Phase Behaviour of Lactose as Affected by Presence of Lactic Acid and Minerals

By

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A thesis submitted in fulfilment of the requirements for the degree of

Master of Science (Research)

College of Health and Biomedicine

Faculty of Health, Engineering and Science

Victoria University

2015

To my beloved parents, Dimuthu and Vinara

I. Abstract

Owing to growing markets for Greek yoghurts and soft cheeses, dairy industry generates huge quantity of acid whey creating a significant environmental problem globally. This by-product cannot be simply disposed of due to its toxicity during decomposition, robbing oxygen from rivers and streams creating health and environmental concerns. Currently, there is no solution to acid whey waste, but it seems the behaviour of lactose in presence of LA and Ca in acid whey restricts its further processability due to the failure of lactose to crystallize which thus remains in its amorphous form during concentration and further processing, including spray drying.

Hence, the present study aims at fundamentally understand the concentration and crystallisation behaviour of lactose and its interactions with other constituents of acid whey especially LA and Ca. A model based study was thus implemented with varying concentrations of Ca (0.12, 0.072 or 0.035% w/w) and LA (0.05, 0.2, 0.4 or 1% w/w) in establishing behaviour of lactose during concentration up to 50% (w/w). Different concentrations of LA and Ca were selected to imitate the concentrations originally present in acid whey, nanofiltered retentates and sweet whey. Furthermore, the crystallization nature of freeze dried lactose in presence of LA and/or Ca were investigated. Differential scanning colorimetry (DSC) was used to determine the phase transitions while, fourier transform infrared spectroscopy (FTIR) was used to determine the structural changes and screens the molecular vibrations shown by various compounds.

L + 1% (w/w) LA solution exhibited a higher enthalpy of water dehydration in comparison to pure lactose indicating that the presence of LA hindered removal of water from lactose. Formation of a strong hydration layer consisting LA and H_3O^+ ions around lactose resulted in restricted water mobility and/or a change in a structure of lactose. Thus a higher energy input appeared necessary to crystallize lactose. In addition, delayed crystallisation and less crystalline lactose were obtained in the presence of LA as compared to pure lactose.

Presence of Ca in lactose solution increased the evaporation enthalpy of water as compared to pure lactose. This effect was likely caused by the ability of ionic Ca^{2+} to organize the structure of water. The strong dipole-ion interactions between water and Ca^{2+} the water molecules may be densely packed within the hydration layers which may leads to restricted mobility. The presence of 1% (w/w) LA and 0.12% (w/w) Ca in lactose solution significantly increased the evaporation enthalpy of water, delayed and increased the energy required for L crystallization as compared to pure L. Furthermore, the crystallinity of lactose has been reduced significantly. However, reduction of concentration of both LA and Ca led to gradual decrease in water evaporation enthalpy and increased crystallinity.

The present study revealed that the presence of high concentrations of both LA and Ca hinders the removal of water from lactose due to formation of strong hydration layer around lactose molecules, which restricts their molecular movements. This appears to be responsible for governing the behavior of lactose. Consequently lactose molecules require greater energy for proper alignment and initiation of nucleation and crystallization. It was found that by manipulating Ca concentration would be possible to predict phase behavior of lactose and thus a partial removal of Ca from acid whey may be a feasible strategy in improving the crystallization behaviour of lactose and thereby enabling better processing of acid whey.

II. Declaration

“I, Rangani Wijayasinghe, declare that the Master by Research thesis entitled “Phase Behaviour of Lactose as Affected by Presence of Lactic Acid and Minerals” is no more than 60,000 words in length including quotes and exclusive of tables, figures, appendices, bibliography, references and footnotes. This thesis contains no material that has been submitted previously, in whole or in part, for the award of any other academic degree or diploma. Except where otherwise indicated, this thesis is my own work”.

Signature: Date:

Rangani Wijayasinghe

III. Acknowledgements

Foremost, I would like to express my sincere gratitude to my principle supervisor, Professor Todor Vasiljevic for his professional guidance, valuable suggestions and encouragement given me strength and confidence necessary for the development of the study. His continuous diligence and patience over these years are genuinely appreciated.

I would also like to thank my co-supervisor, Dr. Jayani Chandrapala, for her contribution and valuable discussions during the studies. Her immeasurable guidance and support helped me to develop my research abilities throughout the study.

Professor Chris Perera and Dr. Prashanthi Kodikara helped me a lot providing valuable advices and support on numerous occasions even before the start of this study. My sincere thanks to them.

I wish to sincerely thank the laboratory staff at Victoria University, Werribee for their technical help and patience especially Stacey Lloid, Joseph Pelle, Dr. Sudinna Hewakapuge, Min Nguyen, Charmaine DiQuattro and Mary Marshall.

I am grateful to Dr. Muditha Dissanayake, Dr. Marlen Cran, for all the support given me throughout the study.

I appreciate the great friendship I have made during my research studies at Victoria University specially, Chathuri Piyadasa, Nuwan Vithanage, Gangani Uduwerella, Manjula Nishanthi, Samantha Liyanarachchi, Kasup Munaweera, Rabia Ashraf, Manpreet Grewal, Uditha Bandara and all others. Thank you all for the support given me during my studies.

I would like to express my profound gratitude to my late mother, my father, sisters and brother for their endless encouragement and loving support during my whole life.

Most importantly, I would like to express my deepest gratitude to my husband Dimuthu for his understanding, support and unlimited patience. He shared the burden, anxieties and pleasures of this study. I owe an immeasurable debt and deep affection to my little daughter Vinara, who lost a lot due to my research studies and for her unknowing inspiration to me.

Table of Contents

List of Figures

List of Tables

Chapter 1

Introduction

1.1 Background

The dairy industry is founded on processing of raw milk into final products such as yogurt, cheese, ice cream, butter, and various types of desserts with use of numerous processes such as pasteurization, coagulation, filtration, centrifugation, chilling, etc. Throughout these processes, large volumes of by-products are removed as effluents. Whey is such a spinoff from the dairy industry. It is the main by-product of cheese and yoghurt productions which is universally defined as the serum or liquid portion remaining after the milk has been curdled and strained (de Wit, 2001; Jelen, 2009).

Whey has been generally classified into two major groups depending on the manufacturing process. The whey expelled from hard and semi-hard cheeses and rennet casein production is known as "*sweet whey*" with a pH of above 5.6. Conversely, the whey with a pH of 4.3 - 4.6 released during production of acid coagulated dairy products such as soft cheeses or strained (Greek style) yoghurts is referred to as "*acid whey*" (Schmidt et al., 1984). These two main streams mainly consist of lactose, whey proteins and minerals apart from water being the majority (de Wit, 2001) and differ from the origin (Table 1-1).

Table 1-1: Approximate composition of liquid whey by type (Jelen, 2009; Gosta, 1995)

Constituent	Sweet whey % (w/w)	Acid whey % (w/w)
Total solids	6.3-7.0	6.5-7.0
Water	93.6	93.5
Protein	0.6-1.0	0.6-0.8
Lactose	4.6-5.2	4.4-4.9
Calcium	0.04-0.06	0.09-0.12
Lactic acid	0.05-0.2	0.4-0.64

The prompt responses by dairy industries towards changing their traditional processes for more sustainable and environmentally friendly processes, led to find solutions for a zero discharge strategy. In early 70's, sweet whey disposal was a huge problem for dairy industries with increased production of cheese. However, the invention of new technologies was able to resolve the problem, where the sweet whey waste stream was concentrated through evaporation to ~45 - 60% (w/w) solids followed by crystallization of lactose and spray drying to produce a non-sticky free flowing whey powder, which is currently used in different food applications (Chandrapala et al., 2015).

Nowadays the consumer preferences are growing more towards the acid coagulated dairy products due to health benefits generating a vast production of acid whey where industry is struggling to find solutions for proper disposal and utilization avenues for the stream. The waste stream has been trialed successfully as an animal feed and as a soil fertilizer. However, care must be taken of the quantity being fed to the animals due to health concerns and the type of plant being fertilised. Additionally, converting whey into biogas requires a big capital cost and is not feasible for small scale farmers. Thus, a major portion of the world acid whey production is thrown away as a sewage creating a serious pollution problem for the surrounding environment (Panesar et al., 2007; Saffari & Langrish, 2014).

In contrast to sweet whey, an industrial processing method for acid whey still has not been found and is considered a hard to process stream mainly because of inability to obtain crystalline lactose, which in concentrated acid whey remains in its amorphous form. Some of the approaches reported low rate of crystallization, lumping and caking of whey powder particles during spray drying of acid whey due to the crystallisation behaviour differences of lactose in comparison to sweet whey (Dec & Chojnowski, 2006).

In comparison to sweet whey, acid whey contains high levels of calcium and more importantly high amounts of lactic acid. Some researchers have postulated that the formation of calcium lactate crystals may be the origin of the thickening behaviour of lactose during spray drying of acid whey (Mimouni et al., 2007). Thus, it appears that the role of lactic acid and/or calcium is crucial for the behaviour of lactose and subsequently unprocessability of acid whey. Presumably lactic acid and calcium affect associations between lactose and water, thus rendering removal of water molecules difficult. There is anecdotal evidence in favour of the behaviour of lactose in presence of lactic acid and calcium but, the science in this regard is not known, which limits the solutions to the current and growing problem. However due to lack of evidence, it is thus of utmost importance to understand the behaviour of lactose in the presence of lactic acid and calcium. A systematic study is lacking in this regard especially during the concentration step, since this is also considered a crucial step during processing of acid whey and thus recovery of lactose crystals. This study would assist in proposing approaches in minimizing the disposal of acid whey as waste and at the same time creating value added products where companies can achieve a higher return.

1.2 Research Aim and Objectives

The main aim of the present study was to establish the physico-chemical and thermal behaviour of lactose model solutions as affected by presence of lactic acid and calcium during the concentration step.

The objective of the project was thus to answer the following fundamental questions:

- What are the physico-chemical and thermal characteristics of model lactose solutions during the concentration step?
- How these physico-chemical and thermal characteristics differ with the presence of different concentrations of lactic acid and/or calcium?
- Are there any interactions between lactose, lactic acid and calcium in model lactose systems during the concentration process which lead to unprocessability issues?
- Can these interactions be manipulated by modifying the equilibrium via removal of a key compound?

1.3 Structure of the thesis

The objective of the present study was to examine the physico-chemical and thermal behavior of model lactose solutions in presence of lactic acid and calcium. The concentrated model lactose solutions ranging from 15% to 50% (w/w) with different concentrations of lactic acid (0.05% to 1% w/w) and calcium (0.035% w/w to 0.12% w/w) were used as the main systems for the proposed study. The thesis consists of following chapters:

Chapter 1 - Provides general information containing background of the study, research aim, objectives and the structure of the thesis.

Chapter 2 - Presents a literature review explaining more detailed background information on lactose and lactose crystallization.

Chapter 3 - Focuses on the physico-chemical and thermal properties of model lactose solutions as a function of concentration in the absence/presence of lactic acid.

Chapter 4 - Focuses on the phase behavior of lactose in presence of varying concentrations of lactic acid and calcium.

Chapter 5 - Concludes the overall chapters and presents the future directions, which can be used to optimize the processing conditions in order to obtain maximum use of acid whey and thereby minimize the environmental pollution.

Chapter 2

Literature review

2.1 Lactose

Lactose (Figure 2-1) is a reducing disaccharide having an empirical formula of $C_{12}H_{22}O_{11}$ and consisting of glucose and galactose molecules as the principal monosaccharides combined through β 1 - 4 glycosidic bond (Fox & McSweeney, 1998). Lactose synthesized in epithelial cells in mammary glands of most mammalian species serves as the primary source of energy and its concentration in milk differs from species to species. As an example, human milk contains ~7% (w/w) of lactose, while only 4.6% (w/w) is present in bovine milk (Anand et al., 2013).

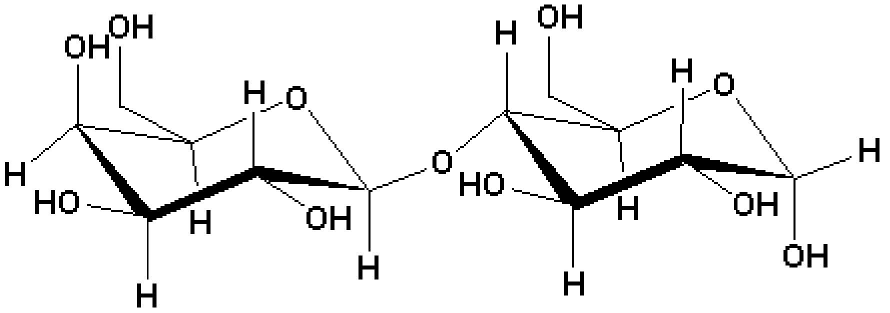


Figure 2-1: Schematic representation of the lactose molecule (Vasiljevic, 2003)

2.2 Physico-chemical properties of lactose

Lactose possesses some favorable functional properties such as relative low sweetness, flavour emphasizing and protein stabilization abilities, which in turn make this sugar an important ingredient for various food and pharmaceutical applications (Dissanayake et al., 2013). However, these properties are affected by various physico-chemical characteristics of lactose such as mutarotation, solubility and crystallization (Lefort et al., 2006).

2.2.1 Mutarotation

The lactose molecule has a hemiacetal ring structure and the hydroxyl group attached to the 1st carbon of the glucose moiety is free to lie above or below the plane of the ring. This structure is responsible for the two anomeric forms (α and β) of lactose (Figure 2-2). Both forms change into one another continuously and this process is known as mutarotation (Fox, 2009). Mutarotational reactions are generally following first-order kinetics (Harper, 1992).

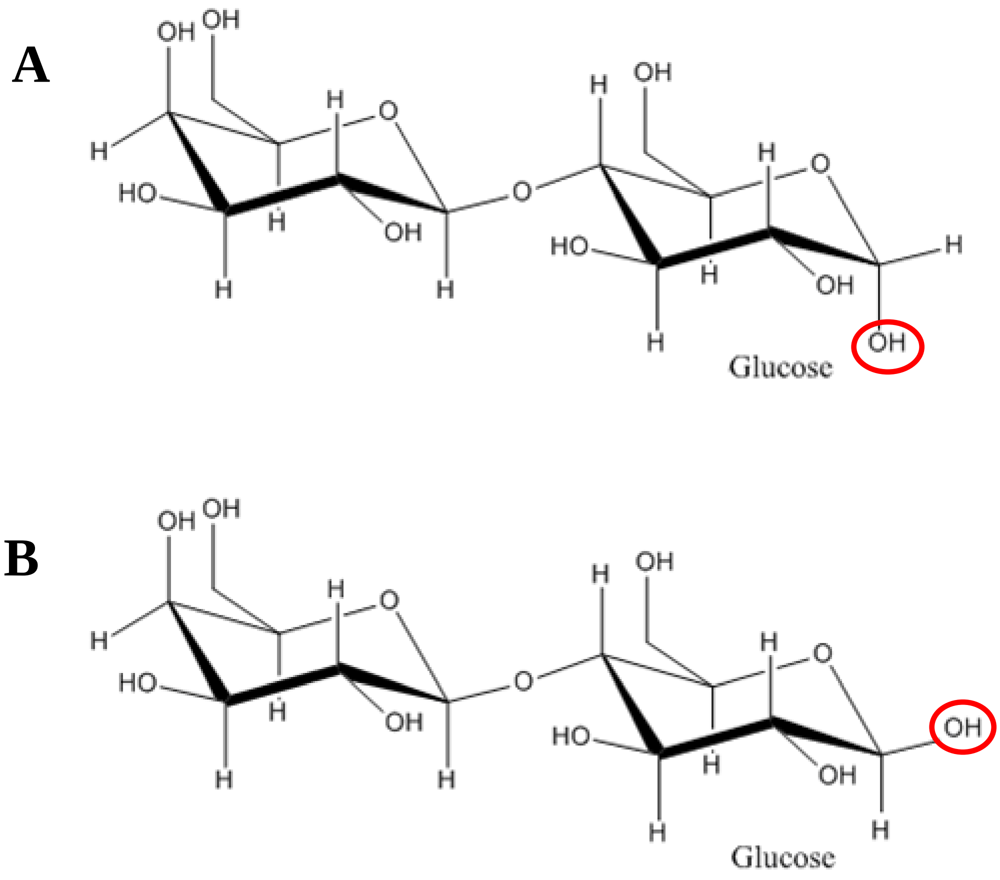


Figure 2-2: (A) α and (B) β anomeric forms of lactose

The two forms of lactose have different physical characteristics as listed in Table 2-1. In an aqueous system of lactose, the equilibrium ratio of α to β is 37.3:62.7% at room temperature (Vasiljevic, 2003). This equilibrium is affected by temperature (Roetman & Buma, 1974), concentration of lactose and presence of impurities (Hartel & Shastry, 1991) and is independent of pH (Harper, 1992). High temperatures and low concentrations of lactose are preferred to reach the equilibrium in quick time. For instance, only few minutes were taken to reach the equilibrium of α and β at 70 °C while it extended to few hours at 20 °C (Harper, 1992). Most importantly, α and β forms of lactose have a great influence on the physico-chemical and functional properties of lactose such as crystallization, solid state properties and solubility.

Table 2-1: Physical properties of α and β anomers of lactose (Harper, 1992)

	α Lactose Monohydrate	**β Lactose Anhydride**
Molecular Weight (g/mol)	360	342
Melting point (°C)	202	252
Density (g/mL)	1.54	1.59
Specific optical rotation	+89.4 °	+35.0 °
Initial solubility in water (g/g)	0.07	0.5

2.2.2 Solubility

Lactose has a relatively low solubility compared to other common sugars (Harper, 1992). Lactose possesses an overall equilibrium solubility of 18 g per 100 g of water at 20 °C (Fox & Mcsweeny, 1998), although the extent of the solubility is governed by the presence and quantity of the two anomeric forms: hydrated α- lactose and anhydrous β-lactose (Hunziker & Nissen, 1926). The initial solubility of hydrated α-lactose is approximately 7 g per 100 g of water at 20 °C while the solubility of anhydrous β-lactose is 50 g per 100 g of water (Hunziker & Nissen, 1926).

Furthermore, the presence of impurities in solution appears to influence the equilibrium solubility of lactose mainly due to the changes in supersaturation of the solution (Bhargava & Jelen, 1996). In addition, the change in structure of bulk water molecules due to impurities can sequentially increase/decrease the solubility of lactose (Bhargava & Jelen, 1996). As an example, some of the calcium salts such as nitrate, bromide or chloride significantly increases the solubility of lactose resulting very stable solutions (Herrington, 1934b; Harper, 1992). Herrington (1934b) postulated this increase to the formation of a molecular compound consisting lactose, salts and water molecules. Hence, formation of chemical complexes between lactose, salts and water molecules can result in changes in solubility of lactose (Nickerson, 1974; Harper, 1992).

2.3 Production process of lactose from whey

In an industrial scale, lactose is mostly produced from whey/whey permeates obtained as by products from production of cheese and yoghurt. Three steps namely as concentration, crystallization and separation are involved in the production of lactose. The traditional methods used to make edible and pharmaceutical grade lactose are presented in Figure 2-3.

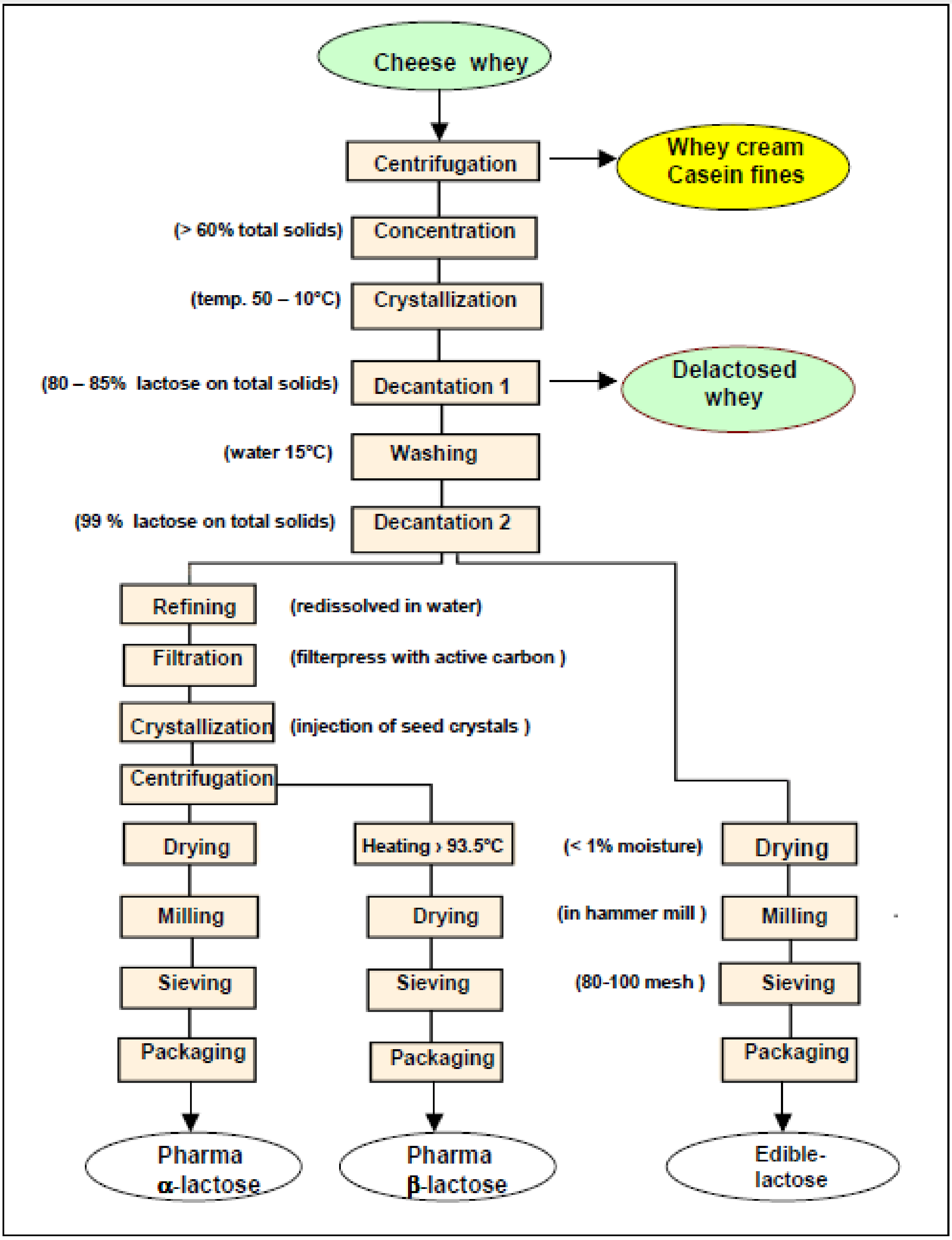


Figure 2-3: Traditional methods use for the recovery of lactose from sweet cheese whey (de Wit, 2001)

2.3.1 Step 1: Concentration

The concentration process involves evaporation of water from whey/whey permeate streams and eventually to supersaturate the lactose in solution with relation to the content of water. Generally, the whey streams will be concentrated 8-10 times the original solid content of whey (6.5% w/w) resulting a 50-60% (w/w) concentrated whey solution at the end. Evaporation is most commonly done by means of falling-film evaporator with two or more stages. The energy required for the evaporation is obtained through the steam under reduced pressure (de Wit, 2001). Usually the evaporator is designed to reduce the required steam as much as possible. Due to the use of multiple stages, heat is used only at the first stage and in subsequent stages the vapor from the previous stage is used as the heat source. Hence, the use of falling film evaporators for concentration is economical in energy vise.

2.3.2 Step 2: Crystallization

Crystallization is one of the mostly used techniques in dairy industry as a method of production, purification and recovery of solid materials. Regardless of being one of the best and cheapest methods available for the recovery of pure solids from even mixed solutions, crystallization provides an additional advantage of producing a final product with many desired characteristics (Mullin, 2001). Crystallization process generally composed of two basic steps: “nucleation” which is the production of zero size crystals/nuclei and “crystal growth” where the growth of nuclei happens depending on available supersaturation of the solution (Wong & Hartel, 2014).

2.3.2.1 Nucleation

Nucleation is the formation of a solid crystal from a liquid solution in a small region. Concentrated whey is subjected to rapid cooling followed by slow cooling in order to induce the nucleation of lactose. Clustering and aggregation of molecules/ions in a solution is the starting point of nucleation. These clusters of molecules are then stable and grow in to detectable size crystals (Wong & Hartel, 2014). These zero size crystals/nuclei act as main centres for the crystallization. Total nucleation is the sum effect of two categories named as “primary” and “secondary”.

Primary nucleation can be further subdivided into homogeneous and heterogeneous. The main driving force for the primary nucleation is the supersaturation of the solution (Herrington, 1934a). In homogeneous nucleation, ions or molecules in a solution can interact to form short lived clusters, where short chains or flat monolayers may be formed originally and ultimately a crystalline lattice structure is formed. The process of formation of a crystalline lattice structure occurs rapidly and can only continue in local regions with very high supersaturation spots (Mullin, 2001). Homogeneous nucleation needs high energy for nuclei formation thus occurs only in highly supersaturated solutions at high temperature conditions (Shi et al., 1989). Heterogeneous nucleation occurs in presence of foreign materials such as dust particles or any other minor impurities in the system. Thus, this would reduce the required energy for nuclei formation and can occur under low supersaturation levels. Homogeneous nucleation rarely occurs in practice due to difficulties to initiate nucleation without a solid surface to catalyze nucleation (Agrawal & Paterson, 2015). Primary nucleation only occurs at the startup phase of the crystallization process.

Secondary nucleation occurs only in presence of suspended solute particles or seed crystals. This is the dominant mechanism for the crystallization process. Secondary nucleation at lower supersaturation levels reduces the production of large number of small crystals from excessive nucleation. However, with increase in supersaturation the rate of nucleation increases. Nuclei must need to reach a critical size to grow in to a steady crystal. While below this level, the nuclei will dissolve back in the solution (Mandare & Pangarkar, 2003). The Embryo Coagulation Secondary Nucleation (ECSN) theory by Qian and Botsaris, (1997) states that solute clusters are attached by van der Waals forces of the seed crystals. The high concentration of solute clusters (embryos) in the surrounding area of crystals results in rapid attractions between each other, leading to formation of nuclei which are greater than the critical size. The nuclei should be released to the bulk solution through a collision impact or fluid shear in order for these crystals to grow as individual crystals.

Secondary nucleation can happen either through fluid shear or collision between already existing crystals and the solid surface of the crystallizer. Fluid shear nucleation occurs when the high speed travelling liquid sweeping away some pre-ordered solute layers near the crystal surface, which acts to form as new crystals. Collisions lead to physical damage to the crystals, thus resulting fragments which act as secondary nuclei. Secondary nucleation is the most commonly used technique for industrial lactose production process (Shi et al., 1989; Wong & Hartel, 2014).

2.3.2.2 Crystal growth

Growth of lactose crystals follows few steps (Hartel, 2001; Rjabova et al., 2013). The first step is the diffusion of lactose molecules from the bulk solution to the solid interface. This is followed by the mutarotation of lactose molecules to appropriate anomeric form. The second step is surface reaction or integration, where the removal of all or some of the hydration water molecules associated with lactose occurs. The third step is the orientation of lactose molecules with each other followed by diffusion of water molecules away from the crystals. Surface diffusion of the lactose molecules to an appropriate crystal lattice incorporation site occurs in the fourth step. Finally the removal of latent heat away from the growing crystals happens (Wong & Hartel, 2014).

2.3.3 Step 3: Separation

Decanter centrifuge (Figure 2-4) is most commonly used for the separation of lactose crystals from the slurry (Harper, 1992). The main advantage of this method is that it can be used continuously for the production of lactose and at the same time the crystal size distribution can be better controlled (Harper, 1992). The amount of washing required to remove the impurities, viscosity of the solution, homogeneity of crystals, and the design of the centrifuge are the main factors to be considered in the separation step. It is essential to remove impurities from lactose crystals for the production of high quality lactose. 80-85% total solid content of lactose can be achieved after the first decantation, while the purity can be increased to ~99% through washing in a second decanter (de Wit, 2001).

Lactose refining is the process which used to further purify the food grade lactose to be used in pharmaceutical applications. In the refining process, food grade lactose is redissolved in hot water to obtain a lactose solution with 50% concentration (de Wit, 2001). Decolorizing carbon is added afterwards to remove any defective colour while reducing some impurities to some extent. Then the carbon and the flocculated impurities are removed by filtration (Harper, 1992). The purify lactose solution is then subjected to another crystallization process.

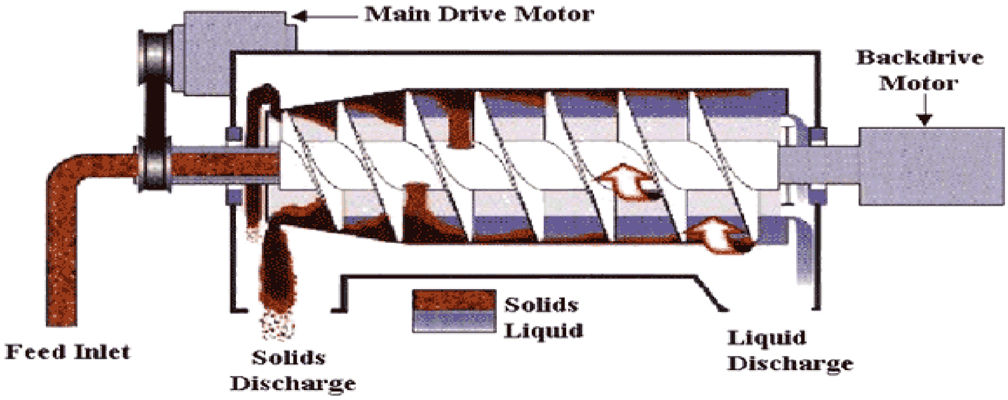


Figure 2-4: Decanter centrifuge (*www.hutch-hayes.com*)

2.4 Forms of lactose

There are two main forms of lactose depending on their crystalline characteristics, namely as crystalline and amorphous (Figure 2-5).

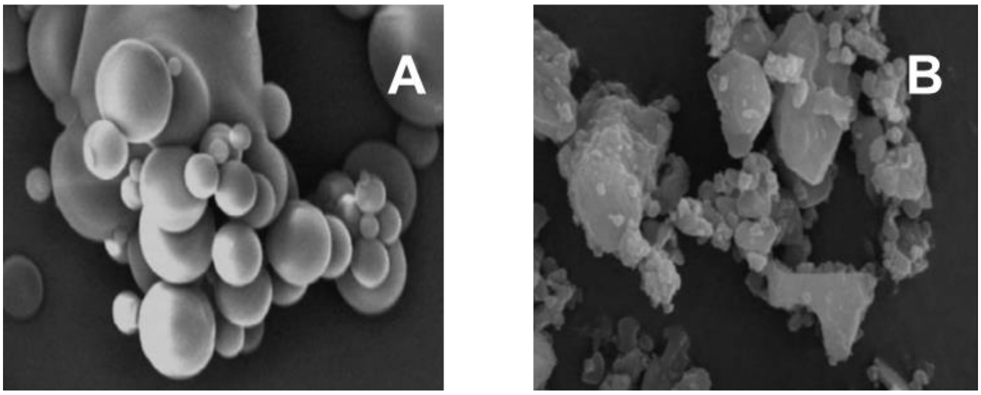


Figure 2-5: Scanning electron micrographs of (A) amorphous & (B) crystalline spray-dried lactose (Haque & Roos, 2006)

2.4.1 Crystalline lactose

Lactose molecules are arranged in a definite 3D structure in a crystal lattice in crystalline state. Crystalline lactose is further subdivided as α-Lactose monohydrate, β-Lactose/β-Anhydride, α-Stable anhydrous and α-Unstable anhydrous (Kirk et al., 2007).

α-Lactose monohydrate (α-Hydrate) crystals grow out from supersaturated solutions at crystallization temperatures below 93.5 °C and is the only stable form of lactose crystals under ambient temperature conditions. In the crystalline state, each lactose molecule is associated with one molecule of water which is known as crystallization water. Thus the normal water content of α-Lactose monohydrate is about 5% (Fox & McSweeney, 1998). All the other forms of lactose crystals convert in to the α-Hydrate form even in the presence of small amounts of water at temperatures below 93.5 °C. α-Hydrate form exists in variety of crystal shapes depending on the conditions of the crystal growth. Tomahawk shape is the most common (Figure 2-6). These crystals are monoclinic sphenoidal and have only one axis of symmetry. They have trapezoidal side faces, rhombic tops and bottoms, and beveled faces at the base and apex, giving the crystal a distinct tomahawk appearance (Hunziker & Nissen, 1927). These crystals are hard, brittle and dissolve very slowly.

Unstable anhydrous α-form can be prepared by dehydrating α-Lactose monohydrate *in vacuo* at temperatures between 65 and 93.5 °C and is only stable with the absence of water (Fox & McSweeney, 1998). Stable anhydrous α-form is produced by heating α-lactose monohydrate at temperatures between 100 – 190 °C. This form of lactose is not hygroscopic and is more soluble than the other forms.

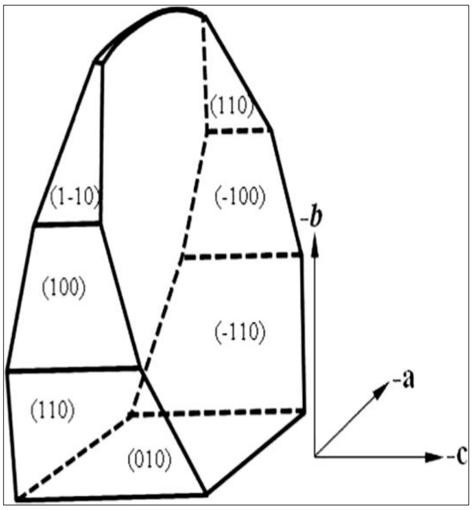


Figure 2-6: Tomahawk shaped crystal of α-Hydrate showing faceted structure (Wong & Hartel, 2014)

β-Anhydride lactose crystals are formed from a supersaturated solution at temperatures above 93.5 °C. β-Lactose is less soluble above 93.5 °C and substantially sweeter than α-Lactose (Fox & McSweeney, 1998). Moreover, β-Lactose crystals possess a characteristic uneven-sided diamond shape (Listiohadi et al., 2009).

2.4.2 Amorphous lactose

Amorphous lactose is a metastable state and is present in a glassy, solid state or in a syrup-like, super-cooled liquid state where no definite long range arrangement of molecules is present which defines the crystalline state. When a lactose solution is dried quickly, viscosity increases so rapidly where there is no time for the crystallization to take place and thus an amorphous form of lactose is formed. The structure of this form is expected to change with time, temperature and water contents (Roos & Karel, 1991a, 1991b; Slade et al., 1991). The molecular mobility and the intermolecular distances are high in amorphous state due to the lack of long range arrangement of molecules. Thus, it has a higher potential energy compared to the crystalline state.

Amorphous lactose is highly hygroscopic leading to a sticky nature and causes problems during storage and handling of many food products (Aguilera et al., 1995). Hence the presence of amorphous lactose in many food products is not desirable since stickiness influences significantly the storage stability and quality of the final product. Therefore, it is essential to take appropriate measures to crystallize lactose in a pre-crystallization step in order to minimize the possible problems.

2.4.2.1 Glass transition

The temperature at which an amorphous material transforms its physical state from glassy solid state or in a syrup-like, super-cooled liquid state to rubbery state is known as the glass transition temperature (T_g). Change of physical state of an amorphous glass through rubbery (transitional) to crystalline state is illustrated in Figure 2-7.

The glass transition generally occurs over a temperature range and is controlled by the heterogeneity of the system, where increased heterogeneity of a system leads to an increase in the glass transition temperature range (Bosma et al., 1988; Roudaut et al., 2004). Thus presence of impurities may increase the temperature range. Glass transition temperature involves no latent energy. However, it can be determined by the physical changes occurring at temperatures above T_g such as increase in heat capacity, free molecular volume, thermal expansion coefficient, dielectric coefficient, and changes in viscoelastic properties (Bhandari & Howes, 1999; Roos, 2009). Generally a high viscosity is experienced in the glassy state, where it decreases slowly allowing sufficient time to form a proper crystalline state. However, above T_g the viscosity decreases drastically and reduces the contact time which causes stickiness hindering the further processing of powders (Roos, 2009).

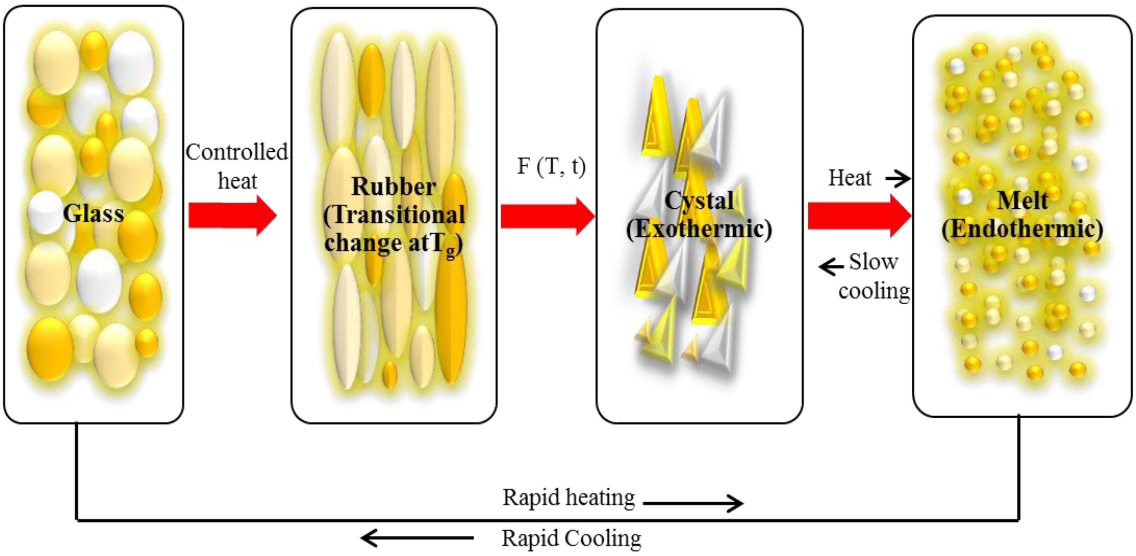


Figure 2-7: Change of physical state of an amorphous glass through rubbery (transitional) to crystalline state; where T and t are temperature and time respectively. (Adapted from Bhandari & Howes, 1999)

In general, high molecular weight polymers/long chain molecules such as lactose, sucrose and maltose have high glass transition temperatures while low molecular weight compounds such as lactic acid, water and simple monosaccharide sugars in uncontaminated form have low glass transition temperatures (Table 2-2).

Typically, lactose has a high glass transition temperature of 101 °C. However components with low molecular weight and T_g values such as water and lactic acid has the ability to reduce the typical T_g value of lactose (Shrestha et al., 2006). Furthermore, mineral salts also influence the T_g of lactose. The influence of bivalent salts such as Ca and Mg on T_g of lactose is higher than the effect of monovalent salts (Omar & Roos, 2007). They have reported a T_g of ~121 °C for lactose in presence of $CaCl_2$ while, a T_g of ~99 °C was observed for lactose in presence of KCl at zero relative vapor pressure. Thus, it is evident that the presence of impurities greatly influences the T_g of lactose either positively or negatively.

Table 2-2: Glass transition temperatures of some food components (Roos, 1993; Roos & Karel, 1991b; Maltini et al., 1967)

Food component	**Molecular weight (g/mol)**	**T_g (°C)**
Water	18	-135
Lactic acid	90	-60
Fructose	180	5
Glucose	180	31
Galactose	180	32
Sucrose	342	62
Maltose	342	87
Lactose	342	101

The glass transition temperatures of carbohydrates are affected by water, with greater water content resulting in decreased glass transition temperatures. Water acts as a plasticiser enabling the mobilisation of amorphous components. Consequently, a lower temperature is required to initiate the onset of the glass transition for higher water contents. Both higher temperature and water content can enable molecular mobility and the onset of the glass transition as can be seen by Figure 2-8. The region beyond the glass transition curve is the glassy state (Figure 2-8). In this region molecular mobility is very low and limits the crystallization. Thus, glass transition curve can be used to determine the storage temperatures. Increase in temperature increase the molecular mobility of amorphous lactose which leads to time-dependent structural changes such as stickiness, collapse, and crystallization when T_g falls below the ambient temperature (Roos & Karel, 1993). Thus, state diagram and T_g is often seen as an important tools to indicate of product properties and stability of foods (Levine & Slade, 1992).

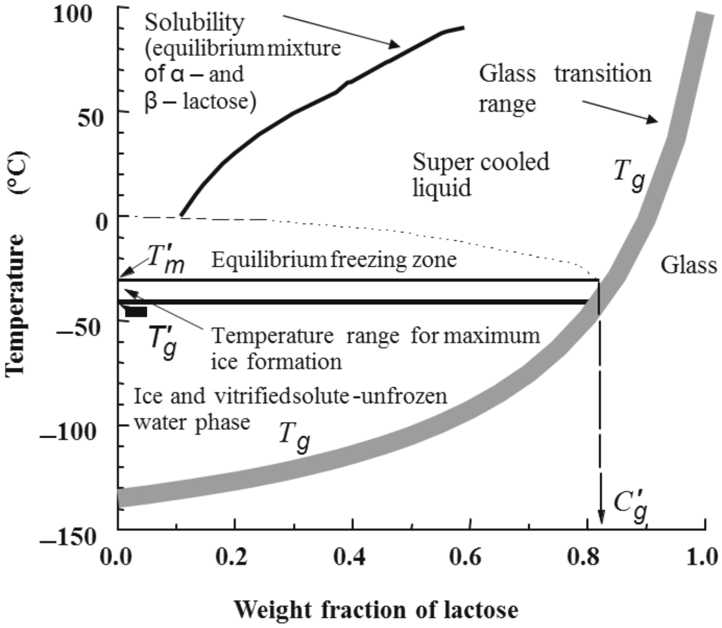


Figure 2-8: State diagram of lactose (Roos, 2009)

2.4.2.2 *Sticking and Caking*

Generally stickiness and caking in dairy powders are used to describe milk/whey powder cohesion (Paterson et al., 2005) which is often related to water plasticization of amorphous lactose present in powders. Water plasticization allows a sufficient decrease in surface viscosity, enhances liquid-like behaviour and develop surface tension favourable for adhesion (Roos, 2009). This happens at temperatures above glass transition temperature. Thus, pre-crystallization of lactose has been recommended to overcome the stickiness problem during spray drying (Fox & McSweeney, 1998).

Transformation of amorphous material from glassy to a less viscous liquid-like state allows liquid flow and the formation of inter-particle liquid bridges which results in caking of amorphous powders (Roos, 2009). There are few stages in the process of caking which includes bridging, agglomeration, compaction and liquefaction. Caking in amorphous powders occur when powder transformed into lumps and then agglomerated to a solid (Aguilera et al., 1995). Storing at relative humidity (RH) below 25% was recommended to avoid caking in amorphous powders with amorphous lactose (Bronlund, 1997) while others recommend storing below 30% RH (Che & Chen, 2009). However, the most desired way to overcome this problem is to control the pre-crystallization process conditions of lactose in concentrated whey which is still far from optimized.

2.5 Factors affecting lactose crystallization

Lactose recovery from whey is affected by numerous factors including process parameters and properties of the solution such as degree of supersaturation, solution temperature, viscosity, concentration of lactose, presence of impurities and pH (Jelen & Coulter, 1973a; Bhargava & Jelen, 1996; Ganzle et al., 2008; Rjabova et al., 2013).

2.5.1 Supersaturation and temperature

A lactose solution can be supersaturated by either increasing the amount of lactose through evaporation or by cooling the solution where lactose becomes less soluble in water at low temperatures of below 15 °C. The effect of supersaturation and temperature on lactose crystallization has been researched widely both qualitatively and quantitatively (Twieg & Nikerson, 1968; Jelen & Coulter, 1973a; Raghavan et al., 2001; Wong et al., 2012). These studies indicated that the supersaturation acts as the leading factor that controls the growth rate directly where it increased with increase in supersaturation (Herrington, 1934a; Valle-Vega & Nickerson, 1977; Visser, 1982). However, effects of temperature, is highly interactive where higher temperatures were preferred depending on the size of the crystals obtained (Jelen & Coulter, 1973a; Butler 1998).

2.5.2 pH

High ($pH > 7$ up to pH 10) and low ($pH < 1$) pH accelerate mutarotation creating favourable conditions for an efficient crystallization, while no influence within the range of 4-7 (Jelen & Coulter, 1973a) was observed. The presence of lactic acid appears to act as an inhibitor hindering the crystallization process (Jelen & Coulter, 1973b; Nickerson & Moore, 1974b; Ganzle et al., 2008) and was attributed to its inability to create a very low pH level ($pH < 1$) which promotes the crystallization. In contrast, high alkali conditions ($pH > 10$) were found to catalyze the formation of lactose degradation products which in turn may hinder the crystallization (Nickerson & Moore, 1974b; Ganzle et al., 2008).

2.5.3 Effect agitation

It is of utmost importance that the content of the crystallization tank needs to be vigorously agitated continuously in order to avoid sedimentation of lactose crystals throughout the whole crystallization period. Sedimentation leads to the growth of crystals only through the surface layer resulting irregular shapes. If crystals were agitated at a rate that keeps them in suspension, the growth rate was increased due to increased surface area (Haase & Nickerson, 1966b) with reducing the time required for nuclei formation (Raghavan et al., 2001). Furthermore, agitation is important to transport the supersaturated solution to the surface of the crystals which helps to maintain an even supersaturation throughout the crystallizer.

2.5.4 Effect of impurities

Whey, the primary source of lactose production contains numerous components such as proteins, acids, salts and vitamins. Presence of these components in a lactose solution deeply affects the crystallization kinetics of lactose via changes in solubility, nucleation and crystal growth (Botsaris, 1982). Solubility changes are directly affecting the supersaturation of the solution and thereby affect the nucleation and crystal growth (Mullin, 1979). This change in solubility can be as a consequence of impurities on the structure of bulk water (Von Hippel & Schleich, 1969). As an example, some ions with low charge density such as Cl^-, Br^- and I^- lead to perturbation and structure breaking of water molecules while, ions with high charge density such as Ca^{2+}, Ba^{2+}, Sr^{2+} and Li^{2+} may lead to reorganization of the water structure in lactose solutions (Bhargava & Jelen, 1996).

Nucleation, the first step of lactose crystallization is found to be affected significantly by the presence of impurities. In some instances impurities may inhibit the nuclei formation (Raghavan et al., 2001; Ganzel et al., 2008) while the secondary nucleation can occur based on the impurity, taking it as a starting point for the crystal growth (Mullin, 2001). In addition secondary nucleation increases by adsorbing some impurities onto the existing crystal surfaces (Mullin, 2001). According to Mimouni, et al., 2005) presence of proteins as an impurity promotes the nucleation step. Owing to the hydrophilic nature of proteins, water affinity is high and proteins present in the solution make local lactose supersaturation spots which promote nucleation and eventually this may resulted with large number of small crystals.

Furthermore, the crystal growth step is highly influenced by the presence of impurities. Addition of metal ions (Pb^{2+}) with high molecular weight as an impurity reduces the growth rate of nuclei which in turn enhances the time period between the formation of nucleus and its growth to a detectable size (Agrawal & Paterson, 2015). Furthermore, impurities may adsorb on the faster growing faces of the crystals leading to strong inhibitions of crystal growth rates (Michaels & van Kreveld, 1966). Riboflavin was also found to be an impurity which has an ability to block the growth sites retarding the growth of a particular face (Michaels & van Kreveld 1966; Smart & Smith 1992). In addition presence of proteins reduces the crystal growth (Mimouni et al., 2005). Moreover, lactic acid was considered as an inhibitor of lactose crystallization (Jelen & Couleter, 1973b).

2.6 Unprocessability of acid whey

The application of spray drying for the production of acid whey powders is restricted by the uncontrolled thickening of liquid acid whey during the lactose crystallization step. Spray drying of acid whey is also limited due to the highly sticky nature of the resultant acid whey powders which sticks to the dryer and cyclone walls (Mimouni et al., 2007; Shrestha et al., 2007; Saffari & Langrish, 2014). Moreover, lumping and caking of the acid whey powder particles during storage deteriorate the quality of the powders (Dec & Chojnowski, 2006). Highly variable composition of acid whey especially lactic acid and calcium contents as compared to sweet whey was hypothesized as responsible for the varying behaviour of acid whey (de Wit, 2001; Gernigon et al., 2013).

2.6.1 Effect of lactic acid on lactose and acid whey processing

Processing of whey powders is mainly governed by the behaviour of lactose during concentration and crystallization steps of whey. Presence of whey components or impurities affects the lactose crystallization either positively or negatively and thus LA was found to play a major role (Michaels & van Kreveld 1966; Jelen & Coulter, 1973b; Nickerson & Moore 1974; Smart & Smith 1992; Shrestha et al., 2006; Saffari & Langrish, 2014). However, most of the studies have concentrated on model systems in determining the effect of lactic acid on crystallization behaviour of lactose and are in disagreement which highlighted the fact that use of different experimental methods and conditions do affected the effects exerting. For example, the presence of lactic acid was found to have no effect on lactose crystallization by (Michaels & van Kreveld 1966), while Jelen and Coulter (1973b) and Nickerson and Moore (1974) have observed a decrease in lactose crystallization in presence of lactic acid 2.5% and 5% impurity levels. In contrast, Smart and Smith (1991) have observed an increase in lactose crystallization in presence of lactic acid in model lactose solutions (40% w/w) containing 5% (w/w) lactic acid.

There are very limited studies conducted on spray drying of lactose solutions in presence of lactic acid. Saffari and Langrish (2014) have studied the lactose crystallization in presence of varying concentration of lactic acid (1-20% w/w) and have found that the yield of the spray dried powders have decreased with the increase in concentration of lactic acid. Moreover, Shrestha et al. (2007) have studied spray drying of lactose with varying concentrations of lactic acid (0-12% lactic acid/100g lactose) and found that > 4.2% lactic acid started to wall deposition and agglomeration of powder and 12% no powder was recovered when lactic acid concentration reached 12%. Furthermore, due to the presence of lactic acid caking of acid whey powder can occur at much lower moisture content during storage which can be due to the highly hygroscopic nature of lactic acid.

Though there are many studies on lactose crystallization in presence of lactic acid, there is no scientific literature on lactose crystallization in presence of lactic acid during the concentration step.

2.6.2 Effects of calcium on lactose and acid whey processing

The components present in whey have been comprehensively studied alone or in combination in order to determine their effect on the lactose crystallisation kinetics. Effect of calcium as a whey component is important to discover the behaviour of lactose in acid whey as it contains higher concentration of calcium as compared to sweet whey.

Jelen and Coulter (1973a) have studied the lactose crystallization kinetics in presence of calcium and found that calcium accelerate the crystal growth rate in the absence of other minerals. This can be probably due to the acceleration of the surface deposition step of the crystallization process with the presence of salts rather than increasing the mutarotation and/or reducing the lactose solubility (Bhargava & Jelen, 1996). However, this acceleration depends on the concentration of $CaCl_2$ present in the solution. For example: impurity level more than 10% with $CaCl_2$, the crystal growth rate of lactose was retarded (Jelen and Coulter, 1973b). Furthermore, in presence of $CaCl_2$ the solubility of lactose found to be increased retarding the lactose crystal growth (Herrington 1934b). In contrast, Visser (1984) has obtained contradicting results where the effect of $CaCl_2$ found to increase the crystal growth rate while increasing the solubility of lactose. However, Ca in the form of calcium phosphate found to have no effect on lactose crystallization kinetics (Smart & Smith, 1992), which thus highlights the importance of the form of Ca towards lactose crystallizations kinetics.

Acid whey unprocessability studies are hardly found in open literature except for the study conducted on thickening behaviour of concentrated acid whey (Mimouni et al., 2007). They have discovered that the presence of higher concentration of calcium in acid whey leads to formation of filamentous structures corresponding to Ca-lactate crystals which are responding to the sharp increase in viscosity and the thickening behaviour of acid whey. The increase in viscosity and the thickening behaviour of acid whey depends on the concentrations of Ca and lactate.

However there is no study reported about the combination effect of lactic acid and calcium on lactose crystallization during the concentration step. Hence it is utmost important to discover the effect of lactic acid and calcium on lactose behaviour during the concentration step.

Chapter 3

Water behaviour as a function of concentration and presence of lactic acid in lactose model systems

3.1 Introduction

Acid whey is considered a hard to process stream mainly due to the failure of lactose to crystallize, where lactose remains in its amorphous form during concentration and hinders further processing (Shrestha et al., 2007; Saffari & Langrish, 2014). It was hypothesized such behaviour of lactose to the presence of lactic acid (Shrestha et al., 2007; Saffari & Langrish, 2014). Presence of LA which has a low glass transition temperature, reduces the glass transition temperature of lactose when present in a mixture together (Shrestha et al., 2007) and thereby substantial structural property changes within the components can happen leaving detrimental issues for further processing. In addition, the highly hygroscopic nature of LA may also influence the unprocessability issues.

The effect of LA on lactose crystallization has been studied by few researchers (Jelen & Coulter, 1973b; Shrestha et al., 2006; Saffari & Langrish, 2014). It was found that the presence of lactic acid act as an inhibitor hindering the crystallization process (Jelen & Coulter, 1973b), while the presence of LA has led to increase stickiness of spray dried lactose powders (Shrestha et al., 2006). Moreover, Saffari and Langrish (2014) found that, increased LA concentration decreased the yield of spray dried lactose powders. However, all of these studies have conducted on powder samples which still leave the question of the influence of LA towards the behaviour of lactose in liquid acid whey solutions during the concentration process prior obtaining lactose crystals.

Current understanding highlights the importance of the presence of LA for elucidating kinetics of lactose crystallization. Since no study has been conducted on liquid samples, it would be beneficial to establish the influence of the presence of LA towards the change in behaviour of water and thereby predict the crystallization behaviour of lactose in liquid lactose model solutions. The main hypothesis of this study is the presence of lactic acid may influence the structural associations of lactose and water by changing the structure of water due to its highly hygroscopic nature. A systematic study is lacking in this regard especially during the concentration step, since this is also considered a crucial step during processing of acid whey and thus recovery of lactose crystals. Hence, this chapter aimed at establishing the structure and behaviour of water molecules surrounding the lactose molecules as a function of its concentration (15% to 50% w/w) in the presence or absence of LA. Furthermore, the crystallization nature of lactose with/without LA was established.

3.2 Materials and Methods

3.2.1 Materials

A commercial food grade lactose powder (purity ≥ 98%) and an analytical grade lactic acid solution were obtained from Murray Goulburn Co-operative Co. Ltd (Brunswick, Australia) and Sigma-Aldrich Pvt. Ltd. (Castle Hill, NSW, Australia) respectively. Milli-Q water was used at all times for the preparation of solutions.

3.2.2 Preparation of solutions

Lactose powder (5 g) was dissolved in 95 g of Milli-Q water at room temperature to obtain a 5% (w/w) lactose solution (L). Lactose plus lactic acid (L+LA) solution was prepared by the addition of 1 g of LA to the 5 g of lactose powder and made upto a total weight of 100 g. 5% and 1% were selected to imitate the lactose and LA concentrations present in acid whey respectively. The solutions were stirred using a magnetic stirrer for about 30 minutes until clear solutions were obtained where no crystals were visually observed. The pH of lactose solution was 5.8 while the pH of L + LA solution was 4.9. All the solutions were filtered using 0.45 µm Whatman filter papers to remove any undissolved particles (Wijayasinghe et al., 2015).

3.2.3 Concentration of lactose and L+LA solutions

Concentration of lactose and L + LA solutions was performed using a rotary evaporator (EYELA, Tokyo. Rikakikai Co. Ltd. Japan). Temperature of the rotary evaporator water bath was adjusted to 55 °C to imitate the industrial conditions. The stock solutions were kept at this temperature until required concentrations (15, 20, 30, 40 or 50 % w/w) of lactose were achieved. The concentration was terminated when the concentration was confirmed by a refractometer.

Refractometry measurements were performed measuring the refractive index (Brix value) using a laboratory refractometer (Atago abbe, Tokyo, Japan) for concentration determinations.

3.2.4 Freeze-drying

50% w/w concentrated solutions of lactose and L + LA were poured in to 15 mL eppendorf tubes. All samples were frozen at -20 °C for 24 h. The samples were freeze-dried (Dynavac FD 300 freezedryer; Dynavac Engineering Pty, Australia) for 72 h and the residual water was removed by keeping samples in vacuum desiccators over P_2O_5 at room temperature for at least 5 days.

3.2.5 Differential scanning calorimetry (DSC)

Concentrated L and L+LA solutions were transferred into pre-weighed DSC aluminium pans (40 μL; ME-26763, Schwerzenbach, Switzerland) and the pans were hermitically sealed. Subsequently, samples were transferred into a DSC (DSC 1 STAR^e System, Gas controller - GC 200, Mettler Toledo Schwerzenbach, Switzerland). An empty pan was used as a reference. The DSC was calibrated using indium (melting point, 156.6 °C and ΔH_m, 28.45 J g^{-1}). All samples were scanned with a starting temperature of 25 °C and end temperature of 180 °C at a heating rate of 5 °C min^{-1}, which was a modified version of the method described by Haque and Roos (2006). Onset and end set temperatures of water evaporation and related enthalpies (area under the curves) were analyzed using STARe thermal analysis software, 12.1 (Mettler Toledo).

Freeze-dried samples were also subjected to the same program with slight modifications in which samples were scanned to 120 °C at a heating rate of 10 °C min^{-1}, and then cooled at 5 °C min^{-1} to 25 °C, followed by a second heating scan at 10 °C min^{-1} to 200 °C. The scans were normalized by the system according to sample mass and the integrated peak energies were used to compare the differing degrees of crystallinity.

3.2.6 Fourier transform infrared spectroscopy (FTIR)

The FTIR spectra of pure lactose solutions with 5% (w/w) initial concentration and 5% (w/w) L + 1% (w/w) LA samples were obtained in the range of 4000 - 400 cm^{-1} immediately after achieving relevant concentrations from 15% to 50% (w/w) using fourier transform infrared spectroscopy (Shimadzu IR Affinity-1, Shimadzu corp., Kyoto, Japan) with the combined software of IR Solution (Shimadzu Corp.), Version 1.40. Sample spectra were collected in the absorbance mode after background detraction. Each spectrum was a result of an average of 32 scans recorded at 4 cm^{-1} resolution. The spectra were analyzed over the wavelength ranges of interest 4000 - 2600 cm^{-1}, 2000 - 950 cm^{-1} and 1200 - 950 cm^{-1}.

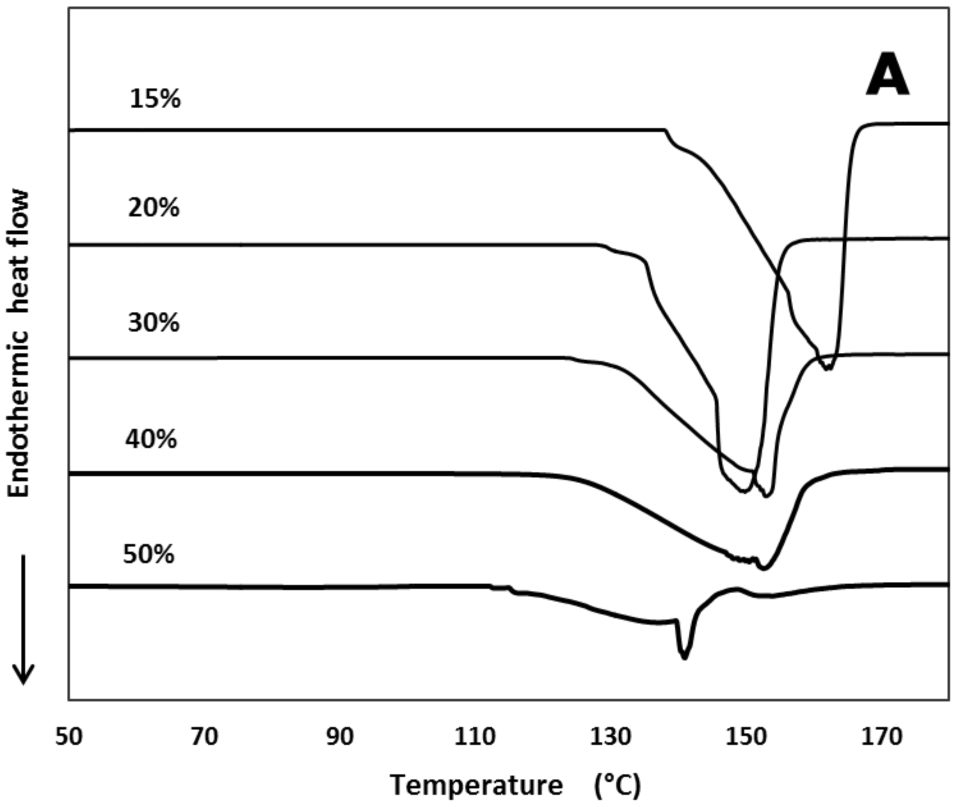
3.2.7 Statistical Analysis

All experiments were arranged in a randomized split plot block design, in which lactose concentration (5 levels) was the main factor and the replication served as blocks. The whole design was replicated with a subsequent subsampling resulting in at least 4 independent observations ($n \geq 4$). The least square difference (LSD) was used to differentiate the means with the pre-set probability at $p<0.05$.

3.3 Results and Discussion

3.3.1 Behaviour of water molecules around lactose in presence of lactic acid as a function of concentration

Concentrating lactose solutions by evaporation usually results in formation of highly viscous, metastable, amorphous lactose concentrate (Roos & Karel, 1990). Thermographic analysis of lactose solutions during concentration from 15% to 50% (w/w) is shown in Figure 3-1 (A). The obtained thermograms resulted an endothermic peak around 130-140 °C which can be attributed as the removal of water molecules from the lactose solutions (Yazdanpanah & Langrish, 2011). The recorded onset, end-set and peak temperatures were not impacted on with the change in lactose concentration ($p>0.05$). However, as expected increasing concentration significantly ($p<0.05$) lowered the total enthalpies of dehydration (Table 3-1). As shown, 15% (w/w) pure lactose solution had an enthalpy of ~1588 J g^{-1}. During concentration, the enthalpy declined resulting in a value of ~679 J g^{-1} for the 50% (w/w) lactose solution. The amount of free water available for removal was reduced by 35% when increasing the lactose concentration from 15% to 50% (w/w). However, almost a 57% reduction in total enthalpy was observed which indicated that reduced availability of free water molecules for dehydration is not only the cause for decreased enthalpy. During concentration, physical changes may occur in biological materials (i.e. lactose) which in turn results in different water molecular mobility due to varying degrees of associations (Roos & Karel, 1990). Variances in inter molecular distances and order of molecular arrangements may also lead to differences in potential energy (Anslyn & Dougherty, 2006).



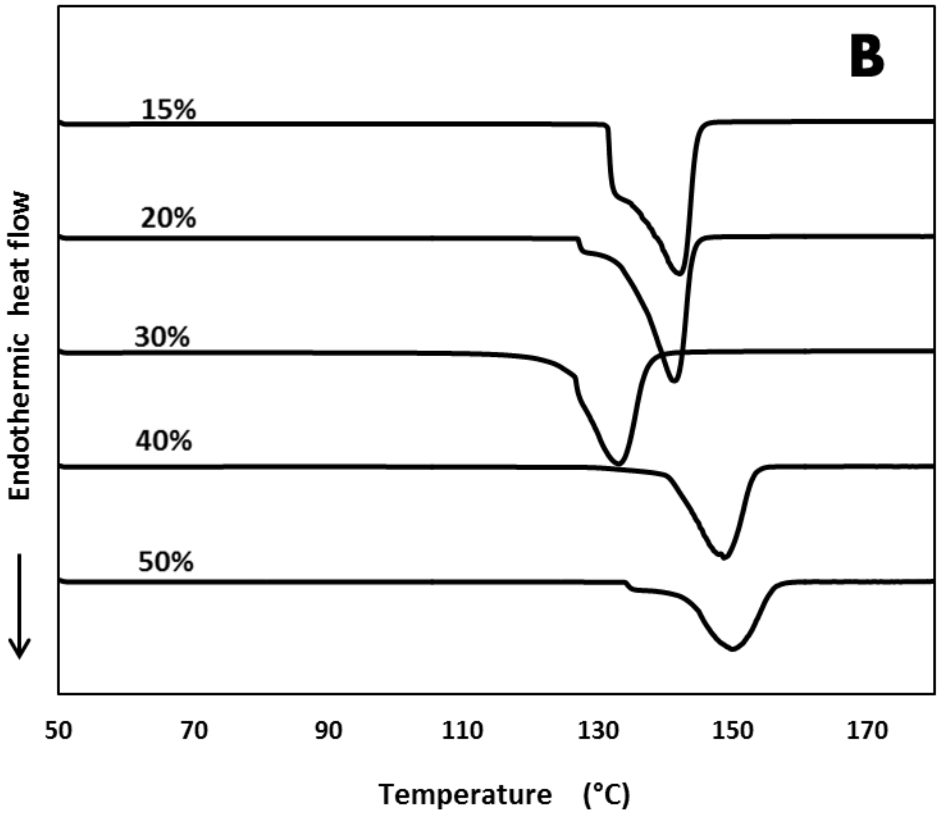


Figure 3-1: Dynamic DSC curves for lactose solutions as a function of concentration: (A) without LA, (B) in the presence of 1% (w/w) LA.

Water dehydration behaviour of lactose has changed substantially upon addition of LA (Figure 3-1 B). LA is miscible with water and has the ability to attract and hold water molecules from the surrounding environment (Jamshidian et al., 2010). Increasing concentration of L + LA solution had no observable ($p>0.05$) effect on the onset, end-set and peak temperatures, although the dehydration enthalpies were significantly ($p<0.05$) reduced (Table 3-1). For example, 15% (w/w) L + LA solution had an enthalpy of 1621 J g^{-1} whereas an enthalpy ~965 J g^{-1} was observed for the 50% (w/w) L + LA solution. Furthermore, these enthalpies were higher than those of pure lactose solutions, which further highlight the fact that the reduced availability of free water molecules for removal with increase in concentration is not the sole factor, but molecular arrangements and mobility do play an important role. LA is a highly hydrophilic compound, which strongly interacts with water. This in turn induces changes in the structural associations and mobility of adjacent water molecules (Reid & Fennema, 2008) extending distances between lactose molecules. In addition, reactivity of water molecules may be changed due to strong attraction with LA (Reid & Fennema, 2008). LA forms hydrated H_3O^+ ions with water via exceptionally strong H bonds (Belitz et al., 2009). Hence, rise in enthalpies of L+LA solutions indicate that a high energy was required to remove water molecules from lactose solutions in the presence of LA.

Table 3-1: Onset, end-set, peak temperatures and enthalpy of lactose solutions as a function of concentration and presence of lactic acid

Concentration % (w/w)	Onset temperature °C	End set temperature °C	Peak temperature °C	Enthalpy J g^{-1}
LACTOSE				
15	135.04	148.16	138.80	1588.68^{a}
20	129.42	145.55	135.80	1479.04^{a}
30	140.37	157.89	150.61	1002.02^{b}
40	140.12	155.09	147.59	1000.69^{b}
50	133.49	142.35	134.01	679.03^{c}
LACTOSE+LACTIC ACID				
15	128.48	137.29	130.66	1621.51^{a}
20	121.03	130.54	124.05	1465.90^{a}
30	129.41	139.85	131.63	1376.33^{a}
40	134.67	146.84	140.00	845.17^{cb}
50	129.20	144.17	136.29	965.09^{b}
*SEM	14.17	5.28	5.09	101.61

Values are means of at least 4 independent observations ($n \geq 4$). Means in the same column with different alphabets are significantly different ($p < 0.05$). *SEM denotes pooled standard error of the mean, $p < 0.05$.

3.3.2 Glass transition and crystallinity of freeze dried samples

Thermal analysis of concentrated liquid samples showed that presence of LA had a great influence on the removal of water from lactose. This in turn may lead to change in glass transition (T_g) and crystallinity of lactose. Hence, freeze dried lactose and L + 1% LA samples were tested for the changes in their crystallinity and glass transition temperatures (Figure 3-2).

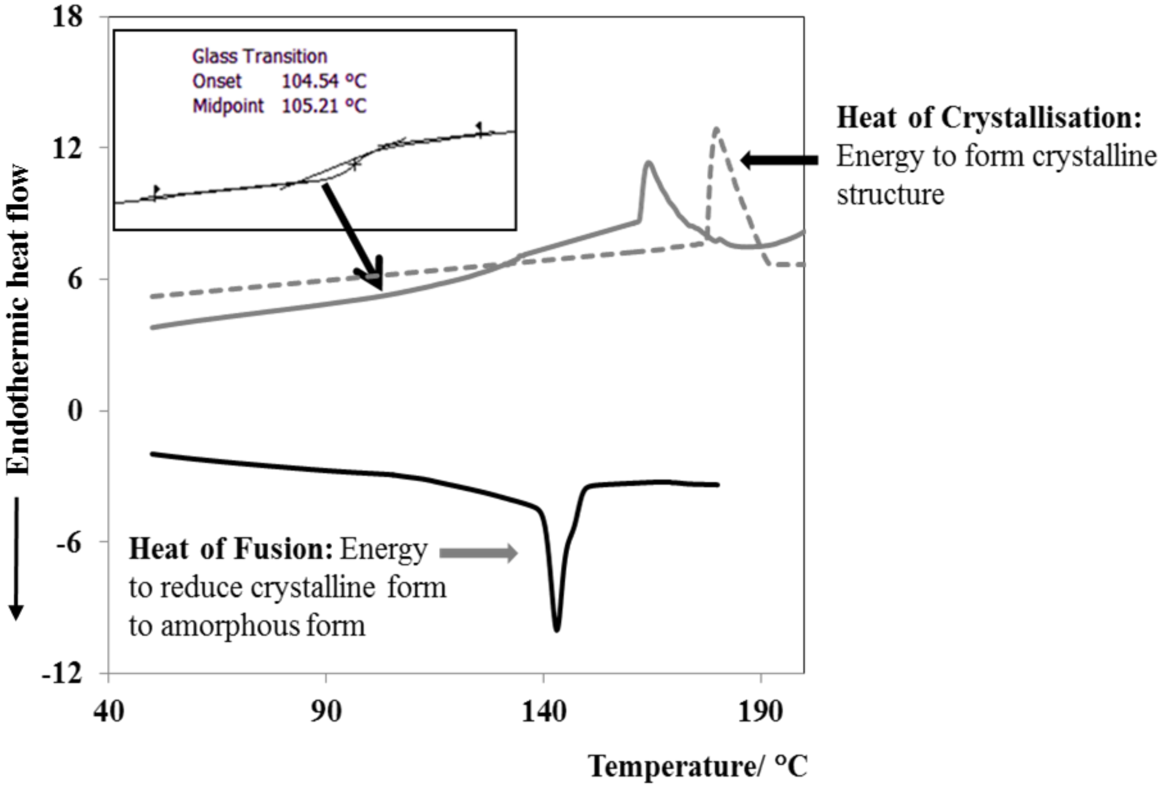


Figure 3-2: Dynamic DSC curves for freeze-dried lactose (grey solid) and L + 1% (w/w) LA (grey dashed) and lactose monohydrate (solid black) in the temperature region of 50 to 170 °C

Amorphous lactose often exists in a glassy, solid state or in a syrup-like, super-cooled liquid state and the phase transition is a second-order (Roos, 2009). Glass transitions are an important parameter for understanding the mechanisms of transformation processes in foods. This transition may be impacted by various factors and in this study, the effect of LA presence on phase behavior of lactose was examined by thermographic analysis of powdered lactose. In the DSC analysis, an endothermic relaxation peak was associated with the glass transition of pure lactose even in an immediate rescan of samples (Figure 3-2). The recorded glass transition temperature for the pure lactose was 105.2 °C. This value is similar to the glass transition observed by Omar and Roos (2007) as 105.5°C, but quite higher than the value recorded by Roos and Karel (1990) highlighting likely the different nature of experiments and the compositional variations of samples towards the absolute T_g values. LA is highly hygroscopic, exists in liquid state at room temperature, and has a very low T_g at -60 ºC (Maltini et al., 1967). The temperature range that was used within the present study (due to limitations of the instrument) did not show a T_g value for L + 1% LA sample indicating that T_g of lactose might have probably occurred at a temperature below the level of instrument detection due to the presence of LA. The presence of vaporized water molecules absorbed by the powders significantly plasticize the material, thereby lowering T_g (Aubuchon & Thomas, 2007). Since LA is a highly hygroscopic compound, its presence would draw water molecules which would result in a low T_g.

Amorphous lactose is metastable with a tendency to absorb large amounts of water, resulting in plasticization and increased molecular mobility, which eventually leads to the rearrangement of lactose molecules. Exceeding glass transition temperature of lactose, result in dramatic changes in the flow properties of powders and the time dependent crystallization of lactose. The instant crystallization temperature (T_{cr}) of lactose measured using the DSC was 163.7 °C, while L+ 1% LA appeared around 179.1 °C (Figure 3-2). The reported crystallization temperature (T_{cr}) of dry lactose varies in literature, depending on moisture content, DSC scanning rates and type of DSC pan (sealed/puncture) (Haque & Roos, 2004). The addition of LA led to higher crystallization temperatures showing a delay in crystallization with the addition of LA. Furthermore, the onset and end set temperatures of lactose crystallization were 161.7 °C and 170.6 °C respectively, for pure lactose samples. When LA was present, the onset and endset temperatures were 177.0 °C and 188.9 °C respectively. The broader peak observed in the thermograph of L + 1% w/w LA shows an obvious difference in the crystallization temperature range between lactose and L + 1% w/w LA. lactose sample have crystallized within ~9 °C, while L + 1% w/w LA sample showed a ~12 °C temperature window. A greater peak was observed by L + 1% LA sample in comparison to pure lactose indicating a large amorphous content (Chiou et al., 2008). In addition, the enthalpy associated with crystallization termed as heat of crystallization (ΔH_{cr}) can be used to quantify the amorphous content (Aubuchon & Thomas, 2007). The heat of crystallization was ~112 J g^{-1} and ~239 J g^{-1} for L and L + 1% LA respectively. Hence, this clearly shows that more energy is required to induce lactose crystallization in the presence of 1% LA.

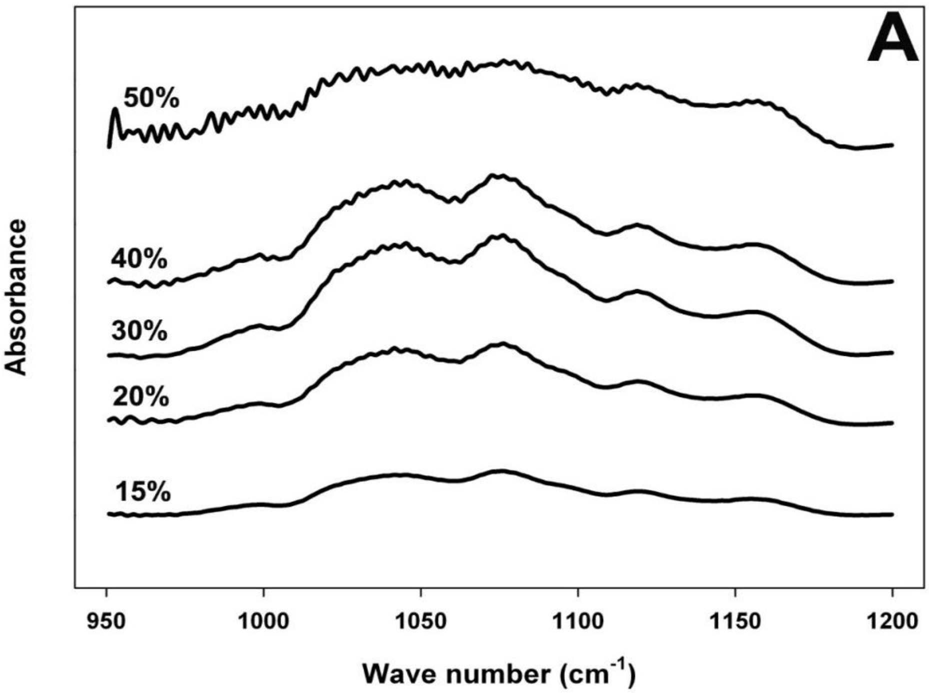
In the present study, lactose monohydrate was used as 100% crystalline with crystallization energy of ~626 J g^{-1}. This was measured by subtraction of heat of crystallization from heat of fusion values as stated by Chiou et al., (2008). Freeze dried lactose sample of the present study produced an average energy of ~314 J g^{-1}, while L + 1% LA sample showed an average of ~74 J g^{-1}. This shows that, freeze dried lactose sample consist of ~50% crystalline L. Furthermore, in the presence of LA the crystallinity of lactose declined, down to only 12%. This reinforces the understanding that, inability to process acid whey is due to presence of LA as it prevents crystallization of lactose.

3.3.3 Fourier transform infrared spectroscopy (FTIR)

Above a certain temperature (glass transition temperature) amorphous lactose transfers through the rubbery zone into a crystalline state due to increased molecular mobility and as a result of water plasticization. During this phase transition the chemical and physical properties of lactose occur. The results of thermographic analysis confirmed that the behaviour of water molecules in lactose was substantially influenced by lactose concentration and presence of LA. The presence of LA hindered the evaporation of water molecules to a greater extent at 50% (w/w) concentration in comparison to other concentrations. Earlier, it was hypothesized that this could possibly be due to certain structural changes of lactose in presence of LA, which could be identified by infrared spectroscopy. FTIR is a simple and rapid technique that screens molecular vibrations shown by various compounds (Subramanian et al., 2009). Generally, 1200 - 800 cm^{-1} range is used to determine phase changes of carbohydrates as C-C and C-O stretching and vibrations are influenced by their physical state (Ottenhof et al., 2003). Similarly, 3600 - 3200 cm^{-1} region is used to determine the structure of water around carbohydrate molecules.

Figure 3-3 shows the FTIR spectra of lactose with and without addition of LA in the IR range of 1200 - 950 cm^{-1}. Two prominent peaks originating around 1040 cm^{-1} and 1075 cm^{-1} were observed in the 20% - 40% (w/w) lactose solutions. The corresponding peaks were found to be associated with C-C stretching vibrations and C-O stretching of glucose molecule of lactose respectively (Solis-Oba et al., 2011). On the other hand, these two peaks were almost absent in the 50% (w/w) lactose solution (Figure 3-3 A). During concentration, the intermolecular H-bonds become stronger between lactose and water molecules (Punitha et al., 2014), which consequently results in reduced stretching vibrations.

The addition of LA led to further decline in intensities of the peaks around 1040 cm^{-1} and 1075 cm^{-1} with respect to lactose solutions at all concentrations (Figure 3-3 A). As explained prior, the formation of hydrated H_3O^+ ions with strong H bonds in presence of LA may lead to this further disappearance of stretching vibrations. This effect was more pronounced with 50% (w/w) L + LA solution in comparison to lactose solution where the peaks were undefinable (Figure 3-3 B). Furthermore, the poorly defined peaks within this range correspond to the presence of amorphous lactose (Islam & Langrish, 2010). This observation thus further highlights reduced crystallinity nature of lactose molecules in the presence of LA, which also confirms the observations obtained by DSC.



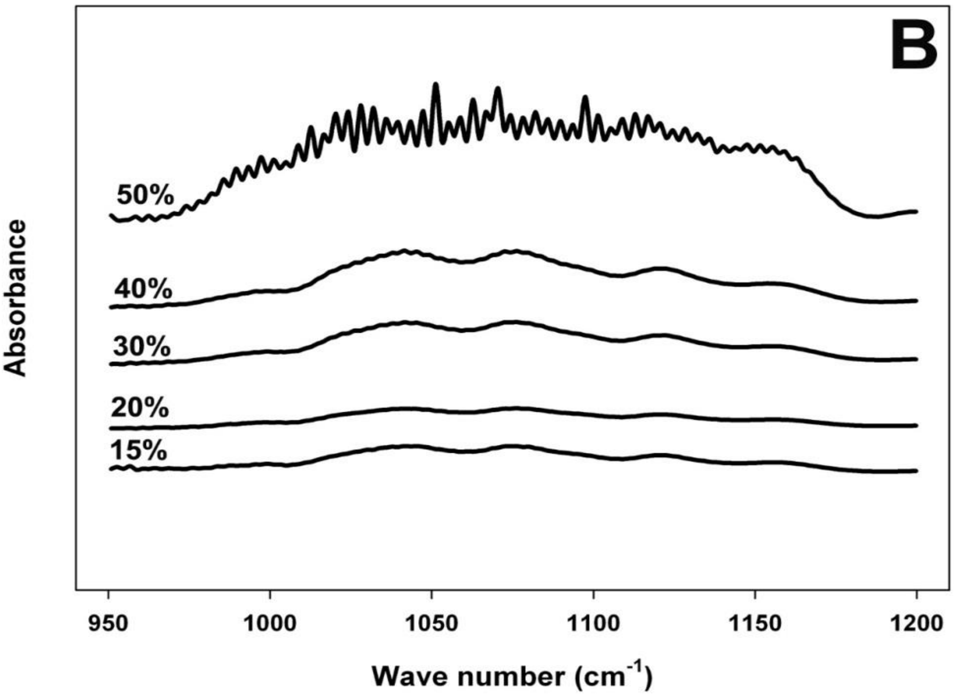


Figure 3-3: FT-IR spectra of 1200 - 950 cm^{-1} region in lactose (A) and L + LA (B) as a function of concentration

A protruding peak around 1650 cm^{-1} was observed for pure lactose solutions, which may correspond to the bending mode of water molecules (Velazquez et al., 2003). This peak appeared at all concentrations, although its intensity was reduced as a function of concentration (Figure 3-4 A). This may likely be due to reduced water content in the system with greater lactose concentration. L + LA solution containing 15% (w/w) also showed a prominent peak around 1650 cm^{-1}. However, this peak has shifted towards lower wave number ~1570 cm^{-1} with rise in the lactose concentration. In addition, the intensity of the peak was reduced substantially up to 40% (w/w) concentration, while the 50% (w/w) sample showed an increase in intensity, which confirms that movement of water molecules, was restricted in the presence of LA. In addition, bands around 1359 cm^{-1}, 1408 cm^{-1} and 1448 cm^{-1} were observed in the 50% (w/w) L + LA solution, which could be due to the C-C/C-H aliphatic and C-CH_3 aliphatic stretching vibrations of LA, and C-C/C-H ring stretching vibrations of lactose respectively (Pamula et al., 2001). Moreover, two other sharp peaks originating around 1248 cm^{-1} and 1307 cm^{-1} in the 50% (w/w) L + LA solution (Figure 3-4 B) were observed, which were absent in the 50% (w/w) lactose solution. Information about origin of these peaks is lacking in literature, although Pamula et al., (2001) assigned these peaks to bending vibrations of O-H groups. Considering that these peaks were absent in the pure lactose solutions, their appearance may indicate a change in structure of lactose in presence of LA.

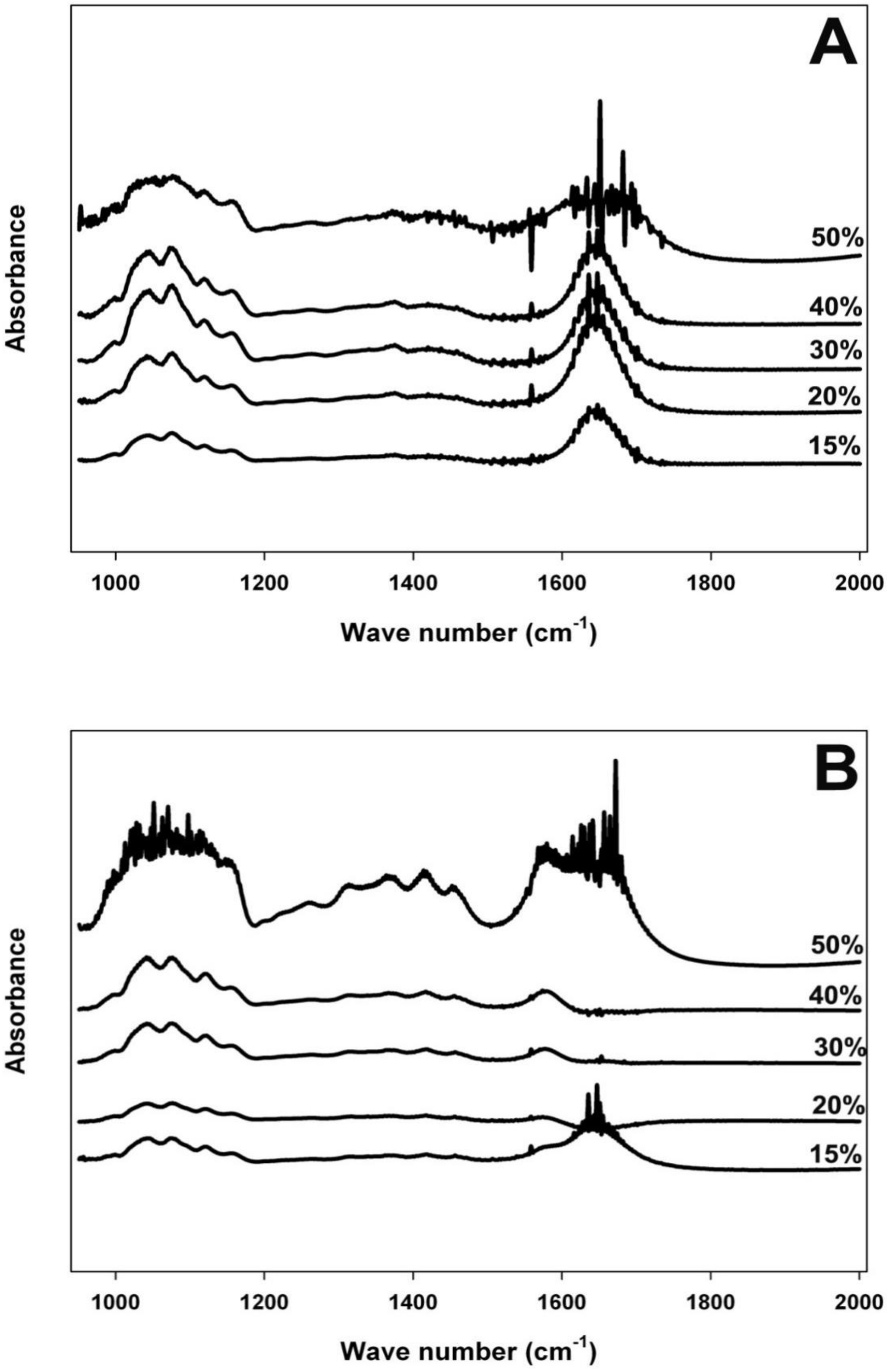


Figure 3-4: FT-IR spectra of 2000 - 950 cm^{-1} region in lactose solutions without (A) and with (B) addition of LA as a function of concentration

One of the regions of a particular interest is between 3600 - 3200 cm^{-1}, which represents the H-bonding region and the behaviour of water molecules (Wolkers et al., 1998). The presence of LA hindered the removal of water from lactose molecules. This observation may be possibly due to a change in water activities around lactose molecules due to strong interactions with LA and water molecules, which are represented by the H-bonding patterns. In carbohydrates, hydroxyl groups are hardly present in isolation as they are usually involved in intra and inter molecular H-bonding with other hydroxyl groups (Ottenhof et al., 2003). The shape of these peaks is indicative of constrained water molecules. As the concentration of pure lactose solution rose, the absorbance in the 3600 - 3200 cm^{-1} region declined (Figure 3-5 A). A large volume of freely available water molecules exists in the 15% (w/w) lactose solution in comparison to the 50% (w/w) lactose solution, which results in a higher mobility of the water molecules. This is reversed as the lactose concentration is increased as in the 50% (w/w) lactose solution showing restricted water mobility and likely stronger attractions with lactose molecules. Stronger H-bonding may distort the water molecules (Sharp et al., 2001) inducing the O-H stretching vibrations that may lead towards either an asymmetrical or symmetrical stretching. Concurrently, the H-O-H bending can also lead to certain structural changes. The appearance of a small peak around 3600 cm^{-1} in the 50% (w/w) lactose solution confirmed that the distortion of water molecules around lactose molecules occurred due to symmetrical O-H stretching vibrations with gradual increase in concentration likely caused by decline in number of water molecules competing for available sites.

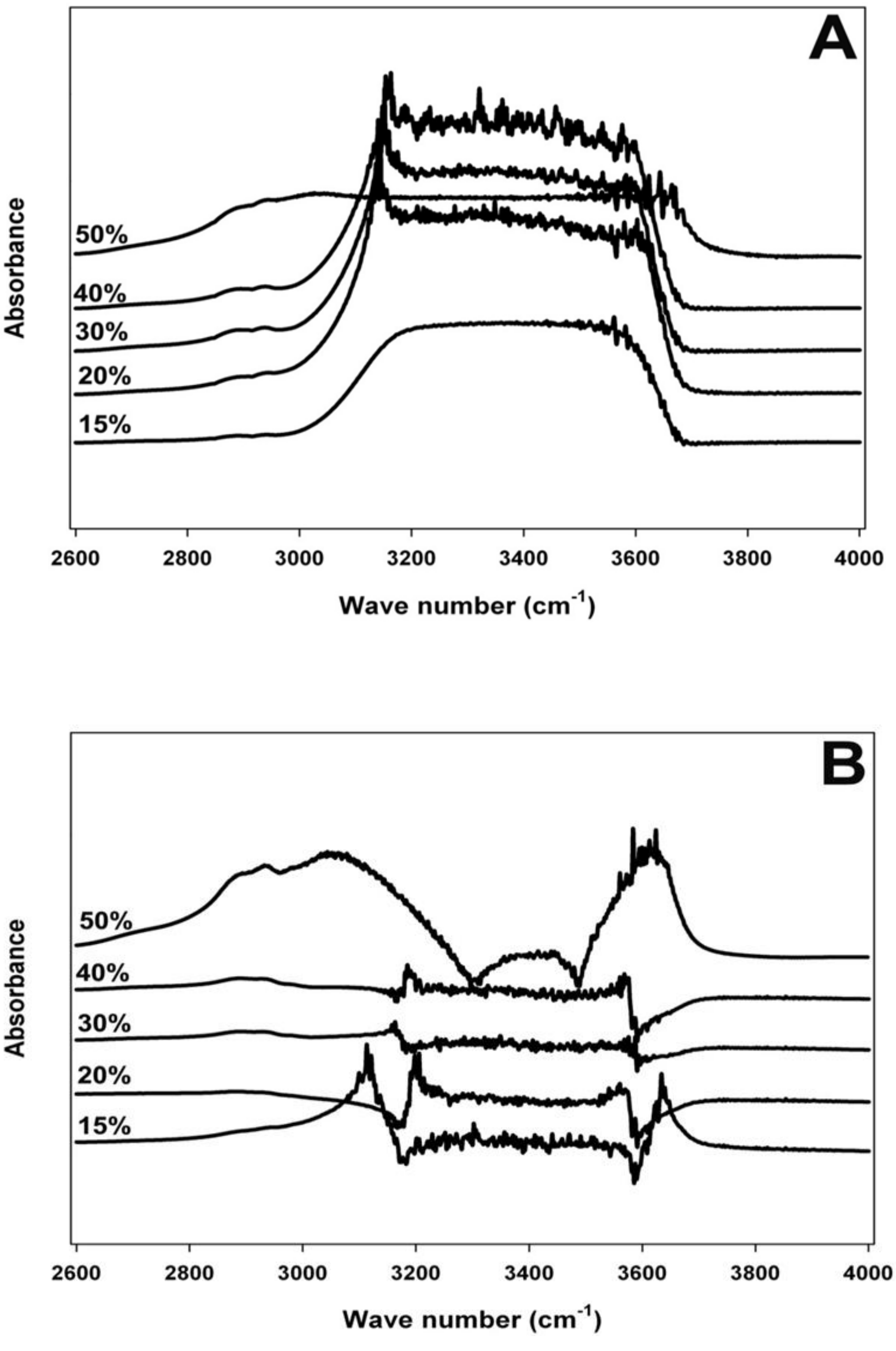


Figure 3-5: FT-IR spectra of lactose (A) and L + LA (B) as a function of concentration in the range of 4000 - 2600 cm^{-1}

Addition of LA resulted in disappearance of almost all peaks around 3600 - 3200 cm^{-1} indicating strong attraction between LA and water molecules. On the other hand, two prominent peaks around 3000 - 2900 cm^{-1} and 3600 cm^{-1} respectively, were observed in the 50% (w/w) L + LA solution and the former peak was absent in the pure lactose solution at the corresponding concentration (Figure 3-5 B). The peak around 3000 - 2900 cm^{-1} can be described as the stretching vibrations of hydrocarbon groups of LA (Pamula et al., 2001). The latter peak (around 3600 cm^{-1}) was higher in intensity in comparison to what was observed in the pure lactose solution, further highlighting distortion of water molecules and their mobility in presence of LA. During concentration, the amount of LA increases, which consequently results in stronger interactions with a fewer water molecules remaining in the 50% (w/w) solution. This also leads to a greater competition between lactose and LA molecules for available water molecules and subsequently a more disordered physical state.

3.4 Lactose hydration – a hypothesis

According to the present study, two mechanisms may be proposed to describe the behavior of water molecules around lactose molecules during concentration of pure lactose and lactose solutions with LA. The first mechanism depicts the behavior of lactose during concentration of pure lactose solution (Figure 3-6). The tendency of water to associate with hydrophilic substances is generally defined as hydration or the water binding. Lactose is a hydrophilic solute with an ability to interact strongly with water (Reid & Fennema, 2008). The water molecules can lead to formation of several hydration layers around lactose molecule (Figure 3-6 A). However, increase in lactose concentration during concentration reduces the amount of available water molecules limiting to only one hydration layer and/or a partially completed hydration layer around lactose molecule (Figure 3-6 B). In addition, these water molecules are now strongly attracted to lactose as indicated by the reduced stretching vibrations. These strong interactions can induce partial structural changes of the hydrophilic solute itself as it was observed with the FTIR results and was consistent with Reid & Fennema (2008). This effect was more pronounced with rise in concentration and limited amount of available water to engage in these attractions, which consequently lead to a distortion effect of lactose molecules attempting to occupy their hydrophilic sites and overall a structural change. These structurally changed L molecules have shown a great tendency towards accelerating water removal.

The second mechanism describes behaviour of lactose in presence of LA during concentration. Addition of a third component (LA) to the lactose and water solution enhances the attraction forces between LA and water molecules, which in turn reduces the mobility of the water molecules (Timasheff, 2002). Subsequently, LA further interacts with lactose, resulting in change of the chemical potentials of both compounds. Furthermore, formation of hydrated H_3O^+ ions in the presence of LA leads to strong H bonding with lactose molecules and subsequently creation of a strong hydration layer (Figure 3-6 C). This effect is more pronounced at higher concentrations where fewer water molecules are available to form a hydration layer. These water molecules are also strongly attached to the lactose molecules due to increased reactivity of the H_3O^+ ions. The hydration layer would consequently be strongly attracted to the lactose molecules, which in turn requires greater energy to remove the hydration layer from lactose molecules. These strong interactions induce an evident structural change of lactose molecules, which are quite different and prominent than those observed for pure lactose systems. Furthermore, the affinity of LA to bind water molecules is much greater than that of lactose. This may lead to strong attractions between water molecules and LA and creation of a hydration complex, which consequently may attach to lactose molecule (Figure 3-6 D).

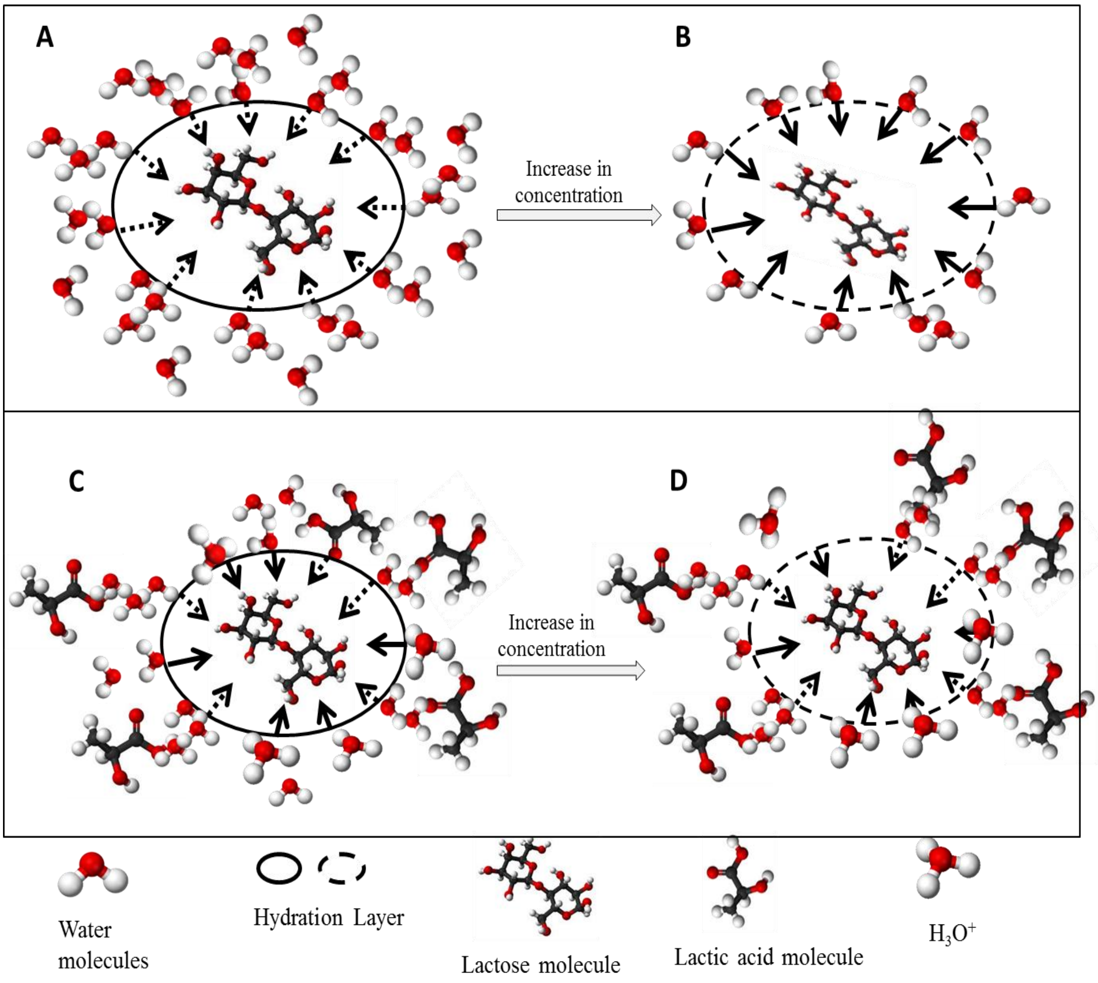


Figure 3-6: Schematic representation of the lactose hydration mechanism 1 (A & B) of pure lactose solutions and lactose hydration mechanism 2 (C & D) with the addition of LA as a function of concentration

3.5 Conclusion

Compositional differences between acid and sweet whey hinder further processing of acid whey mainly due to presence of amorphous lactose. In pure lactose solution, enthalpy of water dehydration was reduced with increase in lactose concentration. The addition of LA led to increase in water dehydration enthalpy indicating that the presence of LA hindered removal of water from lactose. LA as a highly hydrophilic compound appears to strongly interact with water molecules. This may lead to formation of a strong hydration layer consisting LA and H_3O^+ ions around lactose molecules via exceptionally strong H bonds, which thereby result in restricted water mobility and/or a change in a structure of lactose. This phenomenon appears responsible for limited lactose crystallization where a high energy input appeared necessary to crystallize lactose. The observations indicate that partial or complete removal of LA from acid whey may improve lactose crystallization and thereby processability of acid whey. LA removal could be achieved by membrane processing however, several questions still remain to be answered. Obviously from the current data LA concentration, which is frequently present in acid whey and concentrated during evaporation process, plays an important role in lactose crystallization. Therefore a threshold of LA concentration that would allow for proper lactose crystallization needs to be established. Moreover the role of the main minerals such as Ca may need to be determined as it may hinder or facilitate lactose crystallization.

Chapter 4

Lactose phase behavior in presence of varying concentrations of lactic acid and calcium

4.1 Introduction

The use of whey powder as a food ingredient in different food products is directly connected to the extent and efficiency of the lactose crystallization step during manufacture of whey powders. Crystallization of lactose is affected by several factors such as presence of impurities, degree of supersaturation, solution temperature, viscosity, concentration of lactose, presence of proteins and pH (Herrington, 1934a & b; Bhargava & Jelen, 1996; Mimouni et al., 2005; Ganzle et al., 2008; Rjabova et al., 2013). Among these factors presence of impurities has been considered as a major concern (Herrington, 1934b).

Presence of minerals as impurities in lactose solutions have contradicting outcome, resulting in an enhanced or diminished crystallization rates depending on the concentration and the type of the salt present (Jelen & Coulter, 1973b). For example, the impurity level more than 10% with $CaCl_2$, the crystal growth rate of lactose was retarded while, the addition of NaH_2PO_4 to deproteinated whey increased the growth rate of lactose crystals by 30%. A change in solubility of lactose was assumed to be the cause for the crystallization behaviour changes in presence of mineral impurities (Mullin, 1979). These solubility changes can be due to structural changes of water molecules in bulk water. Presence of ions with high charge density (Ca^{2+}, Ba^{2+}) is believed to orient the water structure due to their ability to electrostatically organize the structure of water. However, some ions with low charge density (Cl^-, Br^-, I^-) has been found to lead perturbation and structure breaking of water molecules (Von Hippel & Schleich, 1969). Hence, changes in water structure directly affect the solubility and thereby, the supersaturation of the lactose solution which is one of the leading factors governing lactose crystallization (Mullin, 1979).

The presence of organic acids mainly LA directly affect the lactose crystallization in acid whey solutions (Shrestha et al., 2006). For example presence of LA appears to act as an inhibitor slowing down the crystallization process (Jelen & Coulter, 1973b; Nickerson & Moore, 1974; Ganzle et al., 2008). Furthermore, chapter 3 of this study reported that the presence of 1% (w/w) LA directly hindered the water evaporation behaviour of lactose, increased crystallization temperature and most importantly increased the amount of amorphous lactose in model lactose solutions.

Whey is mainly a mixture of water, lactose, LA, Ca and proteins. The main compositional differences found in sweet whey and acid whey with regards to LA and Ca concentrations may possibly be the most considerable reason for the improper lactose crystallization. As an example, Mimouni et al., (2007) discovered that the formation of filamentous structures corresponding to Ca-lactate crystals is responsible for the thickening behaviour of acid whey and it depends on the concentrations of Ca and lactate. However, a systematic study is missing with regards to the lactose crystallization kinetics in presence of LA and Ca especially during the concentration step. A good understanding of lactose crystallization kinetics may help to maximize the quality and minimize the cost of production. Thus, the fundamental understanding of the behaviour of lactose in presence of different concentrations of LA and Ca concentrations would be beneficial in order to resolve the acid whey processing problems satisfactorily.

Hence, this study aimed at establishing the behaviour of lactose and water in the presence/absence of different concentrations of LA and/or Ca using model systems having lactose, LA and Ca with an overall goal of postulating a mechanism for lactose crystallization in acid whey. Different concentrations of Ca and LA for the model systems were selected to imitate the concentrations originally present in acid whey, nanofiltered retentates and sweet whey. Concentrated samples via vacuum evaporation were analyzed through differential scanning colorimetry (DSC) for their thermal behavior and the effects of LA and Ca on the structural changes of lactose were observed through fourier transform infrared spectroscopy (FTIR) in detail. Furthermore, freeze dried samples have been analyzed for changes in glass transition temperatures and the crystallization behavior of lactose through DSC in order to predict the extent of crystallinity/amorphicity of lactose present in model lactose solutions with varying amounts of LA and Ca, which in turn is important in predicting the spray drying ability of acid whey.

4.2 Materials and Methods

4.2.1 Materials

A commercial food grade lactose powder was obtained from Murray Goulburn Co-operative Co. Ltd (Brunswick, Australia). An analytical grade LA (98% w/w) and $CaCl_2$ were obtained from Sigma-Aldrich Pvt. Ltd. (Castle Hill, NSW, Australia). Milli-Q water was used for the preparation of solutions.

4.2.2 Preparation of model solutions

A 5% (w/w) lactose solution (L) was prepared by dissolving 5 g of lactose powder in 95 g of Milli-Q water at room temperature. Similarly, a 24% (w/w) $CaCl_2$ stock solution was prepared by dissolving 24 g of $CaCl_2$ in 76 g of Milli-Q water. The model solutions, depicted in Table 4-1. Concentrations for LA as 1% (w/w) and Ca as 0.12% (w/w) were selected to imitate the amounts present in the real acid whey waste stream while, 0.4% (w/w) and 0.2% (w/w) LA and 0.072% (w/w) Ca were selected to imitate the concentrations present in nanofiltered acid whey retentates. A model lactose system resembling the sweet whey waste stream (0.05% (w/w) LA and 0.035% (w/w) Ca) was used for comparison purposes.

The solutions were stirred using a magnetic stirrer for about 30 minutes until clear solutions were obtained with no visually observable crystals. All solutions were filtered using 0.45 µm Whatman filter papers to remove any undissolved particles.

4.2.3 Concentration

Concentration of all model solutions containing lactose, LA, and Ca to obtain 50% (w/w) were performed as described previously in chapter 3 section 3.2.3.

4.2.4 Freeze-drying

A portion of concentrated samples were freeze-dried as described in chapter 3 section 3.2.4.

4.2.5 Differential scanning calorimetry (DSC)

DSC measurements on liquid and freeze dried samples were performed as described in section 3.2.5.

Table 4-1: Lactose model systems used within the study

Sample Name	**Lactose (%**	**LA (%**	**Ca (% w/w)**
L	5	-	-
L + 1% LA	5	1.00	-
L + 0.4% LA	5	0.40	-
L + 0.2% LA	5	0.20	-
L + 0.05% LA	5	0.05	-
L + 0.12% Ca	5	-	0.120
L + 0.072% Ca	5	-	0.072
L + 0.035% Ca	5	-	0.035
L + 1% LA + 0.12% Ca	5	1.00	0.120
L + 0.4% LA + 0.12% Ca	5	0.40	0.120
L + 0.2% LA + 0.12% Ca	5	0.20	0.120
L + 1% LA + 0.072% Ca	5	1.0	0.072
L + 0.4% LA + 0.072% Ca	5	0.40	0.072
L + 0.2% LA + 0.072% Ca	5	0.20	0.072
L + 0.05% LA + 0.0.035% Ca	5	0.05	0.035

4.2.6 Fourier transform infrared spectroscopy (FTIR)

The FTIR spectra of model solutions containing lactose, LA and Ca were obtained in the range of 4000 - 600 cm^{-1} immediately after achieving the concentration of 50% (w/w) using PerkinElmer frontier FTIR spectrometer (PerkinElmer, MA, USA) with the combined software of IR Solution (Shimadzu Corp.), Version 1.40. Sample spectra were collected in the absorbance mode after background subtraction. Each spectrum was a result of an average of 16 scans recorded at 4 cm^{-1} resolution. The spectra were vector normalized over the wavelength ranges of interest 1700 - 800 cm^{-1} and 3800 - 2600 cm^{-1}.

4.2.7 Statistical Analysis

All experiments were arranged in a randomized split plot block design, in which treatment for the lactose was the main factor and the replication served as blocks. The whole design was replicated with a subsequent subsampling resulting in at least 4 independent observations ($n \geq 4$). The least square difference (LSD) was used to differentiate the means with the pre-set probability at $p<0.05$.

4.3 Results and Discussion

4.3.1 Water - lactose interaction in the presence of lactic acid and calcium

The effect of LA and Ca presence on water binding to lactose molecules was examined using DSC (Figure 4-1) by obtaining onset, peak and end-set temperatures and enthalpies of water evaporation (Table 4-2). These changes depend on a number of variables including degree of lactose super-saturation, solution temperature, viscosity, pH and presence of various impurities (Rjabova et al., 2013). In this study it was hypothesized that the presence of LA and Ca would influence the behavior and molecular mobility of water molecules around lactose molecules.

The DSC analysis revealed an endothermic peak around 140 - 150 °C which has been attributed to phase transition of water (Yazdanpanah & Langrish, 2011). The recorded average onset, end-set and peak temperatures after four replicates appeared unaffected ($p>0.05$) by the presence of LA and/or Ca (Table 4-2). However, the enthalpy related to evaporation of water increased significantly with the presence of varying amounts of LA and/or Ca (Table 4-2).

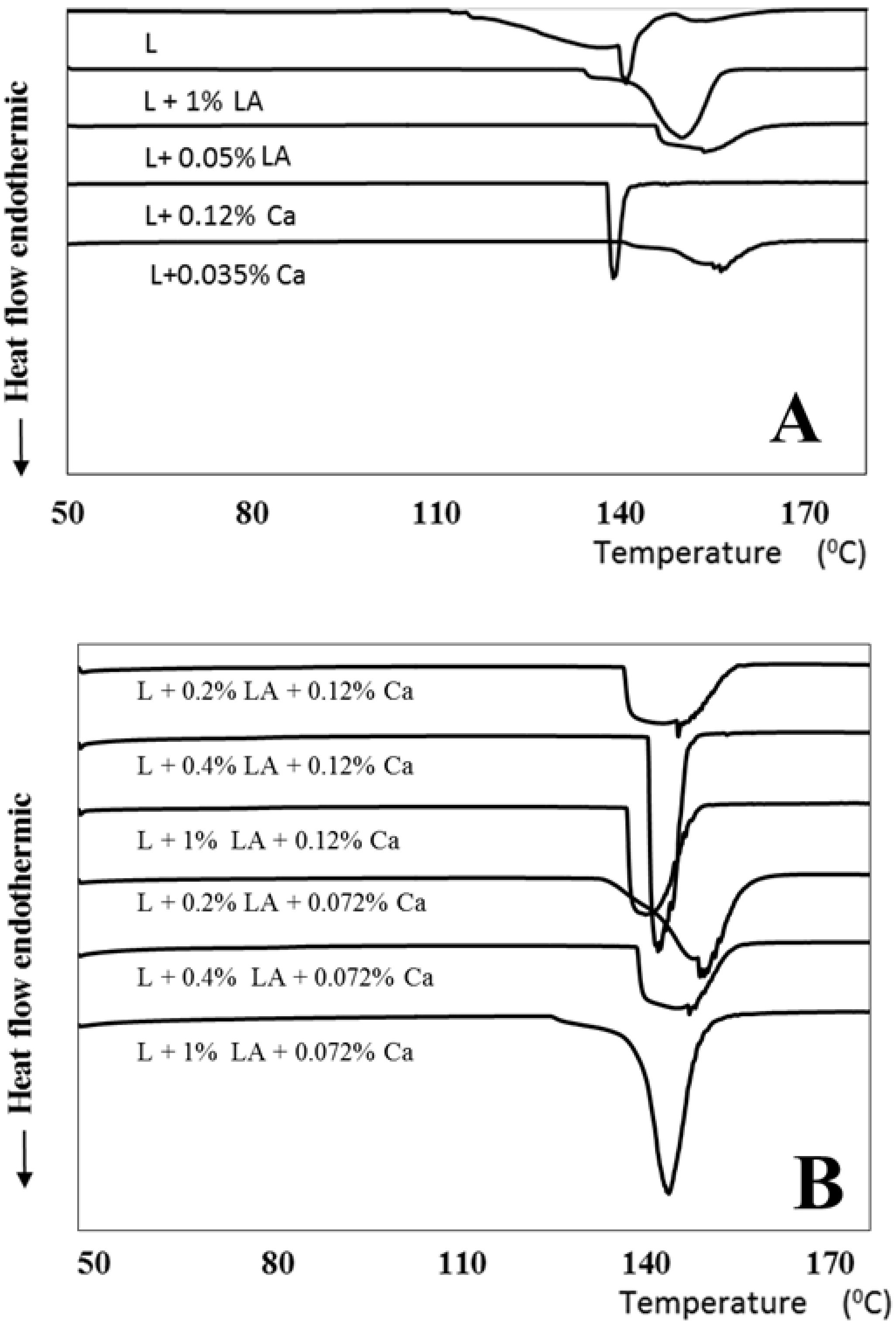


Figure 4-1: Dynamic DSC curves for lactose solutions: (A) with and without addition of LA (1% w/w & 0.05% w/w) or Ca (0.12% w/w & 0.035% w/w), (B) with addition of varying concentrations of LA (1%, 0.4% & 0.2% w/w) and Ca (0.12% & 0.072%)

Table 4-2: Enthalpy of water evaporation of lactose solutions in presence/absence of varying concentrations of LA and Ca

Sample Name	**Enthalpy J g^{-}**
L	679.0
L + 1% LA	965.1
L + 0.2% LA	865.8
L + 0.05% LA	772.0
L + 0.12% Ca	932.0
L + 0.072% Ca	946.9
L + 0.035% Ca	813.7
L + 1% LA + 0.12% Ca	1022.0
L + 0.4% LA + 0.12% Ca	1038.6
L + 0.2% LA + 0.12% Ca	978.9
L + 1% LA + 0.072% Ca	852.3
L + 0.4% LA + 0.072% Ca	890.7
L + 0.2% LA + 0.072% Ca	921.8
L + 0.05% LA + 0.035% Ca	803.5
SEM	54.86

**Values are means of at least 4 independent observations ($n \geq 4$). SEM denotes pooled standard error of the mean

The energy required to evaporate water from the pure lactose solution was ~679 J g^{-1}. The addition of 1% (w/w) LA increased the required energy for water evaporation by 30% with respect to pure lactose. As explained in chapter 3, presence of LA prompts changes in the structural associations of lactose and thus reduces the mobility of water molecules by lengthening the distances between lactose-lactose molecules, complexing with water molecules due to its high hydrophilic nature and affecting the availability of water molecules towards the formation of the hydration layer of lactose molecules. All these in combination, led to a strong hydration layer around lactose molecules and thus in turn need more energy to break the strong hydration layer, resulting in increased water evaporation enthalpy. Even though, LA was found to affect the behaviour of lactose, it is important to establish a minimum amount of LA that needs to be present for proper crystallization of lactose. The main aim of the first part of this chapter was to establish a threshold limit for LA that would allow proper crystallization of lactose.

Reduction of LA concentration from 1% (w/w) to 0.05% (w/w) led to a gradual decrease in water evaporation enthalpy (Table 4-2) indicating that LA present in the solution changes water-lactose binding behavior. For instance, presence of 0.05% (w/w) LA required ~772 J g $^{-1}$, which is about 20% reduction in enthalpy in contrast to L + 1% (w/w) LA (Table 4-2). The strength of the hydration layer consisting H_3O^+, increased distances between lactose-lactose molecule and lactose-lactic acid complex formation are all diminished by the reduction of LA concentration. This may in turn resulted in reduce enthalpy required for removal of water.

Apart from LA, Ca has also been considered as a key compound influencing phase behavior of lactose (Mimouni et al., 2007). In this study, 0.12%, 0.072% or 0.035% (w/w) Ca was added to lactose solutions in order to imitate the amounts of Ca present in acid whey (Gosta, 1995), acid whey retentates obtained through nanofiltration (Nguyen et al., 2003) and sweet whey (Gosta, 1995) respectively. Addition of 0.12% and 0.072% (w/w) Ca led to a significant ($p < 0.05$) increase in the enthalpy of water evaporation in comparison to pure lactose solution. On the other hand, presence of 0.035% (w/w) Ca led to decrease in enthalpy required for water evaporation in compared to 0.072% and 0.12% Ca (w/w) additions. However, it was larger than pure lactose (Table 4-2). This effect was likely caused by the ability of ionic Ca^{2+} to organize the structure of water (Bhargava & Jelen, 1996). Ca^{2+} is a divalent ion with a strong electric field which acts as a water structure promoter. Furthermore, Ca^{2+} ions strongly interact with four to six layers of water molecules through dipole-ion interactions (Reid & Fennema, 2008). Hence, the water molecules may be densely packed within these hydration layers in comparison to water molecules in pure water solutions leading to restricted mobility (Reid & Fennema, 2008). Concurrently, formation of calcium-lactose complexes (Herrington, 1934b; Charley & Saltman, 1963) takes place where, hydroxyl groups of lactose molecules bind with the water molecules present in the hydration layer of Ca (Cook & Bugg, 1973). Thus, the lactose molecule may be surrounded by a strong hydration layer creating a shielding effect towards easy removal of water and thus, preventing the orderly arrangement of lactose molecules towards forming a crystalline material.

The main compositional difference between sweet and acid whey is the levels of LA and Ca. Therefore combined effects of these two components on the water evaporation behavior of lactose were further examined. The results are presented in Figure 4-1B. Presence of 1% (w/w) LA and 0.12% (w/w) Ca resulted in ~1022 J g^{-1} of water evaporation enthalpy while decreasing the LA concentration up to 0.2% (w/w) at the same level of Ca, reduced the enthalpy to ~978 J g^{-1}. The reduction appeared fairly minor although, the energy required for evaporation of water in both cases were significantly ($p<0.05$) greater than that of the pure lactose solution. This observation indicated that for a substantial change in water behaviour and likely lactose phase behavior, a change in both LA and Ca concentration would thus be required. Another instance, lowering Ca concentration further, to 0.072% (w/w) decreased the enthalpy of water evaporation to ~852 J g^{-1} when 1% (w/w) LA was present. Interestingly, the enthalpy increased to ~921 J g^{-1} when LA concentration was lowered to 0.2% (w/w) and by keeping the same concentration of Ca (0.072% w/w). These results highlight that the presence of both LA and Ca highly influence the water behaviour of lactose.

As described in chapter 1, problems associated with lactose crystallization and consequently spray drying of acid whey is not encountered during processing of sweet whey. Therefore, a solution containing 5% (w/w) lactose, 0.05% (w/w) LA and 0.035% (w/w) Ca was prepared and assessed for its phase behavior. The determined evaporation enthalpy was ~803 J g^{-1} which, is only about 18% greater than the pure lactose and most importantly ~21% lower than the 1% (w/w) LA + 0.12% (w/w) Ca system. Hence, the present result shows that a reduction of concentrations of both components at least to concentration levels of ≤ 0.05% and ≤ 0.03% respectively may diminish the strong hydration layer formation and may facilitate easy removal of water from lactose.

4.3.2 Phase behavior of dried samples

The glass transition ($T_{g)}$ of freeze dried food materials is an important factor for understanding the phase transitions in food systems and for maintaining the quality (Bhandari & Howes, 1999). These phase changes are also related to the molecular mobility and phase transition temperatures during and after a concentration process (Roos & Karel, 1990), which was earlier hypothesized to be affected by the presence of LA and Ca. Thus, freeze dried model solutions were analyzed to determine the effect of Ca and LA on the crystallization behavior of lactose. Figure 4-2 shows a representative graph for crystallization behaviour of lactose in presence of LA or Ca in freeze dried samples.

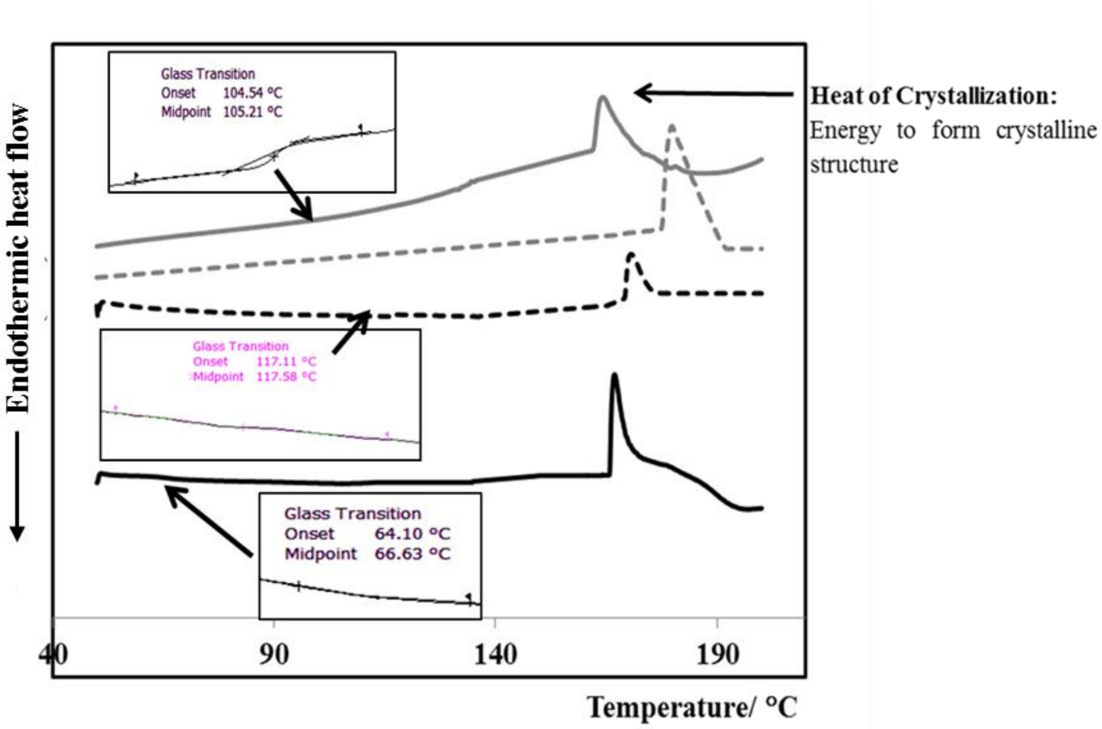


Figure 4-2: Dynamic DSC curves for freeze-dried lactose (grey solid) and L+1% (w/w) LA (grey dashed) L + 0.2% (w/w) LA (solid black) and L + 0.12% (w/w) Ca (black dashed) in the temperature region from 50 to 200 °C

Table 4-3 summarizes the thermal properties (crystallization enthalpy, onset, end set, peak temperatures and T_g) of freeze dried lactose samples with varying concentrations of Ca and LA. Freeze dried pure lactose sample showed a T_g value of ~105 °C. This value is in line with the value reported (105.5 °C) by Omar and Roos (2007). However, the dry sample containing 1% (w/w) LA did not exhibit a T_g value within the scanned temperature region. This may most probably be due to the low T_g value of LA at -60 °C (Maltini et al., 1967) as discussed in chapter 3. However, reduction of LA concentration to 0.2% (w/w) increased the T_g value to ~66.6 °C. This is consistent with Shrestha et al., (2006). LA is a highly hygroscopic compound and when present in high concentrations it enhances water-lactose interactions in the lactose system. This also increases the plasticization effect of water reducing the T_g of lactose.

On the other hand, addition of 0.12% (w/w) Ca has increased the T_g value of L by ~12 °C (Table 4-3), which was fairly close to a previously reported value of ~121 °C (Omar & Roos, 2007). The reduction of Ca concentration to 0.072% (w/w) did not show marked change in T_g compared to L + 0.12% Ca (~117 °C). Moreover, presence of 0.2% (w/w) LA with 0.12% (w/w) Ca also had no apparent effect on the T_g value. However, with the reduction of Ca concentration to 0.072% (w/w) with 0.2% (w/w) LA reduced the T_g value to ~95 °C indicating the interactive nature of LA and Ca towards the T_g of lactose.

The crystallization of pure lactose occurred within a temperature window of ~9 °C. In the presence of 1% (w/w) LA, this window has broadened to ~12 °C while it was narrowed down to ~5 °C in the presence of 0.12% (w/w) of Ca. In contrast, presence of both components 0.2% (w/w) LA and 0.12% (w/w) Ca led to an increase in the temperature window to ~10 °C highlighting that both components do contribute towards the crystallisation behaviour of lactose. The presence of both LA and Ca possibly may increases the distances between lactose molecules and may delay the required orderly arrangement of lactose molecules to form the crystalline structure.

High energy may require to crystallize the lactose in presence of high concentrations of Ca and LA. Hence, the observed crystallization peaks were integrated to obtain the total area under the peak, which is defined as crystallisation enthalpy in Table 4-3. This crystallization enthalpy was used to estimate the level of amorphicity of lactose according to Saffari and Langrish (2014). Pure lactose samples of freeze dried powders showed an enthalpy of ~112 J g^{-1} while the powdered samples with added 1% (w/w) LA showed ~239 J g^{-1} indicating that more amorphous lactose exists in the presence of LA with compared to pure lactose. Further reduction of LA concentration to 0.2% (w/w) reduced the enthalpy to ~184 J g^{-1}, which is about ~23% reduction of amorphicity compared to amorphicity of 1% (w/w) LA. Thus, the concentration of LA present in lactose solutions directly affect the amount of amorphous lactose. Furthermore, addition of 0.12% (w/w) Ca to 0.2% (w/w) LA + L system resulted with ~351 J g^{-1} of crystallization enthalpy which is about 3 times more than pure lactose indicating that Ca also plays a major role in determining the amount of amorphous lactose in a model lactose system containing both LA and Ca. Decreasing Ca concentration to 0.072% (w/w) keeping the same concentration of LA reduced the enthalpy to ~159 J g^{-1}. This is only ~1.4 times than pure lactose. It further highlights the fact that there is an interactive effect between LA and Ca. However, this interactive effect is solely concentration dependent.

Table 4-3: Tg, crystallization onset, end-set and peak temperatures and enthalpy of lactose freeze dried powders in presence of LA and Ca

Sample	**Tg (°C)**	**Onset temperature (°C) Crystallization**	**Endset temperature (°C) Crystallization**	**Peak temperature (°C) Crystallization**	**Enthalpy (J g^{-1}) Crystallization**
L	105.2	161.7	170.6	163.7	111.9
L + 1% LA	ND*	177.0	188.9	179.1	238.7
L + 0.2% LA	66.6	165.7	169.8	166.5	184.3
L + 0.12% Ca	117.4	169.1	174.8	170.5	106.7
L + 0.072% Ca	116.6	174.1	180.2	179.2	112.6
L + 0.2% LA + 0.12% Ca	117.3	182.9	192.6	191.6	350.8
L + 0.2% LA + 0.072% Ca	94.8	144.4	169.8	159.5	159.1

*ND = Not detected

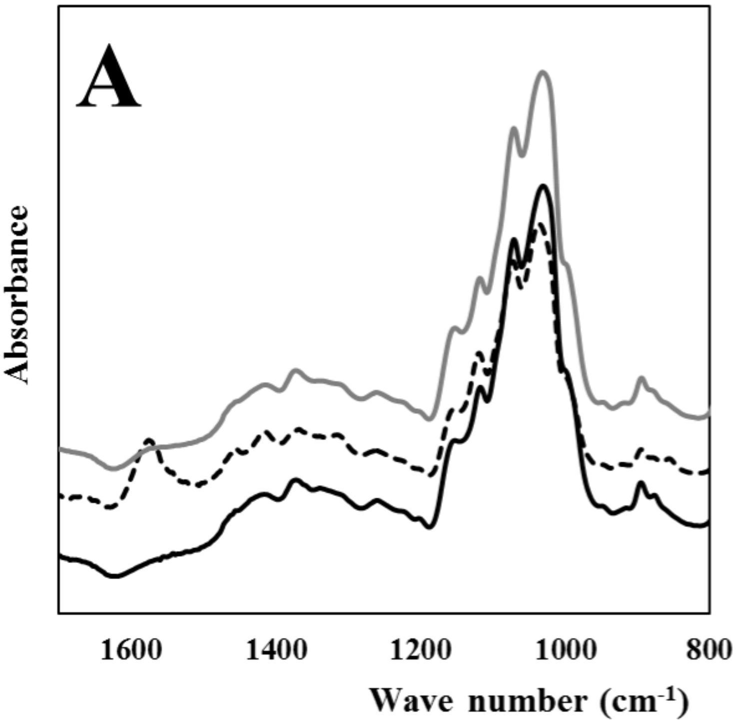
4.3.3 Fourier transform infrared spectroscopy (FTIR)

DSC results established that the lactose crystallization was influenced considerably by the presence of LA and Ca in lactose solutions. The resulted changes in evaporation enthalpy and crystallinity with the presence of LA and Ca possibly may be due to the structural changes occurring in lactose and/or water molecules around the lactose molecules. FTIR is a simple and rapid technique which can detect changes on molecular vibrations of compounds as influenced by presence of another component or two, as well as detect any structural changes (Subramanian et al., 2009). Thus, the FTIR spectra of lactose in presence of different concentrations of Ca and LA were recorded in the region 4000–400 cm^{-1}. Generally, 1200 - 800 cm^{-1} region is used detect phase changes of carbohydrate compounds owing to C-C and C-O stretching and vibrations which are influenced by presence of varying physical states (Ottenhof et al., 2003). Similarly the behavior of water molecules is determined in the range of 3600 - 3200 cm^{-1}. As a consequence the results for two different regions, 3800 - 2600 and 1800 - 800 cm^{-1} are discussed separately.

4.3.3.1 FTIR frequency region 1800-800 cm^{-1}

Figure 4-3 (A) shows the FTIR spectra of lactose with and without addition of 1% (w/w) LA and 0.05% (w/w) LA. Two protruding peaks initiating around 1022 cm^{-1} and 1065 cm^{-1} were observed for all 3 solutions, which were related to be associated with C-C stretching vibrations and C-O stretching of glucose molecule of lactose respectively (Solis-Oba et al., 2011). Addition of 1% (w/w) LA to the lactose solution drastically reduced the intensity of the peaks (Figure 4-3A). This reduction might have been a result of a restricted movement of the lactose molecule due to formation of a strong hydration layer around the lactose molecule. Reduction of LA concentration to 0.05% (w/w) resulted in similar peak intensities obtained for pure lactose indicating the presence of a weaker hydration layer. The presence of high amounts of LA resulted in a third peak originating around 1575 cm^{-1} which was previously attributed to the lactate ion (Sedlarik et al., 2006).

Similarly, two prominent peaks initiating around 1022 cm^{-1} and 1065 cm^{-1} were observed for lactose solutions in the presence of varying concentrations of Ca (Figure 4-3B). The presence of 0.12% and 0.072% (w/w) Ca reduced the peak intensities while, 0.035% (w/w) Ca addition did not induce any change in intensities with respect to pure lactose solution. The appearance of the peak around 1022 cm^{-1} is due to the presence of -OH groups of L (Sedlarik et al., 2006). As discussed prior in section 4.3.1, the formation of the lactose-Ca complex is directly related to the binding of hydroxyl groups of lactose with the water molecules present in the Ca hydration layer (Cook & Bugg, 1973). Thus, in presence of high amounts of Ca and the availability of -OH groups of lactose may be limited. This resulted in reduce peak intensities. Moreover, no peak originating around 1575 cm^{-1} (assigned to lactate ion peak in section 4.3.3.1) was observed for lactose solutions containing Ca only (Figure 4-3B).



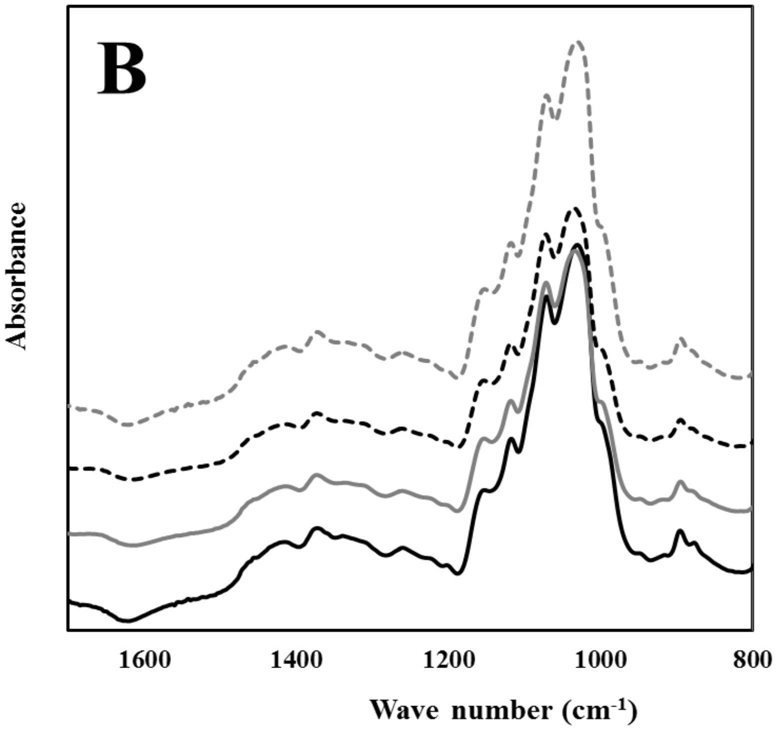


Figure 4-3: FTIR spectra of lactose (Black solid), L+1% LA (Black dashed) and L + 0.5% LA (grey solid) [A], and FTIR spectra of L (Black solid), L + 0.12% Ca (grey solid), L + 0.072% Ca (Black dashed) and L + 0.035% Ca (grey dashed) [B] in the region of 1600-800 cm^{-1}

Figure 4-4 represents the FTIR spectra (1600 – 800 cm^{-1}) of lactose in presence/absence of varying concentrations of LA and Ca. The two protruding peaks initiating around 1022 cm^{-1} and 1065 cm^{-1} were also observed for all systems, although the peak intensities differed substantially and were totally dependent on the concentrations of the components present. The lowest intensity was observed for 1% LA and 0.12% Ca sample indicating the strong hydration layer around lactose molecules which restricts its movement as discussed in section 4.3.1. The highest intensity was observed in the spectra of pure lactose followed by 0.05% (w/w) LA + 0.035% (w/w) Ca and 0.2% (w/w) LA + 0.072% (w/w) Ca. These results further highlighted the fact that there is an interactive effect with Ca and LA towards the behaviour of lactose molecules. However, reducing the concentration of both components found to be beneficial for the movements of lactose molecules. In addition, a clear peak was present around 1575 cm^{-1} in presence of LA which was attributed to lactate ion peak (section 4.3.3.1). This peak was gradually increased with rise in LA concentration (Figure 4-4).

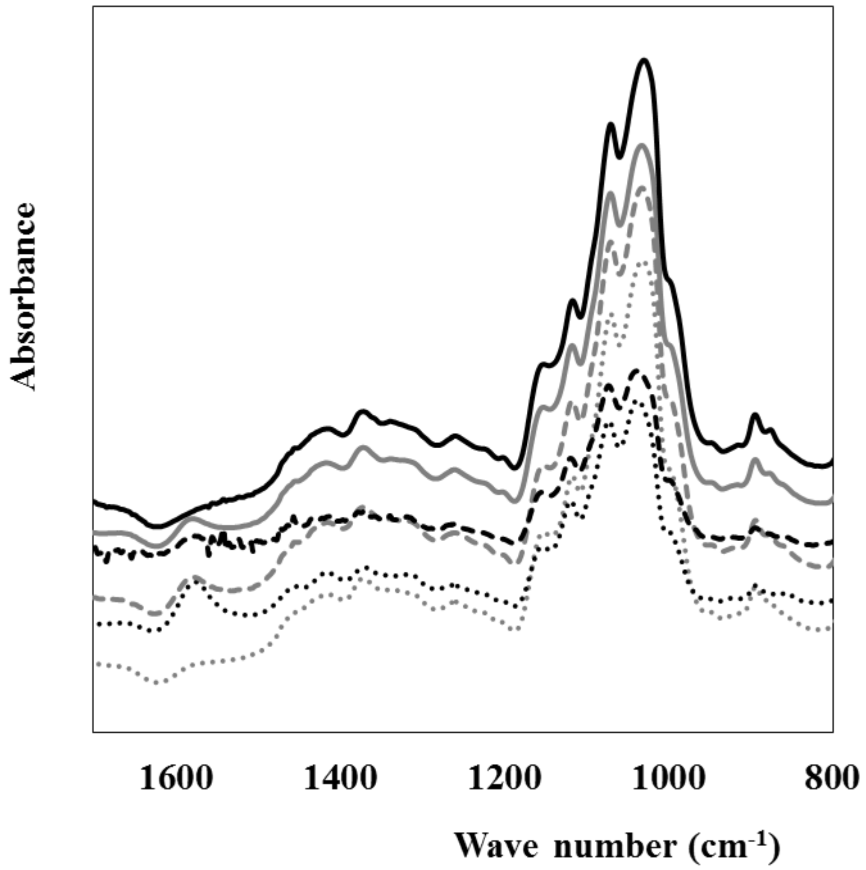


Figure 4-4: FTIR spectra of lactose (black solid), L + 0.2% LA + 0.12% Ca (grey solid), L + 1% LA + 0.12% Ca (black dashed), L + 0.2% LA + 0.072% Ca (grey dashed), L + 1% LA + 0.072% Ca (Black dots), L + 0.05% LA + 0.035% Ca (grey dots) in the region of 1600-800 cm^{-1}

4.3.3.2 FTIR frequency region 3800-2600 cm^{-1}

Properties of many food products are dominated by the presence and behavior of water molecules. Lactose crystallization is also governed by behavior of water molecules around lactose molecules. The presence of impurities may change the behaviour of water molecules which would influence kinetics of lactose crystallization. As discussed in above sections 4.3.1, 4.3.2 and 4.3.3.1 that the presence of Ca and/or LA influences the behavior of water molecules around lactose molecules and thereby, changes the crystallization behaviour.

The FTIR spectra of lactose in presence/absence of varying concentrations of Ca and LA in the region of 3800-2600 cm^{-1} are presented in Figure 4-5. This region is characteristic of O-H and C-H stretching vibrations of water and molecular compounds such as sugars (Punitha et al., 2014). Variations in wave number and intensity are interpreted as being controlled by changes in hydrogen bonding.

The peak originating around 3100 cm^{-1} is assigned to the hydrogen-bonded O-H groups (Lu et al., 2003). A clear reduction in the peak intensity was observed by the presence of 1% (w/w) LA regardless of the Ca concentration. This effect was thus, mainly due to restrictions in molecular mobility of water molecules due to the presence of strong H-bonds of the hydration layer in the presence of LA as discussed prior in chapter 3. The corresponding peak disappeared completely when 1% (w/w) LA was accompanying with 0.12% (w/w) Ca indicating highly restricted movement of water molecules. Already strong hydration layer around lactose molecules created in the presence of LA is likely further strengthened due to the strong ion-dipole interactions of Ca and water molecules. This was further confirmed by FTIR spectra showing the reduced peak intensities of O-H stretching vibrations as highlighted by the reduction of the O-H stretching peak at around 3507 cm^{-1} (Kirk et al., 2007).

Furthermore, the absorption bands in between 3000 - 2600 cm^{-1} are related to the C-H stretching vibrations of free sugars (Lu et al., 2003) and LA (Pamula et al., 2001). An apparent peak around 2900 cm^{-1} was observed which can be due to the stretching vibrations of C-H bond of free lactose (Lu et al., 2003) and/or due to the C-H group of

LA. The intensity of the band around 2900 cm^{-1} reduced with increase in LA and Ca concentration. This further highlighted the reduced amounts of available free lactose. Interestingly, a peak around 3747 cm^{-1} was observed for the L + 1% LA + 0.12% Ca sample while, it was absent in the other samples. This may be due to distortion of water molecules in the presence of high amounts of Ca and LA (Sharp et al., 2001).

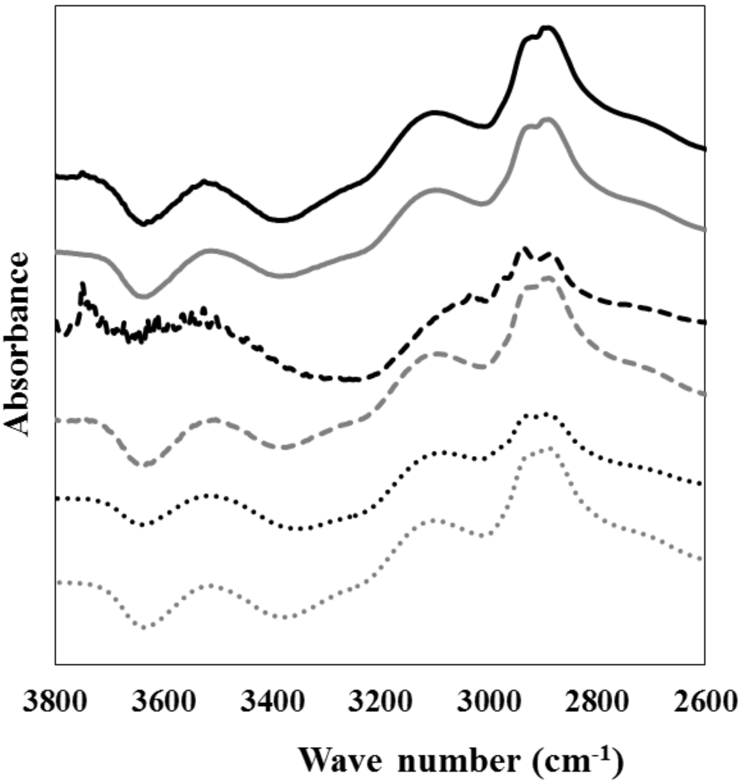


Figure 4-5: FTIR spectra of lactose (black solid), L + 0.2% LA + 0.12% Ca (grey solid), L + 1% LA + 0.12% Ca (black dashed), L + 0.2% LA + 0.072% Ca (grey dashed), L + 1% LA + 0.072% Ca (Black dots), L + 0.05% LA + 0.035% Ca (grey dots) in the region of 3800-2600 cm^{-1}

4.4 Conclusion

Presence of high amounts of LA and Ca hinders further processing of acid whey mainly due to the presence of amorphous lactose. Hence, a threshold limit for LA and Ca is of a necessity to postulate a strategy. The addition of 1% w/w LA and 0.12% w/w Ca led to an increase in water evaporation enthalpy indicating that the presence of high concentrations of LA and Ca hindered removal of water from lactose. Presence of LA and Ca in high concentrations led to a formation of a strong hydration layer around lactose molecules, which thereby result in restricted water and lactose mobility. This effect further led to a limited lactose crystallization where a high energy was required to crystallize lactose. Thus, the present chapter found that a partial removal of LA and Ca from acid whey at least to concentrations ~0.05% (w/w) and ~0.035% (w/w) respectively, may improve the crystallization of lactose and thereby, the processability of acid whey. The study expanded our understanding of behaviour of lactose in presence of varying concentrations of LA and Ca in order to predict controlling conditions for crystallization of lactose from acid whey.

Chapter 5

Conclusions and future directions

5.1 Conclusions

Further processing of acid whey to produce a non-sticky, free flowing whey powder has long been a problem for the dairy industry which leads to a huge environmental issue. Failure of lactose crystallization, which thus remains in its amorphous form during concentration, hinders further processing of acid whey. It appears that the role of LA and minerals especially, calcium are crucially influential for the behaviour of lactose and consequently the unprocessability of acid whey. Hence, the main objective of the proposed research was to find answers to a number of fundamental questions relating to the crystallization behaviour of lactose and its interactions with other constituents of acid whey. The model systems consisting of LA and Ca in lactose and lactose alone were used to investigate the lactose behaviour. These findings may eventually lead to the successful utilization of acid whey, thus creating a base for zero discharge dairy facilities.

Enthalpy of water evaporation was found to decrease with the increase in concentration of lactose solutions. The addition of 1% (w/w) LA led to an increase in water evaporation enthalpy signifying that the presence of LA restricted the removal of water from lactose due to the formation of a strong hydration layer consisting LA and H_3O^+ ions around lactose molecules via strong H bonds. FTIR was used to monitor the structural changes of lactose and the behaviour of water molecules. The FTIR spectrums confirmed the restricted molecular mobility of water and a change in structure of lactose signifying the presence of LA-stimulated changes in structural associations. This provided evidence for the main hypothesis of the study. Furthermore, freeze dried sample of pure lactose exhibited in a 50% crystallinity while, the presence of 1% (w/w) LA led to a decrease in crystallinity to ~12% providing evidence for the presence of more amorphous lactose in the presence of LA. The data obtained through the study of chapter 3 revealed that the presence of 1% (w/w) LA plays an important role towards lactose crystallization. Therefore, it was primarily required to find a threshold limit of LA concentration that would allow for proper lactose crystallization. Hence, a study of lactose with varying concentrations of LA was established. Moreover, a study of the effect of Ca on lactose behaviour was also established.

Reduction of LA concentration from 1% - 0.05% (w/w) decreased the enthalpy related to water evaporation of the concentrated samples by ~20%. This reduction of enthalpy further confirmed that the nature of water binding behaviour depends on the concentration of LA present in the solution. Moreover, reduction of LA concentration from 1% - 0.2% (w/w) reduced the level of amorphicity by ~22%. This explains the behaviour of lactose was directly influenced by the LA concentration which has affected the strength of the lactose hydration layer.

The addition of 0.12% (w/w) Ca to lactose solution increased the water evaporation enthalpy by ~27% compared to pure lactose. Further reduction of Ca concentration to 0.035% (w/w) led to a decrease in the water evaporation enthalpy by ~11% compared to 0.12% (w/w) Ca. This indicated that the presence of Ca and its concentration had impacted on the removal of water from lactose. These changes attributed the ability of Ca ions to organize the structure of water molecules and the immobilization of water due to the strong dipole – ion interactions between Ca and water.

Acid whey is a mixture of components where LA and Ca are the two main species which differ compositionally from sweet whey. Thus, it was required to examine the combine effect of LA and Ca. lactose samples containing both 1% (w/w) LA and 0.12%

(w/w) Ca resulted in a 50% higher water evaporation enthalpy in comparison to pure lactose. The water evaporation enthalpy resulted in the reduction of both LA and Ca to 0.05% and 0.035% respectively, was insignificant in compared to that of pure lactose. This highlighted the fact that removal of water from lactose can be successfully achieved by removing LA and Ca from acid whey mixtures to concentrations of ~0.05% and 0.03% respectively.

Overall, this study strengthened the idea that presence of high concentrations of LA and Ca highly influence the mobility of water molecules and consequently increase the amorphicity of lactose leading to improper crystallization. Thus, these findings enhanced our understanding of the behaviour of lactose in the presence of LA and Ca providing proven evidences for the tested hypothesis of the present study, “lactic acid and calcium affect the associations between lactose and water, thus rendering removal of water molecules difficult and diminish the crystallinity of lactose”. Hence, it is vital to remove these two components fully or partially to certain concentrations either through membrane filtering or with other techniques in order to achieve proper crystallization of lactose present in acid whey as a useful approach to improve the processability of acid whey.

5.2 Future Directions

This research has thrown up many questions in need of further investigation. Present study expanded our knowledge with regards to the behaviour of lactose in presence of varying concentrations of LA and Ca. Acid whey is a multicomponent solution more importantly containing high amounts of proteins. Presence of proteins may also influence the behaviour of lactose. Thus, further studies regarding the role of proteins on the behavior of lactose in the presence of LA and Ca during the concentration step would be worthwhile which may help to establish a greater degree of accuracy on this matter.

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