Dynamic high pressure–induced gelation in milk protein model systems

E. Venir,¹ G. Marchesini, M. Biasutti, and N. Innocente

Department of Food Science, University of Udine, Udine, Italy

ABSTRACT

The structure-functional properties of milk proteins are relevant in food formulation. Recently, there has been growing interest in dynamic high-pressure homogenization effects on the rheological-structural properties of food macromolecules and proteins. The aim of this work was to evaluate the effects of different homogenization pressures on rheological properties of milk protein model systems. For this purpose, sodium caseinate (SC) and whey protein concentrate (WPC) were dispersed at different concentrations (1, 2, and 4%), pasteurized, and then homogenized at 0, 18 MPa (conventional pressure, CP), 100 MPa (high pressure, HP), and 150 MPa (HP+). Differences in viscosity were observed between WPC and casein dispersions according to concentration, heat treatment, and homogenization pressure. Mechanical spectra described the characteristic behavior of solutions except for the WPC 4% pasteurized sample, in which a network formed but was broken after homogenization. Dispersions with different ratios of WPC and SC were also made. In these systems, pasteurization alone did not determine network formation, whereas homogenization alone promoted cold gelation. A total concentration of at least 4% was required for homogenization-induced gelation in pasteurized and unpasteurized samples. Gels with higher elastic modulus (G') were obtained in more concentrated samples, and a bell-shaped behavior with the maximum value at HP was observed. The HP treatment produced stronger gels than the CP treatment. Similar G' values were obtained when different concentrations, pasteurization conditions, and homogenization pressures were combined. Therefore, by setting appropriate process conditions, systems or gels with tailored characteristics may be obtained from dispersions of milk proteins.

Key words: high-pressure homogenization, rheology, sodium caseinate, whey protein concentrate

INTRODUCTION

The structural properties of whey and casein proteins are known to be affected by high temperatures and high pressure processes. Upon heat treatment, whey proteins form aggregates and irreversible heat-set gels (Aguilera, 1995; Iordache and Jelen, 2003; Mahmoudi et al., 2007). According to the classification of Clark and Ross-Murphy (1987), thermally denatured globular proteins lead to gel networks involving specific interactions between denser and less flexible particles. The gelation of whey proteins occurs through the following steps: unfolding, which leads to exhibition of reactive sites, aggregation of partially unfolded proteins, and polymerization of the protein network (Ferry, 1948; Aguilera, 1995; Totosa et al., 2002; Foegeding, 2005). Heat-induced gelation of whey proteins depends on many factors including composition, extent of denaturation, pH, temperature, concentration, heating rate, ionic strength, and the presence of specific ions. Both fine-stranded and particulate gels may be obtained from globular proteins and whey proteins, according to gelling conditions and the extent of denaturation (Rojas et al., 1997; Foegeding, 2005). Fine-stranded gels (also called “fibrillar” or “string of beads”) are transparent or translucent and are formed under conditions of pH greater or less than the isoelectric point (pI) and at low ionic strength; particulate gels (also called “aggregated” or “coagulated”) are opaque and are formed under conditions where there is minimal charge repulsion, such as at a pH close to pI or at high ionic strength (Foegeding, 2005). Aguilera (1995) reported that unfolding-aggregation of globular proteins can also be pressure induced. High pressure would modify the native volume of proteins and induce hydrophobic interactions and form disulfide bonds between protein molecules, resulting in the rearrangement of the gel structure (Galazka et al., 2000; Totosa et al., 2002). Isostatic high pressures do not affect covalent bonds and, in turn, the primary structure. At pressures >300 MPa, hydrogen bonds are reported to be broken down (Rodiles-López et al., 2008) or disrupted and reformed (López-Fandiño, 2006), whereas moderate pressures have little influence on hydrogen bonds (Balny and Masson, 1993; Masson and Cléry, 1996). Therefore, depending on the applied

Received June 8, 2009.

Accepted October 29, 2009.

¹Corresponding author: elena.venir@uniud.it

pressure, the secondary structure may be differently affected by isostatic treatments, and moderate pressures are recognized to slightly influence secondary structure (Silva and Weber, 1993; Galazka et al., 2000; Bouaouina et al., 2006; López-Fandiño, 2006), whereas nonreversible denaturation takes place >700 MPa (Balny and Masson, 1993). The main targets of isostatic high pressure treatments are the electrostatic and hydrophobic interactions with disruption of the tertiary (>200 MPa) and quaternary (<150 MPa) structure of globular proteins (Balny and Masson, 1993; Huppertz et al., 2006a; López-Fandiño, 2006). Of all the major whey proteins, β -LG appears to be the most sensitive to pressure (Patel et al., 2005; Considine et al., 2007) leading to β -LG aggregation through both thiol-disulfide interchanges and hydrophobic interactions (Funtenberger et al., 1995, 1997). High-pressure-induced gels show different properties compared with heat-set gels (Hoover, 1993; Van Camp and Huyghebaert, 1995a; Ngarize et al., 2005; López-Fandiño, 2006). The heat-induced whey protein concentrate (WPC) gels are reported to be less porous with a more compact network structure. In this case, more long-term, intermolecular network bonds form between the adjacent polypeptide side chains (Van Camp and Huyghebaert, 1995a). In contrast, high-pressure-induced WPC gels are characterized by a porous, finely stranded network structure in which the holes around the polypeptide chains are presumably filled with nonincorporated liquid (Van Camp and Huyghebaert, 1995a). The authors attributed this structure to a greater number of weaker, short-term bonds.

Keim and Hinrichs (2004) found that the gel strength of high-pressure-induced whey protein gels increased with increasing pressure holding time because of the formation of stabilizing covalent disulfide interactions linking the network. Protein concentration also affects gelation with higher gel strength at higher concentrations (Van Camp and Huyghebaert, 1995b; Famelart et al., 1998).

Dynamic high pressure treatment is of growing interest with a continuous development in results. In early studies it was reported that high pressure (140 to 200 MPa) did not produce changes in the secondary structure of β -LG (Subirade et al., 1998; Allain et al., 1999; Hayes and Kelly, 2003). Nevertheless, the architecture of β -LG before and after high-pressure treatment is reported to be stabilized by slightly different interactions (Subirade et al., 1998), and Krimm and Bandekar (1986) hypothesized a decrease of hydrogen bonding in the β -sheet structure. More recently, Pereda et al. (2009) observed that homogenization at 200 MPa of milk induced ~32% β -LG denaturation. Ultra-high-pressure (300 MPa) treatment of whey protein isolate (WPI) dispersion caused dissociation of large protein

aggregates leading to unmasking of buried hydrophobic groups and resulting in an increase of surface hydrophobicity (Paquin, 1999; Bouaouina et al., 2006) as well as aggregation through hydrophobic interactions (Gracia-Juliá et al., 2008). Gel formation was observed upon microfluidification of WPI dispersion (Paquin, 1999).

With respect to thermal effects on caseinate, Lee et al. (1992) found an increase in the hydrophobicity after heating an aqueous solution of sodium caseinate (pH 7) at 65°C for 30 min. They also observed an inverse relation between temperature and the concentration of active SH (thiol) groups, possibly because of the formation of S-S bonds. At neutral pH, sodium caseinate may be present as polydispersed mixture of polymers of the 3 major subunits (Lee et al., 1992) or as individual casein molecules. The latter can be found in dispersions at low ionic strength (3 mM) and far from the pI, at least for concentrations less than 50 g/L (HadjSadok et al., 2008). Moreover, at high ionic strength (>100 mM), caseinate can form small aggregates that appear to be similar to native casein submicelles (Panouillé et al., 2005). Isostatic high pressures are reported to scarcely affect the functional properties of solutions of individual pure casein or sodium caseinate (Galazka et al., 2000), whereas they can cause extensive micellar dissociation at pressures above 200 MPa (Huppertz et al., 2006b) or reassociation after prolonged treatment at 200 to 300 MPa (Huppertz et al., 2006a).

Sodium caseinate suspensions are found to be Newtonian at low concentrations, whereas they become shear thinning at concentrations from >8 to 12% (wt/wt) up to 22% (wt/wt; Hermansson, 1975; Konstance and Strange, 1991; Carr et al., 2002). It has been reported that caseinate dispersions did not show yield stress, and their apparent viscosity increased as a function of solid and salt concentration and pH (Carr et al., 2002), whereas, at pH 7.00, viscosity decreased with increasing temperature from 30 to 70°C (Hayes et al., 1968).

Dispersions containing whey and casein proteins may form mixed dairy gels (Aguilera and Kessler, 1989; Aguilera and Stanley, 1999). In contrast, sodium caseinate did not form large aggregates or gel when heated at high concentrations in the absence of WPI (Guyomarc'h et al., 2009). Caseins and whey proteins are known to interact upon heating as widely reported in the literature (Mottar et al., 1989; Corredig and Dalgleish, 1999; Livney et al., 2003; Young and Foegeding, 2008; Guyomarc'h et al., 2009). Mottar et al. (1989) and Beaulieu et al. (1999a) reported that upon heating at 90°C for 10 min and 95°C for 5 min, respectively, a proportion of β -LG and α -LA may bind to casein with disulfide bonding or via β -LG/ κ -casein complexes, leading to aggregates or gels. Beaulieu et al. (1999b) suggested that, in the case of a low casein:whey protein

Table 1. Summary of the sodium caseinate (SC)–whey protein concentrate (WPC) dispersions tested

Sample code	SC (% wt/vol)	WPC (% wt/vol)
1SC+2WPC	1	2
2SC+1WPC	2	1
1SC+1WPC	1	1
2SC+2WPC	2	2
2.5SC+1.75WPC	2.5	1.75
2.5SC+2.5WPC	2.5	2.5
2.5SC+1.25WPC	2.5	1.25

ratio, 2 species of proteinaceous particles would exist: casein micelles covered and sometimes bridged by denatured whey proteins, and irregularly shaped particles composed of whey proteins. Complexation of whey proteins can also occur via α -LA/ α -LA, β -LG/ β -LG and β -LG/ α -LA, with aggregate formation dependent on their relative proportions (Rojas et al., 1997).

Until now, most work has been done on the effect of dynamic high pressure on functional properties of proteins, but less is known about the gelation of biopolymer solutions induced by homogenization. Dynamic high- and ultra-high-pressure homogenization (up to 350 MPa) technologies are of increasing interest in the dairy area because of the efficient reduction in size of the fat globules, oil droplets, and fragment particles in dispersions and emulsions (Floury et al., 2000; Hayes and Kelly, 2003; McClements, 2005; Perrier-Cornet et al., 2005; Gracia-Juliá et al., 2008). The aim of this work was to evaluate the effect of different homogenization pressures on the rheological properties of milk protein aqueous model systems. For this purpose, both sodium caseinate and WPC were dispersed at different concentrations and either pasteurized and homogenized, or both, at 0, 18, 100, and 150 MPa.

MATERIALS AND METHODS

Milk Proteins

Commercial WPC (HP880) and sodium caseinate (SC) powders (Borculo Domo Ingredients, Zwolle, the Netherlands) were dissolved in water at pH 7.00 ± 0.05 . The chemical characterization of the WPC was reported in a previous paper (Innocente et al., 2009). The WPC contained 97.34 g of dry solids per 100 g of powder and, on a dry basis (wt/wt), 77.98% total protein, 6.50% fat, 6.20% lactose, and 6.66% ash. α -Lactalbumin and β -LG accounted for about 30% and 27% per 100 g of total protein, respectively. Immunoglobulins, proteose peptone fraction, and residual casein were 23.42, 12.64, and 5.18%, respectively.

The SC contained 93.30 g of dry solids per 100 g of powder and, on a dry basis (wt/wt), 4.03% ash and 92% total proteins consisting of approximately 86% caseinate and 6% residual whey proteins. Residual fat and lactose were approximately 1.50 and 0.20% on a dry basis, respectively.

To calculate the amount of powders to be dissolved to obtain a known final protein concentration (wt/vol), the powders were dispersed in water at the final concentration of 1% (wt/vol) and pH 7.00 ± 0.05 and stored overnight at 4°C. The protein dispersions were then centrifuged ($3,000 \times g$ for 15 min at 25°C) and the total nitrogen content of the supernatant was determined by Kjeldahl method. We calculated that amounts of 1.23 and 1.45 g for SC and WPC powders, respectively, were required to obtain 1 g of final protein concentration.

Protein Dispersions

Single protein dispersions were prepared by dissolving SC and WPC in water at final protein concentrations of 1, 2, and 4% (wt/vol). Mixed protein dispersions were prepared by dissolving WPC and SC at different concentrations, according the scheme summarized in Table 1. All samples were stored overnight at 4°C to allow protein hydration before pasteurization, homogenization treatment, and rheological analyses.

Pasteurization and Homogenization Treatments

Dispersions were pasteurized (82°C for 8 min), homogenized, or both. A 2-stage mode homogenizer (Panda 2K, Niro Soavi spa, Parma, Italy) was used, and 3 passages through the homogenizer were performed for a total recycling time of 90 s. Selected homogenization pressure were as follows: conventional pressure at 15/3 MPa (CP) and high pressure at 97/3 MPa (HP) and 147/3 MPa (HP+). Inlet temperatures of 65°C for CP and 45°C for both HP and HP+ treatments were selected to achieve the same outlet temperature, which did not exceed 70°C. After homogenization, all samples were held at 4°C for 4 h without stirring before rheological analyses. The pH of dispersions was almost steady after thermal and homogenization treatments.

For each protein dispersion (SC and WPC) the following samples were considered: a control sample, neither pasteurized nor homogenized (CON); a control sample pasteurized but not homogenized (CON-P); samples homogenized at 15/3 MPa (CP), 97/3 MPa (HP), and 147/3 MPa (HP+); samples pasteurized and homogenized at 15/3 MPa (P-CP), 97/3 MPa (P-HP), and 147/3 MPa (P-HP+).

Rheological Analyses

Rheological tests were carried out by means of a controlled stress rheometer (StressTech rheometer, Reologica Instruments AB, Lund, Sweden) at $4.0^{\circ}\text{C} \pm 0.2^{\circ}\text{C}$ by using bob cup (25 mm diameter) sensor geometry. Before analysis, the sample placed in the rheometer cell rested for 5 min allowing the stress induced during loading to relax.

Steady shear flow curves were determined at shear rates ($\dot{\gamma}$) ranging from 4.410 s^{-1} to 194 s^{-1} . Mechanical spectra were obtained in a frequency range of 0.05 to 1 Hz at shear stresses within the linear viscoelastic regions that were previously determined by stress sweep tests at fixed frequencies of 0.1 and 1 Hz. The shear stress for the mechanical spectra were 0.2 Pa for WPC 4% CON-P and 0.3 Pa for all the other viscoelastic samples [2SC+2WPC, 2.5SC+1.75WPC and 2.5SC+2.5WPC [where values before SC and WPC indicate the % (wt/vol) of that component; see Table 1 for definitions of sample codes]; CP, HP and HP+; and P-CP, P-HP, and P-HP+].

Statistical Analyses

The data shown are the averages of at least 4 values. Student's *t*-test was used to compare 2 means, and one-way ANOVA (*F*-test) and Tukey's Honestly Significant Difference test were used for multiple comparison. In both cases, the differences between the means were considered statistically significant for *P*-values <0.01 . All statistical analyses were conducted using the software Statistical Discovery JMP 3.0 for Windows (SAS Institute Inc, Cary, NC).

RESULTS AND DISCUSSION

Single Protein Dispersions

Newtonian behavior at steady shear flow was observed in SC and WPC water dispersions at 1 and 2% (wt/vol) total protein, and viscosity values are reported in Table 2. The viscosity of both protein dispersions was higher in more concentrated systems, and SC dispersions showed higher values compared with WPC samples, as expected. The viscosity of WPC dispersions at 1% did not change after thermal and homogenization treatments. On the contrary, in 2% dispersions, viscosity increased after pasteurization and decreased following homogenization to values lower than those of the untreated samples. It is well known that thermal treatments lead to globular protein aggregation, depending on different factors, including concentration (Aguilera, 1995), which can cause the viscosity increase

Table 2. Mean values ($\pm$ SD) of viscosity of whey protein concentrate (WPC) and sodium caseinate (SC) dispersions

Sample ¹	Viscosity (mPa·s)	
	1% (wt/vol)	2% (wt/vol)
WPC CON	2.10 ^a $\pm$ 0	2.50 ^b $\pm$ 0
WPC CON-P	2.17 ^a $\pm$ 0.05	4.10 ^a $\pm$ 0.05
WPC P-CP	2.14 ^a $\pm$ 0.05	2.40 ^c $\pm$ 0
WPC P-HP	2.07 ^a $\pm$ 0.05	2.37 ^{cd} $\pm$ 0.05
WPC P-HP+	2.06 ^a $\pm$ 0.05	2.30 ^d $\pm$ 0
SC CON	6.05 ^a $\pm$ 0.13	11.17 ^b $\pm$ 0.15
SC CON-P	6.12 ^a $\pm$ 0.05	12.27 ^a $\pm$ 0.15
SC P-CP	4.92 ^b $\pm$ 0.15	9.10 ^c $\pm$ 0.08
SC P-HP	3.70 ^c $\pm$ 0.08	6.12 ^d $\pm$ 0.05
SC P-HP+	3.30 ^d $\pm$ 0.06	5.65 ^e $\pm$ 0.06

^{a-e}Different letters within the same column refer to statistical differences (Tukey's Honestly Significant Difference test, *P* < 0.01).

¹CON = control, no pasteurization or homogenization; CON-P = sample pasteurized but not homogenized; P-CP = pasteurized and homogenized at conventional pressure (18 MPa); P-HP = pasteurized and homogenized at high pressure (100 MPa); P-HP+ = pasteurized and homogenized at high pressure (150 MPa).

observed in pasteurized samples. Conversely, a reduction in viscosity after homogenization may be ascribed to the disruption of the aggregates formed, as reported by some authors (Aguilera, 1995; Iordache and Jelen, 2003). With respect to SC dispersions, thermal treatment did not affect the viscosity of the 1% sample, whereas a slight viscosity increase was observed in the 2% sample. It should be noted that pasteurization accounted for a 64% increase in viscosity in the 2% WPC samples, whereas it was equal to 10% in the 2% SC dispersions. Pasteurization slightly affects caseinate as reported by Chu et al. (1995) and HadjSadok et al. (2008). Heating overnight at 70°C leads to precipitation of a small weight fraction ($<5\%$) of material. Most of the caseinate is heat stable and the aggregation number increases reversibly up to 50°C , remaining constant at higher temperatures up to at least 70°C (HadjSadok et al., 2008). Conversely, milk globular proteins are unfolded at temperatures in the range from 59 to 82°C (Aguilera, 1995). In both 1% and 2% SC solutions (Table 2), homogenization decreased viscosity below initial values, with lower viscosity at higher pressures, possibly due to disaggregation. The diameter of caseins micelles is known to decrease following high-pressure homogenization (Roach and Harte, 2008). Viscosity of 2% WPC and 2% SC diminished from 2.5 to 2.3 mPa·s and from 11.17 to 5.65 mPa·s respectively. The greatest viscosity change of sodium caseinate would indicate a different behavior to high-pressure homogenization with respect to WPC, probably because of disaggregation of the submicellar fraction of caseinate.

Flow curves and viscosity values of single SC and WPC samples at 4% (wt/vol) are shown in Figure

Figure 1. Flow curves and mean viscosity values of 4% (wt/vol) sodium caseinate (SC) and whey protein concentrate (WPC) samples. CON = untreated; CP = homogenized at 18 MPa; HP = homogenized at 100 MPa; HP+ = homogenized at 150 MPa; CON-P: pasteurized; P-CP = pasteurized and homogenized at 18 MPa; P-HP = pasteurized and homogenized at 100 MPa; P-HP+ = pasteurized and homogenized at 150 MPa.

1. For all 4% SC samples, Newtonian or slight shear-thinning behavior was observed. Similarly, Hermansson (1975) and Konstane and Strange (1991) observed Newtonian behavior for caseinate dispersions at concentrations <12%. Pitkowsky et al. (2008) observed the viscoelastic behavior of sodium caseinate suspensions above approximately 100 g/L. Comparing the curve slopes in Figure 1A, a viscosity increase resulting from thermal treatment was observed. Homogenization of the SC dispersion led to a decrease in viscosity and the flow curves of P-CP, P-HP, and P-HP+ were lower than that of CON. The slope of P-CP was higher than the slopes of the P-HP and P-HP+ curves, which were superimposable. The CON and homogenized WPC samples showed Newtonian behavior, whereas the pasteurized (CON-P) solution deviated from linearity and displayed shear-thinning behavior (Figure 1B). The non-Newtonian curve was fitted with Herschel Bulkley equation ($R^2 = 1$) and the yield stress (1 ± 0.17 Pa), flow index (0.68 ± 0.01), and consistency index (0.17

± 0.02 Pa·sⁿ) were determined. The consistency index indicated higher viscosity with respect to Newtonian samples.

Conversely, homogenization led to a viscosity decrease close to the CON value. Shear-thinning behavior generally arises from nonsymmetrical, flow-orienting particles or from gel networks that confer viscoelastic properties. The latter are evaluated in the dynamic mode by mechanical spectra.

Mechanical spectra of 4% pasteurized WPC sample showed values of elastic modulus (G') higher than viscous modulus (G''), both being slightly frequency dependent; phase angle was about 30 to 40 degrees, also slightly frequency dependent; complex viscosity (η^*) decreased with increasing frequency. These rheological data indicated that development of an elastic component, with an improvement of viscoelastic properties, occurred in 4% pasteurized WPC. These results are consistent with a weak gel behavior, according to the conditions given by Ross-Murphy (1995).

It has been reported that whey protein gelation is the result of both physical (electrostatic and hydrophobic) and chemical (disulfide) interactions between the constituting protein molecules. The destabilization of the native tertiary folding of the proteins promotes the interactions between them to a level that ultimately causes the formation of a stable network and gelation (van Vliet et al., 2004). Havea et al. (2004) found that WPC dispersions heated at 85°C for 10 min contained both disulfide bonds and noncovalent linkage, the latter influencing the properties of heat-induced WPC gels.

Heat-induced gelation of whey proteins is a well-known phenomenon related to their thermal denaturation, which occurs at 76 to 82°C for β -LG, 59 to 62°C for α -LA, and 72 to 74°C for BSA (Bernard and Jelen, 1985). Heat denaturation consists mainly of the unfolding of the globular whey proteins, which may undergo to aggregation leading to gel formation (Aguilera, 1995). Fine-stranded gels were obtained at pH ~7 after heat treatment at 80 to 85°C by Rojas et al. (1997). Gelation occurred at minimum concentrations of 80 g/L of β -LG + 20 g/L of α -LA. Compared with the β -LG alone, replacing β -LG with an equal amount of α -LA enhanced gelation of the blend. As the amount of α -fraction replacing the β -fraction increased beyond 40 g/L, gelation decreased below the level of the β -fraction alone and approached that of the α -fraction, suggesting that an optimum value for gelation enhancement exists (Rojas et al., 1997). In the present work, the 4% WPC dispersion contained more α -LA (approximately 12.8 g/L) than β -LG (approximately 10.6 g/L), which could account for gelation at different concentrations, which vary from those appearing in the literature.

Flow curves of the 4% WPC pasteurized sample showed Newtonian behavior after homogenization (Figure 1B), indicating that gel network breaking had occurred. Because gelation may be pressure induced (Hoover, 1993; Anema, 2008), the effect of homogenization pressure was also tested on the 4% WPC non-pasteurized dispersions (CP, HP, and HP+). Without pasteurization, the sample did not form a gel network and exhibited Newtonian behavior with viscosity values lower than the CON samples (Table 3). Results for single milk protein dispersions proved that heat treatment was efficient in promoting gel formation of 4% WPC dispersion, whereas homogenization, independently of concentration and applied pressures, did not produce a gel-like network and possibly destroyed the existing one. Similarly, Grácia-Juliá et al. (2008) did not find viscosity changes after homogenization at 100 and 150 MPa of WPI dispersions at 6% (pH 6.5), whereas a small but significant increase was caused by short-time thermal treatment at 82°C.

Table 3. Mean values ($\pm$ SD) of viscosity of 4% (wt/vol) whey protein concentrate (WPC) samples not pasteurized

Sample ¹	Viscosity (mPa·s)
CON	3.80 ^a $\pm$ 0.00
CP	3.30 ^b $\pm$ 0.10
HP	3.17 ^b $\pm$ 0.06
HP+	3.23 ^b $\pm$ 0.15

^{a,b}Different letters refer to statistical differences (Tukey's Honestly Significant Difference test, $P < 0.01$).

¹CON = control, no pasteurization or homogenization; CP = sample homogenized at 15/3 MPa; HP = sample homogenized at 97/3 MPa; HP+ = sample homogenized at 147/3 MPa.

Mixed Protein Dispersions

On the basis of the well-known interaction between caseins and whey proteins reported in the literature (Mottar et al., 1989; Singh and Latham, 1993; Dalgleish et al., 1997; Beaulieu et al., 1999a,b; Corredig and Dalgleish, 1999; Young and Foegeding, 2008; Guyomarc'h et al., 2009), mixed dispersions of WPC and SC were prepared at different concentrations. Table 4 shows the viscosity values of SC-WPC dispersions at concentrations of 1+1, 1+2, and 2.5+1.25 (% wt/vol). All samples exhibited concentration-dependent Newtonian behavior at steady shear flow. A slight viscosity increase was observed after pasteurization of 1+1 and 1+2, whereas it was more pronounced in the 2.5+1.25 (% wt/vol) dispersion. The effect of homogenization varied from that seen in single dispersions. In fact, homogenization led to a viscosity increase, more evident in the 1+2 dispersion, where a maximum at P-HP was observed, possibly as a consequence of protein interactions promoted by high pressures. The viscosity increase in mixed dispersions after homogenization suggests that the mechanisms involved are different from those in single dispersions, where viscosity increased after heat treatment but decreased after homogenization.

Similarly to the single (Table 2) and mixed (Table 4) dispersions, the CON-P samples were more viscous than the CON samples (Table 5) and both were characterized by Newtonian behavior. The SC/WPC 2+2, 2.5+1.75, and 2.5+2.5 homogenized samples deviated from linearity. The flow curves are reported in Figure 2: the left column refers to homogenized dispersions, the right to pasteurized + homogenized systems. In all cases, the CON dispersions were Newtonian, whereas homogenized samples presented shear-thinning behavior that was more pronounced at higher applied pressures. As shown in Figure 2, the homogenization pressure effect was related to total protein content and inlet sample viscosity. Homogenization was more effective on the more viscous and concentrated sample, namely 2.5SC+2.5WPC. The relation between inlet

Table 4. Mean values ($\pm$ SD) of viscosity of sodium caseinate (SC)–whey protein concentrate (WPC) dispersions at concentrations (% wt/vol) of 1+1, 1+2, and 2.5+1.25

Sample ¹	Viscosity (mPa·s)		
	1SC+1WPC	1SC+2WPC	2.5SC+1.25WPC
CON	4.13 ^b $\pm$ 0.10	5.23 ^b $\pm$ 0.12	5.40 ^b $\pm$ 0.00
CON-P	4.48 ^a $\pm$ 0.21	5.40 ^b $\pm$ 0.69	6.43 ^a $\pm$ 0.12
CP	4.58 ^a $\pm$ 0.05	6.80 ^a $\pm$ 0.00	6.67 ^a $\pm$ 0.06
HP	4.07 ^b $\pm$ 0.06	7.30 ^a $\pm$ 0.00	6.37 ^a $\pm$ 0.12

^{a,b}Different letters within the same column refer to statistically significant differences (Tukey's Honestly Significant Difference test, $P < 0.01$).

¹CON = control, no pasteurization or homogenization; CON-P = sample pasteurized but not homogenized; CP = sample homogenized at 15/3 MPa; HP = sample homogenized at 97/3 MPa.

viscosity and homogenization pressure effect was shown by Phipps (1975). The author reported that, at a same operating pressure, the increase in viscosity of the medium led to smaller mean drop diameters.

The sample viscosity increase due to pasteurization would indicate that protein–protein interactions occurred. Nevertheless, Newtonian behavior, or negligible deviation from it, was still observed (CON-P in Figure 2). The P-CP samples achieved higher viscosity with respect to CP, whereas P-HP samples were comparable to the nonpasteurized samples. Therefore, pasteurization and conventional pressure homogenization were synergistic in determining viscosity increase, whereas at higher pressures (HP samples), homogenization predominated on heat treatment effects. In the specific case of 2.5SC+2.5WPC systems, the flow curves of pasteurized CP and non-heat-treated HP samples were comparable. At pressures >100 MPa (HP+), a lowering of flow curves was observed possibly because of overpressure effects. Homogenization always determined deviations from linearity leading to shear-thinning behavior. The latter may arise from viscoelastic gel systems, where a network has to be broken before flow (Ross-Murphy, 1995). The slope change observed at shear rates between 50 and 100 s^{-1} in 2.5SC+2.5WPC treated samples (HP, P-CP, and P-HP in Figure 2) may be related to network disruption. Before 50 s^{-1} , the slope of the flow curve was high because the system

contained a network. With increasing shear rates, the network broke; above approximately 100 s^{-1} , the slope of the flow curve was lower because the system was de-structured. The viscoelastic properties, investigated by means of oscillatory tests, yielded mechanical spectra consistent with weak gel systems (Figure 3). With the exception of the 2SC+2WPC unpasteurized CP dispersion, homogenization led to gelation of the 2.5+2.5, 2.5+1.75, and 2+2 SC-WPC systems. It may be suggested that dynamic high pressure treatment promoted partial unfolding of milk proteins and the exposition of junction zones more suitable for intermolecular rather than intramolecular interactions. Moreover, dynamic high pressure would force a closer contact between caseinate and whey proteins, leading to the formation of a interpenetrating polymer network. The latter consists of 2 or more polymer networks that are at least partially interlaced on a molecular scale but not covalently bonded to each other and they cannot be separated unless chemical bonds are broken (Work et al., 2004). This type of network would not occur in the single protein dispersions, namely 4% WPC, which exhibited viscoelastic properties after pasteurization but lost them after homogenization, nor in the solely pasteurized, mixed dispersions. It should be noted that samples were stored and analyzed at 4°C; hence, gelation was enhanced by the favored interactions taking place at low temperatures.

Table 5. Mean values ($\pm$ SD) of viscosity of CON and CON-P samples¹ of sodium caseinate (SC)–whey protein concentrate (WPC) dispersions at concentrations (% wt/vol) of 2+2, 2.5+1.75, and 2.5+2.5

SC	WPC	Total concentration	Viscosity (mPa·s)	
			CON	CON-P
2	2	4	7.06 ^b $\pm$ 0.47	9.77 ^a $\pm$ 1.76
2.5	1.75	4.25	9.08 ^b $\pm$ 0.71	13.38 ^a $\pm$ 2.97
2.5	2.5	5	12.43 ^b $\pm$ 1.62	19.37 ^a $\pm$ 1.76

^{a,b}Different letters within rows refer to statistically significant differences (Tukey's Honestly Significant Difference test, $P < 0.01$).

¹CON = control, no pasteurization or homogenization; CON-P = sample pasteurized but not homogenized.

Figure 2. Flow curves of sodium caseinate (SC)-whey protein concentrate (WPC) dispersions at concentrations (% wt/vol) of 2.5+2.5, 2.5+1.75, and 2+2. Left column = unpasteurized; right column = pasteurized; CON = untreated; CP = homogenization pressure of 18 MPa; HP = homogenization pressure of 100 MPa.

The strength of the gel systems was characterized by G' and the ratio G''/G' ($= \tan \delta$; loss tangent). In Figure 4A the G' of gelled systems are shown as a function of homogenization pressure. Two main groups may be observed, the first included samples with low G' , consisting of low concentrated systems in which different pressures did not cause significant changes in the elastic modulus. The second group included the more concentrated samples with higher G' and a bell-shaped behavior with the maximum value at HP. Pasteurized samples set mostly at higher G' than nonthermally treated systems because of the heat-induced activation of reactive sites, which can form a stronger gel after homogenization.

High pressure homogenization necessarily involves an increase in temperature; nevertheless, the effects of heat and other forces (e.g., pressure, shear, turbulence, cavitation) related to homogenization can be distinguished based on the following considerations. Inlet

Figure 3. Representative mechanical spectrum of 2.5% sodium caseinate (SC)-2.5% whey protein concentrate (WPC) gelled sample after homogenization at 100 MPa. G^* , G' , and G'' = complex, elastic, and viscous moduli, respectively.

Figure 4. A) Mean values and standard deviations of elastic modulus (G') of gel systems as function of homogenization pressure; B) mean values and standard deviations of loss tangent (G''/G') of 2.5% sodium caseinate (SC)-2.5% whey protein concentrate (WPC) gel.

temperatures were selected to achieve the same heat treatment during homogenization (maximum outlet temperature of 70°C; recycling for 90 s) for CP, HP, and HP+ samples. In unpasteurized 2+2 systems, HP and HP+ led to gelation but CP did not, although all samples underwent the same heating during homogenization. Pereda et al. (2009) observed that homogenization of milk at 200 MPa, where an increase up to 78.5°C for less than 1 s was involved, induced approximately 32% β -LG denaturation. A comparable heat treatment (72°C for 15 s) of milk determined denaturation of just 8% β -LG. The authors attributed the denaturation not only to the thermal effect of the treatment, but to the high homogenization pressure-related forces. Moreover, in the pasteurized (82°C for 8 min) samples, the effects of the pretreatment overcame those of short-time heating both in CP and high pressure (HP and HP+) treat-

ments. In the present work, pasteurization (82°C for 8 min) would produce denaturation to greater extent with respect to the short-time heating of homogenization, both in P-CP and P-HP samples. Nevertheless, the increase in pressure from 18 MPa (CP) to 100 MPa (HP) yielded higher G' values (2.5+2.5 in Figure 4A), indicating a substantial effect of the pressure-related forces.

The β -LG and α -LA ratio is of relevance because, depending on the heat process, a proportion of β -LG and α -LA bind to casein (Mottar et al., 1989; Corredig and Dalgleish, 1999), preventing aggregation and favoring gelation (Mottar et al., 1989). Hydrophobic-hydrophilic properties of caseins were influenced by the β -LG/ α -LA ratio present at the micellar surface. The lower the ratio, the higher the water-holding capacity and the lower the surface hydrophobicity (Mottar et al., 1989).

The α -LA to β -LG ratio in the WPC powder used in the present work was approximately 1 (Innocente et al., 2009).

Homogenization was less effective when reactive sites were not heat-activated; thus, the CP sample at 2SC+2WPC did not form a gel unless heat treatment preceded homogenization (Figure 4A). Nevertheless, HP can induce activation because denaturation of β -LG began at 50 MPa (Dumay et al., 1994) and, in general, increasing pressure led to higher denaturation (Hinrichs et al., 1996) and to an increase in the strength of the gel network (Van Camp and Huyghebaert, 1995b). In the hydrostatic high pressure field, gel strength is known to increase as a result of an increase in pressure (within the range of 200–400 MPa) and solids concentration; milk protein dispersions were found to form gels at concentrations of at least 9.7 to 11% depending on pH (Van Camp and Huyghebaert, 1995b; Famelart et al., 1998). In contrast, in this experimentation, the gelation of mixed SC-WPC dispersions by homogenization occurred at a minimum total concentration of 4%.

Figure 4A shows that heat treatment enhanced gel strength especially at 18 MPa (CP), but this effect decreased with increasing pressure. It can be inferred that increasing pressure overcomes the effects of heat, related to the pretreatment or the homogenization process, on gelation. Nevertheless, homogenization was less efficient above 100 MPa, possibly because of the effects of overpressure. In Figure 4A, comparable G' values of CP 2.5SC+2.5WPC and the less concentrated samples may be observed.

In addition to the rheological parameters obtained from mechanical spectra, the loss tangent (G''/G') is an index of the solid- or liquid-like behavior of systems. In Figure 4B the loss tangent of the 2.5SC+2.5WPC gel versus homogenization pressure is reported. As expected, the loss tangent was higher at 18 MPa for the unpasteurized samples and achieved a minimum at 100 MPa for P-HP and HP, indicating more liquid- or solid-like systems, respectively. These data are in agreement with the G' trends shown in Figure 4A.

CONCLUSIONS

In single dispersions of WPC and SC, pasteurization (82°C for 8 min) produced a viscosity increase, which was more evident at higher concentrations of WPC or SC. The subsequent homogenization lowered viscosity below the values observed before pasteurization. In the concentration range adopted (1–4%), SC dispersions did not produce gels after thermal and homogenization treatments. Conversely, pasteurization of the 4% WPC dispersion led to an improvement of viscoelastic properties, which were lost after the homogenization

treatment. In mixed SC and WPC dispersions, an increase in viscosity or a change in flow properties from Newtonian to non-Newtonian occurred following homogenization. Homogenization-induced gelation was observed in mixed samples at a total concentration of at least 4% in both pasteurized and unpasteurized samples. In unpasteurized samples, CP, HP, and HP+ treatments promoted cold gelation, possibly through the formation of caseinate-whey protein interpenetrating network. Gels with higher G' were obtained in more concentrated samples homogenized at 100 MPa (HP). Gels with similar G' (in the range of 0.3–0.85 Pa) were obtained by appropriately combining concentrations, pasteurization conditions, and homogenization pressures. Therefore, by setting appropriate process conditions, systems or gels with tailored characteristics may be obtained from dispersions of milk proteins. Future perspectives may include the development of the specific textural-structural properties of milk proteins by dynamic high pressure homogenization. This could allow the exploitation of intrinsic milk proteins as fat mimetics or as thickening or gelling agent replacers in low additive formulations.

ACKNOWLEDGMENTS

The authors are grateful to Enrico Maltini (Department of Food Science, University of Udine, Italy) for his helpful advice.

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