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STUDY OF THE EFFECT OF INDUCED CRYSTALLIZATION OF LACTOSE AND MANUFACTURING TECHNOLOGY ON THE PHYSICOCHEMICAL, COLORIMETRIC, TEXTURAL AND RHEOLOGICAL CHARACTERISTICS OF DULCE DE LECHE

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

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I dedicate to my grandmother Edite that was
an artisanal candy maker

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ABSTRACT

NETTO, Gabriel Gama, D.Sc., Universidade Federal de Viçosa, October, 2022. **Study of the Effect of Induced Crystallization of Lactose and Manufacturing Technology on the Physicochemical, Colorimetric, Textural and Rheological Characteristics of Dulce de Leche**. Advisor: Ítalo Tuler Perrone. Co-advisors: Antônio Fernandes de Carvalho and Rodrigo Stephani.

Dulce de leche (DL) is a very popular dairy product in Latin American countries and its production ranges from small artisanal producers using only two ingredients, milk and sucrose in a simple pan, to large dairy companies with different technologies and scale production. DL can be consumed directly or used in the preparation of dessert recipes. One of the great demands is the confectionery segment in which the DL is used as a topping and filling. For this type of application, the proper viscosity and consistency of the DL is important because it must remain structured in the confectionery products. The induced crystallization of lactose in DL is a common practice used by some dairy industries with the main objective of preventing the appearance of large lactose crystals that affect the sensory perception of the product in a defect known as sandiness. Larger dairy industries that have a vacuum concentration line for the manufacture of powdered milk and/or sweetened condensed milk use concentrated milk in the production of DL with the objective of reducing cost through energy savings. No studies were found in the literature that correlated the induced crystallization of lactose or a vacuum concentration step on the rheological properties of DL. Thus, this thesis was structured in two articles with the aim of evaluating the effect on DL properties caused by different manufacturing technologies commonly used by the dairy industry and using a standard DL recipe. The first article evaluated on a laboratory scale the effect of lactose induced crystallization under different conditions of temperature and stirring time on the physicochemical and colorimetric characteristics, texture profile and rheological behavior of DL. The second article evaluated in a pilot scale the effect of the manufacturing technology with different equipment and the induced crystallization of lactose on the physicochemical, colorimetric, texture profile and rheological behavior and sensory analysis of the DL. In both articles, the rheological behavior of the treatments was determined and adjusted to the mathematical model that best explains the flow profile in the flow curve analysis and

the values of the storage modules (G') and loss (G") of energy were determined in oscillatory rheological analysis. Both the induced crystallization of lactose and the type of manufacturing technology were determinant in the properties of DL.

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Keywords: dulce de leche, lactose, crystallization, manufacturing technology.

RESUMO

NETTO, Gabriel Gama, D.Sc., Universidade Federal de Viçosa, Outubro, 2022. **Estudo do Efeito da Cristalização Induzida da Lactose e Tecnologia de Fabricação sobre as Características Físico-Quimicas, Colorimétricas, Texturais e Reológicas do Doce de Leite.** Orientador: Ítalo Tuler Perrone. Coorientadores: Antônio Fernandes de Carvalho e Rodrigo Stephani.

O doce de leite (DL) é um produto lácteo muito apreciado nos países Latino-americanos e sua produção é pulverizada compreendendo desde pequenos produtores artesanais usando apenas dois ingredientes o leite e açúcar em uma panela simples aos grandes laticínios com diferentes tecnologias e com produção em escala. O DL pode ser consumido direto ou utilizado no preparo de receitas de sobremesas. Uma das grandes demandas é o segmento de confeitaria em que o DL é utilizado como cobertura e recheio. Para este tipo de aplicação, a viscosidade e consistência adequada do DL é importante porque tem que se manter estruturado nos produtos de confeitaria. A cristalização induzida da lactose em DL é uma prática comum usada por algumas indústrias de laticínios com o objetivo principal de evitar o aparecimento de grandes cristais de lactose que afetam a percepção sensorial do produto em defeito conhecido como arenosidade. Indústrias de laticínios de maior porte que têm linha de concentração a vácuo para fabricação de leite em pó e\ou leite condensado usam leite concentrado na produção de DL com objetivo de redução de custo através de economia energética. Não foram encontrados na literatura estudos que correlacionassem a cristalização induzida da lactose nem de uma etapa de concentração à vácuo sobre as propriedades reológicas do DL. Dessa forma, esta tese foi estrutura em dois artigos com o objetivo de avaliar o efeito nas propriedades do DL causados pelas diferentes tecnologias de fabricação comumente usadas pela indústria de laticínios e usando uma receita padrão de DL. O primeiro artigo avaliou em escala laboratorial efeito da cristalização induzida de lactose em diferentes condições de temperatura e tempo de agitação sobre as características físico-químicas, colorimétricas, perfil de textura e comportamento reológico do DL. O segundo artigo avaliou em escala piloto o efeito da tecnologia de fabricação com diferentes equipamentos e da cristalização induzida da lactose sobre as características físico-químicas, colorimétricas, perfil de textura e comportamento

reológico e análise sensorial do DL. Em ambos os artigos foi determinado o comportamento reológico dos tratamentos e ajustados ao modelo matemático que melhor explica o perfil de escoamento na análise de curva de fluxo e, também foi determinado os valores dos módulos de armazenamento (G') e perda (G") de energia na análise reológica oscilatória. Tanto a cristalização induzida da lactose quanto o tipo de tecnologia de fabricação foram determinantes nas propriedades do DL.

Palavras-chave: doce de leite, lactose, cristalização, tecnologia de fabricação.

SUMMARY

1. GENERAL INTRODUCTION

According to the Technical Regulation for Fixing the Identity and Quality of Dulce de Leche approved by the Brazilian inspection service (MAPA), *Dulce de Leche* (DL) is the product, with or without the addition of other food substances, obtained by concentration and action from heat at normal or reduced pressure of milk or reconstituted milk, with or without addition of milk solids and/or cream and added sucrose (partially substituted or not by monosaccharides and/or other disaccharides) (Brasil, 1997).

DL is a dessert highly appreciated by consumers in Latin American countries. DL can be consumed directly, with cheese or used in the preparation of a multitude of dessert recipes. The confectionery segment plays a key role in the demand for DL consumption that can be used as a topping and/or felling in the preparation of cakes and pies. For confectionery application the texture and consistency of the DL is important as it needs to be able to structure on the cake surface and is also used to design decoration on confectionery.

The mixture of milk with sucrose and the other ingredients and additives is subjected to continuous evaporation of water through the transfer of energy in the form of heat indirectly with steam from boilers, having a pressure between 100 kPa to 600 kPa. The steam used in the process is transformed into steam condensate, water at 85–90 ºC, and its quantification makes it possible to determine the total mass of steam used in the manufacture. The process time can vary from 30 minutes to 4 hours, depending on the relationship between the area of energy exchange in the form of heat and the volume of milk, as well as the steam pressure used (Perrone et al. 2019). Normally, two types of evaporators can be used in the manufacture of dulce de leche, the first being the atmospheric pressure evaporator (pan) and the reduced pressure evaporator, known in the industry as vacuum evaporators. The two devices can also be combined.

The technology using only the pan is more used and has the advantage of simple operation and maintenance, however there is a limitation in working with large volumes of milk. Vacuum evaporators allow working with large volumes of milk while reducing the cost of evaporating water (Perrone et al. 2019).

Among the constituents of DL, lactose deserves attention due to its contributions to the color, flavor and texture of the product. At the end of cooking, lactose is the third

largest component, behind only sucrose and water, being in a supersaturated concentration in the aqueous phase of the DL. Naturally some of this lactose will migrate from the soluble state to the crystalline form until an equilibrium is established. In the supersaturation condition, the rate and velocity at which lactose migrates from the aqueous phase to the formation of nuclei and to the crystal surface is dependent on variables such as final lactose concentration, product viscosity, product cooling rate, shear, addition of lactose crystals (Schuck, 2011).

Lactose, or chemically called O-4-D-galactopyranosyl-(1,4)-D-glucopyranose, is the main carbohydrate in milk. This sugar has been found in the milk of almost all mammals and is unique to milk. This carbohydrate is a disaccharide composed of D-glucose and D-galactose and exists in two anomeric forms, α-Lactose and β-Lactose. The aldehyde group of C^1 galactose is linked to the C^4 group of glucose through a β-1,4-glycosidic bond, as shown in Fig. 1 (Walstra, Wouters & Geurts, 2006).

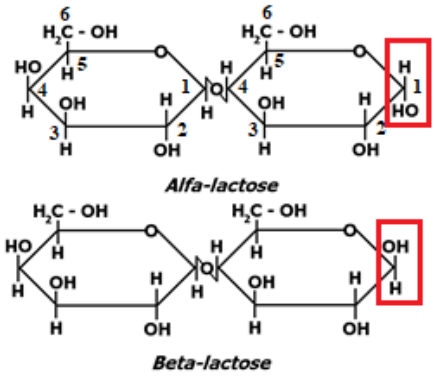

Figure 1 – Chemical structure of lactose (source: adapted from Perrone et al., 2019)

Lactose is a reducing sugar. It can be observed that the conversion of the α-lactose anomer into the β-lactose anomer, and vice versa, occurs through the open-chain form. This phenomenon is called mutarotation. In fresh milk 0.1% of the lactose is in the open chain form. At high temperatures and at high pH values, this proportion is much higher, reaching between 1 and 10%. As the aldehyde group is by far the most reactive of lactose, this means that sugar reactivity is greatly increased (Walstra, Wouters & Geurts, 2006). The high reactivity of lactose at high temperatures is crucial in the development of sensory characteristics in the DL. One of these aspects is the development of brownish coloration due to the dark pigments formed mainly during the cooking process. These compounds are formed in a non-enzymatic reaction known as

the Maillard reaction that occurs between proteins (primarily with the amino acid lysine) and reducing sugars such as lactose (Stephani et al., 2019).

The alpha and beta anomers of lactose have very different properties, the most important being the specific rotation, which for the alpha form at 20 °C is [α]D = +89º and for the beta form [β] = +35º and solubility 70 and 500 g L^{-1}, for alpha and beta lactose, respectively (Fox and McSweeney, 2009).

The α and β-lactose fractions are soluble in water to the extent of about 70 and 500 g L^{-1}, respectively, at 20 °C; at equilibrium, the alpha:beta ratio is about 37:63, giving a total solubility of about 180 g L^{-1} at 20 °C. The solubility of α-lactose is more temperature dependent than that of the beta-anomer, as it is more soluble at temperatures lower than 93.58 °C. Therefore, α-lactose is the form of lactose that crystallizes below 93.58 °C and is the usual commercial form of lactose; β-lactose can be prepared by crystallization at a temperature higher than 93.58 °C (Fox & McSweeney, 2009).

The crystallization of lactose in dulce de leche is commonly associated with the defect known as sandiness, in which the lactose crystals grow to a size that they are perceptible sensory, however sensory perception is dependent on the size and quantity of crystals formed (Hough, Martinex and Contarine, 1990). Some mechanisms can be industrially employed to control or delay the defect, such as partial hydrolysis of milk lactose before starting to cook, increase in product viscosity through the addition of thickening agents or the addition of lactose crystals (Perrone et al. 2019). These crystallization control mechanisms change the quantity and size of lactose crystals and can impact the rheology and texture of the DL.

The lactose crystallization profile (number, size and shape of crystals) plays a fundamental role in the conservation, texture, acceptance and quality of DL. This study aimed to investigate how different controlled conditions of induced crystallization of lactose and the main manufacturing technologies used by the industry affect the physicochemical, colorimetric, rheological and sensory characteristics of DL.

In view of the above considerations, the thesis aimed to analyze and meet two current demands by two different papers:

- Understand if there is a more favorable process condition to carry out the induced crystallization of lactose and if these conditions modify the properties of the DL**.**

PAPER 1: The Effect of Induced Crystallization of Lactose on Dulce de Leche properties.

- How lactose induced crystallization combined with different DL manufacturing technologies used by the industry affect product properties. **PAPER 2:** Influence of Induced Lactose Crystallization and Manufacturing Technology on the Physicochemical, Rheological, Colorimetric and Sensory Characteristics of *Dulce de Leche*

** The papers presented below are in the format requested by the different journals in which the works were published.

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2. PAPER 1

Netto, G.G., Francisquini, J.d., de Carvalho, A.F. *et al.* The effect of induced crystallization of lactose on *dulce de leche* properties. *Eur Food Res Technol* (2022). https://doi.org/10.1007/s00217-022-04115-7

The effect of induced crystallization of lactose on dulce de leche properties

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Abstract

This study evaluated the effect of induced crystallization of lactose, by adding lactose crystals at different temperatures, on the texture and physicochemical, colorimetric, rheological behavior of *dulce de leche*. Three different samples of *dulce de leche* were produced: control TRT1 (filling 70-75 °C after cooking) and two treatments varying the conditions of forced crystallization of lactose TRT2 (25 °C/90 min) and TRT 3 (35 °C/30

min. and at 25 °C/60 min). There were no significant changes in physicochemical characteristics, but crystallization altered the *dulce de leche* colors parameters. Microscopy indicated the formation of three groups of crystallization profiles according to the size and distribution of lactose crystals, which showed a significant decrease ($p<0.05$) in some textural parameters in the treatments with induced lactose crystallization. All treatments showed pseudoplastic behavior in the flow curves and the data were adjusted to the Power Law model, but there were significant differences in the consistency index (K) and flow behavior index (n). The treatments with induced crystallization showed different behavior in the dynamic rheological test, however, the TRT3 was the closest to the control, possibly due to its crystallization profile.

Key-words: Lactose crystals, physicochemical analyzes, texture, rheology, browning index

1. Introduction

Dulce de leche (DL) is a concentrated dairy product appreciated in most Latin American countries and its consumption can be direct, as an ingredient in desserts, or used by the confectionery segment in fillings and toppings. In Brazil, a large portion of the manufactured DL is consumed in the confectionery market and for this segment, the texture and rheology characteristics are very important for the product functionality [1].

DL is made by mixing milk, sucrose, sodium bicarbonate and other additives subjected to continuous evaporation of water under normal or reduced pressure through the indirect transfer of thermal energy with steam from boilers to reach a concentration close to 70% (w/w) [2]. Heating and maintaining an elevated temperature for an extended period produce changes in the milk composition that develop the sensorial and physicochemical characteristics of the DL [3,4].

The milk evaporation step can result in physicochemical and structural changes that can alter the flow properties of the product, removing water and reducing the space between the particles (casein micelles, fat globules, whey proteins, lactose and minor constituents) [5,6]. This greater interaction between milk components, which protein and fat are the most altered and the physicochemical changes within the elements strongly affect the rheological behavior of concentrated milk [7].

Among the constituents of DL, lactose deserves a spotlight for its contributions to the color, flavor and texture of the product. At the end of the evaporating step, lactose is the third-largest component behind sucrose and water, being in supersaturated concentration in the aqueous phase of DL [2].

Crystallization is a physicochemical process resulting from the supersaturation of solutions with the formation and growth of crystals, such crystallization is dependent on a number of factors such as temperature, pH, presence of salts, agitation, time, cooling rate, viscosity and mutarotation. [2,8,9]. Lactose supersaturation is the driving force for state change, causing lactose to shift to a crystallized state [8]. Lactose crystallization in DL is commonly associated with the defect known as sandiness, in which lactose crystals grow to a size that becomes sensory perceptible [10]. Some mechanisms may be industrially employed to control or delay this defect, such as 1) partial lactose hydrolysis of milk prior to evaporating; 2) increased product viscosity by adding thickening agents or; 3) the induction of crystallization by the addition of lactose crystals [2]. Among these mechanisms, induced lactose crystallization through lactose

addition alters the quantity and size of the crystals that impact the texture of the DL and probably has great relevance in the rheological behavior of the product [11]. However, no studies were found evaluating the effect of induced crystallization of lactose on the rheological characteristics of DL.

Lactose crystallization is not a traditional practice in the production of DL, unlike the production of condensed milk, in which crystallization is a mandatory and important step [12,13]. The biggest problem in crystallizing lactose in DL corresponds to the fact that the time (before, during or after production) and the temperature of lactose addition can interfere with the sensory (color, texture, and flavor) and microbiological (fungi and bacteria such as *Escherichia coli*) characteristics of the product [2,14]. Therefore, lactose is usually added at the end of production and at lower temperatures to facilitate the formation of small lactose crystals (not perceptible to the taste) [12,15,16]. However, during the production of DL, packaging at lower temperatures can result in microbial contamination of the product. Thus, for the company to carry out this crystallization of lactose, it is required satisfactory hygienic control on the part of the company [2,14].

In summary, the aim of this work was to evaluate the influence of induced lactose crystallization, at different temperatures and, at the same final time, by the addition of lactose crystals on the physicochemical, colorimetric, texture and rheological behavior of DL and try to understand if the control or manipulation of the lactose crystallization conditions may contribute to a product with specific functionalities.

2. Material and methods

2.1- *Dulce de Leche* Manufacture

For DL manufacture were used pasteurized standardized milk LTST (3% fat, Laticínios Escola at the Universidade Federal de Viçosa, Viçosa-MG, Brazil), whole milk powder (Itambé, Brazil), sucrose (180 g/L milk, Delta, Brazil), sodium bicarbonate (1000 mg/L milk, Carbonor, Camaçari-BA, Brazil), potassium sorbate (200 mg/kg product, Wanglong Group CO LTD, China) and micronized α-Lactose Monohydrate (50 mg/kg of product, Sigma Aldrich, Saint Louis, USA, according to the lactose supplier the lactose applied in this study had d_{90} equal to 10 micrometers). The DL was manufactured using a system developed by the research group itself, which simulates

industrial evaporation devices on a lab bench scale. The system comprises a commercial Thermomix TM5 processor (Vorwerk, Wuppertal, Germany) coupled to a load cell (Ramuza IDR 7.500, Santana de Parna ba, Brazil) with 1 g precision and a PT-100 temperature sensor. The function of the load cell is to monitor water evaporation throughout the process. This evaporation system allows us to monitor the loss of water mass and stop the process at the desired final composition, based on previous mass balance calculations [17].

Sucrose was mixed with milk powder and sodium bicarbonate and added to the fluid milk. Then, heating to concentration was initiated and the soluble solids content (Refractometric Index, Digital Refractometer, 2WAJ-D Biobrix, Brazil) were monitored until the end (68-69°Brix). For microbiological control, potassium sorbate was added during concentration when the refractive index range was between 58-60º Brix. At the end of the concentration process, one part of the DL was filled into 150 mL plastic containers (control) and the remaining amount of DL was divided into three parts to vary the crystallization conditions forming the remaining three treatments. The process of adding microcrystalline lactose to DL accompanied by agitation was defined, in this study, as induction of lactose crystallization. The portioned DL for crystallization induction was cooled to the target temperature and then micronized α-lactose crystals were added and kept in a temperature-controlled bath under stirring at standard speed for 90 minutes (min) and then filled into 150 mL plastic containers. After manufacturing, all treatments were kept at room temperature during storage.

The conditions of each treatment were (n=3): Treatment 1 (TRT 1 - control) - cooling to 70-75ºC and directly packaged without lactose addition and stirring; Treatment 2 (TRT 2) - cooling to 25 °C and lactose induced crystallization for 90 min; Treatment 3 (TRT 3) - cooling to 35 °C and lactose induced crystallization for 30 min followed by cooling to 25 °C and lactose induced crystallization for 60 min. Each treatment was made in 3 repetitions.

2.2 Physicochemical, colorimetric analysis and browning index

After the production of the DL, moisture content was analyzed by gravimetric method at 102ºC [18], soluble solids content (Biobrix digital refractometer, model 2WAJ-D) and pH (Kasvi pH meter, Curitiba, Brazil).

For color analysis a CIE Lab ($L^*a^*b^*$) color scale with D65 illuminant (6900° K) with 10 ° aperture was used (Color Quest II, Hunterlab). The value L^* represents the brightness of the sample, ranging from black (0) to white (100); the value of a^* represents the color ranging from red (+) to green (-); and the value of b^* represents the color ranging from yellow (+) to blue (-). The equipment was calibrated in the excluded specular reflectance mode using white (C6299 Hunterlab Color Standard) and gray (C6299G Hunterlab Color Standard) reference plates. Analyzes were performed in duplicate.

The browning index (BI), used to evaluate the brown color intensity of the samples, was calculated by Equation (1), which correlates the parameters L^*, $a^*$and b^*[19,20].

Equation 1:

$$BI = 100 \cdot (x - 0.31)/0.17$$

where $x = (a^* + 1.750 \cdot L^*)/(5.645 \cdot L^* + a^* - 3.012 \cdot b^*)$

2.3 Optical microscopy

The microscopic analysis was performed with the aid of an optical microscope (Olympus, model BX41 TE) using a 10x ocular lens and a 25x objective lens, with a camera attached connected to a computer containing the MSI Ima Win software for photographic recording and measurement of the size of the lactose crystals. The images were obtained in duplicate and in 4 different fields.

2.4 Instrumental texture profile

The texture profiles (TPA) of the *dulce de leche* were evaluated in duplicate using a Brookfield CT3 texture analyzer (Brookfield Engineering Laboratories, Middleborough, MA, USA) to obtain the force-time curves. The method for texture profile analysis (TPA) was used as recommended [21]. The Texture Profile was supplied with a 4.5 kg load cell and application software (Brookfield Texture PRO CT®). Two successive compressions were performed in each sample. Through force-time curves, the results of hardness, adhesiveness, cohesiveness, elasticity and gumminess were calculated by the software. The samples were previously transferred

to a cylindrical acrylic container (4.0 cm in diameter and 7.0 cm in height). The following parameters were adopted to test the texture: mode TPA; test and return speed 1 mm s^{-1}; target depth 20 mm; trigger load 5.0 g; pre-test speed 1 mm s^{-1}; data rate 10 points/s; probeTA4/1000 (38.1 mm diameter).

2.5 Rheological evaluation

2.5.1 Steady shear flow

The flow behavior of the samples was determined using a rotary rheometer (R/S plus SST 2000; Brookfield) with a CC45 sensor (concentric cylinders), connected to the RHEOCALC V 1.1 software. The analysis was taken at 25 °C in three steps, initially using a constant shear rate of 100 s^{-1} by 420 s for loss of thixotropy of the sample and then a decreasing ramp ranging from 100 to 0.5s^{-1} and an increasing ramp of 0.5 to 100 s^{-1} for 180s each ramp. The third step was used to plot data on the graphs of shear rate (X axis) versus shear stress (Y axis).

2.5.2 Dynamic test

Oscillatory tests were performed with a rheometer (Discovery Hybrid Rheometer 1, TA Instruments, The United States of America) equipped with a stainless-steel parallel plate geometry (diameter = 25 mm; gap = 1 mm), maintained at 25.0 ± 0.1 ºC.

Before starting the oscillatory test, a simulation was performed at a constant frequency of 2.53/s (1 Hz), varying the amplitude of the stress applied to the samples to determine the linear viscosity region (LRV). The criterion established for its determination was the linear relationship between strain and stress. In the linear viscoelastic range, material functions, such as storage modulus G′ and loss modulus G″ do not depend on the magnitude of the applied stress, the magnitude of deforming strain or application strain rate.

The dependence of the storage modulus (G′) and loss modulus (G″) on angular frequency (w) was determined at a small magnitude of stress in the linear viscoelastic region (LRV). In the experiments, shear stress (б) was applied as a sinusoidal time function over a small fixed amplitude. The stress frequency was increased step by

step, and at any frequency step, the resulting signal was transformed into the elastic and viscous components. The frequency range used was from 2.5 to 100 s^{-1}.

2.6 Statistical analysis

Analysis of variance (ANOVA) was performed using the SAS software (version 9.3, SAS Institute Incorporation, The United States of America), licensed by the University of Viçosa (UFV). For the mathematical modeling, nonlinear regression was applied to the experimental data to estimate the parameters of different rheological models.

3. Results and Discussion

3.1 Physical-chemical characteristics, color and microscopy

Table 1 shows the physicochemical results of the DL samples. There was not a significant difference ($p<0.05$) in the moisture content (values between 27.98 and 28.54 g/100 g), soluble solids content (values between 68.77 and 69.00 °Brix) and pH (values between 6.10 and 6.15), regardless of the presence or absence of crystallization. According to requirements established by Mercosur legislation, the moisture content of DL must be a maximum of 30.00 g/100g [22] and requirements established by literature, a recommended soluble solids content within the range of 68.00 – 70.00°Brix [2]. There is still no ideal range regarding the pH parameter. In a study carried out by [23], a compositional analysis was performed on seven DL samples acquired in commercial establishments located in different states of Brazil. In this same study, moisture content was found with a minimum value of 17.49 g/100 g and a maximum of 29.67 g/100 g, as well as a pH with a minimum value of 6.14 and a maximum of 6.37. In Francisquini et al. [4], the authors investigated the evaporation rates and evaporative capacity of evaporators at atmospheric pressure during the production of DL by evaluating the content of soluble solids throughout the manufacture of three different DL formulations and, in the end, the products obtained soluble solids content with a minimum value of 66.37°Brix, maximum of 70.30°Brix and an average of 68.98°Brix. The reported values for the physical-chemical parameters

are within the same range of values found in the requirements established by Mercosur legislation and by literature as mentioned above.

Considering that it was used 2000 g milk + 100 g whole milk powder + 498.6 g sucrose + 2.77 g sodium bicarbonate + 0.175 g micronized lactose for the manufacture of DL, it resulted in 2601.54 g of raw materials. After concentration, it resulted in a final amount of approximately 1120 g of dulce de leche and 1481.54 g of evaporated water. Therefore, considering that these DL present 30% moisture, it could be inferred that the amount of water is approximately 258.46 g and the amount of lactose (milk + powdered milk + micronized lactose) is approximately 130.58 g. Resulting in 50.52 g lactose/100 g water in the DL.

DL is a brown-colored product that can have its color intensity controlled by the manipulation of ingredients and processing conditions. The brownish coloration is due to dark pigments formed mainly during the cooking process. These compounds are formed in a non-enzymatic reaction known as the Maillard reaction that occurs between proteins (mainly with lysine amino acid) and reducing sugars such as lactose [2]. The formation of these dark pigments is enhanced by the increase in temperature and exposure time at high temperatures, conditions observed during the manufacture of DL. The intensification of the Maillard reaction in foods such as DL can result in interferences from a nutritional point of view (such as a decrease in amino acids and proteins available for absorption and/or an increase in the endogenous pool of advanced glycation products) and sensory (such as more intense color and/or modification of texture and flavor) decreasing its acceptance by consumers [24]. There are some studies that performed analyzes of indicators of the Maillard reaction in DL to verify the influence of heat treatment in different products with different formulations and modifications of factors that can influence the higher or lower speed of MR, such as pH, lactose hydrolysis, increased concentration of reducing sugars [3,4]. However, in this research, the main processing variables that could affect the intensity of the Maillard reaction between different treatments were minimized through the standardization of boiling temperature, evaporation rate and standardization of formulation ingredients.

It is worth mentioning that the treatments were submitted to the same process conditions until the end of the evaporation step in which they were fractionated into TRT1 (control), TRT2 (cooling to 25 °C and lactose-induced crystallization for 90 min.) and TRT3 (cooling to 35 °C and lactose-induced crystallization for 30 min followed by

cooling to 25 °C and lactose induced crystallization for 60 min.). Therefore, the main effect on the observed color difference between treatments is correlated with the crystallization process.

The colorimetric results are presented and visually supported by the images in Figures 1B and 1C. The lightness parameter value (L^*) increased for samples that had induced lactose crystallization, with the value of the control treatment (TRT 1) increasing from 41.39 to 47.07 and 45.58 for treatments TRT 2 and TRT 3 respectively. Although L values increased for the two treatments that induced crystallization, only treatment TRT 2 differed statistically from control TRT 1 ($p<0.05$). Increasing the value of L^* indicates a color change in the white scale revealing increased lightness. The induced crystallization did not influence statistically the redness (a^*) parameter ($p<0.05$) with values: 5.73 (TRT1); 7.08 (TRT2) and 7.01 (TRT3). The redness parameter indicates a change in color toward the red scale. In contrast, the yellowness parameter (b*) showed a statistical difference ($P <0.05$), with an increase in value through the induction of lactose crystallization. The control sample TRT 1 (11.98) statistically differed from treatments TRT 2 (15.83) and TRT 3 (15.22), while the two treatments with lactose crystallization induction remained statistically equal. Increasing the yellowness parameter indicates a color shift toward yellow on the colorimetric scale.

Fig. 1 (A) Registration of lactose crystals after 30 days of manufacture (images in 250x and the size of the two crystals on their longest sides were measured and reported in µm). (B) Images of the three samples of DL for visual assessment of the effect on the color of induced crystallization of lactose. (C) Colorimetric analysis (L* – lightness, a* – redness and b* – yellowness) and browning index (BI), used to evaluate the brown color intensity of the samples.

Different superscripts letter within the same line (same parameter L, a* or b*) indicate significant differences by the Tukey test ($P<0.05$).

**Treatment 1 (TRT 1) - cooling to 70-75 °C and directly packaged without lactose addition and stirring; Treatment 2 (TRT 2) - cooling to 25 °C and lactose induced crystallization for 90 min; Treatment 3 (TRT 3) - cooling to 35 °C and lactose induced crystallization for 30 min. plus cooling to 25 °C and lactose induced crystallization for 60 min.).

Francisquini et al. [4] performed a color analysis in DL with and without lactose hydrolysis, with L^* values ranging from 31.44 to 61.23; a^* between 5.26 and 10.6; b^* between 7.00 and 23.40 conforming to the results found in the study developed here. Also, Leddomado et al. [25] produced six different formulations of DL by modifying the type of milk used (whole or skimmed) and the type of prebiotic (inulin or xylooligosaccharides) and found minimum and maximum values respectively L^*: 44.02 and 47.00; a^*: 12.62 and 13.95; b^*:17.12 and 19.43 values are slightly higher than the minimum and maximum values found in the present work, which demonstrates that the modification of the formulation and/or process can influence the final color of the DL.

The browning index is used to assess the brown color intensity of different food products such as Kiwifruit during hot air and microwave drying with BI values of 42.11 to 78.93 [19], fresh sliced mushrooms with BI values of 16.10 to 26.30 [26], whey protein isolate with BI values between 10 – 20 [27], buffalo milk powder with and without lactose hydrolysis with BI values between 5.87 and 32.28 [28], goat milk powder with and without hydrolysis of lactose with BI values between 7.37 and 60.72 [29]. No significant statistical difference was found in the darkening index of the samples analyzed here (TRT1 50.29; TRT2 52.17; TRT3 50.60), demonstrating that they are not different from each other in terms of brown color intensity.

For DL, only one study has determined the BI [30] so far. However, in that study, the value range found for BI was 2000 - 3700, a value much higher than the one found in the present study. This difference must be inferred by multiplying the parameter a* in the formula to obtain the BI, which does not exist in the original equation [19,20]. Thus, this makes it impossible to compare the results.

Lactose crystals can be observed in all treatments within 30 days of manufacture in the microscopic records of Figure 1A. The control treatment had larger crystals compared to the other treatments with an average dimension of 79 µm. A predominance of trapezoid-shaped crystals can be observed. The TRT2 treatment showed crystals with dimensions between 30 and 40 µm with a predominance of trapezoid-shaped crystals, but some pyramid-shaped crystals can also be seen. The TRT3 treatment presented a distinct crystallization profile with a large number of small crystals with a dimension below 10 µm and the larger crystals had a dimension of 18 to 25 µm and a similar distribution between trapezium and pyramidal shape can be found. Based on the microscopy results, the crystallization profile can be divided into three groups, the first formed by TRT1 with large crystals, the second by TRT2 with

medium crystals profile and the third group formed by TRT3 with a very high number of small crystals.

There is a trend of a sensory deficiency, related to the perception of lactose crystals during product consumption, which is called sandiness [25,31]. From a size range between 15 µm and 20 µm the lactose crystals become perceptible to the palate in condensed milk [32]. Therefore, there are no studies for DL that show which size and shape the lactose is perceptible to the palate. However, it is probable that DL with lactose crystallization induction (TRT2 and TRT3) results in DL with crystals in smaller quantities and smaller sizes, which could reduce this defect. Crystallization occurs by two consecutive events: nucleation - the generation of new crystals (nuclei), followed by growth where the nuclei become larger crystals [8]. It is likely that after manufacturing the control sample, nuclei for crystal growth have not yet been formed. Lactose in solution moves from the aqueous phase of the DL to the surface of the crystals by diffusion, and the treatments TRT 2 and TRT 3 that had lactose nuclei added with the agitation of the crystallization favored the contact between the lactose in the aqueous phase of DL and the surface of the crystals.

The TRT3 treatment presented a different crystallization profile (higher number of crystals and smaller size) than TRT2, possibly due to the two cooling stages during crystallization. In the first cooling stage, the product reaches a temperature of 35 °C, in which the rate of mutarotation is higher than 25 °C. However, at 35 °C the solution saturation force for crystallization is lower than 25 °C. In the first stage, it is stirred for 30 minutes, a time that did not favor the mutation of lactose and discouraged the force of saturation for crystallization. In the second stage of crystallization, the product reached a temperature of 25 °C, after being stirred for 60 minutes. This second stage occurs with a decrease in the rate of mutarotation and an increase in the saturation force for crystallization [8].

Lactose is present in milk and dairy products in two anomeric forms, alpha and beta, which have very different properties, the most important being the specific rotation, which for the alpha form at 20 ºC is [α]D = +89º and for the beta form [β] = +35º and the solubility. The α and β-lactose fractions are soluble in water to the extent of about 70 and 500 g L^{-1}, respectively, at 20 °C; at equilibrium, the alpha: beta ratio is about 37:63, resulting in a total solubility of about 180 g L^{-1} at 20 °C. Due to this balance, it is possible to observe the behavior of conversion of the α-lactose anomer to the β-lactose anomer, and vice versa in a phenomenon called mutarotation [33]. The

solubility of α-lactose depends more on the temperature than beta-anomer, and it is more soluble at temperatures below 93.58 °C. Therefore, α-lactose is the form of lactose that crystallizes below 93.58 °C and it is, for example, the usual commercial form of lactose [33].

By lowering the temperature, the supersaturation of lactose increases while the rate of mutarotation is reduced. By adding the lactose crystals, crystallization occurred spontaneously forming a large number of nuclei and as the lactose crystallized as alpha crystals, the beta lactose was converted to alpha lactose by mutarotation equilibrium. This step was common to treatments TRT2 and TRT3, but when the second cooling step from 35 °C to 25 °C was performed in TRT3, there was a further increase in the supersaturation curve, leading to the maintenance of a large number of lactose crystals, which showed limited growth. The TRT2 treatment was performed direct cooling to 25 ºC which greatly increased the supersaturation, but with limited mutarotation speed leading to crystals larger than TRT3. In the meantime, at 25ºC the product had higher viscosity limiting the contact among lactose in solution and the lactose nucleus.

3.2 Texture profile

The results of the instrumental texture profile indicated a significant difference ($p<0.05$) between the samples for the parameters: hardness, adhesiveness, cohesiveness, elasticity and gumminess, with the formation of two groups of samples according to the result range. The first group is formed by the control sample (TRT 1) and the second by TRT 2 and TRT 3 samples. It's possible to observe that all samples that had the induction of lactose crystallization presented statistically lower results for hardness, adhesiveness, cohesiveness, elasticity and gumminess. When relating the texture parameters to the chewing process, the TRT1 sample, compared to the other treatments, presents greater resistance to breaking of DL structure during chewing until the moment of swallowing, greater adherence in the mouth and greater ability to return to the original shape after compression removal. Silva et al. (2015), in the evaluation of the effect of adding modified starch on the characteristics of the DL, obtained for the control sample (no starch addition) texture parameter, present results compatible with those found in the control sample TRT 1.

Induced crystallization of lactose alone is not enough to explain the reduction in texture parameter values. The induced crystallization of lactose added to the shear during the crystallization step is probably the main contribution in the process that altered the structure of the DL and reduced the parameters when compared to the TRT1 sample. The highest values of hardness and gumminess of the control sample (TRT 1) corroborate the estimated model parameters adjusted from the flow behavior curve (Table 3) in which the control treatment presented the highest consistency index value k and higher apparent viscosity as a function of shear rate. It is not the crystallization itself that reduces the values of texture parameters, but the sum of events in the crystallization step. Shear during the crystallization step can be considered one of the factors that most impact the reduction of texture parameters. When the flow curve (Figure 2) is observed with increasing sweet shear, it starts to exhibit a pseudoplastic behavior as can be explained by the adjusted mathematical models. However, the product cannot be considered thixotropic, that is, it does not recover its initial condition after being subjected to shear. Therefore, the shear effect of the crystallization step is the possible main cause of the reduction of texture parameters and also of the shear stress in the flow curve.

Francisquini et al. [34] evaluated the texture parameters of commercial brands of traditional DL and the authors found mean values of 1015.58 ± 900.35 g for hardness, 619.67 ± 400.75 g for gumminess, 16.13 ± 4.34 mJ for elasticity and 59.20 ± 69.79 mJ for adhesiveness. It is worth noting that the high standard deviation found in the cited work is inferred to the de-standardization of DL commercialized in the Brazilian market (type and amount of ingredients added, type of equipment used, time and type of processing). In a study carried out by Pinto et al. [17], texture analysis was performed on DL with and without lactose hydrolysis produced through milk with and without homogenization. The study found hardness values from 437 to 1400 g; adhesiveness from 20 to 77 mJ; elasticity from 10 to 19 mm; gumminess from 386 to 1321 g. The difference in the reported values and those found in the present work demonstrates that modifications in the formulations, as well as differences in the DL manufacturing process, can change the texture of this product. In conclusion, it appears that the values of hardness, adhesiveness and gumminess exhibit lower values in the present work when compared to the cited literature works. The only exception concerns the elasticity parameter, which presented a higher result in the present work than in the studies in the literature compared.

3.3 Rheological Characterization

The differences observed in the different treatments in the flow curves and in the oscillatory test were mainly due to the accomplishment or not of the crystallization step, with greater shear effect and secondly, with less intensity the effect of the different crystallization profiles of the treatments. As there was no significant difference between the physicochemical results, the differences observed in the rheological data are justified by the effect of the crystallization step, as will be discussed below.

3.3.1 Steady-state flow

The first step of the flow curve analysis method was to submit the treatments to a high shear condition for a time of 420 s in order to reduce the thixotropic behavior of the samples. The pre-analysis shear flow curves clearly demonstrate the effect of agitation in reducing the apparent viscosity of the three treatments over time (data not shown) and which allows us to extrapolate that also the shear during the crystallization step had a great influence of values lower shear stress for treatments TRT2 and TRT3 when compared to control TRT1.

Figure 2 shows the flow curves obtained from the different DL samples (TRT 1, TRT 2 and TRT 3). All three samples exhibited qualitatively similar behavior, with the initial shear stress starting near the axis origin with an upward concave curve forming over the shear rate. Increasing the shear rate causing a less proportional increase in shear stress is a shear-thinning characteristic, also known as pseudoplastic behavior. This behavior is the disruption of interactions between the structural units in the food due to the hydrodynamic forces generated during the shear that the product undergoes [35]. Ranalli, Andrés, & Califano [7] also observed pseudoplastic behavior in the largest extension of the flow curves for commercial samples of DL.

Fig. 2 Steady-state flow curves of DL samples

*Treatment 1 (TRT 1) - cooling to 70-75 °C and directly packaged without lactose addition and stirring; Treatment 2 (TRT 2) - cooling to 25 °C and induced crystallization of lactose for 90 min; Treatment 3 (TRT 3) - cooling to 35 °C and induced crystallization of lactose for 30 min plus cooling to 25 °C and induced crystallization of lactose for 60 min.)

The flow curves results were fitted to the Power Law model represented by Equation 2, also known as the Ostwald de Waele equation:

Equation 2:

$$\sigma = K.\ddot{\Upsilon}^{n}$$

Where σ is shear stress (Pa.s), K is consistency index (Pa.s), $\ddot{\Upsilon}$ is the shear rate (s^{-1}) e n is the flow behavior index (dimensionless).

The flow curves of the three samples of DL were properly adjusted in the Power Law model and the estimated parameters of the model (k and n) can be seen in Table 3. The TRT 1 control was the most consistent (highest k value) and lower values were found in the TRT 2 and TRT 3 treatments. The values of n <1 for all treatments confirmed the pseudoplastic behavior of the samples and the TRT 1 sample presented lower values of n being more pseudoplastic than the TRT 2 and TRT 3 treatments. Another aspect that can be observed in Table 3 is the apparent viscosity (η_a) at the shear rate of 100 s^{-1}. The apparent viscosity of treatments that had induced crystallization of lactose (TRT 2 and TRT 3) became less viscous than the control sample TRT 1, although there were no statistical differences. These lower viscosity results from TRT 2 and TRT 3 treatments are consistent with the lower values of hardness, adhesiveness, cohesiveness and gumminess observed in the instrumental texture profile of Table 2 and are probably associated with induced lactose crystallization and shear during the crystallization step.

It was previously inferred that the difference observed between the TRT1 control treatment and the treatments with induced lactose crystallization is the sum of the shear during crystallization with profile and amount of crystals formed. However, when we compare the results of treatments only TRT2 and TRT3, it can be concluded that the difference between the two treatments is due to the crystallization profile, since both treatments were submitted to the same shear time during the crystallization step. Possibly the higher incidence of crystals and the proximity between them favored a greater contact between the crystals and structuring of the DL in the TRT3 treatment, corroborated by the superior consistency index result (45.90 ± 4.56 Pa s-1) when compared to the TRT 2 consistency index (30.11 ± 3.50 Pa s^{-1}). This greater structuring

of the TRT3 treatment reflected in a greater shear stress along the shear rate compared to TRT2.

3.3.2 Oscillatory test

The results of the oscillatory tests of the samples that had induced the crystallization of lactose were compared graphically with the control sample, as can be observed in graphs a and b of Figure 3. In all three samples of DL, there was a predominance of the storage module. (G') over the loss modulus (G”) over the entire frequency range, ie, the DL behaved more like a solid than a liquid in all treatments.

Fig. 3 Frequency dependence of storage (g′) and loss (g″) moduli for the different samples of DL. A = treatment TRT1 compared to TRT 2 and B = treatment TRT 1 compared to TRT 3

*Treatment 1 (TRT 1) - cooling to 70-75 °C and directly packaged without lactose addition and stirring; Treatment 2 (TRT 2) - cooling to 25 °C and induced crystallization of lactose for 90 min; Treatment 3 (TRT 3) - cooling to 35 °C and induced crystallization of lactose for 30 min plus cooling to 25 °C and induced crystallization of lactose for 60 min.)

In the intermediate region of the mechanical frequency spectrum, there is a region with a slight “plateau” that could be observed in both modules and was more evident in the loss tangent curve (tg [δ] = G”/ G'). This region has been assigned as a transition zone characterized by a system highly structured by the rearrangement of polymer chains [36]. This same behavior in commercial samples of DL was previously described in the research of Navarro, Ferrero, & Zaritzky [37] and Ranalli, Andrés, & Califano [7].

For all treatments, it is possible to observe a reduction in the difference between the storage modulus (G') and the loss modulus (G”) along the frequency spectrum, with a sharp reduction in the higher frequency values. This visual observation is confirmed by increasing the loss tangent value tg (δ) across the frequency spectrum for all treatments. The TRT 2 treatment was the sample that most differed from the TRT 1 control sample for both storage (G') and loss module (G”), evidencing the largest consistency difference among the three treatments, as can be seen in Figure 3a. These

results are consistent with the lowest viscosity in the flow curves (Figure 2) and lowest consistency index k (Table 3). The TRT 3 treatment was closest to the TRT 1 control sample in the storage modules (G') and loss module (G''), as well as the sample that had the smallest difference in the flow curve compared to the control treatment. However, these results are antagonistic to the parameters of the instrumental texture profile (Table 2) for which the sample presented statistically lower values. This behavior may be related to the crystallization profile of the TRT 3 treatment, as this sample presented a very small number of lactose crystals, as can be seen in Figure 2a. The large number of crystals in the TRT3 treatment seems to have contributed to a greater structuring of the DL, leading to an approximation of the behavior of the control DL TRT1. Unlike the flow curve analysis, the oscillatory test is performed in the region of linear viscoelasticity (linear viscoelastic range) there is no effect of mechanical forces of the equipment, the results of expression of storage modulus and energy loss are exclusively because of the characteristics intrinsic to each treatment. This is possibly the reason that the TRT3 treatment is closer to the behavior of TRT1 than the TRT2 treatment.

4. Conclusion

The induced crystallization of lactose in the DL at different temperatures did not cause significant changes in the physicochemical characteristics of the product, but altered the form of interaction of the product with light, leading to a change in color with treatments with induced lactose crystallization (changes in the values of L^* and b^*). The addition of lactose crystals in the DL changed the lactose crystallization profile favoring the formation of a large number of small crystals. It is worth noting that crystallization using two stages of cooling in the TRT3 treatment (at 35 °C/30 min. and at 25 °C/60 min.) provided greater efficiency in the number of crystals and also showed less growth.

The induced crystallization of lactose altered the instrumental texture profile and the rheological behavior of the DL samples. Treatments with induced crystallization showed a decrease in texture parameters. All treatments showed pseudoplastic behavior in the flow curve and were properly fitted to the Power Law model, although the induced crystallization treatments showed flow behavior index (n) and consistency index (k) lower than the control treatment. In the dynamic test, there was a

predominance of the energy storage module (G') over the loss modulus (G”) for all treatments and a magnitude reduction was observed in both modules in the treatments that induced crystallization. There was a smaller difference between both modules comparing the control with TRT3 probably due to the crystallization profile of TRT 3, which showed many small crystals that had little evolution in size over 30 days in the microscopic evaluation.

Valuable differences were found in the characteristics of the DL treatments that had lactose crystallization induced compared to the control. Changes in texture parameters and rheological behavior can affect the application of DL, but further studies are suggested, mainly in crystallization conditions, for a better understanding of the effect of the product in the confectionery segment.

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Figure legends:

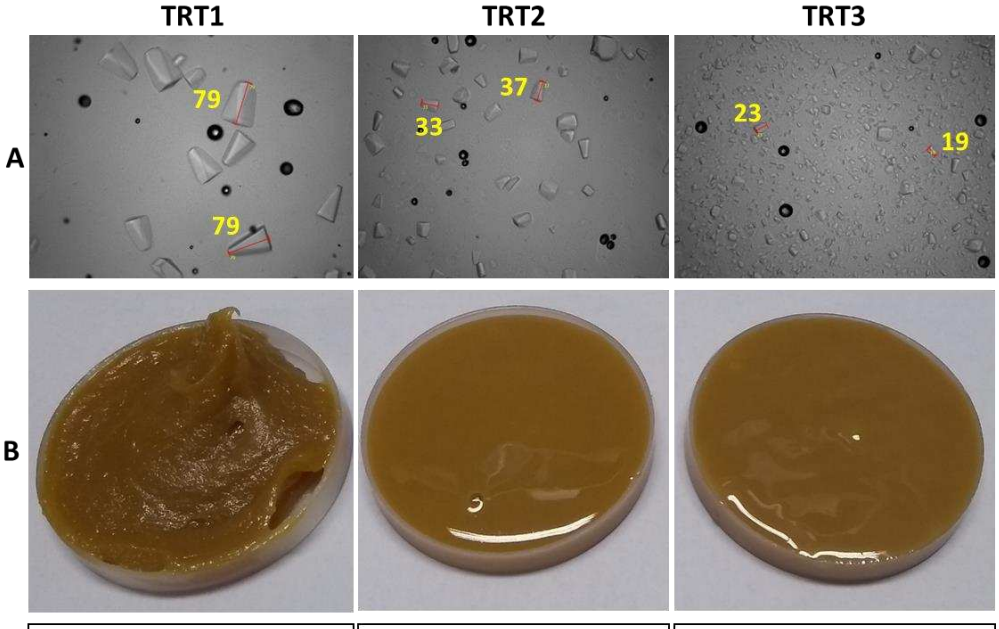
Figure 1:

(A) Registration of lactose crystals after 30 days of manufacture (images in 250x and the size of the two crystals on their longest sides were measured and reported in µm). (B) Images of the three samples of DL for visual assessment of the effect on the color of induced crystallization of lactose. (C) Colorimetric analysis (L* – lightness, a* – redness and b* – yellowness) and browning index (BI), used to evaluate the brown color intensity of the samples.

Different superscripts letter within the same line (same parameter L, a* or b*) indicate significant differences by the Tukey test (P<0.05).

**Treatment 1 (TRT 1) - cooling to 70-75 °C and directly packaged without lactose addition and stirring; Treatment 2 (TRT 2) - cooling to 25 °C and lactose induced crystallization for 90 min; Treatment 3 (TRT 3) - cooling to 35 °C and lactose induced crystallization for 30 min. plus cooling to 25 °C and lactose induced crystallization for 60 min.).

Figure 1:



	TRT1	TRT2	TRT3
C	L* = 41.39 ± 2.98^{a} a* = 5.73 ± 0.62^{a} b* = 11.98 ± 2.05^{a} BI = 50.29 ± 5.43^{a}	L* = 47.07 ± 1.16^{b} a* = 7.08 ± 0.71^{a} b* = 15.83 ± 0.32^{b} BI = 52.17 ± 1.26^{a}	L* = 45.58 ± 1.41^{ab} a* = 7.01 ± 0.63^{a} b* = 15.22 ± 0.70^{b} BI = 50.60 ± 3.07^{a}

Figure 2:

Steady-state flow curves of DL samples

*Treatment 1 (TRT 1) - cooling to 70-75 °C and directly packaged without lactose addition and stirring; Treatment 2 (TRT 2) - cooling to 25 °C and induced crystallization of lactose for 90 min; Treatment 3 (TRT 3) - cooling to 35 °C and induced crystallization of lactose for 30 min plus cooling to 25 °C and induced crystallization of lactose for 60 min.)

Figure 2:

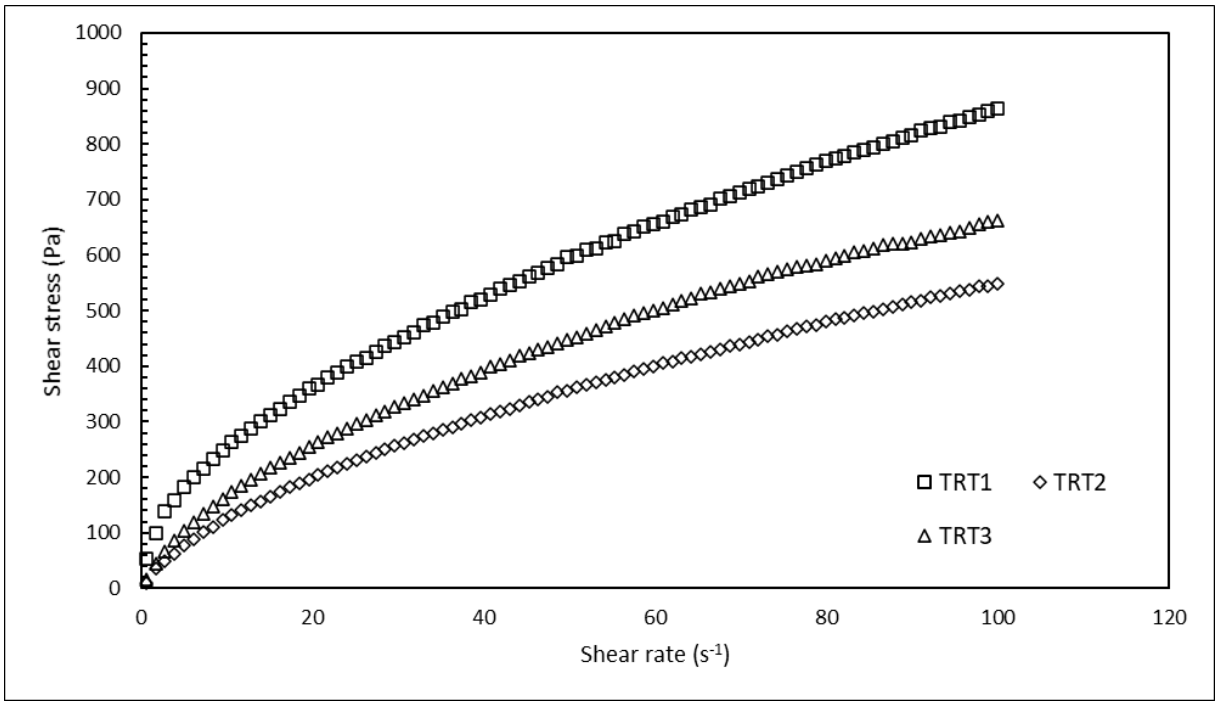
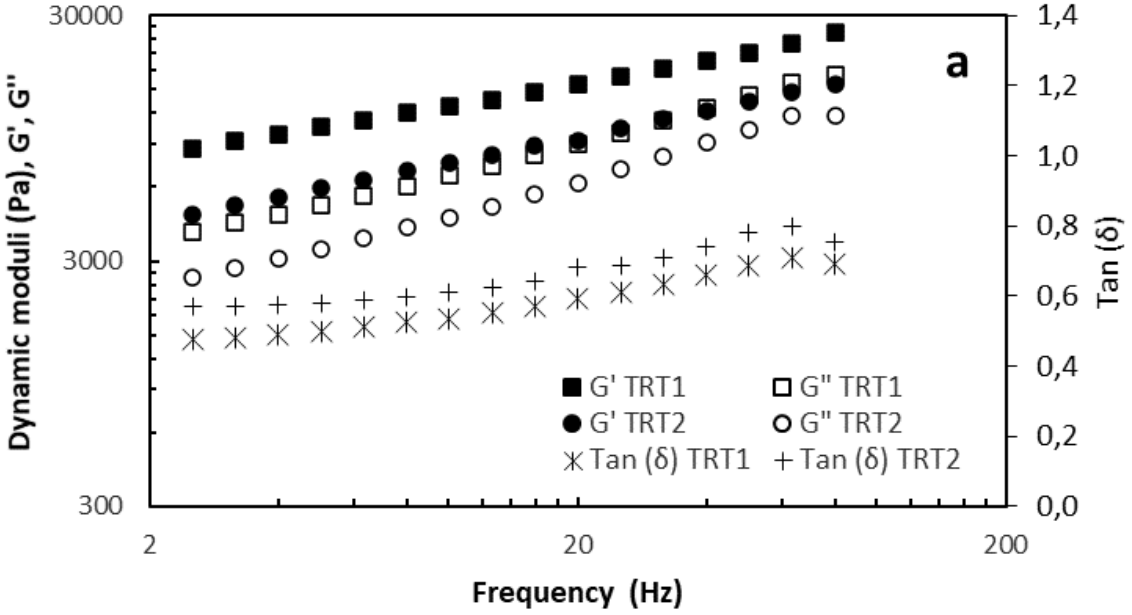


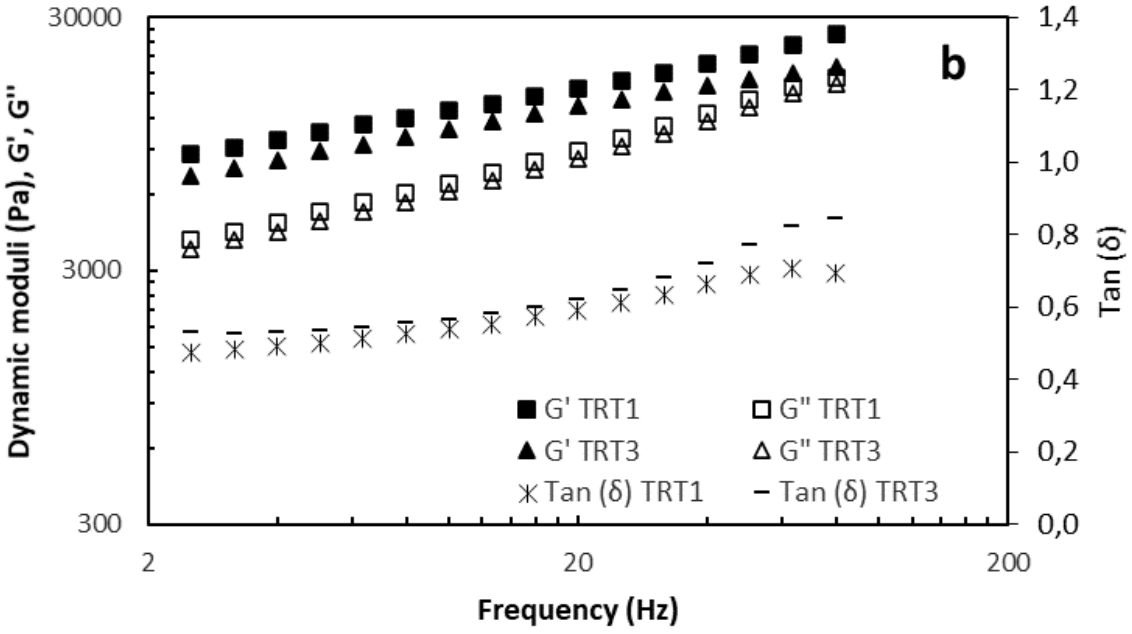
Figure 3:

Frequency dependence of storage (g′) and loss (g″) moduli for the different samples of DL. A = treatment TRT1 compared to TRT 2 and B = treatment TRT 1 compared to TRT 3

*Treatment 1 (TRT 1) - cooling to 70-75 °C and directly packaged without lactose addition and stirring; Treatment 2 (TRT 2) - cooling to 25 °C and induced crystallization of lactose for 90 min; Treatment 3 (TRT 3) - cooling to 35 °C and induced crystallization of lactose for 30 min plus cooling to 25 °C and induced crystallization of lactose for 60 min.)

Figure 3:


a
Dynamic moduli (Pa), G', G''
Tan (δ)
Frequency (Hz)
30000
3000
300
2
20
200
1,4
1,2
1,0
0,8
0,6
0,4
0,2
0,0
G' TRT1
G'' TRT1
G' TRT2
G'' TRT2
Tan (δ) TRT1
Tan (δ) TRT2


b
Dynamic moduli (Pa), G', G''
Tan (δ)
Frequency (Hz)
30000
3000
300
2
20
200
1,4
1,2
1,0
0,8
0,6
0,4
0,2
0,0
G' TRT1
G'' TRT1
G' TRT3
G'' TRT3
Tan (δ) TRT1
Tan (δ) TRT3

Tables legends:

Table. 1 Mean values followed by their standard deviations from the physicochemical analyzes of the formulated products

Sample	Moisture (g/100 g)	Soluble Solids (°Brix)	pH
TRT 1	28.54 ± 0.52[a]	69.00 ± 0.64[a]	6.10 ± 0.17[a]
TRT 2	27.98 ± 1.20[a]	68.77 ± 0.58[a]	6.12 ± 0.15[a]
TRT 3	28.09 ± 0.08[a]	68.93 ± 0.46[a]	6.15 ± 0.10[a]

*Different superscripts letter within the same column indicate significant differences by the Tukey test (P<0.05)

**Treatment 1 (TRT 1) - cooling to 70-75 °C and directly packaged without lactose addition and stirring; Treatment 2 (TRT 2) - cooling to 25 °C and lactose induced crystallization for 90 min; Treatment 3 (TRT 3) - cooling to 35 °C and lactose induced crystallization for 30 min followed by cooling to 25 °C and lactose induced crystallization for 60 min.

Table 2 Instrumental texture profile of dulce de leche samples

Sample	Parameters				
	Hardness (g)	Adhesiveness (mJ)	Cohesiveness	Elasticity (mm)	Gumminess (g)
TRT 1	190.77 ± 25.86[a]	15.39 ± 2.71[a]	0.740 ± 0.026[a]	16.10 ± 0.44[a]	140.63 ± 22.21[a]
TRT 2	46.27 ± 11.88[b]	1.73 ± 0.95[b]	0.520 ± 0.010[b]	12.97 ± 0.57[b]	23.97 ± 5.88[b]
TRT 3	45.93 ± 14.44[b]	2.07 ± 2.69[b]	0.520 ± 0.030[b]	13.10 ± 0.73[b]	23.87 ± 7.31[b]

*Different superscripts letter within the same column indicate significant differences by the Tukey test (P<0.05)

**Treatment 1 (TRT 1) - cooling to 70-75 °C and directly packaged without lactose addition and stirring; Treatment 2 (TRT 2) - cooling to 25 °C and lactose induced crystallization for 90 min; Treatment 3 (TRT 3) - cooling to 35 °C and lactose induced crystallization for 30 min plus cooling to 25 °C and lactose induced crystallization for 60 min.)

Table 3 Flow behavior index (n) and consistency index (k) obtained by fitting the Power Law model and apparent viscosity (η_a) at 100 s^{-1} shear rate for different *dulce de leche* treatment

Sample	N	k (Pa s^{-1})	η_{a100} (Pa s^{-1})	Adequacy Fitting R^2
TRT 1	0.54 ± 0.02[a]	72.83 ± 4.27[a]	8.64 ± 1.49[a]	0.9997
TRT 2	0.64 ± 0.03[b]	30.11 ± 3.50[b]	5.48 ± 1.56[a]	0.9997
TRT 3	0.63 ± 0.10[b]	45.90 ± 4.56[b]	6.62 ± 1.84[a]	0.9989

*Different superscripts letter within the same column indicate significant differences by the Tukey test (P<0.05)

**Treatment 1 (TRT 1) - cooling to 70-75 °C and directly packaged without lactose addition and stirring; Treatment 2 (TRT 2) - cooling to 25 °C and lactose induced crystallization for 90 min; Treatment 3 (TRT 3) - cooling to 35 °C and lactose induced crystallization for 30 min plus cooling to 25 °C and lactose induced crystallization for 60 min.)

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3. PAPER 2

Netto, Gabriel et al. **Influence of Induced Lactose Crystallization and Manufacturing Technology on the Physicochemical, Rheological, Colorimetric and Sensory Characteristics of *Dulce de Leche*,** 2022

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Influence of Induced Lactose Crystallization and Manufacturing Technology on the Physicochemical, Rheological, Colorimetric and Sensory Characteristics of *Dulce de Leche*

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Abstract

This study evaluated the influence of induced crystallization of lactose and manufacturing technology on physicochemical, colorimetric characteristics, lactose crystals formation, rheological behavior and sensory acceptance of Dulce de Leche (DL). DL was produced using four different procedures: 1- DL Cont –DL made with fresh milk without lactose addition; 2 - DL Cryst – DL made with fresh milk and lactose addition and stirring; 3 - EDL Cont – DL made with evaporated milk without lactose and stirring and 4 - EDL Cryst – DL made with evaporated milk with lactose addition and

stirring. There was no significant difference in the moisture, pH, soluble solids and water activity (Aw) for the four DL treatments, but there was a change in the size distribution of the lactose crystals and the color of the product due to the induced crystallization of lactose. The texture profile of DL made using fresh milk technology and evaporated milk technology were different, with the evaporated milk treatments presenting some texture parameters in the order of 10 times smaller. The flow curve data of the treatments manufactured using fresh milk were adjusted to the Herschel-Bulkley model and the data from the treatments made with evaporated milk were adjusted to the Power Law model. In the oscillatory tests, the viscoelastic behavior of all treatments was characterized by the predominance of the energy storage module (G') over the energy loss module (G”) along the frequency spectrum and the crystallization profile of DL affected the results. There was a difference in sensory parameters ($P < 0.05$) due to induced crystallization of lactose and manufacturing technology, whose results showed that the more consistent and darker (DL Contr treatment) had the highest acceptance.

1. Introduction

Dulce de leche (DL) is a concentrated dairy product much appreciated in most Latin American countries and its consumption can be direct, as an ingredient in the preparation of desserts or by the confectionery segment for use in fillings and toppings. In Brazil, a large portion of the manufactured DL is consumed in the confectionery market and for this segment the texture and rheology characteristics are very important for the product functionality (Perrone et al. 2019).

In Brazil, DL is manufactured in plants that range from small, artisanal plants to small, medium, and large industrial dairy factories. The raw material quality, the type of formulation, and the technology hurdles related to a lack of standardization of time and temperature during the manufacturing process have made it difficult for brands on the Brazilian market to be standardized (Stephani, et al. 2019).

DL is obtained by mixing milk, sucrose, sodium bicarbonate, and other additives subjected to continuous evaporation of water under normal or reduced pressure by indirectly transferring heat energy with steam from boilers to reach a concentration close to 70% (w / w) (Perrone et al., 2019). Heating and maintaining the elevated temperature for an extended period generate changes in the milk constituents that develop the sensorial and physicochemical characteristics of the DL. Total processing time can vary from 40 minutes to 4 hours. Processing time depends on the type of equipment and the amount of steam injected. Processing time plays an important role in a product viscosity, color, flavor and ultimately determines the characteristics of the final product (Perrone, 2007). Sensory characteristics (flavour, colour, aroma) are mainly due to the Maillard reaction that occurs during production (Mehta & Deeth, 2016).

During the processing of DL, the evaporation stage at high temperatures causes the main physicochemical and structural changes in the milk components (Stephani et al., 2019), which can affect the flow properties of the DL due to the removal of water and reduction of the space between the milk components, denaturation of whey proteins and interactions with caseins. This greater changes and interaction between milk components can strongly affect the rheological behavior of DL (Sulejmani et al., 2021).

Among all the constituents of DL, lactose deserves to be highlighted due to its contributions in the color, flavor and texture of the product. At the end of the

evaporation step, lactose is the third component in quantity behind only sucrose and water, it is in supersaturated concentration in the aqueous phase of DL (Perrone et al, 2019). Lactose supersaturation is the driving force for state change, causing lactose to shift to crystallized state (Shuck, 2011). Lactose crystallization in DL is commonly associated with the defect known as sandiness, in which lactose crystals grow to a size that becomes sensory perceptible (Hough, Martinez and Contarini, 1998). Some mechanisms may be industrially employed to control or delay this defect, such as partial lactose hydrolysis of milk prior to evaporating, increase in product viscosity by the addition of thickening agents or the induction of crystallization by the addition of lactose crystals (Perrone et al., 2019). Among these mechanisms, crystallization induced through lactose addition alters the quantity and size of the crystals, impacts on the texture of the DL and probably has great relevance in the rheological behavior of the product. However, no studies were found in the literature evaluating the effect of induced crystallization of lactose on the rheological characteristics of DL and the effect of vacuum evaporation as part of process.

The objective of this work was to evaluate the influence of induced crystallization of lactose and manufacture technology on the physicochemical, colorimetric, texture, rheological behavior, and sensorial acceptance of DL and try to understand how the control of these variables can help in the final product application properties.

2. Material and Methods

2.1- Dulce de Leche Manufacture

The different DL treatments were performed in the dairy factory of Cândido Tostes Dairy Institute in the city of Juiz de Fora-MG during December 2018. The milk used in the production of the DL was collected at the school's dairy receiving platform after the physicochemical analysis. The milk used in the production of different DL repetition presented the following variation on composition and analytical parameters: 34.0± 0.2 g of fat\L milk, 117.60± 1.05g of total dry solids\L milk, 83.30± 1.10 g of total free fat dry solids\ L milk, 1.030± 0.001 g/mL density, 16.50±0.25 ºD acidity and pH 6.79 ±0.04.

Part of the milk was concentrated using single stage vacuum evaporator (APV, Junior Model) at a concentration factor of 2.7± 0.2 times.

For the manufacture of the DL it was used fresh or evaporated milk, sucrose (180 g/L milk - Delta, Brazil), sodium bicarbonate (1000 mg/L milk - Carbonor, Camaçari-BA, Brazil), potassium sorbate (200 mg/kg DL - Wanglong Group CO LTD, China). The treatments with lactose addition were used α-Lactose Monohydrate (50 mg/kg DL Sigma Aldrich, Saint Louis, USA, according to the lactose supplier the lactose applied in this study had d_{90} equal to 10 micrometers). For the production of DL with evaporated milk, the concentration factor was considered to calculate the amount of ingredients. Thus, the production with fresh and evaporated milk had the same formulation.

The DL was manufactured using a double wall stainless steel equipment with indirect heating and a nominal capacity of 50 liters (Inoxul, Brazil). Sucrose and sodium bicarbonate were added to the fresh milk or evaporated milk, then heating to concentration was initiated and the soluble solids content was monitored (Refractometric Index, Digital Refractometer, 2WAJ-D Biobrix, Brazil) until the end point 69-70 ºBrix. To control fungal and yeast growth, potassium sorbate was added during concentration in all treatments in the refretometric index range 58-60 ºBrix. At the end concentration point, part of DL was cooled to 70 – 75 °C and filled and the other part was cooled at 35 ºC for lactose addition. After reaching the target temperature the α-lactose crystals were added and the samples were kept at controlled temperature under stirring condition for 90 min and then bottled in approximately 0.8 kg cans. The DL treatments were kept at room temperature during storage. Each treatment was manufactured in 2 repetitions as described below:

Dulce de Leche (DL Cont) – DL made with fresh milk and cooling to 70 - 75 ºC and filling without lactose addition and stirring;

Crystallized Dulce de Leche (DL Cryst) – DL made with fresh milk and cooling to 35 ° C, lactose addition and stirring for 90 min.;

Evaporated Dulce de Leche (EDL Cont) – DL made with evaporated milk and cooling to 70 - 75 ºC and filling without lactose addition and stirring;

Evaporated Crystallized Dulce de Leche (EDL Cryst) – DL made with evaporated cooling to 35 ° C and lactose addition and stirring for 90 min.

2.2 Physicochemical and colorimetric analysis

After the manufacture of the DL, moisture content was analyzed by gravimetric method at 102 ºC (IDF 15B: 1991), refractometric index (Digital Refractometer, 2WAJ-D Biobrix, Brazil), pH (Kasvi pH meter, Curitiba, Brazil) and water activity Aw (Aqualab, Decagon Devices Inc., USA).

For color analysis a CIE Lab (L * a * b) color scale with D65 illuminant (6900 ° K) with 10 ° aperture was used (Color Quest II, Hunterlab). The value L * represents the brightness of the sample, ranging from black (0) to white (100); the value of a * represents the color ranging from red (+) to green (-); and the value of b * represents the color ranging from yellow (+) to blue (-). The apparatus was calibrated in the excluded specular reflectance mode using white (C6299 Hunterlab Color Standard) and gray (C6299G Hunterlab Color Standard) reference plates. The analyzes were performed in quartz cuvettes.

The browning index (BI), used to evaluate the brown color intensity of the samples, was calculated by Equation (1), which correlates the parameters L^*, $a^*$and b^*(Maskan, 2001; Mohapatra et al., 2010)

Equation 1:

$$BI = 100 \cdot (x - 0.31)/0.17$$

where $x = (a^* + 1.750 \cdot L^*)/(5.645 \cdot L^* + a^* - 3.012 \cdot b^*)$

2.3 Microscopy

The microscopy of the lactose crystals in the DL over 60 days was performed using an electron microscope (Olympus, model BX41 TE). Standardized amount of DL sample was transferred to slide and observed at 200 times magnification. The images were obtained in duplicate and in 4 different fields.

2.4 Instrumental Texture Profile

The texture profiles (TPA) of the DL were evaluated in duplicate using a Brookfield CT3 texturometer (Brookfield Engineering Laboratories, Middleborough, MA, USA) to obtain the force-time curves. The method for texture profile analysis (TPA) was used as recommended in Sczesniak (1963). The Texture profile was supplied with a 4.5 kg load cell and application software (Brookfield Texture PRO CT®). Two

successive compressions were performed in each sample. Through force-time curves the results of hardness, adhesiveness, cohesiveness, elasticity and gumminess were calculated by the software. The samples were previously transferred to a cylindrical acrylic vessel (4.0 cm in diameter and 7.0 cm in height). The following parameters were adopted to test the texture:

Mode: TPA;

Test and return speed: 1 mm s-1;

Target depth: 20 mm;

Trigger load: 5.0 g;

Pre-test speed: 1 mm s-1;

Data rate: 10 points / s;

Probe: TA4 / 1000 (38.1 mm diameter)

2.5 Rheological Evaluation

2.5.1 Stead Shear Flow

The flow behavior of the samples was determined using a rotary rheometer (R/S plus SST 2000; Brookfield) with a CC45 sensor (concentric cylinders), connected to the RHEOCALC V 1.1 software. The analysis were carried out at 25 ° C in three steps, initially using a constant shear rate of 100 s^{-1} by 420 s for loss of thixotropy of the sample, then a descending ramp ranging from 100 to 0.5 s^{-1} and, finally, a rising ramp of 0.5 to 100 s^{-1} for 180 s each ramp.

2.5.2 Dynamic Test

Oscillatory tests were performed on rheometer (Discovery Hybrid Rheometer 1, TA Instruments, The United States of America) equipped with a stainless steel parallel plate geometry (diameter = 25 mm; gap = 1 mm), maintained at 25.0 ± 0.1 ºC. with parallel plate geometry.

Before starting the oscillatory test, a simulation was performed at a constant frequency of 2.53 / s (1 Hz), varying the amplitude of the stress applied to the samples to determine the linear viscosity region (LRV). The criterion followed for its determination was the linear relationship between strain and stress. In the linear viscoelastic range, material functions such as storage modulus G ′ and loss modulus

G ″ do not depend on the magnitude of the applied stress, the magnitude of deforming strain or the rate of application strain.

The dependence of the storage modulus (G ′) and loss modulus (G ″) on angular frequency (w) was determined at a small magnitude of stress in the linear viscoelastic region (LRV). In the experiments, shear stress (Ϭ) was applied as a sinusoidal time function over a small fixed amplitude. The stress frequency was increased step by step, and at any frequency step, the resulting signal was transformed into the elastic and viscous components. The frequency range used was from 2.5 to 100 s^{-1}.

2.6 Sensory Evaluation

In the sensory evaluation, acceptance test was performed in a laboratory with six individual cabins. The samples were provided randomly to judges in plastic cups and coded with three digits. Water was also provided to clean off taste buds after the evaluation of each sample. The acceptance test was conducted to evaluate the attributes of color, texture, flavor, taste and overall aspect. It was applied to 128 untrained judges, composed of 61 men and 67 women, aged between 18 and 68 years. Hedonic scale of 9 points, whose ends refer to extremely disliked (1) and extremely liked (9), was used. The samples were presented to the judges and they were asked to score them according to the proposed scale. The results were statistically analyzed by analysis of variance. The test was performed between 8 and 11 am.

2.7 Statistical Analysis

Analysis of variance (ANOVA) was performed using the SAS software (version 9.3, SAS Institute Incorporation, The United States of America), licensed by the Universidade Federal de Viçosa (UFV). For mathematical modelling a nonlinear regression was applied to the experimental data to estimate the parameters of the different rheological models.

3. Results and Discussion

Physical–chemical characteristics, color and microscopy

For the production of DL using fresh milk, 20 kg of milk, 3.6 kg of sucrose, 20 g of sodium bicarbonate, and 9 g of potassium sorbate were used at the beginning of the process. Whereas to produce DL using evaporated milk with concentration factor of 2.7, 17.26 kg of concentrated milk, 8.375 kg of sucrose, 46.4 g of sodium bicarbonate and 15 g of potassium sorbate were used. It took the DL using fresh milk 110.2±1.4 min to reach a solids content of 69° Brix, while the process using evaporated milk was performed in 151.1 ± 4.2 min. In each of the manufacturing processes, half of the DL mass was cooled to 70 °C and transferred to the packaging, while the other half was cooled to 35 °C (in the equipment) and added to the α-lactose crystals, then kept under stirring for 90 min before being packaged.

The production process time of DL made with evaporated milk was 37% longer to reach 69 °Brix than with fresh milk. This longer time is explained by the characteristic of evaporated milk during heating, as it was very rapid to reach boiling and formed a lot of foam, more than in the process with fresh milk. Then, it was necessary to apply lower steam pressure during heating (300 kPa), while the treatments made with fresh milk used a steam pressure of 500 kPa. Perrone et al. (2019), recommend using 100 to 600 kPa of pressure during the manufacture of DL using a DL cook equipment. The concentrated milk at the beginning of the process contained approximately 83.7 g of protein L^{-1}, while the fresh milk contained approximately 31.0 g of protein L^{-1}, with proteins being the main components of milk with high foaming capacity. Increasing the amount of caseins and whey proteins increases foaming intensity as well as improves stabilization (Xiong et al. 2020), however, for the production of DL it has been shown to have an effect on the heating and water removal step. The greater amount of proteins and the proximity between them favored boiling and foaming, as a consequence, heating with lower steam pressure was necessary and contributed to a lower temperature and water evaporation rate than the DL made with fresh milk.

The DL obtained from fresh milk at the end of the heating process removed 16.14 kg of water, which is equivalent to an evaporation rate of 0.146 kg of water\min, while the DL made with concentrated milk removed 7.80 kg of water equivalent to an evaporation rate of 0.052 kg of water\min. The lactose content in the DL at the end of the heating step was 49.06 g\100g of water in the DL produced with fresh milk and 48.08 g\100g of water. The value of 50.52 g of lactose/100g was found by Gabriel et al., (2022) who also studied the effect of induced crystallization of lactose on DL

produced under different crystallization conditions. Both studies obtained similar lactose values in the LD.

Table 1 shows the physicochemical results of the four manufactured DL treatments. There was no significant difference for the parameters moisture (values between 24.74 and 26.84%), soluble solids (values between 69.50 and 71.00), pH (values between 6.26 and 6.38) and water activity (values between 0.805 and 0.829) among the four treatments. Mercosur legislation requires a maximum DL moisture content of 30.00 g/100g (Brazil, 1997) and the literature on DL technology recommends a soluble solids content in the range of 68.00 – 70.00 °Brix (Perrone et al. 2019). There is no ideal range regarding the pH parameter, but it is influenced by the acidity of the milk after neutralization with sodium bicarbonate. In a survey carried out on trademarks obtained from different regions in Brazil, Gaze et al. (2015) determined the composition of 7 DL samples. In this study, a minimum moisture content of 17.49 g/100 g and a maximum of 29.67 g/100 g were found, as well as a pH with a minimum value of 6.14 and a maximum of 6.37.

Table 1 - Physicochemical results of dulce de leche samples

Sample	Moisture	Soluble Solids (° Brix)	pH	Aw
DL Contr	24.74^{a} ± 0.79	71.00^{a} ± 0.00	6.38^{a} ± 0.21	0.805^{a} ± 0.017
DL Cryst	25.75^{a} ± 1.04	70.50^{a} ± 0.71	6.26^{a} ± 0.06	0.812^{a} ± 0.011
EDL Contr	26.84^{a} ± 0.89	69.50^{a} ± 0.71	6.32^{a} ± 0.71	0.829^{a} ± 0.013
EDL Cryst	25.47^{a} ± 1.75	70.25^{a} ± 0.35	6.36^{a} ± 0.01	0.818^{a} ± 0.014

Different superscripts letter within the same column indicate significant differences by the Tukey test ($P<0.05$).

Figure 1A shows the lactose crystals formed in the 4 DL treatments after 60 days of manufacture. The DL Cont treatment (Fig. 1_A1) presented large crystals, but less quantity than the other treatments and it is possible to notice the presence of crystal fragments at the bottom of the image. Fragmentation occurred during slide preparation at the time the coverslip was placed over the DL sample as it is noticed that some larger crystals were broken. The crystals that remained intact the predominance of trapezoid-shaped crystals can be perceived in. The DL Cryst treatment (Fig.1_A2) showed several small crystals demonstrating that the addition of α-lactose was able to control the evolution in crystal size over the storage period. It is possible to notice the presence of a few crystals in the trapezoid-shape, however, it is

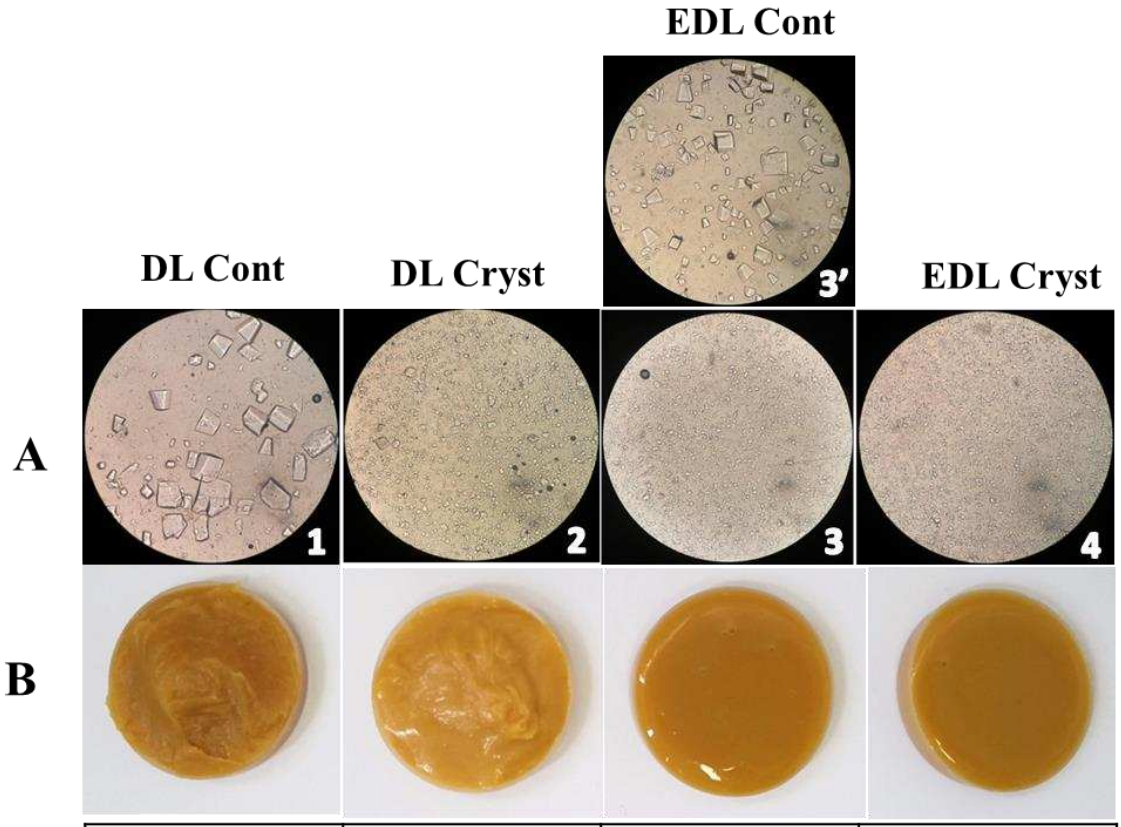
not possible to identify the shape of the smaller crystals. The first four samples taken from the EDL Cont treatment (Fig.1_A3) showed many small crystals similar to the samples with induced crystallization, so a new sample were collected from the bottom of the package (Fig.1_A3') and then it was possible to visualize larger crystals. In (Fig.1_A3') there was a predominance of trapezoid-shaped crystals, but smaller than to the DL Cont treatment. The difference in the distribution of crystals in the EDL Cont treatment is explained by the low viscosity of the product, as can be seen in the results of the flow curves (Fig.2) and the low value of the viscosity at 50 s^{-1} shear rate (Tab. 3), and by the texture profile parameters results (Tab. 2). The product was fluid in appearance, then the larger lactose crystals that are heavier and denser settled to the bottom of the package, while the small crystals were evenly distributed in the DL. The EDL Cryst treatment (Fig_1A4) presented many small crystals evenly distributed throughout the product and, due to the small size, there was no sedimentation at the bottom of the package. It was not possible to observe the predominant shape of the crystals and it is also noted that the EDL Cryst treatment presented even smaller lactose crystals than the DL Cryst treatment. The DL Cryst and EDL Cryst treatments were submitted to the same lactose induced crystallization procedure, however the difference in the crystallization profile seems to be associated with the difference in viscosity between them. The lower viscosity of the EDL Cryst treatment favored the spreadability of the lactose crystals added to the DL and a higher rate of displacement of soluble lactose to the surface of the crystals and consequently more crystals and of smaller size than the DL Cryst treatment.

The results observed in microscopy are complementary to the information obtained in the colorimetric analysis of the different treatments. The Fig 1C shows the results of the colorimetric analysis and it can be perceived that the traditional manufacturing technology using fresh milk and the technology with evaporated milk did not statically change the color parameters. The treatments DL Cont and EDL Cont showed no statistical difference. However, the results obtained in the samples that had induced lactose crystallization (DL Cryst and EDL Cryst) presented different behavior compared to their control versions using the same process technology. DL Cryst treatment presented statistical difference ($P < 0.05$) by Tukey test in parameters L, a, and b as comparing with DL Cont treatment. The induced crystallization provided the product with the lightest shade with the L value increasing from 39.71 (DL Cont) to 44.14, and parameter a from 5.23 to 6.74, and parameter b from 10.32 to 14.09. This

change in parameters indicates that the sample has become lighter and closer to red and yellow on the colorimetric scale.

The EDL Cont and EDL Cryst samples did not show statistical difference between them for any of the colorimetric parameters. This can be explained because in both treatments the lactose crystals suspended in the DL did not show great visual differences in distribution, as can be seen in the microscopy.

The difference in the results of the colorimetric parameters can be supported by the Fig.1B images. For the treatments made with evaporated milk Figure 1_A3 and 1_A4 no color difference was visually observed. The treatments made with evaporated milk (EDL Cont and EDL Cryst) did not differ from the treatment DL Cont made with fresh milk. Different manufacturing technologies could result in products with different color as the process time was different. The possible reason that the DL Cont treatment and the EDL Cont and EDL Cryst treatments did not show differences is because the first treatment was processed at a higher temperature (higher vapor pressure), although the time was shorter, while the last two had longer process time and lower temperature (lower vapor pressure). The results of the colorimetric analysis and visual observation of the samples are consistent with the results of the sensory analysis, in which only the DL Cryst sample presented statistical difference from the other treatments by Tukey test ($P < 0.05$) as presented in Table 4.



DL Cont	DL Cryst	EDL Cont	EDL Cryst
L* $39.71^{b} \pm 0.01$	**L*** $44.14^{a} \pm 0.54$	**L*** $41.45^{ab} \pm 2.36$	**L*** $42.08^{ab} \pm 1.14$
a* $5.23^{b} \pm 0.12$	**a*** $6.74^{a} \pm 0.63$	**a*** $4.33^{b} \pm 0.24$	**a*** $4.96^{b} \pm 0.61$
b* $10.32^{b} \pm 0.23$	**b*** $14.09^{a} \pm 0.82$	**b*** $12.31^{ab} \pm 0.50$	**b*** $12.79^{a} \pm 1.18$
BI $0.393^{a} \pm 0.010$	**BI** $0.491^{a} \pm 0.030$	**BI** $0.424^{a} \pm 0.015$	**BI** $0.442^{a} \pm 0.036$

Fig. 1 – (A) Microscopy of dulce de leche samples after 60 days of manufacture (increased by 200 times); (B) Image of the four samples of DL for visual assessment of the effect on the color of induced lactose crystallization and processing technology and (C) Colorimetric analysis (L* – lightness, a* – redness and b* – yellowness) and browning index (BI), used to evaluate the brown color intensity of the samples.

*The DL sampling of images A1, A2, A3 and A4 were random and chosen among the different images of each treatment. The sampling of the A3' image had the position on the package determined. The sample was collected from the bottom of the package.

**Different superscripts letter within the same line (same parameter L*, a*, b* or BI) indicate significant differences by the Tukey test ($P<0.05$).

*** DL Cont - DL made with fresh milk and cooling to 70 - 75 ºC and filling without lactose addition and stirring; DL Cryst – DL made with fresh milk and cooling to 35 ° C, lactose addition and stirring for 90 min.; EDL Cont – DL made with Evaporated Milk, cooling to 70 - 75 ºC and filling without lactose addition and stirring and EDL Cryst – DL made with Evaporated Milk, cooling to 35 ° C, lactose addition and stirring for 90 min.

Instrumental Texture Profile

Table 2 shows significant difference (P<0.05) between the results of the treatments of the instrumental texture profile for the parameters hardness, adhesiveness, cohesiveness, elasticity and gumminess, with formation of three groups of samples. The first group is formed by the treatment DL Cont, the second by sample DL Cryst, and the third by the samples EDL Cont and EDL Cryst. It can be observed that the treatment with induced lactose crystallization, DL Cryst presented significant difference (P <0.05) by Tukey test for the parameters hardness, adhesiveness and gumminess comparing with control DL Cont. Relating the texture parameters to the chewing process, the DL Cont treatment compared to the others treatments presents greater resistance to the breaking of the structure of DL during chewing until the moment of swallowing, greater adherence in the mouth and greater ability to return to the original shape after compression removal. The results of the texture parameters of DL Cont and DL Cryst are in accordance with the results of hardness (297 to 457g), adhesiveness (20.54 to 34.18 mJ) and gumminess (253.9 to 431.1g) found by Silva et al. (2015) during the evaluation of the effect of different modified food starch addition on the characteristics of the DL. Induced crystallization of lactose alone is not enough to explain the reduction in texture parameter values of DL Cryst treatment compared with DL Cont. The induced crystallization by the added lactose and the shear during the crystallization stage are probably the main contributions in the process that altered the structure of DL and reduce the parameters.

There was no significant difference between EDL Cont and EDL Cryst treatments made with evaporated milk in any of the texture parameters. These two treatments presented very low consistency and this probably explains the fact that the sample with induced lactose crystallization (EDL Cryst) does not differ from the EDL Cont. These two treatments presented texture and consistency similar to sweetened condensed milk than DL texture. There was a difference of 10 times in the hardness and gumminess parameters results between the DL made with fresh milk compared to the DL made with evaporated milk. This difference is possibly due to the lower vapor pressure during the concentration stage of evaporated milk DL. Heating plays a key role in denaturing whey proteins and opening casein chains and reaction between them, which leads to enhancing water absorption, and consequently increasing

viscosity (Anema, 2009). A lower vapor pressure in the DL produced with evaporated milk means that the process temperature was lower, reducing the intensity of viscosity development in the DL. This behavior is also observed in the flow curves results discussed in the next topic.

Changes in protein conformation due to heat exposure can affect water binding thermodynamics by altering the availability of polar sites or hydration sites. The transition from compact globular conformation of the protein molecule to random conformation results in increased available surface area and exposure of peptides and amino acid (previously protected) side chains, thus interacting with water (Kinsella, 1982). Denatured protein is generally less soluble or even insoluble, promotes an increase in food viscosity and has increased reactivity of its side groups (Araújo, 2004). Thus, proteins play a key role in the development of consistency in DL and the lower processing temperature during the manufacture of DL treatments using evaporated milk may have led to lower exposure of hydrophilic radicals that bind water causing lower consistency development in DL.

Table 2 – Instrumental texture profile of Dulce de Leche treatments

	Parameters				
Sample	Hardness (g)	Adhesiveness (mJ)	Cohesiveness	Springiness Index	Gumminess (g)
DL Contr	$396.90^{a} \pm 29.90$	$35.37^{a} \pm 0.68$	$0.688^{a} \pm 0.007$	$0.84^{a} \pm 0.01$	$272.63^{a} \pm 24.13$
DL Cryst	$229.63^{b} \pm 30.13$	$22.54^{b} \pm 5.33$	$0.723^{a} \pm 0.008$	$0.84^{a} \pm 0.03$	$165.23^{b} \pm 20.78$
EDL Contr	$28.00^{c} \pm 0.75$	$0.63^{c} \pm 0.04$	$0.483^{b} \pm 0.018$	$0.63^{b} \pm 0.02$	$13.60^{c} \pm 0.90$
EDL Cryst	$27.50^{c} \pm 1.75$	$0.60^{c} \pm 0.03$	$0.493^{b} \pm 0.023$	$0.63^{b} \pm 0.02$	$13.68^{c} \pm 1.58$

Different superscripts letter within the same column indicate significant difference by the Tukey test ($P<0.05$)

Rheological Characterization

Steady-state flow

Figure 2 shows the flow curves obtained from the four DL (DL Cont, DL Cryst, EDL Cont and EDL Cryst) and can be observed that treatments produced by different technology (fresh milk and evaporated milk) showed different rheological behavior. The DL Cont and DL Cryst treatments required initial shear to start the flow and after that showed a thinning behavior. This behavior is typical of Herschel-Bulkley fluid. The treatments EDL Cont and EDL Cryst showed thinning behavior with shear stress starting near origin. This behavior is known as pseudoplastic. Both technologies, DL

with fresh milk and DL with evaporated milk, have a similar profile with a formation of an upward concave curve forming over the shear rate and an increasing in the shear rate causing a less than proportional increase in shear stress. This behavior can be understood as the rupture of interactions between the structural units in the food due to the hydrodynamic forces generated during the shear that the product undergoes (Rao, 2013).

The flow curves results of the DL treatments manufacture with fresh milk (DL Cont and DL Cryst) were fitted to the Herschel-Bulkley model represented by Eq. 1:

$$\sigma = \sigma o + K.\ddot{\Upsilon}^{n} \quad (1)$$

Where σ is shear stress (Pa.s), σ_0 is initial shear stress (Pa.s) K is consistency index (Pa.s), $\ddot{\Upsilon}$ is the shear rate (s^{-1}) e *n* is flow behavior index (dimensionless).

The induced lactose crystallization in the treatment DL Cryst made the product less consistent than the control treatment DL Cont over the whole shear rate, as can be seen in Fig.2. This visual observation in the graph is confirmed by the parameters estimated from the data adjusted from the Herschel-Bulkley model (Tab. 3) in which there was a reduction in the consistency index (k) from 36.31 for DL Cont treatment to 10.93 for DL Cryst treatment and greater approximation of flow behavior index of 1, increasing from 0.71 to 0.90, although the crystallized treatment had a higher estimated initial shear stress value (σ0). Value n <1 is characteristic of pseudoplastic behavior, while Newtonian and Bingham plastic fluids have a value of n = 1, and the smaller value of *n* indicates the more fluid is the product. These results are consistent with the lower values of the instrumental texture profile parameters between the two samples (Tab. 2). Stirring for 90 min during the crystallization step appears to be the main effect that contribute to the reduction in viscosity and consistency of the DL Cryst treatment compared to the DL Cont. This assumption is supported by the results observed while performing the flow curve analysis (data not shown). The first part of the method is to subject each treatment to a shear rate of 100 s^{-1} for 420 s for loss of thixotropy of the material. The combination of shear rate and time was defined through previous tests and lower shear intensity or shorter time were not enough to eliminate all LD thixotropy.

The flow curves results of the DL treatments manufacture with evaporated milk (EDL Cont and EDL Cryst) were fitted to the Power Law model represented by Eq. 2, also known as the Ostwald de Waele equation:

$$\sigma = K.\dot{\Upsilon}^{n} \quad (2)$$

Where σ is shear stress (Pa.s) , K is consistency index (Pa.s), $\dot{\Upsilon}$ is the shear rate (s^{-1}) and *n* is flow behavior index (dimensionless).

The EDL Cont and EDL Cryst treatments had a similar flow curve with the EDC Cont becoming slightly less viscous than the crystallized version. The observation of similar behavior in the graph is confirmed by the estimated parameters obtained from the Power Law model where the consistency index (k) was close between the two treatments (Tab. 3) and showed the same value of flow behavior index (n). The comparison between treatments made with fresh milk and treatments made with evaporated milk shows a big difference in viscosity and consistency. Treatments made with evaporated milk showed much lower viscosity and consistency possibly due to the use of lower vapor pressure during manufacture. The apparent viscosity (ηa) at the 50 s^{-1} shear rate was 10.94, 8.59, 3.74 and 4.29 Pa s^{-1} for the treatments DL Cont, DL Cryst, EDL Cont, and EDL Cryst, respectively.

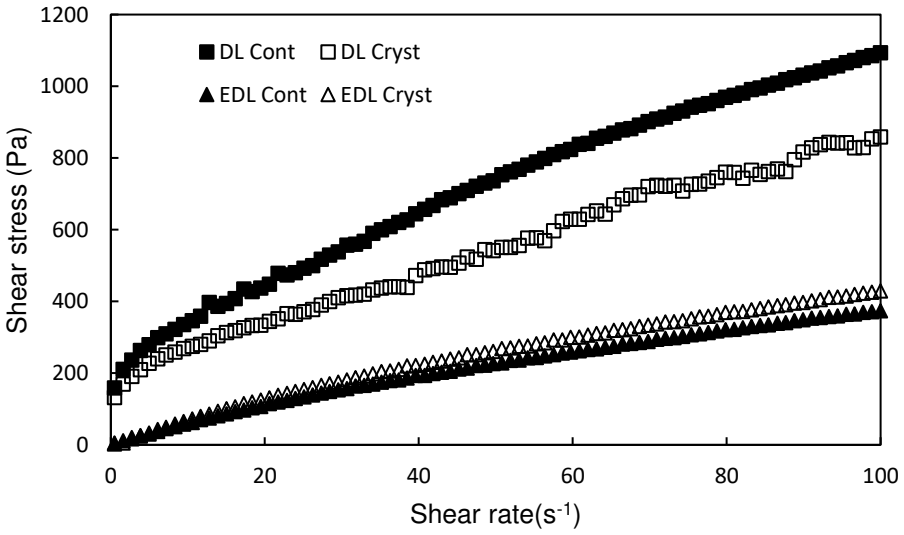


Fig 2 – Steady-state flow curves of Dulce de Leche treatments

*DL Cont - DL cooling to 70 - 75 ºC and filling without lactose addition and stirring; DL Cryst - Dulce de Leche cooling to 35 ° C, lactose addition and stirring for 90 min.; EDL Cont – DL made with Evaporated Milk, cooling to 70 - 75 ºC and filling without lactose addition and stirring and EDL Cryst – DL made with Evaporated Milk, cooling to 35 ° C, lactose addition and stirring for 90 min.

Table 3 - Flow behavior index (n) and consistency index (k) obtained by fitting the Power Law model and initial shear stress (σ0) flow behavior index (n) and consistency index (k) obtained by fitting the Herschel-Bulkley model and apparente viscosity (ηa) in the 50 s^{-1} shear rate to different Dulce de Leche treatment

	Treatment	σ_0 (Pa)	K (Pa s-1)	n	Adequacy Fitting R^2	ηa (Pa s^{-1})
H.B. model	DL Contr	151.33 ± 16.31	37.50 ± 9.35	0.71 ± 0.07	0.9984	10.94 ± 0.51
$\sigma = \sigma_0 + K.\dot{\gamma}^n$	DL Cryst	161.27 ± 15.07	14.59 ± 8.61	0.87 ± 0.04	0.9945	8.59 ± 0.59
Power Law model	EDL Contr	-	11.77 ± 0.25	0.75 ± 0.00	0.9990	3.74 ± 0.04
$\sigma = K.\dot{\gamma}^n$	EDL Cryst	-	13.88 ± 1.85	0.75 ± 0.02	0.9984	4.29 ± 0.07

Oscillatory test

The results of the oscillatory tests were grouped into three graphs, the first comparing treatments made with fresh milk (Fig.3A), the second comparing treatments made with evaporated milk (Fig.3B), and the third comparing treatments with induced crystallization. For all four treatments there was a predominance of the energy storage module (G') over the energy loss module (G”) in the whole mechanical frequency spectrum, with a reduction in the difference between the modules at the highest frequency values. These values indicate that DL treatments behave more similar to a solid than a liquid, however with reduction of the solid characteristic with the increase of the frequency.

In the intermediate region of the mechanical frequency spectrum of treatments DL Cont and DL Cryst there is a region with a slight “plateau” could be observed in both modules and was more evident in the loss tangent curve (tg [δ] = G”/ G'). This region has been assigned as a transition zone characterized by a system highly structured by the rearrangement of polymer chains (Ferry, 1980). This same behavior in commercial samples of DL was previously described in the research of Navarro, Ferrero and Zaritzky (1999) and Ranalli, Andrés and Califano (2012). It can be seen in Fig. 3A that the plateau is more accentuated in the DL Cryst treatment, possibly because of the crystallization profile with a large number and distribution of lactose crystals in the DL (Fig. 1_A2). The organization of lactose crystals with increasing in

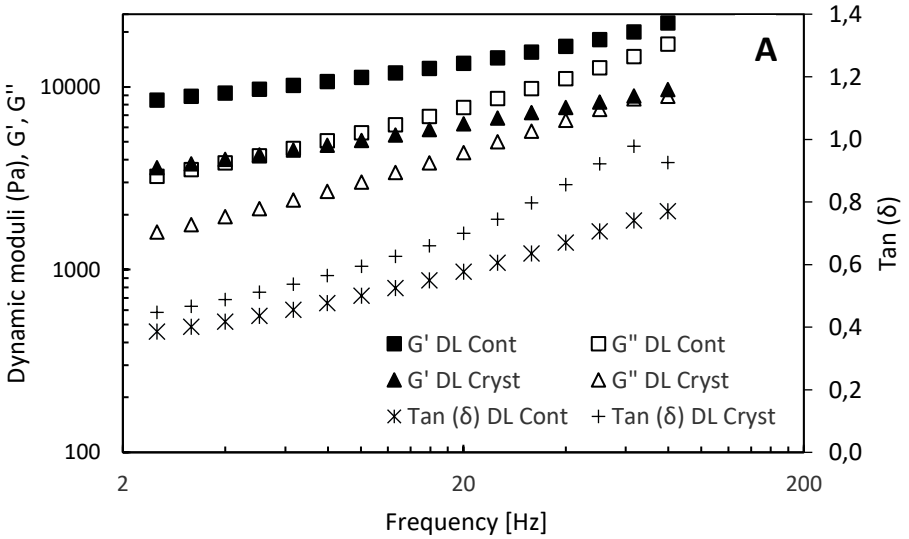
frequency seems to contribute to the values between the storage module (G') and loss module(G”) to reduce more sharply in the DL Cryst than the DL Cont.

Figure 3A shows that induced lactose crystallization (DL Cryst) led to a reduction in storage modulus (G') and loss modulus (G”) values compared to DL Cont treatment indicating a loss of consistency due to performing the lactose- induced crystallization step. At higher frequency values there is a marked reduction in the difference between the module values in the two treatments, which is characterized by the increased slope of the loss tangent tg [δ], which for the DL Cryst treatment is close to 1. The shear suffered during oscillatory test causes a disturbance in the structure of the DL, leading to an increase in the loss modulus (G”) greater than the increase that occurs in the storage modulus (G').

Figure 3B shows similar behavior of treatment EDL Cont and EDL Cryst and there was no effect of lactose crystallization in the second. The behavior of the storage modulus (G') and the loss model (G”) of both treatments were similar with overlapping values over the entire frequency spectrum. This result can be explained given that the dispersion of suspended lactose crystals in these two treatments was similar as can be seen in Fig. 1_A3 and Fig.1_A4. 1, although large crystals were formed in the EDL Cont treatment, the larger crystals settled at the bottom of the package. (Fig.1_A3 ') due to the low product consistency.

There is a noteworthy aspect observed in the characteristics of the DL Cryst treatment. In the results obtained in the texture parameters (Tab.2) and in the flow curves (Fig. 2) it is easily identified the effects of the manufacturing technology using fresh milk and evaporated milk on the characteristics of the DL. The DL Cont and DL Cryst treatments presented much higher values compared to the EDL Cont and EDL Cryst treatments, justified mainly by the difference between the vapor pressure used during the production of DL. In view of all the textural and rheological results discussed so far, the data in Fig.3C demonstrates a certain controversy, as the DL Cryst treatment presented the lowest values in the storage modulus (G') and loss modulus (G”) over the entire frequency range. While the DL Cont treatment showed the highest values, the DL Cryst treatment showed the greatest decrease, which was below the EDL Cont and EDL Cryst treatments that had the lowest values of texture, viscosity and consistency parameters. The explanation for the behavior of the DL Cryst treatment is the lactose crystallization profile, whose lactose crystals might have been oriented in an organized way towards the direction of rotation of the parallel plates

during the analysis, leading to a reduction in both the storage modulus (G') and the loss module (G”). The difference between the treatments made with fresh milk was not observed between the EDL Cont and EDL Cryst treatments because the crystallization profile was similar for the lactose crystals that remained suspended in the DL. In the study of the effect of different conditions of induced crystallization of lactose on DL. Netto et al. (2022) observed that the TRT 3 treatment that was subjected to induced crystallization at 35 °C for 30 min. and 25 °C for 60 min. showed a crystallization profile with a large number of small crystals reduced the difference between storage modulus (G') and loss modulus (G”) compared to the treatment without TRT1 induced crystallization, while the TRT2 treatment (lactose-induced crystallization at 25 °C for 90 min) showed a greater difference in the storage modulus (G') and loss modulus (G”) compared to the non-crystallized treatment. In this study, the TRT2 treatment presented a crystallization profile closer to the DC Cryst treatment and similar behavior in the oscillatory test. It appears that a homogeneous and controlled distribution of small lactose crystals formed by induced crystallization alters the way in which the polymeric chains of proteins and other components of the DL contribute to the structuring of the product.



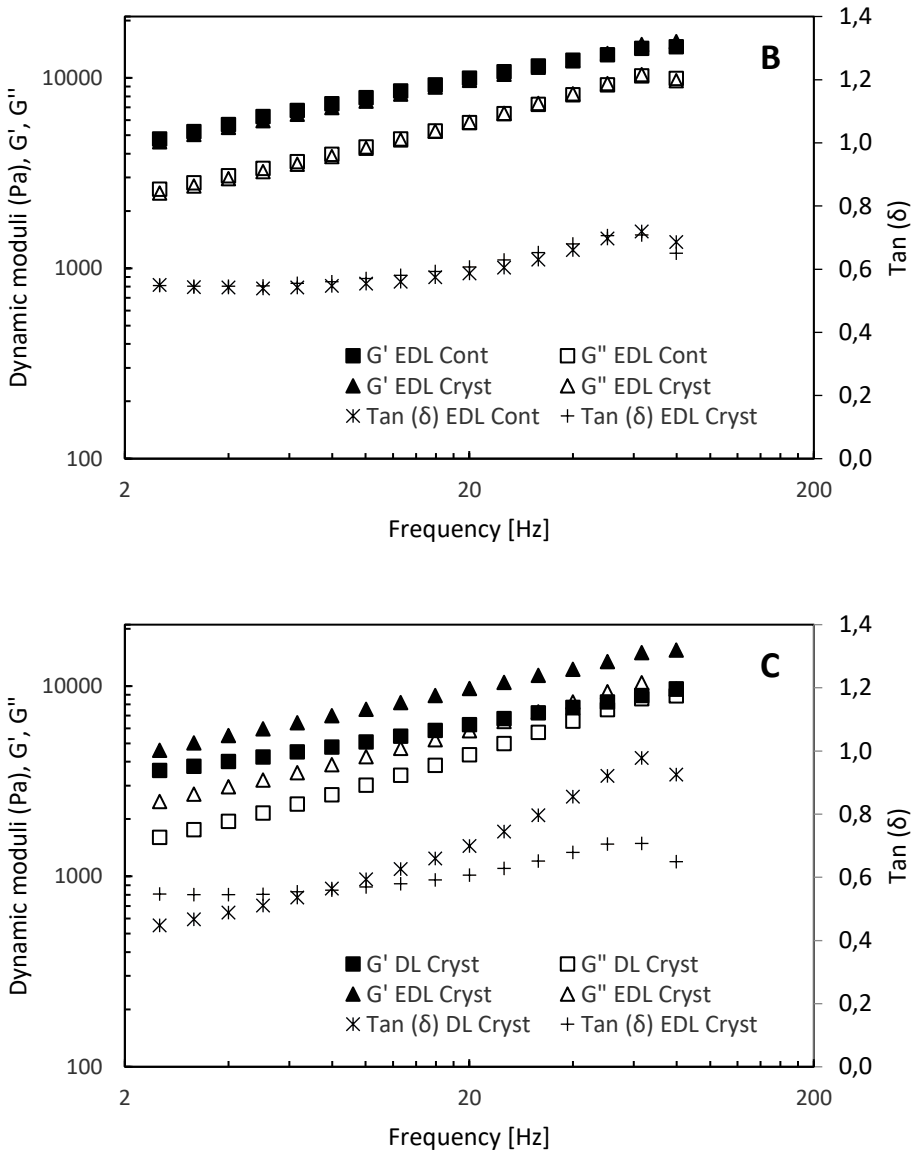


Fig 3 – Frequency dependence of Storage (g′) and Loss (g″) moduli for the different treatments of dulce de leche. A = Dulce de Leche treatments manufactured with fresh milk; B = Dulce de Leche treatments manufactured with evaporated milk and C = Comparison of the two treatments with induced crystallization DL Cryst and EDL Cryst

*DL Cont - DL cooling to 70 - 75 ºC and filling without lactose addition and stirring; DL Cryst - Dulce de Leche cooling to 35 ° C, lactose addition and stirring for 90 min.; EDL Cont – DL made with Evaporated Milk, cooling to 70 - 75 ºC and filling without lactose addition and stirring and EDL Cryst – DL made with Evaporated Milk, cooling to 35 ° C, lactose addition and stirring for 90 min.

Sensorial Evaluation

Table 4 - Sensory evaluation results of the dulce de leche acceptance test

Parameters	Sample

	DL Cont	DL Cryst	EDL Cont	EDL Cryst
Color	7.72[a]	6.95[b]	7.57[a]	7.55[a]
Flavor	6.95[a]	6.92[a]	6.89[a]	7.13[a]
Texture	7.18[a]	7.33[a]	6.11[b]	6.15[b]
Taste	7.70[a]	6.94[bc]	6.91[c]	7.36[ab]
Overall Impression	7.46[a]	7.05[b]	6.87[b]	7.04[b]

Different superscript letters on the same line differ statistically ($p < 0.05$) by the Tukey test. Test with 128 untrained evaluators. DL Cont - Control Dulce de Leche; DL Cryst - Induced Crystallized Dulce de Leche. EDL Cont - Control Dulce de Leche with Evaporated Milk and EDL Cryst - Induced Crystallized Dulche de Leche with Evaporated Milk.

Table 4 shows the results of the sensory evaluation of the four DL samples. There was a significant difference ($P < 0.05$) for the color parameter between DL Cryst treatment and the other treatments. The color change due to induced crystallization of lactose and reduced product acceptance. No difference in color was observed between samples made with evaporated milk. There was no significant difference between all treatments for flavor parameter. The EDL Cont and EDL Cryst treatments have lower acceptance for the texture parameter probably due to the lower consistency of these treatments. For the taste parameter, DL Cont and EDL Cryst treatments had greater acceptance, while induced lactose crystallization decreased acceptance of DL Cryst treatment. The DL Cont treatment was the sample with the greatest acceptance in overall impression, although it had sensorially lactose crystals perceived (some judges registered in the sensory form the presence of large crystals). The other treatments showed no statistical difference among them. From the results it is evident that both the lactose-induced crystallization and the manufacturing technology using fresh or evaporated milk influenced the sensory acceptance of the DL. Samples with lighter color and less consistency had less acceptance from the public.

4. Conclusion

The manufacturing technology using fresh milk and evaporated milk led to the production of DL with completely different characteristics. The main differences are in the instrumental texture profile, the rheological behavior, size and distribution of lactose crystals, and sensory acceptance of the product. There was no change in the physicochemical characteristics due to the lactose crystallization step or the use of fresh milk or evaporated milk.

Treatments made using fresh milk presented higher values of instrumental texture profile parameters than treatments made with evaporated milk. The lactose

crystallization step led to reduced product consistency, change in size and distribution of lactose crystals and altered the color of the DL. The lactose induced crystallized DL showed a lighter color.

The flow curve data of the DL samples produced using fresh milk had been adequately adjusted to the Herschel-Bulkley model with the induced lactose crystallization sample showing lower consistency index and higher fluid behavior index. The flow curve data of treatments manufactured using evaporated milk were adequately adjusted to the Power Law model and both treatments showed similar behavior. In the oscillatory tests the viscoelastic behavior of all treatments was marked by the predominance in the energy storage module (G') over the loss module (G”) along the frequency spectrum. The treatment using fresh milk without induced crystallization showed the highest storage modulus (G') and loss modulus (G") throughout the entire frequency range, while the DL Cryst treatment showed the lowest results. The treatments with evaporated milk showed similar results in the oscillatory tests.

There was a difference in sensory acceptance of the samples of DL due to lactose- induced crystallization and manufacturing technology. The combination of more consistent sample and darker coloration was more widely accepted by the public.

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4. OTHER PUBLICATIONS DEVELOPED DURING THE DOCTORATE

4.1 PUBLISHED PAPERS

1. Souza, L. B. A et al. Gordura Superficial em Leite em Pó. **Revista do Instituto de Laticínios Cândido Tostes**, v. 76, n. 2, p. 107-117, 2021.
2. Silva, K. C. M et al. Determinação da Lactose Ante às Metodologias Contemporâneas. **Revista do Instituto de Laticínios Cândido Tostes**, v. 75, n. 1, p. 59-71, 2020.
3. Netto, G. G. ; et al. Proposta de tecnologia para produção de queijo Minas Padrão sem lactose. **Indústria de Laticínios**, v. 136, p. 98-104, 2019.
4. Netto, G. G. et al. Efeito da cristalização induzida da lactose em doce de leite produzido utilizando diferentes tecnologias de fabricação sobre as caracteristícas físico-químicas, sensoriais e perfil instrumental de textura (TPA). In: 32 CNL, 2019, Juiz de Fora. Anais do 32 CNL, 2019. v. 1. p. 1-161.
5. Silva, K. C. M et al. Determinação da lactose em produtos comerciais utilizando diferentes métodos analíticos. **Indústria de Laticínios**, v. 139, p. 102-107, 2019.
6. Souza, L. B. A et al. Chips Fit: Brigadeiro Liofilizado de Alto Teor Proteico. In: Minas Láctea, 2019, Juiz de Fora. Congresso Nacional de Laticínios, 2019.
7. Souza, L. B. A et al. Aceitação Sensorial de Queijo Minas Padrão com Adição de Bebida Destilada Comparada a Três Tipos de Queijos. In: Minas Láctea, 2019, Juiz de Fora. Congresso Nacional de Laticínios, 2019.

4.2 PUBLISHED BOOK

1. Perrone, Ítalo Túler et al. JD. Doce de Leite: Química e Tecnologia. Cap-Lab, 2019.

5. GENERAL CONCLUSIONS AND PERSPECTIVES

The induction of lactose crystallization in dulce de leche does not change the physicochemical results of the product, however it had an influence on color, texture and rheological behavior. The different lactose crystallization conditions directly affected the crystallization profile such as quantity, size and shape of lactose crystals. The **paper 1** helped to understand that performing the induced crystallization reduces the texture parameters, the viscosity values in the flow curve test and in the G' storage modules and G" energy loss. These changes were due to the combination between the shear during the crystallization step and the crystallization profile of the treatments. There is an understanding that the application properties of DL in confectionary can be improved by carrying out the induced crystallization of lactose, however more studies are needed.

The type of technology used in the manufacture of DL can dramatically influence the texture and rheological properties of the product. **Paper 2** showed that the use of evaporated milk for the manufacture of DL needs adjustments so that the milk components have a heating intensity necessary for the development of viscosity and consistency, important parameters for the application of DL in the confectionery segment. DL at the end of the heating step, which showed lower viscosity, showed greater efficiency in forming large amounts of small-sized crystals. Although the DL manufactured using traditional technology presented lactose crystals large enough to be detected by the panelists, it was the treatment that obtained the greatest acceptance in the global evaluation.

Preliminary studies indicated that the amount of sucrose used in the formulation of DL has great relevance in the viscosity and textural parameters of the product. Assessing the influence of the amount of sucrose in relation to the amount of milk solids on the properties of DL is a recommendation for the sequence of research carried out in this thesis.