TÉCNICO LISBOA

Melting Cheese: Understanding and Optimization

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November 2017

Abstract

The demand for melted cheese as an ingredient pressures cheese manufacturers to provide cheese with perfect melting properties for each application. The goal of this project was to work towards a more complete understanding of the process and what influences it. The first part of the project aimed to deepen the understanding of micellar behavior under cheese conditions. For that, micellar casein isolate (MCI) solutions adjusted in terms of pH and salt, were subjected to turbidity measurements (20°C to 80°C and back to 20°C) and the heat-induced changes in protein distribution were studied. The results showed that adjusting pH to 5.7 and 5.3 in combination with the addition of 2 or 5% NaCl led to disintegration of the casein micelles. Upon heating, samples with adjusted pH showed aggregation of the casein particles in solution, this being more accentuated for the samples with adjusted salt. The second part of the project was focused on cheese to understand its melting and cooling process and how composition influences it. CLSM observation showed changes in fat distribution after melting. Rheology measurements were performed in cheese with adjusted composition. The results showed that increasing the NaCl content and the pH of the cheese to 5.7 retarded the onset of the melting and decreased the fluidity reached. Lowering the pH to 5.1 had an impact on the maximum tan δ, lowering it, but did not influence the temperature at which the melting happens. Lowering pH to 4.7 impaired the melting. Calcium chloride and citric acid were added to cheese and the first showed to hinder the melting and the latter appeared to help it. The obtained results on cheese melting could allow manufacturers to tailor/differentiate the products according to specific requirements by customers.

Keywords: Milk Proteins, Casein Micelles, Cheese, Cheese Melting, Cheese Rheology

1. Introduction

1.1. Milk Proteins

Casein is the major protein component of bovine milk, covering approximately 80% of the total milk protein and there are four individual types of casein molecules, the α_{s1}-, α_{s2}-, β-, and κ-casein. In milk, the caseins, together with calcium phosphate, form aggregates known as casein micelles. They are easily destabilized, either by treatment with proteolytic enzymes or by acidification, to give the coagula, which is the base of cheeses and yogurt-type products [1]. When milk is converted into cheese, casein and fat are concentrated, whereas the other milk components, especially water, lactose and whey protein, are mainly removed along with the whey, making casein the major protein component in cheese [2]. Regarding the study of the micelle structure, very little can be concluded with certainty [3]. However, it is well established that, most, if not all, of the κ-casein, is found on the surfaces of the micelles. [4]. This κ-casein causes the micelle to be stable against aggregation [1]. Casein micelles in milk are remarkably stable systems that can withstand the rigorous conditions applied during commercial processing. However, under certain conditions of temperature and pH, the colloidal integrity of the casein micelles can be disrupted, resulting in decreased stability [5]. When the pH of milk is reduced, the different acido-basic groups of milk's constituents (organic and inorganic phosphate, citrate, carboxylic residues) become increasingly more protonated. Consequently, micellar calcium phosphate is dissolved and the caseins are released into the diffusible fraction of milk. The addition of NaCl to milk leads to a slight decrease in pH and an increase in Ca^{2+} concentration in the diffusible phase. Casein micelles are remarkably stable against heat treatments. However, several changes were documented, depending on the intensity of the heat treatments. Beliciu *et al.* [6] reported that sterilization affected the colloidal stability, viscosity, and flow behavior of micellar casein concentrate. The observed effects were credited in part to a loss in solubility of calcium phosphate as a result of the high heat treatment, but it was hypothesized that casein micelle dissociation also likely played a role in this instability. Whey proteins constitute 20% of the proteins in milk and include β-lactoglobulin (β-Lg), α- lactalbumin (α-La), bovine serum albumin (BSA), and immunoglobulins [7]. Denaturation of whey proteins and their association with casein micelles via intermolecular –SH/S–S interchange reactions occur during heating of milk. In theory, prevention of heat-induced aggregation of whey

proteins, in particular, β-Lg, can be achieved by inhibiting S–S bond formation. To block the -SH groups N-ethylmaleimide (NEM) can be used [8].

1.2. Cheese Rheology

Cheese is a viscoelastic material because it exhibits viscous and elastic properties at the same time. [9]. The rheology of cheese is a function of the interactive effects of several factors, including composition, microstructure, macrostructure and the physicochemical state of the components (e.g., the degree of casein hydrolysis, the degree of fat crystallization). Small amplitude oscillatory tests are commonly used to measure properties of cheeses during gelation and ripening as well as the change as affected by ingredients (i.e. fat or calcium phosphate) and processing parameters [10] and a very logical application of small strain oscillatory tests is the characterization of melting properties of cheese [11]. It is difficult to elucidate the direct effects of altering the concentration of any compositional component on the rheology of cheese since the levels of the different components tend to vary simultaneously. Upon the application of a stress to a cheese product, the calcium phosphate para-casein network is the principal structural component that controls the extent of deformation for a given stress. Hence, reduced fat cheese, in which the network occupies a volume fraction greater than in full-fat cheese is firmer than the latter. Increasing the calcium content of cheddar cheese while retaining moisture content relatively constant increased the firmness of cheese due to the increase in the extent of calcium-induced crosslinking of the para-casein molecules comprising the para-casein network [12]. Generally, as the pH decreases, the calcium phosphate dissolves from the casein. There is an increase in melt and stretch as the pH decreases. However, if the pH falls too low, the stretch characteristic can be lost due to the proximity to the isoelectric point of the caseins. [13] [14]. By convention, melting of cheese is the ability of the cheese to flow and spread as well as the loss of (visual) integrity of the individual cheese shreds. Heating cheese to temperatures encountered during baking and grilling (90–98 °C), results in two major microstructural changes [15]: Contraction and shrinkage of the *para*-casein micelles (owing to a temperature-induced increase in the extent of hydrophobic interactions between the casein molecules) and a simultaneous expulsion of moisture from the casein network; Liquefaction and coalescence of fat globules.

2. Materials and Methods

2.1. MCI samples

Preparation

Micellar casein isolate (MCI) powder from Friesland Campina (The Netherlands) was dissolved at 3.5% (m/m) protein content in ultrapure MilliQ water and stirred for 30 min at 50°C in a water bath. Sodium azide (NaN_3) was added (0.02% (m/m)) to prevent microbial spoilage. The MCI solution was kept stirring overnight (~5°C) to ensure complete rehydration. In cold conditions (~3℃), provided by a bath with ice, the pH was adjusted to 5.7 and 5.3 with 1M lactic acid (Sigma Aldrich, USA). After that, the salt content was adjusted to 2 and 5% (m/m) with sodium chloride (Sigma-Aldrich, USA) and finally, the final protein concentration was adjusted to 3.0% (m/m) with MilliQ water. A different type of MCI samples was also prepared with 0.4 g/kg of n-ethylmaleimide (NEM) from sigma-Aldrich.

Turbidity measurements

Turbidity measurements were performed in the Carry 4000 UV-Vis spectrophotometer (Agilent Technologies, USA). The selected wavelength was 800 nm and the samples were placed in 2 mm glass cuvettes. The temperature ramp was from 20℃ to 80℃ and back to 20℃ with a heating and cooling rate of 2℃/min. MilliQ water was used as reference.

SDS-PAGE

SDS-PAGE was performed in the supernatant from the ultracentrifugation (100,000 g for 1h, 20℃) of the MCI samples. The experimental setup consists in diluting the samples to 2 mg of protein/ml using MilliQ water and mix it with Laemmli buffer (50 μL each). To guarantee reducing conditions, dithiothreitol (DTT) is added to the Laemmli buffer (15 mg/ml). The samples are incubated for 5 min at 90℃ and centrifuged for 4 min at 15000 g in a 5417C Centrifuge (Eppendorf, Germany). After the preparation of the samples, 10 μL of each are pipetted into the 12+2 gels. In the present work, a 4–20% gradient Criterion TGX Precast Midi Protein Gel (BIO-RAD, USA) was used and ran at 200 V. The gel was placed into Instant blue from Expedeon immediately after stopping the electrophoresis and left staining in the dye overnight. The gel was rinsed with water a few times before scanning. Precision Plus Protein Standards from BIO-RAD were used.

Calcium ion activity

The calcium ion activity values were calculated using the following equation:

$$a = \gamma_{ca} C_{ca} \quad \text{(Eq. 1)}$$

Where a is the calcium ion activity, γ_{ca} the divalent ion activity coefficient and C_{ca} the free calcium concentration. The conductivity (K) measurement allows the calculation of the ionic strength (I) with the following equation:

$$\mathrm{K} = \mathrm{a} \times \mathrm{I} + \mathrm{b} \quad \text{(Eq. 2)}$$

Where a and b are coefficients. The obtained ionic strength (I) is used for the calculation of the divalent ion activity coefficient (γ_{ca}),

$$\log(\gamma_{Ca}) = \frac{-0.5 \times (Z)^2 \times \sqrt{I}}{1+\sqrt{I}} \quad \text{(Eq. 3)}$$

Where Z is the valence number, which is +2 for Ca^{2+}. The equation 4 exhibits the relation between the measured Ca-potential (E_{ca}) and the free calcium concentration (C_{ca}).

$$E_{Ca} = c \times \log(C_{Ca}) + d \quad \text{(Eq. 4)}$$

Where the coefficients c and d are calculated by two logarithmic functions,

$$c = e \times \ln(I) + f \quad \text{(Eq. 5)}$$

$$d = g \times \ln(I) + h \quad \text{(Eq. 6)}$$

Where e, f, g and h are coefficients. To calculate the previous parameters an experimental setup with a conductivity meter (Radiometer Copenhagen, Denmark), and a calcium electrode potential meter (Schott instruments, Germany) was put together and the values were measured simultaneously. These parameters are measured with a tri-electrode setup, for the conductivity measurement, for the reference electrode and the Ca-potential measurement electrode. Before and after each measurement, the electrodes are washed with MILLIQ water and dried with a tissue. During this calculation method, six unknown coefficients are used. These are determined based on the linear (coefficients a and b) and logarithmic curves (coefficients e, f, g, and h) obtained after a calibration step with 25 standards solutions divided into five groups of five samples with the same Ionic strength and a gradient of Ca-concentration. The standard solutions and the solutions to be studied were analyzed at a constant temperature of 20°C.

2.2. Cheese

Sample preparation

Gouda-type cheese produced at NIZO (The Netherlands) was the used cheese. Cheese blocks were cut into small discs with a diameter and thickness of, approximately, 27 and 2 mm, respectively. The cutting of the cheese was performed in cold conditions (~5°C) to prevent the melting of fat at room temperature. The first step was to cut a cheese cylinder with a diameter of 27 mm, then the cheese cylinder was cut into discs by wire cutting. The cheese discs were kept at 5°C wrapped in plastic wrap and foil paper until analysis An important part of the project was to study the influence of the cheese composition in the melting properties. The composition was adjusted in terms of moisture, NaCl, calcium and whey protein content, calcium distribution and pH using two different methods. To adjust to higher values of moisture and different values of Salt (NaCl from Sigma-Aldrich, USA), calcium (calcium chloride dihydrate from Merck, Germany) and whey protein (Bipro from Agropur, Canada) content, solutions with the required characteristics were prepared and 5 droplets of 5 μL were added to each side of the discs (total of 50 μL of solution). The solutions were prepared with milk permeate (mixture of neutral and acidic milk permeate to a final pH of 5.3). The discs were left equilibrating overnight at 5°C in small containers. To lower the moisture content, the discs were left in a desiccator containing silica gel. To adjust the pH of the cheese, a desiccator where the atmosphere was altered with ammonia (12% v/v, BDH Chemicals, England) or acetic acid (99.85% v/v, Scharlab, Spain) was used. The characteristics of each adjustment are described in table1. Note that, the final composition values for moisture and NaCl content were calculated based on the weight difference of the cheese discs before and after the adjustment. The pH of the cheese discs was measured using a surface electrode (632 pH-meter, Metrohm, Switzerland)

Table 1- Description of the different solutions used for the droplet deposition technique and characteristics of the altered atmosphere technique.

Final composition	Droplet Deposition	Altered Atmosphere
33% Moisture	--	Desiccator with silica gel -1 h (turned after 30 min)
46% Moisture	50 μL of milk permeate	--
1.9% Nacl	50 μL of milk permeate, 5% NaCl	--
2.6% NaCl	50 μL of milk permeate, 10% NaCl	--
3.4% NaCl	50 μL of milk permeate, 15% NaCl	--
3.8% NaCl	50 μL of milk permeate, 18% NaCl	--
pH 4.7	--	Desiccator with acetic acid -14 min (turned after 7 min)
pH 5.1	--	Desiccator with acetic acid -10 min (turned after 5 min)
pH 5.7	--	Desiccator with ammonia (12%) - 3 min (turned after 1.5 min)
3.8% NaCl, pH 5.0	50 μL of milk permeate, 15% NaCl	Desiccator with acetic acid -10 min (turned after 5 min)
3.8% NaCl, pH 5.7	50 μL of milk permeate, 15% NaCl	Desiccator with ammonia (12%) - 3 min (turned after 1.5 min)
w/ Calcium chloride surplus	50 μL of 1M calcium chloride solution	--
w/ Citric acid	50 μL of 1% citric acid solution	--
w/ Whey protein surplus	50 μL of 5% whey protein solution	--

CLSM

The confocal laser scanning microscopy instrument used was the Leica TCS SP5 (Leica microsystems, Germany). Argon laser with the power set at 20% was used. The images obtained were collected with the 20x immersion objective and the image format is 1024x1024 pixels. The selected speed was 200 Hz. The preparation of the samples consisted of cutting a thin and flat (best as possible) slice of cheese and color the samples with Nile Blue. The selected emission and absorbance wavelengths were 488 and 633 nm.

Rheology measurements

The rheology measurements were performed in a AR-G2 rheometer (TA Instruments, USA). Low-amplitude oscillatory rheology measurements were performed in cheese discs with diameter and thickness of, approximately, 27 and 2 mm, respectively. The measurements were performed as temperature sweeps at a strain of 0.1% and frequency 1Hz, with an equilibration time of 5 minutes and a selected gap height of 1.75 mm. These conditions ensured that the samples were within the linear viscoelastic range during testing [16]. The selected heating and cooling rate was 2°C/min. Parallel-plate geometry was the used geometry with a 40mm serrated top plate. The discs were placed in the center of the bottom plate and surrounded by a paraffin oil (Merck, USA) before the beginning of the measurement to prevent water evaporation during heating. The measured variables were the elastic or storage modulus, G', the viscous or loss modulus, G'' and the loss tangent, tan δ, where δ is the phase angle.

3. Results and Discussion

3.1. MCI Samples

Unheated samples (20°C)

Adjusting the MCI sample conditions to lower pH and higher salt content changes micelle structure and behavior. When both the pH and salt were adjusted, the initial state of the solution becomes obvious. The low initial values of optical density (OD_{800}) (Table 2), the high values of calcium ion activity (Figure 1) and the dark bands presented in the SDS-PAGE results (Figure 2), show that after preparation almost all the casein micelles have fallen apart, mainly due to calcium phosphate solubilization, which increases with the increase in salt content and decrease in pH.

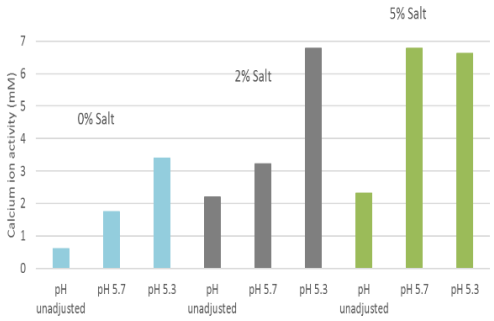

Figure 1- Calcium ion activity values, at 20℃, for the different MCI samples. The blue, grey and green columns correspond to the 0, 2 and 5% of NaCl content, respectively.

Table 2- OD_{800} values for each solution at 20℃.

Sample	Initial OD Value (20℃)
0% NaCl, pH unadjusted	1.11
0% NaCl, pH 5.7	1.03
0% NaCl, pH 5.3	0.87
2% NaCl, pH unadjusted	0.71
2% NaCl, pH 5.7	0.18
2% NaCl, pH 5.3	0.29
5% NaCl, pH unadjusted	0.61
5% NaCl, pH 5.7	0.05
5% NaCl, pH 5.3	0.03

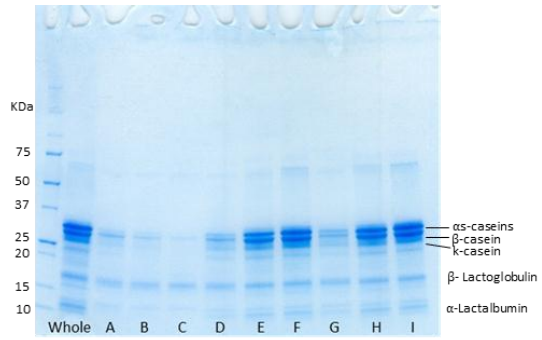

Figure 2- SDS-PAGE results from the supernatants that resulted from the ultracentrifugation at 100,000 g for 1 hour for MCI samples at unadjusted pH (A, D, G), pH 5.7 (B, E, H) or pH 5.3 (C, F, I) and containing 0 (A, B, C), 2.0 (D, E, F) or 5.0% (G, H, I) added NaCl. The first lane (whole) corresponds to the whole sample at unadjusted pH and without added NaCl.

Heat-induced changes

An increase in OD_{800} value on heating (Figure 3) suggests increased aggregation of the casein particles present in the solution. Aggregation was observed in almost all conditions, the intensity being most extensive in solutions with both elevated NaCl content and reduced pH. This suggests that on increasing temperature, the primary casein particles remaining after micellar disruption are prone to aggregation. This may be the result of a combination of the low net-negative charge (due to pH reduction), the low Debye length (due to salt addition) and the increased association of Calcium and phosphate with the caseins (due to elevated temperature) jointly reducing inter-particle repulsion. An important aspect is the reversibility of the protein interactions leading to aggregation. If reversible, a significant decrease in OD_{800} would occur. This was not observed, except for the solution with pH 5.3 and 5% NaCl. However, in the latter case after heating and cooling a deposit was formed, showing that the protein aggregation was not reversible. To understand the impact of whey proteins being present in small proportions in MCI, solutions were also prepared with 0.4g/kg of n-ethylmaleimide (NEM), thus preventing heat-induced irreversible whey protein denaturation and interaction of whey proteins with casein by thiol-disulfide group reactions. Figure 3 shows that, for most conditions, the addition of NEM did not influence the aggregation in a noteworthy way, which suggests that the whey protein present is not responsible for the aggregation observed.

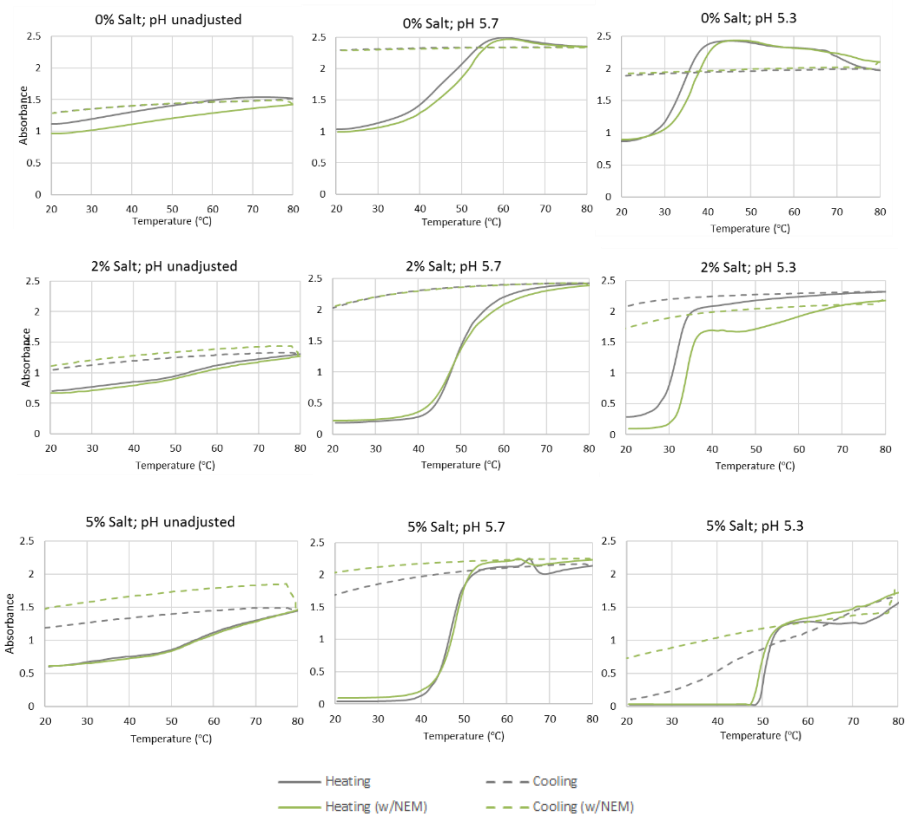

Figure 3- Turbidity measurements performed in the standard MCI samples and MCI samples complemented with NEM to a final concentration of 0.4g/kg. The temperature ramp used was from 20℃ to 80℃ and back to 20℃ with a heating and cooling rate of 2℃/min, with values taken every degree. Optical density was measured at 800 nm.

3.2. Cheese

CLSM observation

Microstructure changes were studied with confocal scanning laser microscopy observations of cheese that showed characteristic changes in microstructure as a result of heating (Figure 4): Fat coalescence occurred and persisted after cooling resulting in a more heterogeneous distribution.

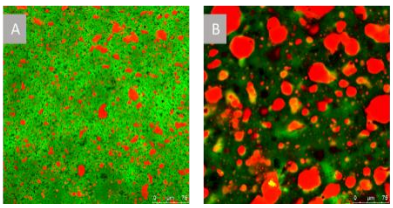

Figure 4- Images obtained by CLSM. A- Fat containing cheese before heating; B- Fat containing cheese after heating. Temperature ramp: heating from 5℃ to 80℃ and cooling to 5℃ with a heating and cooling rate of 2℃/min The red color corresponds to fat phase and the green to the protein matrix.

Rheology measurements

Figure 5 shows the rheology results for the non-adjusted fat-containing cheese over the 5℃ to 80 and back to 5℃ temperature ramp. The trends observed are typical of cheese melting curves. It is possible to see that the values of G' and G'' decrease upon heating and increase in cooling and the opposite happens for the value of tanδ that increases during heating and decreases upon cooling. From Figure 5, it is possible to see that when cheese is heated, there is a dramatic decrease in the total number and/or strength of bonds in the cheese matrix, which is indicated by the steady decrease by several orders of magnitude in the dynamic moduli (G' and G''), and an increase in tan δ. This change in the dynamic viscoelastic parameters indicates that, at elevated temperatures, cheese changes to a more viscous-like material compared with unmelted cheese. Lucey *et al*. [17] report that hydrophobic interactions play a key role in determining the conformation and interaction of protein molecules. Hydrophobic interactions tend to increase in strength with temperature which may reduce the size of the contact area between casein particles (as these individual molecule contract on themselves), and the net result is a reduction in overall gel strength. Several types of electrostatic interactions are also likely to be important in cheese and are manifested as charge repulsion between similar charges on proteins, salt bridges, and CCP bridges. Hydrogen bonding (attractive) also decreases with increasing

temperature. This suggests that the balance would be shifted towards more repulsion and a weakening of the matrix, which is what is observed in Figure 5C.

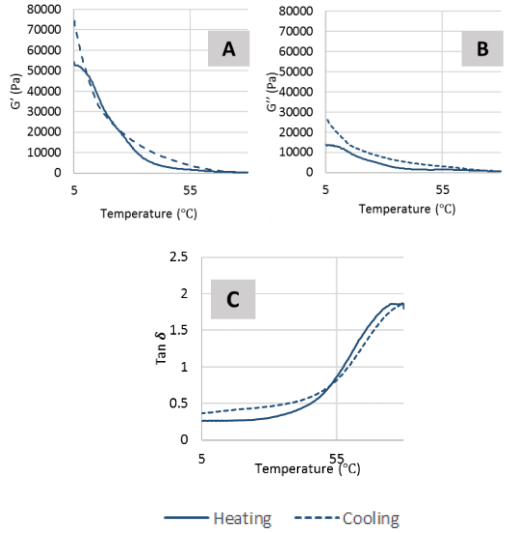

Figure 5- Average curves of elastic modulus, G' (A), viscous modulus, G'' (B) and loss tangent, Tan δ (C) for the non-adjusted fat-containing cheese. Rheology measurements over a temperature sweep (5°C-80°C-5°C) with a heating and cooling rate of 2°C/min, strain of 0.1% and frequency of 1Hz of cheese discs with a diameter and thickness of, approximately, 27 and 2 mm, respectively.

Composition adjustment

The composition of the fat-containing gouda cheese was adjusted in terms of moisture, salt, calcium and whey protein content, calcium distribution and pH as described in the materials and method section.

Moisture content

The moisture content of the non-adjusted cheese is approximately 40%. To study the influence of the moisture in the melting properties of cheese, the moisture content of cheese discs was adjusted to 33% and 46%. The resulting composition and the resulting tan δ curves are presented Table 4 and Figure 6, respectively. In Table 3, are some parameters taken from Figure 6. From the obtained results, it is possible to see that with the decrease in moisture content, the melting happens at higher temperatures. Indeed, increasing the moisture content or increasing the ratio of moisture to protein in cheese weakens the rigidity as the volume fraction of protein decreases [17]. On the other hand, as the concentration of casein increases, the intra- and inter-strand linkages become more numerous and the network displays greater elasticity and is more difficult to deform [18], and this influences a wide range of textural characteristics (e.g., softness, shreddability, and meltability). However, it is important to highlight the fact that, in this trial, the moisture was not the only variable to change but also salt and fat content, so the observed results may not be only due to the change in moisture.

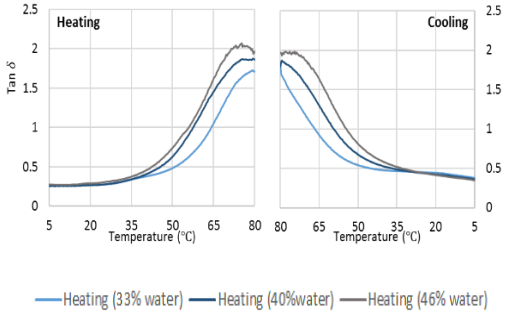

Figure 6- Average curves for tan δ for different moistrue contents (33,40 and 46%). Rheology measurements over a temperature sweep (5°C-80°C-5°C) with a heating and cooling rate of 2°C/min, strain of 0.1% and frequency of 1Hz with cheese discs with a diameter and thickness of, approximately, 27 and 2 mm, respectively.

Table 3- Summary table with parameters from Figure 6.

Water content	(tan δ)i	(tan δ)f	T(tanδ=1) Heating	T(tanδ=1) Cooling
33%	0.28	0.38	64.7	66.9
40%	0.26	0.36	57.9	59.3
46%	0.27	0.35	56.1	55.5

Table 4- Composition of the cheese discs with adjusted moisture. Note that the composition for the 33% and 46% moisture cheese discs was calculated based on the difference of moisture and the composition of the non-adjusted cheese given by the reference methods.

Moisture (% m/m)	NaCl (% m/m)	Fat (% m/m)	$\frac{moisture}{protein}$	$\frac{NaCl}{moisture}$
33	~1.7	~34	1.13*	5.2%
40	1.54	31	1.57*	3.9%
46	~1.4	~27	1.95*	3.0%

*2% of dry matter was considered to be mineral material and lactate.

Salt Content

The non-adjusted NaCl content was 1.54% (m/m). Cheese discs were adjusted to 1.4, 1.9, 2.6, 3.4 and 3.8% of NaCl. The resulting composition and the resulting tan δ curves are presented in Table 5 and Figure 7, respectively.

Table 7 shows some parameters taken from Figure 7. From the results, it is possible to extrapolate that the increase in salt content retarded the onset of melting of the cheese since the melting happens at higher temperatures and reaches lower maximum tanδ values. When the concentration of salt is increased several mechanisms can take place. The solubilization of calcium phosphate that is associated to the micelles can happen which will lead to weaker linkages in the cheese matrix. However, when the salt is increased there is also an increase in ionic strength and in protein hydration and a decrease in electrostatic repulsion, which will lead to stronger linkages in the cheese matrix. Apparently, there is a balance between these opposite effects which is shifted to stronger linkages and that is why the melting happens at higher temperatures for the more salted cheese discs. Indeed, Rulikowska *et al*. [19] report that the reduction of NaCl directly leads to less displacement of Ca, and less casein solubilization, thus lower NaCl cheeses have less casein hydration than higher NaCl cheeses.

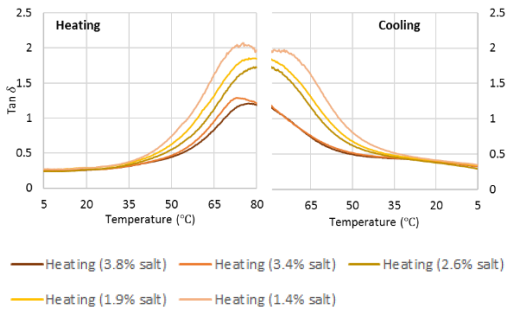

Figure 7- Average curves for tan δ for different salt contents (1.4, 1.9, 2.6, 3.4 and 3.8%). Rheology measurements over a temperature sweep (5°C-80°C-5°C) with a heating and cooling rate of 2°C/min, strain of 0.1% and frequency of 1Hz with cheese discs with a diameter and thickness of, approximately, 27 and 2 mm, respectively.

Table 5- Composition of the cheese discs with adjusted NaCl content. Note that the composition was calculated based on the difference of moisture and the composition of the non-adjusted cheese given by the reference methods.

NaCl (% m/m)	Moisture (% m/m)	Fat (% m/m)	$\frac{moisture}{protein}$	$\frac{NaCl}{moisture}$
1.4	~46	~27	1.95*	3.0%
1.9			1.99*	4.1%
2.6			2.05*	5.7%
3.4			2.13*	7.4%
3.8			2.17*	8.3%

pH

Cheese discs were adjusted to lower pH value, 4.7 and 5.1 and to higher values, 5.7. The resulting tan δ curves are presented in Figure 8 and some parameters taken from this figure in Table 7. The effect of pH is probably a consequence of its influences on (1) the ratio of soluble-to-colloidal Ca, (2) the degree of para-casein hydration and (3) the type/extent of protein interactions [18]. Raising the pH of the cheese to 5.7 retarded the onset of melting and decreased maximum tanδ, indicating an increase in particle interactions. Lowering pH to 5.1 had little effect on heating behavior, but when further lowering to pH 4.7 melting was inhibited due to the proximity to the isoelectric point of the caseins.

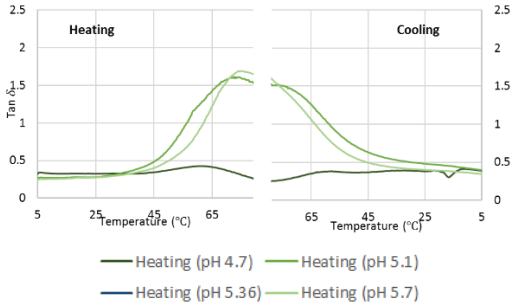

Figure 8- Average curves for tan δ for different pH values (4.7, 5.1, 5.36 and 5.7). Rheology measurements over a temperature sweep (5°C-80°C-5°C) with a heating and cooling rate of 2°C/min, strain of 0.1% and frequency of 1Hz with cheese discs with a diameter and thickness of, approximately, 27 and 2 mm, respectively.

Table 6- Composition of the cheese discs with adjusted pH. Note that the composition was calculated based on the difference of moisture and the composition of the non-adjusted cheese given by the reference methods

pH	Moisture (% m/m)	NaCl (% m/m)	Fat (% m/m)
4.7	~36	~1.6	~33
5.1	~40	~1.54	~31
5.36			
5.7			

Calcium content

A surplus of calcium phosphate was added to the cheese discs. Also added to the cheese discs was citric acid. This will not modify the calcium content but it is known to sequester calcium to form the soluble calcium citrate anion ($CaCit^-$), which increases calcium solubility [20]. The resulting curves are presented in Figure 9 and some parameters taken from this figure are in Table 7. From Figure 9, it is possible to infer adding the calcium chloride leads to a melting

that happens at higher temperatures and reaches lower maximum tanδ values, whereas with the citric acid the melting happens earlier. Perhaps this happens because when the calcium chloride is added, there is an increase in the insoluble calcium content and because of that some soluble calcium phosphate will become insoluble and associate with the micelles leading to stronger linkages in the cheese matrix. On the other hand, citrate is known to promote colloidal calcium phosphate solubilization and by that should decrease the content of bound calcium in cheese. This would decrease protein-protein interactions leading to increased emulsification of fat by caseins, and it would affect the hardness and melting properties of cheese. This goes in agreement with the observed results.

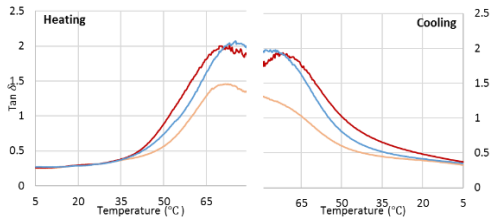

Figure 9- Average tan δ curves for cheese discs to which calcium chloride and citric acid solutions were added. The blue curve corresponds to cheese discs with milk permeate added. The three curves have, roughly, the same moisture content.

Table 7- Summary table with parameters from figure 7, 8 and 9.

Parameter	NaCl% (m/m) (Figure 7)					pH (Figure 8)				Figure 9		
	1.4	1.9	2.6	3.4	3.8	4.7	5.1	5.36	5.7	w/Calcium chloride	w/Citric acid	w/ Milk permeate
(tan δ)i	0.27	0.27	0.24	0.25	0.24	0.33	0.27		0.24	0.25	0.26	0.27
(tan δ)f	0.34	0.34	0.30	0.32	0.32	0.39	0.38		0.35	0.32	0.37	0.35
T(tanδ=1) Heating	56.1	59.2	62.9	65.9	68.9	--	56.5		62.5	60.2	53.4	56.1
T(tanδ=1) Cooling	55.5	59.6	61.6	70.5	73.8	--	58.5		64.3	64.5	51.4	55.5

Conclusions and Further Work

Adjusting MCI solutions to pH 5.7 and 5.3 combined with NaCl adjustment to 2% and 5% decreased micellar integrity, mainly by calcium phosphate solubilization. Upon heating, solutions with adjusted pH showed aggregation of the casein particles, the more so at higher salt content. Changes were mainly irreversible. The melting behaviour (onset temperature, maximum fluidity, decrease in fluidity upon further temperature increase, magnitude of irreversible change) can be influenced by adjusting pH, salt content, calcium content and its distribution, all factors affecting casein particle interactions and their mobility, and aggregation at elevated temperatures. Such adjustments can be of industrial relevance for optimizing process efficiency, yield in pasta-filata processing and for tuning cheese 's hot functionality

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