Uniaxial Compression of Thermal Gels Based on Microfluidized Blends of WPI and Heat-Denatured WPI

Christian Sanchez,*,† Michel Pouliot,‡ Denis Renard,§ and Paul Paquin‡

Laboratoire de Physico-Chimie et Génie Alimentaires, ENSAIA-INPL, 2 avenue de la Forêt-de-Haye, 54500 Vandoeuvre-lès-Nancy, France, Centre de Recherches STELA, Faculté des Sciences de l'Agriculture et de l'Alimentation, Université Laval, Pavillon Paul-Comtois, local 1316, Québec, Canada G1K 7P4, and INRA, Centre de Recherches Agro-Alimentaires, Laboratoire de Physico-Chimie des Macromolécules, BP 1627, 44316 Nantes Cedex 03, France

The effect of heat-denatured whey protein isolate (dWPI)/whey protein isolate (WPI) ratio (0−0.6), microfluidization pressure (0−1000 bar), and number of passes (1−10) on the uniaxial shear stress at 10% (σ_{10}) and 80% (σ_{80}) relative deformation of dWPI/WPI heat-induced gels (14% total protein, w/w) was studied. No correlation between the average diameter of aggregates and the dWPI/WPI ratio, microfluidization pressure, or number of passes was found. However, increasing the microfluidization pressure or the number of passes resulted in a narrower size distribution of aggregates. Increasing the dWPI/WPI ratio and the number of passes resulted in a decrease and an increase of gel hardness, respectively. The results were interpreted in terms of more random aggregation/gelation of proteins in the presence of aggregates that could result in localized heterogeneities into gels and more dissipation of the deformation energy during compression. The positive effect of the number of passes on the gel hardness was also considered to be due to a more homogeneous aggregation/gelation of proteins in the presence of smaller aggregates.

Keywords: *Whey proteins; aggregates; gels; heat-treatment; microfluidization*

INTRODUCTION

Protein microparticles are engineered aggregates used by industry either as fat substitute or as texturing agent in foods such as dairy products (e.g. ice cream, yogurt, cream, cream cheese) and oil-based products (salad dressing, French dressing, mayonnaise), i.e. in ready-to-eat foods. More specifically, these new food ingredients are highly structured complexes formed from protein solutions by a combination of treatments such as acidification, heating, shearing, and/or high-pressure homogenization (Sanchez and Paquin, 1997). Generally protein microparticles cannot be used easily in heated food products because of their heat sensitivity that causes the formation of large aggregates and impart grittiness (Cheftel and Dumay, 1993). This is a serious drawback, and it would be interesting to better understand how protein microparticles affect the structuring and physical properties of heated foods in order to optimize their use.

The problem is complex since microparticulated proteins are in fact a heterogeneous population of native soluble proteins and colloidal species that can be defined as soluble and insoluble aggregated proteins. A soluble aggregate refers to particles that do not sediment under defined centrifugation conditions. Thus it has been shown that the presence of more than 3.5% microparticulated whey proteins (average particle diameter: 0.33 μm) accelerated thermal aggregation of diluted whey protein solutions (0.08% total protein, pH 6.0), with a shift of aggregation mechanism from predominantly a one-stage to a two-stage process, and that both the amount of microparticles and the insoluble/soluble aggregated protein ratio were important in modifying such a thermal aggregation (Sanchez et al., 1997a). It is important to note that, very recently, the acceleration of protein aggregation in the presence of compatible particles has also been demonstrated theoretically through Brownian dynamics simulations (Wijmans and Dickinson, 1998). Specifically, major changes in aggregation of whey proteins were observed at insoluble/soluble aggregated protein ratios of 0.5 and a relative total amount of 20% aggregates (Sanchez et al., 1997a). In a similar way, the insoluble/soluble whey protein ratio considerably affects mechanical properties of thermally induced whey protein concentrate (WPC) gels as demonstrated previously by Beuschel et al. (1992) and Hung and Smith (1993). At constant total protein concentration (16% w/w), an increasing insoluble/soluble protein ratio induced the formation of softer gels. The structural features accounting for the obtained results were not given. Furthermore, the authors varied the insoluble/soluble protein ratio without specifying how much soluble proteins were in an aggregated state. It would be also surprising that the different aggregates (soluble and insoluble) could display the same size distribution since three of their four ratios were obtained by heating WPC at different temperature and time of heating (78.2 °C/30 s; 92.2 °C/30 s; 126.7 °C, 30 min).

To our knowledge, the impact of the size of microparticulated proteins on the physical properties of foods has not been studied experimentally. With this background, specific objectives of the present paper were to provide

* Corresponding author.
† ENSAIA-INPL.
‡ Université Laval.
§ INRA.

10.1021/jf980577o CCC: $18.00
Published on Web 02/03/1999

a *first global approach* to the effects of both heat-denatured whey protein isolate (dWPI)/whey protein isolate (WPI) ratio and microparticle size on the uniaxial compression properties of thermally induced WPI gels. Thermally induced gels have been chosen as a valuable example of heated-based structured food.

MATERIALS AND METHODS

Preparation of Heat-Denatured Whey Protein Isolate (dWPI). Commercial whey protein isolate (WPI) was given by Protose Inc. (Ottawa, Canada). The WPI chemical composition was 89.1% protein (N × 6.38), 6.7% ash (0.12% Ca^{2+}, 0.63% Na^{+}), 0.4% lactose, 0.4% fat, and 4.9% moisture. The WPI was suspended at room temperature in distilled water (5% protein, w/v) under stirring for 90 min. The pH of the WPI suspension was adjusted to 6.00 with 1.0M HCl, and the suspension was stored overnight at 10 °C. The day after, the pH of the WPI suspension was readjusted to 6.00 with 1.0 M HCl, and the suspension was heat-treated at 85 °C (±2 °C) for 20 s in a lab model Spiratherm heat-exchanger (Cherry-Burrel, Cedar Rapids, IA). The heat-denatured WPI (dWPI) was concentrated 5 times and diafiltered with distilled water (1 diavolume) at 25 °C through a hollow-fiber polysulfone membrane Romicon PM10 (Romicon Inc., Woburn, MA) and then spray-dried using a Niro type atomizer (Niro Atomizer, Soeborg, Denmark) with an inlet temperature of 200 °C (±2 °C) and an outlet temperature of 88 °C (±2 °C). The dWPI chemical composition was 93.1% protein (N × 6.38), 6.2% ash (0.12% Ca^{2+}, 0.36% Na^{+}), and 3.9% moisture (lactose and fat contents were not determined). Among 93.1% whey proteins, 90.6% were proteins in an aggregated state (soluble aggregates, 22.7%; insoluble aggregates, 67.9%) as estimated previously (Sanchez et al., 1997a).

Microparticulation of Composite dWPI/WPI Blends. Composite suspensions (in deionized water) of WPI and dWPI at different dWPI/WPI ratios (0−0.6) and 15% (w/w) total protein were mixed for 60 min at 22 °C. Sodium azide (0.02%, w/w) was added to the suspensions in order to prevent microbial spoilage. The initial pH value of the suspensions (6.4−6.6) was adjusted to 6.00 with 1 M HCl. The total protein content of blends was consequently set to 14% (w/w). Composite blends were degassed in a flask under vacuum for 90 min with gentle stirring to avoid sedimentation of protein aggregates. The different blends were immediately microparticulated at different pressures (0−1.0 kbar) and number of passes (1−10) using a Microfluidizer M-110 Y (Microfluidics Corp., Newton, MA). The reaction chambers of the microfluidizer were previously immersed in an ice bath, and the temperature of the microfluidized suspensions was maintained at 20 °C (±3 °C) by a coiled heat exchanger immersed in cold water (20 ± 3 °C) and connected to the outlet of the microfluidization chambers. The microparticulated dWPI/WPI blends were used without further treatment. The level of pressure 0 bar was set by recirculating the suspensions in the microfluidizer without applying any counterpressure. The size distribution of the microparticulated proteins was determined by photon correlation spectroscopy (PCS), after dilution of the suspension at around 0.01% protein (v/v) with deionized water, using a laser light scattering apparatus type Nicomp (Pacifics, CA) as previously reported (Sanchez et al., 1997a).

Preparation of Microparticulated dWPI/ WPI Based Heat-Set Gels. Microfluidized dWPI/ WPI suspensions were poured immediately into glass tubes (5 mm inner diameter and 30 mm length) already coated with Sigmacote (Sigma Chemical Co., St. Louis, MO) and closed at both ends with rubber stoppers. Gels were formed by heating the suspensions at 90 °C for 30 min in a temperature-controlled water bath and then stored at 5 °C for 16−18 h. Prior to rheological testing, gels were equilibrated at 22 °C for 90 min, carefully removed from glass tubes, and cut with a template holding two parallel razor blades into 5 mm diameter and 5 mm length cylinders. The size of gel samples was chosen according to the sensitivity of the texture analyzer load cell as described in the following section.

Uniaxial Compression of Microparticulated dWPI/ WPI Based Heat-Set Gels. Mechanical responses of the different gels were evaluated using uniaxial compression. Practically, gel samples were compressed once at 80% relative deformation between two parallel plates coated with mineral oil, using a Stable Micro System texture analyzer (Surrey, U.K.) equipped with a 5 kg load cell. Constant cross-head speed of the compressing plates was set at 0.5 mm·s^{-1}, which corresponds to an initial strain rate of 0.1 s^{-1} for a 5 mm length sample. Around 5−7 cylinders of each gel were analyzed per experimental condition. The following parameters were extracted from force-relative deformation compression profiles: (i) σ_{10} (Pa), the shear stress at 10% relative deformation; (ii) σ_{80} (Pa), the shear stress at 80% relative deformation or at the gel fracture.

Experimental Design and Statistical Analysis. The experimental treatment of the study (dWPI/WPI ratio, microfluidization pressure, and number of passes) was performed according to a central composite experimental design (Montgomery, 1976; Thompson, 1982). This design is based on a central point repeated several times on which the variance is estimated. The evolution of the variance on the experimental space is a function of its distance from the central point and not of the direction. A partial factorial is built over the central point, and extreme conditions are added on every independent variable. For this particular experiment, the different conditions were defined according to Table 1. All statistical analyses were performed on the SAS software (SAS Institute Inc., Cary, NC, version 6.12). Response surface analysis (RSREG procedure) was performed on all the determined parameters along with an analysis of the residuals to try to detect any residual tendency in data. A correlation matrix was produced (CORR procedure) to identify the possible correlations between the determined parameters.

RESULTS

Size of the Produced Whey Protein Microparticles. One of the major objectives of the present study was to analyze the effects of whey protein microparticle size on the mechanical properties of dWPI−WPI gels. The measured average diameter (d_{av}) of protein aggregates obtained through microfluidization of dWPI−WPI suspensions (14% w/w proteins; 0−0.6 dWPI/WPI weight ratio) at different homogenization pressures (0−800 Pa) and number of passes (1−10) is reported in Table 1. The d_{av} has to be considered as an "apparent" average diameter rather than an absolute value since optical properties of WPI aggregates and their shape were unknown and PCS measurements were performed at only one scattering angle (90°). Keeping in mind these restrictions, the determined d_{av} of particles in the experimental system ranged from 210 to 480 nm (Table 1), with a mean diameter of 337 nm and a root mean standard error of 41 nm. The 210 nm value corresponded to suspensions based on the commercial WPI, i.e., without added dWPI and microfluidized at 500 bar/5 passes, and the 480 nm value corresponded to mixed suspensions (dWPI/WPI ratio: 0.23) microfluidized at 500 bar/1 pass.

A preliminary remark is that commercial WPI contained a fraction of polymerized proteins since the diameter of a β-lactoglobulin monomer, the main protein in WPI, is in the order of 3−4 nm (Haque et al., 1993). Aggregated material in our WPI has been estimated to represent 12% of the total protein content at pH 6.0 (Sanchez et al., 1997a). Aggregated whey proteins can arise from thermal treatments of whey during processing and both pH- and concentration-induced interactions of proteins in "solution". An important point to note is that the size of protein aggregates from 0.23/0/5 suspen-

Table 1. Experimental Design Used in the Present Study (Coded and Experimental Values) and Experimental Parameters Obtained (Average Diameter of Protein Particles, d_{av}; Shear Stress at 10% (σ_{10}) and 80% (σ_{80}) Relative Deformation of Microfluidized dWPI/WPI Based Thermal Gels at pH 6.0)

coded values				exptl values			determined params		
no. of points	dWPI/WPI ratio	microfluidization pressure (bars)	no. of passes	dWPI/WPI ratio	microfluidization pressure (bars)	no. of passes	d_{av}	σ_{10} (kPa)	σ_{80} (kPa)
1	1	1	1	0.43	800	8	302	10.9	630.2
1	1	1	−1	0.43	800	2	339	13.4	744.3
1	1	−1	1	0.43	200	8	357	13.3	681.4
1	1	−1	−1	0.43	200	2	389	13.4	784.1
1	−1	1	1	0.08	800	8	303	22.4	932.6
1	−1	1	−1	0.08	800	2	333	20.4	903.1
1	−1	−1	1	0.08	200	8	363	22.4	974.8
1	−1	−1	−1	0.08	200	2	387	23.2	917.9
6	0	0	0	0.23	500	5	332	18.2	839.3
1	−1.628	0	0	0.00	500	5	210	24.9	1021.8
1	1.628	0	0	0.60	500	5	333	10.6	698.6
1	0	−1.628	0	0.23	0	5	nd[a]	13.3	459.2[b]
1	0	1.628	0	0.23	1000	5	304	19.2	858.1
1	0	0	−1.628	0.23	500	1	480	13.4	644.1
1	0	0	1.628	0.23	500	10	308	15	667.9

[a] nd: not determined (see text for details). [b] Shear stress at gel fracture.

sions (suspensions with 0.23 dWPI/WPI ratio, microfluidized at 0 bar, and 5 passes) could not be determined using PCS because of the extensive sedimentation of large aggregates during the measurement (duration of measurement: 20 min). Heterogeneous size distribution of particles was observed by optical microscopy with apparent diameters up to 100−300 μm (large aggregates were able to plug microfluidization chambers without continuous stirring of 0.23/0/5 suspensions in the feeding glass vessel). Microfluidization of the same suspension at 500 bar/1 pass was sufficient to obtain measurable small aggregate size (d_{av}: 480 nm), demonstrating the efficiency of microfluidization technology in reducing the size of thermally induced whey protein aggregates. No significant model was found ($P > 0.05$) relating d_{av} to the number of passes, the dWPI/WPI ratio, or the pressure (without considering the 0 bar pressure level). According to our experimental design, we studied 15 suspensions but we only found 5 significantly different d_{av} (Table 1; $P < 0.05$). One value was unknown, as above-mentioned, but certainly above 1000 nm; the others were 480, 390, 360, 300−310, and 210 nm. The size distributions of the latter five are plotted in Figure 1. It can be observed that, except for the two extremes corresponding to d_{av} of 210 nm (0/500/5 suspension) and 480 nm (0.23/500/1 suspension), the obtained size distributions exhibited only small differences. As a general observation, large aggregates in suspensions containing dWPI are easily broken by microfluidization at any pressure. Provided that above 1 pass is applied, the microparticle dispersion displays a narrower size distribution, but the size of the microparticles does not change markedly *in average*. For instance, in our experimental system, the 0.23 dWPI/WPI ratio suspension displays a d_{av} value of 480, 333, and 310 nm after 1, 5, or 10 passes, respectively (indicating that microfluidization above 5 passes is mostly unnecessary). This is probably the main explanation for the lack of correlation found between d_{av} and the three independent variables, especially the microfluidization pressure and number of passes. However, the small differences observed in the Figure 1 do not preclude the observation that increasing the number of passes at one microfluidization pressure, or increasing the microfluidization pressure at one number of pass, increases the percentage of aggregates with diameters in the range of d_{av} and decreases the percentage of large particles (diameters above 1000 nm essentially).

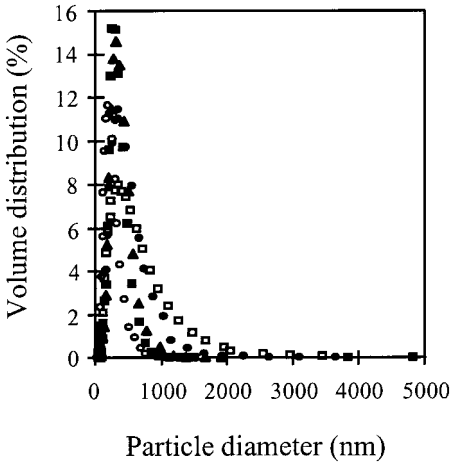

Figure 1. Volume size distributions of 0.43/200/2 (●), 0/500/5 (○), 0.23/500/10 (■), 0.23/500/1 (□), and 0.23/500/5 (▲) microfluidized 0.01% (w/w) dWPI/WPI suspensions as determined at 20 °C by laser light scattering. Key: WPI, whey protein isolate; dWPI, heat-denatured whey protein isolate; X/Y/Z, dWPI/WPI ratio/microfluidization pressure/number of passes.

Uniaxial Compression of Gels Based on Microfluidized dWPI/WPI Suspensions. The numerical values obtained from uniaxial compression of dWPI/WPI gels are reported in Table 1. The σ_{10} and σ_{80} parameters have been obtained at 10% and 80% relative deformation, respectively, and are indicative of gel "hardness" at small and large deformation. The two parameters are purely empirical since the values depend on the experimental methodology, e.g. compression rate or gel sample dimensions, but are very useful for purposes of comparison.

We first observed that gels based on 0.23/0/5 mixed suspensions were the only one that macroscopically fractured during the compression cycle (at around 60% relative deformation). Considering further that the size (distribution) of the very large nonmicrofluidized aggregates was unknown, we only carried out statistical analyses on results obtained on the 14 not fracturable gels containing microfluidized aggregates (corresponding to 14 conditions of the statistical design). The σ_{10} values of the selected dWPI/WPI mixed gels ranged from

Table 2. Magnitude (*F*-Statistic) of the Effects of dWPI/WPI Ratio, Microfluidization Pressure, and Number of Passes on the Compressive Shear Stress at 10% (σ_{10}) and 80% (σ_{80}) Relative Deformation Obtained on Microfluidized dWPI/WPI Based Thermal Gels at pH 6.0, As Obtained by Statistical Analysis of Experimental Data

compression params	dWPI/WPI ratio	microfluidization pressure (Pa)	no. of passes
σ_{10}	63	ns[a]	5.8
σ_{80}	18	3.8	6.3

[a] ns: nonsignificant effect.

10.6 to 24.9 kPa (Table 1), with mean response of 17.3 kPa and a root mean standard error of 1.95 kPa. Response surface analysis of data indicated that the dWPI/WPI ratio was the most important variable affecting σ_{10} (Table 2). The number of passes played only a minor, but significant, role (≈10 times less important than the dWPI/WPI ratio). The microfluidization pressure did not have a significant effect on σ_{10}. No interaction (no crossing effects) was found among the independent variables (dWPI/WPI ratio, pressure of homogenization, number of passes). The evolution of σ_{10} could be modelized with an adjusted $R^2 = 0.94$. The lack of fit was not significant ($P > 0.05$), indicating that the model effectively took into account the whole structure of data. Figure 2, at a microfluidization pressure $P = 500$ bar, clearly shows that increasing the dWPI/WPI ratio in mixed gels caused a decrease of σ_{10}. In other words, an increasing amount of whey protein microparticles renders mixed dWPI−WPI gels softer than WPI gels. At any dWPI/WPI ratio, an increasing number of passes promoted an increase in σ_{10}. However the shear stress gain was small compared to the energy applied from 2 to 10 passes.

The σ_{10} and σ_{80} parameters were very well correlated ($P < 0.0001$), and one could expect for similar statistical results because both parameters are coming from the same compression tests. However, some characteristic features have been observed for σ_{80}, that ranged from 630.2 to 1021.8 kPa with a mean response of 797.7 kPa and a root mean standard error of 104.9 kPa. Response surface analysis of σ_{80} values indicated that the three independent variables (dWPI/WPI ratio, microfluidization pressure, and number of passes) had a significant effect on σ_{80} (Table 2). The dWPI/WPI ratio was again the most important variable affecting σ_{80} ($F = 18$), followed by the number of passes ($F = 6.3$) and the microfluidization pressure ($F = 3.8$), which remains a poorly influencing parameter. The evolution of σ_{80} could be modeled with an adjusted $R^2 = 0.84$. Crossing effects were significant (dWPI/WPI ratio × pressure of homogenization, $P < 0.05$; pressure of homogenization × number of passes, $p < 0.03$) indicating some synergy between independent variables. However the lack of fit was significant ($P < 0.05$) indicating that the model did not take into account the total variability of data. The modeled surface at a pressure P = 500 bar is shown in Figure 3. Similar surfaces could be shown at other pressures. Although the shape of the surface resembles that calculated for σ_{10}, the σ_{80} value decreased faster with the increase of dWPI/WPI ratio. The number of passes significantly and positively affected σ_{80}, particularly for mixed gels containing high dWPI/WPI ratios (Figure 2). In that case, σ_{80} increased rapidly from 2 passes to 5 passes and then leveled off from 5 passes to 10 passes indicating again that microfluidization of aggregates is essentially effective below 5 passes. It is

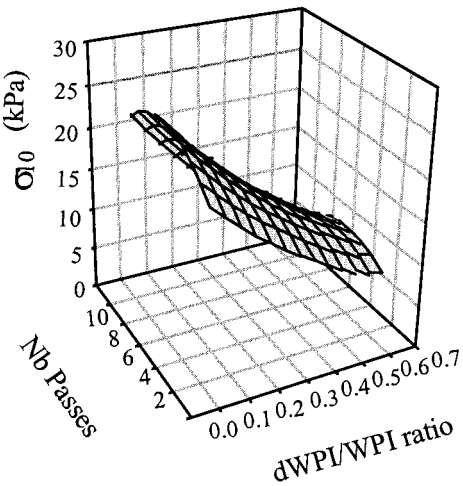

Figure 2. Effects of the dWPI/WPI ratio and number of passes on the shear stress at 10% relative deformation (σ_{10}) obtained at 20 °C on heat-induced gels (90 °C/1 h) containing 14% (w/w) total proteins (pH 6.0). The microfluidization pressure is set to 500 bar.

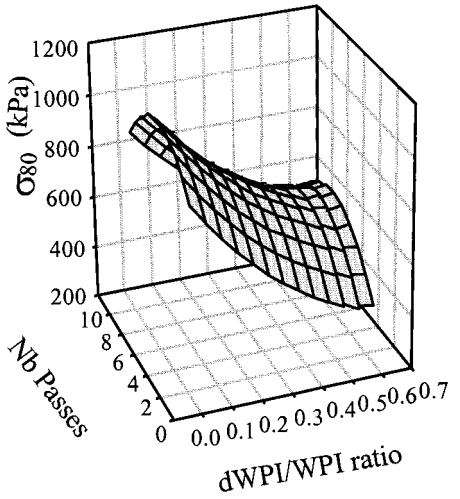

Figure 3. Effects of the dWPI/WPI ratio and number of passes on the shear stress at 80% relative deformation (σ_{80}) obtained at 20 °C on heat-induced gels (90 °C/1 h) containing 14% (w/w) total proteins (pH 6.0). The microfluidization pressure is set to 500 bar.

interesting to note that a suspension with a 0.6 dWPI/WPI ratio microfluidized at 500 bar/5 passes produced a gel with comparable hardness than a gel issued from a suspension with a low dWPI/WPI ratio microfluidized at 500 bar/1 pass. Thus, repeated microfluidization of whey protein aggregates could allow one to incorporate more microparticles into thermal gels without significantly affecting their mechanical properties at small and large deformation.

DISCUSSION

Mechanical properties of mixed dWPI/WPI gels can be modified depending on the dWPI/WPI ratio and the state of dispersion of aggregates obtained through microfluidization. Unambiguously, the dWPI/WPI ratio is the most influencing parameter. Increasing the dWPI/WPI ratio into gels, at constant total protein concentration, means in the present study to increase the relative content in WPI microparticles. Similar results were found by de Wit et al. (1988) and Beuschel et al. (1992) using nonmicrofluidized whey protein aggregates. The

reasons for the softer characteristics observed on WPI gels containing microparticles lie certainly in the specific thermal aggregation/gelation of the different protein species in blends. In first appoximation we only consider the presence in blends of native whey proteins and (microfluidized) thermally aggregated whey proteins. In other words, we consider that microfluidized soluble and insoluble WPI aggregates display the same thermal aggregation/gelation behavior. This point will be adressed at the end of the discussion. Thus, to qualitatively explain our results, we have first to remember the broad lines of native whey proteins thermal aggregation/gelation and then to estimate how WPI aggregates may modify the latter.

Thermal gelation of whey proteins is a multistage reaction involving first partial unfolding of proteins, then aggregation of these partly unfolded proteins through nonspecific (mainly hydrophobic) and specific (e.g. covalent disulfide bonds) interactions, and finally formation of a network stabilized by electrostatic and hydrogen bonding above a critical protein concentration (Aguilera, 1995). Depending on the balance between protein−protein and protein−solvent attractive and repulsive forces, which are modulated by pH and ionic strength of solutions, transparent or opaque gels can be obtained (Hermansson, 1979; Stading and Hermansson, 1990; Langton and Hermansson, 1992). At low ionic strength, transparent gels are produced at pH values where repulsive (e.g. electrostatic) forces between whey proteins overcome hydrophobic attractive forces, i.e., far from the isoelectric region (pH $\approx$ 4−6). Under the same conditions, opaque highly aggregated gels are produced in the isoelectric region, where protein−protein attractive forces overcome repulsive forces. Although transparent gels are homogeneous gels structured by linear aggregates resulting from an ordered aggregation, opaque gels are heterogeneous gels structured by large globular aggregates resulting from random aggregation (Langton and Hermansson, 1992). Interestingly, whey protein solutions at pH values in the range 4−6 display at room temperature a high turbidity revealing that aggregates are formed prior to gelation (Stading and Hermansson, 1990; Sanchez et al., 1997b). These aggregate-containing suspensions aggregate/gel considerably faster than transparent solutions, forming the larger aggregates and heterogeneous gels just mentioned above (Xiong, 1992; Langton and Hermansson, 1992; Stading et al., 1993; Tang et al., 1993). Finally, although transparent gels, for instance at pH <6.0, are rigid, brittle, and exhibit low syneresis, opaque gels are soft, rubbery, and exhibit high syneresis (Stading and Hermansson, 1990; Stading et al., 1993; Sanchez et al., 1997b).

Let us turn now to the thermal aggregation/gelation of whey protein suspensions containing WPI microparticles. In these suspensions, the same is true in simple whey protein aggregate suspensions; protein−protein interactions are detected at lower temperatures than in solutions not containing added microparticles or simple aggregates (Barbut and Foegeding, 1993; McClements and Keogh, 1995; Sanchez et al., 1997a). According to McClements and Keogh (1995), such observation can be partly explained by the presence at the surface of WPI aggregates of nonpolar amino acids, which are normally located in the hydrophobic interior of native globular proteins. As reported in the Introduction of this paper, this results in a heterogeneous aggregation mechanism and formation of larger aggregates, as compared to the aggregation mechanism of WPI solutions (Sanchez et al., 1997a). Thus, by analogy to the different thermal aggregation/gelation mechanism of native whey protein solutions at pH value within the isoelectric region and far from this region (see above), mixed dWPI/WPI blends probably exhibited both ordered and random aggregation of proteins. In that case, the resulting gels may contain heterogeneous areas with large globular aggregates (resulting from localized phase separation phenomenom) and more homogeneous areas with more linear structures (Langton and Hermansson, 1992). A direct consequence of such likely structures is that the energy of deformation applied during uniaxial compression is more dissipated than for whey protein gels not containing initially aggregates or microparticles. In our opinion, this is the main explanation for the continuous decrease of σ_{10} and σ_{80} observed for gels containing increasing dWPI/WPI ratios. In this case, the contribution of random protein aggregation to the final mechanical properties is enhanced. Also the faster decrease of σ_{80} with increasing dWPI/WPI ratios, as compared to σ_{10}, may be explained on that basis since if more energy is applied to the system, then more energy is dissipated due probably to the increase of the flow contribution.

The number of passes applied during microfluidization also plays a minor but significant role on the rheological properties of heat-induced gels. In fact, at any dWPI/WPI ratio, repeated microfluidization of dispersions up to 5 passes enhances rheological parameters of the resulting gels. The effect is stronger for both σ_{10} and σ_{80} at high dWPI/WPI ratio than at low ratio, which seems logical since microfluidization affects the size of microparticles. Even whether no significant statistical model was found relating d_{av} of particles to the number of passes, the determined effects are believed to be caused primarily by a better dispersion of WPI microparticles. Indeed, Brownian dynamics simulations have shown quite clearly that two particle gels can differ strongly if their constituant particles have different size distributions (Wijmans and Dickinson, 1998). However, the structural reasons accounting for the enhancement of gel mechanical properties through repeated microfluidization of the starting mixed suspensions remain unclear. Following the above hypothesis, we suggest that the increase of σ_{10} and σ_{80} determined on mixed dWPI−WPI gels with the increase of number of passes is primarily due to a more homogeneous aggregation/gelation of whey proteins. The latter could induce the formation of smaller whey protein aggregates that contribute to a lower dissipation of deformation energy during uniaxial compression of the different gels. It is noteworthy, following a suggestion of one reviewer, that other possible influencing parameters could be the different shape or surface properties of aggregates microfluidized at different pressures or number of passes.

We have focused in the discussion on the significant effects of dWPI/WPI ratio and microparticle size on hardness of dWPI−WPI mixed gels without any distinction between soluble and insoluble aggregates. Yet we have shown in a preliminary study that the soluble/insoluble aggregate ratio seems to affect the thermal aggregation kinetics of diluted WPI suspensions (Sanchez et al., 1997a). Thus, we think that the specific thermal aggregation/gelation behavior of soluble and insoluble

WPI microparticles, as compared to that of native whey proteins, must be clearly and fundamentally established. Specifically, our future objectives are (i) to study more accurately both the effect of microparticle size and the soluble/insoluble aggregate ratio on thermal aggregation kinetics of diluted whey protein suspensions and (ii) to study the effects of the same parameters on the mechanical properties of mixed microfluidized dWPI−WPI gels. We also hope in the near future to define what are the structural and physicochemical differences (whether differences exist) between soluble and insoluble whey protein aggregates (shape, surface properties) so as to provide a means to crack these aggregates into various functional forms.

CONCLUSION

We have shown in this study that the presence of heat-induced whey protein aggregates into whey protein dispersions can modify the mechanical properties of the resulting heat-induced gels. A few percent of aggregates was sufficient, which is in the range of the amount of aggregates typically found in commercial whey protein concentrate (WPC) or isolate (WPI). This is a very important trend industrially, because a part of the variability observed in the gelation properties of WPC and WPI could be ascribed to differences in the initial aggregate content of protein dispersions (in other terms, the initial structure of the dispersion could play an important role in determining the functional properties of the system). On the other hand, the size distribution of aggregates also affects the mechanical properties of gels. Therefore the microfluidization technology could become an interesting tool to standardize the gelation properties of (or minimize the differences of quality between) whey protein dispersions, as well as to change these properties modifying the size distribution and structure of aggregates.

LITERATURE CITED

Aguilera, J. M. Gelation of whey proteins. *Food Technol*. **1995**, *10*, 83−89.

Barbut, S.; Foegeding, E. A. Ca^{2+}-induced gelation of preheated whey protein isolate. *J. Food Sci.* **1993**, *58*, 867−871.

Beuschel, B. C.; Culbertson, J. D.; Partridge, D. M.; Smith, D. M. Gelation and emulsification properties of partially insolubilized whey protein concentrates. *J. Food Sci*. **1992**, *57*, 605−609, 634.

Cheftel, J. C.; Dumay, E. Microcoagulation of proteins for development of "creaminess". *Food Rev. Int*. **1993**, *9*, 473−502.

De Wit, J. N.; Hontelez-Backx, E.; Adamse, M. Evaluation of functional properties of whey protein concentrates and whey protein isolates. 3. Functional properties in aqueous solutions. *Neth. Milk Dairy J*. **1988**, *42*, 155−172.

Haque, Z.; Casai, G. A.; Wilson, W. W.; Antila, P.; Antila, V. Effect of casein hydrolysates on association properties of milk proteins as seen by dynamic light scattering. *J. Agric. Food Chem*. **1993**, *41*, 203−207.

Hermansson, A. M. Aggregation and denaturation involved in gel formation. In *Functionality and Protein Structure*; Pour-El, E., Ed.; ACS Symposium Series 92; American Chemical Society: Washington, DC, 1979; pp 82−103.

Hung, T. Y.; Smith, D. M. Dynamic rheological properties and microstructure of partially insolubilized whey protein concentrate gels. *J. Food Sci*. **1993**, *58*, 1047−1049, 1054.

Langton, M.; Hermansson, A. M. Fine-stranded and particulate gels of β-lactoglobulin and whey proteins at varying pH. *Food Hydrocolloids* **1992**, *5*, 523−539.

McClements, D. J.; Keogh, M. K. Physical properties of cold-setting gels formed from heat-denatured whey protein isolate. *J. Sci. Food Agric*. **1995**, *69*, 7−14.

Montgomery D. C., In *Design and analysis of experiments*; John Wiley & Sons: New York, 1976; Chapter 14, pp 340−368.

Sanchez, C.; Paquin, P. Protein and protein-polysaccharide microparticles. In *Food Proteins and Their Applications in Foods*; Damodaran, S., Paraf, A., Eds.; Marcel Dekker: New York, 1997; Part III, Chapter 17, pp 503−528.

Sanchez, C., Pouliot, M.; Gauthier, S. F.; Paquin, P. Thermal aggregation of whey protein isolate containing microparticulated or hydrolyzed whey proteins. *J. Agric. Food Chem*. **1997a**, *45*, 2384−2392.

Sanchez, C.; Schmitt. C.; Babak, V.; Hardy, J. Rheology of whey protein isolate-xanthan mixed solutions and gels. Effect of pH and xanthan concentration. *Nahrung* **1997b**, *41*, 336−343.

Stading, M.; Hermansson, A. M. Viscoelastic behaviour of β-lactoglobulin gel structures. *Food Hydrocolloids* **1990**, *4*, 121−135.

Stading, M.; Hermansson, A. M. Microstructure and rheological properties of particulate β-lactoglobulin gels. *Food Hydrocolloids* **1993**, *7*, 195−212.

Tang, Q.; McCarthy, O. J.; Munro, P. A. Oscillatory rheological study of the gelation mechanism of whey protein concentrate solutions: effects of physicochemical variables on gel formation. *J. Dairy Res*. **1993**, *60*, 543−555.

Thompson D. Response surface experimentation. *J. Food Process. Preserv*. **1982**, *6*, 155−188.

Wijmans, C. M.; Dickinson, E. Brownian dynamics simulations of filled particle gels. *J. Chem. Soc., Faraday Trans*. **1998**, *94*, 129−137.

Xiong, Y. L. Influence of pH and ionic environment on thermal aggregation of whey proteins. *J. Agric. Food Chem*. **1992**, *40*, 380−384.

Received for review June 1, 1998. Revised manuscript received October 23, 1998. Accepted November 25, 1998. This research was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC grants) and by the program Synergie (Ministère de l'Enseignement Supérieur et de la Science du Québec). We also thank the "Centre Québécois de Valorisation de la Biomasse" (CQVB) and the Agropur, Parmalat, and Novalait companies for financial support.

JF980577O