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Sequence-dependent kinetic trapping of biphasic structures in maltodextrin–whey protein gels

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The structural properties of maltodextrin in mixtures with native whey protein have been investigated using dynamic oscillation and a sequence of experimental time–temperature, frequency and strain sweeps. Cooling of the binary system results in a gelled maltodextrin matrix surrounding the liquid whey protein inclusions. Subsequent heating denatures the protein and the formation of a gelled filler changes the viscoelastic functions of the mixture dramatically. Modelling of the two distinct microstructures shows a clear transition from an isostrain condition with a solid-like maltodextrin matrix to an isostress arrangement where the protein filler forms the strongest phase. Estimation of the pattern of solvent partition between the two phases suggests that there is a linear, positive dependence between the relative amount of solvent kept in the liquid inclusions of whey protein and its polymer concentration. However, gelation of the protein immobilizes the mixed system and prevents, within the experimental timescale, solvent diffusion into the maltodextrin network, thus creating a constant value for the relative solvent distribution between the two phases. © 1997 Elsevier Science Ltd

INTRODUCTION

In recent years there has been much interest in the structural properties and molecular demixing phenomena of protein–protein and protein–polysaccharide mixtures. In the spreads and soft cheeses area, an inclusive patent, based largely on gelatin–maltodextrin composite gels, sets the scene for the formulation of water-continuous products with less than 5% fat (Cain *et al.*, 1989). Several developments of this have now emerged, and mixtures of maltodextrin with milk protein have also been used in an attempt to create spreadable textures suitably similar to those of butter and margarine, with pleasant flavour release (Gupta & Kasapis, 1995).

For some time now, the physical properties of biopolymer mixtures with utility as fat replacers have engaged our attention in an effort to interpret experimental observations in terms of underlying fundamentals such as molecular organisation and functions. A wide range of complementary experimental techniques are used such as centrifugation, calorimetry, light microscopy, NMR, a laser-diffraction particle size analysis, optical rotation, UV turbidimetry, visual observations and small/large deformation mechanical measurements.

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Firstly, we monitored the behaviour of gelatin–maltodextrin systems as a function of temperature over a wide concentration range (Kasapis *et al.*, 1993a). Quench cooling of transparent, single-phase solutions yields a gelatin supporting matrix which, upon ordering of the carbohydrate, shrinks gradually and allows slow diffusion of water to the maltodextrin assemblies. Well established ideas of polymer deswelling were used to rationalise the kinetics of solvent repartition between the two components (Tanaka, 1978; Ilavsky, 1981). Controlled cooling at 1°/min slows down the process of gelation that freezes the system and encourages phase separation with a concomitant reinforcement of storage modulus in the gelatin–maltodextrin gels (Alevisopoulos *et al.*, 1996). Concentrated preparations develop turbidity and phase separate in solution at equilibrium, thus allowing the construction of a phase diagram. However, the resultant microstructure of composite gels is heavily under kinetic control, since fast cooling (quenching) produces maltodextrin continuous phases whereas slower cooling (1°/min) allows phase inversion in the system and the creation of a gelatin continuous matrix. In the latter case, the composite gel collapses at a temperature congruent with the melting temperature of gelatin (≈33°C), as opposed to that of maltodextrin (≥75°C), thus offering compelling evidence that the protein component forms the only continuous phase.

Gelatin or maltodextrin phase continuity was also observed by light microscopy. Finally, application of the Takayanagi *et al.* blending laws (1963) to the mechanical properties of our mixtures indicates an isostress arrangement. Bicontinuous networks, instead, enforce isostrain conditions in the same model.

The second area of research activity, in this laboratory, dealt with the conformational influence on shaping a general outline for the interpretation of phase phenomena in mixed biopolymer gels. Commercially available soya and milk protein products undergo thermal denaturation during industrial processing, but are capable of gelation upon reconstitution of the powders (Ladd, 1994; Goodenough, 1995). Besides the soya/milk-protein mixtures, the effect of steric exclusion on the state of phase separation was examined in maltodextrin/milk-protein systems, and maltodextrin in combination with sodium caseinate, a protein that can form structures with a high viscous component ('pasty' solids). It was found that conformational similarities between the globular molecules of milk and soya proteins (Chronakis & Kasapis, 1993), and for that matter the disordered coils of gelatin and maltodextrin (see preceding section) tolerate each other and remain in a single-phase solution before the inevitable phase separation in concentrated regimes. By contrast, the conformationally dissimilar species of disordered maltodextrin chains and the thermally-unfolded milk-protein molecules (Chronakis *et al.*, 1996) or the caseinate particles (Manoj *et al.*, 1996) promote an early phase separation in solution. Cooling of these blends results in composite gels which, over a narrow concentration range, phase invert from a maltodextrin to a protein-continuous arrangement. Modelling of the phase behaviour allowed resolution of the pattern of relative water partition between the two polymer phases. It was stated that gels are kinetically trapped into arrangements where the proportion of solvent associated with one polymer is reduced as it ceases to be the supporting phase and becomes the discontinuous filler.

The above research, however, is lacking in understanding of phase behaviour in mixtures with a native globular protein. The present work focuses on the mechanical properties of native whey protein in combination with maltodextrin. Heating of the protein solution disrupts the native conformation and induces gelation, a result which can be exploited to manipulate molecular organisation in the mixtures and to test the suitability of the isostrain and isostress blending laws to biphasic maltodextrin–whey protein networks.

EXPERIMENTAL SECTION

Materials

The maltodextrin sample used was provided by Avebe, 9607 PT Foxhol, The Netherlands. It is an enzymatically converted potato starch, commercially available under the name Paselli SA-2. It has been characterised using NMR for dextrose equivalent (DE = 2.9, i.e. a number average molecular weight of ≈5600) and degree of branching (3.7%) by Kasapis *et al.* (1993b). Analysis of the same sample by gel permeation chromatography (GPC) produced a DE value of 2.7 and a symmetrical curve in the molecular weight distribution. The following slicing in molecular weight has been taken from the area observed by plotting the GPC signal intensity vs retention times (F. Deleyn, personal communication):

MW	Area (%)
<1000	2.59
$10^3-5\times10^3$	10.88
$5\times10^3-25\times10^3$	21.93
$25\times10^3-2\times10^5$	43.76
$2\times10^5-10^6$	20.27
$10^6-5\times10^6$	0.57

The moisture content of the sample is 8%. It contains 0.06% fat and 0.1% protein (N*6.25) on a dry weight basis. Atomic absorption analysis produced the following: 800 ppm phosphorus, 450 ppm potassium, 400 ppm calcium, 200 ppm sodium, 100 ppm magnesium, 5 ppm maximum sulphite content (as SO_2) and between 0.5 and 2 ppm iron.

The sample of whey protein was supplied in the form of a creamy white powder by Le Sueur Isolates, P.O. Box 144, Le Sueur, MN 56058, USA. It is a protein isolate commercially available as Bipro. Kjeldahl analysis produced a protein content (N*6.38) of 95% (dry basis), which consists of β-lactoglobulin (71%; ratio of monomer to dimer is 5:1), α-lactalbumin(18%) and 6% bovine serum albumin. Molecular weights of the proteins are 18.6 (monomer), 14.2 and 66.0 kD, respectively (Aguilera, 1995). The sample also contains ash (<3%), 1% fat, 1% lactose with the moisture content being about 4%. The isoelectric pH (pI) ranges from 4.8 for α-La and 5.1 for BSA to 5.3 for β-Lg (Kinsella & Whitehead, 1989). The pH value of solutions used is 6.8±0.1, which should ensure good solubility for the water-holding modelling of the present work. The method of production involves ultrafiltration through cellulose acetate membranes at ambient temperature which leaves the final product in the native form.

Methods

Solutions of maltodextrin (SA-2) were made by dissolving the powder in distilled water at 90°C using vigorous agitation for 15 min. NaCl (Analar grade) was then added at 1% (0.17 M). Samples were loaded on the preset platen of an oscillatory rheometer at 5°C, the top plate was lowered, and the periphery of the sample was coated with light silicone oil (100 cSt) to prevent evaporation of water. This experimentally expedient

method of quench cooling was preferred, as opposed to controlled cooling (e.g. at 1°/min), since there is an induction period before the onset of gelation during our isothermal run (3 h at 5°C; frequency of 1.6 Hz; 0.5% strain). Following this, mechanical spectra were taken within the 0.01–10 Hz frequency range, and gels were then heated to 95°C to record their melting profile at the scan rate of 1°/min. Alternatively, SA-2 gels were heated to 75°C, held there for 60 min and then cooled down, thus implementing an experimental routine analogous to the time–temperature course used for protein samples (see below). Finally, selected samples at the end of frequency sweeps (5°C) were subjected to increasing amplitude of oscillation and the pattern of network breakage was followed (frequency of 1.6 Hz).

Whey protein solutions were readily prepared by dispersing the powder in distilled water with gentle stirring at ambient temperature. NaCl was then added at 1%. Samples were loaded onto a rheometer at 45°C, heated to 75°C at a scan rate of 1°/min (frequency of 1.6 Hz; 0.5% strain), and held there for 60 min to approach asymptotically a constant storage modulus value ('pseudoequilibrium modulus'). These settings were chosen following the work of Hermansson (1986), who obtained whey protein gels with good water-holding properties by heating them to 75°C, at pH 7. Mechanical spectra were recorded at 75°C (0.01–10 Hz) after the completion of the 60 min run, and then samples were cooled to 5°C (1°/min). It was verified that the viscoelastic properties of whey protein gels show no variation with time at 5°C, and for the majority of samples we proceeded, immediately, with frequency sweeps followed by strain sweeps.

Binary mixtures were made by combining appropriate amounts of maltodextrin and protein stock preparations at 45°C, a temperature at which both components remain stable in solution. The mixed solutions were loaded onto a rheometer at 5°C (quench cooling), left there for 3 h, subjected to an increasing frequency of oscillation (0.01–10 Hz), heated to 75°C (1°/min), kept there for 60 min, subjected to another frequency sweep, cooled to 5°C at the above scan rate, held there for a second 3 h period, monitored as a function of frequency for a third time and, finally, measured under increasing amplitude of oscillation until non-linear wave forms were observed. Full spectra were, thus, obtained to show the time, temperature, frequency (ω) and strain dependence of storage modulus (G'), loss modulus (G''), tan δ (G''/G') and complex dynamic viscosity ($\eta^* = (G'^2 + G''^2)^{1/2}/\omega$). The measuring geometry was either a parallel-plate (40 mm diameter; 1 mm gap) on a commercial Carri-Med CSL500 rheometer, or a cone-and-plate (50 mm diameter; 0.05 rad cone angle) on a sensitive prototype rheometer designed and constructed in this department (Richardson & Goycoolea, 1994).

RESULTS AND DISCUSSION

Résumé of the viscoelastic functions of maltodextrin gels

The gelation process of maltodextrin samples with a dextrose equivalent (DE, the amount of measured reducing power relative to glucose as 100) in the range from 2 to 12 has been recently investigated using dynamic oscillation. Controlled cooling of solutions reveals that network formation is heavily determined by the amount of polymer, with 'semi-dilute' preparations requiring long holding periods e.g. 67 min at 5°C for a 15% C*1906 (Cerestar) sample, whereas at 50% solids the onset of gel formation is observed at ≈50°C in the cooling run (scan rate of 1°/min; Chronakis *et al.*, 1996). The effect of concentration on the development of storage modulus can also be seen in Fig. 1 where samples of SA-2 maltodextrin were quenched from 45 to 5°C. A steep rise in G' demarcates the beginning of gelation and shifts smoothly from long (55 min) to short (5 min) holding times as concentration rises from 22 to 35%. The holding stage at 5°C lasted for 3 h, thus allowing a significant further increase in the rigidity of networks. Mechanical spectra at the end of the isothermal runs argue for a solid-like response (Fig. 2) with $G' \gg G''$ and the storage modulus showing a flat frequency dependence (0.01–10 Hz). The general form of G'' trace changes as a function of SA-2 concentration from a curvature with a maximum depth at about 0.1 Hz to a signal with little frequency dependence (Fig. 2(a) and (b), respectively). It has been suggested by Manoj *et al.* (1996) that the dip at levels just above the minimum critical gelling concentration (e.g. 21.5% from Fig. 3) is due to a network comprising of mainly long helices with relaxation lifetimes longer than the experimental frequency of measurements (also discussed for mechanical spectra of swollen gelatin gels; Higgs &

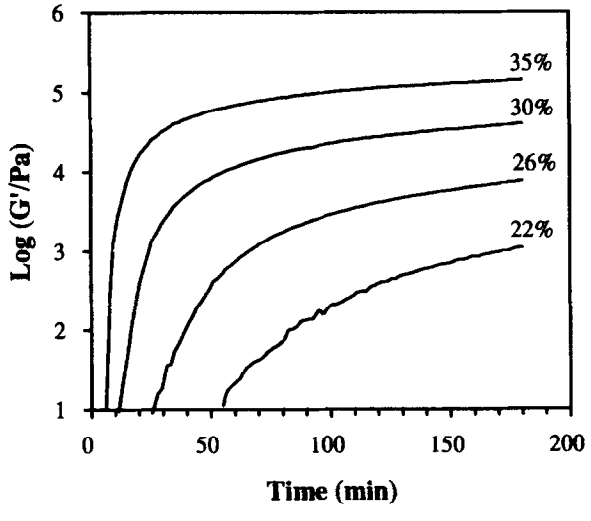

Fig. 1. The development of storage modulus for several maltodextrin samples (3 h at 5°C; 1·6 Hz; 0·5% strain).

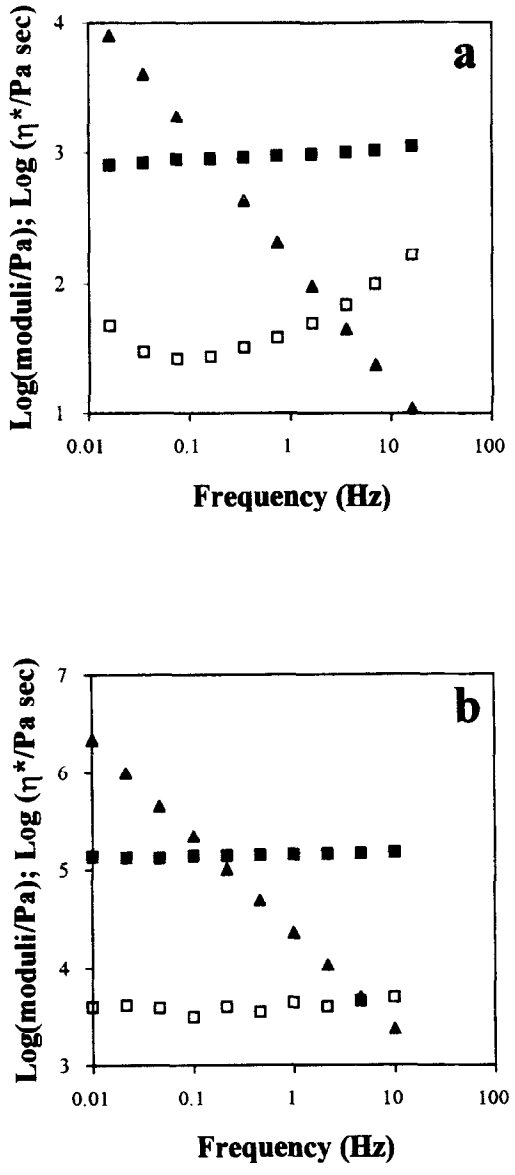

Fig. 2. Frequency variation of G'(■), G''(□), and η^* (▲) for (a) 21·5% and (b) 35% maltodextrin gels (3 h at 5°C; 0·5% strain). From Manoj *et al.*, 1996.

Ross-Murphy, 1990). Using a specially fractionated C*1906 maltodextrin sample, Chronakis *et al.* (1996) demonstrated that higher concentrations (for example 35% polymer in Fig. 2(b)) force the shorter chains to aggregate on the pre-formed nucleus, thus creating a plethora of shorter-timescale processes which are seen partly as transient at the experimental frequency range, with concomitant increase and flattening out of the G'' trace. As an index of differentiation from the viscoelastic parameters of the whey protein networks of our investigation (75°C; pH 6.8; 1% NaCl), the tan δ value of maltodextrin networks at the experimentally convenient frequency (1.6 Hz) of cooling and heating runs is 0.038±0.07.

The above frequency sweeps provided values of storage modulus which were plotted against the corresponding sample concentration (C) in Fig. 3. The fit shown was obtained using the cascade theory as proposed by Clark and Ross-Murphy, 1985. Briefly, the network connectivity in this model is defined by the valency of binding sites per macromolecule (functionality, f), the degree of cross-linking of functionalities (conversion factor, α), the probability of a chain, active or inactive, terminating (extinction probability, ν), and the equilibrium constant (K) between ordered and disordered chain segments. Based on these parameters the G' vs C function is given by the following mathematical expression:

$$KG'(f-1)(f-2)/gRT = \{C/C_o\} \{[(f-1)^2\alpha(1-\nu)^2(1-\beta)]/[2(f-2)]\} \quad (1)$$

where g is the 'front factor' which scales the absolute value of modulus, C_o is the minimum critical gelling concentration, R is the gas constant, T is the absolute temperature of the gel, and the β parameter is a function of α, f and ν. The values of C_o and f (19.3% and 2.7, respectively) both reflect the low number average molecular weight of a maltodextrin chain; about 5600 daltons (dextrose equivalent of 2.9), as in the Materials section. This contrasts strongly, for example, with at least 20 potentially available binding sites in linear and long (MW of 7×10^5) kappa-carrageenan chains which are capable of gelation at concentration of 0.5% (Rochas *et al.*, 1990; Clark, 1994). The above quantitative description of a network formed by a combination of disordered segments and associated junction zones is dramatically altered at lower molecular weight fractions of starch hydrolysates. Thus, a linear modulus-concentration fit is observed for samples with DE of 4.3 (SA-6/Avebe)

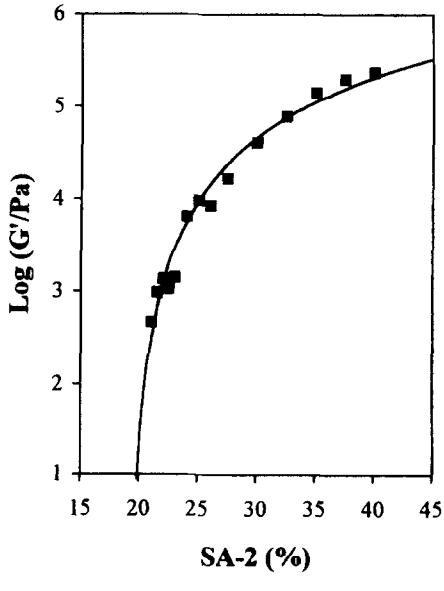

Fig. 3. Storage modulus at the end of experimentation at 5°C (3 h time and frequency sweeps) plotted against the corresponding maltodextrin concentrations (frequency of 1·6 Hz; 0·5% strain).

attributed to agglomeration of aggregated helices (Kasapis *et al.*, 1993c). The linear relationship is maintained in extensively trimmed maltodextrin chains (TrimChoice with a DE of 12/A.E. Staley) which require a solids content of about 37% to form self-supporting structures (Chronakis & Kasapis, 1995).

Mechanical spectra at 5°C were followed by heating runs to 95°C at a scan rate of 1°/min. As shown in Fig. 4(a), the intermolecular associations are reversible at high temperatures, exhibiting substantial thermal hysteresis (contrast with the extended 'lag period' preceding gelation in Fig. 1), and the melting points shift gradually from about 77 to 95°C at 23 and 40% solids, respectively. This behaviour is the result of ever growing aggregation with rising maltodextrin concentration, and has also been observed for networks of increasingly concentrated agarose gels (Watase *et al.*, 1989). Since the present study is mainly concerned with the structural characteristics of maltodextrin–whey protein mixtures, single SA-2 gels were also exposed to the heat treatment of whey protein samples in an attempt to create a baseline of behaviour for the quantitative analysis of the composites. Figure 4(b) reproduces a typical temperature–time course of G' and G'' on heating from 5 to 75°C (1°/min), and holding at 75°C for 60 min. As indicated by the pronounced reduction in modulus values, systems exhibit partial melting which stops short of complete network liquefaction at 75°C, resulting in a vestigial network with a large associated viscous component; the tan δ value derived from the flat G' and G'' traces is about 0.41. Subsequent cooling (1°/min in Fig. 4(c)) results in a rapid development of network rigidity, whereas the rise of G' for a 30% maltodextrin sample during the initial quench cooling occurs 11 min within the time sweep, at 5°C (Fig. 1). Similarly, control-cooled maltodextrin solutions at a C/C_0 ratio of 1.6 (30%/19.3% for SA-2) start gelling at about 5°C (Chronakis *et al.*, 1996). Almost certainly, the accelerated gelation in Fig. 4(c) is due to residual unmelted structures which act as templates for the crystallisation of disordered species and the rapid propagation of maltodextrin aggregates. The same phenomenon was observed by Schierbaum *et al.* (1986), whereby addition of retrograded amylose led to notable acceleration of the gelation process in maltodextrin solutions. Recently, Chronakis & Kasapis (1995) used the above approach in an effort to induce rapid gelation and minimise bulk phase separation in the production of low fat spreads, and showed that 'seeded' maltodextrin solutions form self-supporting gels in half the time needed for the unseeded counterparts.

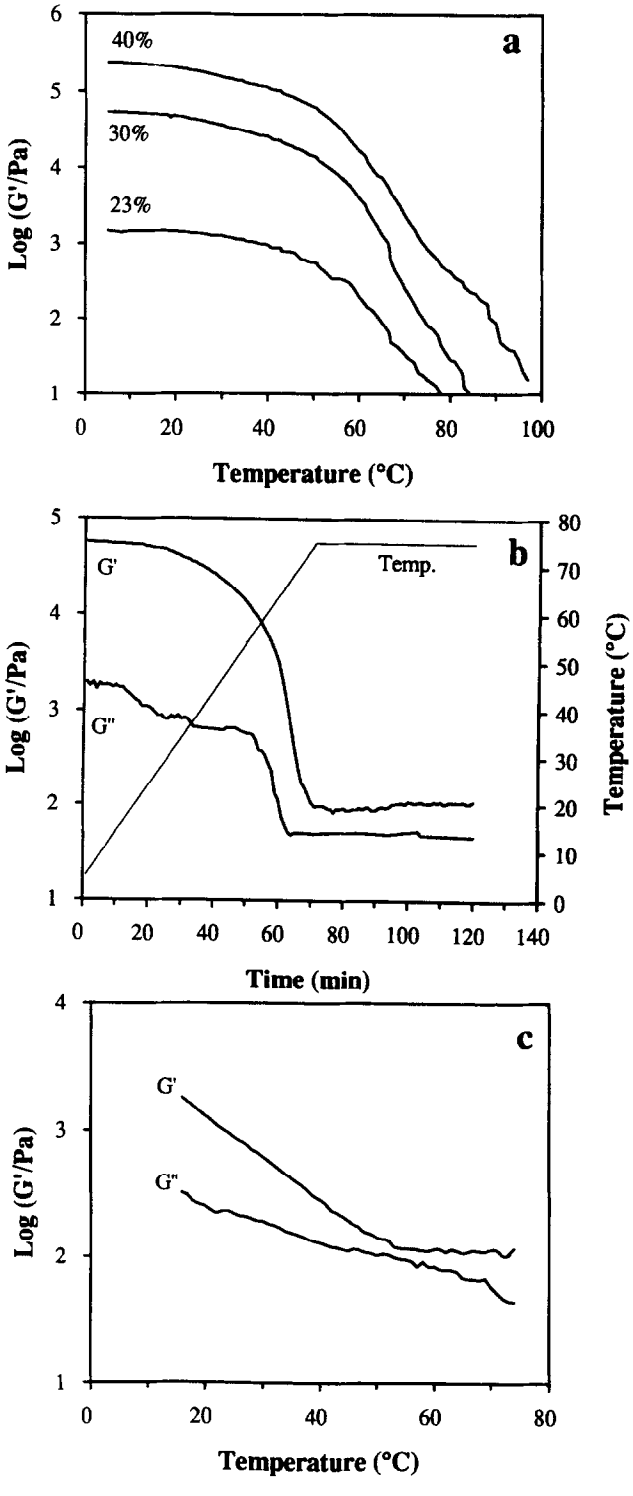

Fig. 4. Modulus variation for maltodextrin gels during: (a) controlled heating to 95°C (scan rate of 1°/min); (b) heating to 75°C (1°/min) and holding for 60 min (30% SA-2); and (c) subsequent cooling at the above scan rate (30% SA-2).

Small deformation mechanical properties of whey protein gels

The term whey protein over the years, has become a definition of convenience in numerous studies of the water- and fat-holding, viscoelastic and textural properties of its concentrated extracts from milk (McL. Whitney, 1988). It is now well established that temperature, pH and ionic strength profoundly affect the structural properties of a whey protein gel. Increasing temperature usually from 60 to 85°C induces

unfolding (denaturation) and subsequent association of the unfolded molecules (Parris & Baginski, 1991). At high enough concentrations, networks are formed which are stabilised by secondary forces (hydrogen bonding, ionic, and non-specific hydrophobic interactions, in Clark & Ross-Murphy, 1987), and specific covalent bonds (disulphide bridging, Watanabe & Klostermeyer, 1976). Thermal denaturation is pH sensitive, with minimal coagulation occurring at pH above 6.5 and below 3.7 (De Wit, 1981; Bernal & Jelen, 1985). The transparent, fine stranded whey protein gels are converted to opaque, aggregated structures at pH close to pI (4–6) or by adding cations (Hermansson, 1988; Fernandes, 1994). In the case of sodium chloride (used in this investigation), increasing concentrations cause an initial strengthening and subsequent weakening with maximum gel strength occurring between 150 and 250 mM (Mulvihill & Kinsella, 1988).

In the context of this investigation, the gelation of whey protein samples was monitored, in combination with the data on single maltodextrin systems, to assist in the proper understanding of their composites' microstructure. Dissolution of the powder in 1% NaCl solution (pH 6.8) produced clear solutions over a wide range of concentrations (from 4 to 22.5%) and a salt content typical of low fat savoury products. Heating at 75°C, however, turned the transparent solutions into cloudy gels. Electron microscopy studies by Hermansson (1986) under similar experimental conditions (pH 7), have shown the formation of a randomly aggregated network whose structure becomes coarser with increasing temperature (from 75 to 95°C) and levels of added sodium chloride (from 0 to 3%).

Figure 5(a) shows the variation in G' and G'' on heating from 45 to 75°C at 1°/min and holding there for 60 min. Judging from the steep rise in the magnitude of storage modulus, the onset of gelation is observed at about 70°C and becomes noticeable by eye in the form of substantial turbidity and the formation of a milky gel. After 60 min at 75°C the values of G' approach, asymptotically, a constant value (pseudoequilibrium modulus), and a following frequency sweep reveals gel-like spectra with flat G' and G'' traces, and a tan δ ratio of 0.095 (Fig. 5(b)). On subsequent cooling (from 75 to 5°C) a monotonic increase in G' and G'' is recorded (Fig. 6(a)), with no further change of moduli at 5°C with time. Similarly, a denatured soya protein (Supro 760/Protein Technologies International) has shown a linear gradient in the development of modulus with decreasing temperature, and this effect was attributed to a reduction in entropy which consolidated the attractive forces (hydrogen bonding, van der Waals forces) between the protein particles (Chronakis & Kasapis, 1993). Mechanical spectra of the denatured whey protein recorded at 5°C are similar to those at 75°C, but with a smaller separation between the G' and G'' traces, i.e. the tan δ value is now 0.127 (Fig. 6(b)). A simple

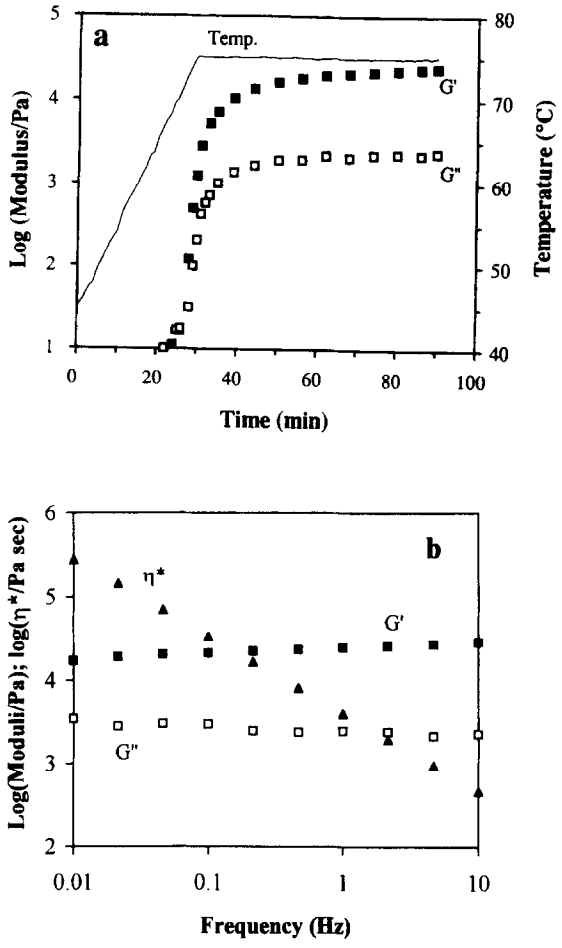

Fig. 5. Modulus development for a 15% whey protein system as a result of: (a) heating to 75°C at 1°/min and holding for 60 min; and (b) increasing the frequency of oscillation from 0·01 to 10 Hz (0·5% strain).

explanation of the upward trend in tan δ values is that the extra stabilisation due to the short-lived, non-covalent forces is seen largely as temporary within the time-scale of measurement, thus contributing mainly to the viscous component of these globular protein networks. Both the denatured whey proteins of this study and the Supro 760 (tan $\delta = 0.120$) possess viscoelastic ratios and, therefore, a viscous component, well above the values reported in the preceding section for maltodextrin gels (tan $\delta \approx 0.038$ at 1.6 Hz in Fig. 2).

The values of G' from the frequency sweeps at 75 and 5°C were plotted against the polymer concentration in Fig. 7. In both cases the development of storage modulus could be fitted by the cascade formalism in the form given by Eqn 1. This approach is derived from the Hermans (1965) theory for gelation, and both have been used to monitor the concentration dependence of shear modulus in protein gels where physical aggregates contribute to the development of an elastic component. These investigations included gels of the main component of whey protein, β-lactoglobulin, at pH 7.5 and 5.3 (Stading *et al.*, 1993), up to 22% BSA heated for 3 h at

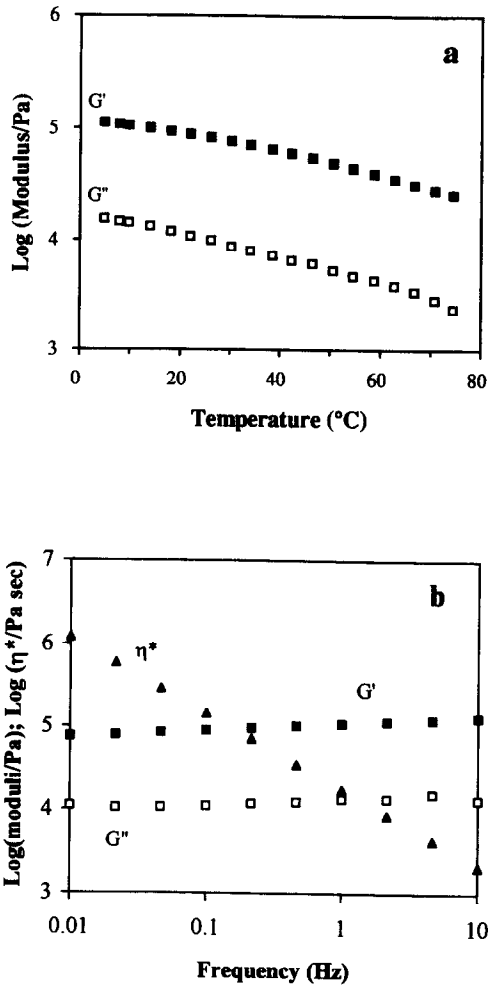

Fig. 6. Following the temperature–time treatment of Fig. 5, the 15% whey protein gel was: (a) cooled from 75° to 5°C at 1°/min; and (b) subjected to a frequency ramp at 5°C (0·5% strain).

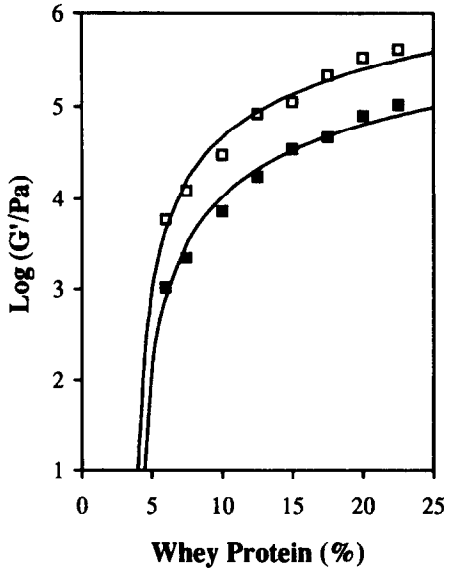

Fig. 7. The concentration dependence of storage modulus for whey protein gels left for 60 min at 75°C (■), and cooled subsequently to 5°C (□).

85°C (Richardson & Ross-Murphy, 1981), and denatured soy gels (Bikbov *et al.*, 1979; Chronakis *et al.*, 1995). Random aggregation must prevail for supramolecular webs to appear homogeneous allowing a valid, albeit simplified, quantification of the gelling properties of the systems. Moreover, the assumption of cross-linking equilibrium between free and bound sites with a constant K would be affected by the presence of covalent bonds. We have verified that heat-treated whey protein preparations in urea create transparent gels, thus making the disulphide bridges an important factor in the stabilisation of our samples; the importance of disulphide bonds in whey protein gelation has been thoroughly documented by Hillier *et al.* (1980). This aspect might cause a deviation between theory and experiment in terms of the derived parameters. Despite this, however, the following interesting points are noted from the analysis of Fig. 7.

The two curves differ slightly in shape, but substantially in magnitude of G' with increasing protein concentration, and the functionality values reflect the stronger networks at 5°C ($f=3.3$ as compared with a value of 2.8 at 75°C). Clark and Lee-Tuffnell (1986) have predicted this behaviour by feeding the cascade theory of gelation with higher values of functionality, thus creating a family of theoretical master curves which varied significantly in absolute values with increasing ratios of C/C_o. Therefore, reduction in the distances between sites with potential for non-covalent interactions (van der Waals, hydrogen bonding, electrostatic interactions) might allow them to represent a significant part of the network strength in the concentrated preparations of this investigation. However, fits converge at the 'semi-dilute' end of polymer concentration and a common minimum critical gelling concentration is obtained at 4±0.1%. Reduction in the number of protein particles/points in the three-dimensional matrix might dilute down the effectiveness of secondary bonds and expose the contribution of intermolecular –S–S– bridges created as a result of the thermal treatment at 75°C.

Observations on the structural behaviour of maltodextrin–whey protein mixtures

Following the analysis of single component gels, a series of binary mixtures was prepared at 45°C, keeping the concentration of maltodextrin constant at 20% (just above the C_o value of 19.3%) and varying the amount of whey protein from 0 to 15% in steps of 2.5%. Centrifugation of the blends at the above temperature yields a single-phase solution up to a protein level of 2.5%, whereas in the remaining samples bulk phase separation is observed in the form of a precipitate and a liquid phase. Having detected phase separation under conditions (temperature, concentration) where each

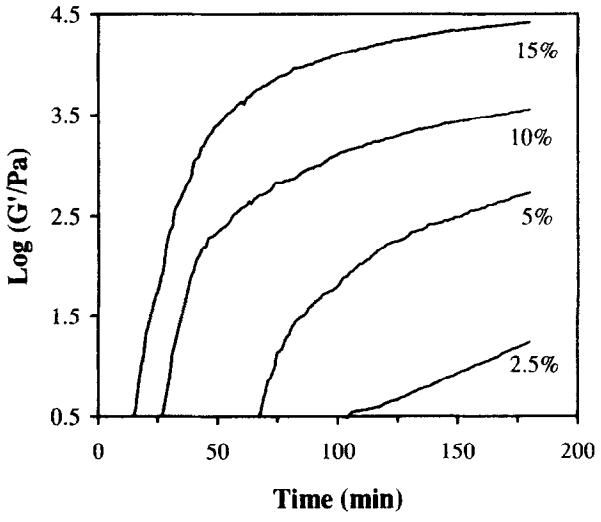

Fig. 8. Gelation for the 20% maltodextrin mixtures with the whey protein concentrations shown by the individual traces (3 h at 5°C; 1·6 Hz; 0·5% strain).

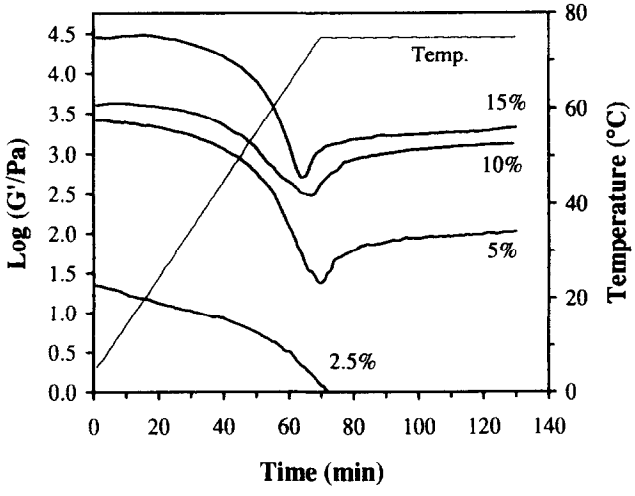

Fig. 9. Following the experiment of Fig. 8, the maltodextrin–whey protein gels were heated from 5 to 75°C (1°/min) and held for 60 min (1·6 Hz; 0·5% strain).

polymeric constituent forms a stable solution in isolation, freshly made mixed systems were quenched to 5°C and the measuring routine was implemented.

Figure 8 shows the time-dependence of storage modulus (3 h at 5°C) for selected combinations of our mixtures. At 2.5% whey protein slow augmentation of the storage modulus results in a weak maltodextrin network (G' is about 26 Pa). Taking into account that a single phase behaviour was observed in the centrifuged solution, it seems that in the absence of steric exclusions in the gel state SA-2 forms a network at its nominal concentration (20%). However, thermodynamic incompatibility between the two components at higher concentrations leads to phase separation at 45°C, and a different pattern of modulus development with time at 5°C. The outcome of steric exclusion between the two polymers is a more concentrated maltodextrin phase (i.e. the effective concentration is >20%) which is manifest in networks of enhanced rigidity at 5°C. In the absence of heat treatment whey proteins remain, of course, in the native non-gelling conformation, but are capable of holding water, thus concentrating up the continuous maltodextrin matrix. As a result the gelling transition appears earlier and earlier with increasing amounts of protein, and follows the smooth progression observed for the single maltodextrin gels (Fig. 1).

In Fig. 9 the variation of G' for the above samples is reproduced as a function of heating from 5 to 75°C (scan rate of 1°/min). This is followed by an isothermal run at 75°C for 60 min. At 2.5% whey protein, the soft maltodextrin network loses strength rapidly and eventually collapses at about 72°C. The heating regime will denature the protein, but in the absence of separation phenomena its concentration remains below the C_0 (≈4% in Fig. 7), thus being unable to form a three-dimensional network. Steric exclusion at higher levels of protein, however, results in concentrated maltodextrin networks which should withstand the thermal treatment, as illustrated for the 30% preparation in Fig. 4(b). Meanwhile, the liquid inclusions of protein transform gradually into gelled fillers which are surrounded by the weakened maltodextrin matrix, and this is monitored as a rise in G' values along the isothermal curve of Fig. 9.

Finally, the experimental procedure was concluded by cooling the blends to 5°C at the standard scan rate, and allowing the gels to mature at this temperature for 3 h (Fig. 10). Surviving amylose-like helices in solution should act as nucleation sites for aggregation thus accounting for the early development of the 20% maltodextrin network in the presence of 2.5% whey

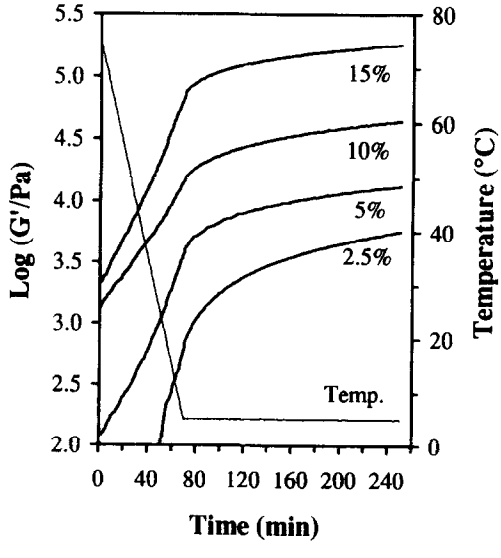

Fig. 10. Following the experiment of Fig. 9, the maltodextrin–whey protein gels were cooled at 1°/min from 75 to 5°C and kept for 3 h (1·6 Hz; 0·5% strain).

protein; according to Fig. 1, a 20% maltodextrin solution should go through conformational transition much later, towards the end of the holding period at 5°C. The bimodal profile of structure development observed in the remaining compositions can be rationalised if we consider the pattern of network formation of the individual components. Thus, the first step should be the result of attractive forces generated between adjacent polypeptide chains, since an immediate increase in storage modulus is observed upon cooling of single whey protein gels following denaturation at 75°C (Fig. 6a). The following 'wave' of structure development becomes apparent halfway through the cooling stage, which agrees well with the onset of gelation for the 'seeded' maltodextrin system of Fig. 4c. Furthermore, the second development continues during the isothermal run at 5°C, with the overall modulus of the composite growing smoothly, a property observed for ageing maltodextrin gels (Fig. 1).

Finally, Fig. 11 illustrates a representative strain sweep for a maltodextrin continuous gel supporting the inclusions of denatured whey protein. Clearly, the experimental strain of time/temperature/frequency ramps (0.5%) lies well within the linear viscoelastic region which extends up to about 2% strain. The overall breaking profile is close to that of a maltodextrin network (Manoj *et al.*, 1996), which is more strain sensitive than the structure of a whey protein gel (linear viscoelastic region extends to 40% in Steventon *et al.*, 1992).

Modelling of the storage modulus in maltodextrin–whey protein gels

The development of G' as a function of polymer concentration in single preparations (cascade fits of Figs 3 and 7), and the understanding achieved in the preceding section on the macromolecular organisation of constituent phases were employed in the quantitative analysis of the binary mixtures. This approach has already been applied to composites of maltodextrin–gelatin, maltodextrin–sodium caseinate, maltodextrin–denatured milk protein and denatured milk–soya proteins, thus providing a general outline of phase separation in terms of polymer composition, conformation and kinetics of gelation (Kasapis, 1995). A missing link in our database is the subject of this investigation, i.e. the behaviour of native globular proteins which very often are blended with other ingredients at ambient temperature and become thermally denatured during manufacturing of the commercial product (Thomsen, 1995).

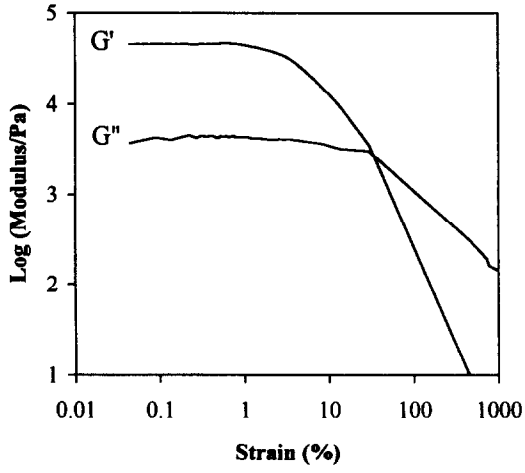

Fig. 11. Following the experimental procedure of Fig. 10, a strain sweep is shown for a 20% maltodextrin with 10% denatured milk protein mixture at 5°C (1·6 Hz).

Computations are based on the following equations which relate the moduli of the individual networks to those of the composite gels (Takayanagi *et al.*, 1963; Clark *et al.*, 1983):

$$G'_{C(U)} = \phi_x G'_x + \phi_y G'_y \quad (2)$$

$$1/G'_{C(L)} = \phi_x/G'_x + \phi_y/G'_y \quad (3)$$

where G'_C is the modulus of the composite, and ϕ_x, G'_x are the phase volume and modulus of polymer X, with ϕ_y, G'_y denoting the corresponding parameters for polymer Y. For $G'_x > G'_y$, and the polymer X forming the supporting matrix, Eqn 2 sets an upper limit or bound (isostrain case), whereas phase inversion in the system with the polymer X being now the discontinuous filler, describes via Eqn 3 a lower limit or bound (isostress case). For each experimental combination (e.g. 20% maltodextrin plus 10% whey protein) the computerised algorithm considers solvent distributions from $S_x = 1$ and $S_y = 0$ to $S_x = 0$ and $S_y = 1$ in steps of 0.005, where S_x and S_y are the solvent fractions in phases X and Y so that S_x plus S_y is always equal to one (Morris, 1992). At each solvent partition the effective polymer concentration is estimated and related to a value of storage modulus through the theoretical parameters of the cascade formalism (K, α, f) used to fit the data in Figs 3 and 7. The values of phase volume, derived from solvent fractions taking into account the contribution of polymer chains to phase density (Kasapis *et al.*, 1993a), and the calculated moduli are both used in Eqns 2 and 3, thus generating arrays of data for the moduli of composite gels.

Figure 12 illustrates the computerised output of G'_C vs solvent fraction in the maltodextrin phase (polymer X) for the first part of the experimental routine at which gels were quenched and held for 3 h at 5°C. Under these conditions a continuous solid-like matrix (maltodextrin) is penetrated by liquid inclusions (whey protein); an isostrain arrangement. Therefore, G'_y was set to zero and Eqn 2 was used to produce the upper bounds of Fig. 12. In the absence of phase separation at 2.5% whey protein, the strength of a 20% maltodextrin network falls below the values generated for a composite. For the remaining combinations, however, a

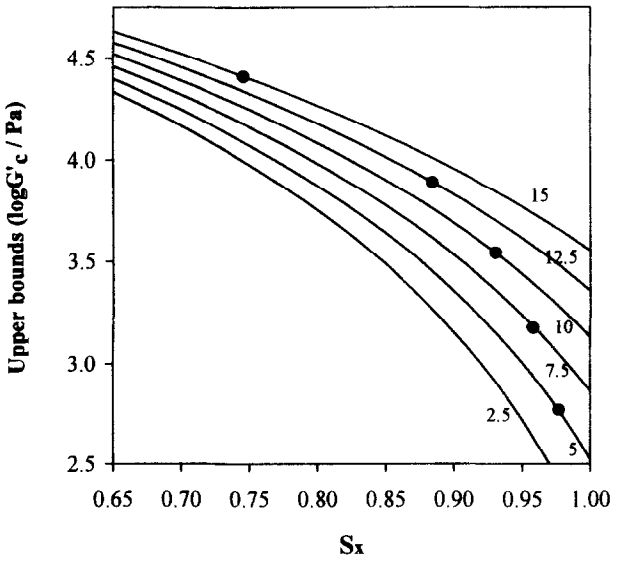

Fig. 12. Isostrain predictions for the 20% maltodextrin mixtures having been quenched and kept at 5°C for 3 h. Experimental composite moduli (●) are shown to intercept the calculated upper bounds at the appropriate whey protein concentrations (%).

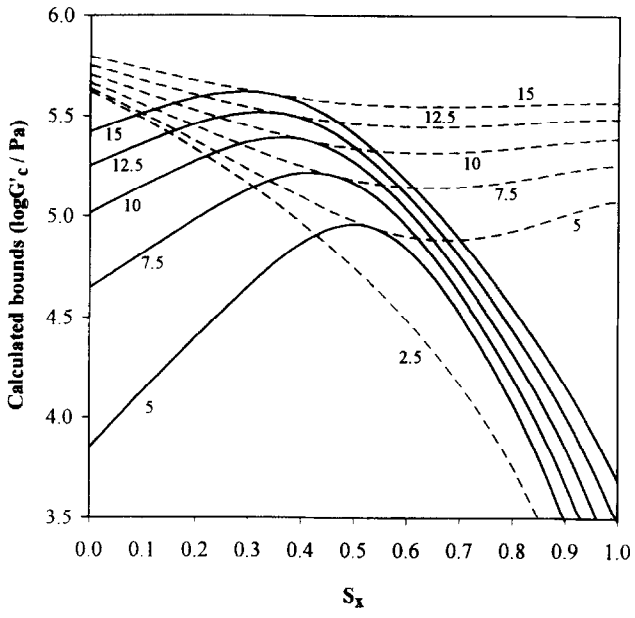

Fig. 13. Calculated master curve for the 20% maltodextrin series with the whey protein concentrations (%) shown at the corresponding bounds. The upper and lower bounds are illustrated as dashed and solid lines, respectively. This time the mixed gels were left at 5°C for 3 h, heated and held at 75°C for 1 h, and finally cooled and kept at 5°C for 3 h.

smooth trend to smaller S_x values with increasing amounts of protein is observed when experimental moduli are plotted on the corresponding bounds.

Figure 13 is the computerised output designed to check the applicability of blending laws to the mixed systems that have been taken through the full thermal treatment, i.e. loading at 5°C and keeping there for 3 h, heating to 75°C and leaving at this temperature for 1 h, cooling to 5°C and holding for a second 3 h period. As discussed in Fig. 9, whey protein denatures during heating, but does not form a continuous network at 2.5% level of solids and, therefore, the upper bound of Fig. 12 has been replotted to reflect the solid-like maltodextrin matrix formed on subsequent cooling. In the remaining combinations, however, the two polymers phase separate (centrifugation, reinforced SA-2 modulus in Fig. 8) and as a result whey proteins form structures of considerable strength (bimodal oscillatory profiles in Figs 9 and 10), thus making the consideration of both bounds necessary. At very low values of S_x, where most of the water is with the whey protein phase, the maltodextrin is extremely concentrated and, thus $G'_x \gg G'_y$. Conversely, at very high values of S_x, $G'_x \ll G'_y$. At one critical value of S_x the moduli of the two phases cross over, and under these conditions it becomes apparent from Eqns (2) and (3) that the isostrain and isostress blending laws also yield this common value ($G'_x = G'_y = G'_{C(U)} = G'_{C(L)}$). Up to this point, the upper bound value corresponds to a maltodextrin continuous system, and the lower bound value to whey protein continuous. At higher values of S_x, $G'_{C(U)}$ relates to a whey protein continuous phase and $G'_{C(L)}$ to maltodextrin continuous. As a result, maltodextrin continuous curves run from the top left to the bottom right, whereas whey protein continuous curves extend from the bottom left to the top right of the graph.

The composition of Fig. 13 is thorough, but there is too much detail. To clearly show the distribution of solvent between the two phases, part of it has been enlarged and the experimental G'_C values at the end of the second isothermal run at 5°C are shown to intercept the appropriate bounds (Fig. 14). At 2.5% whey protein in the blend, denaturation leads to more expanded (open) polypeptide chains which can exclude, effectively, the polysaccharide segments, thus creating a more concentrated and stronger maltodextrin continuous phase. As a result the experimental modulus of the composite can now mount the upper bound in Fig. 14 with the whey protein retaining about 20% of the solvent in its phase. At higher concentrations of whey protein, however, the rigidity of composite networks falls below the isostrain arrangements; this becomes clear if the intercepts of Fig. 14 are compared with the upper limits in Fig. 13. Furthermore the storage modulus values fall short of the isostress bounds for a whey protein continuous gel, a result which is in accordance with the experimental evidence of a maltodextrin supporting phase surrounding the protein filler. Instead, the moduli of mixed gels with levels of protein between 5 and 15% cross smoothly the isostress bounds for a maltodextrin continuous phase, hence creating a pattern of diminishing S_x values with increasing concentration of whey protein.

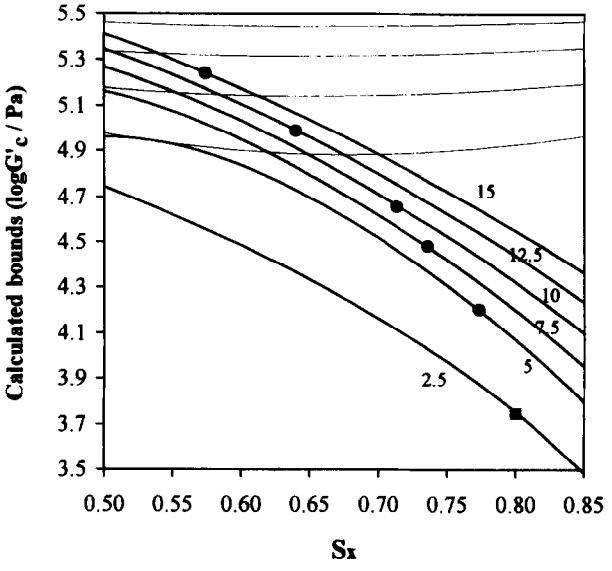

Fig. 14. An expansion from Fig. 13 showing the upper bound behaviour (maltodextrin continuous) for the 2·5% whey protein combination (■), the isostress conditions for the remaining maltodextrin continuous blends (●), and the physically unrealistic isostrain limits for whey protein continuous preparations (thin lines).

CONCLUSIONS

Thermal denaturation triggers drastic changes in the viscoelasticity of whey protein whose phase behaviour, in mixtures with maltodextrin, was examined using the blending laws. According to Eqns 2 and 3 the mechanical strength of a composite material is mainly derived from the rigidity of the continuous network, altered in a consistent manner by the presence of filler (isostrain or isostress conditions). In most examples to date, the isostress model has been more suitable for describing the properties of biopolymer composites where the moduli of the constituent phases do not differ significantly (McEvoy *et al.*, 1985; Kasapis *et al.*, 1993a). Upper bound behaviour is observed in synthetic polymer work where one component can be several orders of magnitude stronger than the other at equal concentrations, for example in dispersions of polybutadiene (PBD) in styrene-acrylonitrile copolymer (STAN) and temperatures above the glass transition temperature of PBD, but below that of STAN (Takayanagi et al., 1963; Nielsen, 1979). In the present work we have designed an upper bound behaviour for a mixture of biological macromolecules by entrapping the watery solution of native whey protein (5–15% solids range) within a gelled maltodextrin phase. Protein denaturation in these mixtures demonstrates the requirement for a versatile blending law which now models an isostress arrangement, i.e. a weaker maltodextrin continuous network surrounding the stronger beads of gelled whey protein.

As discussed in Figs 12–14 changes in the conformation of whey protein create two composites with each one revealing a distinct pattern of water partition between the constituent phases. A convenient parameter compares the amount of solvent kept in each phase per unit of the original (nominal) concentration of the appropriate polymer:

$$p = (S_x/x)/(S_y/y) \quad (4)$$

where x and y are, respectively, the percentage weights of polymers X and Y in a blend (Clark, 1987). Obviously for extensively associated biopolymer chains the solvent kept in the protein phase of a mixture and, therefore, the p value, is an average of the aggregated and non-aggregated parts of its network, which can differ significantly from the corresponding estimate for the polysaccharide network (Clark *et al.*, 1982).

Recent work by Durrani *et al.* (1993), on gelatin–amylopectin solutions, confirmed that the p factor is directly related to the Flory–Huggins interaction parameter between the two polymers (χ_{GA}). Using a least squares fit, tie-line calculations produced a phase diagram where the values of χ_{GA}, and hence of p, varied systematically with starting concentrations in the binary solution. In a personal communication to the present authors, A.H. Clark showed that for the phase diagram of gelatin–maltodextrin solutions, p values were also expected to vary systematically with polymer composition (Kasapis *et al.*, 1993b). This result was at odds with the constant value of p (1.8 in favour of gelatin), obtained upon quenching the same solutions to maltodextrin continuous composite gels (Kasapis *et al.*, 1993a). To rectify the apparent discrepancy, an extensive investigation was carried out in this laboratory which produced constant p values for several phase separated gels (Kasapis, 1995). Furthermore, it was found that phase inversion in the system, due to increasing concentration of one component, resulted in two different p values, one for the X-continuous and the other for the Y-continuous composite gels. Eventually, Alevisopoulos *et al.* (1996) manipulated the viscoelastic properties of gelatin–maltodextrin samples by controlled, slow cooling and achieved gelatin continuous composite gels which yielded a p value of 7.6 in favour of the protein. It was then clear that gelation demolishes the state of phase separation at equilibrium between two biopolymers in solution, and that in the gel state p does not reflect the relative affinity of the two biopolymers for solvent. Instead, it was proposed that gels are kinetically trapped in the composite, with the continuous network always holding disproportionate amounts of solvent. Either side of the phase inversion point, constant p values were observed which were not influenced by polymeric composition.

We feel that the solvent partition results of this investigation present a helpful picture of the importance of conformational changes in the phase behaviour of a mixed system. Rapid diffusion of water molecules from the whey protein solution to the gelling maltodextrin phase would accumulate a large body of solvent within

the polysaccharide network and create a high p value in its favour, as shown in Fig. 15. Furthermore, in the absence of a long range structure the amount of solvent kept in the whey protein phase (polymer Y) is directly related to the volume occupied by clusters of globular molecules which become more effective in excluding maltodextrin chains (polymer X), thus reducing p, at higher concentrations. In the language of polymer physics this is equivalent to a lattice model, where by increasing the chain length or the concentration of one component a relatively larger number of lattice points become unavailable to the second species thus reducing miscibility and creating a concentration or molecular weight dependence of χ (Koningsveld, 1989). Subsequent heating to 75°C results in partial melting of the maltodextrin network which allows diffusion of solvent to the gelling whey protein phase (5–15% solids in the blend). This time the enclosure of water molecules into the interior of the protein network seems permanent within the experimental timescale, and no significant redistribution of solvent is observed during subsequent cooling which, of course, reforms the maltodextrin matrix. Thus, the denaturation of whey protein has effectively frozen the system which is seen in a constant solvent partition ($p = 1 \pm 0.1$ i.e. equal solvent distribution) for the above concentration range. Along these lines, complete melting of the maltodextrin network in a mixture with 2.5% whey protein allowed the latter to entrap even more solvent, resulting in a whey protein phase now containing twice as much water per unit polymer as in the maltodextrin phase, i.e. $p = 0.5$.

Finally, experimental evidence and mechanical-analogue modelling argue for a non-interactive phase separation where the structural properties of the individual components (e.g. G' vs C relationships) are imitated in the composite gels. In the following up of this study, we have reversed the sequence of network formation, applying initial heating, instead of cooling, to the mixed solutions. This time, observations support a destructive phase separation and a co-gelling situation which cannot be visualised using the structural properties of the single maltodextrin and denatured whey protein gels.

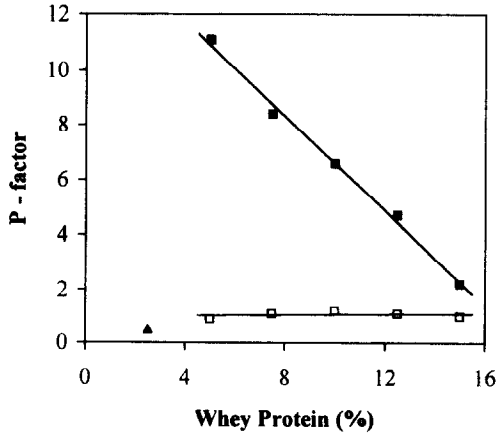

Fig. 15. Relative solvent partition data plotted against whey protein concentration for the mixed gels of Fig. 12 (■), and Fig. 14 (▲;□).

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