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Technological aspects of lactose-hydrolyzed milk powder

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ABSTRACT

Few reports describe the effect of lactose hydrolysis on the properties of milk powder during production and storage. Hence, the aim of this study was to evaluate the effects of five different levels of enzymatic lactose hydrolysis during the production and storage of milk powder. As the lactose hydrolysis rate increased, adhesion to the drying chamber also increased, due to higher levels of particle agglomeration. Additionally, more brown powder was obtained when the lactose hydrolysis rate was increased, which in turn negatively affected rehydration ability. Using Raman spectroscopy, crystallization of the lactose residues in various samples was assessed over 6 weeks of accelerated aging at a room temperature environment with 75.5% of air moisture. Products with 25% or greater lactose hydrolysis showed no signs of crystallization, in contrast to the non-hydrolyzed sample.

1. Introduction

Lactose maldigestion in human adults can result in gastrointestinal discomfort after consuming lactose-containing products (a condition known as lactose intolerance) which is why many people avoid dairy products. The important nutritional value of dairy products has therefore led to the production of low-lactose or lactose-free products. (Bailey et al., 2013; Rong et al., 2011; Troise et al., 2016). In Brazil, the lactase persistence allele, LCT-13910T, was found in about 43% of both white and “pardo” (mixed ethnicity) Brazilians and 20% of black Brazilians, but was absent among all Brazilians of Japanese descent studied (Mattar et al., 2009). Lactose-free and low-lactose products such as yoghurt, UHT milk and beverages, as well as cheese, are well-established on the market in many countries (Moreira et al., 2017; Milkovska-Stamenova & Hoffmann, 2017; Ruiz-Matute et al., 2012; Adhikari, Dooley, Chambers, & Bhumiratana, 2010), but the production of lactose-free milk powder remains an under-studied area.

Spray drying is widely used in the food industry and is applied mainly for food preservation, constituent and emulsion stabilizing, and microencapsulation of microorganisms, enzymes and molecules (Janiszewska-Turak, 2017; Sánchez, Cuvelier, & Turchiuli, 2016; Noello, Carvalho, Silva, & Hubinger, 2016; Zheng, Fu, Huang, Jeantet, & Chen, 2016, Pinto et al., 2015, chap. 5; Estevinho, Damas, Martins, & Rocha, 2014).

The glassy state formation and the degree of crystallinity in dried dairy powders are essential steps for controlling properties such as stickiness, caking, porosity, solubility and dissolution rates, flowability, and bioavailability (Carpin et al., 2016; Islam & Langrish, 2010; Langrish, 2008; Roos, 2010; Schmitz-Schug, Gianfrancesco, Kulozik, & Foerst, 2013). The relationships among product composition, glass transition temperature (related to the glassy state formation and the degree of crystallinity), spray-drying settings, and powder properties play a key role in the industrial production of foods and have been widely researched. (Carpin et al., 2016; Norwood et al., 2017; Sadek et al., 2016; Schuck et al., 2009; Schuck et al., 2016; Schuck, le Floch-Fouere, & Jeantet, 2013; Zhu, Méjean, Blanchard, Jeantet, & Schuck, 2011). In the case of hydrolyzed milk powders, spray drying is very difficult because most of the powder sticks to the insides of the dryer even at very low inlet/outlet air temperatures (Shrestha, Howes, Adhikari, & Bhandari, 2007). During the production and storage of low-lactose powdered milk (LLPM), technological problems may occur, including unwanted adhesion of agglomerated particles to the equipment, caking, and darkening. These issues can lead to low production yield, operational problems and difficulty in powder handling (Fernández,

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Schebor, & Chirife, 2003). According to Schuck et al. (2015), lactose-hydrolyzed milk has a greater number of molecules (glucose and galactose, as opposed to lactose) in its amorphous state during drying and the product becomes highly hygroscopic, which makes processing productivity challenging due to chamber clogging and powder conservation.

Therefore, this study's objective was to elucidate the technological and storage characteristics of various LLPMs produced by spray-drying after concentration, using enzymatic lactose hydrolysis. This work contributes to the development of technology for hydrolyzed milk powder manufacturing.

2. Materials and methods

2.1. Obtaining powder products

Concentrated whole milk was obtained from the reconstitution of milk powder in water at 25 °C and contained approximately 40% total solids. The trials were performed using whole milk powder from the Brazilian dairy producer Itambé. This whole milk powder was produced without agglomeration and without lecithin addition. The composition of the powder was solely milk. In order to hydrolyze the milk, 0.2% w/w of Lactomax Super enzyme by Prozyn® (São Paulo, Brazil) was added, and samples were incubated at 34 °C ± 1 °C. The analytical report of the enzyme applied in the experiment indicated enzymatic activity of 60,000 ONPGU·g^{-1} (*o*-nitrophenyl-b-D-galactopyranoside Units·g^{-1}) and density between 1.1 and 1.3 g·mL^{-1}.

To quantify the degree of lactose hydrolysis, samples were collected before the addition of lactase, then collected every 10 min afterwards until the desired hydrolysis level was achieved. Prior to the cryoscopic lactose hydrolysis degree evaluation, samples of concentrated milk were diluted to 10% w/v total solids using distilled water to achieve the characteristics of fluid milk. The degree of hydrolysis was monitored by measuring the freezing point depression in each diluted sample using an RTI MK540 Flex II Cryoscope (São Paulo, Brazil). The same approach that was applied by Rodrigues Júnior et al. (2016). The enzyme was inactivated by heating the milk to between 90 and 95 °C for 60 s. The percentage of lactose hydrolysis was determined after the heat-induced inactivation of the enzyme.

Five treatments of lactose hydrolysis were obtained: non-hydrolyzed milk concentrate (0H) and 25% (25H), 50% (50H), 75% (75H) and > 99% (99H) hydrolyzed milk concentrates. Four replicates (n = 4) of each condition were performed, totaling 20 experiments.

All products were dried in a Spray Dryer MSD 1.0 (using a flow rate of 1.0 ± 0.1 kg·h^{-1}, with compressed air flow rate of 30 L·min^{-1} and blower air flow rate of 2.0 m^3·min^{-1} through a pressure nozzle (1 mm diameter) atomization system LabMaq (Ribeirão Preto, Brazil). The drying parameters were set to an inlet air temperature of 170 °C ± 5 °C and an outlet air temperature of 85 °C ± 5 °C. At the end of the drying process, the powder from each sample was collected from the equipment, vacuum-packed, protected from light, and stored in a temperature-controlled location (25 °C).

Photo documentation of the drying equipment was gathered with a Motorola Moto G 13 Megapixel model camera at the end of each sample preparation.

2.2. Physical-chemical analyses

The milk powder samples obtained were analyzed for moisture, protein, lipid, ash, and water activity according to Zenebon, Pascuet, and Tiglea (2008). Moisture was determined using a gravimetric oven technique at 105 °C. Total protein was determined using the micro-Kjeldahl method; ash content was determined using a gravimetric method. Weight loss of the material subjected to incineration in a muffle furnace at 550 °C was recorded, and the lipid content was determined using the Gerber method. Powders were analyzed for water activity (a_w) using a Decagon3TE Aqualab instrument (Pullman, USA). The lactose amounts in the powders were determined using the enzymatic method (McCleary & Charnock, 2004) using the kit K-Lacgar (Megaenzymes, USA).

2.3. Particle size distribution of the rehydrated powders by laser diffraction

The size distribution of the powder particles during rehydration was obtained using a Beckman Coulter LS 13 320 laser-diffraction analyzer (Beckman Coulter, Miami, FL, USA) coupled to an aqueous liquid module (Beckman Coulter, Miami, FL, USA).

A sufficient amount of sample to generate turbidity readings was added to the liquid analysis module tank, which contained water at room temperature. Samples were added slowly to prevent the formation of agglomerates. The rehydration process was monitored every 3 min for 15 min. During this time, the samples remained under recirculation into the equipment. At the end of the data collection, stable particle size distributions were obtained. Data were collected in the particle size region of 0.04 to 2000 μm, with an acquisition time of 100 s. The results were obtained using 1.332 as the refractive index for the dispersing medium (water) and 1.57 for particles. This method, as described by Mimouni, Deeth, Whittaker, Gidley, and Bhandari (2009), has been used to measure the size of dairy components in suspension in individually fat globules, lactose crystals, casein micelles, or composite media such as skim milk or whole milk. Following the administration of this method, the results were represented as the volume (%) occupied by the particles in relation to their size.

2.4. Scanning electron microscopy

The morphology and agglomeration characteristics of sample particles were evaluated without prior preparation using scanning electron microscopy (Hitachi TM 3000, Hitachi Ltd., Tokyo, Japan). A magnification of 400 × was used to characterize the samples.

2.5. Obtaining Raman spectra during accelerated aging

Approximately 5 g of each of the five LLPM treatments were stored in a vacuum desiccator in a saturated NaCl solution to obtain a relative humidity of 75.5% at approximately 23 °C. Raman spectra of these samples were obtained weekly for 6 consecutive weeks. The Raman spectra of all LLPM samples represented in this study were obtained using a Bruker FT-Raman RFS 100 spectrometer equipped with a liquid nitrogen-cooled Ge detector and a Nd:YAG laser. Spectra were collected using a 100 mW laser beam with near-infrared excitation at 1064 nm, and the scattered radiation was collected at 180°. For all spectra, good signal/noise ratios were obtained by performing an average of 512 scans, which were collected with a spectral resolution of 4 cm^{-1} in the region from 3500 cm^{-1} to 50 cm^{-1}. OPUS platform 6.0 was used for the acquisition of Raman spectra. All spectra were obtained in duplicate to ensure that the intensity and spectral regions of the respective vibrational modes were reproducible.

2.6. Chemometrics

To perform the exploratory analysis, the Raman spectra were evaluated using Matlab software version 7.10.0 (R2010a). A potential complication in the interpretation of Raman spectra is the contribution and effect of factors such as particle size and morphological differences. These effects are dependent on experimental conditions and must be removed before the use of chemometric tools. To address these effects, Raman spectra were preprocessed using a weighted least-squares baseline, Raman intensities were normalized to a unit vector length and mean centering, and principal components analysis was employed. The number of main components was chosen according to the explained variance.

2.7. Mass balance

The mass loss (q_{mass}; $kg{\cdot}h^{-1}$), refers to the amount of powder lost during the drying process due to adhesion of particles to equipment or loss by cyclone, can be estimated by follow equation:

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

where m_{CM} is the flow rate of the concentrated milk to be dried ($kg{\cdot}h^{-1}$); $DM_{CM}$is the solids content in the product to be dried ($40.0 \pm 0.2\ kg{\cdot}kg^{-1}$); $m_{DMP}$is the flow rate of the milk powder ($kg{\cdot}h^{-1}$); DM_{DMP} is the solids content of the dairy powder ($kg{\cdot}kg^{-1}$).

2.8. Statistical analysis

The results were evaluated by variance analysis (ANOVA) and Tukey's test for comparison of means ($p < 0.05$). Data were analyzed using the Statistical Analysis System statistical program (version 9.2, SAS Institute Inc., 2006) licensed to the Federal University of Viçosa.

3. Results and discussion

The correlation coefficient between calculated hydrolysis by freezing point depression and determined hydrolysis by enzymatic method was 0.992, indicating a strong linear relationship, but different results are described in the literature. The formation of galacto-oligosaccharides (GOS) is described as a limiting factor for application of freezing point depression as an analytical tool to quantify lactose hydrolysis (Baer, Frank, & Loewenstein, 1980; Mahoney, 1998; Martínez-Villaluenga, Cardelle-Cobas, Corzo, Olano, & Villamiel, 2008).

LLPM samples were characterized with respect to moisture, total protein, lipid, and ash content and a_w. The product compositions obtained are presented in Table 1.

There was no significant difference ($p > 0.05$) in a_w, total lipid content or total protein content among treatments, indicating that the drying process simulated industrial processing and occurred in a controlled manner.

There was a significant difference ($p < 0.05$) in moisture content among treatments. As the hydrolysis levels increased, higher moisture levels were obtained.

This hydrolysis-related increase in moisture, however, had no direct influence on the protein, lipid, and ash contents of the LLPMs (Table 1). Thus, this effect was not directly associated with the product, but with the analytical technique used to obtain moisture content, which is better suited to traditional whole milk powder (containing lactose) than LLPM. At present, there is still no specific methodology for moisture content measurement in powders with low and/or reduced lactose content by enzymatic hydrolysis. It is believed that the use of analytical parameters recommended for moisture analysis of traditional whole milk powder with LLPM may promote the Maillard reaction during dehydration of the sample. Thus, a higher weight loss is obtained, which is characterized as moisture.

3.1. Drying ability

As shown in Fig. 1, the lactose-hydrolyzed LLPM treatments (25H, 50H, 75H and 99H) showed greater adherence to the drying chamber as the lactose hydrolysis level increased compared to the control product (0H). Similar behavior was also observed in relation to the color of the treatments, which progressively darkened as hydrolysis increased.

In addition to low concentrations of lactose, the LLPM samples contain glucose and galactose. These monosaccharides have a glass transition temperature that is lower than that of lactose. It is worth noting that the T_g of a carbohydrate is inversely proportional to its molecular mass. The T_g values for lactose, glucose and galactose are 98 °C, 31 °C and 30 °C, respectively (Roos, 1993). According Couchman and Karasz (1978) Eq. (2) allows determine the theoretical glass transition temperature of the product and Eq. (3) establish the relation between calculated and real T_g (Schuck, Méjean, Dolivet, & Jeantet, 2005).

$$T_g = \frac{w_1\Delta c_{p_1}T_{g_1} + w_2\Delta c_{p_2}T_{g_2} + w_3\Delta c_{p_3}T_{g_3}}{w_1\Delta c_{p_1} + w_2\Delta c_{p_2} + w_3\Delta c_{p_3}} \quad (2)$$

where T_{gi} is the glass transition temperature of a component i (K); w_i is its weight fraction; Δcp_i is the change in heat capacity of this component at T_{gi} ($J{\cdot}kg^{-1}{\cdot}C^{-1}$).

$$TT_g = 1.23\ RT_g - 13.99 \quad (3)$$

where TT_g is the calculated or theoretical T_g obtained by Eq. (1); RT_g is the real or measured T_g.

As to the final composition of the products (Table 1), the reduction of the glass transition temperature according to the increase in lactose

Table 1
Physicochemical data for the reduced and low lactose whole milk powder (n = 4).

Products	Moisture ($g{\cdot}100\ g^{-1}$)	a_w	Total fat ($g{\cdot}100\ g^{-1}$)	Total protein ($g{\cdot}100\ g^{-1}$)	Ash ($g{\cdot}100\ g^{-1}$)	Lactose ($g{\cdot}100\ g^{-1}$)	TTg (°C)	RTg (°C)
0H	4.1 ± 2.1^{a}	$0.19 \pm 0,04^{a}$	$23.7 \pm 0,8^{a}$	23.0 ± 0.8^{a}	$6.0 \pm 0,0^{ab}$	31.64 ± 0.73^{e}	60.6	60.6
25H	5.7 ± 2.0^{ab}	$0.17 \pm 0,04^{a}$	$23.3 \pm 0,8^{a}$	25.0 ± 0.5^{a}	$6.0 \pm 0,0^{b}$	22.22 ± 0.04^{d}	52.2	53.8
50H	6.5 ± 2.0^{ab}	$0.18 \pm 0,05^{a}$	$23.9 \pm 2,4^{a}$	23.7 ± 0.9^{a}	$5.9 \pm 0,0^{ab}$	13.30 ± 0.06^{c}	41.8	45.4
75H	9.2 ± 2.6^{b}	$0.18 \pm 0,02^{a}$	$25.1 \pm 1,1^{a}$	23.2 ± 1.9^{a}	$6.0 \pm 0,0^{ab}$	7.33 ± 0.14^{b}	34.2	39.2
99H	10.1 ± 1.7^{b}	$0.21 \pm 0,03^{a}$	$24.3 \pm 1,1^{a}$	23.2 ± 0.9^{a}	$5.9 \pm 0,1^{a}$	1.86 ± 0.51^{a}	26.9	33.2

Products	Mean (μm)	S.D. (μm)	Size distribution[a] (μm)	% volume	Treatments
			D_{10}	D_{90}	< 0.8 μm
Control	4.8	6.5	0.1	16.3	37.0
25H	9.1	9.6	0.2	22.9	19.1
50H	16.5	16.8	2.4	37.6	9.1
75H	16.0	14.9	3.4	35.1	7.2
99H	19.1	19.2	4.2	39.7	4.4

Statistical analyses of the average attributes, where the same letter does not differ ($p < 0.05$) by Tukey test. TTg is the calculated or theoretical Tg, RTg is the real or measured Tg. For the calculation, the following values of Tg and ΔCp were used: lactose Tg 98 °C, ΔCp 0,38 $J{\cdot}kg^{-1}{\cdot}°C^{-1}$ (Senoussi, Dumoulin, & Berk, 1995); casein Tg 132 °C, ΔCp 0,26 $J{\cdot}kg^{-1}{\cdot}°C^{-1}$ (Matveev, Grinberg, Sochava, & Tolstoguzov, 1997); whey proteins Tg 127 °C, ΔCp 0,09 $J{\cdot}kg^{-1}{\cdot}°C^{-1}$ (Kalichevsky et al. 1993; Matveev et al., 1997; Mauer, Smith, & Labuza, 2000); maltodextrin Tg 142 °C, ΔCp 0,30 $J{\cdot}kg^{-1}{\cdot}°C^{-1}$ (Roos & Karel, 1991; Zhu et al., 2011); inulin Tg 120 °C, ΔCp 0,65 $J{\cdot}kg^{-1}{\cdot}°C^{-1}$ (Zimeri & Kokini, 2002).

[a] Note: D_{10}, D_{90} represent 10% and 90%, respectively, of the cumulative particle size distribution.

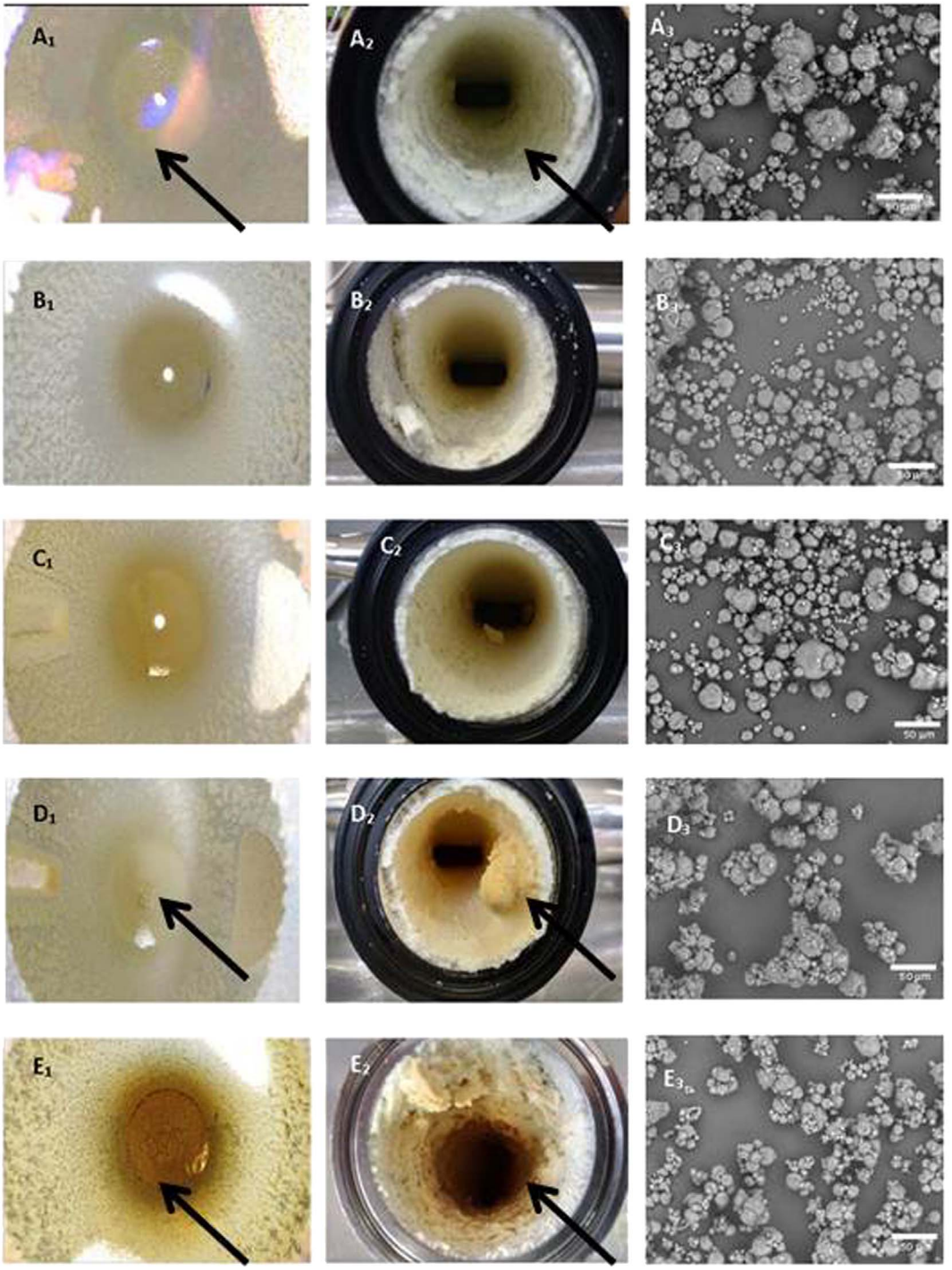

Fig. 1. Comparison in images on the influence of hydrolysis level in the drying of the product by spray-dryer. Panel A represents control product without hydrolysis (0H); B 25% (25H); C 50% (50H); D 75% (75H); E > 99% (99H) lactose hydrolysis. Respectively, inside the drying chamber after 1 h of operation (1); cyclone inlet after 1 h of operation (2); and SEM micrographs of reduced and low lactose milk powder at magnification of 400 ×, showing particles agglomeration of the powders (3).

hydrolysis is determined by applying Eqs. (2) and (3) and is presented in Table 1. The hydrolysis of lactose promoted a reduction of 27.4 °C in the glass transition temperature from treatment 0H to the treatment 99H. The calculated values for T_g are in accordance to Fernández et al. (2003). To evaluate the effect of the glass transition temperature on the drying capacity of the product, the stickiness and caking sensitivity index was established (SCSI) (Schuck et al., 2005). Using this index, it is possible to evaluate the behavior of the powders during drying (stickiness) and storage (caking) according to the variation range in the parameters [T_p - T_g] (T_p corresponding to the temperature of the powder) and Δc_p (is the change in heat capacity during glass transition). The temperature of the particle during spray drying falls between the outlet air temperature and the wet-bulb temperature of the outlet air, which corresponds to 10 °C to 20 °C below the outlet air temperature (Písecký, 1997; Westergaard, 2001, chap. 6). The outlet air temperature during the trials was 85 °C ± 5 °C, indicating a variation of product particle temperature between 60 °C to 80 °C during spray drying. When the given temperatures of the powders during drying are taken into consideration, the values for Δc_p lower than 0.3 $J \cdot g^{-1} \cdot °C^{-1}$ during glass transition (Schuck et al., 2005) and the final composition of the

products (Table 1) make it possible to determine the SCSI for all the treatments. The calculated SCSI values are 4 to treatment 0H, 6 for 25H and 50H, and, 7 for 75H and 99H. SCSI values less than or equal to 4 indicates stickiness and/or caking are not expected. SCSI values equal or higher than 6 indicate high to very high tendency to stickiness and/or caking hazard (Schuck et al., 2005). Lactose hydrolysis promotes higher SCSI values, which in turn the products' behavior from not expected to high/very high tendency to stickiness and/or caking hazard. When producing LLPM in the food industry using the same drying parameters as used for traditional milk powder, agglomeration, chamber gripping, caking, darkening and increased hygroscopicity may occur. Foster, Bronlund, and Paterson (2006) studied the time-dependent nature of glass transition-related cohesion for amorphous sucrose, maltose, glucose, galactose and fructose powders. The results of this work confirm that the rate of cohesiveness development is proportional to the [T_p - T_g] value, that is, the greater the temperature above the T_g, the quicker the powders will develop liquid bridges which may result in stickiness and caking.

Shrestha et al. (2007) concluded that spray drying skimmed milk powder containing hydrolyzed lactose resulted in low yield, with large amounts of powder remaining adhered to the equipment, and only 25% of powder recovered in the cyclone. The T_g of the hydrolyzed lactose dry powder was 49 °C.

Fernández et al. (2003) observed that hydrolyzed lactose in milk powder was darker than traditional milk powder due to the greater availability of reducing sugars, which favors the Maillard reaction.

These production issues for hydrolyzed lactose milk powder lead to a reduction in powder quality, including difficulty in rehydration, altered sensory aspects and low yield (Fernández et al., 2003). To address these issues, the dairy industry continues to investigate the significant changes in composition that occur for lactose-hydrolyzed milk powder processed using traditional milk drying conditions (Jouppila & Roos, 1994).

The scanning electron microscopy images (Fig. 1) showed that the behavior of the microscopic structures of the LLPMs corroborates the findings of the images captured inside the spray dryer: agglomeration increases as the level of hydrolysis increases.

According to Schuck et al. (2005), the loss of mass related to milk powder production can reach 10% in industry production. Under the conditions of the experiment, the treatment with 0% of hydrolysis reached 13 ± 2% of mass loss. All lactose hydrolyzed milk productions from verified the tendency to adhere inside the equipment in lesser or greater degrees, depending of the percentage of hydrolysis. The treatment 99H with 1.86% of residual lactose presented mas loss of 33 ± 4%.

3.2. Rehydration of the powder

Particle size distribution of the samples after rehydration was determined by laser diffraction. For each sample, Fig. 2 shows the distribution of the percentage of volume occupied by the particles according to their hydrodynamic diameter. Typically, particle size distribution of raw milk contains two populations: one corresponding to casein micelles (centered at approximately 150–200 nm) and another corresponding to fat globules (centered at approximately 5 μm). All samples showed a population of particles centered at approximately 200 nm, which may correspond to the casein micelles, as found in raw milk. The intensity of this population decreases with increasing rates of lactose hydrolysis in the samples. All samples, except for the non-hydrolyzed sample, showed another large population of particles between 1 and 200 μm, with larger diameters for the samples with higher rates of lactose hydrolysis. In addition to the population centered at 200 nm, the non-hydrolyzed sample showed three other particles populations distributed between 0.8 and 40 μm. Thus, higher rates of lactose hydrolysis decrease the rehydration capacity of the powders.

Additionally, increasing the level of lactose hydrolysis had a direct

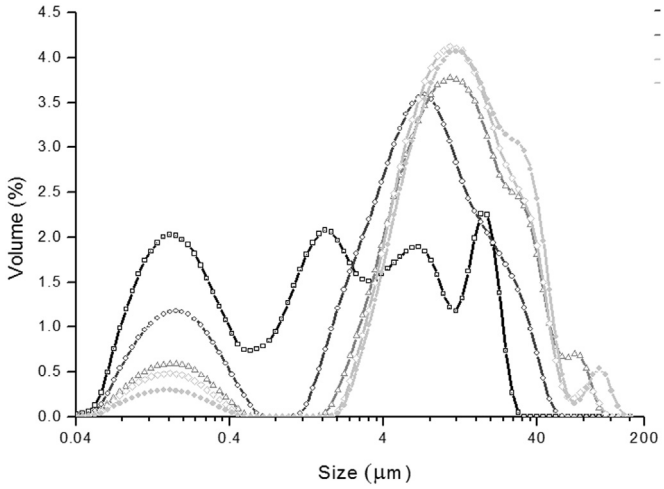

Fig. 2. Distribution of the size of the particles of rehydrated whole milk powder presenting different rates of lactose hydrolysis: 0% (□), 25% (○), 50% (△), 75% (◌) and 100% (●).

effect on particle size. Values obtained for D10 were 0.131, 0.190, 2.42, 3.36 and 4.22 μm for 0H, 25H, 50H, 75H and 99H, respectively, as shown in Table 1. A similar effect was also observed in D90, reinforcing the observation of increased hydrodynamic diameter size of the particles.

There is a direct relationship between the viscosity of concentrated milk before spray drying and the size of the powder particles. An increase in viscosity leads to an increase in particle size (Písecký, 1997; Schuck et al., 2005; Westergaard, 2001, chap. 6). Abbasi and Saeedabadian (2015) showed that increasing lactose hydrolysis (0, 25, 50, 75, and > 75% of hydrolysis) increases the apparent milk viscosity. The increase in the level of lactose hydrolysis showed a direct effect on particle size and could be related to changes in milk viscosity.

Table 1 shows the volume (%) of particles smaller than 0.8 μm. It is essential to emphasize the relationship between the size reduction observed and the increase in lactose hydrolysis level: sample 0H had a value of 37.0% compared to 4.4% for sample 99H. In the particle size region smaller than 0.8 μm, the greater contribution to the hydrodynamic diameter was related to casein micelles (centered at approximately 150–200 nm), which showed a clear reduction corresponding to increasing lactose hydrolysis after hydration of the powders in water.

If the rehydration process occurs in two steps, as described by Mimouni et al. (2009), the different morphologies of the powder particles could explain the different rehydration capacities observed. These authors described the process of milk protein concentrate (MPC) powder rehydration as occurring in two simultaneous steps: the rupture of agglomerates of particles into individual particles, and the release of the particle material to the aqueous phase (Mimouni et al., 2009). As shown in Fig. 1, the increase in the rate of lactose hydrolysis resulted in a larger agglomeration of particles. This could explain the decrease in the rehydration capacity of the powders.

Naranjo, Gonzales, Leiva, and Malec (2013) analyzed the kinetics of the Maillard reaction in lactose-hydrolyzed skim milk powder and related model systems containing carbohydrate mixtures (lactose, galactose and glucose). In all systems analyzed, a decrease in carbohydrates showed similar correlations for zero and first-order kinetics. In the samples containing casein and one reducing sugar, the ratio among the rate constants for the decrease of carbohydrates (10^5 mol·h^{-1}) was 20:10:1 for k_{gal}:k_{glu}:k_{lac}, where k_i is the Zero order rate constants for carbohydrate decrease in model systems with lysine and, respectively, with galactose, glucose and lactose (Naranjo et al., 2013). The same work demonstrated that the kinetic constants for available lysine loss and sugar decrease in the system with lactose were 10-fold lower than in the system with the monosaccharides. Milkovska-Stamenova and

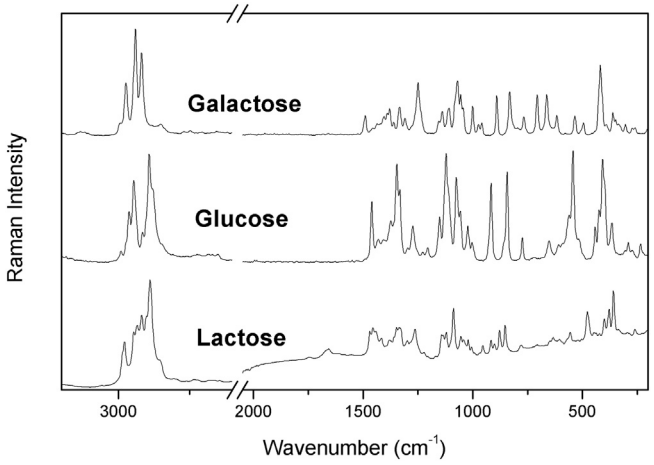

Fig. 3. Raman spectra of crystalline structures: galactose, glucose and lactose.

Hoffmann (2017) studied the effect of lactose hydrolysis in the Maillard reaction during dairy product processing. They demonstrated in quantitative terms that storage increased lactosylation up to fourfold in UHT milk and infant formula. Exosylation increased up to elevenfold in lactose-free UHT milk and threefold in infant formula. Furthermore, the lactose-free UHT milk showed a strong increase in the number and quantity of hexosylated peptides during three months of storage, which was further enlarged by mild heating (Milkovska-Stamenova & Hoffmann, 2017). The increase in the kinetic constant for carbohydrate decrease and in the kinetic constant for available lysine loss is the main reasons which explain the browning observed in Fig. 1, as a consequence of the increasing in lactose hydrolysis in the powders.

3.3. Raman spectra and principal component analysis of the powders

Fig. 3 shows the Raman spectra of lactose, glucose, and galactose in crystalline form. The 3000 cm^{-1} spectral region corresponds to symmetrical and asymmetrical C—H stretching (Mahdad-Benzerdjeb, Taleb-Mokhtari, & Sekkal-Rahal, 2007), while the region between 1500 and 200 cm^{-1} presents vibrational features. The region between 1500 and 1250 cm^{-1} corresponds to CH_2 and CH_2OH deformation modes (Mahdad-Benzerdjeb et al., 2007; Shih, Lupoi, & Smith, 2011). C—C and C—O stretching are observed in the spectral range between 1200 and 950 cm^{-1} (De Gelder, De Gussem, Vandenabeele, & Moens, 2007; Mahdad-Benzerdjeb et al., 2007). In the region from 950 to 800 cm^{-1}, C—O—H, C—C—H and O—C—H side group deformations are observed (de Gelder et al., 2007; Mahdad-Benzerdjeb et al., 2007). Endocyclic and exocyclic deformation modes appear at 543 and 410 cm^{-1}, respectively, for glucose, and at 477 and 377 cm^{-1}, respectively, for lactose. For the region from 1500 to 800 cm^{-1}, the lactose spectrum shows more bands than the spectra of glucose and galactose (de Gelder et al., 2007).

The characteristic bands for lactose at 2982, 2885, 1086, 876, 851, 479 and 357 cm^{-1} can be used to monitor low-lactose milk powder during production and aging. Changes in spectral characteristics are indicative of the degree of lactose hydrolysis and/or crystallization. Low-lactose milk powder contains a mixture of lactose, glucose, and galactose. The Raman spectrum of glucose presents bands at 2947, 2896, 1350, 1125, 1074, 917, 844, 540 and 405 cm^{-1} (Cerchiaro, Sant'Ana, Temperini, & da Costa Ferreira, 2005). The main Raman bands for galactose are found at 2973, 2939, 2920, 1250, 1071, 890, 833, 704, 661 and 420 cm^{-1} (Cerchiaro et al., 2005).

Fig. 4a shows the Raman spectra of LLPM milk powders after production, with varying degrees of lactose hydrolysis. The Raman spectrum of milk powder is composed of the characteristic bands for its three main compounds: proteins, fats and carbohydrates. A detailed

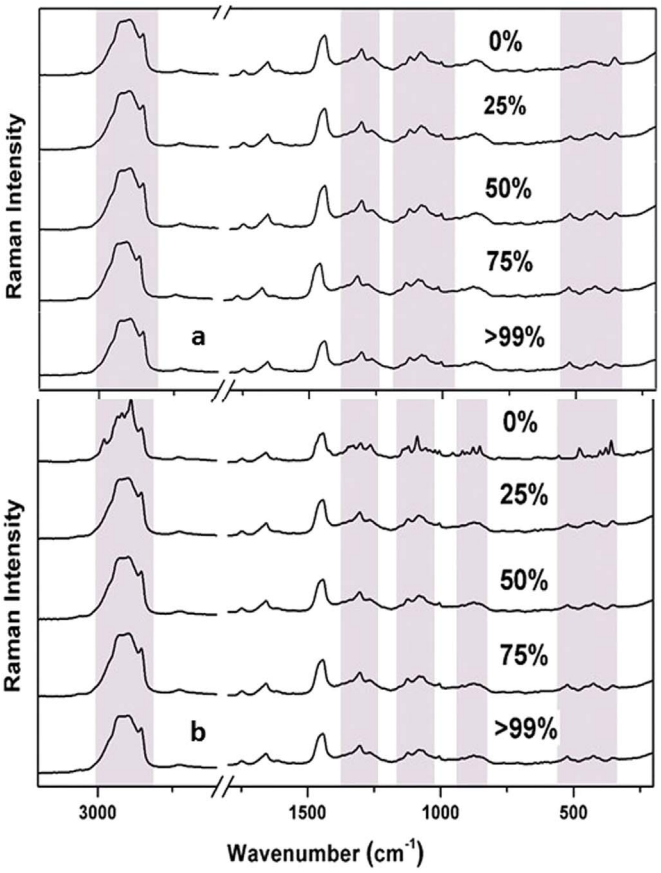

Fig. 4. (a) Raman spectra of RLL samples immediately after production; (b) Raman spectra of RLL samples 6 weeks of storage at 75.5% relative humidity.

discussion of the FT-Raman spectrum of milk powder can be found in previous work (Almeida, Oliveira, Stephani, & de Oliveira, 2011). The main changes in the Raman spectra for the lactose-hydrolyzed milk powders can be observed in the regions of 2900 cm^{-1} and 1350 cm^{-1}, from 1200 to 800 cm^{-1} and in the low wavenumber range of 480 to 410 cm^{-1}. The lactose Raman band intensities decrease with hydrolysis, and the glucose- and galactose-related bands at 525 and 424 cm^{-1} increase in intensity (Shih et al., 2011). Notably, the saccharides are present in amorphous form in the powdered milk immediately after production.

Fig. 4b shows the Raman spectra of LLPM milk powders after 6 weeks of storage at 75.5% relative humidity. As shown in this figure, a change in the spectral profile for the sample without hydrolysis is observed in the regions of 2900 cm^{-1} and 1350 cm^{-1}, from 1200 to 800 cm^{-1} and in the low wavenumber range of 480 to 410 cm^{-1}. For samples without hydrolysis, new and better-defined bands in these spectral regions indicate the crystallization of lactose (Hogan & O'Callaghan, 2010; Yazdanpanah & Langrish, 2011).

An exploratory analysis, based on principal component analysis, was performed for the Raman spectra of the samples after production and from 1 to 6 weeks of storage at 75.5% relative humidity. The PCA model was built using the spectral region from 1200 to 400 cm^{-1}. The score plot of PC1 versus PC2 (Fig. 5a), which explains 96.52% of the total variance of the data, reveals the groups cluster according to degree of lactose hydrolysis. PC1 separates LLPM milk powder samples from traditional milk powder samples (0% hydrolysis). PC2 distinguishes the lactose hydrolysis content of the samples. For the samples without lactose hydrolysis, the sample from the first week of storage appears distinct from the other samples. The lactose in this sample is present in amorphous form, but in the samples from 2 to 6 weeks, the lactose is present in crystalline form.

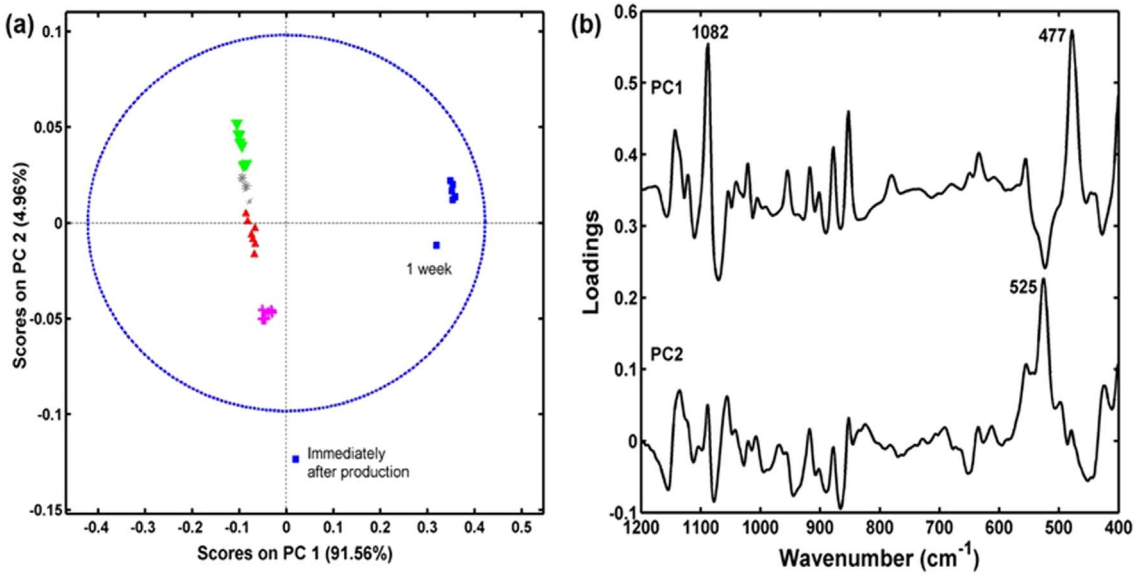

Fig. 5. (a) PCA: graph of PC1 vs. PC2 scores of the 1200–400 cm^{-1} spectral region of the RLL samples during storage: 0% (■), 25% (+), 50% (▲), 75% (*) and > 99% of lactose hidrolysis (▼); (b) PCA: graph of loadings of the PC1 and PC2 versus Raman shift for the PCA model.

The Raman bands primarily responsible for sample grouping were identified by plotting loadings, as shown in Fig. 5b. The PC1 loadings show that the Raman bands with the highest contribution were at 1082 and 477 cm^{-1}, which indicate the presence of crystalline lactose in the milk powder. The Raman bands that contribute the most to PC2 correspond mainly to bands related to the presence of glucose, localized at 525 cm^{-1}.

The primary phenomenon identified by Raman spectroscopy was that lactose hydrolysis (25H, 50H, 75H and 99H) leads to powders with no carbohydrate crystallization (lactose, glucose and galactose) during storage. Milk powder containing hydrolyzed lactose has a lower T_g (50 °C) than ordinary milk powder (92 °C). Galactose and glucose are known to be more hygroscopic than lactose. Consequently, absorbed water should have a plasticizing effect that logically promotes the crystallization of the remaining lactose. Rather, Jouppila and Roos (1994) reported that no indication of crystallization (e.g., water release) was observed in the sorption isotherm of hydrolyzed lactose milk powder. Shrestha et al. (2007) determined the moisture sorption behavior and glass transition temperature of spray dried skim milk powder with hydrolyzed lactose. One of the interesting aspects of this hydrolyzed product was that the water content continued to increase with increasing storage a_w, unlike the lactose product, which started to release water at $a_w \geq 0.432$ and began to absorb water again $a_w \geq 0.529$, due to crystallization. There was no visible crystallization in skim milk powder with hydrolyzed lactose, as had been observed in the lactose product. No crystallization peaks were seen when SMPHL was scanned through a differential scanning calorimeter (Shrestha et al., 2007). The crystallization process consists of two steps, nucleation and crystal growth. Nucleation is the formation of the crystalline phase from supersaturated solutions and can occur by either a spontaneous or a forced nucleation mechanism (Hartel & Shastry, 1991). Following the formation of the crystal nuclei, the growth rates for the nuclei (crystal growth) depend on the physical conditions of the amorphous powder or saturated solution (Das & Langrish, 2012). Gabarra and Hartel (1998) studied the effects of corn syrup saccharides on glass transition (T_g) and crystallization of freeze-dried sucrose using differential scanning calorimetry. According to these authors, the addition of corn syrup and their fractions were sufficient to reduce or even completely inhibit the crystallization of sucrose. This suggests that corn syrup saccharides contributed in other ways, to the inhibition of crystallization of sucrose beyond a general effect on molecular mobility, free volume, and T_g. This supports the possibility of surface incorporation effects limiting the rate of sucrose crystal growth from these amorphous mixtures (Gabarra & Hartel, 1998). Das, Wang, and Langrish (2014) studied the solid-phase crystallization growth kinetics of spray-dried sugar powders, including glucose, lactose and sucrose. According to their results, the height of the activation energy barrier for crystallization appears to be linked to the molecular weight, with larger molecular weight sugars (sucrose and lactose) showing lower activation enthalpies as compared to glucose. The enthalpies of activation for glucose, lactose and sucrose crystallization have been found to be $58 \pm 8\ kJ{\cdot}mol^{-1}$, $39 \pm 2\ kJ{\cdot}mol^{-1}$ and $68 \pm 4\ kJ{\cdot}mol^{-1}$, and the entropies of activation have been calculated as $-92 \pm 27\ J{\cdot}mol^{-1}{\cdot}K^{-1}$, $-156 \pm 6\ J{\cdot}mol^{-1}{\cdot}K^{-1}$ and $-60 \pm 5\ J{\cdot}mol^{-1}{\cdot}K^{-1}$, respectively (Das et al., 2014). The highest value for the activation energy barrier for glucose crystallization (compared to lactose) and the inhibitory effect of corn syrup (made up primarily of glucose) addition to the sucrose crystallization could be related to the absence of crystals in LLPM milk powders. Thus, in hydrolyzed lactose milk powder, glucose and galactose seem to have the ability to inhibit nucleation and/or crystal growth. This effect has not been clearly explained in scientific literature, but it is corroborated by the data obtained from the LLPM samples.

The Raman spectra of the LLPM milk powders and the PCA exploratory analysis provide information about the crystallization of lactose and the degree of lactose hydrolysis.

According to the results of this work, the production of hydrolyzed lactose milk powders should be based on the reduction of the powder temperature (if it is possible keep the temperature of the powder lower than its glass transition temperature) during spray drying and storage to avoid stickiness, caking and browning. To reduce the temperature of the powder during production, spray dryer settings should lower the inlet and outlet air temperatures and reduce the product flow rate. Storing the product under refrigeration is also recommended.

4. Conclusions

Lactose hydrolysis of glucose and galactose impacts the drying of milk products by promoting greater adhesion to equipment, greater tendency to browning, decreased solubility and modification of microstructure. Dairy products containing lactose can promote the formation of lactose crystals when subjected to conditions conducive to glass transition. The Raman spectroscopy results combined with chemometric analyses reveal that lactose-hydrolyzed dairy products do not crystallize when subjected to conditions favorable for glass transition.

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