JOÃO PABLO FORTES PEREIRA

LACTOSE CRYSTALS: DETERMINATION AND FORMATION IN DIFFERENTS CONCENTRATED DAIRY PRODUCTS

Thesis presented to Universidade Federal de Viçosa as part of the requirements for the Graduate Program in Food Science and Technology to obtain the title of *Doctor Scientiae.*

Advisor: Antônio Fernandes de Carvalho

Co-advisors: Ítalo Tuler Perrone
Rodrigo Stephani
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APPROVED: November 22th, 2019.

Assent:

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Advisor

I dedicate to the persons more important of my life
My parents, Diego, and my wife, Isabel.

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ABSTRACT

PEREIRA, João Pablo Fortes, D.Sc., Universidade Federal de Viçosa, November, 2019. **Lactose crystals: determination and formation in differents concentrated dairy products** Advisor: Antônio Fernandes de Carvalho. Co-advisors: Ítalo Tuler Perrone, Rodrigo Stephani and Pierre Schuck.

The objective of this work was to develop the study of sugars crystallization in differents concentrated dairy products (study of lactose crystallization in concentrated whey important step before the drying of whey), using different analytical tools. For sweetened condensed milk, the behavior of commercial samples of the product in the laser diffraction particle analyzer was analyzed using two differents dispersing materials (water and lactose solution) in the liquid module of the equipment, also evaluating the influence of recirculation time for sample dispersion in particle size distribution. It was observed that the use of lactose solution preserved the characteristics of the sweetened condensed milk particles for a longer time when compared to the analysis performed with water as solvent in the equipment. It was also concluded that a recirculation time of two minutes was important to ensure the dispersibility of the studied samples. Lactose crystallization in concentrated whey it was evaluated if the addition of sodium citrate would influence the crystallization of this sugar, analysing this behavior through different analytical tools. In this work it was observed that sodium citrate accelerates the lactose crystallization in concentrated whey and better control the size of crystals formed. It was also studied the crystallization of sucrose and lactose in model fat free tablet dulce de leche, aiming to monitor the influence of lactose, skimmed milk constituents and heating of the product on the sucrose crystallization during the crystallization step. In this work it was noticed that the lactose interferes on sucrose crystallization and the presence of skimmed milk constituents changed the rheological characteristics of the product when compared to the model solutions produced. The heating step accentuated this effect. These changes increased the viscosity making it difficult to crystallize sugars, thus interfering with the hardness of the samples.

Keyword: Lactose. Sucrose. Crystallization.

RESUMO

PEREIRA, João Pablo Fortes, D.Sc., Universidade Federal de Viçosa, novembro de 2019. **Cristais de lactose: determinação e formação em diferentes produtos lácteos concentrados.** Orientador: Antônio Fernandes de Carvalho. Coorientadores: Ítalo Tuler Perrone, Rodrigo Stephani e Pierre Schuck.

O objetivo deste trabalho foi desenvolver o estudo da cristalização de açúcares em variados produtos lácteos concentrados (estudo da cristalização da lactose em soro concentrado etapa fundamental e anterior à secagem de soro), tendo auxílio de diferentes ferramentas analíticas. Para o leite condensado foi analisado o comportamento de amostras comerciais do produto no analisador de partículas por difração a laser usando dois diferentes materiais dispersantes (água e solução de lactose) no módulo líquido do equipamento, avaliando também a influência do tempo de recirculação para dispersão da amostra na distribuição do tamanho de partículas. Foi possível observar neste trabalho que o uso de solução de lactose preservou por mais tempo as características das partículas do leite condensado quando comparado com as análises realizadas tendo água como solvente no equipamento. Concluiu-se também que um tempo de recirculação de dois minutos era importante para garantir a dispersibilidade das amostras estudadas. No estudo da cristalização da lactose em soro de leite concentrado foi avaliado se a adição de citrato de sódio influenciaria a cristalização deste açúcar, analisando este comportamento por meio de diferentes ferramentas analíticas. Neste trabalho observou-se que o citrato de sódio, independente da concentração utilizada no estudo, acelera a cristalização da lactose em soro de leite concentrado além de diminuir a variação do tamanho dos cristais formados quando 0,10% de citrato de sódio foi adicionado. Também foi estudada a cristalização da sacarose e lactose em um modelo de doce de leite em barra sem gordura, tendo como objetivo monitorar a influência da lactose, dos constituintes do leite desnatado e do cozimento do produto sobre a cristalização da sacarose durante a etapa de cristalização. Neste trabalho percebeu-se que a lactose interfere na cristalização da sacarose, e a presença de constituintes do leite desnatado alteraram a características reológicas do produto quando comparado às soluções modelos produzidas. A etapa de aquecimento acentuou este efeito. Esta alteração aumentou a

viscosidade dificultando a cristalização dos açúcares, consequentemente, interferindo na dureza das amostras.

Palavras-chave: Lactose. Sacarose. Cristalização.

THESIS OUTPUT

Publications (papers)

João Pablo Fortes Pereira, Igor Lima de Paula, Rodrigo Stephani, Ítalo Tuler Perrone, Luiz Fernando Cappa de Oliveira, Antônio Fernandes de Carvalho. (Chapter 2). Water versus lactose solution as a dispersion medium for particle analysis in sweetened condensed milk by laser diffraction. Published in: **Química Nova,** 2019. 42(8): p.928-931. http://dx.doi.org/10.21577/0100-4042.20170406

Júlia d'Almeida Francisquini, João Pablo Fortes Pereira, Michele da Silva Pinto, Antônio Fernandes Carvalho, Ítalo Tuler Perrone, Paulo Henrique da Fonseca da Silva. Evolution of soluble solid content and evaporation rate curves during the manufacture of dulce de leche. Published in: **Food Science and Technology**, 2019. 39(1): p.78-82. http://dx.doi.org/10.1590/fst.26617

João Pablo Fortes Pereira; Laís Lima Melquíades; Rodrigo Stephani; Luiz Fernando Cappa de Oliveira; Ítalo Tuler Perrone; Antônio Fernandes de Carvalho. (Chapter 3). Effect of sodium citrate on lactose crystallization in concentrated whey. Submitted in: April 2019 at **International Journal of Dairy Technology.**

João Pablo Fortes Pereira; Natália Maria Germano Alves; Rodrigo Stephani, Luiz Fernando Cappa de Oliveira, Ítalo Tuler Perrone, Antônio Fernandes de Carvalho. (Chapter 4). Influence of lactose, skimmed milk components and heating step on sucrose crystallization in model free fat tablet dulce de leche. In preparation

Oral presentation.

Lactose crystallization in sweetened condensed milk and whey. **I Simposium Inovaleite.** October, 2019 – Viçosa, Brazil.

Avaliação físico-química e nutricional de fórmulas infantis submetidas a diferentes tratamentos térmicos. **III Workshop de Ciência e Tecnologia de Leite e Derivados, Inovaleite.** August, 2016 – Viçosa, Brazil.

Poster presentation

João Pablo Fortes Pereira; Laís Lima Melquíades; Rodrigo Stephani; Luiz Fernando Cappa de Oliveira; Ítalo Tuler Perrone; Antônio Fernandes de Carvalho. Influência do citrato de sódio na cristalização da lactose em soro de leite concentrado. **30° Congresso Nacional de Laticínios.** July, 2019 – Juiz de Fora, Brazil.

João Pablo Fortes Pereira; Laís Lima Melquíades; Rodrigo Stephani; Luiz Fernando Cappa de Oliveira; Ítalo Tuler Perrone; Antônio Fernandes de Carvalho. Efeito do citrato de sódio no crescimento de cristais de lactose em soro de leite concentrado. **30° Congresso Nacional de Laticínios.** July, 2019 – Juiz de Fora, Brazil.

João Pablo Fortes Pereira, Igor Lima de Paula, Rodrigo Stephani, Ítalo Tuler Perrone, Luiz Fernando Cappa de Oliveira, Antônio Fernandes de Carvalho. Behavior of sweetened condensed milk samples submitted to analysis of particles by laser diffraction with different dispersing materials. **I Simpósio brasileiro sobe materiais e pesquisas relacionadas.** April, 2018 – Juiz de Fora, Brazil.

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LIST OF ABBREVIATIONS

α - Alpha
AOAC – Association of Official Agricultural Chemists
β – Beta
B1 - Initial soluble solids content (°Brix time zero)
B2 - Final soluble solids content (°Brix after crystallization)
CAPES – Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
°C – Celsius
CIT – Citrate
cm - Centimeter
CO_2 – Carbon Dioxide
CNPq – Conselho Nacional de Desenvolvimento Científico e Tecnológico
CW – Concentrated whey
DL – Dulce de Leche
Fapemig - Fundação de Amparo à Pesquisa do Estado de Minas Gerais
FMR - Fixed mineral residue
g - Gram
H_2O - Water
IDF – International Dairy Federation
L – Liter
LC - Lactose crystallization
mL – Milliliter
µm - Micrometer
mW – Milliwats
NEEM - Espectroscopy and Molecular Structure Nucleo
PCA – Principal component analysis
pH – Potential of hydrogen
QUIMTEC - Laboratory of Chemistry and Technolgy
rpm – Rotation per minute
SCM – Sweetened condensed milk

TS – Total solids

$w \cdot w^{-1}$ – Weight per weight

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GENERAL INTRODUCTION

The process of crystallization of some sugars is a natural step to stabilization of supersaturation solutions (unstable thermodynamics) arising from the concentration of sugar solutions, either by cooling the solution or by addition of an anti-solvent.

Sugar crystallization is important to modify the texture and sweetness of the food. Sugar crystallization is also important for the conservation of powdered product resources during shelf life. When obtained without previous crystallization, whey powder is a very fine, hygroscopic powder, with a tendency for aggregation of colloidal particles due to the presence of lactose in an amorphous or glassy state.

The crystallization process takes place by two main mechanisms that occur simultaneously: the nucleation and the growth of the crystals that, along with other factors of minor relevance, provide the size distribution of the crystals formed.

The main objective of this work was to study the crystallization of lactose and/or sucrose in different dairy matrices, as well to apply different analytical tools to greater understanding the said process. Specific objectives were grouped into three chapters:

Chapter 1 – Literature Review.

Chapter 2 – Water versus lactose solution as a dispersion medium for particle analysis in sweetened condensed milk by laser diffraction.

Chapter 3 – Effect of sodium citrate on lactose crystallization in concentrated whey.

Chapter 4 – Influence of lactose, skimmed milk constituents and heating step on sucrose crystallization in model free fat tablet dulce de leche.

In the Chapter 1, a short review was made of the sugars studied and the dairy products used as matrices.

In the Chapter 2, it was characterized the behavior of commercially-distributed sweetened condensed milk samples via laser diffraction analysis using different dispersant media (water and lactose solution) and determined the influence of solubilization time on the distribution size of particles.

In the Chapter 3, it was evaluated if the addition of sodium citrate influenced lactose crystallization in concentrated whey using many tools as refractometry,

microscopy, Raman spectroscopy followed by chemometric data analysis and particle size analysis by laser diffraction.

In the Chapter 4, it was made a study to monitor the influence of lactose, skimmed milk constituents and heating step on sucrose crystallization during the crystallization step of model free fat tablet dulce de leche.

The development of scientifically based parameters for the processing of dairy products whose crystallization is a fundamental step in the process, whether due to sensory or technological reasons. It is also important for the development of the Brazilian concentrated and dehydrated industry, contributing to a greater competitiveness of these industries in domestic and foreign markets.

CHAPTER 1

LITERATURE REVIEW

Carbohydrates

Carbohydrates are the most important energy source in our diet. Roots, tubers, and bread, for example, are rich in carbohydrates and provide a reservoir of energy. The main function of carbohydrates in food is provide energy for the body, especially the brain, which uses only the glucose as a source of energy. These nutrients after digested and absorbed, enter the bloodstream for use by cells in each 1g of metabolized carbohydrate generates 4 kcal of energy (Slavin and Carlson, 2014). They are metabolized into high energy compounds that can participate in all biochemical reactions, where they provide the necessary energy. Carbohydrates also provide material for the synthesis of some important chemical compounds in the body. They are present in the liver as liver glycogen and also in the muscles as muscle glycogen. Glycogen is an example of high molecular weight carbohydrate as well as starch and cellulose. The recommended daily intake of carbohydrate is around 45 to 65% of the diet (Mahan, 2012), depending of each organism.

Carbohydrates are the most abundant biomolecules on Earth and each year, photosynthesis converts more than 100 billion metric tons of CO_2 and H_2O into cellulose and other plant products (Lehninger et al. 2005). Carbohydrates as sugar and starch are a dietary staple and their oxidation is the central energy-yielding pathway in most heterotrophic cells (Slavin and Carlson, 2014). Insoluble carbohydrate polymers are used as structural and elements of protection in the cell walls of bacteria and plants and in the connective tissues of animals. Carbohydrate polymers also bind to proteins and lipids to perform a variety of functions in living things (Lehninger et al. 2005).

Carbohydrates are organic molecules containing carbon, hydrogen and oxygen. Carbohydrates are predominantly cyclized polyhydroxy aldehydes or ketones, or substances that yield such compounds on hydrolysis (Vaclavik and Christian, 2008). Sugars that have asymmetric carbon atoms are optical actives, their solutions deviate or vibration plane from the polarized light that crosses them (Perrone, 2010). Many

carbohydrates have the empirical formula $(CH_2O)n$; some also contain sulfur, nitrogen, or phosphorus. Three classes stand out of carbohydrates group: monosaccharides, oligosaccharides, and polysaccharides (Cummings and Stephens, 2007).

Monosaccharides, or simple sugars, consist of a single polyhydroxy aldehyde or ketone unit. The most abundant monosaccharide in nature is the six-carbon sugar D-glucose, sometimes referred to as dextrose. Others monosaccharides are fructose and galactose (Lehninger et al. 2005; Cummings and Stephen, 2007).

Oligosaccharides consist of short chains of monosaccharide units, or residues, joined by characteristic linkages (glycosidic bonds). The most abundant are the disaccharides, (two monosaccharide units). Typical is sucrose, or cane sugar, which consists of the six-carbon sugars D-glucose and D-fructose. Are also largely found lactose (D-glucose + D-galactose) and maltose (D-glucose + D-glucose). In cells, most oligosaccharides having three or more units do not occur as free entities but are joined to nonsugar molecules (lipids or proteins) in glycoconjugates (Lehninger et al. 2005; Cummings and Stephen, 2007).

Carbohydrate polymers are found in a range of sizes. The polymers that containing more than 20 monosaccharide units are called polysaccharides. Polysaccharides may have hundreds or thousands of monosaccharide units. Some polysaccharide molecules, such as cellulose, are linear chains, whereas others, such as glycogen, are branched chains. The vegetables, starch and cellulose both consist of units of D-glucose, but they differ in the type of glycosidic linkage, and consequently have different properties and biological roles (Sackheim and Lehman, 2001; Lehninger et al. 2005; Cummings and Stephen, 2007).

The properties of sugars not only make them useful as sweeteners but also for the structural development and for processing of formulated foods as the food transforms from more fluid to solid during heating, freezing, and storage. Viscosity, solubility, hygroscopicity and crystallinity are examples of physical properties of sugars that are important in their use as ingredients. The effect these properties have on other molecules and how they influence component interactions is important to elaboration of a product of quality (Davis, 1995).

Sweet taste is perceived in the tongue of the interaction between sugars and proteins in the presence of water. Nofre et al. (1996) and Eggers et al. (2000) have suggested that for a sweet taste, a sugar molecule needs to occupy a specific location

within the appropriate receptor where it associates with a protein via hydrogen bonds. This bond exposes a hydrophobic substance bound to a sugar region within the not clear why a specific sugar is perceived as sweeter than others, since almost all chemical structure is identical to all sugar molecules. Sweetness in monosaccharides seems to be correlated with small differences in glucose ring stereochemistry. At sweetness scale where sucrose has an arbitrary sweetness of 1.00, the single ring of mannose is considered tasteless, while glucose is 0.47 and the fructose is 1.74.

The scale of sweetness is showed in de Table 1.

Table 1. The scale of sweetness (sucrose relative)

Substance	Sweetness (sucrose relative)
Lactose	0.16
Galactose	0.32
Maltose	0.33
Glicose	0.74
Sucrose	1.00
Frutose	1.74
Sodium Ciclamate	30
Aspartam	180
Sacarin	300
Sucralose	650
Alitame	2,000
Taumatin	3,000
Monelin	3,000

Source: Damodaran, S.; Parkin, K. (2017).

Sucrose

Sucrose is an important commodity in world trade. Although competition from alternative sweeteners and starch-derived isoglucose is substantial, over 100 million tons of sugar have been produced worldwide annually in recent years (Mathlouthi and Reiser, 1995).

Sucrose is a disaccharide composed of two monosaccharides: glucose and fructose. Sucrose is naturally produced through the metabolism of some plants. From the extraction and purification of this carbohydrate table sugar is produced. It has the molecular formula $C_{12}H_{22}O_{11}$ (Lehninger et al. 2005). For human consumption, sucrose is extracted and refined from sugarcane or beet, mainly (Mathlouthi and Reiser, 1995).

Sucrose is sold, mainly, in the crystal form. In the crystalline state, a structure analysis will provide accurate description of the individual three-dimensional arrangement. In the liquid state, the sugar is associated to a one-dimensional, probably. Therefore, a dynamic rather than a static description must be sought, especially when geometries are changing rapidly. Hence, the term 'structure' must also encompass the dynamic fluctuations that the molecule may undergo (Hynes and Le page, 1991).

Most monosaccharides exist in the form of heterocyclic rings or cyclic hemiacetals, such as five-membered furanoses or six-membered pyranoses (Lehninger et al. 2005). The centre of chirality generated by hemiacetal ring closure is the only carbon bound to two oxygen atoms. It is labelled C-1, and the others are numbered sequentially around the ring. The D or L designation of the configuration refers to the position of the hydroxyl group on the asymmetric carbon farthest from the C-1, i.e. the C-5 of hexoses and the C-4 of pentoses (Lehninger et al. 2005; Cummings and Stephen, 2007). The official name of sucrose, according to the IUPAC-IUB Commission of Biochemical nomenclature is D-fructofuranosyl-a-D-glucopyranoside. In the Figure 1 is showed the sucrose’s structure.

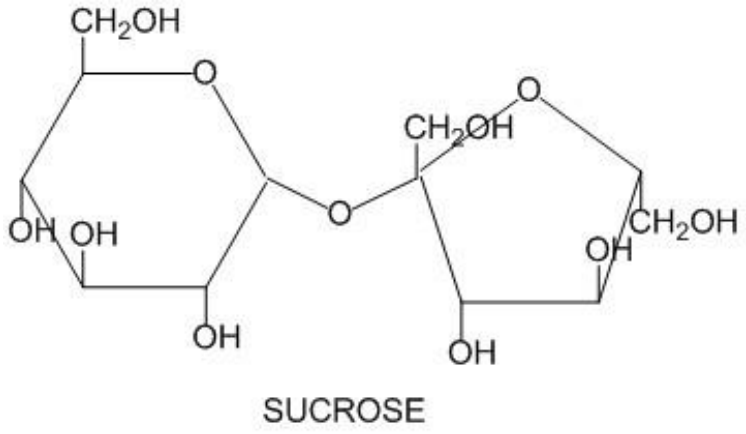

Figure 1. Sucrose's structure.

Unlike most disaccharides, the glycosidic bond of the sucrose is formed between the reducing ends of both glucose and fructose, and not between the reducing end of one and the non-reducing end of the other, forming a non-reducing disaccharide. This linkage inhibits further bonding to other saccharides units (Lehninger et al. 2005; Cummings and Stephen, 2007).

Sugar beet plants are located in colder climates, where sugar beet is grown and processed directly into refined sugar. In warm climates sucrose is extracted from sugar cane. To process of the sugar, first, the raw sugar crystals is washed, after the crystals are dissolved in a sugar syrup that is filtered. The sugar syrup is then concentrated under vacuum and crystallized as the final purification process to produce pure sucrose crystals (Mathlouthi and Reiser, 1995).

In addition to the ability to sweeten foods and drinks, the sucrose can also act as a food preservative when used in sufficient concentrations. Sucrose is strongly link to food water, decreasing the water activity of these foods. Sucrose is important to the structure of many foods, including biscuits and cookies, cakes and pies, candy, and ice cream.

Sucrose crystallizes in the monoclinic space group P21 with room-temperature lattice parameters a = 1.08631 nm, b = 0.87044 nm, c = 0.77624 nm, β = 102.938°. Sucrose crystallization is anhydrous, unlike other sugars that crystallize as mono or dihydrates (Bottelheim et al. 2009). Primary nucleation is characterized by mechanisms in which crystals form in the absence of pre-existing crystals. If the solution is absolutely pure, nucleation is homogeneous, while nucleation is heterogeneous in the presence of foreign solid substances (Thakur and Kessler,

2015). If crystallization occurs in a crystalline suspension, as normally encountered in crystallization equipment, it is referred to as secondary nucleation (NÝVLT et al., 2001). Although sucrose crystal can have many shapes, the most common shape has a ratio of 0.6, 1.0 and 0.8 in its three dimensions (Saska and Myerson, 1983). In the Figure 2 is showed the sucrose crystal in tablet dulce de leche.

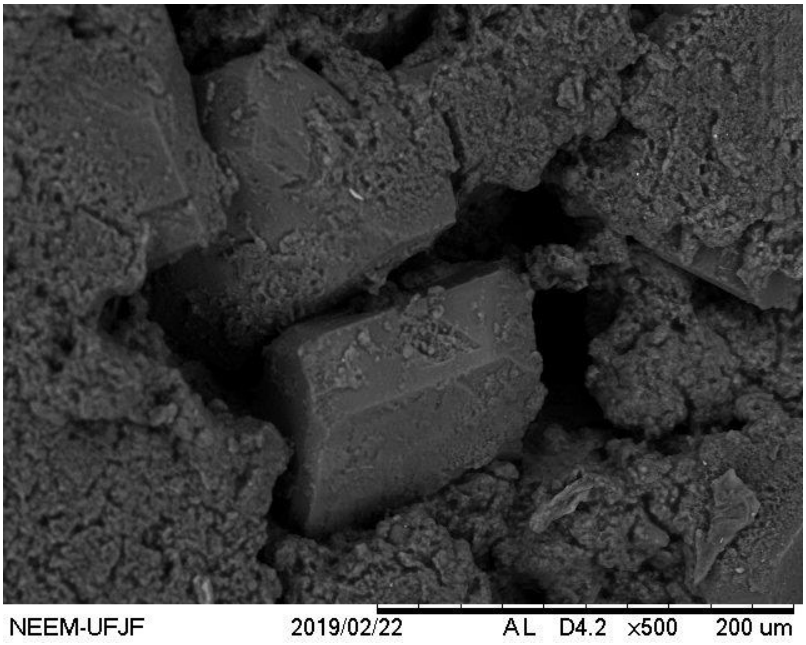

Figure 2. Sucrose crystal in tablet dulce de leche.

The purity of sucrose is measured by polarimetry, through the rotation of plane-polarized light by a solution of sugar. The specific rotation at 20 °C using yellow "sodium-D" light (589 nm) is +66.47° (Desai et al. 2013). Commercial samples of sugar are assayed using this parameter. Sucrose does not deteriorate at ambient conditions.

Lactose

Lactose is the predominant carbohydrate in the milk, in which there are also low concentrations of glucose and galactose (Robinson, 1981). According to Damodaran and Parkin (2017), the lactose concentration in milk varies according to origin between 2.0 and 8.5%. Cow's milk contains more lactose than any other solid component, with an almost constant concentration between 45 to 50 g · 1000 mL^{-1} (Walstra and Jenness, 1984). Lactose is not as sweet as other sugars; it is about 30 times less sweet than cane sugar, for example.

Carbohydrates that have asymmetric carbon atoms are optically active, and, when in solution, they deviating from vibration plane of the polarized light that crosses them (Lehninger et al. 2005). The isomers are α lactose and β lactose that differ in their specific rotation. Figure 3 shows the lactose isomers.

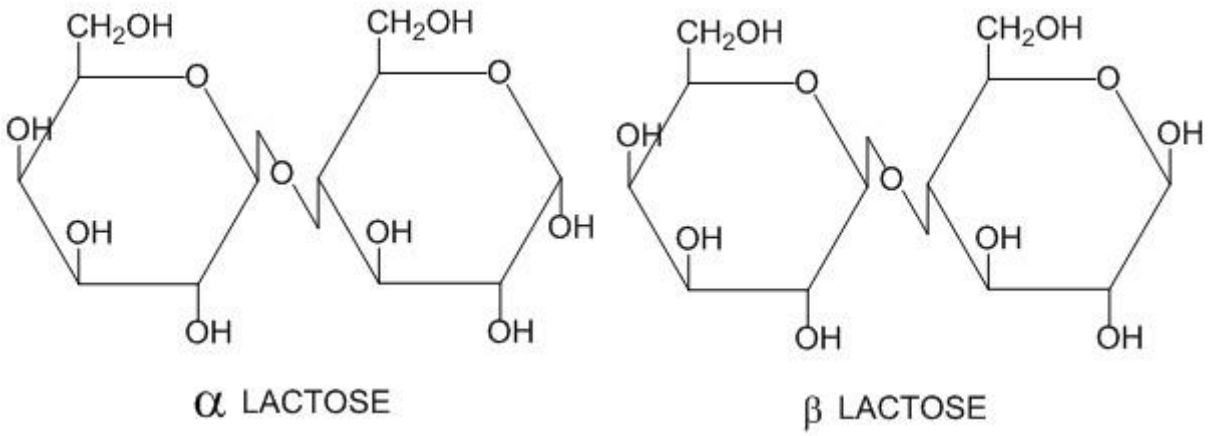

Figure 3. Lactose's structure isomers.

Average solubility of lactose at 20°C is 20g · 100 g^{-1} of water, solubility of glucose is 107g · 100 g^{-1} of water and of the galactose is 50g · 100 g^{-1} of water (Bobbio and Bobbio, 1992). A freshly prepared solution of β lactose will change its rotation over time as balance with α lactose takes place. In dairy products, two crystalline forms of lactose can occur, an anhydrous and another hydrated. Also occur an amorphous glassy mixture of α lactose and β lactose. The α lactose structural form can be converted to the beta structural form by changing the position of hydroxyl and hydrogen in the reducing group. This change in rotation and transformation into solution from one form to another is called mutarotation. According to Whittier (1944), mutarotation

is a characteristic phenomenon of all reducing sugar in aqueous solution and, in some instances, is attributed to changes in concentrations of α and β forms.

Lactose solution in equilibrium state, at 25 °C has 62.25% of its lactose in β form and 37.75% in α form (Whittier, 1944). Alpha and β forms have distinct physical properties. According to Nickerson (1974), α lactose at 20 ºC has a specific rotation in water of $[\alpha]_D$ = 89.4 º and melt point of 201.6 ºC. While β lactose has specific rotation in water of $[\beta]_D$ = 35.0 º and melt point of 252.2 ºC.

For mutarotation, the reaction activation energy is 75 kJ mol^{-1} and under conditions of high sugar concentration, such as in sweetened condensed milk and dulce de leche, a significant decrease in mutarotation rate occurs (Walstra and Jenness 1984). The α and β lactose fractions have distinct solubilities and mutorotation becomes an important factor in crystallization (Holsinger, 1997).

Usually, α lactose crystallizes as a hydrate, containing equimolar amounts of lactose and water, and its crystals are non-hygroscopic. At room temperature, anhydrous β lactose dissolves faster than α lactose hydrate and its solubility is approximately ten times higher, its crystals being smaller but having a larger surface area (Walstra and Jeness, 1984).

According to Whittier (1944), when an excess of α lactose is added in water at 15 °C, approximately only 7 g · 100 g^{-1}, it is dissolved and is defined as the true solubility of the α form. The increase in solubility over time is due to mutarotation, as the α form is converted to β form, making the solution unsaturated in relation to α lactose, and a greater amount of α lactose may be dissolved. The process continues until the equilibrium, (approximately 17 g · 100 g^{-1}). According to Holsinger (1997), β lactose, under similar conditions, has an initial solubility of approximately 50 g · 100 g^{-1}. According to Walstra et al. (2001), if β lactose is added in water, the solubilization process starts very fast, but slows down over time. As a consequence of mutarotation, more α lactose is formed than the solubility limit of the solution, resulting in crystallization of the α form. Alpha lactose crystal has characteristic shape, called tomahawk crystals (Pisponen, 2017). Figure 4 shows lactose crystals in concentrated whey.

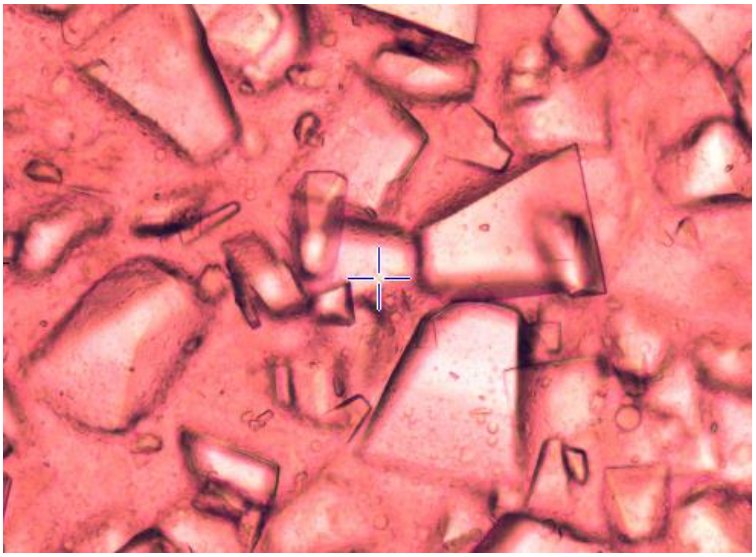

Figure 4. Lactose crystals in concentrated whey.

Sweetened condensed milk

The sterilization of evaporated milk in closed containers was developed in the finish of nineteenth century, being a very efficient method of preservation. By the 1950s, conservation through the addition of sugar had already been improved. The combination of these conservation methods (barrier technology) made it possible to manufacture condensed milk on a large scale. True evolution occurs when continuous evaporative production becomes possible, followed by aseptic packaging technology (Tetra Pak, 2015).

Evaporated milk can be classified into two different types: unsweetened and sweetened condensed milk (SCM). Both products can be made with fresh milk or reconstituted milk from milk powder (Hess, 2003).

Talking specifically about SCM, it is known that the product can be made from whole or skimmed milk. In the manufacture of SCM, heat-treated milk is pumped into the evaporator. When sugar is added in solution, it occurs during evaporation, but sugar can also be added dry before evaporation. After concentration, the product is cooled in such a way that lactose is crystallized into small particles in the supersaturated solution. These crystals must be so small, less than 10 µm, that they cannot be detected by the tongue. After cooling and crystallization, the SCM is packed (Tetra Pak, 2015).

SCM should be yellowish in color and has a high viscosity. The product is packed in aseptic paperboard packages or in cans, which in this case must be cleaned and sterilized before filling, as no sterilization takes place after canning (Renhe et al. 2017).

For the manufacture of quality product, the milk and sugar mixture must be standardized, especially fat, protein and sugar content. It is important to heat treat the milk to be used in SCM manufacturing to destroy microorganisms and enzymes that could cause product problems. Heat treatment is also important for increasing product viscosity during storage (consequence of protein denaturation). Milk is generally heat treated at 82°C for 10 minutes is a product with a high viscosity is required. If a low viscosity product is required, the temperature and time combination should be at a higher temperature for a shorter time, for example 116°C for 30 seconds. The shelf life of SCM depends on several factors, including product composition. The osmotic

pressure of product is high and this minimizes the proliferation of microorganisms (Tetra Pak, 2015).

Sucrose is mainly responsible for increasing osmotic pressure. A sugar content of at least 62.5% in the aqueous phase is required to produce an osmotic pressure high enough to inhibit to growth of bacterial. The sugar can add before heat treatment (dry sugar) or added in the evaporator (sugar syrup). The stage at which sugar is added affects the viscosity of the final product. Early addition of sugar may cause increased viscosity during storage. SCMs maybe homogenized at 5 – 7.5 mPa in sequence after evaporation to regulate the viscosity of the end product (Renhe et al, 2011).

A critical step in the SCM production process is product cooling. At the end of the concentration process the SCM will become a supersaturated solution as there will be little water for lactose solubilization (Renhe et al, 2011). Part of the sugar will therefore be precipitated as crystals. If excess lactose can crystallize freely, sugar crystals will slowly increase and the product will be rough and unsuitable for many applications. Therefore, the crystallization of lactose should be controlled to obtain very small crystals. The largest crystal size allowed in first class SCM is 10 µm. These crystals will remain dispersed in the product under normal storage temperatures, 15 - 25 °C, and are not be felt on the tongue. Rapid cooling is required under vigorous agitation without air retention to have a quality product. Some manufacturers do instant cooling, others use seed crystals in the form of finely ground lactose crystals and are added at a rate of about 0.05% of the total mixture when the product reaches crystallization temperature (about 30 °C). It is the temperature at which the sugar solution is supersaturated, so that the added lactose does not solubilize. However, the temperature should not be so low that spontaneous nucleation may occur prior to mixing of the crystals (Renhe et al 2011; Tetra Pak, 2015).

Whey Powder - production depends on lactose crystals formation in concentrated whey

Whey was once considered a high cost by-product for the dairy industry. Today, it is regarded as a valuable co-product of cheese making. However, with the scientific proof of the high nutritional values of its constituents and the advancement of fractionation and purification techniques, whey has come to be considered by many industries as an important raw material for production of ingredients in the food industry (Gernigon *et al*. 2010). Whey can be used in various dairy derivatives but is widely used in the composition of fermented or unfermented dairy drinks and their drying to produce whey powder.

Whey products enhance flavor and color, improve texture, have the capacity of to emulsify and stabilize formulates, improve flow properties and dispersibility in dry mixes, can help extend shelf-life, and exhibit a range of other properties that increase food product quality (Perrone, 2010).

Advances in technology and investments in research and development have enabled the whey industry to expand its product line from commodities to a variety of higher valued products, including whey protein concentrates, whey protein isolates and fractions. The industry continues to innovate, while at the same time focusing on finding new uses and new markets for these value-added ingredients (Macgibbon et al. 2014).

Drying is a method of food preservation that inhibits the growth of micro-organism like yeasts and mould through the removal of water. Drying is one of the oldest preservation processes available to the mankind. Many products can be prepared by dehydration using dryers that are in operation in different industries like chemical, pharmaceutical, process and dairy (Masters, 2002).

Foods are dehydrated for two main reasons, to inhibit the growth and activity of micro-organisms and hence preserve the food, and/or to reduce the weight and bulk of food for cheaper transport and storage (Masters, 2002).

In foods, to a quality dried product and to ensure safe storage the final moisture content of the food should be less than 20 % for fruits and meat, less than 10 % for vegetables, and 2.5-5 % for dairy products. Low moisture content is only an indication of food stability and not a guarantee. It is the availability of moisture for microbial growth that is more important and the term water activity (aw) is used to describe this. Water

activity varies from 0 to 1. The lower the value the more difficult it is for micro-organisms to grow on a food (Tetra Pak, 2015).

According to Knipschildt and Andersen (1994), the drying method is the most important dairy preservation method, because the use of this technique enables the conversion of milk or whey into milk or whey powder with minimal nutritional losses.

According to Codex Alimentarius (1999), whey powder is the product obtained by dehydrating whey or acid whey using technologically appropriate processes and is suitable for human consumption. Whey powder is produced using a combination of membrane concentration or vacuum evaporation processes and spray drying.

The dairy drying process involves the formation of lactose in the amorphous state which is highly hygroscopic. According to Hynd (1980), whey powder has the tendency to absorb water from ambient air, resulting in aggregation of colloidal particles of the product during storage, caused by the replacement of part of the amorphous lactose by crystalline lactose,

Whey powder obtained without prior lactose crystallization becomes a fine, hygroscopic powder which aggregates quickly because lactose remains in an amorphous state in whey (Masters 2002). According to Fox and McSweeney (1998), in order to increase the shelf life of whey powder, the acidity of the whey, the crystallization of lactose and the outlet air temperature should be controlled. Sorption curves, according to Jouppila and Roos (1994), are drastically affected by the presence of amorphous lactose.

The crystallization of amorphous material occurs when amorphous solid molecules reorganize in an ordered structure under specific conditions such as alteration of relative humidity, elevation of storage temperature among other factors (Jouppila and Roos 1994). The crystallization process is essential to producing whey powder because amorphous lactose is the most hygroscopic and unstable form found in whey. Prior lactose crystallization can reduce the risk of stickiness and caking in the whey powder (Saffari and Langrish 2014; Tham *et al.* 2017). According to Knipschildt and Andersen (1994), crystallization of concentrated whey can be performed by the addition of crystallization nuclei, microcrystalline lactose or whey powder, followed by stirring and temperature control.

Tablet dulce de leche

Dulce de leche (DL) is a product from Latin America. In this region, the product is one of the most widely manufactured dairy products in South America, mainly in Argentina and Brazil, where it is marketed as a paste or tablet. Due to dulce de leche's low moisture content, the product can be safely stored at room temperature. The primary ingredients used to manufacture dulce de leche are milk, sucrose and an acidity reducer (Perrone et al. 2011).

Total processing time can vary from 40 min to 4 h. Processing time depends on the type of equipment used and the amount of steam injected. Processing time plays an important role in a product's viscosity, color and flavor and ultimately determines the characteristics of the final product (Perrone, 2007).

Processing end point for dulce de leche can be measured by determining the soluble solids content (above 66 °Brix) or by observing how a small droplet of dulce de leche behaves when submerged in water. Once the desired solid content / consistency is reached, the final product (paste dulce de leche) should be cooled to 75 °C to 80 °C then packaged while still warm in cans or glass containers that have been filled to the top to eliminate any air and prevent contamination. Tablet dulce de leche is crystallized with stirrers while the cooling occur. The product is then stored at room temperature for a period ranging from 160 to 180 days (Francisquini et al. 2019).

Tablet dulce de leche is crystallized into blocks which have a uniform coloration and texture. The Tablets are typically eaten in pieces (Stephani et al. 2019).

The difference in sweetness between paste and tablet dulce de leche is determined by the total solid and sucrose content and from the manufacturing process. For tablet dulce de leche, production involves a slow, manual process that induces lactose and sucrose crystallization in the product (Perrone et al. 2011; Stephani et al. 2019).

Sucrose and lactose content is higher in the syrup used to make tablet dulce de leche than it is in that used to paste dulce de leche and consequently increases crystallization. The increased crystallization is necessary for the product to become solid and firm enough to be cut and served in pieces. To this end, the original solution is subjected to a controlled-crystallization process or batching. In controlled crystallization or churning, the product undergoes intense stirring as it is slowly cooled.

This process induces the formation of numerous sucrose and lactose crystals which ultimately alter the texture of the product (Stephani et al. 2019).

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CHAPTER 2

WATER VERSUS LACTOSE SOLUTION AS A DISPERSION MEDIUM FOR PARTICLE ANALYSIS IN SWEETENED CONDENSED MILK BY LASER DIFFRACTION

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ABSTRACT

Sweetened condensed milk contains various dispersed particles, such as proteins, fats, and lactose, all of which make it highly viscous. During sweetened condensed milk production, microcrystalline lactose is added in order to control the product crystallization. The purpose of this study was to characterize the behavior of commercially-sold sweetened condensed milks submitted to laser particle size analysis. To this characterization, were used two different dispersion media: water and a lactose solution. The lactose solution dispersion medium extended the length of time of the sweetened condensed milk samples' original characteristics were present during the laser diffraction particle sizing measurements that were carried out. Significant differences were observed ($p < 0.05$) between analysis times when water was used as a dispersing medium. When lactose solution was used, not had difference ($p > 0.05$). Using a lactose solution as a dispersion medium when determining particle size in sweetened condensed milk prolongs the original characteristics of the sample during laser diffraction particle analysis.

Key words: concentrated dairy products; particle size; characterization.

INTRODUCTION

Laser diffraction particle analysis is a technique which involves dispersing a sample in a flowing liquid or it could be done by dry via. The particles cause discontinuities in the fluid flow which are detected by an incident laser, and correlated with particle size (Brar and Verma, 2011). Once a certain number of particles is reached, the incident light undergoes different phenomena (diffraction, refraction, reflection and absorption) which in turn create a three-dimensional light envelope (Jillavenkatesa, 2001). The detectors measure the intensity and the angle of scattered light, then use mathematical algorithms to convert the signal to particle size (Brar and Verma, 2011). Prior knowledge of the sample matter's refractive indexes is necessary in order to analyze the dispersant medium. Laser diffraction analysis is non-specific and the particle distribution present in the samples is determined by grouping the particles by size rather than chemical composition or other segregation criteria. This technique is widely used in food matrix studies, as preparation of size controlled starch nanoparticles, characterizations of emulsions, studies of the presence of micro- and nanoparticles in drinks and foods and quantitative determination of fat and total protein (Bogomolov et al. 2012; Chang et al. 2017; Ge et al. 2017; De La Calle et al. 2018; Hart et al. 2018).

Codex Alimentarius defines sweetened condensed milks as dairy products that can be obtained by partially removing water from a milk and sugar solution or any other process that produces a product with the same composition and characteristics. The product must present a minimum of 8 g · 100 g^{-1} of fat, 28 g · 100 g^{-1} of milk solids and 34 g · 100 g^{-1} of protein in non-fatty milk solids (Codex Alimentarius, 1999).

Sweetened condensed milk contains dispersed proteins, fats, and lactose which give it a high viscosity (Renhe at al. 2017).

The lactose content in commercial sweetened condensed milk is 10.16 g · 100 g^{-1} of the product (37.6 g· 100 g^{-1} of water), resulting a saturated solution of lactose in water (Renhe et al. 2017). When lactose is present in the solution in excess of 2.1 times the saturation value (20 g · 100 g^{-1} water at 20 °C), spontaneous crystallization occurs (Walstra et al. 2001). Determining the number of nuclei formed or induced during the lactose crystallization is important in order to control crystal growth up until the sugar supersaturation decreases. The available surface area for deposition,

viscosity, temperature and mutarotation are also factors that affect crystal growth (Fox and McSweeney, 1998). Controlling the size of lactose crystals during the crystallization in sweetened condensed milk is important in order to obtain a product with desirable sensorial characteristics. Laser particle analysis can be used to determine crystal size during the production and shelf storage when certain conditions have been established. The usual liquid for particle size determination in foods is water, the hypothesis of this work is: for sugar crystallized foods solubilization would happens during analysis affecting the results. The aim of the present work was to characterize the behavior of commercially-distributed sweetened condensed milk samples via laser diffraction analysis using different dispersant media (water and lactose solution) and determine the influence of solubilization time on the distribution size of particles.

MATERIAL AND METHODS

Samples from four different brands of sweetened condensed milk purchased in Juiz de Fora, MG, Brazil were analyzed gravimetrically for moisture content, by Kjeldahl method (the total nitrogen in the samples was determined in duplicate, and the nitrogen content was multiplied by the conversion factor 6.38 to obtain the equivalent amounts of protein) (IDF, 1993), for protein content and using the Gerber method (based in acid digestion coupled to centrifugal separation in specific laboratory glassware) (IDF, 2008), for fat content. The content of the fixed mineral residue (FMR) was determined gravimetrically by incinerating the sample in a muffle oven at 550 °C for 10 hours (AOAC, 1997). Sugar content (sucrose and lactose) was obtained by mathematical calculation.

Microphotographs of the samples were taken with an Olympus BX51 optical microscope equipped with a bright halogen light source. Average crystal size was determined using OPUS software. The crystals in the images were counted to calculate the number of crystals present per gram of sweetened condensed milk. In total, 60 micrographs were taken for each sample (six slides x 10 fields per slide) (Martinez et al. 1990).

Particle size distribution during the dissolution of the sweetened condensed milk samples was determined using a Beckman Coulter LS 13 320 Particle Sizing Analyzer

(Beckman Coulter, Miami, FL, USA) coupled to a liquid module (Beckman Coulter, Miami, FL, USA).

Approximately one drop of each sample was added to the liquid module filled with room temperature water to facilitate 47 % ± 5 % of obscuration in the PIDS (Polarization Intensity Differential Scattering System) photo detectors. The dissolution process was monitored over an eight minutes period and measured every two minutes for a total of five successive measurements. Samples of the same five sweetened condensed milk brands were submitted to the same procedure and conditions using a lactose solution instead of water in the liquid module (necessary adjustments to the apparatus were made). The lactose solution was prepared with a concentrated solution of lactose 23.0 % w · w^{-1}, cooled to 25 °C, filtered, then added to the water in the analyzer reservoir to obtain a concentration of 13.0 ± 0.5 % w · w^{-1} lactose (to approximate the sample lactose). Approximately 750 mL of concentrated solution was used to test each sample. Data were collected at 0.04 to 2000 µm for 90 seconds. The results were obtained using the standard refractive index of the dispersing medium (1.332 water) or (1.352 lactose solution) and the lactose particle refractive index (1.53) (Minouni et al. 2005).

Statistical analysis were performed using Graph Pad Prism 5 software (Graph Pad Software Inc., San Diego, CA, USA). For the treatment variable (water or lactose solution), the Student's t-test was performed simultaneously. For the time variable, a one-way analysis of variance was performed followed by verification using the Tukey test.

RESULTS AND DISCUSSION

The results of the centesimal composition of the market sweetened condensed milks tested are described in Table 1.

Table 1. Composition of sweetened condensed milk (n=4)

Attribute	Mean	Standard Deviation	Standard Error	Variation Coefficient (%)
Moisture ($g \cdot 100g^{-1}$)	27.83	0.61	0.30	2.18
Fat ($g \cdot 100g^{-1}$)	7.92	0.32	0.16	3.99
Protein ($g \cdot 100g^{-1}$)	6.13	0.30	0.15	4.93
FMR ($g \cdot 100g^{-1}$)	1.30	0.12	0.06	9.32
Sugars ($g \cdot 100g^{-1}$)	56.83	0.43	0.22	0.76

Based on the data obtained, it is worth noting product composition standardization for the four sweetened condensed milk samples tested. Composition is just one quality attribute in the production of sweetened condensed milk. Viscosity, flavor, odor, and crystal size are other important quality attributes in commercially-sold sweetened condensed milks (Walstra et al. 2001). The data in this study corroborated the results obtained by others works (Renhe et al. 2017; Siddique et al. 2017). In the latter study, the centesimal composition of 27 samples of sweetened condensed milk produced in Brazil and 12 samples commercialized in Bangladesh, respectively were evaluated.

The results along with average crystal size are presented in Table 2.

Table 2. Number and average size of sweetened condensed milk sample lactose crystals by optical microscopy.

Sample	Amount (n° crystals $\cdot g^{-1}$)	Size (µm)
A	$1.23 \times 10^8 \pm 2.14 \times 10^6$	11.2 ± 0.6
B	$1.74 \times 10^8 \pm 1.96 \times 10^6$	11.1 ± 0.3
C	$1.07 \times 10^9 \pm 2.87 \times 10^7$	9.8 ± 0.8
D	$9.29 \times 10^8 \pm 1.44 \times 10^6$	8.8 ± 1.4

An inverse relationship between number and mean size of the crystals is expected for sweetened condensed milk (Walstra et al. 2001). Lactose crystallization takes place due to nucleation crystal growth. During the nucleation, lactose molecules organize and aggregate in order to minimize chemical potential in the solution and balance interfacial tension caused by crystal formation in the new phase. During the

crystal growth stage, small particles cluster and/or α-lactose molecules bind to the formed nuclei causing them to grow (Wong and Hartel, 2014). In the present study, it is not possible to conclude that the reduced size of certain sample crystals was due to the greater number of nuclei present because the samples have been manufactured for commercial sale and the technology used (addition or not of cores by dispersion and, if added, in what quantity) is not known. However, based on the low standard deviation found the sugar contents of the samples, it can be assumed that the correlation may be true.

As to particle size distribution throughout dissolution time, the dissolution profile difference is shown in the Figure 1.

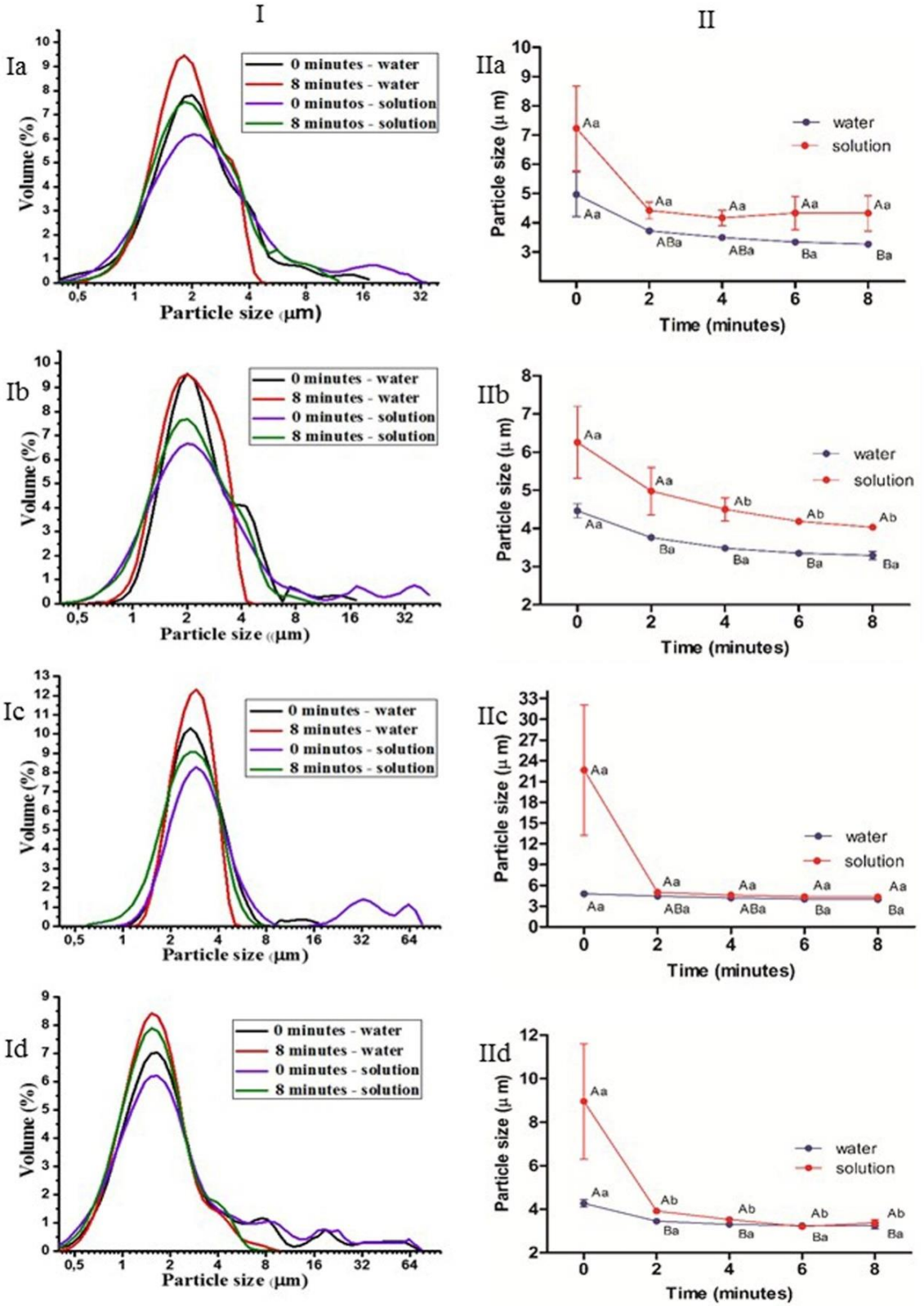

Figure 1. (I) Graphs of particle size distribution mean in sweetened condensed milk samples (Ia, Ib, Ic and Id), analyses carried out at 0 and 8 minutes when samples were dispersed in water and in lactose solution (II) Particle size distribution (d_{90}) of the

sweetened condensed milk samples (IIa, IIb, IIc and IId) over five successive measurements (0, 2, 4, 6 and 8 minutes). The results are presented by mean ± standard error of the mean. Means followed by the same capital letter do not differ within the same treatment for the variable time, as determined by one-way variance analysis using the Tukey test ($p>0.05$). Means followed by the same lowercase letter do not differ within the same time for period the treatment variable, as determined by the Student's t-test ($p>0.05$).

The value of d_{90} shows the maximum particle size and represents 90% of the volume percentage distribution in the sweetened condensed milk samples during laser diffraction particle analysis.

For most of the sweetened condensed milk samples studied, a significant difference was observed ($p <0.05$) between analysis times when water was used as a dispersing medium versus when a lactose solution was used ($p> 0.05$). The first two reading of sample particle solubility/dispersion in water (0 minutes and 2 minutes) were emphasized. There was no difference ($p> 0.05$) between the measurements performed at 6 and 8 minutes for any of the sweetened condensed milk samples studied. Analysis performed immediately after adding the sample hinders the ability to perform subsequent readings because of the short particle dispersion time. To ensure reliability, the sample needs to be recirculated, regardless of the liquid used for sample dispersion. Therefore, the first laser diffraction particle analysis must be performed after 2 minutes of sample recirculation. Even though non-specified particles, such as the sweetening components, in sweetened condensed milk present a complex analysis matrix and the particle distribution of other components are affected during recirculation, the lactose molecules in the solution play a key role in laser diffraction particle analysis.

The addition of a lactose solution as a dispersion medium promoted disturbances that differ from those found when water was used. The water can be substituted for a lactose solution in order to preserve the crystallized lactose present in the samples and in tern determine particle size. The initial analysis (when the sample profile is as close as possible to the original sample with no dispersant effect) was used to plot the dissolution profile of the samples at 0 and 8 minutes and the final analysis after the dispersion medium had a maximum effect on the sample.

Using lactose solution instead of water as dispersing medium allowed for a slower dissolution of the analyzed substance and thus a more reliable measurement of particle size in the substance's natural state. The d_{90} in Figure 1 shows particle size at 90 % distribution per volume (this was higher when measured using a lactose solution); it comes to an approximation of "natural" conditions of the matrices obtained. How concentration differences affect rates of matter migration has been widely demonstrated for different food matrices in scientific studies (Rastogi and Raghavarao 1997; Reynier et al. 2002; Hartel et al. 2011).

CONCLUSION

Using a lactose solution as a dispersion medium when determining particle size in sweetened condensed milk prolongs the original characteristics of the sample during laser diffraction particle analysis. Samples dissolve faster when water is used as the dispersant. For greater reliability of results, a two-minute recirculation of the product in the analyzer is necessary. The laser diffraction particle analyzer is an important complementary tool for studying particle behavior in the product. The adoption of the methodology studied in this article by the food industry needs more studies because sweetened condensed milk vary significantly in composition and in technologies of production.

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CHAPTER 3

EFFECT OF SODIUM CITRATE ON LACTOSE CRYSTALLIZATION IN CONCENTRATED WHEY

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ABSTRACT

Lactose crystallization in concentrated whey is a determinant step for drying efficiency because it impacts both yield and spray dryer adhesion. Studies on model solutions indicate that adding sodium citrate to whey enhances and accelerates crystallization. This study aimed to evaluate how the addition of sodium citrate influences lactose crystallization in whey concentrate using refractometry, microscopy, Raman spectroscopy followed by chemometric data analysis, and particle size analysis by laser diffraction. Sodium citrate increased crystallization rate and decreased both variability and crystal size in concentrated whey, increasing the effectiveness of the whey powder production.

Key Words*:* whey; sodium citrate; lactose; crystallization

INTRODUCTION

Whey is obtained by draining after casein destabilization by chymosin proteolysis or acidification of milk (Siso, 1996). Lactose is the main solute in whey (Walstra et al. 2001; de Souza et al. 2010; Amado et al. 2016; Kondor and Hogan 2017).

According to Damodaran and Parkin (2017), lactose concentration in milk varies from species to species and ranges from 2.0 to 8.5%. Cow's milk contains more lactose than any other dry matter, with a concentration between 45 and 50 $g \cdot L^{-1}$ (Walstra and Jenness, 1984).

According to Walstra et al. (2001), average lactose solubility at 20 °C is $20g \cdot 100g^{-1}$ of water, while glucose solubility is $107g \cdot 100g^{-1}$ of water and galactose is $50g \cdot 100g^{-1}$ of water. Sugars' asymmetric carbon atoms are optically active, their solutions deflect the vibration plane from polarized light when it passes through them. α-lactose and β-lactose differ in their specific rotations, and a freshly prepared solution of either will alter its rotation to attain an equilibrium state. The structural form of one lactose isomer can be converted to the structural form of another by changing the positions of the hydroxyl radicals and the hydrogen cells in the reducing group. This rotation alteration and subsequent transformation from one form to another is called mutarotation. Alpha and β lactose fractions have different solubilities (Holsinger, 1997).

Alpha lactose commonly crystallizes as a hydrate which contains equimolar amounts of lactose and water and has crystals that are hard and non-hygroscopic (Walstra and Jenness, 1984). The same authors stated that anhydrous β-lactose dissolves more rapidly than α-lactose hydrate at room temperature.

Whey powder obtained without prior lactose crystallization becomes a fine, hygroscopic powder which aggregates quickly because lactose remains in an amorphous state in whey (Masters, 2002). The crystallization of amorphous material occurs when amorphous solid molecules reorganize in an ordered structure under specific conditions such as alteration of relative humidity, elevation of storage temperature among other factors (Jouppila and Roos, 1994). The crystallization process is essential to producing whey powder because amorphous lactose is the most hygroscopic and unstable form found in whey. Prior lactose crystallization can reduce

the risk of stickiness and caking in the whey powder (Saffari and Langrish, 2014; Tham et al. 2017).

The presence of salts or sucrose has been shown to influence in lactose crystallization (Gernigon et al. 2013). Certain calcium salts such as nitrate increase lactose solubility. Alternately, the use of calcium hydroxide solutions has been shown to reduce lactose solubility (Harper, 1992; Holsinger, 1997). Patel and Nickerson (1970) demonstrated the complex interaction between salts and lactose during the crystallization. A study published by Gernigon et al. (2013) showed the positive influence of sodium citrate on the rate of lactose crystallization in model solutions. Patel and Nickerson's (1970) work affirmed the catalytic effect of sodium citrate on lactose mutarotation when the concentration of 1.79 $g \cdot L^{-1}$ was added in solution with 60 $g \cdot L^{-1}$ of lactose.

The objective of this study was to evaluate if the addition of sodium citrate influences lactose crystallization in concentrated whey (CW) by refractometry, microscopy, Raman spectroscopy followed by chemometric data analysis and particle size analysis by laser diffraction.

MATERIALS AND METHODS

Obtaining the sample

The whey used in the experiment was obtained from the production of curd cheese. It was concentrated in a single-stage APV® Junior plate evaporator until a lactose concentration of 42.6 $g \cdot 100\ g^{-1}$ of water was reached. The whey was packed in 1000 mL plastic bottles and frozen in a Thermo Scientific® Model 900 Series ultra-freezer at -75 °C. Fractions of the samples were thawed, filtered and concentrated in a rotary evaporator (60 °C) to bring the lactose concentration to approximately 77.4 $g \cdot 100\ g^{-1}$ of water, being cooled for approximately 10 minutes until the temperature of 22 °C was reached. The concentrated was then subjected to a crystallization process in a lab bench process simulator (Thermomix® at 22 °C and at 67 rpm agitation). The laboratory-scale process is important for experimental control, such as temperature,

stirring speed and weighing on analytical scales, which are more difficult to control on larger work scales.

Samples were taken every 30 minutes from time 0 minute (exit time from the Rotavaporator) to 240 minutes of crystallization to determine Raman spectroscopy, optical microscopy, particle analysis by laser diffraction and refractometry measurements. In this way, nine samples were collected from each replicate. In 0 minutes were observed a few crystals, being disconsidered. All analyses for the study were performed in triplicate. The three treatments were: control, addition of sodium citrate 0.05g·100g^{-1} (CIT 0.05%) and addition of sodium citrate 0.10g·100g^{-1} (CIT 0.10%). Sodium citrate was added to the treatments using a previously prepared 10g·100g^{-1} of solution. The amount of Sodium citrate solution added to the experiment obeys the good manufacturing practices stipulated so it is safe for human consumption.

Moisture

Moisture was determined by gravimetric method, where 2.0 grams of the sample was distributed on a plate containing sand and brought to the oven at 105 °C until the constant mass is achieved. The mass difference before and after evaporation allows the calculation of moisture.

Potential of hydrogen

The potential of hydrogen (pH) of the samples and of the sodium citrate solution were measured with direct reading on digital potentiometer (PG 1800 Gehaka, Brazil).

Lactose crystallization percentages

Total lactose was determined using a Megazyme® enzymatic kit for lactose and galactose. Lactose crystallization percentages were determined by refractometry (Reichert® digital refractometer model AR 200). The data obtained were used in the calculation described by Equation 1 suggested by Westergaard (1994):

Equation 1: $\% \text{ LC} = \frac{(B_1 - B_2)\ 950000}{L \cdot TS\ (95 - B_2)}$

Where: LC = lactose crystallization B_1 = initial soluble solids content (time 0 minute) (ºBrix), B_2 = final soluble, solids content (ºBrix), L = % lactose in dry matter, and TS = total solids.

Raman spectroscopy

To obtain the Raman spectra, the Bruker FT-Raman RFS 100 spectrometer was equipped with a liquid nitrogen-cooled GE detector, and laser Nd: YAG emission with excitation at 1064 nm. The samples were placed in a 10 x 10 mm quartz cuvette where a laser beam set at 200 mW power passed through. 512 scans were performed and collected with spectral resolution of 4 cm^{-1} in a region between 3200 cm^{-1} and 200 cm^{-1} (Stephani et al. 2017). The OPUS 6.0 (Bruker Optik, Ettlingen, Germany) software was used to obtain the Raman spectra. The data were submitted to the chemometric analysis using Matlab software version 7.10 (R2010a) for principal component analysis (PCA). A pre-treatment consisting of the baseline correction, a second derivative using the Savitzky-Golay (1964) smoothing algorithm with 15-point window and 2nd-order polynomial, and the data average made to decrease contributions and unwanted effects was performed.

Microphotographs

Microphotographs of the samples were obtained using an Olympus BX51 optical microscope with a bright halogen light source. Crystal size was determined using OPUS software. The microphotograph of $10g \cdot 100g^{-1}$ sodium citrate solution was performed to verify the total salt solubilization.

Laser diffraction particle size analysis

For laser diffraction particle size analysis during the process of whey concentrate dissolution, a Beckman Coulter LS 13 320 laser diffraction analyzer was coupled to the liquid analysis module (Beckman Coulter, Miami, FL, USA). Approximately five drops of the samples were added to the liquid module containing approximately 800 mL lactose solution at 13.0% ± 0.5% at 25 °C. Sample additions

were controlled by the level of observed obscurence (47% ± 5%). Data were collected in the region of 0.04 to 2000 μm with collection time established in 90 second intervals. The results were obtained using the refractive index of 1.352 for the dispersing medium (lactose solution) and 1.53 for the target particles (lactose crystals) according to Mimouni et al. (2009) and were represented by the occupied volume percentage by the particles in function of their size.

Statistical analyses

Statistical analyses were performed using the Statistical Analysis software (SAS Institute Inc. 2006). One-way variance analyses (ANOVA) for mean comparison followed by Tukey's tests were carried out throughout the study.

RESULTS AND DISCUSSION

Moisture and lactose mean contents found in the CW samples were 42.0g·100g^{-1} and 32.8g·100mL^{-1}, respectively, resulting in 77.4g of lactose in 100g of water. The pHs measured in samples were: 5.56 ± 0.2, 5.56 ± 0.1 and 5.57 ± 0.2 respectively for the control, 0.05% CIT and 0.10% CIT. The pH of concentrated whey of each treatment did not change statistically, thus not being considered as a variable during the experiment. The pHs of citrate solution were 8.15 for all treatments. The average lactose crystallization percentage curves for the three treatments calculated from equation 1 over time are presented in Figure 1.

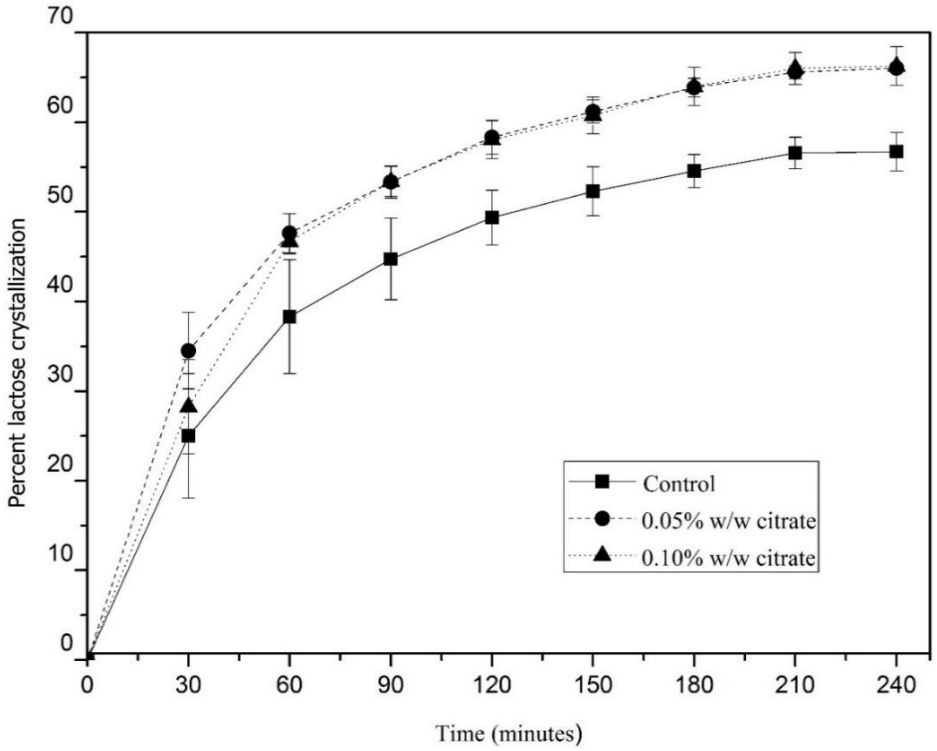

Figure 1. Lactose crystallization percentage in concentrated whey.

Figure 1 shows that the use of sodium citrate favours lactose crystallization in whey concentrate, reaching 56.7%, 66.0% and 66.3% of crystallization respectively for the control, 0.05% CIT and 0.10% CIT.

From time T180 (180 minutes of crystallization) onward, a significant difference in the crystallization percentage between treatments ($p < 0.05$) was observed; the percentage increased with the addition of sodium citrate. The results corroborate the work of Gernigon et al. (2013) where sodium citrate and other organic salts, minerals and proteins were added to a supersaturated solution to test their influence on lactose crystallization.

Polynomial regressions are presented in Equations 2, 3 and 4 for the control treatments, CIT 0.05% and CIT 0.10%, respectively.

Equation 2: $y = 9.16E^{-6}x^3 - 0.0048x^2 + 0.8516x + 1.3557$, were $R^2 = 0.9906$

Equation 3: $y = 1.32E^{-5}x^3 - 0.0065x^2 + 1.0729x + 2.8201$, were $R^2 = 0.9736$

Equation 4: $y = 1.16E^{-5}x^3 - 0.0060x^2 + 1.0362x + 1.1198$, were $R^2 = 0.9908$

Equations 2, 3 and 4 can be used to predict crystallization percentages (y) under the conditions tested in function of time (x). The first-time derivatives of these equations

provide the lactose crystallization rates represented by Equations 5, 6 and 7 for the control treatments, CIT 0.05% and CIT 0.10%, respectively

Equation 5: $y = 6.45E^{-5}x^2 + 0.0166x + 0.8193$

Equation 6: $y = 1.04E^{-4}x^2 + 0.0244x + 1.0853$

Equation 7: $y = 7.56E^{-5}x^2 + 0.0196x + 0.9566$

Crystallization rate is shown to be higher when the whey concentrate was added of sodium citrate in that way sodium citrate increase lactose crystallization under the same conditions of stirring and temperature applied in this experiment. There is no difference between the final percentage of lactose crystallization comparing the addition of 0.05% and 0.10% sodium citrate in concentrated whey. In the beginning of the lactose crystallization process the addition of 0.10% of sodium citrate leads to a higher rate of crystallization but this effect occurs only before the first hour of the process.

Figure 2 shows the microphotographs of lactose crystallization over time for the three treatments. Table 1 shows the average size of the lactose crystals over time for all three treatments.

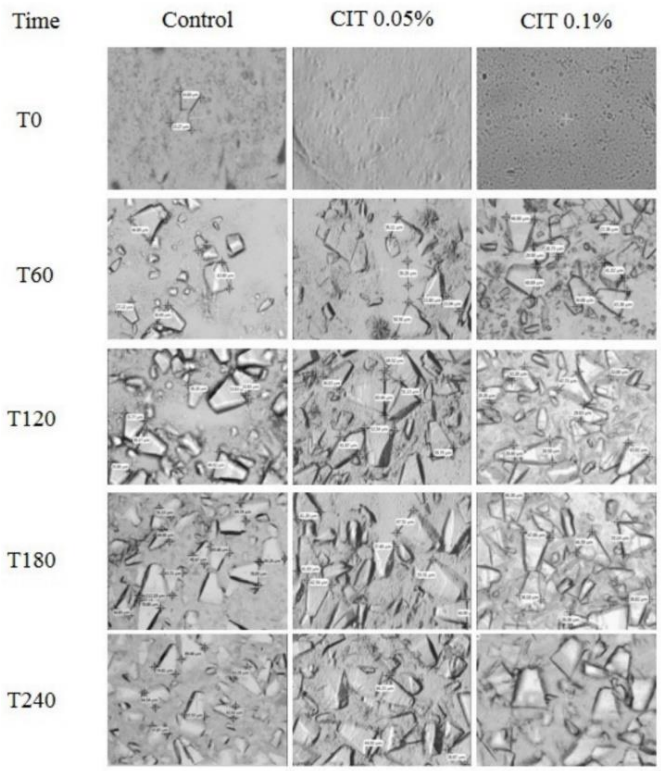

Figure 2. Optical microscopy images of lactose crystallization over time: Control = control; CIT 0.05% = addition of sodium citrate 0.05% w · w^{-1}; CIT0.10% = addition of sodium citrate 0.10% w · w^{-1}. Increase of 1000X in T0 and 200X for T60 to T240.

Table 1. Mean size of lactose crystals throughout the lactose crystallization period in whey concentrate in the different treatments (control, addition of sodium citrate 0.05% w · w^{-1} and addition of sodium citrate 0.10% w · w^{-1}). n = 3.

Time	Control (µm)	CIT0.05% (µm)	CIT 0.10% (µm)
T0	18 ± 1[a]	23 ± 3[a]	11 ± 1[b]
T30	33 ± 1[a]	43 ± 3[b]	36 ± 1[ab]
T60	39 ± 2[a]	47 ± 4[a]	44 ± 2[a]
T90	53 ± 3[a]	49 ± 3[a]	40 ± 1[b]
T120	46 ± 3[a]	50 ± 3[a]	42 ± 2[a]
T150	49 ± 3[a]	51 ± 4[a]	41 ± 1[a]
T180	52 ± 4[a]	51 ± 3[a]	44 ± 2[a]
T210	56 ± 4[a]	46 ± 3[ab]	44 ± 2[b]
T240	58 ± 5[a]	50 ± 4[ab]	41 ± 1[b]

Represented result by mean ± standard error of the mean.
Means followed by the same letter, at the same time, did not differ significantly from each other by the Tukey test (p >0.05). Control = control; CIT 5% = addition of sodium citrate 0.05% w · w^{-1}; CIT0.10% = addition of sodium citrate 0.10% w · w^{-1}.

The addition of sodium citrate induced an increase in crystal formation rate from the moment it was added (T0). The salt also promoted an average lactose crystal size reduction (perceived from T90), as shown in Figure 2 and Table 1. The smaller average size of the lactose crystals favors the whey drying process especially when it is in the range of 30 µm and 50 µm (Simeão et al. 2017). Crystals larger than 50 µm were found at the end of crystallization for the control treatment.

The work carried out in model solutions by Gernigon et al. (2013) found that larger lactose crystals formed when sodium citrate was present. The present work has demonstrated how the addition of sodium citrate salt influences lactose crystallization in whey concentrate which, unlike model solutions, has other constituents that influence crystallization, such as whey proteins, casein residues, and other salts present. The study by Mimouni et al. (2005) demonstrated the retarding effect of whey proteins on the growth of lactose crystals when added in the proportion of 5g.100g^{-1} of water. The same authors attributed to the whey proteins acting as crystallization nuclei,

thus favoring the formation of a larger number of smaller crystals since the crystallization rate remained the same.

Figure 3 shows the spectroscopic profiles of the samples throughout lactose crystallization; it is represented by one of the repetitions of the 0.10% CIT treatment (3A). In the model constructed by the PCA 97.9% of the total variance captured. Concentrated whey samples submitted to crystallization presented a grouping profile (as shown in the chart) of scores of the principal component corresponding to 89.0% (3B). Figure 3 also shows the weight graph for the principal component (3C) which demonstrates the Raman displacements responsible for the separation of the samples in the score graphs. The variables that contributed most are those that have greater weight in the separation and grouping of the studied samples.

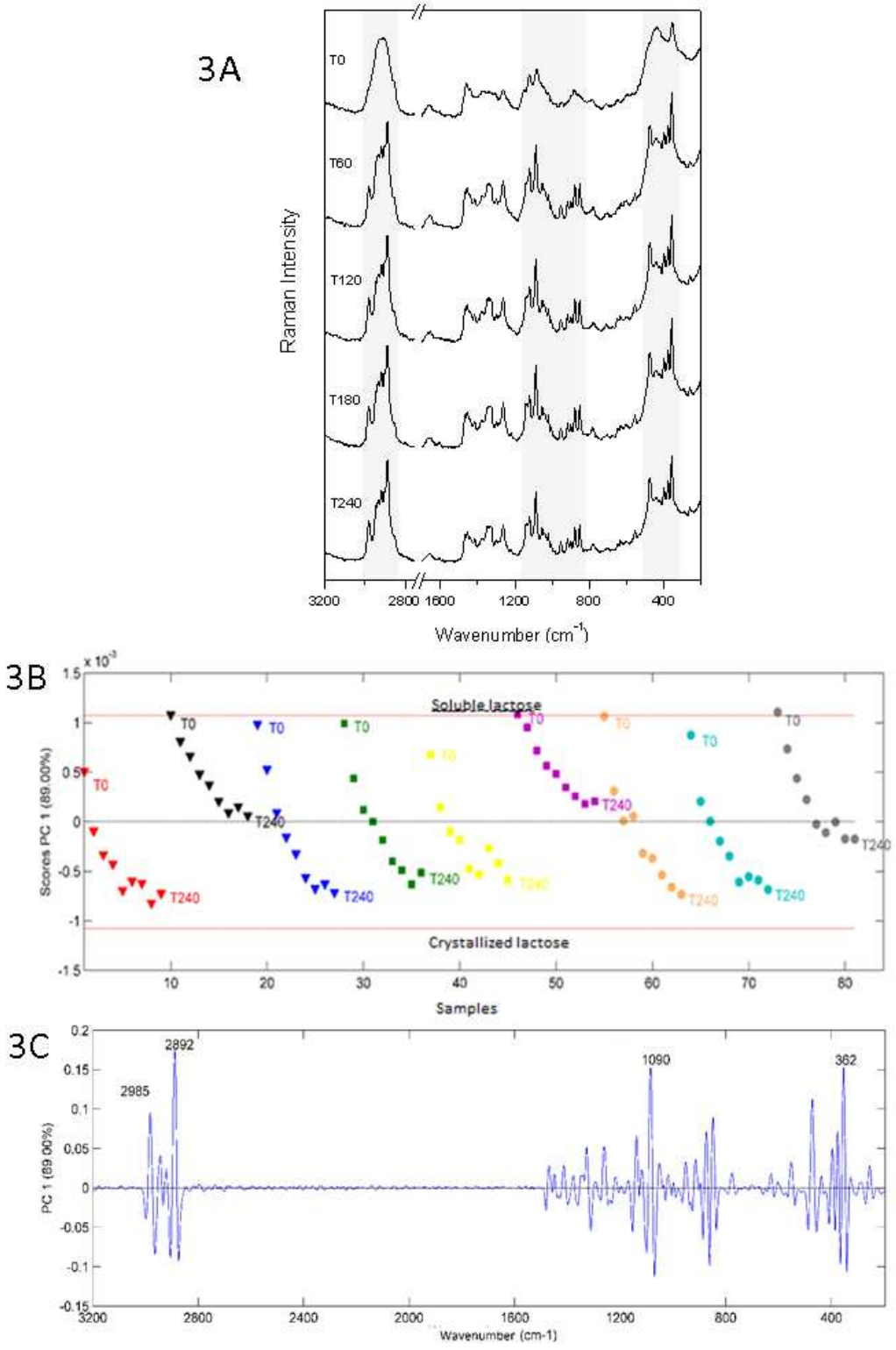

Figure 3. (3A) Spectroscopic profile throughout lactose crystallization; (3B) CP1 scoring plot of the spectral region (3200-200 cm^{-1}) of the samples (control (∇), addition of sodium citrate 0.05% m / m (□) and addition of sodium citrate 0.10 % m / m (◊)). The three replicates of each treatment are represented; (3C) Graph of the weights in CP1 versus number of spectral region waves.

In (3A), spectroscopic profile changes were observed in all treatments between T0 (after removal from the rotaevaporator) and other times. The new bands present in the spectroscopic profile were formed because of an orderly three-dimensional molecule formation during crystallization. This decreased the frequency distribution of each bond and in turn reduced the peak width of Raman spectrum vibrations. The main differences are found in the 2900 cm^{-1} region and can be attributed to the C-H bond stretch. Changes were noted in the regions of 1125 cm^{-1} and 1054 cm^{-1} involving the modes [v (C-O) + v (C-C) + δ (C-O-H)], and also in the 477 cm^{-1} region involving [δ (C-C-C) + T (C-H)] (Stephani et al. 2017). Spectral changes in the same regions were attributed to lactose in the work of Almeida *et al.* (2012). Their study characterized the bands corresponding to the main constituents of milk powder and modified milk powder by means of Raman spectroscopy analysis of different samples with different levels of lipids and carbohydrates. Results obtained by Stephani et al. (2017) demonstrated the spectroscopic profile modification of a sample of whey protein concentrate in bands similar to those found in the present work. Their results compared the profiles after six months of storage to the spectroscopic profile of α-lactose monohydrate crystals and demonstrated the vitreous transition of lactose characterized by the spectroscopic profile of crystalline lactose.

Raman spectroscopy identifies lactose crystallization by analyzing the laser scattering that occurs contact with the crystals; it can be used to construct sensors that will definitively identify the start of crystallization. Refractometry can trace the crystallization process through refractive index reduction.

The initial spectra points (T0) depart from a region attributed to soluble lactose and migrate to a region that is generally characterized by crystalline lactose over time. Based on the principal component (3B) scores obtained through analysis of 60,750 spectroscopic data using repetitions of each of the treatments, it is possible to observe a similar profile between the treatments and their replicates. An exception is found for the second replicate of the control and third replicate of treatment CIT 0.05%, showing differences between repetitions not found in refractometric analysis. This differentiation shows the high sensibility of this method to identify and monitor lactose crystallization behavior in concentrated whey. The distribution profile difference found in these scores is due to the higher average crystal sizes in the replicates of the cited treatments throughout the crystallization process. Viana et al. (2013) demonstrated the

relationship between the nanocrystal size of titanium dioxide and the number of Raman waves recorded. Gao et al. (2016) proposed a theoretical method to determine the effects on the Raman spectrum for semiconductor nanocrystals. The method postulates that the displacement the Raman spectra exerts on nanocrystals can result in overlapping effects: quantum effect displacement and surface effect displacement. Further analysis showed that size-dependent Raman variations in silicon nanocrystals resulted mainly from quantum effect changes. According to the same authors, for nanodiamonds, the surface effect ratio changes in Raman displacement reached about 40%, concluding that the model provides a strong argument for using Raman spectroscopy as a tool to measure material size.

In (3C), the weight graph for the principal component shows the Raman displacements responsible for sample separation in the score graphs. The variables that contribute most to these displacements are those that have greater weight in the separation and grouping of the studied samples. The regions that demonstrate vibrational modes of lactose (2985, 2892, 1090 and 362 cm^{-1}) show greater weight in the separation of the samples.

Similar results have been found in recent studies (Almeida et al. 2012; Rodrigues Júnior et al. 2016; Stephani et al. 2017). Thus, the exploratory analysis performed using the PCA with the obtained data by Raman spectroscopy allows profile determination in lactose crystallization in CW.

Table 2 shows the percentage volume of particles greater than 10μm.

Table 2. Particle size distribution of the whey concentrate showing the percentage of particle volume greater than 10 μm throughout the lactose crystallization time in the

different treatments (control, addition of 0.05% w / w sodium citrate and addition of sodium citrate 0.10% w · w^{-1}). n = 3.

Time	Control >10µm (% volume)	CIT0.05% >10µm (% volume)	CIT 0.10% >10µm (% volume)
T0	26.7±1.1 [a]	19.6±0.1 [b]	17.9±0.6 [b]
T30	27.4±0.4 [a]	29.5±0.1 [a]	18.5±2.0 [b]
T60	24.9±0.9 [a]	22.7±0.0 [a]	24.5±1.3 [a]
T90	29.6±1.2 [a]	23.6±0.5 [b]	22.5±1.4 [b]
T120	34.1±1.5 [a]	26.7±0.9 [b]	24.3±1.5 [b]
T150	27.7±1.5 [a]	20.0±0.7 [b]	21.0±0.7 [b]
T180	30.6±0.3 [a]	24.1±0.9 [b]	21.2±0.9 [b]
T210	27.7±3.0 [a]	25.7±0.6 [a]	22.4±0.5 [a]
T240	22.9±3.4 [a]	29.8±1.5 [a]	22.1±0.6 [a]

Result represented by mean ± standard error of the mean.

Means followed by the same letter and taken at the same time, did not differ significantly from each other when verified by Tukey test (p >0.05). Control = control; CIT 0.05% = addition of sodium citrate 0.05% w · w^{-1}; CIT0.10% = addition of sodium citrate 0.10% w · w^{-1}.

Because a laser diffraction analyzer recognizes nonspecific particles as well as those being studied, other constituents found as agglomerates or isolated in the whey concentrate contributed to the particle distribution results. However, due to similar analysis conditions and an intense change in the lactose's physical state (soluble / crystalline), volume distribution changes occur mostly as a result of lactose crystallization.

Therefore, it can be inferred that the addition of sodium citrate to whey concentrate allows a greater control over the size of the formed crystals, corroborating the microscopic analysis results. The smaller standard error of the mean observed for the treatments where sodium citrate was added also confirms this inference. Due to the lower variation in the values when 0.10% w · w^{-1} of sodium citrate was added to whey concentrate found over time, there seems to be a better balance between lactose crystal growth phases.

CONCLUSION

The crystallization stage is determinant during the whey powder production to reduce the amount of amorphous lactose in the powder and as a consequence to decrease the incidence of stickiness and caking. To achieve a high percentage of lactose crystallization industries controls lactose supersaturation after evaporation, the rate of cooling and stirring in the crystallization vat and in some cases the addition of nuclei. According to the conditions described in this experiment the sodium citrate addition (0.05% or 0.10% of addition) increases the final percentage of lactose crystallization in the concentrated whey. In the practical point of view dairy industries could add sodium citrate to the concentrated whey to improve lactose crystallization and powder stability (regarding to stickiness and caking). The reasons of the positive effect of sodium citrate in the final percentage of lactose crystallization (after 4h of crystallization process) were not investigates in this article, but this described phenomenon could support new technological approaches during the whey powder production. Raman spectroscopy followed by a chemometric analysis of data and particle analysis by laser diffraction enable lactose crystallization in whey to be monitored.

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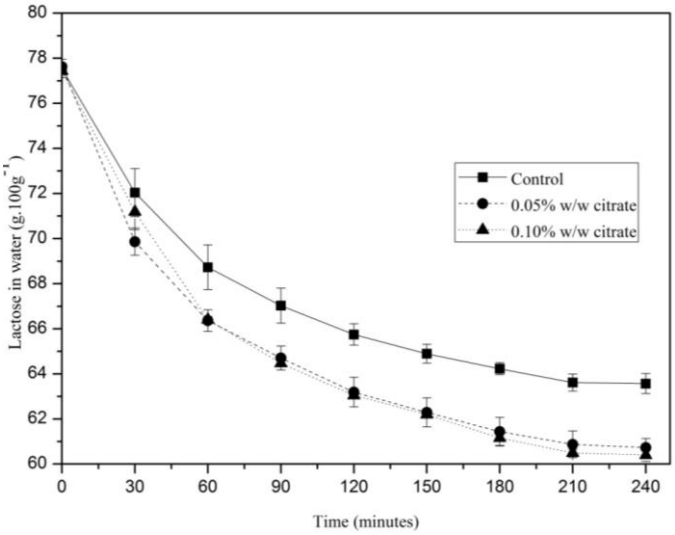
SUPPLEMENTARY MATERIAL



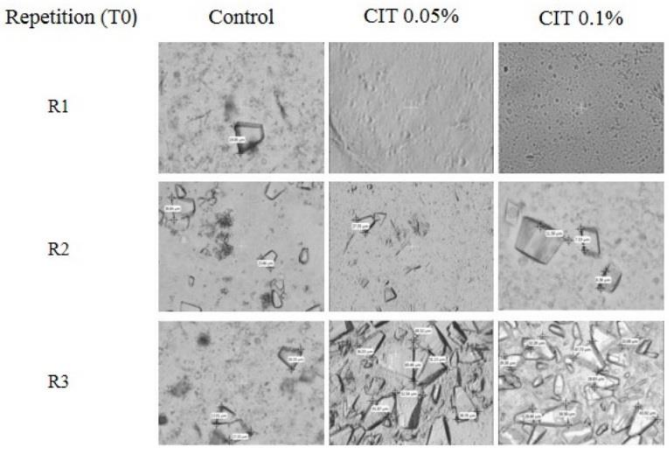
Supplementary Figure 1. Lactose solubilization percentage in concentrated whey.

Supplementary Table. Solubility of lactose in water at 20°C.

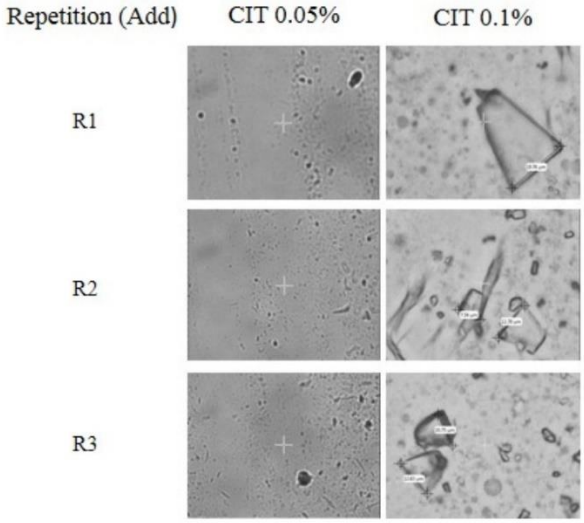
Sample	Control	CIT 0.05%	CIT 0.10%
pH	5.83 ± 0.01[a]	6.14 ± 0.01[b]	6.40 ± 0.02[c]
Brix	18.1 ± 0.2[a]	17.5 ± 0.1[b]	17.1 ± 0.2[b]
Lactose in water (g.100g^{-1})	22.1 ± 0.3[a]	21.2 ± 0.1[b]	20.6 ± 0.3[c]

Result represented by mean ± standard error of the mean.

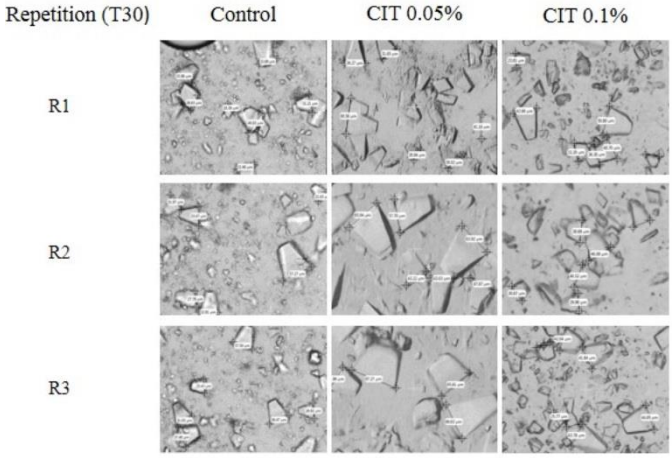
Means followed by the same letter, did not differ significantly from each other when verified by Tukey test ($p > 0.05$). Control = control; CIT 0.05% = addition of sodium citrate 0.05% w · w^{-1}; CIT0.10% = addition of sodium citrate 0.10% w · w^{-1}.



Supplementary Figure 2. Optical microscopy images of lactose crystallization in the repetition: Control = control; CIT 0.05% = addition of sodium citrate 0.05% w · w^{-1}; CIT0.10% = addition of sodium citrate 0.10% w · w^{-1}. Increase of 1000X in T0.



Supplementary Figure 3. Optical microscopy images of lactose crystallization in the repetition: CIT 0.05% = addition of sodium citrate 0.05% w · w^{-1}; CIT0.10% = addition of sodium citrate 0.10% w · w^{-1}. Increase of 1000X in added moment of sodium citrate.



Supplementary Figure 4. Optical microscopy images of lactose crystallization in the repetition: Control = control; CIT 0.05% = addition of sodium citrate 0.05% w · w^{-1}; CIT0.10% = addition of sodium citrate 0.10% w · w^{-1}. Increase of 200X in T30.

CHAPTER 4

INFLUENCE OF LACTOSE, SKIMMED MILK CONSTITUENTS AND HEATING STEP ON SUCROSE CRYSTALLIZATION IN MODEL FAT FREE OF TABLET DULCE DE LECHE

João Pablo Fortes Pereira; Natália Maria Germano Alves; Rodrigo Stephani, Luiz Fernando Cappa de Oliveira, Ítalo Tuler Perrone, Antônio Fernandes de Carvalho

ABSTRACT

Dulce de leche (DL) is a product from Latin America. It is marketed as a paste or tablet. The tablet DL is crystallized into blocks, which have a uniform coloration and texture. Sucrose and lactose content is higher in the tablet DL than it is in that used to paste DL and consequently increases crystallization. The aim of this study was to monitor the influence of lactose and heating on sucrose crystallization in model fat free of tablet DL. Booth factors strongly modify the sucrose crystallization behavior. A significant reduction in the sugars mass crystallized was found when sucrose + lactose solution (up to 120 minutes), skimmed milk powder and heating were research. Moreover, the heating step accentuated this effect. The presence of skimmed milk constituents in the mixture, mainly proteins, change the rheological characteristics of product. These changes increase the viscosity hampering the sugar crystallization, consequently, interfering of hardness of samples.

Key Words*:* sugars; solubilization; texture.

INTRODUCTION

Dulce de leche (DL) is a product from Latin America. In this region, the product is one of the most widely manufactured dairy products in South America, mainly in Brazil and Argentina, where it is marketed as a paste or tablet (Perrone et. al. 2011).

DL is defined as a product made with or without the addition of other food substances that is obtained from milk or reconstituted milk and added sucrose (either partially substituted or not by monosaccharides and/or other disaccharides) via concentration and heat action at normal or reduced pressure (Brasil, 1997).

Basically, the ingredients used to manufacture the DL are milk, sucrose, and an acidity reducer. Acidity reducers can be used to reduce or avoid precipitation of proteins, as well as enhance product color. Sodium bicarbonate is one of the most common acidity reducers used in DL production (Stephani et. al. 2019). The color of DL is brown due the Maillard's reaction, but the intensity of this color can changed according to dairy industries and consumer public. The amount of acidity reducer and the intensity of thermal treatment to which the ingredients are submitted during manufacturing alters of intensity of Maillard's reaction and, consequently, the changed of color and flavor of product (Perrone, 2007).

Paste DL, is produced as a creamy paste, with uniform texture, no crystals, a distinctive brownish color, and a characteristic taste. It is generally made to be eaten as a dessert and used in pie fillings, roll cakes, and tablets, among other sweets. The tablet DL is crystallized into blocks, which have a uniform coloration and texture. For this type of Dl, production involves a slow process that induces lactose and sucrose crystallization in the product. The tablets are typically eaten in pieces (Stephani et. al. 2019).

Sucrose and lactose content is higher in the tablet DL than it is in that used to paste DL and consequently increases crystallization. The increased crystallization is necessary for the product to become solid and firm enough to be cut and served in pieces. To this end, the original solution is subjected to a controlled-crystallization process or batching. In controlled crystallization or churning, the product undergoes intense stirring as it is slowly boiled. This process induces the formation of numerous sucrose and lactose crystals, which ultimately alter the texture of the product.

Sugar crystallization is important to caracteristics of the tablet DL, therefore, the knowledge of sugar crystallization kinetics is important and justificated.

The presence of salts or sucrose has been shown to influence in lactose crystallization (Gernigon et al. 2013). According to Walstra et al. (2001), average lactose solubility at 20 °C is $20g \cdot 100g^{-1}$ of water, while glucose solubility is $107g \cdot 100g^{-1}$ of water and galactose is $50g \cdot 100g^{-1}$ of water. The average sucrose solubility at 20 °C is $200g \cdot 100g^{-1}$ of water (Mathlouthi and Reiser, 1995), therefore is about 10 times more soluble than lactose.

Lactose is most frequently found as hydrated α-lactose monohydrate crystal (McSweeney and Fox, 2009), while sucrose generally crystallizes in anhydrous form (Kaletunc and Breslauer, 2003).

The aim of this study was to monitor the influence of lactose and heating on sucrose crystallization during the crystallization step of the model fat free of tablet DL.

MATERIALS AND METHODS

Centesimal content analysis (Moisture, Lactose, Protein Fat and Minerals)

Moisture, lactose, protein, fat and mineral content were determined in skimmed milk powder to do the mass balance of the treatments. Moisture was determined by gravimetric method, where 2.0 grams of the sample was distributed on a plate containing sand and brought to the oven at 105 °C until the constant mass is achieved. The mass difference before and after evaporation allows the calculation of moisture. Total lactose was determined using a Megazyme® enzymatic kit for lactose and galactose. The total nitrogen in the samples was determined by Kjeldahl method, and the nitrogen content was multiplied by the conversion factor 6.38 to obtain the equivalent amounts of protein (IDF, 1993). The fat content was determined by Gerber method (based in acid digestion coupled to centrifugal separation in specific laboratory glassware) (IDF, 2008). The content of the fixed mineral residue (FMR) was determined gravimetrically by incinerating the sample in a muffle oven at 550 °C for 10 hours (AOAC, 1997).

Obtaining the samples

Having the results of centesimal composition of the skimmed milk powder, the samples used in the experiment were formulated to have the same concentration of sucrose and/or lactose (% lactose or sucrose in water) in all treatments (n = 4). Thus, the amounts addition of ingredients were different to each treatment, because was respected the ratio of each soluble sugar in water. The Table 1 showed the formulation, the final composition and solubility of each sugar (lactose and sucrose) of four treatments used in the study.

Table 1. Demonstration of the experimental treatments (formulation, the final composition and solubility).

Added Amount - Formulation- (g·100 g^{-1})				
Ingredient	Sucrose solution	Sucrose + Lactose solution	Tablet DL without heating	Tablet DL with heating
Lactose	---	14.97	---	---
Skimmed Milk Powder	---	---	25.07	25.07
Sucrose	80.00	68.02	59.94	59.94
Water	20.00	17.01	14.99	14.99
Total	100.00	100.00	100.00	100.00
Final Composition (g·100 g^{-}r)				
Attribute	Sucrose solution	Sucrose + Lactose solution	Tablet DL without heating	Tablet DL with heating
Fat	---	---	0.22	0.22
Lactose	---	14.97	13.19	13.19
Minerals (FRM)	---	---	1.78	1.78
Moisture	20.00	17.01	16.01	16.01
Protein	---	---	8.86	8.86
Sucrose	80.00	68.02	59.94	59.94

Total	100.00	100.00	100.00	100.00
Sugar solubility (g·100 g^{-1}of water)				
Sugar	Sucrose solution	Sucrose + Lactose solution	Tablet DL without heating	Tablet DL with heating
Lactose	---	88.02	88.03	88.03
Sucrose	399,95	399,95	400,00	400,00

All treatments were manufactured in a process simulator with warming (105°C) and stirring speed (67 rpm) to solubilization of constituents (the mass evaporated water was added to reposition). The DL need heating for developed color and flavor, therefore in this treatment 570 g of water was used to heat for about 100 minutes until have the composition showed in table 1. The process control was done by mass balance.

After solubilization step concluded, started the crystallization step. The solutions were transferred to another pan being stirred at 90 rpm for 30 minutes cooling at 80°C to 25°C. Later, the DL was packed and kept to crystallize. Sugars' crystallization were determined by refractometry. Eleven times were measured during the crystallization step.

Hardness analysis

Texture analysis was performed using a texturometer (Amatek Brookfield CT3). The samples were placed on Becker of 70.0 mm diameter. Sample were placed until reaching the heigth of 60.0mm. A 25.4 mm diameter (TA 11/1000) cylindrical probe was used, operating at a speed of 2 mm·s^{-1} to a depth of 15 mm and a contact force of 10.0 g.

Data were collected with Texture Pro CT V1.4 Build software. Texture Profile Analysis (TPA) was performed, in which the parameters hardness (maximum compression force) was evaluated.

RESULTS AND DISCUSSION

The skimmed milk powder used in the formulations had as composition: 0,8 g·100 g-1 of fat, 52,6 g·100 g-1 of lactose, 4,1 g·100 g-1 of moisture, 35,5 g·100 g-1 of protein, and 7,0 g·100 g-1 of RMF.

The Figure 1 show the behavior of sugars solubility during the crystallization step in different mixtures. The samples used in the experiment were formulated to have the same concentration of sucrose and/or lactose (% lactose or sucrose in water) in all treatments.

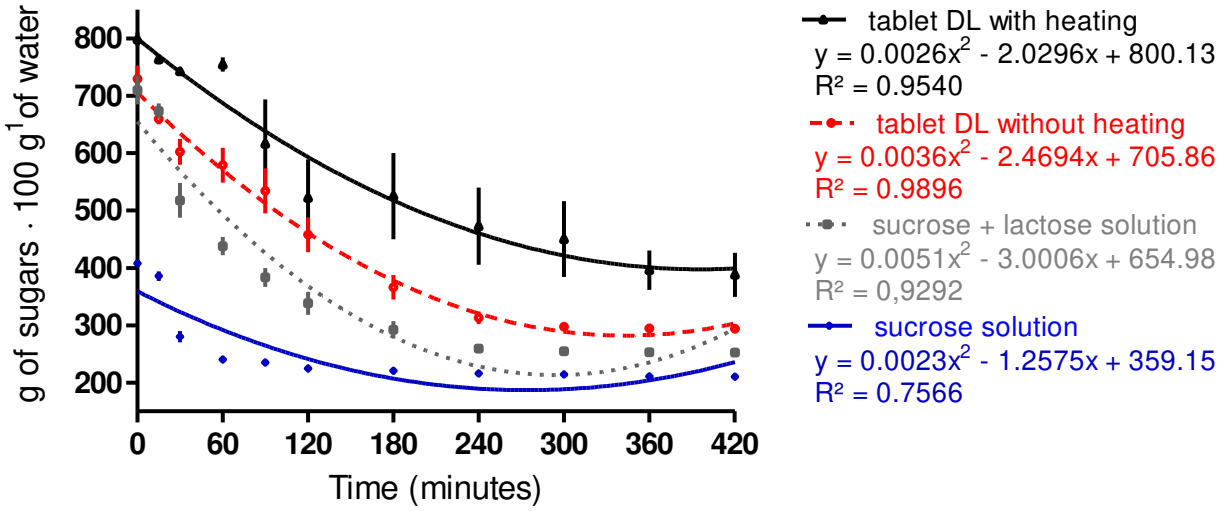


Figure 1. solubility curve of sugars during the crystallization step.

Crystallization was a natural process due to the high supersaturation of sucrose and/or lactose under the experimental conditions (400g · $100g^{-1}$ H_2O to sucrose, and 88g · $100g^{-1}$ H_2O to lactose), leaving the solution thermodynamically unstable.

According to Hartel (2001), the crystallization can be divided in four steps: the first is generation of a supersaturated phase, followed of nucleation, crystal growth and recrystallization. Once the solution has been become supersaturated, there is thermodynamic driving force for crystallization, leaving less unstable the solution.

The curves that represent the crystallization models of all treatments have polynomials behavior. The mean error decrease when sugar is dissolved only in water (model solutions). When milk powder is solubilized with sugar, experimental reproducibility decreases, increasing the error bar. This is explained by the increase of

chemical species in the mixture, increasing the possibility of molecular interactions, changing the chemical balance of the medium in different ways.

The first-time derivatives of the equations provide the sugars crystals formation rates represented by Equations 1, 2, 3 and 4 for the all treatments: tablet DL with heating, tablet DL without heating, sugars solution and sucrose solution, respectively.

Equation 1: $y = 0{,}0052x - 2{,}0296$

Equation 2: $y = 0{,}0072x - 2{,}4694$

Equation 3: $y = 0{,}0102x - 3{,}0006$

Equation 4: $y = 0{,}0046x - 1{,}2575$

This way it is possible to calculate the mass of sugars leaving of the solution per minute. For example, considering the time of 200 minutes, the rates mentioned are 0,9896 g, 1,0294 g, 0,9606 g and 0,3375 g, corresponding to the treatments tablet DL with heating, tablet DL without heating, sugars solution and sucrose solution.

Table 2 shows the interference of lactose, milk compounds and heating on sucrose crystallization. Mean value found for 3 repetitions of each treatment was considered.

Table 2. Sugars mass ($g \cdot 100g^{-1}$) crystalized during the crystallization in tablet DL.

Time (min)	Sucrose crystallization ($g \cdot 100g^{-1}$)	Sucrose + Lactose crystallization ($g \cdot 100g^{-1}$)	tablet DL without heating crystallization ($g \cdot 100g^{-1}$)	tablet DL with heating crystallization ($g \cdot 100g^{-1}$)
0	0.00	0.00	0.00	0.00
15	1.16	0.65 (-44%)*	0.93 (-19%)*	0.49 (-58%)*
30	8.30	4.45 (-46%)*	2.19 (-74%)*	0.83 (-90%)*
60	11.83	7.11 (-40%)*	2.76 (-77%)*	0.94 (-92%)*
90	12.66	9.55 (-25%)*	4.05 (-68%)*	3.56 (-72%)*
120	13.86	12.02 (-13%)*	6.48 (-53%)*	6.00 (-57%)*
180	14.36	15.02 (5%)*	10.48 (-27%)*	6.00 (-42%)*
240	14.90	17.61 (18%)*	13.56 (-9%)*	7.65 (-51%)*
300	15.10	18.07 (20%)*	14.62 (-3%)*	8.48 (-44%)*
360	15.52	18.18 (17%)*	14.81 (-5%)*	10.39 (-67%)*
420	15.64	18.26 (17%)*	14.85 (-5%)*	10.80 (-31%)*

* Crystal mass reduction comparing to the crystal mass obtained from the sucrose solution.

In the solution containing sucrose and lactose, a decrease (13%) of crystallization was observed up to 120 minutes, with a subsequent increase until the end of experiment (17%). At the beginning of the crystallization step, the crystal mass difference formed between the solution is increase. This is due to the lower mobility of molecules in lactose and sucrose solution (higher supersaturation). With crystallization, the mobility of molecules increases in this solution.

In the treatments in which the milk powder was added to the mixture (without and with heating) noticed a decrease in sugar crystallization during the whole experiment, 5% and 31%, respectively.

Lactose is less soluble than sucrose and is further reduced in solubility in the presence of high concentrations of sucrose (Peter, 1928; Nickerson and Moore, 1972). According to Laos et. al. (2007), studying the influence of fructose, glucose and corn syrup on the crystallization of sucrose by applying the polarized light microscopy and measuring the water activity concluded that the presence of fructose, glucose and corn syrup inhibited the sucrose crystallization in supersaturated solution.

Thorat et. al. (2018), when studied the effect of addition of mono-, di-, and tri-saccharide on sucrose crystallization in solution and mixtures previously lyophilized, showed that all sugars added delayed the sucrose crystallization. The same authors observed too that disaccharides and trisaccharides were found to be more effective than monosaccharides for delaying sucrose crystallization.

Hartel (2001) explain that monosaccharides, glucose and fructose and glucose polymers adsorb to the sugar crystal surface and inhibit incorporation of the sucrose molecules, inhibiting of crystallization rates of sucrose. Levenson and Hartel (2005), when researched the nucleation of amorphous sucrose-corn syrup mixtures, suggested that added corn syrup increased the viscosity and reduced the molecular mobility of the sugar matrix, which required additional energy for crystallization to occur. Corn syrup may have also interacted with the sucrose molecules by hydrogen bonding, affecting the diffusion. Thus, more energy would thus be required for the sucrose molecules to diffuse through the bulk solution for nucleation to occur. The same reasoning can be explained for the addition of lactose in the study.

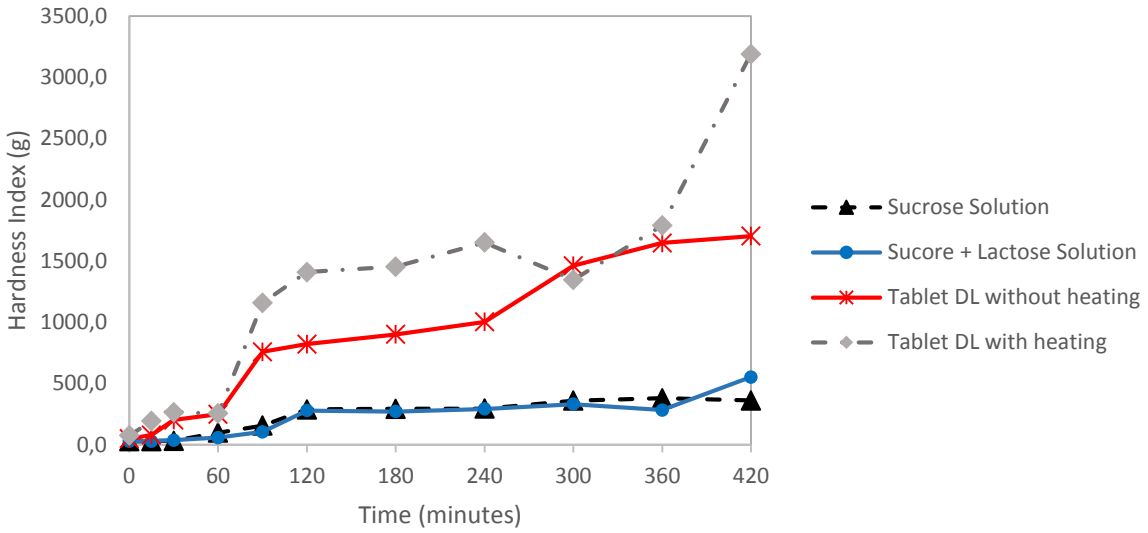


Figure 2. Hardness idex (g) of the samples during crystallization step.

Based on the hardness analysis data showed in Fig. 2, it is possible to group the treatments into two different hardness profiles. One grouping of solutions and the other grouping treatments that add skimmed milk powder to the mixture.

The presence of milk constituents in the mixture, especially proteins and salts, change the characteristics and interaction intra and inter molecules. These changes increase the viscosity difficulting the sugar crystallization, consequently, interfering of hardness of samples. Proteins are a classic example of increased viscosity when heated as they are denatured and more exposed to water binding. Many authors described milk proteins denaturation and influence the viscosity (Damodaran and Parkin, 2017; Walstra and Jenness, 1984; Walstra et.al. 2001)

The study was conducted with rehydrated skimmed milk powder, so even the unheated treatment has a large amount of denatured protein. Others compounds of mixture contribute whit hardness index.

Nickerson and Patel (1972) evaluate the influence of sucrose addition on lactose crystallization and the influence of lactose addition on sucrose crystallization. They showed that the type and number of crystals influenced by variation in sowing plays a role in determining the hardness of the crystallizing mass. This is smaller, however, compared to the influence of composition.

CONCLUSION

The presence of lactose, skimmed milk constituents and heating of milk influence the sucrose crystallization in the tablet DL.

The presence of skimmed milk constituents in the mixture, mainly proteins, change the rheological characteristics of product. These changes increase the viscosity hampering the sugar crystallization, consequently, interfering of hardness of samples.

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GENERAL CONCLUSION AND PERSPECTIVES

The work addressed the crystallization of lactose and / or sucrose in different concentrated dairy products, seeking under different chemical environments to understand this behavioral / state change. For the dairy industry, domain and control over these event are important. Lactose is the main dairy solid, which is involved in several physicochemical and microbiological reactions, and together with sucrose, when present in the formulation, is fundamental for the stability, conservation and sensory characteristics of the products. For example, crystallization of lactose in concentrated whey is a critical step to the quality of whey powder. It is a long step and costly for industries by high consume of energy. Because of this it is important accelerate this step. In the present work it was concluded that the use of sodium citrate, in concentrations already commonly used in other dairy products, was sufficient to increase the stage performance by approximately 17%.

Throughout the work it also sought to evaluate the viability of using different analytical tools, up to now little known and used by industries, but which may prove to be very useful due to the ease of execution of the analytical march, minimization of waste generation, increased of methodological sensitivity, and decreased time necessary to obtain and interpret data.

In the study with commercial samples of sweetened condensed milk, it was sought to evaluate the influence of the sample dispersing material on the liquid module and also the minimum recirculation time required to obtain the sweetened condensed milk particle distribution by laser diffraction. The purpose of the study was to generate a protocol to be adopted that may replace microscopic analyses to obtain the size of lactose crystals, which are currently adopted, but which require a long time to determine. Another important analytical tool also used in the thesis was Raman spectroscopy, which, if properly used, increases the sensitivity of identifying the beginning of formation of lactose crystals in concentrated whey, allowing greater control of this step of production of whey powder.

Scientific studies that seek or develop academic knowledge and, at the same time, industrial applicability, are fundamental for technical scientific development, being very relevant for food industries. As a perspective for future research, it is thought that new analytical tools should be studied and implemented to improvement of the quality control of products and process, which is vital for maintaining brands and products in the market.

The studies presented subsidize of information to the scientific community and at the same time the industries, stimulating complementary studies. The alteration of the dispersant material in the liquid module of particle analyzer opens the possibility of studies with other food matrices until then, having the maintenance of the size and quantity of particles (original condition of the product) impaired by the use of water as dispersing material.

The detailed performance of sodium citrate in lactose crystallization in concentrated whey00000000000, without response in the present thesis, stimulates the execution of complementary works on the subject. Another perspective for future work on this topic would be to change other conditions in the lactose crystallization step in concentrated whey in order to improve the performance of the whey powder producing industries.

The use of tablet DL as a research object enables the dissemination of this product to the international scientific community. Few works of tablet DL, mainly written in English, are found in the literature. Therefore, different studies and characterizations of this product should be performed to consolidate scientific information about this food matrix. To continue of the study, formulations using micellar casein and whey protein isolate, using ultrafiltration permeate and also, using standard milk with fat would interesting to research the protein and salts effects on sucrose crystallization in tablet DL. The tablet DL is a product widely made in Brazil, but unknown outside the country. Further studies on this dairy product are extremely necessary for the dissemination of the product.

The search to generate knowledge and the distribution of this knowledge to improve the quality of products and processes is becoming increasingly necessary. It is a continuous demand and must be fed to the evolution of society.