pH influences hydrolysis of sodium polyphosphate in dairy matrices and the structure of processed cheese

A. P. Barth,* C. F. Tormena,† and W. H. Viotto*¹

*School of Food Engineering, University of Campinas (UNICAMP), Campinas, Brazil, 13083-862

†Institute of Chemistry, University of Campinas (UNICAMP), Campinas, Brazil, 13083-970

ABSTRACT

We investigated the effect of pH (5.2 to 6.8) on the hydrolysis of a sodium polyphosphate in water, milk, calcium caseinate, and spreadable processed cheese, as well as the effect of pH on the cheese structure. Monitoring of the hydrolysis in water and the different milk matrices was carried out using ³¹P nuclear magnetic resonance technique. In general, the decrease in pH increased the hydrolysis of polyphosphates in all matrices. The presence of calcium in milk increased the rate of hydrolysis. Hydrolysis in milk was higher than in calcium caseinate, probably due to lower molecular mobility in concentrated systems with high viscosity. Increasing the pH decreased the hardness and adhesiveness of the cheeses. At low pH (5.2 and 5.6), the cheeses presented a granular structure, although, at more neutral pH (6.0 to 6.8), the structure was continuous, homogeneous, and more fluid. These results highlight the importance of precise pH control in the manufacture of processed cheeses.

Key words: ³¹P nuclear magnetic resonance, hydrolysis, sodium polyphosphate, processed cheese

INTRODUCTION

The extensive use of sodium phosphates in the processed cheese manufacture is due to their ability to sequester calcium and to adjust pH. The complexation with calcium causes casein dissociation and increases its solubility and hydration. Under the action of heat and shear, the dissociated casein emulsifies the fat, favoring the formation of a homogeneous structure (Guinee et al., 2004; Mizuno and Lucey, 2005).

Usually, the commercial emulsifying salts are mixtures of long- and short-chain-length sodium phosphates to ensure a balance between the chelating action and the

buffering effect (Schär and Bosset, 2002; Guinee et al., 2004).

Temperature and pH conditions, used in the manufacture of processed cheeses, may allow the hydrolysis of polyphosphates (Molins, 1991; McBeath et al., 2007). Hydrolysis occurs through the nucleophilic attack of water on the terminal phosphate unit resulting in a break of P-O-P bond and formation of monophosphates as an end product of reaction (Jager and Heyns, 1998). Rulliere et al. (2012) demonstrated that the hydrolysis occurs above 100°C in aqueous solutions, a temperature close to those used in the manufacture of processed cheeses. According to Torres-Dorante et al. (2005), the hydrolysis is slow at neutral pH, even at temperatures of 100°C. In acid solutions, the increase in the concentration of H⁺ ions becomes a strong catalyst for hydrolysis reactions (Jager and Heyns, 1998). Hydrolysis may cause decreased water binding capacity of the matrix, shifting the final cheese pH and changing the cheese texture (Liu et al., 2015).

Requeijão cremoso is a Brazilian spreadable processed cheese obtained from the fusion of fresh cheese, made by direct acidification of heated milk or enzymatic coagulation (Cunha et al., 2013). The main difference between them is the pH, which can vary from 5.4 (direct acidification) to 6.8 (enzymatic coagulation). Changes in pH influence the microstructure and texture of processed cheeses, due to the effects on protein interactions, fat emulsification, and hydration of casein (Marchesseau et al., 1997).

³¹P Nuclear magnetic resonance (NMR) spectroscopy has been used for the determination of phosphorus compounds in foods (Hrynyszyn et al., 2010; Rulliere et al., 2013) and monitoring of the hydrolysis of polyphosphates in fish (Liu et al., 2015). Nuclear magnetic resonance spectroscopy can simultaneously detect monophosphates, diphosphates, triphosphates, and polyphosphates in different food matrices. This user-friendly technique brings advantages, such as speed, precision, and ease of analysis (Liu et al., 2015).

Studies on hydrolysis of polyphosphates are mainly focused on the effect of temperature on such reactions,

Received February 20, 2017.

Accepted July 17, 2017.

¹Corresponding author: walkyoto@unicamp.br

and most of them are performed on simple systems such as water (Jager and Heyns, 1998; Rulliere et al., 2012). No studies were found that evaluate the effect of pH on the rate of hydrolysis of polyphosphates in dairy products, such as milk and processed cheeses. Also, the change in the phosphate chain length can shift the texture and microstructure of processed cheese. Understanding the hydrolysis process of polyphosphates is necessary for the development of emulsifying salts that meet the quality and functionality requirements of processed cheeses. The objective of this study was to investigate the effect of different pH (5.2, 5.6, 6.0, 6.4, and 6.8) on the hydrolysis of a commercial polyphosphate salt in water, milk, calcium caseinate, and a model processed cheese, and evaluate the effect of pH on the texture and structure of spreadable processed cheese.

MATERIALS AND METHODS

Materials

Sodium monophosphate, disodium diphosphate, tetrasodium diphosphate, and pentasodium tripolyphosphate were purchased from Sigma Chemical (St. Louis, MO), and commercial sodium polyphosphate was obtained from Budenheim (Columbus, OH). The ingredients used were skim milk powder (moisture $90.07 \pm 0.04\%$, fat $0.4 \pm 0.36\%$, protein $3.24 \pm 0.14\%$, calcium 148.81 ± 9.87 mg/100 g; Nestlé, São Paulo, Brazil), calcium caseinate (moisture $5.7 \pm 0.1\%$, fat $4.2 \pm 0.4\%$, protein $86.8 \pm 0.6\%$) (ALIBRA, Campinas, Brazil), and butter oil (fat $99.66 \pm 0.01\%$, moisture $0.26 \pm 0.03\%$; NZMP, Fonterra Cooperative Group Ltd., Auckland, New Zealand). Lactic acid (85%) was purchased from ECIBRA (São Paulo, Brazil) and NaOH $0.1 \text{ mol}\cdot\text{L}^{-1}$ from Dinâmica (São Paulo, Brazil).

Effect of pH on the Hydrolysis of Polyphosphate in Different Matrices

Preparation of Standard Solutions. Solutions of sodium monophosphate, disodium diphosphate, tetrasodium diphosphate, and pentasodium tripolyphosphate were prepared by dissolving each salt in distilled water to a final concentration of 10% (wt/vol).

Preparation of Samples in Water and Milk Matrices. In all samples, the concentration of the commercial polyphosphate salt added was 2.3% (wt/vol), and the pH of each sample was adjusted to the following values: 5.2, 5.6, 6.0, 6.4, and 6.8 using 85% lactic acid. The pH measurements were performed in pHmeter Digimed DM22 (Digicron Analítica Ltd., São Paulo, Brazil). Aqueous solutions were prepared by dissolving

commercial polyphosphate salt in distilled water. Milk solutions were prepared by dissolving skim milk powder 10% (wt/vol) in distilled water. The solutions were kept under constant stirring at 20°C until complete salt dissolution and pH adjustment. The calcium caseinate solutions were prepared by mixing the calcium caseinate in distilled water to a final protein concentration of 10.5%. The homogenization was made with the aid of a Mondial 500 W mixer (Manaus, Brazil). After homogenization, the pH was adjusted and the calcium caseinate mixture was allowed to cool (7°C) for 8 h, for complete hydration of the protein. An IKA LR100 reactor (IKA, Staufen, Germany), which has controlled temperature and speed settings, was used to stir the calcium caseinate gel with the polyphosphate salt at 3,000 rpm. The reactor temperature was adjusted to 25°C ; the commercial salt was then added and the mixture homogenized at 5,000 rpm for 3 to 5 min.

Model spreadable processed cheese was formulated according to Cunha and Viotto (2010), using an IKA LR 100 (IKA). Distilled water, lactic acid (amount predetermined to reach the desired pH), and calcium caseinate (10.5%) were stirred with a mixer until obtaining a homogeneous mixture. The mixture was left overnight in a refrigerator for complete hydration of the calcium caseinate. After hydration, the mixture was transferred to the automated reactor. The polyphosphate blend was added to the mixture (25°C), previously diluted in water, and stirred at 5,000 rpm for 5 min. After that, the temperature was raised to 70°C , butter oil was added, and the mixture stirred for 2 min at 15,000 rpm. The temperature was then increased to 90°C for 3 min, with constant stirring at 10,000 rpm. Finally, the product was hot packed in 200-mL plastic cups previously sanitized with 200 ppm sodium hypochlorite solution and sealed with equally sanitized aluminum caps. After cooling in an ice bath, the product was stored at 10°C . The procedure was performed in duplicate, resulting in 10 experiments.

All samples were, immediately after preparation, transferred to glass NMR tubes with 5 mm diameter and placed in liquid nitrogen to avoid any polyphosphate degradation until the analysis.

Determination of Polyphosphate Hydrolysis by ^{31}P NMR. The NMR experiments were carried out on a Bruker Avance III spectrometer (Karlsruhe, Germany) operating at the frequency of 202.35 MHz for the ^{31}P nuclei. The chemical shift and multiplicity of the signals in the ^{31}P NMR spectrum are influenced by the pH and number of phosphate groups of the polyphosphates (Li et al., 2001). In the milk matrices, the ionic interference causes a signal amplification and chemical shifting, which is why we used pure phosphate spectra as a reference for signal assignment.

The 1-dimensional NMR spectra of ^{31}P NMR with ^1H decoupling were obtained with a spectral width of 21,306.8 Hz and acquisition time of 0.3845 s. The recycle delay between each transient was 60 s, which was essential to achieve the quantitative condition necessary to determine the relative amounts of the polyphosphates with a different number of phosphate units in the commercial blend. For samples of the polyphosphate salt in milk, calcium caseinate solution, and processed cheese, an exponential function was used to multiply the free induction decay with a line width (lb) of 20 Hz. In all cases, the reference frequency was defined as the peak corresponding to the monophosphate, around 1 ppm, depending on pH and temperature. All spectra were processed using TOPSPIN 3.1 software (Bruker). Capillaries containing D_2O were used to deuterium lock.

Solutions of the polyphosphate in water, milk, and caseinate at different pH were subjected to the same conditions of time and temperature that occur during the manufacture of processed cheese. The samples were placed on the spectrometer at 25°C , heated to 90°C for 3 min, and then cooled to 10°C . The ^{31}P NMR spectra were obtained at the beginning (25°C) and the end (10°C) of the process. The total process time was 90 min. Processed cheeses samples were taken at the beginning and end of the process [i.e., immediately after addition of the polyphosphate (25°C) and after 10 d refrigerated storage (10°C) for ^{31}P NMR analysis]. The hydrolysis rate was considered as the increase in the percentage of the monophosphate at the end of each process. The transfer of protons is facilitated by the presence of 4 OH groups in the terminal phosphate groups, rather than 2, in nonterminal phosphate units (Jager and Heyns, 1998). Thus, the hydrolysis of polyphosphates occurs mainly by the breakdown of the $(\text{PO}_4)_3$ terminal groups, with the formation of monophosphates as a final product.

The integration and normalization of the signal areas in the ^{31}P NMR spectrum allowed the determination of the polyphosphate composition. The relative percentage of each phosphate species (monophosphate, diphosphate, tripolyphosphate, and polyphosphate) was expressed as the ratio between the integration area of the peak of each component and the total area of the species ^{31}P NMR spectra.

Effect of pH on the Structure of Spreadable Processed Cheese

Physical-Chemical Characterization of Processed Cheeses. Samples were evaluated, in triplicate, after 10 d of refrigerated storage, in relation to the following parameters: pH by the potentiometric method (Digimed DM22 pH meter, Digicron Analytical Ltd.,

São Paulo, Brazil); moisture, total nitrogen, and NPN, according to AOAC procedure (AOAC International, 2006). Total protein was obtained by multiplying the total nitrogen content by the conversion factor 6.38. For determination of the fat content, the Gerber method, AOAC 2000.18 (AOAC International, 2006), was used and total calcium according to Rand et al. (1975).

Texture Profile Analysis. The texture profile analysis of the processed cheeses was determined using a universal TAXT2 texturometer (Stable Micro Systems Ltd., Surrey, UK), equipped with a 5.0-kg load cell. The tests were carried out according to the methodology described by Cunha and Viotto (2010). The samples were maintained overnight in a refrigerator and analyzed at 10°C . The analyses of the spreadable processed cheese were performed on its own packaging to avoid any changes in the product structure. Force as a function of time was obtained for the 2 compression–decompression cycles and the parameters obtained were gumminess, adhesiveness, and hardness. All determinations were carried out in quadruplicate at 10 d of refrigerated storage.

Cheese Microstructure. The microstructure of the processed cheese was evaluated by scanning electron microscopy. The preparation of the samples followed the procedure described by Marchesseau et al. (1997), adapted by Gallina (2005). Samples were dried in a CPD 030 critical dryer (Baltec AG, Balzers, Liechtenstein). They were then fractured at room temperature, mounted on specific aluminum cylindrical supports with silver glue, and metallized with gold 80% and 20% palladium using metallizer SCD 050 (Baltec AG). The observation was taken in a scanning electron microscope (QUANTA, Thermo Fisher Scientific, Hillsboro, OR) at 5 kV. Samples were evaluated at 10 d of refrigerated storage.

Experimental Design and Statistical Analysis

A randomized complete block experimental design was adopted. The factor studied was the pH at levels 5.2, 5.6, 6.0, 6.4, and 6.8. The results were evaluated using the ANOVA, using the Tukey test to compare the means ($P < 0.05$). The analyses were performed using the program Statistica 8.0 (Statsoft Inc., Tulsa, OK).

RESULTS AND DISCUSSION

Hydrolysis of Polyphosphate in Water, Milk, Calcium Caseinate, and Processed Cheese

^{31}P NMR Measurements in Aqueous and Dairy Matrices. Polyphosphate spectrum in water presented several signals, identified in 3 distinct regions

of chemical shift: (1) around 1 ppm (a), (2) between -5 and -9 ppm (c and b) and (3), between -21 ppm and -24 ppm (d; Figure 1). The singlet at 1.44 ppm (^3P) corresponds to the anion monophosphate (PO_4^{3-}). Centered at -7.44 ppm appears a doublet that corresponds to the phosphorus atoms of terminal positions of the long-chain polyphosphates ($P > 3$), identified as ^cP . According to Turner et al. (2003), the phosphorus atoms in the terminal positions resonate between -4 and -10 ppm as a function of the pH of the sample. The singlet at -7.73 ppm (^bP) corresponds to the 2 phosphorus atoms of the diphosphate ($\text{P}_2\text{O}_7^{4-}$), due to its conformation and symmetrical structure.

The spectrum region between -21 and -24 ppm exhibits 6 overlapping signals (multiplet) at -21.45, -21.65, -22.44, -22.92, -23.69, and -23.88 ppm corresponding to the internal phosphorus atoms (^dP) of

the long-chain polyphosphates, as shown by Liu et al. (2015). The sum of the doublet area, corresponding to the terminal phosphorus groups, and the multiplet corresponding to the internal phosphorus units, was used to represent the percentage of long-chain polyphosphates ($P > 3$). Thus, the commercial emulsifying salt composition was 24.50% of monophosphates, 23.52% of diphosphates, and 51.98% of long-chain polyphosphates ($P > 3$). The ^{31}P NMR spectrum of the polyphosphates in milk matrices was similar to that found in the aqueous solutions, except for the signal corresponding to monophosphate (Figure 2). All milk matrices (milk, calcium caseinate, and processed cheese) have, in their composition, phosphorus residues that appear in the spectrum in the region corresponding to monophosphates, around 1 ppm (values taken into account when integrating spectral signals). So, when polyphosphate

Figure 1. ^{31}P Nuclear magnetic resonance spectra of commercial polyphosphate salt (2.3%) in water at pH 6.8 and 25°C. a - d Phosphorus atoms that appear in spectra.

Figure 2. ^{31}P Nuclear magnetic resonance spectra of polyphosphate salt (2.3%) in water (a), milk (b), calcium caseinate (c), and processed cheese (d) at pH 6.8 and 25°C.

was added to the water, milk, and calcium caseinate, any variations in the monophosphate ratio are due to the hydrolysis of the added polyphosphate salt.

Effect of pH on Polyphosphate Hydrolysis. The pH and different matrices influenced the hydrolysis of polyphosphates. At low pH (5.2 and 5.6), monophosphate formation was more pronounced than at pH close to neutrality (6.0 to 6.8; Figure 3). In water, the polyphosphate was quite stable at neutral pH. As the pH decreased, a small increase occurred in the rate of hydrolysis, reaching a maximum of 7.59% at pH 5.2 (Figure 3). Other studies (Hrynczyszyn et al., 2010) also found similar results, showing increased hydrolysis with decreasing pH.

For the polyphosphate to be hydrolyzed, it must be susceptible to the nucleophilic attack of water. The decrease in pH causes protonation of the terminal phosphate group on the polyphosphate chains. At low pH solutions, protonation of the double-bond oxygen activates the polyphosphate for the nucleophilic attack of water. The protonation pathway, which activates the terminal polyphosphates units, has lower activation energy ($57 \text{ kJ}\cdot\text{mol}^{-1}$) compared with the nonprotonation pathway ($104 \text{ kJ}\cdot\text{mol}^{-1}$; Jager and Heyns, 1998), that is, in neutral solutions. As the activation energy decreased, the percentage of hydrolysis increased.

In milk, the hydrolysis followed the same tendency of the water, increasing the hydrolytic degradation with

the reduction of the pH. However, rates were much higher, with a maximum of 17.02% at pH 5.2 (Figure 3). The higher rate of hydrolysis in milk is due to the catalytic effect of metal ions, such as calcium (on average 119 mg of calcium/100 g of milk). It is due to the chelating effect of this cation on the oxygen atoms, which causes a change in the conformation of the polyphosphate chain. The conformational change leads to a greater susceptibility of the phosphorus atom to the nucleophilic attack of water (Molins, 1991). Ruliere et al. (2012) also observed increased hydrolysis of short- and long-chain polyphosphates in solutions with calcium when compared with aqueous solutions at 100°C.

The calcium caseinate hydrolysis behavior was different. There was an increase in hydrolysis with a decrease in pH, but only up to pH 5.6 (from 1.79% at pH 6.8 to 14.91% at pH 5.6). At pH 5.2, the hydrolysis decrease to 8.19% (Figure 3). It may be related to the lower mobility of phosphate in a more viscous system than water and milk. According to Jager and Heyns (1998), the rate of hydrolysis does not depend only on the activation energy. Another factor that interferes with the hydrolysis of the polyphosphates involves the collision between the different particles, that is, the polyphosphate, the H^+ and Ca^{2+} ions, and the water molecules. In aqueous solutions, the molecules have free movement and the chance of collision between the particles increases.

In concentrated solutions of proteins, like calcium caseinate solution (10.5% in water), the molecules have more difficulty of movement, and therefore, the chance of collision between particles decreases. It explains the lower hydrolysis rate of polyphosphate in calcium caseinate when compared with that in milk. The pH 5.2, also, is closer to the isoelectric point of casein (pH 4.6), which means a decrease of the electrostatic repulsion of the protein chains, making the network more compact (Marchesseau et al., 1997). More dense protein network hinders the diffusion and resulting collision of the polyphosphates with the water molecules, which may have caused a decrease in the hydrolysis rate in this system because there was also no stirring during the ^{31}P NMR analysis.

In the processed cheese, the ^{31}P NMR spectrum of the final product revealed the presence of monophosphates (from 0 to 2 ppm) and long-chain polyphosphates ($P > 3$), between -21 and -24 ppm (Figure 2c). The hydrolysis occurred more strongly in the more acidic pH (pH 5.2 and 5.6) than in pH closer to neutrality (6.0 to 6.8).

Hydrolysis in processed cheese was higher than in calcium caseinate, probably due to the vigorous stirring that occurs during the manufacture of the processed

cheese. The agitation favors the collision between the particles of the environment (i.e., the polyphosphates and the H^+ and Ca^{2+} ions), increasing the rate of hydrolysis of the polyphosphates.

Similar to calcium caseinate, the hydrolysis in processed cheese at pH 5.6 ($19.90\% \pm 0.19$) was higher than at pH 5.2 ($15.30\% \pm 0.51$). Again, this fact can be explained by the more dense protein network at pH 5.2 and slower diffusion of the particles. In the range of pH 6.0 to 6.8, the percentage of hydrolysis was low and remained almost constant (around 4%).

The results showed the effect of pH decrease on the hydrolysis of polyphosphate in milk matrices, with changes in salt composition and formation of short-chain phosphates. Processed cheeses structure depends on the composition of the emulsifying salts, and the hydrolysis during processing may explain the differences in structure and functionality of the final product.

Effect of pH on the Structure of Processed Cheese

Table 1 shows the composition of processed cheese at different pH values. As expected, the use of anhydrous ingredients resulted in a constant composition, with

Figure 3. Hydrolysis of the commercial polyphosphate salt in water and dairy matrices at different pH values.

Table 1. Effect of initial pH on composition of processed cheese ($n = 2$)¹

Item	Initial pH				
	5.2	5.6	6.0	6.4	6.8
Moisture (%)	62.20 ± 0.47 ^a	64.99 ± 1.4 ^a	63.14 ± 0.1 ^a	61.20 ± 0.70 ^a	62.10 ± 0.10 ^a
Fat (%)	22.25 ± 0.17 ^a	21.75 ± 1.06 ^a	23.25 ± 0.17 ^a	23.00 ± 0.35 ^a	22.00 ± 1.06 ^a
Fat in DM (%)	59.11 ± 0.93 ^a	62.40 ± 0.03 ^a	62.52 ± 0.32 ^a	62.07 ± 0.76 ^a	60.20 ± 0.58 ^a
Protein (%)	10.89 ± 0.10 ^a	10.58 ± 0.53 ^a	10.92 ± 0.12 ^a	10.96 ± 0.10 ^a	10.96 ± 0.29 ^a
NPN (%)	0.02 ± 0.00 ^a	0.02 ± 0.00 ^a	0.02 ± 0.00 ^a	0.02 ± 0.00 ^a	0.02 ± 0.00 ^a
Calcium (mg/100 g)	197.91 ± 1.29 ^a	192.77 ± 1.99 ^a	196.96 ± 2.78 ^a	192.69 ± 1.84 ^a	194.32 ± 2.08 ^a
pH	5.19 ± 0.01 ^a	5.59 ± 0.02 ^b	5.75 ± 0.02 ^c	6.08 ± 0.01 ^d	6.22 ± 0.00 ^e

^{a-e}Means within a row with different superscripts differ ($P < 0.05$).

¹Means ± SD.

differences only in the final pH of the cheese. The pH of the cheeses adjusted to 6.0, 6.4, and 6.8 decreased after 10 d of refrigerated storage. However, in cheeses at initial pH of 5.2 and 5.6, the final pH remained practically constant. The higher rate of hydrolysis at low pH (pH 5.2 and 5.6) increased the percentage of monophosphates and potent buffering agents, maintaining constant the pH values of the cheeses.

The decrease in pH increased the hardness, adhesiveness, and gumminess of the cheese ($P < 0.05$). The decrease in pH values decreased the electrostatic repulsion between caseins, increasing the protein-protein interactions, which resulted in a more compact structure with formation of large agglomerates, as shown by the microstructure (Figure 4). Cheeses produced at lower pH (pH 5.2) exhibited a harder and more brittle appearance when compared with samples made at higher pH values (pH 6.0 to 6.8), which presented a more fluid texture, resembling a very viscous liquid.

At higher pH values (above 6.0), the processed cheese presented a behavior of a concentrated protein emulsion, with a “long texture” and less adhesive than cheeses made with lower pH. At pH 6.4, Figure 4 shows cheese with a continuous, homogeneous, and more fluid network, with the fat globules homogeneously distributed in the protein matrix. The increase of the negative charges of the caseins allows the formation of different types of interactions, such as hydrogen bonds and electrostatic interactions. The presence of these

bonds enhances the absorption and binding of water by the protein matrix. The microstructure of the cheese processed at pH 6.0 was very similar to pH 6.4 (results not shown).

Hydrolysis may also explain the lower hardness found in cheeses with higher pH [i.e., pH of 6.0 to 6.8 (Table 2)]. In this pH range, there was a lower rate of hydrolysis, that is, a greater proportion of long polyphosphate chains ($P > 3$). The long polyphosphate chain added multiple negative charges to caseins, which probably led to a lower intensity of the hydrophobic interactions between the dispersed chains and thus decreased the hardness of the matrix. Moreover, the absence of or a small ratio of tetrasodium diphosphate and disodium diphosphate was observed in the ³¹P NMR spectrum of the samples (Figure 2c). According to Mizuno and Lucy (2007), there should be an optimum concentration of diphosphates for gel formation. The authors claim that excessive or insufficient amounts of diphosphates lead to the formation of fragile gels.

CONCLUSIONS

The hydrolysis of the sodium polyphosphate increased with the decrease in pH. The presence of calcium in milk matrices increased the rate of hydrolysis. In highly viscous systems, such as calcium caseinate and processed cheese, the hydrolysis decreased due to the low mobility of the molecules. Small variations in

Table 2. Effect of initial pH on processed cheese texture profile ($n = 4$)¹

Item	pH				
	5.2	5.6	6.0	6.4	6.8
Hardness (N)	10.82 ± 0.34 ^a	4.01 ± 0.44 ^b	2.15 ± 0.32 ^c	0.85 ± 0.12 ^d	1.13 ± 0.38 ^d
Gumminess (–)	5.49 ± 0.54 ^a	2.58 ± 0.23 ^b	1.48 ± 0.41 ^c	0.57 ± 0.16 ^d	0.81 ± 0.23 ^d
Adhesiveness (N-s)	28.06 ± 4.30 ^a	9.70 ± 1.63 ^b	4.36 ± 0.60 ^c	4.17 ± 0.69 ^c	2.59 ± 0.84 ^c

^{a-d}Means within a row with different superscripts differ ($P < 0.05$).

¹Means ± SD.

Figure 4. Scanning electron micrographs of processed cheese manufactured with different pH values (5.2, 5.6, 6.4, and 6.8). Void spaces (white arrows) indicate the presence of fat particles in the processed cheese. Bar = 3 µm.

pH affected the structure of processed cheeses. Cheeses manufactured at pH 5.2 and 5.6 presented a firm and brittle texture. At more neutral pH (6.0 to 6.8), the cheeses were soft and presented a more fluid and continuous structure.

ACKNOWLEDGMENTS

The São Paulo Research Foundation (FAPESP, São Paulo, Brazil), grant 14/07291–3, and National Council for the Improvement of Higher Education (CAPES, Brasília, Brazil) provided financial support.

REFERENCES

- AOAC International. 2006. Official Methods of Analysis. 18th ed. AOAC International, Arlington, VA.
- Cunha, C. R., R. Grimaldi, M. R. Alcântara, and W. H. Viotto. 2013. Effect of the type of fat on rheology, functional properties and sensory acceptance of spreadable cheese analogue. *Int. J. Dairy Technol.* 66:54–62. <https://doi.org/10.1111/j.1471-0307.2012.00876.x>.
- Cunha, C. R., and W. H. Viotto. 2010. Casein peptization, functional properties, and sensory acceptance of processed cheese spread made with different emulsifying salts. *J. Food Sci.* 75:C113–C120. <https://doi.org/10.1111/j.1750-3841.2009.01444.x>.
- Gallina, D. A. 2005. Influence of UHT processing on the quality of full fat and reduced fat requeijão cremoso. PhD Thesis. Faculty of Food Engineering, State University of Campinas, Campinas, Brazil.
- Guinee, T. P., M. Caric, and M. Kaláb. 2004. Pasteurized processed cheese and substitute/imitation cheese products. Pages 349–394 in *Cheese: Chemistry, Physics, and Microbiology*. Vol. 2. P. F. Fox, P. L. H. McSweeney, T. M. Cogan, and T. P. Guinee, ed. Elsevier, London, UK.
- Hrynyszyn, P., A. Jastrzebska, and E. Szlyk. 2010. Determination of phosphate compounds in meat products by ³¹-Phosphorus Nuclear Magnetic Resonance spectroscopy with methylenediphosphonic acid after alkaline extraction. *Anal. Chim. Acta* 673:73–78. <https://doi.org/10.1016/j.aca.2010.05.018>.

- Jager, H., and A. M. Heyns. 1998. Kinetics of acid-catalyzed hydrolysis of a polyphosphate in water. *J. Phys. Chem. A* 102:2838–2841. <https://doi.org/10.1021/jp9730252>.
- Li, R. R., W. L. Kerr, R. T. Toledo, and Q. Teng. 2001. ^{31}P NMR analysis of chicken breast meat vacuum tumbled with NaCl and various phosphates. *J. Sci. Food Agric.* 81:576–582. <https://doi.org/10.1002/jsfa.846>.
- Liu, W., M. Xu, Y. Zhang, F. Wang, T. Hui, B. Cui, X. Guo, and Z. Peng. 2015. Mechanism of polyphosphates hydrolysis by purified polyphosphatases from the dorsal muscle of silver carp (*Hypophthalmichthys molitrix*) as detected by ^{31}P NMR. *J. Food Sci.* 80:2413–2419. <https://doi.org/10.1111/1750-3841.13080>.
- Marchesseau, S., E. Gastaldi, A. Lagaude, and J. L. Cuq. 1997. Influence of pH on protein interactions and microstructure of processed cheese. *J. Dairy Sci.* 80:1483–1489. [https://doi.org/10.3168/jds.S0022-0302\(97\)76076-4](https://doi.org/10.3168/jds.S0022-0302(97)76076-4).
- McBeath, T. M., E. Lombi, M. J. McLaughlin, and E. K. Bumann. 2007. Polyphosphate-fertilizer solution stability with time, temperature, and pH. *J. Plant Nutr. Soil Sci.* 170:387–391. <https://doi.org/10.1002/jpln.200625166>.
- Mizuno, R., and J. A. Lucey. 2005. Effects of emulsifying salts on the turbidity and calcium-phosphate-protein interactions in casein micelles. *J. Dairy Sci.* 88:3070–3078. [https://doi.org/10.3168/jds.S0022-0302\(05\)72988-X](https://doi.org/10.3168/jds.S0022-0302(05)72988-X).
- Mizuno, R., and J. A. Lucey. 2007. Properties of milk protein gels formed by phosphates. *J. Dairy Sci.* 90:4524–4531. <https://doi.org/10.3168/jds.2007-0229>.
- Molins, R. A. 1991. The chemistry of phosphates in foods. Pages 7–38 in *Phosphates in Food*. CRC Press, Boca Raton, FL.
- Rand, M. C., A. E. Greensberg, and M. J. Taras. 1975. Standard methods for the examination of water and wastewater. American Public Health Association, Washington, DC.
- Rulliere, C., L. Perenes, D. Senocq, A. Dodi, and S. Marchesseau. 2012. Heat treatment effect on polyphosphate chain length in aqueous and calcium solutions. *Food Chem.* 134:712–716. <https://doi.org/10.1016/j.foodchem.2012.02.164>.
- Rulliere, C., C. Rondeau-Mouro, S. Raouch, M. Dufrechou, and S. Marchesseau. 2013. Studies of polyphosphate composition and their interaction with dairy matrices by ion chromatography and ^{31}P NMR spectroscopy. *Int. Dairy J.* 28:102–108. <https://doi.org/10.1016/j.idairyj.2012.09.005>.
- Schär, W., and J. O. Bosset. 2002. Chemical and physico-chemical changes in processed cheese and ready-made fondue during storage. A review. *J. Food Sci. Technol.* 35:15–20. <https://doi.org/10.1006/fstl.2001.0820>.
- Torres-Dorante, L. O., N. Claassen, B. Steingrobe, and H. W. Olf. 2005. Hydrolysis rates of inorganic polyphosphates in aqueous solution as well as in soils and effects on P availability. *J. Plant Nutr. Soil Sci.* 168:352–358. <https://doi.org/10.1002/jpln.200420494>.
- Turner, B. L., N. Mahieu, and L. M. Codron. 2003. Phosphorus-31 nuclear magnetic resonance spectral assignments of phosphorus compounds in soil NaOH-EDTA extracts. *Soil Sci. Soc. Am. J.* 67:497–510.