et al., 2007).

Boreddy et al. (2016) studied the effects of RFDH-treatment of EWP (27.12 MHz; 60, 70, 80, and 90°C followed by a hold period in a hot air oven at 4 h at 90°C to 72 hr at 60°C) on functional and quality properties compared to standard EWP processing (58°C for 14 d). Researchers discovered that RFDH-treated EWP samples had similar color and solubility compared with control EWP. Further, the optimal RFDH-treatment was 90°C for ≥ 8 hr, which resulted in EWP that had improved functional properties (foaming stability, foaming capacity, water holding capacity, and firmness) compared with control EWP (Boreddy et al., 2016).

1.5.2 Radio Frequency Dielectric Heating in the Dairy Industry

The dairy industry utilizes RFDH processing to improve functional properties and produce new dairy protein ingredients (Chen et al., 2013). Dairy protein ingredients, such as whey protein isolates and sodium casienates, are practical due to their accessible nature, nutritional properties,

and versatile capabilities within a wide range of food products (Corzo-Martínez, Soria, Villamiel, Olano, Harte, and Moreno, 2011).

Radio frequency dielectric heating of dairy products, such as dried dairy products, may be possible as a food safety precaution against harmful pathogens such as *Cronobacter sakazakii* and *Salmonella spp.* As well as being an effective heating method, RFDH can be a post-process lethality treatment to prevent pathogenic contamination in dried milk such as infant formulas. However, the functional properties of dried milk can be altered with RFDH treatments (Chen et al., 2013).

Chen et al. (2013) studied time and temperature effects of RFDH-treated NFDM on WPNI, nitrogen solubility index (**NSI**), and color. High heat-NFDM and LH- underwent RFDH-treatments ranging from 75 to 90°C for five to 125 min. All samples displayed decreased solubility in HH (1.3 to 3%) and LH (3 to 5%) compared with controls (not RFDH-treated) except for HH-treated at 75°C at zero min. Overall, NSI values of LH were greater than the NSI of HH-, which verified that the pre-spray-drying treatment affected solubility. Nonfat dry milk that was RFDH-treated displayed significantly decreased WPNI in LH if the treatment was ≥ 80°C. These lower values of WPNI suggest the LH had greater amounts of native whey protein prior to processing or greater dissociations of α-LA and β-LG from the casein micelles. Color changes were observed in RF-treated LH, which may be interpreted as Maillard browning reactions being initiated (Chen et al., 2013). In a following study, RFDH-thermal processing parameters (decimal reduction time, **D**-value, at 75, 80, 85, and 90 °C; and thermal resistance constant, ***z***-value) in HH- and LH were determined in order to achieve thermal destruction of harmful pathogens (Michael, Phebus, Thippareddi, Subbiah, Birla, and Schmidt, 2014).

Radio frequency dielectric heating affects the heat stability of proteins within reconstituted NFDM. Sanchez Alan, Wang, and Schmidt (2017) studied the effects of RFDH on the heat stability of HH- and LH at 75, 80, and 85°C for different holding time intervals (between 43 and 125 min) in an oven of corresponding RFDH-treatment temperature (75, 80, and 85°C). The heat stability was assessed using an oil bath (140°C) and the heat coagulation times (**HCT**) were recorded. LH-RFDH-treated samples of 75°C or 80°C had greater heat stability when the pH was below pH 6.8 compared with (non-treated) LH samples. The greater HCT in RFDH-treated LH-NFDM samples could be the result from induced reactions other than whey protein denaturation reactions (Sanchez Alan et al., 2017).

In a subsequent study, Sanchez Alan, Subbiah, and Schmidt (2019) further examined improved foaming, heat stability, and emulsifying functionalities of RFDH-treated LH. The LH- was heated to 80, 85, and 90°C and held for 60 or 90 min in a convection oven preset at the target temperature (80/60, 80/90, 85/60, 85/90, 90/60, and 90°C /90 min). Researchers reported improved foaming properties with increased overrun (~11, 12, 14, and 15%) and stability (~14, 33, 48, and 49%) of 85/60, 85/90, 90/60, and 90/90 compared with untreated LH- due to increased concentration of DWPs following RFDH treatments; thus, increasing the water holding abilities (Sanchez Alan et al., 2019).

In addition to improved foaming, RFDH-treated LH samples exhibited improved heat stabilities at pH 6.4 (~51%) and pH 6.6 (~57%) when compared with untreated LH due to increased associations of β-LG/κ-casein contributing to the higher stability of the casein micelle (Singh, 2004; Sanchez Alan et al., 2019). When compared to the heat stability of HH-, all RFDH-treated LH, except for 85/60, displayed significantly greater heat stability at pH 6.8, 7.0, and 7.2 (Sanchez Alan et al., 2019). In terms of emulsifying properties of RFDH-treated LH, all samples displayed

significantly greater emulsion activity index (**EAI**) compared to HH. When comparing with HH, an increased EAI was observed in 85/60 (43%), 85/90 (52%), 90/60 (43%), and 90/90 (41%) resulting from increased fat droplet sizes and concentrations of DWP caused from increased dry-heating (Sanchez Alan et al., 2019).

Due to the improved functional properties of RFDH-treated LH, food systems typically incorporating HH (i.e. baked goods, sauces, and confectionaries) can possibly use RFDH-treated LH as an alternative during the formulation and production process. Since HH- can be difficult to manufacture due to increased DWP, RFDH-treated LH can produce similar functional properties required for these food systems. Based upon previous literature, it is hypothesized that utilizing RFDH technology to feasibly heat LH to a specified temperature can improve functional properties; therefore, producing similar physical properties in other food systems, which typically incorporate HH (Chen et al., 2013; Michael et al., 2014; Sanchez Alan et al., 2017; Sanchez Alan et al., 2019). Thus, the objective of this study were to study the physical properties in other specified food systems (i.e. bread, caramel, and white sauce) and determine if RFDH of LH can be used as an alternative to HH.

Chapter 2 - RFDH of LH-NFDM and Food Systems Incorporation

2.1 Experimental Design and Statistical Analysis

For the experiment, extra-grade LH- produced by Dairy America (1H-41316-01, Fresno, CA) and extra-grade HH- produced by ConAgra Foods (B150/1000913, Menomonie, WI) were obtained. Both HH and LH were used for comparison. Three replications of the RFDH treatments were completed and all products were analyzed in Manhattan, KS. All data were analyzed using SAS v. 9.4 (SAS Institute Inc.). The proximate analysis between the compositional values of low heat (LH) and high heat (HH) nonfat dry milk (NFDM) were analyzed using Tukey's significant difference test (Kuehl, 1999). Each NFDM composition had three repeated measurements (n = 3) A Tukey procedure was selected due to equal group sizes for pairwise accuracy (Kuehl, 1999). All tests were determined at a $p \leq 0.05$. The SAS code for compositional analysis of LH- and HH-NFDM can be found in Appendix E.

With a radio frequency dielectric heating (**RFDH**) unit (Food Engineering Lab, University of Nebraska, Lincoln), approximately 350 g of LH was heated to 85°C and then held for 90 min (**85/90**) or 180 min (**85/180**) at 85°C in a convection oven (Thelco, GCA/Precision Scientific, Schaar Scientific Company, Chicago, IL). The four NFDM samples – HH, LH, 85/90 and 85/180 were rehydrated and assessed for their pH and titratable acidity. Due to pH being logarithmic scale to measure lognormal distribution of hydrogen (H^+) activity, the titratable acidity was assessed with a one-way ANOVA using Tukey's significant difference test at a $p \leq 0.05$ with SAS (SAS Version 9.4) (Kuehl, 1999; Kuna-Broniowska & Smal, 2017). The SAS code for titratable acidity analysis of the rehydrated NFDM can be found in Appendix F.

The four NFDM samples were analyzed for nitrogen fractions, whey protein nitrogen index (**WPNI**), particle size, and were subjected to gel electrophoresis. Data were subjected to a one-

way ANOVA using SAS (SAS Version 9.4) and determined with a $p \leq 0.05$ significance level. The SAS code for NFDM protein profiles can be found in Appendix G.

The four types of NFDM were formulated within a bread, white sauce, or caramel food system. The bread samples were assessed the day after bread-making for volume, color, and firmness. The dough properties (i.e. water absorption, dough development time, and degree of softening) of flour and NFDM blends were measured with a computerized farinograph (Farinograph-E, C.W. Brabender Instruments® Inc., Hackensack, NJ, USA). Three replications were done and each replicate had three measurements for bread loaves and dough. For bread, the two bread machines were accounted for variances between bread loaves.

For white sauce and caramels, the proximate analyses results for the HH and LH were used, so that all NFDM samples were reconstituted to achieve approximately 3.5% protein content (weight/volume). White sauces were formulated, prepared, stored at 4 ± 1°C for 16 hr and then assessed for color, firmness, syneresis, and rheological properties. Caramels were formulated, prepared, stored at 4 ± 1°C for 16 hr and then assessed for color, water activity ($\mathbf{A_w}$), hardness, and stickiness. Three replications were done and each replicate had three measurements.

For bread, white sauce, and caramel, the three replicates measurements were averaged and replications statistically analyzed with a randomized block design. Replication (3) was the block factor. Data were analyzed as a one-way ANOVA using SAS (SAS Version 9.4, SAS Institute, Inc., Cary, NC). Significant means were differentiated by the use of a Tukey test (Kuehl, 1999) and determined with a $p \leq 0.05$ significance level. Appendix J, Appendix K, and Appendix L displays SAS code for bread, white sauce, and caramels respectively.

2.2 NFDM Source

Extra-grade LH and extra-grade HH were obtained from commercial suppliers and within the day of arrival, approximately 0.5 kg samples were removed and placed in a 0.93L zip-top package (Ziploc® freezer bags, S.C. Johnson & Son Inc., Racine, WI), then another 0.93L zip-top package and stored at a -2 ± 1°C (Equatherm, Lab-Line Instruments Inc., Melrose Park, IL) until use. About 10 kg from each original bag of NFDM was re-packaged into 0.5 kg lots.

2.3 RFDH Treatment

The RFDH unit (Strayfield Limited, Theale, Berkshire, England) (Figure 2.1) was programmed at 27.12 MHz frequency with a 120-mm electrode distance from the surface of the sample, which was placed in the center of the oven (Figure 2.2). LH was transferred into a circular high-density polyethylene tray (0.3 cm thick, 19.0 cm diameter) (Dynalab Corp., Rochester, NY) and leveled with a straight-edge to ensure complete distribution in tray. The LH trays (one at a time) were placed in the RFDH unit. To monitor the temperature throughout the RFDH process, four fiber-optic probes (T1, Neoptix Inc., Québec, Canada) were placed at the center, 3-cm from the center, and 3-cm from the edge, and at the edge of the plastic tray, and then connected to a data logger (Reflex, Neoptix Inc.) Once the center point achieved the target temperature of 85°C, the polyethylene tray was immediately transferred to a convection oven (Thelco, GCA/Precision Scientific, Schaar Scientific Company, Chicago, IL) (Figure 2.3) pre-set to 85°C for the designated holding times, 90 min (85/90) and 180 min (85/180). The time to reach the target temperatures in the RFDH unit was a monitored dependent variable. A typical heating profile for LH is shown in Figure 2.4.

Following the hold time, the tray was removed from the convection oven and cooled at 23 ± 1°C, and ~200 g of dry-heated LH was removed from the center of the tray, placed into separate, re-sealable polyethylene bags (0.93 L Ziploc freezer bags, S.C. Johnson & Son Inc.) and stored at −2 ± 1°C (Equatherm, Lab-Line Instruments Inc.) until assessment. These four NFDM samples – HH, LH, 85/90 and 85/180 were used to manufacture breads, caramels, and white sauces. The ability to compare to control samples – HH or LH would provide key insights into the RFDH impact on LH and if/how any change to the LH may carry through into food systems.

Figure 2.1. Radio frequency dielectric unit (Strayfield Limited, Theale, Berkshire, England) at University of Nebraska-Lincoln.

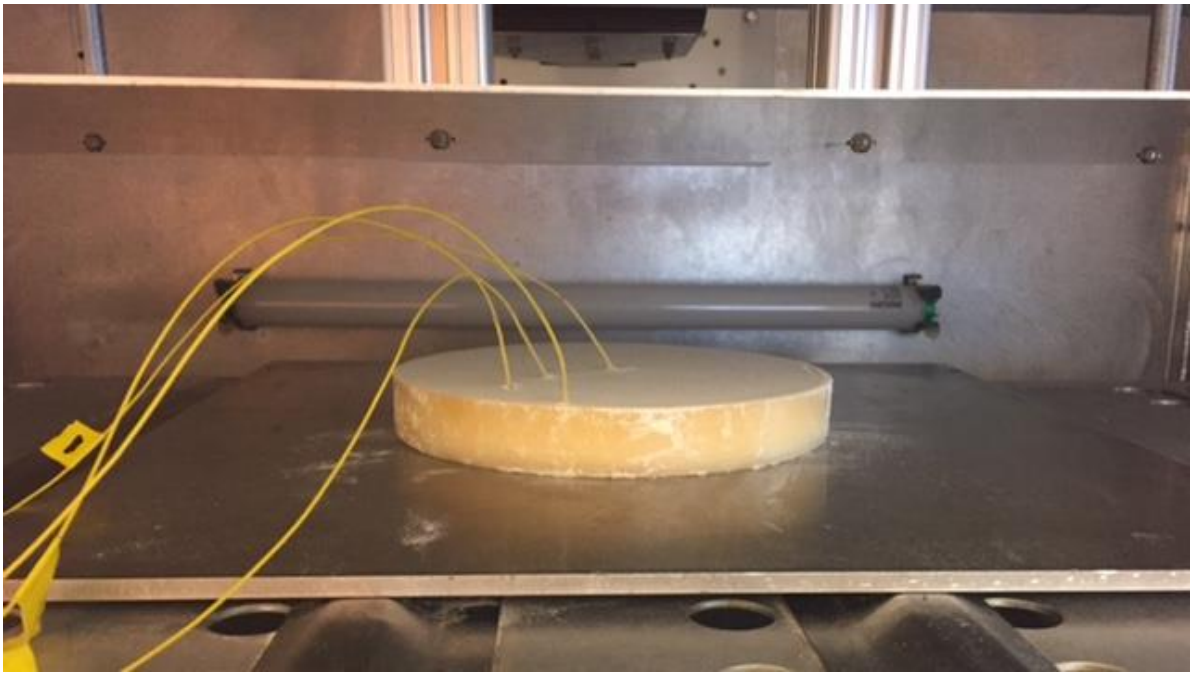

Figure 2.2. Radio frequency dielectric unit (Strayfield Limited, Theale, Berkshire, England) at University of Nebraska-Lincoln with ~ 350 g of nonfat dry milk samples placed in circular high-density polyethylene tray (0.3 cm thick, 19.0 cm diameter) (Dynalab Corp)

Figure 2.3. The convection oven (Thelco, GCA/Precision Scientific, Schaar Scientific Company, Chicago, IL) pre-set to 85°C for the designated holding times, 90 min and 180 min.

2.4 Methods

2.4.1 NFDM: Proximate Analysis

2.4.1.1 Nitrogen

NFDM samples were reconstituted to achieve approximately 3.5% protein content (w/v). Total nitrogen content of HH and LH was performed according to the IDF Standard 185 (IDF, 2002) by the combustion method using a LECO® FP- 2000 Nitrogen/Protein Determinator (Laboratory Equipment Co., St. Joseph, MI). The total nitrogen content was converted to total protein with the nitrogen conversion multiplication factor of 6.38 (IDF, 2002).

2.4.1.2 Moisture

The moisture contents of NFDM were determined using the forced air-drying method 990.20 (AOAC International, 2002) using an Isotemp atmospheric oven (Model 750F, Fisher Scientific).

2.4.1.3 Ash

Ash contents of NFDM samples were determined by incinerating the milk powders at 550°C in a Thermolyne™ tabletop muffle furnace (Cat. F30428C, Thermo Fisher Scientific) following AOAC method 954.46 (AOAC International, 2002; method 954.46).

2.4.1.4 Fat

Fat contents of the NFDM samples were determined by rehydrating 10 g of NFDM with 90 g of DI water. The fat contents were measured by microwave drying followed by nuclear magnetic resonance using the CEM, SMART Trac™ 41 Fat and Moisture Analyzer (CEM Corporation, Matthews, NC) (Kamruzzaman, ElMasry, Sun, & Allen, 2012).

2.4.1.5 Carbohydrates

The carbohydrates contents of the NFDM samples were calculated by the difference (AOAC International, 2002; method 986.25):

Carbohydrate % = 100- (protein % - moisture % - fat % - ash %)

2.4.2 Reconstitution of NFDM

NFDM samples were reconstituted with deionized (DI) water to produce a total protein content of 3.5% (w/v). Based upon the total weight of 800 g of DI water and NFDM, HH (79.82 g) and LH (80.36 g) were weighed and immediately transferred to separate 1000 mL glass beakers and DI water was added (HH = 720.70 g; LH = 722.42 g). The HH and LH milks were covered with aluminum foil (Reynolds Kitchens, Richmond, VA) and placed on hot plates (Fischer Scientific) while continuously mixing at 700 rpm with magnetic stir bars (Fischer Scientific) for 1 hr at 24 ± 1°C. The rehydrated milks were stored for 16 hr at 4 ± 1°C (Westinghouse 18.0 Cu. Ft. Top Freezer Refrigerator) before heating to 40 ± 1°C (~8 min) on a hot plate with continuous stirring with magnetic stir rods.

2.4.3 pH and Titratable Acidity of Reconstituted NFDM Samples

For the reconstituted NFDM samples, the pH was measured using a pH/mV/Ion meter (Accumet ® portable AP63, Fisher Scientific) that had been calibrated with standardized buffer solutions, pH 4.00, 7.00, and 12.00 (S25849A/B, Fisher Science Education) (Hooi, Barbano, Bradley, Budde, Bulthaus, Chettair, Lynch, & Reddy, 2004).

After measuring the pH, the titratable acidity (**TA**) was measured by weighing 9 g of the rehydrated milks into a 100 mL glass, graduated beaker using a pipette. Samples were mixed on a stir plate with magnetic stir rods (Fisher Scientific) for five min prior to adding 0.5 mL drops of 1% phenolphthalein indicator and titrated with 0.1N sodium hydroxide (SS276-1, Fisher

Scientific) until the first permanent color change to pink (~30 sec). Acidity is expressed as the percentage of lactic acid ((1 mL of 0.1 N sodium hydroxide (NaOH) = 0.009 grams of lactic acid)). Initial volume and final volume from the burette was recorded and calculated using the following formula (Hooi et al., 2004):

$$\% \text{ acidity} = \frac{(\text{mL NaOH}) \text{ x } (\text{Normality of NaOH}) \text{ x } 9}{\text{Sample weight in grams}} \times 100$$

2.4.4 Protein Profiles of the NFDM Samples

2.4.4.1 Whey protein nitrogen index (WPNI)

In order to generate a standard curve [transmittance (**T%**) vs. whey protein nitrogen (**WPN**) (mg/g)], standard references HH and LH samples (~125 g each) were obtained from the American Dairy Products Institute (**ADPI**) (Chicago, IL) and prepared following the ADPI method for WPNI (ADPI, 2009). The standard curve equation was calculated as y = -5.9635x + 97.608 (R^2 = 0.99) by Excel 2013 (Microsoft, Redmond, WA) (shown in Appendix A) where *y* is the T% and *x* is the concentration (mg/g). This standard curve was used for the NFDM samples – HH, LH, 85/90 and 85/180. These data were used to confirm or indicate a change in NFDM classification.

2.4.4.2 Protein fractionation

Rehydrated NFDM samples were fractionated using a method developed by Rowland (1938). To determine whey protein denaturation of HH, LH 85/90, and 85/180, total nitrogen (**TN**), non-casein nitrogen (**NCN**), and non-protein nitrogen (**NPN**) fractions were obtained and assessed for nitrogen content by the Kjeldahl method at the Soil Testing and Plant Testing Laboratory at Kansas State University (Manhattan, KS) The Kjeldahl protocol followed by the Soil Testing and Plant Testing Laboratory can be found in Appendix B (AOAC International, 2010, methods

numbers 991.20, 991.21, and 998.05, respectively). The nitrogen conversion multiplication factor of 6.38 was used for each protein fraction (IDF, 2002).

According to Rowland (1938), the process to obtain the NCN fraction requires the precipitation of the caseins and denatured whey proteins (**DWP**). The remaining supernatant will contain soluble, heat sensitive native whey proteins, whey protein aggregates, and other nitrogen fractions that were not denatured (Rowland, 1938; Lacotte, Gomez, Bardeau, Muller, Acharid, Quervel, Trossat, & Birlouez-Aragon, 2015).

The NCN fraction was prepared by adding and mixing 10 mL of each rehydrated NFDM sample, 70 mL of deionized water at 40°C, and 1 mL of 10% of acetic acid solution (Fisherbrand™, Fischer Scientific) into a 100 mL volumetric flask. After 10 min, one mL of 1 N sodium acetate (Sigma Aldrich, St. Louis, MO) was added while continuously stirring on a hot plate. Solutions were cooled to 23 ± 1°C, made to volume with deionized water, mixed, and set quiescently until precipitate formed. The pH was 4.6 after the addition of sodium acetate. The solutions were filtered through Whatman No.1 filter paper (Fisherbrand™, Fischer Scientific) and the supernatant was analyzed for the nitrogen content.

The NPN fraction was prepared by adding 10 mL of rehydrated HH, LH, 85/90 or 85/180 to a 50 mL volumetric flask and diluted with 1 mL of 15% trichloroacetic acid solution (Fisherbrand™, Fischer Scientific) to volume at a pH 4.6. Once the precipitate had settled, the solution was filtered through Whatman No.1 filter paper (Fisherbrand™, Fischer Scientific) and the supernatant was analyzed for the nitrogen content.

The following calculations were performed to determine how the dry heat treatment impacted the protein associations, and hence, the nitrogen-containing fractions in the NFDM. The

method of Sodini et al. (2005) was used to calculate the protein denaturation as seen with equation four below:

(1) True protein (**TP**) = (TN-NPN) x 6.38

(2) Protein soluble at pH 4.6 (**SP**) = (NCN-NPN) x 6.38

(3) Protein insoluble at pH 4.6 (**IP**) = (TN-NPN-NCN) x 6.38

$$(4)\ \text{Protein denaturation} = \frac{SP_{LH}\text{-}SP_{85/90\ \text{or}\ 85/180}}{SP_{LH}} \times 100\ \%$$

2.4.4.3 Particle size

Following Dumpler and Kulozik (2016), particle size (reported as average mean diameter) measurements of rehydrated NFDM were made by dynamic light scattering (**DLS**) using a Malvern Zetasizer 4 instrument with an attached ZET5110 particle sizing cell (Malvern Instruments Ltd., Malvern, Worchester, England) with minor modifications. By understanding the conditions of the test and size of the components in the rehydrated milks, the particle size obtained will represent the average mean of the casein micelle size (Taterka & Castillo, 2015). Due to the nature of casein micelles to aggregate and form large particles, the small particles of casein micelle fragments formed may not have been detected by the Zetasizer. Therefore, samples were diluted 1:50 in a simulated milk solvent, Ca-imidazole ((20 mM-imidazole, 5 mM calcium chloride, 30 mM sodium chloride) (Sigma Aldrich, St. Louis, MO)) at a pH 7.0, which was indistinguishable from water (Anema & Li, 2003), to explain the casein micelle aggregation phenomenon.

The Nano ZSP (Malvern Instruments, Ltd.) at 20 ± 0.5°C was set for an equilibrium time of 10 sec, coupled with a dynamic light scattering (DLS) set at an angle of 90°, a refractive index of 1.450, and a backscattering angle of 173°. The particle absorption index was set at 0.001. The rehydrated milks were diluted (1:50) in the CA imidazole buffer immediately prior to evaluation to suspend the casein micelles and allow stability during measurements. The autocorrelation

function of the Zetasizer automatically calculated the mean hydrodynamic diameter (Z-average mean) of the milk. Several measurements were done and the average diameter of the particle size distribution, on 11 runs per measurement was reported (Taterka & Castillo, 2015; Dumpler & Kulozik, 2016).

2.4.4.4 Sodium dodecyl sulfate – polyacrylamide gel electrophoresis (SDS-PAGE)

Lastly, the rehydrated NFDM milks were subjected to SDS-PAGE as described by Liu, Dunstan, and Martin (2012) with some modifications. The NFDM dispersions were prepared, one with and one without a reducing agent ((β-mercaptoethanol (**BME**) (0.05%) (Bio-Rad Laboratories, Hercules, CA)). The reducing agent was used in order to better separate casein micelles by the dissociation of the disulfide bonds between the α_{s2}-casein and κ-casein (Nguyen, Anema, Havea, Guyomarc'h, & Wong, 2012; Sanchez Alan et al., 2017) The NFDM was diluted to 2 mg/mL protein with 2×Laemmli sample buffer (Bio-Rad Laboratories). If the reducing agent was added, it was added before this process step. The diluted samples were heated for 5 min at 95°C using an AccuBlockTM digital dry bath ((Model D1100, Labnet International, Inc., Woodbridge, NJ) and immediately cooled in a freezer at –18°C for 3 min (Liu et al., 2012).

The SDS-PAGE was carried out on a 4-15% Mini-PROTEAN® TGXTM precast polyacrylamide gel (Bio-Rad Laboratories) using a Precision Plus ProteinTM Dual Color Standards (Bio-Rad Laboratories) as the marker. The protein bands were stained with a Bio-SafeTM Coomassie G-250 Stain (Bio-Rad Laboratories). To scan the gels, a Syngene Model Gbox Chemi XR (Syngene Ltd, Frederick, MD) scanner was used (Liu et al., 2012).

2.4.5 Bread Flour: Proximate Analysis

2.4.5.1 Protein

The total nitrogen content of the bread flour (Gold Medal Bread Flour, General Mills Sales Inc., Minneapolis, MN) was determined according to the AAAC combustion method (Method 46-30.01) using a LECO® FP- 2000 Nitrogen/Protein Determinator (Laboratory Equipment Co., St. Joseph, MI) (AACC International, 2010). The protein was calculated by a multiplication factor of 5.70 (AACC International 2010) (Table 2.1).

Table 2.1. Proximate analysis of bread flour

Ingredient	Protein (%)	Moisture (%)
Bread Flour	12.50 ± 0.08	7.08 ± 1.43

2.4.5.2 Moisture

The moisture contents of bread flours were determined using the forced air-drying method (Method 44-11.01) (AACC International 2010) using an Isotemp atmospheric oven (Model 750F, Fisher Scientific) (Table 2.1).

2.4.6 Rheological Properties of Bread Dough

The rheological properties of the dough were determined with a computerized Brabender Farinograph (Farinograph-E, C.W. Brabender Instruments® Inc.) with a 300 g flour mixing bowl according to Method 54-21.02 (AACC International, 2010) based upon optimum water absorption (500 BU) for all flour and NFDM mixtures. From the farinogram, consistency, dough development time (min), stability, softening degree (**ICC**) farinograph units (**FU**), water absorption (14% moisture basis), and farinograph quality number (**FQN**) were obtained (Madenci & Bilgiçli, 2014).

2.4.7 Physical Properties of Bread

2.4.7.1 Bread formulation

The bread formulation was prepared following the optimized straight dough bread making recipe with modifications. This method evaluates wheat flour quality and the influence of ingredients incorporated within the dough and bread loaf (Method 10-10.03, AACC International 2010). The bread formulation included deionized water and LH ((LH, 85/90, 85/180 (Dairy America, 1H-41316-01, Fresno, CA) and HH (ConAgra Foods, B150/1000913, Menomonie, WI)). Other ingredients were purchased from a local grocery store (Dillons, Manhattan, KS) included: wheat flour (Gold Medal Bread Flour, General Mills Sales Inc.), unsalted butter (Land O' Lakes, Arden Mills, MN), granulated sugar (C&H, Domino Foods Inc., Yonkers, NY), fast rise yeast (Kroger, The Kroger Co., Cincinnati, OH) and salt (Morton, Morton Salt Inc., Chicago, IL) (Table 2.2).

Table 2.2. Optimized bread formulation recipe with modifications

Ingredient Component	Amount (w/w) (%)
Bread Flour, 14% moisture basis	300.00
Deionized Water	177.00
Sugar	11.11
Yeast	5.56
Salt	4.17
Butter	10.41
Nonfat Dry Milk	5.56

[1]Adapted from AACC International Method 10-10.03 (2000).

2.4.7.2 Bread preparation

All breads were prepared by carefully weighing and transferring all ingredients into the bread maker (Sunbeam Programmable Breadmaker-5891, Sunbeam Products Inc.) in order to ensure consistent quality with all bread samples. For all bread samples, a "basic program", "medium color", and "2-pound load" parameters were selected. After the baking time was

completed, bread loaves set at 27 ± 1°C until the internal temperature reached 27 ± 1°C. Bread loaves were then wrapped with plastic wrap (GLAD ClingWrap 90M, The Glad Products Company), stored at 27 ± 1°C for 16 hr, and assessed the following day for weight, volume, color, and firmness (Gallagher et al., 2005).

2.4.7.3 Bread weight and volume displacement

The volume of bread samples was measured using the rapeseed displacement method 10-05.01 (AACC International, 2000). Calculations included:

$$(1)\ W_{seeds} = W_{total} - W_{sample} - W_{container}$$

$$(2)\ V_{seeds} = \frac{W_{seeds}}{\rho_{seeds}}$$

$$(3)\ V_{sample} = V_{container} - V_{seeds}$$

Where, W=weight (g),

V=volume (cm^3),

ρ=density (g/cm^3)

2.4.7.4 Bread crumb firmness

Bread crumb firmness was measured with a texture analyzer (Stable Micro System, Model TZXT2, Texture Expert, Surrey, England) with a 25 kg load cell and a 36 mm diameter probe attached. All measurements were made on "Measurement Force in Compression" mode, pre-test speed, test speed and post-test speed were set to 1.0, 1.7, and 10.0mm/s, respectively, and a compression ratio was set to 40% (Liu, Tang, Mao, Mah, Jiao, & Wang, 2011). The thickness of bread slices was measured at 25 mm and the pressure related to a compression ratio of 25% was defined from the bread firmness provided (Stable Micro System) (AACC International Method 74-10.02, 2000).

2.4.7.5 Bread color

The color of bread loaves and slices were measured using a Hunter Lab MiniScan EZ 4500L spectrophotometer (Hunter Associates Laboratory Inc., Reston, VA) on the CIE *L**, *a**, *b** scale; *L** represents the lightness versus darkness scale; *a** represents red-green color; and *b** represents yellow-blue color. All bread measurements were conducted in average daylight 24 hr after bread making. The colorimeter was placed directly on the bread surfaces prior to cutting to ensure an unaltered surface. Afterwards, bread loaves were cut into 25 mm slices for texture analysis and the colorimeter was placed directly on the bread crumb portions for color analysis (Gallagher et al., 2005). Color difference (**ΔE***) was calculated based upon the difference between the HH bread samples (1) with one of the other bread samples made with LH, 85/90, or 85/1180 (2) (Gallagher et al., 2005):

$$\Delta E^{*}=[(L_1{}^{*}-L_2{}^{*})^2+(b_1{}^{*}-b_2{}^{*})^2+(a_1{}^{*}-a_2{}^{*})^2]^{1/2}$$

Where ΔE^{*} represents the total color difference between HH (1) and LH, 85/90, or 85/180 (2),

$L_1{}^{*}, L_2{}^{*}$ represent lightness (L = 0, black; L = 100, white),

$b_1{}^{*}, b_2{}^{*}$ represent blueness or yellowness (−b = blue ; +b = yellowness),

$a_1{}^{*}, a_2{}^{*}$ represent greeness or redness (−a = green ; +a = red)

2.4.8 White Sauce: Formulation, Preparation, and Evaluation Properties

2.4.8.1 White sauce formulation

The white sauce formulation was prepared following a typical commercial white sauce or béchamel formula but without the addition of guar/xanthan gum or a preservative such as potassium sorbate (Heyman et al., 2010). The white sauce mix included deionized water and NFDM ((LH, 85/90, 85/180), HH)). Other ingredients were purchased from a local grocery store

(Dillons, Manhattan, KS) and included all-purpose wheat flour (Gold Medal All Purpose Flour), unsalted butter (Land O' Lakes), and salt (Morton, Morton Salt Inc.) (Table 2.3).

Table 2.3. Model white sauce formulation[1]

Ingredient	Amount (w/w) (%)
Deionized water	75.90
NFDM	10.00
Wheat flour	5.00
Unsalted Butter	8.00
Salt	1.10

[1]Adapted from Heyman et al. (2010).

2.4.8.2 White sauce preparation

Prior to preparing sauces, NFDM was rehydrated (250 rpm, 30 min) with deionized water using a hot plate as described with the reconstitution method in section 2.4.2 without the last step, heating the milk to 40°C. The reconstituted milk was stored at 4 ± 1°C for 16 hr to ensure complete hydration of the milk proteins. The roux was prepared separately by mixing butter and wheat flour in a stainless steel pan (0.93 L) on a hot plate while continuously stirring and heating at 100 ± 1°C for 2 min. Rehydrated NFDM and salt were added to the roux and further heated to 85 ± 1°C with continuous stirring by the same individual (~6 min). After sauces reached 85 ± 1°C, pans were removed from the heat and cooled to 60 ± 1°C. Sauces were poured in 739-mL plastic containers (Glad, The Glad Products Company), cooled to 30 ± 1°C, stored at approximately 7 ± 1°C for 16 hr prior to assessment (Heyman et al., 2010).

2.4.8.3 White sauce color

The color of white sauce samples was assessed at 27 ± 1°C using the Hunter Lab MiniScan EZ 4500L spectrophotometer as described in Section 2.4.7.5, except that white sauces were spooned into 60 ML aluminum sample pans (1.5X 6.35 cm (h x dia) (Fisherbrand™) and

covered with plastic wrap (Glad, The Glad Products Company). The colorimeter was placed on the protected surface and assessed (Bortnowska et al., 2016).

2.4.8.4 White sauce firmness

The extrusion properties of the white sauces were assessed with a texture analyzer (Stable Micro System, Model TZXT2) equipped with a back-extrusion rig cell (Texture Technologies) and a 40 mm diameter compressing disk forming a 10 mm annulus gap. The compression rate for the texture analyzer was 10 mm/s, a trigger force of 10 g, and an extrusion force of 4 s. The white sauce samples were placed inside the extrusion cell at 30 ± 1°C and quickly measured (Stable Micro System, Texture Expert). The peak positive force was recorded as the firmness based upon the calculation provided by the manufacturer software (Stable Micro System) (Arocas et al., 2009).

2.4.8.5 White sauce water holding capacity

The water holding capacity (**WHC**) of white sauces were evaluated by the application of centrifugal forces. Fifty mL conical, plastic centrifuge tubes (Fisherbrand™, Fischer Scientific) were filled with 20 g of white sauce and sealed with caps. The centrifuge tubes were stored at 7 ± 1°C at an upright position. At an equilibration temperature of 20 ± 1°C, samples were centrifuged (Centrifuge Model J2-21, Beckman) at 6,000 x *g* for 15 min. The supernatant was drained and the pellet weight determined. The WHC was calculated as (Heyman et al., 2010):

$$\%\ \text{Water Holding Capacity} = \frac{\textit{weight of decanted liquid}}{\textit{total weight before centrifuge}} \times 100$$

2.4.9 Caramel: Formulation, Preparation, and Evaluation Methods

2.4.9.1 Caramel formulation

The caramel formulations were prepared following a typical commercial caramel formula with modifications without the addition of a sweetened condensed milk or soy lecithin (Minifie, 1999; Mendenhall & Hartel, 2016). The caramel ingredients were purchased from a local grocery

store (Dillons, Manhattan, KS) and included granulated sugar (C&H, Domino Foods Inc., Yonkers, NY), light corn syrup (Karo, ACH Food Companies Inc., Memphis, TN), vegetable oil (Crisco, The J.M. Smucker Company, Orrvile, OH), unsalted butter (Land O' Lakes), and salt (Morton, Morton Salt Inc. (Table 2.4).

The NFDM samples were reconstituted as described in section 2.4.2. A control formulation was set to 2.5% protein, 12.5% fat in caramels, and a 10% final moisture content. The total sucrose to corn syrup ratio was set at 50:50 (w/w%), 56:44 on a dry solid basis (Mendenhall & Hartel, 2016).

Table 2.4. Model caramel formulation[1]

Ingredient	Amount (w/w%)
Sugar, granulated	35.40
Corn syrup	34.00
Vegetable oil	12.10
NFDM	8.40
Water	7.00
Butter	3.00
Salt	0.10

[1]Adapted from Mendenhall & Hartel (2016).

2.4.9.2 Caramel preparation

Prior to preparing caramels, NFDM was reconstituted (250 rpm, 10 min) with deionized water as described in white sauce preparation. The following day, caramels were prepared using a starting pre-mix weight of 200 g to yield 100 g of finished caramel. The butter, salt, and reconstituted NFDM were thermally monitored (Digi Thermo, Fischer™ Scientific) and heated to 60 ± 1°C or until butter had melted in a 0.93 L saucepan. In a stainless steel saucepan (0.93 L), sugar, corn syrup, vegetable oil, and water were mixed until ingredients were fully dispersed (8 min). The sugar, corn syrup, vegetable oil, and water were heated on a different hot plate and monitored with a confectionary thermometer (CDN Digital Candy/Deep Fry/Pre-Programmed &

Programmable Thermometer, CDN, Qualitas Products, Torrance, CA) until a temperature of 135 ± 1°C and then held for 10 min until a browned, caramel-like color was formed. At this point, the pan was removed from the heat and placed on an unheated surface. The butter, salt, and milk mixture was poured slowly into the sugar syrup and whisked gently until tripled in size (~200 mL). The saucepan was returned back the hot plate and boiled until a temperature of 119 ± 1°C. The liquid caramel was poured into square, silicone molds (33 x 25 x 16 mm) (O'Crème Silicon Molds, BakeDeco, Brooklyn, NY) set at 27 ± 1°C, and assessed the following day.

2.4.9.3 Caramel color

The color of caramel samples were measured using a Hunter Lab MiniScan EZ 4500L spectrophotometer as described in Section 2.4.7.5 except for the following. (All caramel measurements were conducted in average daylight ~16 hr after caramel processing. The colorimeter was placed directly on the caramel samples prior to cutting to ensure an unaltered surface (Miller & Hartel, 2015).

2.4.9.4 Caramel water activity

For water activity (a_w), an AquaLab 3TE water activity meter (Decagon, Pullman, WA) was used at a controlled temperature of 25 ± 1°C. Caramel samples were obtained from the center of molds (~8 g), placed in the sample cups with a depth of 3 mm, and then the sample cups were placed into the water activity meter (Levin et al., 2016). Due to the slow-diffusing nature of caramel, the water activity meter was set to a continuous reading mode while the chamber of the meter remained sealed and the meter repeated consecutive readings. The water activity meter ran until two consecutive readings (~10 min) were similar (Miller & Hartel, 2015).

2.4.9.5 Caramel firmness and stickiness

Caramel firmness and stickiness were measured with a texture analyzer (Stable Micro System, Model TZXT2. For compression samples, silicon molds (O'Crème Silicon Molds, BakeDeco, Brooklyn, NY) measuring 33 mm (l) x 10 mm (w) x 16 mm (h)) were filled with caramel with an excess to form a small dome above the top lip of the mold (Mendenhall & Hartel, 2016). Immediately before testing, the excess caramel was cut from the top of the silicon mold to expose a fresh surface and leaving a caramel sample 10 mm thick. A TA-8 stainless steel, spherical probe (Texture Technologies) was used for the firmness and stickiness tests. The probe descended at a 10 mm/s pretest speed until the probe experienced a trigger force of 5 g. The probe then descended at a rate of 5 mm/s for a penetration depth of 8 mm, paused for 1 s, and then raised to the initial height at a rate of 5 mm/s. The peak positive force was recorded as the firmness, and the peak negative force recorded as the stickiness based upon the calculation provided by the manufacturer software (Stable Micro System) (Levin et al., 2016).

2.5 NFDM and Protein Profiles Results and Discussion

2.5.1 NFDM: Proximate Analysis and Classification

Based upon Table 2.5, the HH and LH possessed the expected composition except for fat contents (ADPI, 2009). According to APDI (2009), average fat percentages for NFDM should be around 0.6 to 1.25%. The higher than average fat percentages observed in HH and LH could be contributed to improper standardization during NFDM production or probably more likely, the CEM analysis of the reconstituted NFDM samples (ADPI, 2009; Kamruzzaman, et al., 2012). Hovever, based on these results, both NFDM samples exceeded standard fat contents: hence, these powders did not meet Extra Grade Standards.

Table 2.5. Composition of high heat nonfat dry milk (HH) and low heat nonfat dry milk (LH)a

Component	HH	LH
Total protein (%)	36.41 ± 0.12^{a}	35.99 ± 0.10^{b}
Moisture (%)	3.96 ± 0.16^{a}	2.31 ± 0.16^{b}
Fat (%)	1.53 ± 0.09	1.60 ± 0.00
Ash (%)	8.07 ± 0.32	8.26 ± 0.52
Carbohydrates (%)	51.46 ± 0.21	50.95 ± 0.63

[a] Means (n=3) ± SD within a row with different superscripts, differ (P <0.05)

2.5.2 RFDH Heating of LH

2.5.2.1. RFDH heating curves

When monitoring the RFDH time to reach desired temperature of 85°C, it has been reported that the RFDH treatment of NFDM is rapid (~15 min) (Michael et al., 2014; Sanchez Alan et al., 2017; Sanchez Alan et al., 2019). To standardize the timing process, the start time of the RFDH treatment began when the NFDM (center probe) achieved 30°C and ended when the NFDM achieved 85°C. For this experiment, the target temperature was based on the center fiber optic probe, due to being the last of the four probes to reach the desired temperature.

Figure 2.4 shows a representative RFDH trial for LH. The remaining RFDH heating curves can be seen in Appendix C. As shown in Figure 2.4, an increased temperature was observed at the edge compared with the center probes; thus, ~200 g of LH from the tray edges were discarded after the designated hold time. During the test runs, the edge probes did display increased temperature compared with the other probe values. Similar inconsistencies in the probes were observed by Sanchez Alan et al. (2019) who reported the edge probe lagged compared with the center probe when RFDH-heating NFDM. On the other hand, Boreddy et al. (2016) reported a 19°C increase in temperatures from the edge probe compared with the center probe when heating EWP in the RFDH unit.

Overall, the time to reach the target temperature of 85°C was rapid and consistent with other published reports. In this experiment, the average RFDH time was 19.18 min, with a range of 17.00 to 23.20 min (Figure 2.4, which shows the average of the RFDH treatment). When considering the overall heat exposure for the 85/90 and 85/180 samples, the 85/90 sample received an average of 109.92 min vs. 198.44 min for the 85/180 sample (Table 2.6). The RFDH time as well as the total heating time are consistent with previous research (Boreddy et al., 2014; Wang, 2016; Sanchez Alan et al., 2017; Sanchez Alan et al., 2019).

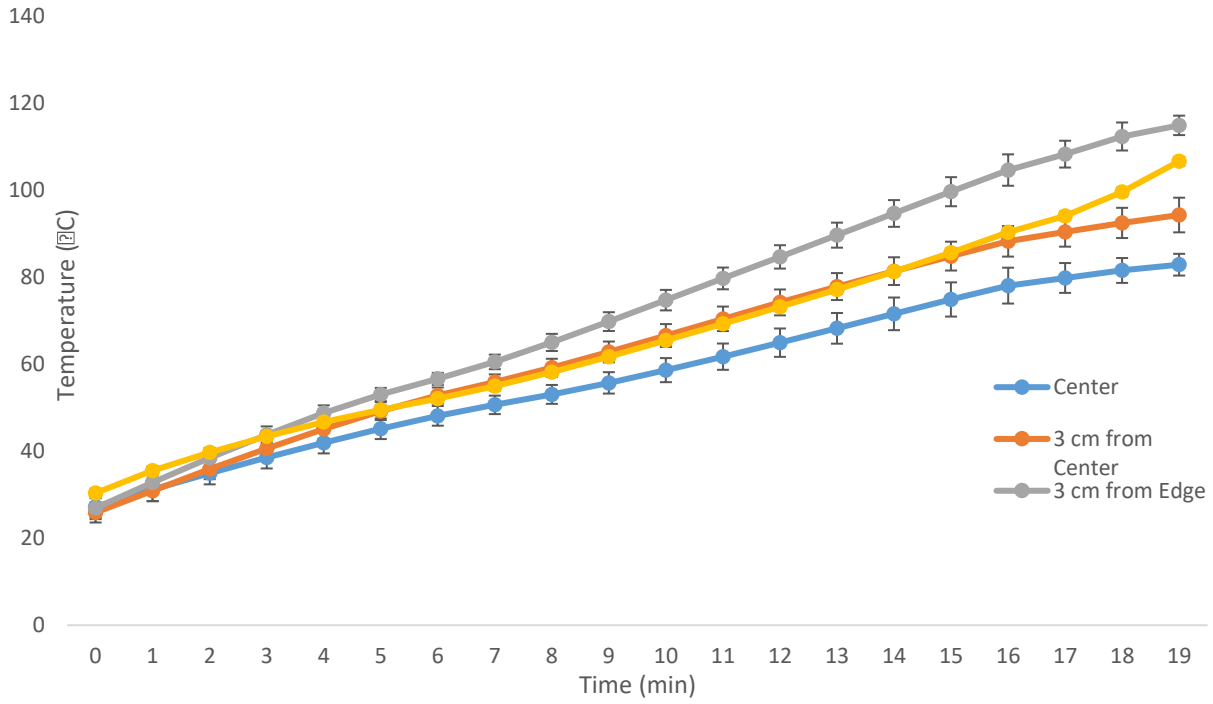

Figure 2.4. Radio frequency dielectric heating of low heat nonfat dry milk (LH) from 30 to 85°C (target temperature for the center probe) in a circular polypropylene tray and then held for 90 min in a convection oven. The legend shows the location of four fiber-optic probes within the LH

Table 2.6. Average heating times in the RFDH-and total heating time of low heat nonfat dry milk (LH-NFDM)

LH-NFDM[1]	RFDH time (min)[2]	Total heating time (min)
85/90	19.92 ± 2.54	109.92
85/180	18.84 ± 0.68	198.44

[1]Treated in RFDH unit for 85°C with subsequent holding time in (90 or 180 min) in a convection oven
[2] Mean ± SD (n = 3)

2.5.3 pH and Titratable Acidity of Reconstituted NFDM Samples

For all reconstituted samples - LH, HH, 85/90, and 85/180, the pH and TA can be seen in Table 2.7. Due to pH using a logarithmic scale, the pH was not statistically assessed; whereas, the titratable acidity concentration were (Kuna-Broniowska & Smal, 2017). The pH of the rehydrated heat-treated LH, 85/90 and 85/180, had an average pH of 6.81 and 6.79, respectively. The 85/90, 85/180, and HH displayed lower pH values when compared with LH (0.29%, 0.59%, and 0.15%, respectively).

However, there was a significant difference with the titratable acidity between LH, 85/90, and 85/180 when compared with HH (20.7%, 26.7%, and 32.3%, respectively). The dry-heated samples, 85/90 and 85/180, were significantly similar.

Table 2.7. pH and Titratable Acidity of reconstituted nonfat dry milk types (NFDM)

NFDM[1]	pH	Titratable Acidity (%)
LH	6.83 ± 0.01	$0.16 \pm 0.00^{b,c}$
85/90	6.81 ± 0.00	$0.17 \pm 0.00^{a,b}$
85/180	6.79 ± 0.00	0.18 ± 0.00^{a}
HH	6.82 ± 0.04	0.13 ± 0.02^{d}

[a,b]Means (n=3) ± SD within the titratable acidity column with different superscripts, differ (P <0.05)
[1]85/90 = low heat (LH) NFDM heated in a radio frequency dielectric heating (RFDH) unit to 85°C and held for 90 min; 85/180 = LH NFDM heated in RFDH to 85°C and held for 180 min; HH = high heat NFDM

2.5.4 Whey Protein Nitrogen Index (WPNI)

According to Table 2.8, the dry-heat treatment significantly decreased WPNI values (35.74% and 48.15%, respectively for 85/90 and 85/180 compared with LH). Since the WPNI represents the native whey proteins, these data suggest the overall dry-heat treatment of the LH denatured native whey proteins (APDI, 2009). However, both 85/90 and 85/180 displayed higher WPNI values when compared with HH (120.64% and 112.06%, respectively) suggesting the dry-heat treatment of LH does not denature native whey proteins to the same extent as does the

manufacture of HH. From the WPNI results, the LH and HH samples met the classification criteria as defined by ADPI, whereas the 85/90 and 85/180 lost their LH status and would be classified as Medium Heat (**MH**) NFDM (ADPI, 2009).

It should be noted the WPNI method is used as a heat classification of NFDM made through the traditional process – e.g., standardization, pasteurization, evaporation, spray drying, and may have limited value as an index of heating history if a different heating regimen is used or different products. For example, soluble whey protein content can be influenced by natural variation of protein concentration in milk samples (Sikand, Tong, & Walker, 2008). Thus, when interpreting these data, limitations may exist when used to comparing the WPNI of the dry-heated samples once rehydrated, because of the nature of protein aggregates to release particles when rehydrated (Sing et al., 2008; Crowley, Desautel, Gazi, Kelly, Huppertiz, & O'Mahony, 2015).

Table 2.8. Whey protein nitrogen index (WPNI) of reconstituted nonfat dry milk (NFDM) types

NFDM[1]	WPNI
LH	7.19 ± 0.004^{a}
85/90	5.01 ± 0.015^{b}
85/180	4.40 ± 0.026^{c}
HH	1.24 ± 0.71^{d}

[a,b]Means (n=3) ± SD within a column with different superscripts, differ (P <0.05)
[1]85/90 = low heat (LH) NFDM heated in a radio frequency dielectric heating (RFDH) unit to 85°C and held for 90 min; 85/180 = LH NFDM heated in RFDH to 85°C and held for 180 min; HH = high heat NFDM

2.5.5 Protein Fractionation

Table 2.9 displays the significantly different protein fractions of the rehydrated NFDM samples. All protein fractions can be found in Appendix H. The 85/90 and 85/180 had significantly greater NCN protein concentrations compared with LH (8.6% and 10.0%, respectively) and HH (84.11% and 85.19%, respectively) milks. According to Rowland (1938), NCN requires the

precipitation of the caseins and denatured whey proteins (**DWP**). The remaining supernatant will contain soluble, heat sensitive native whey proteins, whey protein aggregates, and other nitrogen fractions that were not denatured (Rowland, 1938; Lacotte, Gomez, Bardeau, Muller, Acharid, Quervel, Trossat, & Birlouez-Aragon, 2015). As seen in Table 2.9, the SP amounts were significantly greater in 85/90 and 85/180, when compared with LH (16.22%) and HH (115.79%). The SP fraction includes soluble heat-sensitive native whey proteins and whey protein aggregates (Patel & Patel, 2015). Dry-heating conditions have been shown to induce greater aggregations between DWP, as well as, associations between DWP and casein micelles in SMP (Singh & Creamer, 1991; Jovanovic et al., 2007). The increase of SP as a function of the overall RFDH treatment in this study may be due to increased aggregation between whey proteins during the dry-heating process, while maintaining their solubility (Singh & Creamer, 1991; Gulzar, Lechevalier, Bouhallab, Croguennec, 2012; Sanchez Alan et al., 2019). As the 85/90 and 85/180 had greater contents of SP –, using Sodini et al. (2005) calculation for percent denaturation was not possible. More interestingly, these data suggest whey protein denaturation reaction differs dependent upon the system when heating – e.g., dry heat vs. a wet heat system.

Insoluble proteins (**IP**) are proteins not soluble at pH 4.6 (i.e. casein and associated DWP on the micellar surface) (Smithers & Augustin, 2012). With extensive thermal processing, insoluble protein aggregate concentrations can increase through sulfhydryl-disulfide interchange and hydrophobic interactions between denatured whey proteins (Gulzar et al., 2012; Patel & Patel, 2015) The 85/90 and 85/180 displayed significantly lower concentrations of IP when compared with HH (11.19% and 10.82%, respectively). The HH milk displayed greater levels of IP due to its manufacturing process, which causes increased concentrations of DWP and their associations with casein micelle or whey-whey aggregation creating an IP fraction that is larger when compared

with LH (Patel & Patel, 2015). When compared with LH, the 85/90 and 85/180 displayed significantly greater concentrations of IP (3.39% and 3.76%, respectively) and soluble proteins (16.20%). The RFDH-treatment of LH does produce increased amounts of insoluble and soluble proteins, while reducing the amount residual native proteins seen with the WPNI (Gulzar et. al, 2012).

Table 2.9. Protein contents in various fractions (%) of nonfat dry milk (NFDM) types

NFDM[1]	True Protein Nitrogen (%)	Non-Casein Nitrogen Protein (%)	Soluble Protein (%)	Insoluble Protein (%)
LH	3.12 ± 0.005^{b}	0.70 ± 0.00^{b}	0.51 ± 0.002^{b}	2.61 ± 0.07^{c}
85/90	3.29 ± 0.0001^{a}	0.76 ± 0.002^{a}	0.60 ± 0.0005^{a}	2.70 ± 0.007^{b}
85/180	3.31 ± 0.0002^{a}	0.77 ± 0.00^{a}	0.60 ± 0.00^{a}	2.71 ± 0.0002^{b}
HH	3.18 ± 0.0034^{b}	0.31 ± 0.00^{c}	0.16 ± 0.01^{c}	3.02 ± 0.002^{a}

[a,b]Means (n=3) ± SD within a column with different superscripts, differ (P <0.05)
[1]85/90 = low heat (LH) NFDM heated in a radio frequency dielectric heating (RFDH) unit to 85°C and held for 90 min; 85/180 = LH heated in RFDH to 85°C and held for 180 min; HH = high heat NFDM

2.5.6 Particle Size

The particle size is presented as the average mean diameter of the casein micelle distribution (Taterka & Castillo, 2015). As displayed in Table 2.10, a holding time of 90 min did not seem to cause major differences in average size when compared with LH; however, the longer holding time of 180 min did increase the average casein micelle size. According to Anema & Li (2003), the increase of the casein micelle size will be caused from association between the DWP on the casein micellar surface rather than increased concentrations of whey protein aggregates. Overall, the RFDH-treatment of LH-NFDM may cause associations of DWP with the micellar surface of the casein micelle as exhibited with increased particle distribution (Schorsch, Wilkins, Jones, & Norton, 2001; Anema & Li, 2003).

Table 2.10. Particle analysis (nm) of reconstituted nonfat dry milk (NFDM) types

NFDM[1]	Particle Analysis (nm)
LH	$212.35 \pm 7.57^{b,c}$
85/90	212.00 ± 4.60^{c}
85/180	231.00 ± 13.30^{b}
HH	259.00 ± 19.90^{a}

[a,b]Means (n=3) ± SD within a column with different superscripts, differ (P <0.05)
[1]85/90 = low heat (LH) NFDM heated in a radio frequency dielectric heating (RFDH) unit to 85°C and held for 90 min; 85/180 = LH heated in RFDH to 85°C and held for 180 min; HH = high heat NFDM

2.5.7 Sodium Dodecyl Sulfate – Polyacrylamide Gel Electrophoresis (SDS-PAGE)

To understand the impact of the bonding mechanisms between proteins that might occur due to the dry heat reatment, the rehydrated NFDM samples were analyzed under reducing and non-reducing SDS-PAGE. Figures 2.5a and Fig 2.5b show the protein profile of the rehydrated NFDM samples without a reducing agent and with a reducing agent – specifically β-mercaptoethanol (**BME**)-, respectively. In both gels, Lane 1 displays the markers for identification of the major milk proteins: α_s–casein (**αs-CN**) (37,000 Da), β-casein (**β-CN**) (26,000-33,000 Da), κ-casein (**κ-CN**) (23,000 Da), β-LG (15,000 Da), and α–LA (10,000 Da). Also, two minor bands for immunoglobulins (**IG**) (150,000 Da) and blood serum albumins (**BSA**) (65,000 Da) fractions are present as well (Varnam & Sutherland, 2001; Jovanovic et al., 2007).

Analysis of the gel without the reducing agent added to the rehydrated NFDM, (Fig 2.5a) shows a stark difference between LH (lane 2) and HH (lane 5) especially around the whey protein bands - α–LA and β-LG. These bands are very distinctive in the LH and less noticeable in the HH. These results correlate to the manufacture of these two products – one with significant denaturation of whey protein (HH) and one to minimize this reaction (LH). This would be in agreement with the WPNI values displayed in Table 2.8, as HH should contain extensively denatured whey

proteins due to the manufacturing conditions selected during its process, which corresponds to low WPNI (ADPI, 2009; Parris et al., 1990). Not too surprisingly, the 85/90 and 85/180 fall between these two lanes for the α–LA and β-LG. Moreover, another difference in the gel are the bands at 100,000 and 150,000 Da. The 85/90 and 85/180 milks have more intense bands than does the LH; further the 150,000 Da band is absent in the HH milk. It has been suggested that the larger band could represent some of the soluble aggregate formed during the dry heat process (Jovanovic et al., 2007; Sanchez Alan et al., 2017).

In contrast, the gel in which the rehydrated milks were subjected to a reducing agent also is important to identifying the impact of the RFDH treatment on LH. The BME will cleave disulfide bonds, which in this case can be present in protein-protein aggregates (Jovanovic et al., 2007; Sanchez Alan et al., 2017). In the reducing SDS-PAGE, (Figure 2.5b), the RFDH-treated samples 85/90 and 85/180 (lane 3 and lane 4, respectively) displayed more diffusion in the bands corresponding with the β-LG (15,000 Da) and α-LA (10,000 Da) compared to the LH (lane 2). This diffusion suggests increased whey protein denaturation (**WPD**) during the dry heat process (Wang, 2015; Sanchez Alan et al., 2017). The HH sample (lane 5) displayed faint bands corresponding to the β-LG and α-LA, but less diffusion compared to 85/90 and 85/180. This would be in agreement with the WPNI values displayed in Table 2.8, as HH should contain extensively denatured whey proteins due to the manufacturing conditions selected during its process, which corresponds with the lower WPNI values suggesting increased whey protein denaturation during the manufacturing (ADPI, 2009; Parris et al., 1990). The BSA bands (75,000 Da) appeared more diffused in the 85/90 and 85/180 than the LH and the HH suggesting the formation of disulfide or hydrophobic linked soluble aggregates occurred during the dry heat process (deWit & Klarenbeek, 1984; Patel & Patel, 2015). Blood serum albumin denatures at a lower temperature (62°C) and will

form aggregates prior to α–LA and β-LG aggregation temperatures (64°C and 78°C, respectively) (Havea, Singh, & Creamer, 2001; Ryan, Zhong, & Foegeding, 2013).

Considering the 85/90 and 85/180 lanes, the diffused zones were not affected by **BME** (Fig. 2.5b, lanes 3 and 4), which can be interpreted as the use of covalent bonds, other than disulfide bonds, as aggregates formed during the dry heat process (Wang, 2015; Sanchez Alan et al., 2017). Soluble protein can aggregate through intermolecular and intramolecular bonds that include covalent bonds or noncovalent bonds, such as van der Waals forces and electrostatic interactions (Patel & Patel, 2015; Sanchez Alan et al., 2017). Even with the addition of the BME, there was less visual band intensity on the gel suggesting that the increased formations of soluble protein aggregates formed during the dry heating process are through bonds other than disulfide (Dissanayake & Vasiljevic, 2009; Wang, 2015; Sanchez Alan et al., 2017).

All other SDS-PAGE results can be found in Appendix I. All samples were reconstituted to an equivalent protein concentration (2 mg/ml), so that band intensities could be qualitatively compared.

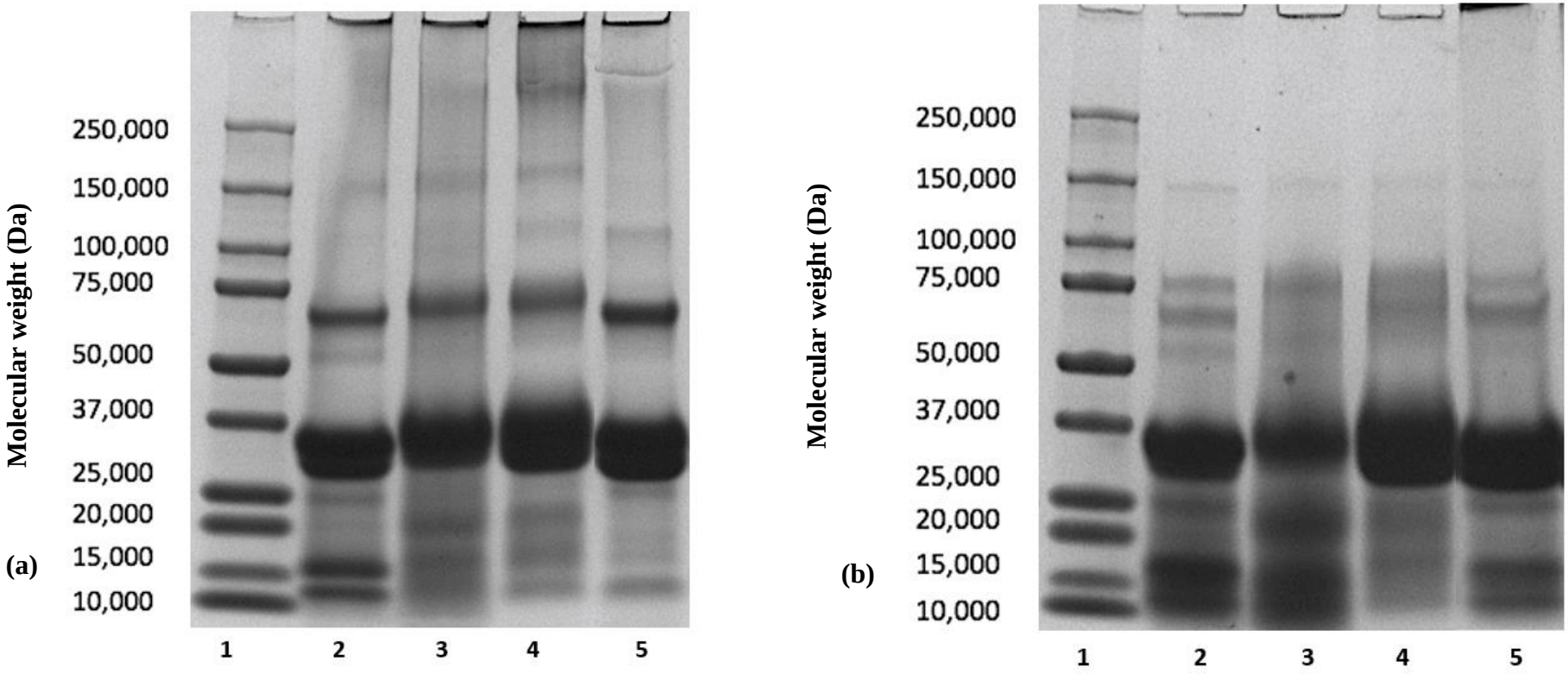

Figure 2.5. SDS-PAGE of standard protein markers (lane 1) and reconstituted nonfat dry milk (NFDM) (10 µL) (lanes 2-5). All NFDM samples were rehydrated to 3.5% (w/v) protein. Low heat (LH) NFDM (lane 2), LH-NFDM RFDH-treated to 85°C and held for 90 min (85/90) (lane 3), LH-NFDM RFDH-treated to 85°C and held for 180 min (85/180) (lane 4), and high heat (HH) NFDM (lane 5). All NFDM samples were reconstituted to 3.5% protein, and diluted to 2 mg mL^{-1} protein. (a) without β-mercaptoethanol (b) with β-mercaptoethanol

2.6 Bread Results and Discussion

2.6.1 Rheological Properties of Dough

The bread flour and NFDM mixtures were assessed with a Farinograph for the following rheological properties: consistency, development time, stability, degree of softening, water absorption, and farinograph quality number (**FQN**) (Table 2.11). The only significant rheological property affected by NFDM type was FQN. Dough mixtures formulated with HH (46.0 min) displayed a significantly lower FQN (194.2%; 205.8%, respectively) than the doughs containing 85/90 (135.3 min) and 85/180 (140.7 min). The FQN represents the ability of the dough to retain overall structure during mixing time. Thus, the higher FQN displayed in doughs formulated with 85/90 and 85/180 are interpreted to mean that these doughs were stronger compared with the HH dough (Kinsella, 1971). When compared the dough formulated with LH, 85/90 and 85/180 displayed significantly similar FQN. Overall, the LH and RFDH-treated LH samples displayed greater viscoelastic properties during the mixing process when compared to HH.

Table 2.11. Rheological properties of bread doughs made with various types of nonfat dry milk (NFDM)

	Nonfat Dry Milk Type[1]			
Rheological Properties	**LH**	**85/90**	**85/180**	**HH**
Consistency	517.67 ± 11.73	530.67 ± 9.57	519.67 ±12.50	522.67 ± 18.12
Development time (min)	2.40 ± 0.37	1.60 ± 0.14	2.00 ± 0.14	2.00 ± 0.14
Dough stability (min)	13.70 ± 1.07	12.83 ± 1.14	13.63 ± 0.62	13.87 ± 0.45
Softening Degree (ICC) (FU)	33.33 ± 6.80	44.33 ± 16.44	45.33 ± 16.86	35.00 ± 2.94
Water absorption (14%)	63.67 ± 0.31	64.00 ± 0.22	63.40 ± 0.37	63.80 ± 0.45
Farinograph Quality Number	142.00 ± 11.34[a]	135.33 ±14.29[a]	140.67 ± 7.04[a]	46.00 ± 8.52[b]

[a,b] Means (n=3) ± SD within a row with different superscripts, differ (P <0.05)
[1] LH = low heat NFDM; 85/90 = low heat (LH) NFDM heated in a radio frequency dielectric heating (RFDH) unit to 85°C and held for 90 min; 85/180 = LH heated in RFDH to 85°C and held for 180 min; HH = high heat NFDM

2.6.2 Physical Properties of Bread

2.6.2.1 Bread weight, volume displacement, and firmness

Based upon data presented in Table 2.12, the bread containing 85/90 had a similar loaf weight as bread loaves formulated with HH. However, the bread loaf containing 85/180 had a significantly decreased weight (0.15%) when compared with breads formulated with HH. For the volume and specific volume, the breads containing LH were not as great as the bread containing HH. In terms of bread loaf firmness, the 85/90 displayed a significantly similar firmness as HH, whereas, 85/180 was significantly less firm than 85/90 (22.53%) and HH (30.77%).

When comparing against LH, the bread loaf weight was significantly less in breads formulated with 85/90 (0.30%), 85/180 (0.52%), and HH (0.37%). In comparison, specific volume was greater in the breads containing 85/90, 85/180 or HH (157.73%, 109.79%, and 51.55%, respectively) when compared with bread loaves containing LH as seen in Table 2.12. Bread

firmness significantly decreased in bread loaves formulated with 85/180 (20.25 to 30.76%) compared with breads formulated with 85/90 (20.3%), LH, and HH, respectively (Table 2.12).

Table 2.12. Physical properties of breads made with various nonfat dry milk (NFDM) types

	Nonfat Dry Milk Type[1]			
Rheological Properties	**LH**	**85/90**	**85/180**	**HH**
Weight (g)	446.00 ± 0.00[a]	444.67 ± 0.47[b]	443.67 ± 0.47[c]	444.33 ± 0.47[b]
Volume (cm^3)	865.66 ± 14.50[d]	1306.06 ± 154.30[c]	1807.58 ± 86.89[b]	2222.22 ± 3.11[a]
Specific volume (g/cm^3)	1.94 ± 0.03[d]	2.94 ± 0.35[c]	4.07 ± 0.19[b]	5.00 ± 0.01[a]
Firmness (g)	479.41 ± 19.79[a]	416.21 ± 45.31[a]	331.93 ± 29.00[b]	452.62 ± 23.77[a]

[a,b] Means (n=3) ± SD within a row with different superscripts, differ (P <0.05)
[1] LH = low heat NFDM; 85/90 = low heat (LH) NFDM heated in a radio frequency dielectric heating (RFDH) unit to 85°C and held for 90 min; 85/180 = LH heated in RFDH to 85°C and held for 180 min; HH = high heat NFDM

2.6.2.2 Bread color

In terms of color properties (Table 2.13), significant differences were observed on the bottom of bread loaves only. Bread loaves formulated with HH, 85/90 and 85/180 were considered to have the same color properties. Bread loaves formulated with LH displayed significantly increased darkness (L^*) and redness (a^*) compared with bread loaves formulated with 85/90 (3.2%, 7.2%), 85/180 (3.8%, 6.7%), and HH (3.2%, 10.2%), respectively.

Table 2.13. Color properties of breads made with various nonfat dry milk (NFDM) types

		Nonfat Dry Milk Type[1]			
Area of measurement	**Color Properties**	**LH**	**85/90**	**85/180**	**HH**
Bottom of loaves	L^*[2]	50.37 ± 0.84[b]	51.78 ± 0.59[a,b]	52.30 ± 0.23[a]	51.99 ± 0.24[a,b]
	a^*[2]	18.81 ± 0.52[a]	17.51 ± 0.36[a,b]	17.59 ± 0.41[a,b]	16.98 ± 0.33[b]
	b^*[2]	35.44 ± 0.93	35.08 ± 0.51	34.16 ± 0.54	33.86 ± 0.56
	$\Delta E^{2.3}$	0.43 ± 0.03	0.88 ± 0.01	0.93 ± 0.02	-
Top of loaves	L^*[2]	59.89 ± 0.55	59.56 ± 2.17	60.96 ± 0.72	61.55 ± 0.34
	a^*[2]	16.00 ± 0.61	15.68 ± 0.81	15.50 ± 0.30	15.41 ± 0.24
	b^*[2]	34.70 ± 0.40	34.70 ± 0.80	35.55 ± 0.38	35.46 ± 1.01
	$\Delta E^{2.3}$	3.40 ± 0.05	2.42 ± 0.04	2.55 ± 0.02	-
Side of loaves	L^*[2]	48.54 ± 1.99	47.67 ± 0.56	48.32 ± 0.92	47.08 ± 0.63
	a^*[2]	18.11 ± 0.53	18.52 ± 0.46	18.57 ± 0.38	17.75 ± 0.62
	b^*[2]	32.34 ± 0.39	33.14 ± 0.66	33.63 ± 1.04	32.39 ± 0.37
	$\Delta E^{2.3}$	0.77 ± 0.07	1.56 ± 0.04	0.92 ± 0.03	-
Crumb of loaves	L^*[2]	76.19 ± 0.98	76.69 ± 0.74	75.59 ± 1.88	76.62 ± 1.04
	a^*[2]	1.44 ± 0.24	1.35 ± 0.19	1.59 ± 0.13	1.60 ± 0.36
	b^*[2]	22.54 ± 0.61	22.15 ± 0.84	22.84 ± 0.17	22.89 ± 1.30
	$\Delta E^{2.3}$	1.34 ± 0.06	1.68 ± 0.07	0.88 ± 0.03	-

[a,b] Means (n=3) ± SD within a row for a specific bread location with different superscripts, differ ($P < 0.05$)

[1] LH = low heat NFDM; 85/90 = low heat (LH) NFDM heated in a radio frequency dielectric heating (RFDH) unit to 85°C and held for 90 min; 85/180 = LH heated in RFDH to 85°C and held for 180 min; HH = high heat NFDM

[2] L^* represents lightness (scale 0 to 100) a^* represents red to green (100 to -100) and b^* represents blue to yellow (-100 to 100)

$\Delta E^{2.3}$represents color difference compared to HH bread

2.7 White Sauce Results and Discussion

2.7.1 White Sauce Color

When comparing color similarities between white sauces made with 85/90 or 85/180 with the white sauces produced with HH, the 85/90 displayed similar yellowness (4.8%), while 85/180 produced similar darkness (1.5%) (Table 2.14). Additionally, white sauces produced with 85/90 exhibited significantly more yellowness (10.7%) when compared with white sauces formulated with 85/180. However, the white sauce produced with the 85/180 showed a significantly lighter (higher L^*) (2.3%) color in comparison to white sauces made with 85/90.

The decrease of yellowness and lightness in white sauces produced with 85/180 could have occurred during the heating step when preparing white sauces, if the Maillard reactions were activated and initiated to expose free amine group (Nishimura, Goto, Higasa, Kawase, & Matsumura, 2001). Although the RFDH treatment did cause significant color differences of (L^*) and (b^*) in the white sauce, both white sauces formulated with 85/90 and 85/180 are considered visually similar to humans, as their overall color difference was below the humanly visible difference threshold of ΔE (3.7 units) (Sun-Waterhouse, Yeoh, Massarotto, Wibisono, & Wadha, 2010).

Table 2.14. Color properties of white sauces made formulated with various nonfat dry milk (NFDM) types[1]

	Nonfat Dry Milk Type[1]			
Color Properties	**LH**	**85/90**	**85/180**	**HH**
*L**[2]	86.76 ± 0.96[b,c]	85.62 ± 1.19[c]	87.61 ± 2.55[a,b]	88.91 ± 1.25[a]
*a**[2]	-1.13 ± 0.39	-1.30 ± 0.42	-1.43 ± 0.18	-1.30 ± 0.63
*b**[2]	14.60 ± 0.94[a]	15.28 ± 0.33[a]	13.65 ± 0.70[b]	14.58 ± 0.39[a]
$\Delta E^{2.3}$	2.60 ± 0.63	3.37 ± 0.60	2.54 ± 0.89	-

[a,b] Means (n=3) ± SD within a row with different superscripts, differ (P <0.05)
[1]LH = low heat NFDM; 85/90 = low heat (LH) NFDM heated in a radio frequency dielectric heating (RFDH) unit to 85°C and held for 90 min; 85/180 = LH heated in RFDH to 85°C and held for 180 min; HH = high heat NFDM
[2]*L** represents lightness (scale 0 to 100) *a** represents red to green (100 to -100) and *b** represents blue to yellow (-100 to 100)
$\Delta E^{2.3}$represents color difference compared to HH white sauce

2.7.2 White Sauce Firmness and Water Holding Capacity

White sauces displayed significant differences in firmness (Table 2.15). The white sauce formulated with 85/90 had significantly greater firmness (84.7%; 46.9%) and lower WHC (58.5%; 51.8%) when compared with white sauces formulated with HH and 85/180, respectively. However, the white sauce formulated with 85/180 exhibited the same firmness and WHC as the sauce formulated with HH.

Table 2.15. Physical properties of white sauces made formulated with various nonfat dry milk (NFDM) types[1]

	Nonfat Dry Milk Type[1]			
Physical Properties	**LH**	**85/90**	**85/180**	**HH**
Firmness (g)	87.38 ± 22.60[a]	82.57 ± 3.92[a]	56.20 ± 8.34[b]	44.7 ± 5.20[b]
Water holding capacity (%)	7.08 ± 0.39[c]	7.10 ± 0.46[c]	14.71 ± 1.41[b]	17.08 ± 1.65[a]

[a,b] Means (n=3) ± SD within a row with different superscripts, differ (P <0.05)
[1]LH = low heat NFDM; 85/90 = low heat (LH) NFDM heated in a radio frequency dielectric heating (RFDH) unit to 85°C and held for 90 min; 85/180 = LH heated in RFDH to 85°C and held for 180 min; HH = high heat NFDM

2.8 Caramel Results and Discussion

2.8.1 Caramel Color

When comparing caramels for color (*L**, *a**, *b**, and *ΔE**), the 85/180 caramels were significantly darker (lower *L**) (13.1%; 14.3%) compared with caramels made with HH and 85/90, respectively (Table 2.16). However, the caramels formulated with 85/90 displayed significantly similar color properties when compared to caramels produced with HH. Several individual factors can contribute to the color of finished caramel such as increased cooking temperatures and times during caramel processing or greater concentrations of reducing sugars, which could lead to more Maillard browning reactions (Miller & Hartel, 2015; Levin et al., 2016).

As well as imparting an overall darker color in the finished caramel, the incorporation of 85/90 caused similar redness (*a**) as caramels formulated with HH (1.9%) (Table 2.16). However, caramels produced with 85/180, produced caramels that displayed significantly lower redness compared with caramels formulated with 85/90 (8.6%) and HH (10.6%). Additionally, the 85/180 caramel displayed significantly different color properties (*L** and *a**) than HH and/or LH.

The ΔE* for 85/90 and 85/180 (Table 2.16) caramels was greater than the set acceptance limit for visual color matching at 3.7 units (ΔE*), indicating that these color of these caramels would be noticeably different to the human eye when compared with the HH caramel (Sun-Waterhouse et al., 2010).

Table 2.16. Color properties of caramel made formulated with various nonfat dry milk (NFDM) products[1]

	Nonfat Dry Milk Type[1]			
Color Properties	**LH**	**85/90**	**85/180**	**HH**
*L**[2]	45.91 ± 1.24[a]	47.25 ± 1.46[a]	40.48 ± 1.41[b]	46.61 ± 1.12[a]
*a**[2]	12.66 ± 0.58[a]	11.72 ± 0.96[b]	10.75 ± 0.65[c]	11.95 ± 0.36[a,b]
*b**[2]	26.93 ± 0.62[a]	27.47 ± 3.76[a]	22.38 ± 2.42[b]	25.56 ± 1.48[a,b]
$\Delta E^{2.3}$	2.55 ± 0.51	4.46 ± 0.88	8.77 ± 0.26	-

[a,b] Means (n=3) ± SD within a row with different superscripts, differ (P <0.05)
[1]LH = low heat NFDM; 85/90 = low heat (LH) nonfat dry milk heated in a radio frequency dielectric heating (RFDH) unit to 85°C and held for 90 min; 85/180 = LH heated in RFDH to 85°C and held for 180 min; HH = high heat NFDM
[2]*L** represents lightness (scale 0 to 100), *a** represents red to green (100 to -100), and *b** represents blue to yellow (-100 to 100)
$\Delta E^{2.3}$represents color difference compared to HH caramel

2.8.1 Caramel Water Activity

Water activity (**a_w**) is a colligative property meaning that a direct interaction occurs between molecules and free water. When the amount of dissolved solids increases, a_w decreases. During caramel processing, the finished caramel product will be significantly impacted by smaller molecules compared with larger molecules (Miller & Hartel, 2015). The water activity of caramels formulated with LH, 85/90, and 85/180, displayed significantly lower a_w (34.3%, 29.7%, and 16.6%, respectively) compared with caramels produced with HH (Table 2.17).

Table 2.17. Chemical and physical properties of caramel made formulated with various nonfat dry milk (NFDM) types[1]

	Nonfat Dry Milk Type[1]			
Physical Properties	**LH**	**85/90**	**85/180**	**HH**
Water activity	0.41 ± 0.04^{c}	0.43 ± 0.03^{c}	0.49 ± 0.04^{b}	0.58 ± 0.04^{a}
Firmness	786.29 ± 190.77^{a}	123.90 ± 11.30^{b}	93.26 ± 12.12^{b}	124.42 ± 33.38^{b}
Stickiness	-418.34 ± 136.28^{b}	-73.80 ± 23.32^{a}	-54.48 ± 6.18^{a}	-74.87 ± 17.13^{a}

[a,b] Means (n=3) ± SD within a row with different superscripts, differ (P <0.05)
[1]LH = low heat NFDM; 85/90 = low heat (LH) nonfat dry milk heated in a radio frequency dielectric heating (RFDH) unit to 85°C and held for 90 min; 85/180 = LH heated in RFDH to 85°C and held for 180 min; HH = high heat NFDM

2.8.1 Caramel Firmness and Stickiness

From a texture perspective, no significant differences were found between the stickiness (overall average of ~67 g) and firmness (overall average of ~113 g) of caramels made with HH, 85/90, and 85/180 (Table 2.17). However, caramels formulated with LH displayed significantly greater firmness and stickiness compared with caramels produced with 85/90 (145.6% and 140.2%, respectively), 85/180 (157.6% and 153.9%, respectively), or HH (124.4% and 139.3%, respectively). Due to the higher firmness and stickiness of caramel formulated with LH, larger standard deviations were observed in these caramels, probably because of the viscosity and surface chemistry affecting the data collection of the texture analysis probe during the penetration of the caramel itself (Foegeding & Steiner, 2002). As the texture analysis probe lowers, the caramel's characteristics can affect probe data collection due to increased likelihood to stick with the probe.

Chapter 3 - Summary

The dry heat process of LH-NFDM at 85°C for either 90 or 180 min (85/90 and 85/180, respectively) caused significantly decreased WPNI when compared with LH, suggesting that the process itself (a quick increase in temperature, followed by a hold period at the elevated temperature) will induce whey protein denaturation. Within the protein fractions, the dry-heated LH-NFMD displayed significantly increased protein contents in the soluble protein and insoluble protein compared with LH. This was interpreted as the dry heating process, produces greater concentrations of soluble aggregates and insoluble aggregates within the proteins, and may in fact bond with some non-protein fraction as well, e.g., lactose. The increased average mean diameter of the casein micelle size of the 85/180 sample supports the increase of insoluble aggregates seen with the protein fractionation. Additionally, the SDS-PAGE analyses suggests the formation of soluble aggregates may have different bonding mechanism's as the diffused protein bands suggested that bonds other than disulfide s occurred in the dry-heated LH. Overall these day may hint at the fact the protein denaturation in a dried skim milk product may differ from protein denaturation in the liquid system – milk.

The dry-heated LH produced similar quality properties as HH when formulated within food systems such as bread, white sauce, and caramel. When formulating the RFDH-treated LH, 85/90 and 85/180, within different model food systems that would typically utilize HH, many similar qualities were observed. For bread, bread loaves formulated with 85/90, displayed similar weight, firmness, and color properties as bread loaves formulated with HH. In comparison, specific volume and volume of bread loaves formulated with 85/180 and HH were similar.

However, in a white sauce food system, white sauces formulated with 85/180 exhibited the most similar quality attributes as white sauces formulated with HH in terms of color lightness,

firmness, and water-holding capacity. The 85/90 produced white sauces with significantly similar *b** values. Finally in a caramel system, both 85/90 and 85/180 could replace HH in caramel formulations. In terms of caramel texture, both 85/90 and 85/180 displayed similar firmness and stickiness as caramels formulated with HH. However, caramels produced with 85/90 produced the most similar color values as caramels formulated with HH.

Overall, the RFDH-treatment, 85°C, with a combined holding temperature of 90 or 180 min, produced some similar physical properties food systems as food systems formulated with HH. Although the amount of denatured whey proteins were not similar in these samples, further research, the RFDH-temperature and holding time could be adjusted for each food system to find the most desirable ratio depending on the desired finished quality of the food system. Additionally, further testing involving food systems typically incorporating LH (i.e. yogurt, ice cream, cheese) with RFDH-treated LH could be investigated.

Chapter 4 - Conclusion

Through the application of radio frequency dielectric heating (RFDH) on low-heat (LH) nonfat dry milk (NFDM) at 85°C with a holding time at 90 minutes or 180 minutes, many food systems, such as bread, white sauce, and caramels, displayed significantly similar quality finished food products when compared to those foods formulate with HH-NFDM. By controlling the RFDH temperature and time, it may be possible to form soluble aggregates that impart improved functionality into the food systems. Thus, the formation of soluble aggregates during RFDH produce similar physical properties of food systems with HH-NFDM incorporations. However, further research needs to be done to understand the physicochemical interactions occurring between the RFDH-treated LH-NFDM and other ingredients within formulations.

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Appendix A - WPNI Standard Curve and Equation

Table A.1. Whey protein nitrogen index (WPNI) standardization values.

Tube	T%				Average T%	WPNI (mg/g)
	R1*	R2	R3	R4		
1	54.60	54.20	54.30	54.40	54.38	7.44
2	60.70	61.20	60.80	60.80	60.88	6.08
3	68.90	68.10	68.20	68.60	68.45	4.72
4	76.80	77.10	76.80	77.20	76.98	3.38
5	85.50	86.00	85.40	85.90	85.70	2.03
6	94.10	94.30	94.20	94.60	94.30	0.66

*R1-4 represent measurement repetitions 1 through 4

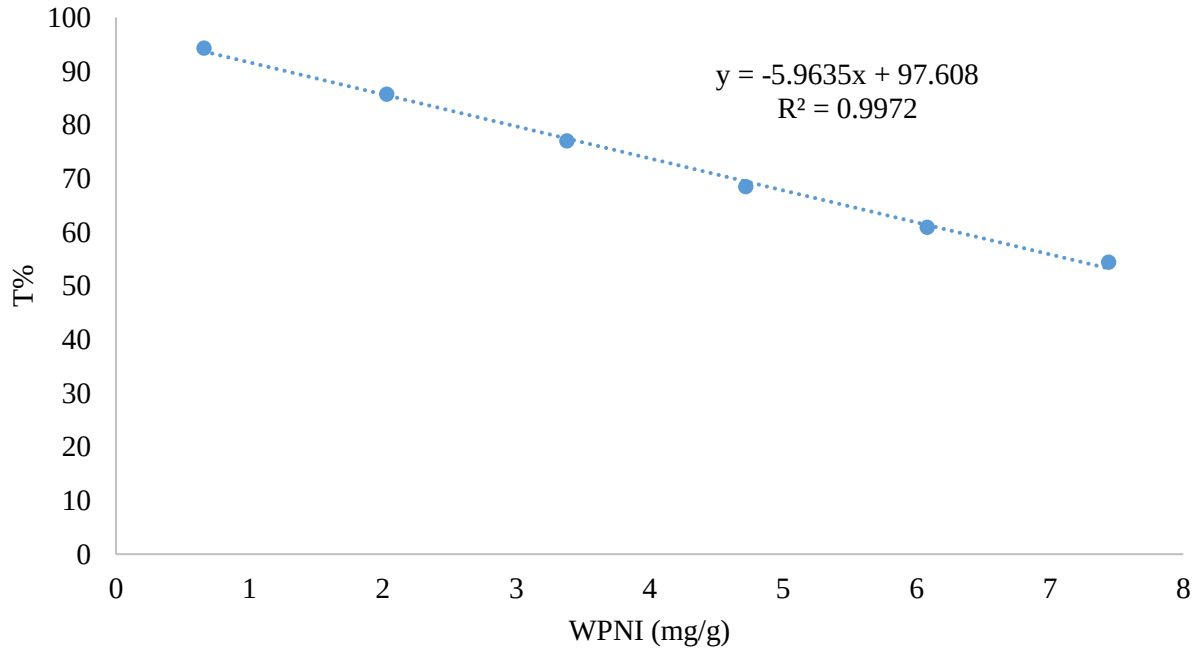


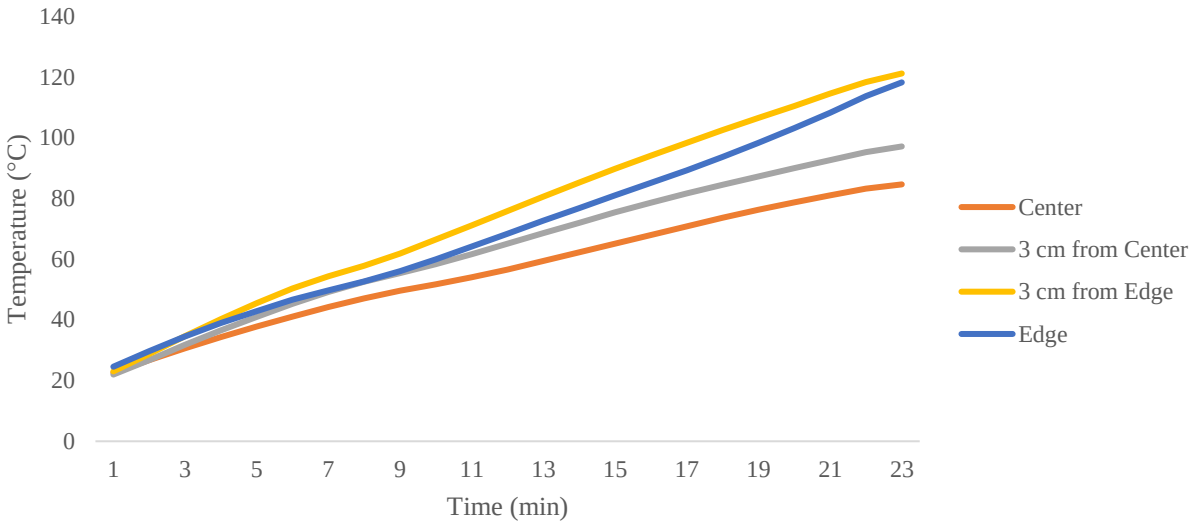
Figure A.1. Whey protein nitrogen index (WPNI) standard curve plotted by turbidity transmittance (T%) vs. whey protein nitrogen index (WPNI) (mg/g)

Appendix B - Kjeldahl Method

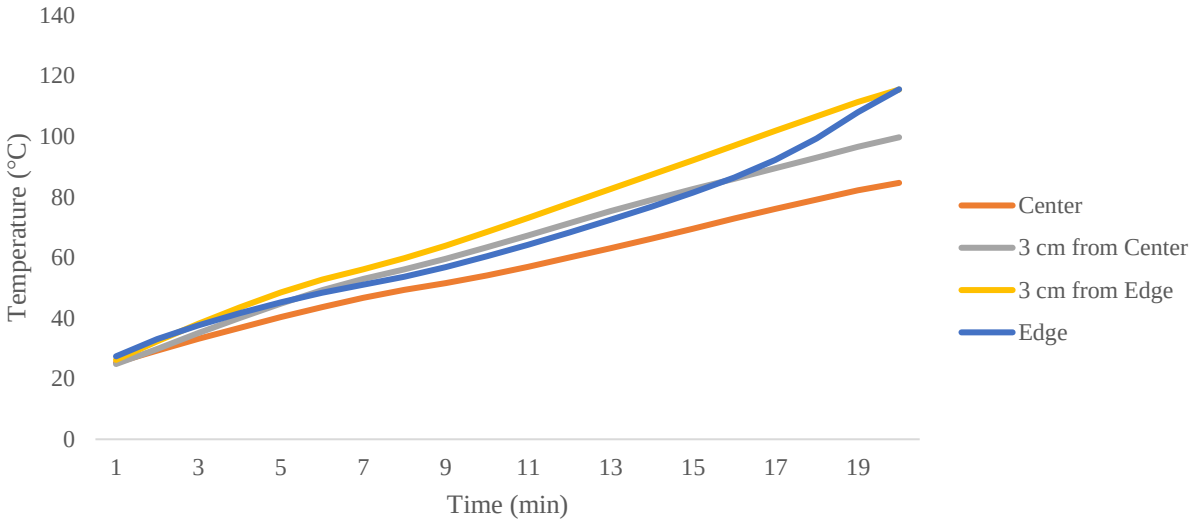
The Kjeldahl procedure for protein fractionation was tested at the Soil Testing and Plant Testing Laboratories at Kansas State University in Manhattan, KS. To find the TPN, 20 mL of reconstituted NFDM were pipetted into 200 mL Kjeldahl flask and 5 mL of nitrogen-free sulfuric acid (Fischer Scientific), 2 g of potassium sulfate (Fischer Scientific), 0.2 g of copper sulfate (Fischer Scientific), and two drops of selenium oxychloride (Fischer Scientific) were added to samples. The mixtures were heated for 15 min until contents in the flask were clear, cooled, and 50 mL of deionized water and 15 mL of 50 % sodium hydroxide solution were added to the Kjeldahl flasks. By performing steam distillation, ammonia was released directly from the Kjeldahl flasks through a small bulb trap and condenser and 25 mL of N/50 sulphuric acid (Fischer Scientific) and two drops of 0.1% methyl red solution (Fischer Scientific) were added. After 15 min, all of the ammonia was removed and the distillate was boiled to remove all CO_2, cooled, and excess acid was titrated with carbonate-free *N*/50 sodium hydroxide (Fischer Scientific) from a 10 mL burette reading to 0.02 mL. A multiplication factor of 6.38 was used to convert the nitrogen concentrations to protein (Rowland, 1938).

For the NCN and NPN, the Kjeldahl described to find the total nitrogen content was repeated. A multiplication factor of 6.38 was used to convert the nitrogen concentrations to protein (Rowland, 1938).

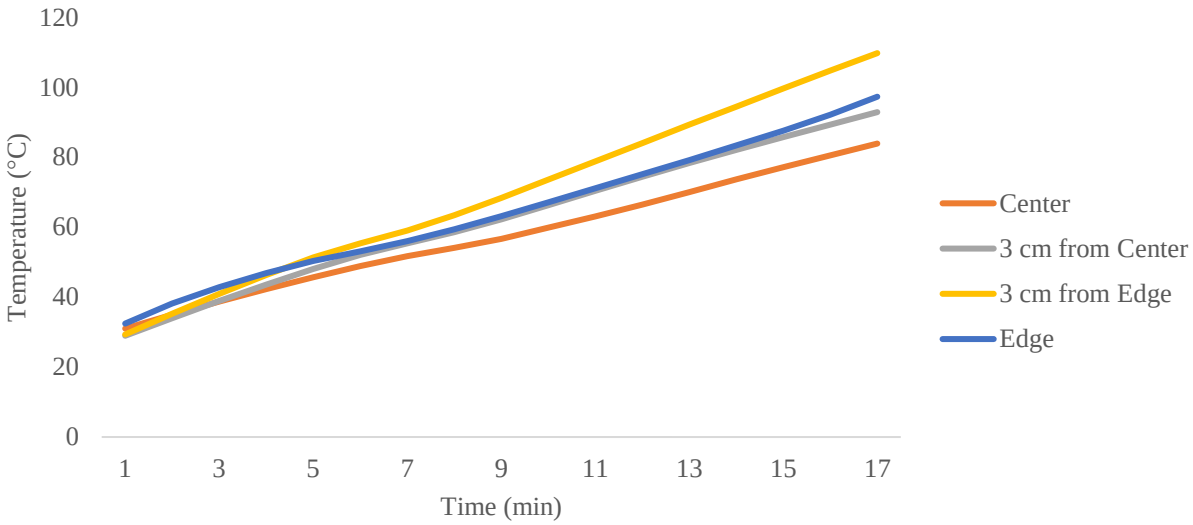
Appendix C - Radio Frequency Dielectric Heating (RFDH) Curves



(a)

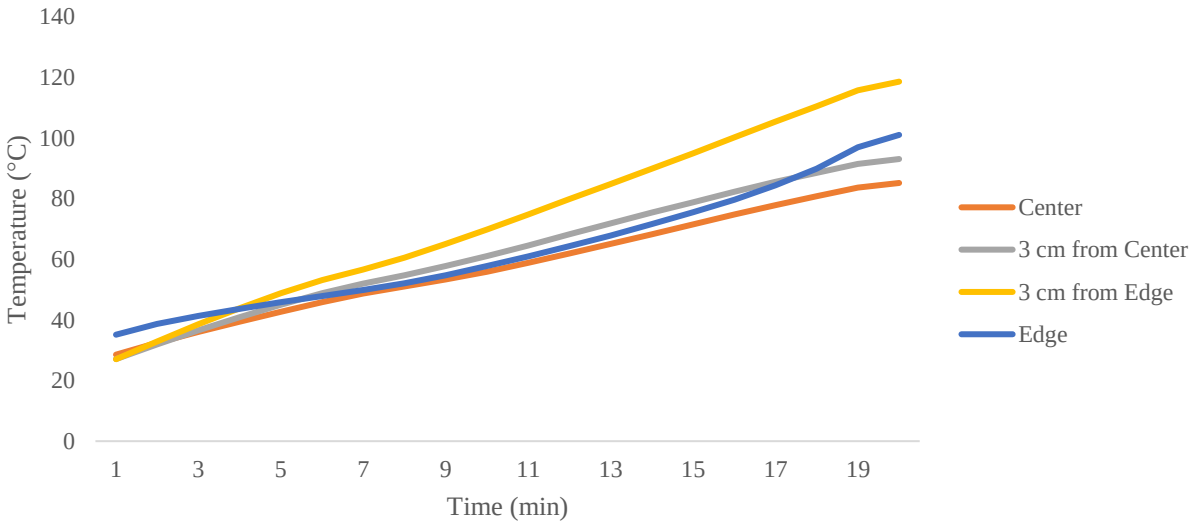


(b)

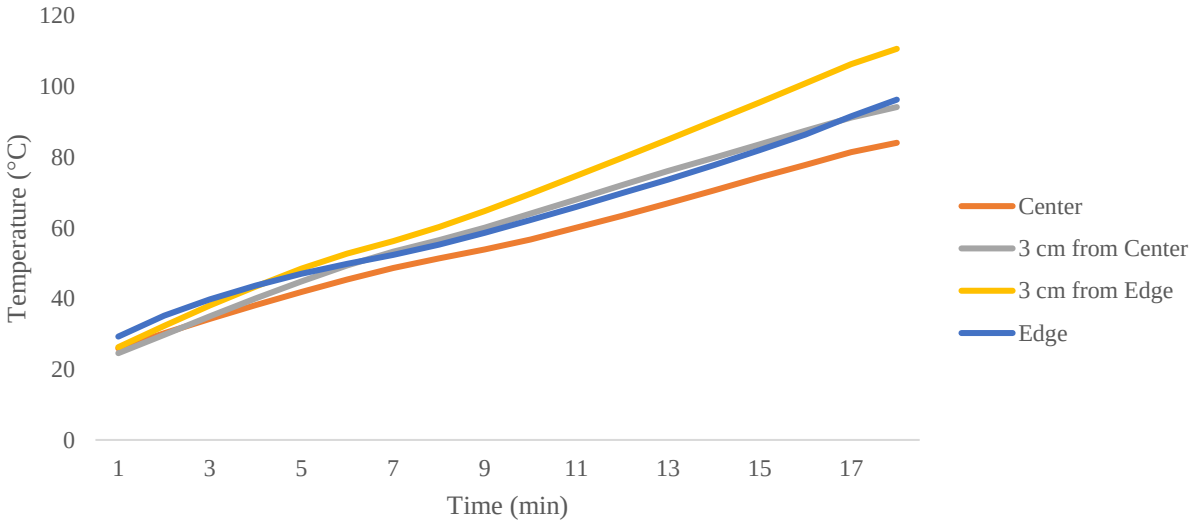


(c)

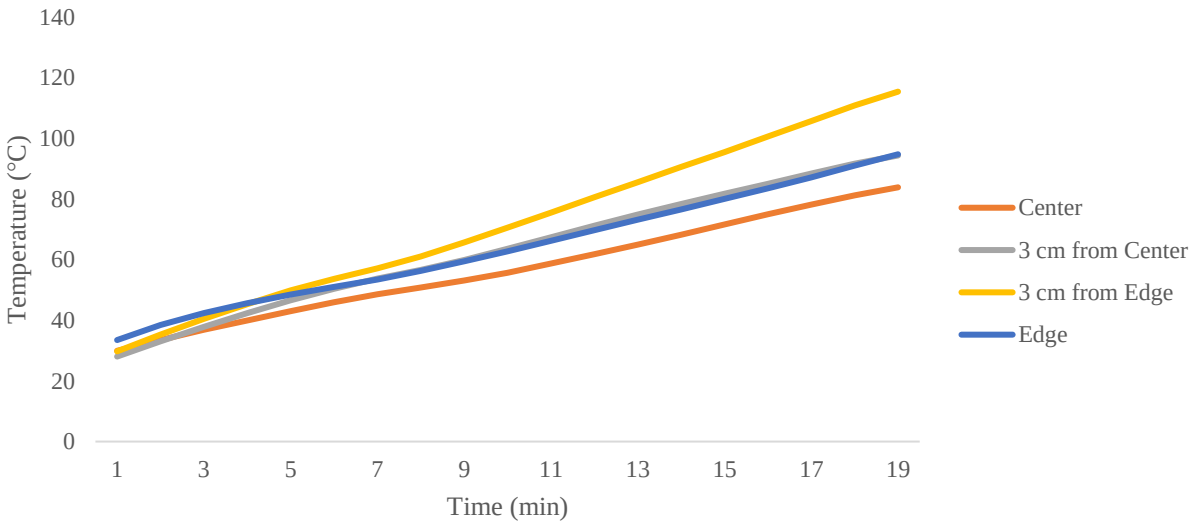
Figure C.1. Radio frequency dielectric heating of low heat nonfat dry milk from 30 to 85°C (target temperature for the center probe) in a circular polypropylene tray followed by continued heat exposure in a convection oven for 30 min. The legend shows the location of four fiber-optic probes for monitoring temperatures. (a) Rep 1; (b) Rep 2; (c) Rep 3.


140
120
100
80
60
40
20
0
Temperature (°C)
1
3
5
7
9
11
13
15
17
19
Time (min)
Center
3 cm from Center
3 cm from Edge
Edge

(a)


120
100
80
60
40
20
0
Temperature (°C)
1
3
5
7
9
11
13
15
17
Time (min)
Center
3 cm from Center
3 cm from Edge
Edge

(b)



(c)

Figure C.2. Radio frequency dielectric heating of low heat nonfat dry milk from 30 to 85°C (target temperature for the center probe) in a circular polypropylene tray followed by continued heat exposure in a convection oven for 180 min. The legend shows the location of four fiber optic proves for monitoring temperatures. (a) Rep 1, (b) Rep 2, (C) Rep 3.

Appendix D - Radio Frequency Dielectric Heating (RFDH) Times

Table D.1. Radio frequency dielectric heating (RFDH) of low-heat (LH) nonfat dry milk (NFDM) from 30 to 85°C (target temperature for the center probe) in a circular polypropylene tray followed by continued heat exposure in a convection oven for 90 min of Repetition 1.

Time (min)	Probe (°C)			
	Center	3 cm from Center	3 cm from Edge	Edge
0.00	22.62	21.98	23.03	24.56
1.00	26.73	26.77	28.72	29.72
2.00	30.62	31.74	34.64	34.51
3.00	34.32	36.51	40.24	38.90
4.00	37.78	41.00	45.51	42.90
5.00	41.10	45.26	50.33	46.60
6.00	44.22	49.18	54.39	49.72
7.00	47.07	52.58	57.83	52.67
8.00	49.55	55.39	61.84	56.03
9.00	51.76	58.31	66.42	59.97
10.00	54.04	61.63	71.11	64.11
11.00	56.60	65.12	75.89	68.39
12.00	59.42	68.56	80.59	72.64
13.00	62.26	71.98	85.22	76.84
14.00	65.09	75.40	89.76	81.01
15.00	67.96	78.63	94.10	85.13
16.00	70.80	81.67	98.32	89.26
17.00	73.63	84.44	102.49	93.61
18.00	76.24	87.18	106.49	98.30
19.00	78.65	89.93	110.42	103.16
20.00	81.01	92.64	114.50	108.22
21.00	83.17	95.23	118.36	113.72
22.00	84.61	97.11	121.14	118.21
0.00	22.62	21.98	23.03	24.56

Table D.2. Radio frequency dielectric heating (RFDH) of low-heat (LH) nonfat dry milk (NFDM) from 30 to 85°C (target temperature for the center probe) in a circular polypropylene tray followed by continued heat exposure in a convection oven for 90 min of Repetition 2.

Time (min)	Probe (°C)			
	Center	3 cm from Center	3 cm from Edge	Edge
0.00	25.29	24.83	26.20	27.37
1.00	29.28	29.80	32.28	33.01
2.00	33.16	35.03	38.15	37.60
3.00	36.80	40.03	43.53	41.61
4.00	40.29	44.77	48.50	45.23
5.00	43.60	49.18	52.73	48.40
6.00	46.66	52.93	56.09	51.02
7.00	49.30	56.07	59.76	53.69
8.00	51.58	59.53	63.92	56.76
9.00	54.09	63.39	68.44	60.39
10.00	56.90	67.31	73.06	64.20
11.00	59.96	71.30	77.82	68.24
12.00	63.07	75.26	82.54	72.42
13.00	66.22	78.99	87.29	76.73
14.00	69.50	82.63	92.11	81.41
15.00	72.83	86.01	96.90	86.40
16.00	76.04	89.45	101.80	92.18
17.00	79.06	92.93	106.57	99.24
18.00	82.14	96.55	111.36	107.94
19.00	84.62	99.66	115.42	115.50

Table D.3. Radio frequency dielectric heating (RFDH) of low-heat (LH) nonfat dry milk (NFDM) from 30 to 85°C (target temperature for the center probe) in a circular polypropylene tray followed by continued heat exposure in a convection oven for 90 min of Repetition 3.

Time (min)	Probe (°C)			
	Center	3 cm from Center	3 cm from Edge	Edge
0.00	31.01	28.95	29.33	32.44
1.00	34.98	33.96	35.21	38.13
2.00	38.73	38.90	40.93	42.85
3.00	42.30	43.59	46.33	46.89
4.00	45.71	48.10	51.35	50.31
5.00	48.92	52.10	55.40	53.10
6.00	51.70	55.36	59.01	56.01
7.00	54.10	58.55	63.46	59.43
8.00	56.69	62.28	68.39	63.16
9.00	59.83	66.27	73.56	67.08
10.00	63.10	70.36	78.82	71.09
11.00	66.47	74.44	84.02	75.10
12.00	70.04	78.32	89.28	79.16
13.00	73.67	82.13	94.46	83.33
14.00	77.13	85.78	99.64	87.56
15.00	80.53	89.31	104.77	92.17
16.00	83.90	92.90	109.76	97.31

Table D.4. Radio frequency dielectric heating (RFDH) of low-heat (LH) nonfat dry milk (NFDM) from 30 to 85°C (target temperature for the center probe) in a circular polypropylene tray followed by continued heat exposure in a convection oven for 180 min of Repetition 1.

Time (min)	Probe (°C)			
	Center	3 cm from Center	3 cm from Edge	Edge
0.00	28.54	27.09	27.05	35.13
1.00	32.46	31.97	32.92	38.71
2.00	36.05	36.58	38.57	41.33
3.00	39.45	40.87	43.87	43.69
4.00	42.71	44.99	48.85	45.91
5.00	45.86	48.78	53.13	47.90
6.00	48.72	52.03	56.67	49.87
7.00	51.09	54.76	60.57	52.12
8.00	53.37	57.76	65.13	54.78
9.00	55.93	61.12	69.86	57.76
10.00	58.84	64.61	74.81	60.96
11.00	61.94	68.24	79.86	64.38
12.00	65.06	71.82	84.80	67.89
13.00	68.29	75.43	89.92	71.62
14.00	71.56	78.86	95.03	75.50
15.00	74.84	82.26	100.26	79.71
16.00	77.84	85.55	105.41	84.40
17.00	80.83	88.53	110.49	89.91
18.00	83.72	91.52	115.76	96.98
19.00	85.20	93.10	118.64	101.06

Table D.5. Radio frequency dielectric heating (RFDH) of low-heat (LH) nonfat dry milk (NFDM) from 30 to 85°C (target temperature for the center probe) in a circular polypropylene tray followed by continued heat exposure in a convection oven for 180 min of Repetition 2.

Time (min)	Probe (°C)			
	Center	3 cm from Center	3 cm from Edge	Edge
0.00	25.90	24.55	26.25	29.24
1.00	30.22	29.71	32.23	35.18
2.00	34.29	34.98	38.01	39.76
3.00	38.16	40.02	43.39	43.68
4.00	41.88	44.87	48.43	47.05
5.00	45.40	49.38	52.73	49.84
6.00	48.65	53.26	56.24	52.39
7.00	51.37	56.52	60.20	55.28
8.00	53.87	60.08	64.80	58.59
9.00	56.73	63.99	69.66	62.21
10.00	60.01	68.04	74.72	65.94
11.00	63.40	72.06	79.82	69.79
12.00	66.88	76.05	84.90	73.67
13.00	70.54	79.82	90.16	77.68
14.00	74.29	83.58	95.42	81.92
15.00	77.83	87.44	100.85	86.42
16.00	81.37	91.21	106.34	91.54
17.00	84.06	94.16	110.63	96.26

Table D.6. Radio frequency dielectric heating (RFDH) of low-heat (LH) nonfat dry milk (NFDM) from 30 to 85°C (target temperature for the center probe) in a circular polypropylene tray followed by continued heat exposure in a convection oven for 180 min of Repetition 3.

Time (min)	Probe (°C)			
	Center	3 cm from Center	3 cm from Edge	Edge
0.00	29.95	28.06	29.77	33.50
1.00	33.55	33.07	35.28	38.49
2.00	36.86	37.89	40.46	42.39
3.00	40.01	42.36	45.33	45.69
4.00	43.04	46.57	49.85	48.56
5.00	45.94	50.46	53.74	51.00
6.00	48.64	53.76	57.18	53.49
7.00	50.83	56.61	61.10	56.29
8.00	53.12	59.96	65.72	59.45
9.00	55.74	63.63	70.55	62.82
10.00	58.72	67.37	75.57	66.27
11.00	61.82	71.17	80.60	69.79
12.00	64.99	74.93	85.55	73.18
13.00	68.27	78.43	90.60	76.60
14.00	71.63	81.80	95.56	80.08
15.00	75.00	85.10	100.64	83.55
16.00	78.15	88.40	105.73	87.13
17.00	81.30	91.67	110.89	91.07
18.00	83.90	94.41	115.47	94.80

Appendix E - NFDM Compositional Analysis SAS Code

```
data composition;
input rep $ type $ Protein Moisture Ash Carbohydrate Fat;
datalines;
1	HH	36.39	4.62	7.62	51.75	1.6
2	HH	36.47	3.61	8.25	51.24	1.4
3	HH	36.36	3.64	8.33	51.39	1.6
1	LH	35.99	2.00	8.47	50.63	1.6
2	LH	35.95	1.99	8.76	50.39	1.6
3	LH	36.04	2.94	7.55	51.84	1.6
;
run;

** Protein;
proc glimmix data = composition plots = all;
class rep type;
model protein = Type / ddfm = KR;
random rep;
	lsmeans type / pdiff adjust = tukey;
run;

** Moisture;
proc glimmix data = composition plots = all;
class rep type;
model moisture = Type / ddfm = KR;
random rep;
	lsmeans type / pdiff adjust = tukey;
run;

** Ash;
proc glimmix data = composition plots = all;
class rep type;
model ash = Type / ddfm = KR;
random rep;
	lsmeans type / pdiff adjust = tukey;
run;

** Carbohydrate;
proc glimmix data = composition plots = all;
class rep type;
model Carbohydrate = Type / ddfm = KR;
random rep;
	lsmeans type / pdiff adjust = tukey;
run;
```

**** Fat;**

```
proc glimmix data = composition plots = all;
class rep type;
model Carbohydrate = Type / ddfm = KR;
random rep;
        lsmeans type / pdiff adjust = tukey;
```

Appendix F - Reconstituted NFDM pH and Titratable Acidity

Analysis SAS Code

```
data TA;
input rep $ type $ TA @@;
datalines;

1 HH 1.117
2 HH 1.153
3 HH 1.117
1 LH 1.153
2 LH 1.162
3 LH 1.153
1 8590 1.162
2 8590 1.171
3 8590 1.162
1 85180 0.18
2 85180 0.18
3 85180 0.18;

run;

** TA;
proc glimmix data = TA plots = all;
class rep type;
model TA = Type / ddfm = KR;
random rep;
        lsmeans type / pdiff adjust = tukey;
run;
```

Appendix G - NFDM Protein Characterization SAS Code

```
data protein;
input Block $ Rep $ Type $ TotalProteinNitrogen NonCaseinNitrogen NonProteinNitrogen
SolubleProtein InsolubleProtein WheyProteinNitrogenIndex
@@;
datalines;
3 1 HH 3.28 0.30 0.16 0.15 2.97 1.17
6 2 HH 3.38 0.32 0.15 0.17 3.07 1.30
9 3 HH 3.33 0.31 0.15 0.16 3.02 1.25
3 1 LH 3.35 0.71 0.16 0.55 2.64 7.13
6 2 LH 3.23 0.70 0.17 0.53 2.52 7.33
9 3 LH 3.37 0.69 0.23 0.46 2.68 7.11
3 1 8590 3.45 0.76 0.17 0.59 2.69 5.20
6 2 8590 3.43 0.74 0.15 0.59 2.69 4.90
9 3 8590 3.50 0.79 0.16 0.63 2.71 4.94
3 1 85180 3.50 0.76 0.17 0.59 2.73 3.94
6 2 85180 3.46 0.77 0.17 0.60 2.70 4.48
9 3 85180 3.48 0.77 0.17 0.60 2.71 4.77
;
run;
proc sort data=protein out=yosort;
  by block rep type;
run;

**Total Protein Nitrogen;
proc glimmix data = protein plots = all;
class block rep type;
model TotalProteinNitrogen = Type / ddfm = KR;
random Block;
output out = Diagnostics student = student predicted = predicted;
        lsmeans type / pdiff adjust = tukey;
run;

** Non-Casein Nitrogen;
proc glimmix data = protein plots = all;
class block rep type;
model NonCaseinNitrogen = Type / ddfm = KR;
random Block;
output out = Diagnostics student = student predicted = predicted;
        lsmeans type / pdiff adjust = tukey;
run;

** Non-Protein Nitrogen;
proc glimmix data = protein plots = all;
class block rep type;
```

```
model NonProteinNitrogen = Type / ddfm = KR;
random Block;
output out = Diagnostics student = student predicted = predicted;
        lsmeans type / pdiff adjust = tukey;
run;
```

**** Soluble Protein;**

```
proc glimmix data = protein plots = all;
class block rep type;
model SolubleProtein = Type / ddfm = KR;
random Block;
output out = Diagnostics student = student predicted = predicted;
        lsmeans type / pdiff adjust = tukey;
run;
```

**** Insoluble Protein;**

```
proc glimmix data = protein plots = all;
class block rep type;
model InsolubleProtein = Type / ddfm = KR;
random Block;
output out = Diagnostics student = student predicted = predicted;
        lsmeans type / pdiff adjust = tukey;
run;
```

**** Whey Protein Nitrogen Index;**

```
proc glimmix data = protein plots = all;
class block rep type;
model WheyProteinNitrogenIndex = Type / ddfm = KR;
random Block;
output out = Diagnostics student = student predicted = predicted;
        lsmeans type / pdiff adjust = tukey;
```

Appendix H - NFDM Protein Fractions

Table H.1. Protein contents in various fractions (%) of nonfat dry milk (NFDM) types.

NFDM[1]	True Protein Nitrogen (%)	Non-Casein Nitrogen Protein (%)	Non-Protein Nitrogen (%)	Soluble Protein (%)	Insoluble Protein (%)
LH	3.12 ± 0.005[b]	0.70 ± 0.00[b]	0.19 ± 0.02	0.51 ± 0.002[b]	2.61 ± 0.07[c]
85/90	3.29 ± 0.0001[a]	0.76 ± 0.002[a]	0.16 ± 0.01	0.60 ± 0.0005[a]	2.70 ± 0.007[b]
85/180	3.31 ± 0.0002[a]	0.77 ± 0.00[a]	0.17 ± 0.00	0.60 ± 0.00[a]	2.71 ± 0.0002[b]
HH	3.18 ± 0.0034[b]	0.31 ± 0.00[c]	0.15 ± 0.00	0.16 ± 0.01[c]	3.02 ± 0.002[a]

[a,b]Means (n=3) ± SD within a column with different superscripts, differ (P <0.05)

[1]85/90 = low heat (LH) NFDM heated in a radio frequency dielectric heating (RFDH) unit to 85°C and held for 90 min; 85/180 = LH heated in RFDH to 85°C and held for 180 min; HH = high heat NFDM

Appendix I - Protein Analysis by SDS-PAGE

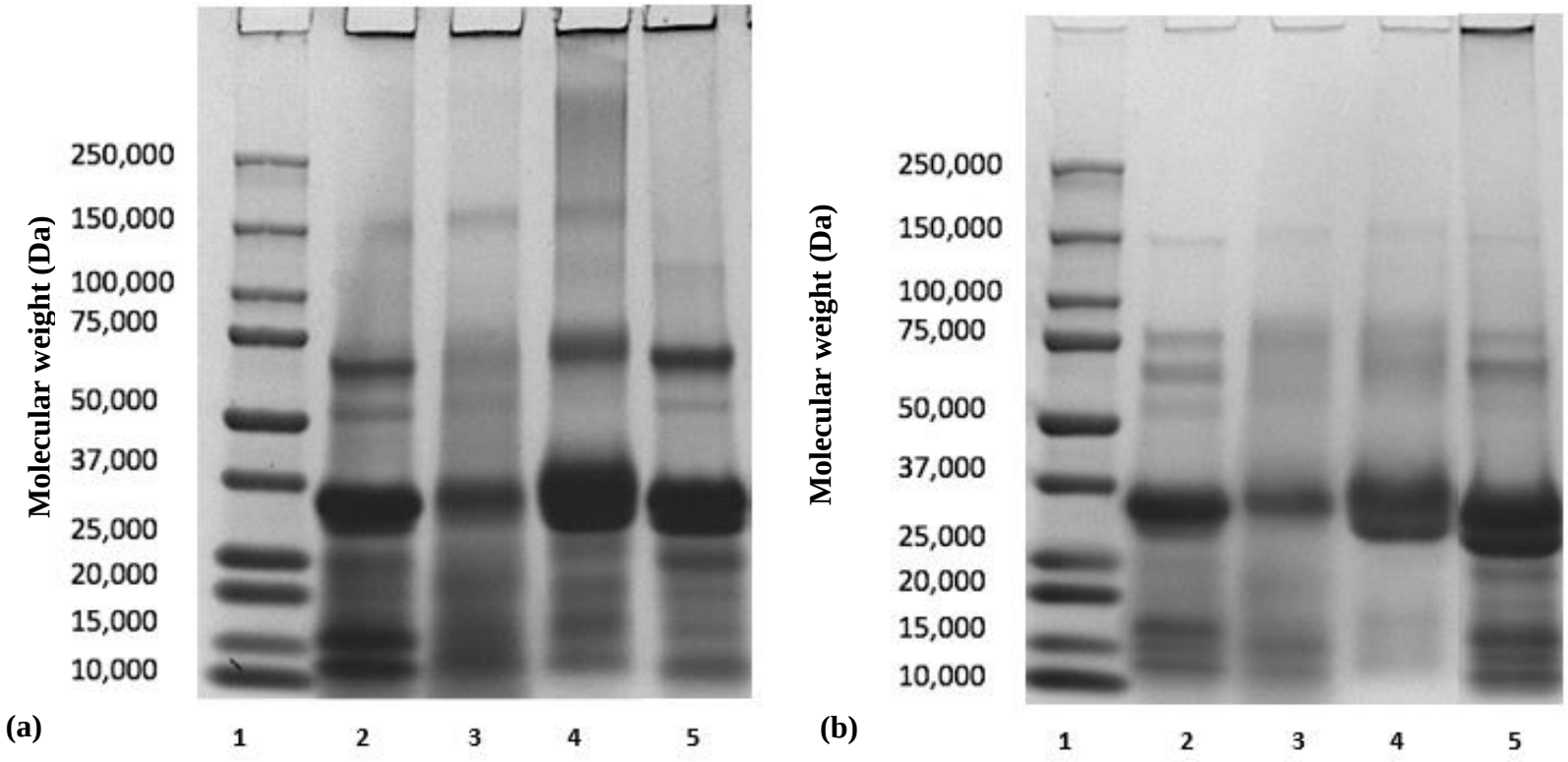


Figure I.1. Rep 2 SDS-PAGE of standard protein markers (lane 1) and reconstituted nonfat dry milk (NFDM) (10 µL) (lanes 2-5). All NFDM samples were rehydrated to 3.5% (w/v) protein. Low heat (LH) NFDM (lane 2), LH-NFDM RFDH-treated to 85°C and held for 90 min (85/90) (lane 3), LH-NFDM RFDH-treated to 85°C and held for 180 min (85/180) (lane 4), and high heat (HH) NFDM (lane 5). All NFDM samples were reconstituted to 3.5% protein, and diluted to 2 mg mL^{-1} protein. (a) without β-mercaptoethanol (b) with β-mercaptoethanol.

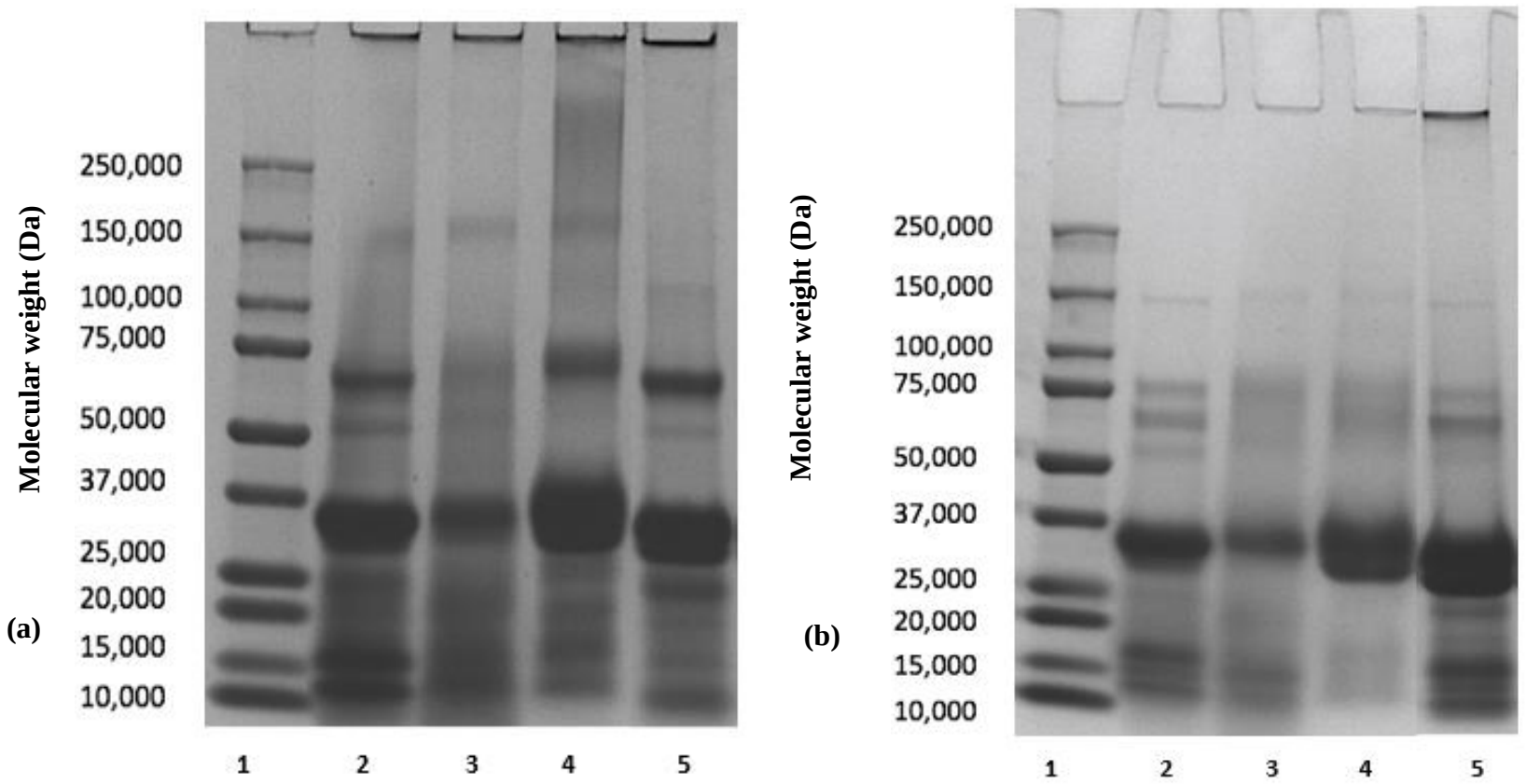


Figure I.2. Rep 3 SDS-PAGE of standard protein markers (lane 1) and reconstituted nonfat dry milk (NFDM) (10 µL) (lanes 2-5). All NFDM samples were rehydrated to 3.5% (w/v) protein. Low heat (LH) NFDM (lane 2), LH-NFDM RFDH-treated to 85°C and held for 90 min (85/90) (lane 3), LH-NFDM RFDH-treated to 85°C and held for 180 min (85/180) (lane 4), and high heat (HH) NFDM (lane 5). All NFDM samples were reconstituted to 3.5% protein, and diluted to 2 mg mL^{-1} protein. (a) without β-mercaptoethanol (b) with β-mercaptoethanol.

Appendix J - Bread SAS Code

```
data bread;
input Block $ Rep $ Type $ OvenRun $ TopL Topa Topb SideL Sidea Sideb BottomL Bottoma
Bottomb CrumbL Crumba Crumbb Weight Volume SpecificVolume Firmness Consistency
DevelopmentTime Stability DofSoftening DofSoftening WAbsorption_500 WAbsorption_14
FQNumber
;
datalines;
1 2 HH 1 61.07 15.08 34.03 46.38 18.07 31.88 51.67 16.65 33.11
77.32 1.79 23.19 444.00 2218.18 5.00 466.99 543.00 1.90 14.40 29.00
31.00 71.10 64.30 39.00
2 4 HH 2 61.79 15.65 36.06 46.97 16.88 32.77 52.26 17.44 34.47
75.14 1.91 24.31 444.00 2225.76 5.01 471.75 499.00 1.90 13.30 39.00
38.00 70.00 63.20 41.00
3 6 HH 1 61.79 15.50 36.28 47.90 18.31 32.53 52.05 16.85 34.00
77.39 1.10 21.18 445.00 2222.73 4.99 419.12 526.00 2.20 13.90 32.00 36.00
70.70 63.90 58.00
1 2 LH 1 60.66 15.15 34.23 49.95 17.36 32.86 49.18 19.02 34.38
77.27 1.77 23.19 446.00 845.45 1.90 491.09 507.00 2.30 15.20 15.00 24.00
70.20 63.40 158.00
2 4 LH 2 59.65 16.28 34.67 49.94 18.43 32.23 50.94 19.31 35.28
74.89 1.26 22.70 446.00 872.73 1.96 495.60 512.00 2.00 12.80 17.00 36.00
70.30 63.50 133.00
3 6 LH 1 59.37 16.58 35.20 45.73 18.55 31.92 50.98 18.09 36.65
76.40 1.28 21.72 446.00 878.79 1.97 451.54 534.00 2.90 13.10 11.00 40.00
70.90 64.10 135.00
1 2 8590 2 56.49 16.80 33.79 47.45 18.62 33.76 52.26 17.14 35.13
76.80 1.52 22.77 444.00 1200.00 2.70 457.37 526.00 1.70 14.40 11.00 23.00
70.70 63.90 153.00
2 4 8590 1 61.07 14.96 35.75 48.44 19.02 33.44 52.13 17.39 35.68
77.54 1.45 22.71 445.00 1193.94 2.68 438.16 522.00 1.70 11.70 25.00 47.00
70.60 63.80 118.00
3 6 8590 2 61.13 15.26 34.56 47.13 17.92 32.22 50.94 17.99 34.42
75.74 1.09 20.96 445.00 1524.24 3.43 353.11 544.00 1.40 12.40 9.00 63.00
71.10 64.30 135.00
1 2 85180 2 61.90 15.07 35.01 49.48 19.01 35.10 52.62 17.07 33.53
77.91 1.43 22.95 443.00 1751.52 3.95 276.20 502.00 1.90 13.80 10.00 69.00
70.10 63.30 150.00
2 4 85180 1 60.82 15.71 35.78 47.12 18.61 33.03 52.13 18.08 34.10
75.55 1.75 22.96 444.00 1740.91 3.92 310.46 528.00 1.90 12.80 12.00 36.00
70.70 63.00 133.00
3 6 85180 2 60.16 15.71 35.85 48.35 18.08 32.77 52.15 17.63 34.84
73.30 1.59 22.60 444.00 1930.30 4.35 372.93 529.00 2.20 14.30 8.00
31.00 70.70 63.90 139.00
;
```

```
run;

** Top L*;
proc glimmix data = bread plots = all;
class block rep type OvenRun;
model TopL = Type / ddfm = KR;
random rep OvenRun(rep);
        lsmeans type / pdiff adjust = tukey;
run;

** Top a*;
proc glimmix data = bread plots = all;
class block rep type OvenRun;
model Topa = Type / ddfm = KR;
random rep OvenRun(rep);
        lsmeans type / pdiff adjust = tukey;
run;

** Top b*;
proc glimmix data = bread plots = all;
class block rep type OvenRun;
model Topb = Type / ddfm = KR;
random rep OvenRun(rep);
        lsmeans type / pdiff adjust = tukey;
run;

** Side L*;
proc glimmix data = bread plots = all;
class block rep type OvenRun;
model SideL = Type / ddfm = KR;
random rep OvenRun(rep);
        lsmeans type / pdiff adjust = tukey;
run;

** Side a*;
proc glimmix data = bread plots = all;
class block rep type OvenRun;
model Sidea = Type / ddfm = KR;
random rep OvenRun(rep);
        lsmeans type / pdiff adjust = tukey;
run;

** Side b*;
proc glimmix data = bread plots = all;
class block rep type OvenRun;
model Sideb = Type / ddfm = KR;
```

```
random rep OvenRun(rep);
        lsmeans type / pdiff adjust = tukey;
run;

** Bottom L*;
proc glimmix data = bread plots = all;
class block rep type OvenRun;
model BottomL = Type / ddfm = KR;
random rep OvenRun(rep);
        lsmeans type / pdiff adjust = tukey;
run;

** Bottom a*;
proc glimmix data = bread plots = all;
class block rep type OvenRun;
model Bottoma = Type / ddfm = KR;
random rep OvenRun(rep);
        lsmeans type / pdiff adjust = tukey;
run;

** Bottom b*;
proc glimmix data = bread plots = all;
class block rep type OvenRun;
model Bottomb = Type / ddfm = KR;
random rep OvenRun(rep);
        lsmeans type / pdiff adjust = tukey;
run;

** Crumb L*;
proc glimmix data = bread plots = all;
class block rep type OvenRun;
model CrumbL = Type / ddfm = KR;
random rep OvenRun(rep);
        lsmeans type / pdiff adjust = tukey;
run;

** Crumb a*;
proc glimmix data = bread plots = all;
class block rep type OvenRun;
model Crumba = Type / ddfm = KR;
random rep OvenRun(rep);
        lsmeans type / pdiff adjust = tukey;
run;

** Crumb b*;
proc glimmix data = bread plots = all;
```

```
class block rep type OvenRun;
model Crumbb = Type / ddfm = KR;
random rep OvenRun(rep);
        lsmeans type / pdiff adjust = tukey;
run;
```

**** Weight;**

```
proc glimmix data = bread plots = all;
class block rep type OvenRun;
model Weight = Type / ddfm = KR;
random rep OvenRun(rep);
        lsmeans type / pdiff adjust = tukey;
run;
```

**** Volume;**

```
proc glimmix data = bread plots = all;
class block rep type OvenRun;
model Volume = Type / ddfm = KR;
random rep OvenRun(rep);
        lsmeans type / pdiff adjust = tukey;
run;
```

**** Specific Volume;**

```
proc glimmix data = bread plots = all;
class block rep type OvenRun;
model SpecificVolume = Type / ddfm = KR;
random rep OvenRun(rep);
        lsmeans type / pdiff adjust = tukey;
run;
```

**** Firmness;**

```
proc glimmix data = bread plots = all;
class block rep type OvenRun;
model Firmness = Type / ddfm = KR;
random rep OvenRun(rep);
        lsmeans type / pdiff adjust = tukey;
run;
```

**** Consistency;**

```
proc glimmix data = bread plots = all;
class block rep type OvenRun;
model Consistency = Type / ddfm = KR;
random rep OvenRun(rep);
        lsmeans type / pdiff adjust = tukey;
run;
```

```
** Development Time;
proc glimmix data = bread plots = all;
class block rep type OvenRun;
model DevelopmentTime = Type / ddfm = KR;
random rep OvenRun(rep);
        lsmeans type / pdiff adjust = tukey;
run;

** Stability;
proc glimmix data = bread plots = all;
class block rep type OvenRun;
model Stability = Type / ddfm = KR;
random rep OvenRun(rep);
        lsmeans type / pdiff adjust = tukey;
run;

** Degree of Softening;
proc glimmix data = bread plots = all;
class block rep type OvenRun;
model DofSoftening = Type / ddfm = KR;
random rep OvenRun(rep);
        lsmeans type / pdiff adjust = tukey;
run;

** Water Absorption (500);
proc glimmix data = bread plots = all;
class block rep type OvenRun;
model WAbsorption_500 = Type / ddfm = KR;
random rep OvenRun(rep);
        lsmeans type / pdiff adjust = tukey;
run;

** Water Absorption (14);
proc glimmix data = bread plots = all;
class block rep type OvenRun;
model WAbsorption_14 = Type / ddfm = KR;
random rep OvenRun(rep);
        lsmeans type / pdiff adjust = tukey;
run;

** Farinograph Quality Number;
proc glimmix data = bread plots = all;
class block rep type OvenRun;
model FQNumber = Type / ddfm = KR;
random rep OvenRun(rep);
        lsmeans type / pdiff adjust = tukey;
```

```
run;
```

Appendix K - White Sauce SAS Code

```
data sauce;
input Block Rep Type $ Syneresis Firmness L a b WaterActivity
;
datalines;
1       1       HH      18.97   39.40   87.83   -0.45   15.08   0.984
2       2       HH      15.23   45.15   88.79   -1.95   14.22   0.989
3       3       HH      17.05   49.54   90.12   -1.51   14.42   0.981
1       1       LH      7.27    62.86   85.76   -1.38   13.35   0.985
2       2       LH      6.86    117.23  87.26   -1.43   14.85   0.983
3       3       LH      7.13    82.03   87.27   -0.59   15.60   0.983
1       1       8590    7.57    82.88   84.45   -0.75   15.73   0.989
2       2       8590    6.94    78.57   85.23   -1.72   15.02   0.988
3       3       8590    6.78    86.25   87.18   -1.43   15.08   0.981
1       1       85180   15.65   47.32   86.07   -1.21   13.43   0.983
2       2       85180   13.50   65.79   85.56   -1.62   14.56   0.984
3       3       85180   14.99   55.49   91.19   -1.47   12.98   0.986
;
run;

proc sort data=sauce out=sausort;
  by block  type;
run;

** Syneresis;
proc glimmix data = sauce plots = all;
class block rep type;
model syneresis = Type / ddfm = KR;
random Block;
        lsmeans type / pdiff adjust = tukey;
run;

** Firmness;
proc glimmix data = sauce plots = all;
class block rep type;
model Firmness = Type / ddfm = KR;
random Block;
        lsmeans type / pdiff adjust = tukey;
run;

** L*;
proc glimmix data = sauce plots = all;
class block rep type;
model L = Type / ddfm = KR;
random Block;
```

```
        lsmeans type / pdiff adjust = tukey;
run;

** a*;
proc glimmix data = sauce plots = all;
class block rep type;
model a = Type / ddfm = KR;
random Block;
        lsmeans type / pdiff adjust = tukey;
run;

** b*;
proc glimmix data = sauce plots = all;
class block rep type;
model b = Type / ddfm = KR;
random Block;
        lsmeans type / pdiff adjust = tukey;
run;

** Water Activity;
proc glimmix data = sauce plots = all;
class block rep type;
model WaterActivity = Type / ddfm = KR;
random Block;
        lsmeans type / pdiff adjust = tukey;
run;
```

Appendix L - Caramel SAS Code

```
data caramel;
input Block $ Rep $ Type $ WaterActivity L a b Firmness Stickiness
;
datalines;
1       1       HH      0.55    46.99   11.97   24.88   98.53   -66.71
2       2       HH      0.57    47.39   11.77   24.60   130.40  -82.47
3       3       HH      0.61    45.46   12.11   27.20   144.33  -75.44
1       1       LH      0.38    45.67   12.33   26.63   615.19  -353.25
2       2       LH      0.42    44.92   12.20   26.71   950.22  -553.20
3       3       LH      0.42    47.13   13.45   27.46   793.47  -348.56
1       1       8590    0.42    48.48   11.90   28.26   119.03  -74.49
2       2       8590    0.46    46.31   12.65   31.23   73.70   -48.50
3       3       8590    0.41    46.97   10.61   22.91   178.97  -98.41
1       1       85180   0.44    41.26   11.13   23.93   93.38   -57.41
2       2       85180   0.52    41.45   11.20   24.08   95.23   -54.61
3       3       85180   0.52    38.73   9.93    19.13   91.16   -51.42
;
run;

proc sort data=caramel out=carasort;
  by block type;
run;

** Water Activity;
proc glimmix data = caramel plots = all;
class block rep type;
model WaterActivity = Type  / ddfm = KR;
random Block;
        lsmeans type / pdiff adjust = tukey;
run;

** L*;
proc glimmix data = caramel plots = all;
class block rep type;
model L = Type  / ddfm = KR;
random Block;
        lsmeans type / pdiff adjust = tukey;
run;

** a*;
proc glimmix data = caramel plots = all;
class block rep type;
model a = Type  / ddfm = KR;
random Block;
```

```
        lsmeans type / pdiff adjust = tukey;
run;

** b*;
proc glimmix data = caramel plots = all;
class block rep type;
model b = Type  / ddfm = KR;
random Block;
        lsmeans type / pdiff adjust = tukey;
run;

** Firmness;
proc glimmix data = caramel plots = all;
class block rep type;
model Firmness = Type  / ddfm = KR;
random Block;
        lsmeans type / pdiff adjust = tukey;
run;

** Stickiness;
proc glimmix data = caramel plots = all;
class block rep type;
model Stickiness = Type  / ddfm = KR;
random Block;
        lsmeans type / pdiff adjust = tukey;
run;
```