Impact of sodium orthophosphate, sodium pyrophosphate or sodium citrate addition via dry-salting on the properties of low-moisture part skim mozzarella

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Abstract

Low-moisture part-skim mozzarella was dry-salted with sodium orthophosphate, sodium pyrophosphate or sodium citrate to examine the impact on its functional properties after 14 d of storage at 4 °C. Relative to the control, dry-salting the cheese with sodium pyrophosphate impaired its flow and extensibility after heating, whereas those dry-salted with sodium orthophosphate or sodium citrate had greater flow and required less work to extend after heating. Elevated levels of insoluble Ca and P were found in all cheeses except for the cheese with sodium citrate which had lower insoluble P. The findings suggested that the addition of sodium pyrophosphate enhanced the interactions between Ca, P and para-casein possibly by formation of Ca-caseinate phosphate complexes, whereas the effects of sodium orthophosphate may be explained by displacement of colloidal Ca-P and subsequent re-association to the casein micelle, and those of sodium citrate by solubilization of colloidal Ca-P and formation of insoluble Ca-citrate complexes.

1. Introduction

Low-moisture part-skim (LMPS) mozzarella is a firm to semi-hard, unripened cheese characterized by a long stranded parallel-orientated fibrous protein structure (CXS 262-2006; European Commission, 2017). The orientation of the protein fibers is obtained after kneading and stretching curd of a suitable pH in hot water, dilute brine, or steam until a smooth and lump-free consistency is reached (CXS 262-2006; Fox, Guinee, Cogan, & McSweeney, 2017; Kindstedt, Carić, & Milanović, 2004; McMahon & Oberg, 2017). The cheese has excellent shreddability, i.e., the shredded cheese does not mat together or form clumps, shows moderate flow on heating, and has the capability to form strings on extending the molten cheese, which makes the cheese highly desirable in the global pizza market (Everett & Auty, 2008; Guinee, Harrington, Corcoran, Mulholland, & Mullins, 2000a; Kindstedt, 1999, 2004; Yang, Watkinson, Gillies, & James, 2016). Previous research investigated the variations in the functional quality of LMPS mozzarella (extensibility or flow of the heated cheese) (To et al., 2020, 2022), and corroborated the findings of existing research showing that reducing insoluble Ca, i.e., the number of Ca-P nanoclusters cross-linking the casein, enhances the functional properties of the cheese (Guinee, Feeney, Auty, & Fox, 2002; Joshi, Muthukumarappan, & Dave, 2004; Kern, Weiss, & Hinrichs, 2018; Kindstedt et al., 2004; Metzger, Barbano, & Kindstedt, 2001). Various methods to control the level of insoluble Ca in cheese have been identified, and include pH alteration of the milk at renneting, lowering scald temperature, and/or reducing the pH at whey drainage (Fox et al., 2017; Johnson & Lucey, 2006; McMahon & Oberg, 2017). In commercial practice, lowering the pH at milk renneting is frequently encountered as it enables the manufacturer to cut back on the overall manufacturing costs, i.e., less coagulant is required to coagulate the milk (Montilla, Calvo, & Olano, 1995; St-Gelais, Champagne, & Bélanger, 1997), and curd fermentation time can be shortened or eliminated (McMahon & Oberg, 2017). However, the concomitant displacement of soluble caseins

and minerals to the serum phase has a detrimental impact on the processing of whey (Chandrapala et al., 2015; Jeurnink & Brinkman, 1994), e.g., increased fouling of the membranes during whey filtration, scaling of the evaporator walls during whey concentration, and hence, limits the use of milk acidification as a tool to adjust cheese functionality.

Alternatively, commercial manufacturers may consider salting the curd with Ca-sequestrants to alter the amount of colloidal Ca-P cross-links in LMPS mozzarella. The potential use of Ca-sequestrants (sodium phosphates, sodium pyrophosphates or sodium citrates) has been outlined since several decades in a considerable number of industrial patents for the manufacture of LMPS mozzarella or pizza cheese (Barz & Durkin, 1996; Barz et al., 1999; Dahlstrom, Wiegand, & Aimutis, 2001; Rizvi, Shukla, & Srikiatden, 1999). However, few studies have reported on the effects thereof. One particular publication investigated the effects of tetrasodium pyrophosphate or trisodium citrate added to the milled curd (~42 °C) of nonfat *Pasta Filata* cheese made from milk acidified to pH 5.8 using lactic acid, and stored for 1 or 2 d at 4 °C (Mizuno & Lucey, 2005b). A second paper examined the addition of sodium phosphate or sodium citrate to the milk during the manufacturing of LMPS mozzarella but did not successfully make cheeses with normalized gross composition (Cheng, Augustin, McKinnon, & Sutherland, 1997). The majority of the research concerning the use of Ca-sequestrants in cheese, is, moreover, largely applicable to processed cheese of which the manufacturing differs strongly from that of LMPS mozzarella (Carić, Gantar, & Kaláb, 1985; Chen & Liu, 2012; Kapoor & Metzger, 2008; Mekmene & Gaucheron, 2011; Shirashoji, Aoyagi, Jaeggi, & Lucey, 2016). Processed cheese involves the blending of one or more varieties of natural cheese combined with a mixture of one or multiple Ca-sequestrants at temperatures of at least 66 °C for at least 30 s into a homogeneous plastic mass, and subsequent hot-filling of the cheese (CXS A-8(b)-1978; Fox & Guinee, 2017; 21 C.F.R. § 133.169, 2022). Expanding the existing knowledge on the influence of Ca-sequestrants on the functional properties

of LMPS mozzarella made using a cultured cheese-making process is therefore of interest. These cheeses are made by inoculating the milk with a starter culture, fermenting the curd to pH 5.05 to 5.20, salting the curd after plasticization, and holding the cheese for at least 2 weeks at 4 °C (Fox et al., 2017; Kindstedt et al., 2004; McMahon & Oberg, 2017).

The objective of the current study is to investigate the effects of dosing Ca-sequestrants (sodium orthophosphate, sodium pyrophosphate or sodium citrate) to the fermented, plasticized curd of LMPS mozzarella on its biochemical and functional properties after 14 d of storage at 4 °C. These Ca-sequestrants are commonly used in the manufacture of processed cheese to attain cheese with specific functional properties (Dimitreli, Thomareis, & Smith, 2005; Guinee & O'Kennedy, 2012). Hence, they can be potentially used to alter the functional quality of LMPS mozzarella. Moreover, by dosing the Ca-sequestrants to the plasticized curd (rather than to the milk or the curd directly after whey drainage), the quality of the whey can be maintained.

2. Materials and methods

2.1. Raw ingredients used for cheese-making

Standardized, pasteurized milk (12.3 % w/w dry matter (DM), 2.8 % w/w fat, 3.8 %, w/w protein) was obtained from Milcobel CV, Langemark, Belgium. Freeze-dried thermophilic starter culture (*Streptococcus thermophilus*; strength: 500 units) and Chymosin (EC 3.4.23.4; 200 IMCU ml^{-1}) were obtained from Chr. Hansen, Hørsholm, Denmark. $CaCl_2$ (33%, w/w) was obtained from Brouwland, Beverlo, Belgium. NaCl was obtained from Zoutman, Roeselare, Belgium. Lactic acid (80%, w/w) was obtained from Corbion, Amsterdam, The Netherlands. Sodium dihydrogen orthophosphate (98% NaH_2PO_4, MFCD00149209) and disodium hydrogen orthophosphate (98% Na_2HPO_4, MFCD00003496) were purchased from Acros Organics, Geel, Belgium. Disodium pyrophosphate (99% $Na_2H_2P_2O_7$; MFCD00014246), tetrasodium pyrophosphate decahydrate (99% $Na_4P_2O_7.10H_2O$; MFCD00149200) and sodium hydrogen citrate sesquihydrate (99% $C_6H_6Na_2O_7.1.5H_2O$; MFCD00150445) were purchased from Sigma-Aldrich, Overijse, Belgium. Trisodium citrate dihydrate (99% $C_6H_5Na_3O_7.2H_2O$; MFCD00150031) was purchased from VWR, Leuven, Belgium.

2.2. Preparing starter culture solutions for cheese-making

One liter of standardized, pasteurized milk was divided into 2 equal portions, placed in a water bath and heated to 35.5 °C. Once the milk acquired the desired temperature, the freeze-dried starter culture (500 units) was removed from the freezer at -80 °C, and the contents were poured into 1 portion of the heated milk using a funnel. The remaining portion was used to rinse the funnel and the empty bag, and was combined into the first milk portion to a final concentration of 500 units L^{-1} milk. The inoculated milk was stirred for 5 min on a stirring plate until a lump-free and

116 homogeneous consistency was obtained. Aliquots of the mixture (1.6 mL) were transferred into 2
117 mL Eppendorf tubes, which were transferred immediately to the freezer at -80 °C.
118
119 *2.3. Cheese production and treatments*
120 Standardized, pasteurized milk was removed from the cold storage (4 °C; pH ~ 6.70), and 10.3 kg
121 milk was poured into a 10 L double-walled cheese vat equipped with a stirrer paddle (10L, Pierre
122 Guerin SAS, Le Mignon, France). The pH was measured using a portable pH meter for food
123 applications (HI98161; Hanna Instruments, Temse, Belgium) equipped with a puncture probe
124 (FC2023; Hanna Instruments, Temse, Belgium). The milk was gradually heated to 35.5 °C in about
125 30 min under continuous agitation (30 RPM) which gradually decreased the pH to about 6.60.
126 When the milk reached 35.5 °C, an Eppendorf tube containing 1.6 mL of starter culture solution
127 (500 units L^{-1} milk) was removed from the freezer and dissolved into the milk (final concentration
128 = 8 units 100 L^{-1} milk).
129 The start of cheese-making (t = 0 min) was chosen as the moment when the Eppendorf tube was
130 completely rinsed with warm milk. $CaCl_2$ and chymosin were dosed at a concentration of 1.03 mM
131 and 0.51 IMCU g^{-1} milk protein at t = 5 or 44 min, respectively, after which the milk was stirred
132 for another 2 min at 20 RPM. At t = 46 min, the agitation was stopped, and the stirrer paddle was
133 replaced with the curd cutters (Pierre Guerin SAS, Le Mignon, France). The gel was set for about
134 37 min, after which the curd was cut at t = 81 min for 4 min at 10 RPM. After cutting, the curd
135 cutters were replaced with the stirrer paddle, and the curd was allowed to heal for 15 min in the
136 absence of any agitation. At t = 100 min, the curd-whey mixture was stirred at 10 RPM for 39 min
137 and gently heated to 39 °C. At t = 139 min, the curd-whey mixture was drained at a pH of about
138 6.30, and the curd grains were held at 39 °C for about 90 min to promote curd dehydration and
139 fermentation to a pH of about 5.20.

140 The fermented curd was cut into particles of roughly 1 x 1 x 6 cm^3 which were submerged into 1.5
141 L hot water (75 °C). The submersion of curd particles into 1.5 L hot water (75 °C) was repeated
142 until the curd temperature reached 55 °C. The increase in curd temperature enabled the particles to
143 fuse, and the curd was stretched manually for 5 min. After stretching, the hot curd (~1140 g) was
144 removed from the stretch water, divided into two equal portions of 550 g, and dry-salted by
145 sprinkling the salt on top of the curd mass while gently kneading. An overview of the different salt
146 treatments and their added amounts is presented in Table 1.
147 The control cheese was obtained by dry-salting 550 g of curd with NaCl to 1.2 %, w/w (CTL). In
148 respective order, the experimental cheeses containing sodium orthophosphate (OP), sodium
149 pyrophosphate (PP) or sodium citrate (CIT) were obtained by dry-salting 550 g of curd with a
150 mixture of sodium orthophosphate (NaH_2PO_4, Na_2HPO_4), a mixture of sodium pyrophosphate
151 ($Na_2H_2P_2O_7$, $Na_4P_2O_7.10H_2O$), or a mixture of sodium citrate ($C_6H_6Na_2O_7.1.5H_2O$,
152 $C_6H_5Na_3O_7.2H_2O$). A preliminary study was conducted in which 0.51, 1.01 or 1.14 g of each salt
153 mixture (without NaCl) was dissolved into 48 g water at 55 °C to mimic the dissolution into 100 g
154 of curd (52 %, w/w DM), and the pH of the salt solution was about 5.20. Hence, the addition of
155 each salt mixture to the curd of LMPS mozzarella after plasticization was not expected to
156 significantly alter curd pH (about 5.20).
157 The PP cheese was salted to about 1.0 %, w/w sodium pyrophosphate which corresponded to about
158 23.5 mmoles of calcium-sequestrant (CS) in 550 g curd (Table 1). To enable a fair comparison
159 between the different treatments, also OP and CIT cheeses (550 g curd) were salted with 23.5
160 mmoles of CS, which corresponded to 0.5 %, w/w sodium orthophosphate and 1.1 %, w/w sodium
161 citrate, respectively. OP, PP and CIT cheeses were furthermore dry-salted with NaCl until the
162 amount of added Na was similar level to that of the CTL cheese (472 mg total Na 100 g^{-1} curd)
163 (Table 1).

After salting, the curd was molded into a block (~ 550 g; 14 cm × 10 cm × 4 cm), and cooled in dilute brine (4 °C; 2.5%, w/w NaCl, 0.06 M $CaCl_2$, pH adjusted to 5.1 using lactic acid) for about 2 h. The brine strength was similar to the salt-in-moisture (S/M) content of the CTL cheese (1.2%, w/w NaCl; 48%, w/w moisture) to minimize diffusion of Na from the cheese to the brine, and vice versa. The concentration of $CaCl_2$ was kept similar to that of an undiluted brine to avoid the occurrence of a soft rind defect (Guerts, Walstra, & Mulder, 1972). After brining, the cheeses were vacuum-packed and stored at 4 °C for 14 d prior to analysis. Each cheese treatment was made in quintuplicate.

2.4. Cheese characterization

2.4.1. Cheese composition

Grated LMPS mozzarella was analyzed for moisture, fat, crude protein and total Cl using standard International Dairy Federation methods (IDF 2004, 2006, 2008, 2014). Moisture was determined per cheese block in triplicate, whereas fat, crude protein, total Cl were determined per cheese block in duplicate.

2.4.2. Total cheese contents of Na, Ca and P

Na, Ca and P Plasma HIQU 10.000 mg L^{-1} stock solutions were purchased from Chemlab-Analytical, Zedelgem, Belgium (CL01.1404.0500; CL01.0643.050, CL01.0643.050). Stock solutions were diluted to 15, 30, 45, 60, and 75 mg L^{-1} in 1 %, w/w HNO_3 for calibration. A dilute nitric acid solution (1 %, w/w HNO_3) and a certified reference skim milk powder for major and trace elements (ERMBD151-20G, Sigma-Aldrich, Overijse, Belgium) were chosen as the negative and positive controls, respectively. A quality control comprising 40 mg L^{-1} analyte was injected every 10^{th} measurement to detect baseline drift.

Grated cheese (0.5 g) was weighed in 16 x 125 mm glass vials (L00079D, Milestone, Sorisole, Italy), and acid digested with 3 mL 65% HNO_3 in a single reaction chamber (UltraWAVE ECR, Milestone, Sorisole, Italy) at 240°C for about 30 min. The liquid digestate was diluted to 100 mL using 1 %, w/w HNO_3, filtered with a 20/25 Chromafil Xtra 0.45 µm PA syringe filter (729213400; Filter-service, Eupen, Belgium), and analyzed for Na, Ca and P by inductively coupled plasma atomic emission spectroscopy (iCAP 7000, Thermo scientific, USA) (ISO 11885). Total Na, Ca and P were determined per cheese block in duplicate, and expressed as mg 100 g^{-1} cheese.

2.4.3. Biochemical properties

pH of the water-soluble cheese extract. Water-soluble cheese extracts were made in duplicate by preparing a mixture of grated cheese (m_{cheese} = 20 g) and distilled water (m_{H2O} = 40 g at 50 °C) in a Stomacher bag (A11048, Novolab, Geraardsbergen, Belgium). The amount of WSE (m_{WSE}) was considered as the sum of the water added to the cheese, and the amount of moisture in the cheese (Eq. 1).

$$m_{WSE} = m_{H20} + m_{cheese} * (100 - DM) \quad \text{Eq. 1}$$

The mixture was macerated using a mortar and pestle, and held for 1 h in a water bath set at 50 °C. Then, the mixture was centrifuged at 3000 *g* for 20 min at room temperature, and the supernatant was filtered through glass wool (Acros Organics, Geel, Belgium) to yield the water-soluble extract (WSE). The pH of the WSE was measured using a benchtop meter (HI5222, Hannah Instruments, Temse, Belgium) and considered as the cheese pH. Preliminary trials compared the pH of the cheese measured via the current approach with that measured via the method described by the British Standards Institution (BSI, 1976), i.e., measuring the pH on a slurry of cheese (20 g) and distilled water (12 g), and found that the former method resulted in slightly higher cheese pH values (0.05 to 0.10 units) for all comparisons.

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212 *2.4.3.1. Water-soluble Na, Ca and P content.* Aliquots (~3 mL) of each WSE were weighed in 16
213 x 125 mm glass vials (L00079D, Milestone, Sorisole, Italy), and acid digested with 3 mL 65%
214 HNO_3 in a single reaction chamber (UltraWAVE ECR, Milestone, Sorisole, Italy) at 240°C for
215 about 30 min. The liquid digestate was diluted to 100 mL using 1% HNO_3, filtered with a 20/25
216 Chromafil Xtra 0.45 µm PA syringe filter (729213400; Filter-service, Eupen, Belgium), and
217 analyzed for Na (Na_{WSE}), Ca (Ca_{WSE}) and P (P_{WSE}) by inductively coupled plasma atomic emission
218 spectroscopy (iCAP 7000, Thermo scientific, USA) (ISO 11885).
219 The water-soluble Na (WSNa), Ca (WSCa) and P (WSP) were determined (Eq. 2-4), and expressed
220 in mg 100 g^{-1} cheese. From these values, the insoluble Ca and P content of the cheese were
221 determined (Eq. 5-6), and expressed in mg g^{-1} protein (with CP = crude protein content of the
222 cheese). Measurements were conducted per cheese block in duplicate.

$$WSNa = Na_{WSE} * m_{WSE} / m_{cheese} * 100 \quad \text{Eq. 2}$$

$$WSCa = Ca_{WSE} * m_{WSE} / m_{cheese} * 100 \quad \text{Eq. 3}$$

$$WSP = P_{WSE} * m_{WSE} / m_{cheese} * 100 \quad \text{Eq. 4}$$

$$\text{Insoluble Ca} = [\text{Total Ca} - \text{WSCa}] / \text{CP} \quad \text{Eq. 5}$$

$$\text{Insoluble P} = [\text{Total P} - \text{WSP}] / \text{CP} \quad \text{Eq. 6}$$

223

224 *2.4.3.2. Water-soluble N and pH 4.6 soluble N content.* An aliquot (~2 mL) of the WSE was
225 analyzed for total N (IDF 2014) to determine the level of water-soluble N in the cheese (WSN),
226 expressed as a percentage of total cheese N. The remaining portion of the WSE was pH-adjusted
227 to 4.6 using 10%, w/w HCl (Honeywell Fluka™ Chemicals, Offenbach, Germany), centrifuged at
228 3000 *g* for 20 min at room temperature, and filtered through glass wool to obtain the pH 4.6 WSE.
229 The water extract was analyzed for total N (IDF 2014) to determine the level of pH 4.6 soluble N

(pH4.6SN). The pH4.6SN, an index of primary proteolysis, was expressed as a percentage of total cheese N. The amount of cheese soluble protein, expressed as percentage of total N, was estimated by subtracting the pH4.6SN from the total WSN content.

2.4.4. Cheese functional properties

2.4.4.1. Surface hardness of the unheated cheese. A cheese block was removed from the cold storage at 4 °C, and placed on the loading cell of an Instron 5942 texture meter (Instron, Boechout, Belgium) fitted with a spherical ball probe (diameter = 25.4 mm) (CNS Farnell, Borehamwood, Hertfordshire, UK). The probe was lowered at a rate of 15 mm s^{-1}, and indented the surface of the cheese block to a depth of 5 mm. The mean peak force taken from 5 equidistant points measured at the top and 5 equidistant points at the bottom surface of the cheese block was determined as a measure for the surface hardness of the unheated cheese.

2.4.4.2. Heat-induced changes in viscoelastic properties of the cheese. A cheese cylinder (diameter = 50 mm, h = 2 mm) was placed on the loading platform of an MCR302 Anton Paar Rheometer (Anton Paar, Graz, Austria) between two parallel cross-hatched plates (PP50/P2; INSET I-PP50/SS/P2; Anton Paar, Graz, Austria), and analyzed using small-strain oscillation rheology. A loading force of 20 N was chosen to set the gap. After setting the gap, the outer exposed surface of the cheese cylinder was lightly brushed with sunflower oil to prevent loss of moisture during the analysis. The sample was equilibrated at 25 °C for 10 min, and heated to 90 °C at a rate of 3.28 °C min^{-1} while subjected to a shear strain (γ) = 0.0063 at constant frequency (f) = 1 Hz. The cross-over temperature (COT), defined as the temperature at which $G' = G''$, and hence, a measure for the melting point of the cheese, and the maximum loss tangent (LT_{max}), a measure for the maximum

fluidity attained during heating to 90 °C, were determined. Each analysis was performed per cheese block in duplicate, and the mean values were determined.

2.4.4.3. Extensibility of the heated cheese. Shredded cheese (50 g) was placed in a microwaveable receptacle containing a comb (Stable Microsystems Ltd., Godalming, UK) and melted at 500 W for 60 s to a temperature of 93 ± 4 °C. The molten cheese mass was allowed to equilibrate at room temperature for 2.5 min during which the cheese temperature decreased to 71 ± 5 °C. The receptacle containing the molten cheese mass was loaded on an A/CE cheese extensibility rig (Stable Microsystems Ltd., Godalming, UK) which was customized to fit an Instron 5942 texture meter (Instron, Boechout, Belgium). Hereafter, the molten cheese mass was uniaxially extended to a height of 340 mm at a rate of 10 mm s^{-1}. From the resultant force-distance curve, the cumulative work required to extend the molten cheese to a height of 340 mm was determined ($EW_{2.5}$; extensional work after 2.5 min equilibration at room temperature). Measurements were conducted per cheese block in triplicate, and the mean values were determined.

2.4.4.4. Flow of the heated cheese. A cheese cylinder (diameter = 45 mm, h = 4 mm) was placed inside a Duroplan borosilicate Petri dish covered with a borosilicate lid (diameter = 100 mm, h = 17 mm; VWR, Overijse, Belgium), and heated for 8 min at 220 °C in a UF160 convection drying oven (Memmert, Mariakerke, Germany). After cooling to room temperature, the flow of the heated cheese was determined as the percentual increase in mean diameter of the heated disc measured along 4 equidistant lines. Measurements were performed per cheese block in quadruplicate, and the mean values were determined.

2.5. Statistical analysis

277 A One-Sample T-test was used to compare the total Na, Cl and P content of each cheese treatment
278 with the theoretical value at $\alpha = 0.05$. One-way ANOVA and Tukey's HSD post-hoc test were used
279 to determine significant differences between mean compositional, biochemical and functional
280 values of the different cheese treatments at $\alpha = 0.05$ throughout. The statistical software package
281 used was JMP 15 (SAS Institute Inc., Cary, NC, United States).

3. Results and discussion

3.1. Gross and mineral composition

All cheeses had a similar DM (52 %, w/w; $P > 0.05$), fat-in-dry matter (FDM) (41 %, w/w; $P > 0.05$) and crude protein content (25 %, w/w; $P > 0.05$), indicating that salting the cheese after curd plasticization, and before molding and cooling in brine, did not significantly alter the gross composition. The DM and FDM of all cheeses conformed to the specification for LMPS mozzarella, as defined by the Code of Federal Regulations (USDA, 2020). By comparison, Mizuno & Lucey (2005b) observed a decrease in the crude protein content in cheese upon salting the curd with a mixture of tetrasodium pyrophosphate and glucono-δ-lactone before plasticization, which most likely was due to enhanced protein solubilization, and hence, increased losses of protein into the stretch water.

The total Cl content differed significantly between the different cheese treatments (Table 2; $P < 0.05$). The CTL had the highest total Cl content, whereas the PP and CIT cheeses had the lowest. This trend reflected the amount of NaCl added to each cheese (Table 1). The total Cl contents of the CTL, OP, PP and CIT cheeses were not significantly different from the added amounts (737, 584, 417 and 423 mg 100 g^{-1} cheese, respectively).

All cheeses had a similar total Na content (Table 2; $P > 0.05$) which reflected the addition of similar amounts of Na to the curd (Table 1). Although the Na content of the CTL, OP, PP and CIT cheeses were numerically lower than the added amounts (472 mg Na 100 g^{-1} curd), only that of the OP cheese was found to be slightly significant ($P = 0.033$).

All cheeses had a similar total Ca content (Table 2; $P > 0.05$), which indicated that there was no loss of solubilized Ca in the manufacturing steps after curd dry-salting (e.g., molding and cooling in weak brine).

The total P content of the CTL cheese in this study was similar to that of the CIT cheese ($P > 0.05$) (Table 2), but was lower than those of OP ($P < 0.05$) or PP ($P < 0.001$) cheeses. The amount of added P in the PP cheese was higher than that permitted by the American and European standards for unripened cheese (~154 mg P 100 g^{-1} cheese, CXS 221-2001; ~87 mg P 100 g^{-1}, European Commission, 2017), but cheeses with a similar total P content have been made before (Mizuno & Lucey, 2005b). The increase in total P in OP (52 mg P 100 g^{-1} cheese) and PP (166 mg P 100 g^{-1} cheese) was, however, significantly less ($P < 0.001$ and $P < 0.01$, respectively) than the theoretical amounts added during salting (133 and 265 mg P 100 g^{-1} cheese, respectively). The P content of the brine used to cool down the cheeses was examined, which showed that only 27 and 16 % of the loss in total P was explained by loss of soluble orthophosphate and pyrophosphate in the brine, respectively (data not included). It was unlikely that the remaining P was lost in the expelled moisture during curd dry-salting and molding as the content of total Na or total Cl in each cheese was not significantly different from their added amounts ($P > 0.05$) (Tables 1 and 2). The brine strength was furthermore too weak to salt the cheeses to the same total Cl content (Table 2), and by extension, to the same total Na content. Combining the findings above, we hypothesize that the remaining P was likely lost in the brine as insoluble Ca-P complexes which could not be sampled. Overall, we succeeded in obtaining cheeses with a similar gross composition, and similar total Na and Ca contents ($P > 0.05$) (Table 2). Equalizing the contents of total Na is necessary to normalize its influence on the protein's water-binding capacity and proteolysis (Guinee, 2004; Guinee, Mulholland, Mullins, & Corcoran, 2000b; Guo, Gilmore, & Kindstedt, 1997; Smith, Hindmarsh, Carr, Golding, & Reid, 2017). Hence, any influence of the treatment in the current study on the functionality of the cheese is likely related to biochemical changes in the calcium-phosphate para-casein matrix (pH, level of insoluble Ca or P, pH4.6SN).

329 *3.2. Biochemical properties*

330 Figure 1 illustrates the mean values for pH, pH4.6SN, insoluble Ca and insoluble P content of each
331 cheese. Overall, the mean values for the CTL after 14 d of storage at 4 °C are in agreement with
332 previous research on LMPS mozzarella (i.e., pH ≈ 5.40 to 5.50; pH4.6SN ≈ 2 to 4 % TN; insoluble
333 Ca ≈ 17 to 20 mg g^{-1} protein) manufactured on pilot-scale (Feeney, Fox, & Guinee, 2001; Metzger
334 et al., 2001; Guinee et al., 2002) or obtained from commercial plants (Guo, Gilmore, & Kindstedt,
335 1995; To et al., 2020; 2022). After 14 d of storage at 4 °C, the pH of the CTL cheese increased
336 from about 5.20 (curd milling) to about 5.50. This increase in pH is commonly found for cheeses
337 manufactured at our facilities, and may be explained by the solubilization of colloidal Ca-P, and
338 the protonation of released phosphate during cooling to and storage at 4 °C (Guinee et al., 2002;
339 Upreti, Bühlmann & Metzger, 2006; van Hooydonk, Hagedoorn & Boerrigter, 1986). An increase
340 in pH as a result of extensive protein hydrolysis, and the release of NH_3, was unlikely in the current
341 study considering the short period of time the cheese was stored at 4 °C, as evidenced by the
342 relatively low value for pH4.6SN (Fig. 1B).

343 Relative to the CTL after 14 d of storage at 4 °C, PP cheese exhibited a lower pH (~0.20 units) (P
344 < 0.001) (Fig. 1A), had 15% less pH4.6SN (P < 0.05) (Fig. 1B), and increased levels of insoluble
345 Ca (P < 0.01) and P (P < 0.01) (Fig. 1C-D). The cause of the relatively low pH in PP cheese was
346 uncertain. Pyrophosphate has been found to associate with casein-bound Ca, and hence, less
347 phosphate may have been released from the para-casein matrix which could explain the weak
348 increase in pH during 14 d of storage at 4 °C (Shirashoji, 2016; Upreti et al., 2006). We examined
349 the level of soluble protein content in the WSE, but found no significant differences between the
350 different treatments (results not shown), which indicated that the lower contents of pH4.6SN in PP
351 cheese was likely caused by impaired casein hydrolysis. This finding was unexpected as variations
352 in solubilization of colloidal Ca were thought to affect soluble protein content in the WSE (Guinee

& O'Kennedy, 2012; Mizuno & Lucey, 2005b). In addition, pyrophosphate has a higher peptization coefficient than orthophosphate or citrate (Dimitreli et al., 2005; Guinee et al., 2017). This discrepancy may be related to the cooking of LMPS mozzarella curd at about 60 °C which inactivates the majority of the residual enzymes, and hence, the cheese has a relatively high amount of intact casein. The hydrophobic interactions between intact caseins combined with hydrogen bonding in natural cheese may explain their limited release from the cheese matrix to the serum phase (Dimitreli et al., 2005; Vilela, Gomes & Fereira, 2020).

Previous research showed that the level of insoluble Ca in the CTL remained relatively constant throughout one month of storage at 4 °C (To et al., 2020), which led us to believe that the increase in insoluble Ca content in PP cheese was likely caused by association/precipitation of serum-soluble Ca to/on the para-casein matrix. In fact, visual inspection of the cheese after 14 d of storage at 4 °C revealed the appearance of a white precipitate throughout the cheese (Fig. 2), confirming the presence of salt precipitation. The increase in insoluble P measured in PP cheese, relative to that of the CTL, however, accounted for 68 % of the P added to PP cheese, whereas 100 % of the total Na content in the cheese was recovered in the water-soluble extract (Supplementary Figure A.1), indicating that the majority of the white precipitate comprised calcium-pyrophosphate complexes.

Although not significant ($P > 0.05$), OP and CIT cheeses also had higher levels of insoluble Ca than the CTL, but lower levels than the PP cheese (Fig. 1C). Relative to the CTL, the numerically lower contents of insoluble P content in CIT cheese (Fig. 1D) combined with the higher levels of insoluble Ca (Fig. 1C), suggested that citrate increased solubilization of colloidal Ca-P, and formed Ca-citrate complexes which interacted/precipitated with/on the casein micelles. Indeed, a closer examination of the CIT cheese after 14 d of storage at 4 °C revealed salt precipitation throughout (Fig. 2), which could explain the increase in insoluble Ca vs. the decrease in insoluble P as observed

in the current study. The deposition of insoluble calcium citrate crystals (Carić et al., 1985; Shirashoji et al., 2016), and of calcium phosphate in processed cheese manufacturing has also been reported after visualization using scanning or transmission electron microscopy (Carić et al., 1985) or visually in processed cheese at a pH of about 6.0 (favorable for calcium phosphate precipitation) made with sodium phosphate (> 1 %) (Brickley et al., 2008). No visual salt precipitation was, however, observed in OP cheeses (Fig. 2), but it is possible that small crystals had formed in the casein micellar network.

The increase in insoluble Ca on addition of sodium citrate in this study (Fig. 1C) was in contrast with Mizuno & Lucey (2005b), who found that the addition of sodium citrate decreased insoluble Ca, and hence, no salt precipitation occurred. The discrepancy is likely due to differences in salting stage and in storage period of the cheese. Mizuno & Lucey (2005b) salted the curd directly after vat drainage, and likely washed out the majority of the salt during curd plasticization, and hence, did not observe salt precipitation after 1 d of storage at 4 °C. In contrast, blooming of small white crystals on the surface of processed cheese may occur during storage (Carić et al., 1985). Injecting a solution of sodium citrate in Cheddar cheese was found to promote curd syneresis, decrease WSCa, and increase WSP, and likely induced precipitation of calcium citrate crystals at the surface of the cheese due to the concentrated environment (Pastorino, Hansen, & McMahon, 2003).

The experimental solubilities of calcium citrate tetrahydrate or calcium citrate hexahydrate in water from 0 to 100 °C was found to range from 3 to 8 mM at pH 6.7, and increased to about 15 mM on reducing the pH to 4.90 at 21 °C (Boulet & Marier, 1959; Vavrusova & Skibsted, 2016), whereas those in milk or milk ultrafiltrate were estimated to range from 6 to 10 mM (Boulet & Marier, 1959; Mekmene, Le Graët, & Gaucheron, 2009). The WSCa content in the aqueous phase of the CTL cheese in this study was 137 mM, whereas the curd of the CIT cheese was salted to about 90 mM citrate (assuming no losses during cheese making). As such, the increase in insoluble Ca in the

current study is likely explained by the precipitation of calcium citrate. Unfortunately, we could not distinguish between the different forms of insoluble Ca, i.e., colloidal Ca-P, casein-bound Ca attached electrostatically to dissociated carboxyl groups of acidic amino acids or to phosphoserine residues, or precipitated insoluble Ca complexes, and hence, it was not possible to quantify the changes in colloidal Ca-P cross-links in the para-casein matrix.

The absence of significant differences in pH or pH4.6SN between CTL, OP and CIT cheeses, however, suggested that the calcium-phosphate para-casein matrix interacted differently with sodium orthophosphate or sodium citrate than with sodium pyrophosphate.

3.3. Functional properties

The surface hardness of PP cheese was significantly higher ($P < 0.01$) than that of the CTL (Table 3), suggesting that the addition of pyrophosphate to the curd tightened the calcium-phosphate para-casein network, and increased its resistance towards mechanical displacement. The tightening of the calcium-phosphate para-casein network was in agreement with the changes in its viscoelastic behavior during heating from 25 to 90 °C (Fig. 3A-B). The LT_{max} of PP cheese was consistently lower than 1.0, i.e., the elastic modulus, *G'*, remained larger than the viscous modulus, *G"*, and hence, the cheese did not melt when heated to 90 °C (COT > 90 °C).

In contrast, cheese containing sodium citrate had a lower surface hardness ($P < 0.05$) (Table 3) and COT ($P < 0.001$) (Fig. 3B) when compared to those of the CTL, suggesting that the calcium-phosphate para-casein network was weakened, and that the heat-induced displacement thereof was facilitated. Relative to the CTL, salting the curd with sodium orthophosphate also reduced the mean numerical value of the surface hardness (Table 3) and COT ($P > 0.05$) (Fig. 3B), but not as strongly as salting the curd with sodium citrate. No significant differences in LT_{max} ($P > 0.05$) (Fig. 3A) between CTL, OP or CIT cheese were found.

425 Although PP cheese did not melt during gradual heating from 25 to 90 °C, melted cheese was
426 obtained when examining the extensibility and flow of the heated cheese, which likely is explained
427 by the more pronounced heating intensity (60 s at 500 W or 8 min at 220 °C, respectively) applied
428 in these analyses. Relative to the CTL, all experimental cheeses had reduced $EW_{2.5}$ values ($P <$
429 0.05) (Fig. 3C) and greater flow ($P < 0.05$) (Fig. 3D). CIT cheeses, in particular, had the lowest
430 $EW_{2.5}$ ($P < 0.001$), and hence, required the least work to stretch the molten cheese 2.5 min after
431 heating, and the greatest flow ($P < 0.001$).
432 Figure 4 shows the visual appearance of each cheese after stretching the molten cheese to a height
433 of 340 mm. It is clear that the addition of CS to the curd of LMPS mozzarella did not alter its ability
434 to stretch, i.e. the microstructure of the cheese still resembled a parallel-aligned fibrous protein
435 structure, typically known for LMPS mozzarella, and enabled the molten cheese to form long,
436 lump-free strands on extension, except for cheese containing sodium pyrophosphate, which had a
437 gooey appearance interspersed with disparate blobs of coalesced casein and fat. In addition, heating
438 the PP cheese at 220 °C for 8 min (Fig. 5) resulted in a highly irregular melt with random patches
439 of unmolten cheese. Hence, a pronounced difference in the visual appearance of heated PP cheese
440 was found when compared to that of OP cheese even though they did not quantitatively differ in
441 terms of $EW_{2.5}$ ($P > 0.05$) or flow ($P > 0.05$) (Fig. 3C-D). To the best of our knowledge, the visual
442 defects of heated cheese containing sodium pyrophosphate have not been reported before; they
443 might be related to the tightening of the calcium-phosphate para-casein network.
444
445 *3.4. Comparison between cheeses manufactured from curd salted with different types of CS.*
446 A clear effect of the type of CS on the biochemical and functional properties of LMPS mozzarella
447 was found. The presence of pyrophosphate resulted in cheese with a relatively low pH (Fig. 1A),
448 and increased insoluble Ca and P contents (Fig. 1C-D). Combined with the tightening of the

449 calcium-phosphate para-casein network, as derived from the impaired casein hydrolysis (Fig. 1B),
450 increases in cheese surface hardness (Table 3), overall non-melting behavior during heating from
451 25 to 90 °C (Fig. 3A-B), and the irregular melt after more intense heat treatments (Fig. 4-5), we
452 hypothesize that pyrophosphate was able to form complexes with Ca which partially precipitated
453 (Fig. 2), and partially formed new cross-links throughout the para-casein matrix. These interactions
454 are likely related to the gooey appearance of PP cheese after melting (Fig. 4), which suggested a
455 greater immobilization and structuring of the fat or serum phase.
456 Indeed, the addition of pyrophosphate to concentrated milk protein solutions at pH 7.0 or 5.8,
457 depending on the salt concentration, increased the level of casein-bound Ca and P_i, augmented
458 protein-protein interactions, shifted the peak buffering capacity to a lower pH, and even induced
459 gel formation, suggesting that the addition of pyrophosphate resulted in the formation of new
460 caseinate-Ca phosphate complexes (Mizuno & Lucey, 2005a, Saricay, Hettiarachchi, Culler, &
461 Harte, 2019). In addition, our findings corroborate those of Mizuno & Lucey (2005b) who
462 examined the addition of 1, 3 or 5%, w/w tetrasodium pyrophosphate to chemically acidified curd
463 (pH 5.8) prior to plasticization, and suggested that newly formed caseinate-Ca phosphate
464 complexes altered protein-protein interactions, and hence, impaired cheese functionality. There
465 was, however, no mention of salt precipitation in the unheated cheese despite dosing the salt at 3
466 or 5 %, w/w, or of visual defects in the heated cheese, which likely reflects the difference in salting
467 stage, whereby the majority of the salt added before curd plasticization was washed away during
468 plasticization and brining. Shirashoji et al. (2016) prepared processed Cheddar cheese containing
469 tetrasodium pyrophosphate (0.25 to 2.75%) with similar pH, and found that increasing the level of
470 pyrophosphate in the cheese increased the insoluble Ca and P content. The authors suggested that
471 insoluble calcium pyrophosphate complexes were formed of which some were likely associated
472 with caseins. However, the flow of the heated cheese was minimal at about 1.0 % pyrophosphate,

473 and increased at a lower or higher salt concentration. This discrepancy was also reported by Saricay
474 et al. (2019) who found an optimal gel strength when concentrated micellar casein solutions
475 contained 10 mM tetrasodium pyrophosphate. It was suggested that higher salt concentrations may
476 weaken the interactions between caseins by excessive charge repulsion between newly formed
477 casein calcium pyrophosphate complexes (Shirashoji et al., 2016; Saricay et al., 2019).
478 Citrate increased the solubilization of colloidal Ca-P (Fig. 1D), and the formed Ca-citrate
479 complexes precipitated on the casein during 14 d of storage at 4 °C (Fig. 1C, Fig. 2). The weakening
480 of the calcium-phosphate para-casein matrix was reflected by the production of the softest cheese
481 (Table 3) with the lowest melting temperature (Fig. 3A). In addition, the molten cheese required
482 the least work to stretch to a height of 340 mm (Fig. 3C), and had the greatest flow (Fig. 3D). It
483 was clear that salting the curd with sodium citrate, instead of sodium pyrophosphate, resulted in
484 nearly opposite effects.
485 Similarly, mixing milk protein concentrate solutions with trisodium citrate improved the
486 functionality of cheeses derived thereof. This finding was associated with citrate-induced
487 solubilization of colloidal Ca-P which lowered the levels of casein-bound Ca and P_i (Mizuno &
488 Lucey, 2005a,b). Although increasing the level of sodium citrate in nonfat *Pasta Filata* cheese was
489 found to decrease cheese hardness, and increase flow (Mizuno & Lucey, 2005b), opposite effects
490 were found in processed Cheddar cheese on increasing sodium citrate from 0.25 to 2.75 %
491 (Shirashoji et al., 2006) or from 2 to 4 % in processed cheese made from nonfat cheese base
492 (Brickley et al., 2008). This discrepancy likely reflects the differences in make procedure between
493 natural and processed cheeses. The addition of larger quantities of sodium citrate during processed
494 cheese manufacturing improves the dispersion of casein and the formation of emulsified fat
495 globules (pseudo-protein particles), and improves the homogeneity of the newly-built casein
496 network of processed cheese and reinforces it. In contrast, the addition of sodium citrate to the curd

497 of nonfat *Pasta Filata* cheese solubilizes the colloidal Ca-P of the existing casein network, which
498 weakens casein-casein interactions (Brickley et al., 2008; Chen & Liu, 2012; Mizuno & Lucey,
499 2005b, Shirashoji et al., 2016).
500 Salting the curd of LMPS mozzarella with sodium orthophosphate after plasticization resulted in
501 similar, but less strong effects as compared to salting the curd with sodium citrate on the
502 biochemical and functional properties of the cheese, and without visual salt precipitation (Fig. 2).
503 Considering the slight increase in insoluble Ca (Fig. 1C), the association of 76% of the added P to
504 the insoluble fraction (insoluble P), and the non-significant effects on cheese pH or pH4.6SN (Fig.
505 1A-B), it was thought that the addition of orthophosphate to the curd weakened the calcium-
506 phosphate para-casein matrix by sequestering colloidal Ca, and induced re-
507 association/precipitation of the newly formed complexes to/on the para-casein matrix without
508 creating new cross-links. Other works have described the displacement of Ca^{2+} and inorganic
509 phosphate from the serum to the casein micellar phase, and the formation of new micellar calcium-
510 phosphate that was different from the native micellar calcium phosphate after milk treatment with
511 $CaCl_2$ (10 mM) (Philippe, Gaucheron, Le Graët, Michel, & Garem, 2003).

4. Conclusions

The addition of sodium orthophosphate, sodium pyrophosphate or sodium citrate to the curd of LMPS mozzarella via dry-salting altered its functional properties (firmness, COT, extensibility, flow). All observed properties were determined after 14 d of storage at 4 °C. Relative to the control, cheese with sodium pyrophosphate had a lower cheese pH and pH4.6SN, and impaired melting characteristics and extensibility. The increase in the levels of insoluble Ca and P in the cheese suggested that sodium pyrophosphate induced the formation of insoluble caseinate-Ca phosphate complexes, and enhanced protein-protein interactions.

In contrast, cheeses with sodium orthophosphate or sodium citrate had similar cheese pH and pH4.6SN, and improved functionality when compared to the control. The cheeses containing sodium citrate had higher insoluble Ca but lower insoluble P, suggesting that the improved functionality may be explained by enhanced solubilization of colloidal Ca-P and precipitation of insoluble Ca-citrate complexes. The improved functionality of cheeses containing sodium orthophosphate likely reflected a weakening of the calcium-phosphate para-casein matrix, possibly by displacement of colloidal Ca-P and subsequent re-association to the micellar phase.

Overall, the current study revealed that the functional properties of LMPS mozzarella cheese could be altered relatively easily by addition of CS via curd dry-salting. The appearance of a white precipitate in cheese containing sodium pyrophosphate or sodium citrate, however, remains undesirable, and additional studies involving the effects of CS dosage or mixtures of CS in an attempt to mitigate this issue, or to further differentiate cheeses with altered functionality, remain of interest.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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LIST OF CAPTIONS

Fig. 1 pH (A), pH 4.6 soluble nitrogen (pH4.6SN) (B), expressed as a percentage of total cheese nitrogen (% TN), insoluble Ca (C) and insoluble P (D) content of cheeses salted with NaCl (CTL), a mixture of NaCl and sodium orthophosphate (OP), a mixture of NaCl and sodium pyrophosphate (PP) or a mixture of NaCl and sodium citrate (CIT). Different superscripted letters denote a significant difference at $P < 0.05$.

Fig. 2 Visual appearance of cheeses after 14 d of storage at 4 °C. The cheeses were salted with NaCl (CTL), a mixture of NaCl and sodium orthophosphate (OP), a mixture of NaCl and sodium pyrophosphate (PP) or a mixture of NaCl and sodium citrate (CIT).

Fig. 3 Functional properties [maximum fluidity (LT_{max}) (A), cross-over temperature (COT) (B), work required to extend molten cheese to a height of 340 mm 2.5 min after heating ($EW_{2.5}$) (C), flow of the heated cheese (D)] of cheeses salted with NaCl (CTL), a mixture of NaCl and sodium orthophosphate (OP), a mixture of NaCl and sodium pyrophosphate (PP) or a mixture of NaCl and sodium citrate (CIT). Different superscripted letters denote a significant difference at $P < 0.05$.

Fig. 4 Visual appearance of cheeses during extension to a height of 340 mm 2.5 min after melting. The cheeses were salted with NaCl (CTL), a mixture of NaCl and sodium orthophosphate (OP), a mixture of NaCl and sodium pyrophosphate (PP) or a mixture of NaCl and sodium citrate (CIT).

Fig. 5 Visual appearance of cheeses after heating for 8 min at 220 °C and cooling to room temperature. The cheeses were salted with NaCl (CTL), a mixture of NaCl and sodium

768 orthophosphate (OP), a mixture of NaCl and sodium pyrophosphate (PP) or a mixture of NaCl and
769 sodium citrate (CIT).
770
771 **Supplementary Figure A.1** Water-soluble Na (WSNa), expressed as a percentage of total cheese
772 Na, of cheeses salted with NaCl (CTL), a mixture of NaCl and sodium orthophosphate (OP), a
773 mixture of NaCl and sodium pyrophosphate (PP) or a mixture of NaCl and sodium citrate (CIT).
774 Different superscripted letters denote a significant difference at $P < 0.05$.
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776 **To et al. (2022) Fig. 1**

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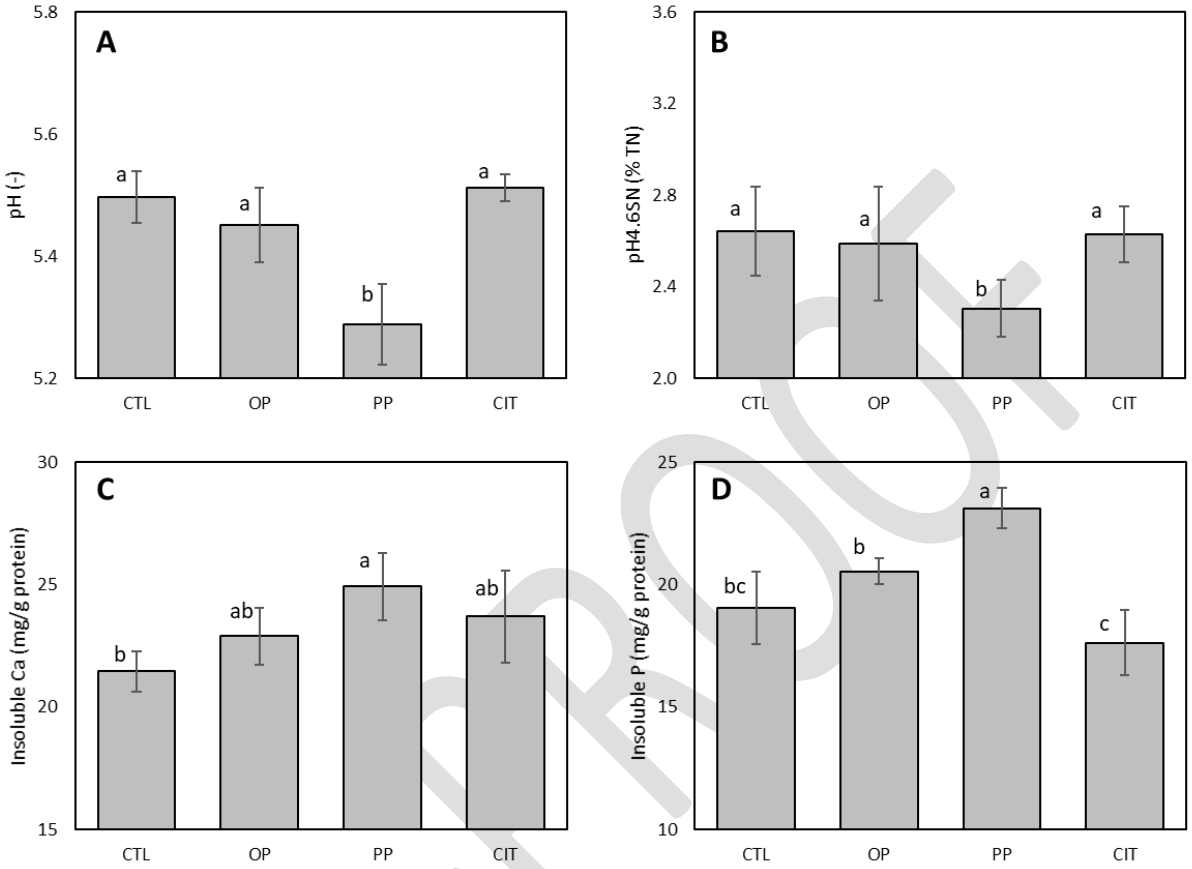

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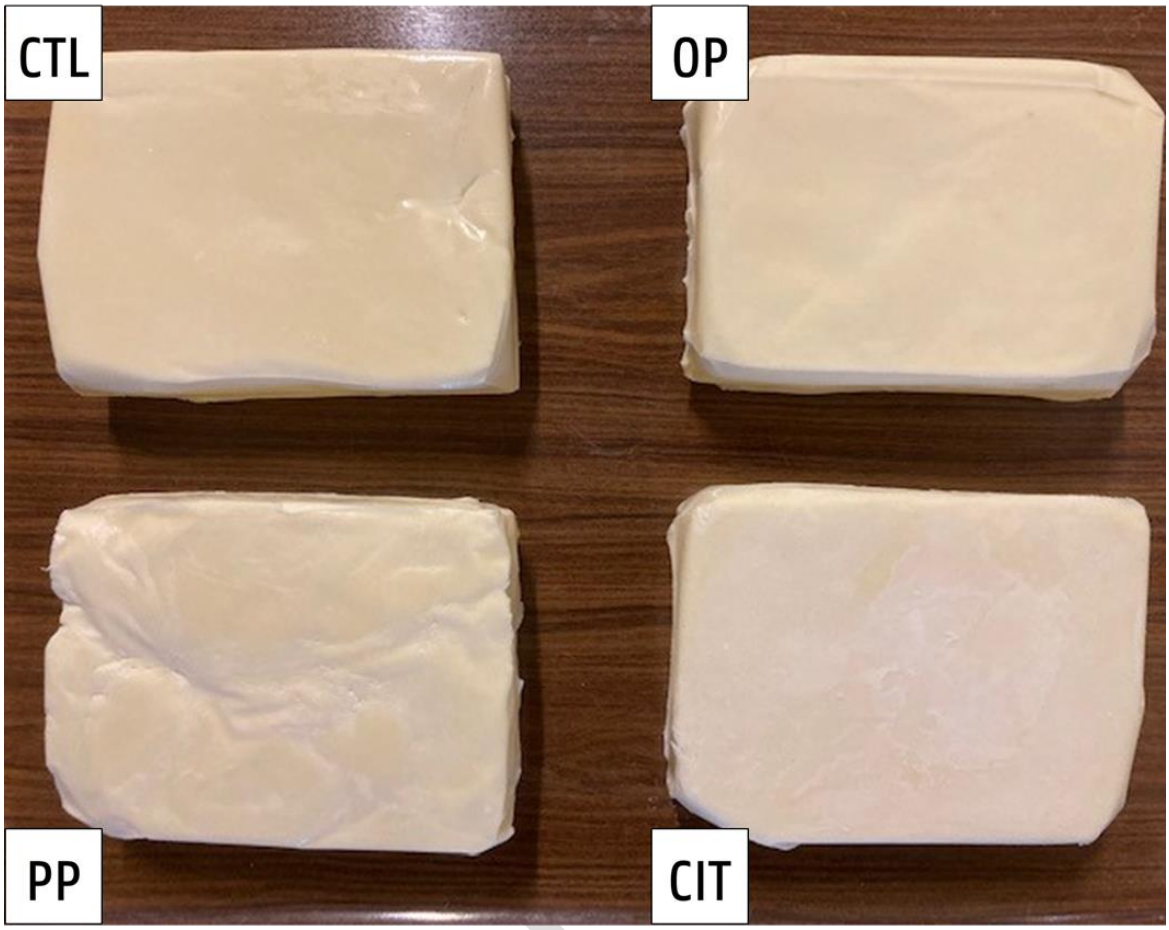
780 **To et al. (2022) Fig. 2**



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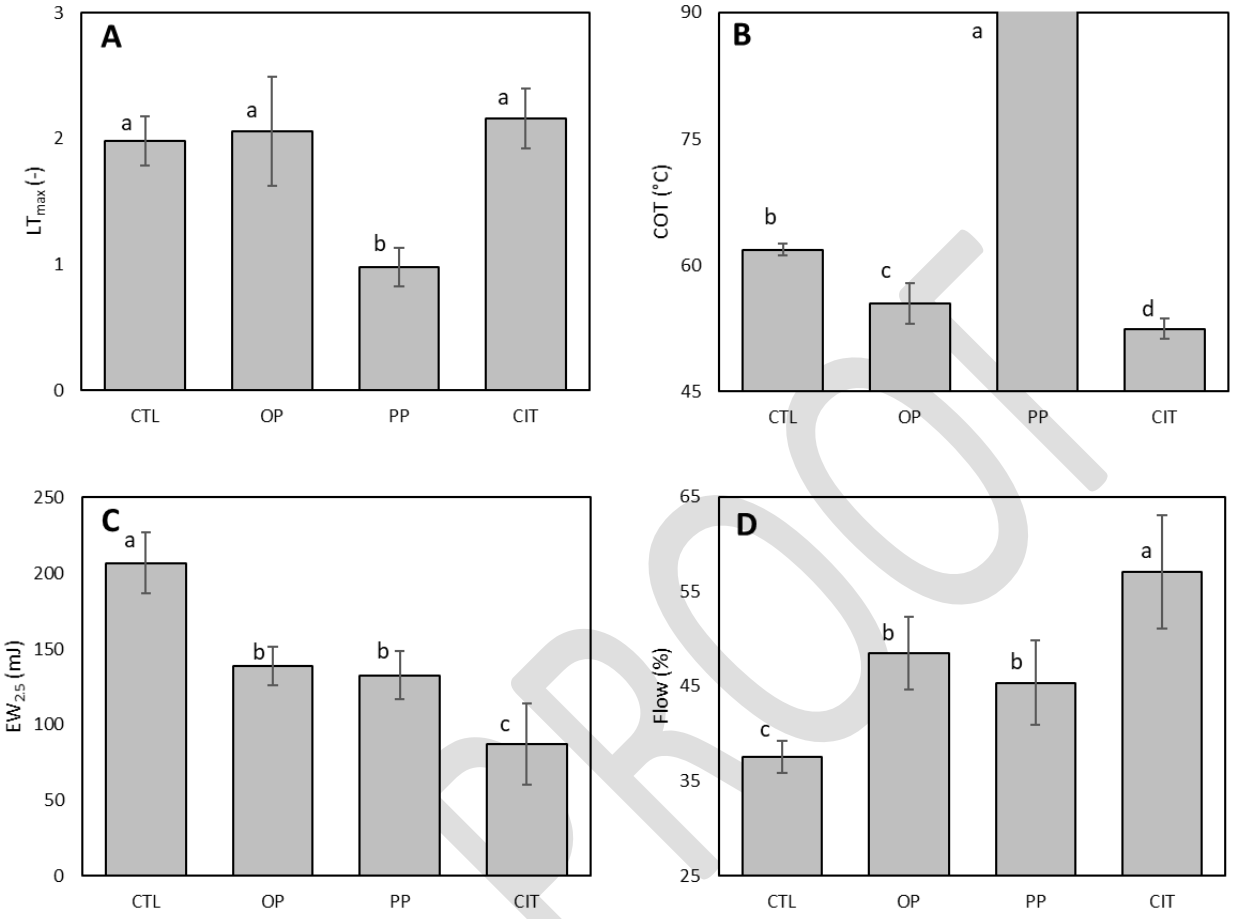
783 **To et al. (2022) Fig. 3**



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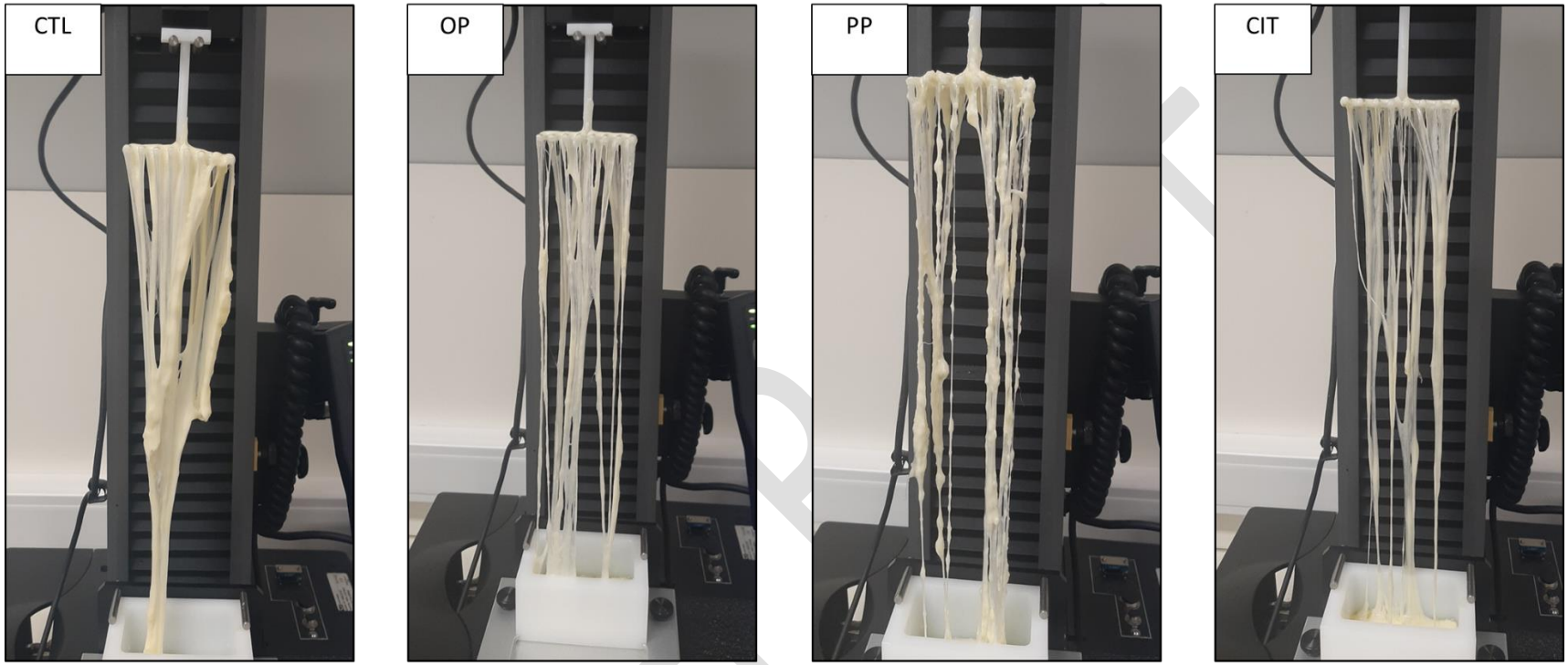
786 **To et al. (2020) Fig. 4**



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789 **To et al. (2022) Fig. 5**

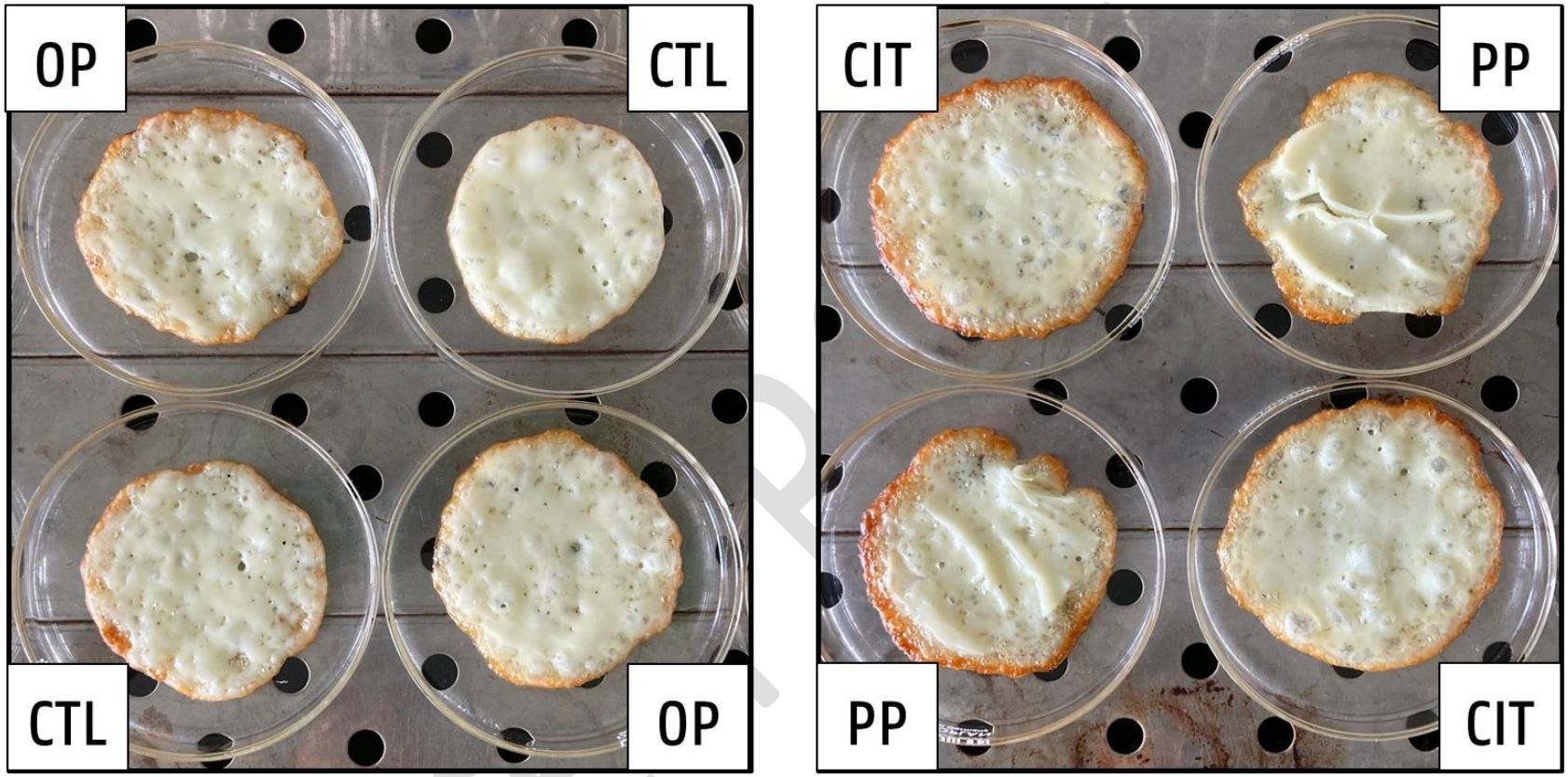

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Table 1. Overview of the added amount of electrolytes to 550 g of curd by dry-salting for each cheese treatment[1]

Electrolyte	Amount of electrolyte added to 550 g of curd (g)			
	CTL	OP	PP	CIT
NaCl	6.68	5.29	3.78	3.83
NaH_2PO_4	-	2.77	-	-
Na_2HPO_4	-	0.07	-	-
$Na_2H_2P_2O_7$	-	-	4.88	-
$Na_4P_2O_7 \cdot 10H_2O$	-	-	0.68	-
$C_6H_6Na_2O_7.1.5H_2O$	-	-	-	5.57
$C_6H_5Na_3O_7.2H_2O$	-	-	-	0.69

[1]The different treatments are: cheese made from curd salted with NaCl (CTL), a mixture of NaCl and sodium orthophosphate (OP), a mixture of NaCl and sodium pyrophosphate (PP), or a mixture of NaCl and sodium citrate (CIT).

797 **Table 2. Mineral composition of the different cheese treatments[1, 2]**

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Treatment	Total Cl (mg $100g^{-1}$)	Total Na (mg $100g^{-1}$)	Total Ca (mg $100g^{-1}$)	Total P (mg $100g^{-1}$)
CTL	649[a]	427[a]	823[a]	543[a]
OP	543[ab]	397[a]	790[a]	595[ab]
PP	468[b]	454[a]	803[a]	709[b]
CIT	465[b]	439[a]	779[a]	540[a]

[1]The different treatments are: cheese made from curd salted with NaCl (CTL), a mixture of NaCl and sodium orthophosphate (OP), a mixture of NaCl and sodium pyrophosphate (PP), or a mixture of NaCl and sodium citrate (CIT).

[2]Different superscripted letters within a column denote a significant difference at $P < 0.05$. Each cheese treatment was prepared in quintuplicate (n = 5).

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800

801 **Table 3. Surface hardness of the different cheese treatments[1, 2]**

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Treatment	Surface hardness (N)
CTL	42.3[b]
OP	36.9[bc]
PP	66.8[a]
CIT	31.6[c]

[1]The different treatments are: cheese made from curd salted with NaCl (CTL), a mixture of NaCl and sodium orthophosphate (OP), a mixture of NaCl and sodium pyrophosphate (PP), or a mixture of NaCl and sodium citrate (CIT).

[2]Different superscripted letters within a column denote a significant difference at $P < 0.05$. Each cheese treatment was prepared in quintuplicate (n = 5).

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