AN ABSTRACT OF THE THESIS OF

Melissa L. Nonnemacher for the degree of Master of Science in Food Science and Technology presented on August 11, 2004.

Title: Effect of whey concentrate crystallization parameters on lactose crystal forms

Abstract Approved:

Redacted for Privacy

Lisbeth Meunier-Goddik

Effects of whey concentrate crystallization parameters of temperature, pH, and length of time of crystallization on lactose crystal forms and amount of lactose crystallized were investigated. Effect of crystallization parameters on freeze-dried powder quality was also investigated. Lactose crystal forms were observed in x-ray crystal diffraction patterns of freeze-dried whey concentrate and quantification of amount of lactose crystallized was performed using peak areas. Crystallization parameters had no significant effect on lactose form or amount crystallized. Lactose crystallized at 55% (SD 4%) in the α-lactose monohydrate form with

crystals of similar dimensions. Powder properties of solubility index, free moisture, and angle of repose (flowability) were not significantly affected. Conclusions were that normal commercial processing parameter fluctuations do not affect whey concentrate cystallization.

Effect of Whey Concentrate Crystallization Parameters on

Lactose Crystal Forms

by

Melissa L. Nonnemacher

A THESIS

Submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Presented August 11, 2004

Commencement June 2005

Master of Science thesis of Melissa L. Nonnemacher
Presented on August 11, 2004.

APPROVED:

Redacted for Privacy

Major Professor, representing Food Science and Technology

Redacted for Privacy

Head of the Department of Food Science and Technology

Redacted for Privacy

Dean of the Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Redacted for Privacy

Melissa L. Nonnemacher, Author

ACKNOWLEDGEMENTS

I would like to thank my advisor, Dr. Lisbeth Goddik, and committee members Dr. Mike Penner and Dr. Alix Gitelman for their willingness to listen to and discuss my questions. They all made time for me when I needed it. I would also like to thank committee members, Dr. Bob McGorrin and Dr. Jean Hall, who stepped in at the last minute to attend my defense after summer vacation conflicts occurred.

I couldn't have constructed the hygroscopicity apparatus without the help of James Batdorff, who also helped with other equipment and labware problems and questions that are too numerous to mention. Additional thanks go to Jeff Clawson, for assistance with the freeze-dryer and other questions, Alex Yokochi for assistance with the x-ray diffractometer, Melynda Cheng for help with transporting the whey concentrate and preparing it for crystallization, and Marcia Walker and Rhoda Sithole for general support.

The greatest thanks go to my family, especially my mom, who have supported me every step of the way- from helping me move across the country, twice, to the belief that I could finish despite all the setbacks. I would also like to thank Brian for his patience. Although he missed the research, he was there for the writing process and I really appreciate his support.

TABLE OF CONTENTS

Page

CHAPTER 1: GENERAL INTRODUCTION..... 1

CHAPTER 2: LITERATURE REVIEW..... 8

EFFECT OF PROCESSING STEPS..... 10

Effects of thermal processes on whey powder quality..... 12
Effects of crystallization on whey powder quality... 16
Effects of spray drying on whey powder quality..... 22

LACTOSE CRYSTAL GROWTH AND RATE OF CRYSTALLIZATION..... 25

X-RAY CRYSTAL DIFFRACTION..... 28

CHAPTER 3: EFFECT OF WHEY CONCENTRATE CRYSTALLIZATION PARAMETERS ON LACTOSE CRYSTAL FORM..... 29

ABSTRACT..... 30

INTRODUCTION..... 31

MATERIALS AND METHODS..... 34

Whey Samples..... 34
Crystallization of Whey Concentrate..... 34
Viscosity Measurements..... 37
Freeze Drying..... 37
Compositional Analysis..... 38
Sample Preparation for Further Analysis..... 38
Hygroscopicity Measurements..... 38
Flowability (Angle of Repose)..... 39
Titratable Acidity..... 39
Solubility Index..... 40
X-ray Crystal Diffraction..... 40
Size Determination of Lactose Crystals..... 41
Estimation of Commercial Powder Variability..... 41

TABLE OF CONTENTS (Continued)

Page

MATERIALS AND METHODS (Continued)

Enzymatic Analysis of Lactose Hydrolysis..... 42
Statistical Analysis..... 42

RESULTS AND DISCUSSION..... 44

CONCLUSION..... 66

REFERENCES..... 67

CHAPTER 4: CONCLUSIONS..... 69

BIBLIOGRAPHY..... 72

LIST OF FIGURES

Figure		Page
2.1	Basic process for the manufacture of whey powder	11
2.2	Alpha-lactose	17
2.3	Change in whey concentrate crystallization time based on whey volume	23
2.4	Common shape of the α-lactose monohydrate crystal	27
3.1	Flow chart of research performed	36
3.2	Rate of cooling for whey concentrate during crystallization at a) 10 °C and b) 15 °C	45
3.3	X-ray crystal diffraction pattern for pure α-lactose monohydrate	50
3.4	X-ray crystal diffraction patterns for freeze-dried whey concentrate samples crystallized at different a) Temperatures, b) pH, and c) Lengths of crystallization (time)	51
3.5	Hygroscopicity (%) of freeze-dried whey concentrate samples crystallized at different conditions	58
3.6	Hygroscopicity (%) and amount of lactose crystallized in freeze-dried whey concentrate samples crystallized at different conditions	60
3.7	Lactose crystal dimensions in whey concentrate samples crystallized at different conditions	63
3.8	Lactose crystals in α-lactose monohydrate form	65

LIST OF TABLES

Table Page

3.1 Whey concentrate composition for three separate trials..... 44

3.2 Solubility index and % titratable acidity for whey concentrate samples..... 47

3.3 Viscosity of whey concentrate before and after crystallization at different conditions..... 48

3.4 a) Amount of crystalline lactose (%) determined using x-ray crystal diffraction for selected freeze-dried whey concentrate samples crystallized at different conditions..... 54

b) Amount of crystalline lactose (%) determined using x-ray crystal diffraction for commercial spray-dried powders from different lots..... 54

3.5 a) Free moisture, hygroscopicity, and angle of repose values for selected freeze-dried whey concentrate samples crystallized at different conditions..... 56

b) Free moisture, hygroscopicity, and angle of repose values for commercial spray-dried whey powders samples from one day's production..... 57

3.6 Mean crystal dimensions for lactose crystals in whey concentrate samples crystallized at different conditions..... 62

CHAPTER 1

GENERAL INTRODUCTION

GENERAL INTRODUCTION

Whey is the liquid substance obtained by separating the coagulum from milk, cream, or skim milk during the cheesemaking process (21 CFR Sec. 184.1979). Processing liquid whey, using reverse osmosis filtration and evaporation, produces whey concentrate. The final concentrate contains approximately 60% solids, the majority of which is lactose. To make commercial whey powder, whey concentrate is crystallized to encourage the formation of the desired α-lactose monohydrate and then spray dried. The final dried product is required to be composed of 0.2-2.0% fat, 10-15% protein, and 61-75% lactose (21 CFR Sec. 184.1979).

In 2003, dairy companies throughout the United States produced 1.09 billion pounds of whey powder (USDA 2004). It is often sold cheaply as animal feed, but has recently been found useful as a food ingredient in a wide variety of products, from soups to baked goods to meat products (Spreer 1998). Whey powder is commonly used in these products for its functional properties, which include imparting dairy flavor and contributing to desireable Maillard color formation, in addition to being an inexpensive source of carbohydrates (Caric 1994b; Fennema 1996b).

Utilization of whey powder as an ingredient requires that the powder be of high quality and consistency as manufacturers require an ingredient to be consistent to maintain their own predictable and stable process and end product. A high quality whey powder is non-hygroscopic, free flowing, white in color, and free of undesireable off-flavors. A hygroscopic powder is one that takes up water from the surrounding air, leading to a perceptible, change in the powder's properties (ie. flowability).

Previous research has shown that lactose forms, specifically amorphous and crystalline forms, are largely responsible for hygroscopicity and caking of dairy powders (Troy and Sharp 1930; Sharp and Doob 1941; Saltmarch and Labuza 1980). Lactose is a disaccharide naturally present in milk that consists of D-glucose and D-galactose (β-D-galactopyranosyl (1-4)-D-glucopyranose). In aqueous solution, lactose has two isomeric forms, α-lactose and β-lactose. Mutarotation occurs until the forms reach a temperature dependent equilibrium (Walstra 1999).

In the solid state, lactose can be amorphous or crystalline. Amorphous lactose is in the glassy state with molecules packed in random order. Crystalline lactose contains ordered molecules and can be present in whey powder as four different forms: anhydrous β-lactose, unstable anhydrous α-lactose, stable anhydrous α-lactose, and α-lactose monohydrate. Mixed crystalline forms can also occur (ex. 5α/3β) (Drapier-Beche et al 1997).

The different lactose forms have different hydration properties that affect water uptake and therefore the hygroscopic nature of the powder (Drapier-Beche et al 1997). Amorphous lactose is very hygroscopic. Any moisture near crystalline α-lactose monohydrate can only be adsorbed on the crystal faces in very small amounts, making this form stable (Walstra 2003c).

The processes and conditions (heat, pre-crystallization, etc.) used to produce whey powder affect which lactose forms are present in the final spray dried product. Controlling the crystallization conditions prior to spray drying can help promote the formation of the desired α-lactose monohydrate crystals. The majority of lactose crystallization occurs between concentrating and spray drying. Lactose crystallization involves four important steps. First, the whey concentrate is rapidly cooled to 30 °C to saturate the solution and favor nucleation. Lactose nuclei provide the seeds from which new crystal structures grow. Second, whey concentrate is seeded with powdered α-monohydrate crystals. This lactose seeding material acts as pre-formed nuclei that provide existing crystalline surface structures on which lactose can readily crystallize (Jayaprakasha et al 1995). The seeds promote the formation of many more small α-monohydrate crystals than would be observed without seeding (Jayaprakasha et al 1995). Third, the seeded concentrate is slowly cooled to 10 °C to increase the saturation. Last, the concentrate is continuously stirred for 8-24 hours. Stirring during crystallization breaks apart undesireable larger sized crystals and brings solubilized lactose into

contact with lactose nuclei for crystallization (Walstra 2003a). The rate at which crystallization occurs depends on a number of additional variables including total available crystal surface (increased by seeding), degree of supersaturation, temperature, presence of interfering salts, and viscosity of the solution (Caric 1994c).

When conducting experimental analysis to determine the amount and types of lactose crystals produced during the commercial whey crystallization process, the crystals must be "locked" into their solid forms by segregating the water. Any water that is present can allow the crystals to dissolve and mutarotate, which would give an incorrect view of the types and amount of crystallized lactose present at the end of the crystallization process. Lactose crystal forms can be maintained by rapid freezing of the samples followed by freeze drying as performed by Jouppila et al (1997; 1998) and Drapier-Beche et al (1997) on lactose and skim milk powder samples. Rapid freezing crystallizes the water in the sample preventing it from interacting with other components. Freeze drying then removes the water in the sample by sublimating the solid water into a gaseous form. As the water is not present in the liquid phase, little alteration in the sample is seen. Freeze dried samples are very similar in composition to their pre-freezing states.

X-ray diffraction (XRD) is a common technique used to identify the various crystal forms of lactose. The different lactose forms have different angles

of diffraction that are seen as peaks in XRD patterns. The intensities of the peaks relate to the amount of each crystal form present.

Much of the previous research regarding lactose crystallization was performed on pure lactose solutions and in liquid whey to discover how to maximize the amount of lactose recovered. Additional research has focused on the development of lactose crystals in skim milk powder and whey powder, which can be a problem during powder storage. Temperature, pH, and total solids have been previously determined to affect lactose crystallization and size of crystals. The pH and total solids of whey concentrate are approximately 5.4 and 60 %, respectively, with crystallization taking place at 10 ^{0}C for a minimum of 4 hours, but as long as 24 hours, for most operations (Honer 1985; Patel 1991). Nickerson and Moore (J Dairy Sci 1974) found that acidity outside the normal processing range (pH < 1) accelerated lactose crystallization in pure lactose solutions. Modler and Lefkovitch (1986) found that pH had no effect on lactose crystal size. Hargrove et al (1975) saw no significant increase in crystallization by crystallizing whey concentrate for longer than one hour at 42 ^{0}C or seeding the whey concentrate. Jayaprakasha et al (1995) determined that total solids and amount of seeding material affected the amount of lactose crystallized and the average crystal size. Saito et al (1988) found that α-lactose monohydrate crystallized in whey powder exposed to 75 % humidity. However, little is known about how pH, length of

crystallization time, and temperature affect lactose crystallization within the whey concentrate system and within normal, commercial processing parameters.

The objective of the research was to determine whether altering the crystallization conditions of whey concentrate, within normal processing parameters, affects the amount and structure of lactose crystals. Parameters investigated include sample pH, crystallization time, and crystallization temperature.

This research is intended to be of benefit to the commercial whey processing industry. The manufacturing process sees fluctuations in: pH of the whey due to activity of cheddar cheese starter cultures and types of cheese whey used; crystallization temperatures, due to different rates of cooling; and length of time to crystallize, as crystallization is a batch process within a continuous process with crystallization time often adjusted to accommodate changes in the volume of incoming whey from cheese vats. The treatments chosen for this research are extremes generally seen in commercial whey processing and results of the experiments will show whether these normal variations have an effect on the final whey powder quality.

CHAPTER 2

LITERATURE REVIEW

LITERATURE REVIEW

Whey is the greenish-yellow liquid that is separated from curd during the manufacture of cheese. The cheese curd contains most of the fat and the casein protein, while the whey contains lactose and the proteins β-lactoglobulin, α-lactalbumin, immunoglobulin, proteose peptone, and other minor proteins, and minerals (de la Fuente et al 2002). There are two different types of whey produced: acid whey and sweet whey. Acid whey is a by-product of the direct addition of acid to coagulate milk, as is used in the manufacture of cottage cheese or ricotta cheese. Sweet whey is the by-product of the addition of proteolytic enzymes to milk. These enzymes break down caseins, resulting in agglomeration of the casein micelles to produce curd. The majority of sweet whey is recovered from the production of cheddar cheese. Acid whey has a final pH of 4.4-4.6 while sweet whey has a higher pH of 5.5-6.0 (Chandan 1997). Frequently, both types of whey are dried into a powder which are sold as food ingredients.

Whey powder made from sweet whey is required to be 61-75% lactose, 10-15% protein, 0.2-2.0% fat, 7-14% ash, 1-8% moisture (21 CFR Sec. 184.1979). Utilization of whey powder as a food ingredient requires that it be of high quality and have consistent properties. A high quality whey powder will have all of the desireable properties for use which include flow properties, keeping quality, and ability to easily reconstitute (Hardy 2002). The powder should be non-

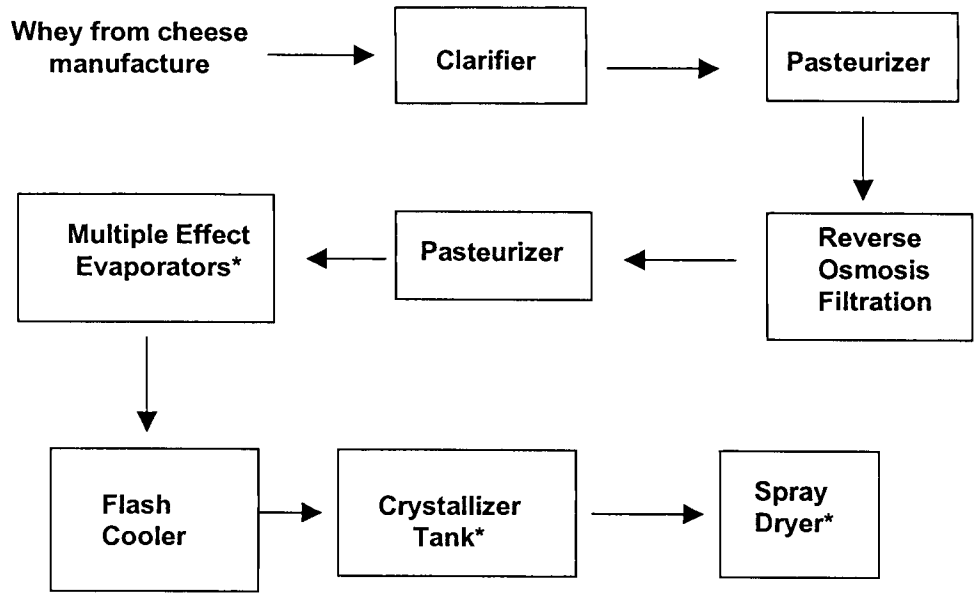
hygroscopic and free flowing for ease of use, white in color and free of undesireable off-flavors throughout the shelf-life of the powder, and able to be reconstituted as needed. Despite the similarities in composition of all whey powders, those manufactured using different processing variables produce products with different characteristics. Processes must be chosen correctly for the desired product and closely monitored to produce a consistent and high-quality product.

EFFECT OF PROCESSING STEPS

The production of whey powder requires three general operations: evaporation, crystallization, and drying (Figure 2.1). The specific technology, processing steps, and the monitoring and control of these steps can affect the hygroscopicity, color, flavor, and/or solubility of the powder (Caric 1994c).

Caking tendency, lactose crystallization rate, and free water content are important characteristics that vary greatly depending on the technology and temperatures used in the manufacture of whey powder. The tendency for a powder to cake can vary from approximately 0 %, where the powder maintained at normal storage conditions will experience practically no caking, to 100 %, where the powder begins immediately to cake after production is finished. Lactose crystallization can vary from 0 to 95 % and free moisture content can range from 1 to 4 % based on production methods used (Caric 1994c).

Figure 2.1 Basic process for the manufacture of whey powder



Steps indicated with an * are general operations required for the manufacture of whey powder

Effects of thermal processes on whey powder quality

Pasteurization and evaporation are both thermal processing steps required in the production of whey powder. These processes utilize the minimum temperatures required to efficiently process the whey while still maintaining a safe product. However, the heat applied to the whey concentrate during these steps can potentially cause protein denaturation, which can have a large effect on the quality of the whey powder (Schmidt et al 1984).

Pasteurization is a relatively mild heat treatment that is used to destroy heat-sensitive microorganisms, specifically pathogens and most spoilage bacteria, and inactivate enzymes (Fellows 1997). Whey pasteurization is generally performed at 72 °C for 15 sec to inactivate the enzymes and microorganisms that were naturally present in the milk and those added to the milk during cheese manufacture (21 CFR 1240.61). Omitting the pasteurization step can allow for the survival of these enzymes and microorganisms in the final whey powder which can result in a shorter shelf-life due to off-flavors, lipid oxidation, etc., for the powder (Walstra 1999).

During processing, the whey is concentrated from 6.35 % total solids to the 42 – 60 % total solids required for crystallization and rapid spray drying (Caric 1994c). Evaporation is a common process used to first remove water from whey before finishing using a spray drying. Evaporation is used, even though it can cause some thermal damage, as it is more economical than removing the water from the entire whey stream using the less damaging process of spray drying

(Gillies 1974). Temperatures in the range of 55-75 °C are generally used for the process of evaporation. Multiple effect evaporators are often chosen to efficiently remove water from the liquid whey without exposing the product to high temperatures for an extended period of time.

Protein denaturation can have effects on the quality of the whey powder based on the extent to which denaturation has occurred. The two main effects include decreased solubility and increased or decreased water binding capacity. Additional effects on viscosity and foamability are more of a concern in higher protein concentrates (Schmidt et al 1984; Hawks et al 1993).

A native protein is the most thermodynamically stable form of a protein at normal conditions of pH and temperature (Fennema 1996a). The stable form is made up of specific conformations of its polypeptide(s). The native state is maintained through a balance of many peptide chain interactions, including electrostatic and hydrophobic, in addition to hydrogen bonding and disulfide bonds (Mangino 1984).

A denatured protein is considered to be one that has undergone major changes in the secondary, tertiary, and quaternary structures without cleavage of backbone peptide bonds (Fennema 1996a). This change in structure modifies a number of important functional properties of the protein: viscosity, solubility, foaming, and fat and flavor (Schmidt et al 1984; Fennema 1996b). These functional properties are affected due to differences in how the protein reacts with other proteins and/or solvent water (Damodaran 1997).

Whey proteins β-lactoglobulin and α-lactalbumin are globular proteins, a type of secondary structure, with a net negative charge at pH 6.6 (pH of milk) (de Wit and Klarenbeek 1984). Most of their hydrophobic residues are buried, due to intramolecular folding, which prevents much self-association or interaction with other molecules (Fennema 1996a).

Denaturation of whey proteins occurs within a range of temperatures, starting at 62 °C. Most dairy processing operations heat to temperatures that are high enough to cause some protein denaturation. Of all the whey proteins, α-lactalbumin is the least heat stable with denaturation occurring at approximately 62 °C (de Wit and Klarenbeek 1984). However, the structural unfolding that occurs is reversible except at very high temperatures (Fennema 1996a). Serum albumin denatures at 64-66 °C, immunoglobulins at 72 °C, and β-lactoglobulins at 78-82 °C (de Wit and Klarenbeek 1984; Jelen 1991). Proteose-peptones are essentially heat stable (Jelen 1991). Alpha-lactalbumin comprises 25% and β-lactoglobulin 50 % of the whey proteins found in cheese whey (Robinson 1994).

The amount of lactose and milk salts present in whey may have an affect on the amount of whey protein denaturation that occurs during heating (Schmidt et al 1984). Beta-lactoglobulin has been found to be stabilized against thermal denaturation by increasing amounts of lactose, but destabilized by increasing amounts of ionic calcium (Schmidt et al 1984; Parris 1993). Higher levels of calcium ions can lead to an increase in the formation of insoluble protein

aggregates from unfolded proteins (de Wit 1990). The amount of protein aggregation that occurs has also been found to be dependant on the pH of the whey, with more aggregation occurring at low pH (Parris 1993). Increased denaturation of β-lactoglobulin has been found to occur at alkaline pH while increased denaturation of α-lactalbumin has been observed at acid pH conditions (de Wit 1990). Since the pH and titratable acidity of the whey is closely controlled during the cheese-making process, large variations in pH are not usually much of a concern in whey powder manufacturing using whey from one type of cheese, like cheddar. However, processes that use whey from a variety of cheeses, for example mozzarella and cheddar cheese wheys combined for processing, may encounter larger pH fluctuations.

Heat denaturation can also result in reduced protein solubility. In order for a protein to be soluble, electrostatic and hydration repulsive forces need to be greater than attractive hydrophobic reactions. Increased temperature can disrupt ionic and hydrogen bonding and cause a decrease in protein-water interactions and an increase in protein-protein interactions. These changes upset the surface hydrophilicity/hydrophobicity balance, thereby decreasing solubility (Damodaron 1997). Fewer protein-solvent interactions also causes a decrease in water binding capacity while more hydrophobic protein-protein interactions leads to the aggregation of protein molecules (Fennema 1996b). Decreased solubility of the

final whey powder is undesireable as it makes the powder difficult to use as it will not mix easily with water.

Denaturation can have positive or negative affects on the water binding capacity of whey proteins (Mangino 1984). The type of effect is based on the amino acids in the protein and their arrangement. If a protein has been unfolded, groups that can hydrogen bond may be able to make better contact with water than was possible in the native state, increasing water binding capacity (Mangino 1984). However, if the unfolded state causes these groups to be in closer contact with one another, hydrogen bonding could occur between the groups and decrease the binding with water.

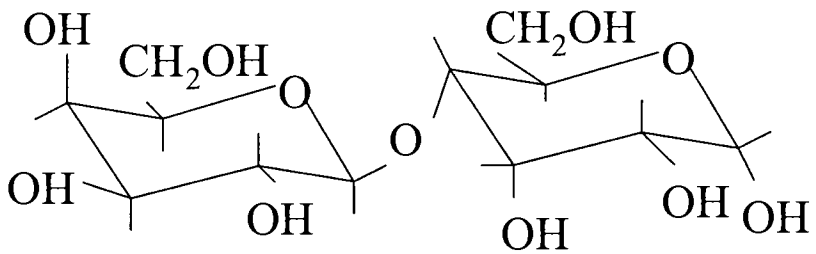
An increase in the hydrophobicity of thermally denatured proteins can increase the ability of proteins to bind to volatile flavors while in a high moisture state (Fennema 1996a). Whey proteins are susceptible to binding aldehydes, ketones, and alcohols generated by the oxidation of unsaturated fatty acids (Fennema 1996a). Free fatty acids may have a undesireable affect on the flavor and functional properties of the whey powder (Tomaino et al 2001).

Effects of crystallization on whey powder quality

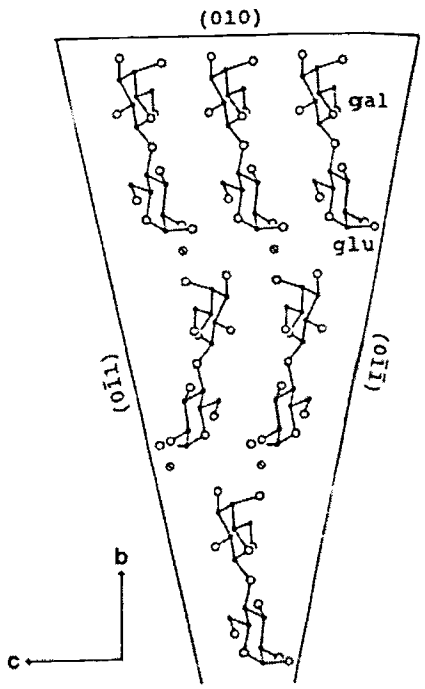
The form of lactose desired in the final whey powder product is crystalline α-monohydrate. Usually, α-monohydrate crystallizes as a hydrate containing one α-lactose molecule and one water molecule (Figure 2.2) (Walstra 1999). Water is

Figure 2.2. Alpha-lactose.

a) Chemical structure of α-lactose



b) Packing arrangement of α-lactose monohydrate (Mak 1992)



packed between α-lactose molecules and hydrogen bonding occurs between the water and the lactose molecules in a three-dimensional network (Fries 1971). The crystals are fairly stable, as they have few hydrophillic groups and are not readily soluble, and non-hygroscopic. While β-lactose crystals are very stable as well, α-lactose monohydrate crystals predominately crystallize at temperatures below 93 °C which are the temperatures used during processing (Zadow 1984). Final crystal size is desired to be less than 25 μm as the small final crystal size prevents a sandy texture when utilized as an ingredient in other foods (Early 1998).

A whey powder with a high concentration of amorphous lactose is the result of omitting the lactose crystallization step during whey powder production (Saito 1988). The reason the step is sometimes omitted is to decrease production costs (Gillies 1974). However, omitting the crystallization step does not allow time for lactose to naturally crystallize. Amorphous lactose is highly hygroscopic and will easily take up water from the surrounding environment (Caric 1994). This increase in water content of the powder can be very detrimental to the powder quality. The uptake of water by the hygroscopic powder leads to it becoming tacky, which can result in undesirable caking, making it difficult to pour or transfer the powder (Walstra 1999).

Caking, or a solidification of powder, is specifically due to a greater number and intensity of particle-particle interactions (Hardy et al 2002). Dry whey particles are composed of lactose, either amorphous or partially crystalline,

whey protein, fat globules, and air as spherical cells (Saltmarch and Labuza 1980). Moisture taken up from the surrounding air increases the molecular mobility of the lactose in the whey powder particle, resulting in the rearrangement of the lactose into a regular crystal lattice (Saltmarch and Labuza 1980). Crystallization of amorphous lactose is thought to occur due to water plasticization and depression of the glass transition temperature (T_g) to below the storage temperature (Jouppila 1997). T_g, which decreases as moisture content increases, is the temperature at which the sugar transitions between a glassy and a rubbery state. As crystallization occurs, the lactose on the particles become tightly packed. The packing of the lactose causes water to desorb, releasing it to other powder components (Berlin et al 1968; Saltmarch et al 1980). The released water can absorb on particle surfaces and create a saturated solution. This results in the particles becoming sticky and forming liquid bridges (Downton et al 1982; Wallack and King 1988). As evaporation occurs, the liquid bridges become solid, resulting in caked powder (Hardy et al 2002).

Higher water content, due to moisture picked up by amorphous lactose, also increases the possibility that microorganisms will grow in the powder during storage. Depending on the microorganisms present, off-flavors and rancidity could develop, while safety could be jeopardized. Molecular mobility, or the ability of lactose and protein molecules, specifically, to move within the powder, will also be increased due to higher moisture content. Interactions between the reducing sugar, lactose, and whey proteins can lead to increased nonenzymatic

Maillard browning reactions and unwanted color formation during storage (Burin et al 2000). Amorphous lactose also encapsulates milk fat which, like water, can be released during lactose crystallization (Roos 2002). The fat then becomes susceptible to rapid lipid oxidation, which can lead to off-flavors and an overall deterioration of the powder (Ferretti and Flanagan 1971; Roos 2002).

The addition of a step between evaporation and spray drying allows time for lactose crystallization to occur and increases the powder quality. Saturation of lactose occurs too rapidly during spray drying to allow crystallization (Schuck and Dolivet 2002). Higher quality powder is accomplished by increasing the concentration of lactose in the stable α-monohydrate form and decreasing the concentration of undesireable amorphous lactose. To crystallize the whey concentrate, the whey is pumped into a flash cooler and quickly cooled to 30 °C, the optimal temperature for mutarotation of β-lactose into α-lactose (Caric 1994c). The whey is then slowly cooled to 10 °C, taking care to control the cooling (Patel et al 1991). If cooling occurs too quickly, only small amounts of β-lactose will have had time to mutarotate and few α-lactose molecules will be available to bind with one water molecule to form α-lactose monohydrate crystals (Zadow 1984).

Additionally, if too much β-lactose is present, it can also interfere with the growth of the desired α-lactose monohydrate crystal (Saito 1988). Because β- and α-lactose are anomers and structurally very similar, β-lactose can adsorb onto the crystal face and prevent incorporation of α-lactose by blocking attachment sites

(Walstra 2003a). The α-lactose molecules cannot attach to the β-lactose, therefore further crystallization is prevented at that site until the β-lactose molecule moves (Walstra 2003a). This competition between β-lactose and α-lactose, in addition to the presence of other non-lactose components like mineral salts that influence mutarotation, can slow crystal growth if β-lactose is present at a high enough concentration (Patel et al 1991).

Adding approximately 0.1% pure α-monohydrate seed crystals with sizes between 10-15 μm favors the formation of small crystals with a final size of about 25 um or less. Crystals larger than 25 μm can be detected on the palate and are unacceptable for human consumption (Early 1998).

In addition to favoring mutarotation of β-lactose into α-lactose, the decrease in temperature during crystallization also causes the supersaturation of the lactose which, with the high total solids present, again promotes the crystallization of many small lactose crystals (Caric 1994c, Early 1998). If the concentration of lactose were low, the formation of fewer larger sized crystals would be favored (Walstra 2003b).

Agitating the whey concentrate during crystallization also favors crystal formation as small lactose nuclei are brought into contact with the supersaturated solution (Early 1998). In addition, the constant agitation breaks down larger crystals, prevents crystals from depositing on the bottom of the tank, and helps to maintain viscosity (Walstra 2003b). Without agitation, the whey concentrate

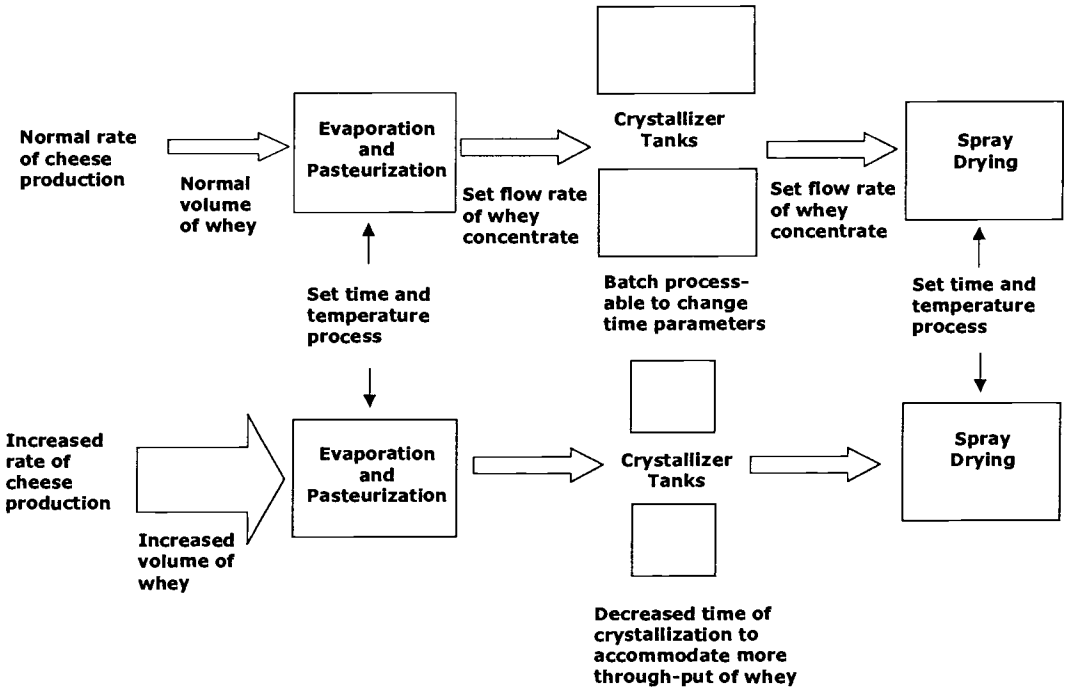
becomes very viscous which decreases the amount of lactose crystallization (Modler et al 1986; Jayaprakasha et al 1995). Increased viscosity also makes the whey concentrate difficult to pump and spray dry, which can result in a powder with high moisture content and decreased powder quality (Hargrove et al 1975).

The crystallization of lactose is a batch step in the middle of a continuous process. The time of crystallization can be altered to accommodate more or less volume of incoming whey (Figure 2.3). When there is extra whey needing to be processed from cheese-making, the crystallization time can be decreased to allow for more product through-put. It is also the least controlled process in the manufacture of whey powder as it is much more difficult to cool at a specific rate than it is to heat at a certain temperature and pressure. Industrial crystallization tanks can accommodate as much as 20,000 gallons of whey concentrate. Cooling of the whey concentrate from the flash cooler temperature of approximately 31 ^{o}C to the crystallization temperature of 10 ^{o}C in the tank is done by circulating cooled water within the tank walls. Although the whey concentrate is agitated, bringing it within contact of the cool walls, the cooling is not instantaneous. The temperature slowly decreases over a few hours before reaching a final temperature of 10 ^{o}C.

Effects of spray drying on whey powder quality

After crystallizing for 4-24 hours, the whey concentrate is spray dried. The type of spray drying system used and the rate at which the whey is dried can affect the final product quality, specifically in terms of the concentration of amorphous

Figure 2.3. Change in whey concentrate crystallization time based on whey volume



lactose. Powders produced using single stage drying which includes spray drying at an inlet temperature of 200 °C and pneumatic transport for cooling results in an unagglomerated, dusty powder with a high bulk density (Early 1998, Caric 1994c). Customers find this type of powder undesireable as it is difficult to use. Unagglomerated powder can be difficult to reconstitute as the cakes are less porous (Downton et al 1982). The powder also includes very fine particles that can cause dust clouds when transferring from the bag.

If a two-stage drying system is used, the resulting powder is agglomerated and free-flowing with few dusty particles (Early 1998). The powder particles are larger in size and are porous, allowing for better rehydration (Downton et al 1982). This non-hygroscopic, caking-resistant powder is produced by decreasing the spray-drying inlet temperature to approximately 185 °C to allow the powder to have about 5-7 % more moisture (Caric 1994c). The powder is then transferred to a fluid bed where ambient air (30 °C) is blown on the powder in the first section to agglomerate. This process is controlled so only the surface of the particle becomes sticky, allowing small clusters of powder to form due to particle collisions and adherence (Downton et al 1982). Air in the second section is approximately 100 °C to remove excess moisture and air in the third section is approximately 11 °C to cool the powder and prevent additional moisture absorption (Patel et al 1991).

To increase the lactose crystallization in the powder even more after spray drying, an inlet temperature of 150 °C is used to produce a powder with 14 % higher moisture content (Caric 1994). At this higher moisture content, the lactose

crystallization can continue on a crystallization conveyor belt before moving to the fluid bed to remove the excess moisture as described in the two-stage evaporation process (Patel et al 1991).

Research performed by de la Fuente, et al (2002) showed no significant alterations in amount of whey protein aggregations after spray drying. The rapid rate of drying that occurs during spray drying (1-10 seconds) and the product remaining at the wet-bulb temperature of the drying air results in minimal protein denaturation (Fellows 1997).

Additional factors that may affect final whey powder quality include use of different types of coagulant during cheese-making, use of annatto as a colorant, and use of bleaching agent to whiten the whey to produce a non-colored final powder. No affect on foamability, which is related to the degree of protein denaturation, was seen in sweet whey protein isolate with use or exclusion of starter media, annatto, pasteurization, bleaching agent, or type of coagulant (Hawks et al 1993).

LACTOSE CRYSTAL GROWTH AND RATE OF CRYSTALLIZATION

The formation and growth of lactose crystals occurs through the process of nucleation and crystallization. Nucleation, or the formation of a new phase within the existing phase, needs to occur before crystallization can take place. Temperature, solute concentration, and impurities all have an effect on the rate of

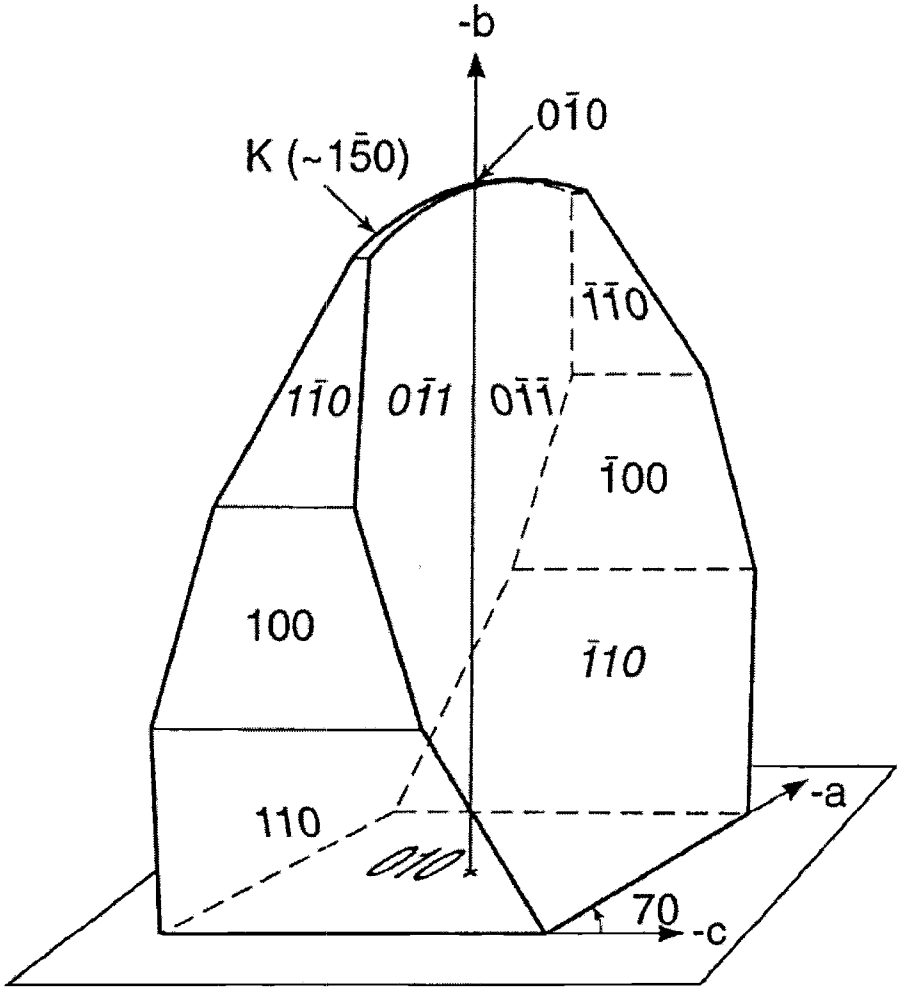
nucleation (Walstra 2003b). Generally, low temperatures, high solute concentration, and catalytic impurities increase the nucleation rate.

Crystallization is a fairly slow process that requires specific conditions and interactions. The rate of crystallization of α-lactose monohydrate involves two equilibria (Zadow 1984). The first involves the conversion of soluble β-lactose to soluble α-lactose while the second involves the conversion of soluble α-lactose into α-lactose monohydrate. The slow rate of crystal formation is partially due to difficulty in incorporating molecules into a crystal lattice. However, the amount and rate of lactose crystallized also depends upon a variety of variables: temperature, pH, presence of crystal nuclei (like seeding material), presence of interfering substances, and time allowed for crystallization (Walstra 2003a).

Lactose crystals grow into different shapes based upon the solution environment and the effect the solution has on the growth rate of the crystal faces. The presence of β-lactose has been found to affect the growth rate of specific faces of the α-lactose monohydrate crystal. This interference is believed to be due to the fact that the α- and β- lactose isomers are very similar in shape and β-lactose is incorporated as easily as α-lactose into the crystal lattice. The adsorption of β-lactose onto the $0\bar{1}1$ and $0\overline{1\bar{1}}$ faces blocks the incorporation of α-lactose and growth on those faces is prevented (Figure 2.4) (Walstra 2003a). The growth that appears to occur on these faces is actually due to growth from adjacent

Fig. 2.4 Common shape of the α-lactose monohydrate crystal.

(Walstra 2003a)



faces. Growth also does not occur on the $0\bar{1}0$ face (in the direction of the b axis). The different growth rates on the crystal faces result in a tomahawk shaped crystal. If little β-lactose is present, needle-like crystals form due to rapid growth on the $0\bar{1}1$ and $0\bar{1}\bar{1}$ faces (Walstra 1999).

X-RAY CRYSTAL DIFFRACTION

X-ray crystal diffraction (XRD) is a non-destructive analytical procedure that is a commonly used to identify and quantify the various crystal forms of lactose in powder samples (Drapier-Beche et al 1997; Jouppila et al 1998). The different lactose forms have different angles of diffraction that are seen as peaks in XRD patterns. The same crystalline substances always give the same pattern. Characteristic peaks have been determined for each crystal form. Alpha-lactose monohydrate has a characteristic peak at the diffraction angle (2θ) 16.4°. Beta-lactose has a characteristic peak of 10.4°. Mixed forms, for example 5α-/3β-lactose found in water plasticized lactose and skim milk powder samples, have a characteristic peak of 18.2° (Jouppila et al 1997; Drapier-Beche 1998; Jouppila et al 1998;). The intensities of the peaks relate to the amount of each crystal form present. Quantification can be performed by analyzing pure form samples and then comparing the characteristic peak area of the mixture to the pure form samples (Drapier-Beche et al 1998).

CHAPTER 3

EFFECT OF WHEY CONCENTRATE CRYSTALLIZATION PARAMETERS ON LACTOSE CRYSTAL FORMS

M.L. Nonnemacher and L.M. Goddik

To be submitted to Journal of Dairy Science

American Dairy Science Association

1111 N. Dunlap Avenue

Savoy, IL 61874

ABSTRACT

Effects of whey concentrate crystallization parameters of temperature, pH, and length of time of crystallization on lactose crystal forms and amount of lactose crystallized were investigated. Effect of crystallization parameters on freeze-dried powder quality was also investigated. Lactose crystal forms were observed in x-ray crystal diffraction patterns of freeze-dried whey concentrate and quantification of amount of lactose crystallized was performed using peak areas. Crystallization parameters had no significant effect on lactose form or amount crystallized. Lactose crystallized at 55% (SD 4%) in the α-lactose monohydrate form with crystals of similar dimensions. Powder properties of solubility index, free moisture, and angle of repose (flowability) were not significantly affected. Conclusions were that normal commercial processing parameter fluctuations do not affect whey concentrate cystallization.

(Key words: sweet whey powder, lactose crystalline forms, x-ray diffraction)

INTRODUCTION

Whey concentrate of approximately 60% solids, often produced through reverse osmosis and evaporation of whey, is commonly spray dried to produce a stable, dried powder which contains 61-75% lactose (21 CFR 184.1979). Before spray drying, the concentrate is usually crystallized to encourage the formation of desired α-lactose monohydrate crystals. The stability of the final powder depends greatly on the amount of lactose crystallized and lactose forms present.

In aqueous solution, lactose (β-D-galactopyranosyl (1-4)-D-glucopyranose) has two isomeric forms, α-lactose and β-lactose. Mutarotation occurs until the forms reach a temperature dependent equilibrium (Walstra 1999). Alpha-lactose is favored at temperatures of less than 93 °C (Zadow 1984). In the solid state, lactose can be in amorphous or crystalline forms. Crystalline lactose occurs as four different forms: anhydrous β-lactose, unstable anhydrous α-lactose, stable anhydrous α-lactose, and α-lactose monohydrate. Mixed crystalline forms can also occur (ex. 5α/3β). Lactose structural forms are important as they are largely responsible for the hygroscopicity of whey powders (Troy and Sharp 1930; Sharp and Doob 1941; Saltmarch 1980). Hygroscopicity is described as the ability of a material to take up water from the surrounding air, leading to a perceptible change in the material's properties. Amorphous lactose is very hygroscopic while the crystalline forms of lactose are only slightly or non-hygroscopic. The amount and

rate of lactose crystallized depends upon a variety of variables: temperature, pH, presence of crystal nuclei, presence of interfering substances, and time allowed for crystallization (Nickerson and Moore 1974, Modler and Lefkovitch 1986, Jayaprakasha et al 1995).

Much of the previous research regarding lactose crystallization was performed on pure lactose solutions and in liquid whey to discover how to maximize the amount of lactose recovered. Temperature, pH, and total solids have been previously determined to affect lactose crystallization and size of crystals. Nickerson and Moore (1974) found that acidity outside the normal processing range (pH < 1) accelerated lactose crystallization in pure lactose solutions. Modler and Lefkovitch (1986) found that pH had no effect on lactose crystal size. Jayaprakasha et al (1995) determined that total solids and amount of seeding material affected the amount of lactose crystallized and the average crystal size. However, little is known about how pH, length of crystallization time, and temperature affect lactose crystallization within the whey concentrate system and within normal, commercial processing parameters.

The objective of the research was to determine whether altering the crystallization conditions of whey concentrate, within normal processing parameters, affects the amount and structure of lactose crystals. Parameters investigated include sample pH, crystallization time, and crystallization temperature.

This research is intended to be of benefit to the commercial whey processing industry. The manufacturing process of whey concentrate experiences fluctuations in the following parameters: pH of the whey due to activity of cheddar cheese starter cultures and types of cheese whey used; crystallization temperatures, due to different rates of cooling; and length of time to crystallize, as crystallization is a batch process within a continuous process with crystallization time often adjusted to accommodate changes in the volume of incoming whey from cheese vats. The treatments chosen for this research are extremes generally seen in commercial whey processing and results of the experiments will indicate whether these normal variations have an effect on the final whey powder quality.

MATERIALS AND METHODS

Whey Samples

The whey concentrate of 62 % total solids, which had been pasteurized, was obtained from a local cheddar cheese processor. The sample was taken from the line between the flash cooler and crystallizer tank. The concentrate was stored in a sealed container and insulated to maintain the temperature for the two hour transport to Oregon State University.

Crystallization of Whey Concentrate

Control: 829 g (500 ml) of whey concentrate, pH 5.6 +/- 0.1, was placed into an electric ice cream maker (Rival 4 quart, model 8704, The Holmes Group, El Paso, TX) used as a crystallizer. Alpha-lactose monohydrate (Foremost Farms, Baraboo, WI) was added at a level of 0.8 g/ 500 ml as seeding material. The seeded container was mixed by hand for 30 seconds until the seed crystals were dispersed. The container was then placed in tempered water inside a 10 °C incubator and allowed to crystallize for 12 hours before freeze-drying. The mechanical rate of stirring for the sample was approximately 40 rpm. Samples cooled to 15 °C in 30 minutes and reached a final temperature of 12 °C after an additional 4 hours. Rate of cooling was determined using thermocouples and

BoxCar Pro software (Version 4.0.7.0, Onset Computer Corporation). Temperature data was collected at a rate of every two seconds.

Treatments

Eight different treatments were applied to the whey concentrate during crystallization (Figure 3.1).

•*No Seeding Material Variation:* One sample was prepared as the control with seeding step omitted.

•*pH Variations (2 levels):* One sample was adjusted to a pH of 4.5 using 13.0 +/- 1.0 ml of 5 N HCl and another was adjusted to pH 6.5 using 11.0 +/- 2.0 ml of 5 N NaOH before adding seeding material. Crystallization conditions were the same as for the control.

•*Temperature Variations (2 levels):* Samples were placed at 5 °C and 15 °C for the 12 hour crystallization step.

•*Crystallization Time Variations (2 levels):* Samples were crystallized for 9 and 15 hours.

•*Pure lactose sample:* One sample was prepared with pure lactose, and no whey concentrate, to observe lactose crystallization under control conditions without additional whey components. Three-hundred fifty-six grams of alpha-lactose monohydrate (Foremost Farms, Baraboo, WI) was added to 330 g of distilled water (at 31 °C), to give the same amount of lactose as in the whey concentrate but

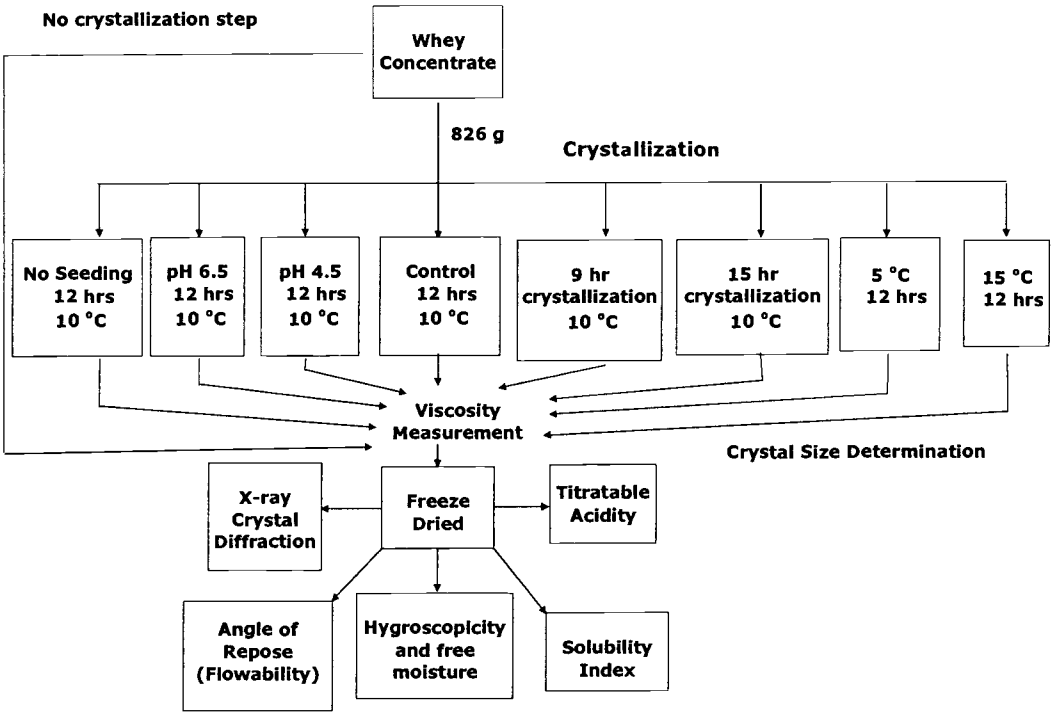


Figure 3.1. Flow chart of research performed.

with different total solids content, and crystallized at the same temperature and time as the control.

Viscosity Measurements

Initial viscosity (in cp) was determined using a Brookfield Viscometer model DV-III (Brookfield Engineering Labs, Inc., Middleboro, MA) and spindle numbers RV 6 or 7. The leg guard and 600 ml plastic Griffin beaker were used. Initial viscosity measurements were measured at the arrival temperatures, which were 36 +/- 0.5 °C. The viscosity of each sample was again measured upon completion of crystallization at the final equilibrium temperature.

Freeze Drying

Forty grams (+/- 0.5) of each of the crystallized whey concentrate samples were transferred to plastic petri dishes and frozen at –35 C for at 24 hours. Frozen samples were freeze dried for 3 days (pressure < 13 Pa) using a Virtis Console 4.5 Freeze Dryer (The Virtis Company, Inc., Gardiner, NY). Upon removal from the freeze dryer, half of each sample and a desiccant packet was placed in a three-layer nylon bag with oxygen barrier, vacuum-sealed, and stored at room temperature. The other half of the sample was placed in a sealed glass jar with a desiccant packet for storage until x-ray diffraction (XRD) analysis was performed.

Compositional Analysis

Protein was analyzed by the Kjeldahl method according to the Official Methods of Analysis, (AOAC method number 991.20. 33.2). Lactose was analyzed using the Lactose/D-glucose enzyme test reference (Boehringer Mannheim/Roche Diagnostics Corporation, Indianapolis, IN). Ash content was determined using the gravimetric method according to the Official Methods of Analysis, (AOAC method number 930.30).

Sample Preparation for Further Analysis

Powder samples were ground using mortar and pestle and sifted through a 0.589 mm sieve to break up lumps that would interfere with powder testing.

Hygroscopicity Measurements

The hygroscopicity of the freeze-dried samples was determined as described by Method No. A 14 a in "Analytical Methods for Dry Milk Products" (A/S Niro Atomizer 1978a). Modifications to the method were the replacement of a vacuum pump with pulling a water vacuum and using a flow rate of 50-60 (mm). Degree of hygroscopicity was determined according to the following table included with the procedure.

Class	Hygroscopicity (%)
Non-hygroscopic	≤ 10.0
Slightly hygroscopic	10.1 – 15.0
Hygroscopic	15.1 – 20.0
Very hygroscopic	20.1 – 25.0
Extremely hygroscopic	25.1 – 30.0

Flowability (Angle of Repose)

The angle of repose was determined using the "Fixed funnel and free standing cone" method (Train 1958). Modifications were made to the procedure. The funnel was set at a height of 0.8 cm above the lab bench. Sample was spooned into the funnel with the funnel stem blocked. Once the funnel was filled, the stem block was removed and the powder was allowed to flow. Flow stopped automatically upon reaching the bottom of the funnel stem. Samples were tapped once to re-start any stopped flow, if needed.

Titratable Acidity

Titratable acidity was determined by titration of 17.6 ml of reconstituted freeze-dried whey concentrate against 0.1 N NaOH with 1% phenolphthalein indicator, as described in Method No. A 19 a in "Analytical Methods for Dry Milk Products" (A/S Niro Atomizer 1978b). A modification of hand mixing the samples (25 shakes per 7 seconds) was performed instead of using a mixer.

Solubility Index

Method No. A 3 a (A/S Niro Atomizer 1978c) was modified to measure the solubility of the samples. One-and-a-half milliliters were transferred into tared 1.5 ml centrifuge tubes. Samples were centrifuged for 5 minutes at 2000 RPM using a Labnet Spectrafuge 16M (radius = 7.3 cm) (Labnet International, Inc., Edison, NJ). Sediment-free liquid was removed using a pipette.

X-ray Crystal Diffraction

XRD patterns were recorded for the freeze-dried powders using Siemens Diffraktometer D5000 (Bruker AXS, Madison, WI). Detector slits of 0.2 mm were used. Data was collected over the diffraction angles (2θ) of 10 to 60° using variable incident and anti-scatter slits set to illuminate 20 mm of the sample. Collection steps of 0.02° with 0.6 second step time were used. Samples were ground using a mortar and pestle and then pressed into sample container using a spatula.

Percentage of lactose in the α-lactose monohydrate crystalline form was determined using the spectrum analysis for pure α-lactose monohydrate and each sample. A characteristic peak for α-lactose monohydrate is at 16.4°. The peak area of the pure α-lactose monohydrate lactose was considered to be 100 % α-lactose monohydrate. A ratio of the peak areas of the α-lactose monohydrate in

the sample to the pure sample was calculated and the value was used as the amount of lactose crystallized in the α-monohydrate form (Drapier-Beche et al 1998).

Size Determination of Lactose Crystals

Dimensions of lactose crystals in the whey concentrate samples were determined by a Nikon Eclipse E400 microscope (Nikon, Japan) at 10x for all samples, except the pure lactose which was viewed at 4x. Images of each slide were taken using a CoolSNAP photometrics camera (Roper Scientific Photometrics, Tucson, AZ) and images were viewed using CoolSNAP software (Version 1.2). Viewing of crystals and measurement of crystal length and width were performed using the program Image J (1.30 V, shareware). The screen was divided into 48 sections and a random number table was used to determine sections from which to measure crystals. Ten length and width measurements were obtained for each sample.

Estimation of Commercial Powder Variability

Whey powder samples produced during one day's production were obtained from a local cheddar cheese processor. Hygroscopicity testing was performed on these samples to determine the normal variability of whey powder to compare to the variability seen in the hygroscopicity measurements of the freeze-dried whey concentrate samples. Additionally, three spray dried powder samples

from three different lots were obtained. X-ray crystal diffraction was performed on these samples to determine the variability in lactose forms and amount of lactose crystallized in commercial samples to compare to the variability seen in the freeze-dried whey concentrate samples.

Enzymatic Analysis of Lactose Hydrolysis

Lactose/D-glucose enzyme test reference (Boehringer Mannheim/Roche Diagnostics Corporation, Indianapolis, IN) was performed to determine whether lactose hydrolysis occurred upon pH adjustment to 4.5. A Shimadzu recording spectrophotometer (Shimadzu, Columbia, MD), model type UV-1601, was used.

Statistical Analysis

One-way analysis of variance (ANOVA) was used to test for different means in the amount of lactose crystallized, viscosity, crystal dimensions, solubility index, hygroscopicity, free moisture, and angle of repose of the samples under the different treatment conditions. Significance was determined at $p < 0.05$. Dunnett's procedure, a multiple comparisons method, was performed to compare treatments to a control. SPlus statistical software was used (Splus 6.1, academic version). Pure lactose samples were excluded from the statistical analysis because of the difference in composition of the lactose mixture and the whey concentrate liquid. The difference would have resulted in a skewing of the data and it's

analysis because the pure lactose data points were outliers for a majority of the tests.

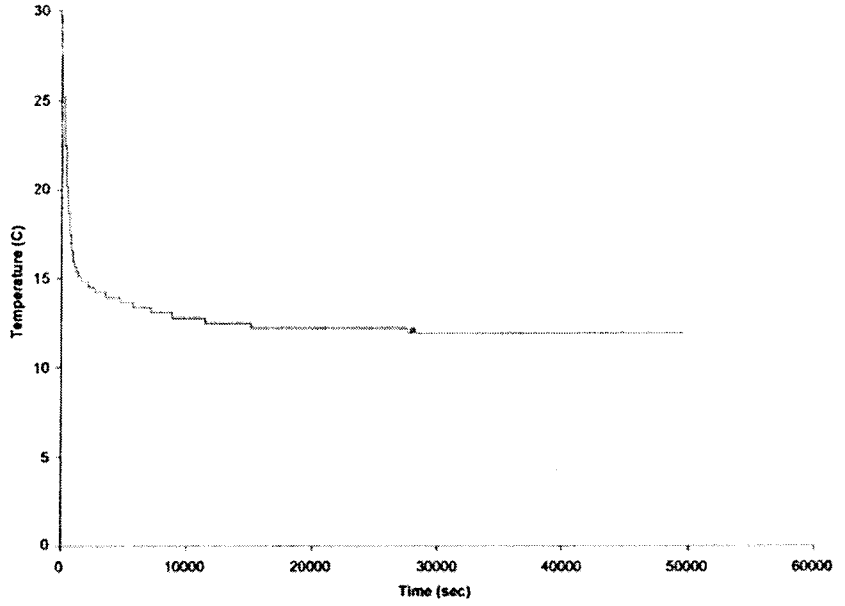
RESULTS AND DISCUSSION

Composition of the freeze-dried whey concentrate used for the three trials is shown in Table 3.1 and all components were within the ranges required for sweet whey powder. The rates of cooling for a 10 °C and 15 °C sample are shown

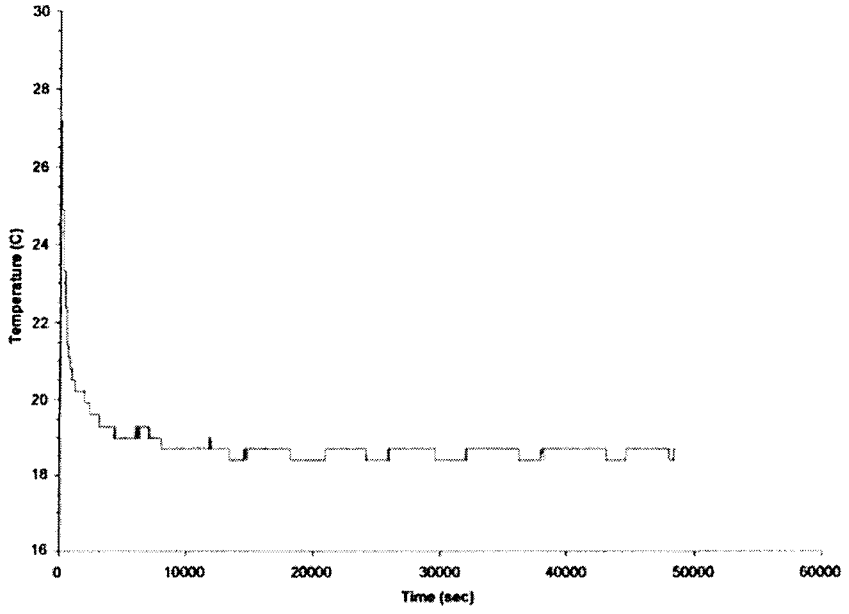
Table 3.1. Whey concentrate composition for three separate trials

	Trial 1	Trial 2	Trial 3	Ave	SD
% Lactose	68.94	70.97	70.13	70.01	1.02
% Ash	7.42	7.38	7.39	7.40	0.02
pH	5.73	5.59	5.53	5.62	0.10
% TA	0.09	0.10	0.10	0.10	0.01

in Figure 3.2 a and b. The sample incubated at 10 °C cooled to 15 °C, from a starting temperature of 36 °C, in 30 minutes and reached a final temperature of 12 °C after an additional 4 hours (Fig. 3.2a). The sample remained at approximately 12 °C for the remainder of the crystallization process. A final sample temperature of 10 °C was never reached possibly due to heat from the agitator motor. A 15 °C incubated sample cooled to 19 °C in approximately 2.5 hours and remained at that temperature (Figure 3.2b). The rates of cooling for the whey concentrate samples were similar to what occurs in industry. Commercially, whey concentrate is slowly cooled to a final temperature over a few hours during the crystallization step. Cooling of the whey concentrate occurs slowly due to the large volume of



a)



b)

Figure 3.2. Rate of cooling for whey concentrate during crystallization at a) 10 °C and b) 15 °C

product held in each crystallizer and the use of water-jackets which cool only the walls of the tank.

Mean titratable acidity and solubility index of the whey concentrate samples are listed in Table 3.2. Titratable acidity for pH 4.5 and pH 6.5 differ from the other samples due to the addition of acid or base needed for pH adjustment.

There is evidence that the mean solubility index of the samples is significantly different as determined by ANOVA ($p < 0.01$, F-test). However, multiple comparison analysis indicates that the difference does not occur between any of the treatments and the control, which are of interest. The solubility index is an indication of how well a powder will mix into solution. No differences in means indicates that this aspect of powder quality is not affected by the treatments.

Whey concentrate viscosity before and after crystallization under different conditions is shown in Table 3.3. There is convincing evidence that mean sample viscosities are different (ANOVA, $p < 0.001$, F-test). Evidence also indicates that there is a significant difference between the mean viscosities of the sample that was analyzed prior to crystallization and the control ($p < 0.05$, Dunnett's method). For the sample in which the whey concentrate did not go through the crystallization step, the viscosity increased by an estimated 9450 centipoise in comparison to the control (95% confidence interval, Dunnett's method: 3060 to 15800 cp). This is likely due to the fact that no agitation of the sample occurred during transportation from the plant to the lab. Lack of agitation has been

Table 3.2. Solubility index and % titratable acidity for whey concentrate samples

Sample	TA (% lactic acid)	Solubility Index Final ht (cm)
No crystallization step	0.10 (0.01)	0.55 (0.12)
Control	0.10 (0.00)	0.48 (0.05)
No seeding material	0.10 (0.00)	0.50 (0.05)
pH 4.5	0.17 (0.02)	0.70 (0.16)
pH 6.5	0.05 (0.01)	0.45 (0.08)
5 C	0.10 (0.01)	0.43 (0.04)
15 C	0.10 (0.00)	0.46 (0.02)
9 hr crystallization	0.10 (0.01)	0.38 (0.00)
15 hr crystallization	0.10 (0.01)	0.43 (0.12)
Lactose	0.00 (0.00)	0.00 (0.00)

Values are reported as mean (SD) of triplicate samples

Table 3.3. Viscosity of whey concentrate before and after crystallization at different conditions

Sample	Mean Viscosity (cp)
No crystallization step	14120 (4808)
Control	4700 (1454)
No seeding material	4613 (1443)
pH 4.5	6418 (1877)
pH 6.5	5227 (1565)
5 C	3302 (1095)
15 C	7006 (2209)
9 hr crystallization	4240 (1294)
15 hr crystallization	4673 (1472)
Lactose	N/A

Values are reported as mean (SD) of triplicate samples

Lactose solution gave no reading using viscometer

observed to solidify whey concentrate (Hargrove et al 1975). No significant differences were seen in the treatments when compared to the control ($p > 0.05$, Dunnett's method). The viscosity of whey concentrate at total solids greater than 40% has been observed to change with temperature (Hargrove et al 1975). Higher viscosity has also been noted to have a negative effect on rates of lactose crystallization (Modler and Lefkovitch 1986, Jayaprakasha et al 1995). Therefore, viscosities were measured at the final equilibrium temperature as the viscosity of the whey concentrate during the crystallization process was of interest. The similar viscosities in samples that were crystallized helps to support the fact that any crystallization differences observed are due solely to the treatment and are not additionally affected by variations in viscosities.

X-ray crystal diffraction was performed to determine the x-ray diffraction pattern for α-lactose monohydrate for use in comparison to peaks observed in the sample diffraction patterns (Figure 3.3). The peak of 16.4° is characteristic of α-lactose monohydrate (Drapier-Beche 1998). A characteristic peak for lactose crystal forms of 5α-/3β-lactose and 3α-/2β-lactose is 18.2° and the crystalline form of β-lactose has a characteristic peak of 10.4° (Drapier-Beche 1998). These characteristic peaks are noted to be missing in the graphs in Figure 3.4. Stable and unstable anhydrous lactose contribute to peaks near the diffraction angles of 12.1°

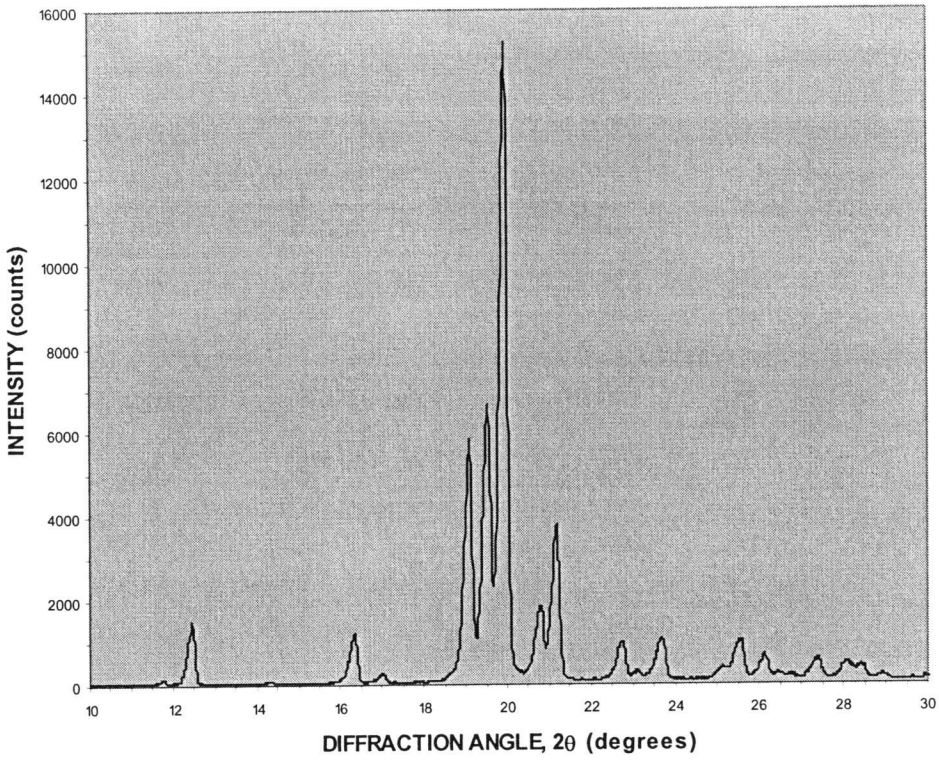
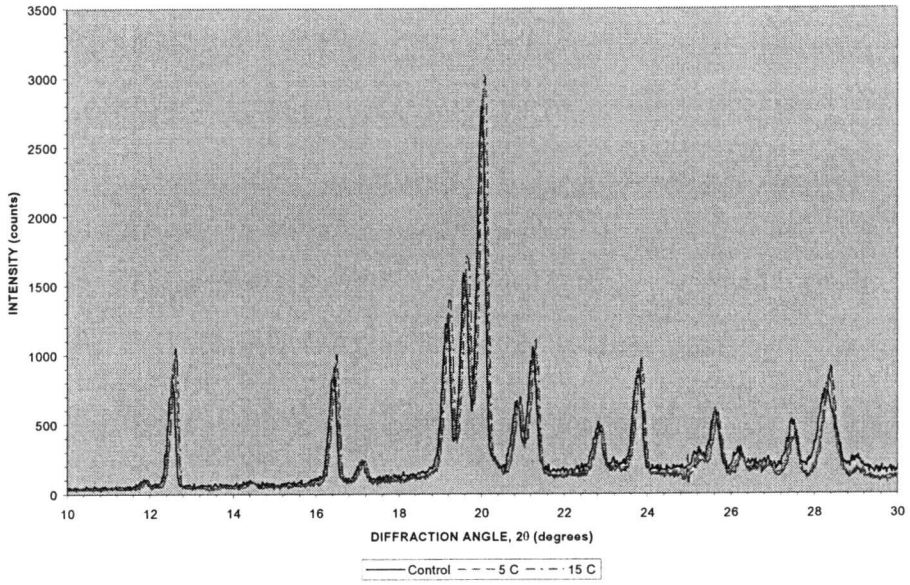


Figure 3.3. X-ray crystal diffraction pattern for pure α-lactose monohydrate. The peak at 16.4° is characteristic of α-lactose monohydrate.

Figure 3.4. X-ray crystal diffraction patterns for freeze-dried whey concentrate samples crystallized at different a) Temperatures, b) pH, and c) Lengths of crystallization.

a) Temperature



b) pH

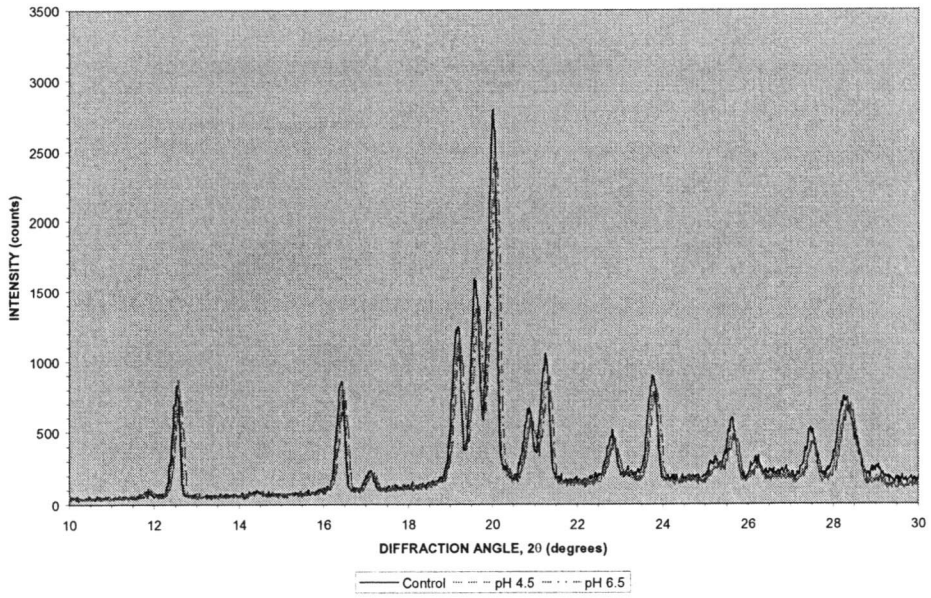
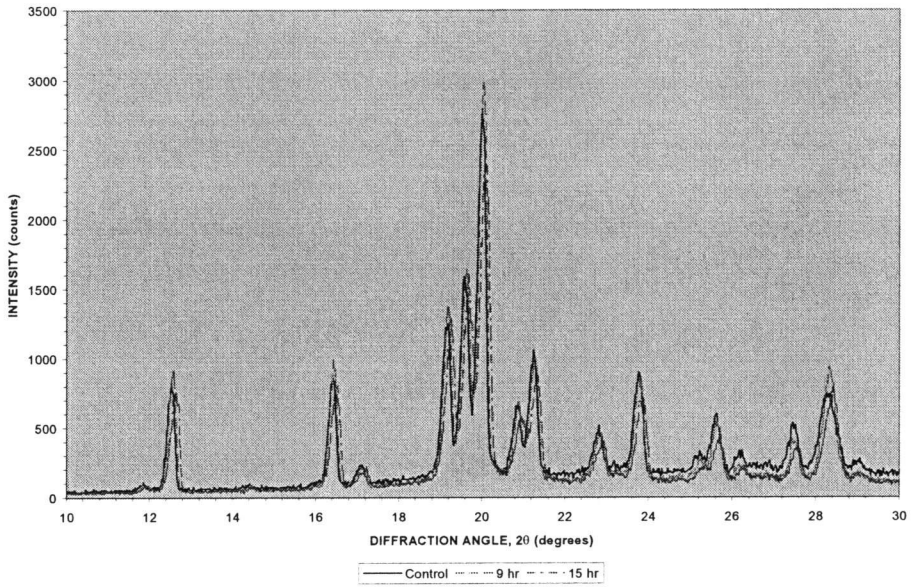


Figure 3.4 (continued)

c) Lengths of crystallization (time)



and 18.2° (Jouppila et al 1998), which are also not observed in the sample graphs (Fig. 3.4), indicating that these other crystalline forms were not present in measurable amounts in the samples. Therefore, the only crystallized lactose form present in measurable amounts is α-lactose monohydrate and only the area of the 16.4° peak was used in lactose quantification. X-ray crystal diffraction patterns of samples with altered temperature, pH, or time of crystallization indicate the same peaks as seen in the pure sample (Figure 3.4 (a,b,c)).

Results of x-ray crystal diffraction analysis, in terms of amount of crystallized lactose, are listed in Table 3.4a. There is no evidence to suggest that any of the mean amounts of lactose crystallized are different (ANOVA, $p > 0.05$, F-test). The normal fluctuations that occur during the crystallization of commercial whey concentrate do not individually appear to affect the amount of lactose crystallized. Results of the enzymatic lactose hydrolysis indicated that lactose hydrolysis did not occur upon the addition of 5 N HCl to adjust the sample to pH 4.5. This confirms that the pH 4.5 sample did not contain a smaller concentration of lactose than the other samples. If lactose hydrolysis had occurred, the sample would have less lactose and more of the hydrolyzed products of galactose and glucose, which would not be able to take part in crystallization. This could have affected the amount of lactose crystallized if significant amounts of lactose hydrolysis had occurred.

Table 3.4a. Amount of crystalline lactose (%) determined using x-ray crystal diffraction for selected freeze-dried whey concentrate samples crystallized at different conditions

Sample	Crystalline Lactose (%)
No crystallization step	55 (5)
Control	53 (9)
No seeding material	57 (5)
pH 4.5	45 (14)
pH 6.5	54 (5)
5 C	54 (13)
15 C	57 (13)
9 hr crystallization	57 (14)
15 hr crystallization	55 (12)
Lactose*	57 (7)

Values are reported as mean (SD) of triplicate samples, except the one sample with an asterisk, which is a duplicate sample.

Table 3.4b. Amount of crystalline lactose (%) determined using x-ray crystal diffraction for commercial spray-dried powders from different lots.

Sample	Crystalline Lactose (%)
1	66
2	44
3	64
Ave	58
SD	12

Table 3.4b indicates the amount of lactose crystallized in the α-lactose monohydrate form in commercial spray-dried whey powder samples. The standard deviation for the commercial samples is very similar to what was seen in all of the freeze-dried crystallized whey concentrate samples. The 12% standard deviation of the amount of lactose crystallized in the commercial samples is similar to the largest standard deviation of 14% for the pH 4.5 sample.

Angle of repose, percent hygroscopicity, and percent free moisture for the freeze-dried samples are shown in Table 3.5a. The levels of hygroscopicity corresponding to the numerical values were determined according to A/S Niro Atomizer (1978a). Different levels are indicated in the table by different letters. Pure lactose, 9 hour crystallization, and 15 hour crystallization samples were categorized as non- hygroscopic; no crystallization, pH 6.5, 5 °C, and 15 °C samples were slightly hygroscopic; and the control, no seeding material, and pH 4.5 samples were hygroscopic.

Angle of repose, percent hygroscopicity, and percent free moisture for the commercial spray-dried samples obtained from one day's production are listed in Table 3.5b. Angle of repose values are slightly higher for these samples in comparison to the freeze-dried whey concentrate samples. However, the percent hygroscopicity and percent free moisture values, and the corresponding standard deviation, are similar.

Table 3.5a. Free moisture, hygroscopicity, and angle of repose values for freeze-dried whey concentrate samples crystallized at different conditions.

Sample	Mean % Free Moisture	Mean % Hygro	Mean Angle of Repose
No crystallization step	1.580 (0.265)	10.5[b] (1.6)	22.5 (1.9)
Control*	0.923 (0.087)	**	22.3 (1.3)
No seeding material	1.068 (0.136)	15.2[c] (2.2)	21.7 (0.8)
pH 4.5*	1.034 (0.675)	15.5[c] (1.0)	22.4 (0.7)
pH 6.5	1.273 (1.213)	13.5[b] (1.2)	22.0 (0.7)
5 C	1.798 (1.140)	14.9[b] (1.8)	22.2 (0.4)
15 C	0.921 (0.127)	10.3[b] (0.4)	22.6 (0.3)
9 hr crystallization	1.088 (0.172)	7.0[a] (0.1)	22.8 (0.4)
15 hr crystallization	1.306 (0.321)	8.2[a] (0.8)	22.6 (0.2)
Lactose*	0.531 (0.187)	1.5[a] (0.4)	25.3 (3.8)

Values are reported as mean (SD) of duplicate samples, except those indicated with an asterisk, which are triplicate samples.

a = non-hygroscopic according to Niro Atomizer hygroscopicity procedure
b = slightly hygroscopic
c = hygroscopic

** = No results were obtained for this sample

Table 3.5b. Free moisture, hygroscopicity, and angle of repose values for commercial spray-dried whey powders sampled from one day's production.

Sample	% Free Moisture	% Hygro	Angle of Repose
1	1.529	13.6	29.54
2	1.349	11.9	27.93
3	1.335	15.2	26.57
4	1.259	16.1	27.19
5	1.026	12.3	27.10
6	1.323	15.5	27.53
Ave	1.304	14.1	27.64
SD	0.163	1.8	1.03

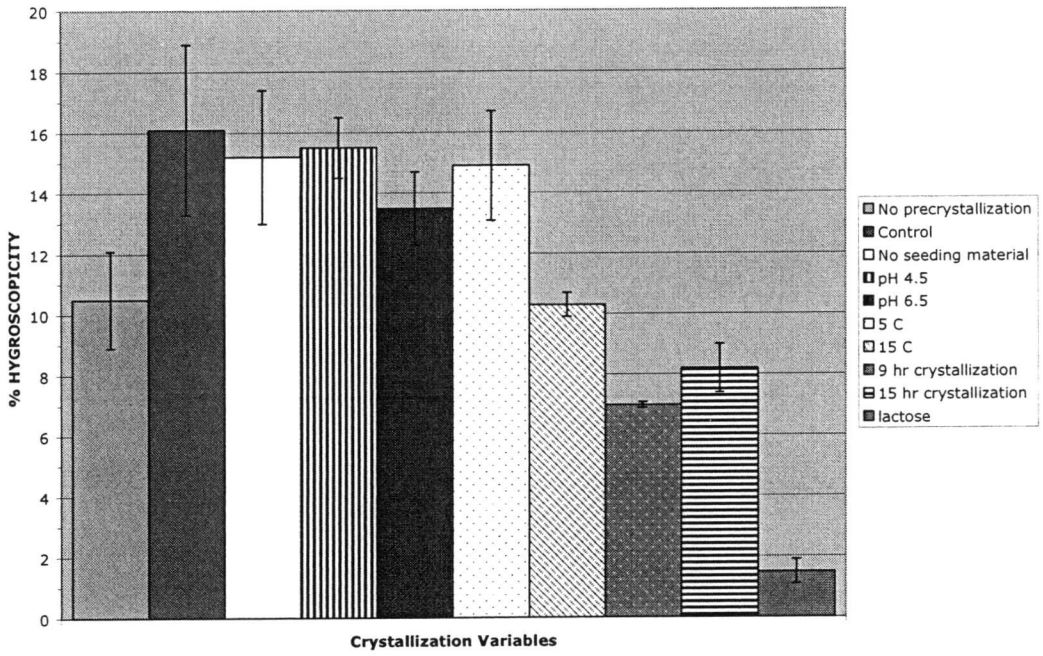


Figure 3.5. Hygroscopicity (%) of freeze-dried whey concentrate samples crystallized at different conditions. Error bars indicate standard deviations of the samples.

Figure 3.5 indicates the percent hygroscopicity of the samples and the corresponding standard deviation. Significant differences were observed in sample mean hygroscopicities (ANOVA, $p < 0.001$, F-test). Since all of the samples contain the same lactose crystal form and approximately the same amount of α-lactose monohydrate, according to XRD results and quantification, amount of crystallized lactose and lactose form are likely not the cause of the hygroscopicity differences as has been observed in previous research (Troy and Sharp 1930, de Vilder 1975, Jayaprakasha et al 1995). However, no correlation between amount of lactose crystallized and hygroscopicity was observed by Hargrove et al, 1975.

A tentative relationship exists between the amount of lactose crystallized (XRD data) and the hygroscopicity (%) of the sample (Figure 3.6). Hygroscopicity of the freeze-dried whey concentrate appears to decrease as the amount of crystallized lactose in the sample increases. The linear regression model for the data was estimated to be $\text{mean}\{H| L\} = 21.1550 - 0.1496*L$, where H is percent hygroscopicity and L is amount of lactose crystallized. The intercept has a standard error of 4.4705 and the coefficient has a standard error of 0.0780. The p-value for the model is 0.07 which is suggestive, but not conclusive, of a relationship between hygroscopicity and amount of lactose crystallized.

Despite the differences observed in the hygroscopicity of the samples, the angle of repose values, which measure the free-flow of a powder sample, were not found to significantly differ (ANOVA $p > 0.05$) (Table 3.5). The treatments had no

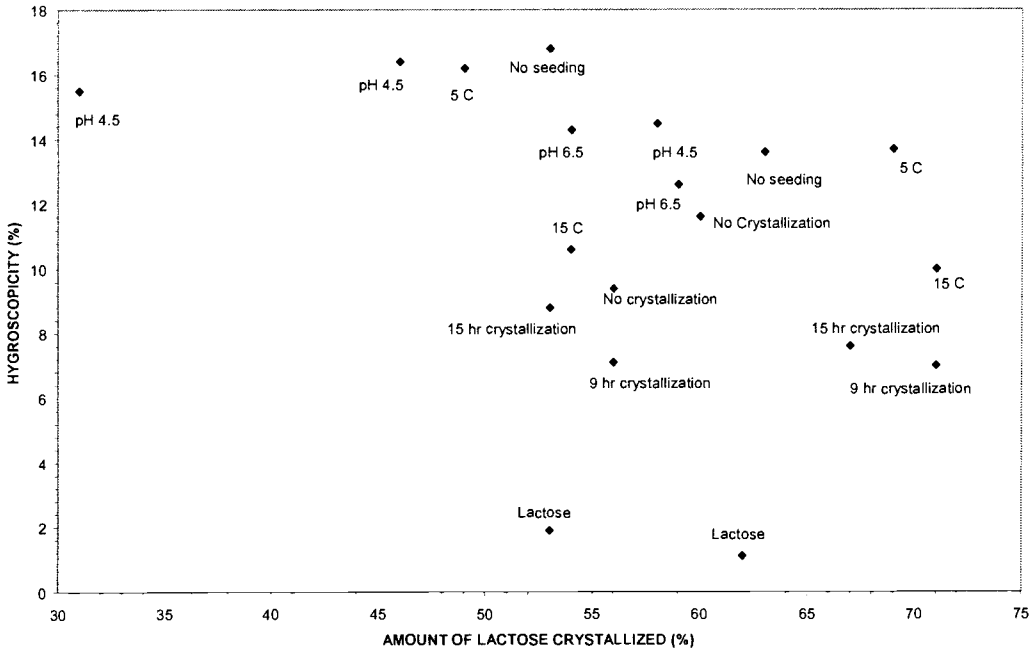


Figure 3.6. Hygroscopicity (%) and amount of lactose crystallized in freeze-dried whey concentrate samples crystallized at different conditions.

effect on the flowability of the freeze-dried powders. It was expected that if differences in hygroscopicity were observed in the samples that angle of repose values would also differ. Angle of repose estimates the adhesive force between particles. The greater the angle, the greater the adhesive force. More hygroscopic powders tend to be stickier, which would in turn be expected to decrease the flowability of the powder.

Since free moisture contributes to the hygroscopicity of a powder, it was possible that differences observed in hygroscopicity would parallel differences in free moisture. However, there is no evidence of differences in the mean percent free moisture of the samples, indicating that the hygroscopicity differences were not due to differences in amount of free moisture (ANOVA, $p > 0.05$, F-test) (Table 3.5).

Mean crystal dimensions for lactose crystals, both length and width, were not significantly different indicating that the treatments had no measurable effect on crystal size (ANOVA, $p > 0.05$, F-test) (Table 3.6). The length and width of all samples, except the pure lactose, were approximately the same (Figure 3.7). The majority of crystals were in a truncated tomahawk shape usually seen with α-monohydrate crystal growth (Figure 3.8a). The truncated shape is likely due to interference from other molecules in the whey concentrate. When other molecules (ex) β-lactose, minerals) are present, they prevent growth along certain faces of the

Table 3.6. Mean crystal dimensions for lactose crystals in whey concentrate samples crystallized at different conditions

Sample	Mean Width (mm)	Mean Length (mm)
No crystallization*	0.018	0.020
	(0.003)	(0.006)
Control*	0.026	0.030
	(0.009)	(0.015)
No seeding material	0.023	0.028
	(0.003)	(0.008)
pH 4.5	0.028	0.034
	(0.005)	(0.009)
pH 6.5	0.027	0.030
	(0.006)	(0.010)
5 C	0.023	0.028
	(0.003)	(0.007)
15 C	0.026	0.029
	(0.006)	(0.009)
9 hr crystallization	0.028	0.030
	(0.008)	(0.010)
15 hr crystallization	0.023	0.025
	(0.004)	(0.006)
Lactose	0.082	0.192
	(0.005)	(0.018)

Values are reported as mean (SD) of triplicate samples, except those indicated with an asterisk, which are duplicate samples.

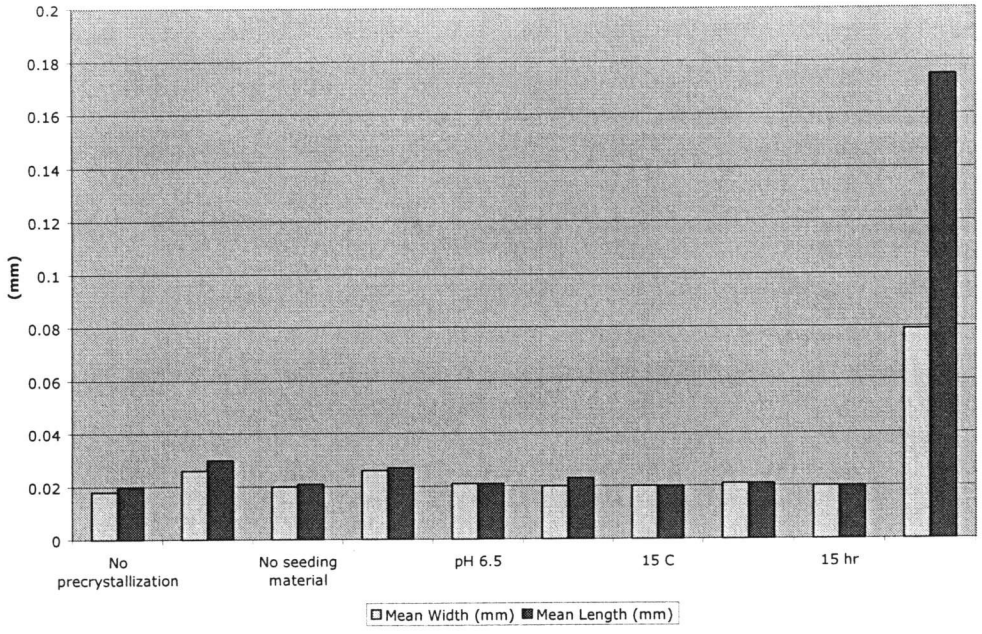


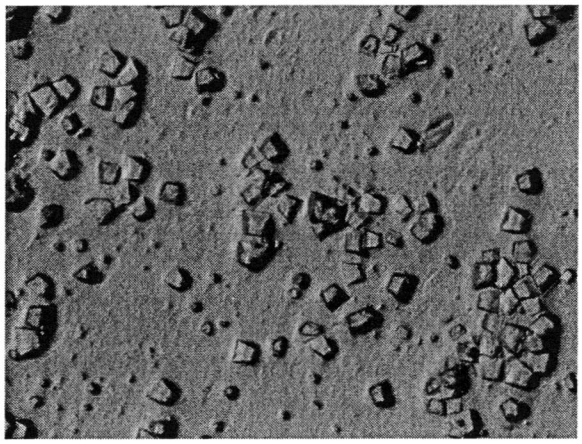
Figure 3.7. Lactose crystal dimensions in whey concentrate samples crystallized at different conditions.

α-monohydrate crystal (Walstra 2003a). Since the crystal size and shape were approximately the same for all of the samples, the interfering components are likely in similar concentrations in the samples. The crystals formed in the pure lactose solution are the characteristic tomahawk shape, but without a truncated end, and much larger in size than the crystals observed in the whey concentrate samples (Figure 3.8b). Growth along all of the crystal faces, except the $0\bar{1}1$, $0\bar{1}\bar{1}$, and $0\bar{1}0$, occurred as in the other samples. Tomahawk crystals were still formed in the pure lactose solution despite the fact that no whey components were present.

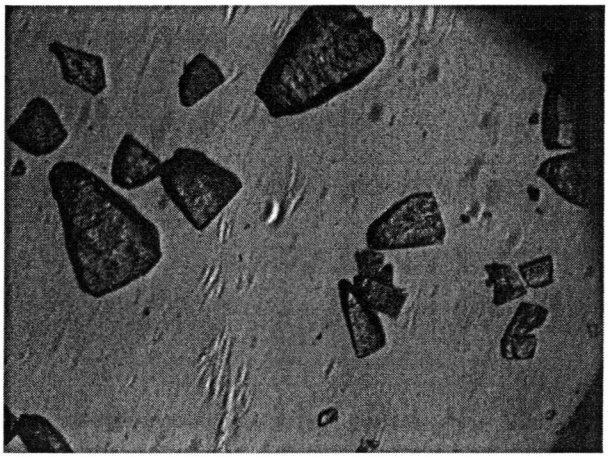
Since the shapes of the α-lactose monohydrate crystals in the crystallized whey concentrate and the crystallized pure lactose were the same tomahawk shape, it is believed that the interfering substances preventing growth on certain faces was likely due to individual β-lactose molecules, the only other component present in all of the samples. According to Walstra, 1999, it is very difficult to convert all of the lactose into the alpha form for crystallization. Much remains in the beta form and available to inhibit crystal growth. The smaller crystal sizes observed in the whey concentrate was possibly due to the presence of other components, like proteins and minerals, that interfered with the movement of α-lactose. Interfering components could block access to the α-lactose monohydrate crystal and slow the rotational movement of the α-lactose needed to fit the molecule into the crystal lattice.

Figure 3.8. Lactose crystals in α-lactose monohydrate form.

a) Crystals crystallized in whey concentrate (10x magnification)



b) Crystals crystallized in pure lactose solution (4x magnification)



CONCLUSION

Altering the crystallization conditions of whey concentrate had no significant effect on the forms of lactose observed or the amount crystallized. Lactose crystallized in the α-lactose monohydrate form as crystals of similar dimensions in all of the samples. Viscosity of the whey concentrate decreased with crystallization.

Quality of the freeze-dried whey concentrate powder was minimally affected by the treatments. Solubility index, angle of repose, and free moisture were not significantly affected by altered crystallization conditions.

Since composition and processing of whey concentrate is approximately the same for different manufacturers, we expect these results to be applicable to other whey concentrate processes. The research shows that whey powder quality appears to be maintained at altered conditions of temperature, pH, and time of crystallization. Commercial whey processors can maintain desired whey powder quality with normal fluctuations in the processing parameters of whey concentrate.

REFERENCES

A/S Niro Atomizer (1978a). Analytical Methods for Dry Milk Products. A 19 a Determination of hygroscopicity.

A/S Niro Atomizer (1978b). Analytical Methods for Dry Milk Products. A 14 a Determination of titratable acidity in milk powder (as % lactic acid).

A/S Niro Atomizer (1978c). Analytical Methods for Dry Milk Products. A 3 a Determination of solubility index.

Boxcar Pro software. Version 4.0.7.0, Onset Computer Corporation.

CoolSNAP software. Version 1.2, Roper Scientific, Inc.

21 CFR 184.1979. Whey standard of identity. Title 21, Sec 184.1979.

De Vilder, J. 1975. Influence de la cristallisation du lactose dans le concentré de sérum sur l'hygroscopicité et l'agglomération de la poudre de sérum. Revue de l'Agriculture. 4: 963-975.

Drapier-Beche, N., J. Fanni, M. Parmentier. 1998. Kinetics of the synthesis of lactose molecular compounds. J Dairy Sci. 81: 2826-2832.

Hargrove, R., F. McDonough, D. LaCroix, and J. Alford. 1975. Production and properties of deproteinized whey powders. J. Dairy Sci. 59 (1): 25-33.

Image J software. Version 1.30, shareware.

Jayaprakasha, H.M., R.S. Ratel, and E. Renner. 1995. Optimization of Precrystallization Process for Nonhygroscopic Whey Powder by Using Reverse Osmosis Concentrate. Japanese Journal of Dairy and Food Science. 44 (3): A-113 - A121.

Jouppila, K., J. Kansikas, and Y. Roos. 1998. Crystallization and x-ray diffraction of crystals formed in water-plasticized amorphous lactose. Biotechnol Prog. 14: 347-350.

Modler, H. and L.P. Lefkovitch. 1986. Influence of pH, Casein, and Whey Protein Denaturation on the Composition, Crystal Size, and Yield of Lactose from Condensed Whey. J Dairy Sci. 69:684-697.

Nickerson, T.A. and E.E. Moore. 1974. Factors Influencing Lactose Crystallization. J Dairy Sci. 57:1315-1319.

Saltmarch, M. and T.P. Labuza. 1980. Influence of relative humidity on the physicochemical state of lactose in spray-dried sweet whey powders. Journal of Food Science. 45:1231-1236.

Sharp, P. and H. Doob. 1941. Effect of humidity on moisture content and forms of lactose in dried whey. J Dairy Sci. 24: 679-690.

SPlus. Academic Version 6.1.

Train, D. 1958. Some aspects of the property of angle of repose of powders. J. Pharm. Pharmacology. 10 (pt 2): 127T-134T.

Troy, H. and P. Sharp. 1930. Alpha and beta lactose in some milk products. J Dairy Sci. 13: 140-157.

Walstra, P., T. Geurts, A. Noomen, A. Jellema, and M. van Boekel. 1999. Dairy Technology: Principles of Milk Properties and Processes. New York, New York: Marcel Dekker, Inc.

Walstra, P. 2003a. Crystallization. In "Physical Chemistry of Foods" (pp 583-649). New York, New York: Marcel Dekker, Inc.

Walstra, P. 2003b. Nucleation. In "Physical Chemistry of Foods" (pp 548-582). New York, New York: Marcel Dekker, Inc.

Zadow, J. 1984. Lactose: properties and uses. J Dairy Sci. 67: 2654-2679.

CHAPTER 4

CONCLUSION

CONCLUSION

The commercial whey manufacturing process sees fluctuations in: pH of the whey, crystallization temperatures, and length of time to crystallize. The treatments chosen for this research investigated parameters coinciding with extremes generally seen in commercial whey processing. Results of the experiments show that these normal variations have a minimal effect on the final whey powder quality.

Altering the crystallization conditions of temperature, pH, and length of crystallization time for whey concentrate has no significant effect on lactose crystal forms observed or the amount of lactose crystallized. X-ray crystal diffraction indicated that the only form of crystalline lactose present in measurable amounts is the desired α-lactose monohydrate. Quantification of crystalline lactose present indicates that approximately 55% (SD 4% including all samples) of the lactose is crystallized in the α-lactose monohydrate form. The process of crystallization and the addition of 0.1% α-lactose monohydrate as seeding material also have no significant effect on lactose crystal forms or amount crystallized.

Lactose crystal dimensions are not significantly affected by altering the crystallization conditions.

Viscosity is found to significantly decrease with the addition of a crystallization step. However, none of the other treatments have an additional affect on the viscosity.

Properties related to the quality of the powder do not appear to be significantly affected by alteration of crystallization conditions. Solubility index, free moisture, and angle of repose (flowability of the powder) are not affected. The amount of lactose present is tentatively related to the hygroscopicity of the powder.

Since composition and processing of whey concentrate is approximately the same for different manufacturers, we expect these results to be applicable to other whey concentrate processes. The results of this research indicate that the amount of fluctuation seen in the commercial processing parameters of whey concentrate produce a powder with acceptable quality. Altering the pH to be within a narrower range before whey concentrate is crystallized and decreasing the extremes of temperature and length of time during whey concentrate crystallization for each batch do not appear to be needed to produce a quality product.

BIBLIOGRAPHY

A/S Niro Atomizer (1978a). Analytical Methods for Dry Milk Products. A 19 a Determination of hygroscopicity.

A/S Niro Atomizer (1978b). Analytical Methods for Dry Milk Products. A 14 a Determination of titratable acidity in milk powder (as % lactic acid).

A/S Niro Atomizer (1978c). Analytical Methods for Dry Milk Products. A 3 a Determination of solubility index.

Berlin, E., B. Anderson, and M. Pallansch. 1968. Comparison of water vapor sorption by milk powder components. J. Dairy Sci. 51 (12): 1912 – 1915.

Boxcar Pro software. Version 4.0.7.0, Onset Computer Corporation.

Burin, L., K. Jouppila, Y. Roos, J. Kansikas, and M. Pilar Buera. 2000. Color formation in dehydrated modified whey powder systems as affected by compression and Tg. J. Agric. Food Chem. 48: 5263-5268.

Caric, M. 1994a. Whey- Commercial Applications. In "Concentrated and Dried Dairy Products" (pp 160-162). New York, New York: VCH Publishers, Inc.

Caric, M. 1994b. Lactose- Principles. In "Concentrated and Dried Dairy Products" (pp 227-228). New York, New York: VCH Publishers, Inc.

Caric, M. 1994c. Whey- Manufacturing Procedures. In "Concentrated and Dried Dairy Products" (pp 155-162). New York, New York: VCH Publishers, Inc.

21 CFR 1240.61. Control of communicable diseases. Title 21, Vol 8, Chpt 1, Sec 1240.61

21 CFR Sec. 184.1979. Whey. Title 21, Sec 1.

Chandan, R. 1997. Whey Products and Lactose. In "Dairy-based Ingredients" (pp 30-34). St. Paul, Minnesota: American Association of Cereal Chemists, Inc.

CoolSNAP software. Version 1.2, Roper Scientific, Inc.

Damodaran, S. and A. Paraf, eds. 1997. Food Proteins and their Applications (pp 232-246). New York, New York: Marcel Dekker, Inc.

de la Fuente, M., Y. Hemar, M. Tamehana, P. Munro, and H. Singh. 2002. Process-induced changes in whey proteins during the manufacture of whey protein concentrates. Int. Dairy J. 12: 361-369.

de Vilder, J. 1975. Influence de la cristallisation du lactose dans le concentré de sérum sur l'hygroscopicité et l'agglomération de la poudre de sérum. Revue de l'Agriculture. 4: 963-975.

de Wit, J. and G. Klarenbeek. 1984. Effects of various heat treatments on structure and solubility of whey proteins. J Dairy Sci. 67: 2701-2710.

de Wit, J. 1990. Thermal stability and functionality of whey proteins. J. Dairy Sci. 73: 3602-3612.

Downton, G., J. Flores-Luna, and C. King. 1982. Mechanism of stickiness in hygroscopic, amorphous powders. Ind. Eng. Chem. Fundam. 21: 447-451.

Drapier-Beche, N., J. Fanni, M. Parmentier, and M. Vilasi. 1997. Evaluation of Lactose Crystalline Forms by Nondestructive Analysis. Journal of Dairy Science. 80:457-463.

Drapier-Beche, N., J. Fanni, M. Parmentier, and M. Vilasi. 1998. Kinetics of the synthesis of lactose molecular compounds. Journal of Dairy Science. 81:2826-2832.

Early, R., ed. 1998. Milk Concentrates and Milk Powders. In "The Technology of Dairy Products" (pp 289-290). London, England: Blackie Acedemic & Professional.

Fellows, P. 1997. Equipment- spray dryers. In "Food Processing Technology: Principles and Practice" (pp 300-302). Cambridge, England: Woodhead Publishing, Ltd.

Fennema, O., ed. 1996a. Amino Acids, Peptides, and Proteins. In "Food Chemistry" (pp 321-429). 3rd ed. New York, New York: Marcel Dekker, Inc.

Fennema, O., ed. 1996b. Characteristics of Milk- The Whey Proteins. In "Food Chemistry" (pp 858-861). 3rd ed. New York, New York: Marcel Dekker, Inc

Ferretti, A. and V. Flanagan. 1971. Volatile constituents of whey powder subjected to accelerated browning. J. Dairy Sci. 54 (12): 1764-1768.

Fries, D., S. Rao, and M. Sundaralingam. 1971. Structural Chemistry of Carbohydrates. III. Crystal and molecular structure of 4-O-β-D-Galactopyranosyl-α-D-glucopyranose monohydrate (α-lactose monohydrate). Acta Crys. B27: 994-1005.

Gillies, M. 1974. Whey Processing and Utilization: Economics and Technical Aspects. Parkridge, New Jersey: Noyes Data Corporation.

Hardy, J., J. Scher, and S. Banon. 2002. Water activity and hydration of dairy powders. Lait. 82: 441-452.

Hargrove, R., F. McDonough, D. LaCroix, and J. Alford. 1975. Production and properties of deproteinized whey powders. J. Dairy Sci. 59 (1): 25-33.

Hawks, S., L. Phillips, R. Rasmussen, D. Barbano, and J. Kinsella. 1993. Effects of processing treatment and cheese-making parameters on foaming properties o whey protein isolates. J. Dairy Sci. 76: 2468-2477.

Honer, C. 1985. Food automation. Prepared Foods. 154(4): 119-120.

Image J software. Version 1.30, shareware.

Jayaprakasha, H.M., R.S. Ratel, and E. Renner. 1995. Optimization of Precrystallization Process for Nonhygroscopic Whey Powder by Using Reverse Osmosis Concentrate. Japanese Journal of Dairy and Food Science. Vol. 44, No. 3: A-113 – A121.

Jelen, P. 1991. Whey: Composition, Properties, Processing and Uses. In "Wiley Encyclopedia of Food Science and Technology". New York, New York: John Wiley & Sons, Inc.

Joupilla, K., J. Kansikas, and Y.H. Roos. 1997. Glass Transition, Water Plasticization, and Lactose Crystallization in Skim Milk Powder. Journal of Dairy Science. 80:3152-3160.

Joupilla, K., J. Kansikas, and Y.H. Roos. 1998. Crystallization and X-ray Diffraction of Crystals Formed in Water-Plasticized Amorphous Lactose. Biotechnology Progress. 14:347-350.

Mak, T., and G. Zhou. 1992. Crystallography in Modern Chemistry: A resource book of crystal structures. New York, New York: John Wiley & Sons, Inc.

Modler, H. and L.P. Lefkovitch. 1986. Influence of pH, Casein, and Whey Protein Denaturation on the Composition, Crystal Size, and Yield of Lactose from Condensed Whey. Journal of Dairy Science. 69:684-697.

Nickerson, T.A. and E.E. Moore. 1974. Factors Influencing Lactose Crystallization. Journal of Dairy Science. 57:1315-1319.

Parris, N., S. Anema, H. Singh, and L. Creamer. 1993. Aggregation of whey proteins in heated sweet whey. J. Agric. Food Chem. 41: 460-464.

Patel, R., H. Jayaprakasha, and S. Singh. 1991. Recent advances in concentration and drying of whey. Indian Dairyman. 43 (9): 417-421.

Robinson, R. 1994. Modern Dairy Technology: Vol. 1 Advances in Milk Processing, 2nd ed. London, UK: Chapman & Hall.

Roos, Y. 2002. Importance of glass transition and water activity to spray drying and stability of dairy powders. Lait. 82: 475-484.

Saito, Z. 1988. Lactose crystallization in commercial whey powders and in spray-dried lactosse. 1988. Food Microstructure. 7: 75-81.

Saltmarch, M. and T.P. Labuza. 1980. Influence of relative humidity on the physicochemical state of lactose in spray-dried sweet whey powders. Journal of Food Science. 45:1231-1236.

Schmidt, R., V. Packard, and H. Morris. 1984. Effect of processing on whey protein functionality. J Dairy Sci. 67: 2723-2733.

Schuck, P. and A. Dolivet. 2002. Lactose crystallization: determination of □-lactose monohydrate in spray-dried dairy products. Lait. 82: 413-421.

Sienkiewicz, T. and C. Riedel. 1990. Whey and Whey Utilization (pp 87-90). 2nd ed. Gelsenkirchen-Buer, Germany: Verlag Th. Mann.

Singh, T., M. Drake, and K. Cadwallader. 2003. Flavor of cheddar cheese: a chemical and sensory perspective. Comp. Reviews in Food Sci. and Food Safety. 2: 139-162.

Spreer, E. 1998. Whey and Whey Utilization. In "Milk and Dairy Product Technology" (pp 405-422). New York, New York: Marcel Dekker, Inc.

SPlus. Academic version 6.1.

Tomaino, R. J. Parker, and D. Larick. 2001. Analysis of free fatty acids in whey products by solid-phase microextraction. J. Agric. Food Chem. 49: 3993-3998.

Train, D. 1958. Some aspects of the property of angle of repose of powders. J. Pharm. Pharmacology. 10 (pt 2): 127T-134T.

USDA. 2004. Dairy products 2003 summary. Da 2-1 (04)a. Available online: www.usda.gov/nass.

Wallack, D. and C. King. 1988. Sticking and agglomeration of hygroscopic, amorphous carbohydrate and food powders. Biotechnology Progress. 4 (1): 31-35.

Walstra, P., T. Geurts, A. Noomen, A. Jellema, and M. van Boekel. 1999. Dairy Technology: Principles of Milk Properties and Processes. New York, New York: Marcel Dekker, Inc.

Walstra, P. 2003a. Crystallization. In "Physical Chemistry of Foods" (pp 583-649). New York, New York: Marcel Dekker, Inc.

Walstra, P. 2003b. Nucleation. In "Physical Chemistry of Foods" (pp 548-582). New York, New York: Marcel Dekker, Inc.

Walstra, P. 2003c. Water Relations- "Water Binding". In "Physical Chemistry of Foods" (pp 265-270). New York, New York: Marcel Dekker, Inc.

Zadow, J. 1984. Lactose: properties and uses. J Dairy Sci. 67: 2654-2679.