VALÉRIA MARIA DOS SANTOS

HOMOGENIZATION AND CRYSTALLIZATION TO IMPROVE THE PROPERTIES OF WHOLE MILK POWDER FOR CHOCOLATE PRODUCTION

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos para obtenção do título de *Magister Scientiae.*

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ABSTRACT

SANTOS, Valéria Maria dos Santos, M.Sc., Universidade Federal de Viçosa, July, 2019. **Homogenization and crystallization to improve the properties of whole milk powder for chocolate production.** Adviser: Ítalo Tuler Perrone. Co-advisers: Antônio Fernandes de Carvalho and Rodrigo Stephani.

Milk powder has a wide use as a raw material or an ingredient to produce several products such as bread, cakes, cookies, dairy products and chocolate. It is desired, to manufacture chocolate, high free fat content and control of the particle size of the milk powder. The aim of this study was to evaluate the influence of different pressures of homogenization on whole milk powder with the crystallized lactose on free fat content and particle size, in order to develop a product with favorable properties for application in the chocolate industry. It was produced milk powders at three homogenization pressure levels (0 MPa, 20 MPa and 80 MPa) and with crystallization of lactose from concentrated whole milk (46.0% ± 1.0%). The powder produced at homogenization pressure of 80 MPa presented a smaller amount of particles in the region <1 μm (39.28%) and higher free fat content (13.78% ± 0.14%), when compared to the powder produced at conventional homogenization pressure (20 MPa), which presented 59.40% particles in the region <1 μm and 5.03% ± 0.17% of free fat. In addition, the milk powder produced at 0 MPa (control) presented the lowest amount of particles in the region <1 μm (24.60%) and highest free fat content (19.39% ± 0.16%) among the three treatments. For chocolate manufacturing, it is desirable that the milk powder has a larger average particle size and a higher free fat content. Given that, and according and to the results of this study, the crystallized whole milk powder (control) homogenized at 0 MPa presented the best properties for application in the chocolate industry, followed by milk powder produced at homogenization pressure of 80 MPa. This study also showed that the powder produced at homogenization pressure of 20 MPa, which is commonly produced in the industry, produces a milk powder with less suitable properties for chocolate production.

Keywords: Milk powder. High pressure homogenization. Free fat. Crystallization of lactose. Particle size. Chocolate.

RESUMO

SANTOS, Valéria Maria dos Santos, M.Sc., Universidade Federal de Viçosa, julho de 2019. **Homogeneização e cristalização para melhorar as propriedades do leite em pó integral para a produção de chocolate.** Orientador: Ítalo Tuler Perrone. Coorientadores: Antônio Fernandes de Carvalho e Rodrigo Stephani.

O leite em pó tem larga utilização como matéria prima para elaborar diversos produtos como pães, bolos, biscoitos, derivados lácteos e chocolate. Deseja-se para a fabricação de chocolate elevado teor de gordura livre e controle do tamanho das partículas do leite em pó. O objetivo do trabalho foi avaliar a influência de diferentes pressões de homogeneização em leite em pó integral com a lactose cristalizada sobre o teor de gordura livre e tamanho das partículas visando o desenvolvimento de um produto com propriedades para aplicação na indústria de chocolates. Foram produzidos leites em pó com três níveis de pressão de homogeneização (0 MPa, 20 MPa e 80 MPa) e com cristalização da lactose a partir de leite de vaca integral concentrado (46,0% ±1,0%). Dentre os 3 tratamentos, o leite em pó Controle (0 MPa) apresentou a menor quantidade de partículas na região <1 μm (24,60%) e maior teor de gordura livre (19,39% ± 0,16%). O leite homogeneizado a 80 MPa apresentou menor quantidade de partículas na região <1 um (39,28%) e maior teor de gordura livre (13,78% ± 0,14%) quando comparado com a homogeneização clássica a 20 MPa que apresentou 59,40% de partículas na região <1 μm e 5,03% ± 0,17% de gordura livre. Para a fabricação de chocolate é desejável que os leites em pó possuam maior tamanho médio de partículas e maior teor de gordura livre. De acordo com as condições do experimento o leite em pó integral cristalizado não homogeneizado apresentou as melhores propriedades para aplicação na indústria de chocolates, seguido do leite homogeneizado a 80 MPa. A pressão de 20 MPa usualmente empregada na indústria produz um leite em pó com as propriedades menos adequadas à produção de chocolates.

Palavras-chave: Leite em pó. Alta pressão de homogeneização. Gordura livre. Cristalização da lactose. Tamanho de partícula. Chocolate.

SUMÁRIO

1.0. Introduction

Milk powder is a food with high nutritional added value, and occupies a large fraction of the international market due to the practicality of consumption, transportation, storage and due to its longer shelf life, conferred by its low water activity (Liang, 2000; Ceballos et al., 2009; Karoui & Blecker, 2011). Milk powder is obtained by atomizing concentrated milk into small droplets. During this process, partial evaporation of the water occurs through contact with the drying air (low relative humidity and high temperature). The drying rate can be attributed to the evaporation surface; the difference of water pressure between the droplet and the air, and the migration rate of the water from the droplet to the surface (Schuck, 2009; Keshani et al., 2013). In addition to direct consumption, milk powder can be used as a raw material or ingredient in the preparation of many products such as bread, cakes, cookies, cheese, ice cream, yogurts and chocolate (Beckett, 2011; Russell et al., 2011).

Several authors have studied the effect of the properties of dehydrated dairy products on the characteristics of chocolate (Liang & Hartel, 2004; Franke & Heinzelmann, 2008; Beckett, 2011; Glicerina et al., 2015; Vásquez et al., 2019). It is known that, for chocolate manufacturing, the milk powder must have a high free fat content (fat which is not fully protected by the native fat globule membrane or covered by amphiphilic molecules) and larger average particle size (Holm et al., 1925; Vignolles et al., 2007). Reaching these parameters, there will be a reduction of the quantity of cocoa butter used in the formulations and an increase in the softness of the final product, thus, lowering the production cost of the chocolate (Twomey & Keough, 1998; Liang & Hartel, 2004). Design new products is a big challenge for researchers and industries considering the theorical approach, laboratory scale productions and sensorial analyses (Amaral et al., 2017; Amaral et al., 2018; Guimarães et al., 2018; Norberto et al., 2018; Oliveira et al., 2018).

The kinetic stabilization of milk, which is one of the technological stages in milk powder manufacturing, is based on the homogenization, being 20 MPa the most common pressure industrially used. This process of homogenization consists of the breakdown, size reduction and strengthening of the encapsulation of the fat globules by the adsorption of proteins (mainly casein micelles) in their interface (Walstra et al., 2006). Due to the increase in kinetic stability, there is a reduction in free fat content in milk powder, which is not favorable for chocolate manufacturing (Vignolles et al., 2009).

In addition, there are high pressure homogenization (HPH) - at pressures up to 200 MPa, and ultra-high pressure homogenization (UHPH), which occurs at pressures ranging from 400 MPa to 600 MPa (Dumay et al., 2013; Schlender et al., 2015). HPH affects the viscosity and functionality of the product by altering the fat globules diameters with a decrease of the particle size, and it occurs for as long as there are sufficient phospholipids and proteins for adsorption and stabilization of such fat globules. With the absence of these surfactants (phospholipids and proteins), a decrease of the kinetic stability occurs due to the high increase of the interfacial area. Consequently, there is a greater exposure of the fat, causing a higher free fat content and coalescence of the fat globules, which lead to an increase of the size of these particles in the product (Dumay et al., 2013; Martínez-Monteagudo et al., 2017; Mercan et al., 2018; Rodarte et al., 2018).

In addition to homogenization, another step that can be studied in the manufacture of milk powder to produce chocolate is the crystallization of lactose. During this process, the crystals of lactose rupture the fat globule membrane, resulting in an increase of the free fat content in the final product (Aguilar & Ziegler, 1994; Faldt & Bergenstahl, 1996; Vignolles et al., 2009).

Therefore, the aim of this study was to evaluate the influence of different pressures of homogenization on whole milk powder with the crystallized lactose on free fat content and particle size, in order to develop a product with favorable properties for application in the chocolate industry.

2.0. Material and Methodology

Milk powders were obtained by drying concentrated whole milk, as shown in Figure 1.

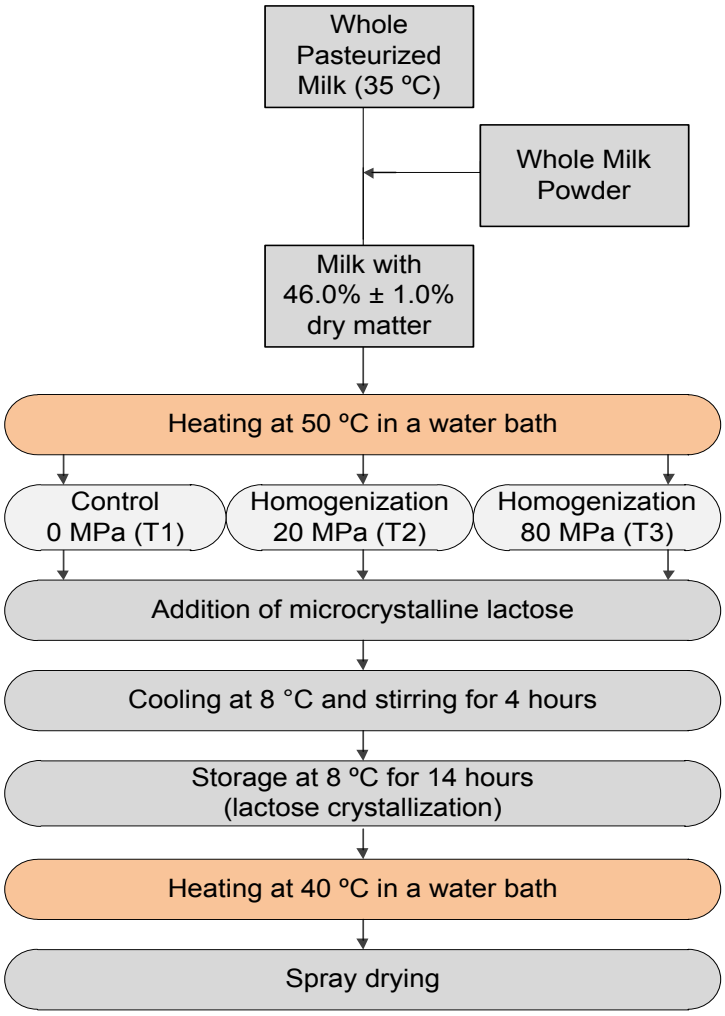

Figure 1: Experimental flowchart.

For each treatment, 625 g of pasteurized whole milk was heated to ± 35 ºC in a water bath (Q334M-28, Quimis, Diadema, Brazil). Then, 375 g of whole milk powder were added under stirring, totalizing approximately 1 kg of concentrated milk sample with 46% total solids. All the concentrated milk samples were heated to 50 ° C and then, subjected to the homogenization process. The first treatment (T1, which is the control) was homogenized (NS2006H, GEA Niro Soavi, Søborg, Denmark) without pressure (0 MPa). The second and third treatments (T2 and T3, respectively) were homogenized at 20 MPa and 80 MPa, respectively. After the homogenization process, the seeding step was performed for the crystallization of lactose, and 0.5% ($m \cdot m^{-1}$) of microcrystalline lactose was added on each sample. Thereafter, the concentrated milk samples were cooled and maintained at 8 °C, under stirring, for 4 hours. After this period, the milk samples were stored at 8 ºC in a refrigerator for 14 hours.

Subsequently, the concentrated milk samples were heated to 40 °C and dried in a mini spray dryer (B-290, Buchi Labortechnik AG, Flawil, Switzerland). In the T3, it was added 10% of

distilled water, aiming to enable the atomization, as the sample presented high viscosity. The drying parameters were: Air flow ($L \cdot min^{-1}$): 40 - 50; Product flow ($L \cdot min^{-1}$): 0.45 - 0.95; Inlet temperature (°C): 150 – 160; Outlet temperature (°C): 80 – 90. Where, airflow is total flow of air used in the heating system of the spray dryer, inlet temperature is the temperature of the air used to dry the product, outlet temperature is air temperature after product drying.

At the end of the drying process, the powders were collected, packed and stored in a temperature-controlled (25 °C) place and protected from light.

Concentrated milk before drying was analyzed for viscosity, optical microscopy and particle size analysis in solution. On the other hand, the powders were analyzed for particle size in solution, fat and free fat content, scanning electron microscopy, Raman spectroscopy, zeta potential and pH.

2.1. Fat and Free Fat Content Analyses

The fat content was determined using the Gerber method, as described by the Adolfo Lutz Institute. The sample (11 mL of reconstituted milk powder at 12% of dry matter) was added in butyrometer (Original, São Paulo, Brazil) along with addition of sulfuric acid (10 mL) and isoamyl alcohol (1 mL). The reading was done on the scale of the butyrometer after centrifugation (ThermoScientific™ Heraeus™ Biofuge™ Stratos™ ThermoFisherScientific, EUA) and immersion in water bath at 65 °C for 5 minutes (Q334M-28, Quimis, Diadema, Brazil).

The free fat method is based on the quantification and extraction of free fat through the use of a hydrophobic organic solvent. For that, 10 g of whole milk powder and 50 mL of petroleum ether were added to the beaker under stirring for 5 minutes. This suspension was filtered then added of 40-50 mL of petroleum ether and allowed to stir again for 5 minutes. This extraction process was repeated until the maximum free fat recovery was reached. Subsequently, the solvent was distilled off in a rotary evaporator (R-300, Fisher Scientific, Leicestershire, England). After extraction, the sample was submitted to oven (100 ºC) for 1 hour for complete removal of the solvent. Then it was put in a desiccator for cooling, weighed and submitted to oven again for 30 minutes, with subsequent weighing until it presents constant mass. The free fat was quantified by gravimetric method (Equation A.1) and expressed as percentage of total fat (Schuck et al., 2012).

Eq. (A.1): $\text{Free fat} = [(M1 - M2) / GT] * 1000$

Where: M1 = Glass balloon mass + sample; M2 = Empty glass balloon mass; GT = Total fat.

Such analyses were performed in duplicate.

2.2. Optical Microscopy

After the seeding stage of the concentrated milk, the lactose crystals in the milk were visualized in Optical Microscope (CX40; Olympus Optical Co., Tokyo, Japan), which has an area of $1.06 \cdot 10^{11}$ nm², and using the objective lens of 40 x 0.85.

2.3. Scanning Electron Microscopy (SEM)

The powders were analyzed by scanning electron microscopy (Hitachi TM 3000, Hitachi Ltd., Tokyo, Japan) at magnitude of 250x. To evaluate the length of the particles obtained from the SEM images, the ImageJ software was used (Abramoff et al., 2004; Marcomini & Souza, 2011). The images were analyzed in duplicate at magnitude of 850x.

With such an evaluation of the particle length, the SPAN value was obtained. SPAN value corresponds to the width or interval of the size distribution based on the volume, being calculated by Equation A.2:

$$\text{Eq. (A.2):} \quad SPAN = (d_{90} - d_{10})/d_{50}$$

Where: d_{90}: 90% of the particles have values equal to or less than the result found.

d_{10}: 10% of the particles have values equal to or less than the result found.

d_{50}: 50% of the particles have values equal to or less than the result found.

The SPAN value was used to calculate the Polydispersivity Index (PDI) value (Horiba, 2014). The PDI refers to the degree of nonuniformity of the particle size distribution. A monodisperse sample shows a high degree of uniformity (PDI value less than 0.4). A polydispersed sample has a low degree of uniformity (PDI value greater than 0.4).

2.4. RAMAN Spectroscopy

For this analysis, it was used a Raman spectrometer (RFS 100, FT-Raman Bruker, Massachusetts, EUA) equipped with a Ge detector, using liquid nitrogen as the cooling fluid, and excited at 1064 nm from a Nd:YAG beam. The samples were placed in aluminum capsules, irradiated with a laser pulse with power of 200 mW, and the scattered radiation was collected at 180º. For each spectrum, an average of 512 scans were collected at 4 cm^{-1} resolution across 3200 cm^{-1} to 200 cm^{-1}. OPUS 6.0 software (Bruker Optik, Ettlingen, Germany) was used to collect the analytical data. All spectra were obtained in duplicate.

2.5. Particle size by laser diffraction

The rehydration capacity of the powders was studied by particle size distribution obtained at Beckman Coulter LS 13 320 laser diffraction analyzer (Beckman Coulter, Miami, FL, USA) coupled to the liquid analysis module (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. Under recirculation data were collected until stable particle size distributions were obtained. The data were collected in the region of 0.375 μm to 2000 μm with time of each collection established in 90 seconds. Results were calculated with refractive indices of 1.332 for the dispersant (water) and 1.57 for the casein micelles (Mimouni et al., 2009), and 1.47 for the fat globules (Michalski et al., 2001) aiming at the observation of total solubility. Data were represented by the percentage of occupied volume by the particles as a function of their size. Statistical analyses were conducted with the equipment software (version 5.03). The analyses were performed over two times (1.5 and 3.0 minutes) and in duplicate.

2.6. Viscosity

The viscosity analysis was performed in viscometer (Q860M, Quimis, Diadema, Brazil). For concentrated milk samples of the control and 20 MPa treatments, probe 1 was used, while for the concentrated milk samples of the 80 MPa treatment, probe 4 was used. The viscosity result (in $MPa \cdot s^{-1}$) was obtained after reaching about 50% of the motor force. The analysis was performed in duplicate.

2.7. Measurements of the zeta potential

The surface charge of the powder particles during the rehydration process was obtained using the Zetasizer equipment (Nano ZS90, Malvern Instruments Ltd, Worcestershire, UK). The samples were diluted (1:25) and the analysis was performed in triplicate. The zeta potential of the powder particles was measured at 23 °C ± 2 °C.

2.8. pH

The pH of the milk powder samples was obtained by direct reading in the potentiometer (K39-1014B, Kasvi, São José dos Pinhais, Brazil). The powders were reconstituted in water

(10% of dry matter) with immersion of the electrode and the potentiometer thermometer in such solution. The analysis was performed in triplicate.

3.0. Results

Table 1 shows the particle size distribution by laser diffraction, zeta potential and pH of the milk powder with high free fat content, after rehydration in water as well the hydrodynamic/SPAN size results by Scanning Electron Microscopy of milk powder particles; the viscosity and hydrodynamic/SPAN size results of the concentrated milk samples, as well particle size distribution by laser diffraction of the concentrated milk after homogenization at different pressures.

The figure 2 shows particle size distribution curves of the concentrated whole milk with different levels of homogenization pressure and curves of milk powder with high free fat content and different levels of homogenization pressure.

The figure 3 shows the optical microscopy images of the lactose crystals of the concentrated milk samples, images of milk powder particles by Scanning Electron Microscopy and Raman spectra of milk powder samples analyzed.

Table 1: Particle size distribution by laser diffraction, zeta potential and pH, hydrodynamic/SPAN size results and viscosity of samples.

	Concentrated Milk						
Particle size distribution of the concentrated milk; particle size distribution, zeta potential and pH of the milk powders with high free fat content, after rehydration in water.	Sample	Mean (μm)	Size distribution (μm)		% Volume <1 μm	Zeta Potential (mV)	pH
			d_{10}	d_{90}			
	Control	7.20[a]	0.26	22.39[a]	25.90[b]	_____	_____
	20 Mpa	0.68[b]	0.19	1.17[b]	78.10[a]	_____	_____
	80 Mpa	0.59[b]	0.17	1.20[b]	81.55[a]	_____	_____
	Milk Powder						
	Control	7.56[a]	0.26	21.85[a]	24.60[c]	-26.43 ± 0.38[a]	6.81 ± 0.26[a]
	20 Mpa	2.37[b]	0.20	5.52[b]	59.40[a]	-27.68 ± 1.06[a]	6.88 ± 0.08[a]
	80 Mpa	7.26[a]	0.18	21.79[a]	39.28[b]	-25.37 ± 0.48[a]	6.85 ± 0.04[a]
	Concentrated milk						
Diameter/SPAN of the lactose crystals and viscosity of the concentrated milk samples and of the particles size of the milk powders obtained in different pressures of homogenization.	Sample	d_{10} (μm)	d_{50} (μm)	d_{90} (μm)	SPAN	Classification	Viscosity (MPa·s^{-1})
	Control	7.17 ± 0.50[a]	16.10 ± 1.70[a]	44.93 ± 2.20[a]	2.29 ± 0.07[a]	Polydispersed	277.85 ± 78.75[b]
	20 Mpa	6.37 ± 0.59[a]	13.64 ± 0.64[a]	28.60 ± 0.24[b]	1.60 ± 0.10[b]	Polydispersed	193.05 ± 2.75[b]
	80 Mpa	7.00 ± 0.27[a]	17.71 ± 0.56[a]	50.27 ± 3.03[a]	2.49 ± 0.05[a]	Polydispersed	3772.70 ± 220.00[a]
	Milk powder (MEV)						
	Control	2.28 ± 0.14[a]	4.93 ± 0.13[a]	19.06 ± 0.21[a]	3.41 ± 0.16[a]	Polydispersed	_____
	20 Mpa	2.02 ± 0.01[a]	4.15 ± 0.17[a]	11.24 ± 0.47[b]	2.33 ± 0.11[b]	Polydispersed	_____
	80 Mpa	2.13 ± 0.01[a]	4.93 ± 0.65[a]	9.82 ± 0.60[b]	1.57 ± 0.09[c]	Polydispersed	_____

Means followed by the same letter, in the same column, do not differ from each other, according to Tukey test at 5% probability level. d_{10}, d_{50}, d_{90} represent the hydrodynamic diameter in which the samples have respectively 10%, 50% and 90% of the particles with values lower than these percentages.

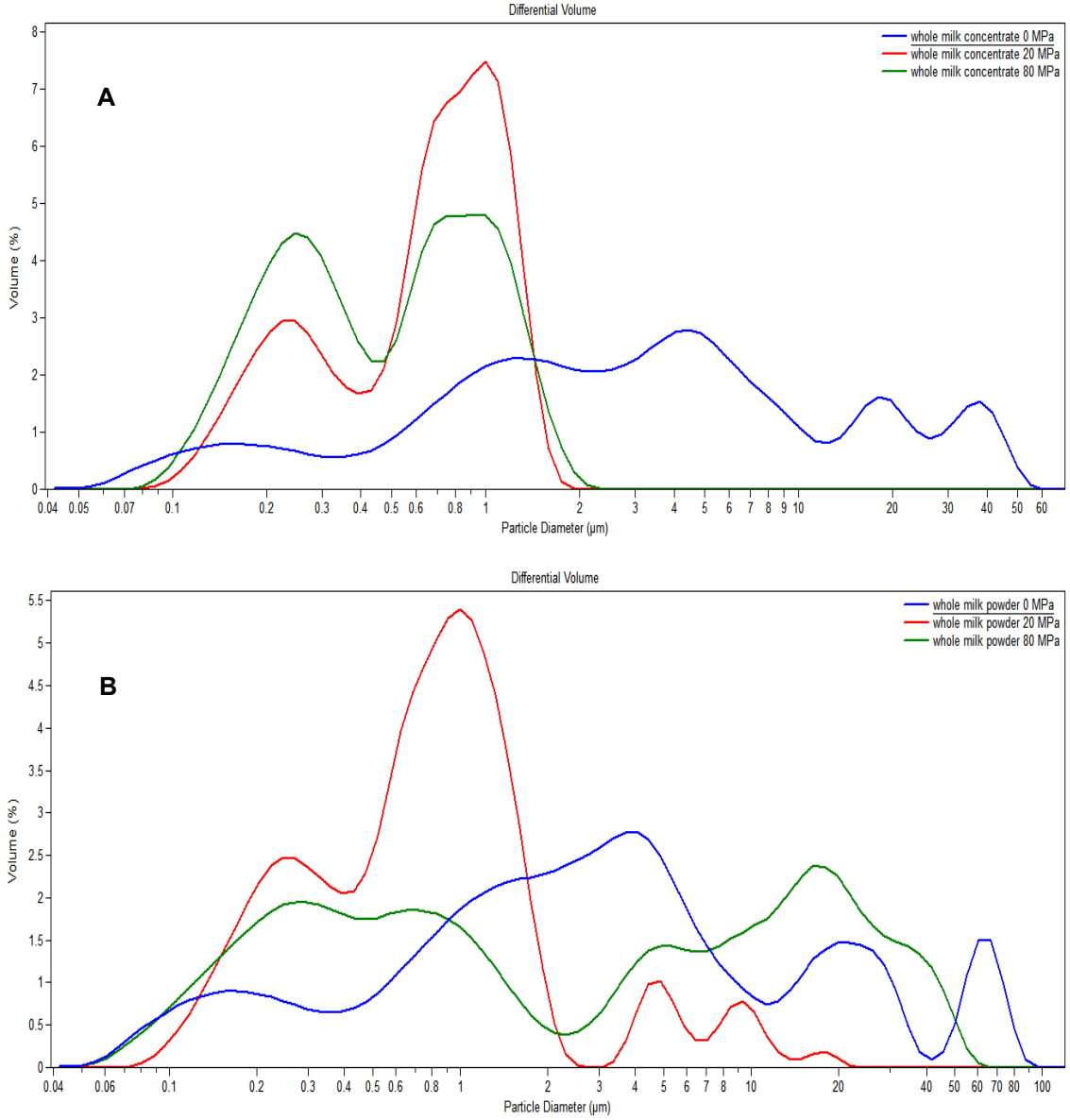

Figure 2: A: Particle size distribution curves of the concentrated whole milk with different levels of homogenization pressure. **B**: Curves of milk powder with high free fat content and different levels of homogenization pressure.

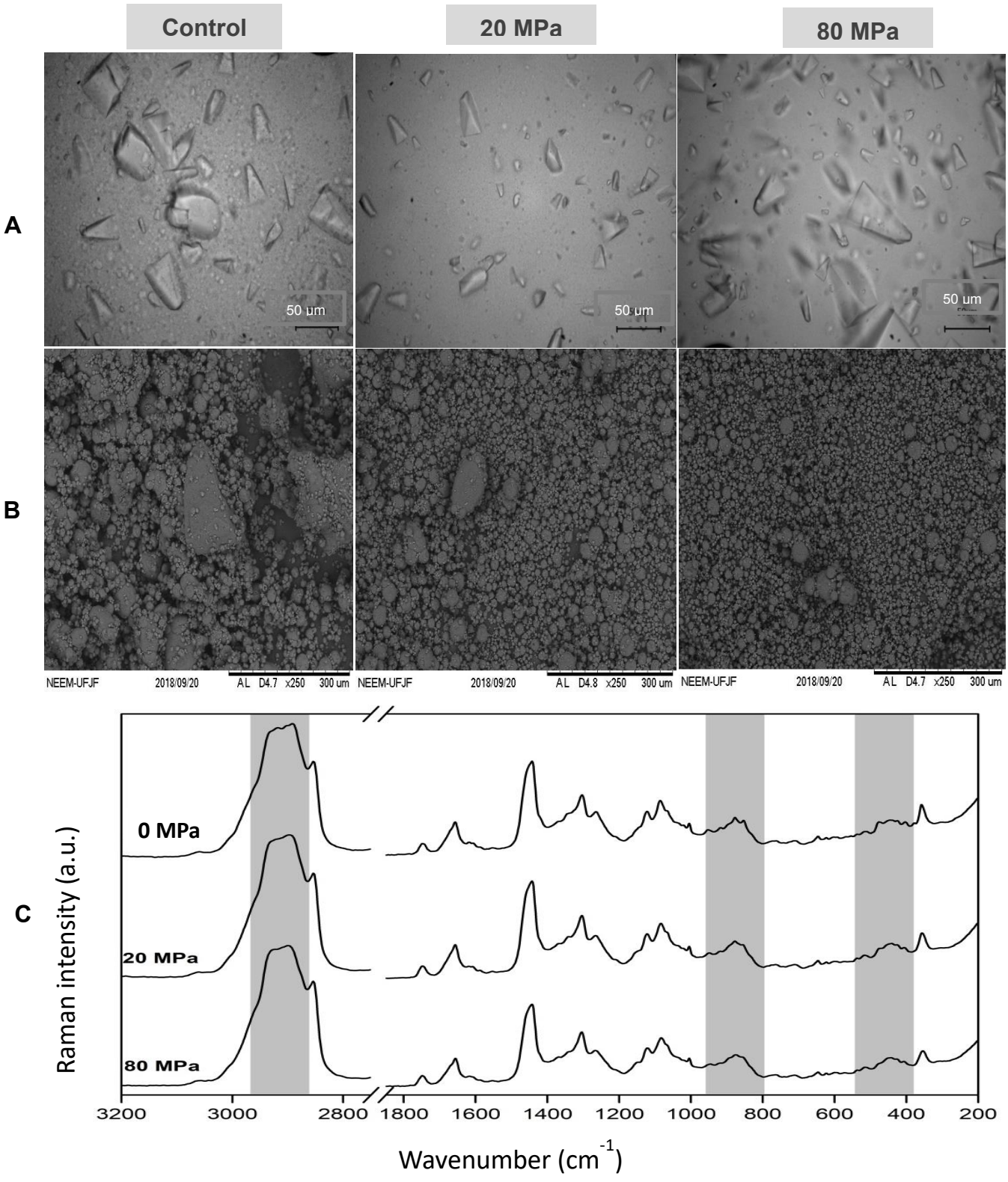

Figure 3: A: Images of milk crystals concentrated by Optical Microscopy. **B**: Images of milk powder particles by Scanning Electron Microscopy. **C**: Raman spectra of milk powder samples analyzed.

4.0. Discussion

4.1. Concentrated milk

The average particle size, as well as the amount of particles in the region below <1 μm, were statistically different ($p < 0.05$) between the control and the 20 MPa treatment samples of concentrated milk. However, there was no significant difference by the Tukey test in the particle size distribution of the concentrated milk samples homogenized at 20 MPa and 80 MPa.

The particle size distribution curve (Figure 2) exposes a population of casein micelles (150-200 nm) and a population corresponding to fat globules and other larger particles of difficult rehydration (1-4 μm).

With the results obtained from the particle size analysis (Table 1 and Figure 2), it was verified that control treatment of the concentrated milk had a small amount of particles in the region <1 μm. After homogenization at 20 MPa, almost all particles were reduced to diameters smaller than 2 μm. The results for treatment at 80 MPa show that the diameter of the particles in solution decreased further (0.25 μm), shifting to the nanometric region. Thus, it can be seen that the increase in the homogenization pressure led to an improvement in the distribution of the emulsion particles. Nonetheless, as the determination of the particle size distribution was performed soon after the homogenization process, it cannot be said that the emulsion remained stable for longer periods of time.

In spite of the statistical difference between the viscosity of the control treatment and concentrated milk homogenized at 80 MPa (table 1), the largest crystals were found in these samples, which can be confirmed by the higher hydrodynamic value (d_{90}). The lower viscosity of the concentrated milk homogenized at 20 MPa, although not presenting statistical difference when compared to the control treatment, was enough to favor the formation of smaller crystals of lactose. Mercan et al., (2018) studied the effect of HPH on the viscosity of concentrated whole milk and observed that, up to a pressure of 20 MPa, there was a decrease in product viscosity when compared to concentrated milk without homogenization. At pressures up to 35 MPa, the viscosity increased in proportion to the homogenization pressure level applied. Finally, pressures above 40 MPa resulted in a large viscosity increase, which may be related to changes in the properties of casein micelles (Mohan et al., 2016).

Concentrated milk in the control and 80 MPa treatments showed the highest SPAN values (more polydisperse), resulting in a lower distribution uniformity of the lactose crystals, when compared to the concentrated milk homogenized at 20 MPa.

4.2. Milk Powder

According to the results presented in Table 1, there was no significant difference in particle size distribution between the powders of the treatments T1 and T3 (mean and d_{90}). The milk powder homogenized at 20 MPa showed the lowest average particle diameter and the lowest d_{90}. Regarding to the distribution of particles in the region <1μm, the milk powder homogenized at 20 MPa presented the greatest amount of particles in this region, followed by the milk powder homogenized at 80 MPa and the milk powder homogenized at 0 MPa.

It is known that the high homogenization pressure (80 MPa) results in particles with smaller diameters (Dodds, 2013). Nevertheless, due to the large rupture of fat globules, there is not enough protein to cover the surface of the membrane of this globule. Thus, these globules are unprotected and the encapsulation coalesce weakens (Hayes et al., 2005; Martínez-Monteagudo et al., 2017). This may explain the fact that milk powder homogenized at 80 MPa presented less amount of particle in the region <1μm when compared to milk powder homogenized at 20 MPa. Mercan et al., (2018) analyzed concentrated whole milk treated with HPH and observed that the particle diameter reduced when homogenized at pressures up to 25 MPa; at pressures higher than that, the particle diameter increased.

The milk powders presented different profiles of particle distribution curves, as showed on Figure 2. The control treatment milk powder presented a curve of a dispersion form, with a population of particles in the region of 0.1 to 0.2 μm (casein micelles) and another population in the region of 1 to 5 μm (fat globules). This powder presented the worst rehydration characteristic when compared to the other treatments, a fact confirmed by the lower amount of particles in the region <1 μm and the higher d_{90}.

For the milk powder homogenized at 20 MPa, a curve with better dispersion was observed, where a large amount of the particles were reduced to diameters smaller than 2 μm, presenting a modal distribution in the region of 1 μm attributed to the homogenization. An increase in the percentage of volume occupied in the region between 0.2 and 0.3 μm, which may refer to the population of casein micelles, was also observed. Therefore, the rehydration capacity of the milk powder homogenized at 20 MPa improved, confirmed by the higher percentage of particles in the region <1 μm and lower value of d_{90}, as showed in Table 1.

In contrast, the milk powder homogenized at 80 MPa showed a worsening in the rehydration capacity, when compared to the milk powder homogenized at 20 MPa (lower percentage of particles in the region <1 μm). This can be explained by the weakening of the encapsulation of fat globules and by the high free fat content in the powder, which leads to the

increase of the particle size (Vignolles et al., 2009). It is inferred that the homogenization at 80 MPa caused a great rupture in the fat globules and there was not enough protein to constitute the globule membrane (Hayes et al., 2005).

In the present study, the higher free fat content directly affected the rehydration of the powder, as the milk powders homogenized at 0 MPa, 20 MPa and had a free fat content of 19.39% ± 0.16%, 5.03% ± 0.17%, and 13.78% ± 0.14%, respectively. In other words, the powder that presented the best rehydration capacity (T2) was the one with the lowest free fat content. The milk powder that exhibited intermediate rehydration capacity (T3) had an intermediate free fat content, while the milk powder that had the worst rehydration capacity (T1) exhibited the highest free fat content.

Such free fat results can be explained by particle size analysis. A higher amount of fat globule particles in the region between 1 μm and 5 μm was observed for the milk powder homogenized at 0 MPa, which may have contributed to the higher free fat content, when compared to the other treatments. On the other hand, the milk powder homogenized at 20 MPa presented smaller fat globules and with greater kinetic stability due to homogenization, resulting in a lower free fat content. Finally, milk powder homogenized at 80 MPa showed a higher free fat content than the milk powder homogenized at 20 MPa, which can be explained by the weakening of the kinetic stability of the fat globule, as a result of the lack of phospholipids and proteins to adsorb on surface of the membrane, thereby forming a more exposed fat interface. (Keogh & O'Kennedy, 1999; Vignolles et al., 2007, Vignolles et al., 2009). It can be seen that the rehydration of the powders is directly associated to the free fat content, which significantly differed ($p < 0.05$) among them according to the Tukey's test. It is noteworthy that the high amount of free fat obtained in this study resulted from the pre-crystallization stage of lactose.

It is known that a cluster of smaller particles promotes a greater amount of air in their spaces, which allows a better rehydration of the powders. Notwithstanding, for the chocolate manufacturing, milk powder with such added air particles may rupture and raise the viscosity of the product. This occurs by increasing of the interfacial area, resulting in need of a greater addition of fat, such as cocoa butter, to reduce such viscosity. This addition of cocoa butter raises the cost of chocolate (Liang & Hartel, 2004). Therefore, a milk powder with desirable characteristics for chocolate production should contain lactose crystals (to increase of free fat content), absence of homogenization (to increase free fat content and average particle size), or

homogenization at high pressures (to increase the free fat content and average particle size, when compared to homogenization at 20 MPa).

Table 1 presents the results of hydrodinamic diameter and SPAN value of the milk powders. The milk powder homogenized at 80 MPa presented the lowest SPAN value, followed by the milk powder homogenized at 20 MPa and the milk powder homogenized at 0 MPa. This suggests that the milk powder with higher homogenization pressure had a more uniform (more polydispersed) particle distribution. Scanning Electron Microscopy images (Figure 3) confirmed this behavior. That is, the powders homogenized at 0 MPa and at 20 MPa presented more agglomerates, when compared with the powder homogenized at 80 MPa. However, even if the uniformity of the particles is an effect of increasing the homogenization pressure, as for the milk of powder homogenized at 80 MPa, the microscopic profile of the same can also be correlated with the drying process by spray drying, since it was necessary to add distilled water to reduce the viscosity and favor the drying process.

In Figure 3, well-defined bands in the regions can be observed at 3000 cm^{-1}, 1650 cm^{-1}, 1450 cm^{-1}, 1350 cm^{-1}, 1150 cm^{-1}, 900 cm^{-1} and 350 cm^{-1}, which indicate crystallization of lactose (Hogan & O'Callaghan, 2010; Yazdanpanah & Langrish, 2011). The Raman spectroscopy results confirm that the lactose crystals produced in the concentrated milk remained in the dehydrated products. Since Raman spectroscopy is a non-destructive, rapid and flow-through analysis tool, the bands indicating the crystallization of lactose can be used as markers for crystallized milk powders directed to the production of chocolate.

From the results presented (table 1), it was concluded that there was no significant difference ($p > 0.05$) for the zeta potential and pH between the different treatments.

The milk powders presented a negative zeta potential due to milk proteins, mainly caseins (80% of total bovine milk protein), which are negatively charged at the natural pH of the milk (6.7) (Philippe et al., 2005).

The analysis of zeta potential is determinant for several studies as it evaluates intermolecular interactions and predicts the stability of colloidal systems. Particles with higher zeta potential, which is higher surface charge (in modulus), usually promote greater stability to colloidal suspensions. In order to predict the stability of samples, a difference of at least 10 mV in the zeta potential is required (Roland et al., 2003). As there was no statistical difference between the samples of this study, it is not possible to affirm stability difference between the analyzed samples.

5.0. Conclusions

It can be inferred that the absence of homogenization or HPH, as well as the crystallization of lactose, are undesirable in the production of milk powder for direct consumption, as they impair the rehydration; nonetheless, for the manufacture of milk powder for use in chocolate production, they are relevant. This relevance results from the increase of the free fat content and achievement of a larger average particle size, which contribute to reduction of costs and improvement of the softness of the product.

In order to meet these desirable requirements for chocolate production, the powder milk homogenized at 0 MPa was the one that presented the most suitable characteristics for this industrial application, followed by milk powder homogenized at 80 MPa.

6.0. References

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APPENDIX:

Milk Powder

Drying parameters

Table 2 presents the mean values of the drying parameters, obtained at every 5 minutes during drying.

Table 2 – Mean values of the parameters, obtained at every 5 minutes during drying.

Parameters	Milk Powder		
	0 MPa	**20 MPa**	**80 MPa**
Total time (min.)	45	45	70
Relative humidity of the spray dryer inlet air (%)	70.10	64.31	63.62
Ambient air temperature (°C)	23.97	25.79	25.21
Relative humidity of the spray dryer outlet air (%)	16.78	17.14	15.41
Spray dryer outlet air temperature (°C)	65.84	65.24	65.97
Spray dryer inlet absolute humidity (g/kg)	12.93	13.26	12.64
Spray dryer outlet absolute humidity (g/kg)	27.79	26.79	25.71
g of evaporated water / kg of dry air	14.86	13.52	13.06
Product flow rate (L/min)	994.17	876.78	790.85

According to these results, it is inferred that the drying process performed in this study occurred in a controlled manner, simulating the industrial process. It could be observed a standardization of the process attributes (temperatures, relative humidity, absolute humidity and gram of water evaporated by kg of dry air). It is noteworthy that the concentrated milk homogenized at 80 MPa had higher viscosity than the other treatments, thus, the drying process took longer and the product flow rate was lower.

Centesimal composition of milk powder

The samples of milk powder dried at different levels of homogenization pressure were characterized for moisture, water activity, total protein, total fat, free fat, ash, carbohydrates and acidity values, as presented in Table 3.

Table 3: Physicochemical analyses of milk powder dried at different levels of homogenization pressure.

Parameter	Control	20 MPa	80 MPa
Moisture (g/100 g)	3.3 ± 0.29 [a]	3.05 ± 0.15 [a]	3.2 ± 0.16 [a]
Water activity	0.2908 ± 0.07 [a]	0.2446 ± 0.05 [a]	0.2745 ± 0.05 [a]
Protein (g/100 g)	25.69 ± 0.13 [a]	25.81 ± 0.10 [a]	25.90 ± 0.04 [a]
Total fat (g/100 g)	24.3 ± 0.11 [a]	25.0 ± 0.05 [a]	24.6 ± 0.44 [a]
Free fat (%)	19.39 ± 0.16 [a]	5.03 ± 0.17 [b]	13.78 ± 0.14 [c]
Ash (g/100 g)	6.57 ± 0.23 [a]	6.48 ± 0.11 [a]	6.54 ± 0.02 [a]
Carbohydrates (g/100 g)	39.99 ± 0.07 [a]	39.60 ± 0.35 [a]	39.75 ± 0.32 [a]
Acidity (%)	0.12 ± 0.0 [a]	0.12 ± 0.0 [a]	0.12 ± 0.1 [a]

Data of two repetitions, analyses performed in duplicate.
Means followed by the same letter in the same raw, for each variable, do not differ from each other at the 5% probability level by Tukey's test.

From the results, it was observed that there was no significant difference ($p > 0.05$), by Tukey test, in relation to the parameters of moisture, water activity, protein, total fat, ash, carbohydrates and acidity between the three treatments, which shows that the homogenization pressure levels studied did not interfere with these physicochemical parameters, as expected. As for the free fat parameter, the three treatments differed from each other.

According to Normative Instruction No. 53 of October 1, 2018, whole milk powder must have at least 26% of fat, maximum acidity of 18 ºD, and up to 3.5% of moisture. Thus, the three treatments analyzed are in accordance with the legislation in relation to acidity and moisture, while fat content of all treatments are slightly below the value required by the Normative Instruction.

Regarding the protein and ash content, the results found for all treatments are close to the values of the average composition of whole milk (24.3% and 6.3% respectively) (Walstra et al., 2006). On the other hand, carbohydrate content was slightly higher than the average carbohydrate content for whole milk (38%). In relation to water activity, the powders presented higher values than the ideal water activity value (0.200 ± 0.020).

Sorption isotherm of the powders

Water activity has a major influence on the shelf life of the food products. Sorption Isotherm means the relationship between a_w and water mass per 10g of food solids at constant temperature (Abramovic & Klofutar, 2006). Figure 4 shows the sorption isotherms of the milk powders from the three treatments.

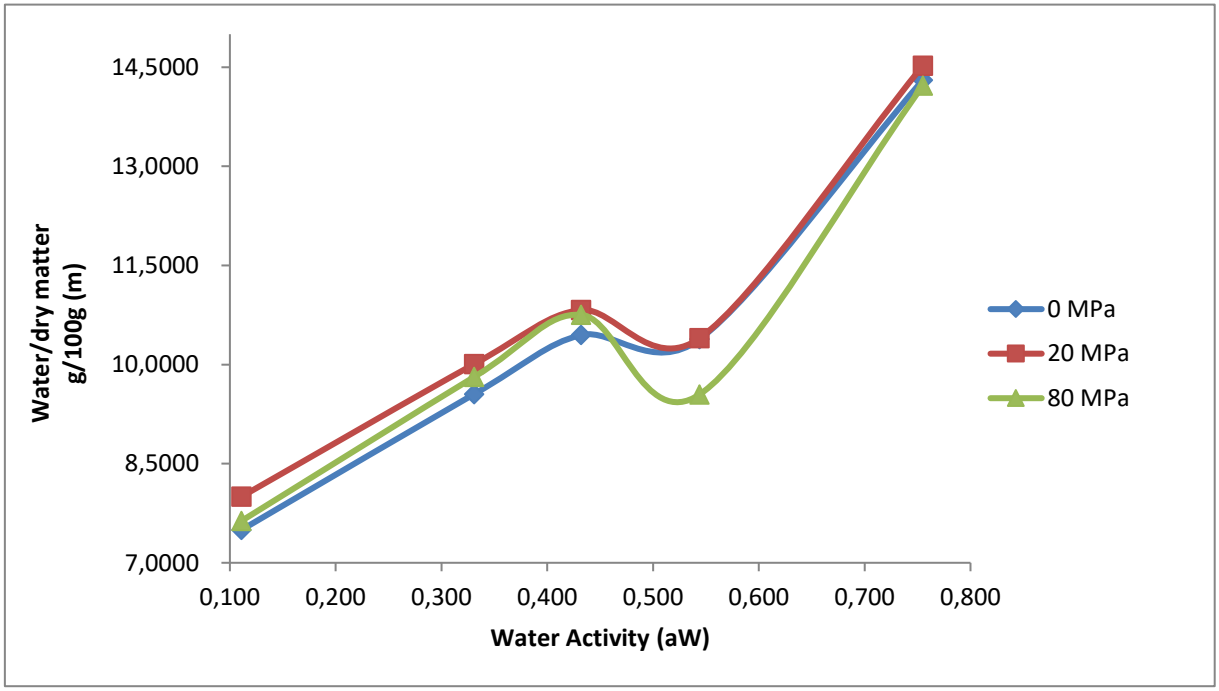

Figure 4: Sorption isotherms of the milk powders from the three treatments.

The three treatments presented a sigmodal behavior, characteristic for most food products. Moreover, in the graph, the isotherms of the powder from the treatments Control and 20 MPa were practically overlapping, which shows that they absorbed values very close of water content. Diversely, milk powder from the treatment at 80 MPa showed a greater depression in the region of water activity from 0.4 to 0.6, which implies that a larger amount of amorphous lactose passed to the crystalline state. This behavior is justified by the crystallization of lactose during the production of the powder, which becomes the most significant factor in sorption behavior.

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