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The influence of alkaline earth metal equilibria on the rheological, melting and textural properties of Cheddar cheese

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The total calcium content of cheese, along with changes in the equilibrium between soluble and casein (CN)-bound calcium during ripening can have a major impact on its rheological, functional and textural properties; however, little is known about the effect of other alkaline earth metals. NaCl was partially substituted with $MgCl_2$ or $SrCl_2$ (8·7 and 11·4 g/kg curd, respectively) at the salting stage of cheesemaking to study their effects on cheese. Three cheeses were produced: Mg supplemented (+Mg), Sr supplemented (+Sr) and a control Cheddar cheese. Ca, Mg and Sr contents of cheese and expressible serum obtained therefrom were determined by atomic absorption spectroscopy. Addition of Mg^{2+} or Sr^{2+} had no effect on % moisture, protein, fat and extent of proteolysis. A proportion of the added Mg^{2+} and Sr^{2+} became CN-bound. The level of CN-bound Mg was higher in the +Mg cheese than the control throughout ripening. The level of CN-bound Ca and Mg decreased during ripening in all cheeses, as did % CN-bound Sr in the +Sr cheese. The presence of Sr^{2+} increased % CN-bound Ca and Mg at a number of ripening times. Adding Mg^{2+} had no effect on % CN-bound Ca. The +Sr cheese exhibited a higher G' at 70 °C and a lower LT_{max} than the control and +Mg cheeses throughout ripening. The +Sr cheese had significantly lower meltability compared with the control and +Mg cheeses after 2 months of ripening. Hardness values of the +Sr cheese were higher at week 2 than the +Mg and control cheeses. Addition of Mg^{2+} did not influence the physical properties of cheese. Supplementing cheese with Sr appeared to have effects analogous to those previously reported for increasing Ca content. Sr^{2+} may form and/or modify nanocluster crosslinks causing an increase in the strength of the *para*-casein matrix.

Keywords: Calcium equilibrium, colloidal calcium phosphate, Cheddar cheese, rheology.

It is well recognised that the physical properties of cheese are influenced by numerous factors such as pH, calcium content, composition (fat, moisture, protein and salt) and proteolysis. Physical properties of cheese such as hardness, melt, stretch and sliceability are of great importance to both consumers and industry alike. The calcium present in milk and cheese exists in two primary phases: insoluble casein-bound Ca phosphate (CCP), known in milk as colloidal calcium phosphate, and soluble calcium in the aqueous phase. CCP is one of the primary structural elements of the casein micelle (Horne, 1998). CCP exists as nanoclusters several nanometres in size, consisting of a calcium phosphate core linked to numerous organic phosphates from phosphorylated serine residues of casein molecules, and are distributed throughout the protein matrix of the casein micelle (Holt, 2004). Lucey & Fox (1993) first suggested that the level of CCP in cheese is more important than the total calcium content in relation to influencing cheese texture and functionality. Subsequent studies have shown that during cheese ripening (especially during the first month), there is partial solubilisation of CCP and a pseudoequilibrium of calcium phosphate between the soluble and insoluble phases is reached (Hassan et al. 2004; Lucey et al. 2005). Increasing the total calcium content in cheese promotes casein-casein interactions through CCP bridging and charge neutralisation leading to increased hardness (Pastorino et al. 2003). O'Mahony et al. (2006) developed a novel model system using a synthetic Cheddar cheese aqueous phase to study the effects of CCP concentration on the rheological properties of Cheddar cheese independent of proteolysis. In this study, increasing the CCP content of cheese led to an increase in storage modulus (G') at 70 °C and a decrease in maximum loss tangent (LT_{max}). Increasing the total calcium content of cheese by addition of calcium chloride at the salting stage (Brickley et al. 2009) or by injecting calcium chloride after

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manufacture (Pastorino et al. 2003) has been found to increase hardness and decrease the meltability of cheese. Thus, an increase in calcium level can enhance the rigidity of cheese.

Calcium is an element in Group 2 of the Periodic Table (alkaline earth metals) and forms divalent cations (Ca^{2+}) in aqueous solution. Magnesium and strontium are also in this group and have similar chemical properties to calcium. Magnesium occurs naturally in milk and is present in bovine milk at a level of 4–6 mmol/kg (Lucey & Horne, 2009). In bovine milk, approximately one-third of the total magnesium and two-thirds of the total calcium are associated with casein micelles (Gaucheron, 2005). There is still uncertainty as to the location and role of casein-bound magnesium. Strontium can occur in milk at trace levels and as with magnesium and calcium, strontium can associate with casein micelles (Zhang & Aoki, 1995; Rosskopfova et al. 2011). The level of soluble calcium throughout ripening has a major influence on physicochemical properties of cheese (Lucey et al. 2005; O'Mahony et al. 2005; Lee et al. 2010); however, little information is known about the effect of magnesium equilibrium throughout ripening on the physical properties of cheese and to the best of our knowledge, there has been no research published on supplementation of Cheddar cheese with strontium and its effect on cheese physical properties.

The objective of the present study was to investigate if addition of magnesium or strontium could influence the textural, functional and rheological properties of Cheddar-style cheese in a manner similar to that of calcium by evaluating their partition between the soluble and casein-bound phase and also their influence on calcium equilibrium.

Materials and methods

Cheese manufacture

Raw bovine milk was standardised to 3·5% fat and pasteurised. Three Cheddar-style cheeses were manufactured according to standard protocol on a 50 kg scale in the food processing facilities at University College, Cork. R-604Y (Chr. Hansen Ltd., Little Island, Co. Cork, Ireland) was used as the starter culture at a level of 0·02% (w/v). Chymosin (Maxiren 180; DSM Food Specialities, Delft, Netherlands), at a strength of 180 IMCU/ml, was added to the cheesemilk at a level of 0·3 ml/l. Coagulum was cut at equal firmness (measured subjectively). Curd was cooked from 31 to 39 °C over 30 min. Whey was drained at pH 6·2. The curd was cheddared until pH 5·4 was reached and was then milled and dry salted. The control curd was salted with NaCl at a level 2·5% w/w. The cheese curd supplemented with magnesium (+Mg) was salted with 0·87% $MgCl_2{\cdot}6H_2O$ + 1·75% NaCl. The cheese curd supplemented with strontium (+Sr) was salted with 1·14% $SrCl_2{\cdot}6H_2O$ + 1·75% NaCl. These salt treatments were calculated to ensure the ionic strength of the +Mg and +Sr cheeses were equal to the control cheese and to supplement these cheeses with the same molar quantity (43 mmol/kg) of $MgCl_2$ or $SrCl_2$. The salted curd was transferred to rectangular moulds 25·4 cm × 20·3 cm and pressed overnight at 490 kPa. The cheeses were vacuum packaged and ripened at 8 °C for a period of 8 months. Three independent cheesemaking trials were performed.

Chemical analysis

Compositional analysis was performed on the cheeses at day 14 of ripening. The moisture contents of the cheeses were determined by an oven drying method (IDF, 1982), protein by the macro-Kjeldahl procedure (IDF, 1986), fat by the Gerber method (IIRS, 1955), salt by a titrimetric method using potentiometric end-point determination (Fox, 1963). Cheese pH was determined by measuring the pH of homogenised cheese slurry made from 10 g cheese and 10 g water at room temperature. Proteolysis was assessed by determining the levels of pH 4·6-soluble nitrogen as % of total nitrogen (pH4·6SN%TN) at 8 weeks of ripening. Urea-polyacrylamide gel electrophoresis was carried out directly on the cheeses using the procedure described in O'Mahony et al. (2005).

Extraction of cheese juice and determination of casein-bound Mg, Ca and Sr

This method was based on the method developed by Morris et al. (1988) and Hassan et al. (2004). The extraction apparatus consisted of a stainless steel mould, a perforated stainless steel base plate, a stainless steel top plate on which pressure was exerted by a hydraulic ram. Freshly grated cheese (100 g) was mixed with sea sand (150 g) and placed in the stainless steel mould lined with nylon cheese cloth. The cheese-sand mixture was subjected to high pressure using a hydraulic press at room temperature. Pressure was gradually increased up to a maximum of ∼ 37 MPa over 3 h. Liquid from the cheese was collected in a vessel below the perforated base plate. The collected juice and liquid fat were centrifuged at 2000 ***g*** for 10 min at 4 °C to separate fat and curd particles from the juice. Three cheese juice extractions were made from each cheese. The cheeses and their juices were analysed for Mg and Ca content using flame atomic absorption spectroscopy according to IDF (2007) and their Sr content was analysed based on the same method for Ca determination according to IDF (2007) except with a wavelength of 460·7 nm and a lamp current of 10 mA. The concentration of each cation in the cheese juice was taken as the percentage of soluble cation in the cheese. Previous studies (Hassan et al. 2004; Lucey et al. 2005) have equated insoluble Ca with CCP; however this is not totally accurate as Ca^{2+} alone can bind directly to caseins (Dickson & Perkins, 1971; Gaucheron et al. 1997). Hence, we refer to the insoluble Mg, Ca and Sr as CN-bound Mg, Ca and Sr. This definition takes into account all possible forms of the bound cation that is associated with casein. Estimation of percentage CN-bound Mg, Ca and Sr of cheese was calculated according to Hassan et al. (2004).

Texture profile analysis

Texture profile analysis (TPA) was performed using a Texture Analyser TA-XT2i (Stable Micro Systems, Godalming, Surrey, UK) according to the method of O'Mahony et al. (2005), except cylindrical samples of dimensions: height 20 mm, diameter 20 mm were used. Hardness was defined according to Bourne (1978). Five replicate samples from each cheese were compressed at each ripening time point.

Dynamic small amplitude oscillatory rheology

Rheological properties of the cheese samples were measured using a Carri-Med CSL^2/100 Controlled Stress Rheometer (TA Instruments, Leatherhead, UK). Measuring geometry consisted of a 40 mm serrated stainless steel parallel plate above, a flat base plate below and a gap size of 1·8 mm. Cheese discs (40 mm diameter, 2 mm height) were glued to the base plate of the Rheometer using cyanoacrylate glue in order to prevent slippage during measurement. The sample was compressed to the gap size and allowed rest for 10 min at 20 °C in order to allow stress relaxation prior to oscillation. Storage modulus (G'), loss modulus (G'') and loss tangent (LT) were recorded continuously at a low amplitude shear strain (0·05) at a frequency of 6·283 rad/s over 20 min during which the temperature was increased from 20 to 82 °C. The frequency and strain values chosen were found to be within the linear viscoelastic range for the cheese samples. Each sample was analysed in triplicate.

Melt analysis

Melt analysis was carried out using a covered Schreiber test (Altan et al. 2005). Each cheese cylinder (5 mm height, 35 mm diameter) was placed in a covered glass petri dish and then placed in an oven at 232 °C for 5 min. These were then removed and cooled for 30 min at room temperature. Measurements of the melt distance were made using electronic callipers. The diameter of the melted sample was measured at 5 different points and an average diameter was determined. Results were expressed as percentage increase in cheese diameter. Analysis on each cheese sample was performed in triplicate.

Statistical analysis

ANOVA was carried out using the PASW Statistics Version 18 program (IBM, Armonk, NY, USA). The level of significance was determined at $P<0·05$.

Results and discussion

Chemical composition of cheeses

The composition of the cheeses is shown in Table 1. Addition of $MgCl_2$ or $SrCl_2$ at the salting stage had no appreciable effect on the percentage moisture, protein and fat in the cheese. Cl^- levels were slightly lower in the +Mg and +Sr cheeses compared with the control. The pH values of the experimental cheeses throughout ripening are shown in Fig. 1. Addition of $MgCl_2$ and $SrCl_2$ led to a reduced pH throughout ripening in the +Mg and +Sr cheeses compared with the control cheese. Addition of Ca^{2+} to milk can cause a decrease in pH due to formation of calcium phosphates and calcium citrates and by exchanges between added Ca^{2+} and micellar H^+ (Philippe et al. 2003). Pastorino et al. (2003) observed a decrease in cheese pH after injecting a concentrated $CaCl_2$ solution into the cheese. This effect was attributed to binding of Ca^{2+} to caseins promoting the release of protons, thereby decreasing pH in a similar way to the mechanism that occurs in milk. Based on these studies, it is possible that some of the added Mg^{2+} and Sr^{2+} formed complexes with inorganic phosphates and may have also formed CCP and thereby decreased pH.

There were no consistent differences in levels of pH4·6SN%TN between the three cheeses (Table 1), and urea-PAGE electrophoretograms (not shown) displayed no differences in proteolytic patterns throughout ripening. As the cheeses were manufactured with all salting treatments calculated on the basis of equal ionic strength, it is to be expected that no great effect on proteolysis would be observed due to NaCl substitution. The level of total calcium generally remained the same in all cheeses at a level of ~800 mg/100 g cheese regardless of salt substitution treatment (Table 1). Addition of $MgCl_2$ increased the magnesium level in the +Mg cheese to more than double that of the control and +Sr cheeses. Addition of $SrCl_2$ led to a residual strontium level of ~200 mg/100 g cheese in the +Sr cheese, whereas the control and +Mg cheeses did not contain detectable levels of strontium.

Calcium equilibrium

The % CN-bound Ca in the cheeses is shown in Fig. 2. The % CN-bound Ca in the control cheese decreased during ripening from ~62–65% on day 1 to ~56–59% by day 60 and remained relatively constant up to day 224. This is generally in agreement with previous studies (Hassan et al. 2004; Lee et al. 2005; Lucey et al. 2005; O'Mahony et al. 2005). It is thought that this reduction in CN-bound Ca reflects the slow attainment of a pseudoequilibrium between CCP and soluble Ca during the early stages of ripening (Hassan et al. 2004). Added Mg^{2+} appeared to have no impact on % CN-bound Ca. Zhang & Aoki (1995) suggested that Mg^{2+} alone cannot crosslink caseins directly, but could promote calcium crosslinking when forming artificial casein micelles. In the present study,

Table 1. Chemical composition of experimental Cheddar-style cheeses during ripening (mean ± SD)

Trial	Parameter†	Treatment		
		Control	+Mg‡	+Sr‡
1	Moisture (%)	$37·37 \pm 0·34^{a}$	$37·68 \pm 0·22^{a}$	$36·76 \pm 0·11^{a}$
	Fat (%)	$31·83 \pm 0·29^{a}$	$31·00 \pm 0·50^{a}$	$31·08 \pm 0·14^{a}$
	Protein (%)	$25·74 \pm 0·68^{a}$	$25·28 \pm 0·13^{a}$	$25·84 \pm 0·34^{a}$
	Cl^{-} (%)	$0·86 \pm 0·01^{a}$	$0·80 \pm 0·01^{b}$	$0·78 \pm 0·01^{b}$
	Ash	$2·94 \pm 0·13^{a}$	$2·89 \pm 0·06^{a}$	$3·14 \pm 0·13^{a}$
	pH4·6SN%TN	$15·07 \pm 0·22^{b}$	$15·76 \pm 0·05^{a}$	$15·14 \pm 0·22^{b}$
	Calcium	$843·20 \pm 7·54^{a}$	$810·76 \pm 13·90^{b}$	$794·40 \pm 8·54^{b}$
	Magnesium	$32·15 \pm 0·71^{b}$	$79·42 \pm 2·05^{a}$	$31·10 \pm 0·13^{b}$
	Strontium	/	/	$197·23 \pm 5·68$
	Cheese juice moisture at d 1 (%)	$87·64 \pm 0·45^{aA}$	$87·87 \pm 0·31^{aA}$	$88·82 \pm 0·64^{aA}$
	Cheese juice moisture at mo 8 (%)	$70·65 \pm 0·24^{bB}$	$72·59 \pm 0·46^{aB}$	$68·52 \pm 0·30^{cB}$
2	Moisture (%)	$37·93 \pm 0·12^{a}$	$37·66 \pm 0·19^{a}$	$38·08 \pm 0·20^{a}$
	Fat (%)	$31·50 \pm 0·43^{a}$	$31·33 \pm 0·29^{a}$	$31·50 \pm 0·50^{a}$
	Protein (%)	$25·40 \pm 0·30^{a}$	$25·49 \pm 0·08^{a}$	$25·67 \pm 0·65^{a}$
	Cl^{-} (%)	$0·87 \pm 0·02^{a}$	$0·83 \pm 0·01^{b}$	$0·81 \pm 0·02^{b}$
	Ash	$3·45 \pm 0·11^{ab}$	$3·26 \pm 0·09^{b}$	$3·77 \pm 0·28^{a}$
	pH4·6SN%TN	$17·07 \pm 0·17^{a}$	$16·77 \pm 0·05^{ab}$	$16·51 \pm 0·19^{b}$
	Calcium	$838·82 \pm 23·34^{a}$	$809·93 \pm 18·32^{a}$	$803·74 \pm 17·15^{a}$
	Magnesium	$30·62 \pm 0·57^{b}$	$80·24 \pm 0·25^{a}$	$29·7 \pm 0·84^{b}$
	Strontium	/	/	$254·67 \pm 3·30$
	Cheese juice moisture at d 1 (%)	$85·81 \pm 0·43^{bA}$	$86·34 \pm 0·35^{bA}$	$89·88 \pm 0·19^{aA}$
	Cheese juice moisture at mo 8 (%)	$73·11 \pm 0·28^{aB}$	$72·40 \pm 0·43^{aB}$	$73·00 \pm 0·39^{aB}$
3	Moisture (%)	$37·93 \pm 0·43^{b}$	$38·96 \pm 0·26^{a}$	$38·21 \pm 0·12^{b}$
	Fat (%)	$32·42 \pm 0·72^{a}$	$32·58 \pm 0·63^{a}$	$31·92 \pm 0·38^{a}$
	Protein (%)	$25·46 \pm 0·94^{a}$	$25·51 \pm 0·62^{a}$	$25·38 \pm 0·05^{a}$
	Cl^{-} (%)	$0·86 \pm 0·01^{a}$	$0·80 \pm 0·01^{b}$	$0·78 \pm 0·01^{c}$
	Ash	$3·32 \pm 0·04^{a}$	$3·14 \pm 0·17^{a}$	$3·25 \pm 0·03^{a}$
	pH4·6SN%TN	$16·56 \pm 0·13^{c}$	$17·05 \pm 0·10^{b}$	$17·80 \pm 0·13^{a}$
	Calcium	$787·25 \pm 15·25^{a}$	$769·06 \pm 30·85^{a}$	$773·72 \pm 12·87^{a}$
	Magnesium	$28·43 \pm 1·63^{b}$	$79·59 \pm 0·65^{a}$	$30·49 \pm 1·17^{b}$
	Strontium	/	/	$197·77 \pm 3·57$
	Cheese juice moisture at d 1 (%)	$87·64 \pm 0·47^{aA}$	$86·41 \pm 0·62^{aA}$	$87·91 \pm 0·58^{aA}$
	Cheese juice moisture at mo 8 (%)	$73·72 \pm 0·40^{aB}$	$72·89 \pm 0·52^{aB}$	$74·00 \pm 0·38^{aB}$

[a,b,c] Different lower case superscript letters in the same row within a trial indicate that values are significantly different ($P < 0·05$)
[A,B] Different upper case superscript letters in the same column within a trial indicate that values for the same parameter at different ripening times are significantly different ($P < 0·05$)
† pH4·6SN%TN = pH 4·6 soluble nitrogen as a % of total nitrogen. Calcium, magnesium and strontium expressed as mg/100 g cheese
‡ +Mg = cheese supplemented with 43 mmol/kg $MgCl_2$; +Sr = cheese supplemented with 43 mmol/kg $SrCl_2$
d = day; mo = month

however, increasing the Mg^{2+} level did not appear to influence the ability of calcium to form CCP in cheese curd. In Trials 1 and 2, the % insoluble Ca was higher at day 1 in the +Sr cheese compared with the control and +Mg cheeses. The presence of Sr^{2+} promoted more Ca to become CN-bound at day 1 in Trials 1 and 2. At day 224 of ripening, the +Sr cheese had higher % CN-bound Ca in Trials 2 and 3. Comparing pH values and % CN-bound Ca (Figs. 1 & 2), suggests that the presence of Sr^{2+} may promote Ca binding to casein at lower pH values. This phenomenon of Sr^{2+} promoting calcium binding to caseins was also reported by Zhang & Aoki (1995), who found that addition of Sr^{2+} could increase the level of micellar calcium in artificial micelles.

Magnesium equilibrium

The level of CN-bound Mg decreased in all cheeses during ripening (Fig. 3). At day 1 of ripening, the % CN-bound Mg in the control cheese was ~ 20% and decreased to ~ 10% by day 14. Morris et al. (1988) reported that 23% of the total magnesium was casein-bound in a 1 month old Cheddar cheese. A lower association of magnesium with casein micelles compared with calcium also exists in milk and is thought to be due to magnesium phosphates being more soluble than the corresponding calcium salts and not being at saturation levels in the aqueous phase of milk (Philippe et al. 2005). The +Mg cheese had a higher level of CN-bound Mg than the control cheese up to day 60 of ripening.

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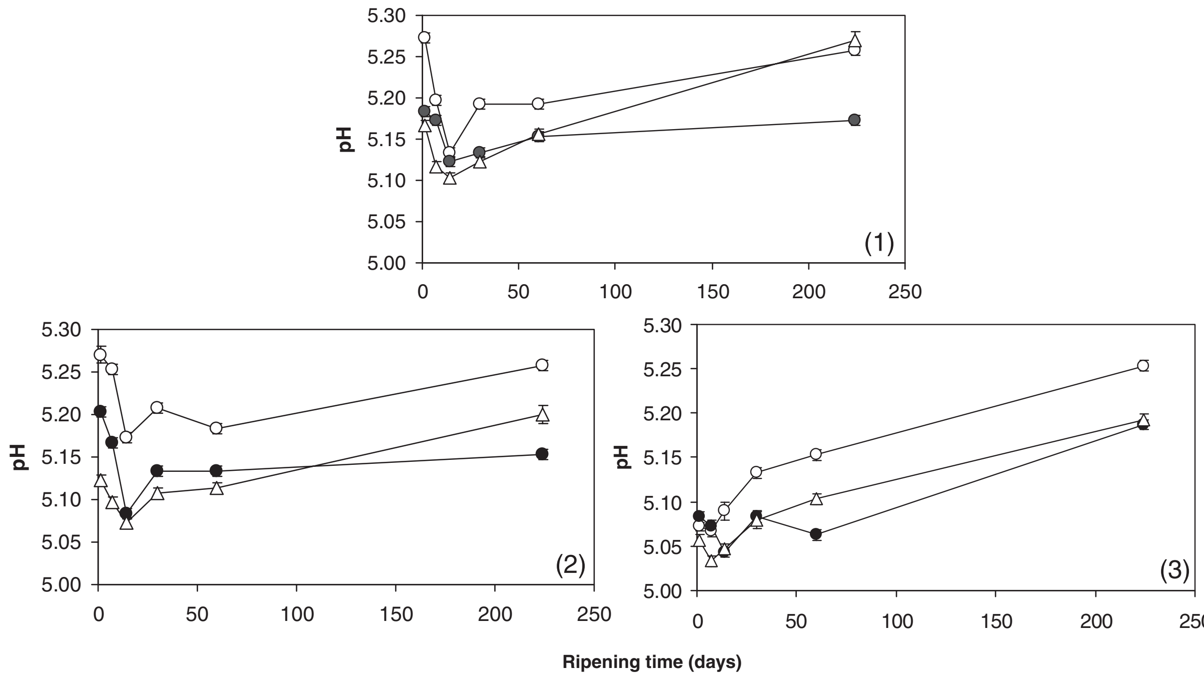

Fig. 1. pH values of control cheese (○); cheese supplemented with 43 mmol/kg $MgCl_2$ (●); cheese supplemented with 43 mmol/kg $SrCl_2$ (△) during ripening in cheesemaking trials (1), (2) and (3).

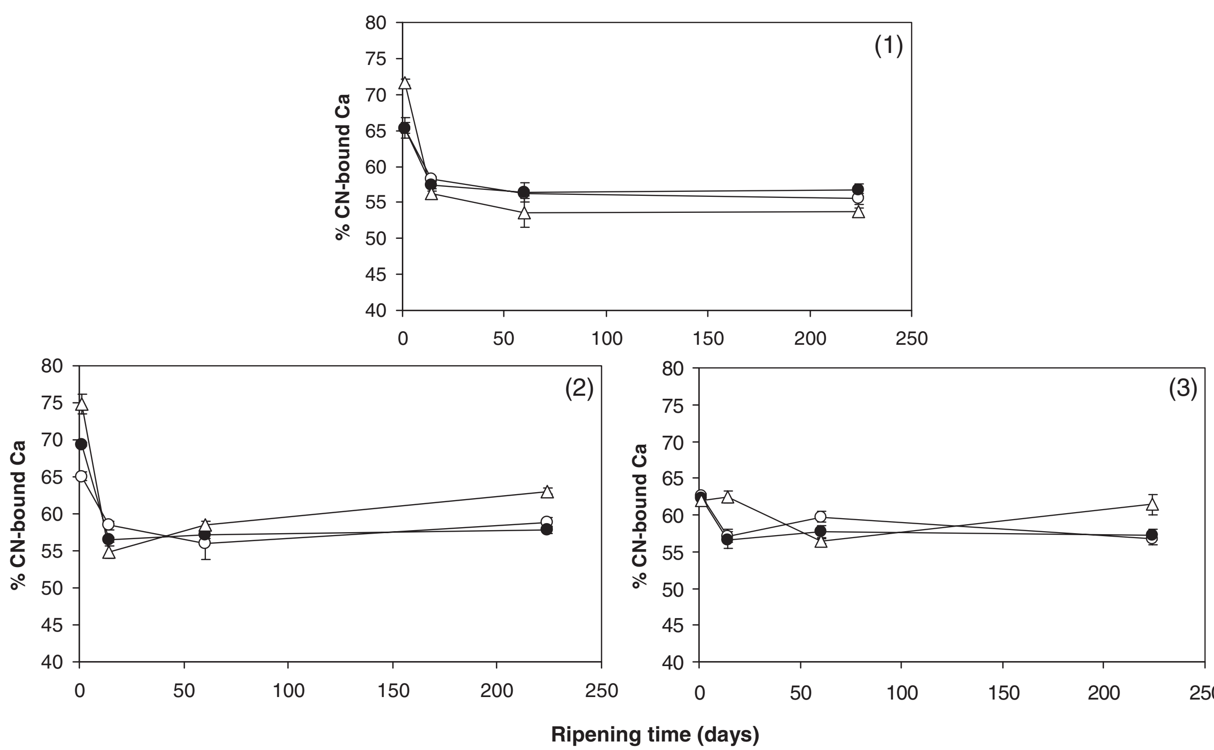

Fig. 2. % CN-bound Ca in control cheese (○); cheese supplemented with 43 mmol/kg $MgCl_2$ (●); cheese supplemented with 43 mmol/kg $SrCl_2$ (△) in cheesemaking trials (1), (2) and (3).

This indicates that during early ripening, a proportion of the added Mg^{2+} was bound to casein. Zhang & Aoki (1995) suggested that Mg^{2+} alone has no crosslinking ability in casein solutions, i.e., replacing Ca^{2+} with Mg^{2+} when formulating artificial casein micelles does not lead to micelle formation. There are no published data supporting the

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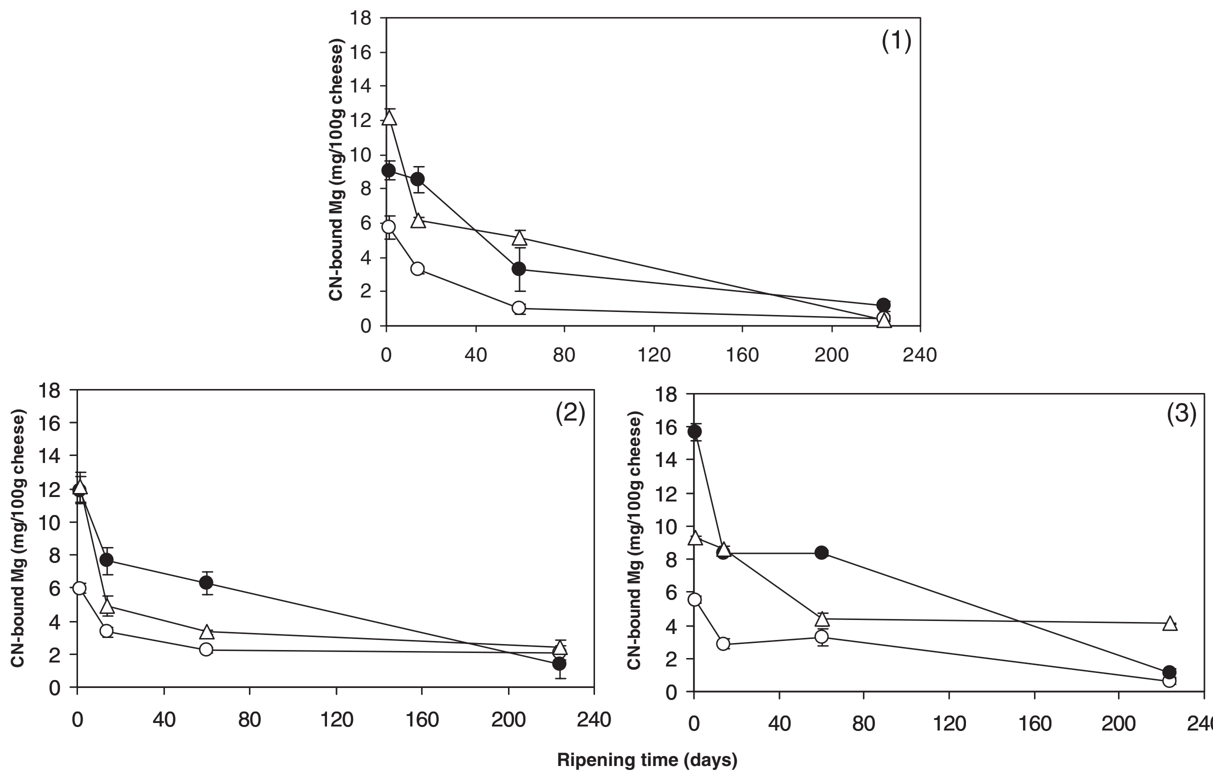

Fig. 3. CN-bound Mg in control cheese (○); cheese supplemented with 43 mmol/kg $MgCl_2$ (●); cheese supplemented with 43 mmol/kg $SrCl_2$ (△) in cheesemaking trials (1), (2) and (3).

existence of magnesium phosphate nanoclusters in milk or any other casein solution/gel, and so it would be unwise to assume the CN-bound Mg necessarily exists in this state. Possible binding sites for Mg^{2+} on caseins other than nanoclusters include monoester phosphate groups on serine and threonine residues and carboxylic groups of aspartic acid and glutamic acid (Dickson & Perkins, 1971) and also phenolic, sulphhydryl and imidazole groups (Gaucheron et al. 1997). However, since Mg^{2+} is a constituent of CCP nanoclusters (Holt, 2004; Lucey & Horne, 2009), it is reasonable to assume that some of the added Mg^{2+} will contribute to CCP crosslinks during cheese ripening. The binding capacity of divalent cations to either α_{s1}- or β-casein is in the order $Mg^{2+} > Ca^{2+} > Sr^{2+}$ (Dickson & Perkins, 1971). This suggests that more Mg^{2+} can be CN-bound compared with Ca^{2+} and Sr^{2+} without necessarily being involved in nanoclusters. The +Sr cheese also had a higher level of CN-bound Mg than the control cheese up to day 60 of ripening. This suggests that the added Sr^{2+} caused more innate Mg^{2+} to associate with casein than in the control (see below).

Strontium equilibrium

The % CN-bound Sr in the +Sr cheeses in all three trials is shown in Fig. 4. The % CN-bound Sr decreased rapidly from ~68–78% on day 1 to ~36–45% by day 14. Values decreased further during ripening to ~7–32% by day 224. As with the % CN-bound Ca, the CN-bound Sr solubilised during ripening but unlike calcium it did not reach a state of equilibrium during early ripening. Zhang & Aoki (1995) observed that artificial casein micelles could be formed when Ca^{2+} was replaced by Sr^{2+} during preparation, suggesting that strontium phosphate has casein crosslinking ability. These crosslinks are likely to be strontium phosphate nanoclusters. The high proportion of added Sr^{2+} in the present study that is CN-bound may therefore exist as strontium phosphate nanoclusters or perhaps mixed cation nanoclusters containing both Ca^{2+} and Sr^{2+} in their structure. The solubility products (K_{sp}) with phosphate from greatest to least are Mg^{2+}, Ca^{2+} and Sr^{2+} (Zhang & Aoki, 1995), so it is likely that Sr^{2+} precipitates to the casein-bound phase as strontium phosphate. As outlined previously for magnesium binding (see *Magnesium equilibrium* section), Sr^{2+} may also bind directly to caseins. In native casein micelles, an estimated 10% of phosphoserine centres are unreacted, i.e., not involved in stabilising nanoclusters (Holt, 2004). Interactions between *para*-casein particles in cheese curd during manufacture and ripening should create more possible sites for nanocluster formation. Philippe et al. (2003) speculated that new CCP formed at these unreacted phosphoserine centres would be different to the native CCP form. When Sr^{2+} is added at salting, it is possible that new strontium-based CCP nanoclusters formed due to this availability of nucleation sites.

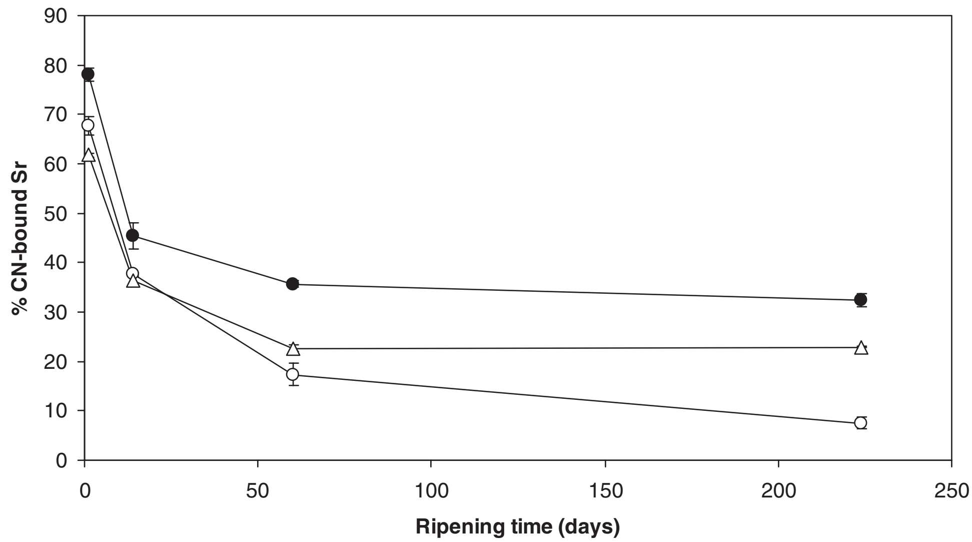

Fig. 4. % CN-bound Sr in cheese supplemented with 43 mmol/kg $SrCl_2$ in cheesemaking trial 1 (○); trial 2 (●); and trial 3 (△).

At day 1 of ripening, the presence of Sr^{2+} in the +Sr cheese caused an increase in the level of CN-bound Ca and Mg compared to the control cheese. Substitution of Ca^{2+} with Sr^{2+} in hydroxyapatite lattice structure has been found to increase the lattice dimensions and volume as Sr^{2+} has a larger ionic radius than Ca^{2+} (Wang & Ye, 2008). Rosskopfova et al. (2011) found that Sr^{2+} could exchange with Ca^{2+} in hydroxyapatite and casein micelles. Cross et al. (2005) proposed a model of the bound calcium phosphate core consisting of two calcium phases based on their Ca:P ratio: a calcium poor phase in the interior and a calcium-rich phase in contact with the phosphoserine groups. The possible existence of strontium phosphate nanoclusters and/or mixed Ca and Sr phosphate-based nanoclusters along with pre-existing Ca phosphate nanoclusters in the +Sr cheese may account for the unusual Ca and Mg equilibria during early ripening as nanocluster ion ratios would likely be different in nanoclusters with Sr^{2+} as a major constituent. Such strontium-based nanoclusters may accommodate more Mg^{2+} in their structure than conventional nanoclusters in the control cheese and the increase in CN-bound Ca due to Sr^{2+} addition may support the existence of the proposed mixed Ca-Sr nanoclusters. Lucey et al. (2003) suggested that larger CCP nanoclusters may grow at the expense of smaller ones through a type of Ostwald ripening. As the % CN-bound Sr decreased extensively by 8 months of ripening, a situation may arise where the most stable form of CCP in the +Sr cheese at this stage is a Sr^{2+} depleted form.

Small amplitude dynamic oscillatory rheology

As can be seen from Fig. 5, the values for *G′* at 70 °C were higher in the +Sr cheese compared with the control and +Mg cheeses throughout ripening. The control and +Mg cheeses had statistically similar values ($P>0{\cdot}05$) for this parameter throughout ripening. O'Mahony et al. (2006) observed an increase in *G′* at 70 °C in cheese with increasing CCP concentration and attributed this to increased CCP bridging between casein molecules which increased the rigidity of the cheese matrix. As the presence of Sr^{2+} led to increased *G′* at 70 °C, it is likely that strontium-based CCP crosslinks increased the strength and rigidity of the *para*-casein matrix. This hypothesis is supported by results shown in Fig. 4 which indicate that a large proportion of added Sr^{2+} was bound to casein during ripening. A higher level of CN-bound Mg is also present in the +Sr cheese (Fig. 3) and this may have also contributed to increased rigidity of this cheese.

The LT_{max} values of experimental cheeses throughout ripening are shown in Fig. 6. The +Sr cheese had lower LT_{max} values than the control and +Mg cheeses throughout ripening. As LT_{max} can be used as an index of melt, these results indicate that the +Sr cheese had the lowest meltabilty. This observation can be explained using the same proposed mechanism as described above for *G′* at 70 °C. Strontium crosslinks increased the strength of interactions between caseins resulting in less melt. The similarity of the viscoelastic properties between the +Mg cheese and control infer that added Mg^{2+} did not sufficiently form or enhance CCP crosslinks. Figure 3 shows that a proportion of added Mg was CN-bound during ripening; however, it is likely that this CN-bound Mg exists at binding sites like carboxylic groups, free phosphoserine groups, etc, rather than forming magnesium phosphate nanocluster crosslinks analogous to conventional CCP. Added Mg^{2+} may contribute in some way to CCP nanoclusters, but not as much as added Sr^{2+}. Zhang & Aoki (1995) speculated that the similarity of the hydrodynamic radii of Ca^{2+} and Sr^{2+} may help explain their similar casein crosslinking abilities.

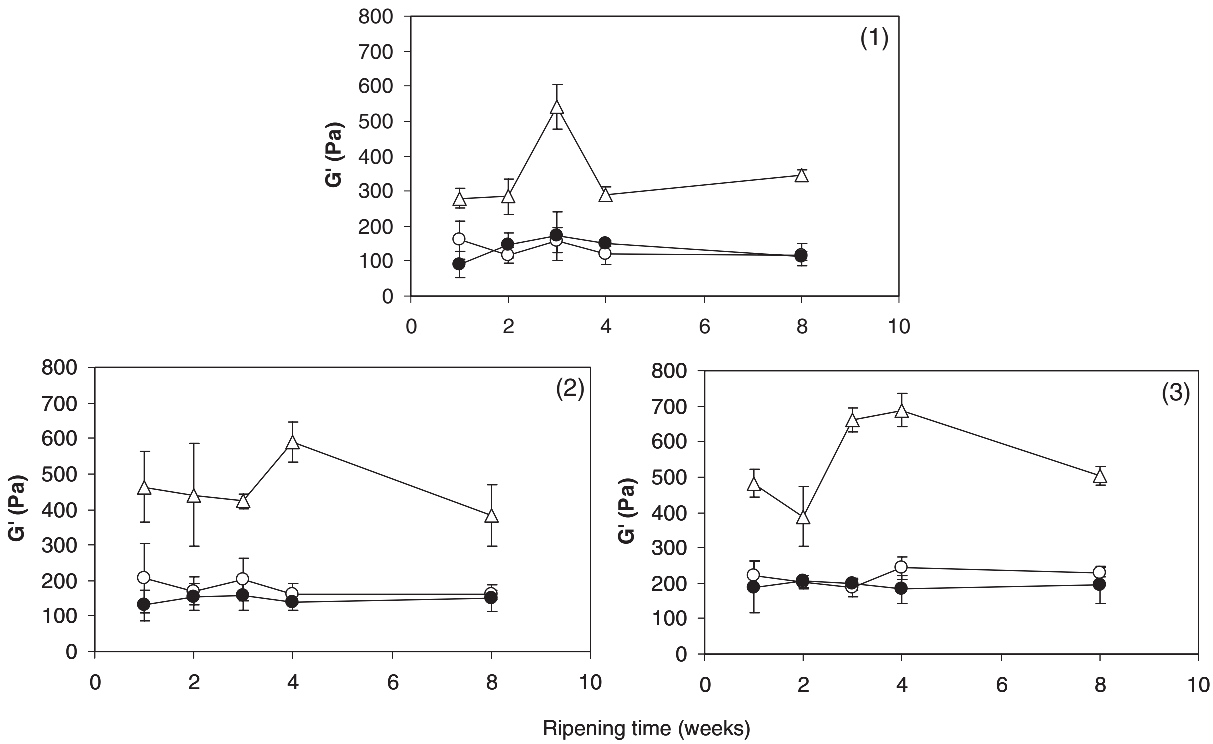

Fig. 5. Storage modulus (*G′*) at 70 °C of control cheese (○); cheese supplemented with 43 mmol/kg $MgCl_2$ (●); cheese supplemented with 43 mmol/kg $SrCl_2$ (△) during ripening in cheesemaking trials (1), (2) and (3).

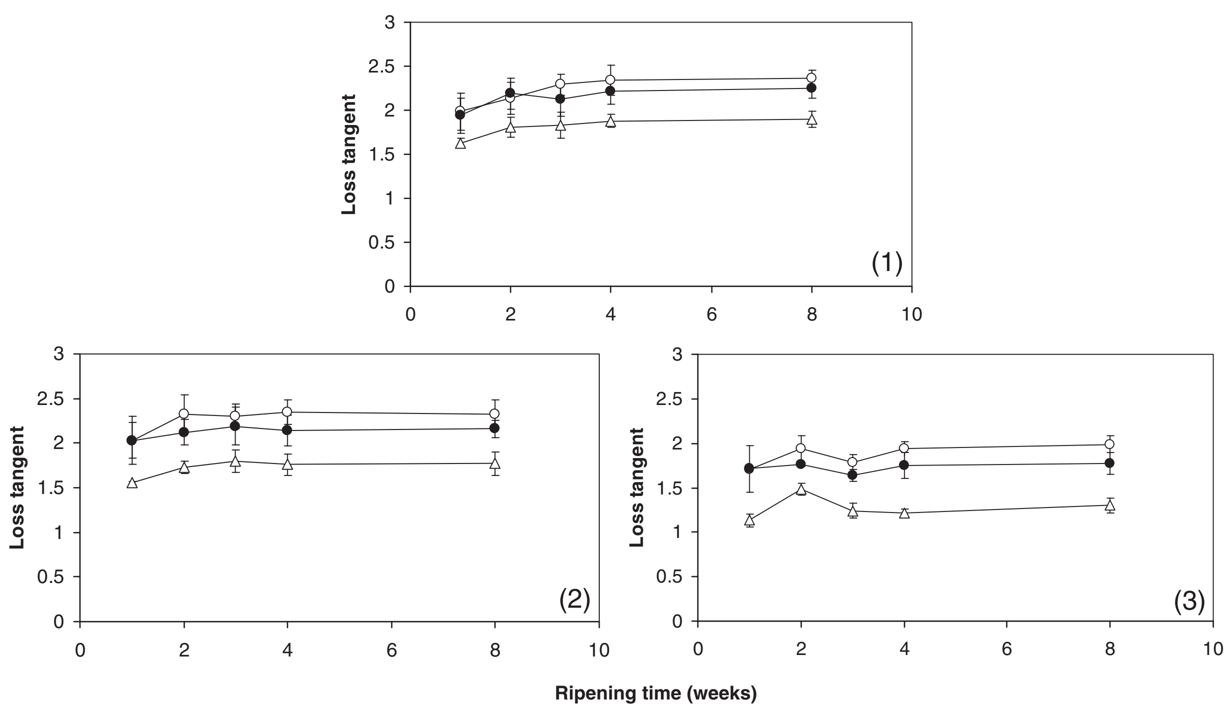

Fig. 6. Maximum loss tangent (LT_{max}) of control cheese (○); cheese supplemented with 43 mmol/kg $MgCl_2$ (●); cheese supplemented with 43 mmol/kg $SrCl_2$ (△) during ripening in cheesemaking trials (1), (2) and (3).

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Table 2. Hardness values (g) as determined by texture profile analysis of experimental cheeses during ripening

		Treatment		
Trial	Ripening time (weeks)	Control	+Mg†	+Sr†
1	1	$12\,659 \pm 1248^{aAB}$	$12\,013 \pm 1192^{aAB}$	$12\,204 \pm 1229^{aB}$
	2	$12\,925 \pm 745^{bA}$	$12\,411 \pm 804^{bA}$	$16\,156 \pm 1415^{aA}$
	4	$11\,936 \pm 396^{bAB}$	$10\,728 \pm 788^{cB}$	$14\,766 \pm 652^{aA}$
	8	$12\,084 \pm 620^{aAB}$	$10\,494 \pm 710^{bB}$	$12\,293 \pm 797^{aB}$
	32	$11\,337 \pm 815^{aB}$	$10\,791 \pm 1007^{aB}$	$10\,823 \pm 446^{aB}$
2	1	$12\,814 \pm 1307^{aB}$	$12\,670 \pm 912^{aA}$	$13\,287 \pm 1233^{aA}$
	2	$14\,653 \pm 738^{aA}$	$12\,603 \pm 1087^{bA}$	$14\,576 \pm 1067^{aA}$
	4	$13\,077 \pm 985^{bAB}$	$12\,492 \pm 861^{bA}$	$14\,810 \pm 943^{aA}$
	8	$11\,426 \pm 1207^{aB}$	$10\,626 \pm 1058^{aB}$	$10\,720 \pm 661^{aB}$
	32	$12\,611 \pm 700^{aB}$	$10\,856 \pm 874^{bB}$	$10\,049 \pm 455^{bB}$
3	1	$14\,049 \pm 1480^{aA}$	$13\,150 \pm 1095^{aA}$	$13\,573 \pm 528^{aA}$
	2	$12\,993 \pm 621^{aAB}$	$11\,292 \pm 919^{bBC}$	$13\,957 \pm 896^{aA}$
	4	$12\,232 \pm 448^{bB}$	$11\,893 \pm 331^{bAB}$	$13\,446 \pm 944^{aA}$
	8	$12\,204 \pm 349^{aB}$	$10\,140 \pm 737^{bC}$	$10\,449 \pm 578^{bB}$
	32	$11\,700 \pm 631^{aB}$	$10\,916 \pm 626^{aBC}$	$10\,020 \pm 370^{bB}$

a,b,c Different lower case superscript letters in the same row within a trial indicate that values are significantly different ($P<0{\cdot}05$)

A,B,C Different upper case superscript letters in the same column within a trial indicate that values for the same parameter at different ripening times are significantly different ($P<0{\cdot}05$)

† +Mg = cheese supplemented with 43 mmol/kg $MgCl_2$; +Sr = cheese supplemented with 43 mmol/kg $SrCl_2$

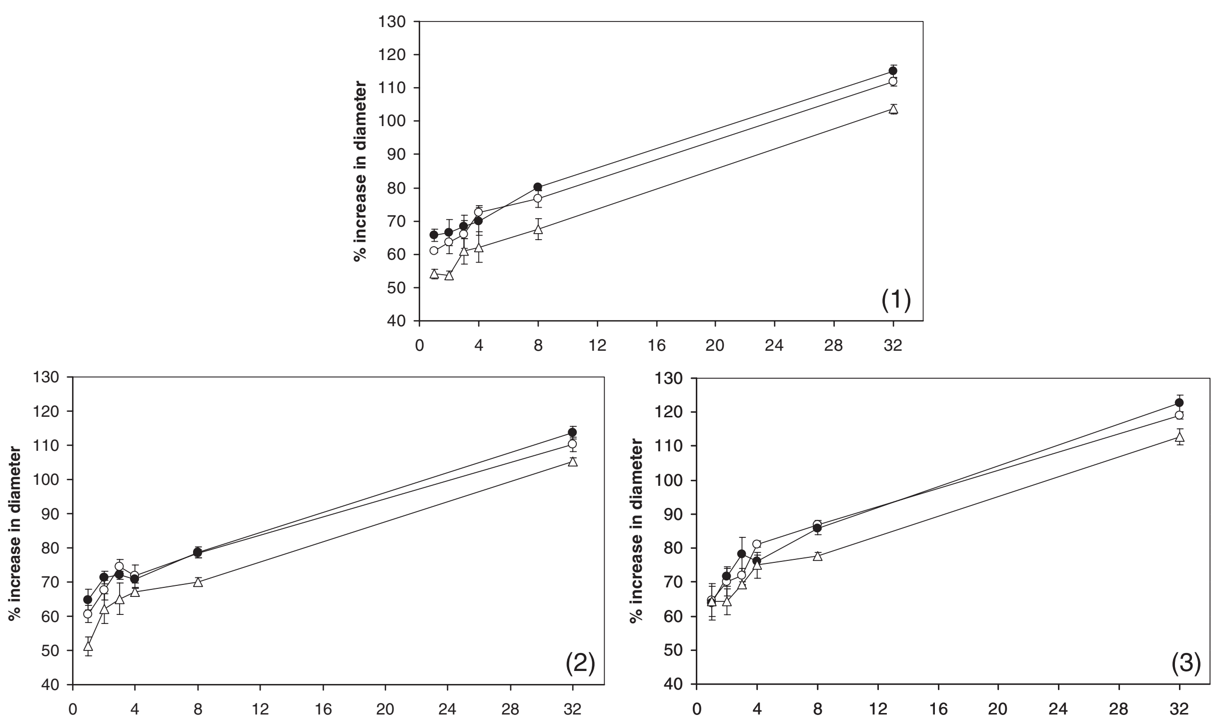

Fig. 7. Percentage increase in cheese diameter from Schreiber melting test for the control cheese (○); cheese supplemented with 43 mmol/kg $MgCl_2$ (●); cheese supplemented with 43 mmol/kg $SrCl_2$ (△) in cheesemaking trials (1), (2) and (3).

Schreiber melting test

Changes in cheese meltability are shown in Fig. 7. The meltability of all cheeses increased during ripening. During the first few weeks of ripening, solubilisation of CCP nanoclusters increased the localised electrostatic repulsion due to exposure of negatively charged phosphoseryl groups which increase melt (Lucey et al. 2003). In conjunction with

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CCP solubilisation, proteolysis will also increase melt by reducing the level of intact casein during ripening. At most time points across all trials, the control and +Mg cheeses had similar meltability. After 2 months of ripening, the +Sr cheese had significantly lower meltability ($P<0{\cdot}05$) compared with the control and +Mg cheeses in all trials. This reduction in true meltability is in agreement with the melt index LT_{max} from dynamic small amplitude oscillatory rheology analysis (Fig. 6). As can be seen in Figs. 3 & 4, the +Sr cheese had CN-bound Sr together with more CN-bound Mg than the control. As discussed above, these results suggest that a denser *para*-casein matrix was present in the +Sr cheese, inducing less meltability than the control and +Mg cheeses. CCP solubilisation slows down and reaches a pseudoequilibrium within the first 2 months of ripening (Hassan et al. 2004; Lucey et al. 2005; O'Mahony et al. 2005), and so it is likely that the increase in melting from week 8 onwards in all cheeses is primarily due to proteolysis. However, the lower meltability of the +Sr cheese compared with the control and +Mg cheeses cannot be attributed to the extent of proteolysis as pH 4·6SN%TN levels were similar at week 8 for all cheeses (Table 1). When cheese is heated above 70 °C, it has been proposed that heat-induced CCP can form (Udayarajan et al. 2005) which may also account for the lower meltability in the +Sr cheese.

Texture profile analysis hardness

TPA hardness values are shown in Table 2. Hardness values generally decreased in all cheeses as ripening time increased. The decrease in hardness during ripening is attributed to solubilisation of CCP during early ripening (O'Mahony et al. 2005) and to a lesser extent the proteolytic breakdown of α_{s1}-casein in the protein matrix (Creamer & Olson, 1982). At week 4 of ripening, the +Sr cheese had significantly higher hardness ($P<0{\cdot}05$) than both the control and +Mg cheeses in all 3 trials. At weeks 2 and 4 of ripening, the +Sr cheese had greater hardness than +Mg cheese. As Sr^{2+} appears to form CCP crosslinks, the +Sr cheese would be expected to be harder than the control. Brickley et al. (2009) observed increased hardness in cheeses after 28 d of ripening when the same molar quantity of $CaCl_2$ was added as $SrCl_2$ in the present study. In the +Sr cheese, at day 14 the % CN-bound Sr was ~36–45% and the level of CN-bound Mg was higher than the control. This suggests that a higher level of casein association and a denser *para*-casein matrix existed in the +Sr cheese during early ripening, leading to higher hardness values. As can be seen in Figs. 2–4, the % insoluble Ca tends to stabilise after day 60, whereas the level of insoluble Mg and Sr continue to decrease up to day 224 in the +Sr cheese. Therefore, the contribution of Mg^{2+} and/or Sr^{2+} to the structural integrity of the cheese matrix decreased as ripening progressed and may account for the lower influence of Sr on hardness values by late ripening.

Conclusion

Supplementing Cheddar cheese with $SrCl_2$ can dramatically alter its physical properties. A proportion of the added Mg^{2+} and Sr^{2+} became CN-bound. The nature of the binding appeared to be the factor that dictated the ability of these ions to alter the physical properties of cheese. It is suggested that the ability of added Sr^{2+} to form CCP nanoclusters increased the strength and density of the *para*-casein matrix, thereby increasing rigidity and reducing melt. Even though some added Mg^{2+} became CN-bound, this did not alter meltability or rheological parameters. This is likely due to the inability of Mg^{2+} to form nanoclusters. Strontium appeared to exhibit behaviour analogous to calcium when added to cheese. It is likely that the solubility of the cation salts in the serum phase of cheese is the principal factor determining their ability to form CCP and thereby modulate textural, rheological and functional properties of cheese. A better understanding of the form of CCP in cheese will enhance our ability to manipulate the physical properties of cheese.

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References

Altan A, Turhan M & Gunasekaran S 2005 Short communication: comparison of covered and uncovered schreiber test for cheese meltability evaluation. *Journal of Dairy Science* **88** 857–861

Bourne MC 1978 Texture profile analysis. *Food Technology* **32** 62–66, 72

Brickley CA, Lucey JA & McSweeney PLH 2009 Effect of the addition of trisodium citrate and calcium chloride during salting on the rheological and textural properties of Cheddar-style cheese during ripening. *International Journal of Dairy Technology* **62** 527–534

Creamer LK & Olson NF 1982 Rheological evaluation of maturing Cheddar cheese. *Journal of Food Science* **47** 631–636, 646

Cross KJ, Huq NL, Palamara JE, Perich JW & Reynolds EC 2005 Physicochemical characterization of casein phosphopeptide-amorphous calcium phosphate nanocomplexes. *Journal of Biological Chemistry* **280** 15362–15369

Dickson IR & Perkins DJ 1971 Studies on interactions between purified bovine caseins and alkaline-earth-metal ions. *Biochemical Journal* **124** 235–240

Fox PF 1963 Potentiometric determination of salt in cheese. *Journal of Dairy Science* **46** 744–745

Gaucheron F 2005 The minerals of milk. *Reproduction Nutrition Development* **45** 473–483

Gaucheron F, Le Graet Y, Boyaval E & Piot M 1997 Binding of cations to casein molecules: importance of physicochemical conditions. *Milchwissenschaft* **52** 322–327

Hassan A, Johnson ME & Lucey JA 2004 Changes in the proportions of soluble and insoluble calcium during the ripening of Cheddar cheese. *Journal of Dairy Science* **87** 854–862

Holt C 2004 An equilibrium thermodynamic model of the sequestration of calcium phosphate by casein micelles and its application to the calculation of the partition of salts in milk. *European Biophysics Journal with Biophysics Letters* **33** 421–434

Horne DS 1998 Casein interactions: casting light on the black boxes, the structure in dairy products. *International Dairy Journal* **8** 171–177

IDF 1982 *Cheese and Processed Cheese. Determination of the Total Solids Content*. Brussels, Belgium: International Dairy Federation. Standard No. 4a

IDF 1986 *Determination of the Nitrogen Content (Kjeldahl method) and Calculation of Crude Protein Content*. Brussels, Belgium: International Dairy Federation. Standard No. 28a

IDF 2007 *Milk and Milk Products – Determination of Calcium, Sodium, Potassium and Magnesium Contents – Atomic Absorption Spectrometric Method*. Brussels, Belgium: International Dairy Federation. Standard No. 119

IIRS 1955 *Determination of the Percentage of Fat in Cheese*. Dublin, Ireland: Institute for Industrial Research and Standards. Irish Standard No. 69

Lee MR, Johnson ME & Lucey JA 2005 Impact of modifications in acid development on the insoluble calcium content and rheological properties of Cheddar cheese. *Journal of Dairy Science* **88** 3798–3809

Lee MR, Johnson ME, Govindasamy-Lucey S, Jaeggi JJ & Lucey JA 2010 Insoluble calcium content and rheological properties of Colby cheese during ripening. *Journal of Dairy Science* **93** 1844–1853

Lucey JA & Fox PF 1993 Importance of calcium and phosphate in cheese manufacture – a review. *Journal of Dairy Science* **76** 1714–1724

Lucey JA & Horne DS 2009 Milk salts: technological significance. In *Advanced Dairy Chemistry*, Vol 3, pp. 351–389 (Eds PLH McSweeney & PF Fox). New York: Springer

Lucey JA, Johnson ME & Horne DS 2003 Invited review: perspectives on the basis of the rheology and texture properties of cheese. *Journal of Dairy Science* **86** 2725–2743

Lucey JA, Mishra R, Hassan A & Johnson ME 2005 Rheological and calcium equilibrium changes during the ripening of Cheddar cheese. *International Dairy Journal* **15** 645–653

Morris HA, Holt C, Brooker BE, Banks JM & Manson W 1988 Inorganic constituents of cheese – analysis of juice from a one-month-old Cheddar cheese and the use of light and electron-microscopy to characterize the crystalline phases. *Journal of Dairy Research* **55** 255–268

O'Mahony JA, Lucey JA & McSweeney PLH 2005 Chymosin-mediated proteolysis, calcium solubilization, and texture development during the ripening of Cheddar cheese. *Journal of Dairy Science* **88** 3101–3114

O'Mahony JA, McSweeney PLH & Lucey JA 2006 A model system for studying the effects of colloidal calcium phosphate concentration on the rheological properties of Cheddar cheese. *Journal of Dairy Science* **89** 892–904

Pastorino AJ, Ricks NP, Hansen CL & McMahon DJ 2003 Effect of calcium and water injection on structure-function relationships of cheese. *Journal of Dairy Science* **86** 105–113

Philippe M, Gaucheron F, Le Graet Y, Michel F & Garem A 2003 Physicochemical characterization of calcium-supplemented skim milk. *Lait* **83** 45–59

Philippe M, Le Graet Y & Gaucheron F 2005 The effects of different cations on the physicochemical characteristics of casein micelles. *Food Chemistry* **90** 673–683

Rosskopfova O, Galambos M & Rajec P 2011 Distribution of strontium in milk component. *Journal of Radioanalytical and Nuclear Chemistry* **290** 601–606

Udayarajan CT, Lucey JA & Horne DS 2005 Use of Fourier transform mechanical spectroscopy to study the melting behavior of cheese. *Journal of Texture Studies* **36** 489–515

Wang X & Ye J 2008 Variation of crystal structure of hydroxyapatite in calcium phosphate cement by the substitution of strontium ions. *Journal of Materials Science: Materials in Medicine* **19** 1183–1186

Zhang ZP & Aoki T 1995 Effect of alkaline earth metals on the crosslinking of casein by micellar calcium phosphate. *Journal of Dairy Science* **78** 1665–1672