PROPERTIES OF SIMPLE SUGARS AND THEIR INTERACTIONS AS AFFECTED BY PRESENCE OF ACIDS AND SALTS

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B.Sc. (Hons), M.Sc.

Thesis submitted for the fulfilment of the requirements for the degree of

Doctor of Philosophy

Victoria University, Australia

Institute for Sustainable Industries & Liveable Cities

January 2024

Dedicated to my family
Dimuthu, Vinara and Ryan

Abstract

Lactose stands as the primary carbohydrate and predominant solute in both milk and whey. While sucrose is identified as the most abundant among low molecular weight carbohydrates. The role of these simple disaccharides is crucial for human life, the environment, and the dairy industry. The dairy industry encounters challenges in extracting lactose from acid whey, a by-product of Greek yoghurt and soft cheese manufacturing, primarily attributed to the inadequacy of lactose to crystallize appropriately. The presence of lactic acid and Ca and their interaction have been found to hinder the crystallization of lactose and negatively impact the environmental issues associated with the acid whey waste stream. Sucrose on the other hand is present in combination with acids and salts in most food products including sugar-sweetened carbonated drinks. Sucrose-acid interactions on the properties of sucrose in soft drinks during the production process remain unclear, which is critical to the health and nutritional value of the product. Therefore, it is crucial to examine the interaction among prevalent disaccharide sugars, acids, and salts to comprehend their behaviour adequately. Therefore, the present study aimed to investigate the physico-chemical and thermal behaviour of simple disaccharide sugars under different compositional and processing conditions to gain a molecular-level understanding of the interactions between sugars and acids and salts.

Understanding the properties of the concentrated lactose solution is paramount in determining the crystallization behaviour of lactose in the presence of different components. Initially, the physicochemical and thermal characteristics of lactose in concentrated solutions were established while considering various acids (lactic, citric, and phosphoric acid) and their concentrations (0.05, 1, or 4% (w/w)), commonly present in acid whey and other food systems. Thermographic analysis found that water evaporation from lactose solutions was hindered by water-acid interaction. This was due to the formation of a strong hydration layer around lactose

molecules through hydrogen bonding. The extent of lactose hydrolysis into glucose and galactose varied depending on factors like acid concentration and molecular interactions.

The influence of all three acids on the crystallization patterns of lactose exhibited variations, predominantly contingent on their concentrations. The addition of 1% or 4% citric or phosphoric acid led to a notable reduction in crystal yield, diminishing by at least 18% compared to the crystal yield of pure lactose, which stood at approximately 82%. The observed acid-induced hydrolysis during the concentrated solution phase has the potential to influence the crystallization behaviour of lactose. This impact is attributed to the presence of glucose and galactose in the solution, influencing supersaturation. The thermographic analysis also indicated that the inclusion of 1% lactic acid, 0.05% and 1% citric acid, and 4% phosphoric acid in lactose solutions resulted in the formation of amorphous lactose.

The hindrance of lactose crystallization in the presence of elevated salt concentrations poses significant challenges for downstream processing in acid whey. Hence, a meticulous study was conducted using a model-based approach, utilizing various cations (Mg, Ca, K, and Na) at concentrations (8, 30, 38, and 22 mM, respectively) that are typically encountered in acid whey. The thermal analysis of concentrated solutions discovered, the introduction of individual cations and their combinations led to an increase in the enthalpy of water evaporation, in contrast to pure lactose. Consequently, there was a significant decrease in crystal yield, which exhibited an exact reversal of the enthalpy order. The salt mixture resulted in the most substantial reduction (63%), followed by Ca (67%), compared to pure lactose (79%). This reduction in yield was inversely related to lactose solubility. It was observed that the involvement of divalent cations played a crucial role in the isomerization of lactose molecules.

The structural properties of sucrose molecules are subject to modifications under the influence of acids and salts. In the relevant study, we established the behaviour of sucrose in the presence of citric acid, phosphoric acid, and sodium. The primary objective of this research was to

elucidate the underlying molecular mechanisms that govern the structural modifications of sucrose in the presence of such additives. The behaviour of sucrose exhibited distinct responses to the presence of acids and salts. The addition of phosphoric acid and citric acid-induced structural modifications in sucrose molecules via a concentration-dependent hydrolysis process. The hydrolysis was notably facilitated by the presence of citric acid and phosphoric acid. Specifically, in the presence of citric acid, lactose hydrolysis resulted in a higher release of fructose, whereas in the presence of phosphoric acid, sucrose underwent separation into its constituent monosaccharides, yielding more glucose than fructose. The alterations in the hydration water layer, stemming from the molecular rotation of sucrose, fructose, and glucose molecules, coupled with the formation of robust hydrogen bonds, played a pivotal role in modifying the enthalpies of water evaporation (ΔH) in the presence of sodium, phosphoric acid, and citric acid. Notably, the influence of phosphoric acid in the sucrose solution demonstrated a more pronounced impact on sucrose behaviour compared to citric acid and sodium.

The behaviour of lactose and sucrose is influenced by a complex interaction of acids and salts, which involves interactions between water, acids, cations, and sugar molecules. This interaction leads to changes in sugar solubility, ion-dipole interactions between water and cations, and alterations in the structure of water molecules. The creation of a strong hydration layer hinders the removal of water. Therefore, the present study established that for lactose to be crystallized in acid whey the composition should be altered. By manipulating the Ca concentration and the lactic acid concentration, lactose crystallization can be improved and sequentially, achieve the better processability of acid whey. Furthermore, the reduction of acid concentration and the use of an alternative for phosphoric acid may be a feasible strategy in beverage production to mitigate the health implications.

Declaration

“I, Rangani Wijayasinghe, declare that the PhD thesis entitled “Properties of Simple Sugars and their Interactions as Affected by Presence of Acids and Salts” is no more than 80,000 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”.

“I have conducted my research in alignment with the Australian Code for the Responsible Conduct of Research and Victoria University’s Higher Degree by Research Policy and Procedures.

[redacted]

19/01/2024

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Acknowledgements

Foremost, I extend my sincere gratitude to my principal supervisor, Professor Todor Vasiljevic, for his exceptional professional guidance, valuable suggestions, and unwavering encouragement that provided me with the strength and confidence needed for the development of this study. His continuous diligence and patience over the years are genuinely appreciated, and I am proud and honoured to be supervised by a scholar of his caliber. Thank you for all the opportunities and understanding.

I also express my gratitude for the invaluable contributions and insightful discussions offered by my co-supervisor, Associate Professor Jayani Chandrapala, throughout my studies. Her guidance and support have played a crucial role in enhancing my research skills on this academic journey.

I extend my sincere thanks to Prof. Thom Huppertz, for his guidance and contribution in my academic journey. I am thankful to Professor Chris Perera, Dr. Prashanthi Kodikara, Dr. Muditha Dissanayake, and Dr. Marlen Cran, for all the support given to me throughout the study.

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

I appreciate the great friendship I have made during my research studies at Victoria University especially, Nuwan Vithanage, Gangani Uduwerella, Manjula Nishanthi, Samantha Liyanarachchi, Anushka Mediwaththe, Tatijana Markosa, and all the others. Thank you all for the support given to me during my studies.

My heartfelt appreciation goes to my family for their unwavering encouragement and loving support during my whole life.

I sincerely thank Australian Postgraduate Award (APA) scheme for granting funds for this study.

Finally, and most importantly, I would like to express my heartiest regards to my husband Dimuthu, my daughter Vinara, and my son Ryan for the sacrifices made during my studies. Thank you all very much.

DETAILS OF INCLUDED PAPERS: THESIS WITH PUBLICATION

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Chapter No.	**Publication Title**	**Publication Status** • Published • Accepted for publication • In revised and resubmit stage • Under review • Manuscript ready for submission	**Publication Details** • Citation, if published • Title, Journal, Date of acceptance letter and Corresponding editor's email address • Title, Journal, Date of submission
2B	Effects of solutes on solution phase properties and crystallization behaviour of sugars	Manuscript ready for submission	
3	Influence of lactic, citric and phosphoric acids on the properties of concentrated lactose solutions	Published	Wijayasinghe, R., Bogahawaththa, D., Huppertz, T., Chandrapala, J., & Vasiljevic, T. (2019). Influence of lactic, citric and phosphoric acids on the properties of concentrated lactose solutions. Food chemistry, 293, 247-253
4	Crystallization behavior and crystal properties of lactose as affected by lactic, citric, or phosphoric acid	Published	Wijayasinghe, R., Bogahawaththa, D., Chandrapala, J., & Vasiljevic, T. (2020). Crystallization behavior and crystal properties of lactose as affected by lactic, citric, or phosphoric acid. Journal of dairy science, 103(12), 11050-11061
5	Unraveling the Influences of Sodium, Potassium, Magnesium, and Calcium on the Crystallization Behavior of Lactose	Published	Wijayasinghe, R., Vasiljevic, T., & Chandrapala, J. (2023). Unraveling the Influences of Sodium, Potassium, Magnesium, and Calcium on the Crystallization Behavior of Lactose. Foods, 12(24), 4397
6	Behaviour of Sucrose as Affected by Presence of Acids and Salts	Under review	Submitted to the journal Food Chemistry on 04 December 2023

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List of Abbreviations and Units

˚C = degree Celsius

ΔH = enthalpy change

μm = micrometre

ANOVA = analysis of variance

a_w = water activity

CA = citric acid

DL = dulce de leche

DSC = Differential Scanning Calorimetry

FTIR = Fourier Transform Infrared Spectroscopy

g = gram

h = hour

HPLC = High Performance Liquid Chromatography

J/g = joules per gram

L = lactose

LA = lactic acid

LSM = least squares mean

min = minute

mL = millilitre

mM = millimolar

MIR = mid-infrared

NIR = near-infrared

PA = phosphoric acid

pH = potential of hydrogen

pKa = the negative base -10 logarithm of the acid dissociation constant (Ka) of a solution

SAS = statistical analysis software

w/v = weight per volume

w/w = weight per weight

XRD = X-Ray diffraction

α = alpha

β = beta

Chapter 1: Introduction

This chapter presents an introduction to the thesis including background information, aim and specific objectives, and structure of the thesis.

1.1 Background

Simple sugars are considered as an integral part in human lives. In general, simple sugars (mono and disaccharides) can be found naturally in various plants mainly fruits and milk or can be produced commercially. They are the simplest form of carbohydrates which are consisting of one or two sugar molecules. Sugars with one sugar molecule are called as monosaccharides while, sugars with two sugar molecules are called as disaccharides. Among these simple sugars, disaccharides especially lactose and sucrose are considered commercially important ingredients in day-to-day operations and the human diet. These sugars have specific roles including the provision of energy for the metabolic activities of the body, act as ingredients for different food products which improve taste and textural attributes, act as natural preservatives and are also used in the pharmaceutical industries.

Lactose or 4-β-D-galactopyranosyl-D-glucopyranose is the main sugar of mammalian milk (Fox, 2009). It is a disaccharide made up of linking glucose and galactose molecules as the main monosaccharides. The main three steps involved in lactose manufacturing include the concentration of whey or whey permeates through membrane filtration and evaporation, lactose crystallization and finally separation of crystallized lactose. Whey is the serum phase of milk which is expelled as a by-product during cheese and yoghurt manufacturing that contains all the soluble nutrients present in milk (lactose, soluble salts, globular proteins)(De Wit, 2001). Whey can be categorized as sweet and acid whey depending on the method applied in the production process of cheese and yoghurts (Chandrapala et al., 2016). Due to the current trend towards the consumption of soft/cream cheeses and Greek-style yoghurts, acid whey has become a soaring problem for the dairy industry. It has been estimated that the production of cream cheese by three Australian plants generates over 134 million litres of acid whey per year leading to a serious environmental issue. This presents a challenge for the industry since it needs to find a solution for acid whey by either sustainable disposal or processing into value added products. Compared to sweet whey, acid whey processing is far from optimized. It is considered as challenging and hard to process stream because concentrated acid whey remains in its amorphous form without producing crystalline lactose (Wijayasinghe, Vasiljevic, & Chandrapala, 2015). Differences in crystallization behaviour of acid whey compared to sweet whey leads to low crystallization rate and lumping and caking of whey powder particles during spay drying (Saffari & Langrish, 2014) are some of the main concerns in acid whey processing (Dec & Chojnowski, 2006). Lactose crystallization is the limiting factor for unprocessability of the acid whey stream. It is well established that lactic acid and calcium affect associations between lactose and water, thus rendering the removal of water difficult (Chandrapala, Wijayasinghe, & Vasiljevic, 2016). The inherent compositional differences found in acid whey with regards to the levels of salts and acids as compared to sweet whey could be the reason for limited success in acid whey processing and variances of crystallization between two whey streams. For example; acid whey contains a higher amount of lactic acid, more minerals and less proteins in comparison to sweet whey. According to (Mimouni, Bouhallab, Famelart, Naegele, & Schuck, 2007) formation of calcium lactate crystals may be the cause for the thickening behaviour of lactose in the process of acid whey spray drying. Moreover, recently published studies indicate that the presence of lactic acid and or calcium influences the physico- chemical properties of lactose via subtle conformational changes that create substantial processing problems and, consequently, an environmental issue (Chandrapala & Vasiljevic, 2017; Wijayasinghe et al., 2015; Wijayasinghe, Vasiljevic, & Chandrapala, 2016). Recent evidence suggests that the presence of lactic acid led to higher crystallization temperatures, delaying the on-set temperature of crystallization and a large percentage of amorphous lactose. In addition, the enthalpy associated with the crystallization or the heat of crystallization, which is used to quantify the amorphous content, showed that the lactose fraction consisted of 50% crystalline lactose, while in the presence of lactic acid, the crystallinity of lactose declined to 12%. Furthermore, it is well established that the inability to process acid whey is due to the presence of both lactic acid and calcium which inhibits the crystallization of lactose (Chandrapala et al., 2016). These studies clearly indicate that obtaining crystalline lactose is a crucial factor in order to process acid whey properly. However, still there are some fundamental issues in lactose crystallization to be answered. A comprehensive fundamental study has not yet been conducted to investigate the molecular level interaction between sugar-acid-water and/or sugar-salt-water. Therefore, it is important to understand down to the molecular level the detail behaviour of lactose in the presence of acids and salts.

Sucrose, on the other hand, is also a disaccharide consisting of two monosaccharides, glucose and fructose. Sucrose is still considered as the main sugar used most frequently for the food production (Hartel & Shastry, 1991). Annually, about 180 million metric tonnes of sugar is producing worldwide (Saini, Nagpal, Garg, & Kumar, 2023) either by sugar cane or sugar beet. Recently, epidemiological evidence suggests that sugars, especially sucrose, are an underlying reason for several chronic diseases. Several influential studies emphasize that sucrose containing soft drinks are one of the key contributors to the aetiology of overweight and obesity among people (Qiang, YongLie, & QianBing, 2009; Tam et al., 2006). Some possible structural changes of sucrose molecules in the presence of organic acids during the production process may likely be the reasons for the occurrence of physiological condition such as obesity where the structurally changed sucrose may metabolize differently in the body. Apart from that, crystallization of sucrose is important in food industry as in many processes the degree of crystallinity would govern properties and acceptability of the final product. For instance, sucrose crystallization is undesirable in beverages, whereas the presence of fine crystals in fondant icing is desirable. Thus, the control and understanding of sucrose solution behaviour along with crystallization during processing appears imperative (Kedward, MacNaughtan, Blanshard, & Mitchell, 1998). A considerable amount of literature has been published on sucrose behaviour in food products in the solution phase and crystalline phase. For example; In presence of organic acids, sucrose increases its hygroscopicity and shows enhanced browning of candies. However, the type of organic acid appeared to govern the extent of these changes (Shin, Lim, & Son, 1998). Presence of impurities affects sucrose crystallization via affecting the mass transfer of sucrose from the bulk to the crystal or adsorption of the impurity onto the surface hindering incorporating of sucrose molecules into the crystal lattice (Smythe, 1967). There is still uncertainty, however, whether the presence of acids and salts changes the molecular structure of the sucrose molecule and the water which may ultimately lead to changes in the structure of food products and also metabolize differently causing physiological conditions such as obesity. There are potential alternatives to sucrose, such as erythritol, that can be used in various food systems (Nastaj et al., 2022). However, the unique properties of sucrose play a critical role in ensuring food quality and safety. Thus, it is of utmost important to determine the structural changes of sugars present in acidified food products such as carbonated soft drinks, and naturally acidified food products in order to develop a better fundamental understanding of how different acids impact the behaviour of sugar molecules in different food systems.

1.2 Research aim and objectives

The main aim of the proposed study was to establish the physico-chemical and thermal behaviour of lactose and sucrose under different compositional and processing conditions in order to fundamentally understand the nature of sugar-acid-water and/or sugar-salt-water interactions. Consequently, it was aimed to understand the behaviour of water molecules around sugar molecules in the presence of organic acids and salts which may lead to structural changes of the sugar molecules.

The specific objectives of the study were

- *Establish the physico-chemical & thermal characteristics of commonly used simple disaccharide sugars (lactose and sucrose) in the presence of different acids (lactic acid, citric acid and phosphoric acid) and salts (Ca, Mg, Na and K).*
- *Establish the nature of the interactions of lactose and sucrose with other components prevailing in the system.*
- *Determine the structural changes of the sugar molecules in the presence of acids and or salts during processing.*
- *Determine the structural changes of water molecules which lead to behave differently within the food system.*

1.3 Thesis outline

Chapter 1 is the introduction to the thesis, which reflects the entire thesis. This chapter discusses the purpose of the study or the background, research objectives and an overview of the thesis. Chapter 2 presents a review literature on current knowledge on the behaviour of the simple sugars (lactose and sucrose) and physico- chemical properties of sugars in presence of various impurities (proteins, acids and salts). Chapter 3 explains about influence of acids on the properties of lactose. This chapter discusses the impact of lactic, citric and phosphoric acids on the physico-chemical and thermal properties of lactose in concentrated solutions. Chapter 4: Crystallization behaviour of lactose with and without addition of acids is discussed in this chapter. Chapter 5 focuses on the behaviour of lactose in presence of calcium, magnesium, sodium and potassium in concentrated solutions and impact of salts on crystallization behaviour. Chapter 6 discusses the behaviour of sucrose in the presence of acids (Phosphoric and Citric acid) and salts (sodium). Finally, Chapter 7 summarizes the research findings, reiterates the consequences and importance of the study and finally suggestions for future studies.

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Chapter 2: Review of literature

This chapter discusses an overview of simple sugars (lactose and sucrose) and the physico-chemical properties of sugars in the presence of various impurities. This has been divided into two chapters, 2A and 2B.

2A: Physicochemical properties of simple sugars

This review extensively examines the physical and chemical characteristics of simple sugars. The chapter commences by elucidating the physicochemical properties of simple sugars, and subsequently, it provides a comprehensive overview of the existing literature on the behaviour of lactose and sucrose in the presence of different solutes.

2A.1 Introduction

Simple sugars are the most basic form of carbohydrates, consisting of either one or two sugar molecules. Monosaccharides refer to sugars with a single sugar molecule mainly glucose. Monosaccharides, being the fundamental building blocks of carbohydrates, exhibit characteristics such as being devoid of colour, soluble in water, and existing in the form of crystalline solids. Essentially, monosaccharides serve as the foundational units from which other carbohydrate polymers, including di-, tri-, and oligosaccharides, are constructed. Disaccharides refer to sugars with two sugar molecules. Sucrose, a disaccharide, is formed by the combination of glucose and fructose, both of which are monosaccharides while lactose, a milk sugar, consists of glucose and galactose monomeric units (Figure 1). These sugars play a crucial role in the lives of humans. They can be naturally found in fruits and milk, or they can be manufactured commercially. Among these simple sugars, lactose, and sucrose, which are disaccharides, are particularly important for human life. These sugars serve specific purposes, such as providing energy for the body's metabolic activities, enhancing the taste and texture of various food products, acting as natural preservatives, and finding applications in the pharmaceutical industry.

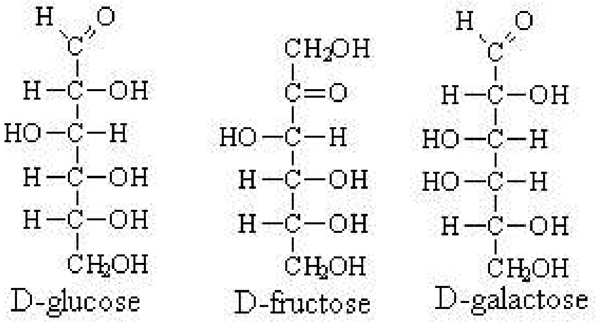

Figure 1: Schematic representation of the glucose, fructose, and galactose

Lactose (β-d-galactopyranosyl-(1→4)-d-glucose), with an empirical formula of $C_{12}H_{22}O_{11}$ (Figure 2), is a disaccharide that exhibits reducing properties. The composition of the molecules consists of glucose and galactose, which are linked together through a β 1 - 4 glycosidic bond, making them the primary monosaccharides present in lactose (Fox, 2009). Lactose is the most abundant carbohydrate in milk which is synthesized in epithelial cells in mammary glands in the majority of mammalian species, acts as the main energy source and its concentration varies across different species in milk (Huppertz & Gazi, 2016). For instance, lactose constitutes approximately 7% (w/w) of human milk, whereas bovine milk contains only 4.6% (w/w) of this carbohydrate (Anand, Som Nath, & Chenchaiah, 2013). In the dairy industries, lactose is extracted from whey or whey permeate which are by-products from the production of yogurts, cheese, and/or caseinates (Wong & Hartel, 2014). The optimization of the lactose manufacturing process in the food industry is crucial for enhancing the economics of whey utilization and reducing pollution. By recovering lactose from whey, the biochemical oxygen demand of whey can be reduced by over 80%. Nevertheless, the current lactose manufacturing process in the food industry is still far from being optimized (Bund & Pandit, 2007; Gänzle, Haase, & Jelen, 2008; Wijayasinghe, Vasiljevic, & Chandrapala, 2015).

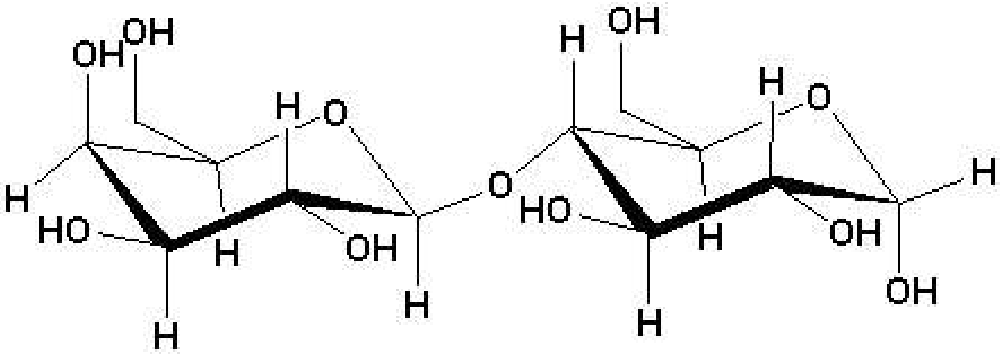

Figure 2: Schematic representation of the lactose molecule (Vasiljevic, 2004)

Sucrose (Figure 3) on the other hand is a disaccharide that does not exhibit reducing properties. It is formed by combining two monosaccharide sugar molecules glucose and fructose. Sucrose is naturally synthesized in various concentrations within numerous plant species and can undergo processing and refining to produce the commonly used table sugar. The extraction of sucrose commercially involves several plants such as sugar maple, sorghum, date palm, carob or locust bean, sugar cane, and sugar beet. Among these, sugar cane and sugar beet remain the primary sources for global sucrose production (Reiser, 1994).

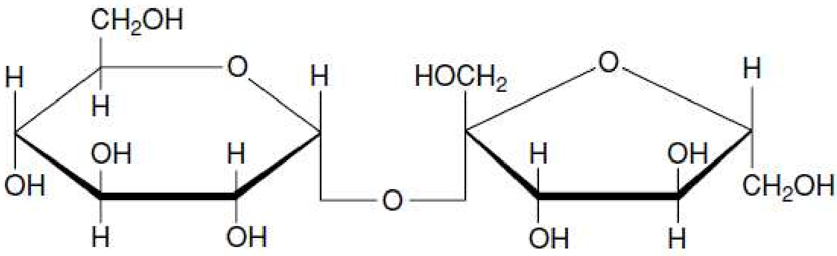

Figure 3: Schematic representation of the sucrose molecule (Macrae, Robinson, & Sadler, 1993)

2A.2 Fundamental properties of lactose and sucrose

Lactose exhibits several advantageous functional characteristics, including a relatively low level of sweetness, the ability to enhance flavors, and the capacity to stabilize proteins. These attributes contribute to the significance of this sugar as a crucial component in a wide range of Applications such as food and pharmaceutical (Dissanayake et al., 2013). Similarly, sucrose present in food contributes to its pleasant taste, making it enjoyable for a wide range of individuals (Tappy & Lê, 2010). In addition to providing a sweet sensation, sugars also play a role in enhancing the texture of certain food products (Hartel & Shastry, 1991). These inherent qualities of sweetness and texture in foods attract people to consume sugary foods and drinks in significant amounts. Nevertheless, the impact of some physico-chemical properties of these sugars, such as mutarotation, solubility, and crystallization, can influence these desirable qualities (Lefort, Caron, Willart, & Descamps, 2006).

2A.2.1 Mutarotation of sugars

Mutarotation refers to the alteration in the physical characteristics of sugar systems caused by a chemical reaction occurring in the solution state of sugars (Nelson & Beegle, 1919). In solution, the optical rotatory powers of sugars such as glucose and fructose change as the substance undergoes a conversion from one form to another. The lactose molecule possesses a hemiacetal ring structure, wherein the hydroxyl group connected to the first carbon of the glucose molecule can freely orient itself above or below the plane of the ring (Figure 4). This particular arrangement accounts for the existence of two anomeric forms, namely α and β, in lactose (Fox, 2009). Mutarotational reactions typically exhibit kinetics that adhere to first-order principles (Harper, 1992).

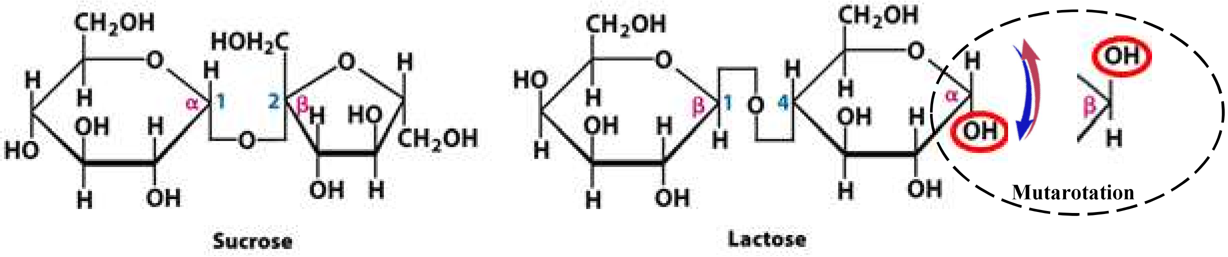

Figure 4: Non reducing sucrose molecule and α/β anomeric forms of lactose

These two forms (α and β) of lactose greatly influence its physical, chemical, and functional properties, such as crystallization, solubility, and solid-state properties. However, researchers have thoroughly investigated the impact of mutarotation on the process of crystallization. Van Hook (1961) delves into the effects caused by the conversion of α to β forms of glucose on the crystallization of glucose. Similarly, Kraus and Nyvlt (1994) also explored these effects, highlighting that only the α form of glucose is capable of crystallizing. Moreover, during the crystallization process, a portion of the β form is converted back to the α form in order to restore equilibrium. However, sucrose does not consist of any anomeric forms or the process of mutarotation because the glycosidic bond is between the anomeric carbon of glucose and the anomeric carbon of fructose where there is no OH group at the anomeric position (Figure

4). As such, sucrose is classified as a non-reducing sugar, resulting in a consistent optical rotation and the absence of mutarotation properties (Reiser, 1994).

2A.2.2 Solubility of sugars

Understanding the solubility properties of sugars, such as sucrose and lactose, is vital for various industries. Factors like temperature, pressure, pH, and presence of impurities can significantly influence the solubility of these sugars. By comprehending the solubility characteristics of these sugars, industries can optimize their use and create a wide range of products to meet consumer demands.

Lactose, in comparison to other commonly found sugars, exhibits a relatively lower solubility (Harper, 1992). At a temperature of 20 °C, the solubility of lactose in water reaches an equilibrium point, with a concentration of 18 g per 100 g of water. However, the degree of its solubility is influenced by the presence and quantity of two distinct forms, hydrated α-lactose, and anhydrous β-lactose (Fox & McSweeney, 1998). The original solubility of hydrated α-lactose is approximately 7 g per 100 g of water at 20 °C, whereas the solubility of anhydrous β-lactose is significantly higher at 50 g per 100 g of water (Hunziker & Nissen, 1926). The equilibrium solubility of lactose can also be affected by the existence of impurities in the solution primarily because of alterations in the supersaturation level. Additionally, the solubility of lactose can be either enhanced or diminished in a sequential manner as a result of the modifications in the structure of bulk water molecules caused by impurities (Bharhava & Jelen, 1996). For instance, the solubility of lactose can be greatly enhanced by certain calcium salts like nitrate, bromide, or chloride, leading to the formation of highly stable solutions (Herrington, 1934). The rise in solubility of lactose could be attributed to the creation of a molecular compound comprising lactose, salts, and water molecules. Consequently, the formation of chemical complexes involving lactose, salts, and water molecules may lead to alterations in the solubility of lactose (Harper, 1992; Herrington, 1934)

Sucrose, on the other hand, possesses a significant characteristic in terms of its solubility in water. The sucrose molecule can easily engage in hydrogen bonding with water molecules owing to its eight hydroxyl groups, three hydrophilic oxygen atoms, and fourteen hydrogen atoms (Engelsen & Pérez, 1996). This interaction ultimately enhances the solubility of sucrose in water. Sucrose solubility has been the subject of extensive research, with numerous authors publishing studies on the solubility of sucrose in pure aqueous solutions since the 1940s (Taylor, 1947). Both experimental data and mathematical modeling pertaining to sucrose solubility can be found in the existing literature. Nevertheless, the solubility of sucrose in impure aqueous solutions is considered a more intricate issue (Crestani, Bernardo, Costa, & Giulietti, 2018).

2A.2.3 Crystallization

Crystallization refers to the phenomenon where solid crystals are formed from a solution, melt, or by direct deposition from a gas phase. This process occurs by altering the solubility conditions of the solute in the solvent, in contrast to precipitation which arises from a chemical reaction (Mullin, 2001). The solid crystalline phase of sugars is of great significance in various food products. In addition to imparting a sweet taste to these products, sugars also contribute to the desired textural properties. The crystallization process of sugars is carefully regulated to either keep them in a dissolved state or control the formation of the solid crystalline phase, thereby achieving the desired textural attributes. Certain food products depend on the existence of a crystalline phase in order to achieve the intended texture. For instance, the delicate crystals found in fondant icing significantly influence the icing's capacity to maintain its form when used in baked goods. However, in the case of hard candies, crystallization is viewed as a flaw rather than a desirable characteristic. Hence, it is crucial to comprehend the principles governing the prevention or formation of the crystalline sugar phase in order to create food products that meet the desired standards.

In dairy industry and sugar industry, crystallization is one of the most used techniques as a means of producing, purifying, and recovering solid materials. It is considered one of the most effective and cost-efficient techniques for obtaining pure solids from mixed solutions. Moreover, crystallization offers the added benefit of yielding a final product with numerous desirable properties (Mullin, 1979). Industrially lactose is recovered from whey permeate through the process of crystallization (McLeod, Paterson, Jones, & Bronlund, 2011) while sucrose is recovered from sugar cane or beet juice crystallization (Austin, 1984) following several clarification steps. The crystallization process typically consists of two fundamental stages: "nucleation," which involves the formation of minuscule crystals or nuclei, and "crystal growth," which occurs as the nuclei expand in size due to the presence of excess solute in the solution.

2A.2.3.1 Nucleation

The initiation of nucleation begins with the gathering and accumulation of molecules/ions within a solution. These molecular clusters subsequently attain stability and gradually develop into crystals of discernible size (Wong & Hartel, 2014). The zero size crystals/nuclei serve as the primary focal points for the process of crystallization. The overall nucleation process is the combined result of two distinct categories referred to as "primary" and "secondary". The process of primary nucleation can be further categorized into two distinct types: homogeneous and heterogeneous. The primary driving factor behind this nucleation process is the state of supersaturation in the solution (Herrington, 1934). The primary driving force behind nucleation is the state of supersaturation in the solution. In the case of homogeneous nucleation, ions or molecules within the solution interact to form temporary clusters. These clusters initially take the form of short chains or flat monolayers, eventually progressing into crystalline lattice structures. The formation of these crystalline lattice structures is a rapid process that occurs solely in localized regions with extremely high levels of supersaturation (Mullin, 1979). Homogeneous nucleation necessitates a significant amount of energy for the formation of nuclei and is primarily observed in solutions that are highly supersaturated and at elevated temperatures (Shi, Hartel, & Liang, 1989). On the contrary, heterogeneous nucleation takes place when foreign substances, such as dust particles or minor impurities, are present. These foreign materials reduce the energy required for nucleus formation, enabling nucleation to occur under conditions of low supersaturation. Homogeneous nucleation is seldom practical due to the challenges associated with initiating nucleation in the absence of a solid surface to catalyze the process (Agrawal & Paterson, 2015). Primary nucleation exclusively takes place during the initial phase of the crystallization process.

Secondary nucleation occurs exclusively when there are suspended solute particles or seed crystals present, and it is the primary mechanism in the process of crystallization. At lower levels of supersaturation, secondary nucleation helps to minimize the formation of numerous small crystals that result from excessive nucleation. However, an increase in supersaturation leads to an enhancement in the rate of nucleation. In order for nuclei to develop into stable crystals, they must reach a critical size; otherwise, they dissolve back into the solution (Mandare & Pangarkar, 2003). The Embryo Coagulation Secondary Nucleation (ECSN) theory (Qian & Botsaris, 1997) proposes that solute clusters are connected to seed crystals through van der Waals forces. The intense clustering of solute particles, also known as embryos, in the vicinity of crystals induces rapid attraction, leading to the formation of nuclei that exceed the critical size. To achieve the formation of a solid crystal structure, it is essential for a nucleus to attain a critical cluster size, typically consisting of approximately 80-100 sucrose molecules

(Mathlouthi & Genotelle, 1998). For the individual growth of these crystals, the nuclei need to be liberated into the surrounding solution by means of collision impact or fluid shear. Secondary nucleation can be initiated through two distinct mechanisms, fluid shear or collision with the solid surface of the crystallizer. In the case of fluid shear nucleation, the rapid movement of a liquid displaces the pre-arranged layers of solute in close proximity to the crystal surface, resulting in the generation of fresh crystals. On the other hand, collisions between existing crystals and the solid surface cause physical damage, resulting in fragments that serve as secondary nuclei. It is worth noting that secondary nucleation is widely employed in the industrial production process of lactose (Wong & Hartel, 2014).

2A.2.3.2 Crystal growth

After the establishment of a stable nucleus, the process of crystal growth takes place. Crystal growth is commonly described as a heterogeneous process consisting of several distinct stages (Hartel, 2001; Rjabova et al., 2013). The initial step involves the diffusion of sugar molecules from the bulk solution to the solid interface. Subsequently, the lactose molecules undergo mutarotation to assume the correct anomeric form. The second step entails surface reaction or integration, during which the hydration water molecules associated with lactose are partially or completely removed. Subsequently, the lactose molecules arrange in alignment with one another, leading to the subsequent dispersion of water molecules from the crystals. In the fourth step, surface diffusion of lactose molecules occurs, leading them to incorporate into the appropriate crystal lattice site. Ultimately, the growing crystals release latent heat as it dissipates from their structure. The growth of sucrose crystals also adheres to the identical sequence. The initial stage involves the diffusion of molecules from the bulk solution towards the immobile mother liquor layer that forms at the surface of the crystal. Subsequently, the sucrose molecules are incorporated into the crystal structure. In industrial settings, multiple crystallization phases are typically required to completely deplete the solution, as sucrose has a relatively high solubility in water. Ultimately, the crystallized sucrose must be separated from the mother liquor.

2A.2.4 Properties of crystalline sucrose

Pure sucrose is characterized by its colorless, odorless, and distinctly sweet properties. Its crystalline structure manifests as an anhydrous monoclinic crystal. The revelation of the crystalline sucrose structure was initially achieved through neutron diffraction (Brown & Levy, 1963), later refined using X-ray (Hanson, Sieker, & Jensen, 1973), and further validated by neutron diffraction (Brown & Levy, 1973). Sucrose molecules exhibit a notably high melting temperature, necessitating increased energy input for the disruption of hydrogen bonding and molecular separation. The crystalline sucrose phase undergoes melting within the temperature range of 160 °C to 186 °C (Pennington & Baker, 1990; Reiser, 1994), with recent findings suggesting a broader melting range of 182-192 °C (Roos, 1993). This temperature range is subject to variation contingent upon the crystallization solvent and purity levels. Sucrose undergoes decomposition near its melting point through a process known as caramelization. This thermal degradation results in the separation of sucrose molecules into glucose, fructose, and ultimately caramel, characterized by a brown hue and a pleasing taste. Caramelization serves as a viable flavor enhancer widely employed in the food industry. The density of a singular sucrose crystal, representing the mass per unit volume, is measured at 1587.1 kg/m3 at 20 °C. Conversely, the bulk density of sucrose is contingent upon crystal size, demonstrating an inverse relationship with crystal size (Asadi, 2006).

2A.2.5 Properties of crystalline lactose

Lactose molecules exhibit a specific three-dimensional arrangement within a crystalline lattice when in the crystalline state. This crystalline state is further categorized into distinct forms, namely α-stable anhydrous, α-unstable anhydrous, α-lactose monohydrate, and β lactose/β-anhydride (Kirk, Dann, & Blatchford, 2007).

α-lactose monohydrate (α-Hydrate) crystals emerge from supersaturated solutions at crystallization temperatures lower than 93.5 °C and represent the sole stable form of lactose crystals under ambient temperature conditions. In this crystalline form, every lactose molecule is bonded with a single molecule of water, commonly referred to as crystallization water. Consequently, the typical water content of α-lactose monohydrate amounts to approximately 5% (Fox, 2009). Other forms of lactose crystals undergo conversion into the α-hydrate form, even in the existence of minimal water, at temperatures lower than 93.5 °C. The α-hydrate form manifests in a range of crystal shapes contingent upon the conditions of crystal growth, with the tomahawk shape being the most prevalent (Figure 5). These crystals exhibit monoclinic sphenoidal characteristics, possessing a sole axis of symmetry, trapezoidal side faces, rhombic tops and bottoms, and beveled faces at the base and apex, resulting in a distinctive tomahawk shape (Hunziker & Nissen, 1926). Notably, these crystals exhibit hardness, brittleness, and slow dissolution.

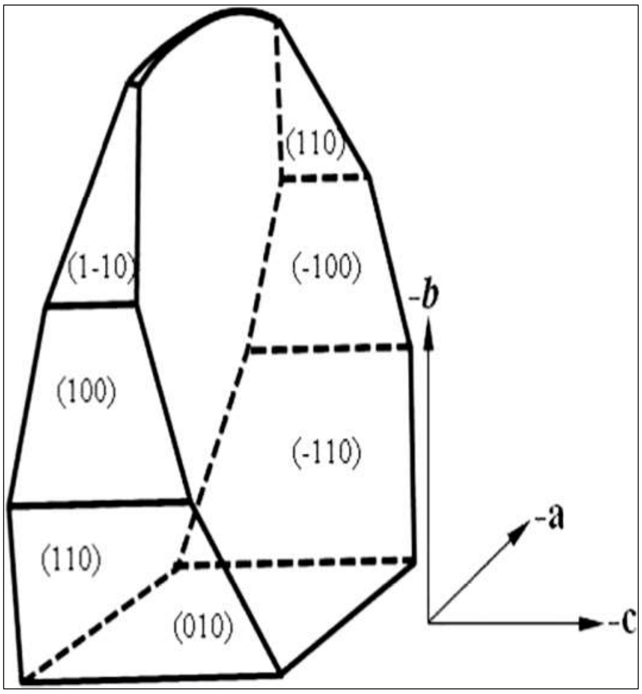

Figure 5: Tomahawk shaped crystal of α-Hydrate showing faceted structure (Wong & Hartel, 2014)

The unstable anhydrous α-form can be synthesized by dehydrating α-lactose monohydrate under vacuum conditions at temperatures ranging from 65 to 93.5 °C and remains stable only in the absence of water (Fox, 2009). Conversely, the stable anhydrous α-form is produced by subjecting α lactose monohydrate to elevated temperatures between 100 – 190 °C. This particular lactose form exhibits non-hygroscopic characteristics and greater solubility compared to other forms.

Crystallization of β-anhydride lactose crystals occurs through the precipitation from a solution exhibiting supersaturation, typically achieved at temperatures exceeding 93.5 °C. Notably, β-lactose exhibits reduced solubility beyond this threshold temperature and is distinguished by its markedly enhanced sweetness when compared to α-lactose (Fox, 2009). Additionally, the crystalline morphology of β-lactose is characterized by a distinctive asymmetrical diamond shape (Listiohadi, Hourigan, Sleigh, & Steele, 2009).

2A.2.6 Properties of amorphous sucrose

Amorphous sugars can be aptly characterized as exhibiting a glassy, vitreous, rubbery, or concentrated system. These forms lack well-defined molecular conformation and are distinguished by short-range molecular order. Various methods contribute to the production of amorphous sugars, including dry-milling, quenching of the melt, rapid drying through processes such as spray-drying, freeze-concentration of aqueous solutions coupled with freeze-drying, as well as boiling or extrusion of highly concentrated solutions followed by subsequent cooling (Reiser, 1994; Yu, 2001). Similar to crystalline sucrose, amorphous sugars possess unique properties. The density of amorphous sucrose, as reported previously, is 1507.7 kg/m^3 (Plato, 1901; Reiser, 1994) however, this value may vary depending on the specific method employed for amorphous sugar production.

Furthermore, the specific heat of amorphous sucrose was determined to be approximately 1.432 kJ/kg K within the temperature range of 22 to 25°C. Differential scanning calorimetry (DSC) was utilized to ascertain critical parameters such as the glass transition (Tg), recrystallization (Tc) induced by moisture sorption, and melting (Roetman & Buma, 1974) of the rearranged amorphous sucrose subjected to varying equilibrium relative humidities (Roos & Karel, 1990).

2A.2.7 Properties of amorphous lactose

The amorphous state of lactose represents a metastable condition, existing either in a glassy, solid state or a syrup-like, super-cooled liquid state, wherein a distinct long-range molecular organization defining crystallinity is absent. Rapid drying of a lactose solution results in an abrupt increase in viscosity, precluding sufficient time for crystallization and facilitating the formation of amorphous lactose. The structural characteristics of this amorphous form are anticipated to undergo alterations in response to temporal, thermal, and water content variations (Bosma, Brinke, & Ellis, 1988; Roos & Karel, 1991; Slade, Levine, & Reid, 1991). Due to the absence of a well-defined long-range molecular arrangement, molecular mobility, and intermolecular distances are elevated in the amorphous state, resulting in higher potential energy relative to the crystalline state.

Remarkably hygroscopic, amorphous lactose exhibits a propensity for stickiness, posing challenges during the handling and storage of numerous food items (Aguilera, del-Valle, & Karel, 1995). Consequently, the existence of amorphous lactose in foods is undesirable, as the adhesive properties significantly impact storage stability and overall product quality. Hence, proactive measures are imperative to induce lactose crystallization in a pre-crystallization step, mitigating potential complications.

2A.3 Factors affecting for sugar crystallization

Sugar crystallization is influenced by a myriad of factors encompassing both process parameters and solution properties. These variables include the degree of supersaturation, temperature of the solution, presence of impurities, and the pH.

2A.3.1 Temperature and supersaturation

The saturation level of the massecuite plays a crucial role in the process of sugar crystallization. It serves as a significant quality indicator as it determines the point at which seeding occurs, influences the quality of the crystals, and impacts the overall production cost (Umo & Alabi, 2016). The sucrose solution necessitates concentration until the metastable zone is attained, as illustrated in Figure 6. At this juncture, the sucrose solution achieves a state of supersaturation to a certain extent, and the introduction of externally added sugar fines serves as a catalyst for crystallization. These externally added sugar fines function as nucleation sites, initiating the process known as secondary nucleation. However, in the labile zone, characterized by a highly supersaturated state, spontaneous nucleation takes place. Due to the solution's supersaturation, the reduced presence of water molecules hinders the formation of bonds with all hydroxyl groups in the sucrose molecules. Consequently, sucrose-sucrose hydrogen bond formation ensues (Mathlouthi & Genotelle, 1998).

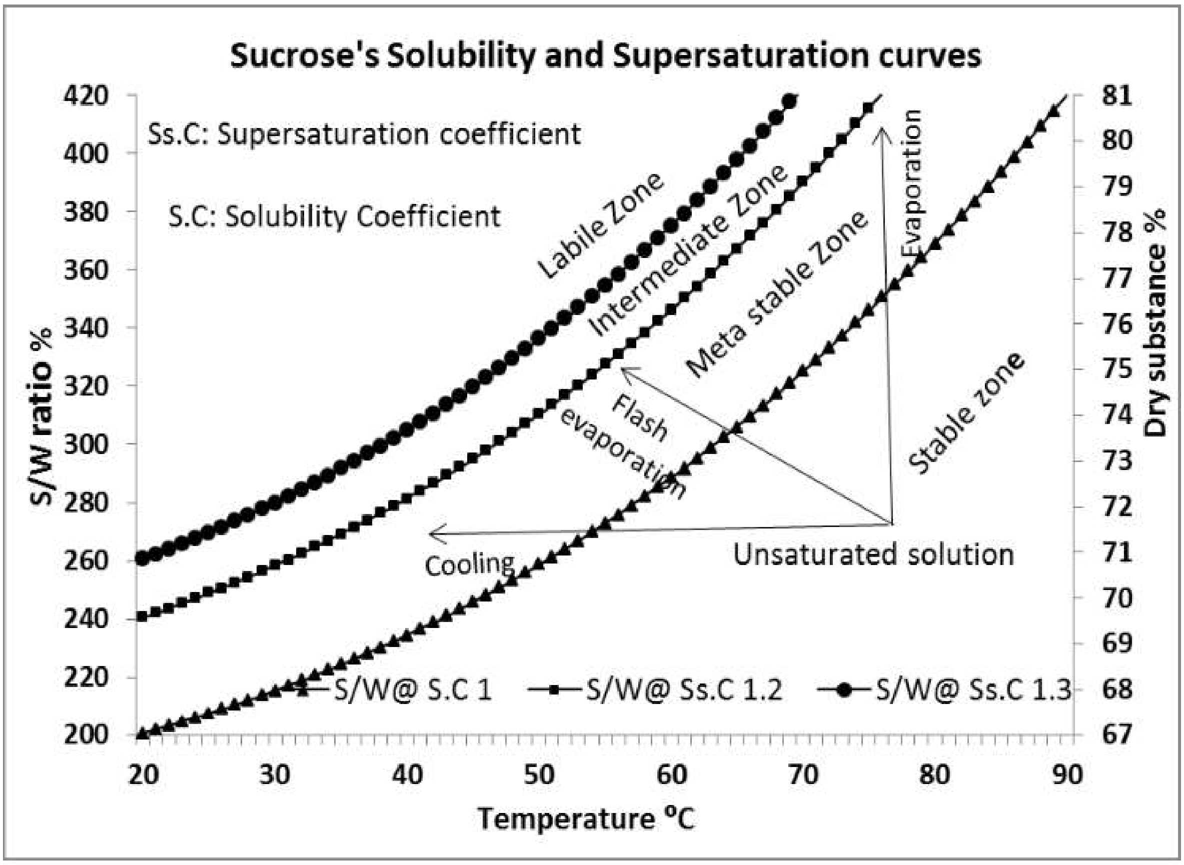

Figure 6: Sucrose solubility and supersaturation curves as a function of temperature (Temerk, El-Abdein, Abdullah, & Amin, 2014)

The lactose solution supersaturation can be achieved through either augmenting lactose concentration via evaporation or lowering the solution temperature, exploiting the reduced solubility of lactose in water at temperatures under 15 °C. Extensive research has been conducted to investigate the qualitative and quantitative impact of supersaturation and temperature on lactose crystallization (Jelen & Coulter, 1973b; Raghavan, Ristic, Sheen, & Sherwood, 2001; Twieg & Nickerson, 1968; Wong, Bund, Connelly, & Hartel, 2012). These investigations have consistently revealed that supersaturation serves as the principal determinant directly influencing the growth rate, with an observed augmentation correlating to an increase in supersaturation (Valle-Vega & Nickerson, 1977; Visser, 1982). Nevertheless, the influence of temperature is notably interactive, with optimal temperatures being contingent upon the desired crystal size obtained (Butler, 1998; Jelen & Coulter, 1973).

2A.3.2 pH

Mutarotation, the process of interconversion between different anomers of a sugar molecule, is influenced by the pH level of the solution. In the case of high pH levels ranging from above 7 to 10, mutarotation is accelerated, creating favorable conditions for efficient crystallization. Similarly, low pH levels, below 1, also enhance mutarotation and promote crystallization. However, within the pH range of 4 to 7, no significant influence on mutarotation and crystallization was observed (Huppertz & Gazi, 2016; Jelen & Coulter, 1973a). In contrast, in high alkali conditions, the formation of degradation products of lactose was catalyzed by pH levels exceeding 10. These degradation products, in turn, may hinder the crystallization process (Gänzle et al., 2008; Nickerson & Moore, 1974). Therefore, it is evident that the pH level of the solution plays a crucial role in mutarotation and the subsequent crystallization process.

2A.3.3 Impurities

The crystallization kinetics of sugars are significantly influenced by the presence of additional components in a sugar solution, which leads to alterations in solubility, nucleation, and crystal growth (Botsaris, 1982). The solubility changes directly impact the level of supersaturation in the solution, consequently influencing both nucleation and crystal growth (Mullin, 1979). The impact of other solutes on sugar behaviour will be reviewed in the next chapter.

2A.4 Analytical methods used in sugar characterization

The significance of understanding sugar crystallization processes, quantifying crystal forms, analyzing thermal characteristics and structural changes, as well as identifying solution phase properties and alterations observed during food processing, is progressively acknowledged. Some of the primary analytical tools employed for the identification and characterization of sugars include differential scanning colorimetry (DSC), Fourier transform infrared spectroscopy, high-performance liquid chromatography, X-ray diffraction, and refractometry.

2A.4.1 Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) serves as a thermal analytical method designed to quantify the disparity in heat flow or temperature variance between a test sample and the reference. This is achieved by subjecting both the sample and reference to temperature variations, typically at a constant rate, and observing the resulting effects. This technique has been widely used in the food and pharmaceutical industry to analyze a range of thermodynamic and transient transitions, as depicted in Figure 7. These transitions encompass the crystallization temperature (Chandrapala, Wijayasinghe, & Vasiljevic, 2016), crystallinity (GombÁs, Szabó-Révész, Kata, Regdon, & Erős, 2002; Saffari & Langrish, 2014), melting temperature (Beckett, Francesconi, Geary, Mackenzie, & Maulny, 2006; Bhandari & Hartel, 2002; Drapier-Beche, Fanni, & Parmentier, 1999), glass transition temperatures (Omar & Roos, 2007), water dehydration of sugars (Raut et al., 2011; Wijayasinghe et al., 2015), polymorph determination (GombÁs et al., 2002). In the majority of cases, these alterations are linked to either first-order or second-order phase transitions. The latter pertains to the glass transition, whereas the former may encompass the melting and/or crystallization of solutes, such as lactose, along with the denaturation/renaturation of proteins.

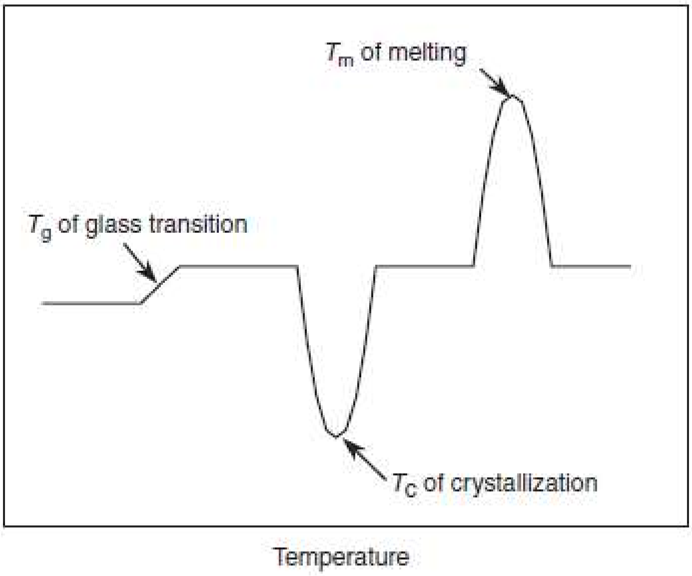

Figure 7: A schematic DSC curve demonstrating the appearance of phase transitions including glass transition, crystallization, and melting (Fuquay, McSweeney, & Fox, 2011)

Differential scanning calorimetry (DSC) has been used to investigate the melting behaviour of sucrose. Bhandari and Hartel, (2002) conducted a study on the co-crystallization of sucrose with either glucose or fructose, and during their analysis, they noticed a minor peak preceding the main melting endotherm of sucrose. They proposed that this phenomenon could be attributed to either the devitrification of amorphous substances or the dissolution of the crystal surface caused by residual moisture (Bhandari & Hartel, 2002). In their research, Beckett et. al. (2006) examined the melting characteristics of sucrose both with and without the inclusion of mineral salts. Their findings indicated that the presence of salts led to a decrease in the melting peak (Beckett et al., 2006).

The DSC analytical technique has been employed by numerous researchers in the examination of the glass transition temperature of sugars. The glass transition temperature (Tg) refers to the specific temperature at which an amorphous material undergoes a change in its physical state, transitioning from a glassy solid state or a syrup-like, super-cooled liquid state to a rubbery state (Roos, 2010). Islam and Langrish, (2010) conducted a study on the crystallinity of lactose using DSC. They investigated how the crystallinity of lactose changes with variations in the inlet gas temperature during the process of spray drying. In the crystalline α-lactose monohydrate sample, no crystallization peak (exothermic) was observed, as anticipated for a fully crystalline material. However, an endothermic peak appeared at approximately 140 °C, indicating the loss of crystalline water and confirming its 100% crystallinity as observed by Gombas et al., (2002) (GombÁs et al., 2002; Islam & Langrish, 2010). By analyzing the thermal behaviour of sugars, researchers can gain insight into its physical and chemical properties, which can have important implications for the food and pharmaceutical industries. Therefore, DSC has become an essential tool for researchers in these fields to deepen their understanding of the thermal properties of sugars.

2A.4.2 Fourier transform infrared spectroscopy (FTIR)

Infrared spectroscopy stands out as one of the extensively utilized spectroscopic techniques, focusing on measuring both the wavelength and intensity of infrared light absorbed by a given sample (Luykx & Van Ruth, 2008). This methodology facilitates the identification of functional groups within a sample, as each functional group possesses a distinctive vibrational frequency. Various factors, such as the mass of the atoms, the bond strength, the spatial arrangement of the atoms, and the duration of the vibrational coupling, can further influence the vibrational frequency of a chemical bond (Karoui, Downey, & Blecker, 2010). Therefore, every compound possesses a distinct IR spectrum, making it unique. The infrared spectrum can be categorized into three spectral regions: near-infrared (NIR), mid-infrared (MIR), and far-infrared, each named in relation to the visible spectrum. These regions encompass the higher-energy near-infrared (NIR) ranging from approximately 14,000 to 4000 cm^{-1}, the mid-infrared (MIR) spanning from about 4000 to 400 cm^{-1}, and the far-infrared covering the range of approximately 400 to 10 cm^{-1}. The commonly used regions for food analysis include NIR and MIR. In comparison to the MIR spectra, NIR exhibits a lower structural selectivity due to the superposition of numerous overtone and combination bands. The MIR region is capable of revealing the structure and conformation of organic molecules. Moreover, the position of bands in the FTIR spectrum (MIR range) is sensitive to the surrounding environment, providing additional insights into the structure and intra- and intermolecular bonding. This characteristic has rendered FTIR spectroscopy a valuable tool for investigating changes in the structure and interactions of food components, particularly lactose, proteins, and lipids, induced by various physicochemical conditions during processing (Kher, Udabage, McKinnon, McNaughton, & Augustin, 2007). Therefore, FRIR is considered a rapid and simple method that examines the molecular vibrations exhibited by diverse compounds. Typically, the spectral range of 1,200 to 800 cm^{-1} is employed to assess the phase transitions of carbohydrates, as the stretching and vibrations of C-C and C-O bonds are affected by their physical state. Similarly, the region between 3,600 and 3,200 cm^{-1} is utilized to examine the water structure surrounding carbohydrate molecules (Ottenhof, MacNaughtan, & Farhat, 2003). However, the precise position and configuration of these bands can be influenced by the existence of solutes, hydrogen bonding, and temperature, potentially complicating the identification of other components within the system.

2A.4.3 High performance liquid chromatography (HPLC)

HPLC, also known as high-performance liquid chromatography, is an analytical technique utilized for the purpose of separating, identifying, and quantifying the various elements present in a mixture. This technique involves introducing the sample mixture into a mobile phase, which then passes through a column. As the components pass through the column, they interact with the stationary phase. The separation of components is dependent on the type of stationary phase employed, which can be chosen based on factors like the chemical nature of the components, their molecular mass, charge, and other relevant properties. In HPLC, various detectors can be employed, however, refractive index detector (RI detector) is the one mostly used in sugar analysis (Luykx & Van Ruth, 2008). HPLC is a widely used method in food analysis for quantifying compounds present in food. For instance, HPLC has been used effectively to investigate the constituents of sugarcane molasses. This method allows for the simultaneous measurement of fructose, glucose, and sucrose levels in the molasses. To achieve this, maltose is used as an internal standard, and a refractive index detector is incorporated into the analytical technique (Xu, Liang, & Zhu, 2015). Similarly, extensive research has been conducted on the hydrolysis of sugars in the presence of various agents, and this investigation has been widely explored using HPLC. A comprehensive review of these studies is available in the existing literature (Ball, 1990; Betschart & Prenosil, 1984; Richmond, Barfuss, Harte, Gray, & Stine, 1982).

2A.4.4 X-Ray diffraction (XRD)

One of the primary analytical instruments employed for the identification and quantification of crystal forms is powder X-ray diffraction (XRD). The examination of increasing intensities and areas of peaks in X-ray diffraction (XRD) patterns through X-ray diffractometry is a crucial method for observing the characteristics of crystalline solid materials, polymers, and food materials which is useful in determination and gather qualitative and quantitative information of the material (Drapier-Beche, Fanni, & Parmentier, 1998; Jouppila, Kansikas, & Roos, 1997). X-ray diffraction patterns play a crucial role in the analysis of crystalline states and crystal structures in various food applications, such as starch and sugars. Additionally, they aid in identifying the presence of crystallinity in partially amorphous food components. This technique involves subjecting a powdered sample to X-rays emitted from different angles in a scanning fashion. Genuine crystalline states exhibit distinct and well-defined peaks of reflection originating from the lattice structure, whereas amorphous food materials lack any discernible X-ray diffraction patterns (Saffari & Langrish, 2014). The prominent representative peaks for crystalline carbohydrates are designated at 2θ = 12.5°, 16.4°, 20.0°, and 20.1° for α-lactose monohydrate; 10.5°, 20.9°, and 21.0° for anhydrous β-lactose; 19.1°, and 21.1° for the blend of anhydrous α:β with a molar ratio of 5:3; and 19.5° for the blend of anhydrous α:β with a molar ratio of 4:1 (Drapier-Beche et al., 1998; Jouppila et al., 1997; Saffari & Langrish, 2014; Wijayasinghe, Bogahawaththa, Chandrapala, & Vasiljevic, 2020).

2A.5 Conclusion

Sugars are universally acknowledged as an essential element of human life. Constituting the elementary structural units of carbohydrates, these sugars play a pivotal role in furnishing the body with its primary source of energy to facilitate various metabolic functions. While extensive research has delved into the properties of sugars, with a particular emphasis on lactose and sucrose, the ever-evolving understanding of their significance continues to be a catalyst for new and ongoing studies. The ongoing exploration of these sugars underscores their enduring relevance and prompts a continual investigation into their multifaceted roles and implications within the realm of human nutrition and physiology.

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Chapter 2B: Effects of solutes on solution phase properties and crystallization behaviour of sugars

This chapter was prepared as a manuscript. This review extensively examines the physical and chemical characteristics of simple sugars. Throughout the analysis, deliberate attempts were made to comprehend the impact of solutes on the behaviour of lactose and sucrose in various food systems, as well as their interactions with the sugars.

Abstract

The effect of solutes on sugars is a fascinating area of study within the field of food science and technology. As an essential component of milk, lactose plays a crucial role in various food products. Sucrose on the other hand is the predominant form of sugar utilized and remains the most commonly employed sweetening agent in the food industry. Since, sugars are mostly present in combination with other solutes such as acids, salts, and proteins in the food systems, understanding the effect of solutes is of great importance for food manufacturers aiming to optimize product quality, texture, stability, and improve the processability. This literature review will delve into the current research and advances in the properties of sugars in the presence of different solutes.

1. Introduction

Sugars play a crucial role in various industries, including food and pharmaceuticals. Sugars are of utmost importance in the food industry as they significantly influence the composition and performance of food products, contributing to specific size and textural properties. The type and amount of sugar used greatly influence the functional properties of bakery, dairy, cereals, and beverage products. The distinct physical properties of different sugars in food, including solubility (Gänzle, Haase, & Jelen, 2008), crystallization (Roos, 2009; Schuck, 2011; Vaccari & Mantovani, 1995), melting (Roos, 1993), amorphosity (Shalaev, Franks, & Echlin, 1996), moisture absorption capacity, and powder flow characteristics, can have a significant impact on the processing, storage, and utilization of food and its ingredients (Hartel & Shastry, 1991). These various physical properties at play are intricately regulated by diverse solutes within the system. Sugars, typically integral components of food systems, exist in conjunction with an array of other constituents, including salts or minerals, acids, additional sugars, proteins, and various others. The interaction of these solutes with sugar molecules and water significantly influences the overall behaviour of sugars, thereby contributing to the complex interplay of factors that shape the dynamic properties of the system. For instance, whey, being the predominant source from which lactose is derived, is a complex matrix encompassing a multitude of components, including proteins, acids, salts, and vitamins (Table 1).

Table 1. Approximate composition of liquid whey by type (P Jelen, 2009)

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 complex composition of a lactose solution, which is affected by these diverse constituents, significantly affects the rate at which lactose crystals form. This impact is particularly evident in changes in solubility dynamics, nucleation processes, and the subsequent growth patterns of lactose crystals (Wong & Hartel, 2014). The complex interactions among these components within the lactose solution play a crucial role in understanding and controlling the crystallization behaviour of lactose (Botsaris, 1982). Additionally, the extraction and refinement processes of sucrose commonly involve sourcing it from either cane or beet sugar extracts, both of which harbor a multitude of non-sugar components. For instance, sodium, potassium, amino-nitrogen, and invert sugar constitute inherent components of the sugar beet root, categorized as impurities (Campbell & Fugate, 2015). These non-sugar constituents contribute to the complexity of the extraction process, and their presence necessitates meticulous refining procedures to isolate sucrose in its pure form. Similarly, sucrose is found in carbonated soft drinks, coexisting with various acids and salts. The interaction of these components plays a pivotal role in influencing the behaviour of sucrose within sugar-sweetened soft drinks, potentially inducing structural deviations in the sucrose molecule. The complex interaction among sucrose, acids, and salts contributes to the overall composition and characteristics of these beverages.

Hence, understanding the effect of solutes on sugar properties is essential for optimizing processes and developing new products. Therefore, this article explores the impact of different solutes, such as salts, acids, and other food components on the behaviour and properties of sugars.

2. The influence of solutes on solution phase properties

Comprehending the dynamics of sugars in the solution phase is imperative for formulating accurate parameters for the crystallization process and achieving a final product of optimal quality. The interactions among sugar, water, and additional constituents assume paramount significance in elucidating the fundamental factors contributing to the challenges encountered within the industry. Broadly, interactions between simple sugars and acids induce alterations in the physicochemical and physiological attributes of sugar solutions, resulting in distinct behaviours and, in numerous cases, operational challenges. Acid whey, for example, has surfaced as a challenging stream for treatment due to its incapacity to attain an adequate amount of crystalline lactose. Consequently, during the concentration process, lactose persists in its non-crystalline state/ amorphous form, hindering the progress of subsequent processing stages (Wijayasinghe, Bogahawaththa, Huppertz, Chandrapala, & Vasiljevic, 2019; Wijayasinghe, Vasiljevic, & Chandrapala, 2015, 2016). A recent investigation into sucrose degradation in the presence of constituents found in sugarcane juice indicates that the presence of organic acids has a mitigating effect on degradation, attributed to a buffering mechanism. Additionally, invert sugars, flavonoids, and minerals exhibit a catalytic influence on sucrose degradation, coinciding with a pronounced decline in juice pH (Marasinghege, Rackemann, Bartley, & Doherty, 2022). Therefore, the precise regulation of physicochemical properties in the solution phase is imperative to achieve the ultimate product with the intended characteristics. The impact of some solutes on solution phase properties are summarised in Table 2.

2.1 Supersaturation and solubility

A supersaturated solution is defined by a solute concentration exceeding its solubility limit. Thermodynamically, a phase transition happens when the chemical potential of the supersaturated solution differs from that of the equilibrium solution, where solute concentration equals solubility (Caballero, Trugo, & Finglas, 2003). This potential difference acts as the driving force for the transition, depending on the disparity between the concentrations and solubility calculated at the same temperature and pressure, termed supersaturation (Schuck, 2011). The quantity of sugar required for complete dissolution and saturation of the solution is highly dependent on the purity level of the solution. Therefore, the impurities present in the solution play a critical role in the supersaturation and the solubility (Rozsa, 2000). Many researchers investigated the impact of different solutes on the solubility behaviour of sugars. For instance, previous studies have substantiated the positive impact of impurities on lactose solubility, indicating an augmentation in the propensity of lactose to dissolve in the presence of calcium nitrate, bromide, or chloride (Nickerson & Moore, 1974b). The effect of phosphates on solubility has contradicting findings. Guu and Zall, (1992) identified phosphates as additional agents that contribute to the elevation of lactose solubility (Guu & Zall, 1992). The addition of potassium phosphate has found as components that increase the solubility of lactose confirming the previous results (Bharhava & Jelen, 1996). In contrast, the presence of sodium phosphate in lactose solutions reduced the solubility of lactose (Smart, 1988; Smart & Smith, 1992). Alterations in solubility can be attributed to the impact of impurities on the structural arrangement of bulk water (Von Hippel & Schleich, 1969). For instance, ions characterized by low charge density, such as Cl^-, Br^-, and I^-, induce perturbations and structural disruptions in water molecules. Conversely, ions with high charge density, including Ca^{2+}, Ba^{2+}, Sr^{2+}, and

Li^{2+}, have the potential to prompt a reorganization of the water structure within lactose solutions (Bharhava & Jelen, 1996). A contemporary investigation into the behavior of lactose in the presence of salts has illuminated that the presence of KCl and NaCl results in an elevation of lactose solubility in comparison to its solubility in a pure lactose environment (Wijayasinghe, Vasiljevic, & Chandrapala, 2023). This phenomenon highlights the complex interaction between ions and water molecules, elucidating the diverse effects that ions of varying charge densities can exert on the solubility behaviour of lactose in solution. The interaction of water molecules with other solutes in the system is contingent on the water affinity of these solutes. This interaction leads to a decrease in the availability of water molecules to interact with lactose, consequently impacting the solubility of lactose.

Table 2. A summary of the impact of impurities/solutes on sugar behaviour in solution phase

Impurity/solute	Sugar's phase/stage of impact	The impact	Reference
Sucrose	Solubility	The presence of sucrose caused a decrease in the solubility of lactose.	(Hunziker & Nissen, 1926; Hunziker & Nissen, 1927)
Dextran	Solubility	Dextran has insignificant impact on the sucrose solubility/Slight decrease in solubility.	(Borji, Borji, & Jourani, 2019)
Glycerol and Propylene glycol	Solubility	Solubility of sucrose hindered in presence of Glycerol and Propylene glycol	(Fey, Weil, & Segur, 1951)
$CaCl_2$, MgCl2, KCl, NaCl	Solubility	The solubility of lactose increased in presence of all four salts.	(Wijayasinghe et al., 2023)
		The viscosity of sucrose found to increase in the presence of all four salts in the order of $K^+ < Na^+ < Ca^{2+} < Mg^{2+}$	(Reiser, 1994)
Alcohols	Solubility	Reduces the solubility of lactose	(Gänzle et al., 2008)
Lactic acid, phosphoric acid and citric acid	Solubility	The solubility of lactose increased in concentration dependant manner	(Wijayasinghe, Bogahawaththa, Chandrapala, & Vasiljevic, 2020)
Sucrose	Mutarotation	The mutarotation of fructose directly proportional to the amount of sucrose in the solution	(Nelson & Beegle, 1919)
Glucose and fructose	Hydrolysis	The presence of glucose or fructose in a cane sugar solution hinders the speed at which it undergoes hydrolysis by invertase.	(Nelson & Beegle, 1919)

It has been found that the effect of pH on the solubility of lactose is insignificant (Smart, 1988). Nevertheless, compounds with surface-active properties tend to exert a discernible influence on the physicochemical behaviour of lactose, indicating potential ramifications for its crystalline structure or solubility properties (Hartel, 1993; Polyanskii, 1987). Furthermore, the elevated concentrations of sucrose and glucose have the potential to markedly diminish the solubility of lactose, thereby fostering an accelerated rate of crystallization. Notably, empirical evidence suggests that a sucrose concentration of 70% can lead to a substantial reduction in lactose solubility, reaching only 42% of its solubility in pure water (Nickerson & Moore, 1974a). Apart from that, solutions containing reducing sugars such as lactose, glucose, and fructose, the existence of different anomers may exert an influence on the solubility of each respective anomer. For instance, in the case of lactose, research indicates that the presence of β -lactose in solution negatively impacts the solubility of α-lactose (Visser, 1982).

The presence of impurities, such as Na, K, and Mg, has been observed to affect the crystallization and solubility of sucrose. The impact of these impurities is contingent upon both the type and concentration of impurity within the solution. When the impurity level is below 10% in the solution, it leads to a decrease in the solubility of sucrose. The presence of additional components in the sucrose solutions can either enhance or hinder the solubility of sucrose, depending on the strength of interactions between the molecules in the solution. This is a complex phenomenon and thus requires further investigation and research about the interactions between solutes and solvents in order to gain a deeper understanding.

The presence of substances like corn syrup, invert sugar, gelatine, proteins, and starches has been observed to hinder the supersaturation concentration of sucrose (Hartel & Shastry, 1991). It is evident that sugars typically induce a reduction in solubility, although certain salts may initially elevate solubility. Furthermore, food constituents like proteins and starches exhibit a propensity to decrease the solubility of sucrose akin to other sugars. These alterations in solubility are linked to the molecular interactions between solutes and water. Generally, it is postulated that solutes forming hydrates in solution tend to diminish the solubility of sucrose, whereas solutes without hydrate formation tendencies tend to enhance sucrose solubility. Extensive research has been conducted to examine the alteration in solubility equilibrium of sucrose when raffinose is present. and the results have been thoroughly documented (Smythe, 1967). Raffinose, a natural impurity found in sugar beets, has a crucial impact on the behaviour of sucrose during beet sugar refining. Moreover, it has been reported that the concentration of invert sugars has an inverse relationship with the equilibrium solubility of sucrose (Hartel & Shastry, 1991).

2.2 Mutarotation

Lactose is a reducing sugar and thus, partially exists in an open-chain configuration featuring an aldehyde group capable of engaging in hemiacetal formation, leading to the establishment of a ring structure. This hemiacetal formation introduces a novel chiral centre (asymmetric carbon), existing in two enantiomorphs denoted as α or β. These two forms of lactose molecule interconvert between α and β anomers through the open chain of the glucose moiety which is known as mutarotation. The establishment of mutarotation equilibrium is significantly influenced by the solution's pH, with the rate of this process being particularly sensitive to variations in pH levels. The minimum in mutarotation rate is observed at pH 5, with notably rapid mutarotation occurring particularly at pH levels below 2 and above 7 (Holsinger, 1997; Huppertz & Gazi, 2016). The minimum value of pH for the mutarotation rate was also reported as a range of 2.5–7.5 (Fox, 2009). It is noteworthy that while the pH of the solution plays a crucial role in governing the kinetics of mutarotation, it does not exert any apparent impact on the resultant equilibrium value of this dynamic process. In the presence of weak acids, bases, and lactate ions the mutarotation rate of lactose in solution increased (Herrington, 1934a). Potassium chloride (KCl) was observed to exert a marginal decelerating influence on the mutarotation rate, while alternative salts, including ammonium chloride, potassium acetate, and ammonium acetate, demonstrated a catalytic effect (Hartel & Shastry, 1991). Research conducted to simulate the compositional characteristics of whey reported an increase in the mutarotation rate in the presence of salts. This study reported that the presence of salt in the solution exhibited a catalytic effect on the mutarotation, approximately doubling the reaction rate, while maintaining the unchanged reaction mechanism (Haase & Nickerson, 1966).

In the presence of high concentrations of sucrose in the in the food system, the rate of mutarotation have found to decrease (Harper, 1992). The presence of sucrose in combination with salts in the system exerts contrasting influences on the kinetics of mutarotation. For example: the presence of citrate and phosphates in milk, at the concentrations typically found, enhances the mutarotation process, causing it to occur at a rate twice as fast as in pure water. Conversely, concentrated sucrose has a contrasting effect, as it hinders the mutarotation reaction. However, this effect is only minimal until a sucrose concentration of 40% (w/v) is reached. Beyond this level, the mutarotation rate decreases, and the catalytic influence of milk salts is counteracted. (Holsinger, 1988). On the other hand, amino acids have been identified as mild catalysts in facilitating the mutarotation of reducing sugars, as reported in the literature (Shallenberger, 1984).

In the study conducted by Kaanane and Labuza (1989), it was determined that various salts, including $FeCl_2$, $CaCl_2$, $CoCl_2$, KCl, or NaCl, did not exhibit a noteworthy impact on the mutarotation dynamics of glucose or fructose. This observation underscores the resilience of the mutarotation process in the presence of these specific salts, as elucidated by the findings of the aforementioned study (Kaanane & Labuza, 1989). The influence on the tautomeric equilibrium of sugar varies depending on the presence of complex-forming configurations and the interaction with ions such as Ca^{2+}, Mg^{2+}, Na^{+}, and K^{+}. Previous studies in the literature have indicated that Ca^{2+} induces a shift in the tautomeric equilibrium of D-glucose towards the α-anomer. For instance, the addition of 1% $CaCl_2$ to hydrolyzed concentrated whey resulted in a slight reduction in the browning rate (Buera, Chirife, & Resnik, 1990). This effect has been attributed to the fact that the complexation reaction between Ca^{2+} and the sugar led to a shift in the anomeric equilibrium, with a more pronounced effect observed in the absence of protein.

Apart from solutes present in the system, the choice of solvent (D_2O or H_2O) played a crucial role, indicating a significant solvent-dependent influence on the kinetics of mutarotation.

3. Crystallization

Sugar crystallization is a process in which sugar molecules transition from a dissolved state in a liquid solution to a solid crystalline form. This phenomenon occurs as the solubility limit of sugar in the solution is exceeded, the sugar molecules interact with each other leading to the formation of sugar crystals. Crystallization is commonly conceptualized as a two-step mechanism comprising nucleation and crystal growth. The initiation phase, involving the formation of minute crystals or nuclei, is termed nucleation. Subsequent growth of these nuclei is contingent upon the prevailing level of supersaturation in the system (Agrawal & Paterson, 2015). This crystallization process is influenced by a range of operational parameters and solution attributes, encompassing factors such as supersaturation, temperature, viscosity, pH, and the existence of impurities (Gänzle et al., 2008; Mimouni, Schuck, & Bouhallab, 2005; Rjabova et al., 2013; Wijayasinghe et al., 2016). Table 3 provides an overview of the influence of impurities/solutes on the crystallization process of sucrose and lactose

Table 3. A summary of the impact of impurities/solutes on the crystallization process of sucrose and lactose

Impurity/solute	Sugar’s phase/stage of impact	The impact	Reference
Calcium phosphate	Nucleation	Calcium phosphate did not catalyse secondary nucleation in a lactose solution with a concentration of 50% w/w of anhydrous lactose solution.	(Kauter, 2003)
Whey proteins	Nucleation	The formation of localized areas with high lactose concentration promotes the occurrence of nucleation, leading to the generation of nuclei and impeding the growth of larger crystals.	(Mimouni et al., 2005)
β lactose	Nucleation	β lactose act as an inhibitor for nucleation in lactose crystallization.	(Raghavan et al., 2000)
Alcohols	Nucleation	Spontaneous nucleation is promoted	(Gänzle et al., 2008)
K_2HPO_4	Crystal growth	The lactose crystal growth rate was decreased with increase salt concentration.	(Bharhava & Jelen, 1996)
Lactose phosphate	Crystal growth	In the presence of lactose phosphate lactose crystallization was decreased with reduced crystal size.	(Lifran, Vu, Durham, Hourigan, & Sleigh, 2007)
Dextran	Crystal growth	Crystal growth rate was reduced in the presence of dextran.	(Borji et al., 2019)
Polysaccharides	Crystal growth	The rate of sucrose crystal growth is diminished as a result of the heightened viscosity of the mother solution and a decrease in the rate at which molecules are integrated into the developing crystal.	(Maurandi, Vaccari, Mantovani, & Rossi, 1989)
NaH_2PO_4	Crystal growth	Promote the lactose crystal growth	(Guu & Zall, 1992)
Lactic acid	Crystal growth	Lactose crystallization retarded in the presence of lactic acid	(Chandrapala, Wijayasinghe, & Vasiljevic, 2016; Pavel Jelen & Coulter, 1973)
Raffinose, KCl, NaBr	Crystal morphology	The fructose unit of the sucrose bound with the impurity and thereby changes the crystalline habit of sucrose.	(Belhamri & Mathlouthi, 2004)
Fructo-oligosaccharides	Crystal morphology	When incorporated in small amounts into sucrose solutions, fructo-oligosaccharides were observed to stimulate atypical rhombic patterns of growth	(Belhamri & Mathlouthi, 2004)
Polysaccharides - Dextrans	Crystal morphology	Elongated crystal growth was observed in the presence of dextran.	(Bubnik, Vaccari, Mantovani, Sgualdino, & Kadlec, 1992)
Riboflavin	Crystal morphology	Inhibits the process of growth, leading to the development of trapezoidal-shaped plates in lactose.	(van Kreveld & Michaels, 1965)

3.1 Nucleation

The initial stage of lactose crystallization, known as nucleation, is notably influenced by the presence of impurities. For example: in the majority of food items, sugars are typically found in conjunction with various additives such as colorants, flavourings, surfactants, and others. The presence of these additives exerts an influence on the nucleation process in specific food products. These effects stem partially from their impact on sugar solubilities and, in part, from inherent effects on nucleation mechanisms. In certain cases, impurities may impede the formation of nuclei (Gänzle et al., 2008; Raghavan, Ristic, Sheen, & Sherwood, 2001) while in some cases secondary nucleation can be triggered by the presence of impurities, which act as a central point for the subsequent growth of crystals (Mullin, 2001). The initiation of sucrose nucleation experiences suppression in the presence of inverted sugar, as documented by Van Hook (1961). Sucrose demonstrates a significant elevation in critical supersaturation, varying from 30 to 40%, when a 40% impurity (maltose) is introduced. On the other hand, glucose displays a moderate increase in the critical supersaturation necessary for secondary nucleation, falling within the range of 3.5 to 5%. Interestingly, at a 20% addition, maltose is observed to effectively impede the growth of glucose crystals (Horn, 1977). The inhibition of nucleation by impurities or additives may be governed by various mechanisms, encompassing mass transfer limitations to bulk molecular motion, molecular charge effects, and steric hindrance effects (Hartel & Shastry, 1991). Furthermore, secondary nucleation experiences augmentation through the adsorption of certain impurities onto existing crystal surfaces (Mullin, 2001). Similarly, the presence of proteins as an impurity fosters the nucleation step. The hydrophilic characteristics of proteins and their high-water affinity, the proteins within the solution create localized lactose supersaturation points, thereby facilitate the nucleation. This process ultimately leads to the generation of a substantial quantity of small crystals (Mimouni et al., 2005).

3.2 Crystal Growth

The stage of crystal growth, constituting the second step in the process of crystallization, is markedly influenced by the presence of a diverse array of solutes within the system. This influence on crystal growth is linked to the specific interactions and dynamic equilibrium established between the solutes and the crystallizing medium. Understanding these complex relationships is crucial for elucidating the mechanisms governing the progression of crystallization through the crystal growth phase. The presence of metal ions, particularly Pb^{2+}, which have a significant molecular weight, as impurities, leads to a decrease in the rate at which nuclei grow. Consequently, this prolongs the time period between the commencement of nucleus formation and its subsequent enlargement to a size that can be observed (Agrawal & Paterson, 2015). The findings of Jelen and Coulter revealed that the presence of calcium accelerates the crystal growth rate in the absence of other minerals (Pavel Jelen & Coulter, 1973). This acceleration of crystal growth is contingent upon the concentration of $CaCl_2$ within the solution. For instance, when the impurity level exceeds 10% in the presence of $CaCl_2$, the crystal growth rate of lactose experiences a deceleration. This phenomenon is likely attributed to the accelerated surface deposition phase in the crystallization process when salts are present, rather than an enhancement of mutarotation and/or a reduction in lactose solubility (Bharhava & Jelen, 1996). The research outcomes regarding the influence of calcium on the rate of crystal growth present conflicting findings. Herrington observed an elevation in the solubility of lactose, leading to a deceleration in the crystal growth of lactose (Herrington, 1934b), aligning with the findings reported by (Wijayasinghe et al., 2023). In opposition to these findings, (Visser, 1984) reported conflicting results, demonstrating that the presence of CaCl2 increased the crystal growth rate while concurrently enhancing the solubility of lactose. These differences can be attributed to the differences in the experimental conditions and other parameters. Notably, calcium in the form of calcium phosphate exhibited no discernible impact on lactose crystallization kinetics, emphasizing the significance of the specific form of calcium in influencing these kinetics (Smart & Smith, 1992). The growth rate of lactose crystals can be either reduced or enhanced by the salts found in a lactose solution (Michaels & Vankreve, 1966; Smart, 1988). This salt-induced alterations in the crystallization of lactose can also be partially ascribed to variations in pH. This is due to the impact of pH on the mutarotation of lactose, whereby a decrease in pH leads to a decline in crystal growth (Nickerson & Moore, 1974b).

The presence of lactic acid seems to function as an inhibitory factor, impeding the crystallization process (Gänzle et al., 2008; Huppertz & Gazi, 2016; Pavel Jelen & Coulter, 1973; Wijayasinghe et al., 2020). This inhibitory effect has been associated with the acid's inability to generate an extremely low pH level (pH < 1), which is conducive to promoting crystallization. This observation underscores the intricate relationship between lactic acid concentration and its impact on the kinetics of crystallization. Moreover, a recent study demonstrated that the introduction of lactic acid prompts the development of a strong hydration layer around lactose molecules. This phenomenon impedes the removal of water molecules and effectively acts as a deterrent to the crystallization process of lactose (Wijayasinghe et al., 2015). The hydration mechanism of lactose pure lactose and in presence of lactic acid is illustrated in Figure 1.

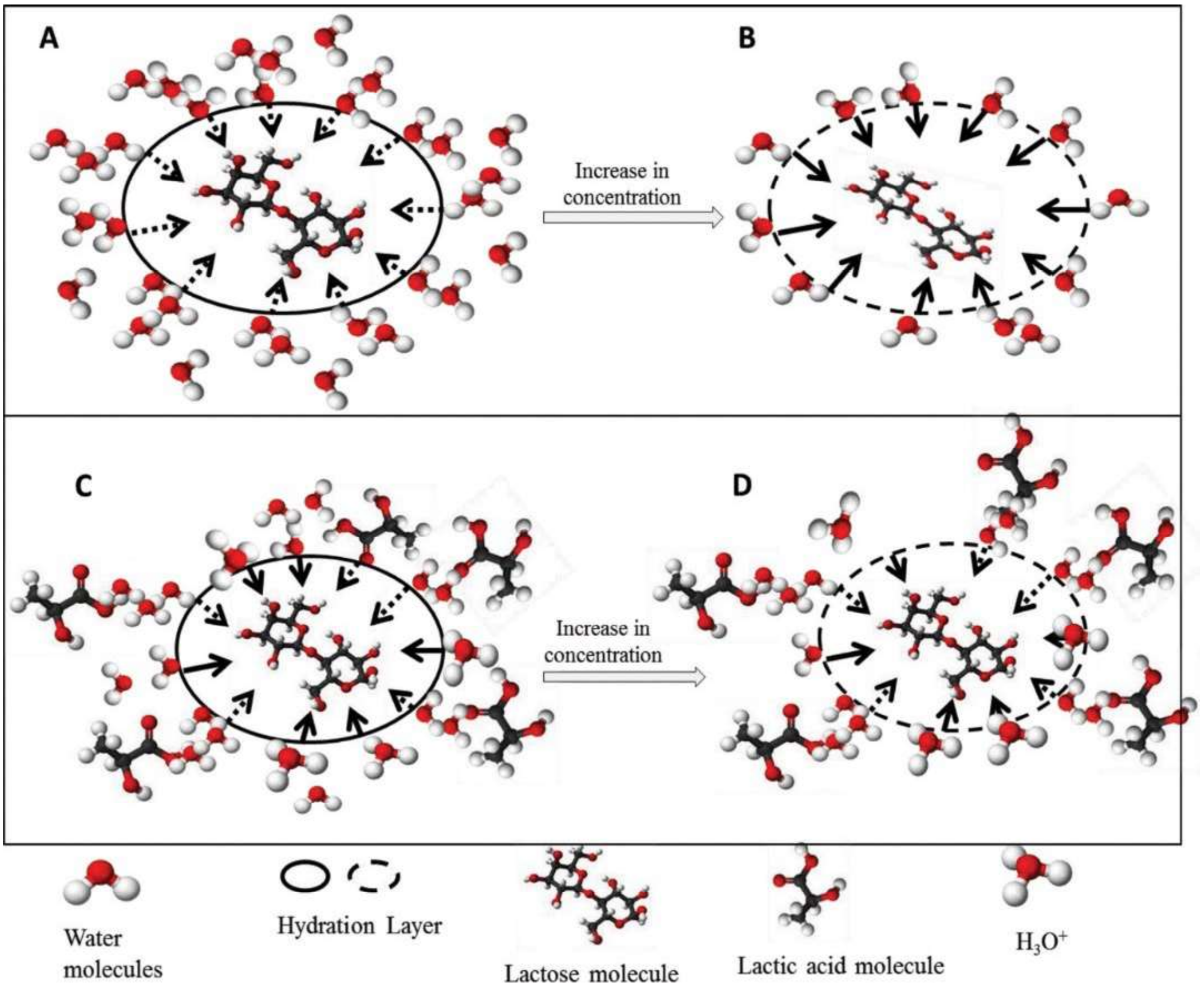

Figure 1. Schematic representation of the lactose hydration mechanism 1 (A and B) of pure lactose solutions and lactose hydration mechanism 2 (C and D) with the addition of lactic acid as a function of concentration. Solid arrows indicate a much stronger attraction, whereas dashed arrows indicate a weaker attraction; a solid circle indicates the stronger hydration layer, whereas a dashed circle indicates the weaker hydration layer (Wijayasinghe et al., 2015).

Moreover, the presence of impurities can result in reduced crystal growth rated due to adsorption onto the rapidly expanding facets of the crystals (Michaels & Vankreve, 1966). Lactose phosphate and riboflavin are among those impurities which were reported to block the growth sites retarding the growth of a particular face. Lactose phosphate is identified as a potent inhibitor for lactose crystal growth, even at lower concentrations of lactose phosphate (Visser, 1980, 1984, 1988). On the other hand, some additives accelerate the growth on specific faces of the crystals (Michaels & Vankreve, 1966).

Crystals of α-lactose hydrate exhibit a diverse range of shapes influenced by the conditions of crystallization. The primary determinant of lactose's crystalline habit is the precipitation pressure, defined as the ratio of the actual concentration to solubility (Herrington, 1934). Under high pressure with rapid crystallization, prisms are the predominant form. As precipitation pressure decreases, crystal morphology shifts to diamond-shaped plates, followed by pyramids and tomahawks, and ultimately, in instances of slow crystallization, fully developed crystals emerge. Nevertheless, the crystalline morphology of lactose crystals is also governed by the presence of other components within the system. For example, lactose monohydrate crystals commonly exhibit a tomahawk shape when cultivated in supersaturated lactose solutions. However, when present in sweetened condensed milk or supersaturated lactose solutions containing 62% sucrose, the crystals deviate from the tomahawk shape and instead resemble short, truncated pyramids with flat rhomboid bases and apices (Hunziker & Nissen, 1927). Moreover, Nickerson also observed that in the presence of impurities, lactose crystals exhibit an irregular and clumped morphology, deviating from the characteristic crystal structure observed in simple lactose solutions (Nickerson, 1962).

The deceleration of lactose crystallization has been documented by increasing the glucose proportion in dulce de leche (DL). This phenomenon is attributed to the viscosity elevation induced by glucose, resulting in a decreased rate of lactose crystal growth (Arobba, Rumbado, & Intorno, 2002; Ferramondo, Chirife, Parada, & Vigo, 1984; Navarro, Ferrero, & Zaritzky, 1999). Furthermore, it was observed that the inclusion of acids, salts, and other sugars diminished the crystallization of sucrose by diminishing viscosity, consequently elevating the diffusion coefficient (Doremus, 1985). An investigation into the co-crystallization of sucrose alongside low molecular weight sugars, like glucose and fructose, demonstrated an inhibitory effect on sucrose crystallization (Bhandari & Hartel, 2002). A comparable impact of lactic acid and calcium on lactose crystallization was observed within a model lactose system (Chandrapala et al., 2016). Likewise, the inclusion of gelatine as an impurity has been observed to possess a hindering effect on crystallization, resulting in a reduced growth rate ranging from one-third to three-fourths of the normal rate, even at low concentrations of gelatine (Michaels & Vankrev, 1966). In highly supersaturated lactose solutions, however, gelatine cannot suppress nucleation, which explains its ineffectiveness in preventing sandiness in ice cream (Nickerson, 1962). The presence of fructose, glucose, and corn syrup inhibited the sucrose crystallization. The crystallization time increased with the concentration of the additive (Laos, Kirs, Kikkas, & Paalme, 2007).

The crystallization of trehalose and sucrose in the presence of salts was investigated by (Longinotti, Mazzobre, Buera, & Corti, 2002). In their study, the researchers deduced that the lower crystallization rates observed in sucrose systems were attributed to the notably high nucleation rate. This finding emphasized the impact of salts on the temperature dependency of the crystallization process. The crystalline habit of sucrose was investigated by (Belhamri & Mathlouthi, 2004) who found an inhibitory effect on various sucrose crystal facets. The influence of raffinose on the morphology of sucrose crystals was ascribed to the harmonious interaction between a raffinose molecule and the structural arrangement of sucrose on various crystal facets (Sgualdino, Aquilano, Tamburini, Vaccari, & Mantovani, 2000)

4. Conclusion

Understanding the effect of solutes on sugar properties is crucial for various industries. The physical, chemical, and sensory attributes of sugars are highly influenced by various components present in food systems such as proteins, acids, and salts. Salts affect the sugar properties through ionic interactions and contribute to the increase in solubility of sugars.

Consequently, it impacts sugar crystallization depending on the type and concentration of salt present in the system. The interactions between proteins and sugars also have a profound impact on the properties of sugars such as viscosity, solubility, crystallization, and the Maillard reaction. The presence of acids in food systems impacts sugar behaviour through structural changes in sugar molecules. Acids also contribute to the formation of inverted sugars, which have unique characteristics in food applications. By considering the role of these solutes, manufacturers can optimize processes and develop products with desired characteristics. Further research in this field will continue to expand our knowledge and lead to innovative applications of sugars in different sectors.

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Chapter 3: Influence of lactic, citric and phosphoric acids on the properties of concentrated lactose solutions

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This chapter has been published as a research article in the journal *Food Chemistry*. Wijayasinghe, R., Bogahawaththa, D., Huppertz, T., Chandrapala, J., & Vasiljevic, T. (2019). Influence of lactic, citric and phosphoric acids on the properties of concentrated lactose solutions. Food chemistry, 293, 247-253.

https://doi.org/10.1016/j.foodchem.2019.04.065

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DECLARATION OF CO-AUTHORSHIP AND CO-CONTRIBUTION: PAPERS INCORPORATED IN THESIS

This declaration is to be completed for each conjointly authored publication and placed at the beginning of the thesis chapter in which the publication appears.

1. PUBLICATION DETAILS (to be completed by the candidate)

Title of Paper/Journal/Book: Influence of lactic, citric and phosphoric acids on the properties of concentrated lactose solutions [redacted]

Surname: Wijayasinghe

First name: Rangani

Institute: Institute for Sustainable Industries and Liveab

Candidate's Co 80%

Status:

Accepted and in press: ☑ Date: 17/

Published: ☑ Date: 19/

2. CANDIDATE DECLARATION

I declare that the publication above meets the requirements to be included in the thesis as outlined in the HDR Policy and related Procedures – policy.vu.edu.au.

Rangani Wijayasinghe Digitally signed by Rangani Wijayasinghe Date: 2024.01.12 00:47:59 +11'00'	12/01/2024
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3. CO-AUTHOR(S) DECLARATION

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Thom Huppertz	3%	Manuscript reviewing and editing	[redacted]	2.01.2024
Jayani Chandrapala	4%	Concept development, and manuscript reviewing and editing	[redacted]	15/01/2024
Todor Vasiljevic	10%	Concept development, manuscript reviewing and editing, and journal submission	[redacted]	12.01.2024

Updated: September 2019

Food Chemistry 293 (2019) 247–253

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Influence of lactic, citric and phosphoric acids on the properties of concentrated lactose solutions

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ARTICLE INFO

Keywords:
Lactose
Citric acid
Phosphoric acid
Lactic acid

ABSTRACT

Physicochemical and thermal characteristics of concentrated lactose solutions containing 0.05, 1, or 4% (w/w) of three acids commonly used in the food industry, i.e., lactic, citric, and phosphoric acid, were studied. Properties of both lactose and water were affected by all acids studied. Thermographic analysis showed that interactions between water and acids hindered evaporation of water from most of lactose solutions. This effect was mostly related to the formation of a strong hydration layer around lactose molecules by hydrogen bonds. Acid-induced hydrolysis of lactose into glucose and galactose varied depending on the concentration, hydrolytic power of acids and molecular interactions in the system. The study concluded that the varying physical, chemical, structural and thermal characteristics of lactose as affected by the presence of different acids was mainly due to the manipulation of water-lactose interactions, whereas the hydrolysis of lactose by the acids plays a smaller role.

1. Introduction

Lactose is the main carbohydrate and the major solute of milk and whey. It is an important pharmaceutical excipient and a major component in many dairy powders and several other food products. Lactose is mainly recovered from whey permeate, which contains around 80% lactose on a dry matter basis. Lactose behaviour, especially during its recovery from whey, is impacted by mineral salts, lactic acid, and other milk components (Wong & Hartel, 2014). In general, interactions between simple sugars and acids modify physicochemical and physiological properties of sugar solutions, leading to a differing behaviour and in many instances processing issues. Acid whey for instance has emerged as a “difficult to treat stream”, owing to its inability to obtain sufficient crystalline lactose, which thus remains in its amorphous form during concentration and hinders further processing (Wijayasinghe, Vasiljevic, & Chandrapala, 2015).

For a sugar to provide required characteristics from appearance to texture, it is necessary to control the state/phase of the sugar (Hartel, Ergun, & Vogel, 2011). Desired properties of the sugar, such as its crystallinity, amorphous state or the combination of both can be achieved by altering the chemical composition of the system in which this sugar is part of. Controlling phase or state transitions in sugar-based foods requires knowledge of equilibrium considerations and kinetic effects. In many products, food grade acids are used to modify the environment but also to control the properties of the sugar. For example, citric acid can be used in candy production in order to obtain proper crystallinity and inversion of the sugar (Childs, 1972). Understanding how certain acids stabilize sugar in confectionaries has opened ways to researchers for development of invert sugars as an ingredient to moderate sucrose graining (Vink, 1998).

Early studies reported that lactose was relatively stable to acid hydrolysis as compared to other disaccharides requiring very low pH (1–2) and high temperatures (100-150°C) (Namvar & Pakizeh, 2012; Vasiljevic, 2004). Moreover, pH < 2 and pH > 7 accelerate mutarotation (Huppertz & Gazi, 2016) creating favourable conditions for an efficient crystallization (Nickerson & Moore, 1974). Very low pH can be achieved in the presence of strong mineral acids such as hydrochloric acid (Coughlin & Nickerson, 1975) and sulphuric acid (Timell (1964). Conversely, Ramsdell and Webb (1945) found that lactose can also be hydrolysed in the presence of citric acid at high temperatures (121°C), although hydrochloric acid was more effective than citric acid. According to Coughlin and Nickerson (1975), hydrolysis of lactose was

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https://doi.org/10.1016/j.foodchem.2019.04.065
Received 19 February 2019; Received in revised form 16 April 2019; Accepted 17 April 2019

insignificant in acid whey and aqueous lactose solutions containing 5 or 10% lactic acid.

Several other studies investigated the impact of lactose concentration on transport properties of aqueous solutions in the presence of minerals such as ferric sulfate, nickel chloride, calcium gluconate, calcium ions and cadmium chloride (Ribeiro, Gomes, Barros, Lobo, & Esteso, 2011; Ribeiro, Rita, Gomes, Lobo, & Esteso, 2011; Verissimo, Gomes et al., 2013; Verissimo, Ribeiro, Ribeiro, Rodrigo, & Esteso, 2014; Verissimo, Valada et al., 2013). Most of these studies revealed that depending on the concentration, lactose can influence the transport behaviour of these systems including molecular mobility affecting the diffusion of minerals. However, there are only limited studies conducted on the behaviour of lactose in aqueous solutions in the presence of different acids. For example, Wijayasinghe et al. (2015) studied the water – lactose behaviour in the presence of lactic acid.

While a body of work exists in this area, behaviour of lactose in the presence of acids (lactic, citric or phosphoric acid) is not fully understood. Thus, it would be important to establish how these acids influence the properties of lactose attempting to elaborate on various complex interactions between lactose and acids. Therefore, the aim of the current study was to use model-based systems to examine the physiochemical and thermal characteristics of lactose and its behaviour in the presence of lactic, citric, and phosphoric acids at the concentrations frequently applied in the industrial systems. Thermal characteristics of lactose were measured by differential scanning calorimetry (DSC), whereas high performance liquid chromatography (HPLC) was used to determine the capacity of the acids to invert lactose. Structural changes of lactose were established by Fourier transform infrared spectroscopy (FTIR). The fundamental knowledge generated by this work would assist manufacturers to optimize the existing processing conditions by manipulating the composition of different acids present in the food systems containing lactose.

2. Materials and methods

2.1. Materials

A commercial food grade lactose powder was obtained from Murray Goulburn Cooperative Co. Ltd (Brunswick, VIC, Australia). A solution of 50% (w/w) citric acid was prepared from citric acid powder (Sigma-Aldrich Pvt. Ltd, Castle Hill, NSW, Australia), while lactic acid (85%, w/w) and phosphoric acid (85%, w/w) solutions were directly sourced from Sigma-Aldrich Pvt. Ltd. Milli-Q water was always used.

2.2. Preparation of solutions

Lactose was dissolved in Milli-Q water at 55 °C ± 3 °C until a clear 40% ± 0.5% (w/w) lactose solution was obtained. The solution was then filtered through a 0.45-µm Whatman filter paper to remove any other undissolved particles. The lactose solution was then divided into ten aliquot parts. Calculated amounts of citric, lactic and phosphoric acids were subsequently added to obtain the concentration ratios of lactose: acid at levels of 100:1 (0.05% w/w acid concentration to 5% w/w lactose solution), 5:1 (1% w/w acid concentration to 5% w/w lactose solution) or 5:4 (4% w/w acid concentration to 5% w/w lactose solution). The lactose sample without any acid added considered as the control. Samples were then concentrated to approximately 55% ± 2% (w/w) of total solids using a rotatory evaporator, as described by Wijayasinghe et al. (2015). Brix values were measured to determine concentration using a laboratory refractometer (Atago Abbe, Tokyo, Japan) after calibration with Milli-Q water.

2.3. Differential scanning calorimetry

Concentrated lactose solutions with approximately 55% ± 2% (w/w) total solids were transferred into pre-weighed aluminium DSC pans (40 µL; ME-26763, Mettler Toledo, Schwerzenbach, Switzerland). The pans were hermitically sealed and then transferred into the DSC instrument (DSC 1 STARe System, gas controller, GC 200, Mettler Toledo). An empty pan was used as a reference. Samples were scanned with a starting temperature of 50 °C and end temperature of 180 °C at a heating rate of 5 °C/min. Onset, mid-point, end-set temperatures of water evaporation and relevant enthalpies (area under the curves) were analysed using a STAR^{e} thermal analysis software v.15 (Mettler Toledo Schwerzenbach, Switzerland) as described by Wijayasinghe, Vasiljevic, and Chandrapala (2016).

2.4. Fourier transform infrared spectroscopy

The FTIR spectra of the samples were obtained in the range of 4000–600 cm^{-1} immediately after reaching the concentration of 55% ± 2% (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 sixteen scans recorded at 4 cm^{-1} resolution (Wijayasinghe et al., 2016).

2.5. High performance liquid chromatography

Lactose hydrolysis was evaluated by HPLC using a Varian 9012 system controller (Agilent Technologies Inc., Santa Clara, CA) coupled with a RI detector (Varian, 9050) and a HPLC column (Aminex HPX-87P, 300 × 7.8 mm, Biorad Instruments, Gladesville, NSW, Australia) as described by Donkor, Henriksson, Vasiljevic, and Shah (2007). The mobile phase used was Milli-Q water with isocratic elution at a flow rate of 0.6 mL/min.

2.6. Determination of water activity and pH

Water activity measurements were performed at a room temperature (20 °C ± 3 °C) using an Aqualab TE water activity meter (Decagon Devices Inc., Pullman, WA, USA). pH was measuremed using an inoLab pH7110 pH meter (WTW, Xylem Inc., Trifthof, Weilheim, Germany).

2.7. Statistical analysis

Results were analysed as a randomised split plot design with lactose and acids as the main plot and the different concentration of the acids as a sub plot. The replicates served as a block. Replication of all experiments was carried out at least twice with subsequent sub-sampling giving at least 4 independent observations ($n \geq 4$). Results were analysed using a General Linear Model of the SAS statistical software (v. 9.2). The least squares mean (LSM) was used to differentiate the means with the pre-set probability at $p \leq 0.05$.

3. Results

3.1. Acid-induced lactose hydrolysis

As indicated in Fig. 1, lactose eluted as a single peak at ~10.7 min in the HPLC chromatogram, which was consistent with the result of Jeon, Galitzer, and Hennessy (1984). This peak was not affected by lactic acid at any concentration studied. In contrast, the elution pattern of lactose substantially changed when lactose solutions contained either citric or phosphoric acid. In the presence of 0.05 or 1% of these acids, lactose was partially hydrolysed into its two constitutive moieties, glucose and galactose, which eluted as two small peaks at ~11.5 and ~13.5 min with almost similar intensities at retention times of ~12 and ~13.5 min, respectively, as previously reported (Jeon et al., 1984). Fig. 2 shows an overview of lactose hydrolysis based on pH and the type of acid. In the presence of citric acid, the degree of lactose hydrolysis

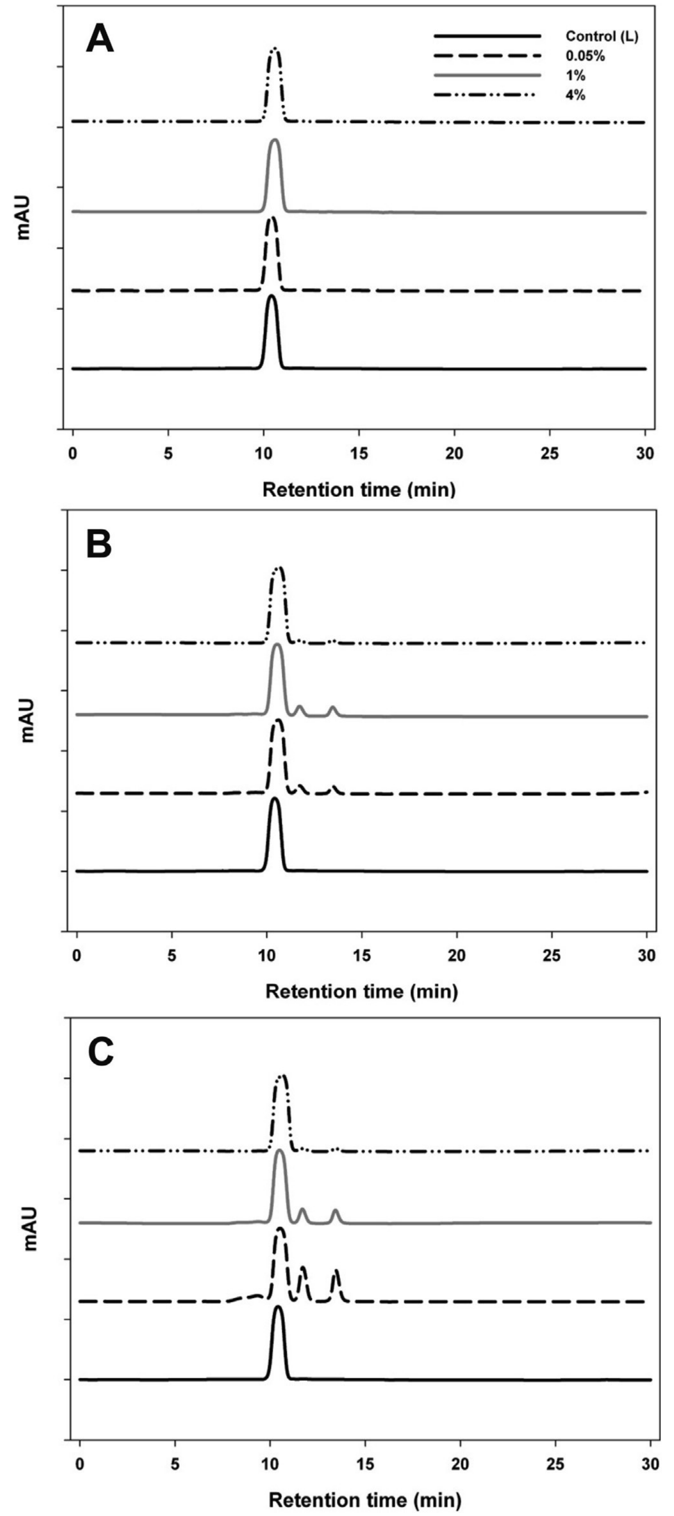

Fig. 1. HPLC chromatograms of lactose solution (~55% (w/w)) containing lactic acid (A), citric acid (B) or phosphoric acid (C) in a concentration of 0.05% (w/w) (▬ ▬), 1% (w/w) (••••••) or 4% (w/w) (▬ ▬▬). The pure lactose solution (control sample) is depicted with the solid line (▬▬▬).

was about 8% at both concentrations (0.05 or 1%). In contrast, the hydrolysis of lactose appeared inversely correlated with the concentration of phosphoric acid. For example, ~27.5% of lactose was hydrolysed when phosphoric acid was present at 0.05%, while only ~ 10% of lactose was hydrolysed with 1% phosphoric acid (Table 1). Neither citric nor phosphoric acid at high concentration (4%) did appear to have any impact on lactose hydrolysis.

3.2. FTIR spectra of lactose solutions in the presence of acids

The FTIR spectrum reflects the molecular fingerprint, which is unique for materials present in the sample. Fig. 3 shows the FTIR spectra of the lactose solutions in the presence of acids at selected concentrations. Two sharp peaks were observed in the control lactose solution, at 1650 and 1372 cm^{-1} correspond to the bending mode of water molecules and bending mode of –CH and –OH groups, respectively (Velazquez, Herrera-Gomez, & Martin-Polo, 2003). Generally, when the concentration of all three acids was 0.05%, scanning yielded almost similar FTIR spectra in comparison to that of the pure lactose. In the presence of either acid at 1%, FTIR spectra of lactose solutions coincided with the spectrum of pure lactose in the range of 1800–1200 cm^{-1} but deviated slightly between 1200 and 800 cm^{-1}. On the other hand, the lactose solutions containing 4% acids had their spectra altered in a wider scanning range (1800–800 cm^{-1}) in comparison to that of the pure lactose.

In the presence of 4% lactic acid, the bending vibrations of O—H molecules changed as indicated by the appearance of another peak at ~1580 cm^{-1}. In addition, several other peaks corresponding to CH_2 bending and CH stretching were observed at 1454, 1415 and 1317 cm^{-1}. In contrast, the bending vibrations of OH molecules in the presence of 4% citric acid exhibited a completely different behaviour. The interferogram of this acid was characterized by appearance of a peak at 1715 cm^{-1} instead of the peak at ~1580 cm^{-1} as in case of lactic acid. In addition, a sharp peak appeared at 1220 cm^{-1} corresponding to the citric acid. Furthermore, phosphoric acid at this concentration had no apparent effect on the bending vibrations of O—H molecules, although changes in vibration of C—C and C—O pyranose ring were noticed by intensified peaks at ~1023 and 995 cm^{-1} in comparison to the spectrum of pure lactose. However, relatively less intensified peaks appeared at same wavelengths (1023 and 995 cm^{-1}), in comparison to pure lactose, when lactose solution contained either 1 or 4% lactic or citric acids, or 1% phosphoric acid.

3.3. Water activity of lactose solutions in the presence of acids

Water activity (a_w) reflects intensity with which water associates with various non-aqueous constituents (Reid & Fennema, 2008). High water activity indicates less interactions between components and vice versa. In the present study, the water activity was studied to predict the nature of interactions between water-lactose and water-acid in the systems. Higher water activity leads to understanding of presence of less interactions between components whereas, low water activity evidences the presence of more interactions between components. Therefore, according to the water activity readings, we can predict the strength of the interactions of lactose, water and acids in the studied systems. It is known that water activity is highly dependent on the concentration of solutes. Even a small change in solute concentration can result in substantial changes of water activity. Hence, the concentration of lactose was maintained at its supersaturated state (55%) (Fox, 2009) and highly plasticized (Roos, 2009) to minimize this effect. Observed water activity of the pure lactose solution was 0.9678 (Table 2), which mostly tallied with 0.9699 reported by Bhandari and Burel (2007). The water activity of lactose solutions, which contained all three acids at different concentrations, was substantially reduced in comparison to pure lactose except the one containing 0.05% of lactic acid. Furthermore, an inverse association was observed between acid concentration and the water activity regardless the type of acids tested (Table 2).

3.4. Thermal properties of lactose solutions in the presence of acids

Lactose solutions with or without addition of acids generated

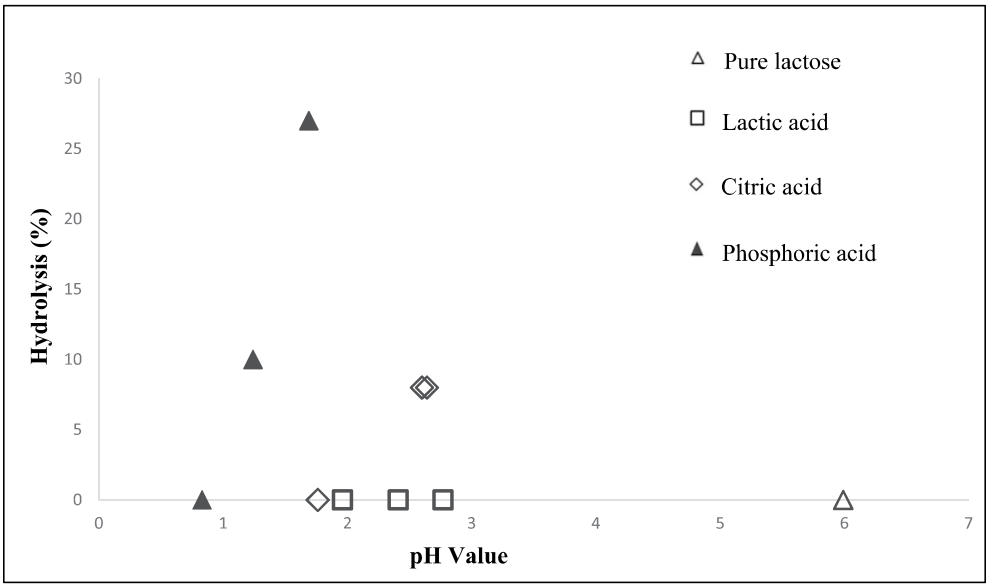

Fig. 2. Hydrolysis of lactose as a function of pH and the type of acid and the acid concentration.

Table 1
pH values and degree of hydrolysis in lactose solutions containing lactic, citric acid phosphoric acids with different concentrations.

Sample concentration		pH	Hydrolysis (%)
Lactose		5.99	0
+ Lactic acid	0.05%	2.77	0
	1.00%	2.41	0
	4.00%	1.96	0
+ Citric acid	0.05%	2.64	8
	1.00%	2.60	8
	4.00%	1.76	0
+ Phosphoric acid	0.05%	1.69	27
	1.00%	1.24	10
	4.00%	0.83	0

endothermic peaks originating around 130–150 °C during the thermographic analysis. These peaks were previously associated with the evaporation of water (Wijayasinghe et al., 2015). The pure lactose solution exhibited a midpoint in the peak at ~137 °C with an enthalpy (ΔH) of ~698 $J{\cdot}g^{-1}$ (Table 2). In the presence of acids, onset temperature of the evaporation of water significantly ($p < 0.05$) increased at every concentration tested. Likewise, the enthalpy (ΔH) of water evaporation was also greater in comparison to that of the pure lactose for all acid types and concentrations tested, except phosphoric acid at 4% (609 $J{\cdot}g^{-1}$). Enthalpy (ΔH) of water evaporation demonstrated an inverse correlation with the concentration of lactic acid (Table 2), but this was not observed for other acids. Notably, enthalpy (ΔH) was always lowest at 4% regardless of the type of acid. Moreover, the water evaporation window, the range between on-set and end-set temperatures of water evaporation (Wijayasinghe et al., 2015), appeared to depend on the type and the concentration of acids. For instance, a relatively wider water evaporation window was observed when lactose solution contained either 1% phosphoric or 4% citric acids.

4. Discussion

4.1. Effects of acids on the behaviour of lactose and water and possible impact on lactose crystallisation

It has been established that the behaviour of lactose is highly impacted by other components present in the system (Hartel & Shastry, 1991; Zeng, Martin, Marriott, & Pritchard, 2000). Lactose (β-D-galactopyranosyl-(1–4)-D-glucose) is a disaccharide formed by a condensation reaction between carbon 1 of a galactose molecule and carbon 4 of a glucose molecule through a β 1–4 glycosidic linkage (Fox, 2009). As per HPLC results in the current study, lactose eluted as the most prominent peak. Due to hydrolytic power of citric and phosphoric acids, even at their low concentrations (0.05 or 1%), the β-(1,4)-glycosidic bond was hydrolysed with a partial release of glucose and galactose.

This reaction appeared to depend on the type and concentration of acid. The effect of acid type on extent of lactose hydrolysis could be attributed to several phenomena. One of them is hydrolytic power of these acids. The Ka values of the acids studied are reported as lactic; pKa 3.86, citric ; pKa_1 3.09, pKa_2 4.74, pKa_3 5.40 and phosphoric ; pKa_1 2.15, pKa_2 7.21, pKa_3 12.32 (Belitz, Grosch, & Schieberle, 2009; Rodrigo, Rebeiro, Verissimo, Esteso, & Leaist, 2019). It is important to mention that citric and phosphoric acids have three pKa values because they are tri-protic acids but lactic acid has only one pKa value. Hence, the strength of these three acids was compared based on their pKa_1 values. Accordingly, phosphoric acid as the strongest acid with the greatest hydrolytic power hydrolysed lactose to a greater extent than other acids. Lactic acid did not hydrolyse lactose under all conditions tested in the current study, which agreed with the results observed by Coughlin and Nickerson (1975). However, fairly similar H^+ ion concentration and/or pH still showed variations in lactose hydrolysis (Fig. 2). For example, we observed 8% hydrolysis by citric acid at pH 2.6 while no hydrolysis was observed with lactic acid at the same pH. Similarly, phosphoric acid hydrolysed 27% of lactose at pH 1.69 while citric acid at pH 1.76 did not hydrolyse lactose at all (Table 1). Thus, pH and concentration of H ion appear to play a minor role in governing the behaviour of lactose and its susceptibility to hydrolysis as low pH does not always create most appropriate conditions for cleavage. On the other hand, the ionic radii of the assessed compounds (lactate, citrate and phosphate) could also play a role in activating lactose molecules. The radius of an atom/molecule increases when it becomes an anion due to increase in its electron density. Consequently, the repulsion exerted by additional electron(s) expands the area of electron cloud around the atom/molecule while maintaining its nuclear charge unchanged (Chang, 2007). For this reason, three acids examined likely had different electron clouds around their anionic form, which may have attenuated lactose molecule, most probably by stretching it and thus allowing it to be cleaved to varying extents. For example, citrate

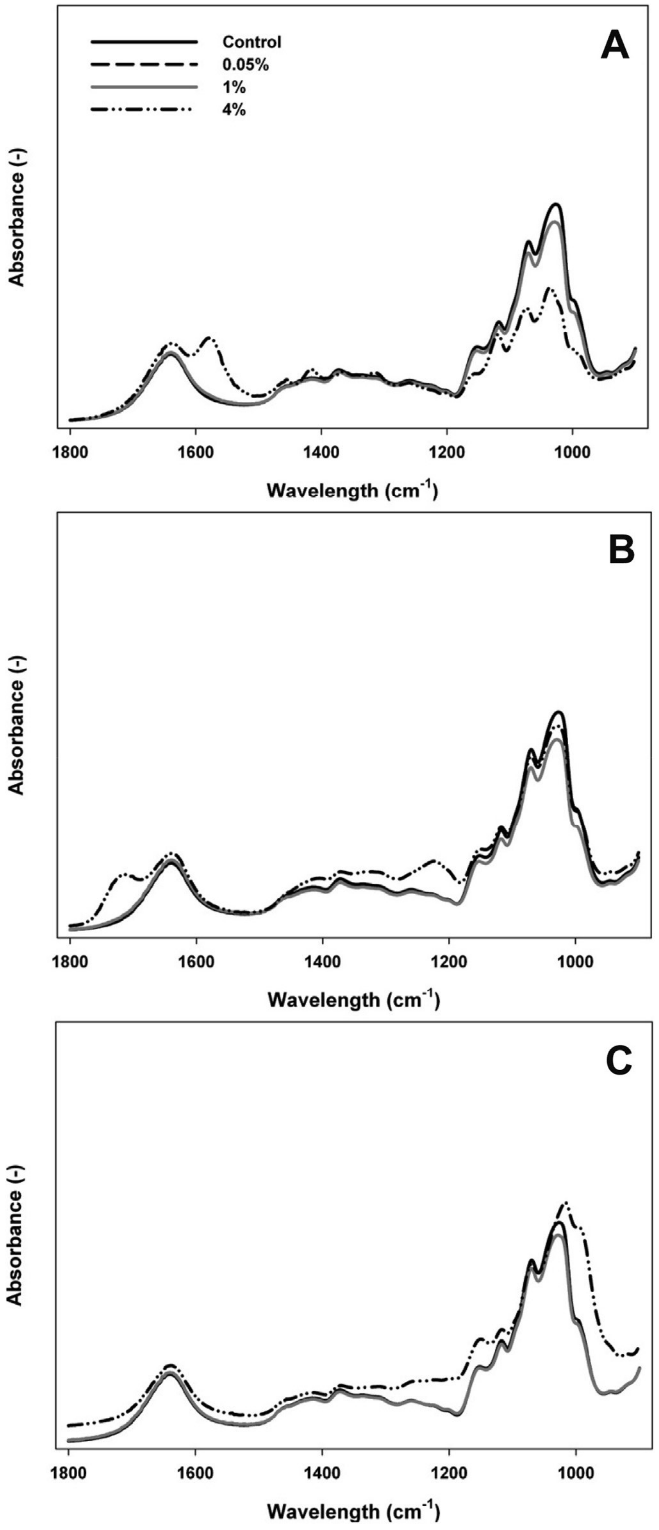

Fig. 3. FTIR spectra of lactose solutions containing either lactic (A), citric (B) or phosphoric acids (C) at a concentration of 0.05% (w/w) (▬ ▬), 1% (w/w) (••••••) or 4% (w/w) (▬ ▬). The pure lactose solution (control sample) is depicted with the solid line (▬▬).

and phosphate ions with larger electron clouds, in comparison to the lactate ion with smaller electron cloud, may have stretched lactose molecules differently leading to its hydrolysis depending on the acid concentration. Furthermore, the inverse association between the extent of lactose hydrolysis and the concentration of citric or phosphoric acids could be ascribed to the differences in molecular arrangement of the lactose molecule due to interaction with these acids and water. These interactions are likely depicted by several changes in the FTIR interferograms in the range of 1,200–800 cm^{-1}. According to Ottenhof, MacNaughtan, and Farhat (2003), the phase changes of carbohydrates can be determined by the spectral changes in this range since C—C and C—O stretching and vibrations are influenced by their physical state. Two prominent peaks appearing at 1023 and 995 cm^{-1} in lactose solutions, with or without acids, are related to C—C stretching vibrations and C—O stretching of glucose moiety of lactose, respectively (Solis-Oba et al., 2011). These changes in C—C stretching vibrations and CO stretching related to glucose indicated that each acid interacts with lactose and water molecules differently in a concentration dependent manner and governs the hydrolysis of lactose. When acid concentration increases intramolecular interactions may become stronger than the intermolecular interactions leading to decline in hydrolysis of lactose. The presence of these acids can also influence the supersaturation by hydrolysis of lactose and thereby change the structure of lactose molecule as observed by FTIR. Furthermore, changes in water behaviour were evident in the FTIR spectra conforming the presence of strong O—H bonds when lactose solutions contained 4% of lactic, citric or phosphoric acid. For instance, O—H bonds were prominent when 4% lactic (1580 cm^{-1}) or citric (1715 cm^{-1}) acid present in lactose solutions in contrast to the pure lactose solution (1650 cm^{-1}).On the other hand, acids dissociate into hydrogen ions and its conjugate base in the solutions. These hydrogen ions lead to formation of hydronium ions with water via exceedingly strong H bonds (Belitz et al., 2009). However, tendency of acids to release hydrogen ions varies depending on their dissociation behaviour. For example, hydrochloric acid dissociates rapidly and completely into H$^+$ and Cl$^-$ ions in water. Conversely, weak acids with very low dissociation constants such as lactic, citric, acetic, amino, and fatty or phosphoric acids dissociate incompletely donating only few of their hydrogen ions into solution. The increase in concentration of hydronium ions (H$_3$O$^+$) in the presence of acids can reduce the amount of free water present in the system, which in turn impede the extent of lactose hydrolysis (Table 1). In this study, the increase in acid concentration of either citric (from 1 to 4%) or phosphoric (from 0.05 to 1 and 4%) resulted in availability of a greater number of hydrogen ions in lactose solutions, which eventually impeded lactose hydrolysis. Moreover, according to the Ostwald dilution law, increasing concentration of a weak acid reduces the dissociation constant due to diminished mobility of the ions (Fuoss, 1959). Since these acids dissociate incompletely in order to maintain the equilibrium in relation to the Le Chatelier's principle (Quílez-Pardo & Solaz-Portolés, 1995), the increase in acid concentration possibly restricts the hydrolysis of lactose. The differences observed between citric and phosphoric acids in terms of lactose hydrolysis can be attributed to their distinctive chemical properties. In an aqueous solution, phosphoric acid behaves as a triprotic acid having three ionisable hydrogen atoms. Each successive dissociation step occurs with declining ease. Due to these reasons, in the present study phosphoric acid hydrolysed lactose to a greater extent at 0.05% than at any higher concentration. Citric acid on the other hand is a weaker acid than the phosphoric acid, thus cleaves comparatively less lactose.

As discussed above, the hydrogen bonding seems to be crucial during concentration of lactose in the presence of acids. Water hydroxyls tend to compete with the sites of carbohydrates for establishing intra- and intermolecular hydrogen bonding and this competition can influence their conformational flexibility (Gallina, Sassi, Paolantoni, Morresi, & Cataliotti, 2006). However, as a consequence of hydrolysis, crystallization behaviour of lactose can be changed as observed by Patocka and Jelen (1988). Partial hydrolysis of lactose, in the presence of either 0.05 or 1% citric or phosphoric acid, can lead to reduction in super-saturation of the system due to differences in solubilities of glucose and galactose, which may interfere with lactose crystallization. At the same time, glucose and galactose possibly start to crystallise, which

Table 2
On-set and end-set temperatures of water evaporation, enthalpy (ΔH) of water evaporation and water activity of lactose solutions containing lactic, citric acid phosphoric acids with different concentrations.

Sample		On-set water evaporation temperature (°C)	End-set water evaporation temperature (°C)	Peak water evaporation temperature (°C)	Enthalpy[a] (ΔH) (Jg^{-1})	Water activity
Lactose		133.9	145.1	137.3	698.0	0.9678
+ Lactic acid	0.05%	145.0	154.5	148.8	838.3	0.9624
	1.00%	144.1	151.8	146.2	741.7	0.9339
	4.00%	146.5	156.3	148.3	710.5	0.9138
+ Citric acid	0.05%	142.6	153.7	147.8	858.8	0.9463
	1.00%	147.9	155.6	149.3	866.7	0.9317
	4.00%	137.4	151.0	143.1	704.14	0.9297
+ Phosphoric acid	0.05%	145.2	152.3	146.5	851.9	0.9574
	1.00%	145.4	158.5	150.0	928.3	0.9351
	4.00%	149.1	159.1	152.7	608.9	0.9105

[a] Enthalpy (ΔH) refers to the area under the peak of the water evaporation obtained by DSC

can also affect lactose crystallisation. The absorption of glucose and galactose crystals onto surface of lactose crystals can prevent integration of lactose molecules and its crystallisation (Laos, Kris, & Kikkas, 2007).

4.2. The thermal behaviour of lactose and water affected by acids

Thermal changes analysed by DSC were characterised by an increased enthalpy (ΔH) of water evaporation in lactose solutions containing all three acids in comparison to that of the pure lactose. The presence of acids has obviously restricted the removal of water from lactose solutions. However, due to hydrolysis of lactose, water evaporation enthalpy (ΔH) appears altered. Restricted water removal can be ascribed to the interaction and hydrogen bonding of lactose, water and acids which depends on the type and concentration of the acid and governs the hydrolysis of lactose. In the presence of acids, oxonium cations (H_3O^+) are formed, which possess a greater potential of creating hydrogen bonds due to their positive charge thus interacting strongly with lactose. Consequently, they established a strong hydration layer around lactose molecules and restricted removal of water during evaporation resulting in higher water evaporation enthalpy (ΔH). The H_3O^+ ions can strongly interact with four to six of first-layer water molecules around lactose molecule, causing them to be less mobile, and pack them more densely than HOH molecules in pure water (Reid & Fennema, 2008), rendering water removal very difficult. The highly hydrophilic compounds such as lactic, citric or phosphoric acids can strongly interact with water molecules since the water affinity for acids is greater than the ability of lactose to interact with water. Thus, the interactions between water and acid restrict movement of water molecules and further change the structural arrangements and mobility of adjacent water molecules leading to compromised lactose crystallization. Generally, when increasing acid concentration, the water activity of respective lactose solutions was lowered indicating greater interactions between lactose, water and acid molecules (Lilley & Sutton, 1991). These interactions between acid and lactose may form complexes and hinder the orderly arrangement of lactose, which in turn interfere with crystallization of lactose. We have already reported that the molecular arrangements and mobility play a significant role during the concentration of lactose solution in the presence of lactic acid thus requiring greater energy for water removal (Wijayasinghe et al., 2015). The driving force for lactose crystallisation is the evaporation of water and transfer of hydration water from the vicinity of the crystal to the bulk of solution. This allows lactose-lactose interactions with minimized free energy leading to formation of crystal lattice (Chandrapala, Wijayasinghe, & Vasiljevic, 2016). Increased enthalpy (ΔH) of water evaporation also indicated that the diffusion of lactose molecules has been restricted due to widened distance between lactose molecules created by strongly attractive lactose-water-acid complexes and thus interfering formation of lactose nuclei. Surprisingly, enthalpy (ΔH) of water evaporation (609 $J{\cdot}g^{-1}$) of lactose solution containing 4% phosphoric acid appears to be significantly below that of the pure lactose (698 $J{\cdot}g^{-1}$), which requires further elaboration.

5. Conclusion

The results obtained in the current study point out that interactions between water and lactose and its behaviour vary with the type and concentration of acids. Presence of all three acids enhanced greater interaction among water molecules in the hydration layer around lactose molecules obstructing water removal. Addition of phosphoric or citric acid to saturated lactose solution leads to a hydrolysis of lactose partially depending on the concentration and the hydrolytic power of acids and molecular interactions in the system. The results thus suggested that the presence of weak acids such as citric or phosphoric in lactose solutions can induce modifications in the structure of both lactose and water molecules and also partial hydrolysis of lactose into its two consecutive moieties, which is beneficial for the dairy manufactures to control their production processes. Therefore, by using citric or phosphoric acids, for example in cheese making or other dairy products, manufacturers may manipulate the properties of lactose via partial hydrolysis and consequently lactose/water interactions.

Conflict of interest

The authors declare that they have no conflict of interests.

Acknowledgement

The authors sincerely acknowledge the financial and technical support from Victoria University. Dr. Muditha Dissanayake is gratefully acknowledged for valuable discussions.

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Chapter 4: Crystallization behavior and crystal properties of lactose as affected by lactic, citric or phosphoric acid

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This chapter has been published as a research article in the *Journal of Dairy Science*. Wijayasinghe, R., Bogahawaththa, D., Chandrapala, J., & Vasiljevic, T. (2020). Crystallization behavior and crystal properties of lactose as affected by lactic, citric, or phosphoric acid. Journal of dairy science, 103(12), 11050-11061.

https://doi.org/10.3168/jds.2020-18375

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1. PUBLICATION DETAILS (to be completed by the candidate)

Title of Paper/Journal/Book: Crystallization behavior and crystal properties of lactose as affected by lactic, citric, or phosphoric acid [redacted]

Surname: Wijayasinghe

First name: Rangani

Institute: Institute for Sustainable Industries and Liveat

Candidate's Co 80%

Status:

Accepted and in press: ☑ Date: 07/

Published: ☑ Date: 14/

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Dimuthu Bogahawaththa	5%	Contribute to data analysis and manuscript reviewing and editing	[redacted]	16/01/2024
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Todor Vasiljevic	10%	Concept development, manuscript reviewing and editing, and journal submission	[redacted]	12.01.2024
			[redacted]	

Updated: September 2019

J. Dairy Sci. 103:11050–11061
https://doi.org/10.3168/jds.2020-18375

Crystallization behavior and crystal properties of lactose as affected by lactic, citric, or phosphoric acid

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ABSTRACT

The presence of acids in a lactose-containing system can affect its crystallization. The crystallization kinetics of lactose solutions were investigated as affected by lactic, citric, or phosphoric acid at a concentration of 0.05, 1, or 4% (wt/wt) as compared with that of pure lactose. The crystallization behavior of lactose was affected differently by the presence of all 3 acids and was mostly concentration dependent. The presence of 1 and 4% citric or phosphoric acid reduced the crystal yield significantly ($\geq$18%) as compared with that of pure lactose (~82%). Thermographic analysis of lactose crystals showed that the presence of 1% lactic, 0.05 and 1% citric, and 4% phosphoric acids in the lactose solutions induced the formation of amorphous lactose. X-Ray diffraction analysis revealed that the lactose crystallized mainly into α-lactose monohydrate, stable anhydrous α-lactose, and anhydrous crystals containing α-lactose and β-lactose in a molar ratio of 5:3 and 4:1. Average size of the lactose particles, comprising of several crystallites, declined depending on the type of the acids and their concentration, but size of a single crystallite was not altered. The findings suggested that the lactose crystallization and crystal properties are governed by the lactose-water interactions, which can be influenced by the presence of acids in a concentration-dependent manner.

Key words: lactose, citric acid, phosphoric acid, lactic acid, crystallization, crystal properties

INTRODUCTION

Crystallization of lactose is an important process in the food industry, especially in the dairy sector, because its physical state can influence the storage stability and quality of various products. In many processes, the degree of sugar crystallinity is critical for the acceptance of the final product. For instance, crystallization of lactose is considered as a desired characteristic in products such as milk chocolate, but the presence of lactose crystals in milk powders, condensed milk, and ice cream hinders the quality of products (Lai and Schmidt, 1990; Martinez et al., 1990). The higher the degree of crystallization of lactose, the lower the proportion of amorphous lactose. Formation of amorphous lactose occurs when a lactose solution is dried quickly, leading to a rapid increase in viscosity of the solution, which results in insufficient time for the crystallization to take place (Roos, 2002). Thus, it is important to understand the principles that govern the formation or prevention of the crystalline sugar phase to develop satisfactory food products.

Lactose is often present in food systems in conjunction with other food constituents such as organic acids, salts, and proteins that can influence the process of lactose crystallization (Nijdam et al., 2007). These components can interfere with lactose crystallization by modifying both nucleation and crystal growth, for example, when manufacturing lactose from whey (Botsaris, 1982). According to Pisponen et al. (2014) and Raghavan et al. (2000), the crystallization of lactose in whey is a long process with an extended induction period for nucleation, which can result in uneven and large crystal size distribution. In some instances, the other food constituents may inhibit nuclei formation of lactose resulting in crystals with irregular-shaped and clumped arrangements (Gänzle et al., 2008). For example, the presence of proteins in a lactose solution can create localized lactose supersaturation spots, which induce nucleation and formation of a great number of small crystals (Mimouni et al., 2005). It has been reported that the presence of lactic acid (**LA**) in lactose solutions prevents lactose crystallization as it does not create a strong acidic environment ($pH < 2$) to promote lactose crystallization (Jelen and Coulter, 1973; Nickerson and Moore, 1974; Huppertz and Gazi, 2016). Moreover, the growth of lactose crystals can be influ-

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Received February 16, 2020.
Accepted August 7, 2020.
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enced by several processing-related and solution conditions including degree of supersaturation, solution pH, temperature, and viscosity (Bhargava and Jelen, 1996; Rjabova et al., 2013). The degree of supersaturation is the primary factor, which directly influences crystallization, whereas other conditions affect the mutarotation and mass transfer rate (Wong and Hartel, 2014). It has also been reported that supersaturation can affect the growth rate and the shape of lactose crystals (Gänzle et al., 2008). The pH appears to affect the rate of mutarotation greatly. For example, the minimum mutarotation rate was observed at pH 5, whereas pH below 2 and above 7 result in a rapid mutarotation rate (Huppertz and Gazi, 2016) confirming the importance of solution pH in lactose crystallization (Gänzle et al., 2008; Wong and Hartel, 2014).

Obviously, these crystallization conditions influence the crystal characteristics including the shape and size, which are important to achieve the ideal characteristics of the lactose-based products (Butler, 1998; Raghavan et al., 2000; Roos, 2009; Parimaladevi and Srinivasan, 2014). For instance, crystal forms or crystal morphology and crystal size are greatest areas of interest in food and pharmaceutical industries because the properties of lactose such as solubility, density, stability, and bioavailability directly depend on the polymorphs and the particle size (Barbosa-Cánovas et al., 2012). The scientific knowledge of kinetics of lactose crystallization, thermal behavior of lactose crystals, and their physical state in various food systems are of utmost importance when developing appropriate processing conditions, minimizing processing cost, and ensuring the quality of food products (Hartel, 2019). Hence, the current study was aimed at investigating thermal and physiochemical characteristics of lactose crystals and their behavior in model-based systems containing acids commonly used in the food industry as modifiers [LA, citric acid (**CA**), or phosphoric acid (**PA**)] at different concentrations. The scientific knowledge provided here may assist manufacturers to develop or optimize processing conditions through modifying the composition of selected acids in food systems comprising lactose.

MATERIALS AND METHODS

Materials

A local manufacturer (Murray Goulburn Co-operative Co. Ltd., Brunswick, VIC, Australia) kindly supplied the lactose powder. Lactic acid and PA (85% wt/wt) were purchased from Sigma-Aldrich Pvt. Ltd. (Castle Hill, NSW, Australia). A CA solution (50% wt/wt) was prepared using a CA powder (Sigma-Aldrich Pvt. Ltd., Castle Hill, NSW, Australia).

Preparation of Model Solutions

Lactose powder (Murray Goulburn Co-operative Co. Ltd., Brunswick, Australia) was dissolved in Milli-Q water (Millipore Corporation, Bedford, MA) at 55°C ± 5°C to obtain a clear 40% ± 0.5% (wt/wt) lactose solution. Then, the solution was filtered to remove any undissolved particles using a 0.45-μm Whatman filter paper (Sigma-Aldrich Pvt. Ltd, Castle Hill, NSW, Australia). The resultant lactose solution was equally divided into 10 aliquots. To achieve the concentration ratios of lactose:acid at levels of 100:1 (0.05% wt/wt acid to 5% wt/wt lactose), 5:1 (1% wt/wt acid to 5% wt/wt lactose), or 5:4 (4% wt/wt acid to 5% wt/wt lactose), calculated amounts of LA, CA, or PA were added to the lactose (**L**) solutions (Wijayasinghe et al., 2019). These samples were coded as L+0.05LA, L+1LA, L+4LA, L+0.05CA, L+1CA, L+4CA, L+0.05PA, L+1PA, and L+4PA. The pure lactose (**PL**) without addition of any acids was considered the control. A rotatory evaporator (EYELA, Rikakikai Co. Ltd., Tokyo, Japan) was then used to concentrate all the samples to achieve ~50% (wt/wt) TS (Wijayasinghe et al., 2015). Lactose concentration was determined using the Brix values measured by a refractometer (Atago Abbe, Tokyo, Japan) after calibration with water. The pH of all the concentrated lactose solutions was measured at 50 ± 2°C using an inoLab pH7110 pH meter (WTW, Xylem Inc., Trifthof, Weilheim, Germany).

Crystallization Procedure

Crystallization of concentrated lactose solutions was performed using the method of Chandrapala et al. (2016). In brief, the concentrated solutions (at 55°C) were rapidly cooled to the crystallization temperature of 30°C within 10 min and equilibrated at the same temperature for 2 h using a water bath consisting of a cooling unit to induce nucleation. Then, the solutions were slowly cooled down to 15°C at a rate of 5°C/h and kept at the same temperature overnight with continuous stirring. The solutions were allowed to establish the mutarotation and attain the solubility equilibrium. The final solubility of the selected samples was determined by measuring concentration of the supernatants. The following day, the lactose crystals were filtered using 0.45-μm filter papers and the separated lactose crystals were oven-dried at 80°C for 4 h. The following equation was then used to calculate the percentage yield of lactose. When calculating the amount of lactose recovered, 5% water of crystallization of α-lactose monohydrate was taken into account. The maximum solubility of lactose was considered as 17.84 g of lactose in 100 g of water at 15 ± 1°C (Adhikari et al., 2018). Therefore, the maximum theoretical yield from 50% solution was considered to be 82.16 g/100 g of water (Wong and Hartel, 2014):

$$\text{yield}\,(\%) = \frac{0.95 \times \text{mass of lactose crystals obtained}}{\text{maximum theoretical crystal yield}} \times 100.$$

Differential Scanning Calorimetry

Differential scanning calorimetry (**DSC**) measurements of crystallized lactose samples were performed using a DSC instrument (DSC 1 STARe System, gas controller, GC 200, Mettler-Toledo, Columbus, OH) as previously explained (Wijayasinghe et al., 2015; Chandrapala et al., 2016) with slight modifications. Crystal samples were scanned at a heating rate of 10°C/min starting from 50°C and ending at 250°C. The STARe thermal analysis software (v. 15.00, Mettler-Toledo, Schwerzenbach, Switzerland) provided relevant information including the on-set, mid-point, and end-set temperatures and enthalpies of lactose crystal samples.

Fourier-Transform Infrared Spectroscopy

A Perkin Elmer Frontier FTIR spectrometer (Perkin Elmer, Waltham, MA) was used to collect the Fourier-transform infrared spectra (**FTIR**) in the 4,000 to 400 cm^{-1} range. Sample spectra were obtained in the absorbance mode after subtracting the background (Wijayasinghe et al., 2016). The spectral data in the region of 3,750 to 2,800 cm^{-1} were initially analyzed to study the interactions among system components. The generated peak areas related to constrained water molecules in the lactose crystals were quantified using the Origin software (Origin Pro 2018, v. 95E, OriginLab Corporation, Northampton, MA). Briefly, peak fitting procedure with the Gaussian function was used to identify and fit the peaks in the Fourier self-deconvoluted spectra and related peak areas were thus able to be quantified (Bogahawaththa et al., 2019).

X-Ray Diffraction

X-Ray powder diffraction patterns of all the samples were obtained using a Rigaku Miniflex 600 X-ray diffractometer (Rigaku Corporation, Wilmington, MA). The lactose powder samples were firmly pressed to form a thin film (0.2 mm) on the glass sample holders before loading them in the instrument. Operating conditions were set at 40 kV and 15 mA and a step size of 0.02° with a speed of 1.2/min from 2θ scanning range from 5° to 30° with Ni Kβ-filter (x2) and a D/tex detector. The X-ray diffraction (**XRD**) data were analyzed by the Origin software (v. 95E) using a peak integration procedure with the Gaussian function. Degree of lactose crystallinity was calculated by dividing total area under the crystalline peaks by total area of the respective diffraction curve. The Scherrer formula was used to calculate average crystallite size (L):

$$\mathrm{L} = \frac{\mathrm{K}\lambda}{\beta \times \cos\theta},$$

where β is the peak width of the diffraction peak profile at half maximum height resulting from small crystallite size in radians, θ is the Bragg angle, λ is the X-ray wavelength (nm), and K is a constant associated with crystallite shape, generally considered as 0.9 (Scherrer, 1918).

Particle Size Determination

Average particle size was measured by a Mastersizer (Malvern-2000, Malvern Instruments Ltd., Malvern, UK) using the method previously reported (Chandrapala et al., 2016).

Crystal Morphology Determined by Optical Light Microscopy

The lactose crystal specimens were examined using a Motic advanced upright microscope (BA310, Motic China Group Co. Ltd., Hong Kong, China). The microscope was coupled with a camera and connected to a PC with a frame grabber to take and analyze images. Each sample was gently spread over a microscopic glass slide and images were captured to analyze crystal morphology. Several images (≥8) were collected from each sample and crystal morphology was analyzed to obtain the most appropriate crystal representation from every sample.

Statistical Analysis

Results were analyzed by 1-way/2-way ANOVA using the SAS statistical software (v. 9.2). The level of significance was considered at $P < 0.05$. The entire experiment was conducted in triplicate.

RESULTS AND DISCUSSION

Crystal Yield of Lactose

The PL solution yielded ~82% of lactose crystals and its solubility was 17.6 g/100 g of water (Table 1). It appeared that the crystal yield of lactose was not significantly ($P > 0.05$) reduced in the presence of LA at all 3 concentrations tested (L+0.0.5LA, L+1LA, and L+4LA) and L+0.05CA and L+0.05PA solutions in comparison to PL, whereas the solubility of the aforementioned samples were also not significantly different. However, when the concentration of CA or PA was increased from 0.05 to 1% and from 1 to 4%, the crystal yield significantly decreased depending on the type of acids, which was directly related to the increased solubility of the same samples. For instance, in the presence of 1 and 4% CA, the yield was significantly reduced by ~18 and 26%, respectively, in comparison to that of L+0.05CA. The PA also hindered the crystal yield with an increase in its concentration from 0.05 to 1% mostly in a similar manner (~19%) to that of CA, but L+4PA resulted in a greater ($P < 0.05$) reduction of lactose crystallization, by ~35%.

Obviously, the presence of acids in a lactose solution can severely affect the crystallization behavior of lactose (Botsaris, 1982) depending on the acid type and concentration (Table 1). This effect can mainly be ascribed to the chemical properties of the acids. Generally, the acids dissociate into hydrogen ions and their conjugate bases in a solution, but the propensity of releasing the hydrogen ions depends on their dissociation behavior. The weak acids with very low dissociation constants, such as lactic, citric, acetic, amino, fatty, and phosphoric acids, dissociate partly releasing only a few of their hydrogen ions into the solution. This leads to formation of hydronium ions with water via exceedingly strong hydrogen bonds (Belitz et al., 2009). According to the pK_a values of LA (pK_a 3.86), CA (pK_a1 3.09, pK_a2 4.74, and pK_a3 5.40), and PA (pK_a1 2.15, pK_a2 7.21, and pK_a3 12.32), PA is the strongest and LA is the weakest acid. Thus, higher H^+ ion concentration (lower pH) created by the PA can lead to formation of a strong hydration layer (H_3O^+ ion layer) around the lactose molecules interfering with integration of lactose molecules into the lactose crystals, resulting in the lowest crystal yield at its highest concentration. On the contrary, LA, being the weakest, resulted in a higher yield in comparison to the other acids, at respective concentration levels. On the other hand, the presence of these acids appeared to influence the solubility of lactose mainly by changing supersaturation of the solution and thereby lactose crystallization. The crystallization is a process composed of 2 basic steps: nucleation and the crystal growth (Wong and Hartel, 2014). The nucleation process in a solution begins with clustering and aggregation of molecules and ions. A nucleus should turn into a critical cluster size (~100–1,000 atoms) to grow into a firm crystal (Sánchez-García et al., 2018). The supersaturation of the solution is the key factor for a nucleus to transform into a critical size.

Table 1. Crystal yield, solubility, and crystal characteristics of lactose (L) in the presence of lactic (LA), citric (CA), or phosphoric (PA) acids determined by differential scanning calorimetry (DSC)[1]

Sample	Concentration of acids (%)	pH of the solutions	Mean yield (%)	Solubility (g of solute/ 100 g of water)	On-set loss of crystalline water (°C)	Peak loss of crystalline water (°C)	End-set loss of crystalline water (°C)	Difference between end-set and on-set temperature	Enthalpy of loss of crystalline water[2] (J/g)	α-Lactose melting peak (°C)
Pure lactose		5.99	82 ± 3.1^{a}	17.6 ± 0.1^{a}	143 ± 0.8^{ab}	147 ± 0.4^{a}	152 ± 1.8^{a}	9 ± 1.0^{a}	167 ± 9.5^{a}	219 ± 0.5^{a}
L+LA	0.05	2.77	81 ± 2.5^{a}	17.9 ± 0.3^{a}	144 ± 0.7^{ab}	152 ± 1.0^{b}	158 ± 1.6^{b}	14 ± 0.9^{b}	159 ± 0.8^{a}	214 ± 0.2^{b}
	1.00	2.41	80 ± 0.8^{a}	18.2 ± 0.6^{a}	144 ± 0.2^{ab}	151 ± 0.7^{b}	155 ± 1.6^{ab}	11 ± 1.4^{a}	141 ± 14^{b}	206 ± 1.1^{c}
	4.00	1.96	78 ± 1.5^{a}	18.5 ± 0.5^{a}	145 ± 0.0^{ab}	151 ± 0.3^{b}	156 ± 0.8^{b}	11 ± 0.8^{a}	143 ± 1.5^{b}	199 ± 0.4^{d}
L+CA	0.05	2.64	82 ± 0.6^{a}	17.75 ± 0.5^{a}	141 ± 1.3^{a}	148 ± 0.8^{ab}	152 ± 0.8^{a}	11 ± 0.5^{a}	156 ± 8.8^{a}	207 ± 0.0^{c}
	1.00	2.60	64 ± 2.3^{b}	22.3 ± 1.2^{b}	145 ± 0.3^{ab}	154 ± 0.1^{c}	157 ± 0.1^{b}	12 ± 0.2^{a}	140 ± 9.3^{b}	210 ± 0.9^{cb}
	4.00	1.76	56 ± 0.1^{c}	22.5 ± 0.9^{c}	147 ± 0.7^{b}	155 ± 0.5^{c}	158 ± 0.8^{b}	11 ± 0.1^{a}	133 ± 3.4^{c}	199 ± 0.4^{d}
L+PA	0.05	1.69	80 ± 0.4^{a}	18.25 ± 0.6^{a}	147 ± 0.1^{b}	157 ± 1.4^{c}	162 ± 1.0^{c}	15 ± 0.9^{b}	160 ± 0.8^{a}	187 ± 1.7^{e}
	1.00	1.24	61 ± 1.1^{b}	22.25 ± 1.0^{b}	147 ± 0.6^{b}	157 ± 0.2^{c}	167 ± 0.1^{d}	20 ± 0.5^{c}	244 ± 0.4^{d}	189 ± 1.8^{e}
	4.00	0.83	45 ± 3.8^{d}	23.5 ± 2.5^{d}	128 ± 12.5^{c}	152 ± 1.1^{b}	161 ± 2.6^{c}	33 ± 9.9^{d}	87 ± 8.3^{e}	NA[3]

[a–e] Values in the same column with different superscript letters are significantly different ($P < 0.05$).

[1] Values are presented as mean peak area ($3 \geq n$) ±SD.

[2] Enthalpy refers to area under the peak obtained by DSC.

[3] NA = not applicable.

Nuclei, which are smaller than the critical size, tend to re-dissolve in the solution (Mandare and Pangarkar, 2003). In the current study, the presence of CA or PA at concentrations $\geq 1\%$ increased the solubility of lactose in comparison to PL, which directly affected the supersaturation of the solution. This in turn altered the nucleation and crystal growth (Mullin, 1979) resulting in reduced yield of lactose crystals. Thus, current results indicate that nucleation and crystal growth were highly impeded by 4% PA in lactose solutions, in comparison to all other lactose solutions, which showed the highest solubility (23.5 g of lactose/76.5 g of water). On the other hand, pH plays an important role in solubility of a solution (Raghavan et al., 2001; Huppertz and Gazi, 2016; Sánchez-García et al., 2019). We observed an inverse relationship between the solubility and the pH in the present study (Table 1). According to Nickerson and Moore (1974) and Huppertz and Gazi (2016), the pH below 2 should enhance a rate of mutarotation and thereby increase the crystallization rate. However, in the present study, the low pH levels (<2) did not enhance the lactose crystallization similar to observations by Visser (1980). Thus, in the present study, especially 1 and 4% CA and PA acted as solvents or crystal growth inhibitors (Visser, 1980), resulting in reduced crystal yield.

Moreover, in our previous study (Wijayasinghe et al., 2019) we reported that, in the presence of CA or PA at $\leq 1\%$ concentration, lactose can be partially hydrolyzed into glucose and galactose, which differ in solubility, leading to reduction of supersaturation of the entire system. Hence, the reduction of crystal yield in the equivalent solutions in the current study, especially L+1CA and L+1PA, could be due to the presence of glucose and galactose molecules that can attach to the surface of the lactose crystals and obstruct the integration of lactose molecules and subsequent crystallization (Laos et al., 2007).

Thermal Behavior of Lactose Crystals

The thermal analysis of lactose crystals determined by DSC is presented in Table 1. These DSC temperature peaks can be related to water of crystallization, behavior of surface water, and the presence or absence of amorphous and crystalline lactose and those of anomers (Listiohadi et al., 2009). Most of the crystal samples were characterized by the absence of an exothermic peak of crystallization, indicating that the obtained solids were in crystalline form (Figure 1). Furthermore, an appearance of an endothermic peak at ~140 to 150°C indicated the loss of crystalline water, which was incorporated into the crystal lattice during crystallization of α-lactose. This endothermic peak further confirmed the crystallinity of those samples (Gombás et al., 2002). However, the crystallized lactose present in L+1LA, L+0.05CA, L+1CA, and L+4PA displayed a small exothermic peak after the first endothermic peak, which may be attributed to recrystallization of amorphous lactose present in the respective samples (Kaialy et al., 2011). The PL and all the other samples containing LA or CA demonstrated an endothermic peak at ~200 to 220°C, which could be attributed to the melting of α-lactose crystals (Gombás et al., 2002).

Moreover, all of the samples, with the exception of L+PA, appeared to have only one melting peak corresponding to the α-lactose. This further highlighted the absence of β-lactose crystals. Interestingly, the melting peak of α-lactose disappeared in the samples contain-

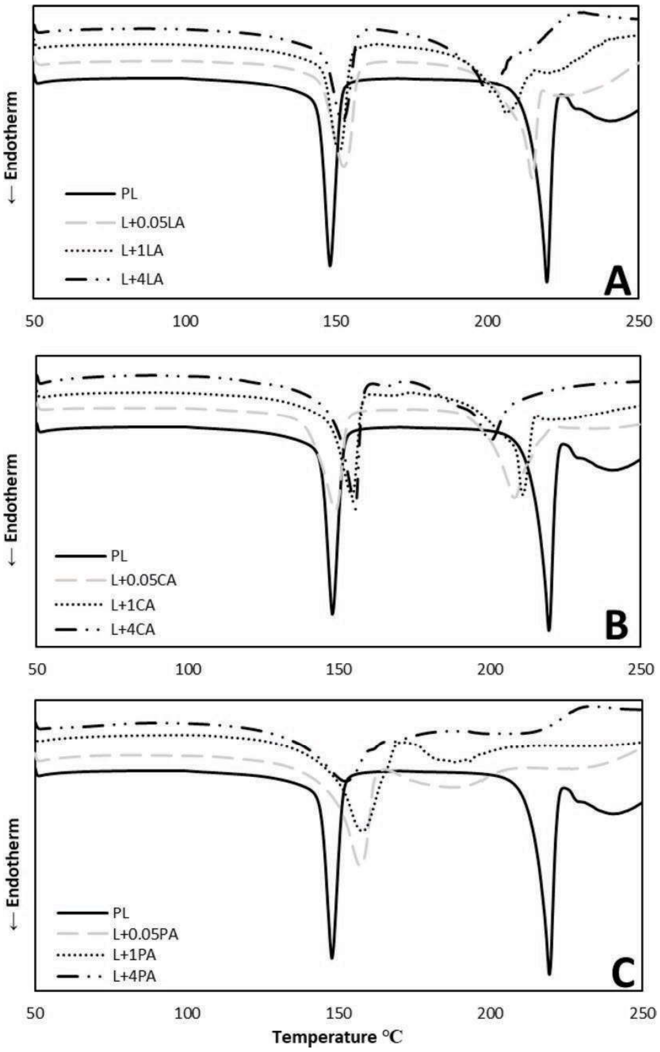

Figure 1. Dynamic differential scanning calorimetry curves for lactose (L) crystals in the presence of lactic (LA; A), citric (CA; B), or phosphoric (PA; C) acids at a concentration of 0.05, 1, or 4% (wt/wt) and pure lactose (PL).

ing PA, possibly due to highly deformed structure, or this peak may have moved to a higher temperature (>250°C) beyond the temperature range applied in the present study. Generally, either type or concentrations of the acids tested did not affect the on-set, end-set, and peak temperatures ($P > 0.05$), but the on-set of crystalline water evaporation was significantly reduced in L+4PA as compared with PL. In contrast, the enthalpies of crystalline water evaporation were reduced in all samples as compared with PL except L+1PA, which requires further elaboration. These reduced enthalpies of crystalline water evaporation could be attributed to obstruction of water removal from the crystal lattice (Wijayasinghe et al., 2015; Chandrapala et al., 2016). Commonly, the temperature range between on-set and end-set temperatures, which is associated with the loss of crystalline water from lactose crystals, widened substantially (11–33°C) in all the samples in comparison to PL (~9°C). The highest range of dehydration temperature (~15–33°C) was reported for the samples containing PA, where higher concentration contributed to the greater association of water with the crystals hindering its evaporation. Widening of the peaks of water evaporation also indicated the deceleration of the crystallization process, which can be attributed to the formation of stronger hydration layer around the lactose molecules.

Additionally, we observed another endothermic peak region at ~160 to 170°C in L+4CA sample, which might be ascribed to the transformation of α-lactose monohydrate into its stable lactose anhydrous form (Kirk et al., 2007) and eventually contribute to formation of more crystalline lactose. This transformation could be induced by high thermal energy generated at high temperatures, but possibly reversible under high humidity (>50%) conditions. However, this peak was also previously suggested to indicate the melting of unstable anhydrous α-lactose (Islam and Langrish, 2010) or as a water dehydration peak (Berlin et al., 1971).

Crystal Morphology of Lactose

The morphology of lactose crystals obtained from the microscopic analysis is depicted in Figure 2. All the samples showed slight deviation in comparison to the characteristic tomahawk morphology of α-lactose crystals (Figure 2A) in the presence of tested acids. Lactose crystals transform into a variety of shapes depending on the solution environment that may affect the growth rate of crystal faces (van Kreveld and Michaels, 1965). Crystals grown in PL solution developed with a tomahawk morphology. In L+0.05LA and L+1CA solutions, crystals were nucleated with fully developed tomahawk morphology with a very small $(0\bar{1}0)$ face and a large $(0\bar{1}\,\bar{1})$ face (Dincer et al., 2009). In L+0.05CA solution, crystals were nucleated with a tomahawk blade sharpened morphology (Figure 2c; Holsinger, 1988), where the $(0\bar{1}0)$ face became sharpened due to slower transport of solvent molecules into the crystal lattice. These crystals have a noticeable $(0\bar{1}\,1)$ face, because it is the slowest developing face. These results indicate that the solution pH above 2 or below 2 can result in crystals with the tomahawk and triangular morphologies with or without irregular faces, respectively (Figure 2 d, e, f, h, i, j). Notably, in the presence of PA at all of the concentrations tested, the lactose crystals were developed with the triangular morphology (Figure 2 d, f, j). This can be related to reduction of the supersaturation of lactose solution due to the hydrolysis as explained by Herrington (1934). Further studies are however required to elucidate how the presence of different acids in the lactose solutions modulate crystallization velocity of the lactose and the crystalline forms. However, it is well established that the presence of impurities in crystallizing solution can result in the morphological changes due to their possible absorption into the faces of growing crystals. The lactose crystals with irregular faces were thus formed in the presence of acids due to incorporation of likely acid molecules into the crystal lattice and consequent interruption of the rhythmic packing arrangement of lactose molecules to form a crystal (Berkovitch-Yellin et al., 1985).

Water Behavior of Lactose Crystals Determined by FTIR

The FTIR analysis was used to elucidate the water behavior of lactose crystals in different samples as shown in Figure 3. The spectral region of 3,600 to 3,200 cm^{-1} depicts the behavior of water molecules (Wolkers et al., 1998). All the samples showed broad vibration bands in this region that correspond to intermolecular stretching vibrations of hydroxyl groups (Kirk et al., 2007; Kaialy et al., 2011). However, variations of these stretching vibrations can be observed depending on the acid type and their concentration in different samples. This highlighted that the vibrations have been either restricted or expanded due to the nature of interactions between acids, water, and lactose. For example, restricted stretching vibrations can be observed in L+4CA and L+1LA indicating the presence of more intermolecular interactions and less water in comparison to PL (Chandrapala et al., 2016), whereas expanded stretching vibrations were observed in all other samples. In the L+4CA sample, the intensity of intermolecular stretching vibrations of hydroxyl groups largely reduced due to increased concentration of CA molecules that resulted in enhanced interactions between acid molecules rather

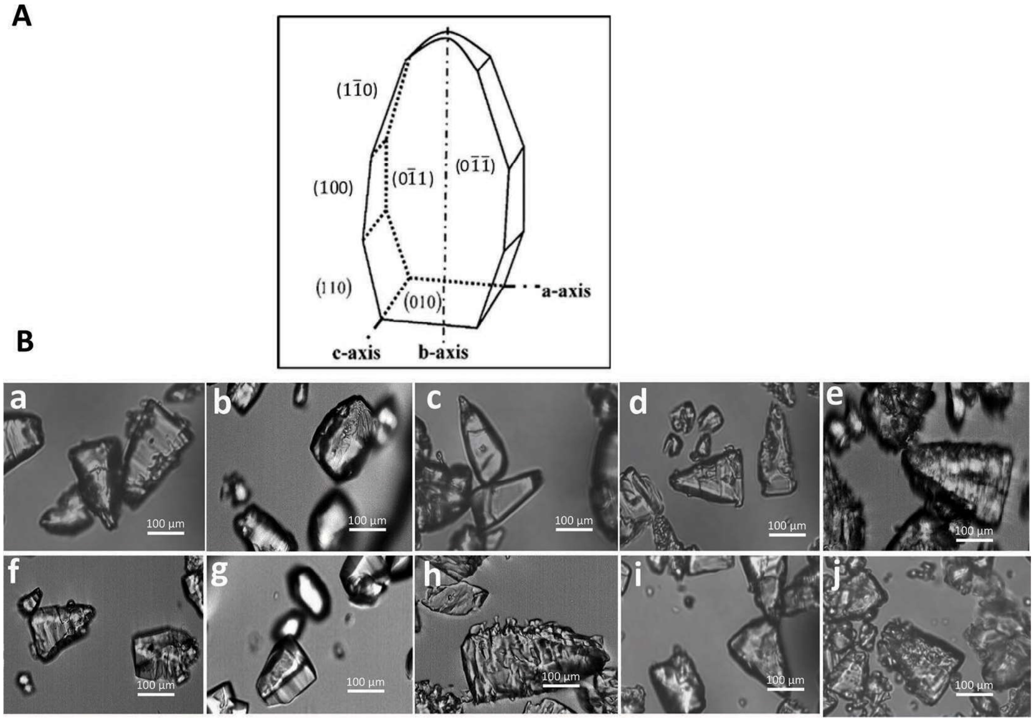

Figure 2. Characteristic tomahawk morphology of α-lactose monohydrate crystal (A) and crystal morphology of lactose in the presence of the acids (B) pure lactose (a), lactose + 0.05% lactic acid (b), lactose + 0.05% citric acid (c), lactose + 0.05% phosphoric acid (d), lactose + 1% lactic acid (e), lactose + 1% citric acid (f), lactose + 1% phosphoric acid (g), lactose + 4% lactic acid (h), lactose + 4% citric acid (i), and lactose + 4% phosphoric acid (j).

than interacting with the water molecule. Similarly, in the presence of 1LA, stretching vibrations of hydroxyl groups were highly reduced, which can be attributed to differences in hydrogen bonding, affected by LA concentration within the crystal lattice (Chandrapala et al., 2016) that limited the mobility of free water molecules. The stretching vibrations were highly expanded in L+4LA, L+0.05CA, and L+4PA displaying greatly restricted mobility of water molecules. These observations apparently indicate changes in molecular mobility of water molecules and structural changes of lactose crystals depending on the acid type and concentration as explained previously by Reid and Fennema (2008). Additionally, the monohydrate form of lactose exhibited a sharp and distinct peak at 3,524 to 3,522 cm^{-1} indicating the stretching of O–H bonding (Kirk et al., 2007). The location and shape of this peak indicate the constrained water molecules contained in the crystal lattice. This peak was more pronounced in L+0.05LA, L+4LA, L+0.05CA, and L+4PA than that of PL, highlighting the presence of more restricted water molecules in the crystal lattice. This was further confirmed by the results of peak area quantification (Table 2) showing a significant ($P < 0.05$) increase in the peak areas in the above samples as compared with PL. Greater peak area indicates the presence of strong interactions between water and acids, indicating the presence of more water molecules in the crystal lattice (Raut et al., 2001) especially in L+4PA sample. This sample also showed a greater association of water with crystals, displayed by DSC results through a wider range of dehydration temperature (~33°C), and a lower water evaporation enthalpy in comparison to other samples, which altogether hindered the evaporation of water from the crystal lattice.

Characterization of Lactose Crystals by XRD

Characteristic XRD patterns of the lactose crystals were depicted in Figure 4. The location of the peaks (2 θ) represents the different types of lactose crystals present in the sample, for instance α-lactose at 12.5°, 16.4°, 20.0°, and 20.1° and anhydrous β-lactose at 10.5°, 20.9°, and 21.0°. A few other peaks have been assigned to the mixtures of lactose crystals, for instance anhydrous α:β with a molar ratio of 5:3 at 19.1° and 21.1° and anhydrous α:β with a molar ratio of 4:1 at 19.5° (Drapier-Beche et al., 1998). In the present study, crystallization of lactose into the form of α-lactose monohydrate can be observed by the peaks at 12.8°–13.3°, 16.7°–16.9°,

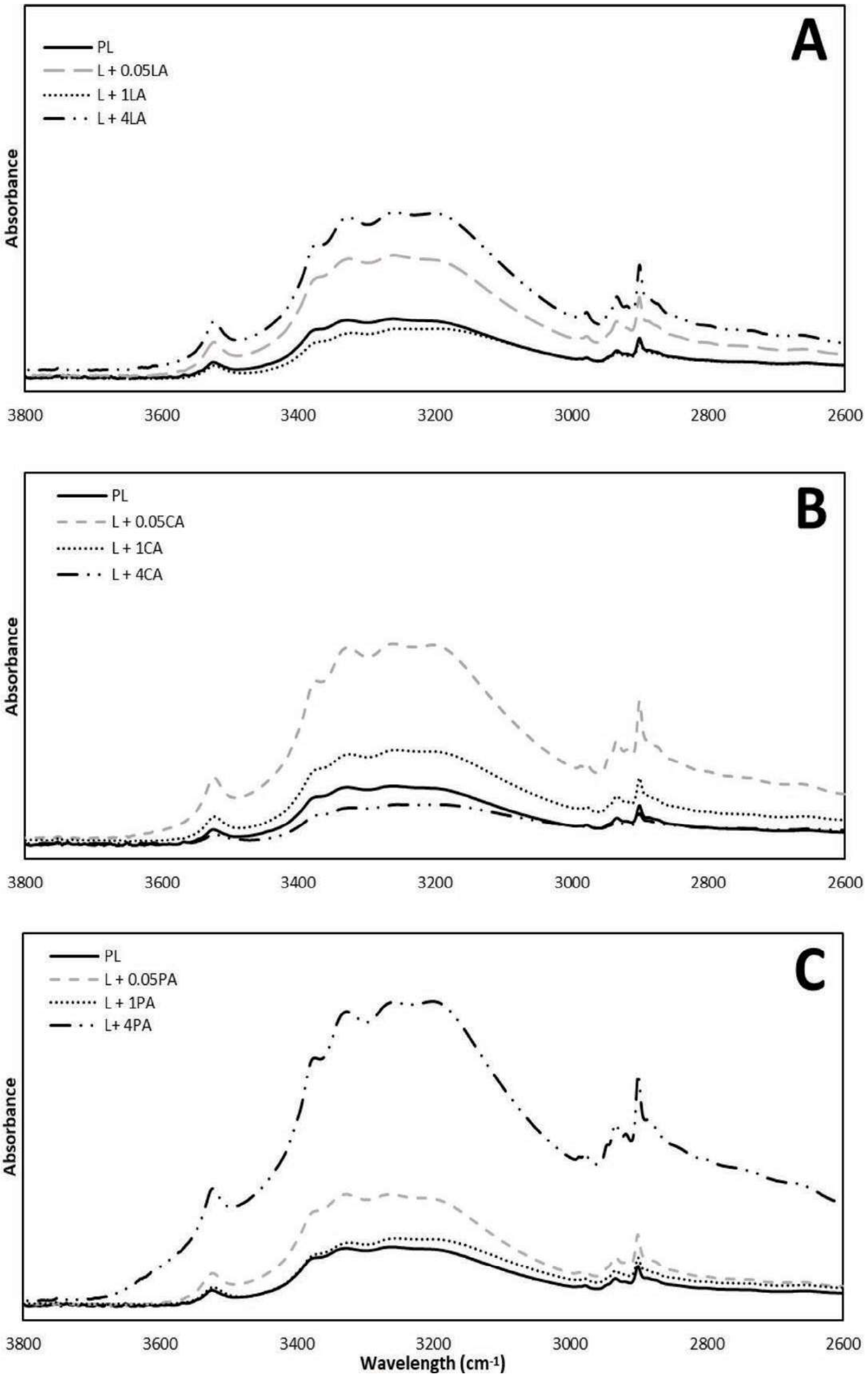

Figure 3. Fourier-transform infrared spectra obtained from the lactose (L) crystals comprising lactic (LA; A), citric (CA; B), or phosphoric (PA; C) acids at a concentration of 0.05, 1, or 4% (wt/wt) and pure lactose (PL).

19.9°, 20.0°, and 20.1° (Table 3) in almost all of the samples tested. However, crystallization of lactose into the anhydrous β-lactose could not be detected in any sample at respective diffraction angles. The slight deviations of the diffraction angles in the present study, in comparison to the previous data (Haque and Roos, 2005), could be related to increased space between crystal layers created by interactions of acids with water and lactose. The peak assigned to the stable anhydrous α-lactose (19.3°) was observed only in L+4CA (Table 3), which coincided with the DSC endothermic peak appeared at ~160 to 170°C in the same sample.

Moreover, we observed a peak related to the mixture of anhydrous α:β with a molar ratio of 4:1 at ~19.5° in all the samples tested including PL, indicating that all the acids tested had no considerable effect on formation of this crystal mixture. However, the crystals with α-lactose and β-lactose in molar ratio of 5:3 were formed only in L+1CA and L+1PA as displayed by a peak at 21.1°. The formation of different types of lactose crystals varies in different food systems mainly due to interactions between lactose and other components present in the system leading to changes in supersaturation, diffusion of lactose molecules, and mutarotation of molecules during nucleation and crystal growth stages (Nijdam et al., 2007). Therefore, variations in the crystal types observed between the present and other studies could be ascribed to the differences in intra- and intermolecular interactions between acids and lactose as already discussed above and differences in crystallization conditions.

The crystalline lactose exhibited several sharp distinct diffraction peaks in the range of 19 to 20° (2θ) as the main signals in all the samples tested (Figure 4). However, the differences in peak intensities could

Table 2. Peak areas of the lactose crystals related to constrained water molecules as determined by the Fourier-transform infrared spectra peak area analysis[1]

Sample	Acid concentration (% wt/wt)	Wave number (cm^{-1})	Mean peak area
Pure lactose	0	3,523	4.9 ± 0.4^{a}
L+LA	0.05	3,523	9.9 ± 0.8^{b}
	1.00	3,522	4.7 ± 0.4^{a}
	4.00	3,523	13.3 ± 1.3^{c}
L+CA	0.05	3,524	15.2 ± 1.2^{c}
	1.00	3,523	8.2 ± 0.8^{b}
	4.00	3,523	3.8 ± 0.2^{a}
L+PA	0.05	3,524	9.0 ± 0.8^{b}
	1.00	3,522	6.1 ± 0.4^{a}
	4.00	3,523	17.7 ± 1.6^{d}

[a–d] Values in the same column with different superscript letters are significantly different ($P < 0.05$).

[1] Values are presented as mean peak area (3 ≥ n) ± SD. L = lactose; LA = lactic acid; CA = citric acid; and PA = phosphoric acid.

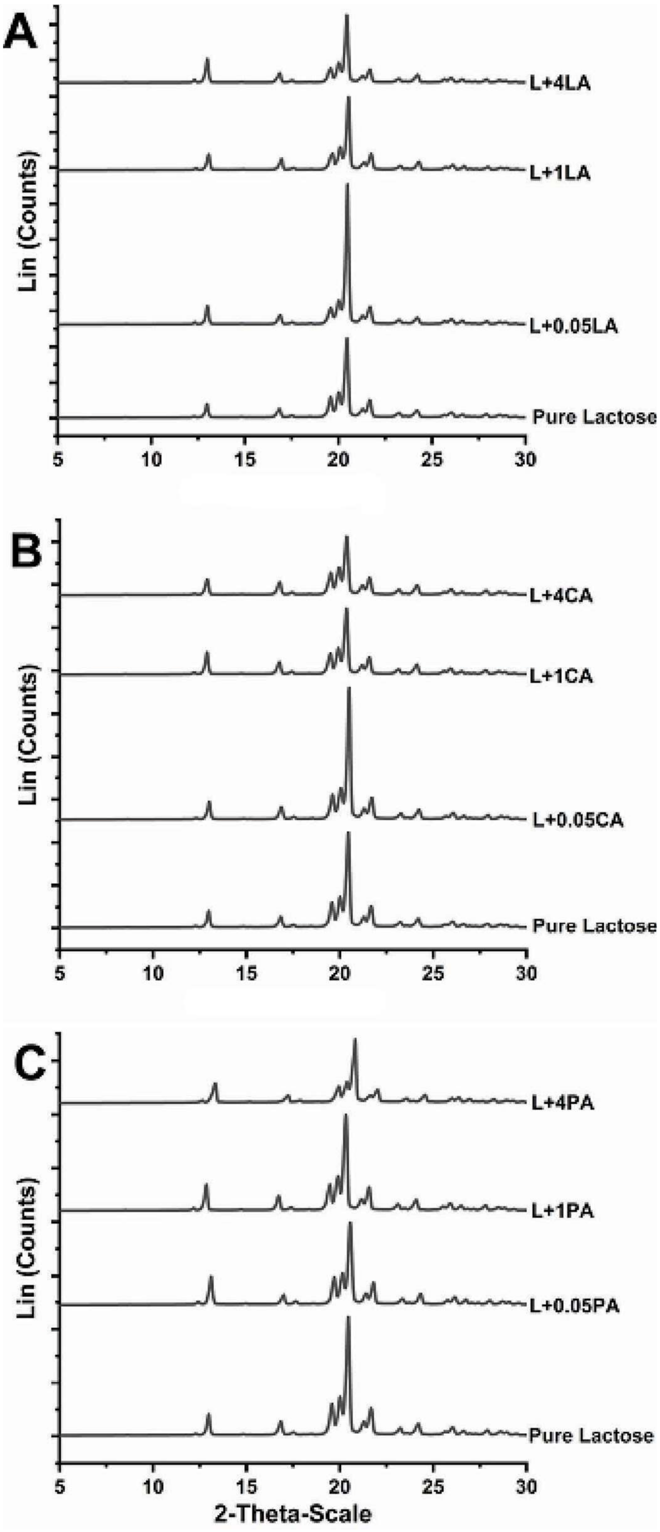

Figure 4. X-Ray diffraction patterns of lactose (L) crystals with addition of lactic (LA; A), citric (CA; B), or phosphoric (PA; C) acids at a concentration of 0.05, 1, or 4% (wt/wt) and pure lactose (PL). Lin = number of reflections raised due to the crystal planes.

Table 3. Characteristics of the different forms of lactose crystals observed by X-ray diffraction patterns at various diffraction angles (2θ)°

		Current study diffraction angle (2θ)°									
			Lactic acid (% wt/wt)			Citric acid (% wt/wt)			Phosphoric acid (% wt/wt)		
Types of crystals	Diffraction angles[1] (2θ)°	Pure lactose	0.05	1	4	0.05	1	4	0.05	1	4
α-Lactose monohydrate	12.5	12.9	13.0	13.0	12.9	13.0	12.8	12.9	13.1	12.8	13.3
	16.4	16.8	16.9	16.9	16.9	16.8	16.7	16.7	17.0	16.7	17.2
	20.0/19.9	20.0	20.0	ND[2]	20.0	20.0	19.9	19.9	ND	19.9	19.9
	20.1	ND	ND	20.1	ND	ND	ND	ND	20.1	ND	ND
Stable anhydrous α-lactose	19.37	ND	ND	ND	ND	ND	ND	19.36	ND	ND	ND
Anhydrous β-lactose	10.5 20.9 21.0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Anhydrous, α:β molar ratio 5:3	19.1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	21.1	ND	ND	ND	ND	ND	21.1	ND	ND	21.1	ND
Anhydrous, α:β molar ratio 4:1	19.5	19.6	19.6	19.7	19.6	19.6	19.5	19.5	19.7	19.4	19.8

[1] Literature data (Haque and Roos, 2005).

[2] ND = not detected.

be related to changes in degree of lactose crystallinity depending on types and concentration of the acids tested as shown in Table 4. The highest degree of lactose crystallinity was recorded in L+4CA while lowest in L+0.05LA in comparison to PL. To form crystalline materials, lactose molecules should be arranged or connected in a regularly repeating pattern or a lattice through van der Waals attractions and hydrogen bonds (Walstra, 2003). However, the interactions between acid molecules and lactose may form lactose-water-acid complexes and obstruct the well-ordered arrangement and bonding pattern of lactose molecules interfering the process of lactose crystallization (Wijayasinghe et al., 2015). For example, in the present study, the LA appeared to highly interact with water and lactose to reduce the orderly arrangement of lactose molecules in L+0.05LA and thereby reduce lactose crystallinity. This could be attributed to the behavior of electron cloud around the LA molecule. Lactic acid is the smallest molecule based on its electron cloud in comparison to other examined acids. Thus, 0.05% of LA strongly interacts with lactose and water, as explained above, leading to formation of lactose-lactic acid-water complexes. The CA possibly increased the water-acid or acid-acid interactions (or both), allowing lactose molecules to interact together under minimal free energy to form crystal lattice (Wijayasinghe et al., 2019).

The average particle size of crystallite lactose (determined by a Mastersizer) was substantially reduced in the presence of all the acids tested in comparison to PL and the trend was mostly concentration dependent (Table 4). The differences in the particle size can be attributed to the hydrophilic nature of the acid molecules and their ability to bind water. However, as discussed earlier, water molecules that are hydrogen bonded with 4 different α-lactose molecules can obstruct removal of water, causing defects in lactose crystals and eventually forming different particle sizes (Nickerson, 1954). Furthermore, reduction of supersaturation of lactose, affected by increase in the acid concentration, may prevent aggregation of crystals, resulting in smaller particle sizes. Additionally, the van der Waals interactions between crystals can be interfered with by acid molecules due to formation of water-acid-lactose complexes. This can contribute to hindering the aggregation of nucleated crystals and loss of their uniformity, resulting in smaller particles. The average crystallite size of lactose (determined by XRD) was not substantially changed across all the samples (Table 4). It appeared that the crystallite size was always smaller than corresponding particle size since the XRD usually captures crystallite contained in the particles (Monshi et al., 2012). However, the crystallite size was not affected by the presence of the tested acids.

Table 4. Crystallinity, particle size, and crystallite size of the lactose

Sample[1]	Acid concentration (% wt/wt)	Average degree of crystallinity[2] (%)	Average particle size[3] (nm)	Average crystallite size[2] (nm)
Pure lactose	0	79.5^{ab}	$142 \pm 4 \times 10^3$	319 ± 61
L+LA	0.05	73.3^{c}	$121 \pm 4 \times 10^3$	347 ± 80
	1.00	79.1^{a}	$97 \pm 3 \times 10^3$	333 ± 71
	4.00	79.9^{b}	$69 \pm 2 \times 10^3$	311 ± 69
L+CA	0.05	77.3^{d}	$118 \pm 18 \times 10^3$	331 ± 65
	1.00	82.3^{f}	$112 \pm 31 \times 10^3$	315 ± 54
	4.00	85.2^{h}	$86 \pm 12 \times 10^3$	283 ± 45
L+PA	0.05	81.2^{e}	$93 \pm 22 \times 10^3$	314 ± 45
	1.00	81.7^{e}	$71 \pm 9 \times 10^3$	317 ± 54
	4.00	77.9^{g}	$87 \pm 16 \times 10^3$	259 ± 43

[a–h] Values in the same column with different superscript letters are significantly different ($P < 0.05$).

[1] L = lactose; LA = lactic acid; CA = citric acid; and PA = phosphoric acid.

[2] Determined by X-ray diffraction.

[3] Determined by Mastersizer (Malvern-2000, Malvern Instruments Ltd., Malvern, UK).

CONCLUSIONS

The crystallization behavior of lactose is mostly affected by the type and concentration of the acids present in the lactose systems. Evidently, interactions of lactose with water and acids modulate the crystalline habits of lactose. The presence of 1% LA, 0.05 and 1% CA, and 4% PA can result in formation of amorphous lactose during crystallization, whereas stable anhydrous α-lactose is produced in the presence of 4% CA. The presence of either CA or PA at ≥1% can cause a greater reduction of crystal yield (≥18%). Moreover, the particle size and crystal shapes are affected depending on the type and concentration of the acid present in the lactose solutions. Therefore, appropriate selection of processing conditions, acid types, and their concentration are important for the crystallization of lactose present in a complex mixture because lactose nucleation and crystallization can be governed by behavior and properties of the solvent water that is considerably influenced by the presence of acids. Therefore, the present study emphasizes the importance of controlling lactose-water interactions and anomeric equilibrium of lactose to achieve a proper crystallization process.

ACKNOWLEDGMENTS

The authors are grateful to the Victoria University for providing financial and technical support. The authors have not stated any conflicts of interest.

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Chapter 5: Unraveling the Influences of Sodium, Potassium, Magnesium, and Calcium on the Crystallization Behavior of Lactose

This chapter has been published as a research article in the Journal *Foods*. Wijayasinghe, R., Vasiljevic, T., & Chandrapala, J. (2023). Unraveling the Influences of Sodium, Potassium, Magnesium, and Calcium on the Crystallization Behavior of Lactose. Foods, 12(24), 4397.
https://doi.org/10.3390/foods12244397

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1. PUBLICATION DETAILS (to be completed by the candidate)

Title of Paper/Journal/Book: Unraveling the Influences of Sodium, Potassium, Magnesium, and Calcium on the Crystallization Behavior of Lactose [redacted]

Surname: Wijayasinghe
First name: Rangani

Institute: Institute for Sustainable Industries and Liveat
Candidate's Co 85%

Status:
Accepted and in press: ☑ Date: 05/
Published: ☑ Date: 07/

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Todor Vasiljevic	10%	Concept development, manuscript reviewing and editing	[redacted]	2.01.2024
Jayani Chandrapala	5%	Concept development, manuscript reviewing and editing, and journal submission	[redacted]	5/01/2024
			[redacted]	
			[redacted]	

Updated: September 2019

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Unraveling the Influences of Sodium, Potassium, Magnesium, and Calcium on the Crystallization Behavior of Lactose

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Abstract: The inability of lactose to properly crystallize due to the presence of high amounts of salts poses significant hurdles for its downstream processing with some dairy waste streams such as acid whey. This study aimed to investigate the physicochemical and thermal behaviors of lactose in the presence of cations commonly present in acid whey. A model-based study was conducted, utilizing various cations (Mg, Ca, K, and Na) at concentrations (8, 30, 38, and 22 mM, respectively) that are typically found in acid whey. The research experiments were conducted using a factorial design. The thermal analysis of concentrated solutions revealed augmentation in the enthalpy of water evaporation in the presence of individual cations and their combinations in comparison with pure lactose (698.4 J/g). The degree of enthalpy increased following the order of Na^+ (918.6 J/g), K^+ (936.6 J/g), Mg^{2+} (987.0 J/g), Ca^{2+} (993.2 J/g), and their mixture (1005.4 J/g). This resulted in a substantial crystal yield decline in the exactly reversed order to that of the enthalpy. The greatest decline was observed in the presence of the salt mixture (63%) followed by Ca (67%) compared with pure lactose (79%). The yield reduction was also inversely related to the solubility of lactose. The presence of divalent cations appeared to play a role in the isomerization of lactose molecules observed using DSC and XRD diffractograms according to the disappearance of peaks related to β lactose. The effect of salts on the crystallization of lactose was a combination of cation–lactose interactions, changes in the solubility of lactose, ion–dipole interactions between water and cations, and changes in the structure of water molecules. By deviating the composition of acid whey, the crystallization of lactose can be enhanced, leading to the improved downstream processing of acid whey.

Keywords: lactose; crystallization; acid whey; salts

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Citation: Wijayasinghe, R.; Vasiljevic, T.; Chandrapala, J. Unraveling the Influences of Sodium, Potassium, Magnesium, and Calcium on the Crystallization Behavior of Lactose. *Foods* **2023**, *12*, 4397. https://doi.org/10.3390/foods12244397

Academic Editors: Shuwen Zhang, Yan Li and Hongjia Li

Received: 2 October 2023
Revised: 16 November 2023
Accepted: 5 December 2023
Published: 7 December 2023

1. Introduction

The process of lactose crystallization in foods is highly complex and involves a multitude of factors that impact the ultimate product quality and processability. Proper control over the crystallization process is essential for preserving the quality, extending the shelf life of specific food products, and increasing downstream processability during the processing of different food streams or even manufacturing of certain food products. For example, lactose crystallization is the first processing step in the separation of lactose from whey solutions originating from cheese and yogurt production. These by-products are commonly referred to as serum that remains subsequent to the coagulation and filtration of milk [1].

Industrially, any whey is concentrated before lactose crystallization to achieve 60–65% of total solids, out of which 50–55% is composed of lactose. This concentrated whey is subjected to spray drying, resulting in the production of powders that find extensive usage in diverse food applications [2]. However, the processing of acid whey, which originates as a by-product of acid-coagulated products such as Greek yogurts or soft cheeses, is widely regarded as a challenging task, primarily due to the inability of lactose to undergo crystallization. This results in lactose persisting in its amorphous state during the concentration process, thereby impeding subsequent processing steps. Due to hindered lactose crystallization in acid whey, the yielded spray-dried powders are hygroscopic with a high propensity to sticking and caking [3]. More recently, it was shown that the presence of lactic acid induced the formation of a strong hydration layer surrounding lactose molecules, thereby hindering water removal and inhibiting lactose crystallization [4] A considerable amount of literature has been published on lactose behavior in the presence of different additives such as lactic acid [5], proteins [6], and calcium [7–9]. Previously, it was established that the salts present in a lactose solution can decrease or increase the growth rate of lactose crystals [10], which was also clear from a previous report [11] showing the rate of lactose crystal growth after the addition of potassium (K) as KCl. Salt-induced changes in lactose crystallization can also partially be attributed to changes in pH, as this affects the mutarotation of lactose, and a reduction in pH reduces the crystal growth [12].

Cationic impurities in lactose solutions also govern lactose crystallization in a direction that is dependent on the concentration and the type of the cation present [8]. It was hypothesized that alterations in the solubility of lactose are responsible for the varying crystallization patterns observed in the presence of mineral impurities [13]. For instance, ionic Ca^{2+} ion has been found to play a significant role in the organization of water structure. Ca^{2+} is a divalent ion that possesses a robust electric field, which acts as a promoter of water structuring. The resulting structural changes in water molecules can have a direct impact on the solubility of lactose, which is a crucial factor governing lactose crystallization [14]. Similarly, the inclusion of sodium phosphate has been observed to decrease lactose solubility [11] due to the high charge density of water-structure-making ions, leading to the reorientation of water molecules, which, in turn, reduces the solubility of lactose. On the other hand, the addition of potassium phosphate has been found to increase lactose solubility [15], which is attributed to the water-structure-breaking capability of potassium. However, lactose's behavior in the presence of different impurities differs according to various phases of the crystallization process, including concentrated solution [4], spray-dried powders [5,16], and crystals [9], due to the different interactions taking place within the system.

Despite the existence of a significant body of literature on this topic, the behavior of lactose in the presence of cations remains a complex and poorly understood phenomenon. As such, more systematic studies on the impact of cations present in acid whey on the crystallization behavior of lactose are needed. Thus, the present study evaluated the physicochemical and thermal behaviors of lactose affected by different cations (Mg^{2+}, Ca^{2+}, K^{+}, and Na^{+}) at concentrations frequently encountered in acid whey waste streams, either individually or in combination. In the context of utilizing salts as additions of various cations, a consistent anion in the form of chloride (Cl^{-}) was upheld to achieve homogeneity in the impact of the anion in this study.

2. Materials and Methods

2.1. Materials

Lactose powder and analytical-grade salts (KCl, NaCl, $MgCl_2$, and $CaCl_2$) were obtained from Murray Goulburn Co-operative Co., Ltd. (Brunswick, Melbourne, Australia) and Sigma–Aldrich Pvt. Ltd. (Macquarie Park, Sydney, Australia), respectively. All solutions were prepared fresh using Milli-Q water at all times.

2.2. Preparation of Lactose Solutions

Lactose solutions were prepared to mirror the concentration of salts and lactose present in acid whey (Table 1). Initially, lactose powder was dissolved in Milli-Q water at 70 °C to achieve a concentration of 40 g of lactose per 100 g of solution. The solution was gently agitated with a magnetic stirrer to accelerate the dissolving process. Any undissolved lactose particles were removed by filtering the solution through 0.45 µm Whatman filter paper. Stock solutions of 5 M composed of calcium chloride ($CaCl_2$), magnesium chloride ($MgCl_2$), potassium chloride (KCl), and sodium chloride (NaCl) were prepared individually. The filtered lactose solution was divided into five parts, and calculated amounts of salts were added to mimic the concentrations of salts present in acid whey to obtain lactose solutions containing the following levels of salts: 38 mM K, 30 mM Ca, 22 mM Na, 8 mM Mg, or a combination of the aforementioned salts at their respective concentrations, as shown in Table 1. A lactose solution containing no added salts was used as the control sample (PL). All the solutions were stirred using a magnetic stirrer to obtain homogenous samples.

Table 1. Combinations of lactose and salt used in this study.

Lactose Salt Combination	Composition of Each Combination
Pure lactose	5 g/100 g solution
Lactose + KCl	5% lactose + 38 mM K
Lactose + $CaCl_2$	5% lactose + 30 mM Ca
Lactose + NaCl	5% lactose + 22 mM Na
Lactose + $MgCl_2$	5% lactose + 8 mM Mg
Lactose + salt mixture	5% lactose + 38 mM K + 30 mM Ca + 22 mM Na + 8 mM Mg

Lactose solutions with and without added salts were subsequently concentrated using a rotary evaporator at 55–60 °C to achieve ~50% (*w*/*w*) total solids, based on refractometry measurements (Atago Abbe, Tokyo, Japan) after calibration with water.

Parts of the concentrated lactose solutions were subsequently subjected to crystallization using a method previously proposed in [17]. The remaining portions of the concentrated solutions were analyzed to establish their properties. The pH of the samples was monitored using a pH meter (inoLab pH7110, WTW, Xylem Inc., Trifthof, Weilheim, Germany).

2.3. Crystallization Procedure

Rapid cooling of the concentrated solutions from 55 °C to 30 °C followed by a 2 h equilibration at the same temperature using a water bath was employed to induce nucleation, with immediate effect. This was followed by slow cooling at a rate of 5 °C/h until the solution temperature reached 15 °C. The solutions were stirred continuously and kept at a constant temperature (15 °C) throughout the night to achieve complete mutarotation. Once the solutions reached solubility equilibrium, the final solubility of all samples was determined by measuring the concentration of the supernatants. The lactose crystals were then separated through 0.45 µm filter paper and dried in an oven at 80 °C for four hours. The percentage yield of lactose was determined using the following equation:

$$\text{Yield}(\%) = \frac{0.95 \times \text{Mass of lactose crystals obtained}}{\text{Maximum theoretical crystal yield}} \times 100$$

In order to accurately calculate the amount of lactose recovered, the crystallization of 50% *w*/*w* α-lactose monohydrate was taken into consideration. The maximum solubility of lactose, which was found to be 17.84 g of lactose in 100 g of water at a temperature of 15 ± 1 °C, was also considered. As a result, the maximum theoretical yield from a 50% solution was determined to be 82.16 g of lactose per 100 g of water. The detailed method used to calculate the crystal yield is explained elsewhere [17].

2.4. Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry (DSC) serves as a fundamental method for quantifying the thermal characteristics of materials, enabling the establishment of a correlation between temperature and specific physical attributes of substances. Moreover, it stands as the sole approach for directly determining the enthalpy linked to the process under investigation. The thermal characteristics of the concentrated lactose solutions were measured immediately after concentration using a DSC instrument (DSC 1 STARe System, gas controller, GC 200, Mettler-Toledo, Columbus, OH, USA) combined with Mettler Toledo STARe evaluation software, version 15.00, according to the improved method [4] with slight modification to the scanning range. All samples were scanned with a starting temperature of 50 °C and end temperature of 200 °C at a heating rate of 5 °C/min. The onset and endset temperatures of water evaporation and related enthalpies (enthalpy is a measure of the total energy stored within a system) were analyzed using STARe thermal analysis software (Version 15.00) (Mettler Toledo).

Lactose crystal samples were also subjected to the same procedure with slight modifications. An amount of 2–5 mg of each crystal sample was scanned with a starting temperature of 50 °C and an end temperature of 250 °C at a rate of 10 °C/min following the method explained elsewhere [9].

2.5. Fourier-Transform Infrared Spectroscopy (FTIR)

The FTIR spectra of the concentrated lactose solutions and lactose crystal samples were collected using an FTIR spectrometer (Frontier 1, Perkin Elmer, Waltham, MA, USA). The concentrated solution spectra were collected instantly after reaching the solution concentration of ~55% (*w*/*w*) in the range of 4000–600 cm^{-1} in absorbance mode after background subtraction [14]. Every spectrum was obtained with an average of sixteen scans recorded at a resolution of 4 cm^{-1}. Similarly, FTIR spectra of lactose crystal samples were also obtained in the range of 4000–600 cm^{-1} in absorbance mode, and the spectra were analyzed within the wavelength ranges of interest, 4000–2600 cm^{-1} and 1200–950 cm^{-1}.

2.6. X-ray Diffraction (XRD)

X-ray powder diffraction patterns of all the samples were obtained using a Rigaku Miniflex 600 X-ray diffractometer (Rigaku Corporation, Wilmington, MA, USA). The lactose powder samples were firmly pressed to form a thin film (0.2 mm) on the glass sample holders before loading them into the instrument. The operating conditions were set at 40 kV and 15 mA and a step size of 0.02° with a speed of 1.2/min in a 2θ scanning range from 5° to 30° with a Ni Kβ-filter and a D/tex detector.

2.7. Statistical Analysis

Two replications of the entire experimental design were carried out. Observational data were analyzed with one-way ANOVA, and 95% confidence intervals were applied. Comparison of the means was assessed using Tukey's test, with $p < 0.05$ considered statistically significant.

3. Results

3.1. pH Values of the Lactose Solutions in the Presence of Salts

The resulting pH of the pure lactose solution was found to be 5.9. The introduction of 22 mM Na into the lactose solution resulted in a decrease in pH to 5.6. The presence of 38 mM K and the cations combination of Mg^{2+}, Ca^{2+}, K^{+}, and Na^{+} further reduced the pH to 5.5. The addition of 8 mM Mg and 30 mM Ca to the lactose solution resulted in further decreases in pH to 4.9 and 4.8, respectively (Table 2).

Table 2. pH values; onset, peak, and endset temperatures; and enthalpy values for loss of water in concentrated lactose solutions, as affected by the presence of different cations in the form of chlorides.

Sample	pH *	Onset Temperature (°C)	End Set Temperature (°C)	Peak Temperature (°C)	Enthalpy Water Evaporation (J/g)
Pure lactose	5.9	143.8 ± 9.1 ^{a}	154.5 ± 10.5 ^{a}	147.6 ± 9.4 ^{a}	698.4 ± 6.5 ^{d}
Lactose + KCl	5.5	147.3 ± 3.9 ^{a}	158.2 ± 2.3 ^{a}	151.8 ± 1.6 ^{a}	936.6 ± 29.8 ^{bc}
Lactose + $CaCl_2$	4.8	143.2 ± 3.4 ^{a}	155.2 ± 0.4 ^{a}	148.2 ± 1.2 ^{a}	993.1 ± 30.8 ^{a}
Lactose + NaCl	5.6	148 ± 2.9 ^{a}	165.2 ± 1.1 ^{a}	156.8 ± 1.6 ^{a}	918.6 ± 4.6 ^{c}
Lactose + $MgCl_2$	4.9	146.4 ± 0.0 ^{a}	164.9 ± 0.0 ^{a}	155.3 ± 0.0 ^{a}	987 ± 0.0 ^{ab}
Lactose + salt mixture	5.5	154.2 ± 1.1 ^{a}	163.8 ± 0.9 ^{a}	155.2 ± 1.0 ^{a}	1005.4 ± 15.5 ^{a}

* SD for pH was ± 0.05. $^{a–d}$ Values in the same column with different superscript letters are significantly different ($p < 0.05$).

3.2. Crystal Yields and Final Solubilities of Lactose Solutions in the Presence of Salts

Pure lactose had a crystal yield of ~79%, which agreed with the results reported previously [9,17]. The final solubility of pure lactose was 17.6 g/100 g of water, which was close to a previously observed value of 17.84 g/100 g [18]. The presence of 8 mM of Mg or 22 mM of Na resulted in ~76% or ~75% crystal yields, respectively, with corresponding solubilities of 19 or 19.5 g/100 g of water. In the presence of 38 mM of K, the crystal yield decreased further to ~70%, which was followed by an increased solubility of 20.2 g/100 g of water. The resulting increased solubility and reduced yield of the lactose in the presence of KCl compared with PL is in agreement with a previous study [15]. Moreover, the presence of 30 mM of Ca yielded ~67% crystals with a resulting final solubility of 22.5 g/100 g of water. Further yield impairment was observed in the presence of cations in combination as the yield was further significantly reduced to ~63%, resulting in 28.5 g/100 g of water as the final solubility. The above-observed solubility results show an inverse relationship with the crystal yield (Table 3).

Table 3. Mean crystal yields, solubilities; onset, peak, and endset temperatures; and enthalpy values for crystallization and melting points of lactose crystals, as affected by the presence of different cations in the form of chlorides.

Sample	Mean Crystal Yield (%)	Solubility (g/100 g of Water)	Onset Loss of Crystalline Water (°C)	Peak Loss of Crystalline Water (°C)	Endset Loss of Crystalline Water (°C)	Enthalpy of Loss of Crystalline Water (J/g) *	α-Lactose Melting Peak (°C)	β-Lactose Melting Peak (°C)
Pure lactose	79	17.6	143.3 ± 0.8 ^{ab}	147 ± 0.4 ^{a}	152.9 ± 1.4 ^{a}	167 ± 8.6 ^{a}	219.5 ± 0.9 ^{a}	230.5 ± 0.9 ^{b}
Lactose + KCl	70	20.2	137.2 ± 1.3 ^{d}	141.2 ± 0.9 ^{c}	145.2 ± 1.1 ^{d}	133.3 ± 4.9 ^{c}	213.6 ± 0.6 ^{b}	228.5 ± 0.6 ^{a}
Lactose + $CaCl_2$	67	22.5	144.5 ± 0.3 ^{a}	148.1 ± 0.9 ^{a}	154.4 ± 1.2 ^{a}	138.1 ± 3.8 ^{bc}	207.3 ± 0.8 ^{c}	ND
Lactose + NaCl	75	19.5	138.4 ± 0.8 ^{cd}	143.9 ± 0.7 ^{b}	148.8 ± 1.3 ^{bc}	133.2 ± 0.9 ^{c}	214.6 ± 0.6 ^{b}	230.7 ± 1.4 ^{b}
Lactose + $MgCl_2$	76	19.0	142.1 ± 0.2 ^{b}	146.5 ± 0.7 ^{a}	151.6 ± 0.7 ^{ab}	148.8 ± 0.2 ^{b}	205.1 ± 0.3 ^{d}	ND
Lactose + salt mixture	63	28.5	139.7 ± 0.2 ^{c}	142.8 ± 0.2 ^{bc}	148.0 ± 1.4 ^{cd}	129.7 ± 8.5 ^{c}	203.3 ± 0.5 ^{e}	ND

* Enthalpy refers to the area under the peak obtained using DSC. Values are presented as means of three individual observations ($3 \geq n$) plus or minus standard deviation. $^{a–e}$ Values in the same column with different superscript letters are significantly different ($p < 0.05$). ND = not detected.

3.3. Thermal Behaviors of Concentrated Lactose Solutions and Crystals

The DSC analysis of the concentrated lactose solutions showed an endothermic peak at ~147 to ~155 °C, which was associated with the removal of water molecules from the lactose solution [19]. The recorded peak temperature was unaffected by the presence of 30 mM of Ca or 38 mM of K, while the presence of other cations individually and in combination slightly increased the peak temperature. However, the water removal peak temperatures were not significantly changed compared with pure lactose (Table 2). Similarly, the recorded onset and endset temperatures for water evaporation were also not affected ($p > 0.05$) by the presence of individual salts or salt mixture. In contrast, the enthalpy associated with water evaporation noticeably increased in the presence of different salts and the combination of salts. The average energy required to evaporate water from the pure lactose (PL) solution was ~698 J/g, while the addition of 22 mM Na, 38 mM K, 8 mM Mg, 30 mM Ca, and the salt mixture increased the average water evaporation enthalpy to ~918.6, 936.6, 987, 993.1, and 1005.4 J/g, respectively (Table 2). The correlation between the rise in the water evaporation enthalpy and the metal group, as well as the atomic radius of each group element, was noted in the present study. Na and K are classified in the alkali group, whereas magnesium (Mg) and calcium (Ca) belong to the alkaline group and show an increase in atomic radius with an ascending atomic number.

The DSC thermograms of the lactose crystal samples are illustrated in Figure 1, with the parameters describing the thermal behaviors of the lactose crystals in the presence of salts shown in Table 3. In general, the temperature peaks in the DSC analysis were found to be associated with the presence or absence of amorphous lactose, crystalline lactose, and the related anomers (α and β forms) of lactose. The exothermic peak observed at ~167 °C is attributed to the transformation of amorphous lactose into its crystalline form. The endothermic peak detected at ~144 °C indicates the elimination of crystalline water, thereby providing evidence for the existence of the crystalline form of lactose. The α and β forms of lactose can be identified by the presence or absence of α-lactose and β-lactose melting peaks at approximately 213 °C and 224 °C, respectively [20,21]. All the samples in the present study were characterized by an endothermic peak at approximately 140–150 °C, revealing the crystalline nature of the samples. This peak refers to the loss of water molecules from a crystal structure [19,22]. The resulting peak values for 22 mM Na, 38 mM K, and salt mixture were significantly different ($p < 0.05$) from that of PL. On the other hand, the peak values for 8 mM Mg or 30 mM Ca were not significantly different from that of PL (Table 3). The DSC thermograms for the lactose crystals in the presence of 30 mM Ca, 8 mM Mg, and 22 mM Na exhibited a small exothermic peak at ~175 °C, which could be associated with the recrystallization of amorphous lactose present in the relevant samples [23]. The PL crystals had the greatest enthalpy value for the removal of crystalline water (167 J/g), which was significantly diminished in the presence of salts ($p < 0.05$). The greatest decline was observed in the presence of a salt mixture, resulting in an enthalpy value of 129.6 J/g, which presents a ~23% decrease compared with that of PL. When it came to individual salts, the magnitude of reduction was the greatest with monovalent cations with a ~20.2% and 20% decline in the presence of 22 mM Na and 38 mM K. At the same time, 30 mM Ca and 8 mM Mg decreased the enthalpy by 17% and 10.9%, respectively. All the lactose samples exhibited another endothermic peak at ~200–220 °C, which can be assigned as the melting of α-lactose [24]. An additional endothermic peak was observed subsequent to the α-lactose melting peak at approximately 228 °C in the PL sample and samples containing Na and K. This additional peak is likely attributed to the melting of β-lactose [25]. This β-lactose peak disappeared in the DSC thermograms of lactose samples containing the salt mixture, Ca, or Mg.

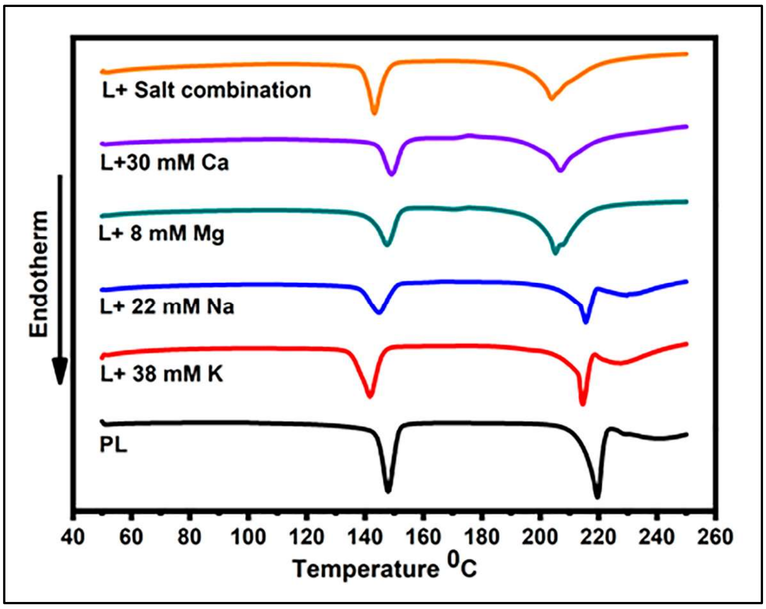

Figure 1. Dynamic DSC curves for lactose crystals in the presence of 8 mM Mg, 30 mM Ca, 38 mM K, 22 mM Na, salt combination, and pure lactose (PL).

3.4. FTIR Spectra of Concentrated Lactose Solutions and Crystals

The behavior of water molecules within a food system is dependent on various interactions taking place between components of the food matrix. As such, understanding how water molecules behave within a food system is crucial to controlling the stability of food systems and crystallization. FTIR is a simple and rapid technique that can be used to determine these changes via the molecular vibrations of various compounds in the food system. In Figure 2 (range 4000–600 cm^{-1}), Figure 2a represents the FTIR spectra obtained for the concentrated lactose solutions in the presence of various cations. The physical state of carbohydrates was determined by analyzing the vibrations and stretching of C-C and C-O bonds in the 800–1200 cm^{-1} range [26]. Figure 2b illustrates the FTIR spectra of lactose with or without the addition of individual salts and their mixture in the range of 1200–850 cm^{-1}. Two strong peaks were observed in all concentrated lactose solutions at ~1030 cm^{-1} and 1070 cm^{-1}. It was found that the corresponding peaks were correlated with C-C stretching vibrations and C-O stretching in lactose, respectively [27,28]. The greatest intensity of these peaks was observed in the presence of 38 mM K, indicating greater stretching vibrations in the lactose molecules, while the lowest was observed for the lactose solution containing 8 mM Mg, which could be attributed to the restricted movements of the lactose molecules. No considerable change, relative to the control, was observed in the intensity of the 1200 to 850 cm^{-1} range for the samples with 30 mM Ca, 22 mM Na, or the salt mixture.

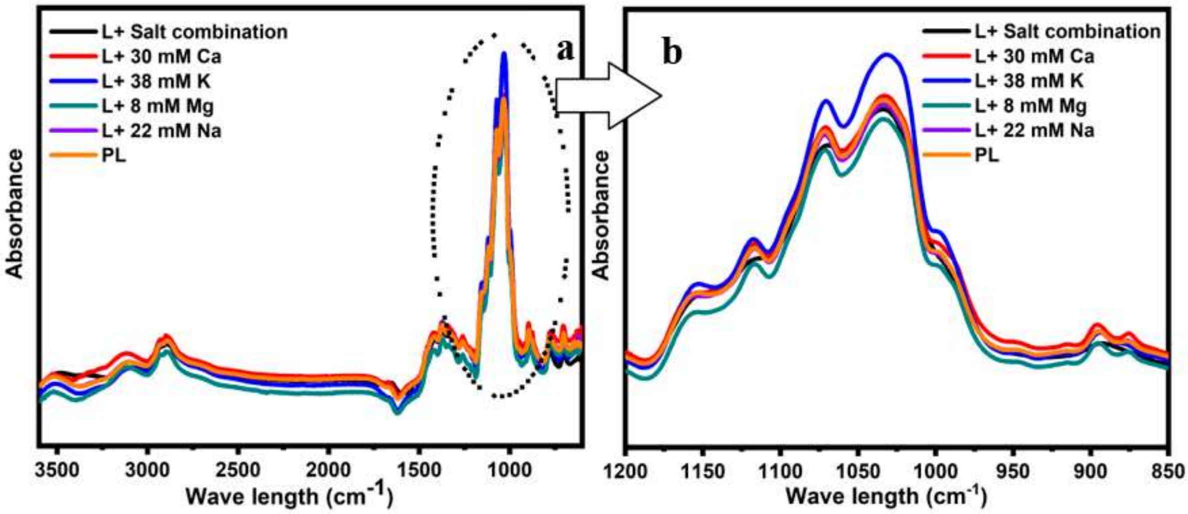

Figure 2. FTIR spectra obtained from the concentrated lactose solutions in the presence of 8 mM Mg, 30 mM Ca, 38 mM K, 22 mM Na, the salt combination, and pure lactose (PL) in the ranges of 4000–600 cm^{-1} (**a**) and 1200–800 cm^{-1} (**b**).

The behaviors of lactose crystals determined using FTIR are shown in Figure 3a,b. The spectral region of 1200–800 cm^{-1} in Figure 3a can be used to differentiate the crystalline form from amorphous lactose [21]. More defined sharp peaks in this range are associated with crystalline lactose, while indistinct peaks symbolize amorphous lactose [9]. All the studied samples in this study were characterized by sharp peaks in the corresponding region, indicating the crystalline nature of lactose in the presence of different salts.

The spectral region between 3600 and 2800 cm^{-1} in Figure 3b shows the behaviors of water molecules and H bonding within the crystal lattices in different hydration states [29]. All samples showed broad bands of vibration corresponding to the intermolecular stretching vibrations of the hydroxyl group [23,30]. However, the deviation in these stretching vibrations appeared to depend on the type of salt present in the samples. The sample containing a concentration of 30 mM Ca, followed by 8 mM Mg, the salt mixture, and 38 mM K, exhibited the most significant increase in the intensity within the region of 3600–2800 cm^{-1} when compared with the reference sample (PL). Conversely, the lactose crystals obtained with a concentration of 22 mM Na displayed the lowest intensity in this region. Furthermore, the shape and location of the sharp, distinct O-H stretching peak at 3520 cm^{-1} [30] are indicative of constrained water molecules in the crystal lattice. This peak is more prominent in the presence of 38 mM K but, in contrast, diminished in the presence of 22 mM Na.

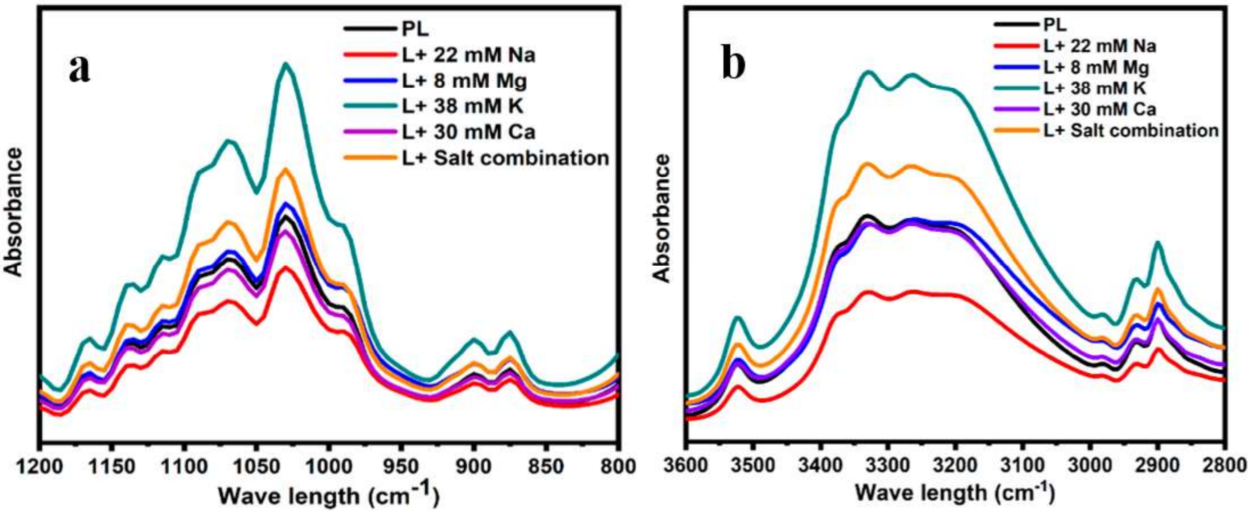

Figure 3. FTIR spectra obtained from the lactose crystals in the presence of 8 mM Mg, 30 mM Ca, 38 mM K, 22 mM Na, the salt combination, and pure lactose (PL) in the ranges of 3600–2800 cm^{-1} (**b**) and 1200–800 cm^{-1} (**a**).

3.5. XRD Patterns of the Lactose Crystals in the Presence of Salts

The XRD technique was used to identify the distinct polymorphic forms of lactose in the presence of different salts (α and β) and determine the purity of the lactose powders. Figure 4 shows the characteristic XRD diffractograms of lactose crystals with or without the addition of different salts. The positioning of the peaks (2θ) denotes the distinct varieties of lactose crystals that are present within the sample. For example, α-lactose is discernible at 12.5°, 16.4°, 20.0°, and 20.1°, while anhydrous β-lactose is identifiable at 10.5°, 19.0–19.5°, 20.9°, and 21.0°. Several additional peaks are attributed to the combinations of lactose crystals, including anhydrous α:β with a molar ratio of 5:3 at 19.1° and 21.1°, as well as anhydrous α:β with a molar ratio of 4:1 at 19.5° [30–32]. In the present study, the crystallization of lactose into the α-lactose monohydrate form is discernible in the peaks observed at 12.9–13.2°, 16.8–17.0°, 20.1°, 20.3°, and 20.5° (as presented in Table 4) in nearly all the samples that were examined.

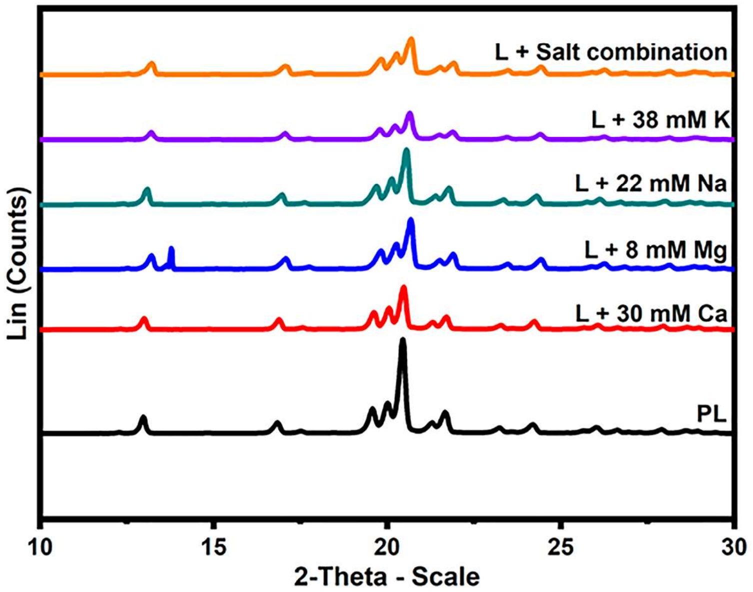

Figure 4. X-ray diffraction patterns of lactose crystals with the addition of 8 mM Mg, 30 mM Ca, 38 mM K, 22 mM Na, salt combination, and pure lactose (PL).

Table 4. Characteristics of the different forms of lactose crystals observed in XRD diffractogram patterns at various diffraction angles (2θ)°.

Types of Crystals	**Diffraction Angles (2θ)° (Literature Data) ***	**Measured Diffraction Angle (2θ)°**					
		Pure Lactose	**Lactose + $CaCl_2$**	**Lactose + $MgCl_2$**	**Lactose + KCl**	**Lactose + NaCl**	**Lactose + Salt Mix**
α-lactose monohydrate	12.5	12.9	13.0	ND	13.0	13.0	13.2
	16.4	16.8	16.8	17.0	16.9	16.8	17.0
	20.0/19.9	ND	ND	ND	ND	ND	ND
	20.1	20.3	20.4	20.1	ND	20.5	ND
Stable anhydrous α-lactose	19.37	ND	ND	ND	ND	ND	ND
Anhydrous β-lactose	10.5 19.0 20.9 21.0	19.5	ND	ND	20.6	20.8	ND
Anhydrous, α:β molar ratio 5:3	19.1	ND	ND	ND	ND	ND	ND
	21.1	ND	ND	ND	21.3	21.4	ND
Anhydrous, α:β molar ratio 4:1	19.5	19.5	ND	ND	ND	ND	ND

* Literature data [30–32]; ND = not detected.

Crystallization of lactose into anhydrous β-lactose was detected in the PL and the samples containing monovalent cations (Na and K) at 19.5°, 20.8°, and 20.6°, respectively, which closely correlate with the diffraction angles reported in the literature (Table 4). Moreover, the samples obtained in the presence of 22 mM Na and 38 mM K resulted in peaks with diffraction angles of 21.4° and 21.3°, which could be related to the mixture of anhydrous α:β with a molar ratio of 5:3. The minor variances observed in the diffraction angles in the current investigation, when contrasted with the literature [33], may be attributed to the augmented interlayer spacing of crystals resulting from the interaction of salts with water and lactose.

4. Discussion

Several factors affect the crystallization of lactose, including impurities, super-saturation, temperature, viscosity, lactose concentration, and pH [34]. Based on the results shown in the tables and figures, lactose crystallization behavior is impacted by the presence of cations in a varying manner depending on the type and likely concentration. The process of lactose crystallization typically consists of two fundamental stages: "nucleation" and "crystal growth" [35]. During crystal growth, the enlargement of nuclei occurs in response to the prevailing level of supersaturation in the solution. Cations as impurities in lactose solutions govern lactose crystallization in a direction that is dependent on the concentration and the type of cation present [8] as it induces alterations in solubility. Alterations in solubility have a direct impact on the state of supersaturation within the solution, consequently influencing the processes of nucleation and crystal growth [36].

Potassium (K^+) is an ion that disrupts the structure of water and has been found to influence the orientation of water molecules [36]. This, in turn, enhances the solubility of lactose and reduces lactose–lactose interactions, disrupting the orderly arrangement of lactose, which results in the hindrance of lactose crystal growth. In contrast, it has been observed that Na^+ ions have the ability to decrease the solubility of lactose when combined with phosphate [37]. Nevertheless, the present investigation revealed that the inclusion of NaCl as an additive led to an increase in solubility when compared with PL. However, the proportional increase in solubility was lower than that observed with KCl, which resulted in a higher yield. This can be attributed to the fact that NaCl is a more soluble salt than KCl and thus possesses a greater water-binding capacity [37], which ultimately leads to a reduction in lactose solubility when compared with KCl. Ions with a very high charge density electrostatically organize water effectively. Ca^{2+} is a divalent ion with a strong electric field, which acts as a water structure promoter [38]. Therefore, the alteration in water structure to varying extents might affect the solubility of lactose and the supersaturation of the solution, and, thereby, the crystal yields. Mg^{2+}, on the other hand, creates lactose-Mg complexes, increasing the solubility of lactose [39]. However, this effect is much lower than the effect of Ca^{2+} on the solubility and crystallization of lactose (Table 3).

Lactose, being a hydrophilic molecule, is enveloped by a hydration layer [4]. Consequently, during nucleation, the water molecules in the hydration layer of lactose undergo de-solvation, thereby enabling lactose–lactose interactions that culminate in the creation of the crystal lattice. The presence of a cation may enhance the bonding between these hydration water molecules and the lactose molecules, thereby impeding the evaporation of these water molecules. This is demonstrated by an increase in water evaporation enthalpies in the presence of different cations, as shown in Table 2. In this table, the greatest enthalpy of 167 J/g for the loss of crystalline water from crystal lattice was obtained for the PL. All other measured enthalpies obtained in the presence of different cations as well as their combination were reduced, indicating the obstruction of water removal from the crystal lattices [40].

Ca^{2+} ions strongly interact with four to six layers of water molecules via dipole–ion interactions [41]. Hence, the water molecules may be densely packed within these hydration layers in comparison with water molecules in the pure lactose solution, leading to restricted mobilities, which may be applied to other cations as well. Plausibly, individual lactose molecules may be surrounded by a strong hydration layer, creating a shielding effect and hindering water removal from the crystal lattice. Interestingly, two protruding peaks initiating at ~1030 cm^{-1} and 1070 cm^{-1}, which are associated with the OH groups of lactose, C-C stretching vibrations, and C-O stretching in the glucose molecule of lactose [27], exhibited significant reductions in intensity in the presence of 22 mM Na. This indicated the restricted movement of the lactose molecules due to the formation of a strong hydration layer around them. The mechanisms by which each individual cation and/or in combination appeared fundamentally different from each other. Cations affect both nucleation and crystal growth rates depending on the radius/charge ratio of the cation due to its hydrophilic and hygroscopic nature [42]. Mg^{2+} has the lowest radius/charge ratio and, thus, a high binding capacity to water. On the other hand, Ca^{2+} has the greatest radius/charge ratio and, consequently, a small binding capacity compared with Mg^{2+}. The lowest yield of lactose crystals was obtained in the presence of Ca^{2+} compared with that in the presence of Mg^{2+}, which was close to that of pure lactose. This indicates that the binding capacity of Mg^{2+} with water allows more lactose molecule interactions and, thereby, crystallization compared with Ca, although this effect cannot be ruled out completely. Furthermore, the incorporation of a cation onto the surface of an existing crystal increases the surface potential difference as a result of the newly charged crystal surface. This, in turn, decreases the thickness of the diffusion layer and, consequently, leads to the acceleration of the diffusion step of the crystallization and, thus, the yield. This effect may have an increased influence in a high-charge-density cation such as Ca^{2+}. In addition, Ca associations with lactose crystals have been found to be enhanced in the presence of other cations [15]. Thus, the further decreased crystal yields in the presence of the salt mixture compared with the presence of Ca may be partly due to the incorporation of the cations into the lactose molecules. This incorporation of cations may also increase the distances between lactose molecules and delay the required orderly arrangement of lactose molecules in forming a crystalline structure.

Previously, it was hypothesized that pH is an important factor in the crystallization of lactose [43]. By altering the pH, the mutarotation of lactose can be accelerated, which favors crystallization [34]. In the presence of all cations, the pH declined, which could have partially played a part in crystallization (Table 2). However, the sample with 8 mM Mg^{2+}

had a pH value close to that of the solution containing 30 mM Ca^{2+}, which also indicates that pH could not be solely responsible for the crystallization patterns. In addition, further research is needed to establish an exact mechanism outlining lactose crystallization as a function of pH.

Water molecules within a crystal lattice can reside either in the lattice, lattice channels, or ion-coordinated sites [9]. Hydrogen bonds are sensitive to charge transfer from donors to acceptors [34]. Water molecules residing in lattice sites are isolated from other water molecules due to contact with lactose, while water molecules residing in lattice channel sites are in contact with other water molecules of adjoining unit cells [44]. These water molecules can interact via H-bonding and ion-coordinated bonds. Ion-coordinated water bonds are thermodynamically preferred and stronger than hydrogen bonds. Thus, the extent of the formation of H bonds and ion-coordinated bonds within these water molecules in the crystal lattice in the presence of different cations may change the behavior of the lactose crystals. The present study clearly showed the changes in H-bonding patterns in the presence of Ca (Figure 3) compared with those in the presence of other cations. However, the presence of the salt mixture showed much greater intensities within the H-bonding region (Figure 3) compared with Ca, highlighting the fact that cations in combination may influence the balance between these two types of bonds and thereby change the lactose crystallization behavior.

Another interesting finding of the current study is that the β-lactose melting peak disappeared in the DSC thermograms of lactose in the presence of 30 mM Ca, 8 mM Mg, and the salt mixture. The disappearance of the β-lactose melting peak may be a direct result of the prevention of lactose isomerization, which can be attributed to the presence of Ca^{2+} and/or Mg^{2+}. The impact of Ca and Mg on the inhibition of lactose isomerization or the impact of mutarotation equilibrium is still unclear as there is no established evidence in the literature. However, this result was further supported by the observed XRD diffraction angles for β-lactose in the presence of Na^{+}, K^{+}, and PL. The aforementioned findings underscore the importance of water molecule behavior and inter- and intra-molecular interactions in governing the behavior of lactose. Overall, the impact of monovalent salts on the crystallization behavior of lactose was found to be comparatively less influential than that of bivalent salts. The change in crystallization behavior can be modulated by adjusting the concentrations of cations present in acid whey. Additional investigations are warranted to assess the behavior of lactose under varying cation concentrations and the impact of anions in acid whey.

5. Conclusions

The process of isolating lactose from a complex mixture like acid whey necessitates precise processing conditions. The formation of lactose crystals and their growth are influenced by the characteristics and actions of the solvent and water, which are significantly affected by the presence of other substances. Crystallization is influenced by the presence of different cations. The presence of the salt mixture containing 8 mM Mg, 30 mM Ca, 38 mM K, and 22 mM Na had the greatest impact on the lactose crystal yield compared with that of pure lactose. This can be attributed to the increased interactions between lactose and cations, resulting in changes in solubility and the structure of water molecules, ultimately affecting the crystallization behavior of lactose. In the presence of all cations, pH declined, which could partially play a part in crystallization. Divalent ions impacted the behavior of lactose by altering the anomerization of lactose as opposed to the monovalent ions. The present study highlighted the fact that the presence of Ca plays a major role in the crystallization of lactose. However, further research is needed to evaluate the effect of the presence of various anions on lactose crystallization.

Author Contributions: Conceptualization, R.W.; methodology, R.W.; software, R.W., T.V. and J.C.; validation, R.W., T.V. and J.C.; formal analysis, R.W., T.V. and J.C.; investigation, R.W.; resources, R.W.; data curation, R.W.; writing—original draft preparation, R.W.; writing—review and editing, T.V. and J.C.; visualization, R.W.; supervision, T.V. and J.C.; funding acquisition, T.V. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: Data are contained within the article.

Acknowledgments: The authors are grateful to Victoria University for providing financial and technical support.

Conflicts of Interest: The authors declare no conflict of interest.

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Chapter 6: Behaviour of Sucrose as Affected by Presence of Acids and Salts

This chapter discusses the behaviour of sucrose in the presence of organic acids and selected salts commonly found in sugar-sweetened soft drinks.

Abstract

This study aimed to establish the behaviour of sucrose in the presence of citric acid (CA), phosphoric acid (PA) and sodium (Na). The presence of acids and salts affected the behaviour of sucrose differently. The addition of PA and CA induced structural changes in sucrose molecules by hydrolyzing the sucrose molecules in a concentration-dependent manner. The addition of CA and PA played a significant role in hydrolysis. In the presence of CA hydrolysis of lactose resulted in the release of more fructose while in the presence of PA sucrose separated into its consecutive monosaccharides producing more glucose than fructose. In addition, the changes of the hydration water layer either due to the molecular rotation of sucrose, fructose and glucose molecules and/or strongly bound H bonds played a significant role in varying water evaporation enthalpies (ΔH) in the presence of Na, PA and CA. The presence of PA in sucrose solution appeared to have a greater impact on the behaviour of sucrose as compared to CA and Na. Thus, it is confirmed that the type of acid/salt present in a certain food system influences the properties of sucrose and thereby it may influence its metabolic fate.

1 Introduction

Sucrose is added to food systems concurrently with acids and salts. Sucrose contributes a sweet sensation to food products, while also providing certain textural properties desirable in foods in some instances (Laos, Kirs, Kikkas, & Paalme, 2007). On the other hand, acids and salts are known to provide various desirable characteristics to beverage manufacturers and are thus widely used across many products including juices and soft drinks. For example, phosphoric (PA) and citric acids (CA) both play roles in preservation and provision of piquant flavour characteristics (Schifferstein & Frijters, 1990). Considering that soft drinks have been identified as key dietary contributors to rise in obesity (Bray, 2010; Merino, Fernández-Díaz, Cózar-Castellano, & Perdomo, 2019), this raises the question of whether sucrose-acid/salt interactions would influence sucrose behaviour to a point where it may not only change its physical characteristics but also metabolic fate. Thus, the control and understanding of sucrose solution behaviour during processing appears imperative. The present study therefore aimed at establishing the physico-chemical and thermal behaviour of sucrose under different compositional and processing conditions in order to fundamentally understand the nature of the interaction of sugars with acids and salts down to the molecular level. This study would help to fundamentally understand the sugar-acid/or sugar-salt interactions and their behaviour in different food systems.

Sucrose is a non-reducing disaccharide composed of glucose and fructose, linked via their anomeric carbons. The structure of sucrose and its behaviour is affected by the surrounding environment, including sucrose supersaturation, temperature and the presence of impurities (Smythe, 1967). In the presence of acids, sucrose increases its hygroscopicity and shows enhanced browning of candies. However, the type of organic acid appeared to govern the extent of these changes (Shin, Lim, & Son, 1998). In addition, the behaviour of water molecules and their mobility plays a crucial role in the behaviour of sucrose (M Mathlouthi & Genotelle, 1998). Sugars exhibit a high susceptibility to alterations in their surroundings, particularly when they are dissolved in an aqueous solution. Therefore, it is crucial to direct our attention towards investigating molecular interactions in such systems.

Vibrational spectroscopy has proven to be a highly suitable method for studying hydrogen bonding, which serves as a key indicator of molecular interactions in sugar-containing aqueous solutions (Mohamed Mathlouthi & Koenig, 1987). It was established that the physico-chemical nature of another disaccharide, lactose, and surrounding water molecules are affected by the presence of lactic acid (LA) and calcium in the system (Chandrapala, Wijayasinghe, & Vasiljevic, 2016; Wijayasinghe, Vasiljevic, & Chandrapala, 2015, 2016). These lactose-water interactions affect mutarotation between α and β anomeric forms of lactose and consequently affected its crystallisation. Due to the significant increase in consumption of sucrose sweetened beverages, it is important to establish whether similar interactions occur with the addition of CA and PA at varying concentrations. It is also thought that changes in sucrose structure may have some influence on metabolism efficiency. At present, there is very little knowledge available on this aspect of sucrose. Thus, the present study aimed at evaluating physico-chemical and thermal characteristics of sucrose using model–based systems in the presence of selected acids (CA and PA commonly found in beverages in concentrations likely to be encountered in these systems (0.05, 0.2 or 1%, w/w) and common salt, NaCl at concentrations of commonly found in soft drinks (30, 60, 120 mg/mL). Thermal characteristics of sucrose were measured by differential scanning calorimetry (DSC), whereas high-performance liquid chromatography (HPLC) was used to determine the capacity of the acids to invert sucrose. Structural changes in sucrose were established by Fourier transform infrared spectroscopy (FTIR). Furthermore, the mobility of water molecules was evaluated under the same conditions. This research provides some insights on whether these interactions would detrimentally impact the properties of a common sweetener that would potentially affect the choice of processing.

2 Materials and Methods

2.1 Materials

An analytical grade sucrose powder, glucose, fructose, citric acid (CA) and phosphoric acid (PA) were obtained from Sigma Aldrich Pty. Ltd (Macquarie Park, NSW, Australia). MilliQ water was used at all times for the preparation of sugar solutions. A 50% w/w CA stock solution was prepared, while PA (85% w/v) and NaCl were directly sourced from Sigma Aldrich Pty. Ltd.

2.2 Preparation of model solutions

Sucrose was dissolved in MilliQ water at room temperature to obtain a 65% (w/w) sucrose solution. The solution was stirred until a clear solution was obtained with no crystals visually observed. Solutions of sucrose with citric acid (S + CA) and phosphoric acid (S + PA) were prepared by direct addition of calculated amounts of PA or CA to achieve a concentration of 0.05%, 0.2 % or 1% w/w. Similarly, a sucrose solution with Sodium (S + Na) was prepared by adding NaCl to achieve 30, 60 and 120 mg/mL concentrations. All the concentrations were selected to mimic the actual concentrations of acids and salts present in sugar-sweetened soft drinks. A sucrose solution containing no added acid/salt was used as the control sample (PS).

2.3 High Performance Liquid Chromatography (HPLC)

Sucrose hydrolysis was assessed using a Shimadzu HPLC system (Model-LC 2030C, Shimadzu Corporation, Kyoto, Japan) coupled with a refractive index detector (Shimadzu Corporation, Kyoto, Japan). Separation was achieved through a Sugar Pak column (Aminex HPX-87P, 300x7.8 mm, Biorad Instruments, Gladesville, NSW, Australia) as previously described (Wijayasinghe, Bogahawaththa, Huppertz, Chandrapala, & Vasiljevic, 2019). The mobile phase consisted of Milli-Q water with isocratic elution at a flow rate of 0.6 mL/min. The column temperature was set at 55-65 °C and each sample with and without addition of CA and PA was run for 30 minutes. A pure glucose and a fructose solution were separately run as reference samples to determine the hydrolysis.

2.4 Differential Scanning Calorimetry (DSC)

Concentrated sucrose solutions (2-5 mg) were transferred into pre-weighed DSC aluminium pans (40 µL; ME-26763, Schwerzenbach, Switzerland) and the pans were hermetically sealed. Subsequently, samples were transferred into a DSC (DSC 1 STARe System, Gas Controller - GC 200, Mettler Toledo Schwerzenbach, Switzerland). An empty pan was used as a reference. All samples were scanned with a starting temperature of 25 °C and end temperature of 200 °C at a heating rate of 10 °C min^{-1} with slight modifications to the method described elsewhere (Wijayasinghe et al., 2015). Onset and end set temperatures of water evaporation, and related enthalpies (area under the curves) were analysed using STARe thermal analysis software, 12.1 (Mettler Toledo).

2.5 Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectra of the sucrose solutions with and without addition of acids and salts were collected using an FTIR spectrometer (Frontier 1, Perkin Elmer, Waltham, MA). The concentrated solution spectra were collected in the range of 4000 - 600 cm^{-1} in absorbance mode after background subtraction. Every spectrum was obtained with an average of sixteen scans recorded at a resolution of 4 cm^{-1}.

2.6 Statistical Analysis

The experimental design was replicated twice on separate occasions, and the samples were sub-sampled. A one-way ANOVA with a 95% confidence interval was employed to compare the means. Statistical significance was determined using Tukey's test, with a significance level of $p < 0.05$.

3 Results and Discussion

3.1 Results

3.1.1 Sucrose hydrolysis induced by citric acid and phosphoric acid as determined by High Performance Liquid Chromatography (HPLC)

HPLC analysis was conducted to examine the impact of acids on the carbohydrate composition of sucrose solutions. It is apparent from the data presented in Figure 1 that PS was separated and eluted as a singular peak at ~10 min. The reference samples including glucose and fructose were eluted at ~ 12 min and ~ 16 min, respectively. In the presence of CA and PA, two additional peaks were identified besides the PS peak, which can be attributed to the presence of glucose and fructose in the samples containing CA and PA. This was very apparent at all concentrations, whereas the peak eluting at ~10 min was diminished with the increase of acid concentration. It is apparent that the addition of CA or PA has induced hydrolysis of the sucrose molecule, cleaving it into glucose (~12 minutes) and fructose (~16 minutes) with remaining sucrose, producing a smaller peak eluting at ~10 minutes. Interestingly, the impact of CA and PA on the cleaving behaviour of sucrose is different as observed by the HPLC chromatograms. In the presence of PA, sucrose hydrolysis resulted in more glucose than fructose while CA-induced hydrolysis tends to produce more fructose.

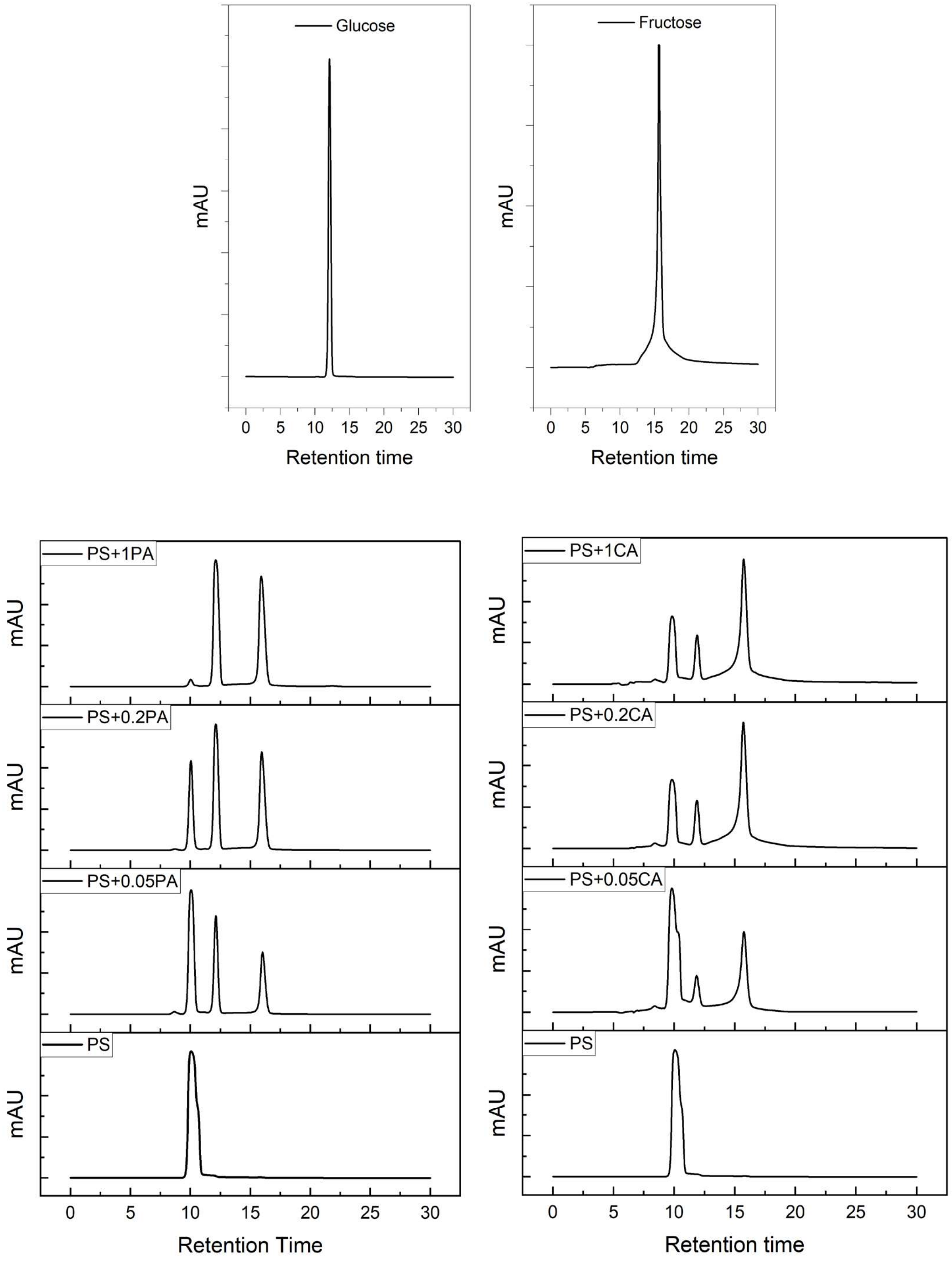

Figure 1. HPLC chromatograms of pure sucrose (PS) solution with and without addition of citric acid (CA) or phosphoric acid (PA) at concentrations 0.05% w/w, 0.2% w/w or 1% w/w.

3.1.2 FTIR measurements

Water-solute interactions govern various physical properties, which may originate in structural changes of the main compounds of the system, usually identified using FTIR (López-Pablos et al., 2018). Figure 2 shows the FTIR spectra obtained for concentrated solutions in the range of 1800 cm^{-1} – 600 cm^{-1} as affected by the presence of varying concentrations of CA, PA and Na. Generally, the spectral range between 1200 cm^{-1} - 800 cm^{-1} encompasses a collection of interconnected bands, primarily arising from the stretching vibrations of C-O and C-C bonds (Cael, Koenig, & Blackwell, 1974). The observed peak intensities were not substantially changed in the presence of Na at any concentration level. Two sharp peaks at ~1051 cm^{-1} and ~ 989 cm^{-1} were observed in all samples, which correspond to the vibrations of the groups of atoms peripheral to the pyranoid or furanoid rings of the sugar (Mohamed Mathlouthi & Koenig, 1987). In the presence of CA, the intensity of these two peaks increased concomitant with the concentration of the acid present in the solution. In the presence of CA, the intensity of these two peaks increased concomitant with the concentration of the acid present in the solution. A similar trend was observed with the presence of PA.

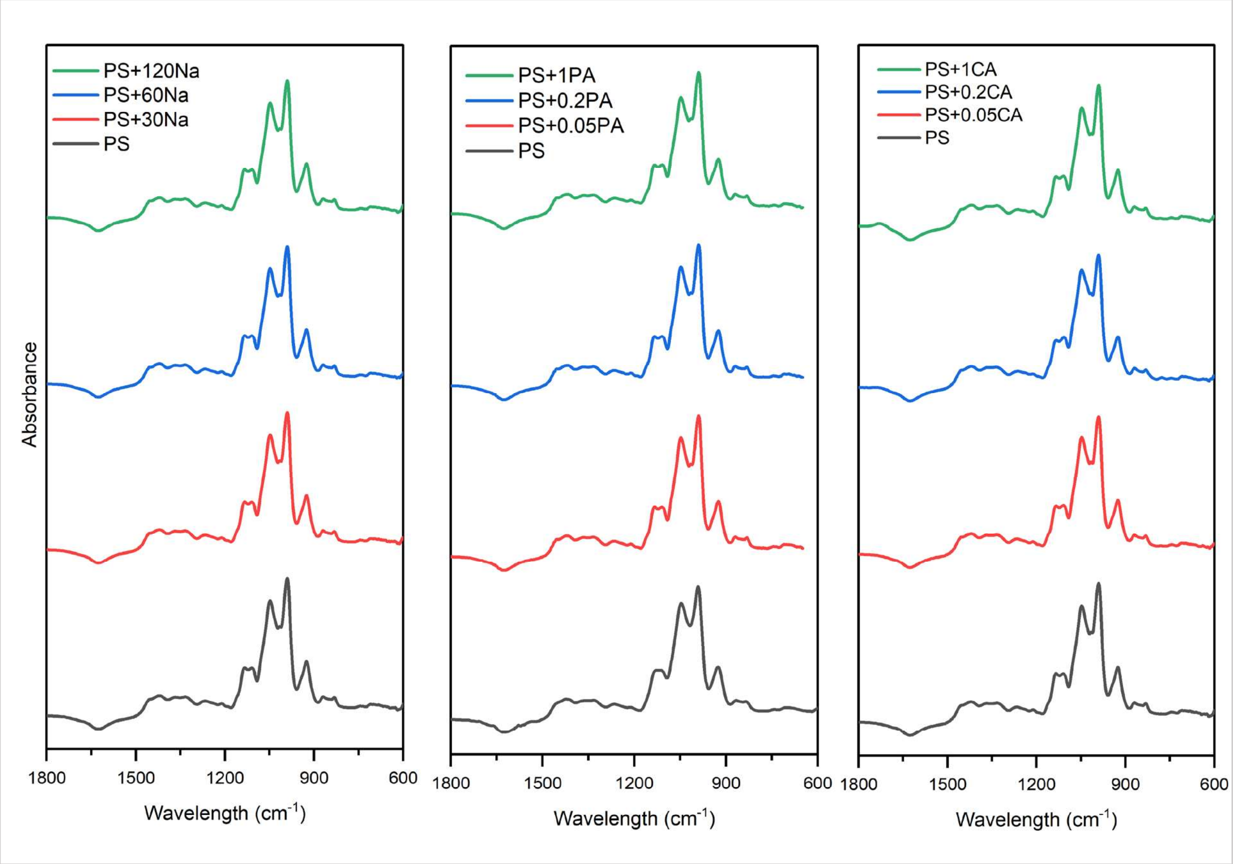

Figure 2. FTIR spectrograms of pure sucrose (PS) solution with and without addition of citric acid (CA) or phosphoric acid (PA) at concentrations 0.05% w/w, 0.2% w/w or 1% w/w and 30, 60 and 120 mg of Na.

3.1.3 Thermal properties of sucrose as determined by Differential Scanning Colorimetry (DSC)

Thermographic analysis of sucrose solutions with or without addition of Na and acids resulted in thermograms with an endothermic peak at around 150-160 °C. This endothermic peak could be attributed to the loss of water from sucrose (Wijayasinghe et al., 2015). The pure sucrose solution exhibited this dehydration peak at ~154 °C with an enthalpy (ΔH) of ~561.5 J/g (Table 1). In the presence of Na, the peak temperature increased as compared to that of PS. However, the enthalpy (ΔH) associated with water evaporation decreased as opposed to PS. The enthalpy

(ΔH) decrease is inversely related to the concentration of the salt (Na) present in the solution. In comparison to PS, the presence of PA resulted in an increase in both the enthalpy (ΔH) associated with water evaporation and the peak temperature of water evaporation. The enthalpy (ΔH) of water evaporation demonstrated an inverse correlation with the concentration of the PA. However, in the presence of CA, the peak temperature and the related enthalpies decreased as compared to these of PS. The decrease in enthalpy (ΔH) demonstrated a direct relationship with the concentration of the acid present in the solution.

Table 1. On-set and end-set temperatures of water evaporation, enthalpy (ΔH) of water evaporation of sucrose solu-tions containing sodium (Na), citric acid (CA) and phosphoric acids (PA) with different concentrations.

Sample	pH	Onset temperature (°C)	End set temperature (°C)	Peak temperature (°C)	Enthalpy (ΔH) water evaporation (J/g)
Pure Sucrose (PS)	6.2	153.3±2.3^{ab}	168.95±16.2^{a}	154.05±1.9^{ab}	561.5±15.4^{abc}
PS + 30Na	5.3	159.65±7^{ab}	167.45±4.0^{a}	161.7±7.2^{ab}	475.45±18.2^{abc}
PS + 60Na	5.1	162.6±6.8^{a}	170.95±5.2^{a}	164.9±6.4^{a}	505.4±11.9^{abc}
PS + 120Na	4.9	157.4±4.0^{ab}	167.2±1.3^{a}	162.55±1.3^{a}	515.05±6.9^{abc}
PS + 0.05PA	2.1	153.55±1.6^{ab}	172.65±1.8^{a}	160.75±0.5^{ab}	702.2±102.5^{a}
PS + 0.2PA	1.8	152.65±2.9^{ab}	159.5±2.0^{a}	156.65±1.5^{ab}	699.9±85.0^{a}
PS + 1PA	1.5	157.25±0.6^{ab}	166.1±0.7^{a}	160.85±0.6^{ab}	697.1±16.3^{ab}
PS + 0.05CA	2.7	150.65±4.2^{ab}	159.7±2.3^{a}	152.9±2.4^{ab}	314.85±148.0^{c}
PS + 0.2CA	2.1	143.85±5.6^{b}	158.5±2.3^{a}	147.95±1.9^{b}	433.15±51.0^{bc}
PS + 1CA	1.8	145.1±5.4^{ab}	159.1±2.0^{a}	152.5±3.2^{ab}	464.65±45.0^{abc}

3.2 Discussion

3.2.1 Effect of citric acid and phosphoric acid on the hydrolysis behaviour of sucrose

The chemical constitution of sucrose refers to β-D-fructofuranosyl α-D-glucopyranoside (Reiser, 1994). Our HPLC data revealed that CA and PA had the ability to hydrolyse sucrose into its constitutive monosaccharide moieties - glucose and fructose as shown in Figure 1. Cleavage of sucrose by an acid follows one of two general proposed mechanisms defined as a unimolecular and a bi-molecular process. While either reaction follows a pseudo-first order kinetics, the rate constants for the reactions change with the type and concentrations of the acid used. Sucrose degradation begins with the initial step of protonating the glycosidic oxygen, resulting in the formation of a sucrose oxonium ion. This ion then proceeds to undergo a scission reaction, leading to the production of fructose carbocation and glucose (Richards, 1988). For example, a unimolecular process is proportional to the proton donating power of the medium, while the bi-molecular process is governed by the concentration of the acid or the hydrogen ions (Dawber, Brown, & Reed, 1966). Glycosyl-oxygen cleavage would thus produce fructose and glucose as fructosyl-oxygen cleavage would yield glucose and fructose. While the ultimate result depends on the type and the concentration of the acid present in the solution. In the presence of PA and CA, sucrose hydrolysis had a direct relationship to the concentration of acid present in the solution. This can be attributed to the presence of more H^+ ions with an increase in acid concentration. However, the hydrolysis power of two acids were different as observed by the HPLC results (Figure 1). This can be attributed to the hydrolytic power of these acids. The Ka values of the acids studied are reported as CA; pK_{a1} 3.09, pK_{a2} 4.74, pK_{a3} 5.40 and PA; pK_{a1} 2.15, pK_{a2} 7.21, pK_{a3} 12.32 (Belitz, Grosch, & Schieberle, 2008; Rodrigo, Ribeiro, Verissimo, Esteso, & Leaist, 2019; Wijayasinghe et al., 2019). It should be noted that CA and PA possess three pK_a values due to their nature as tri-protic acids.

Consequently, the comparison of these two acids' strengths was based on their pK_{a1} value. As a result, PA, being the strongest acid with the highest hydrolytic power, exhibited a greater extent of hydrolysis compared to the CA. It has been noted that when PA is present, the hydrolysis of sucrose is more likely to yield glucose rather than fructose. On the other hand, CA tends to produce more fructose. This difference can be attributed to the distinct cleavage pathways as described earlier. It can be hypothesized that in the presence of PA, fructosyl-oxygen cleavage may take place, leading to increased production of glucose. Conversely, in the presence of CA, glycosyl-oxygen cleavage may occur, resulting in a higher yield of fructose. Thus, undergoing different cleavage processes with the presence of CA and PA may also affect the fructose-glucose, fructose-glucose-water, and fructose-glucose-acid interactions and potentially the metabolization behaviour of sugar in the body. Sucrose, fructose, and glucose are distinct sugar variants that exhibit dissimilar chemical structures. The metabolic characteristics of these sugars exhibit variations within the human body. For instance, sucrose, being a disaccharide, necessitates the cleavage into monosaccharides by the body before its absorption, which requires some energy. Conversely, glucose and fructose, being the most basic forms of sugars, are directly absorbed into the body. There are two main pathways involved in fructose metabolism, the conversion of fructose into glucose and the conversion of fructose into lipids (Mayes, 1993). Hence, excessive consumption of fructose can lead to elevated levels of portal fructose, which in turn triggers the production of glucose and synthesis of lipids within the body. This can also lead to obesity (Johnson, Sánchez-Lozada, Andrews, & Lanaspa, 2017). Similarly, excessive consumption of glucose also prompts the synthesis of lipids by the body. Previous studies demonstrated fructose has a stronger impact on hepatic *de novo* lipogenesis compared to glucose, and this effect is significantly amplified when both monosaccharides are ingested together (Hudgins, Parker, Levine, & Hellerstein, 2011; Softic, Cohen, & Kahn, 2016). Hence, the results indicate that the intake of sugar-sweetened carbonated beverages, which are acidified with CA or PA, may be one of the contributing factors in obesity of individuals as a result of the inadvertent overconsumption of glucose and fructose.

3.2.2 Thermal behaviour and structural behaviour of sucrose

The thermal changes investigated using differential scanning calorimetry (DSC) exhibited distinct characteristics. In sucrose solutions containing PA, there was an observed rise in enthalpy (ΔH) during water evaporation. Conversely, the presence of Na and CA resulted in a decrease in enthalpy compared to that of PS. The limited extraction of water can be attributed to the interplay and formation of hydrogen bonds among sucrose, water, and acids/salt. Formation of a strong hydration layer in presence of PA lead to hindered water evaporation due to the limited removal of water, leading to an increase in the enthalpy of water evaporation (ΔH). The presence of H_3O^+ ions has a significant impact on the surrounding water molecules near the sucrose molecule, resulting in reduced mobility and a denser packing compared to pure water molecules (Fennema, Damodaran, & Parkin, 2008). Consequently, the removal of water becomes exceptionally challenging. Conversely, the water surrounding sucrose may be influenced by the presence of salt in multiple ways. Firstly, salt could modify the overall structure of water and also displace water molecules from the sucrose hydration shell, a phenomenon known as preferential solvation (Eggleston, Vercellotti, Edy, & Clarke, 1996). Additionally, salt may alter the conformation of sucrose, which subsequently impacts the enthalpy of water evaporation. Furthermore, the stability of sucrose can be affected by salts through mechanisms unrelated to water structure. This includes the formation of complexes between salt and sucrose, as well as electrostatic effects that may come into play (Eggleston et al., 1996). This phenomenon is dependent upon the specific type and concentration of the acid/salt, and it regulates the hydrolysis process of sucrose and potentially the crystallinity of the sucrose (Wungtanagorn & Schmidt, 2001). The observed hydrolysis, which resulted in the release of glucose and fructose, lowers the supersaturation of sucrose due to an increase in solubility by the presence of monosaccharides. This could slow down or diminish the crystallization in the presence of CA and PA, especially if a critical supersaturation cannot be reached.

The differences in intensities of the FTIR spectra for CA and PA and the enthalpies associated with the water evaporation suggest that these acids may control different pathways of cleavage that may influence different fructose-glucose, fructose-glucose-water and fructose-glucose-acid interactions.

4 Conclusion

The present study showed that the behaviour of sucrose is governed by the type and concentration of the organic acid present. CA and PA cleave the bond between glucose and fructose. PA had more hydrolytic power as compared to CA and preferentially catalyzes the hydrolysis of glucose over fructose. The impact of Na on the structural behaviour of sucrose was not clearly distinguished from the FTIR study. However, the thermal behaviour of sucrose in the presence of Na demonstrated that the strength of the hydration layer is less strong than the PS as determined by the water evaporation enthalpy. The findings of this research have verified that the nature of the acid that is present can alter the characteristics of sucrose, and this alteration can potentially impact its metabolic destiny. In addition to the aforementioned study's findings, the influence of citric acid and phosphoric acid on the breakdown patterns of sucrose holds significant significance for the food sector. Specifically, the utilization of citric acid in the manufacturing process of high-fructose corn syrup serves as an example. Conversely, the application of phosphoric acid can yield a combination of glucose and fructose with a greater ratio of glucose, which can be employed in industry to achieve desired attributes in food products.

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Chapter 6: Conclusions and future directions

This chapter presents the final findings and outcomes of the entire project. Additionally, it outlines the potential areas for future research and improvement.

7.1 Conclusions

The crystallization of lactose holds significant importance in various food products in the food industry. Specifically, the crystallization of lactose in acid whey plays a crucial role in producing free-flowing powders. However, when lactose is unable to crystallize appropriately, it impedes the processability of acid whey, leading to a significant environmental issue. Therefore, it is essential to ensure that lactose can be crystallized correctly to maintain the efficiency of the process and to ensure the production of high-quality, free-flowing powders. Similarly, increased consumption of sugar-rich soft drinks acidified with organic acids such as citric, carbonic, or phosphoric acid has been blamed for the present and souring health problems such as obesity and diabetes. It appears that the role of acids and salts crucially influences the behaviour of sugars leading to the above issues. Hence, the primary aim of the current investigation was to address several foundational queries concerning the crystallization patterns of lactose, its interactions with various components within acid whey, and the behaviour of sucrose in conjunction with acids and salts.

A model-based study containing lactose, lactic acid, citric acid, and phosphoric acid was established to determine the solution phase properties of lactose. The properties of lactose within the concentrated solution were influenced by the presence of all three examined acids. Thermographic analysis showed that the presence of acids hindered the water evaporation during concentration indicating the strong interaction between acids and water molecules. This effect was related to the creation of a strong hydration layer around lactose molecules impeding the removal of water. The alterations observed through FTIR analysis provided insights into the distinct associations among lactose, water, and acids. The partial hydrolysis of lactose in saturated lactose solutions was observed upon the addition of phosphoric or citric acid, with the extent of hydrolysis being influenced by the concentration and hydrolytic strength of the acids, as well as the molecular interactions within the system. These findings indicate that the inclusion of weak acids such as citric or phosphoric in lactose solutions can lead to structural alterations in both lactose and water molecules, accompanied by the partial hydrolysis of lactose into its constituent parts. Therefore, a study of lactose with the aforementioned acids was required to determine the crystallization behaviour.

The crystallization behaviour of lactose is predominantly influenced by both the type and concentration of acids within the lactose systems. The interactions between lactose, water, and acids play a crucial role in shaping the crystalline characteristics of lactose. Specifically, the presence of 1% lactic acid (LA), 0.05% and 1% citric acid (CA), and 4% phosphoric acid (PA) can lead to the formation of amorphous lactose during the crystallization process, while stable anhydrous α-lactose is generated in the presence of 4% CA. Additionally, when CA or PA is present at concentrations equal to or greater than 1%, a significant reduction in crystal yield (≥18%) is observed. Furthermore, both particle size and crystal shapes are altered based on the type and concentration of the acid present in lactose solutions. Consequently, the judicious selection of processing conditions, acid types, and their concentrations is imperative for the effective crystallization of lactose within complex mixtures. This is essential because the nucleation and crystallization of lactose are intricately linked to the behaviour and properties of the solvent water, which are significantly influenced by the presence of acids. Thus, the present study underscores the critical importance of regulating lactose-water interactions and anomeric equilibrium for achieving optimal crystallization outcomes.

A comprehensive exploration into the crystallization of lactose under the influence of diverse cations was conducted to elucidate the impact of cations found in acid whey on crystallization behaviour. The presence of a salt mixture containing 8 mM Mg, 30 mM Ca, 38 mM K, and 22 mM Na exerted the most significant influence on lactose crystal yield in comparison to pure lactose. This effect is attributed to heightened interactions between lactose and cations, leading to alterations in solubility and the structural configuration of water molecules, thereby influencing the overall crystallization behaviour of lactose. The presence of all cations corresponded to a decline in pH, which could partially contribute to the crystallization process. Divalent ions demonstrated a distinctive impact on lactose behaviour, particularly in altering the isomerization of lactose, in contrast to monovalent ions. The current study underscores the pivotal role of Ca in lactose crystallization. This can be attributed Ca ions to organize the structure of water molecules and the immobilization of water facilitated by robust dipole-ion interactions between Ca and water.

Numerous significant studies highlight that soft drinks containing sucrose are a prominent factor in the aetiology of overweight and obesity in individuals. Structural modifications may arise in sucrose molecules when organic acids are present during the production process. As a result of these alterations, the metabolism of sucrose within the body can be influenced, potentially contributing to physiological conditions like obesity. The current study revealed that the behaviour of sucrose is contingent upon the type and concentration of the acid present. Citric acid and phosphoric acid facilitate the cleavage of the bond between glucose and fructose. Notably, phosphoric acid exhibits greater hydrolytic power compared to citric acid, displaying a preference for catalysing the hydrolysis of glucose over fructose. The influence of sodium (Na) on the structural behaviour of sucrose was not distinctly discovered in the FTIR study. However, thermal analysis demonstrated that the strength of the hydration layer is less robust in the presence of Na than in the case of phosphoric acid, as indicated by the water evaporation enthalpy. These findings emphasize that the nature of the acid present can modify the characteristics of sucrose, potentially influencing its metabolic fate.

These findings enhanced our understanding of sugar-acid and/or sugar-salt interactions and their behaviour in different food systems. The process of isolating lactose from a complex mixture like acid whey necessitates precise processing conditions. The crystallisation of lactose in acid whey can be improved through the alteration of the composition of acid whey. This can be achieved by removing Ca and lactic acid from acid whey either completely or partially. On the other hand, citric acid and phosphoric acid in relevant concentrations can be used to induce structural changes which can be beneficial for the dairy industry to manipulate the properties of lactose. Furthermore, the structural changes of sugar-sweetened soft drinks can be minimised by selecting the proper acid type with reduced concentration which may help to minimize or prevent the health issues associated with sugar-sweetened soft drinks.

Therefore, the combination of these studies in the current research project led to significant outcomes by identifying the modifications in structure and molecular interactions that exist within various food systems. These findings will have implications for streamlining food processing techniques and enhancing our comprehension of sugar metabolism within the human body.

7.2 Future Directions

The study has significantly contributed to our understanding of lactose behaviour under diverse concentrations of lactic, citric, and phosphoric acid and different salts such as calcium, magnesium, potassium and sodium. Similarly, we confirmed the behaviour of sucrose in the presence of acids and salts. However, the current investigation has raised several intriguing questions that warrant further in-depth exploration. Acid whey, being a complex solution, notably features elevated protein levels. The inclusion of proteins and zeta-potential measurements of the solutions may introduce an additional layer of complexity that may impact lactose behaviour. Thus, there is a pressing need for further research to elucidate the specific influence of proteins on the interactions and properties of lactose within the context of acid whey. Future studies should delve into the nuanced role played by proteins, providing a comprehensive understanding of their impact on lactose behaviour and crystallization dynamics in multicomponent solutions like acid whey.

The current study focussed on the individual impact of acids and salts on the behaviour of lactose and sucrose. Therefore, additional research exploring the combined impact of acids and salts on both compounds would enhance our precision in determining processing conditions for acid whey and sugar-sweetened beverages. Moreover, these findings of model systems need to be tested using real systems in order to better understand the complex interactions in these food systems. Apart from that further investigations are warranted to assess the influence of various anions on the crystallization dynamics of lactose.