

## High-Pressure-Induced Rheological Changes of Low-Methoxyl Pectin plus Micellar Casein Mixtures

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The influence of high-pressure treatment (HPT) (200–800 MPa, 5 or 20 min, at 20 °C) on the rheological properties of solutions of amidated low-methoxyl pectin (LMP) and its mixtures with micellar casein (MC) has been investigated in the presence and absence of sucrose. The storage modulus  $G'$  of LMP gels containing 0–55 wt % sucrose and 0.1–1 wt % LMP was found to increase significantly following HPT at  $\geq 400$  MPa. Various concentrations of LMP in the presence of different amounts of MC (0.5–12 wt %) showed contrasting types of rheological behavior. In the presence of a low concentration of LMP (<0.3 wt %), HPT was found to induce a sol–gel transformation at relatively high LMP/MC molar ratios (<4 wt % MC), to reduce values of  $G'$  and the loss modulus  $G''$  at intermediate LMP/MC ratios (4–10 wt % MC), and to increase the values of  $G'$  and  $G''$  at low LMP/MC ratios (>10 wt % MC). In contrast, in the presence of a higher amount of LMP (>0.5 wt %), it was observed that HPT enhances the values of both the storage and the loss moduli over the whole range of MC concentrations.

**KEYWORDS:** Low-methoxyl pectin; micellar casein; high-pressure treatment; rheology; calcium ions; mixed biopolymers; dynamic mechanical testing

### INTRODUCTION

The processing of foods at high pressure, as an alternative or complementary method to conventional heat treatment, is gradually becoming a reality (1, 2). The potential for modifying food texture by this novel technology has become established from studies over the past 20 years of the effects of static high-pressure processing on the functional properties of a range of food biopolymers (3–34). Such studies have especially involved milk proteins (casein micelles and whey protein), as well as pure polysaccharides and their mixtures with milk proteins.

Information on interactions between different food ingredients during processing is essential for understanding the factors controlling food quality (35). Depending on the nature of the like and unlike intermolecular interactions, binary mixtures of proteins and polysaccharides may form single-phase or two-phase systems (36–39). Where the protein–polysaccharide interaction is net attractive, complex coacervation or associative phase separation is likely due to the formation of protein–polysaccharide complexes. The size and stability of such complexes depends on a number of factors such as pH, ionic strength, temperature, high-pressure treatment, flow conditions, and protein/polysaccharide ratio, as well as on the molecular masses and electric charge distributions of the macromolecules. A protein–polysaccharide complex may exhibit better functional properties than either of the two biopolymers used alone (37–39).

Effects of high-pressure treatment (HPT) on globular proteins have received considerable attention over the past several years (4, 7, 10–12, 18). Intramolecular hydrophobic and electrostatic interactions are disrupted by application of pressure, with important consequences for the tertiary and quaternary structures of globular proteins. As with thermal denaturation, these molecular changes can lead to protein aggregation and also to gelation under appropriate conditions. Although the effect of HPT on various polysaccharides has also been investigated, only pectin has been reported to show significant changes in rheological and physicochemical properties (3, 15, 16).

In the case of skim milk, or milk reconstituted from skim milk powder, high-pressure processing leads to substantial changes in both the whey protein fraction and the dispersed casein micelles (5, 6, 8, 9, 17, 19, 20, 23, 28, 32, 33). The lowering of the turbidity of milk on application of high pressure is attributed to the irreversible disintegration of the casein micelles into smaller structural units. Whether HPT actually induces gelation depends on the micellar protein content and the concentration of sugars (32, 33). Electron microscopy has indicated (6, 17, 23, 28) a transformation from spherical micellar particles of 100–300 nm size into chains or clusters of small polydisperse submicelles. Pressurization markedly increases the amounts of casein and minerals in the diffusible phase, but only a small increase in the free  $\text{Ca}^{2+}$  concentration has been observed (6, 23). The lack of a direct correlation between loss of micellar calcium and the level of free  $\text{Ca}^{2+}$  might indicate that pressure-induced micelle dissociation is not exclusively the result of breaking linkages between the casein and the inorganic constituents.

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The present investigation is concerned with mixtures of milk protein with pectin. Previous work in our laboratory (14, 25, 29–31) has studied the effect of HPT on electrostatic complexes of globular proteins and sulfated polysaccharides (dextran sulfate,  $\iota$ - and  $\kappa$ -carrageenans) in aqueous solution and in emulsions. The strength of complexation was found to be sensitive to pH, ionic strength, and polysaccharide charge density. The application of pressure was found to induce the formation of stronger complexes having the capacity to protect the globular proteins against pressure-induced aggregation. We previously also investigated the effect of HPT on  $\beta$ -lactoglobulin–pectin associations in emulsions and gels (26). In this paper we report on the rheology of pressure-treated systems containing a mixture of pectin and micellar casein in the presence and absence of the cosolute sucrose. We therefore describe a potential new processing approach whereby the phenomenon of pressure-induced micellar casein dissociation is exploited for the development of a new kind of microstructure in a mixed casein plus polysaccharide system.

## MATERIALS AND METHODS

**Materials.** Micellar casein (MC) was a native calcium phosphocaseinate powder that had been prepared by INRA (Rennes, France). The native phosphocaseinate was separated from raw milk by tangential membrane microfiltration (pore size = 0.2  $\mu\text{m}$ ), purified by water diafiltration, and then freeze-dried. It had the following composition (40): total protein content, 90.7%; non-casein protein, 5%; lactose, 0.5%; and salts, 8% (calcium, 3.2%). Amidated low-methoxyl pectin (LMP), GENU pectin type 101 AS provided by CPKelco, contained 36% methoxyl (= 100  $\times$  number of ester groups per 100 residues of galacturonic acid) and 14% amide esters (= 100  $\times$  number of amide groups per 100 residues of galacturonic acid). High-purity sucrose (>99.5%, S-7903), imidazole (I-0125), dihydrate calcium chloride ( $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ , C-5080), and hydrochloric acid (35%) were supplied by Sigma Chemical Co. (St. Louis, MO).

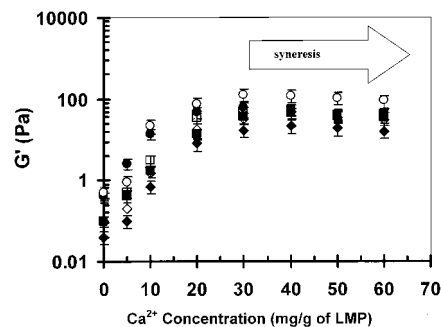
**Preparation of LMP Solution.** The LMP stock solution (5 wt %) was prepared at 25  $^\circ\text{C}$  in 5 mM/L imidazole buffer, pH 6.8, under magnetic stirring for 24 h. For preparing the LMP solution samples, sucrose (up to 55 wt %) was dissolved with stirring in 5 mM/L imidazole buffer, pH 6.8, and then  $\text{CaCl}_2$  (0–60 mg of  $\text{Ca}^{2+}$ /g of LMP) and LMP (0.1–1 wt %) were added. Afterward, the solution was stirred vigorously for 10 min.

**Preparation of Micellar Casein Dispersion.** The MC powder (10–15 wt %) was dispersed in 5 mM/L imidazole buffer, pH 6.8, at 20  $^\circ\text{C}$ . Due to the poor dispersibility of the powder, the dispersion was homogenized for  $\sim$ 15 min using a recirculating laboratory-scale Shields homogenizer (operating pressure = 40 MPa) and then sonicated in an ultrasonic bath for 20 min at 360 W. Particle size distribution was determined by light scattering using a Malvern Mastersizer 2000 (Malvern Instruments, Worcs, U.K.). Average particle diameter ( $d_{43}$ ) was in the range of 5–10  $\mu\text{m}$ .

**Preparation of Casein/Sucrose and Casein/Pectin/Sucrose Dispersions.** The mixed dispersions (MC/sucrose or MC/LMP/sucrose) were prepared at 20  $^\circ\text{C}$  under magnetic stirring conditions for 30 min. To prevent any clumping of gelled pectin, the MC was added gradually and carefully on top of diluted LMP or LMP/sucrose solutions. Some mixed dispersions were aged for 24 h without HPT treatment before rheological testing (see below) for comparison with pressure-treated samples.

**High-Pressure Treatment.** Dispersions and solutions ( $\sim$ 10 mL) were hermetically sealed in polyethylene bags and subjected to HPT at 200–800 MPa for 5–20 min in a laboratory-scale high-pressure processor (Stansted Fluid Power, Stansted, U.K.). Temperature was carefully maintained within the range of 15–30  $^\circ\text{C}$  during compression/decompression cycles and at  $20 \pm 2$   $^\circ\text{C}$  during the pressure-dwell period at the set treatment pressure, as described elsewhere (26, 32).

**Rheological Measurements.** Small-deformation rheological measurements were made in oscillation mode using a controlled-strain



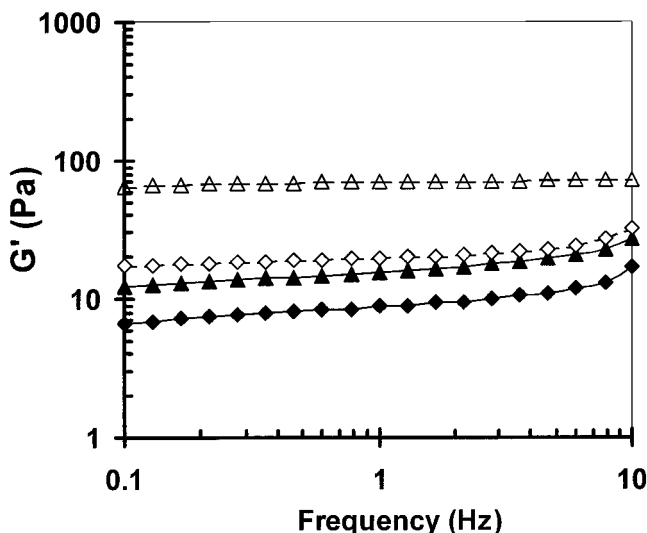
**Figure 1.** Effect of HPT (800 MPa, 20 min, 20  $^\circ\text{C}$ ) and various concentrations of  $\text{Ca}^{2+}$  on the storage modulus  $G'$  (1 Hz) of 0.5 wt % LMP solutions at pH 6.8 containing various concentrations of sucrose ( $\diamond$ ,  $\blacklozenge$ , 0 wt %;  $\square$ ,  $\blacksquare$ , 10 wt %;  $\circ$ ,  $\bullet$ , 30 wt %). Open and solid symbols represent pressurized and untreated samples, respectively. Syneresis occurs at  $\text{Ca}^{2+}$  concentrations  $> \sim 30$  mg/g of LMP.

Bohlin CVO rheometer (Bohlin Instruments, Gloucs, U.K.) with cone and plate geometry (cone angle = 4 $^\circ$ , plate diameter = 40 mm). Measurements were carried out in the linear viscoelastic regime using frequency sweep (0.1–10 Hz) or single-frequency (1 Hz) modes at 5 or 20  $^\circ\text{C}$  after an equilibration period of 15 min. Gel-like character was indicated by a higher value of the storage modulus  $G'$  as compared with the loss modulus  $G''$ . To prevent evaporation, a thin layer of low-viscosity silicone oil was used to cover the exposed edges of the samples.

## RESULTS AND DISCUSSION

**Effect of  $\text{Ca}^{2+}$  Concentration on LMP Gelation.** We first describe the influence of calcium ion concentration on the rheology of aqueous solutions of LMP at pH 6.8 in the absence of micellar casein. **Figure 1** shows how HPT (800 MPa for 20 min) affects the dependence of storage modulus  $G'$  (1 Hz, 20  $^\circ\text{C}$ ) on  $\text{Ca}^{2+}$  concentration for samples with different sucrose concentrations (0–30 wt %). Irrespective of sugar content or processing history (untreated, aged, or pressurized), the storage modulus was found to increase by a factor of  $> 10^2$  with increasing  $\text{Ca}^{2+}$  concentration over the range of 0–30 mg/g of LMP. Any further increase in  $\text{Ca}^{2+}$  concentration was found to lead to a slight reduction in the value of  $G'$ —and, more importantly, to the formation of a nonhomogeneous gel network, as identified visually by a broken loose microstructure, a turbid appearance, and clear evidence of syneresis (macroscopic serum separation).

In agreement with the existing literature (41–46), the results in **Figure 1** imply that formation of an LMP gel of maximum stiffness requires an optimum concentration of ionic calcium. Axelos and Thibault have reported (44) syneresis for  $R = 2[\text{Ca}^{2+}]/[\text{CO}_2^-] > 0.5$ , where the quantities  $[\text{Ca}^{2+}]$  and  $[\text{CO}_2^-]$  denote the molar concentrations of calcium ions and carboxyl groups, respectively. At high ionic calcium levels (high values of  $R$ ) and very low concentrations of LMP, there is no gelation; rather, aggregation of pectin occurs followed by precipitation (44). It would seem that, in the presence of excess ionic calcium, several primary units form sheetlike aggregates (45), with the excess  $\text{Ca}^{2+}$  being weakly bound. These secondary aggregates appear to add relatively little strength to the gels. Higher  $\text{Ca}^{2+}$  concentrations, especially at pH 3–5, can destroy the gel by increasing the degree of cross-linking to such an extent that pectin is precipitated (45). It is noteworthy that HPT can increase the storage modulus of samples with or without added sugars only when the calcium ion content lies below a certain critical minimum value.

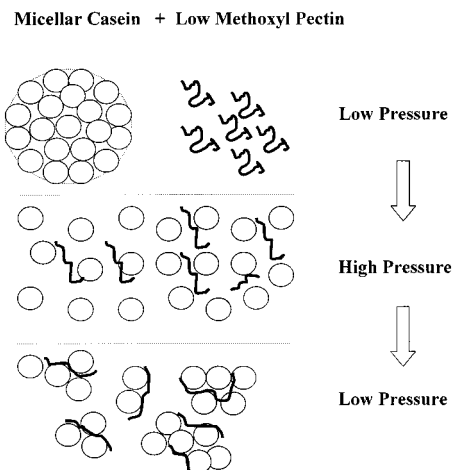


**Figure 2.** Influence of HPT (800 MPa, 20 min, 20 °C) on the frequency-dependent storage modulus  $G'$  of a 0.5 wt % LMP solution at pH 6.8 (containing 25 mg of  $\text{Ca}^{2+}$ /g of LMP) in the absence and presence of sucrose ( $\diamond$ ,  $\blacklozenge$ , 0 wt %;  $\triangle$ ,  $\blacktriangle$ , 30 wt %). Open and solid symbols represent pressurized and untreated samples, respectively.

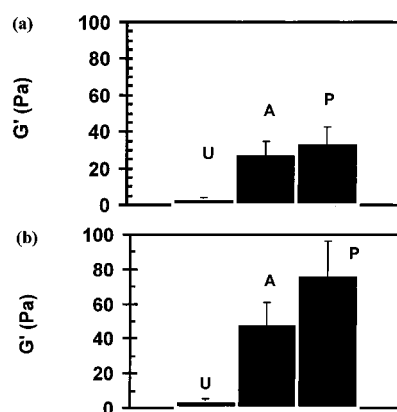
**Gelation of LMP under HPT.** The influence of HPT on the viscoelastic behavior of the LMP gel was investigated over the frequency range of 0.1–10 Hz. The storage modulus of pressurized samples containing 0.1–1 wt % LMP and 0–55 wt % sugar was always greater than that for the equivalent nonpressurized or aged samples. The effect of HPT was much greater in the presence of sucrose than in its absence. Although the storage modulus of each pressurized sample was only slightly higher than for the equivalent one aged for 24 h, it was always substantially higher than that for the nonpressurized one (**Figure 2**). No significant change was found after 20 min of HPT at <400 MPa. At 400–800 MPa, however, the maximum value of  $G'$  was induced very quickly (within 5–20 min) in pressurized samples. The induced viscoelastic changes were almost the same irrespective of the duration of the pressure treatment above 400 MPa. So, despite the time dependency of the normal gelation process, our observations confirm that the structural development of the LMP gel under pressure is mainly dependent on the intensity of pressure rather than its duration. We have observed that pressure-induced pectin gels are more elastic, homogeneous, and clear, and less brittle and sticky, than the equivalent untreated ones.

These results indicate that HPT at room temperature can produce LMP gels of high quality and enhanced rheological properties. The rate of development of the pectin gel network structure is accelerated substantially through HPT. It seems likely that pressure increase/release destroys the original (“egg-box”) structure of the pectin gel network and then re-forms it again through the redistribution of the calcium ions among the polygalacturonan chains. There may also be some simultaneous redistribution of the pectin chains. The higher quality characteristics of the reformed network may be due to a more uniform distribution of the ionic calcium. It is noteworthy that no gel is formed at  $\leq 0.1$  wt % LMP. The limiting lower concentration for gelation has been called the “critical pectin concentration” (44).

These findings are in general agreement with the existing literature (3, 15, 16). Gustin and co-workers (15, 16) found substantial effects of HPT at acidic pH on the gelation behavior of both high- and low-methoxyl pectins, but especially the latter.



**Figure 3.** Schematic representation of the proposed mechanism of pectin-casein association induced by the pressure increase/release cycle.

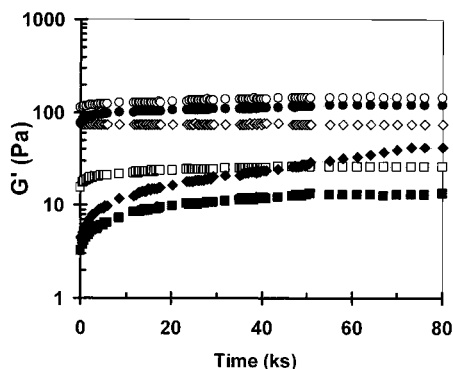


**Figure 4.** Effect of HPT (800 MPa, 20 min, 20 °C) and aging period (16 h) on storage modulus  $G'$  (1 Hz) of mixed LMP/MC dispersions at pH 6.8: (a) 1 wt % LMP + 0.8 wt % micellar casein; (b) 1 wt % LMP + 1.6 wt % micellar casein. Columns U, A, and P represent (fresh) untreated, aged, and pressurized samples, respectively.

It was reported (3) that the strength of heat-set LMP gels increased following pressurization above 100 MPa. Moreover, “crashed” heat-set gels were found to be re-formed above 100 MPa. Differential calorimetry indicated that the same pressure-induced gels were more stable to heating, due to molecular changes occurring within the network structure (as detected by IR and NMR spectroscopy).

**Gelation of LMP/MC under HPT.** We have previously described (32, 34) the effect of HPT on the gelation of dispersions of skim milk powder and native micellar casein in the presence and absence of sugars. Now we consider the influence of HPT on the gelation and rheological properties of the mixed LMP/MC system. Our hypothesis is that, on application of pressure, the protein and polysaccharide components will become much more intimately mixed together as the micellar casein dissociates. When the pressure is lowered, we postulate that the LMP may become incorporated into submicellar casein-pectin complexes or, if the total biopolymer concentration is sufficiently high, into mixed casein/pectin networks. This putative behavior is illustrated schematically in **Figure 3**.

Storage moduli for systems containing 1 wt % LMP + 0.8 or 1.6 wt % micellar casein are compared in **Figure 4**. The value of  $G'$  remains low ( $< 5$  Pa) following initial mixing of the hydrocolloid and protein components, but on quiescent aging the modulus gradually increases with time, reaching a value after  $\sim 24$  h that is some 7–10 times larger (**Figure 5**). The



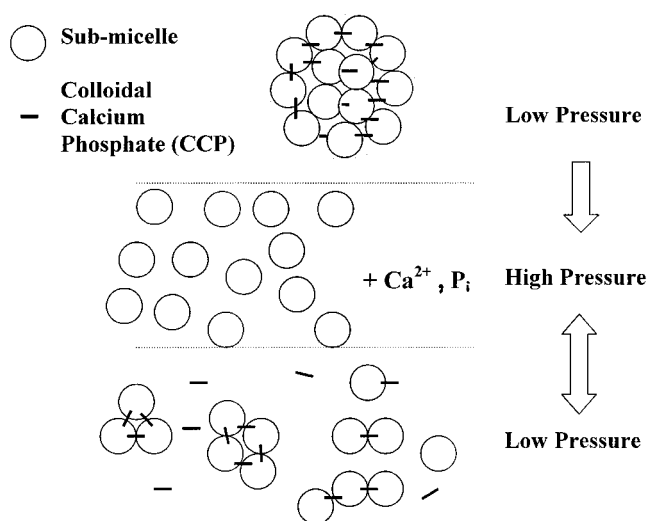
**Figure 5.** Time-dependent storage modulus  $G'$  (1 Hz) for untreated and pressurized (800 MPa, 20 min, 20 °C) mixed LMP (1 wt %) + MC gels: ○, ●, no MC present, but 25 mg  $\text{Ca}^{2+}$  per g of LMP added; □, ■, 0.8 wt % MC; ◇, ◆, 1.6 wt % MC. Open and solid symbols represent pressurized and untreated samples, respectively.

low initial values of  $G'$  in the nonpressurized mixed samples can be attributed to the low content of ionic calcium available for gelation of the LMP. This free ionic calcium corresponds to ~8–10% of the total calcium in milk (23). Typically, the LMP can take 12–16 h to reach its final gel structure (47). During this period in the presence of micellar casein the gel network can strengthen due to increased availability of diffusible calcium ions from within the casein micelles. Approximately one-third of the calcium in bovine milk is present in the diffusible phase either as free  $\text{Ca}^{2+}$  or as calcium complexed predominantly by citrate and phosphate. Through diffusive redistribution, some of this bound calcium becomes slowly available to the pectin during quiescent aging. HPT accelerates this process by rapidly dissociating the casein micelles. Therefore, in pressurized samples (800 MPa, 5 or 20 min, 20 °C), the storage modulus is higher than in nontreated or aged samples. These differences become more apparent at the higher MC concentration.

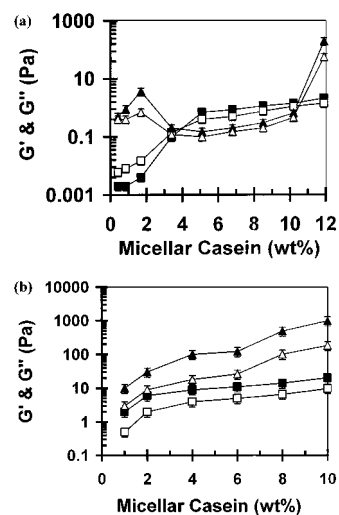
It is well documented (6, 19, 28) that HPT partially dissociates the hydrophobic interactions and the colloidal calcium phosphate (CCP), which together contribute to the internal structure of casein micelles. In other words, as shown schematically in **Figure 6**, HPT facilitates the release of CCP, thereby favoring the redistribution of calcium ions among the polygalacturonan chains to form a firm homogeneous pectin gel network.

Were the HPT to cause the complete disruption of the casein micelles, then we would expect the mixed LMP/MC system to show either the same or a higher storage modulus than that found with LMP alone in the presence of the optimum calcium ion content. (It is supposed that 0.8 or 1.6 wt % MC powder contributes, respectively, 25 or 50 mg of total calcium.) In fact, however, the storage modulus was found to be considerably lower, suggesting that only a fraction of the total calcium is actually released during pressurization. Furthermore, separate experiments have shown that mixing the LMP with pressure-treated MC dispersion does not produce any significant change in  $G'$  as compared with the simple untreated mixture of MC + LMP. That is, the value of  $G'$  is lower than for premixed pressurized samples.

On the basis of these findings, it can be inferred that (a) HPT most likely dissociates only partially the supramolecular structure of the casein micelles into free calcium (and phosphate) ions and casein submicelles (as illustrated in **Figure 6**); (b) at the higher LMP/MC molar ratios, the LMP forms a calcium-induced composite biopolymer gel (involving both free and



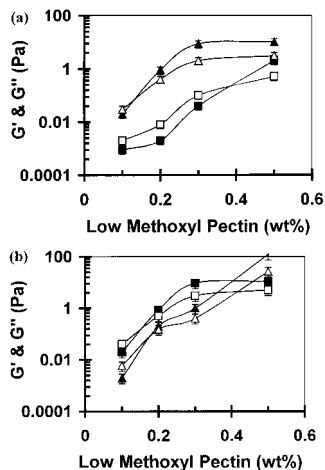
**Figure 6.** Schematic representation of the proposed mechanism of casein micellar dissociation/reassociation induced by the pressure increase/release cycle.



**Figure 7.** Influence of HPT (800 MPa, 20 min, 20 °C) on viscoelastic behavior at 1 Hz of untreated (□, ■) and pressurized (△, ▲) mixed biopolymer systems containing various concentrations of MC in 5 mM/L imidazole buffer (pH 6.8) and different concentrations of LMP: (a) 0.2 wt %; (b) 0.5 wt %. Solid and open symbols represent  $G'$  and  $G''$ , respectively.

colloidal calcium ions) with the polysaccharide network trapping (partially disrupted) casein micelles; (c) the rate of redistribution of ionic calcium in a mixed biopolymer system can be greatly enhanced by HPT; and (d) in a mixed system HPT can be used to enhance the extent of attractive unlike associations between the biopolymers.

**Effect of Biopolymer Concentration on Mixed-System Rheology.** HPT affects the rheology of the casein plus pectin system in a manner that is dependent on the concentrations of both the protein and the polysaccharide components. The behavior is illustrated in **Figure 7** by the set of graphs of storage/loss moduli versus MC concentration (up to 12 wt %) for two different LMP concentrations (0.2 and 0.5 wt %). In nonpressurized samples, the values of  $G'$  and  $G''$  were found to increase strongly with MC concentration in the range of 0.5–5 wt %, but less so for protein concentrations >5 wt %. Moreover, some phase separation became apparent at lower ratios of both biopolymers during the 3 days of monitoring. In addition, only



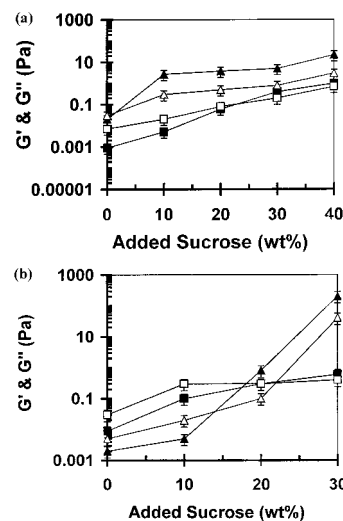
**Figure 8.** Effect of HPT (800 MPa, 20 min, 20 °C) on viscoelastic behavior at 1 Hz of untreated ( $\square$ ,  $\blacksquare$ ) and pressurized ( $\triangle$ ,  $\blacktriangle$ ) mixed biopolymer systems at pH 6.8 containing various concentrations of LMP and (a) 0.84 wt % or (b) 6.8 wt % micellar casein. Solid and open symbols represent  $G'$  and  $G''$ , respectively.

at MC concentrations  $>4$  wt % were gel-like characteristics observed in the untreated samples.

In the data in **Figure 7a** for the pressurized samples, three distinct kinds of effect can be discerned. At low concentrations of micellar casein, HPT substantially increases the moduli values, clearly converting sol-like samples ( $G' < G''$ ) into gel-like ones ( $G' > G''$ ). In contrast, over the intermediate MC concentration range, HPT leads to a marked reduction in the values of the rheological parameters. However, at high MC concentrations, significant increases in  $G'$  and  $G''$  are observed again. The results at high MC concentrations are therefore, as expected, dominated by the behavior of the casein micelles, which dissociate under pressure and then reaggregate after releasing the pressure to form a casein-dominated gel network (32–34). At low and intermediate amounts of MC, however, we postulate that a more important factor is the breakup under pressure of the CCP in the casein micelles and the subsequent binding of the released calcium ions to the polygalacturonan chains, with associated ionic interaction of LMP with fragments of the dissociating casein micelles. It seems reasonable to suppose that the anionic pectin polymers are attracted to positively charged regions on the submicellar casein polymers. This association will tend to become enhanced when the pressure is lowered at the end of the HPT cycle, as the strength of the charged interactions between the well-mixed biopolymers becomes enhanced during casein reaggregation and gelation (34, 38). Consistent with our observations on the inhibiting effect of higher amounts of  $\text{Ca}^{2+}$  on gelation of LMP, it can be assumed that the same mechanisms take place under conditions of intermediate MC concentration.

**Figure 7b** shows that HPT has a completely different effect on the rheological behavior of mixtures containing high concentrations of LMP ( $\geq 0.5$  wt %). In nonpressurized samples, there is a systematic increase in  $G'$  and  $G''$  with casein content and a dominant storage modulus over the wide range of MC. Following HPT, we observe an enhancement of the rheological parameters over the entire MC concentration range with a stronger increase at  $>6$  wt %.

The effect of HPT on the rheology of systems with low (0.84 wt %) and medium (6.8 wt %) concentrations of micellar casein is shown in **Figure 8** over the LMP concentration range of 0.1–0.5 wt %. It can be seen that the behavior is rather complex.



**Figure 9.** Effect of HPT (800 MPa, 20 min, 20 °C) and sucrose concentration on viscoelastic behavior at 1 Hz of untreated ( $\square$ ,  $\blacksquare$ ) and pressurized ( $\triangle$ ,  $\blacktriangle$ ) mixed biopolymer systems containing 0.1 wt % LMP and (a) 1 wt % or (b) 6 wt % micellar casein. Solid and open symbols represent  $G'$  and  $G''$ , respectively.

The storage and loss moduli dramatically increase with HPT at low MC content (**Figure 8a**), but the same parameters decrease in the presence of a moderate concentration of MC. These observations lead us to conclude the existence of two different mechanisms depending on the protein concentration level. At moderate concentrations of MC, the main mechanism is the incorporation of calcium ions released from dissociated casein micelles into gaps between the LMP chains to make a firm and homogeneous filled gel network. Conversely, at low MC concentrations, the main mechanism is postulated to be the increased intensity of interaction of positively charged fragments of casein micelles with negatively charged polygalacturonan chains following pressure release.

**Effect of Sucrose on LMP/MC Interaction under HPT.** **Figure 9** shows the influence of added sucrose on the rheology of mixed LMP plus MC systems before and after HPT. At constant LMP content (0.1 wt %) and low (1 wt %) and moderate (6 wt %) concentrations of casein micelles, the values of the storage and loss moduli for untreated samples were found to increase slightly with increasing sucrose content. Above 20 wt % added sucrose, the untreated samples showed gel-like characteristics ( $G' > G''$ ). Following HPT, substantial increases in  $G'$  and  $G''$  were detected in the presence of a low amount of micellar casein. In contrast, the values of the rheological parameters were observed to decrease in the presence of moderate MC concentration at 20 wt % sucrose. These results indicate that addition of sugar can induce qualitatively different effects depending on the relative proportions of the two biopolymer components.

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