STUDY OF REHYDRATION PROPERTIES OF POWDER PRODUCED FROM
CHELATED SKIM MILK

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by
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ABSTRACT

Study of Rehydration Properties of Powder Produced from Chelated Skim Milk

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Poor rehydration properties of skim milk powder (SMP) can impact processing efficiency and functionality in finished product applications. Rehydration can be split into four stages: wettability, sinkability, dispersibility, and solubility. Previous work has suggested that chelator addition during SMP manufacture leads to higher solubility compared to SMP without chelators. This study focuses on the addition of ethylene diamine tetraacetic acid (EDTA) or sodium citrate dihydrate (SCD) at concentrations of 5, 10, and 15 mM to skim milk prior to evaporating and spray drying. The objective of this investigation was to determine the effects of the chelator additions on each SMP rehydration property (wettability, sinkability, dispersibility, and solubility) during reconstitution to 10% total solids. SCD 15 mM, SCD 10 mM, and SCD 5 mM did not have a significant effect on wettability as measured by IDF method (p-value 0.3234, 0.6376, and 1.0000, respectively). However, SCD 15 mM, SCD 10 mM, and SCD 5 mM had higher levels of solubility as measured by particle size analysis of reconstituted 10%TS samples (p-value <0.0001 for all levels) and by solubility index (p-value <0.0001 for all levels), compared to the control. EDTA 15 mM and EDTA 10 mM treated SMP's had statistically significantly lower initial wettability compared to the control as measured by both contact angle (p-value <0.0001 and 0.0002 respectively) and International Dairy Federation (IDF) method (p-value <0.0001 and 0.0031 respectively). This suggests that EDTA 15

mM and EDTA 10 mM treated SMP's had high surface hydrophobicity. However, both treatments had higher levels of solubility compared to the control, as measured by particle size distribution of a fully solubilized reconstituted 10%TS sample (p-value <0.0001 for both levels) and by solubility index (p-value <0.0001 for both levels). Changes in the rehydration properties may be due to a shift in the calcium and phosphorus behavior of the treated samples, as measured by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). The changes to the solubility index could be due to a sequence of events. First, the chelators chelate all the ionic calcium in the serum. The decrease in ionic calcium shifts the protein-mineral balance between the minerals in the casein micelle and the minerals in the serum, which leads to a depletion of colloidal calcium phosphate, and a subsequent release of caseins from the initial micelle structure. This behavior influenced by EDTA or SCD dissolves the calcium phosphate bridge so that nanoclusters in the casein micelle do not exist (Dalglish and Corredig, 2012). Hence, the casein micelle would be disrupted into individual caseins that may create the appearance that the solution as a whole has higher solubility, as seen in the particle size analyses of fully rehydrated reconstituted 10%TS samples and in the solubility index. Overall, the findings provide insight on how chelators affect the rehydration properties of skim milk powder, when the chelator is added to liquid skim milk, prior to evaporating and spray drying. The findings in effects on rehydration properties may assist dairy powder manufacturers if deciding to add chelators to affect the end product rehydration properties.

Keywords: skim milk powder, chelator, wettability, sinkability, dispersibility, solubility, rehydration properties, reconstitution properties

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CHAPTER 1: INTRODUCTION

Fresh liquid milk is a functional ingredient and good source of nutrition, but can spoil if not maintained properly. Reasons for concentrating milk into powders include to reduce costs for storage and transportation and to reduce water activity in order to increase microbiological and chemical stability. Moreover, milk powder functionality is essential in many applications for nutrition supplementation, browning and flavor development, water binding, emulsification, viscosity modification, and texture. However, poor rehydration properties can impact process efficiency and operating costs of milk powder applications. Thus, it is important to understand and optimize rehydration properties for processing efficiency and for functionality in finished product applications (Bylund, 1995; American Dairy Products Institute, 1990; Chandan and Kilara, 2008).

For different desired functionality, it is possible to disrupt the micelle structure of the most common protein in milk, casein, through the addition of chelators (Gaucheron, 2005). Chelation is defined as the formation of a bond between two or more binding sites within the same compound to a single metal ion, such as the calcium in the casein micelle, to create very stable complexes (IUPAC, 1997). Chelating compounds, or chelators, are legal, safe, and widely used. Thus, calcium chelators can alter the structure and end functionality of casein, which ultimately affects the rehydration properties of SMP.

Rehydration can be described in four stages: wettability, sinkability, dispersibility, and solubility. Wettability is the initial water absorption on the SMP particle surface. Sinkability is when the powder particles drop below the surface of the water, after overcoming the surface tension of the water (Fang, 2008). Sinkability is closely

associated with wettability, sometimes even interchangeable. (ISO 707 | IDF 50:2014).

Dispersibility is defined as the speed with which the powder lumps and agglomerates fall apart when reconstituted with water. The final stage, solubility, is defined as the measure of the final condition of the powder constituents interacting with the water. Overall, the rehydration process is dictated by the functionality of the casein protein, which in turn can be altered by the addition of chelators.

The purpose of this study is to assess the impact of chelators on each rehydration property of SMP. This investigation focuses on the addition of the chelators Disodium Ethylene Diamine Tetraacetic Acid (EDTA) or Sodium Citrate Dihydrate (SCD) at concentrations of 5, 10, and 15 mM to fat free milk prior to evaporating and spray drying.

CHAPTER 2: LITERATURE REVIEW

The overall objective of this literature review is to provide background on the importance of skim milk powder and its functional properties with respect to its rehydration properties (wettability, sinkability, dispersibility, and solubility). This review will first define the skim milk powder system, while providing detail on the mineral and protein components and how changes in those two components can affect the rehydration properties. Each rehydration property will be described and methods of measurement for each, as well as a method to determine the mineral behavior, will be analyzed. Finally, an investigation will be proposed to study the effects of the addition of specific chelators at given levels on each rehydration property during reconstitution to 10% total solids.

2.1 Skim milk powder (SMP)

2.1.1 History and Importance

Skim milk powder dates back to the 13th century, when Marco Polo reported that the soldiers of Kublai Khan carried sun-dried milk. It was not until the early 19th century that the first patents for the modern production of powdered milk were introduced. Roller drying was the popular method before spray drying was established in the 1960s. Milk powders are now widespread around the globe, with products spanning from infant formula to baked goods to recombined dairy products (Pearce, 2015). Overall, milk powders are easily transported, stored, and incorporated into products such as baked goods, confectionary, and ready-to-use therapeutic foods. Skim milk powder is substantial in the global market where New Zealand and the United States of America (USA) are the largest exporters of skim milk powder. Skim milk powder can sometimes be confused with the term Non Fat Dry Milk but there are differences between the two

products that will be later described. One of the largest differences is that powder can be standardized to obtain a skim milk product, but with non fat dry milk, no standardization is permitted. In general, the market in the United States recognizes skim milk powder as Non Fat Dry Milk (NFDm). For the USA market, Dairy Management Inc. (DMI) reports that May 2015 was the second highest ever USA export volume of NFDm, only behind June 2014. Moreover, USA dairy exports of nonfat dry milk, whole milk powder, dry whey, whey protein concentrate/ isolate, and lactose reached 414,462 metric tons between March and May 2015 (DMI, 2015). Thus, skim milk powder and non fat dry milk can be seen as substantial in the dairy industry. This research uses the term skim milk powder over nonfat dry milk.

2.1.2 Manufacture of Skim Milk Powder (SMP)

The manufacturing of SMP is important in providing the nutritional variety of milk in a shelf-stable manner. Compared to other species for sources of milk, bovine milk is widely accepted in providing nutritional and functional needs. Table 2.1 compares bovine milk percent composition to the percent composition of milk of other species.

**Table 2.1. Typical composition of milk of various species (% composition)
(adapted from Chandan and Kilara, 2008)**

Species	Water	Fat	Protein	Lactose	Ash
Cow	86.6	4.6	3.4	4.9	0.5
Goat	86.5	4.5	3.5	4.7	0.8
Human	87.7	3.6	1.8	6.8	0.1
Mare	89.1	1.6	2.7	6.1	0.5

However, varying seasons can cause fluctuation in milk production and composition but this and other factors can be managed through standardizations of SMP. For Codex

Alimentarius (2011), SMP has not more than 5.0% moisture by weight nor more than 1.5% fat by weight and a minimum protein level of 34.0%. The typical percent composition of SMP compared to skim milk and whole bovine milk is shown in Table 2.2.

Table 2.2. Typical percent composition of skim milk powder vs. skim milk vs. bovine milk (Average percentage) (Canadian Dairy Commission, 2011)

Component	Skim milk powder	Skim milk	Bovine milk
Water	3.7	89.0	87.6
Lactose	52.4	5.1	4.7
Fat	1.2	0.2	3.7
Proteins	34.0	4.8	3.3
Ash	8.7	0.9	0.7

Overall, SMP contains lactose and protein in the same relative proportions as the fresh milk from which it was made (Chandan and Kilara, 2008). Approximately 13 kg of whole milk powder (WMP) or 9 kg of skim milk powder (SMP) can be produced from 100 L of whole milk (Pearce, 2015). The manufacture of various milk powders is shown in Figure 2.1.

Figure 2.1. Flow chart of manufacture of various milk powders (Chandan and Kilara, 2008)

Generally, the process to manufacture SMP is to preheat, evaporate, and spray dry pasteurized skim milk. Depending on the end goal, SMP is usually classified by preheat treatment into high-, medium-, and low-heat and further classified as instant (agglomerated) and non-instant (regular or non-agglomerated). Non-instant lasts for 12-18 months where instant lasts for 6-12 months (ADPI 2005). For NFDM, ADPI further separates products by grade. Extra Grade is the highest quality of NFDM, followed by Standard Grade. Standard Grade are products that fail in one or more requirements of Extra Grade. NFDM that is not assigned a grade may be due to high microbiological activity, high fat, moisture, titratable acidity, solubility index, or scorched particles.

The first step in the process is to obtain, pasteurize, and separate raw milk into skim milk and cream using a centrifugal cream separator (Pearce, 2015).

The preheating step is important as this is the highest temperature the milk is exposed to throughout the powder making process (Paracha, 2011). Preheating can be direct (i.e. steam injection), indirect (i.e. heat exchanger), or a combination of direct and indirect methods. Effects of preheating include denaturation of whey proteins, decrease in bacteria, inactivation of enzymes, among others (Pearce, 2015).

Regardless of preheat treatments, all powders must abide by the same the chemical and physical standards. Generally, in industry, companies abide by the standards by a large margin so if there are issues such as accidental moisture or fat, the product still meets the standards. Table 2.3 below shows generally accepted standards for SMP manufacture.

Table 2.3. Chemical and Physical Standards for SMP from ADPI and from Codex Alimentarius (Chandan and Kilara, 2008)

Property	ADPI	Codex Alimentarius
Protein - SNF Basis	Min. 34%	Min. 34%
Moisture	Max. 4.0%	Max. 5.0%
Fat	Max 1.25%	Max 1.5%
Titrateable Acidity	Max 0.15%	Max. 18 mL 0.1N NaOH/ 10 g SNF
Scorched Particles	Max. Disc B (15.0 mg)	Max. Disc B
Solubility Index	Max 1.2 mL	Max. 1.0 mL

As Table 2.3 again indicates, for Codex Alimentarius (2011), SMP has not more than 5.0% moisture by weight nor more than 1.5% fat by weight and a minimum protein level of 34.0%. Moreover, the titrateable acidity, which can measure dissociated and

undissociated hydrogen ions (i.e. important for weak acids such as lactic acid) is under 0.15%. Scorched particles (for organoleptic qualities) are under 18 mL 0.1 N NaOH/ 10g SNF. The solubility index is a maximum of 1.0 mL. Finally, all SMP heat treatments share similar sensory standards in terms of odor (fresh, no off odors), appearance (powder, free of lumps), color (white to light cream), and overall flavor (clean, bland, slightly sweet).

Preheating treatments are checked with whey protein nitrogen index (WPNI). Typical processing treatments are shown in Table 2.4 below with the respective whey protein nitrogen index (WPNI) indicative values.

Table 2.4. Preheating treatment and subsequent WPNI value for powders (ADPI, 2014)

Heat classification	Preheating treatment	WPNI (mg N/g)
High	88°C (190°F) for 30min	<1.5
Medium	71-79°C (160-175°F) for 20min	1.51-5.99
Low	Not exceed 71°C (160°F) for 2min	≥6.0

Higher temperatures and/ or extended holding times directly affect whey protein denaturation. Thus, the heat and holding period depends on the end-use. The Whey Protein Nitrogen Index (WPNI) is used to measure the cumulative heat effects during processing. ADPI respective undenatured whey protein nitrogen (WPN) per gram of milk powder are in units of milligram of undenatured whey protein nitrogen per gram of powder (Chandan and Kilara 2008).

Since the highest temperature in powder production is in the preheating step, it important to understand the denaturation of whey protein because of the subsequent interaction with

casein micelles. The interaction is in the form of disulfide linkages between κ -casein and β -lactoglobulin. Moreover, β -lactoglobulin complexes with α -lactoglobulin which, as a whole, can interact with κ -casein. However, although α_{s2} -casein has one disulfide bridge, it does not interact with β -lactoglobulin. During the preheating step, the minor whey proteins (serum albumin and immunoglobulin) start denaturation at 65°C (149°F), which indicates that the minor whey proteins are denatured in all SMP products. However, α -lactoglobulin and β -lactoglobulin begin denaturation at 70°C (158°F). This means that these two major whey proteins may not be fully denatured in low heat SMP. Overall, changes in casein micelle size during the preheating step can be related to the interaction of whey proteins with the caseins (Paracha, 2011).

The different WPNI values listed in Table 4 can indicate the functionality of the powder and thus lead to different applications for each heat treatment. For example, high-heat powder is the most denatured, least soluble, and has the best heat stability; high-heat powder must demonstrate a minimum stability of 40 minutes at 120°C without gelation of a 20% solids solution. Thus, it is generally more suitable for breadmaking because the high-heat treatment enhances water binding and minimizes gluten interference. Medium- or low- heat powder depresses the loaf volume. High-heat powder is also best used in the manufacture of recombined evaporated skim milk.

Low-heat powder is treated as high temperature, short time (HTST) pasteurization which leaves the proteins in their most native state. Thus, low-heat powder is the most soluble and is the most applicable for products that require maximum protein reactivity such as yogurt, cheese, and infant or animal nutrition. The low-heat treatment also optimizes

sensory properties in dairy foods and beverages. Low-heat powder is the most commonly used in industry.

Since medium-heat powder covers a range in the WPN Index, there is a wide range of heat treatments and the properties can vary from batch to batch with respect to flavor, viscosity, etc. Medium-heat powder has improved heat stability and slightly more developed flavor over low-heat powder. Thus, medium-heat powder applications are generally in confectionary, bakery, meat or UHT products.

Since medium-heat powder characteristics can vary, this research uses low-heat powder. Moreover, for the research conducted here, it was important to use low heat skim milk powder because low heat SMP is closest to skim milk in terms of quality, is the most commonly used, and is the most soluble.

Note that before the evaporation, there is no homogenization step since there is such a low fat level in skim milk. Sometimes reverse osmosis is used to concentrate the milk to about 25% total solids, which can cut energy costs in the evaporation and spray drying steps. Evaporation is a useful step in minimizing the amount of energy used to manufacture SMP; compared to the spray dryer, in general, evaporators require less energy. Moreover, without the evaporation step, the powder particles would have a high air content, poor wettability, and a short shelf life. Evaporation is considered a gentle concentration process since it is under vacuum – there is little additional heat damage after the preheat treatment because the residence times in each stage of the evaporator is short and the temperature exposure is 72°C or less. However, as the milk is more and more concentrated, the viscosity increases and the efficiency of this step decreases rapidly (Chandan and Kilara 2008).

When the milk concentrate is about 40-50% total solids, it is then spray dried to powder. Generally, spray drying takes place in three steps. The first step is the dispersion of the concentrate into very fine droplets through the spray atomizer. The second step is the mixing of the finely dispersed concentrate into a stream of hot air which quickly evaporates the water. The final step is the separation of the dry milk particles from the drying air into the cyclone.

At times, there is an extra step involving a fluid (moving) bed where hot air is blown through the powder, to further reduce the moisture content of the powder. Removing the water increases shelf-life because microbial growth is prevented; this eases the distribution and storage of SMP for wide application. Since there are low levels of fat compared to whole milk powder (WMP), skim milk powder has a longer shelf life, lasting for up to 2 years compared to 6-12 months for WMP. (Bylund, 1995; Chandan and Kilara, 2008).

The manufacturing of SMP can influence its rehydration properties through the physical properties as well the chemical properties.

2.1.3 Casein

The two main proteins, casein and whey protein, are differentiated by the chemical composition and physical properties. In centrifugation, casein results in a pellet while the supernatant (also called whey or serum), contains water, lactose, and whey protein. Thus, whey proteins are generally soluble while caseins are in suspension. Casein can be differentiated by molecular mass as caseins are usually 25-35 kDa while whey proteins are 14-18 kDa (Bylund, 1995). Forney et al. (2011) note that proteins are complex food molecules. This is because environmental conditions such as pH, mineral concentration,

and temperature can affect their tertiary structure, which in turn, affects their interaction with other substances such as the interaction with water. Thus, the majority protein, casein, is important as a lot of the functionality of the SMP is influenced by the behavior of the casein.

Casein proteins are found in milk and make up about 80% of milk proteins. Casein are organized into micelles. There are two main models for the structure of the casein micelle: submicellar, nanocluster, and dual-binding. The submicellar model predicts that micelles formed from submicelles that are linked by CCP. The nanocluster model predicts that there are calcium phosphate/ casein particles linked by noncovalent bonds. However, more recent studies with electron microscopy give images that appear to not support the submicellar model (Dalglish and Corredig, 2012).

While the micelle structure is not fully understood, there are a few key agreements. First, the micelle is generally seen as a porous, spherical, and non-homogenous structure with an average diameter of 150-200 nm. The micelle is about 94% protein and 6% salts on a dry weight basis. The micelles have about 3.5 kg of water/ kg of protein. This means that although caseins are about 2.5% of the total weight of milk, the micelles are about 10% of the volume. The main salts in the micelle are calcium, phosphorous, magnesium, and citrate; these four ions are known as colloidal calcium phosphate (CCP). The micelle is held together by these CCP bridges, protein-protein interactions (including electrostatic and hydrophobic interactions), and hydrogen bonding Dalglish and Corredig, 2012; Chandan and Kilara, 2008).

Out of twenty-one variants, the four main casein genetic fractions are α_{s1} -, α_{s2} -, β -, and κ -casein. These four are in a percentage ratio of 4:1:4:1. Table 2.5 shows the casein make up in milk (% of total protein w/w).

Table 2.5. Casein make up in bovine milk (% of total protein w/w) (Bylund, 1995)

Casein component	% (w/w)
Total casein in milk	79.5%
α_{s1} -casein	30.6
α_{s2} -casein	8.0
β -casein	30.8
κ -casein	10.1

α_{s1} -, α_{s2} -, and β -casein proteins are almost insoluble in water whereas κ -casein is readily soluble in water; it follows that the more hydrophilic κ -casein is found on the outside of the micelle. Since κ -casein is found on the outside of the micelle, the whole micelle is soluble as a colloid. The proportion of κ -casein indicates average size of the micelles; higher proportion leads to a smaller average diameter of casein micelle. Furthermore, on the κ -casein, there is carbohydrate group with a strong negative charge projecting from the exterior of the micelle to stabilize the micelle. This protein-protein interaction, plus the combination of the calcium phosphate and the hydrophobic interactions between sub-micelles, hold together the fairly heat stable casein micelle structure (Dalglish and Corredig, 2012; Bylund, 1995). Figure 2.2 below shows the schematic of a casein micelle.

Figure 2.2. Schematic of a casein micelle (Dalglish and Corredig, 2012)*

*The α_{s1} -, α_{s2} -, and β -caseins (orange) are attached to the calcium phosphate nanoclusters (gray). Some β -caseins (blue) are binded via hydrophobic interactions to other caseins. κ -casein (green) and the caseinomacropeptide chains (black) are on the surface of the micelle.

The α_{s1} -, α_{s2} -, and β -caseins (orange) are attached to the calcium phosphate nanoclusters (gray). Some β -caseins (blue) are binded via hydrophobic interactions to other caseins. κ -casein (green) and the caseinomacropeptide chains (black) are on the surface of the micelle. It appears that the κ -casein prevent aggregation and the caseinomacropeptide chains create a layer, estimated to be 5-10 nm thick which provides steric stabilization and prevents micelles from getting to close to other micelles.

Most of the functionality of the casein micelle depend on the properties of the surface of the micelle, rather than the interior. This is because different methods cause different changes to the caseinomacropptide chains layer, the “hairy” layer, which can affect the stability of the micelle in different ways (Dalglish and Corredig, 2012). For different desired functionality, it is possible to adjust the structure of the casein through various methods such as acidification, heat treatment, or addition of chelators, among other methods (Gaucheron, 2005). These changes are described further in Section 2.2 and Section 2.3.

2.1.4 Minerals in Milk

Milk is a nutritious fluid delivered to offspring from the female mammal. Milk is about 87.6% water, 4.7% lactose, 3.7% fat, 3.3% proteins, and 0.7% ash. Overall, the more abundant minerals in skim milk powder are potassium, calcium, phosphorous, sodium, and magnesium. Potassium, sodium, and chloride ions are essentially diffusible while calcium, phosphate, and magnesium are non-diffusible. Non-diffusible indicates that the ions are partly bound to the casein micelles. Calcium and phosphorous are important because of their roles with the casein protein – about two-thirds of the total calcium and about half the inorganic phosphate are found in the casein micelle structure (Gaucheron, 2005). Minerals are also found in whey protein. Cations can form an ionic bridge between adjacent carboxyl groups from different peptide chains in whey protein. The minerals in milk are dynamic between the serum and the micelle (Udabage et al., 2000). By centrifugation and subsequent analysis of the supernatant, it is possible to measure the calcium activity in the serum phase. Table 2.6 shows the calcium partition in

bovine milk, noting that 31% of the total calcium is in the serum and 69% of the total calcium is in the micelle.

Table 2.6. Calcium partition in bovine milk (adapted from Gaucheron, 2005)

Component	Concentration
Casein	26.1 g/L
Total Ca	29.4 mM
Soluble	9.2 mM
% of soluble Ca	31 %
Miceller Ca	20.2 mM
% of Total Ca that is in casein	69%
Micellar calcium x g ⁻¹ of casein	0.77 mM

The minerals can be influenced to migrate between the serum and the micelle by changes in temperature, pH, or the addition of chelators. By the addition of 10 mM CaCl₂, Udabage et al. (2000) measured 72% of the added calcium was found in the pellet. Moreover, 59% of the added calcium was found in the pellet via the addition of 30 mM CaCl₂. Therefore, chelators affect the calcium equilibrium, which affects the casein micelle structure.

2.2 Chelators

2.2.1 Overview

It is possible to adjust the micelle structure of the most common protein in milk, casein, through various methods such as acidification, heat treatment, or addition of chelators, among other methods (Gaucheron, 2005). Chelating compounds, or chelators, are legal, safe, and widely used. Chelation is defined as the formation a bond between two or more binding sites within the same compound to a single metal ion, such as calcium, to create

very stable complexes (IUPAC, 1997). Chelators tend to be thermodynamically stable and displace other ligands in entropy-favoring reactions (Zeng and Jadir, 2015). The Greek word “chele” means the pincers of crabs, lobsters, and scorpions. This image indicates the shape and tight grip of the formation of bonds between a chelator and an ion (Flora and Pachauri, 2010). Chelators can be classified as strong or weak depend on the ion-binding capacity. The pH of the system also has an effect on chelators, as chelators can be unstable at low pH. In general, chelators are in the form of a salt and are thus known as chelating salts. In Codex Alimentarius (2011), chelating salts listed under food additives include trisodium citrate, tricalcium citrate, dicalcium phosphate, and tripotassium phosphate; these chelators are used as emulsifying salts, stabilizers, or thickeners. Sodium citrates are allowed as a stabilizer additive and potassium phosphates are allowed as acidity regulators. Both are allowed at a maximum level of 5,000 mg/kg (0.5%) or in combination (Codex Alimentarius, 2011). As for the effect on dietary bioavailability, certain chelating salts such as tricalcium citrate are used for fortification of beverages such as orange juice or grape juice. Tricalcium citrate has a high calcium level (31%) and moderate solubility (0.9 g/L); its solubility improves at pH below 4.5 and at low temperatures (Gerstner, 2002) Furthermore, chelating salts have been used in dairy for decades. Thus, certain chelating salts are ideal to try to alter the reconstitution properties of SMP, such as a higher wettability, sinkability, dispersibility, and solubility. When chelating salts are added to casein micelle, the chelator can alter the functionality. For example, Sikand et al. (2013) noted that lower values for solubility index were observed for SMP samples with 5mM EDTA and 5mM sodium polyphosphate as compared to the control sample with no chelators. Schuck et al. (2002) found that adding

citrate or phosphate prior to drying or adding NaCl during reconstitution improves the rehydration process of the powder. Furthermore, Konstance and Strange (1991) showed that the solubility of sodium caseinate and calcium caseinate improved when sodium phosphate was added. These changes in functionality may be due to the idea that chelators can cause a mineral imbalance between the minerals in the micelle and the minerals in the serum, namely of the mineral, calcium. For example, Sikand et al. (2011) associated the differences in the solubility of milk protein concentrate (MPC) and milk protein isolate (MPI) with differences in mineral composition. It is generally believed that the chelators first bind to the calcium in the serum. To return to equilibrium, the calcium inside the micelle migrate into the serum; this causes the CCP bridges, which hold the micelle structure together, to collapse. In theory, the micelle separate into smaller, negatively charged micelle structures, which repel each other. The repulsion could affect the reconstitution properties such as dispersibility; since water is polar and the small micelles are negatively charged, there would be a stronger interaction than the one between the water and an average micelle. Gaucheron (2005) notes that the modification of casein micelles by the addition of chelators is irreversible. Thus, by adjusting the concentration of calcium in the solution via chelator addition, we may alter the interaction of calcium in the casein micelle. By understanding the casein micelle structure and by controlling the structural changes, rehydration properties of chelated SMP may be higher compared to SMP without chelator.

Three major groups of chelators include phosphates, Ethylene Diamine Tetraacetic Acid (EDTA), and citrates. In general, polyphosphates are variable and the system cannot be easily defined. Thus, this study only utilizes and discusses EDTA and citrate.

2.2.2 Disodium Ethylene Diamine Tetraacetic Acid (EDTA)

EDTA is a hexadentate chelating agent with a molecular weight of 372.2 g/mol. The chemical formula of a typical EDTA salt, Disodium EDTA, is $C_{10}H_{14}N_2O_8Na_2 \cdot 2 H_2O$. Hexadentate indicates that there are six spots per EDTA molecule to bind with calcium ions: 4 oxygens and 2 nitrogens (Zeng and Jadir, 2015). Figure 2.3 shows the fully protonated form of EDTA.

Figure 2.3. Fully protonated form of Ethylene Diamine Tetraacetic Acid (EDTA)
(Zeng and Jadir, 2015)

As EDTA has a strong ion-binding capacity, EDTA has applications to prevent undesired metals from reacting. For example, in personal care products and laundry detergents, EDTA binds to free metal ions such as calcium and magnesium to reduce the hardness in water in order for the other ingredients in those products to work more efficiently (Zeng and Jadir, 2015). Although Disodium EDTA is GRAS, it is not allowed in milk powders. However, EDTA is allowed in food products such as cabbage, fermented malt beverages,

and mayonnaise to promote color retention, as a preservative, to promote stability, and more (CFR 21, 2015). In the casein micelle, EDTA dissolves the calcium phosphate so that nanoclusters in the casein micelle do not exist (Dalglish and Corredig, 2012). Odagiri and Nickerson (1965) observed that, when Disodium EDTA was added, the pH of milk decreased, probably because the uptake of protons by the phosphate ions was overshadowed by the liberation of protons. EDTA is most commonly used as a chelating salt form, Disodium EDTA (Zeng and Jandir, 2015). Overall, EDTA is considered a stronger chelator than sodium citrate dehydrate (SCD). Sikand et al. (2013) noted that lower values for solubility index were observed for SMP samples with 5mM EDTA as compared to the control sample with no chelators. However, Sikand et al. (2013) did not study the effect of EDTA on all stages of rehydration properties. Furthermore, Sikand et al. (2013) showed that the chelator addition step was to reconstituted SMP, which was evaporated and spray dried a second time. Thus, this investigation further analyzes the effect of EDTA on the rehydration properties of SMP, when the EDTA is added to the fat free milk prior to evaporation and spray drying.

2.2.3 Sodium Citrate Dihydrate (SCD)

Citrates are commonly used in the dairy industry and one of the most commonly used chelating salt is Sodium Citrate Dihydrate. SCD is a conjugate base of a weak acid with a molecular weight of 294.1 g/mol. The chemical formula is $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$. SCD is allowed in milk products as a stabilizing agent, up to 5,000 mg/ kg or 0.5%.

2.3 Rehydration Properties

Forny et al. (2011) noted that in real reconstitution processes, the rehydration properties cannot be clearly isolated from the others. The following methods were developed to attempt to separately measure each rehydration property of wettability, sinkability, dispersibility, and solubility.

Wettability has been measured via the Washburn equation but there can be nuances due to the complex milk system. Therefore, the contact angle measurement test is used in this study to measure “surface hydrophobicity”, rather than the traditional idea of wettability (Crowley, 2015). Since wettability and sinkability are closely tied in the IDF Wettability method, this method is used to relate to the contact angle method for wettability as well as to provide information on wettability and sinkability. Dispersibility is measured via dissolution rate measurements via spectrophotometry and particle size analysis (Ferrer et al, 2008; Millqvist et al., 2001). Solubility is measured by particle size analysis of a rehydrated sample and by the solubility index (ADPI, 2002). Furthermore, the effects on the rehydration properties are theorized to be caused by changes in the calcium balance between the serum and the micelle. Thus, the total calcium and the calcium in the serum after centrifugation is measured by inductively coupled plasma optical emission spectroscopy (ICP-OES).

2.3.1 Overview

Skim milk powder rehydration takes place in four stages: wettability, sinkability, dispersibility, and solubility (Fang, 2008). Figure 4 below illustrates how the stages overlap and are not necessarily discrete.

Figure 2.4. Schematic of dissolution stages for a standard powder particle showing how different rehydration stages overlap with time (adapted from Fang, 2008)

Chandan and Kilara (2008) note that it is important to understand the physical properties of the powder and how they may affect the rehydration properties. This can be done by measuring bulk density, which is the weight of powder that in a fixed volume container. There are various standard methods to measuring bulk density, including one manual method outlined by ADPI (2002) and one automated method such as the Stampvolumeter. Typical results for skim milk powder are in the range of 0.58-0.68 g/mL. Bulk density measures occluded air, interstitial air, and the distribution of the size and shape of the powder particles. For example, powder particles, as measured by a particle size analyzer, that are more homogenous in shape, have higher bulk densities and powder particles that are more homogenous in size, have smaller bulk densities. In general, lower bulk density values are desired. Since bulk density is generally most influenced by the concentrate characteristics, atomization methodology, drying

parameters, and the extent of whey protein denaturation, all of these factors are kept constant throughout manufacturing of the samples. For example, after evaporation and prior to spray drying, all the concentrates are standardized to 40% total solids. For this study, only particle size analysis is utilized for information on the physical properties of the powders.

2.3.2 Wettability

Wettability is the water absorption on the SMP particle surface. This includes the ability of the powder particles to overcome the water surface tension. If the powder cannot overcome the surface tension sufficiently, the powders may stick to the walls of the container and layers form.

Gaiani (2005) notes that, for whey powders, the controlling stage is the wetting stage, but in contrast, the controlling stage of casein proteins is the dispersion. Schuck et al (2007) showed that the low solubility index of micellar casein powder was the result of a slow rate of rehydration rather than the consequence of a high content of insoluble material. Overall, there are many factors that can affect wettability. In general, wettability is lower during storage for six months or more (Litman and Ashworth, 1957). While wettability is most influenced by free fat content of the powder and the state of the lactose, SMP does not have enough free fat on the surface to have large effects for oxidation, so there is minimal alteration of the wettability functionality (Paracha, 2011). Moreover, wettability can be influenced by spray drying atomization methodology and other spray drying parameters so these are kept constant across all samples during manufacturing. Also, wettability is affected by the air between the powder particles, known as interstitial air. In general, wettability is reduced when there is an increase in interstitial air between the

powder particles because the water is more easily diffused through the more porous structure (Fang, 2008). Finally, wettability is dependent on powder particle size (Vega and Roos, 2006), which will be measured in this study.

Studies to measure wettability are usually derived from the Washburn equation (Fang, 2008). From the Washburn equation, wettability can be higher if there are large pores in large particles and high porosity in the particles as well as a small contact angle.

However, the equation may not fit the milk system because the surface tension changes due to the different components in milk that dissolve, including lactose, whey protein, and salts (Fang, 2008). Thus, the contact angle measurement test adapted from pharmaceutical use is used in this study to measure surface hydrophobicity, rather than the traditional idea of wettability (Crowley, 2015). This method is discussed more in detail in Section 3.6.1.1. Overall, a treated sample will show less surface hydrophobicity over untreated SMP if the sample has a smaller contact angle than that for untreated SMP.

2.3.3 Sinkability

Sinkability is when the powder particles drop below the surface of the water (Fang, 2008). Thus, the surface area characteristics are critical in sinkability. The ability to sink is a function of specific volume, density, and particle size. Agglomerated powders normally have the best ability to sink (Bylund, 1995). Sinkability is closely associated with wettability, sometimes even interchangeable. For example, in the International Dairy Federation (IDF) wettability method, the wettability time (wetting time) is defined as “the time required for all the particles of the sample to become wetted, i.e. to sink below the surface of the water and any remaining on the surface to assume a typical “wet”

appearance” (ISO 707 | IDF 50:2014). The biggest influences for sinkability are particle density and occluded air (air content enclosed within the powder particle. Higher density particles with less occluded air are more likely to sink in water due to water more easily diffusing through the porous structure. Since wettability and sinkability are closely tied in the IDF Wettability method, this method is used to relate to the contact angle method for wettability as well as to provide information on sinkability. Since this IDF method can vary widely from sample to sample, it is recommended to analyze in triplicates and to report each time, as well as the mean. Thus, SMP with chelator will show higher sinkability if the powder with chelator shows a smaller mean time to “wet” than the SMP without chelator.

2.3.4 Dispersibility

Dispersibility is defined as the speed with which the powder lumps and agglomerates fall apart when reconstituted with water. In other words, good dispersibility is seen when powders in water are distributed as single particles, leaving no lumps (i.e. scum).

Dispersibility is dependent on the casein dispersing because casein is the non-soluble protein. Moreover, a powder with a high content of denatured proteins is very difficult to disperse (Fang, 2008). Thus, minimizing preheat treatment leads to higher dispersibility (Paracha, 2011; Bylund, 1995). Dispersibility can be measured via dissolution rate measurements via spectrophotometry in units of Optical Density per second (O.D./second) (Ferrer et al, 2008; Millqvist-Fureby et al., 2001). If SMP with chelators had a higher dispersibility, the dissolution rate (O.D./ second) would be higher than SMP without chelator.

2.3.5 Solubility

Solubility is defined as the measure of the final condition of the powder constituents interacting with the water. In other words, solubility describes how well the powders dissolve and form a stable suspension. Solubility is important because, if the powder is not completely solubilized, there are can be problems in processing such as clogging of filters. Solubility is considered the most important property of the rehydration process as it is the final stage (Bylund, 1995; Fang, 2008). To measure solubility, the solubility index is used (ADPI 2002). In this test, the insoluble material is generally denatured protein (β -lactoglobulin), casein, and lactose. Solubility is mostly influenced by preheat treatment, atomization type, the physical properties of the concentrate prior to drying such as viscosity, and the temperature of rehydration. Thus, the preheat treatment, and atomization type, and temperature of rehydration are controlled factors during this testing. The maximum solubility for SMP without chelator is 1.0 mL. Thus, higher solubility would be if SMP with chelators had solubility index levels lower than solubility index levels for SMP without chelator.

Moreover, particle size distribution analysis is conducted of fully solubilized reconstituted treatment samples. Thus, treated powders with higher solubility would show powders that are smaller and more homogenous in size than the data for skim milk powder without chelator.

2.3.6 Calcium and Phosphorus Behavior

When rehydrating the samples, the water quality is important. During evaporation, the milk has been rid of pure (distilled) water. Therefore, the water used to rehydrate the samples must be low in hardness expressed as calcium carbonate (CaCO_3), i.e. <100

mg/L. An excessive mineral content will jeopardize the salt balance of the reconstituted product (Bylund, 1995). Thus, this study also utilizes the ICP-OES to measure the calcium and phosphate levels in the water used for manufacturing powders and in the water used for rehydrating powders, in addition to measuring the total and serum mineral levels in the samples.

2.4 Summary, Hypotheses, and Objectives

From the literature review, Sikand et al. (2013) noted that lower values for solubility index were observed for SMP samples with 5mM EDTA and 5mM sodium polyphosphate as compared to the control sample with no chelators. However, the study had not focused on rehydration properties of SMP. Moreover, the manufacturing process was reconstituting SMP to 30% TS, adding chelator, then evaporating and spray drying. Thus, the focus of this study expands upon the observations of Sikand et al. (2013) by adding chelators to fat free milk prior to evaporating and spray drying and subsequently analyzing the powder on rehydration properties.

The hypotheses of this study are as follows:

- 1) Powder produced from chelated skim milk have higher wettability, sinkability, dispersibility, and solubility than SMP not treated with chelators
- 2) Chelators affect the rehydration properties of SMP by altering the calcium balance between the serum and the casein micelle

The objectives of this study are as follows:

- 1) To incorporate the chelating salts Disodium EDTA or Sodium Citrate Dihydrate at concentrations of 5, 10, and 15 mM to skim milk prior to evaporating and spray drying.

- 2) To determine the effects of the chelator additions on each SMP rehydration property (wettability, sinkability, dispersibility, and solubility) during reconstitution to 10% total solids, compared to the control (SMP with no chelator added) in order to assess changes in rehydration properties.
- 3) To quantify the calcium behavior in the system, for understanding as to how chelators affect the SMP system.

CHAPTER 3: MATERIALS AND METHODS

3.1 Ingredients

3.1.1 Pasteurized Fat Free Milk

Grade A, Pasteurized, Homogenized, Vitamin A&D, fat free milk was obtained from Producers Dairy Foods, Inc. (Fresno, CA 93701) for each block in 6 gallon packages. All packages were stored in 2°C (36°F) refrigerator.

3.1.2 Chelators

The Dissolvine NA 2-P product provided by Akzo Nobel Functional Chemicals LLC (Chicago, IL) (Batch 610E2013) was the chemical Disodium Ethylenediaminetetraacetic acid (EDTA). The chemical formula was $C_{10}H_{14}N_2O_8Na_2 \cdot 2 H_2O$.

Sodium Citrate Dihydrate (SCD) was obtained from Tate & Lyle (Lot #GC1F27BK5A).

The chemical formula was $Na_3C_6H_5O_7$.

Both chelators were stored at room temperature, 20-25°C (68-77°F).

3.2 Powder Making Process

Generally, to produce the chelated skim milk powder samples for this study, the chelator was added to the skim milk. The pH was adjusted with acid or base and the liquid mixture was hydrated overnight in a refrigerator. After a minimum of 14 hours, the mixture was evaporated and the resulting concentrate was adjusted to 40% total solids (%TS). Finally, the concentrate was spray dried, cooled, and stored until analysis. Figure 3.1 shows a schematic of the general process to produce the chelated SMP samples for this study. Process details are found in Section 3.2.2-3.2.6.

Figure 3.1. Schematic of the general process to produce the chelated SMP samples

3.2.1 Experimental Design

This was a randomized complete block design (RCBD). There were two experimental factors of chelator type and chelator concentration. There was one blocking factor with 2 levels. As fat free milk had a two-week shelf life, the powder samples were prepared in blocks where each sample was made in each block to account for any differences in the fat free milk supply. Within each block, there were 7 treatment combinations. The 7 treatment combinations are as follows: 6 combinations (chelator type at 2 levels: EDTA or SCD with chelator concentration at 3 levels: 5 mM, 10 mM, and 15 mM) plus the control treatment. This was done in duplicate for a total of 14 samples. Figure 3.2 below shows a summary of the RCBD.

Figure 3.2. Summary of the Randomized Complete Block Design (RCBD)*

*EDTA=Disodium Ethylene Diamine Tetraacetic Acid, SCD=Sodium Citrate Dihydrate

Once all the samples were produced, the results of the response factors were analyzed as a RCBD using two-factor ANOVA with Dunnett comparisons comparing each of the 6 treatments to the control as this study is focused on having higher levels of each rehydration property compared to the control, not between treatments. Results were expressed as a mean with standard error (S.E.). All graphs for the raw data can be found in the Appendix as noted. Two-way analysis of variance (ANOVA) was carried out by JMP Pro 12.1. Assumptions for each response were checked for the following criteria: 1) each sample is independent and was randomly assigned for testing 2) the distribution of the residuals followed a normal distribution and 3) population variances were equal across responses. Differences were considered to be significant at $p\text{-value} < 0.05$. If statistically different, JMP was used to conduct multiple comparisons of treatments versus control using Dunnett's procedure. Differences were considered to be significant with a family wise error rate (FWER) of $\alpha = 0.05$.

The responses for the 14 samples can be organized into two categories: powder composition and rehydration properties. The responses are summarized in Table 3.1 below.

Table 3.1. Experimental responses including method and units

Property	Sample	Response	Units
Powder properties	Powder sample	%moisture, %ash, %protein	%
		%protein	%
		Mean particle size of powder	μm
Rehydration properties	Powder sample	Contact angle	Degrees
		IDF wetting time	Seconds
		Dissolution rate	O.D./sec
	Reconstituted SMP Sample (10% TS)	Mean particle size of reconstituted sample	μm
		Insoluble sediment	mL
		%soluble calcium and %soluble phosphorus	%

From Table 3.1, for powder composition, the responses were mean particle size, percent moisture, percent ash, and percent protein. Two samples were selected for percent fat and percent lactose. For rehydration properties, the responses analyzed with the powder were contact angle, wetting time, and dissolution rate. The responses that were analyzed with a 10% reconstituted SMP sample were mean particle size, solubility index, %soluble calcium, and %soluble phosphorus.

3.2.2 Chelator Addition into Fat Free Milk

For each sample, 59.9 kg (132 lbs) of free fat milk was weighed out. Using an Orion 350 Advanced PerpHecT LogR pH meter, the pH was measured as a check for microbiological quality. The CEM LabWave 9000 (Figure 3.3a.) was used to measure percent total solids (%TS) with power setting of 45% and a sample size of 2-3 grams for a 5-minute run. The Brookfield DV-II+ Pro Model D (Serial No. 8544431) with spindle

62 at 60 RPM (Figure 3.3b.) was used to measure viscosity of a 200.0 mL sample. %TS and viscosity of the liquid fat free milk were checked once per block with a temperature range of $4.0 \pm 1.0^{\circ}\text{C}$.

Figure 3.3a. Brookfield Viscometer

Figure 3.3b. CEM LabWave 9000

Over one minute, one chelator (EDTA or SCD) at concentration of 5, 10, or 15 mM was stirred into the free fat milk with a stirring wand. The calculations for the weight of each chelator to add was as follows:

Equation 1. Weight of Disodium Ethylenediaminetetraacetic acid (EDTA) (g) used for 15 mM

$$58.1 \text{ L} \times 0.015 \text{ M} \times 372.2 \text{ g/mol} = 324.4 \text{ g EDTA}$$

Equation 2. Weight of Sodium Citrate Dihydrate (SCD) (g) used for 5 mM

$$58.1 \text{ L} \times 0.005 \text{ M} \times 294.1 \text{ g/mol} = 85.4 \text{ g Citrate}$$

Table 3.2 is a summary of the calculated amounts in grams at the desired concentrations of each chelator type.

Table 3.2. Weight (g) of chelator to add for desired concentration (mM) or %(w/w)*

	Desired concentration (mM) or %(w/w)		
Chelator type	5 mM or 0.18%	10 mM or 0.36%	15 mM or 0.54%
EDTA	108.1g	216.2g	324.4g
Chelator type	5 mM or 0.14%	10 mM or 0.28%	15 mM or 0.43%
SCD	85.4g	170.9g	256.3g

*EDTA=Disodium Ethylene Diamine Tetraacetic Acid, SCD=Sodium Citrate Dihydrate

It is important to note that at the time of manufacturing, the %moisture of the EDTA and SCD samples were not accounted for in the calculation of weight (g) of chelator to add to obtain desired concentration (mM). As EDTA was 10% moisture and SCD was 13% moisture, the true concentrations of the samples are presented in the following table.

Table 3.3. True concentration (mM) and %(w/w) of each chelator type*

Chelator type	Concentration (mM)			% (w/w)		
EDTA	4.5	9.0	13.5	0.16	0.32	0.49
SCD	4.3	8.8	13.2	0.12	0.25	0.37

*EDTA=Disodium Ethylene Diamine Tetraacetic Acid, SCD=Sodium Citrate Dihydrate

After all the chelator was mixed into the milk, the mixture was stirred for an additional 2 minutes, then rehydrated for 30 minutes before the pH was adjusted. Another important factor in casein and chelator behavior is the pH level. The pH levels are affected by the

addition of chelator. Chandan and Kilara (2008) note that the concentration causes transfer of calcium and phosphate to the colloidal phase of milk, causing a re-establishment of the mineral salt equilibria, with the release of hydrogen ions. For example, when 5 mM of EDTA is added to a 10% reconstituted SMP system, the pH lowers to 6.36 from 6.68. Thus, it is important to adjust the pH of samples back to 6.65 ± 0.05 after the addition of chelator. The pH was adjusted to ensure that the only difference between samples was the chelator. The addition of EDTA decreased pH while the addition of SCD increased pH. The decrease in pH by EDTA may be due to the release of hydrogen ions as EDTA dissolves (Figure 2.3). Adding SCD initially increases the pH of the liquid skim milk. The general reaction producing this increased pH is thought to be due to destruction of the colloidal tricalcium phosphate by formation of the calcium complex salt and liberation of phosphate ions. Acid phosphate ions would be formed by ionic equilibria through uptake of protons and result in a concomitant pH rise (Odagiri and Nickerson, 1965).

Thus, the pH was adjusted to 6.65 ± 0.05 (6.60-6.70) by stirring in 70.0 – 1000.0 mL of 1.0 M Hydrochloric acid (HCl) or 1.0 M Sodium Hydroxide (NaOH). The 1.0 M hydrochloric acid was prepared by diluting concentrated (12.1 M) HCl. A 1.0 L volumetric flask was filled almost to the top with DI water and 82.64 mL conc. HCl was added before filling the volumetric flask to the line with DI water. The 1.0 NaOH was prepared in a 1.0 L volumetric flask that was filled with DI water. 40.0 g of NaOH pellets (Fisher Scientific Lot 146052) were dissolved before filling the volumetric flask to the line with DI water. After the pH was adjusted, each sample was rehydrated in a 2°C (36°F) refrigerator overnight, for a minimum of 14 hours prior to use.

3.2.3 Evaporation into 40% Total Solids Concentrate

Before evaporation, the samples were stirred for 4 minutes to ensure homogenous solution prior to evaporation. Viscosity and pH were measured with a temperature range of $5.0 \pm 2.0^{\circ}\text{C}$, as described in Section 3.2.2, to ensure no changes during storage. Each sample was introduced into the Custom rising film Marriot Walker Evaporator (Birmingham, MI) (Figure 3.4a).

Figure 3.4a. Custom rising film Marriot Walker Evaporator

Figure 3.4b. Calandria visual inspection

The evaporation temperature was set at 49°C (120°F) and the vacuum pressure was 28 inHg. The process took about 3 minutes to stabilize the temperature although about 30 minutes were needed to pump all of the sample into the evaporator. Evaporation step was

completed when the temperature began increasing, the level in the glass was getting low, and the boiling over slowed down. The evaporation process was completed in about 60 minutes. Prior to cleaning the evaporator, the calandria was visually inspected with a flashlight after each run to check for differences in fouling patterns (Figure 3.4b).

3.2.4 Spray Drying into Powder

Prior to spray drying, the %TS was measured and standardized to $40 \pm 1\%$ as necessary with the softened pilot plant water. Also, the viscosity of the concentrate was checked with a temperature range of $39.0 \pm 2.0^{\circ}\text{C}$, as described in Section 3.2.2. A Niro Filter Lab Spray Dryer (Figure 3.5) was used for the spray drying process, which took about 15 minutes for each trial.

Figure 3.5. Niro Filter Lab Spray Dryer

The feed rate was 500 psi, the inlet air temperature was $204 \pm 3.9^{\circ}\text{C}$ ($400 \pm 25^{\circ}\text{F}$), and the outlet air temperature was 82°C (180°F). The settings for the spray drying step are summarized in Table 3.3 below.

Table 3.4. Spray drying step operating parameters

Operating variable	Setting
Feed pressure	500 psi
Inlet air temperature	$204 \pm 3.9^{\circ}\text{C}$ ($400^{\circ}\text{F} \pm 25^{\circ}\text{F}$)
Outlet air temperature	82°C (180°F)
Atomizer nozzle	Type SX, Orifice No. 67, Core No. 20 (Spraying Systems Co., Wheaton, IL)

Prior to collecting the powder from the spray dryer, the powder was allowed to cool in the cyclone to less than 38°C (100°F), or a minimum of 30 minutes.

3.2.5 Cleaning Procedure

For the evaporator, the cleaning procedure was to rinse out the system with warm water for about 20 minutes, caustic wash (Sodium Hydroxide pH >10) for 60 minutes, rinse with water for 10 minutes, acid wash (Phosphoric Acid pH <4) for 30 minutes, and rinse with water again for 15 minutes. The evaporating system was run prior to introduction of feed to reach temperature settings. For the spray dryer, the cleaning procedure was to rinse thoroughly with hot water until no traces of powder remain. The feed assembly was rinsed with hot water for 10 minutes, then a caustic wash (Sodium Hydroxide pH >10) for 30 minutes, and rinsed for 10 minutes. The spray drying system was started up prior to introduction of feed to dry out system and reach equilibrium. After SMP production and cleaning procedure, system air dried overnight, minimum of 14 hours.

3.2.6 Powder Storage

All powder was stored in 1-2 freezer gallon bags that were each about $\frac{3}{4}$ full. The freezer bag was placed in a Mylar bag at room temperature. The maximum temperature during transport/ storage was 30°C (86°F) (ISO 707 | IDF 50:2014).

3.3 Sampling Procedure

3.3.1 Preparation for Powder Sample

Before opening each bag of powder for analysis, it was made more homogenous by shaking or by inverting container repeatedly (AOAC, 1995a). A clean, dry trier of stainless steel was passed steadily through powder at an even rate of penetration. When the trier reached the bottom of the container, the contents were withdrawn and discharged immediately into a sample container. Care was taken to not touch the powder with fingers, by wearing gloves (AOAC, 1995b). Care was also taken to avoid sampling on excessively hot or rainy days (i.e. when the temperature or humidity was high). If lumps were present, sample was sifted through a No. 20 sieve. The sample was rubbed through the sieve with gloved hands, and the sieve was tapped vigorously to obtain as much sample as possible (AOAC, 1995a).

3.3.2 Preparation for 10% Total Solids Reconstituted Sample

Powder was prepared as in Section 3.3.1. The %moisture data (procedure described in Section 3.4.1) was used to calculate the weight in grams to reach $10 \pm 0.1\%$ Total Solids for each sample. An example calculation is seen in Equation 3 with Control Block 1, which had 4.4% moisture.

Equation 3. Using %moisture data to calculate the weight (g) to reach $10 \pm 0.1\%$

Total Solids for a 50 mL reconstituted sample of Control Block 1 at 4.4% moisture

$$[10\% / (1.000 - 0.044)] / [100 \text{ mL} / 50 \text{ mL}] = 5.2301 \text{ g SMP} + 44.7699 \text{ g deionized water}$$

Weighed out water was added to the weighed out powder in a 100 mL beaker. The mixture was stirred with a 1" stir bar at 700 rpm for one hour. The sample was hydrated overnight in a 39°F refrigerator for a minimum of 14 hours. Prior to analysis, samples were brought to room temperature by stirring at 700 rpm for 1.5 hours.

3.4 Powder Composition

3.4.1 Percent Moisture

Percent moisture was determined using Moisture in Dried Milk (AOAC, 1995c) and calculated using Equation 3 below. Measurements were carried out in duplicate.

Equation 4. To calculate percent moisture after vacuum oven

$$\% \text{ moisture} = 100 - [100 \times (\text{final sample weight (g)} / \text{original sample weight})]$$

3.4.2 Percent Ash

Percent ash was determined using Ash of Dried Milk (AOAC, 1995d) and calculated using Equation 4 below. Samples used for percent moisture could be further used to determine percent ash. Measurements were carried out in duplicate.

Equation 5. To calculate percent ash after ash oven

$$\% \text{ ash} = 100 - [100 \times (\text{Final sample weight (g)} / \text{original sample weight})]$$

3.4.3 Percent Fat

Percent fat method was determined using Fat in Dried Milk (AOAC, 1995e).

Measurements for selected samples were carried out in duplicate.

3.4.4 Percent Protein

Percent protein was determined using Protein in Dried Milk (AOAC, 1995f) with the Nitrogen (Total) in Milk, Block Digestor/ Steam Distillation Method (AOAC, 1995g).

Measurements were carried out in duplicate.

3.4.5 Percent Lactose

Percent lactose was calculated as the difference $[100\% - (\% \text{moisture} + \% \text{ash} + \% \text{fat} + \% \text{protein})]$.

3.5 Powder Size via Powder Particle Size Analysis (Powder PSA)

A Beckman Coulter LS230 with powder module was used to measure the powder particle size distribution (Figure 3.6). The LS230 measures particle diameter from 0.4 μm to 2000 μm by light diffraction of laser light at wavelength of 750 nm. To measure particles 0.04 μm to 0.4 μm , the Polarization Intensity Differential Scattering (PIDS) mode was used. PIDS uses an incandescent tungsten-halogen lamp combined with three sets of polarized filters to provide light at the following wavelengths: 450 nm (blue), 600 nm (orange), and 900 nm (near-infrared, invisible).

Figure 3.6. Particle size analyzer (PSA) with powder module

The instrument was adjusted for electrical offsets, the laser was aligned, and the background was measured prior to each sample measurement. Sample was placed into the sifter with a spatula and entered the sample entry port by gravity and the vibrator was applied to maintain obscuration of 40-60%. As it is difficult to describe a particle size distribution with a single number, the data was analyzed by comparing the volume mean diameter, the volume median diameter, and the overall shape of the Volume (%) vs Particle Diameter distribution curve. $D(4,3)$ (μm) is the volume mean diameter. Horiba Scientific (2014) explain the calculation of the volume mean diameter by imagining a histogram table showing the upper and lower limits of “n” size channels along with the percent within this channel. The D_i represents the geometric mean for each channel. $D(4,3)$ is calculated by taking the D_i to fourth power times the percent in that channel, summed over all the channels and taking the D_i to the third power times the percent in that channel, summed over all the channels and dividing. This is shown in Equation 5.

Equation 6. Calculation for the volume mean diameter, $D(4,3)$ (Horiba Scientific, 2014)

$$D(4,3) = \frac{\sum_1^n D_i^4 v_i}{\sum_1^n D_i^3 v_i}$$

The volume median diameter (μm) represents a central point within the distribution. In other words, where half the population resides above this point and the other half falls below this point (Horiba Scientific, 2014). Measurements were carried out in duplicates.

3.6 Rehydration Properties

Forny et al. (2011) noted that in real reconstitution processes, the rehydration properties cannot be clearly isolated from the others. The following methods were developed to

attempt to separately measure each rehydration property of wettability, sinkability, dispersibility, and solubility.

3.6.1 Wettability

3.6.1.1 Contact Angle Method Overview

Contact angle is a commonly used index for wetting behavior in pharmaceutical sciences. Contact angle is in terms of surface hydrophobicity, not exactly wettability (Crowley et al., 2015). This is because wettability usually defined as the water absorption on the SMP particle surface. This includes the ability of the powder particles to overcome the water surface tension (Fang, 2008).

Contact angle measures the initial contact of compressed powder with water, prior to the sinking, dispersion, and solubilization steps. A droplet of water falls 90° to the normal onto the solid surface of a powder tablet. An interaction develops between the water and the surface molecules. If the interaction is strong, the water spreads across the surface but if the interaction is weak, the water will minimize surface contact. Contact angle measures this solid-liquid interface, where a smaller contact angle means that the interaction between the water and surface is strong, implying that the surface is more hydrophilic (Kim et al. 2002). The compression of powders into tablets minimized the influence of powder cake topography because the surface was more continuous and there were fewer air pockets between particles.

3.6.1.2 Preparation of Powder Tablets for Contact Angle Determination

Measurements of contact angle were carried out on tablets of the powder samples.

Powder samples were compressed into tablets using a 12-ton hydraulic press (Figure 3.7a).

Figure 3.7a. 12 ton hydraulic press.

2.5 g of powder was measured into the die. The crank was pumped, until it was aligned with the die and then the crank was pumped 16 times, until enough pressure was applied to compress the powders. At least 6 tablets were compressed per sample.

3.6.1.3 Contact Angle Measurement

Once the tablet was made, one droplet of 5 μL of deionized water was dropped onto the surface using a Microliter $\text{\textcircled{R}}$ #710 Pat.No. 29330877 0.1 mL syringe. Measurements were carried out in triplicate, where each measurement was a single droplet onto a different tablet. CAM 101 software (Figure 3.7b and Figure 3.7c) was used to take pictures.

Contact angle was measured in degrees, and calculated every 990×10^{-4} seconds for 40 frames (Figure 3.7c)

Figure 3.7b. Contact angle measurement set-up

Figure 3.7c. Contact angle measurement software

Also seen in Figure 3.7c, the contact angle was measured for the right side and the left side; the single reading in degrees ($^{\circ}$) taken for this study was the median of the two.

3.6.2 Wettability and Sinkability

3.6.2.1 Modified IDF Wettability Method

The wettability and sinkability method was modified from the Determination of the wettability (wetting time) of instant dried milk procedure set forth by ISO and IDF, Annex B (2014). The modification was the sample preparation. It was noted that the powder sample would form a film along the water surface and not wet nor sink in a timely manner (≤ 12 hours). Freudig (1999) notes that there are issues in sinkability if there are very fine particles, if the particles are hollow from the spray drying process, or if swelling occurs in the wetting stage. Thus, to correct for the possibility of one of these issues, a 2" stir bar was placed at the bottom and set to 450 rpm. This speed was selected

to ensure no incorporation of air or formation of bubbles. Furthermore, 300.0 g deionized water were weighed into a 600.0 mL beaker instead of 250.0 mL also to ensure no incorporation of air. Figure 3.8 is a visual of the experimental setup.

Powder resting on the glass plate (and within the glass tube) was dropped into the beaker of water once the glass plate was removed

The modification from the original procedure was to add a 2" stirbar at 450 rpm

Figure 3.8. Set up for modified IDF wettability method

The wettability time was defined as the time once all the powder was wetted, i.e. sunk below the surface of the water and any remaining on the surface assumed a typical “wet” appearance. The wettability time (in seconds) was the time once all the powder was wetted minus the initial 60 second wait period. Duplicates were carried out for each sample.

3.6.3 Dispersibility

3.6.3.1 Dissolution Rate

Dissolution rate can provide insight on the dispersibility of powders since good dispersibility is seen when powders in water are distributed as single particles, leaving no lumps. For example, after the addition of powder to water, a powder would disperse into smaller particles and saturate the water, thereby increasing the optical density over time, known as dissolution rate. A treated powder with higher dispersibility might be indicated by a higher dissolution rate, as the powder is able to disperse and saturate the water at a faster rate, thus increasing the optical density over less time (compared to the behavior of a control powder). The dissolution rate method used in this study was modified from the method used by Millqvist-Fureby et al. (2001). The dissolution rate was observed with the Ocean Optics USB 4000 spectrophotometer/ Spectra Suite (Figure 3.9).

Figure 3.9. Dissolution rate measurement set-up

A powder sample of $20.0 \pm 0.5 \mu\text{g}$ powder was carefully dropped into a cuvette (1 x 1 cm cross section), which contained 1.75 mL DI water at room temperature and a stirbar at

1400 rpm. The rate of dissolution was observed as optical density per second (O.D./ sec) and was calculated as shown in Equation 6.

Equation 7. Rate of dissolution calculation

$$\text{Rate of dissolution} = \frac{[\text{Optical density (O.D.)}]}{[\text{time (seconds)}]}$$

The integration time was set to 10 milliseconds. The scans to average was set to 5. The boxcar width was set to 4. The wavelength was set at 620.13 nm as it is long enough to measure light scattering with minimal absorption. The slope (O.D./second) was calculated and compared to the control in order to determine if any of the experimental treatments showed a higher or lower rate of dissolution. Samples were completed in duplicate.

3.6.4 Solubility

3.6.4.1 Reconstituted Particle Size Analysis

Mimouni et al. (2009) noted that measuring changes in particle size during powder rehydration mostly used a single mean diameter which was not reflective of the sample as a whole. Thus, Mimouni et al. (2009) monitored the particle size distribution in volume as a function of time. Similar to the methods for particle size analysis for powder samples in this study as described in Section 3.6.5, the particle size analysis of the reconstituted samples in this study were measured as D(4,3), median, and the overall shape of the Volume (%) vs Particle Diameter distribution curve. The three measurements taken from the reconstituted samples after a rehydration period of at least 14 hours, which can be seen as the final solubilization stage. Similar work was carried out by Crowley et al.

(2015). Crowley et al. (2015) employed liquid PSA measurements for particle size distribution measurements in milk protein concentrate suspensions after rehydration for 90 minutes and compared to the particle size distribution measurements after 24 hours. Sample was prepared as a 10 %TS solution, rehydrated overnight for a minimum of 14 hours. Liquid particle size analysis was carried out with the Beckman Coulter LS230 with liquid module. The instrument adjusted for electrical offsets, the laser was aligned, and the background was measured prior to each sample measurement. The samples were placed into the loading cell with a pipette and entered the sample entry port to maintain obscuration of 40-45%. Light passed through the sample cell stand during measurement. Data were analyzed by comparing D(4,3) (μm), median (μm), and the overall shape of the particle size distribution pattern (Volume vs Diameter). Measurements were carried out in triplicates. All samples were compared to the measurements for the control in order to determine which sample had higher or lower end point solubility.

3.6.4.2 Modified Solubility Index

Solubility index is a commonly used procedure to measure the solubility of dried milk. In general, the procedure is to mix powder sample with water and centrifuge the reconstituted mixture. The solubility index is the volume of sediment (in mL). Since the solubility index measures the insoluble portion of the reconstituted mixture, the method has sometimes been termed “Insolubility index.” Thus, higher levels for the solubility index method indicate lower solubility.

The solubility analysis method used for this study was a modified procedure from the one set forth by the Solubility Index Procedure Standards for Grade of Dry Milks (ADPI, 2009). The modification was the sample preparation. It was noted that the powder sample

would stick to the walls of the blender jar, and lead to inaccurate and inconsistent results.

Thus, the powder was reconstituted as follows.

On day 1, the order of sample preparation was randomized. 50 mL of a 10% w/w TS solution of each solution in a 100.0 mL beaker was prepared, adding DI water into the powder sample. Each mixture was stirred with a thin spatula to ensure that the powder was mixed with the water, not stuck to the sidewall, etc. Then, a one-inch stir bar was used to remove sample from the spatula and the sample was then stirred at 700 rpm for 60 minutes. The samples were stored in the 4°C (39°F) refrigerator overnight for a minimum of 14 hours.

On day 2, the samples were brought to room temperature by stirring at 700 rpm for at least 90 minutes but no more than 2 hours. Then, the standard solubility index procedure was adhered to for the remaining steps. As the Eppendorf Centrifuge 5810 R radius was 13 inches in diameter, the centrifuge settings were 940 rpm at 20°C for 5 minutes, both ramp (up and down) set at max at level 9. Each sample was measured to the nearest 0.1 mL. Measurements were completed in duplicate.

3.6.5 Calcium and Phosphorous Behavior

3.6.5.1 Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES)

In addition to analyzing any differences that the addition of chelators to SMP may have on rehydration properties, it was important to note the effect of the chelators on the system and the mechanism to which the chelators may be influencing the changes in rehydration properties. Thus, ICP-OES results may provide insight on the behavior of the chelators. For example, Udabage et al. (2000) found in their work that a high level of non-pelleted calcium was approximately equal to the amount of added EDTA. Thus, by

analyzing the calcium behavior, it was possible to elucidate the effect of the chelators. The calcium behavior was measured via inductively coupled plasma optical emission spectrometry (ICP-OES). Phosphorus was also measured as about half of the phosphorus is in the micelle, in a calcium-phosphate bridge.

The total phosphorus and total calcium (in mg/L) was measured for each reconstituted sample. The serum phosphorus and serum calcium (in mg/L) was measured for each centrifuged reconstituted sample (Udabage, 2000). Measuring both the total and serum levels allowed for calculation for %soluble, as shown in Equation 7 below.

Equation 8. Calculation of %soluble Calcium or %soluble Phosphorus

$$\% \text{soluble Calcium (or Phosphorus)} = \frac{[\text{serum Calcium } (\frac{\text{mg}}{\text{L}})]}{[\text{total Calcium } (\frac{\text{mg}}{\text{L}})]}$$

ICP-OES is a rapid and precise technique to monitor trace elements. This study utilized the Horiba Scientific Ultima 2 for the measurements (Figure 3.10).

Figure 3.10. ICP-OES measured with Horiba Scientific Ultima 2

Inductively coupled plasma (ICP) is the source to excite electrons in samples. Optical emission spectrometry (OES), sometimes known as atomic emission spectrometry (AES), separates the emitted light from the ICP to discrete wavelength. Since each element in the periodic table has its own set of emission wavelengths, the output is compared and the mass fraction of the desired element in the sample can be calculated (Boss and Fredeen, 2004). In this study, Calcium (Ca) and Phosphorous (P) were the desired elements.

Figure 3.11 shows a basic diagram of how ICP-OES works.

Figure 3.11. Diagram of ICP-OES (USGS, 2013)

In general, ash is collected from the samples and mixed with 0.4% nitric acid. This solution is atomized into a mist that reacts with a stream of argon gas into the argon plasma, reaching temperatures up to 7,000°C. At this temperature, the outer-shell electrons of the elements in the sample are excited and emit light wavelengths which are characteristic of the respective element. The spectrometer for the Horiba Scientific Ultima 2 uses a 1-meter optical pathway with dual monochromator gratings to separate the emitted wavelengths onto the photomultiplier tube (PMT) detector. The detector reads the wavelengths and respective intensity sequentially. The intensity of the light dictates the concentration of each desired element. Thus, this signal is converted through a

calibration curve by the computer to produce the concentration of each element in the ash of the samples (USGS, 2013). Overall, the data from ICP-OES can help elucidate the concentration of the calcium between the serum and the colloid and possibly provide insight as to the effects of the chelators on the skim milk mineral system.

The calibration standards for ICP-OES for Calcium (Ca) and Phosphorus (P) were prepared using an ICP Certified Ca standard of 1000 mg/L (1000 mg/L Ca Lot 18-85CAY by SPEX CertiPrep, Metuchen, NJ) or ICP Certified P standard of 1000 ppm (Phosphorus for ICP – CGP1-1 by Inorganic Ventures, Christiansburg, VA).

Three 1.0 L volumetric flasks of diluted nitric acid (HNO_3) were prepared by adding 4.0 mL concentrated HNO_3 into a 1.0 L volumetric flask and diluted with deionized water to make a 0.4% solution. (AOAC 991.25).

The P calibration standards were 0, 5, 10, 25, 50, 75, 100 ppm. The standards were made by adding 0.0, 0.5, 1.0, 2.5, 5.0, 7.5, or 10.0 mL of the P standard into 100.0 mL volumetric flasks and diluting with 0.4% HNO_3 .

The Ca calibration standards were 0, 5, 25, 50, 100, 150, 200 ppm. The standards were made by adding 0.0, 0.5, 2.5, 5.0, 10.0, 15.0, or 20.0 mL of the C standard into 100.0 mL volumetric flasks and diluting with 0.4% HNO_3 .

It took three days to prepare each sample and testing was on the fourth day, with the procedure as outlined below (AOAC 984.27; AOAC 991.25; AOAC 645.46). On day 1, samples were randomized for order of preparation and prepared in triplicates. 80.0 mL of 10% w/w TS solutions of each sample was prepared by reconstituting water into powder and stirring at 700 rpm for 1 hour. The samples were stored in the 4°C (39°F) refrigerator overnight, for a minimum of 14 hours. On day 2, the samples were stirred at 700 rpm for

1.5 hours. For total P or Ca, 5.0 g was weighed into a crucible. For soluble P or Ca, at least 40 mL of sample was centrifuged at 18,000 rpm (test tube maximum = 39,000 x g) for 60 minutes at 4°C. 5.0 g of supernatant was carefully pipetted into a crucible. All samples were placed in a vacuum oven with vacuum at 25 inHg at 100°C for 4 hours. Then, all samples were placed in an ash oven at 525°C overnight, minimum of 16 hours. On day 3, the ash oven was turned off and allowed to cool to 100°C (~4.5 hours). Samples were transferred to a dessicator and cooled to room temperature for 30 minutes. An addition of 0.2 mL concentrated HNO₃ was made to each container to dissolve the ash. Finally, the mixture was transferred to a 50.0 mL volumetric flask and diluted to volume with diluted (0.4%) HNO₃. On day 4, the operating parameters for ICP-OES were as follows: for P, wavelength 213.618 nm and for Ca, wavelength 317.993 nm. An initial calibration was performed for each element using the previously mentioned standards. A minicalibration was performed after every 10 samples to correct for the drift in measurement over time. The minicalibration included measuring the 50 ppm P calibration standard and the 100 ppm Ca calibration standard. Then, the instrument would adjust the calibration curve to account for drift over time. Four duplicates were also added at the end to remeasure the four samples and ensure consistency. All measurements were in mg/L.

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Powder Making Process Observations

Prior to treatment, the fat free milk was a steady pH of 6.75 ± 0.02 over the two weeks, per block. The % total solids (%TS) was 9.66 ± 0.22 %TS. Thus, the quality of the milk was deemed suitable and consistent.

All treatments within each block were produced from the same milk supply. All treatments were evaporated to 40 ± 1 %TS at a temperature $38 \pm 2^\circ\text{C}$.

Post evaporation, the calandria was visually inspected with a flashlight to check for differences in fouling patterns. No fouling was observed for any treatment.

For spray drying, the outlet air temperature was set to 82°C (180°F). For this spray dryer, only the outlet air temperature could be controlled. The inlet air temperature was maintained by the outlet air temperature setting, the feed rate, the %TS of the concentrate, and the overall composition of the concentrate. Since the feed rate, %TS, and overall composition of the concentrate was as uniform as possible, the outlet temperature had to be adjusted at times to 85°C (185°F) to maintain the inlet air temperature. The burner heated the intake air to about 204°C (400°F). The hot air entered the top of the drying chamber, and pulled out at the bottom of the chamber and exhausted as the air cooled. The inlet air temperature range was usually 193 - 204°C (380 - 400°F). However, for each control treatment in each block, the range was slightly but noticeably lower, at 191 - 202°C (375 - 395°F).

4.2 Powder Composition

Percent composition was measured for the powder samples (Table 4.1). Note that only a selection of samples was used to find the full percent composition.

Table 4.1. Mean $\pm$ standard error (S.E.) of the percent composition of the experimental SMPs*

	Block 1					Block 2				
Treatment	Moisture	Ash	Fat	Protein	Lactose	Moisture	Ash	Fat	Protein	Lactose
Control	4.40 $\pm$ 0.36	7.31 $\pm$ 0.01	1.26 $\pm$ 0.21	34.7 $\pm$ 0.2	52.33	3.43 $\pm$ 0.36	7.55 $\pm$ 0.01	0.99 $\pm$ 0.21	34.9 $\pm$ 0.2	53.13
SCD 5mM	4.54 $\pm$ 0.33	7.83 $\pm$ 0.01	¹	34.5 $\pm$ 0.2	²	3.63 $\pm$ 0.33	8.08 $\pm$ 0.01	¹	33.8 $\pm$ 0.2	²
SCD 10mM	4.15 $\pm$ 0.18	8.48 $\pm$ 0.04	¹	33.8 $\pm$ 0.1	²	4.27 $\pm$ 0.18	8.65 $\pm$ 0.04	¹	33.9 $\pm$ 0.1	²
SCD 15mM	4.06 $\pm$ 0.27	9.45 $\pm$ 0.17	¹	33.3 $\pm$ 0.1	²	4.36 $\pm$ 0.27	9.36 $\pm$ 0.17	¹	33.4 $\pm$ 0.1	²
EDTA 5mM	4.52 $\pm$ 0.10	8.96 $\pm$ 0.14	¹	35.8 $\pm$ 0.2	²	4.07 $\pm$ 0.10	8.03 $\pm$ 0.14	¹	35.2 $\pm$ 0.2	²
EDTA 10mM	4.61 $\pm$ 0.10	8.10 $\pm$ 0.67	¹	34.6 $\pm$ 0.4	²	4.23 $\pm$ 0.07	8.63 $\pm$ 0.67	¹	36.3 $\pm$ 0.4	²
EDTA 15mM	3.91 $\pm$ 0.40	7.70 $\pm$ 0.59	¹	36.0 $\pm$ 0.1	²	4.47 $\pm$ 0.40	9.28 $\pm$ 0.59	¹	35.9 $\pm$ 0.1	²

Data shown as mean $\pm$ standard error (S.E.)

*EDTA = Disodium Ethylene Diamine Tetraacetic Acid, SCD = Sodium Citrate Dihydrate

¹Denotes that the %fat was not measured for the sample.

²Denotes that the %lactose could not be calculated since the %fat was not measured for the sample.

Pooled standard error for %moisture = 0.10, Pooled standard error for %ash = 0.13, Pooled standard error for %protein = 0.2

Differences in %composition properties were seen in Table 4.1. For example, the sample with the smallest mean amount of protein was SCD 15 mM Block 1 at 33.3% while the sample with the largest mean amount of protein was EDTA 10 mM Block 2 at 36.3%. Differences may be due to differences in initial weighing of liquid fat free milk sample. Moreover, the Kjeldahl method, which was used to measure %protein includes non-protein nitrogen in its measurement, such as the extra amine's in EDTA and in urea, which could lead to slightly inaccurate results.

4.3 Powder Size via Powder Particle Size Analysis (Powder PSA)

The physical property of the powders in term of particle size may have an effect on the rehydration properties, specifically wettability. For example, Kim et al. (2002) noted that wetting may be higher for powders with large particles that contain large pores. Table 4.2 shows the mean and standard error (S.E.) for the volume mean diameter (D_{4,3}) and the volume median diameter of the powder particle size distribution.

Table 4.2. Mean and standard error (S.E.) of the volume mean diameter (D_{4,3}) (μm) and volume median diameter (μm) of the powder particle size distribution for each treatment powder, separated by block*

Treatment	Block 1				Block 2			
	D(4,3) (μm)		Median (μm)		D(4,3) (μm)		Median (μm)	
	Mean	S.E	Mean	S.E	Mean	S.E	Mean	S.E
Control	14.88 ^a	0.41	39.99 ^a	0.05	17.96 ^a	0.08	49.84 ^a	0.11
5mM	16.21 ^b	0.10	47.02 ^b	0.40	19.12 ^b	0.04	51.21 ^b	0.199
SCD 10mM	17.13 ^b	0.33	45.75 ^a	2.26	18.79 ^b	0.37	51.02 ^a	0.94
15mM	18.45 ^b	0.25	51.13 ^b	0.77	19.92 ^b	0.13	54.48 ^b	0.04
5mM	16.56 ^a	0.27	46.35 ^b	1.67	18.20 ^a	0.12	51.56 ^b	0.08
EDTA 10mM	17.28 ^b	0.06	48.75 ^b	1.09	18.79 ^b	0.24	54.51 ^b	0.66
15mM	16.53 ^a	0.10	46.24 ^a	0.29	17.59 ^a	0.05	47.65 ^a	0.23

*EDTA=Disodium Ethylene Diamine Tetraacetic Acid, SCD=Sodium Citrate Dihydrate

^{a,b}Different superscripts denote results that are statistically significantly different from the control according to Dunnett procedure with a family wise error rate of $\alpha = 0.05$

Pooled standard error for D(4,3) = 0.25

Pooled standard error for Median = 0.89

For D(4,3), analyzing all the data with a two-way ANOVA on JMP obtained a treatment p-value of <0.0001. This p-value reflected that there is a significant difference in mean D(4,3) between some of the treatment powders compared to the control. With Dunnett procedure, all the treatments were compared to the control. SCD 15 mM, SCD 10 mM, SCD 5 mM, and EDTA 10 mM had significant differences in D(4,3) compared to the

control. The 95% confidence intervals were constructed to analyze the average amount by which treatments would differ from the control mean volume mean diameter. The interval for SCD 15 mM had the largest increase and was in the range of increasing the control mean volume mean diameter by between 1.78 μm and 3.75 μm . Higher mean volume mean diameters compared to control may be seen as higher wettability.

For the volume median diameter, analyzing the data with a two-way ANOVA on JMP obtained a treatment p-value of <0.0001 . This p-value reflected that there is a significant difference in mean volume median diameter between some of the treatment powders compared to the control. With Dunnett multiple comparisons, all the treatments were compared to the control. SCD 15 mM, EDTA 10 mM, SCD 5 mM, and EDTA 5 mM had significant differences in mean volume median diameter compared to the control. The 95% confidence intervals were constructed to analyze the average amount by which treatments would differ from the control mean volume median diameter. The intervals for SCD 15 mM and EDTA 10 mM had the largest increase. The interval for SCD 15 mM is in the range of increasing the control mean volume median diameter by between 4.37 μm and 11.41 μm . The interval for EDTA 10 mM is in the range of increasing the control mean volume median diameter by between 3.20 μm and 10.23 μm . Higher mean volume median diameters compared to control may be seen as higher wettability.

Although for some of the treatments, the mean volume mean diameter and mean volume median diameter were significantly different than the control, all the treatments agreed with literature that spray-dried powder particles usually have a diameter of 10 to 250 μm (Caric, 1994).

The particle size distribution graphs show that the powders have similar trends in their distribution. Figure 4.1a shows the overall shape of the Volume (%) vs Particle Diameter distribution curve for EDTA 10 mM Block 1, which is similar to the distribution of all the other powders.

Figure 4.1a. Powder Particle Size Distribution of Volume (%) vs Particle Size Diameter of Disodium Ethylene Diamine Tetraacetic Acid (EDTA) 10 mM, Block 1

As seen in Figure 4.1a, there are two main peaks in the Volume (%) vs Particle Diameter distribution curve for EDTA 10 mM Block 1, which is similar of all the other experimental powders. There are different sizes of powders due to the time at which the individual powder particles were exposed to the fluctuating drying conditions. The first peak (around 60 μm) agrees with literature that spray-dried powder particles usually have a diameter of 10 to 250 μm (Caric, 1994). The second peak (around 1000 μm) may be due to slight differences of drying conditions experienced by those individual particles.

For example, slightly higher outlet temperatures can produce slightly larger powder particle sizes (Keogh et al., 2004). Figure 4.1b shows the Number (%) vs Particle Diameter distribution curve and indicates that there may be very few particles with diameter around 1000 μm .

Figure 4.1b. Powder Particle Size Distribution of Number (%) vs Particle Size

Diameter of Disodium Ethylene Diamine Tetraacetic Acid (EDTA) 10 mM, Block 1

Overall, while the volume mean diameter and volume median diameter data showed significant differences between some of the treated powders and the control, the powder samples were deemed suitable for testing, because all the powders were still within range of the average particle size as discussed in the literature.

Also from the JMP analysis, both volume mean diameter and volume median diameter data showed a significant block effect, both had a block p-value of <0.0001 . This may be due to many factors in processing, including but not limited to, differences in fat free

milk supply, differences in the operation of the evaporator or spray dryer, or differences in milk powder storage.

4.4 Rehydration Properties

4.4.1 Wettability in Terms of Surface Hydrophobicity via Contact Angle

Kim et al. (2002) and Crowley et al. (2015) noted that a smaller contact angle can indicate higher wettability. Initial wettability was measured by the contact angle method at 0.5 seconds. Analyzing the data with a two-way ANOVA on JMP obtained a treatment p-value of <0.0001. This p-value reflected that there is a significant difference in mean initial contact angle at 0.5 seconds between some of the treatment powders compared to the control. Table 4.3 shows the difference between treatment sample mean initial contact angle and control sample mean initial contact angle.

Table 4.3. Dunnett procedure to compare the contact angle (°) at 0.5 seconds for the treatments to the contact angle (°) at 0.5 seconds for the control^{a*}

Treatment	Difference between treatment mean minus control mean
EDTA 15 mM	+8.1 ^b
EDTA 10 mM	+7.2 ^b
SCD 15 mM	+3.0 ^a
SCD 5 mM	-2.1 ^a
EDTA 5 mM	-5.8 ^b
SCD 10 mM	-7.2 ^b

*EDTA=Disodium Ethylene Diamine Tetraacetic Acid, SCD=Sodium Citrate Dihydrate

^{a,b}Different superscripts denote results that are statistically significantly different from the control^a according to Dunnett procedure with a family wise error rate of $\alpha = 0.05$
Pooled standard error = 1.0

From Table 4.3 the sample mean contact angles for EDTA 15 mM and EDTA 10 mM are larger than the sample mean contact angle for the control (no chelator treatment) by 8.1° and 7.2° , respectively (Table 4.3). EDTA 5 mM and SCD 10 mM have smaller sample mean contact angles than the sample mean contact angle for the control by 5.8° and 7.2° , respectively (Table 4.3). With Dunnett multiple comparisons, all the treatments were compared to the control. The differences in sample means were found to be statistically significant. The 95% confidence intervals were constructed to analyze the average amount by which treatments would differ from the control mean initial contact angle. The interval for EDTA 15 mM is in the range of increasing the control mean initial contact angle by between 4.1° and 12.1° . The interval for EDTA 10 mM is in the range of increasing the control mean initial contact angle by between 3.2° and 11.1° . This data may indicate that higher concentrations of EDTA (15 mM and 10 mM) show a lower initial wettability compared to the control.

The interval for EDTA 5 mM is in the range of decreasing the control mean initial contact angle by between 1.8° and 9.8° . The interval for SCD 10 mM is in the range of decreasing the control mean initial contact angle by between 3.2° and 11.2° . This data may indicate that EDTA 5 mM and SCD 10 mM show a higher initial wettability compared to the control.

Figure 4.2a-d depict the visual representation of the contact angle over time for the treatments, separated by block and chelator type.

Figure 4.2a. Contact angle over time for Control vs Sodium Citrate Dihydrate (SCD), Block 1

Figure 4.2b. Contact angle over time for Control vs Sodium Citrate Dihydrate (SCD), Block 2

Figure 4.2c. Contact angle over time for Control vs Disodium Ethylene Diamine Tetraacetic Acid (EDTA), Block 1

Figure 4.2d. Contact angle over time for Control vs Disodium Ethylene Diamine Tetraacetic Acid (EDTA), Block 2

In Figure 4.2a and 4.2b, it is clear that SCD 15 mM (yellow line) showed a significant lower contact angle for initial wettability compared to the control (blue line), indicating higher wettability. In Figure 4.2c and Figure 4.2d, it is also clear that EDTA 5 mM (orange line) had a significant lower contact angle for initial wettability compared to the control (blue line), indicating higher wettability. It is also clear that EDTA 15 mM (yellow line) and EDTA 10 mM (gray line) showed a significant higher contact angle for initial wettability compared to the control (blue line), indicating lower wettability. Overall, it is interesting that SCD 15 mM showed a higher initial wettability as measured by contact angle compared to the control treatment. This is interesting because this treatment had a significantly higher average level in the volume median diameter of the powder particle size compared to the control. This is in agreement with Kim et al. (2002) that fast wetting may occur with large particles that contain large pores. However, EDTA 10 mM had a lower initial wettability as measured by contact angle compared to the control treatment but had a significantly lower average level in the volume median diameter of the powder particle size compared to the control. Furthermore, although EDTA 15 mM had lower initial wettability as measured by contact angle compared to the control, EDTA 15 mM did not have a significant difference in average level of volume median diameter of the powder particle size compared to the control. Thus, the significant differences in powder particle size may not have been a good indicator for initial wettability as measured by contact angle, for this study.

4.4.2 Wettability and Sinkability via Modified IDF Wettability Method

As the contact angle method is in terms of surface hydrophobicity, not exactly wettability, the IDF method for measuring wettability was also used. Contact angle measurement was a more controlled system as the powders were compressed into tablets with the same press and the same syringe was used, so water droplet size was consistent. This is compared to the IDF method set forth by ISO and IDF, Annex B (2014) which was a comparably more subjective measurement in deciding when all powder particles had sunk below the surface of the water. Thus, it was interesting to run and compare both tests. As discussed in Section 3.6.2.1, the procedure set forth by ISO and IDF, Annex B (2014) was slightly modified. The mean and standard error (S.E.) results are shown in Table 4.4. Table 4.4 also shows the difference between treatment sample mean IDF wettability and control sample mean IDF wettability.

Table 4.4. Mean and standard error (S.E) for each treatment for IDF wettability (seconds) and difference between treatment mean minus control mean^{a*}

Treatment	Wettability (sec)		Difference between treatment mean minus control mean
	Mean	S.E	
Control	173	22	
SCD 5 mM	177	14	+4 ^a
SCD 10 mM	223	21	+50 ^a
SCD 15 mM	243	11	+70 ^a
EDTA 5 mM	241	15	+68 ^a
EDTA 10 mM	333	40	+160 ^b
EDTA 15 mM	440	45	+267 ^b

*EDTA=Disodium Ethylene Diamine Tetraacetic Acid, SCD=Sodium Citrate Dihydrate

^{a,b}Different superscripts denote results that are statistically significantly different from the control^a according to Dunnett procedure with a family wise error rate of $\alpha = 0.05$

Pooled standard error = 28

All data was analyzed with a two-way ANOVA on JMP and obtained a treatment p-value of <0.0001 . This p-value reflected that there is a significant difference in mean IDF wettability between some of the treatment powders compared to the control. With Dunnett procedure, all the treatments were compared to the control. As seen by the superscripts in Table 4.4, only EDTA 15 mM and EDTA 10 mM were significantly different from the control. The 95% confidence intervals were constructed for the treatments to analyze the average amount by which these two treatments would differ from the control mean IDF wettability method response. The interval for EDTA 15 mM is in the range of increasing the control mean IDF wettability method response by between 157 seconds and 376 seconds. The interval for EDTA 10 mM is in the range of increasing the control mean IDF wettability method response by between 50 seconds and 269 seconds. This data may indicate that EDTA 15 mM and EDTA 10 mM show a lower initial wettability compared to the control. However, while EDTA 5 mM and SCD 10 mM had no statistically significant effect on wettability in this IDF method, observations indicate that the IDF method may not have been able to capture the significant lower contact angle (i.e. higher initial wettability) compared to the control. For example, Figure 4.3a shows a photo of SCD 15 mM Block 1 after the timer was stopped. This photo shows how not all the powder was incorporated with the water. This is compared to Figure 4.3b which is a photo of SCD 10 mM, Block 1 after timer was stopped for the IDF wettability method. Figure 4.3b represents how the powder particles for most samples were incorporated into the water before the timer stopped.

Figure 4.3a. Sodium Citrate Dihydrate (SCD) 15 mM Block 1 after timer was stopped for IDF wettability method

Figure 4.3b. Sodium Citrate Dihydrate (SCD) 10 mM Block 1 after timer was stopped for IDF wettability method

Again, the IDF method was not able to capture that both EDTA 5 mM and SCD 10 mM had significantly higher initial wettability as measured by the contact angle method compared to the control.

Comparing the two wettability methods, both the contact angle method and the IDF wettability method indicated that EDTA 15 mM and EDTA 10 mM had lower initial wettability compared to the control. The IDF wettability method did not indicate that EDTA 5 mM and SCD 10 mM had higher wettability, while the contact angle method did indicate that EDTA 5 mM and SCD 10 mM had higher wettability. Observations during

the IDF wettability method indicate that the IDF method may not have been able to capture the significant difference for EDTA 5 mM and SCD 10 mM in wettability that was measured by the contact angle method. This may be because the IDF method is not as sensitive of a test as contact angle or possibly more replicates may have been necessary to find more significant differences. On the other hand, contact angle may have captured statistically significant differences that are not practically significant. For example, in industry, a high shear mixer is used to incorporate bulk powder into liquid, negating slight differences in wettability. All in all, the IDF method may not be as sensitive of a method as contact angle, but is a simpler method if less precise measurements are acceptable to the researcher. At the same time, although the IDF method is widely used and is known as a standard method for measuring wettability, the results in this study may indicate that the IDF method may need further refinement to acquire as sensitive wettability insight as the more complex contact angle method.

4.4.3 Dissolution Rate

The mean and standard error (S.E.) results for dissolution rate (O.D./ second) are shown in Table 4.5. The difference between treatment sample mean dissolution rate minus control sample mean dissolution rate is also shown in Table 4.5.

Table 4.5. Mean and standard error (S.E.) for each treatment of the dissolution rate (O.D./second) and difference between treatment mean minus control mean^{a*}

Treatment	Dissolution rate (O.D./second)		Difference between treatment mean minus control mean
	Mean	S.E	
Control	0.072	0.007	
SCD 5 mM	0.051	0.001	-0.021 ^b
SCD 10 mM	0.050	0.004	-0.022 ^b
SCD 15 mM	0.006	0.003	-0.066 ^b
EDTA 5 mM	0.055	0.002	-0.017 ^b
EDTA 10 mM	0.033	0.003	-0.039 ^b
EDTA 15 mM	0.004	0.003	-0.068 ^b

*EDTA=Disodium Ethylene Diamine Tetraacetic Acid, SCD=Sodium Citrate Dihydrate

^{a,b}Different superscripts denote results that are statistically significantly different from the control^a according to Dunnett procedure with a family wise error rate of $\alpha = 0.05$

Pooled standard error = 0.004

As seen in Table 4.5, EDTA 15 mM had a sample mean dissolution rate that was 0.068 O.D./ second lower than the control sample mean dissolution rate. Analyzing the data with a two-way ANOVA on JMP obtained a treatment p-value of <0.0001. This p-value reflected that there is a significant difference in mean dissolution rate between some of the treatment powders compared to the control. With Dunnett multiple comparisons, all the treatments were compared to the control. All treatments were significantly different from control as indicated in Table 4.5. The 95% confidence intervals were constructed to analyze the average amount by which treatments would differ from the control mean dissolution rate. All treatments showed a decrease in the mean rate of dissolution compared to the control. This may indicate that all treated SMPs show lower dispersibility compared to the control, as discussed in Section 3.6.3.1. However, this method may not have been able to capture true significant differences in dissolution rate.

For example, Figure 4.4 shows that the dissolution rate did not measure all the SCD 10 mM, Block 1 powder because there was some powder that was not incorporated into the water, despite the use of a stirbar.

Figure 4.4. Sodium Citrate Dihydrate (SCD) 10 mM, Block 1 for dissolution rate test

Thus, the measured dissolution rates, although statistically significant, may not have captured the true dissolution rate behavior of the powder. This may be due in part to the changes in wettability and sinkability properties due to the treatments. Millqvist-Fureby et al. (2001) interpreted the results in their study that lower dissolution rates reflected lower wettability. This is compared to this study where the measured average dissolution rates that were lower compared to the control dissolution rate may have been due to lower wettability, although the results are not reflective of wettability analysis. This method did not analyze wettability because the light scattering detection was along the bottom of the cuvette, measuring light scattering after the powder had become wetted and sunk below the surface of the water.

Also from the JMP analysis, the dissolution rate data showed a significant block effect, with a block p-value of 0.0065. This may be due to many factors in processing, including

but not limited to, differences in fat free milk supply, differences in the operation of the evaporator or spray dryer, or differences in milk powder storage.

4.4.4 Reconstituted Particle Size Analysis

The mean and standard error (S.E.) results for volume mean diameter (D_{4,3}) and volume median diameter of the rehydrated particle size distribution are shown in Table 4.6.

Table 4.6. Mean and standard error (S.E.) of volume mean diameter (D_{4,3}) (μm) mean and standard error (S.E.) of volume median diameter (μm) of the rehydrated particle size distribution for each treatment*

		D(4,3) (μm)		Median (μm)	
Treatment		Mean	S.E	Mean	S.E
Control		31.6 ^a	2.1	47.3 ^a	0.65
SCD	5mM	0.5 ^b	1.6	0.5 ^b	1.02
	10mM	0.5 ^b	1.6	0.5 ^b	1.03
	15mM	0.6 ^b	1.6	0.5 ^b	1.03
EDTA	5mM	36.5 ^a	8.8	64.9 ^b	5.4
	10mM	0.5 ^b	1.6	0.5 ^b	1.02
	15mM	0.6 ^b	1.6	0.6 ^b	1.01

*EDTA=Disodium Ethylene Diamine Tetraacetic Acid, SCD=Sodium Citrate Dihydrate

^{a,b}Different superscripts denote results that are statistically significantly different from the control^a according to Dunnett procedure with a family wise error rate of $\alpha = 0.05$

Many data points were near zero, which complicated the mathematical process of an ANOVA analysis. Thus, the two-way ANOVA was conducted on a log₁₀ transformation of the volume mean diameter and on a log₁₀ transformation of the volume median diameter data.

The JMP analysis of the log₁₀ transformation of the volume mean diameter obtained a treatment p-value of <0.0001. This p-value reflected that there is a significant difference in mean volume mean diameter between some of the treatment powders compared to the control. With Dunnett multiple comparisons, all the treatments were compared to the

control. In Table 4.6 show that all but one (EDTA 5 mM) of the treated rehydrated samples had a significant difference in mean particle size volume mean diameter of the rehydrated sample as compared to the control. EDTA 5 mM had no statistical significant difference compared to the control (treatment p-value of 0.9872). The 95% confidence intervals were constructed to analyze the average amount by which treatments would differ from the control mean volume mean diameter. The intervals for all the significantly different treatments showed a decrease in mean volume mean diameter compared to the control mean volume mean diameter.

The JMP analysis of the $\log_{10}$ transformation of the volume median diameter obtained a treatment p-value of <0.0001 . This p-value reflected that there is a significant difference in mean volume median diameter between some of the treatment powders compared to the control. With Dunnett multiple comparisons, all the treatments were compared to the control. In Table 4.6 show that all treatments of the treated rehydrated samples had a significant difference in mean particle size volume median diameter of the rehydrated sample as compared to the control. The 95% confidence intervals were constructed to analyze the average amount by which treatments would differ from the control mean volume median diameter. The intervals for all the treatments, except EDTA 5 mM, showed a decrease in mean volume median diameter compared to the control. The interval for EDTA 5 mM is in the range of increasing the control volume median diameter by between $0.15\ \mu\text{m}$ and $0.43\ \mu\text{m}$. Overall, this particle size data on reconstituted 10% TS samples may indicate that all treatments except EDTA 5 mM show higher solubility compared to the control. The Volume (%) vs Particle Diameter distribution curves are shown in Figure 4.5a-b.

Figure 4.5a. Particle Size Analysis (PSA) for reconstituted samples of Sodium Citrate Dihydrate (SCD) at 5mM, 10mM, and 15mM vs Control

Figure 4.5b. Particle Size Analysis (PSA) for reconstituted samples of Disodium Ethylene Diamine Tetraacetic Acid (EDTA) at 5mM, 10mM, and 15mM vs Control

Figure 4.5a show that SCD at all concentrations shifted the Volume (%) vs Particle Diameter distribution curve to the left compared to the control. Figure 4.5b show that EDTA at 15 mM and EDTA at 10 mM shifted the Volume (%) vs Particle Diameter distribution curve to the left compared to the control. For EDTA 5 mM, the Volume (%) vs Particle Diameter distribution curve did not shift from the control Volume (%) vs Particle Diameter distribution curve. The behavior of EDTA compared to SCD for the Volume (%) vs Particle Diameter distribution curve analyses of a reconstituted 10%TS sample may indicate that EDTA does not chelate as well as SCD. This may be interpreted because the smaller level of EDTA did not disrupt the particle size distribution while the even the smaller level of SCD caused the Volume (%) vs Particle Diameter distribution curve to shift to the left, compared to the control Volume (%) vs Particle Diameter distribution curve. However, this behavior is not consistent with the general knowledge that EDTA is a stronger chelator than SCD. Thus, further replication is needed to assess if the particle size distribution behavior for reconstituted 10%TS samples is true.

Crowley et al. (2015) identified major peaks in the Volume (%) vs Particle Diameter distribution curves in their work, noting that the peak $< 1 \mu\text{m}$ was casein and that the peak $> 10 \mu\text{m}$ represented primary powder particles. Furthermore, in their work, reconstituted samples with a larger peak $< 1 \mu\text{m}$ correlated with smaller absolute value contact angles and also a sharper initial drop in contact angle from 0.0 seconds to 2.0 seconds.

Comparing the work of Crowley et al. (2015) to the work in this study, it is clear that the control samples have many primary powder particles while the chelated samples are mostly casein. However, the reconstituted samples with a larger peak $< 1 \mu\text{m}$ in this study

are the SCD at all concentrations, EDTA 15 mM, and EDTA 10 mM. The contact angle measurements for these treatments are not all significantly different from control, and the treatments that are different from control may be higher or lower with respect to contact angle behavior. Thus, more work is needed to elucidate if the reconstituted particle size distribution behavior can be related with contact angle data.

Also from the JMP analysis, both volume mean diameter and volume median diameter data for a 10%TS reconstituted sample showed a significant block effect. The block p-value for volume mean diameter was 0.0035 and the block p-value for volume median diameter was 0.003. This may be due to many factors in processing, including but not limited to, differences in fat free milk supply, differences in the operation of the evaporator or spray dryer, or differences in milk powder storage.

4.4.5 Solubility Analysis via Modified Solubility Index

The mean and standard error (S.E.) results for solubility index (mL) are shown in Table 4.7. The difference between treatment sample mean solubility index minus control sample mean solubility index is also shown in Table 4.7.

Table 4.7. Mean and standard error (S.E.) for solubility index of each treatment (mL) and difference between treatment mean minus control mean^{a*}

Treatment	Solubility index (mL)		Difference between treatment mean minus control mean
	Mean	S.E	
Control	2.1	0.09	
SCD 5 mM	0.5	0.06	-1.6 ^b
SCD 10 mM	0.2	0.01	-1.9 ^b
SCD 15 mM	0.05	0.04	-2.0 ^b
EDTA 5 mM	0.8	0.01	-1.3 ^b
EDTA 10 mM	0.2	0.06	-1.9 ^b
EDTA 15 mM	0.05	0.04	-2.0 ^b

*EDTA=Disodium Ethylene Diamine Tetraacetic Acid, SCD=Sodium Citrate Dihydrate

^{a,b}Different superscripts denote results that are statistically significantly different from the control^a according to Dunnett procedure with a family wise error rate of $\alpha = 0.05$

Pooled standard error = 0.06

Analyzing the data with a two-way ANOVA on JMP obtained a treatment p-value of <0.0001. This p-value reflected that there is a significant difference in mean solubility index between some of the treatment powders compared to the control. With Dunnett multiple comparisons, all the treatments were compared to the control. All treatments were significantly different from control for the solubility index. The 95% confidence intervals were constructed to analyze the average amount by which treatments would differ from the control mean solubility index. All the intervals show a decrease in mean solubility index compared to the control. This may indicate that all treatments show higher solubility compared to the control. Although trends of the solubility index data can be seen with a graphical representation of the data, the solubility index photographs of the individual samples can also show any trends. Figure 4.6(a-d) are photographs of the solubility index centrifuge tubes at the end of analysis, when the sediment was measured, separated by block and chelator type.

Figure 4.6a. Solubility index for Ctrl vs. SCD, Block 1*

Figure 4.6b. Solubility index for Ctrl vs. EDTA, Block 1*

Figure 4.6c. Solubility index for Ctrl vs. SCD, Block 2*

Figure 4.6d. Solubility index for Ctrl vs. EDTA, Block 2*

*Ctrl = Control, SCD = Sodium Citrate Dihydrate, EDTA = Disodium Ethylene Diamine Tetraacetic Acid

Further discussion of the 95% confidence intervals was necessary to elaborate on the trend seen in Figures 4.6a-d. The intervals for EDTA 15 mM, EDTA 10 mM, SCD 15 mM, and SCD 10 mM are in the range of reducing the control mean solubility index response by between 2.1 mL and 1.7 mL. The interval for SCD 5 mM is in the range of reducing the control mean solubility index response by between 1.8 mL and 1.3 mL. The interval for EDTA 5 mM is in the range of reducing the control mean solubility index response by between 1.6 mL and 1.0 mL. This may indicate that as the level of concentration of chelator increases, the solubility also increases, regardless of chelator type.

Overall, from Figure 4.6a-d, as the level of concentration increased, regardless of chelator type, the solubility was higher. It is interesting to note that in this solubility index test, the concentration seemed more significant than the chelator type, indicating little to no difference in solubility effect between the chelator types. For example, the concentration at 15 mM, regardless of chelator type, achieved a solubility index of 0.05 mL and the concentration at 10 mM, regardless of chelator type, achieved a solubility index of around 0.2 mL. Moreover, powder particle size differences are not reflected in the particle size distribution of a fully solubilized reconstituted sample nor in the solubility index. This may indicate that in this study, the final solubility is more affected by the chemistry of the treatments rather than the physical property of the powder.

The changes to the solubility index could be due to a sequence of events. First, the chelators chelate all the ionic calcium in the serum. The decrease in ionic calcium shifts the protein-mineral balance between the minerals in the casein micelle and the minerals in the serum, which leads to a depletion of colloidal calcium phosphate, and a subsequent

release of caseins from the initial micelle structure. This behavior influenced by EDTA or SCD dissolves the calcium phosphate bridge so that nanoclusters in the casein micelle do not exist (Dalglish and Corredig, 2012). Hence, the casein micelle would be disrupted into individual caseins that may create the appearance that the solution as a whole has higher solubility, as seen in the particle size analyses of fully rehydrated reconstituted 10%TS samples and in the solubility index.

4.4.6 Calcium and Phosphorous Behavior via Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES)

Table 4.8 shows showed the single check for the total calcium and phosphorus levels in the water used during this study.

Table 4.8. Single check of total calcium and phosphorus in the water supply

Water supply	Total Ca (ppm)	Total P (ppm)
Deionized water (Room 103)	0.08	0.35
Softened water (Pilot plant)	0.35	0.42

The deionized water in the laboratory room 103 was used for reconstitution while the softened water in the pilot plant was used for adjusting %TS for the concentrate prior to spray drying into powder. Overall, the single check observes that the water supply may not have contributed to the total calcium and phosphorus levels in the samples.

It was important to analyze the percent soluble calcium and phosphorus levels to perceive the chelators chelating calcium. The mean and standard error (S.E.) results for %soluble Ca and %soluble P are shown in Table 4.9.

Table 4.9. Mean and standard error (S.E.) of %soluble Calcium (Ca) and %soluble Phosphorus (P) for all treatments for ICP-OES*

Treatment	%soluble Ca		%soluble P	
	Mean	S.E	Mean	S.E
Control	39 ^a	0.9	54 ^a	1.0
SCD 5 mM	54 ^b	1.6	64 ^b	1.6
SCD 10 mM	72 ^b	1.7	79 ^b	1.7
SCD 15 mM	80 ^b	0.8	83 ^b	1.1
EDTA 5 mM	57 ^b	0.8	64 ^b	0.7
EDTA 10 mM	81 ^b	0.7	82 ^b	1.2
EDTA 15 mM	87 ^b	1.7	88 ^b	1.1

*EDTA=Disodium Ethylene Diamine Tetraacetic Acid, SCD=Sodium Citrate Dihydrate

^{a,b}Different superscripts denote results that are statistically significantly different from the control^a according to Dunnett procedure with a family wise error rate of $\alpha = 0.05$

Pooled standard error for %soluble Ca = 1.25

Pooled standard error for %soluble P = 1.25

Analyzing the %soluble calcium and the %soluble phosphorus data separately, each with a two-way ANOVA on JMP obtained a treatment p-value of <0.0001 for each analysis. A treatment p-value of <0.0001 reflected that there is a significant difference in both mean %soluble calcium and %soluble phosphorus between some of the treatment powders compared to the control. With Dunnett multiple comparisons, all the treatments were compared to the control. All treatments were significantly different than the control mean %soluble calcium and %soluble phosphorus. Figure 4.7 shows a graphical comparison of each treatment mean %soluble Ca and %soluble P to the control mean %soluble Ca and control mean %soluble P.

Figure 4.7. Graph of %soluble Calcium (Ca) and %soluble Phosphorus (P) for all treatments for ICP-OES*

*EDTA=Disodium Ethylene Diamine Tetraacetic Acid, SCD=Sodium Citrate Dihydrate

Construction of 95% confidence intervals were necessary to elaborate on the trend seen in Figures 4.6a-d. The 95% confidence intervals were constructed to analyze the average amount by which treatments would differ from the control mean %soluble calcium and %soluble phosphorus. All the intervals may indicate that as the level of concentration of chelator increases, there is also an increase in %soluble calcium and %soluble phosphorus compared to the control mean %soluble calcium and %soluble phosphorus. In this study, as the level of concentration increased for each chelator type, the mean %soluble calcium and the mean %soluble phosphorus was higher. Moreover, as expected and seen with the work of Anema (2015), the %soluble calcium and phosphorus were linearly correlated- as calcium had a higher %soluble percentage, phosphorus also had a

higher %soluble percentage. It is important to note the make-up of the %soluble calcium and %soluble phosphorus fractions in this study. For example, EDTA has a strong ion-binding capacity so it follows that EDTA 15 mM and EDTA 10 mM would have chelated the ionic calcium in the serum. Subsequently, the level of ionic calcium in the serum decreased as the ionic calcium became chelated and no longer available. Thus, this chelated calcium would still be captured, along with any remaining ionic calcium, in the %soluble calcium measurement by ICP-OES. Thus, the higher levels of %soluble calcium and of %soluble phosphorus may be due to the increase in chelated calcium in the serum as well as a decrease in colloidal calcium in the serum.

CHAPTER 5: CONCLUSIONS AND LIMITATIONS

The first major hypothesis of the study was that powder produced from chelated skim milk have higher wettability, sinkability, dispersibility, and solubility than SMP not treated with chelators. The goal of this study was to understand how the addition of EDTA or SCD at concentrations of 5, 10, and 15 mM to skim milk prior to evaporating and spray drying affected all the rehydration properties of SMP during reconstitution to 10 %TS, not just the final solubility as observed by Sikand et al. (2016). Overall, EDTA 15 mM and EDTA 10 mM had significantly lower initial wettability compared to the control as measured by both contact angle and IDF method. This suggests that EDTA 15 mM and EDTA 10 mM had high surface hydrophobicity. However, EDTA at all levels had significantly higher levels of solubility compared to the control (treatment p-value of <0.0001). Thus, in this study, early stages of rehydration such as wettability cannot be used a predictor for later stages of rehydration such as solubility. This trend is also seen in the work by Mao et al. (2012). Mao et al. (2012) observed that treated MPC80 with 150 mM sodium chloride during diafiltration had the highest solubility of their treatments but also the highest surface hydrophobicity. This may be due to the modified surface properties by the reduced intermolecular disulfide bonds and the associated decrease in mean particle size observed by Mao et al. (2012). However, this study did not analyze the disulfide bond interactions nor was there a decrease in volume mean diameter particle size of the powder for these treatments. Moreover, powder particle size differences are not reflected in the particle size distribution of a fully solubilized reconstituted sample nor in the solubility index. This may indicate that in this study, the final solubility is more affected by the chemistry of the treatments rather than the physical property of the

powder. It is interesting to note that even though there were large %composition differences such as 33.3% protein for SCD 15 mM Block 1 and 36.4% protein for EDTA 10 mM Block 2, the differences were not reflected in any of the data. Furthermore, this study also found that SCD 15 mM, SCD 10 mM, and SCD 5 mM did not have a significant effect on wettability as measured by IDF method (treatment p-values of 0.3234, treatment p-value of 0.6376, and treatment p-value of 1.0000, respectively). However, SCD 15 mM, SCD 10 mM, and SCD 5 mM also had higher levels of solubility as measured by particle size analysis of reconstituted 10% TS samples (treatment p-value of <0.0001 for all levels) and by solubility index (treatment p-value of <0.0001 for all levels), compared to the control.

The second major hypothesis was that chelators affect the rehydration properties of SMP by altering the calcium balance between the serum and the casein micelle. Thus, a goal of this study was to provide insight on how the chelators were affecting the SMP system via analyzing %soluble calcium and %soluble phosphorus of centrifuged reconstituted 10% TS samples via ICP-OES. With ICP-OES data, all the treated powders had statistically significant higher levels in %soluble calcium and %soluble phosphorus compared to the control. Also seen with the work of Anema (2015), the % soluble calcium and phosphorus were correlated- as calcium had a higher percentage, phosphorus did as well. Anema (2015) noted that the addition of sodium hexametaphosphate shifted the mineral equilibrium which dissociated the casein micelles. This led to a shift to the left in particle size distribution of a 9% TS rehydrated solution compared to the control (as also seen in this study with a 10% TS solution) indicating that the chelated samples are mostly casein micelles (Crowley et al., 2015). Furthermore, the increase in %soluble

calcium as seen with all the treatments compared to the control is consistent with an increased final solubility for all the treatments compared the control. This is because the initially non-available colloidal calcium was driven out of the micelle to reestablish equilibrium from the EDTA or SCD chelating the ionic calcium in the serum. Thus, the casein micelle would be disrupted into individual caseins that can may create the appearance that the solution as a whole has higher solubility, as seen in this study in the particle size analyses of fully rehydrated reconstituted 10%TS samples and in the solubility index. However, this sequence of chelation may have lowered the wettability of the EDTA powders compared to the control, although there was no effect on wettability of the SCD powders compared to the control. This difference between the EDTA and SCD fully rehydrated reconstituted 10%TS samples may be to the behavior of the chelated calcium.

In general in this study, EDTA treated powders had a lower wettability but a higher solubility compared to the control. SCD treated powders had no difference in wettability but a higher solubility compared to the control. Further work is needed to understand the mechanism in which certain chelators may have lower wettability, sinkability, and dispersibility, but higher solubility of skim milk powder.

Overall, the findings provide insight on how chelators affect the rehydration properties of skim milk powder, when the chelator is added to liquid skim milk, prior to evaporating and spray drying. The findings in effects on rehydration properties may assist dairy powder manufacturers if deciding to add chelators to affect the end product rehydration properties.

CHAPTER 6: DIRECTIONS FOR FUTURE RESEARCH

Additional research into the relationship between the effect of chelator type and concentration on the rehydration properties is needed to advance the understanding of the mechanism in which certain chelators may have lower wettability, sinkability, and dispersibility, but higher solubility of skim milk powder. Also, future research can involve further method development to quantify each rehydration property of wettability, sinkability, dispersibility, and solubility.

1. As this study measured the physical property of the powder only with particle size, additional research may be helpful in investigating the surface of the spray dried powder particles via electron microscopy to determine changes in conformation and functional properties as a result of the chelator additions (Vega and Roos, 2006).
2. Since this study measured mineral behavior only with ICP-OES, it may be beneficial to develop methods to quantify the changes in protein conformation and hydrophobic interactions that occur as the casein micelle fragments as the calcium-phosphate bridge splits (Anema, 2015).
3. The addition of chelating agents in this investigation was in three levels of concentration. It would be interesting to study the behavior of one chelating agent over six or more levels of concentration to analyze the mineral behavior in terms of soluble (ionized or chelated and ionized) versus colloidal.
4. This study focused on rehydration properties. Thus, it would be useful to determine the effect of the addition of chelators on other functional properties post-reconstitution, such as rheology. For example, do the chelated reconstituted

samples have similar or different overall functionality as compared to the original milk source?

5. Determine if the chelated skim milk powder has an interaction effect on poorly soluble substances, such as medicine, which utilize skim milk powder as a carrier to achieve a higher level of solubility (Kumar, 2009).

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APPENDICES

APPENDIX A: JMP Output for Volume Mean Diameter [D(4,3)] of Powder

Block 1

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err		
				Mean	Lower 95%	Upper 95%
Control	2	17.9550	0.10607	0.07500	17.002	18.908
EDTA 05mM	2	18.1950	0.16263	0.11500	16.734	19.656
EDTA 10mM	2	18.7900	0.33941	0.24000	15.741	21.839
EDTA 15mM	2	17.5900	0.07071	0.05000	16.955	18.225
SCD 05mM	3	18.1767	1.62562	0.93855	14.138	22.215
SCD 10mM	2	18.7850	0.51619	0.36500	14.147	23.423
SCD 15mM	2	19.9150	0.17678	0.12500	18.327	21.503

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err		
				Mean	Lower 95%	Upper 95%
Control	2	14.8750	0.572756	0.40500	9.729	20.021
EDTA 05mM	2	16.5600	0.381838	0.27000	13.129	19.991
EDTA 10mM	2	17.2750	0.077782	0.05500	16.576	17.974
EDTA 15mM	2	16.5300	0.141421	0.10000	15.259	17.801
SCD 05mM	2	16.2050	0.134350	0.09500	14.998	17.412
SCD 10mM	2	17.1250	0.473762	0.33500	12.868	21.382
SCD 15mM	2	18.4450	0.346482	0.24500	15.332	21.558

APPENDIX A: JMP Output for Volume Mean Diameter [D(4,3)] of Powder (cont.)

APPENDIX B: JMP Output for Volume Median Diameter of Powder

Means and Std Deviations						
Level	Number	Mean	Std Dev	Std Err		
				Mean	Lower 95%	Upper 95%
Control	2	49.8400	0.15556	0.1100	48.442	51.238
EDTA 05mM	2	51.5600	0.11314	0.0800	50.544	52.576
EDTA 10mM	2	54.5050	0.92631	0.6550	46.182	62.828
EDTA 15mM	2	47.6500	0.32527	0.2300	44.728	50.572
SCD 05mM	3	49.6800	2.65684	1.5339	43.080	56.280
SCD 10mM	2	51.0150	1.32229	0.9350	39.135	62.895
SCD 15mM	2	54.4800	0.05657	0.0400	53.972	54.988

Means and Std Deviations						
Level	Number	Mean	Std Dev	Std Err		
				Mean	Lower 95%	Upper 95%
Control	2	39.9850	0.06364	0.0450	39.413	40.557
EDTA 05mM	2	46.3500	2.36174	1.6700	25.131	67.569
EDTA 10mM	2	48.7500	1.54149	1.0900	34.900	62.600
EDTA 15mM	2	46.2350	0.40305	0.2850	42.614	49.856
SCD 05mM	2	47.0150	0.55861	0.3950	41.996	52.034
SCD 10mM	2	45.7500	3.19612	2.2600	17.034	74.466
SCD 15mM	2	51.1300	1.08894	0.7700	41.346	60.914

APPENDIX B: JMP Output for Volume Median Diameter of Powder (cont.)

Response Volume Median Diameter

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	7	346.28584	49.4694	15.6707
Error	20	63.13609	3.1568	Prob > F
C. Total	27	409.42192		<.0001*

Lack Of Fit

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob> t
Intercept	48.9625	0.335772	145.82	<.0001*
Treatment[Control]	-4.05	0.822471	-4.92	<.0001*
Treatment[EDTA 10mM]	2.665	0.822471	3.24	0.0041*
Treatment[EDTA 15mM]	-2.02	0.822471	-2.46	0.0233*
Treatment[EDTA 5mM]	-0.0075	0.822471	-0.01	0.9928
Treatment[SCD 10mM]	-0.58	0.822471	-0.71	0.4888
Treatment[SCD 15mM]	3.8425	0.822471	4.67	0.0001*
Block[1]	-2.503214	0.335772	-7.46	<.0001*

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Treatment	6	6	170.83555	9.0194	<.0001*
Block	1	1	175.45029	55.5785	<.0001*

Effect Details

Treatment

Least Squares Means Table

Level	Sq Mean	Std Error	Mean
Control	44.912500	0.88836990	44.9125
EDTA 10mM	51.627500	0.88836990	51.6275
EDTA 15mM	46.942500	0.88836990	46.9425
EDTA 5mM	48.955000	0.88836990	48.9550
SCD 10mM	48.382500	0.88836990	48.3825
SCD 15mM	52.805000	0.88836990	52.8050
SCD 5mM	49.112500	0.88836990	49.1125

LSMeans Differences Dunnett

$\alpha = 0.050$ Q= 2.80164 Control=Control Adjustment = Dunnett

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
EDTA 10mM	Control	6.715000	1.256345	3.19517	10.23483	0.0002*
EDTA 15mM	Control	2.030000	1.256345	-1.48983	5.54983	0.4168
EDTA 5mM	Control	4.042500	1.256345	0.52267	7.56233	0.0207*
SCD 10mM	Control	3.470000	1.256345	-0.04983	6.98983	0.0543
SCD 15mM	Control	7.892500	1.256345	4.37267	11.41233	<.0001*
SCD 5mM	Control	4.200000	1.256345	0.68017	7.71983	0.0157*

APPENDIX B: JMP Output for Volume Median Diameter of Powder (cont.)

APPENDIX C: JMP Output for Contact Angle at 0.5 Seconds

Means and Std Deviations						
Level	Number	Mean	Std Dev	Std Err		
				Mean	Lower 95%	Upper 95%
Control	6	59.3037	2.02594	0.8271	57.178	61.430
EDTA 10mM	6	66.4542	0.75959	0.3101	65.657	67.251
EDTA 15mM	6	67.4490	2.81569	1.1495	64.494	70.404
EDTA 5mM	6	53.4622	1.40681	0.5743	51.986	54.939
SCD 10mM	6	52.1427	3.29796	1.3464	48.682	55.604
SCD 15mM	6	62.3268	2.48221	1.0134	59.722	64.932
SCD 5mM	6	57.2023	3.61470	1.4757	53.409	60.996

APPENDIX C: JMP Output for Contact Angle at 0.5 Seconds (cont.)

Response 0.5 seconds						
Effect Summary						
Summary of Fit						
Analysis of Variance						
		Sum of				
Source	DF	Squares	Mean Square	F Ratio		
Model	7	1294.1652	184.881	28.1297		
Error	34	223.4629	6.572	Prob > F		
C. Total	41	1517.6281		<.0001*		
Lack Of Fit						
Parameter Estimates						
Term	Estimate	Std Error	t Ratio	Prob> t		
Intercept	59.762976	0.395584	151.08	<.0001*		
Treatment[Control]	-0.45931	0.968979	-0.47	0.6385		
Treatment[EDTA 10mM]	6.6911905	0.968979	6.91	<.0001*		
Treatment[EDTA 15mM]	7.6860238	0.968979	7.93	<.0001*		
Treatment[EDTA 5mM]	-6.30081	0.968979	-6.50	<.0001*		
Treatment[SCD 10mM]	-7.62031	0.968979	-7.86	<.0001*		
Treatment[SCD 15mM]	2.5638571	0.968979	2.65	0.0122*		
Block[1]	0.3244048	0.395584	0.82	0.4179		
Effect Tests						
		Sum of				
Source	Nparm	DF	Squares	F Ratio	Prob > F	
Treatment	6	6	1289.7452	32.7059	<.0001*	
Block	1	1	4.4200	0.6725	0.4179	
Effect Details						
Treatment						
Least Squares Means Table						
		Least				
Level	Sq Mean	Std Error	Mean			
Control	59.303667	1.0466165	59.3037			
EDTA 10mM	66.454167	1.0466165	66.4542			
EDTA 15mM	67.449000	1.0466165	67.4490			
EDTA 5mM	53.462167	1.0466165	53.4622			
SCD 10mM	52.142667	1.0466165	52.1427			
SCD 15mM	62.326833	1.0466165	62.3268			
SCD 5mM	57.202333	1.0466165	57.2023			
LSMeans Differences Dunnett						
α= 0.050 Q= 2.7011 Control=Control Adjustment = Dunnett						
Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
EDTA 10mM	Control	7.15050	1.480139	3.1525	11.1485	0.0002*
EDTA 15mM	Control	8.14533	1.480139	4.1473	12.1433	<.0001*
EDTA 5mM	Control	-5.84150	1.480139	-9.8395	-1.8435	0.0020*
SCD 10mM	Control	-7.16100	1.480139	-11.1590	-3.1630	0.0002*
SCD 15mM	Control	3.02317	1.480139	-0.9748	7.0212	0.1977
SCD 5mM	Control	-2.10133	1.480139	-6.0993	1.8967	0.5333

APPENDIX C: JMP Output for Contact Angle at 0.5 Seconds (cont.)

APPENDIX D: JMP Output for International Dairy Federation (IDF) Wettability

Means and Std Deviations						
Level	Number	Mean	Std Dev	Std Err		
				Mean	Lower 95%	Upper 95%
Control	4	173.250	42.5309	21.265	105.57	240.93
EDTA 10mM	4	332.750	80.6410	40.321	204.43	461.07
EDTA 15mM	4	439.750	90.9192	45.460	295.08	584.42
EDTA 5mM	4	240.500	29.7803	14.890	193.11	287.89
SCD 10mM	4	223.250	41.8332	20.917	156.68	289.82
SCD 15mM	4	243.250	21.5035	10.752	209.03	277.47
SCD 5mM	4	177.250	27.9169	13.958	132.83	221.67

APPENDIX D: JMP Output for International Dairy Federation (IDF) Wettability (cont.)

▼ Response Wettability						
▷ Effect Summary						
▷ Summary of Fit						
▴ Analysis of Variance						
		Sum of				
Source	DF	Squares	Mean Square	F Ratio		
Model	7	218936.50	31276.6	10.1928		
Error	20	61370.36	3068.5		Prob > F	
C. Total	27	280306.86				<.0001*
▷ Lack Of Fit						
▴ Parameter Estimates						
Term		Estimate	Std Error	t Ratio	Prob> t	
Intercept		261.42857	10.46852	24.97	<.0001*	
Treatment[Control]		-88.17857	25.64253	-3.44	0.0026*	
Treatment[EDTA 10mM]		71.321429	25.64253	2.78	0.0115*	
Treatment[EDTA 15mM]		178.32143	25.64253	6.95	<.0001*	
Treatment[EDTA 5mM]		-20.92857	25.64253	-0.82	0.4240	
Treatment[SCD 10mM]		-38.17857	25.64253	-1.49	0.1521	
Treatment[SCD 15mM]		-18.17857	25.64253	-0.71	0.4866	
Block[1]		10.428571	10.46852	1.00	0.3311	
▴ Effect Tests						
		Sum of				
Source	Nparm	DF	Squares	F Ratio	Prob > F	
Treatment	6	6	215891.36	11.7261	<.0001*	
Block	1	1	3045.14	0.9924	0.3311	
▴ Effect Details						
▴ ▼ Treatment						
▴ Least Squares Means Table						
		Least				
Level		Sq Mean	Std Error	Mean		
Control		173.25000	27.697102	173.250		
EDTA 10mM		332.75000	27.697102	332.750		
EDTA 15mM		439.75000	27.697102	439.750		
EDTA 5mM		240.50000	27.697102	240.500		
SCD 10mM		223.25000	27.697102	223.250		
SCD 15mM		243.25000	27.697102	243.250		
SCD 5mM		177.25000	27.697102	177.250		
▴ ▼ LSMeans Differences Dunnett						
α= 0.050 Q= 2.80164 Control=Control Adjustment = Dunnett						
Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
EDTA 10mM	Control	159.5000	39.16962	49.761	269.2392	0.0031*
EDTA 15mM	Control	266.5000	39.16962	156.761	376.2392	<.0001*
EDTA 5mM	Control	67.2500	39.16962	-42.489	176.9892	0.3599
SCD 10mM	Control	50.0000	39.16962	-59.739	159.7392	0.6376
SCD 15mM	Control	70.0000	39.16962	-39.739	179.7392	0.3234
SCD 5mM	Control	4.0000	39.16962	-105.739	113.7392	1.0000

APPENDIX D: JMP Output for International Dairy Federation (IDF) Wettability (cont.)

APPENDIX E: JMP Output for Dissolution Rate

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err		
				Mean	Lower 95%	Upper 95%
Control	4	0.072100	0.015362	0.00768	0.0477	0.09654
EDTA 10mM	4	0.033225	0.006227	0.00311	0.0233	0.04313
EDTA 15mM	4	0.004025	0.006028	0.00301	-0.0056	0.01362
EDTA 5mM	4	0.055000	0.003512	0.00176	0.0494	0.06059
SCD 10mM	4	0.050275	0.009506	0.00475	0.0351	0.06540
SCD 15mM	4	0.006400	0.005955	0.00298	-0.0031	0.01588
SCD 5mM	4	0.050950	0.002747	0.00137	0.0466	0.05532

APPENDIX E: JMP Output for Dissolution Rate (cont.)

Response Dissolution Rate

Effect Summary

Summary of Fit

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	7	0.01639570	0.002342	34.1825
Error	20	0.00137043	0.000069	Prob > F
C. Total	27	0.01776613		<.0001*

Lack Of Fit

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob> t
Intercept	0.0388536	0.001564	24.84	<.0001*
Treatment[Control]	0.0332464	0.003832	8.68	<.0001*
Treatment[EDTA 10mM]	-0.005629	0.003832	-1.47	0.1574
Treatment[EDTA 15mM]	-0.034829	0.003832	-9.09	<.0001*
Treatment[EDTA 5mM]	0.0161464	0.003832	4.21	0.0004*
Treatment[SCD 10mM]	0.0114214	0.003832	2.98	0.0074*
Treatment[SCD 15mM]	-0.032454	0.003832	-8.47	<.0001*
Block[1]	0.0047536	0.001564	3.04	0.0065*

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Treatment	6	6	0.01576300	38.3407	<.0001*
Block	1	1	0.00063270	9.2336	0.0065*

Effect Details

Treatment

Least Squares Means Table

Level	Sq Mean	Std Error	Mean
Control	0.07210000	0.00413889	0.072100
EDTA 10mM	0.03322500	0.00413889	0.033225
EDTA 15mM	0.00402500	0.00413889	0.004025
EDTA 5mM	0.05500000	0.00413889	0.055000
SCD 10mM	0.05027500	0.00413889	0.050275
SCD 15mM	0.00640000	0.00413889	0.006400
SCD 5mM	0.05095000	0.00413889	0.050950

LSMeans Differences Dunnett

$\alpha = 0.050$ Q= 2.80164 Control=Control Adjustment = Dunnett

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
EDTA 10mM	Control	-0.038875	0.0058533	-0.055274	-0.022476	<.0001*
EDTA 15mM	Control	-0.068075	0.0058533	-0.084474	-0.051676	<.0001*
EDTA 5mM	Control	-0.017100	0.0058533	-0.033499	-0.000701	0.0390*
SCD 10mM	Control	-0.021825	0.0058533	-0.038224	-0.005426	0.0066*
SCD 15mM	Control	-0.065700	0.0058533	-0.082099	-0.049301	<.0001*
SCD 5mM	Control	-0.021150	0.0058533	-0.037549	-0.004751	0.0086*

APPENDIX E: JMP Output for Dissolution Rate (cont.)

APPENDIX F: JMP Output for Log₁₀ Volume Mean Diameter [D(4,3)] of Rehydrated Sample

Means and Std Deviations						
Level	Number	Mean	Std Dev	Std Err		
				Mean	Lower 95%	Upper 95%
Control	6	3.4415	0.160909	0.06569	3.273	3.610
EDTA 05mM	6	3.3562	0.646852	0.26408	2.677	4.035
EDTA 10mM	6	-0.6306	0.097137	0.03966	-0.733	-0.529
EDTA 15mM	6	-0.5589	0.086148	0.03517	-0.649	-0.468
SCD 05mM	6	-0.6729	0.109539	0.04472	-0.788	-0.558
SCD 10mM	6	-0.6518	0.160185	0.06540	-0.820	-0.484
SCD 15 mM	6	-0.5838	0.131998	0.05389	-0.722	-0.445

APPENDIX F: JMP Output for Log₁₀ Volume Mean Diameter [D(4,3)] of Rehydrated Sample (cont.)

APPENDIX G: JMP Output for Log₁₀ Volume Median Diameter of Rehydrated Sample

Means and Std Deviations						
Level	Number	Mean	Std Dev	Std Err		
				Mean	Lower 95%	Upper 95%
Control	6	3.8551	0.030564	0.01248	3.823	3.887
EDTA 05mM	6	4.1466	0.200749	0.08196	3.936	4.357
EDTA 10mM	6	-0.6573	0.025307	0.01033	-0.684	-0.631
EDTA 15mM	6	-0.5884	0.031886	0.01302	-0.622	-0.555
SCD 05mM	6	-0.7070	0.038012	0.01552	-0.747	-0.667
SCD 10mM	6	-0.6773	0.076113	0.03107	-0.757	-0.597
SCD 15 mM	6	-0.6209	0.048924	0.01997	-0.672	-0.570

APPENDIX G: JMP Output for Log₁₀ Volume Median Diameter of Rehydrated Sample (cont.)

Response Log[Volume Median Diameter]

Effect Summary

Actual by Predicted Plot

Summary of Fit

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	7	185.84845	26.5498	3437.309
Error	34	0.26262	0.0077	Prob > F
C. Total	41	186.11106		<.0001*

Lack Of Fit

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob> t
Intercept	0.6786939	0.013561	50.05	<.0001*
Treatment[Control]	3.1763985	0.033218	95.62	<.0001*
Treatment[EDTA 05mM]	3.4679383	0.033218	104.40	<.0001*
Treatment[EDTA 10mM]	-1.336	0.033218	-40.22	<.0001*
Treatment[EDTA 15mM]	-1.267062	0.033218	-38.14	<.0001*
Treatment[SCD 05mM]	-1.38572	0.033218	-41.72	<.0001*
Treatment[SCD 10mM]	-1.355982	0.033218	-40.82	<.0001*
Block[1]	-0.054114	0.013561	-3.99	0.0003*

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Treatment	6	6	185.72546	4007.540	<.0001*
Block	1	1	0.12299	15.9232	0.0003*

Effect Details

Treatment

Least Squares Means Table

Level	Sq Mean	Std Error	Mean
Control	3.855092	0.03587943	3.8551
EDTA 05mM	4.146632	0.03587943	4.1466
EDTA 10mM	-0.657306	0.03587943	-0.6573
EDTA 15mM	-0.588368	0.03587943	-0.5884
SCD 05mM	-0.707026	0.03587943	-0.7070
SCD 10mM	-0.677288	0.03587943	-0.6773
SCD 15 mM	-0.620880	0.03587943	-0.6209

LSMeans Differences Dunnett

$\alpha = 0.050$ Q= 2.7011 Control=Control Adjustment = Dunnett

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
EDTA 05mM	Control	0.29154	0.0507412	0.15448	0.42860	<.0001*
EDTA 10mM	Control	-4.51240	0.0507412	-4.64945	-4.37534	<.0001*
EDTA 15mM	Control	-4.44346	0.0507412	-4.58052	-4.30640	<.0001*
SCD 05mM	Control	-4.56212	0.0507412	-4.69918	-4.42506	<.0001*
SCD 10mM	Control	-4.53238	0.0507412	-4.66944	-4.39532	<.0001*
SCD 15 mM	Control	-4.47597	0.0507412	-4.61303	-4.33892	<.0001*

APPENDIX G: JMP Output for Log₁₀ Volume Median Diameter of Rehydrated Sample (cont.)

APPENDIX H: JMP Output for Solubility Index

Means and Std Deviations						
Level	Number	Mean	Std Dev	Std Err		
				Mean	Lower 95%	Upper 95%
Control	2	2.05000	0.121218	0.08571	0.9609	3.1391
EDTA 10mM	2	0.20000	0.090914	0.06429	-0.6168	1.0168
EDTA 15mM	2	0.05000	0.050508	0.03571	-0.4038	0.5038
EDTA 5mM	2	0.75000	0.020203	0.01429	0.5685	0.9315
SCD 10mM	2	0.15000	0.020203	0.01429	-0.0315	0.3315
SCD 15mM	2	0.05000	0.050508	0.03571	-0.4038	0.5038
SCD 5mM	2	0.50000	0.090914	0.06429	-0.3168	1.3168

APPENDIX H: JMP Output for Solubility Index (cont.)

▼ Response Insolubility index						
▷ Effect Summary						
▷ Summary of Fit						
▾ Analysis of Variance						
		Sum of				
Source	DF	Squares	Mean Square	F Ratio		
Model	7	6.1650000	0.880714	142.2692		
Error	6	0.0371429	0.006190		Prob > F	
C. Total	13	6.2021429				<.0001*
▾ Parameter Estimates						
Term		Estimate	Std Error	t Ratio	Prob> t	
Intercept		0.5357143	0.021028	25.48	<.0001*	
Treatment[Control]		1.5142857	0.051508	29.40	<.0001*	
Treatment[EDTA 10mM]		-0.335714	0.051508	-6.52	0.0006*	
Treatment[EDTA 15mM]		-0.485714	0.051508	-9.43	<.0001*	
Treatment[EDTA 5mM]		0.2142857	0.051508	4.16	0.0059*	
Treatment[SCD 10mM]		-0.385714	0.051508	-7.49	0.0003*	
Treatment[SCD 15mM]		-0.485714	0.051508	-9.43	<.0001*	
Block[1]		-0.035714	0.021028	-1.70	0.1403	
▾ Effect Tests						
			Sum of			
Source	Nparm	DF	Squares	F Ratio	Prob > F	
Treatment	6	6	6.1471429	165.5000	<.0001*	
Block	1	1	0.0178571	2.8846	0.1403	
▾ Effect Details						
▾ ▼ Treatment						
▾ Least Squares Means Table						
		Least				
Level		Sq Mean	Std Error	Mean		
Control		2.0500000	0.05563486	2.05000		
EDTA 10mM		0.2000000	0.05563486	0.20000		
EDTA 15mM		0.0500000	0.05563486	0.05000		
EDTA 5mM		0.7500000	0.05563486	0.75000		
SCD 10mM		0.1500000	0.05563486	0.15000		
SCD 15mM		0.0500000	0.05563486	0.05000		
SCD 5mM		0.5000000	0.05563486	0.50000		
▾ ▼ LSMeans Differences Dunnett						
α= 0.050 Q= 3.48878 Control=Control Adjustment = Dunnett						
Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
EDTA 10mM	Control	-1.85000	0.0786796	-2.12450	-1.57550	<.0001*
EDTA 15mM	Control	-2.00000	0.0786796	-2.27450	-1.72550	<.0001*
EDTA 5mM	Control	-1.30000	0.0786796	-1.57450	-1.02550	<.0001*
SCD 10mM	Control	-1.90000	0.0786796	-2.17450	-1.62550	<.0001*
SCD 15mM	Control	-2.00000	0.0786796	-2.27450	-1.72550	<.0001*
SCD 5mM	Control	-1.55000	0.0786796	-1.82450	-1.27550	<.0001*

APPENDIX H: JMP Output for Solubility Index (cont.)

APPENDIX I: JMP Output for Percent Soluble Calcium (Ca)

Means and Std Deviations						
Level	Number	Mean	Std Dev	Std Err		
				Mean	Lower 95%	Upper 95%
Control	6	39.2167	2.14422	0.8754	36.966	41.467
EDTA 10mM	6	80.7833	1.66543	0.6799	79.036	82.531
EDTA 15mM	6	87.3833	4.25178	1.7358	82.921	91.845
EDTA 5mM	6	56.7333	1.95619	0.7986	54.680	58.786
SCD 10mM	6	72.4333	4.09667	1.6725	68.134	76.733
SCD 15mM	6	79.9000	1.94113	0.7925	77.863	81.937
SCD 5mM	6	53.6500	3.95209	1.6134	49.503	57.797

APPENDIX I: JMP Output for Percent Soluble Calcium (Ca) (cont.)

▼ Response Percent Ca

▷ Effect Summary

▷ Summary of Fit

▴ Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	7	11151.056	1593.01	171.0064
Error	34	316.727	9.32	Prob > F
C. Total	41	11467.783		<.0001*

▷ Lack Of Fit

▴ Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob> t
Intercept	67.157143	0.470954	142.60	<.0001*
Treatment[Control]	-27.94048	1.153596	-24.22	<.0001*
Treatment[EDTA 10mM]	13.62619	1.153596	11.81	<.0001*
Treatment[EDTA 15mM]	20.22619	1.153596	17.53	<.0001*
Treatment[EDTA 5mM]	-10.42381	1.153596	-9.04	<.0001*
Treatment[SCD 10mM]	5.2761905	1.153596	4.57	<.0001*
Treatment[SCD 15mM]	12.742857	1.153596	11.05	<.0001*
Block[1]	0.5	0.470954	1.06	0.2959

▴ Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Treatment	6	6	11140.556	199.3196	<.0001*
Block	1	1	10.500	1.1272	0.2959

▴ Effect Details

▴ ▼ Treatment

▴ Least Squares Means Table

Level	Sq Mean	Std Error	Mean
Control	39.216667	1.2460264	39.2167
EDTA 10mM	80.783333	1.2460264	80.7833
EDTA 15mM	87.383333	1.2460264	87.3833
EDTA 5mM	56.733333	1.2460264	56.7333
SCD 10mM	72.433333	1.2460264	72.4333
SCD 15mM	79.900000	1.2460264	79.9000
SCD 5mM	53.650000	1.2460264	53.6500

▴ ▼ LSMeans Differences Dunnett

α = 0.050 Q= 2.7011 Control=Control Adjustment = Dunnett

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
EDTA 10mM	Control	41.56667	1.762147	36.80693	46.32640	<.0001*
EDTA 15mM	Control	48.16667	1.762147	43.40693	52.92640	<.0001*
EDTA 5mM	Control	17.51667	1.762147	12.75693	22.27640	<.0001*
SCD 10mM	Control	33.21667	1.762147	28.45693	37.97640	<.0001*
SCD 15mM	Control	40.68333	1.762147	35.92360	45.44307	<.0001*
SCD 5mM	Control	14.43333	1.762147	9.67360	19.19307	<.0001*

APPENDIX I: JMP Output for Percent Soluble Calcium (Ca) (cont.)

APPENDIX J: JMP Output for Percent Soluble Phosphorus (P)

Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err		
				Mean	Lower 95%	Upper 95%
Control	6	53.8333	2.36455	0.9653	51.352	56.315
EDTA 10mM	6	81.5833	2.90982	1.1879	78.530	84.637
EDTA 15mM	6	87.8667	2.67020	1.0901	85.064	90.669
EDTA 5mM	6	63.5000	1.82205	0.7438	61.588	65.412
SCD 10mM	6	79.0333	4.05494	1.6554	74.778	83.289
SCD 15mM	6	83.2167	2.70801	1.1055	80.375	86.059
SCD 5mM	6	64.4667	3.95412	1.6143	60.317	68.616

APPENDIX J: JMP Output for Percent Soluble Phosphorus (P) (cont.)

Response Percent P

Effect Summary

Summary of Fit

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	7	5792.6281	827.518	88.0353
Error	34	319.5948	9.400	Prob > F
C. Total	41	6112.2229		<.0001*

Lack Of Fit

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob> t
Intercept	73.357143	0.473081	155.06	<.0001*
Treatment[Control]	-19.52381	1.158808	-16.85	<.0001*
Treatment[EDTA 10mM]	8.2261905	1.158808	7.10	<.0001*
Treatment[EDTA 15mM]	14.509524	1.158808	12.52	<.0001*
Treatment[EDTA 5mM]	-9.857143	1.158808	-8.51	<.0001*
Treatment[SCD 10mM]	5.6761905	1.158808	4.90	<.0001*
Treatment[SCD 15mM]	9.8595238	1.158808	8.51	<.0001*
Block[1]	0.247619	0.473081	0.52	0.6041

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Treatment	6	6	5790.0529	102.6622	<.0001*
Block	1	1	2.5752	0.2740	0.6041

Effect Details

Treatment

Least Squares Means Table

Level	Sq Mean	Std Error	Mean
Control	53.833333	1.2516553	53.8333
EDTA 10mM	81.583333	1.2516553	81.5833
EDTA 15mM	87.866667	1.2516553	87.8667
EDTA 5mM	63.500000	1.2516553	63.5000
SCD 10mM	79.033333	1.2516553	79.0333
SCD 15mM	83.216667	1.2516553	83.2167
SCD 5mM	64.466667	1.2516553	64.4667

LSMeans Differences Dunnett

$\alpha = 0.050$ Q= 2.7011 Control=Control Adjustment = Dunnett

Level	- Level	Difference	Std Err Dif	Lower CL	Upper CL	p-Value
EDTA 10mM	Control	27.75000	1.770108	22.96876	32.53124	<.0001*
EDTA 15mM	Control	34.03333	1.770108	29.25210	38.81457	<.0001*
EDTA 5mM	Control	9.66667	1.770108	4.88543	14.44790	<.0001*
SCD 10mM	Control	25.20000	1.770108	20.41876	29.98124	<.0001*
SCD 15mM	Control	29.38333	1.770108	24.60210	34.16457	<.0001*
SCD 5mM	Control	10.63333	1.770108	5.85210	15.41457	<.0001*

APPENDIX J: JMP Output for Percent Soluble Phosphorus (P) (cont.)