UDC 546.48:637.33:539.5
ISSN 1330-9862

original scientific paper

(FTB-983)

Effect of Cd^{2+} Cation on the Rheological Profile of a Model Soft Cheese

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Received: October 23, 1999
Accepted: May 17, 2000

Summary

The aim of this work was to asses the effects of the cadmium, an ion of similar ionic radius and charge as calcium, on the rheology of a model soft cheese at different stages of manufacture. Several batches of reconstituted skim milk with 9 % (w/v) solids and four cadmium concentrations (0.14, 0.35, 0.88, 2.2 mM) were used to make a model mexican soft stretchable cheese whose rheological parameters, G' (storage modulus or elastic component) and G'' (loss modulus or viscous component), at different stages of manufacture, were measured using a Stress Controlled Rheometer.

All milk gels produced behaved as weak viscoelastic systems with G' > G'' over the measured amplitude and frequency ranges. Gradual cadmium addition increased significantly both moduli with no apparent change in the rheological system (short range of loss tangent values).

The comparisson of cadmium effects on the milk gels involved with those of calcium showed that both ions may have similar mode of action.

Key words: stretchable cheese, ionic cadmium addition, rheological properties

Introduction

Dynamic testing is a fundamental method for determining rheological properties of viscoelastic materials. It is normally performed by imposing a sinusoidally varying strain and measuring the resulting stress in the sample. The amplitude of strain is usually kept small to stay within a linear viscoelastic region. In this region, it is possible to distinguish one viscoelastic function from another by different methods (*1*). Measurements of the oscillatory shear moduli are frequently used to continuously monitor the viscoelastic behaviour of systems undergoing a sol-to-gel transition. The gel point corresponds to the intersection of the G' (storage modulus or elastic component) and G'' (loss modulus or viscous component), *i.e.* the point where $\tan \delta = G'/G'' = 1$ (*1*). This method has proved adequate for monitoring the increase of milk gel firmness in the laboratory and when comparing the results to actual cheese making conditions it could be useful to establish correlations between measured reological properties and cheese making parameters.

In the colloidal phase of milk, calcium exists as a tightly bound component of the casein micelle in the form of inorganic calcium phosphate. It has been proposed that two submicelles can be joined *via* $Ca_9(PO_4)_6$ clusters, bound at each end to an ester phosphate group, suggesting that calcium plays an important role in the framework of the micelle, by joining the submicelles (2). Previuos studies on the effect of ions similar to calcium on milk are scarce. Most of the studies on this matter have been done only with ions having similar charge to

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that of Ca^{2+}, irrespectively of their size (e.g. Ca^{2+}, Mg^{2+}, Ba^{2+} and Zn^{2+}) (*3*). To investigate if the changes observed on the rheology of milk gels due to calcium addition are ion specific, the effect of cadmium addition to milk was examined, since it has the same charge and similar ionic radius as calcium (*4*). For that reason the aim of this study was to assess the effect of different levels of cadmium addition, on the rheological profile of a model mexican soft stretchable cheese, at different stages of manufacture and to evaluate any significant differences from those produced by calcium addition.

Materials and Methods

Milk preparation

Skim milk powder was supplied by Kerrygold (Irish Dairy Board). It was used to prepare 1 litre volumes of reconstituted milk with 9 % (w/v) solids to which cadmium chloride ($CdCl_2$) was added according to the batch, making a total of five levels of cadmium (control with no cadmium addition, 0.14, 0.35, 0.88 and 2.2 mM). All milks were prepared using deionised water. A typical stranded white soft cheese similar to »Mozzarella« known by the names of »Asadero« and »Oaxaca« in Mexico, a local »stretchable cheese«, was prepared from each of the milks following a modified traditional method, except that sodium chloride was not added (*5*).

Cheese making

The milk was batch pasteurised in 2 L containers at 63 °C for 30 min; it was then cooled to 32 °C and inoculated with thermophilic starter cultures, 1 % (v/v) of *Streptococcus salivarius* ssp. *thermophilus* and 1 % (v/v) *Lactobacillus delbrueckii* ssp. *bulgaricus* (DRI-VAC, CH–1 and CH–2 from Christian Hansen's Laboratories, Copenhagen, Denmark) prepared from freeze dried culture. Commercial standard rennet (Christian Hansen, Reading, UK) was used at 0.2 mL/L of milk to form the coagulum (coagulation time ranged from 18.9 to 21.9 min) which, one hour after formation, was cut to the size of small »coffee beans« (about 5 mm). The temperature was raised to 42 °C over 35 minutes, and then the whey drained till the pH of the curd reached a value of 5.3– 5.5. The pans were then transferred to a waterbath and the curd was cooked at 75 °C for 15 min to produce small strands of white soft stretchable cheese, cut in small pieces and cooled at room temperature.

Rheological tests

Samples at different stages of manufacture, *i.e.* those of the coagulum obtained after the rennet addition (soft coagulum), those resulted after the drainage of the whey (hard coagulum), and those of the finished cheese were taken for rheological tests. Even when microsyneresis occured in milk gels, the samples were kept as homogeneous as possible when measurements were made. Slices of cheese samples of about 1 mm thick and 20 mm in diameter were carefully cut with a knife and kept refrigerated to avoid drying or any structural disturbance before carrying out the rheological tests. Slices of soft and hard coagulum of about 1 mm thick and 20 mm in diameter were carefully cut with a spatula just before the rheological tests were carried out, to avoid any disturbance of the systems. Samples were carefully transferred to the rheometer, compressed to the measurement thickness (1 mm) and »rested« for 15 min to allow any stresses induced during loading to dissipate.

Dynamic oscillatory measurements were performed on an RTI Controlled Stress Rheometer (CSR), using a 20 mm in diameter parallel plate system with 1 mm gap setting at 25 °C. Measurements were made by taking stress amplitude sweeps (torque range 1 to 3 mN m for the soft coagulum and 1 to 10 mN m for the hard coagulum and the soft cheese) at a frequency of 1 Hz, which ensured practically linear viscoelastic behaviour. Frequency sweeps were performed over the range 0.1 to 10 Hz at a torque of 1 mN m for the soft coagulum; 5 mN m for the hard coagulum and 10 mN m for the soft cheese, using the same configuration and temperature as already mentioned.

Data analysis

Statistical treatment was done on the frequency sweep data, applying simple regression analysis using the SAS statistical package (*6*) to find out how the rheological parameters (G' and G'') were related to the frequency. To compare the effect of each factor involved, 3 models of straight lines were adjusted to the general equation **y = a + bx**, where **y** is the dependent variable, **x** is the independent variable, **a** is the intercept and **b** is the slope (*7*). The frequency was taken as a linear function. To investigate whether the cadmium, had any effect on the above mentioned rheological parameters, analysis of variance (F-test) (*7*) was applied in all cases to compare 3 models of regression which described the data as (*i*) 2 independent lines (for the two treatments), (*ii*) 2 parallel lines and (*iii*) a common line; model 2 was considered as the reference. The residual sums of the squares were compared to the residual mean square value for the reference model and the F-test was used at a significance level $\alpha = 0.05$. A non-significant **F** value between models **a** and **b** means that the data fit both models, while a significant **F** value between **b** and **c** means that the data could fit a common line, with no effect of the requested factor, since there would be no evidence that the data derived from different statistical populations.

Results

The results of the amplitude sweep (storage modulus, G′, and loss modulus, G'') of the milk gels involved in this study (1 to 3 mN m for soft coagulum; 1 to 10 mN m for hard coagulum and soft cheese) were independent of the amplitude applied (e.g. controls, Fig. 1) with the values of G′ being above those of G'' over all the measured amplitude values. All samples (control and with added cadmium) behaved as weak viscoelastic systems, with plots similar in shape to those in Fig. 1. An initial addition of cadmium (0.14 mM) produced some rise in the storage and loss moduli of the milk gels, subsequent additions (0.35 and 0.88 mM) produced further significant increases in overall moduli values. This effect continued up to the maximum concentration

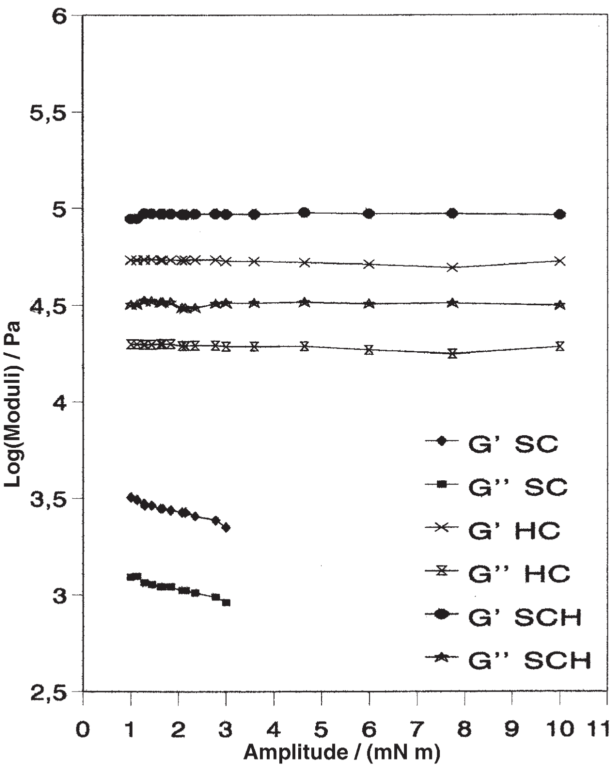

Fig. 1. Rheological behaviour (amplitude) of the controls of the soft coagulum (SC), hard coagulum (HC) and soft cheese (SCH)

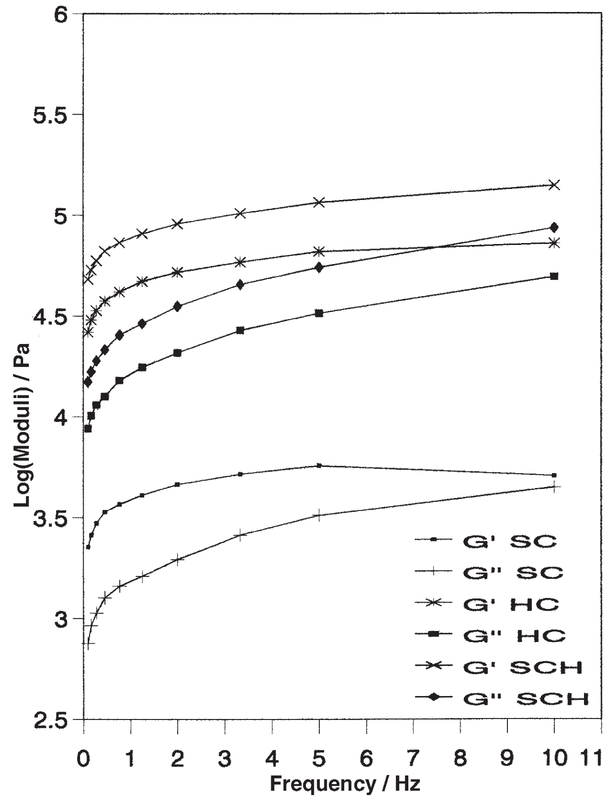

Fig. 2. Rheological behaviour (frequency) of the controls of the soft coagulum (SC), hard coagulum (HC) and soft cheese (SCH)

used (2.2 mM), with no aparent saturation point whithin the range studied. Treated samples (those with added cadmium), in general, were less amplitude dependent than the control material.

The tan δ or loss tangent values (G''/G′) obtained from the amplitude sweeps (soft coagulum) for cadmium treated samples were in a short range, and were similar to those observed in a previous study for calcium (*5*) (Table 1). This suggested that, although some differences in the moduli values were observed, the rheological systems were similar. Fig. 2 shows the typical rheological behaviour (G′ and G''; frequency sweep) of all the involved milk gels (independent controls). All the plots were characteristic of this type of system (*i.e.* G′ > G'' over all ranges measured). The plots initially showed some frequency dependence till about 2 Hz and then some plateau behaviour, where the moduli seemed to be less dependent on the frequency. Gradual addition of cadmium produced a corresponding change in both moduli values, which generally increased along with the ion concentration. Fig. 3 shows the effect of the addition of cadmium (control compared to 2.2 mM added Cd^{2+}) on G′ of the three milk systems involved (all were essentially similar in character to those already described). The frequency data for G″ followed a similar pattern of behaviour (not shown). The overall range of loss tangent values previously obtained for soft coagulum treated with calcium (*5*) (from 0.1–10 Hz) was similar to those of the cadmium treated samples (Table 2). Statistical treatment of the frequency data showed cadmium to increase both the storage modulus and the loss modulus significantly (**F** 2–3 significant), both in overall terms and incrementally (Table 3). The amplitude sweep results for the hard coagulum behaved linearly over all measured ranges (Fig. 1). It should be noted that the overall ranges of the loss tangent data were similar for calcium and cadmium (Table 1). The frequency data showed similar behaviour and were typical of this type of plot (Fig. 2). The overall range of loss tangent re-

Table 1. Overall changes in the loss tangent for the amplitude sweep of the milk gels with cation addition

Ion	Soft coagulum	Hard coagulum	Soft cheese
cd $^{2+}$	0.29–0.34	0.34–0.36	0.31–0.40
Ca^{2+}	0.31–0.37	0.33–0.36	0.30–0.40

All ion concentrations are included, the range values for Ca^{2+} were taken from reference (*5*)

Table 2. Overall changes in the loss tangent for the frequency sweep of the milk gels with cation addition

Ion	Soft coagulum	Hard coagulum	Soft cheese
Cd^{2+}	0.28–.78	0.29–0.64	0.28–0.78
Ca^{2+}	0.25–.80	0.31–0.66	0.25–0.62

All ion concentrations are included, the range of values for Ca^{2+} were taken from reference (*5*)

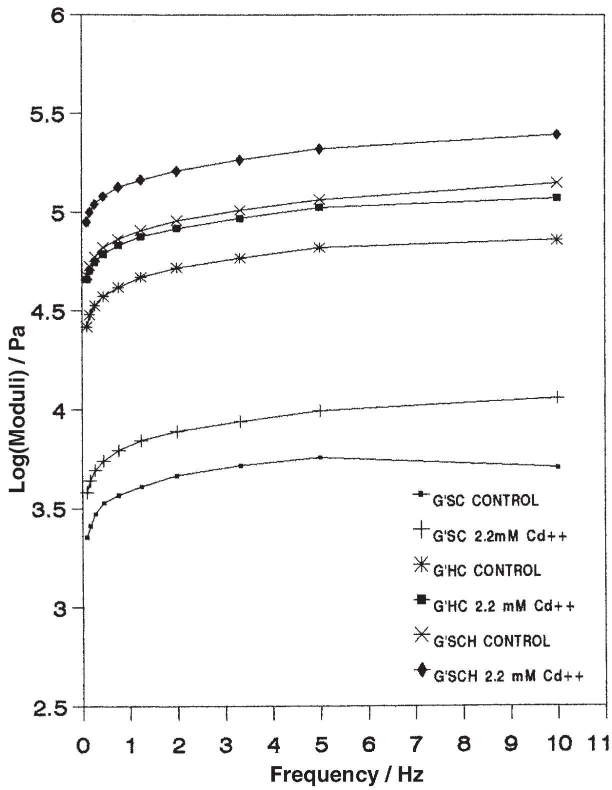

Fig. 3. Effect of 2.2 mM cadmium on the rheological behaviour of the soft coagulum (SC), hard coagulum (HC) and soft cheese (SCH)

Table 3. Effect of cadmium on the rheology of the soft coagulum

Samples	G′	G''
All together	**F**1–2 = 2.31 ns **F**2–3 = 46.4 s	**F**1–2 = 1.4 ns **F**2–3 = 32.2 s
Control/0.14 mM	**F**1–2 = 0.0012 ns **F**2–3 = 85.9 s	**F**1–2 = 0.034 ns **F**2–3 = 33 s
0.14/0.35 mM	**F**1–2 = 0.009 ns **F**2–3 = 32.2 s	**F**1–2 = 0.12 ns **F**2–3 = 29.5 s
0.35/0.88 mM	**F**1–2 = 0.053 ns **F**2–3 = 22.3 s	**F**1–2 = 0.28 ns **F**2–3 = 21.5 s
0.88/2.2 mM	**F**1–2 = 0.027 ns **F**2–3 = 14.1 s	**F**1–2 = 0.007 ns **F**2–3 = 16.8 s

F1–2 = **F** from the comparison of models 1 and 2; **F**2–3 = **F** from the comparison of models 2 and 3; s = significant at $\alpha = 0.05$; ns = nonsignificant at $\alpha = 0.05$

ported previously for calcium treated samples of hard coagulum (*5*) was similar to those found in this study for cadmium treated samples (Table 2, 0.1–10 Hz). Statistical analysis of the data (Table 4) showed that the addition of cadmium produced significant changes in both the storage (G′) and loss (G'') moduli of the hard coagulum. However, individual small additions did not always produce significant changes in the material (e.g. 0.35–0.88 mM and 0.88–2.20 mM, respectively).

Table 4. Effect of cadmium on the rheology of the hard coagulum

Samples	G′	G''
All together	**F**1–2 = 2.69 ns **F**2–3 = 1079 s	**F**1–2 = 0.007 ns **F**2–3 = 26.2 s
Control/0.14 mM	**F**1–2 = 0.38 ns **F**2–3 = 25.2 s	**F**1–2 = 0.016 ns **F**2–3 = 12.9 s
0.14/0.35 mM	**F**1–2 = 0.05 ns **F**2–3 = 8.6 s	**F**1–2 = 0.0013 ns **F**2–3 = 11.62 s
0.35/0.88 mM	**F**1–2 = 0.16 ns **F**2–3 = 2.62 ns	**F**1–2 = 0.004 ns **F**2–3 = 0.79 ns
0.35/2.2 mM	**F**1–2 = 0.07 ns **F**2–3 = 5.81 s	**F**1–2 = 0.006 ns **F**2–3 = 4.97 s
0.88/2.2 mM	**F**1–2 = 0.0006 ns **F**2–3 = 0.2 ns	**F**1–2 = 0.013 ns **F**2–3 = 0.034 ns

F1–2 = **F** from the comparison of models 1 and 2; **F**2–3 = **F** from the comparisons of models 2 and 3; s = significant at $\alpha = 0.05$; ns = nonsignificant at $\alpha = 0.05$

Table 5. Effect of cadmium on the rheology of the soft cheese

Samples	G′	G''
All together	**F**1–2 = 1.74 ns **F**2–3 = 69.3 s	**F**1–2 = 1.27 ns **F**2–3 = 38.5 s
Control/0.14 mM	**F**1–2 = 20.4 s **F**2–3 = 77.9 s	**F**1–2 = 0.32 ns **F**2–3 = 53.4 s
0.14/0.35 mM	**F**1–2 = 0.24 ns **F**2–3 = 1.93 ns	**F**1–2 = 0.004 ns **F**2–3 = 0.68 ns
0.14/0.88 mM	**F**1–2 = 0.13 ns **F**2–3 = 5.47 s	**F**1–2 = 0.47 ns **F**2–3 = 4.95 s
0.35/0.88 mM	**F**1–2 = 0.072 ns **F**2–3 = 13.8 s	**F**1–2 = 0.081 ns **F**2–3 = 0.0005 ns
0.35/2.2 mM	**F**1–2 = 5.63 s **F**2–3 = 20.2 s	**F**1–2 = 0.58 ns **F**2–3 = 11.3 s
0.88/2.2 mM	**F**1–2 = 0.26 ns **F**2–3 = 43.4 s	**F**1–2 = 0.46 ns **F**2–3 = 1.23 ns

F1–2 = **F** from the comparison of models 1 and 2; **F**2–3 = **F** from the comparison of models 2 and 3; s = significant at $\alpha = 0.05$; ns = nonsignificant at $\alpha = 0.05$

The amplitude plots of storage and loss moduli (G′ and G'') for the cheese samples made with added cadmium were similar to those observed previously for calcium (*5*). All the graphs showed a trend to be flat, indicating linear viscoelastic behaviour over all the amplitude range studied (e.g. Fig. 1). The range of the loss tangent for Ca^{2+} samples was of the same order of magnitude as that of Cd^{2+} samples (Table 1), indicating similar rheological behaviour (1–10 mN m). Similar results were observed for the frequency data (G′, Fig. 3). They were typical of this type of plot (Fig. 2), with a corresponding increase in the moduli as the cadmium concentration increased. The range of loss tangent values was also of the same order of magnitude as that of calcium (Table 2).

Table 5 shows that the addition of cadmium produced significant changes in both the storage (G′) and loss (G'') moduli of the soft cheese. However, individual small additions did not always produce significant changes in the rheological profiles at some of the added cadmium levels (e.g. 0.14–0.35 mM, respectively). It is interesting to note that in some cases the cadmium addition affected more especially G′ with no apparent effect on G'', but the reverse situation was never observed (*i.e.* 0.35/0.88 mM and 0.88/2.2 mM, Table 5).

Discussion

Milk gels consist of a network of small aggregates of micelles, alternated by thicker nodes of the same material, with pores of a few micrometers in diameter (*8*). The soft coagulum formed on renneting was not always found to follow the characteristic acidification profile expected for this type of fermentation.This difference may be explained by the toxic effect of cadmium at the highest concentration, whose biochemical properties are strongly connected with blockade of oxidative phosphorilation, not allowing the starter cultures to develop the acid environment for the proper formation of the gel (*9*). The concentration of cadmium considered as »normal« in raw milk and related dairy products ranges from 0.015 to 0.097 μg/g dry weight of samples, usually as a result of contamination (*3,9*). The coagulum produced was a weak viscoelastic gel system (*1*). The maximum stress amplitude that such a system could withstand was about 3.0 mN m, but with greater amplitude values breaking of the structure occured. This suggests that any network structure has a finite domain after which the structure breaks down to give a different rheological system. Measurements made using stress amplitude values lying in the range from 1 to 3 mN m would ensure that the rheological parameters belong to the original material. Such materials have characteristics which are consistent with the proposal that the material behaves as a »particle gel« and not as a gel consisting of cross-linked, randomly coiled macromolecules (*8*). Elimination of water resulted in the formation of the hard coagulum, with an overall strengthening of the structure, which may be due to either an increase in the number of junctions or an increase in the strength of the junctions present (*1,8*). Statistical tests were applied to determine any significant differences in the frequency profile data. When the values of the storage (G′) and loss (G'') modulus were taken as dependent variables, they showed a high degree of correlation with the values of the frequency (correlation coefficient ranged from 0.93 to 0.99). The resulting straight lines, when analyzed using analysis of variance (**F** value), resulted in data which were highly significant at the $\alpha = 0.05$ level, suggesting that the linear regression was appropriate. While a straight-line fit was considered acceptable, it was unclear if the data could always be regarded as »parallel« in nature (*i.e.* all slopes the same). Subsequently, two further models were applied in order to fully examine the data. Parallel line or the independent line models were used to best fit the data, while the »one line« model indicated effectiveness of the factor investigated.

During cheese making, the milk components could manifest some changes in their rheological properties. Initially the liquid milk is pasteurized, that could result in some denaturation of the whey proteins present (*10*), and in some limited cross-linking by disulphide interchange between the κ-casein and whey proteins (β-lactoglobulin) (*11*). The addition of starter cultures can be regarded as an acidification step, intended to produce the appropriate pH conditions for enzymic hydrolysis of the protein. The subsequent rennet addition splits the κ-casein at the junction between the para-κ-casein and caseinomacropeptide moieties, *i.e.* in bovine κ-casein, at the Phe_{105}–Met_{106} bond (*12,13*). As with any other enzymic process other factors such as temperature, pH and ionic conditions have all been reported to have significant effects on the rate of proteolysis and the subsequent rate of coagulum formation (*13–15*). To induce proper stretching of the curd, casein is transformed chemically to another form of the protein, which precipitates out at room temperature as di-calcium paracaseinate. To produce the final smooth, plastic mass, a second treatment is required for the proper stretching, which was brought about by the mentioned bacterial development, or direct introduction of acid (*16*). The rheological behavior of the resulting hard coagulum (curd) would also suggest similar linkages to those found in the soft material. However, the overall effect seems to be that of a concentration of the solids present (*17*). Heat treatment of the hard coagulm was used to produce the final stranded stretchable soft cheese. This process caused considerable denaturation of the protein (*10*) and some binding of denatured serum protein (mainly β-lactoglobulin) to casein micelles (κ-casein). Such severe changes in the biochemistry of the system might have been well expected to produce changes in the physical characteristics of the material. However, while this was observed to be the case, subsequent examination of the rheology of the final cheese indicated that under the test conditions chosen, it behaved practically as a concentrated coagulum (Fig. 2) (*1,18*). With the exception of the differing stresses that the various samples could withstand, all measured rheological profiles were similar in shape (both as functions of amplitude and frequency) and loss tangent values, being different only in the absolute values of their moduli. Both the amplitude (Fig. 1) and frequency profiles (Fig. 2 and 3) showed a significant enhancement in both the storage and loss moduli of the gel samples, made from skim milks, with cadmium addition (Tables 3–5). Statistical analysis showed that in all cases when there was a significant effect observed for the cadmium, G′ was always affected (*i.e.* **F** 2–3 significant). This suggests that the effect of cadmium is mediated *via* the protein network and acts independently. It has previously been postulated that although the chemical nature of the cross-links is not yet entirely clear, phosphoserine side chains of β-casein are probably involved (*19*). All materials showed weak viscoelastic behaviour with no evidence of any sudden changes or breakdown in structure either as a function of stress amplitude or frequency. The increase of cadmium added caused higher strengthening effects. This suggests that the cadmium may be linking »matrix« material to the network as it has been previously proposed for calcium (*20,21*). Rheological studies related to the gels formed after addition of divalent ions to milk are scarce, and those undertaken on milk coagulation (e.g. Mg^{2+}, Ba^{2+}, Mn^{2+} and Zn^{2+}) do not have a similar ionic radius as calcium. In this study, cadmium, which has the same charge as calcium and a similar ionic radius (0.99 and 0.97 Å, for Ca^{2+} and Cd^{2+}, respectively) (*4*), produced some enhancement in the structure of both the soft and hard coagulums and the final soft cheese (Fig. 3). Ranges of the loss tangent values of the milk gels on cadmium addition were similar to that observed for calcium treatment (Tables 1 and 2). This suggests that cadmium has a similar mode of action to that of the calcium.

In conclusion, all the milk gels studied behaved as weak viscoelastic materials, with the storage modulus predominating over all the measured amplitude and frequency ranges. An ion like Cd^{2+}, similar in charge and ionic radius to Ca^{2+}, produces similar effect in the rheological behaviour of milk gels obtained during the manufacture of a typical model mexican stretchable cheese, with an increase in the moduli and no apparent change in the rheological system as the level of ion addition increased. Further investigation is needed to define the mode of action of ionic metals on the milk gels, but so far, these results suggest that the divalent cations $Ca^{2+}$and Cd^{2+} may have similar mode of action.

Aknowledgement

The author greatfuly acknowledges Mr. Andrew Wilbey and Dr. Alan Bell from the Dept. of Food Sci. and Tech., University of Reading (UK), for valuable help and discussion while this work was being undertaken.

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Utjecaj dvovalentnog kationa kadmija na reološka svojstva modelnog mekog sira

Sažetak

U radu je utvrđen utjecaj iona kadmija, sličnog ionskog radijusa i naboja kao što ga ima kalcij, na reologiju modelnog mekog sira u različitim fazama proizvodnje.

Nekoliko pripravaka rekonstituiranog obranog mlijeka s 9 % (w/v) krute tvari i 4 različite koncentracije kadmija (0,14; 0,35; 0,88 i 2,2 mM) upotrijebljeno je kako bi se proizveo model meksičkog mekog rastezljivog sira, čiji su reološki parametri G' (modul skladištenja ili elastična komponenta) i G'' (gubitak modula ili komponenta viskoznosti) mjereni u raznim fazama proizvodnje, koristeći reometar s kontroliranim tlakom. Svi proizvedeni gelovi mlijeka ponašali su se kao slabi viskozno-elastični sustavi s G'>G'' tijekom svih izmjerenih amplituda i područja frekvencije. Postupno povećanje udjela dodanoga kadmija znatno povećava oba modula, bez vidljive promjene u reološkom sustavu (kratko područje gubitka tangentnih vrijednosti). Uspoređujući utjecaj kadmija s utjecajem kalcija na gelove mlijeka, vidi se da oba iona slično djeluju.