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Ecole Doctorale des Sciences de la Matière, du Rayonnement et de l'Environnement - SMRE
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THESIS

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Tatiana LOPES FIALHO

LACTOSE HYDROLYZED MILK POWDER: OPTIMIZATION OF THE DRYING PROCESS AND STUDY OF STRUCTURAL AND FUNCTIONAL PROPERTIES

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THESIS OUTPUT

Review

Tatiana Lopes Fialho, Pierre Schuck, Evandro Martins, Arlan Caldas Pereira Silveira, Carolina Rodrigues de Jesus Silva, Rodrigo Stephani, Ítalo Tuler Perrone, Antônio Fernandes de Carvalho. (2018). "Leite em pó deslactosado: caracterização termodinâmica do processo e avaliação físico-química e tecno-funcional do produto." *Revista Indústria de laticínios*, 132, 129-131.

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Book Chapter

Ítalo Tuler Perrone, Evandro Martins, Tatiana Lopes Fialho, Pierre Schuck, Antônio Fernandes de Carvalho. 'Lactose hydrolyzed Milk Powder.' This book chapter will be published in *Advances in Spray Drying of Dairy Products*.

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ABSTRACT

FIALHO, Tatiana Lopes, D.Sc., Thesis supported in cotutelle between the Université des Sciences et Technologies de Lille and the Universidade Federal de Viçosa, May, 2019. **Lactose hydrolyzed milk powder: Optimization of the drying process and study of structural and functional properties**. Advisor: Antônio Fernandes de Carvalho. Co-advisors: Italo Tuler Perrone, Pierre Schuck and Evandro Martins.

The production technology of lactose hydrolyzed milk powder has been developed to meet the needs of lactose intolerant consumers. Although the product is currently marketed in some countries, the industry faces technological issues during the production and storage of the powder such as agglomeration, caking, browning, high hygroscopicity, low production yield and loss of techno-functional properties. In this context, two main objectives were assigned to the work of this thesis: (i) to optimize the drying process of lactose hydrolyzed milk powder; (ii) to understand the impact of lactose hydrolysis on the internal structure of lactose hydrolyzed milk powder on a molecular scale. In order to optimize the drying process of lactose hydrolyzed milk powder, powder samples were subjected to various drying conditions: concentrated milk flow rates varying from 0.3 to 1.5 $kg{\cdot}h^{-1}$ and inlet air temperature ranging from 115 to 160 °C. Then, a thermodynamic characterization of the drying process was carried out using the equations of mass and energy balance. To understand the impact of lactose hydrolysis on the internal structure of the powder after drying and during storage, the organization and dynamics of the molecules in lactose hydrolyzed milk powder were analyzed by examining appearance and structure of the powder samples and their techno-functional properties. Throughout the experiments, traditional milk powder was used as a control. In this study, it has been observed that the ideal parameters for lactose hydrolyzed milk powder production were: inlet air temperature at 145 ° C and 1.0 $kg \cdot h^{-1}$ flow rate. This finding reinforces the idea that the drying conditions of lactose hydrolyzed milk powder are different from those used to make traditional milk powder. It was also observed that molecules present in milk powder hydrolyzed with lactose presented a more homogeneous molecular organization compared to traditional milk powder and allowed for greater protein-sugar interaction. Under accelerated aging conditions of the hydrolyzed powder, the protein glycation was

the initial process that triggers the main modifications observed in lactose hydrolyzed milk powder during storage.

RESUMO

FIALHO, Tatiana Lopes, D.Sc., Tese em cotutela entre l'université des Sciences et Technologies de Lille e Universidade Federal de Viçosa, maio de 2019. **Leite em pó com lactose hidrolisada: Otimização do processo de secagem e estudo das propriedades estruturais e funcionais**. Orientador: Antônio Fernandes de Carvalho. Coorientadores: Ítalo Tuler Perrone, Pierre Schuck e Evandro Martins.

A tecnologia de produção de leite em pó hidrolisado com lactose foi desenvolvida para atender às necessidades dos consumidores intolerantes à lactose. Embora o produto seja atualmente comercializado em alguns países, a indústria enfrenta problemas tecnológicos durante a produção e armazenamento do pó, como aglomeração, aglomeração, escurecimento, alta higroscopicidade, baixo rendimento de produção e perda de propriedades tecnofuncionais. Neste contexto, dois objetivos principais foram atribuídos ao trabalho desta tese: (i) otimizar o processo de secagem do leite em pó hidrolisado com lactose; (ii) compreender o impacto da hidrólise da lactose na estrutura interna do leite em pó hidrolisado à base de lactose em escala molecular. Para otimizar o processo de secagem do leite em pó hidrolisado com lactose, as amostras de pó foram submetidas a diferentes condições de secagem: fluxo de leite concentrado variando de 0,3 a 1,5 kg h^{-1} e temperatura do ar de entrada variando de 115 a 160 ° C. Em seguida, uma caracterização termodinâmica do processo de secagem foi realizada utilizando as equações de massa e balanço de energia. Para compreender o impacto da hidrólise da lactose sobre a estrutura interna do pó após a secagem e durante o armazenamento, analisou-se a organização e dinâmica das moléculas em leite em pó hidrolisado pela análise do aspecto e estrutura das amostras de pó e suas propriedades tecno-funcionais. Ao longo dos experimentos, o leite em pó tradicional foi usado como controle. Neste estudo, observou-se que os parâmetros ideais para a produção de leite em pó hidrolisado com lactose foram: temperatura do ar de entrada a 145 ° C e vazão de 1,0 kg·h^{-1}. Este achado reforça a ideia de que as condições de secagem do leite em pó hidrolisado à base de lactose são diferentes daquelas usadas para fazer o leite em pó tradicional. Observou-se também que as moléculas presentes no leite em pó hidrolisado com lactose apresentaram uma organização molecular mais homogênea em relação ao leite em pó tradicional e permitiram maior interação proteína-açúcar. Em condições de envelhecimento acelerado do pó hidrolisado, a glicação da proteína foi o

processo inicial que desencadeou as principais modificações observadas no leite em pó hidrolisado de lactose durante o armazenamento.

RÉSUMÉ

FIALHO, Tatiana Lopes, D.Sc., Thèse soutenue en cotutelle entre l'université des Sciences et Technologies de Lille et Universidade Federal de Viçosa, Mai, 2019. **Lait hydrolysé au lactose: Optimisation du processus de séchage et étude des propriétés structurelles et fonctionnelles.** Directeur: Antônio Fernandes de Carvalho. Co-directeur: Italo Tuler Perrone, Pierre Schuck and Evandro Martins.

La technologie de production de la poudre de lait hydrolysée au lactose a été développée pour répondre aux besoins des consommateurs intolérants au lactose. Bien que le produit soit actuellement commercialisé dans certains pays, le secteur est confronté à des problèmes technologiques lors de la production et du stockage de la poudre, tels que l'agglomération, la prise en masse, le brunissement, une hygroscopicité élevée, un faible rendement de production et une perte de propriétés techno-fonctionnelles. Dans ce contexte, deux objectifs principaux ont été assignés aux travaux de cette thèse: (i) optimiser le processus de séchage de la poudre de lait hydrolysée au lactose; (ii) comprendre l'impact de l'hydrolyse du lactose sur la structure interne de la poudre de lait hydrolysée au lactose à l'échelle moléculaire. Afin d'optimiser le processus de séchage de la poudre de lait hydrolysée au lactose, des échantillons de poudre ont été soumis à différentes conditions de séchage: débits de lait concentré variant de 0,3 à 1,5 $kg{\cdot}h^{-1}$ et température d'entrée de l'air comprise entre 115 et 160°C. Ensuite, une caractérisation thermodynamique du processus de séchage a été réalisée en utilisant les équations de bilan massique et énergétique. Pour comprendre l'impact de l'hydrolyse du lactose sur la structure interne de la poudre après séchage et pendant le stockage, nous avons analysé l'organisation et la dynamique des molécules dans la poudre de lait hydrolysée au lactose en examinant l'aspect et la structure des échantillons de poudre et leurs propriétés techno-fonctionnelles. Tout au long des expériences, le lait en poudre traditionnel a été utilisé comme témoin. Dans cette étude, il a été observé que les paramètres idéaux pour la production de la poudre de lait hydrolysée au lactose étaient les suivants: température de l'air entrant à 145°C et débit de 1,0 $kg{\cdot}h^{-1}$. Cette découverte renforce l'idée selon laquelle les conditions de séchage de la poudre de lait hydrolysée au lactose sont différentes de celles utilisées pour la fabrication de la poudre de lait traditionnel. Il a

également été observé que les molécules présentes dans la poudre de lait hydrolysée au lactose présentaient une organisation moléculaire plus homogène par rapport la poudre de lait traditionnel et permettaient une plus grande interaction protéine-sucre. Dans des conditions de vieillissement accéléré de la poudre hydrolysée, la glycation des protéines était le processus initial qui a déclenché les principales modifications observées dans la poudre de lait hydrolysée au lactose pendant le stockage.

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CHAPTER I
INTRODUCTION

1. Context of the project

Drying milk is the most commonly used method in the dehydrated dairy manufacturing sector because it extends storage and conservation time while reducing logistics costs and nutritional losses (Schuck et al., 2005).

Lactose is the most abundant component in milk and skimmed milk powders, 38% and 51%, respectively (Marconi & Panfili, 1998; Schuck et al., 2005). The disaccharide is made up of glucose and galactose, two monosaccharides covalently linked by a β-glycosidic bond (1-4) (Walstra, Wousters, & Geurts, 2006). Lactose intolerance is one dietary issue related to the consumption of foods with high lactose contents.

Recent research has demonstrated that 75% of the world population show some degree of lactose intolerance (Silva, Oliveira, & Perin, 2019), with a higher concentration of lactose intolerant individuals in South America, Africa and Asia (Lule et al., 2016).

In order to meet demand from the lactose-intolerant populations, a wide variety of lactose-free dairy products are now available on the market, such as pasteurized and UHT milk, yogurt, cheeses, ice-cream, *dulce de leche* and others dairy products (Abbasi & Saeedabadian, 2013; Antunes et al., 2014; Mota, et al., 2009; Ruiz-Matute et al., 2012; Vénica et al., 2013).

The production technology for milk powder with low lactose content has been developed quite recent and most of the current industrial processes are based on the enzymatic hydrolysis of this sugar. Although lactose hydrolyzed milk powder is already marketed in some countries, the industry faces several technological drawbacks during production and storage, such as agglomeration, stickiness, caking, browning and elevated hygroscopicity. All of these result in low production yield and loss of techno-functional properties (Fialho et al., 2018; Fialho et al., 2018; Torres et al., 2017).

Currently, few reports have been dedicated to explaining the issues related to he production of lactose hydrolyzed milk powder; nevertheless, existing works designate the glass transition as a key to the problem (Fernández, Schebor & Chirife, 2003; Shrestha et al., 2007; Torres et al., 2017). The glass transition (Tg) consists of a change of the system from a glassy state (high viscosity fluid) to a rubbery state (low viscosity fluid) when the dairy powder particles are heated to the glass transition temperature (T_{Tg}). The rubbery state leads to structural changes in the powder and results in a

product with undesirable physicochemical characteristics (Roos, 2002; Schuck et al., 2005).

The glass transition temperature (T_{Tg}) of dairy powders is linked to the concentration, specific heat, and glass transition temperature of each component. Since water has a low glass transition temperature (T_{Tg}= -135°C), this constituent is the principal component responsible for decreasing the glass transition temperature of dairy powder (Roos & Drusch, 2015).

In the case of lactose hydrolyzed milk powder, lactose (T_{Tg}=101°C) is hydrolyzed into glucose (T_{Tg}= 31°C) and galactose (T_{Tg}= 30°C) (Roos, 1993; Roos & Karel, 1990). As a consequence of carbohydrate hydrolysis, the glass transition temperature of lactose hydrolyzed milk powder (36°C when in equilibrium with air containing 11% relative humidity) is significantly lower than that of traditional milk powder (61°C under the same equilibrated conditions) (Fernández, Schebor, & Chirife, 2003). Because of this drop in T_{Tg}, lactose hydrolyzed milk powder is more prone to a glass transition during drying, transport and storage.

2. Objectives

In this context, two main objectives were assigned to this study: (i) to optimize the drying process of lactose hydrolyzed milk powder; (ii) to understand the impact of lactose hydrolysis on a product's internal structure at a molecular scale.

3. Manuscript organization

This document is divided into the following chapters:

- Chapter II: Review of literature. This section presents a compilation of studies that show the importance of lactose hydrolyzed milk powder production around the world, the principles that govern this production, the technological problems associated with it, and the challenges the market faces to produce lactose hydrolyzed milk powder. This section will be published as a chapter entitled "Lactose hydrolyzed milk powder" in *Advances in Spray Drying of Dairy Products*.
- Chapter III: Optimization of the lactose hydrolyzed milk powder drying process. This chapter seeks to understand drying process characteristics and powder

quality with various drying parameters. The first section focuses on a thermodynamic study of the process and explores different inlet air temperatures and concentrated milk flow rates. The second section evaluates the product's physicochemical and techno-functional characteristics when subjected to inlet air and concentrated milk flow rate variations. These two sections haves been published in *Drying Technology* (Fialho et al., 2018).

- Chapter IV: Study of the structural and functional properties of lactose hydrolyzed milk powder. This section explores the impact of sugars (lactose, galactose and glucose) on the molecular structure of lactose hydrolyzed milk powder. The first part focuses on sugar-sugar and sugar-protein interactions and how they affect the powder's molecular organization. This first part was submitted to *Food Structure*. The second part describes the molecular mechanism of agglomeration in lactose hydrolyzed milk powder.' This second part will be submitted to *Food Structure*.

- Chapter V: Conclusion and perspectives. This section indicates future areas of study to continue to build on the research presented.

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CHAPTER II
REVIEW OF LITERATURE

Lactose hydrolyzed milk powder

The content of this second chapter will be published in book:

Advances in Spray Drying of Dairy Products

Evandro Martins, Tatiana Lopes Fialho, Pierre Schuck, Antônio Fernandes de Carvalho, Ítalo Tuler Perrone

1. Introduction

Recent surveys estimate that 75% of world population show some degree of lactose intolerance (Silva et al., 2019). Although this food sensitivity is widespread around the world, there are higher numbers of lactose-intolerant individuals in South America, Africa and Asia. Lactose intolerance can limit the consommation of dairy products in these regions (Lule et al., 2016)(Figure 1).

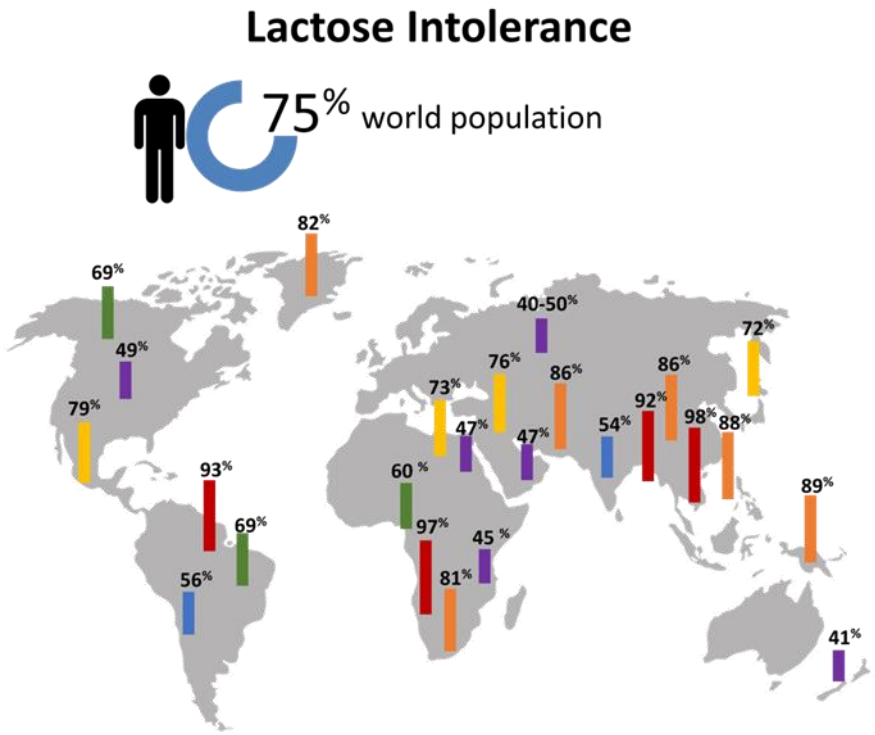

Figure 1. Distribution of world population with some degree of lactose intolerance. Adapted from Valio (2018): https://www.valio.com/consumers/lactose-free/

Lactose intolerance in humans is characterized by the absence or the low production of the β-D-galactosidase enzyme, commonly known as lactase, which hydrolyzes lactose to release galactose and glucose monosaccharides for bloodstream absorption (Gerbault et al., 2011).

Inefficient enzymatic hydrolysis of lactose allows the to sugar arrive intact in the intestinal lumen. This increases local osmolarity and also serves as a substrate for gut microbiota that can then produce acids and gases as CH_4, CO_2 and H_2 (Lule et al., 2016). The most common clinical consequences of low lactose digestion in lactose intolerant individuals are diarrhea, flatulence and abdominal distension (Gerbault et al., 2011).

About 5.7 billion people around the world are potential consumers of low-lactose dairy products, which represents a large market segment. According to the latest report published by *Future Market Insights*, the global lactose-free dairy products market is estimated to reach US$ 17.8 million by the end of 2027. This estimative has driven new product development in the dairy industry (Report, 2018).

A wide variety of lactose-free dairy products are available on the market. These include pasteurized and UHT milk, yogurt, cheeses, ice-cream, and *dulce de leche* (Abbasi & Saeedabadian, 2013; Antunes et al., 2014; Mota et al., 2009; Ruiz-Matute et al., 2012; Vénica et al., 2013).

The technology for producing milk powder with low lactose content is quite recent. Most of the current industrial processes are based on the enzymatic hydrolysis of the sugar. Although this product is already marketed in some countries, the dairy industry faces certain technological drawbacks in using it, such as as low production yield, equipment operation malfunctions, and powder adhesion to the drying tower, plus problems associated with the loss of techno-functional properties during powder storage (Fialho et al., 2018; Fialho et al., 2018; Torres et al., 2017).

In this chapter, the primary techniques for producing lactose hydrolyzed milk powder will be covered. The production challenges and the strategies that can be adopted to overcome them will be emphasized.

2. Enzymatic lactose hydrolysis in milk

The first study involving lactose hydrolysis in raw and pasteurized milks by application of lactase from *Saccharomyces lactis* was published in 1973 (Kosikowski &

Wierbicki, 1973). 18 years later, a patent was registered for the process which uses a sonicated culture medium containing bacterial cells as a source of lactase to produce milk and dairy products with hydrolyzed lactose (Jackson & Jelen, 1989).

β-D-galactosidase is isolated from different sources such as plants (almonds, peaches, apricots, apples), animal organs, yeasts, bacteria and filamentous fungi (Richmond, Gray, & Stine, 1981). Current enzymes produced from bacteria, yeasts or filamentous fungi are more frequently used in foods and can be commercially acquired in powder or liquid form (Table 1).

Table 1. Microbial sources of β-D-galactosidase enzymes.

Source	Species	Reference
Yeast	*Kluyveromyces lactis*	Fialho et al., 2018ª
	Kluyveromyces fragilis	Jurado et al., 2002
	Kluyveromyces marxianus	Brady et al., 1995
Filamentous fungi	*Aspergillus oryzae*	Bosso et al., 2016
	Aspergillus niger	Jones et al., 2017
	Aspergillus sphaericus	Jones et al., 2017
	Claveromycis fragiles	Abbasi & Saeedabadian, 2015
Bacteria	*Escherichia coli*	Mahalakshmi et al., 2013
	Lactobacillus oleracea	Mahalakshmi et al., 2013
	Bacillus subtilis	El-Kader et al., 2012
	Bacillus stearothermophilus	Chen et al., 2008
	Bacillus circulans	Yin et al., 2017

How β-D-galactosidase performs in industrial applications depends on factors such as substrate and enzyme concentrations, process temperature, media pH, enzyme activity and food matrix structure (Bosso et al., 2016; Zolnere & Ciprovica, 2017). In

order to meet dairy industry needs better, companies have commercialized several β-D-galactosidase enzymes that are active in broader temperature and pH ranges. These reduce operational costs and sensorial alterations of the final product.

In general, the amount of enzymes used to hydrolyse lactose in milk is defined by the supplier. The optimal temperature for maitaining maximal enzymatic activity is dependent on the type of enzyme used (Zolnere & Ciprovica, 2017). For example, the optimal process temperature for Klyveromyces lactis, Kluyveromyces fragilis and Kluyveromyces marxianus is around 40°C (Bosso et al., 2016; Brady et al., 1995; Jurado et al., 2002).

This temperature can favor the multiplication of microorganisms present in milk and cause premature deterioration of the raw material. For this reason, it is recommended that the milk be pasteurized beforehand and the process not exceed 4 hours (Ladero, Santos, & Garcia-Ochoa, 2000).

Another possibility consists in pasteurizing raw milk then concentrating it in a vaccum evaporator prior to the enzyme application (Fialho et al., 2018). The vaccum evaporation promotes water remotion from the food matrix by heating it under low pressure conditions. In this technique, the boiling temperature of water is inferior to 100°C and evaporation occurs between 55°C and 75°C. This minimizes thermal damage to milk components. Milk with 40 to 55% ($w \cdot w^{-1}$) dry matter can be obtained by water evaporation at an energy cost that is up to 20 times lower the spray drying (CARIC et al., 2009). The concentrated milk leaves the evaporator and is cooled to the enzyme's optimal action temperature of enzyme. Then, the enzyme is added directly to the liquid. (Figure 2).

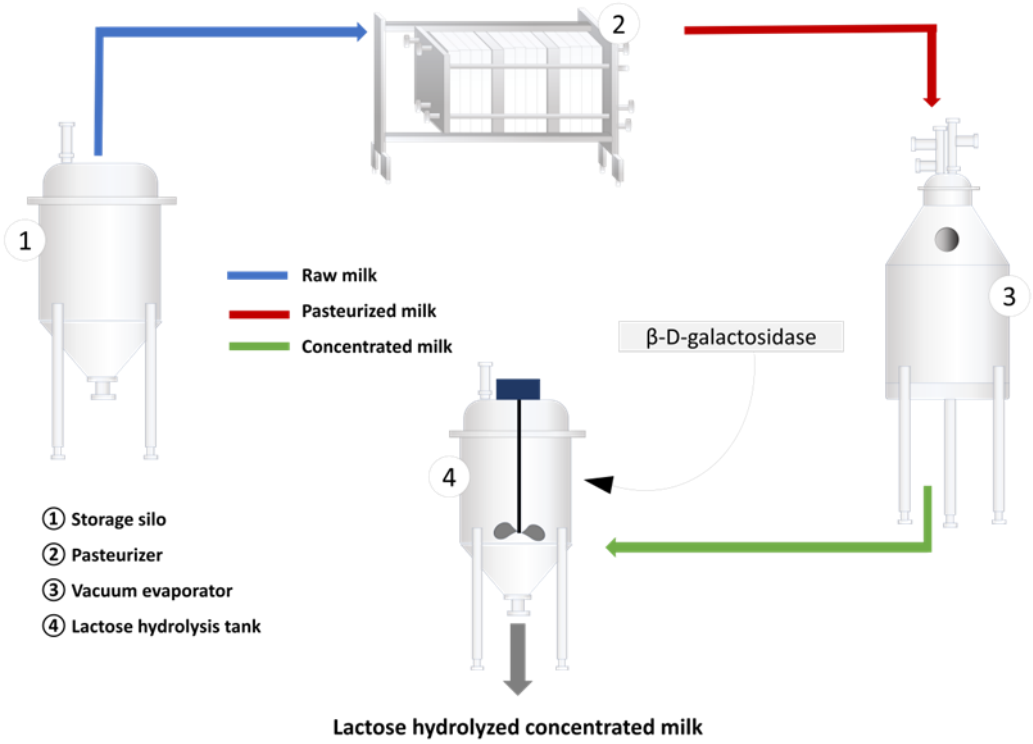

Figure 2. Diagram of lactose hydrolysis in concentrated milk.

To evaluate lactose hydrolysis efficiency in fluid or concentrated milk during or after the process, spectrophotometric analysis kits can be used for quality control (Fialho et al., 2018). These analytical tests are generally expensive and can require equipment that is not frequently used in daily analyses carried out in the dairy industry. This is why certain works have proposed using cryoscopy analysis to quantify lactose in milk because it is a quick and practical option in industrial settings (Rodrigues Junior et al., 2016).

Other means for evaluating lactose hydrolysis in dairy products include chromatographic tecnhiques (Morlock, Morlock, & Lemo, 2014) and, more recently, Raman spectrophotometry analysis (Torres et al., 2017).

3. Spray drying of concentrated milk with hydrolyzed lactose

Spray drying can convert concentrated milk into powder with minimal nutritional losses (Caric et al., 2009). The process yields products with low humidity which ensures

longer storage times and reduces logistic costs associated with packaging and transportation (Schuck, 2002).

When lactose hydrolyzed milk powder is produced, concentrated milk at approximately 50°C is injected into an atomizer nozzle which then sprinkles it in small droplets. Maintaining the concentrated milk temperature at 50°C is important because this temperature reduces fluid viscosity which aids smaller droplet formation during atomization. It also favors water evaporation in the drying chamber (Schuck et al., 2013; Schuck et al., 2009).

The concentrated milk droplets then come into contact with a low-humidity, high-temperature inlet air flow (150°C to 300°C) that promotes instantaneous water evaporation (Schuck et al., 2009). Due to temperature and partial vapor pressure differences between the hot air and the droplets, heat energy is transferred from the air to the droplets while water is removed from the droplets and carried away by airflow.

The evaporation kinetic is related to three main factors: i) the surface evaporation; ii) the difference in water vapor pressure between the food matrix and the air; iii) the water migration rate from the center of the droplet towards the surface. According to Fourrier's law, the larger is the exchange area, the faster the heat energy transference and the greater the drying speed. Similarly, the higher the partial vapor pressure difference between droplets and hot air, the faster the drying.

Once the powder particles have formed inside the drying chamber, they can remain attached to equipment walls or be carried by outlet air flow and collected by cyclone. The powder particles remain in the drying chamber for a short period of time (20 to 60 seconds) and, therefore, no real equilibrium between the hot air humidity and the product can be established. Consequently, when outlet air temperature increases, the energetic efficiency of the equipment is reduced (Schuck, 2002).

Certain drying plants may also include a fluidizer bed at the end of process to reduce product temperature and improve powder rehydration through particle agglomeration (Knipschildt, 1896).

Figure 3 represents the production of lactose hydrolyzed milk powder by spray drying.

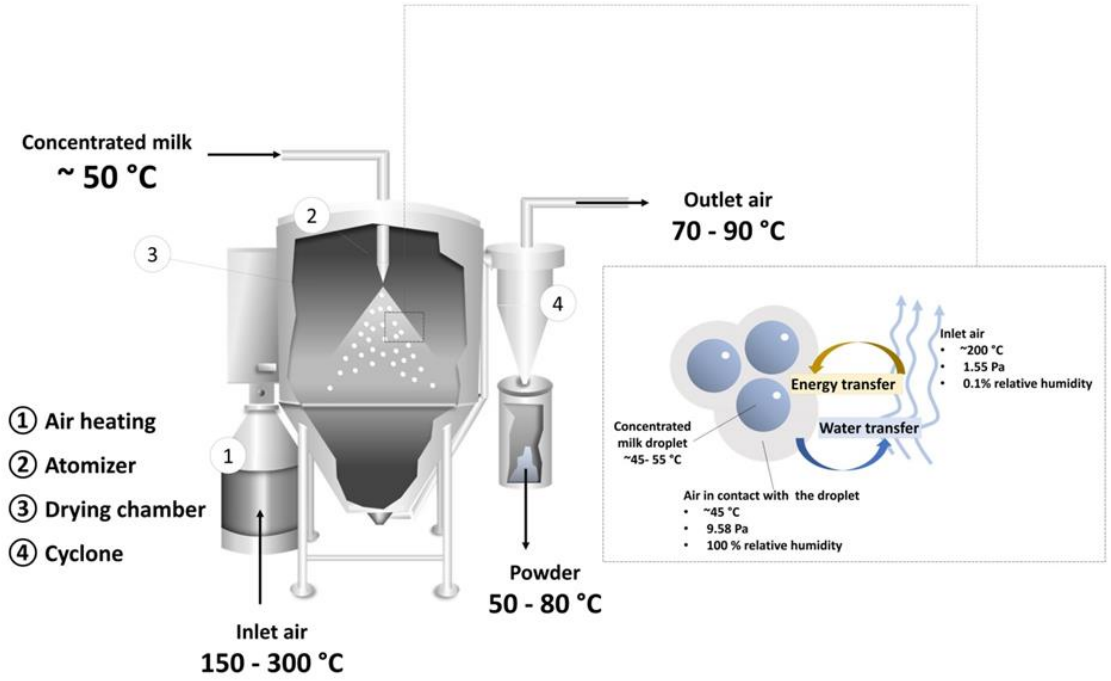

Figure 3. Diagram of production of lactose hydrolyzed milk powder by spray drying.

4. Problems associated with production of lactose hydrolyzed milk powder

Heat-treated dairy products such as milk powder are subjected to sequencing non-enzymatic reactions known as the Maillard reaction. This reaction is characterized by chemical interactions between a carbonyl group from the reducing sugar and a free amino group from the protein or amino acid. During prolonged heating or storage of dairy powders, reactive compounds are formed and polymerize with protein residues forming brown pigments or melanoidins (Fox et al., 1998).

Lactose is a reducing sugar that naturally participates in the Maillard reaction. However when lactose is hydrolyzed, the disaccharide gives rise to two new reducing sugars molecules (glucose and galactose) that intensify the reaction (Figure 4a). Consequently, lactose hydrolyzed milk powders tend to be browner than traditional products and, in pronounced cases; can show lower rehydration degrees (Fernández et al., 2003; Fialho et al., 2018).

Another effect of lactose hydrolysis is that the glucose and galactose molecules express greater sweetening than lactose (Nijpels, 1981). Dairy products treated with β-D-galactose therefore taste sweeter which may sensorially de-characterize the food and result in consumer rejection.

In addition to the aforementioned issues (changes in color and taste), the industrial production of lactose hydrolyzed milk powder has technological drawbacks that include low yield, adherence to drying chambers (Figure 4b) and caking (Figure 4d) (Fialho et al., 2018; Fialho et al., 2018; Torres et al., 2017).

Currently, few studies have examined the issues related to lactose hydrolyzed milk powder production; nevertheless, the existing works designate glass transition as a key to the problem (Fernández et al., 2003; Shrestha et al., 2007; Torres et al., 2017).

Glass transition consists of a system change from a vitreous state (high viscosity fluid) to a gummy state. This final physical state is characterized by a low viscosity solution conducive to structural changes such as particle agglomeration (Figure 4c), chamber adhesion (Figure 4b), and caking (Figure 4d) (Roos, 2002; Schuck et al., 2005).

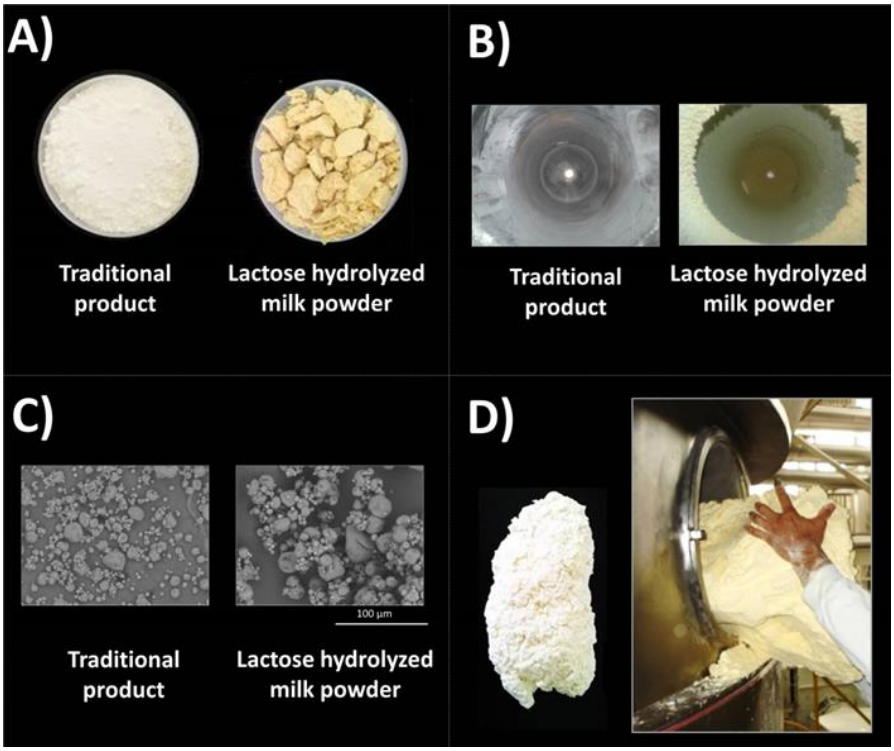

Figure 4. Problems associated with production of lactose hydrolyzed milk powder. A) powder browning; B) drying chamber adhesion; C) powder particle agglomeration; D) powder caking.

The glassy transition of milk powder occurs at the glass transition temperature (T_{Tg}), which varies according to product composition. It can be established by the following equation (Couchman & Karasz, 1978):

$$T_{Tg} = \frac{W1xTTg1x\Delta Cp1 + W2xTTg2x\Delta Cp2 + \cdots + WnxTTgnx\Delta Cpn}{W1x\Delta Cp1 + W2x\Delta Cp2 + \cdots + Wnx\Delta Cpn}$$

where W is the percentage of the component in the powdered milk, T_{Tg} is the glass transition temperature of the anhydrous component, and ΔCp is the specific heat change of the component. The numbers (1, 2, n) correspond to the compounds present in milk, including water.

As observed in Figure 5, the T_{Tg} of lactose is approximately 3 times that of the T_{Tg} of glucose and galactose. For example, a milk powder with 11% $w \cdot w^{-1}$ of humidity would have a T_{Tg}= 61°C while the hydrolyzed product would have a T_{Tg}= 36°C (Figure 5) (Fernández et al., 2003). From a practical point of view, this means that the lactose hydrolyzed milk powder suffers glass transition at lower temperatures than traditional milk powder.

In addition to galactose and glucose, water content in lactose hydrolyzed milk powder represents another factor responsible for the reduction of T_{Tg} (Jouppila, Kansikas, & Roos, 1997). As shown in Table 2, water has a very low T_{Tg} value and, for this reason, water content should be kept low, particularly in lactose hydrolyzed milk powders because they are more susceptible to glass transition.

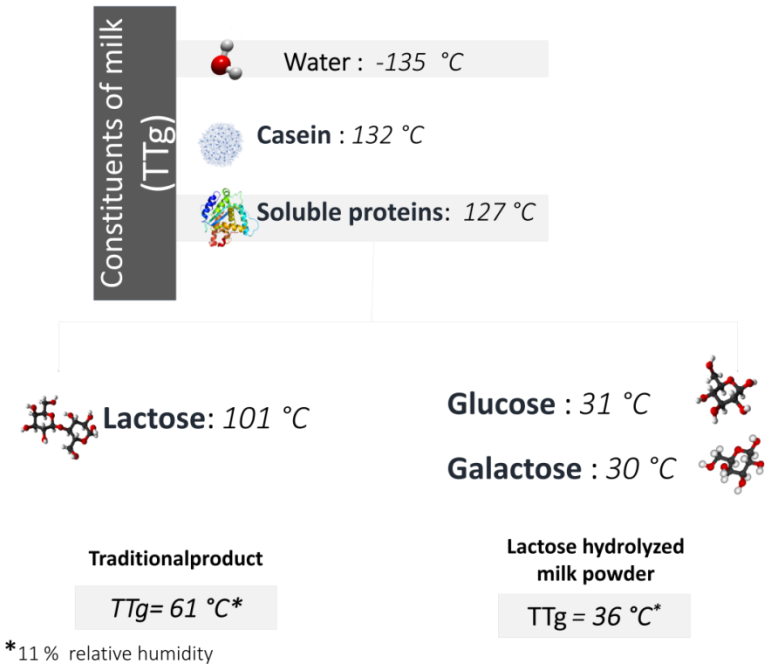

Figure 5. Glass transition temperatures (T_{Tg}) of milk constituents, traditional milk powder and lactose hydrolyzed milk powder. Source: Kalichevsky, Blanshard, & Tokarczuk, 1993; Schuck et al., 2005; Senoussi & Berk, 1995)

During spray drying, milk powder particles are heated inside the drying chamber. In the case of lactose hydrolysed milk powder, if this temperature is inferior to the powder's T_{Tg} it will remain in a vitreous state. By contrast, if this temperature goes above the powder's T_{Tg} it will readily undergo glass transition (Roos, 2002).

In this situation, the powder passes through a series of structural transformations such as a free volume increase, a viscosity decrease, specific heat variations, and an increase in thermal expansion which culminates in powder agglomeration and adhesion to equipment surfaces (Jouppila et al., 1997; Jouppila & Roos, 1994; Torres et al., 2017).

Shrestha et al. (2007) demonstrated that low production yield of lactose hydrolyzed milk powder is primarily due to powder adhesion to equipment. The same work also demonstrated that the T_{Tg} of anhydrous powder was 49°C and only 25% of product was recovered from the cyclone.

The production of lactose hydrolyzed milk powder remains a challenge to the dairy industry which is why it continues to look for ways to improve quality and reduce production costs.

5. Perspectives on the production of lactose hydrolyzed milk powder

Some dairy industries have focused on incorporating high molecular weight compounds to lactose hydrolyzed milk powder in order to decrease the issues of production. Substances with high glass transition temperature such as maltodextrins, inulin and other polysaccharides can increase powder T_{Tg}, and thus overcome the associated technological issues (Rodrigues Junior et al., 2016).

In a pioneering approach, Fialho et al. (2018) determined that controlling the drying parameters represents a promising strategy for the production of lactose hydrolyzed milk powder with quality and productivity levels that come closer to the production of traditional milk powder. According to the authors, when lactose hydrolyzed milk is dried using conventional operating parameters, the powder particles are overheated and bring about the glass transition.

By simultaneously adjusting the concentrated milk injection rate and the inlet air temperature, it is possible obtain a powder with characteristics of color, rehydration, water activity, particle size and morphology that are very similar to traditional milk powder (Fialho et al., 2018).

Despite these successful sensorial and techno-functional result, spray drying requires greater thermal energy compared to traditional milk powder production (Fialho et al., 2018). In other words, more energy is needed to obtain a high-quality product and limit production issues.

Another promising strategy consists in physically removing lactose from milk using membrane technology (Figure 6). Patent nº WO 03/094623 A1 demonstrates lactose removal through successive ultrafiltration and nanofiltration of the milk (Tossavainen & Sahlstein, 2003). Removing the sugar increases the powder T_{Tg} instead of reducing it as explained in the lactose hydrolysis discussion.

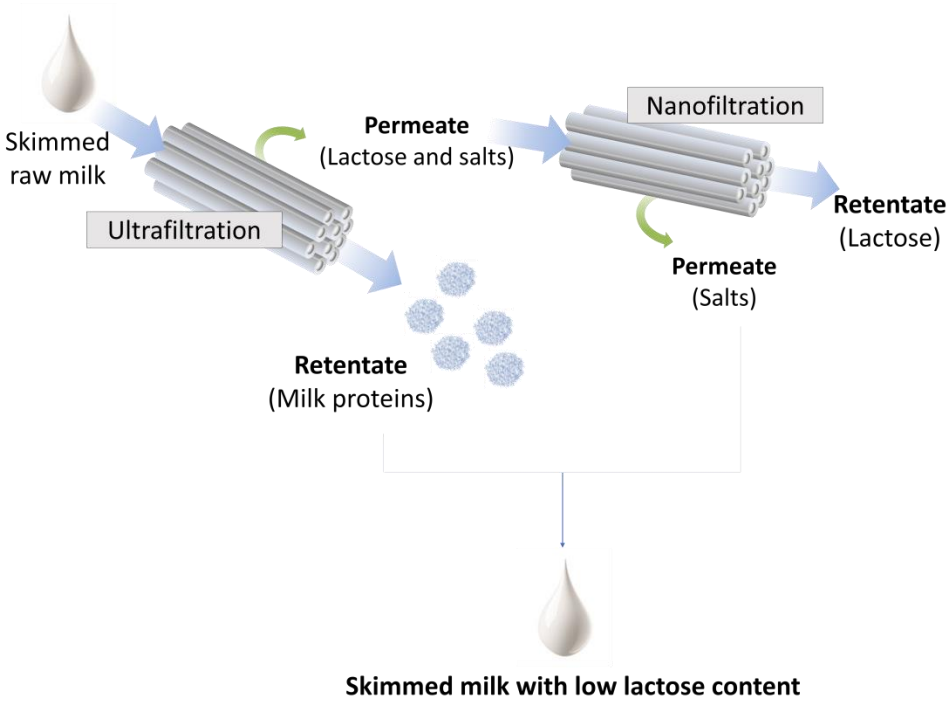

Figure 6. Diagram of the physical removal of lactose using membrane filtration. Source: WO 03/094623, 2003.

Although membrane filtration is an emerging technique, some industries have not yet incorporated the technology due to implementation costs, skilled labor requirements, and industry regulations.

The limitations of the dairy industry in the production of lactose hydrolyzed milk powder interconnected by the scarcity of scientific studies and the search to meet the desires of people who are lactose intolerant led to this thesis. The study is expected to become more widespread in the next few years and will promote significant advances in lactose hydrolyzed milk powder production.

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CHAPTER III

OPTIMIZATION OF THE LACTOSE HYDROLYZED MILK POWDER DRYING PROCESS

PART 1: LACTOSE HYDROLYZED MILK POWDER: THERMODYNAMIC CHARACTERIZATION OF THE DRYING PROCESS

Preamble

In order to meet the needs of lactose-intolerant consumers, the dairy industry has developed lactose hydrolyzed milk powder despite the technological problems that can be associated with its production: unwanted agglomeration, powder adhesion in the drying chamber, caking, browning and elevated powder hygroscopicity levels. These issues are due to the lowering of the milk powder's glass transition temperature (T_{Tg}) caused by lactose hydrolysis. The glass transition (Tg) consists in a change of the powder from an amorphous state to a gummy state and results in operational issues such as low yield and product handling difficulties. The aim of the present study was to determine the ideal drying conditions for lactose hydrolyzed milk powder production.

Our questions:

- Lactose hydrolyzed milk powder can be produced by using the same operating parameters applied to traditional milk powder production?
- Are there drying parameters that can favor or disfavor the drying process of lactose hydrolyzed milk?
- What are the optimal drying parameters to lactose hydrolyzed milk powder?

Lactose hydrolyzed milk powder: thermodynamic characterization of the drying process

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Abstract

Industrial production of lactose hydrolyzed milk powder (LHMP) remains challenging. Due to the presence of the monosaccharides glucose and galactose, lactose-free powders tend to suffer stickiness, caking, and browning during drying and storage.

We sought to find ideal conditions spray dryer inlet air temperature ($\theta_{air,in}$) and concentrated milk flow rate ($\mathbf{m_{CM}}$) for LHMP production. We tested $\theta_{air,in}$ settings of 115–160°C and $\mathbf{m_{CM}}$ of 0.3–1.5 kg·h^{-1}, and also applied mass and energetic balances. Lactose hydrolyzed milk powder generally exhibited higher mass and energetic losses than the reference (milk powder containing lactose), as a consequence of the relatively low dryability of LHMP. For a lab scale spray dryer, the ideal conditions settings for LHMP production were $\theta_{air,in}$ = 145 ± 2°C and $\mathbf{m_{CM}}$ =1.0 kg·h^{-1}, taking into account the mass yield and energetic cost (kJ·kg^{-1} of powder) of the process. These ideal conditions are a potential tool for the industrial development of lactose-free dairy powders.

KEYWORDS: lactose hydrolyzed milk powder, mass and energetic balances, spray drying

1. Introduction

Dairy products with low lactose content can be produced by using of membrane filtration techniques (physical removal of lactose from milk) or by using of enzymatic hydrolysis methods. These strategies are applied by dairy industry for the purpose to increase the variety of available products in the market for consumer diagnosed as lactose intolerant. Approximately 75% of the adult world population shows some kind

of intolerance to lactose, being the Asian countries (about 90% of population) those more contribute for this high statistic. [1]

Lactose intolerance in humans is characterized by absent or low production of the enzyme β-galactosidase (lactase). This enzyme hydrolyzes lactose into the monosaccharides glucose and galactose, which are easily absorbed by the small intestine. If the lactose is not hydrolyzed during digestion, the sugar is fermented in the colon, resulting in gastrointestinal discomfort such as flatulence, abdominal aches, and diarrhea[2].

Although the growing demands of hydrolyzed lactose products, this market faces some technological challenges as, for example, the production of lactose hydrolyzed milk powder (LHMP). During the production and storage of this kind of product, technological drawbacks as stickiness to equipment, caking and browning can occur. [3]

In the drying of dairy products, concentrated milk is atomized into the drying chamber where milk droplets encounter hot air. The difference of temperature and pressure between the hot air and the food matrix make possible fast drying of the material. [4,5] Since the drying time is relatively short (~3-30 seconds), the powder molecules are kept in a glassy state. [6,7] The glassy state is evidenced by a high viscosity solution with limited molecular motility. However, the product in this state is hygroscopic and metastable, which means it is vulnerable to changes in physical state or physicochemical properties during processing, storage, and consumption. [8]

The glass transition (Tg) consists of a change of the system from the glassy to the rubbery state when the dairy powder particles are heated until a temperature known as glass transition temperature (T_{Tg}). The rubbery state is characterized by a low viscosity fluid that can suffer structural changes ranging from agglomeration to caking. [7] The changing of the material state can occur during drying and storage, and is a technological problem facing the dairy drying industry. [3]

The glass transition temperature (T_{Tg}) of dairy powders is linked to the concentration, specific heat, and glass transition temperature of each component. Since water has a low glass transition temperature (T_{Tg}= -135°C), this constituent is the principal component responsible for decreasing the glass transition temperature of dairy powder. [8] For this reason, it is very important the humidity control in dried

products in order to improve their stability during the drying and storage. [9]

In the case of LHMP, lactose (T_{Tg}=101°C) is hydrolyzed into glucose (T_{Tg}= 31°C) and galactose (T_{Tg}= 30°C). [10,11] As consequence of carbohydrate hydrolysis, the glass transition temperature of LHMP (36°C when in equilibrium with air of 11% of relative humidity) is significantly smaller than that of traditional milk powder (61 °C under the same equilibrated conditions). [3] Considering this drop in the T_{Tg}, lactose hydrolyzed milk powder is more prone to suffer glass transition during drying, storage, and transport.

The production of LHMP using the same operational parameters applied for milk drying promotes the heating of powder at a temperature above its T_{Tg}. As a consequence, the product can undergo adhesion and browning. [3,12] These drawbacks lead to low yield, operational problems, and difficult powder handling. [3]

The aim of this work was to evaluate the effect of the operating drying parameters ($\theta_{air,in}$ = inlet air temperature and $\mathbf{m_{CM}}$ =concentrated milk flow rate) on the thermodynamic aspect of the lactose hydrolyzed milk powder production.

2. Materials and Methods

2.1. Materials

Raw skim milk with 9% ($w \cdot w^{-1}$) of dry matter was provided by Laticínios Escola (FUNARBE; Viçosa, Brazil). The skim milk powder containing 96% ($w \cdot w^{-1}$) of dry matter was donated by Tangará Foods (Estrela, Brazil). The enzyme lactase Lactomax Super (Prozyn; São Paulo, Brazil) with 60.000 ONPGU$\cdot g^{-1}$ of enzymatic activity and 1.2 $g \cdot mL^{-1}$ of density was used to hydrolyze the lactose while sodium azide (Vetec Química Fina; Duque de Caxias, Brazil) was added to milk in order to prevent the microbial growth.

2.2. Production of lactose hydrolyzed concentrated milk

Concentrated milk at 40% $w \cdot w^{-1}$ of dry matter was prepared from mixture of skim raw milk and skim milk powder (Figure 1). The mixture was added 0.03% $w \cdot w^{-1}$of sodium azide, submitted to pasteurization (65°C·30 min^{-1}) and then cooled quickly and kept at 40°C (Figure 1). To concentrated milk, we added 0.6% $w \cdot w^{-1}$ lactase enzyme, and the lactose hydrolysis process was carried out over 4 h (≥ 90% hydrolysis).

The hydrolysis was confirmed posteriorly by analysis of the power using the Lactose and D-Galactose enzymatic kit (Megazyme; Ireland, 2014). [13]

The lactose hydrolyzed concentrated milk was cooled at 7°C and kept cool until spray drying.

As an experimental control, concentrated milk was prepared as described previously, without the addiction of lactase enzyme (Figure 1).

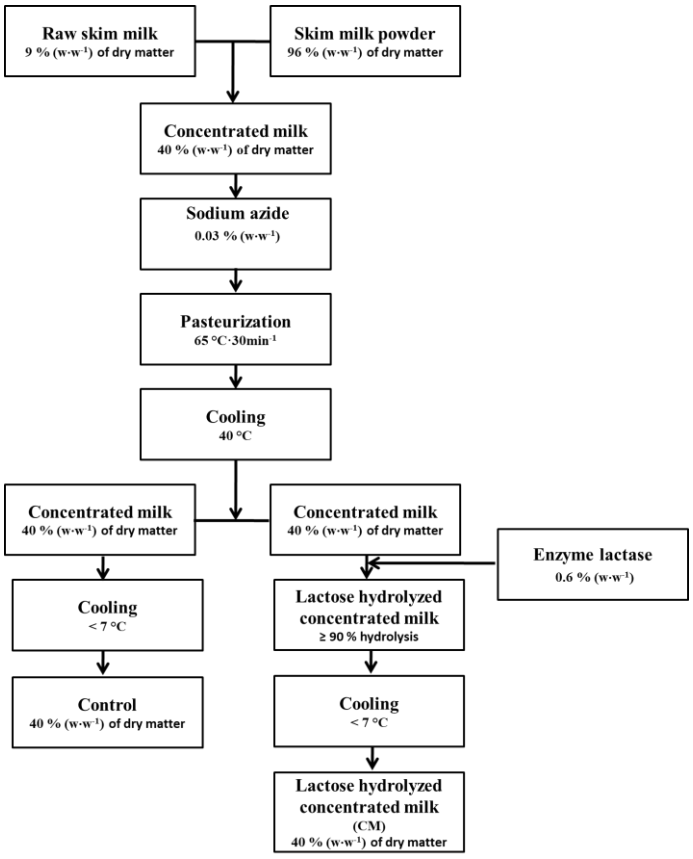

Figure 1. Production of the lactose hydrolyzed concentrated milk and the concentrated milk (control).

2.3. Production of lactose hydrolyzed milk powder (LHMP)

Lactose hydrolyzed concentrated milk or concentrated milk were heated at 40±1 °C, and injected into a pilot scale spray dryer model MSDi 1.0 (Labmaq do Brasil, Brazil), single stage and nozzle atomizer (Figure 2). Probes connected to thermohygrometer HigroFlex 5 (Rotronic; Bassersdorf, Switzerland) were positioned to spray dryer in order to monitor the temperature (°C), relative humidity (%) and the absolute humidity (kg

water·kg^{-1}dry air) in the inlet and outlet air. [14] An anemometer was positioned at the outlet of equipment (Figure 2) to measure the air velocity during drying.

The lactose hydrolyzed concentrated milk was injected into spray dryer at flow rates varying from 0.3 to 1.5 kg·h^{-1} while the inlet air temperature varied from 115 to 160 °C.

To obtain traditional milk powder at 0.20 ± 0.1 of water activity and without stickiness or caking phenomenon, the ideal parameters for drying were previously determined and applied in this study: flow rate of concentrated milk at 1.5 kg·h^{-1}and inlet air temperature at 160°C. The milk powder containing lactose was used as control of the production.

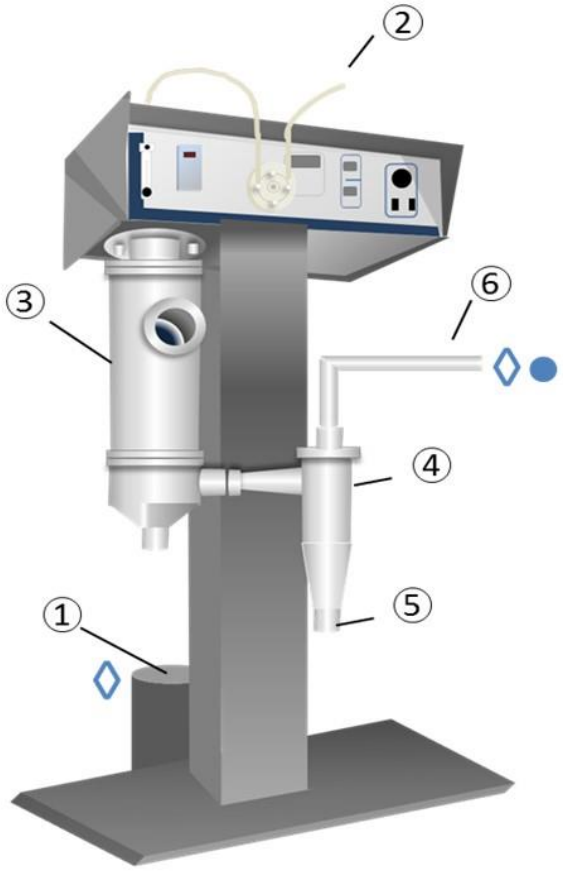

Figure 2. Spray dryer driagram. (1) Inlet air; (2) Hydrolyzed lactose concentrated milk pump; (3) drying camera; (4) cyclone; (5) dust collector; (6) outlet air; (◊) position of thermohygrometer; (●) position of anemometer.

The powders (LHMP and control) were stored in laminated plastic bags under vacuum conditions at 10°C.

2.4. Thermodynamic characterization of the drying process

The thermodynamic characterization of the drying process was performed using the equations of mass and energy balance. [15]

2.4.1. Mass balance

The mass loss (q_{mass}; kg·h^{-1}), that is, the amount of powder lost during the drying process due to adhesion of particles to equipment or loss by cyclone, can be estimated as

$$q_{mass} = \left(1 - \frac{m_{LHMP} \cdot DM_{LHMP}}{m_{CM} \cdot DM_{CM}}\right) \cdot 100 \quad (1),$$

where m_{CM} is the flow rate of the concentrated milk to be dried (kg·h^{-1}); DM_{CM} is the solids content in the product to be dried (40.0±0.2 kg·kg^{-1}); m_{LHMP} is the flow rate of the milk powder (kg·h^{-1}); and DM_{LHMP} is the solids content of the dairy powder (kg·kg^{-1}).

2.4.2. Energy balance

The inlet ($\varepsilon_{total,in}$, kJ·h^{-1}) and outlet ($\varepsilon_{total,out}$, kJ·h^{-1}) total energy to the drying system (Figure 2) are represented as

$$\varepsilon_{total,in} = \left[\theta_{air,in} \cdot (1.01 + 1{,}89 \cdot AH_{in}) + 2500 \cdot AH_{in}\right] \cdot \left[V_{air,out} \cdot A_{out} \cdot \rho_{air} \cdot (1 + AH_{out})^{-1}\right] + \left[(1 - 0.56 \cdot DM_{CM}) \cdot 4.186\right] \cdot m_{CM} \cdot \theta_{CM} \quad (2)$$

and

$$\varepsilon_{total,out} = \left[\theta_{air,out} \cdot (1.01 + 1{,}89 \cdot AH_{out}) + 2500 \cdot AH_{out}\right] \cdot \left[V_{air,out} \cdot A_{out} \cdot \rho_{air} \cdot (1 + AH_{out})^{-1}\right] + \left[(1 - 0.56 \cdot DM_{LHMP}) \cdot 4.186\right] \cdot \frac{m_{LHMP}}{t} \cdot (\theta_{air,out} - 20) \quad (3),$$

where θ_{air} denotes the air temperature (°C); AH is the absolute humidity of air (kg water·kg dried air^{-1}); $V_{air,out}$ is the air velocity (5.6·10^{4}m·h^{-1}); A_{out} corresponds to area of the transversal section of the tube where the air exits the spray dryer (9.6·10^{-4}m^2); and ρ_{air} is the density of the air (1.0 kg·m^{-3}). The prefixes *in* and *out* denote inlet and outlet, respectively.

The percentage of energy loss (q_{energ}) is calculated from the values of inlet total energy ($\varepsilon_{total,in}$, eq. 2) and outlet air energy ($\varepsilon_{air,out}$, eq. 3):

$$q_{energ} = \left(1 - \frac{\varepsilon_{total,out}}{\varepsilon_{total,in}}\right) \cdot 100 \quad (4)$$

The specific energy consumption (SEC, kJ·kg^{-1}) is the inlet total energy over the amount of water evaporated during the drying ($m_{evapwater}$, kg·h^{-1}). This parameter estimates the energy expended per kg of evaporated water:

$$m_{evapwater} = AH_{out} \cdot \left[V_{air,out} \cdot A_{out} \cdot \rho_{air} \cdot AH_{out} \cdot (1 + AH_{out})^{-1}\right] - AH_{in} \cdot \left[V_{air,out} \cdot A_{out} \cdot \rho_{air} \cdot AH_{out} \cdot (1 + AH_{out})^{-1}\right] \quad (5),$$

and

$$SEC = \frac{\varepsilon_{total,in}}{m_{evapwater}} \quad (6)$$

The energy cost (Q, kJ·kg^{-1}) is defined as the reason between the inlet total energy and the mass of produced powder (m_{LHMP}, kg·h^{-1}). This parameter evaluates the amount of energy expended to produce 1 kg of powder:

$$Q = \frac{\varepsilon_{total,in}}{m_{LHMP}} \quad (7)$$

2.5. Powder analyses

2.5.1. Moisture content and dry matter

The moisture content was analyzed of according to the weight loss after drying of 3g of concentrated milk sample and 1.5 g of milk powder with sand in an oven at 105°C for 5 h. [16]

The dry matters (DM) were calculated from equation 8, where M denotes the moisture content in each sample.

$$DM = 100 - M \quad (8)$$

2.5.2. Water activity (a_w)

The water activity (a_w) was determined at 25°C by Aqualab (Decagon 3TE, Decagon Devices Inc., USA).

2.5.3. Glass transition temperature (T_{Tg})

The glass transition temperature was determined by differential scanning calorimetry (DSC) (Q-1000, TA Instruments, Saint Quentin en Yvelines, France). First, the initial scanning rate was 5°C·min^{-1} at -70°C to + 70°C for the lactose hydrolyzed milk powder to eliminate the hysteresis effect of thermal relaxation. The samples were then cooled to 0°C min^{-1} at -70°C and then the T_{Tg} was determined from the DSC curve with scanning at 5°C·min^{-1} from -70°C to + 250°C.

2.5.4. Quantification of the 5-hydroxymethylfurfural (HMF)

The milk powder was diluted in distilled water (1:13) (w·w^{-1}) and deproteinized by the addition of 2.5mL oxalic acid 0.3moL·L^{-1} and 2.5mL trichloroacetic acid 40% (w·v^{-1}). After filtration, 4 mL of the filtrate was collected and 1 mL of 0.05 moL·L^{-1} thiobarbituric acid was added. The solution was heated at 40°C for 30 minutes and cooled to 25°C. The absorbance reading was performed by the spectrophotometer UV visible (Global Trade Technology, São Paulo, Brazil) with wavelength of 443 nm. For determination of the concentration in mg·kg^{-1}, an analytical curve was constructed using different levels of HMF. [17]

2.5.5. Statistical analysis

All experiments were carried out with three repetitions and the results were compared using the Student's t-test statistical method. A significant difference at p-value < 0.05 was assumed.

3. Results and discussion

Considering that the hydrolysis of lactose compromises the physicochemical properties of milk powder as, for example, the lowering of the T_{Tg}, it is expected a

bigger loss mass during the production of lactose hydrolyzed milk powder due to effects of the adhesion into drying tower.

Furthermore, the presence of monosaccharides increases the affinity of the dairy matrix for water, which slows the dehydration process. In other words, it can be imagined that more energy is dispensed in the production of LHMP, relative to the energy expenditure for traditional milk powder (control). To investigate these considerations, the production of LHMP under different operational drying parameters was evaluated in light of mass and energy balances.

3.1. Evaluation of mass loss

The LHMP with not more than 4.8% lactose were produced from the combinations between increasing concentrated milk flow rates ($\mathbf{m_{CM}}$; from 0.3 to 1.5 kg·h^{-1}) and inlet air temperatures ($\boldsymbol{\theta_{air,in}}$; from 115 to 160°C) as shown in the table 1. By the drying data (table 1), it was possible estimate the loss mass ($\mathbf{q_{mass}}$) in each treatment from equation 4.

Table 1. Drying data for production of Lactose hydrolyzed milk powder.

Sample	Inlet		Outlet	
	Air temperature ($\boldsymbol{\theta_{air,in}}$;°C)	Concentrated milk flow rate ($\mathbf{m_{CM}}$;kg·h^{-1})	Milk powder flow rate (m_{LHMP};kg·h^{-1})	Dry matter ($\mathbf{DM_{CM}}$;kg·kg^{-1})
Control*	160	1.5	0.5	96.3
Lactose hydrolyzed milk powder (LHMP)	160	1.5	0.5	94.2
		1.3	0.4	94.3
		1.0	0.3	94.7
		0.8	0.2	95.5
		0.5	0.2	94.3
		0.3	0.1	94.2
		1.5	0.4	93.2
		1.3	0.4	95.3

	1.0	0.4	95.1
145	0.8	0.3	93.8
	0.5	0.2	95.9
	0.3	0.1	92.0
	1.5	0.5	84.5
	1.3	0.4	92.5
	1.0	0.3	92.2
130	0.8	0.3	93.8
	0.5	0.2	94.3
	0.3	0.1	91.8
	1.5	X	X
	1.3	0.4	85.2
	1.0	0.3	92.4
115	0.8	0.3	91.5
	0.5	0.2	94.5
	0.3	0.1	94.0

* Milk powder.
X: Under these conditions, it was impossible to obtain powder.

In theory, all mass entering the spray dryer should go out the equipment, however, in the practice, it never occurs due to effects of powder adhesion or losses into the cyclone.

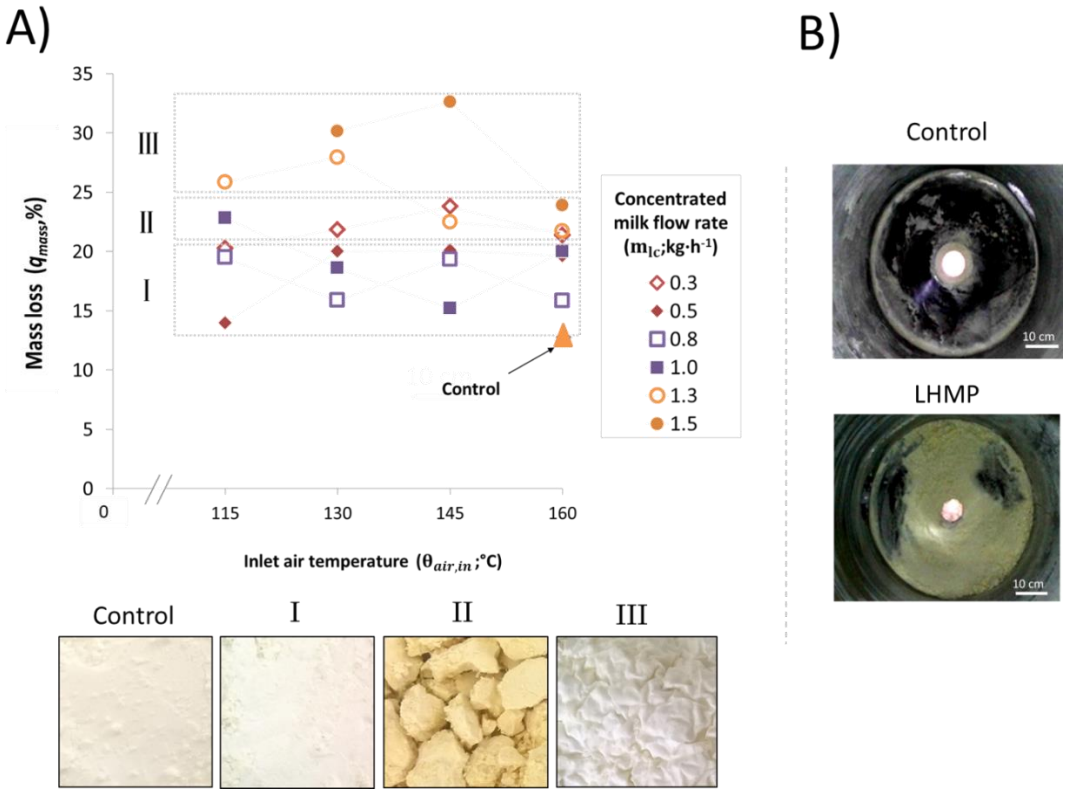

Figure 3. Mass loss during the production of lactose hydrolyzed milk powder (LHMP). A) Effect of inlet air temperature and concentrated milk flow rate on the loss mass; B) Images of powder adhesion in the tower drying.

For the treatment control, *i.e*. milk powder with 51.6% of lactose content, the loss mass was found to be approximately 13%. However, in all treatments applied to produce LHMP, higher values of loss mass (14–33%) were achieved (Figure 3A).

In dairy industries, the loss mass for milk powder production can reach 10%[18] which is a value close to obtained in the control (13%). Already the higher loss mass values for LHMP are associated the lower T_{Tg} due to lactose hydrolysis. In other words, LHMP are more prone to suffer adhesion when compared with the traditional milk powder. In all productions, it was verified that LHMP tends to adhere to equipment in lesser or higher degree, dependent on the drying conditions. A typical adhesion of LHMP can be found in the Figure 3B.

The treatments were arranged in groups in order to better discuss the results. Group I includes all treatments with mass loss of 14–20% (Figure 3A) and powders with a_w = 0.19 ± 0.02. Group II corresponds to mass losses of 22–24% and a_w =0.21± 0.02 , while group III, comprising only the 1.3 and 1.5 kg·h^{-1} flow rates, exhibited relatively

high mass losses during drying (26–33%) and elevated water activity in the powders (a_w =0.44 ± 0.05).

In group III, the combination of low temperatures (115–145°C) with high flow rates (1.3 or 1.5kg·h^{-1}) was responsible for the elevated mass loss. Since the more concentrated milk was injected in the spray dryer by unit of time, the lower temperatures were not able to remove effectively the water from the product which can be observed by higher a_w in the powders. By consequence, the powders showed elevated humidity (6.04 ± 1.42% w·w^{-1}) and reduced T_{Tg} (~2°C). By consequence, the powders showed, in the great majority, a powders with caking aspect (Figure 3A, group III).

In agreement with this reasoning, some authors demonstrated that the increasing of the LHMP humidity from 5.8 to 7.8% w·w^{-1}, resulted in a lowering from 13 to 2°C in the T_{Tg}. [16] Other authors have confirmed that high humidity in dairy powders is responsible for adhesion of material to the drying tower and increased mass loss. [3] It other study was also observed that mass loss in the LHMP production was expressively higher than the milk powder. [12] These authors reported that only 25% of the LHMP was recovered at the end of the drying process.

The group II presents loss mass approximately 2 times bigger than the control and some powders showed brown coloration (Figure 3A, group II) due to the accumulation of products of Maillard reaction. Additional analysis revealed that these powders may contain up to 10 fold the HMF of the control (12.2 mg·kg^{-1}). The brown coloration was more pronounced in powder produced from low flow rates (0.3 kg·h^{-1}) and high temperatures (130–160°C).This coloration may be explained by excessive heating, which probably allowed for glass transition. The glass transition increases molecular mobility due to the decrease of viscosity favoring the Maillard reaction. [19,20,21] The expressive content of reducer sugars and proteins, associated with the heating, seems to be the principal factors that governed the Maillard reaction in the group II. [3,22] Among compounds released from Maillard reaction, the brown coloration is occasioned by accumulation of polymeric substances as the melanoidins. [23] Other than these, water is also produced during the Maillard reaction contributing to increase the humidity of the powders. [21] The samples attributed to group II showed a typical agglomerated aspect as displayed in the Figure 3A.

The group I englobes samples with loss mass inferior or equal to 20% and physical characteristics as color and texture closer to the control (Figure 3A). Furthermore, samples belonging to this group present, in average, water activity similar to control (a_w=0.20). Briefly, this region of flow rate and inlet air temperature is the more appropriated for LHMP production in terms of loss mass and quality of powder.

3.2. Evaluation of energy loss

The presence of the monosaccharides glucose and galactose in lactose hydrolyzed dairy products slows the loss of water during drying, due to the relatively high affinity of these sugars for the aqueous phase. As a consequence, to obtain lactose hydrolyzed powders with low humidity, more thermal energy is spent to remove water. [24]

To evaluate this premise, data recovered from anemometer and thermohygrometer measurements (Table 2) were used to estimate the thermal energy involved in each LHMP production.

Table 2: Data obtained for energy balance calculation.

Sample	Inlet				Outlet		
	Air temperature ($\theta_{air,in}$;°C)	Concentrated milk flow rate (m_{CM};kg·h^{-1})	Absolute air humidity (AH_{in};10^{-2}kg·kg^{-1})	Energy ($\varepsilon_{total,in}$;10^{3}kg·kg^{1})	Air temperature ($\theta_{air,out}$;°C)	Absolute air humidity (AH_{out}; 10^{-2}kg·kg^{-1})	Energy ($\varepsilon_{total,out}$;10^{3}kg·kg^{-1})
Control	160	1.5	1.4	11.0	72.6	3.4	8.8
LHMP	160	1.5	1.6	11.1	76.0	3.4	8.9
		1.3	1.8	11.3	79.1	3.4	9.1
		1.0	1.6	10.9	82.8	2.8	8.4
		0.8	1.6	10.8	85.8	2.4	7.8
		0.5	1.8	10.9	92.8	2.1	7.7
		0.3	1.6	10.7	89.4	1.8	7.2
	145	1.5	1.6	10.8	59.0	3.5	8.5
		1.3	1.4	10.4	63.8	3.4	8.2
		1.0	1.6	10.5	68.7	3.0	8.2
		0.8	1.6	10.4	69.6	2.5	7.4
		0.5	1.6	10.3	79.4	2.2	7.4
		0.3	1.6	10.0	87.9	1.7	7.1

	1.5	1.5	10.0	53.1	3.7	8.5
	1.3	1.6	10.0	58.6	3.4	8.3
	1.0	1.6	10.0	62.0	2.9	7.8
130	0.8	1.5	9.8	66.1	2.9	7.5
	0.5	1.8	9.4	72.7	2.1	7.0
	0.3	1.5	9.5	71.8	1.7	6.8
	1.3	1.5	9.2	50.8	3.3	7.9
	1.0	1.4	8.9	55.0	2.8	7.4
115	0.8	1.4	8.7	59.3	2.5	7.1
	0.5	1.5	8.7	64.5	2.0	6.6
	0.3	1.5	8.8	67.9	1.7	6.3

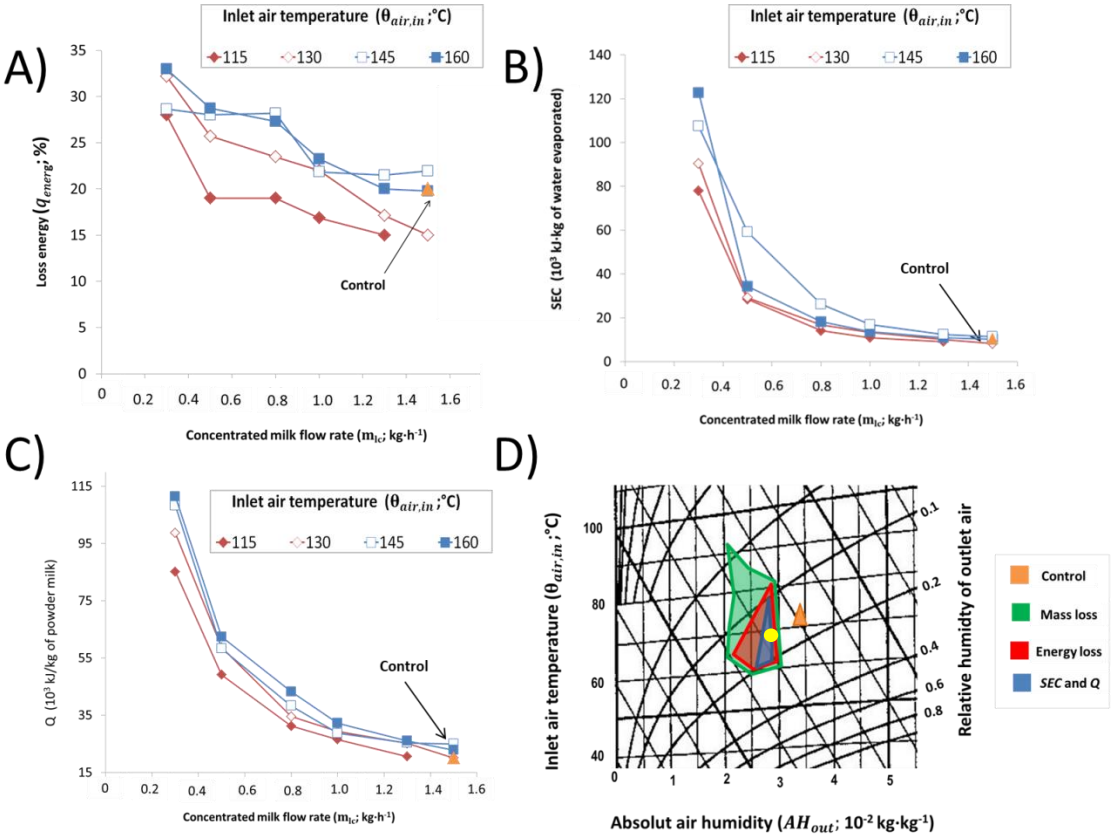

Figure 4. Effect of operational parameters on the energetic balance for lactose hydrolyzed milk powder production. A) Energy loss during the LHMP production; B) Energy needed to evaporate 1 kg of water under different drying conditions; C) Energy dispensed to produce 1 kg of LHMP; D) Mollier-Ramzine diagram showing the optimal drying conditions for LHPM production. The yellow point represents LHMP produced with $\theta_{air,in}$ = 145 °C and m_{CM}= 1.0 kg·h^{-1}.

The energy losses (q_{energ}), displayed in the Figure 4A, were calculated from mathematical considerations proposed by equations 2, 3 and 4. The energy loss calculation helps to demonstrate the amount of thermal energy (in percentage) that was dissipated through the equipment surface.

By increasing the flow rate of concentrated milk (m_{CM}) or by reducing of inlet air temperature ($\theta_{air,in}$), the energy loss tended to decrease (Figure 4A). In both cases, the total energy added to the system is reduced, forcing the best exploitation of heat during drying process. In other words, the thermal energy provided is more efficiently used to transform water in vapor.

The control showed energy loss of 20%, which is an expressive value when compared to the 5% energy loss found by some authors. [25] By contrast, because it is

lab scale equipment, this magnitude of losses can be considered satisfactory since the spray dryer has not ideal thermal isolation and appropriated design. [25] Others authors verified that in this kind of equipment, the energy losses can reach 24%, which is consistent with our finding. [15]

The energy losses for LHMP were 15–33%, depending on the drying conditions (Figure 4A). For m_{CM} between 1.3 and 1.5 kg·h^{-1}, the values of energy losses were near or lower than control (Figure 4A). In this strict gamma, the powder showed particles with agglomerated or stone aspect as described for the samples contained in the groups II and III, respectively (Figure 3A). Thus, the lower energy loss does not in fact represent an advantage.

Surprisingly, for $\theta_{air,in}$ =115°C and $\mathbf{m_{CM}}$ between 0.5 and 1.0 kg·h^{-1}, the energy losses were smaller than control (Figure 4A). This finding indicates that, by adjusting the operational parameters, it is possible improve the energy consummation for production of LHMP. Furthermore, the powders produced from the combination of these operational parameters belonged to group I (Figure 3A), which had properties closer to those of the control.

Besides the energy loss, the other parameter affecting the energetic efficiency of the drying process is the specific energy consumption (SEC), expressed by equation 6. The SEC estimates the amount of energy in kJ needed to evaporate 1 kg of water during the production of LHMP.

By reducing the inlet air temperature, a decrease in the SEC value could be observed (Figure 4B). It seems that for low flow rates (<1.0 kg·h^{-1}), the inlet air temperature is the principal factor that determines the SEC value. The temperature reduction conducts to a decreasing in the inlet total energy ($\varepsilon_{\mathbf{total,in}}$), which directly reduces the SEC value. [18]

On the contrary, at high concentrated milk flow rate (1.5 kg·h^{-1}), the SEC value of LHMP approximates that of the control (1.0 x 10^4 kJ·kg^{-1}), independently of the inlet air temperature (Figure 4B). Under these conditions, more water was injected to the spray dryer, producing higher amounts of vapor. For high $\mathbf{m_{CM}}$ values, the mass of evaporated water seems to govern the SEC value.

However, for the great majority of treatments, the SEC values for LHMP were higher than control.

To estimate the amount of energy necessary to produce 1 kg of powder, the energy cost (Q, kJ·kg^{-1}) was calculated for each treatment from equation 7 (Figure 4C). The energy provided to drying system ($\varepsilon_{total,in}$) is a variable depending of the inlet air temperature and higher temperatures tends to promote higher Q values (Figure 4C).

By keeping the temperature constant, the energy provided to system ($\varepsilon_{total,in}$) hardly varies by increasing of the flow rate (lesser than 1%) can be considered a constant in this case (Figure 4C). On the contrary, the increase in the flow rate increases the powder mass produced (m_{LHMP}) over time due to which $\varepsilon_{total,in} \cdot m_{LHMP}^{-1}$ behaves as an inverse function of type $f(x) = x^{-1}$.

Ideal conditions results were observed for drying carried out with elevated flow rates (1.3 and 1.5 kg·h^{-1}) and inlet temperatures (145 and 160°C), which demonstrated Q values closer to those of the control (2.0 x 10^4 kJ·kg^{-1}) (Figure 4C). In fact, the powders produced under these conditions belonged to groups II or III (Figure 3A); these parameter combinations are not advised for LHMP production.

On the other hand, samples covered by the group I (Figure 3A) showed Q values higher than that of the control and varied from to 2.6 x10^4 to 8.5 x10^4 kJ·kg^{-1} (Figure 4C). This difference between the Q value of the LHMP and the control is associated with the more pronounced adhesion of the former to the drying camera.

3.3. Ideal operational parameters to produce lactose hydrolyzed milk powder

Given the mass and energetic considerations developed above, it is possible to draw a region where ideal LHMP drying conditions can be found.

To help in the determination, the operational parameters will be plotted in the Mollier-Ramzine diagram to facilitate the visualization (Figure 4D).

Considering the lack of data in the literature about mass and energy balances for LHMP production, in this work it was considered as acceptable: mass loss$\leq$ 20%, energy loss $\leq$ 25% and SEC $\leq$ 2.0 x10^4 kJ·kg^{-1}. The drying conditions that attended this established criteria are represented in the Figure 4D through colored zones. The green zone represents the mass loss while the red zone indicates the energy losses (Figure

4D). The region blue, which corresponds to SEC values, is the zone of intercession between all mass and energetic parameters that obey the criteria considered acceptable for LHMP production. Briefly, the blue region contains the ideal conditions for LHMP production.

Since the control shows physicochemical properties very different of the lactose hydrolyzed milk powder, the first one showed ideal operational parameters outside of the area covered by lactose hydrolyzed product (Figure 4D).

Considering only the mass and energetic balances, the best conditions of lactose hydrolyzed milk powder production were binomial temperature/flow rate: 115 °C/0.8 $kg{\cdot}h^{-1}$, 130°C/0.8-1.0$kg{\cdot}h^{-1}$,145°C/1.0$kg{\cdot}h^{-1}$, 160°C/1.0$kg{\cdot}h^{-1}$. On the other hand, powders produced at 115°C/0.8 $kg{\cdot}h^{-1}$ and 130°C/0.8-1.0$kg{\cdot}h^{-1}$ showed water content superior to 6% $w.w^{-1}$ and, for this reason, should be disregarded considering an ideal powder with water content < 5% $w.w^{-1}$.

The powder produced from binomial temperature/flow rate: 145°C/1.0 $kg{\cdot}h^{-1}$, with a_w of 0.20±0.10, exhibited physical characteristics closer to those of the control, and with a relatively low energy cost (Q= $2.9x10^4$ $kJ{\cdot}kg^{-1}$).Under these conditions, the mass loss, energy loss, and SEC were 15%, 22%, and 1.4 $x10^4$ $kJ{\cdot}kg^{-1}$, respectively.

4. Conclusion

The drying of lactose hydrolyzed milk using the same operational parameters applied for traditional product is impracticable for dairy industry and can provoke drawbacks from low yield to clogging of equipment.

In this study, the determination of the ideal operational parameters for LHMP production was carried out by testing drying conditions and analyzing mass and energetic balances. This approach yielded the ideal parameters for LHMP production. We expect that this methodology would readily scale up to industrial spray dryers.

For the lab scale equipment used in this study, the best drying conditions were an inlet air temperature of 145°C and a concentrated milk flow rate of 1.0 $kg{\cdot}h^{-1}$, taking into account the aspects of the powder and the lower energetic cost of the process.

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PART 2: LACTOSE HYDROLYZED MILK POWDER: PHYSICOCHEMICAL AND TECHNO-FUNCTIONAL CHARACTERIZATION

Preamble

The lack of knowledge about lactose hydrolyzed milk powder production along with a growing consumer demand for the product have compelled the dairy industry to produce powders with physicochemical, sensorial and techno-functional characteristics that are very different from traditional milk powder products. Lactose hydrolyzed milk powder most often exhibits the following characteristics: agglomeration, caking, browning and decreased solubility. The results presented in the first part of this chapter showed that the drying operational parameters directly influence the thermodynamic characteristics of the process. The ideal drying parameters for lactose hydrolyzed milk powder production can therefore be determined using mass and energy balance. The objective of this work is to demonstrate the effect of the drying operational parameters on the physicochemical and techno-functional properties of lactose hydrolyzed milk powder.

Our questions:

- Lactose hydrolyzed milk powder produced at same drying parameters as traditional milk powder results in a product with different physicochemical and techno-functional characteristics?
- Are there drying parameters that favor or disfavor the physicochemical and techno-functional properties of lactose hydrolyzed milk powder?
- What are the ideal drying parameters for lactose hydrolyzed milk powder?

Lactose hydrolyzed milk powder: Physicochemical and techno-functional characterization

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Abstract

In order to meet the prospects of the lactose intolerant population, the industry has developed various lactose hydrolyzed dairy products. When the lactose hydrolyzed milk powder (LHMP) is dried using the same operational parameters applied for traditional milk powder (with lactose), the resulting powder tends to suffer a series of problems such as agglomeration, stickiness into spray dryer, caking and elevated hygroscopicity, which promotes decrease physicochemical, sensory and nutritional quality of the product and high rejection at the consumer level. The objective of this work is to is to demonstrate the effect of operational drying parameters ($\theta_{air,in}$= inlet air temperature and m_{CM}=concentrated milk flow rate) on the physicochemical and techno-functional properties of lactose hydrolyzed milk powder. The set parameters for LHMP production were inlet air temperatures ($\theta_{air,in}$) from 115 to 160°C and flow rate (m_{CM}) from 0.3 to 1.5kg·h^{-1} and the powders were evaluated as water content, water activity, color, granulometry and rehydration. In general, LHMP showed water content superior to the control independent of the drying conditions as a consequence of the difficult in drying the product. The physicochemical, sensorial and techno-functional characteristics of the product are extremely dependent on the operational parameters applied to the product. For a lab scale spray dryer, the LHMP produced at $\theta_{air,in}$ = 145°C and m_{CM}= 1.0kg·h^{-1} was the only sample that attended all stipulated quality parameters: water content 93, particle sizes similar to control and complete rehydration. This optimization

is a potential tool for the development of LHMP or new formulations based on whey or not dairy addition in the dairy industry.

KEYWORDS: Physical proprieties; powder technology; spray drying

1. Introduction

The intolerance to lactose present in dairy products is characterized by absence or low production of the enzyme β-galactosidase (lactase) by human organism. The lactase is responsible to hydrolyze the lactose in two monosaccharides (glucose and galactose) which are absorbed by small intestine. If the lactose hydrolysis is not performed by organism, this sugar is fermented in the bowel colon causing gastrointestinal discomforts (GERBAULT et al., 2011).

In order to attend this marketing potential, the dairy industry has constantly developed lactose-free or lactose hydrolyzed products. According to studies carried out by International Dairy Federation (IDF), the consummation of lactose hydrolyzed milk in Italy increased 7.6% only in 2014 (INTERNATIONAL DAIRY FEDERATION IDF, 2015). In South America countries with high incidence of individuals with lactose intolerance, such as the Brazil, recent studies indicate that 5.9% of the new foods or beverage available in the market stamp on their label the information that they are lactose hydrolyzed or lactose-reduced products (MINTEL, 2016). Among the lactose hydrolyzed dairy products currently available in the market, it can be highlighted the production of UHT (Ultra High Temperature) milks, dairy desserts, sweetened condensed milk, yogurt, milk cream, powdered milk mixtures. However, even with the increasing demand, the supply of lactose hydrolyzed milk powder to consumer market is yet a challenge for the dairy industry due to technological drawbacks faced during the production.

When the lactose hydrolyzed milk is dried using the same operational parameters applied for milk drying, the resulting powder tends to suffer a series of problems such as agglomeration, stickiness into spray dryer, caking and elevated hygroscopicity (FERNÁNDEZ; SCHEBOR; CHIRIFE, 2003; JOUPPILA; ROOS, 1994; SHRESTHA et al., 2007). In the case of lactose hydrolyzed milk powder (LHMP), lactose with glass transition temperature (T_{Tg}) equivalent to 101°C is hydrolyzed into glucose (T_{Tg}= 31°C) and galactose (T_{Tg}= 30°C) (ROOS; KAREL, 1990; ROOS, 1993). As a

consequence of carbohydrate hydrolysis, the glass transition temperature of LHMP (36°C when in equilibrium with air of 11% of relative humidity) is significantly smaller than that of traditional milk powder (61°C under the same equilibrated conditions) resulting in a product with physicochemical characteristics quite different of the traditional milk powder (containing lactose) (FERNÁNDEZ; SCHEBOR; CHIRIFE, 2003). Furthermore, the higher content of reducing sugar can favor the browning of powders affecting both the sensory aspect and acceptation by consumers (STAPELFELDT; NIELSEN; SKIBSTED, 1997). As a consequence of these drawbacks, the lactose hydrolyzed milk powder can show less nutritional quality, instability during storage and elevated production cost due to losses during the drying (FERNÁNDEZ; SCHEBOR; CHIRIFE, 2003; NARANJO et al., 2013; SHRESTHA et al., 2007).

Considering that many industries still empirically produce LHMP and these products present a quality inferior to those required by consumers, the objective of this work is to demonstrate the effect of operational parameters of drying on the physicochemical and techno-functional properties of LHMP.

2. Material and Methods

2.1. Material

Raw skim milk with 9% ($w \cdot w^{-1}$) of dry matter was provided by Laticínios Escola (FUNARBE; Viçosa, Brazil). The skim milk powder containing 96% ($w \cdot w^{-1}$) of dry matter was donated by Tangará Foods (Estrela, Brazil). The enzyme lactase Lactomax Super (Prozyn; São Paulo, Brazil) with 60.000 ONPGU$\cdot g^{-1}$of enzymatic activity and 1.2 $g \cdot mL^{-1}$ of density was used to hydrolyze the lactose. A lactase unit (ONPGU) is the amount of enzyme released in a micromole of o-nitrofenil-β-D-galactopiranosídeo for one minute under the assay conditions. The sodium azide (Vetec Química Fina; Duque de Caxias, Brazil) was added to milk in order to prevent the microbial growth.

2.2. Production of lactose hydrolyzed concentrated milk

Concentrated milk at 40% $w \cdot w^{-1}$ of dry matter was prepared from a mixture of raw skim milk and skim milk powder (Figure 1). The mixture was added 0.03% $w \cdot w^{-1}$ of sodium azide, submitted to pasteurization (65°C·30 min^{-1}) and then cooled quickly and kept at 40°C (Figure 1). To concentrated milk it was added 0.6% $w \cdot w^{-1}$ of enzyme lactase

and the lactose hydrolysis process was carried out during 4h (≥ 90 % hydrolysis). The lactose hydrolyzed concentrated milk was cooled at 7°C and kept under cooling until the spray drying. As a control of the process, concentrated milk was prepared as described before without the addition of lactase enzyme (Figure 1).

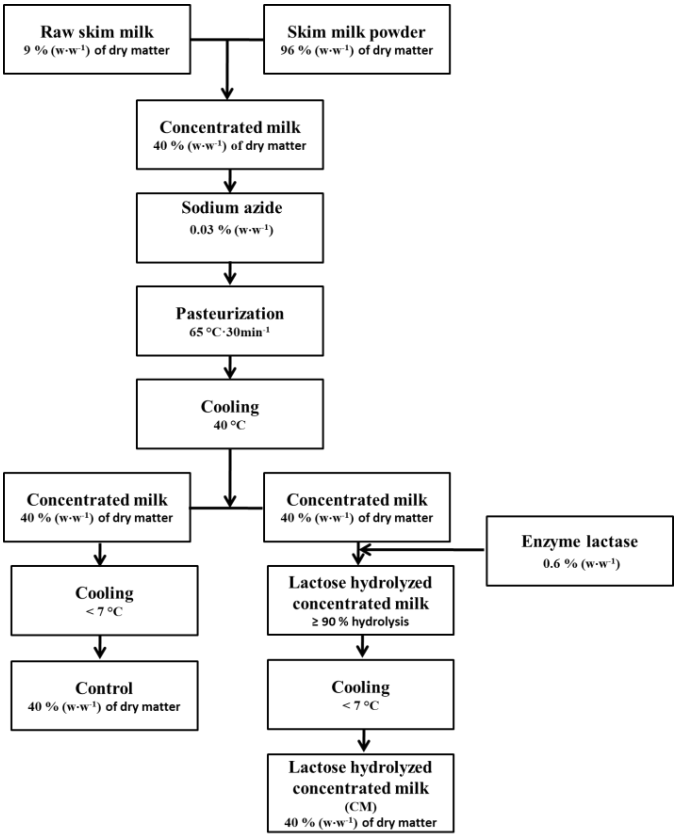

Figure 1. Production of the lactose hydrolyzed concentrated milk and the concentrated milk (control).

2.3. Production of lactose hydrolyzed milk powder (LHMP)

Lactose hydrolyzed concentrated milk or concentrated milk were heated at 40 ± 1°C, and injected into a pilot scale spray dryer model MSDi 1.0 (Labmaq do Brasil, Brazil), single stage and nozzle atomizer belongings to the Inovaleite (Viçosa, Brazil). Probes connected to thermohygrometer HigroFlex 5 (Rotronic; Bassersdorf, Switzerland) were positioned to spray dryer in order to monitor the temperature (°C) in the inlet and outlet air (SCHUCK et al., 2005). The lactose hydrolyzed concentrated milk was injected into spray dryer at flow rates varying from 0.3 to 1.5 $kg \cdot h^{-1}$ while the inlet

air temperature varied from 115 to 160°C. Aiming to obtain traditional milk powder at 0.20 ± 0.1 of water activity and without stickiness or caking phenomenon, the ideal parameters for drying were previously determined and applied in this study: flow rate of concentrated milk at 1.5kg·h^{-1} and inlet air temperature at 160°C. The milk powder containing lactose was used as control of the production. The powders (LHMP and control) were put up in laminated plastic bags under vacuum conditions and storage at 10°C.

2.4. Powder analyses

2.4.1. Moisture content and dry matter

The moisture content was analyzed according to the weight loss after drying 3g of the concentrated milk sample and 1.5g of the milk powder with sand in an oven at 105°C for 5h (SCHUCK et al., 2005a). The dry matter (DM) was calculated from equation 1, where M indicates the moisture content in each sample.

$$DM = 100\% - M \quad (1)$$

2.4.2. Water activity (a_w)

The water activity (a_w) was determined at 25°C by Aqualab (Decagon 3TE, Decagon Devices Inc., USA).

2.4.3. Colorimetry analyses

The color analysis of the powders was carried out by direct lecture of the reflectance using coordinates “L*” (luminosity), “a*” (intensity of red and green) and “b*” (intensity of yellow and blue), applying CIELAB color scale, with illuminant D65 and observation angle of 10°, using a colorimeter Colorquest XE (Hunter Lab, Reston, USA).

2.4.4. Quantification of the 5-hydroxymetylfurfural free (HMF)

The milk powder was diluted in distilled water (1:13) (w·w^{-1}) and deproteinized by the addition of 2.5mL oxalic acid 0.3 moL·L-1 and 2.5 mL trichloroacetic acid 40% (w·v^{-1}). After filtration, 4mL of the filtrate was collected and 1mL of 0.05moL·L^{-1} thiobarbituric acid was added. The solution was heated at 40°C for 30 minutes and

cooled to 25°C. The absorbance reading was performed by UV visible spectrophotometer (Global Trade Technology, São Paulo, Brazil) with wavelength of 443 nm. In order to determine the concentration in $mg \cdot kg^{-1}$, an analytical curve was constructed using different levels of HMF (KEENEY; BASSETTE, 1959).

2.4.5. Scanning electronic microscopy (SEM)

The samples morphology was evaluated using electronic microscopic (Hitachi TM 3000, Hitachi Ltd., Tokyo, Japan). That equipament belongs to the Núcleo de Espectroscopia e Estrutura Molecular of Departamento de Química of Universidade Federal de Juiz de Fora (UFJF).

2.4.6. Particle size analysis by laser diffraction

Lactose hydrolyzed milk powder (LHMP) was rehydrated in distilled water and the size of the particles in suspension was determined using a diffraction laser analyzer (Beckman Coulter LS 13 320, Miami, FL, EUA) coupled with an aqueous liquid module, (Beckman Coulter, Miami, FL, EUA). The results were obtained using an index of 1.33 for dispersant media (water) and 1.57 for particles (casein micelles) (BUSHILL et al., 1965; MIMOUNI; SCHUCK; BOUHALLAB, 2005). That equipament belongs to the Núcleo de Espectroscopia e Estrutura Molecular of Departamento de Química of Universidade Federal de Juiz de Fora (UFJF).

3. Results and discussion

In the milk drying by spray dryer, the intensity of thermal treatment suffered by the milk particles is primarily regulated by the inlet air temperature ($\theta_{air,in}$) and flow rate of concentrated milk (m_{CM}). By the combination of these operational parameters, it is possible to produce milk powders with different characteristics of water content, color, granulometry and rehydration (GRIGIONI et al., 2007; SCHUCK, 2011; STAPELFELDT; NIELSEN; SKIBSTED, 1997).

Table 1. Drying settings (*n* = 3).

Sample	Inlet		
	Air temperature ($\theta_{air,in}$;°C)	Concentrated milk flow rate (m_{CM};kg·h^{-1})	Dry matter (DM_{CM};kg·kg^{-1})
Control*	160 ± 2	1.5 ± 0.1	72.6 ± 1.3
Lactose hydrolyzed milk powder (LHMP)	160 ± 2	1.5 ± 0.1	76.0 ± 1.0
		1.3 ± 0.1	79.1 ± 1.4
		1.0 ± 0.1	82.8 ± 1.7
		0.8 ± 0.1	85.8 ± 1.5
		0.5 ± 0.1	92.8 ± 1.4
		0.3 ± 0.1	89.4 ± 1.7
	145 ± 2	1.5 ± 0.1	59.0 ± 0.9
		1.3 ± 0.1	63.8 ± 1.1
		1.0 ± 0.1	68.7 ± 1.3
		0.8 ± 0.1	69.6 ± 0.9
		0.5 ± 0.1	79.4 ± 1.2
		0.3 ± 0.1	87.9 ± 1.2
	130 ± 2	1.5 ± 0.1	53.1 ± 0.8
		1.3 ± 0.1	58.6 ± 1.1
		1.0 ± 0.1	62.0 ± 1.0
		0.8 ± 0.1	66.1 ± 1.2
		0.5 ± 0.1	72.7 ± 1.5
		0.3 ± 0.1	71.8 ± 1.6
	115 ± 2	1.3 ± 0.1	50.8 ± 0.8
		1.0 ± 0.1	55.0 ± 0.7
		0.8 ± 0.1	59.3 ± 1.0
		0.5 ± 0.1	64.5 ± 1.1
		0.3 ± 0.1	64.9 ± 0.9

3.1. Physicochemical of lactose hydrolyzed milk powders

The spray drying operation aims to remove free water from the skim milk particles to produce powders with low water content (<5% $w.w^{-1}$) and water activity (a_w<0.20) (ALIMENTARIUS, 2011; SCHUCK, 2011). The maintenance of these values is extremely important to guarantee a product with pleasant sensory characteristics and, at the same time, to permit the storage of powders for long periods at room temperature.

For the milk powder (control) produced under precise experimental conditions ($\theta_{air,in}$=160°C; m_{CM}= 1.5kg.h^{-1}), the water content and the water activity assumed values of 3.7% $w.w^{-1}$ and 0.20, respectively, (Figure 2A and B). The lactose hydrolyzed milk powder showed water content superior to 4.1% $w.w^{-1}$ regardless of the drying conditions (Figure 2A); but some powders showed water activity close or inferior to control (0.20; Figure 2B).

Considering that the capacity of water binding of glucose and galactose is higher than lactose, the water content of the lactose hydrolyzed milk powder being superior to milk powder containing lactose, in the same drying conditions, can be considered satisfactory for this kind of product. At the same time, because water is linked to these monosaccharides, the lactose hydrolyzed milk powders can show water activity inferior or equal to that established for control (a_w=0.20). Schuck et al. (2005a) reported productions of lactose hydrolyzed milk powders with water content superior to 5% $w.w^{-1}$ of moisture and water activity inferior to 0.20 which corroborates with our finding.

It was also observed that by increasing $\theta_{air,in}$ or decreasing m_{CM}, the water content and water activity of powders tended, in most cases, to decrease (Figure 2A and B). It was expected that for powders produced at low m_{CM} (0.3 kg.h^{-1}) and high $\theta_{air,in}$ (> 130°C) the water content as well as the water activity should be the smaller among the treatments, but it was not observed by experimental results (Figure 2A and B). This finding can be explained by the rigorous thermal treatments, which probably allowed to glass transition. The glass transition increases molecular mobility due to the decrease of viscosity favoring the glucose and galactose to suffer Maillard reaction producing browning compounds and releasing water (NARANJO et al., 2013; PEREYRA GONZALES et al., 2010; ROOS; JOUPPILA; ZIELASKO, 1996). The water produced by the reaction is probably responsible by increasing water content and activity in these powders

(m_{CM}=0.3 kg.h-1; $\theta_{air,in}$> 130 °C). This finding can be explained by the fact that all treatments with m_{CM}=0.3 $kg.h^{-1}$ e $\theta_{air,in}$=115°C-160°C showed a loss of 65% or more of water during the drying process, thus this increase of water content and activity are due to the Maillard reaction. Furthermore, the lactose hydrolyzed milk powders showed browning coloration (detail figure 2A and figure 2C) which is a good indicative of accumulation of Maillard reaction compounds as dicarbonyl compounds, redutones and derivates of hydroxymethyl furfural (HMF) (LE et al., 2011, 2013; MARTINS; JONGEN; VAN BOEKEL, 2000). To confirm this hypothesis, additional HMF analyses were carried out and revealed that these powders probably contain higher amounts of brown products which are related to the greater intensity of the Maillard reaction (Figure 2D).

Le et al. (2011) also observed a relationship between the increase of the concentration of HMF in milk powder and the increase of the browning colour due to the presence of melanoidines with intensification of the Maillard reaction. The combination of high m_{CM} (1.5$kg.h^{-1}$) with low $\theta_{air,in}$ (<130 °C) resulted in powder with very high moisture (>14.5% $w.w^{-1}$) and water activity (a_w>0.40) (Figure 2A and B). Under these drying conditions, insufficient thermal energy is provided to milk particles and water is not efficiently removed from the product. In addition, it was also observed that the powder with high moisture suffered stickiness and caking during or after drying operation (detail figure 2A). According to SCHUCK et al. (2005a) the water has a very low glass transition temperature (T_{Tg}=-139°C) and its removal is primordial to guarantee the stability of dairy powders. In other words, the water content decreases the T_{Tg} of the lactose hydrolyzed milk powder being the product subject to suffer glass transition during the drying process. As example, for a powder with moisture of 6%, the T_{Tg} is 13 °C while for a powder with moisture of 12%, the T_{Tg} is - 28°C (Schuck et al., 2005a).

Based on reports found in the literature it is possible to infer that the powders with caking aspect (detail figure 2A) were resulting from the glass transition of lactose-hydrolyze milk powder during drying (FITZPATRICK et al., 2007; JOUPPILA; KANSIKAS; ROOS, 1997; ROOS; DRUSCH, 2015; ROOS, 2002). Considering only powders with moisture <5% $w.w^{-1}$, a_w <0.20 and luminosity value > 93 as those with physicochemical properties closer to control, the best combinations of drying temperature and milk flow rates were : $\theta_{air,in}$ = 115°C/m_{CM}=0.5 $kg.h^{-1}$ and $\theta_{air,in}$ = 145°C/ m_{CM}=1.0 to 1.3 $kg.h^{-1}$.

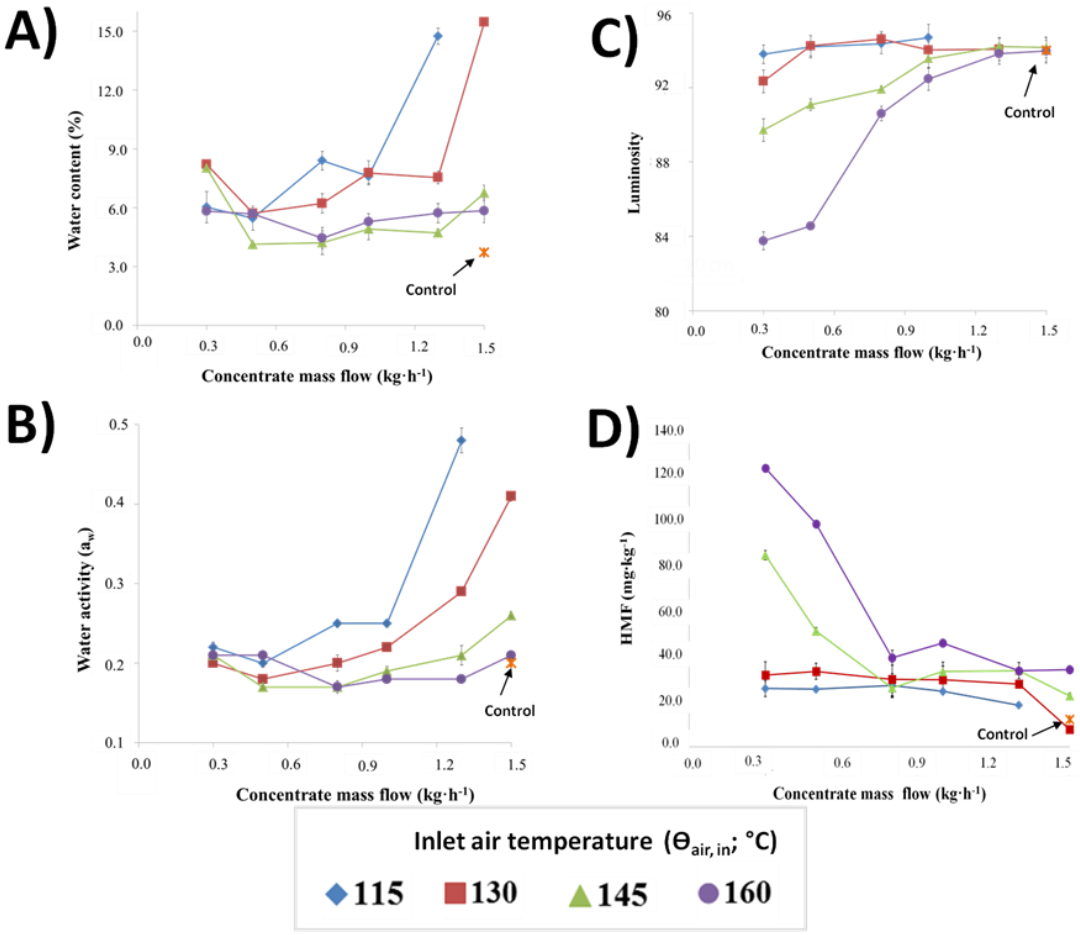

Figure 2. Effect of lactose hydrolyzed milk powder drying parameters. Water content (a), water activity (b), luminosity (c) and hydroxymethylfurfural formation (d). Control: milk powders without lactose hydrolysis. *Some analysis was not performed due to powder caking. Note: HMF, hydroxymethylfurfural.

3.2. Techno-functional properties of lactose-hydrolyze milk powders

The physicochemical analyses allow inferring about some sensory aspects of the powders as well as about its preservation during storage. However, in this section, additional analyses were performed to better understand the behavior of lactose-hydrolyze milk powders during the utilization by consumers.

By scanning electron microscopy (SEM) it was possible observe that the milk powder without lactose hydrolysis (control) showed particles varying from 3.3 to 23.7µm whether or not associated in small agglomerates (Figure 3). The lactose-hydrolyze milk powders that suffered caking in the equipment during drying (Figure 2A) showed a more compact morphology when compared to control (Figure 3A).

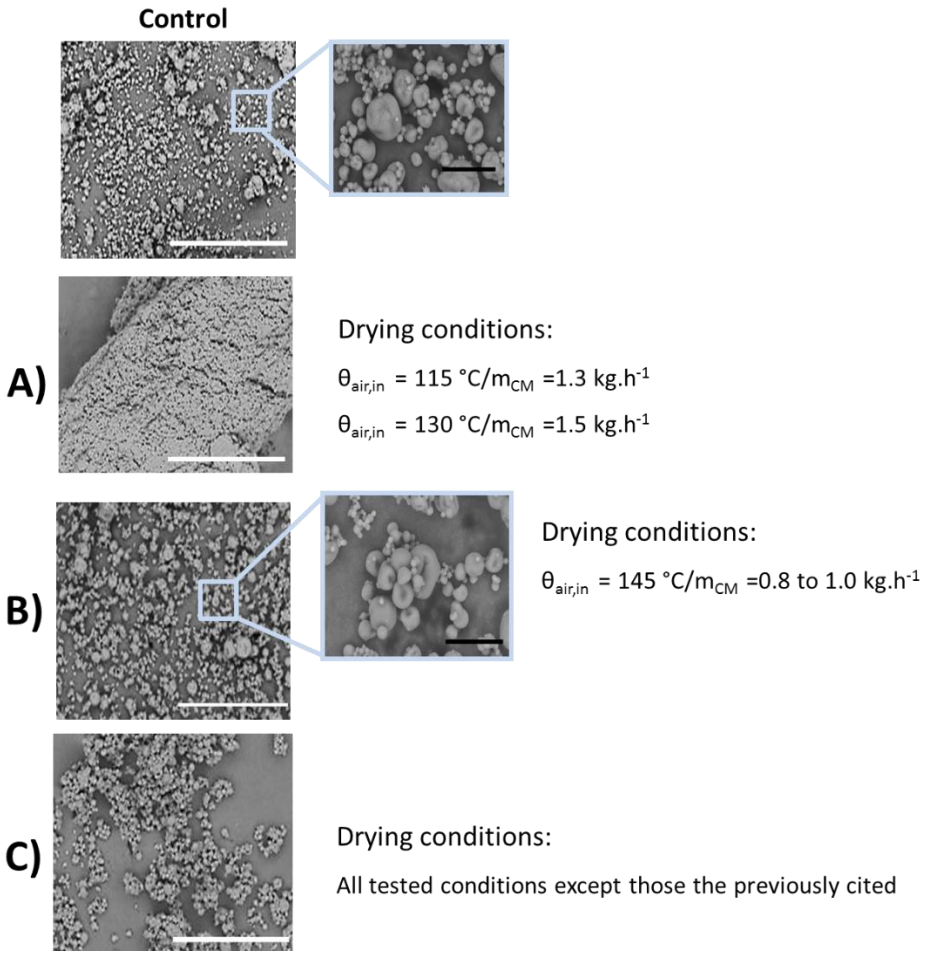

Figure 3. Scanning electron microscopy of lactose-hydrolyze milk powders produced at different drying conditions. (a) Powders with elevated agglomeration; (b) powder with characteristics closer to control and (c) powders with moderate agglomeration. Control: milk powder without lactose hydrolysis. White scale bar: 300 µm, black scale bar: 30 µm.

Probably, the high agglomeration of particles was caused by elevated moisture present in the powders (> 14.5% w.w^{-1}; Figure 2A) which was responsible for the low T_{Tg} of these products (FITZPATRICK et al., 2007; ROOS; DRUSCH, 2015). According to Mimouniet al. (2009), the rehydration process depends on the rupture of agglomerates and posterior exposition of the particles to aqueous phase. From the functional point of view, this can be a drawback for consumers during the preparation of this product at home or during the use of the powder in formulation of foods with lactose free appeal.

Otherwise, lactose hydrolyze milk powders produced under precise conditions ($\theta_{air,in}$ = 145°C/ m_{CM}=0.8 to 1.0kg.h^{-1}) showed particle sizes very similar to control (Figure

3B). Furthermore, it was observed a very high similarity between the morphology of particles with the control (detail in figure 3B).

All other combinations $\theta_{air,in}$ and m_{CM} resulted in powders with moderated agglomeration, however, higher than control (Figure 3C). Despite the agglomeration is not excessive, it was observed a high rehydration time in relation to control.

When dispersed in distilled water, the control showed particles in suspension with size inferior to 0.5 µm indicating an efficiently dispersion of the product in water. Considering that the casein micelles have mean diameter varying between 0.05 and 0.5 µm (FOX; MCSWEENEY, 1998), it is possible affirm that all small agglomerations observed in the control (Figure 3) were completely dispersed in water during rehydration. Fat globules can show higher mean diameters when dispersed in water (4 µm) (BAUMAN et al., 2006), however, once the milk was previously skimmed, fat globules were not detected by laser diffraction analyses.

Powders produced using high temperatures ($\theta_{air,in}$ >145°C) and low flow rate (m_{CM}=0.3kg·h^{-1}), no matter the inlet air temperature, did not show complete dispersion in water (Table 1). Under these drying conditions, the thermal treatment may have been excessive conducting to formation of insoluble compounds or agglomerations. The powders showed browner color (Figure 2C) suggesting, for example, the formation of Maillard products or compounds originated from caramelization reaction. According to Le and collaborators (2011), in dairy powders the evolution of the Maillard reaction and the cross-linking between the casein micelles caused by heat treatment are the main factors that reduce the dispensability of dairy powders in water.

Contrary to expected, powders that suffered caking (figure 2A) were completely dispersed in water during the rehydration (Table 1). According to Schuck et al. (2005a) the lactose hydrolyze milk powder does not crystalize due to high thermoplasticity of the glucose and galactose compared to lactose. Based only on microscopy and rehydration analysis, the powders with characteristics closer to control were produced at $\theta_{air,in}$ = 145°C/m_{CM}=0.8 to 1.0 kg.h^{-1}.

Table 2. Particle size distribution (% volume). Particles after rehydration with hydrodynamic diameter ≤0.5 µm for lactose-hydrolyze milk powders (*n* = 3).

Inlet air temperature ($\theta_{air,\,in}$, °C)	**Lactose hydrolyzed concentrated milk flow rate (m_{CM}, kg·h^{-1})**					
	0.3	**0.5**	**0.8**	**1.0**	**1.3**	**1.5**
115	68	100	100	100	100	*
130	90	100	100	100	100	100
145	0	0	100	100	100	100
160	0	0	0	6	96	100

*It was not possible produce powder at this condition. Lactose hydrolyzed milk powder (LHMP) was rehydrated in distilled water and the size of the particles in suspension was determined using a diffraction laser analyzer coupled with aqueous liquid module (index of 1.33 for dispersant media and 1.57 for particles: casein micelles).

4. Conclusion

Considering the best results for physicochemical and techno-functional analysis, it is possible conclude that the lactose hydrolyze milk powder produced at $\theta_{air,in}$= 145°C/m_{CM}=1.0 kg.h^{-1} was the only sample that attended all stipulated quality parameters. This finding reinforces the idea that the production of lactose hydrolyze milk powder using the same operational drying parameters used to produce the milk powder without lactose hydrolysis results in a food with lower sensory and functional quality. Despite this study has been applied for a production in laboratory scale, this same approach can be used by dairy industries in order to determine the best operational parameters of drying for lactose hydrolyze milk powder or new formulations based on the addition or not of whey or non-dairy additives.

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CHAPTER IV

STRUCTURAL AND FUNCTIONAL PROPERTIES OF LACTOSE HYDROLYZED MILK POWDER

PART 1: SUGAR TYPE MATTERS IN SPRAY DRYING: HOMOGENEOUS DISTRIBUTION IN MILK POWDER FAVORS REPULSIVE INTERACTIONS BETWEEN PROTEINS

Preamble

When subjected to the same drying parameters as traditional milk powder, lactose hydrolyzed milk powder demonstrated different process characteristics and product quality. In addition, lactose hydrolyzed milk powder was more sensitive to drying parameter changes. The first part of Chapter 2 aims to understand the importance of galactose and glucose in the organization and dynamics of milk powder molecules.

Our questions:

- Does the type of sugar present in the concentrated milk impact the organization and dynamics of the molecules present in the powder?
- How the technological problems of lactose hydrolyzed milk powder are related to the presence of galactose and glucose?

Sugar type matters in spray drying:

Homogeneous distribution in milk powder favors repulsive interactions between proteins

The content of this first part of the chapter IV was submitted to Food Structure.

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Abstract

Lactose hydrolyzed milk powder production remains a challenge for the dairy industry because of specific technological problems, such as stickiness and caking during drying. The molecular mechanisms that lead to these problems are poorly understood. The aim of this study is to provide a better understanding of the impact of lactose hydrolysis on milk submitted to spray drying. In order to do so, the organization and dynamics of lactose hydrolyzed milk powder molecules were analyzed after this was being processed. In general, the lactose hydrolyzed milk powder sample structure was characterized by a greater numbers of monosaccharides around proteins compared to the lactose found in a traditional milk powder sample. These differences are explained by a combination of greater repulsive interactions between proteins as well a stronger attractive interaction between proteins and monosaccharides. Our data indicated that this difference in the molecular organization impacted milk powder's hydration kinetics.

KEYWORDS: milk powder, lactose hydrolysis, SAXS, RMN.

1. Introduction

Lactose intolerance is a metabolic food sensitivity that affects approximately 75 % of the world's population in different levels (Lule et al., 2015; Luthy et al., 2017). When intestinal mucosal cells do not produce adequate levels of β-galactosidase

(lactase), lactose reaches the colon and is used as an energy source by local microbiota resulting in abdominal discomfort, bloating, gas and diarrhea (Gerbault et al., 2011; Luthy et al., 2017). The high occurrence of lactose intolerance emphasizes the importance of developing lactose hydrolyzed dairy products to reach this potential market.

Despite increasing demand for lactose hydrolyzed milk powder, consumer supply remains a challenge for the dairy industry due to technological problems during production such as unwanted particle adhesion, caking, stickiness and browning in the spray dryer (Fernández, Schebor, & Chirife, 2003; Fitzpatrick et al., 2007; Torres et al., 2017). These issues can lead to low production yield, operational problems and handling issues (Fialho et al., 2018; Fialho et al., 2018; Shrestha et al., 2007; Torres et al., 2017).

According to Fernández, Schebor, & Chirife (2003), lactose hydrolysis decreases the glass transition temperature (T_{Tg}) of milk powder from approximately 61°C to 36°C when in equilibrium with a 11% relative humidity in ambient air. Because of this significant T_{Tg} reduction, the of lactose hydrolyzed milk powder undergoes easier glass transition in the spray dryer which results in the previous cited technological drawbacks during production.

Recent studies have focused on the drying process and the physicochemical, sensory and technofunctional characteristics of hydrolysed powdered milk (Fernández, Schebor, & Chirife, 2003; Fialho et al., 2018, Fialho et al., 2018; Fitzpatrick et al., 2007; Shrestha et al., 2007, Torres et al., 2017). However, the impact of lactose hydrolysis on the molecular structure of milk powder had not been investigated. The understanding of the effect of the presence of galactose and glucose on the molecular organization of the milk powder particles is important, because through this knowledge it may be possible to search for technology additives and/or process improvements that allow better drying performance of the lactose hydrolyzed milk powder.

Thus, the objective of this study is to understand the importance of galactose and glucose in the organization and physicochemical comportament of the milk powder molecules.

2. Material and methods

2.1. Production of milk powder

Raw skim milk was concentrated at 50 ± 2% ($w \cdot w^{-1}$) of dry matter in a pilot vacuum evaporator (GEA-PE, St Quentin en Yvelines, France) at 65°C with falling stream, without forced recirculation and evaporation capacity of 50$kg \cdot h^{-1}$. The concentrated milk was cooled to 5 ± 2°C and then 0.1 $g \cdot L^{-1}$ of Maxilat LGi lactase enzyme (DSM, The Netherlands) was added until complete hydrolysis after 24h. Experimental enzyme analysis revealed 60,000 ONPGU$\cdot g^{-1}$ (o-nitrophenyl-b-D-galactopyranoside Units$\cdot g^{-1}$) and density between 1.1 and 1.3 $g \cdot mL^{-1}$. Traditional concentrated milk, without addition of the enzyme lactase, was used as control.

Next, traditional and the lactose hydrolyzed concentrated milks were heated to 45 ± 1°C and injected into a Mobile Minor pilot scale spray dryer (GEA-PE, St Quentin en Yvelines, France) fitted with a bi-fluid nozzle atomizer and set to maximum evaporation capacity from 7kg of water per hour.

The drying parameters were: inlet air temperature: 138 ± 1°C, flow rates:1.1 $kg \cdot h^{-1}$ and outlet air temperature: 72 ± 1°C for both traditional and lactose hydrolyzed milk powder samples. Under these conditions, adhesion and the aggregation are not expected to occur. After drying, the milk powders were packed in plastic bags and refrigerated (maximum temperature of 10°C) until the analyses could be carried out.

2.2. Powder analyses

2.2.1. Simulation of the drying process

The drying kinetics of the concentrated milk samples were measured using a gas exchange method (Peixoto et al., 2017). This technique is based on the exchange of water vapor between two recipients; one containing the concentrated milk 40% ($w \cdot w^{-1}$) sample to be tested and the other a solution of polyethylene glycol (PEG) 7% ($w \cdot w^{-1}$), size 3.5 kDa (Sigma Aldrich, USA). Both solutions were placed in a hermetically sealed environment at 20°C until equilibrium was established. The experiment was monitored by weight measurement for 21 days.

2.2.2. Water activity (a_w)

The water activity (a_w) of the sample powders was determined at 25°C using Aqualab Internal Temperature Control (Decagon 3TE, Decagon Devices Inc., USA).

2.2.3. Moisture content

The moisture content of the sample powders was analyzed by weight loss after drying 1.5g of the milk powder with sand in an oven at 105°C for 5h (Schuck et al., 2005).

2.2.4. Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) was used to visualize the surface structure of the samples milk powder particles. The milk powder samples were fixed with carbon double-sided adhesive tape and coated with platinum (conductive material). The images were captured using a FEI Quanta 400 FEG scanning electron microscope (Oregon, United States) operating at an accelerating voltage of 5 kV (Mimouni et al., 2010). The images of all the powdered milk samples inserted in the equipment were collected. Micrographies were measured using ImageJ 1.51K (Wayne rasband, USA).

2.2.5. Nuclear magnetic resonance (NMR)

The solid-state nuclear magnetic resonance analyses were carried out with a Bruker Avance I 400MHz (9,4 T) spectrometer (Bruker, Billerica, USA) fit with a 4mm probe and set to 10000Hz rotation. NMR Proton spectra (^{1}H) of the milk powder samples were obtained using onepulse (zg), with a recycle delay of 5s, a field of radiofrequency of 60kHz and including 16 accumulations and inversion-recovery of 90°-180°. The chemical shifts were given in ppm using to the carbons in tetramethylsilane (TMS) as an external reference for ^{1}H NMR spectra. The spectras were processed with the TopSpin 4.0 software (Bruker, Billerica, USA) in order to obtain the longitudinal relaxation time (T1), a measure of the time required for isotope ^{1}H's nuclear spin under a magnetic field. This procedure was carried out to attain thermal equilibrium after the radiofrequency pulse perturbation in order to evaluate molecule dynamics (Hinrichs et al., 2004). PROMILK®85 (Ingredia, France), an 85% milk protein concentrate was used as

a protein standard to evaluate the internal dynamics of the traditional milk powder sample and the lactose hydrolyzed milk powder samples.

2.2.6. Total rehydration time

Particle size distribution was measured during the dissolution of milk powder in distilled water 8% ($w \cdot w^{-1}$) under agitation 300rpm for 24h. The initial rehydration time was considered at the time the water was added to the dust samples. The aliquots of solution were collected at intervals of 5, 30, 300, 480 and 1440 minutes and analyzed on a Malvern Mastersizer 2000 particle size analyzer (Malvern Instruments Ltd., Malvern, UK) using the refractive index of 1.33 and 1.30 for solvent and particle, respectively (Richard et al., 2013). The casein micelles have average diameters ranging between 0.05 to 0.5µm (Fox et al., 2015). So, the efficient dispersion of the samples in water was obtained when 90% of the sample powder particles were the same size as or smaller than 0.5µm ($D_{90} \geq 0.5\mu m$).

3. Results and discussion

3.1. Impact of Lactose hydrolysis on the osmotic pressure of concentrated milk

To study the impact of lactose hydrolysis on the drying process, the osmotic pressures of the two samples of concentrated milk were measured.

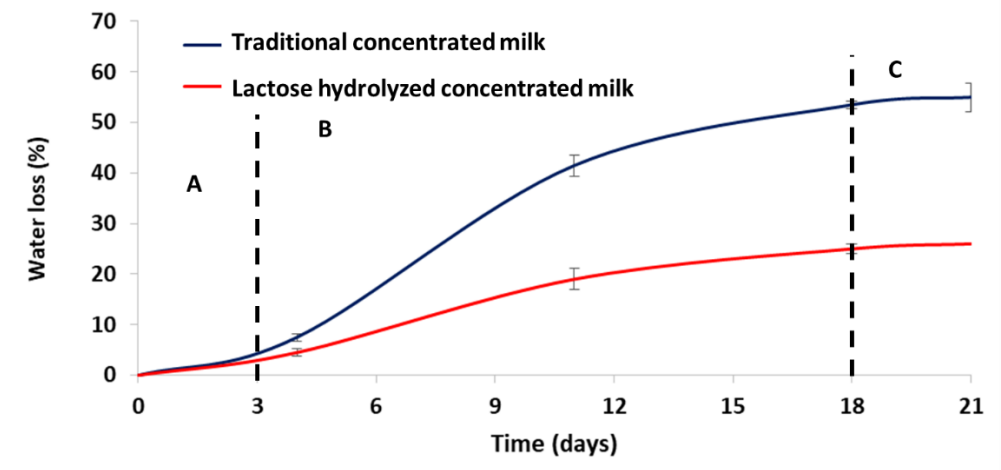

Figure 1. Spray drying water loss: traditional concentrated milk vs. lactose hydrolyzed concentrated milk.

During the dehydration time, using a 7% PEG solution ($w \cdot w^{-1}$), three different stages were observed. In Stage A, the traditional concentrated milk and the lactose

hydrolyzed concentrated milk samples demonstrated slow dehydration kinectics (Figure 1). By Stage B there was an increase in dehydration kinetics for both milk concentrate samples, but the traditional concentrated milk sample showed a faster loss of water than the lactose hydrolyzed concentrated milk sample (Figure 1). At this stage, lactose crystals in the traditional concentrated milk sample were visible to the naked eye (data not shown). In stage C, the dehydration levels in both concentrated milk samples were rougly stabilized. Maximum dehydration data showed that water loss for the traditional concentrated milk sample was approximately twice higher than that of the lactose hydrolyzed concentrated milk sample (Figure 1). Even under conditions of extreme dehydration, monosaccharide crystals in the lactose hydrolyzed concentrated milk were not visible to the naked eye. According to Schuck, et al. (2005), galactose and glucose favor the increasing of the sample heat capacity (ΔCp) that can make monosaccharide crystallization difficult in contrast to lactose.

Actually, it is expected that the lactose hydrolysed concentrated milk displays a greater osmotic pressure than the traditional concentrated milk once the hydrolysis of disaccharide increases the number of free molecules in solution and the osmotic pressure is directly related to the number of free-diffusing molecules in solution (see supporting information for the mathematical description) displaying a repulsive inter or intra interactions.

However, the difference in osmotic pressures for the samples was much greater than expected. Theoretically, the difference in saccharide contents of the two samples should induce a difference in osmotic pressures of about 20% (see supporting information for details). However, our experiments show that the measured difference of osmotic pressure between samples reached about 70%. The discrepancy between theory and practice for these measurements could be related to lactose's greater tendency to display an attractive self-interaction, leading to crystallization.

Noteworthy, right after they were produced, the traditional and lactose hydrolyzed milk powders showed water activity of 0.15 and 0.16, respectively, and moisture of 3.0% and 3.9%, respectively. This behavior has been explained simply by the stronger hydration capacity of monosaccharides (Smith & Lomauro, 1984). The higher content of water in lactose hydrolyzed milk powder corroborates the results found in the osmotic measurement. A higher hydration capacity of the lactose hydrolyzed milk

powder produced by spray dryer as well in concentrated solutions produced by room temperature evaporation (Figure 1). The question now is to check whether this higher water content found during the drying of the lactose hydrolyzed milk powder can also be explained by the lesser tendency of glucose and galactose to display an attractive self-interaction, leading to crystallization.

3.2. Impact of lactose hydrolysis on milk powder particles

3.2.1. Particle microstructure

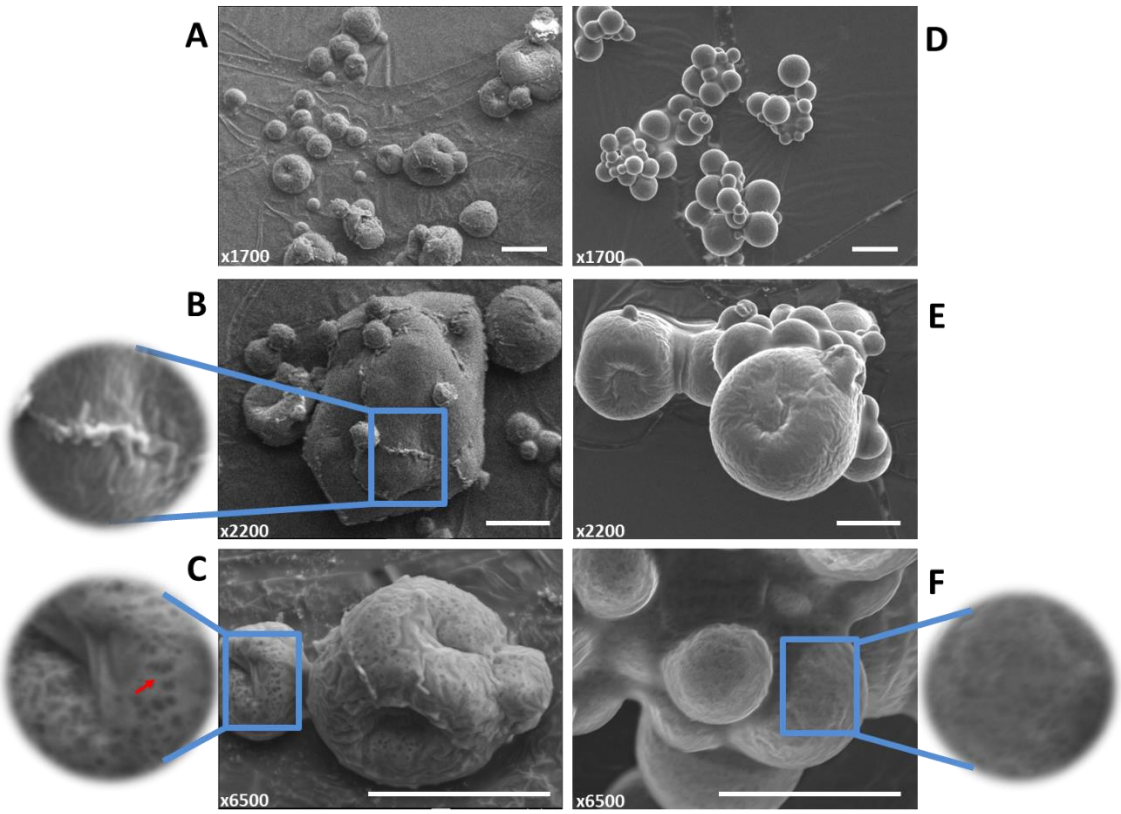

Figure 2. SEM micrographs of traditional milk powder (left) and lactose hydrolyzed milk powder (right). Red arrow: vacuoles; White scale bar: 10µm.

To determine the microstructural impact of hydrolysis on the two milk powder samples, SEM analyses of both samples were carried out.

SEM micrographs showed that the powder particles of the traditional and lactose hydrolyzed milk powder presented different characteristics. Traditional milk powder particles exhibited low agglomeration (Figure 2A) and surface lactose crystals in

triangular, pyramidal and needle-like forms according to Parimaladevi & Srinivasan (2014) and with an average size of 2 µm (Figure 2B) and presence of vacuoles (maximum size 1µm) (Figure 2C). Conversely, the lactose hydrolyzed milk powder particles exhibited agglomeration (Figure 2D), homogeneous surfaces without the presence of vacuoles and sugar crystals (Figure 2E and F).

The presence of nano-scale lactose crystals in traditional milk powder (Figure 2B) soon after manufacture, is common according to Tamime (2007) because skimmed milk powder has a high concentration of lactose. It has been suggested that crystal formation is due to lactose molecules high hygroscopicity as well as ambient humidity which favors crystal formation on the surface of the particles (Fitzpatrick et al., 2007). However, lactose hydrolysis increases the hygroscopicity of the powder (Fernández, Schebor, & Chirife, 2003) and the SEM analysis here show that there is no galactose and/or glucose crystal formation in the lactose hydrolyzed powder. This complies with the interpretation of previous experiments in concentrated solutions.

The vacuoles present on the surface of the traditional milk powder particles (Figure 2C) could be caused by spray dryer water removal. Previous studies have shown that drying at elevated temperatures removes surface water from particles and increases the viscosity of the area resulting in a dry, hard surface (Kim, Chen, & Pearce, 2009; Westergaard, 2004). This barrier hinders water vapor and air diffusion which then causes the particles to increase in size. As the powder moves to the cooler regions of the spray dryer, the particles unswell and enable water vapor condensation inside the vacuoles. This decrease in particle volume damages particle surface by exposing the vacuoles to the external environment (Kim, Chen, & Pearce, 2009; Westergaard, 2004). After spray drying milk powders, Nuzzo et al. (2017) observed the presence of vacuoles (maximum size of 3 µm) on the particle surfaces.

According to Kim et al. (2009) and Roos (2002), when milk powder reaches the glass transition temperature, there is a decrease in particle viscosity. Therefore, the absence of vacuoles on the surface of the lactose hydrolyzed milk powder (Figure 2F) may be caused by a decrease in viscosity during drying which would allow for better component distribution and result in a more closed surface. Another possibility is that this effect is induced by a slower dehydration kinects of the lactose hydrolyzed concentrated milk. Anyway, the absence of such vacuoles in the lactose hydrolyzed

powder demonstrates that the type and the quantity of saccharides in the powder sample have an important impact on the molecular reorganization of the powder particules during the spray drying process.

3.2.2. Molecular organization of saccharides and protein dynamics

Figure 3 shows the spectral profiles of the traditional milk powder and lactose hydrolyzed milk powder samples obtained by NMR spectroscopy.

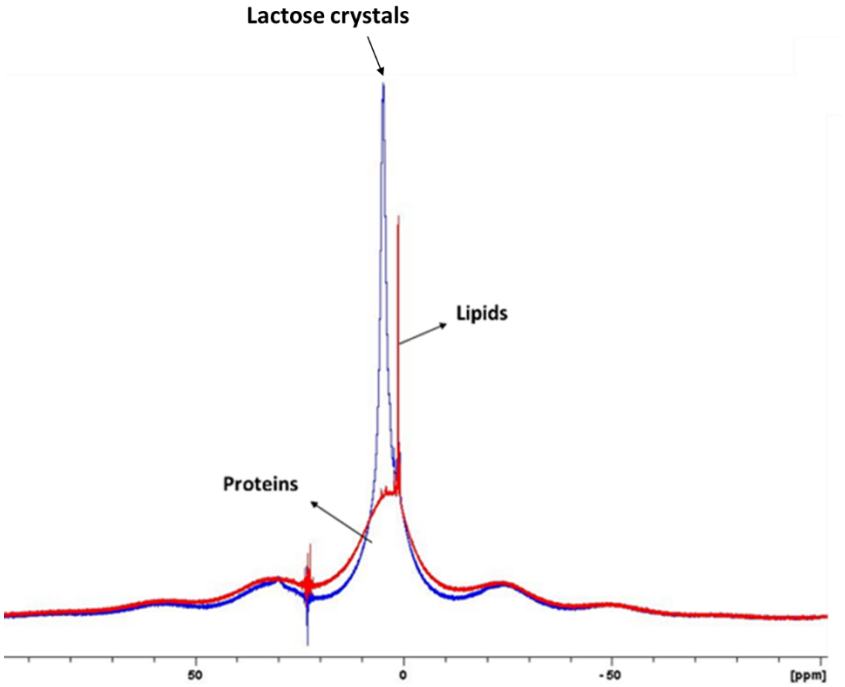

Figure 3. ^{1}H NMR quantitative spectra of traditional milk powder (blue) and lactose hydrolyzed milk powder (red) samples.

Both spectra showed sharper and wider signals in the chemical shift range from 20 to -10 ppm (Figure 3). The sharp signal at this band corresponds to the NMR spectral signature of lipids (Brescia et al., 2004; Nasser et al., 2017). The broad signal width corresponds to the proteins (Nasser et al., 2017). The traditional milk powder sample displayed an additional and relatively sharp peak that was not found in spectral profiles of the lactose hydrolyzed milk powder sample. The peak position and the width Hargreaves (1995) and Nidhi et al. (2011) suggest that this peak corresponds to lactose protons in crystalline form. The absence of a similar signal in the lactose hydrolyzed milk powder sample suggests that galactose and glucose are much more amorphous and produce much broader signals than lactose.

In terms of molecular dynamics, the T1 analysis time observed for protein protons in the traditional and lactose hydrolyzed milk powder were 0.9 and 1.4 seconds, respectively: this represents a 55% difference (Table 1).

Table 1. Longitudinal relaxation time ^{1}H (T1) of milk proteins.

Product	^{1}H T1 - Relaxation time (s)
Traditional milk powder	0.9
Lactose hydrolyzed milk powder	1.4

The longitudinal relaxation time (T1) of ^{1}H was affected by the overall protein dynamics. In such conditions, the dynamic processes are inversely related to T1 (Peixoto et al., 2016). Thus, proteins derived from the lactose hydrolyzed milk powder presented lower dynamic. The molecular dynamic decreases with the increase of intra- or inter-molecular interactions, at angstrom scale (Hinrichs et al., 2004; Rollema & Brinkhuis, 1989). Therefore our results suggest that even though the lactose hydrolyzed milk powder protein displayed higher water content, it demonstrated higher interaction levels with neighboring molecules than that found in the traditional milk powder.

SAXS and gel electrophoresis were used to evaluate the reversible nature of these interactions and determine if there was a modification in chemical structure of the proteins in the samples after rehydration. Both SAXS and gel electrophoresis showed that there was no difference in the casein micelle structure to traditional and lactose hydrolyzed milk powder after rehydration (see supporting information for details). Therefore, it is possible to discard a possibility of protein-protein and protein-saccharide chemical linking in casein micelles that can alter molecular organization of caseins between lactose hydrolyzed and traditional milk powder. Arakawa & Timasheff (1982) have studied the preferential hydration of RNase A protein in lactose and glucose solutions. They observed that the number of glucose molecules interacting on the protein surface was 30% higher than the lactose molecules. Because monosaccharides (glucose and galactose) are smaller than disaccharides (lactose), the protein surface may display more energetically favorable sites for them than for disaccharides. Noting that a very weak physical attractive interaction, based in hydrophilic forces, has already been reported between caseins and polysaccharides

(Peixoto et al., 2015). Protein-saccharide binding is always a water-intermediate interaction and monosacharides requires more hydrogen bonds links with water than disaccharides which affect more protein dynamics (Lerbret et al., 2011) which explains the observed the higher hydration degree of lactose hydrolysed milk powder. Based on these previous data, one can explain such observed slower protein dynamics in lactose hydrolyzed milk powder by a stronger, water mediated, interaction between protein and saccharides.

3.2.3. Rehydration kinetics

The powder sample rehydration kinetics were evaluated to verify the impact of lactose hydrolysis on the techno-functional properties of the the products.

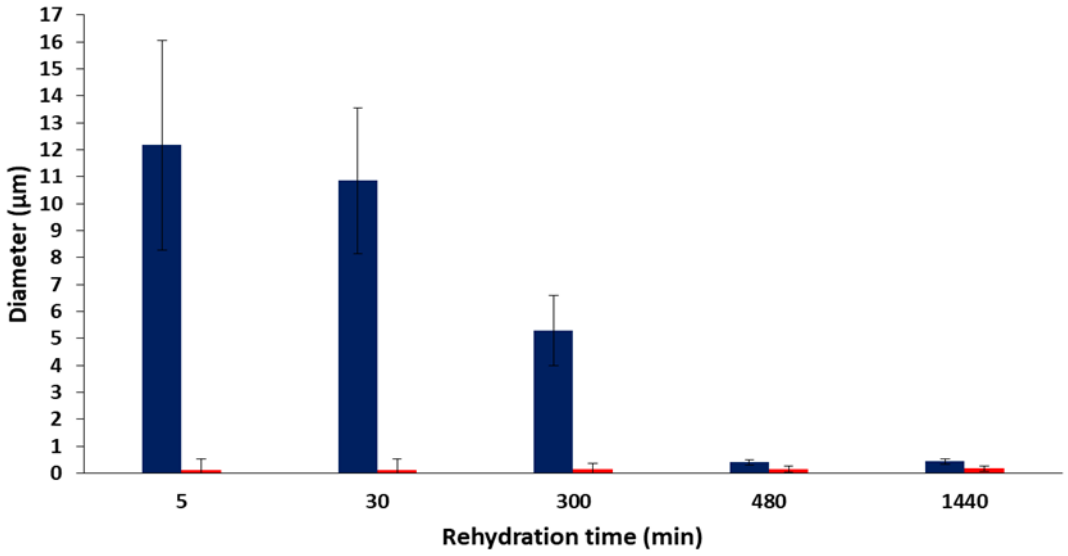

Figure 4. Rehydration kinetics of the traditional milk powder (blue) and the lactose hydrolyzed milk powder (red) samples.

Complete rehydration of the traditional milk powder sample occurred at 480 minutes (Figure 4). The lactose hydrolyzed milk powder sample was completely hydrated in less than 5 minutes (Figure 4).

Because of the lack of vacuoles, one can expect that lactose hydrolyzed milk powder particles should be more compact and display a reduced surface exposure to water and traditional milk powder's vacuoles may have been thought to facilitate water penetration and lead to faster dissolution than that of the lactose hydrolyzed milk

powder. Instead, our results indicate that the specifc molecular organization in the samples containing glucose and galactose between proteins and the higher sugar levels in the sample amorphous states (Figure 3) had a greater impact on protein rehydration kinetics than that of surface water exposure for the sample particles.

3.3. Particle molecular organization

Based on the results obtained in this study, the molecular organization in Figure 5 was suggested for traditional and lactose hydrolyzed milk powder particles.

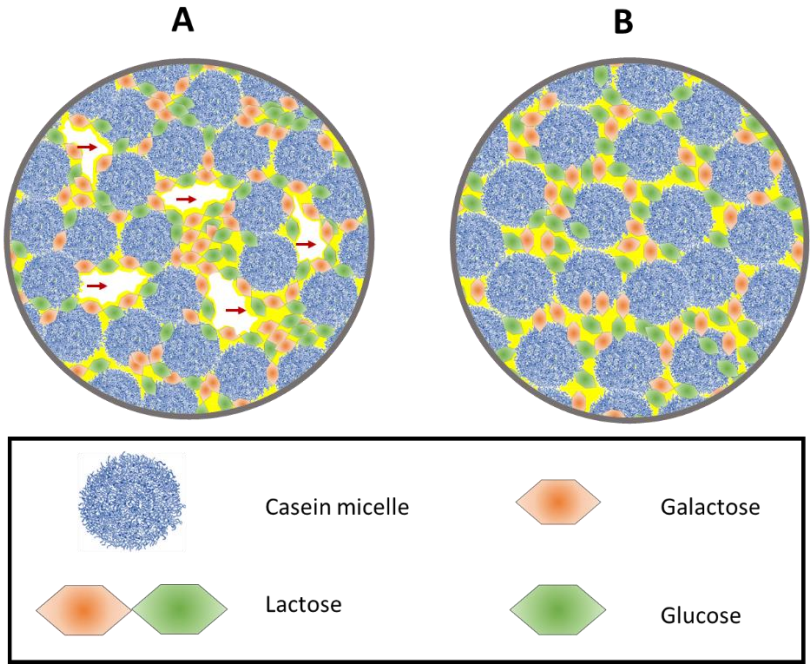

Figure 5. Diagram of traditional milk powder (A) and lactose hydrolyzed milk powder (B) particle molecular organizations. The yellow color indicates water and the red arrows indicate vacuoles.

In Figure 5A, the lactose molecules display a more heterogeneous organization (locally-forming crystallites) which allows for more frequent protein-protein direct contact. By contrast, in Figure 5B, the saccharides (galactose and glucose) display a much more homogenous distribution around proteins and greater hydration. Our data indicates that galactose and glucose displays a higher tendency to forms less monosacharides-monosacharides interactions and more monosacharides-water-proteins interactions. These two tendencies combined may be responsible for the differences in molecular organization and rehydration kinects between the powders:

the specific organization of the powder in protein-water-monosaccharide layers may be the origin of a faster hydration kinects. The question now is how this knowledge of the molecular organization of the two powder samples can impact the understanding of the problems spray drying poses for the dairy industry. Based on our results, further research into the impact of lactose hydrolysis on the behavior of lactose hydrolyzed milk powder in more extreme conditions, comme temperature and storage, is necessary.

4. Conclusion

According to the physicochemical, nanorheological (by NMR) and techno-functional data determined in this study, it can be concluded that the hydrolysis of lactose altered the molecular organization of milk powder. In samples of lactose hydrolyzed milk powder, proteins and monosaccharides showed more homogenous molecular organization, slower dynamics and faster kinetics of hydration compared to traditional milk powder. Based on all these results and explanations, it has been proposed that monosaccharide-monosaccharide interactions are less present and that monosaccharide-water-protein interactions are more present in hydrolyzed powder. This plays a key role in molecular structural organization and in the functional properties of powder, such as rehydration.

This study has enabled us to obtain a better understanding of the influence of galactose and glucose on the molecular structure of lactose hydrolyzed milk powder. Our approach represents an important potential tool to that can be used to improve milk powder production, address manufacturing challenges, and reduce technological issues in the dairy industry.

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PART 2: THE MOLECULAR MECHANISM OF AGGLOMERATION IN LACTOSE HYDROLYZED MILK POWDER

Preamble

Milk drying is a process used by the dairy industry to improve product stability and facilitate product transportation and storage. This practice allows countries with high milk production to export their surplus.

During export, milk powder is subjected to different means of transportation (ship, train and truck), variable transport conditions and storage times, and irregular temperature and relative humidity conditions. Because lactose hydrolyzed milk powder is less stable than traditional milk powder, the product more readily undergoes changes during shelf life such as decreased solubility, browning, agglomeration and caking. In view of this, quality assurance depends on controlling transport/storage conditions.

In the second part of Chapter 2, the objective was to understand the physicochemical mechanisms that occur on a molecular scale when lactose hydrolyzed milk powder is subjected to accelerated aging.

Our questions:

- Does the type of sugar determine the physicochemical changes undergone by the powder when it is subjected to high temperatures?

If yes:

- What are the mechanisms, on a molecular scale, that lead to the observed physicochemical changes?

The molecular mechanism of agglomeration in lactose hydrolyzed milk powder

The content of this first part of the Chapter IV will be submitted to Food Structure

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Abstract

Lactose hydrolyzed milk powder has low stability at high temperatures and high humidity levels, which results in technological problems such as increased rehydration time, decreased solubility, browning and agglomeration. In order to understand how lactose hydrolysis induces these issues over time, the molecular structure of the powder was evaluated during accelerated aging (60 °C for 8h and 24 h). The powders were evaluated for particle morphology, molecular structures and techno-functional properties. This study determined that the higher the tendency of glucose and/or galactose to be distributed around proteins the greater the glycation capacity of the monosaccharides compared to lactose. Protein glycation is the initial process that precedes the primary modifications observed in lactose hydrolyzed milk powder during storage.

1. Introduction

Drying milk one of the most common process in the dairy industry. It is performed to improve product stability and facilitate transport and storage. The milk drying process consists in spraying concentrated milk into small droplets which dry instantaneously upon contact with a stream of hot, dry air due to the difference in temperature and partial vapor pressure between the air and the droplet (Schuck, 2009).

In 2016, China, New Zealand, the European Union, Brazil and Argentina were the main producers of milk powder, totaling 5.390 tons (USDA, 2016). China and Brazil consume almost all of their production, while New Zealand, the European Union and

Argentina consume only a small part of their production and export the surplus. These are considered the 3 main major exporters of milk powder this year (USDA, 2016).

During shipment, milk powder is subjected to different means of transportation (ship, train and truck), transport times and storage conditions, all of which subject it to uncontrolled temperatures and relative humidity levels. According to Nasser, 2017, a load of milk powder traveling from France to Africa over the course of 3 months were subjected to temperature variations that reached peaks of 45°C - 50°C. By contrast, when milk powder is transported from Japan to Memphis (USA), Premititikul (2005) confirmed that extreme temperatures close to 55°C and relative humidity ranging from 30 to 90% are commonly encountered during inter-continental transport.

Although temperature and humidity variations during transportation/commercialization of milk powders occurs regularly, the product remains relatively stable. Temperatures around 20°C will not significantly alter its physicochemical and functional properties (Renner, 1988). However, lactose hydrolyzed milk powder is more sensitive to environmental variations. This sensitivity can cause issues that include increased rehydration time, decreased solubility, browning, agglomeration and caking (Fernández, Schebor, Chirife, 2003; Schuck et al., 2005; Shrestha et al., 2007; Lule et al., 2016). According to Nasser et al., (2017), the number of accidents (high temperature peaks) has a cumulative effect on powder property modifications, regardless of when the accidents occur during storage. Therefore, controlling the transport/storage conditions of lactose hydrolyzed milk powder is fundamental to maintaining product quality.

In our previous study, it has been shown that lactose hydrolyzed milk powder has particles with a more crowded and homogeneous molecular organization than traditional milk powder. This markedly affects the rehydration kinetics of the products (Fialho et al. 2018). Although this discovery establishes a relationship between molecular organization and powder techno-functionality; little is known about how powder molecules reorganize over time and what the consequence of this may be on the product properties. In view of this, the aim of the present study is to provide a better understanding of the molecular reorganization of lactose hydrolyzed milk

powder submitted to accelerate aging and the consequences of this on the product's physicochemical characteristics.

2. Materials and methods

2.1. Production of lactose hydrolyzed concentrated milk

Raw skim milk was added to a pilot vacuum evaporator at 50kg·h^{-1} (GEA-PE, St Quentin and Yvelines, France), heated to 65 °C until 50 ± 2% (w·w^{-1}) dry matter was reached, and then cooled quickly at 5 ± 2°C. 0.1g·L^{-1} of enzyme lactase Maxilat LGi (DSM, Netherlands) was added to the concentrated milk and the lactose hydrolysis process was carried out for 24h (≥ 95% hydrolysis). The hydrolysis was then analyzed using a Lactose and D-Galactose enzymatic kit (Megazyme; Ireland, 2014). The enzyme analytical report applied in the experiment indicated enzymatic activity of 60,000 ONPGU·g^{-1} (o-nitrophenyl-b-D-galactopyranoside units·g^{-1}) with a density between 1.1 and 1.3g·mL^{-1}.

As a control, a traditional concentrated milk sample was prepared as described previously without the addition of lactase enzyme.

2.2. Production of lactose hydrolyzed milk powder

The lactose hydrolyzed concentrated milk was heated at 45 ± 1°C and injected into a Mobile Minor pilot scale spray dryer (GEA-PE, St Quentin en Yvelines, France) with a bi-fluid nozzle atomizer and maximum evaporation capacity of 7kg of water per hour. To obtain the lactose hydrolyzed milk powder, the following drying parameters were used: inlet air temperature : 138 ± 1°C, flow rates: 1.1kg·h^{-1} and outlet air temperature:72 ± 1°C.

The traditional milk powder containing lactose was produced using same drying parameters as those to dry the lactose hydrolyzed milk powder.

2.3. Aging of the powders

The lactose hydrolyzed milk powder was divided into three samples. The first sample was not aged and was used as a control to determine the effects of accelerated aging. The second sample was heated in an oven at 60°C for 8 h. The third sample was

heated in an oven at 60°C for 24 h. The traditional milk powder samples underwent the same treatment as the lactose hydrolyzed milk powder samples.

Soon thereafter, all the powder samples were sealed in laminated plastic bag conditions and stored at 10°C.

2.4. Powder analysis

All powder sample analyses were carried out during storage and performed with three repetitions in triplicate.

2.4.1. Browning index determination

The color measurements were performed on the milk powder samples using a CR-300 Minolta colorimeter (Konica Minolta, Osaka, Japan) run in the L-a-b space.

The values of L*, a* and b* were calibrated with a color standard reference: 96.03, 4.71 and 7.24, respectively. Next, the powders were dispersed on a Petri dish and their colors were measured at 3 different points on the surface of the receptacle. The browning index (BI) used to assess the intensity of the brown color of the samples was calculated using formulas 1 and 2 according to (Nasser et al., 2017).

$$BI = \frac{[100 \times (X - 0.31]}{0.17} \qquad \text{Eq. (1)}$$

where "X" is:

$$X = \frac{(a* + 1.75 \times L*)}{(5.645 \times L* + a* - 3.102 \times b*)} \qquad \text{Eq. (2)}$$

2.4.2. Scanning electron microscopy (SEM)

The milk powder samples were fixed with carbon double-sided adhesive tape and platinum coated (conductive material). The images were captured using an FEI Quanta 400 FEG scanning electron microscope (Oregon, United States) operating at an accelerating voltage of 5 kV (Mimouni et al., 2010). The images of all the powder milk samples inserted into the equipment were collected and analyzed.

2.4.3. Total rehydration time

Particle size distribution was measured from the dissolution of milk powder in distilled water 8% ($w \cdot w^{-1}$) under a 300rpm agitation to 24h. The initial time of

rehydration was considered to be the moment that the powder was added water. Solution aliquots were collected in intervals of 5; 30; 300 and 480 minutes and analyzed in Malvern Mastersizer 2000 equipment (Malvern Instruments Ltd., Malvern, UK) using the refractive index of 1.33 and 1.30 for solvent and particle, respectively (Richard et al., 2013), with a maximum detection of 1mm. The casein micelle had an average diameter between 0.05 to 0.5µm (Fox et al., 2015). Complete product dispersion was set to 90% of the powder particles presented equal or lower size to µm ($D_{90} \leq 0.5\mu m$).

2.4.4. Electrophoretic separation

Samples were prepared according to Pardo & Natalucci (2002), using the protocol of sample reduction per urea. Gel containing stacking layer 4% ($w \cdot w^{-1}$) and run layer 12% ($w \cdot w^{-1}$) and 16% ($w \cdot w^{-1}$) was prepared according to Laemmli (1970). 15µL of each sample was added to the gel coupled with an SE 600 Series vertical using Slab Gel Unit on equipment 80 V (Hoefer Scientific instrument, San Francisco, US). The samples were run in stacking layers at 40 mV and in run layers at 60mV for 360min, with a fixative solution composed of 10% ($w \cdot w^{-1}$) acetic acid, 30% ($w \cdot w^{-1}$) of ethanol and 60% ($w \cdot w^{-1}$) of water added. They were immersed in Comassie-Blue coloring solution for 1.5 h. Discoloration was carried out using acetic acid 10% ($v \cdot v^{-1}$) until the protein bands were visible. The gel was mesured using ImageJ software (Schneider, Rasband, & Eliceiri, 2012).

2.4.5. Small-angle x-ray scattering (SAXS)

SAXS experiments were conducted on the SWING beamline at the SOLEIL synchrotron (l = 1.033 A˚). The Aviex charge-coupled device detector was positioned to collect data in the wave vector (q) range 0.01–0.430 A^{-1}. The milk powders diluted in distilled water (1:5) ($w \cdot w^{-1}$) were flowed through a quartz capillary with a 1.5mm diameter and a 10mm wall thickness, and placed in a vacuum chamber at a flow rate of 0.75µL·min^{-1}. Frames were collected continuously over 500 ms with a dead time of 250ms between frames. Data reduction to absolute units, frame averaging, and subtraction were carried out using the FOXTROT program version 3.3.4. (Synchrotron SOLEIL, France).

During analysis, the lactose hydrolyzed rehydrated milk powder aged at 60°C for 24h clogged the needle used to insert the sample into the equipment, making it impossible to analyze it.

3. Results and discussion

3.1. Aspects of powder particles

3.1.1. Color

Color analyses allow us to infer the sensory aspecst of the powder samples as well as the how well they will remain preserved during storage. The Maillard reaction is composed of a cascade of reactions which begins when the reducing end of a saccharide is linked with a free amino group from a protein, leading to protein glycation (Damodaran, Parkin, & Fennema, 2010). Brown melanoidines are produced at the end of the Maillard reaction. As melanoidine structure has not been fully characterized, powder browning is one of the most common ways of studying the progression of this reaction (Le et al., 2011).

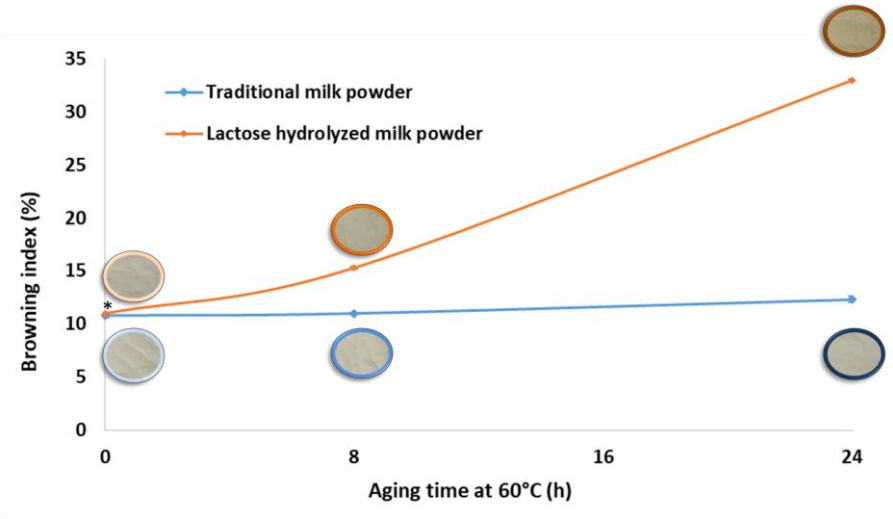

Figure 1. Browning index of traditional milk powder (blue line) and lactose hydrolyzed milk powder (orange line) in aging time. *Non-aged powder.

According to Figure 1, the non-aged traditional milk powder and non-aged lactose hydrolyzed milk powder presented 11% browning. However, throughout the accelerated aging, the powders behaved differently. The traditional milk powder did not

change color as it was aged. The lactose hydrolyzed milk powder showed a browning increase of 32% and 115% at 8h and 24h of accelerated aging, respectively.

While studying the influence of storage conditions on the powder, Nasser et al. (2017) observed that the accelerated aging of micellar casein powder at 60°C for 8 hours corresponded to aging powder stored at 20°C for approximately 12 months. Likewise, accelerated aging at 60°C for 24h corresponded to that of powder stored at 40°C for approximately 1 month. High temperatures favor glycation in the initial stages of the Maillard reaction (Cheison, Josten, & Kulozik, 2013). In more advanced stages, high temperatures influence the formation of intermediate and final products, such as melanoidines (Benzing-Purdie, Ripmeester, & Ratcliffe, 1985).

During accelerated aging, the lactose hydrolyzed milk powder showed more browning than the traditional milk powder. The behavior discrepancy between the two powders can be explained by lactose hydrolyzed milk powder is much more homogenous distribution of saccharides (galactose and glucose) around proteins (part 1 of chapter 4). In addition, galactose and glucose have high glycation capacity when compared to lactose (Cardoso et al., 2018; Naranjo et al., 2013). According to Cardoso et al. (2018), galactose and glucose have a β-casein glycation capacity of 40% and 36% higher than lactose, respectively. Both presented an initial β -casein glycation rate that was 150% higher than lactose, indicating the predominant initial reactivity of monosaccharides. This result concurs with the works of Torres et al. (2017) who observed lactose hydrolyzed milk powder browning increased progressively as hydrolysis increased.

3.1.2. Microstructure

The SEM revealed information about the impact of accelerated aging on the microstructure of the powder particles.

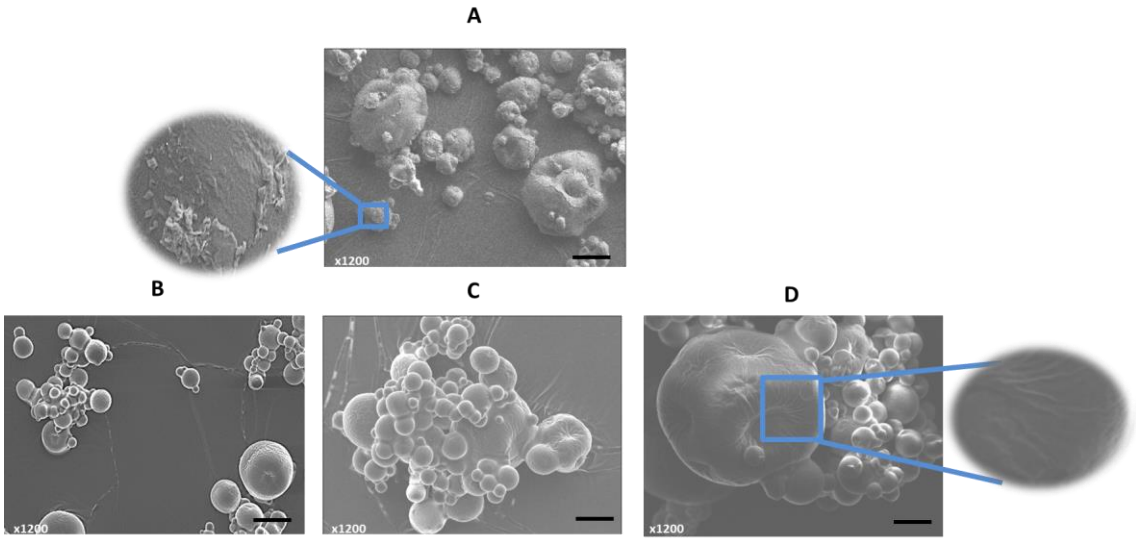

Figure 2. SEM micrographs of the powders. Traditional milk powder (A), non-aged lactose hydrolyzed milk powder (B), lactose hydrolyzed milk powder aged at 8h (C) and lactose hydrolyzed milk powder aged at 24h (D). Black scale bar: 10µm.

SEM micrographs highlighted that traditional milk powder particles showed low agglomeration and low levels of surface lactose crystals (Figure 2A). For non-aged lactose hydrolyzed milk powder, the particles exhibited higher agglomeration and an absence of surface monosaccharide crystals (Figure 2B). As the powder aged at 8h and 24h, particle agglomeration intensified and a complete absence of monosaccharide crystals was observed (Figure 2C and 2D).

In our previous work (Part 1 of Chapter 4) it has been shown that lactose distribution around proteins in traditional milk powder is more heterogeneous than distribution in lactose hydrolyzed milk powder. This more heterogeneous lactose distribution can favor crystal formation during drying.

3.2. Aspects of molecular structure

3.2.1. Rehydration kinetics and micelle size

The rehydration kinetics of the powder samples are presented in Table 1 as a function of the accelerated aging time.

Table 1. D_{90} value relative to the diameter of the particles (µm) of the traditional milk powder and lactose hydrolyzed milk powder over the course of aging.

Rehydration time (minutes)	Aging time at 60 °C					
	Control milk powder			Lactose hydrolyzed milk powder		
	0*	8	24	0	8	24**
5	12.2 ± 3.9	17.0 ± 4.0	64.1 ± 3.7	0.1 ± 0.1	26.5 ± 5.0	60.5 ± 14.3
30	10.9 ± 2.7	12.2 ± 2.3	29.5 ± 2.9	0.1 ± 0.1	0.3 ± 0.2	61.3 ± 12.4
300	5.3 ± 1.3	5.3 ± 2.0	6.7 ± 2.1	0.2 ± 0.2	0.1 ± 0.1	55.9 ± 13.2
480	0.4 ± 0.1	0.4 ± 0.2	0.4 ± 1.4	0.2 ± 0.1	0.2 ± 0.1	261.8 ± 32.1
1440	0.4 ± 0.1	0.3 ± 0.1	0.4 ± 0.3	0.2 ± 0.1	0.1 ± 0.1	164.1 ± 22.5

* Non-aged powder.
** Unreliable data due to equipment detection limitations.

According to Table 1, non-aged lactose hydrolyzed milk powder rehydrated faster than non-aged traditional milk powder. However, with aging, the rehydration time of traditional milk powder samples remained unchanged up to 480 minutes. Conversely, the lactose hydrolyzed milk powder samples presented different behavior with aging. Rehydration time increased when the powder was aged to 8h and complete rehydration was not observed when the sample was aged to 24h. Between the rehydration times 300 and 480min an increase in the D90 value was observed, corresponding to the lactose hydrolyzed milk powder particle diameter when aged to 24h. This behavior could be due to the presence of particles with a diameter greater than the maximum limit the equipment can detect (1mm). It was fragmented and detected only after 300 min of analysis.

The faster hydration of non-aged lactose hydrolyzed milk powder samples compared to traditional milk powder may be related to the arrangement of galactose and glucose in the particles. Our recent studies point out that the lactose hydrolyzed particles display a specific molecular organization where galactose and glucose are more homogenously distributed around proteins. This in turn can facilitate the rehydration process. (Part 1 of chapter 4). In addition, lactose crystals present in traditional milk powder may have made it difficult to rehydrate the powder (Hargreaves, 1995).

The lactose hydrolyzed milk powder aged at 60°C for 24h was not completely rehydrated and the development of protein cross-linking may be the cause. According to Le et al. (2013) and Singh (1991) intermediate and late products of the Maillard reaction such as di-carbonyl glyoxal, methylglyoxal and 3-deoxyglucosone compounds may react with protein amino residues such as lysine and form intra- or inter-molecular cross-linking. However, these reactions can increase the hydrophobicity of the powder particle surfaces (Anema et al., 2006; Havea, 2006) which may cause insolubility of powder. Regardless, the cross-linked protein network formation is related to the partial unfolding of the glycated proteins structure (Closs, Courthaudon, & Lorient, 1990; Hiller & Lorenzen, 2010; Marchin et al., 2007; Morgan et al., 1999; Nacka et al., 1998). Thus, two scenarios may proposed: First, the Maillard reaction directly induces particle agglomeration through the formation of intermolecular covalent links. Second, the Maillard reaction indirectly leads to the formation of non-covalent cross-linking by changing protein conformations.

3.2.2. Covalent links measurement

Figure 3 shows the electrophoresis separation under major protein reduction conditions (α_{s1}-casein, α_{s1}-casein, β-casein and κ-casein) of the powders.

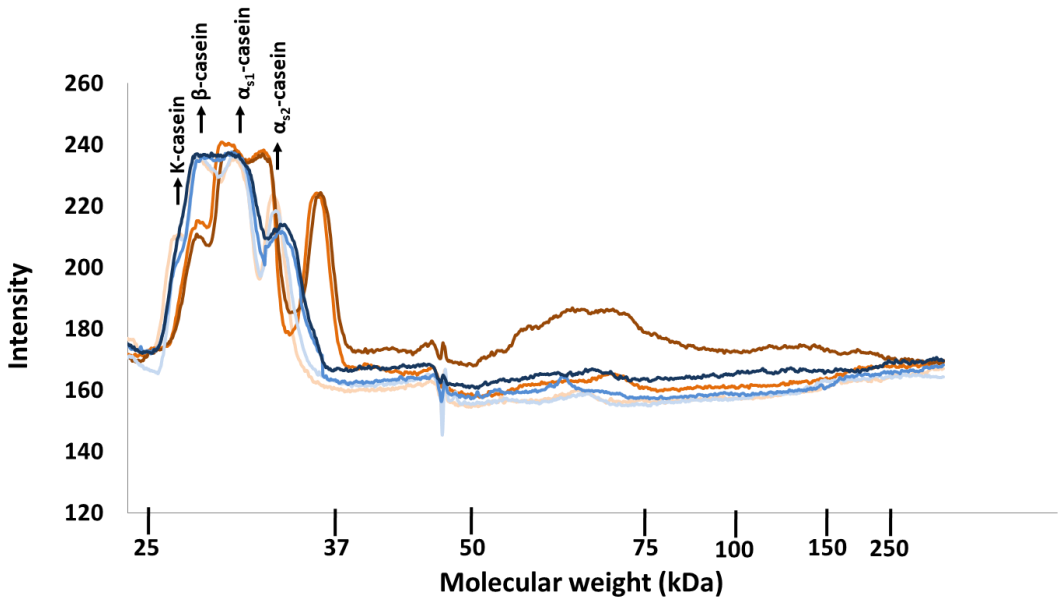

Figure 3. Electrophoretic profile of the powders. Non-aged traditional milk powder ( ), traditional milk powder aged to 8 h ( ), traditional milk powder aged to 24 h ( ), non-aged lactose hydrolyzed milk powder ( ), lactose hydrolyzed milk powder aged to 8 h

( ), lactose hydrolyzed milk powder aged to 24 h ( ). Black arrows represent the protein molecular weight in the non-aged traditional milk powder.

The non-aged traditional milk powder and non-aged lactose hydrolyzed milk powder samples presented the same electrophoretic profiles. The apparent protein molecular weights correspond to those found in literature (Jovanovic et al., 2007). It is possible to observe that the molecular weight of the caseins in the traditional milk powder remained unaltered as the sample was aged. However, lactose hydrolyzed milk powder exhibited a displacement of all protein bands with aging to 8h, which corresponds to an average 8% increase in the protein molecular weight.

The casein band shift reflects casein micelle glycation during the aging of lactose hydrolyzed milk powder. With a molecular mass of 180 Da, galactose and glucose are very small molecules compared to caseins, so monosaccharide-protein interactions contribute to a small increase in protein size. In addition, powder browning during aging (Figure 1) further reinforces the protein glycation.

At the 24h ageing point, the lactose hydrolyzed milk powder maintained the shift of the casein bands observed at 8h aging. This suggests a saturation of glycation sites. A large band of high molecular weight protein complexes appeared, mainly in the region from 50 to 75kDa. Based on the band intensity of these high molecular complexes, it can be estimated that the complexes represent around 17% of total protein molecular weights. The molecular weight of these covalent bound complexes indicates that they are mainly formed by dimmers and trimers of caseins or whey proteins. The modest number of dimmers and trimers complex indicates that the origin of the cohesive force for the very large agglomerates at 24h (observed in the rehydration experiment) is only partially influenced by the direct covalent links between proteins.

3.2.3. Internal molecular organization of micelles after rehydration

The SAXS technique was used to see if the chemical difference between sample casein micelles impacted structural differences in rehydrated samples.

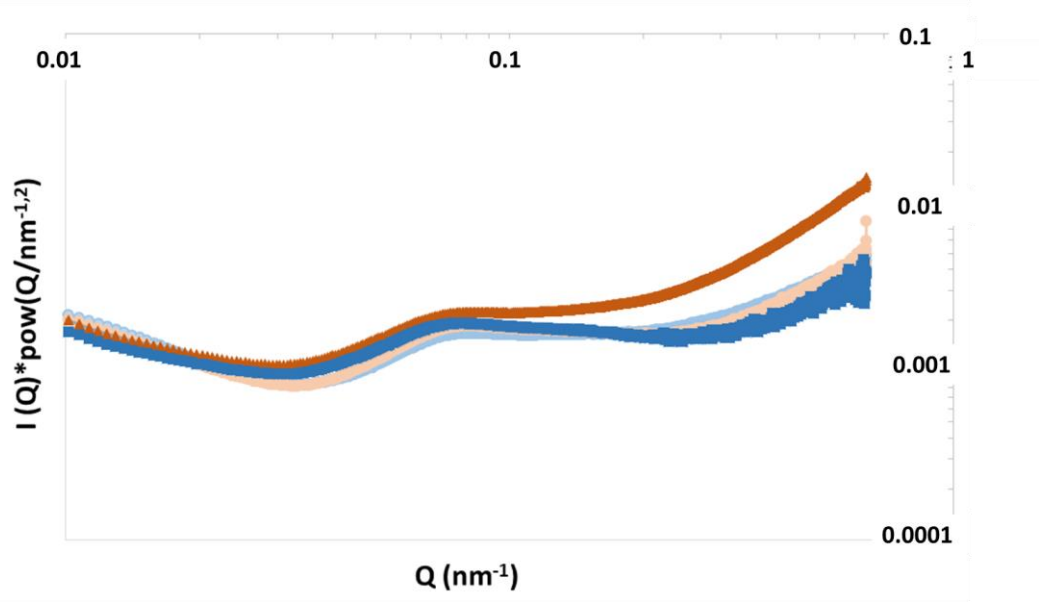

Figure 4. SAXS profile of the powders. Non-aged traditional milk powder (), traditional milk powder aged at 8 h (), traditional milk powder aged at 24 h (), non-aged lactose hydrolyzed milk powder (), lactose hydrolyzed milk powder aged at 8 h ().

As shown in Figure 4, both aged and unaged traditional rehydrated milk powders showed similarities in their SAXS profiles, i.e., powder aging at 60°C for 8 h and 24h did not result in casein micelle structure modifications. By contrast, lactose hydrolyzed milk powder aged at 8h showed an increase in intensity of the intermediate- Q region for the high-Q region (0.1 to 1nm^{-1}) compared to non-aged lactose hydrolyzed rehydrated milk powder.

There is a general agreement that the intermediate and high-Q part of the scattering function reflects the casein micelles internal characteristics as related to internal protein homogeneity or substructures of colloidal calcium phosphate (De Kruif, 2014; Holt et al., 2003; Marchin et al., 2007; Sørensen et al., 2013). It can therefore be inferred that casein micelles present in lactose hydrolyzed rehydrated milk powder have a more heterogeneous internal structure than non-aged lactose hydrolyzed milk powder. This internal structure change of the casein micelle that occurs in lactose hydrolyzed milk powder during aging is probably related to the glycation of the proteins (Figures 1 and 3). Studies suggest that glycation causes partial disorganization in the structures of proteins (Hiller & Lorenzen, 2010; Nacka et al., 1998; Wooster & Augustin, 2007). The covalent attachment of the sugar molecules allows sterically unfolding proteins via diminished intra- and intermolecular interactions and thus slightly increases

the hydrophobicity of the surface of the casein micelle (Hiller & Lorenzen, 2010; Nacka et al., 1998; Wooster & Augustin, 2007). These results support the view that it is glycation is the primarily responsable for generates cohesive forces in the powder particles by modifying protein conformations in the micelle and creating non-covalent linking which in turn results in agglomerated powder.

4. Conclusion

This study indicates that the greater tendency of glucose and/or galactose to be distributed around proteins and allows the monosaccharides a greater glycation capacity than lactose.

Heat exposure, at 60°C for 8h and 24h, resulted in an increase in agglomeration and a reduced rehydration capacity for the lactose hydrolyzed milk powder. No covalent protein-protein complexes were found with 8h of aging and only a modest number (~17%) of protein-protein links were formed with 24h of aging. These results indicate that the decrease in rehydration kinetics cannot be completely assigned to protein cross-linking. Instead, monosaccharide glycation seems to indirectly promote agglomeration by perturbing the native arrangement and/or conformation of proteins (observed using SAXS technology). This leads to the formation of intra-molecular non-covalent links. From this work, it was possible to verify that protein glycation is the initial process that triggers the main modifications observed in lactose hydrolyzed milk powder during storage.

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CHAPTER V
CONCLUSION & PERSPECTIVES

1. Conclusion

Throughout this manuscript, it has been observed that lactose hydrolysis had an impact on the drying process and on the quality of milk powder.

Drying lactose hydrolyzed milk powder using the optimal drying parameters of the control (traditional milk powder) resulted in a process with higher mass loss and a powder with moisture content and agglomeration. This finding reinforces the idea that the production of lactose hydrolyzed milk powder using the same operational parameters for traditional milk powder is impracticable for the dairy industry.

In this study, the ideal operational parameters for the production of lactose hydrolyzed milk powder were determined by testing the drying conditions and analyzing the process characteristics and the powder quality by means of different drying parameters (inlet air temperature ranging from 115 °C at 160 °C and concentrated milk flow rate ranging from 0.3 to 1.5 $kg \cdot h^{-1}$) (Table 1).

Table 1. Influence of the drying parameters under the process and powder characteristics.

Drying parameters	Characteristics of the process	Characteristics of the powder
-High inlet air temperature -Low concentrated milk flow rate	-High mass loss -Low energy loss -Low specific energy consumption (SEC)	-High moisture -High water activity -High browning -High agglomeration -Low rehydration
-Low inlet air temperature -High concentrated milk flow rate	-High mass loss -High energy loss -High specific energy consumption (SEC)	-High moisture -High water activity -High caking

This approach yielded the ideal parameters for production of lactose hydrolyzed milk powder production in a laboratory: inlet air temperature at 145 °C and concentrated milk flow rate at 1.0 $kg \cdot h^{-1}$ (Table 2).

Table 2. Comparison of lactose hydrolyzed milk powders (Inlet air temperature at 145 °C and concentrated milk flow rate at 1.0 kg·h^{-1}) and the traditional milk powder (control).

Parameters	Traditional milk powder (Control)	Lactose hydrolyzed milk powder (145 °C/1.0 kg·h^{-1})
Mass loss (%)	13	15
Energy loss (%)	20	22
Specific energy consumption (10^3 kJ·kg^{-1})	10	14
Moisture content (%)	3.7	4.2
Water activity (a_w)	0.2	0,2
Luminosity (%)	94	94
Rehydratation (%)	100	100

After verifying the impact of lactose hydrolysis on the drying process and on the quality of lactose hydrolyzed milk powder, we sought to understand the mechanisms on a molecular scale that are responsible for the the technological issued observed in the production and shelf life of lactose hydrolyzed milk powder.

In this study, it was observed that molecules present in lactose hydrolyzed milk powder presented a more homogeneous molecular organization than traditional milk powder. Based on this, it has been proposed that the monosaccharide-protein physical interactions through water are more favorable than lactose-protein interactions. Both homogenous molecular organization and the favorable monosaccharide-protein physical interaction explain the difficulties encountered in drying and the greater rehydration of lactose hydrolyzed milk powder compared to traditional milk powder.

When the shelf-life of lactose hydrolyzed milk powder submitted accelerated ageing was analyzed; low product stability was observed (Table 3).

Table 3. Accelerated aging of the lactose hydrolyzed milk powder

Parameters	Accelerated aging		
	No aging	60 °C/8h	60 °C/24h
Color	Yellowish White	Brown	Highly brown
Microstructure	No agglomerated	Agglomerated	High agglomeration
Rehydratation Kinetics	Great rehydratation	Good rehydratation	No rehydratation

This instability of the lactose hydrolyzed milk powder was triggered by the glycation process. The results indicate that the decrease in rehydration kinetics cannot be completely assigned to protein cross-linking. Instead, monosaccharide glycation seems to indirectly promote agglomeration by perturbing the native arrangement and/or conformation of proteins. This leads to the formation of non-native intra-molecular non-covalent links. From this work, it was possible to verify that protein glycation is the initial process that triggers the main modifications observed in lactose hydrolyzed milk powder during storage.

The topics covered in this thesis, optimization of the drying process of lactose hydrolyzed milk powder and its structural and functional properties, on a molecular scale, represent important tools to improve the production of hydrolysed powdered milk.

2. Perspectives

2.1. Optimization of industrial spray drying parameters

In this thesis, the ideal drying parameters of lactose hydrolyzed milk powder have defined using a thermodynamic characterization of the process and a quality determination of the spray dryer powder. The methodology used in this work must be applied on an industrial scale in order to define the ideal drying parameters of lactose hydrolyzed milk powder and minimize technological issues to increase productivity.

2.2. Mapping the composition of lactose hydrolyzed milk powder

This work is based on the physicochemical and techno-functional results observed and proposes that the hydrolysis of lactose impacts the organization and dynamics of the molecules. Lactose hydrolyzed milk powder presents a more homogeneous organization than traditional milk powder, which is why it is interesting to visualize powder composition mapping using a Confocal Raman Microscope.

2.3. Study of the impact of lactose hydrolysis on the molecular organization of lactose hydrolyzed whole milk powder

Examination of the molecular organization of the powders was focused on macromolecules: proteins and sugars (lactose, galactose and glucose). The macromolecules of lactose hydrolyzed whole milk powder are proteins, sugars and fat, and so it is important to understand how fat interacts with proteins and sugars and how it distributes itself inside the particle.

2.4. Study of the stability of lactose hydrolyzed milk powder during the shelf life

This work demonstrated that lactose hydrolyzed milk powder submitted to accelerated aging presents lower physicochemical stability than traditional milk powder. The reduced stability is due to mainly to its composition. The shelf life temperature of the product should be optimized using stability information and taking into account the time and cost of product storage.

Supplementary Materials

CHAPTER IV: STRUCTURAL AND FUNCTIONAL PROPERTIES OF LACTOSE HYDROLYZED MILK POWDER

PART 1: SUGAR TYPE MATTERS IN SPRAY DRYING: HOMOGENEOUS DISTRIBUTION IN MILK POWDER FAVORS REPULSIVE INTERACTIONS BETWEEN PROTEINS

1. Mathematical description of osmotic pressure

1.1. Materials and methods

1.1.1. Theoretical osmotic pressure

The theoretical osmotic pressure of the milk powder samples after stabilization was calculated at 20 °C according to (Bouchoux et al., 2009):

$$\pi = RT\Sigma c \quad \text{Eq.(1)}$$

where:

R= constant (8.314 $J \cdot mol^{-1} \cdot K^{-1}$)

T= temperature in Kelvin

c= osmolality in $mol \cdot L^{-1}$

1.1.2. Experimental osmotic pressure

The experimental osmotic pressure of the milk powder samples was calculated by means of the PEG solution after its stability at 20 °C according to (Money, 1989):

$$log\pi = a + b[PEG]^c \quad \text{Eq. (2)}$$

where: a=0.49; b=2.5; c=0.29

PEG= concentration of polyethylene glycol solution (%, $w \cdot w^{-1}$)

1.2. Results

1.2.1. Calculated theoretical osmotic pressure

Table 1. Osmotic pressure of traditional concentration milk solutions after stabilization.

Traditional concentrated milk	Concentration ($g \cdot L^{-1}$)	Molecular weight ($g \cdot mol^{-1}$)	Concentration ($mol \cdot L^{-1}$)	Osmotic Pressure (MPa)
Lactose	416.33	342*	1.21	2.964
Casein micelles	220.41	280000000**	7.87×10^{-7}	0.000
Soluble proteins	53.88	18400***	2.92×10^{-3}	0.007
		Total		2.971

* Data obtained according to Bobbio & Bobbio (2001)
**Data obtained according to Morris, Foster, & Harding (2000)
*** Data obtained according to Aich, Batabyal, & Joardar (2015). The molecular mass of β-lactoglobulin was used as the most abundant soluble protein in milk powder.
Mineral osmotic pressure was not calculated due to the low mineral concentration in the solution.

Table 2: Osmotic pressure of lactose hydrolyzed concentrated milk solution after stabilization.

Lactose hydrolyzed concentrated milk	Concentration ($g \cdot L^{-1}$)	Molecular weight ($g \cdot mol^{-1}$)	Concentration ($mol \cdot L^{-1}$)	Osmotic Pressure (MPa)
Galactose	130.81	180*	7.26×10^{-1}	1.770
Glucose	130.81	180*	7.26×10^{-1}	1.770
Lactose	14.05	342*	4.11×10^{-2}	0.100
Casein micelles	145.95	280000000**	5.21×10^{-7}	0.000
Soluble proteins	35.68	18400***	1.93×10^{-3}	0.005
		Total		3.645

* Data obtained according to Bobbio & Bobbio (2001)
**Data obtained according to Morris, Foster, & Harding (2000)
*** Data obtained according to Aich, Batabyal, & Joardar (2015). The molecular mass of β-lactoglobulin was used as the most abundant soluble protein in milk powder.
Mineral osmotic pressure was not calculated due to the low mineral concentration in the solution.

1.2.2. Experimental osmotic pressure

Table 3: Osmotic pressure of polyethylene glycol solution after stabilization.

PEG solution after stabilization for	Concentration % ($w \cdot w^{-1}$)	Osmotic Pressure (MPa)
Traditional concentrated milk	0.46	0.024
Lactose hydrolyzed concentrated milk	0.56	0.041

2. Proteins do not display any strutural differences between samples

2.1. Materials and methods

2.1.1. Small-angle x-ray scattering (SAXS)

SAXS experiments were conducted on the SWING beamline using the SOLEIL synchrotron (l = 1.033 A˚). The Aviex charge-coupled device detector was positioned to collect useful data in the q range 0.01–0.430 A^{-1}. The milk powder samples were diluted in distilled water (1:5) ($w \cdot w^{-1}$) and were flowed through a quartz capillary with a diameter of 1.5 mm and a wall thickness of 10 mm, then placed in a vacuum chamber at a flow rate of 0.75 $\mu l \cdot min^{-1}$. Frames were collected continuously during 500 ms with a dead time of 250 ms between frames. Data reduction to absolute units, frame averaging, and subtraction were performed using the FOXTROT program version 3.3.4. (Synchrotron SOLEIL, France).

2.1.2. Electrophoretic separation

Sample preparation was performed according to Pardo & Natalucci (2002), using the sample reduction per urea protocol. The gel containing stacking layer 4 % ($w \cdot w^{-1}$) and run layer 12 % and 16 % ($w \cdot w^{-1}$) was prepared according to Laemmli (1970). Next, 15 µL of sample was added to the gel using a coupled SE 600 Series vertical Slab Gel 80 V Electrophoresis Unit (Hoefer Scientific instrument, San Francisco, US). The sample was tested in a stacked gel at 40 mV and in a run gel at 60 mV. After electrophoresis, the fixative solution 10 % ($w \cdot w^{-1}$) of acetic acid, 30 % ($w \cdot w^{-1}$) of ethanol e 60 % ($w \cdot w^{-1}$) of water) was added and kept during 1 h. The coloring solution Comassie-Blue was added immediately thereafter and kept during 1.5 h. The discoloration was made by adding

acetic acid 10 % (v·v^{-1}) until the protein bands appeared. The gel was mesured using ImageJ software (Schneider, Rasband, & Eliceiri, 2012).

2.2. Result

Small-angle X-ray scattering (SAXS) and electrophoresis have been used in highly diluted samples to check for any structural or chemical differences between the proteins of the two samples.

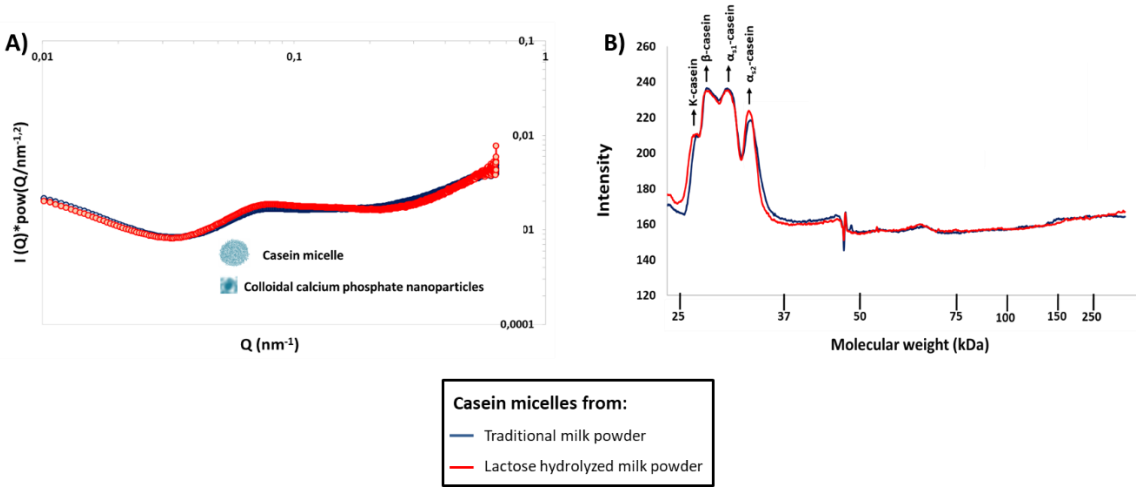

Figure 1. SAXS profil (A) and electrophoretic profile (B) of casein micelles from the traditional milk powder and the lactose hydrolyzed milk powder samples.

As shown in Figure 1A, traditional milk powder and lactose hydrolyzed milk powder diluted in water (1:5) (w·w^{-1}) had the same SAXS intensity in the range of magnitude of the dispersion vector (q) from 0.01 to 1 nm^{-1}. Therefore, lactose hydrolysis did not cause any structural modifications in casein micelles.

Figure 1A displays a spectrum that is very similar to the ones found in samples rich in caseins micelles (Bouchoux et al., 2010). As casein micelles are the largest macromolecules present in skimmed milk powder (diameter ranging from 0.05 to 0.5 µm) (Fox et al., 2015) and that they are found in abundance, this result was to be expected.

Figure 1B shows no significant differences in the size of the proteins present in traditional milk powder versus lactose hydrolyzed milk powder. All proteins presented coherent sizes when compared to previous research (Nasser, et al., 2018). There was no

formation of high molecular weight complexes (HMW), which indicate an absence of cross-linking between casein micelles and between casein micelles and sugars.

These results therefore indicate that the casein micelles present in the traditional milk powder and lactose hydrolyzed milk powder samples exhibit the same structural molecular conformation (Figure 1A and 1B).

3. Reference

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