Conjecturas

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Effect of phosphate addition and homogenization pressure on particle size distribution in pasteurized milk

Efeito da adição de fosfatos e da pressão de homogeneização na distribuição do tamanho das partículas em leite pasteurizado

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ABSTRACT

The aim of the current study is to investigate the effect of analysis times and stabilizing salt blends (LD88, LD89 and KM5), at different doses and homogenization pressures, on particle size distribution (PSD) in pasteurized whole milk. PSD has evidenced that samples not subjected to stabilizing salt addition, heating and homogenization did not show significant variations in storage time. Sixty-seven (67) of the 81 evaluated experiments subjected to stabilizer addition recorded Dv90 values (volume, where 90% of particles were found) higher than those of their corresponding treatments without salt addition. The LD89 blend was the stabilizer recording the lowest Dv90 values at hydration times 0 and 24 hours, regardless of dose level, at homogenization pressure equal to 20 MPa. This very same same blend - at the dose of 1.0 $g.L^{-1}$, 0-hour hydration and homogenization pressure equal to 80 MPa - was the stabilizer recording the lowest overall Dv90 value (0.926 µm) among all experimental conditions. Results enabled concluding that analysis time did not influence PSD, although stabilizer type had influence on PSD, under the homogenization conditions adopted for whole milk in the current study.

Keywords: Nanostructure; Microstructure; Particle Size.

RESUMO

O objetivo deste trabalho foi avaliar o efeito de tempos de análise, mistura de sais estabilizantes (LD88, LD89 e KM5) em diferentes níveis de dosagem e pressões de homogeneização, do leite integral pasteurizado, na distribuição do tamanho das partículas (DTP). Observou-se pela DTP que as amostras sem adição de sais estabilizantes, sem aquecimento e sem homogeneização não obtiveram variações significativas em relação ao tempo de estocagem (Dv90=5,2 µm). Dos 81 experimentos avaliados com alguma adição de estabilizante, 67 apresentaram valores de Dv90 superiores aos seus correspondentes tratamentos sem adição dos sais. O blend LD89 foi o estabilizante que apresentou os menores valores de Dv90 para os tempos de hidratação 0 e 24 horas, independentemente do nível de dosagem, na pressão de homogeneização 20 MPa. O mesmo blend na dosagem de 1,0 $g.L^{-1}$, com 0 horas de hidratação e pressão de homogeneização de 80 MPa, foi o estabilizante que apresentou o menor valor geral de Dv90 (0,926 µm) entre todas as condições experimentais. Conclui-se que o tempo de análise não influenciou a DTP, em contrapartida, o tipo de estabilizante influenciou a DTP nas condições de homogeneização utilizadas neste estudo para leite integral.

Palavras-chave: Nanoestrutura; Microestrutura; Tamanho de partículas.

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INTRODUCTION

Using phosphate salts in milk-producing industries helps saving time, electric power, manpower and chemical products used to clean equipment. In addition, phosphate salts enable increased thermal stability and help minimizing issues such as geling and sediment formation (BRASIL, 1997; WALSTRA et al., 2005; FOX, 2015). The addition of stabilizing salts, mainly of phosphate salts such as sodium monophosphate, diphosphate and triphosphate, to Ultra High Temperature (UHT) products is authorized and regulated in Brazil by Ordinance n. 370 - from September 4th, 1997. However, these salts cannot be added to UHT products in amounts exceeding 0.1 g.100 mL^{-1}. Stabilizing salts are used to increase milk thermal stability, as well as to delay gelling in UHT products. These salts are chelating agents capable of sequestering and complexing calcium ion; consequently, they change the balance between caseins and minerals (AUGUSTIN; CLARKE, 1990; SINGH et al., 2021).

Heating milk to high temperatures in ultra-pasteurization processes can affect milk constituents and likely lead to protein denaturation reactions and to complex formations between whey proteins and caseins. Thus, using these phosphate salts can help maintaining the colloidal stability of these proteins by changing their structure (BUENO et al., 2020; NIEUWENHUIJSE; HUPPERTZ, 2018).

Accordingly, homogenization is a mandatory step in UHT milk production processes, since it provides better product stability under storage conditions, as well as longer shelflife, by applying high pressures capable of reducing the size of milk constituents such as fat globules (D'INCECCO et al., 2018). Homogenization is a processing stage carried out to change the functional or sensory properties of milk with little or no effect on this product's nutritional value. A new membrane is immediately redone during homogenization due to the action of surfactants found in milk; consequently, it reduces the surface tension between globules and the soluble phase. The newly formed membrane presents higher protein concentrations and lower relative phospholipids' participation (MCCRAE et al., 1994; CANO-RUIZ; RICHTER, 1997; YE; ANEMA; SINGH, 2008).

The technology called milkmusion was developed by focusing on the milky matrix in order to get protein-lipid nanoparticles with hydrodynamic radii centered at up to 200 nm. Reducing the size of these fat globules leads to unique emulsification abilities, improves products' digestibility by increasing nutrient absorption and by enabling new

mechanisms for active compounds' release in the body (QUEIROZ et al., 2021; DE PAULA et al., 2021; INOVALEITE, 2021).

Fat globules' natural size is approximately 5 micrometers; however, homogenization can reduce their size to approximately 1 micrometer, which is a viable size to avoid lipid separation. Nevertheless, these fat globules are too big to enable the proper stabilization of emulsions as complex as milk (BAARS et al., 2016; SINGH; GALLIER, 2017; LERMA et al., 2018).

The process adopted to determine particle size distribution (PSD) through phosphate addition to casein and skim milk is described in the scientific literature (UDABAGE; MCKINNON; AUGUSTIN, 2000; PANOUILLÉ; NICOLAI; DURAND, 2004; PITKOWSKI; NICOLAI; DURAND, 2008; DE KORT, 2012; RENHE; INDRIS; CORREDIG, 2018).

The current study is based on the hypothesis that homogenization pressure and stabilizing salt addition to whole milk can influence its particle size distribution. The literature has few studies focused on investigating the way different phosphate salts can influence the particle size of whole milk homogenized at different pressures. Thus, the aim of the present study was to investigate the influence of type, concentration and addition time of different phosphate salts on the PSD of milk pasteurized and homogenized under different pressures.

EXPERIMENTAL PART

Refrigerated milk (5°C ± 2°C) standardized with 30.0 $g.L^{-1}$ of fat matter was subjected to pasteurization (from 72°C to 75°C, for 15 to 20 seconds) and subsequently cooled to 5°C ± 2°C. Samples were added with three different commercial phosphate salt types (LD88, LD89 and KM5) at 4 different doses (0.0 $g.L^{-1}$, 1.0 $g.L^{-1}$, 5.0 $g.L^{-1}$ and 10.0 $g.L^{-1}$). Milk added with salts was grouped into three different treatments: treatment 1 (non-homogenized/non-heated); treatment 2 (non-homogenized/heated in water bath at 80°C ± 2°C); treatment 3 (homogenized at three different pressure levels - 20 MPa, 50 MPa and 80 MPa - in two-stage piston homogenizer APV-1000/heated in water bath at 80°C ± 2°C).

The herein added phosphate salts comprised JOHA LD88 (sodium triphosphate, sodium monophosphate and disodium pyrophosphate) with P_2O_5 (%): 57.5 – 58.5 and pH (1% sol.): 8.5 – 9, 0; JOHA LD89 (sodium tripolyphosphate, sodium hexametaphosphate

and trisodium phosphate) with P_2O_5 (%): 57.5 - 60.0 and pH (1% sol.): 10.1 - 10.9; JOHA KM 5 (sodium hexametaphosphate and disodium phosphate) with P_2O_5 (%): 65 – 67 and pH (1% sol.): 7.0 – 7.6.

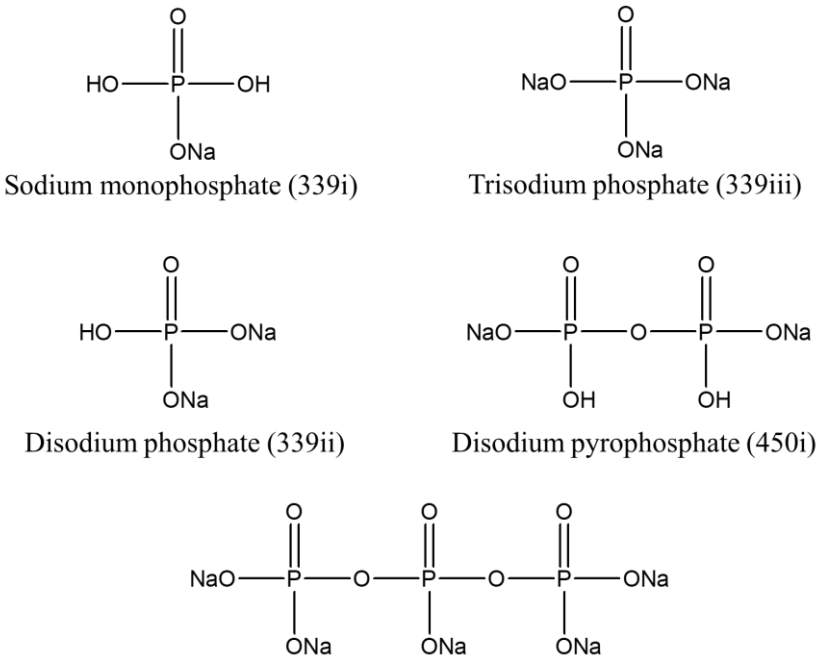
Table 1 shows the INS (International Numbering System) number of different phosphates forming the salts used in the current study, whereas the chemical structures of these phosphates are shown in Figures 1 and 2.

Table 1 – INS (International Numbering System) number of different phosphates forming the herein used salts.

INS	Additive	Salt
339i	Sodium monophosphate (NaH_2PO_4)	LD88
339ii	Disodium phosphate (Na_2HPO_4)	KM5
339iii	Trisodium phosphate (Na_3PO_4)	LD89
450i	Disodium pyrophosphate ($Na_2H_2P_2O_7$)	LD88
451i	Sodium triphosphate or Sodium tripolyphosphate ($Na_5P_3O_{10}$)	LD88 and LD89
452i	Sodium hexametaphosphate (SHMP)	LD89 and KM5

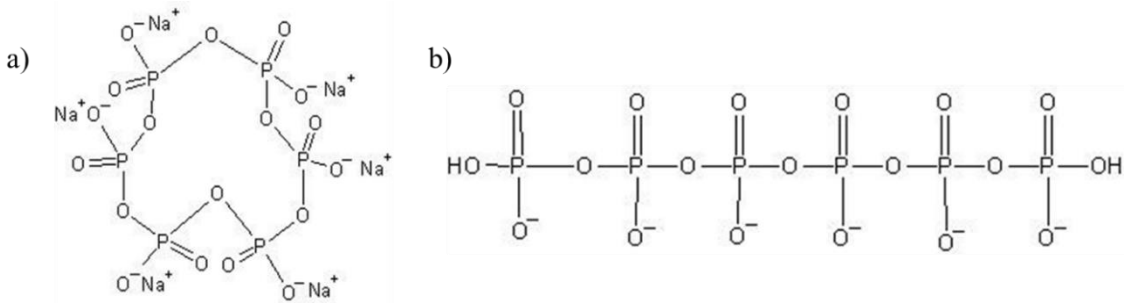
Source: Elaborated by the authors.

Figure 1 – Schematic representation of the structure presented by different phosphates that form the herein used salts.



Source: Elaborated by the authors.

Figure 2 – Schematic representation of the sodium hexametaphosphate (452i) structure - (a) cyclic and (b) linear shape.



Source: Adapted from GAO et al. (2010).

PSD analysis was applied to all samples, in Beckman Coulter LS 13320 Laser Diffraction Particle Size analyzer (Brea, USA) added with Aqueous-Liquid Modulus (ALM), at three different times (0, 24 and 48 hours). Samples were directly and slowly added to the reservoir of the Beckman Coulter LS 13320 Laser Diffraction Particle Size analyzer (Brea, USA) added with Aqueous Liquid Module (ALM), which was filled with water at room temperature (23 °C ± 2 °C), until it reached minimum obscuration level. Data were collected after 90 seconds under recirculation, until stable particle size distributions were observed. Results were calculated by using refractive index 1.332, for the dispersant (water); 1.57, for casein micelles; and 1.47, for fat globule – it enabled finding total solubility (MICHALSKI; BRIARD; MICHEL, 2001; MIMOUNI et al., 2009). Data were expressed as rate of volume taken by particles, based on their size. Beckman Coulter software (particle featuring) version 5.03 was used for statistical analyses applied to the collected data (Dv90 = volume, where 90% of particles were found). Figure 3 shows the schematic summary of the herein adopted methodology.

Finally, Stokes' law was used to find milk particles' displacement speed in 200 mm of milk. Stokes' law was used to find milk particles' displacement speed in 200 mm of milk. Equation 1 was used for theoretical calculations of separation speed, based on (TOWLER; ROBINSON, 1994):

$$v_s = \left[\frac{d^2 \cdot (\rho_p - \rho_1) \cdot g}{18\eta} \right] \quad (1)$$

Wherein:

v_s = particle sedimentation velocity (m/s).

d^2 = particle diameter represented by d_{90} (hydrodynamic diameter observed in particle size distribution, in μm).

ρ_p = particle density represented by milk fat (930 $kg{\cdot}m^{-3}$).

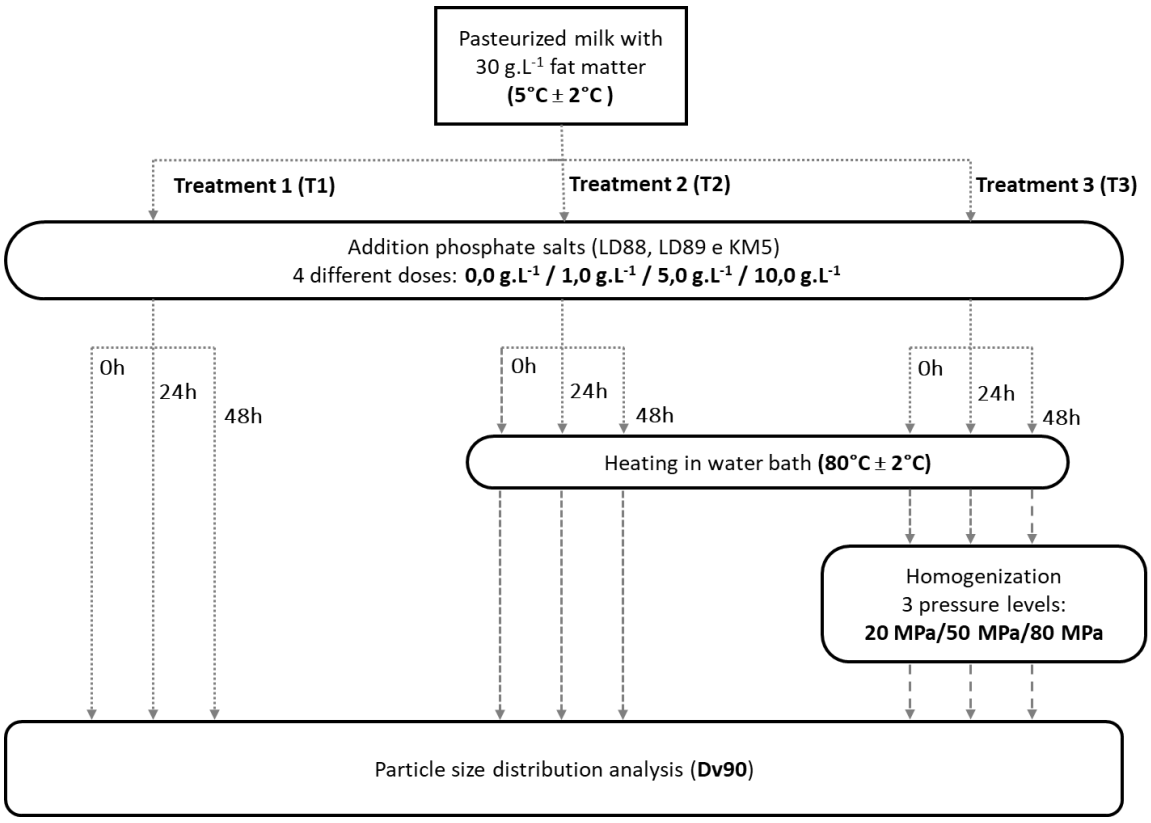
ρ_1 = continuous phase density represented by skimmed milk (1,034 $kg{\cdot}m^{-3}$).

η = continuous phase viscosity represented by skimmed milk (0.00179 $kg{\cdot}m^{-1}{\cdot}s^{-1}$).

g = gravitational acceleration (9.81 $m{\cdot}s^{-2}$).

Particle displacement velocity is often calculated by taking into account milk fat density. However, samples in the current study were subjected to homogenization process at different pressures; this process has changed fat globules' structure, a fact that does not guarantee the same milk fat density in all treatments. Fat density increases after the homogenization process because the membrane formed around the fat globule is formed by proteins.

Figure 3 – Flowchart outlining the methodology adopted for all three treatments carried out in the current study; in total, 180 experiments were subjected to particle distribution analysis in order to find parameter Dv90 (hydrodynamic diameter, expressed in μm).



Source: Elaborated by the authors.

RESULTS AND DISCUSSION

Table 2 shows results recorded for Dv90 in the PSD analysis applied to samples that were not added with salts, at the three analyzed times, in all three treatments.

Table 2 – Mean and standard deviation of parameter Dv90 (μm) in samples without salt addition, at three times in all three treatments: T1 (without heating/without homogenization), T2 (with heating/without homogenization) and T3 (with heating/with homogenization) . n = 3.

Treatment	Pressure (MPa)	Dv90 (μm)		
		0h	24h	48h
Treatment 1 (T1)	NA	5.138 ± 0.091[a]	5.253 ± 0.031[a]	5.256 ± 0.054[a]
Treatment 2 (T2)[2]	NA	6.023 ± 1.502[a]	5.028 ± 0.253[a]	5.213 ± 0.102[a]
Treatment 3 (T3)[3]	20	1.457 ± 0.104[a]	1.517 ± 0.072[a]	1.583 ± 0.046[a]
	50	0.987 ± 0.079[a]	1.066 ± 0.179[a]	1.014 ± 0.056[a]
	80	0.892 ± 0.042[a]	0.938 ± 0.008[a]	0.942 ± 0.023[a]

*means followed by the same letter, at different times, did not significantly differ from each other in the Tukey's test ($p<0.05$). Dv90: hydrodynamic diameter (μm), 90% of particles recorded values lower than the found one.

Source: Elaborated by the authors.

The analyzed times (0, 24 and 48 h) did not influence the hydrodynamic size corresponding to 90% of particles accounting for values lower than the recorded ones (Table 1). In addition, heating (80°C ± 2°C) had low influence on Dv90 values. On the other hand, homogenization led to particles' reduced hydrodynamic diameter. Homogenization at pressure of 20 MPa was enough to reduce particle size by approximately 3 times in comparison to that recorded for non-homogenized samples. However, the smallest hydrodynamic diameters were observed when homogenization pressure increased to 50 MPa and 80 MPa. Among the samples subjected to pressure of 50 MPa, only the one subjected to hydration time 0h presented Dv90 in the sub-micrometric region (<1μm). Finally, increasing the pressure from 50 MPa to 80 MPa enabled 100% of analyzed samples to have particle size allocated in the sub-micro region (<1μm).

Table 3 shows the Dv90 parameter of samples added with phosphate salts at different times and different doses, based on PSD of Treatment 3. Only one sample recorded hydrodynamic diameter value higher than 2 μm; it was the one added with 5

g.L^{-1} of KM5 phosphate salt, at time 0 h and pressure of 80 MPa. On the other hand, the lowest hydrodynamic diameter values observed in the sub-micro region (<1 μm) were recorded for samples subjected to homogenization pressure of 80 MPa, and added with KM5 (24h/1 gL^{-1} and 48 h/1 gL^{-1}), LD89 (0h/1 gL^{-1}) and LD88 (48h/1 gL^{-1}).

Table 3 shows Dv90 values and their respective standard deviations, and it enabled seeing the confidence interval between the minimum and maximum values of such hydrodynamic diameter, at each time and homogenization pressure. Based on results presented in this table, it is possible comparing Dv90 values recorded for samples added with different salt types and concentrations to those recorded for samples without salt addition, at the very same homogenization times and pressures. It is worth mentioning that the current study comprises a significantly large number of variables (combination among 3 salt types, 4 salt doses, 3 homogenization pressures and 3 different hydration times), a fact that highlights its exploratory nature.

Table 3 – Parameter Dv90 (μm) in T3 (with heating/with homogenization) samples added with phosphate salts, at different times and doses.

Pressure (MPa)	Salt ($g.L^{-1}$)	Dv90 (μm) – Treatment 3								
		LD88			LD89			KM5		
		0h	24h	48h	0h	24h	48h	0h	24h	48h
20	1.0	1.684^{+}	1.626^{+}	1.532	1.398	1.540	1.678^{+}	1.927^{+}	1.790^{+}	1.597
	5.0	1.667^{+}	1.658^{+}	1.700^{+}	1.433	1.560	1.578	1.634^{+}	1.683^{+}	1.635^{+}
	10.0	1.439	1.565	1.620	1.501	1.527	1.664^{+}	1.656^{+}	1.455	1.688^{+}
50	1.0	1.141^{+}	1.369^{+}	1.210^{+}	1.076^{+}	1.793^{+}	1.237^{+}	1.390^{+}	1.380^{+}	1.149^{+}
	5.0	1.399^{+}	1.721^{+}	1.705^{+}	1.624^{+}	1.673^{+}	1.637^{+}	1.897^{+}	1.855^{+}	1.698^{+}
	10.0	1.210^{+}	1.390^{+}	1.793^{+}	1.481^{+}	1.930^{+}	1.628^{+}	1.754^{+}	1.601^{+}	1.803^{+}
80	1.0	1.031^{+}	1.044^{+}	0.994^{+}	0.926	1.833^{+}	1.016^{+}	1.870^{+}	0.976^{+}	0.984^{+}
	5.0	1.591^{+}	1.804^{+}	1.836^{+}	1.696^{+}	1.472^{+}	1.607^{+}	2.110^{+}	1.967^{+}	1.700^{+}
	10.0	1.055^{+}	1.739^{+}	1.896^{+}	1.144^{+}	1.968^{+}	1.648^{+}	1.793^{+}	1.637^{+}	1.764^{+}

$^{+}$ Dv90 higher than the maximum values recorded (within the confidence interval) for each corresponding T3 treatment without salt addition, as shown in Table 1. Dv90: hydrodynamic diameter (μm), 90% of particles recorded values lower than the found one.

Source: Elaborated by the authors.

All results recorded for hydrodynamic diameter (Table 3) were either within or above the confidence interval shown in Table 2. Therefore, it is noteworthy that no sample recored hydrodynamic diameter value lower than the confidence interval determined for samples without phosphate salt addition. In total, 83% (67 experiments) of the 81 results (Table 3) presented Dv90 values higher than the maximum value recorded for samples without salt addition. This outcome has evidenced increased particle size in virtually all samples added with salts, resgardless of their type and concentration. Among the other samples added with salts, 17% (14 experiments) presented Dv90 values within the confidence interval (minimum and maximum value) in comparison to samples without salt addition. In other words, phosphate salt addition to samples did not increase particle size. Among the aforementioned 17%, 4 samples were added with LD88 salt (28.6%), 8 samples were added with LD89 salt (57.1%) and 2 samples were added with KM5 salt (14.3%); i.e., it is possible inferring that LD89 was the salt leading to the smallest increase in particle size, whenever it was added to the samples. It is worth mentioning that virtually 100% of samples (except for 1 sample subjected to pressure of 80 MPa) that have recorded Dv90 values within the confidence interval observed for samples without salt addition were the ones subjected to homogenization pressure of 20 MPa. All samples added with salts and subjected to pressure of 50 MPa, and virtually all samples subjected to pressure of 80 MPa (except for 1 sample presenting value within the confidence interval), recorded Dv90 values higher than those observed for samples without salt addition; this outcome has evidenced increase in particle size. In other words, salt addition to samples mainly accounted for the trend to increased particles' hydrodynamic diameter, mainly at pressures 50 MPa and 80 MPa.

Udabage, McKinnon and Augustin (2000) conducted a study to investigate the effects of adding calcium salts and chelating agents in skim milk to help better understanding the reversibility of changes induced in casein micelle at fixed pH. Adding a mix of phosphates (Na_2HPO_4 and NaH_2PO_4), at concentrations of 10, 20 and 30 $mmol.kg^{-1}$, to skim milk did affect the effective diameter of micelles - which was approximately 197 nm - in the current study. However, the addition of 30 $mmol.kg^{-1}$ of these phosphates, which was followed by the addition of 10 $mmol.kg^{-1}$ of $CaCl_2$, has increased the effective diameter of micelles in milk suspensions to approximately 209 nm.

Pitkowski, Nicolai and Durand (2008) investigated casein dissociation resulting from chelating salts' addition by using static and dynamic light scattering and small angle X-ray scattering (SAXS). A mix of sodium polyphosphates was used as chelating agent; it comprised concentrations of each polyphosphate ranging from 10% to 15%, namely: ortho-, pyro-, tri- and tetraphosphate. Different polyphosphate mix concentrations (1.0, 2.0, 3.0, 5.0 and 7.0 $g.L^{-1}$) were added to 11 $g.L^{-1}$ of casein solution; casein complexes were fully dissociated in all cases. Casein that got fully dissociated after polyphosphate addition has formed small micellar particles that presented hydrodynamic radius of approximately 10 nm (PANOUILLÉ; NICOLAI; DURAND; 2004). Furthermore, a mix of intact and dissociated micelles were observed in casein solutions after polyphosphate addition at concentration of 0.5 $g.L^{-1}$.

Sodium hexametaphosphate (SHMP) has the potential to bind to up to three calcium atoms, since its six homogeneously distributed phosphate molecules enable this salt to directly interact with casein amino acid residues through electrostatic interactions. SHMP triggers electrostatic repulsions in micelles and this process leads to κ-casein dissociations (ANEMA, 2015; DE KORT et al., 2011). De Kort (2012) has analyzed variations in casein micelles' particle size at SHMP concentrations ranging from 0 to 100 $mmol.L^{-1}$. The threshold SHMP concentration, at which smaller particles were observed, was 25 $mmol.L^{-1}$. The distribution peak started to change towards particles with smaller diameters (from 30 $mmol.L^{-1}$ on) and reached values lower than 10 nm at SHMP concentrations higher than 50 $mmol.L^{-1}$. The same analyses were performed, after Na_2HPO_4 addition, also at concentrations ranging from 0 to 100 $mmol.L^{-1}$. Results have shown that Na_2HPO_4 has poor ability to dissociate casein micelles into smaller particles, since Na_2HPO_4 addition has increased the diameter of intact micelles from 190 to 220 nm. This outcome suggested that the volume of casein micelles has increased after Na_2HPO_4 addition, which is likely associated with decreased free calcium levels that, in their turn, induced increased repulsion between casein molecules (DE KORT, 2012).

Renhe, Indris and Corredig (2018) have evaluated the stability of micellar casein concentrates subjected to heat with, and without, the addition of calcium chelators (trisodium citrate and Na_2HPO_4). In order to do so, particle size measurements were taken, by taking into consideration pH and chelating salts, after heating at 120°C, for 10 minutes; pH 6.5 did not show significant differences in casein micelle diameter, which ranged from 165 to 190 nm. On the other hand, particle size at pH 6.7 has increased as

calcium chelator concentration also increased, mainly when the citrate-phosphate combination was used. In this case, micelle diameter at concentration of 60 mmol.L^{-1} of citrate-phosphate was 240 nm. Particle size increase at pH 6.9 was more significant at phosphate concentrations higher than 30 mmol.L^{-1}, as well as at citrate-phosphate combinations ranging from 264 to 524 nm. According to Sauer & Moraru (2012), such increase in micellar casein particle size at pH 6.9 is correlated to heating temperature.

Table 4 shows the time (in days) required for particles to cover 200 mm (average size of 1 liter UHT milk carton packs in Brazil).

Table 4 – Time (in days) required to cover 200 mm in samples subjected to T3 (with heating/with homogenization), added with phosphate salts, at different times and doses.

Pressure (MPa)	Salt ($g.L^{-1}$)	Time (in days) required to cover 200 mm – Treatment 3								
		LD88			LD89			KM5		
		0h	24h	48h	0h	24h	48h	0h	24h	48h
20	1.0	25.80	27.70	31.10	37.40	30.80	26.00	19.70	22.80	28.70
	5.0	26.30	26.60	25.30	35.60	30.00	29.40	27.40	25.80	27.30
	10.0	35.30	29.80	27.90	32.40	31.40	26.40	26.70	34.50	25.70
50	1.0	56.20	39.00	49.90	63.10	22.70	47.80	37.80	38.40	55.40
	5.0	37.40	24.70	25.10	27.70	26.10	27.30	20.30	21.20	25.40
	10.0	49.90	37.80	22.70	33.30	19.60	27.60	23.80	28.50	22.50
80	1.0	68.80	67.10	74.00	85.30	21.80	70.80	20.90	76.70	75.50
	5.0	28.90	22.50	21.70	25.40	33.70	28.30	16.40	18.90	25.30
	10.0	65.70	24.20	20.30	55.90	18.90	26.90	22.70	27.30	23.50
Overall Mean		43.81	33.27	33.11	44.01	26.11	34.50	23.97	32.68	34.37
Maximum Value		68.80	67.10	74.00	85.30	33.70	70.80	37.80	76.70	75.50
Minimum Value		25.80	22.50	20.30	25.40	18.90	26.00	16.40	18.90	22.50
Range		43.00	44.60	53.70	59.90	14.80	44.80	21.40	57.80	53.00
Standard deviation		16.81	13.95	17.72	19.96	5.56	15.22	6.23	17.63	18.42
Standard error of the mean		5.60	4.65	5.91	6.65	1.85	5.07	2.08	5.88	6.14
Coefficient of variation (%)		38	42	54	45	21	44	26	54	54

Source: Elaborated by the authors.

The longer the time (in days) required to cover the box, the longer the product's shelflife. On the other hand, the shorter the time (in days) required to cover the box, the shorter the product's shelflife due to particles' deposition at the bottom of the package. The longest time (in days) required to cover 200 mm was observed for the 0-h sample added with LD89 salt (maximum time: 85 days; overall mean time: 44 days). On the other hand, the shortest time (in days) required to cover 200 mm was observed for the 0-h sample added with KM5 salt (maximum time: 16 days; overall mean time: 24 days). This outcome enables inferring that LD89 is likely the salt mostly capable of stabilizing milk particles. The highest time variation coefficient (54 days) was recorded for samples added with LD88 (48 h) and KM5 (24 h and 48 h) salts, whereas the lowest variation coefficient was recorded for samples added with LD89 (24 h) and KM5 (0 h) salts – at 21 and 26 days, respectively.

Moreover, 0-h samples added with LD88 and LD89 salts have shown overall mean time longer than 40 days to cover 200 mm; thus, it was possible classifying these samples as those presenting the highest emulsion physical stability. On the other hand, 24-h samples added with LD88 and KM5, and 48-h samples added with LD88, LD89 and KM5, resulted in mean time to cover 200 mm longer than 30 days; they were classified as presenting intermediate physical stability. Finally, 24-h samples added with LD89, and 0-h samples added with KM5, were the ones showing mean time to cover 200 mm longer than 20 day; they were classified as presenting the lowest physical stability.

According to the scientific literature (UDABAGE; MCKINNON; AUGUSTIN, 2000; PANOUILLÉ; NICOLAI; DURAND, 2004; PITKOWSKI; NICOLAI; DURAND, 2008; DE KORT, 2012; RENHE; INDRIS; CORREDIG, 2018) phosphates enable casein dissociation. Dissociated caseins can interact with fat globules; consequently, they increase particles' hydrodynamic diameter as observed in the current results. Heating can also be correlated to this trend to increased particles' hydrodynamic diameter.

CONCLUSION

Based on results reported in the current exploratory study for treatments without stabilizing salt addition, it is possible concluding that analysis times (0, 24 and 48 h) did not influence particles' hydrodynamic size, whereas heating had little influence on it; however, homogenization was capable of reducing particles' hydrodynamic diameter. On

the other hand, treatments with stabilizing salt addition enalbed concluding that phosphate addition to the samples accounted for the trend to increased particles' hydrodynamic diameter; analysis time did not show any effect on this parameter. The aforementioned increase may be associated with the interaction between dissociated caseins and fat globules, as well as with heating.

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