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Reversible cold gelation of sodium caseinate solutions with added salt

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During a study on the effect of addition of monovalent and divalent salts on the apparent viscosity of sodium caseinate solutions (Carr et al. 2002) it was discovered that many of the high viscosity solutions appeared to gel when refrigerated. Furthermore this cold gelation was found to reverse on heating. The phenomenon of reversible cold gelation of caseinate solutions has not been reported. The most well known example of reversible cold gelation is gelatin solutions, but a number of polysaccharides also form gels on cooling, e.g. agarose, pectin and carrageenan (Evans & Wennerstrom, 1994). Whey proteins also gel at 25 °C in the presence of calcium ions, though not in their absence, and this gelation is not reversible (Barbut & Foegeding, 1993).

The objective of this short study was to use oscillatory rheological methods to confirm the cold gelation of sodium caseinate solutions containing added salts.

Experimental

Sodium caseinate (ALANATE 180, New Zealand Dairy Board, Wellington) with a typical composition 91·3% protein, 3·5% ash, 4·0% moisture, 1·1% fat and 0·1% lactose was dissolved in water at the required solids concentration at 60 °C. To obtain caseinate solutions with a range of added salt concentrations a stock caseinate solution of 20% total solids was first prepared. Solids concentration was checked by overnight oven drying at 105 °C. Weighed quantities of this stock solution were mixed with salt solutions of various concentrations to obtain 14% w/w caseinate solutions with a range of salt concentrations. NaCl was used for most experiments but KCl, NH_4Cl and $ZnCl_2$ were also used in some trials. 1 M NaOH was added to readjust pH to 6·70. This same procedure was used for all salts. Following mixing the solutions were covered and placed in a waterbath at 60 °C for 30 min then centrifuged at 6300 × ***g*** for 10 min. This heating and centrifugation removed any air from the caseinate solutions and destroyed any remaining foam. Air removal was essential to obtain reproducible rheological results.

Rheological measurements were performed with a Bohlin VOR rheometer (Bohlin Rheologi AB, Lund, Sweden) in the oscillatory mode using the C14 coaxial geometry, 14 mm diameter bob, 15·4 mm diameter cup (Carr et al. 2002). After adding a caseinate sample to the rheometer cup the bob was lowered until it was 3 mm from the bottom of the cup and the sample temperature was then monitored. A thin layer of paraffin oil was placed on top of the caseinate solutions to prevent evaporation. After the sample temperature had reached the required test temperature a further 10 min was allowed for temperature equilibration before rheological testing commenced. This required thermal equilibration time was determined from preliminary experiments. In each experiment the solutions were held at 50 °C for 1 h, followed by cooling at 0·7 deg C/min to 5 °C. The solutions were then held at 5 °C for 1 h, followed by heating at 0·7 deg C/min to 50 °C. The frequency and shear strain amplitude were set to 1 Hz and 0·006 respectively. Rheological parameters were determined every 30 s for the duration of each experiment. For clarity only some of the data points are recorded in the figures. All experiments were performed at least twice to ensure reproducibility.

Results and Discussion

Rheological parameters during cold gelation experiments in the presence and absence of added NaCl are shown in Fig. 1. In the absence of added NaCl G′, storage modulus and G″, loss modulus both increased substantially on cooling but gelation did not occur (Fig. 1a). With 1·53 M added NaCl towards the end of the cooling stage G′ exceeded G″ (Fig. 1b), which is often taken as the definition of gel formation (Tung & Dynes, 1982). The phase angle continued to decrease, levelling off at about 12° during the period of holding at 5 °C. When the gel was reheated the phase angle increased rapidly, soon exceeding 45° indicating that the gelation was reversible. Phase angle readings were unreliable for sodium caseinate solutions at

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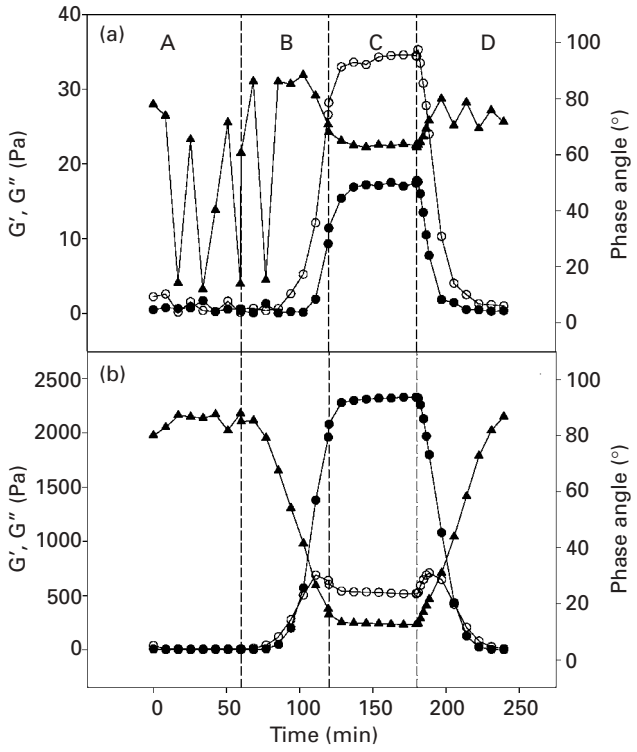

Fig. 1. The rheological parameters G′ (●), G″ (○), and phase angle (▲) during the cooling and heating of a 14% w/w sodium caseinate solution in the absence of NaCl (1a) and containing NaCl at an added ionic strength of 1·53 M (1b). The stages in the experiment were: A, holding at 50 °C; B, cooling to 5 °C; C, holding at 5 °C and D, heating to 50 °C.

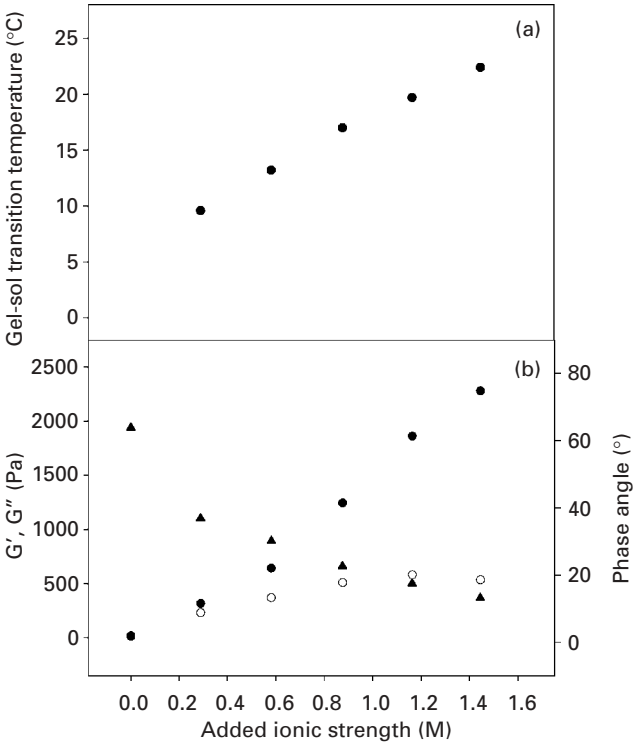

Fig. 2. Effect of NaCl concentration on the gel-sol transition temperature (2a) and on G′ (●), G″ (○), and phase angle (▲) at 5 °C (2b) for 14% w/w sodium caseinate solutions. The plotted data for G′, G″ and phase angle are the average of 120 values. The standard deviations for all the data excluding the control (no added salt) ranged from ±0·79 to ±1·68%. The standard deviations for the control data were ±4·35, 1·98, and 1·15% for G′, G″ and phase angle respectively.

50 °C with no added salt because of the relatively low viscosity but were reproducible under the other conditions examined.

Similar cold gelation behaviour was shown by sodium caseinate solutions with added KCl. However, solutions with added NH_4Cl formed gels with much lower rigidity, and solutions with added $ZnCl_2$ did not form a gel (phase angle greater than 45°) at the added ionic strength (AIS) at which they exhibited highest viscosity, 0·04.

Figure 2 shows the effects of NaCl concentration on the cold gelling characteristics of sodium caseinate. G′ increased roughly linearly with AIS and also increased much more rapidly than G″ so that even at AIS 0·3, G′ exceeded G″, i.e. the solution had gelled at 5 °C. The solution with no added salt did not gel. The gelation temperature recorded in Fig. 2a at each AIS is the mean of the gel-sol transition temperatures measured on cooling of the sol and heating of the gel. The gelation temperature (as measured by the Bohlin waterbath) was consistently 4·6 °C colder than the melting temperature; however, this difference was found to be largely due to the temperature lag between the waterbath and the sample. The gel-sol transition temperature increased approximately linearly with AIS. The generally accepted mechanism for cold gelation of gelatin solutions involves the effect of temperature on protein–protein interactions (Hearle, 1982). At elevated temperatures thermal vibrations enable the protein molecules to slide past one another, but on cooling the thermal vibrations cease to be strong enough to overcome the protein attractive forces at entanglements. The complete movement of whole chains away from one another is thus prevented. The structure solidifies as a gel.

The phase angle decreased roughly linearly with AIS over the range 0·3 to 1·4, whereas below 0·3 the rate of decrease of phase angle was greater (Fig. 2b). The shape of this plot can probably be explained by the mechanisms proposed by Carr et al. (2002) for the effect of AIS on apparent viscosity. The initial addition of salt up to AIS 0·15 suppresses the electric double layer resulting in a change in protein conformation and subsequent salt additions have a dehydrating effect on the protein.

In a review on gelation, Cheftel et al. (1985) suggested that electrostatic repulsions and protein–water interactions tend to keep polypeptide chains apart. Intermolecular protein attraction and hence gelation takes place more readily at high protein concentrations because of the greater probability of intermolecular contacts (Hearle, 1982; Cheftel et al. 1985). Perhaps salt addition to sodium

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caseinate promotes intermolecular protein interactions and so gelation by removing water from the protein backbone allowing easier protein-protein interactions.

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References

Barbut S & Foegeding EA 1993 Ca^{2+} induced gelation of pre-heated whey protein isolate. *Journal of Food Science* **58** 867–871

Carr AJ, Munro PA & Campanella OH 2002 Effect of added monovalent or divalent cations on the rheology of sodium caseinate solutions. *International Dairy Journal* **12** 487–492

Cheftel JC, Cuq JL & Lorient D 1985 Amino acids, peptides and proteins. In *Food Chemistry*, 2nd Edn., pp. 245–369 (Ed. OR Fennema). New York: Marcel Dekker

Evans DF & Wennerstrom H 1994 The colloidal domain – where physics, chemistry, biology and technology meet, pp. 289–324, New York: VCH Publishers

Hearle JW 1982 Polymers and their properties, volume 1, Fundamentals of structure and mechanics, p. 144, West Sussex: Ellis Horwood

Tung C-YM & Dynes PJ 1982 Relationship between viscoelastic properties and gelation in thermosetting systems. *Journal of Applied Polymer Science* **27** 569–574

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AS Foot CBE. MSc – Obituary.

20th January 1909–2nd March 2003

Arthur Samson Foot was born on the family dairy farm in South Dorset, where he gained practical farming and management skills. Much in his career has shown the importance of this background. He always strived to relate his science to practice. He was proud of his roots, and seldom happier than when on a farm.

He was among the first students to receive the BSc degree in agriculture from the University of Reading in 1930. After graduating he took a research assistant post at The National Institute for Research in Dairying. By 1935 Foot had published 5 papers on pig nutrition or husbandry, and one of particular significance on the rate of milking by machine. He received the degree of MSc in 1934.

Meanwhile, Maud Irvine came to Reading to study for the Diploma in Dairying. In 1936 she and two friends decided to form the first ladies team to enter the university's stock judging competition. Coached by Foot, the girls won the Cup: their coach eventually won Maud.

Sam Foot was appointed Head of the restructured Dairy Husbandry Department in 1946. The need for more and better cows, in order to apply statistical methods to experimentation, had long been recognised. Foot led the case for the expansion of the Institute's land and cattle. The purchase of Arborfield Hall Farm in 1947, and the later acquisition of Parrot Farm and Carters Hill, increased the acreage to 1110 by 1971. By 1978 there were 468 Friesian cows, and their followers. Facilities were expanded and the way was paved for the construction later of the Bernard Weitz Centre. These resources allowed significantly meaningful numbers of cows to be used in experiments ranging from extensive feeding trials to detailed investigations into the physiology and metabolism of dairy cows, or the aetiology of mastitis. Foot's vision foresaw the need for this expansion, and his successful planning enabled his colleagues to carry out some of the most comprehensive work ever undertaken in their respective fields. A far cry from 1945 when it was difficult to assemble eighteen cows for a four-month feeding trial.

Sam initiated research on many topics. These included feed for dairy cows from silage, crop drying, kale, new forage species and varieties, and systems of grazing management. Above all, he was justifiably proud that his pre-war milking machine work was the progenitor, through the collaboration of Frank Dodd, Frank Neave, Cliff Thiel and their colleagues, of the most far reaching investigations ever conducted into machine milking and udder disease. The results obtained provided dairy farmers world-wide with enormous savings. No Dorset farmer's son could wish for more.

In 1965 Sam Foot became Deputy Director of the Institute, and he was appointed CBE in 1972. He retired in September 1974. When the Institute closed in 1985 he was deeply saddened by the abandonment of so much he had worked to create. It was a consolation that the University of Reading successfully established CEDAR (the Centre for Dairy Research) at Arborfield.

As Head of Department and Deputy Director Sam Foot was noted for his calm consideration of problems, sound judgement and sage advice. None can remember him showing anger, but his disapproval could be clearly evident, and was usually justified. He was both friend and valued colleague to many.

In the life of the Institute and University and among a wide circle of friends Sam and Maud played a leading role. In retirement, he continued to take a close interest in his brother's, and more recently his nephew's farming. He so much enjoyed his last visit to Dorset.

A succession of health problems afflicted Sam in the 1990's. His courage, and Maud's heroic support in bringing him through to old age, rather bent but with his sharp mind and humour intact, have been an inspiration.

Clive C Balch and Roger Kingwill, July 2003