Viscous Properties of Microparticulated Dairy Proteins and Sucrose¹

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ABSTRACT

Slurries of whey protein concentrate (WPC) or sodium caseinate (Na-CN) mixed with sucrose (36% T.S.) were subjected to microparticulation by a high shear homogenizer operated at 27,000 rpm for 2, 4, and 6 min to facilitate gel formation. After microparticulation treatment, the milk protein and sucrose slurries were evaporated at 85°C for 60 min under a partial vacuum (20 to 45 mm of Hg) to form composite gels. Particle sizes and viscoelastic properties were determined before microparticulation treatment. Microparticulation reduced the particle size of WPC-sucrose slurries from an average size of 330 to 188 nm after 4 min and NaCN-sucrose slurries from 270 to 35 nm after 2 min. The WPC-sucrose composites were gel-like, but NaCN-sucrose composites did not gel. Viscoelastic properties of heated WPC-sucrose composites were liquid-like, exhibiting significant reduction in storage modulus and complex viscosity. Microparticulation reduced particle sizes, which resulted in softer gels as time of shearing increased.

(**Key words:** gel, microparticulation, viscoelasticity)

Abbreviation key: WPC = whey protein concentrate, NaCN = sodium caseinate.

INTRODUCTION

Gels created by microparticulation are binary mixtures of biopolymers with interpenetrating networks. Such gels are mostly heterogeneous composites, containing well-defined boundaries. The rheological properties of composite gels are derived from changes in microstructure (Zeigler, 1991). Microparticulation is accomplished by high shear homogenization to reduce sizes of the mixtures to less than 3 μ m (Singer et al., 1988; Erdman, 1990), and cooking the mixtures to form

a gel network. Milk proteins alone or with sucrose are easily microparticulated to very small spheres (~ 1 μ m or less) composed mostly of small molecules. Stable gels have been created mostly from microparticulation and evaporative cooking processes that increase the interaction of protein and other adjuncts such as polysaccharides (Bernal et al., 1987). Microparticulation of proteins does not result in loss of protein quality as determined by amino acid analysis, protein efficiency ratio, or gel electrophoresis (Erdman, 1990).

Whey proteins and egg proteins have been microparticulated to create new organoleptic properties (Singer and Dunn, 1990). In microparticulated milk and egg protein emulsions, the casein micelles are surrounded by coagulated egg white. Microparticulating whey protein and egg increased hydrophobicity and particle surface areas (Singer and Dunn, 1990). Microparticulated, finely ground particles of protein and egg composites ranging in size from 0.5 to 3.0 μ m have been used as binders and fat replacers in low-fat foods (Hayakawa et al., 1993). Fat replacers are made commercially from two materials, usually proteins and carbohydrates. The carbohydrate-based fat replacers sometimes are combined with food gums such as guar gum to enhance its binding properties (McMahon et al., 1996).

The interaction of milk protein and sucrose to form various matrices is used to create foods such as ice cream, milk syrups, and caramel candies (McMaster et al., 1988). Sugar and milk fat are known to form composite gels that simulate confectionery candies (Onwulata et al., 2000). Milk protein and sucrose can also be processed through microparticulation and heated to form soft gels (McClements et al., 1993). Research on milk protein and sucrose microparticulation to create composite gels is very limited. Therefore, the objectives of this study were to microparticulate whey protein concentrate (WPC) or sodium caseinate (NaCN) in sucrose solutions and evaluate the rheology of composite gels created through thermal evaporation.

MATERIALS AND METHODS

Microparticulated slurries were formulated from sucrose purchased from Domino Sugar Corp. (New York,

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NY); sodium caseinate (Alanate 110; protein content, 91.2%), and whey protein concentrate (Alacen 856; protein content, 80%) purchased from New Zealand Milk Products (Santa Rosa, CA). Eighty grams of NaCN or WPC was mixed with 320 g of finely crushed ice and mixed in a blender for 24 s, using a Waring Lab Micronizer model 31FP93 (Waring Products Division, New Hartford, CT). The protein and ice mixture blend was combined with 320 g of sucrose dissolved in 800 ml of deionized water (protein concentration 25 g/100). The protein and sucrose slurries were mixed and then microparticulated by high shear homogenization at ambient pressure using a PCU-2 homogenizer (Brinkman Instruments, Westbury, NY) for 2, 4, or 6 min. Both slurries were homogenized at 34°C and there was no rise in temperature. The microparticulated slurries (26% solids) were then evaporated to form syrups (42% solids) by heating at 45°C under partial vacuum (5.2 MPa) in a Rotary Evaporator model RE-52 (Yamata Scientific Co., Ltd., Tokyo, Japan) for 40 min. Viscoelastic properties of the microparticulated gels were measured at ambient temperature within 4 h of completion of evaporation. Analyses were replicated three times in a completely randomized order. The characteristics of the slurries were as follows: WPC, pH 6.5; NaCN, pH 6.2 after concentration the syrups (42% solids) had the following composition and characteristics: WPC-sucrose, sucrose 79.3%, protein 16.7%, lipid 0.7%, ash 3.3% and pH 6.3; NaCN-sucrose, sucrose 79.3%, protein 18.6%, lipid 0.2%, ash 1.9%, and pH 6.2.

Moisture Analysis

Moisture analysis was carried out by AOAC Method 991.42 (AOAC, 1998).

Particle Size Determination

The particle size distribution of the samples was determined with an Accusizer Optical Particle Sizer model 770 and a Submicron Particle Sizer model 370 with auto-dilute (Particle Sizing Systems, Santa Barbara, CA). The sizes of the microparticulated slurries before evaporation and the gels after were determined.

Viscosity Measurement

Viscosity was determined with a Brookfield Digital Rheometer model DV-III (Stoughton, MA). The viscosities of the evaporated composite gels were determined with spindle #3, and nonevaporated samples were determined with the ULA spindle. Fifty milliliters of each sample was used. Slurry temperature was maintained at 25°C with a jacketed water bath. Viscosity

was measured with an up-and-down profile that ramped from 60 to 130 rpm in 5 min with 1 min hold. Data were collected every 20 s.

Viscoelastic Properties

A frequency test was made to measure viscous stability and dynamic viscous response to shear and temperature at oscillation frequency (ω) ranges from 0.01 to 100 rad/s with fixed amplitude of the protein-sucrose composites in terms. A strain test was made to evaluate product behavior at increasing strain and to identify the range of linear viscoelasticity. The tests were performed at 25°C with a Rheometrics RDA-700 Dynamic Analyzer (Rheometrics Inc, Piscataway, NJ) using a 0 to 200 g-cm torque transducer equipped with 2.0 cm radius parallel plates with a height of 0.2 cm. The change in viscoelastic properties as a function of time and temperature was determined by dynamic shear measurement. The measurement gives the shear storage modulus (G') and loss modulus (G''). Complex viscosity (η^*) was determined from the frequency sweep at 1.0 rad/s. The syrup samples were analyzed twice for each of the three replicates.

Scanning Electron Microscopy

Stored, frozen (−20°C) samples were thawed to room temperature, and aliquots (1 to 2 ml) were poured into 10 mm diam. Spectrapor dialysis tubing (Spectrum Medical Industries, Inc., Los Angeles, CA) and equilibrated with a fixative solution containing 2% glutaraldehyde and 0.1 M imidazole HCl (pH 7.0) for 24 h. Samples were washed in imidazole buffer and dehydrated by exchange with 50% and absolute ethanol for 24 h. Next, the samples in tubing were frozen in liquid nitrogen and fractured manually with the cooled blade of a surgical scalpel. Fractured fragments were thawed into absolute ethanol and critical point dried in liquid carbon dioxide. Dry fragments were glued to aluminum specimen stubs with colloidal silver paste (Electron Microscopy Sciences, Ft. Washington, PA) and coated by DC sputtering with a thin layer of gold for imaging in a model JSM 840A scanning electron microscope (JEOL USA, Peabody, MA), operated in the secondary electron imaging mode. Digital images were collected with an Imix workstation (Princeton Gamma-tech, Princeton, NJ). Image analysis of digital images (Fast Fourier Transformation) was done as described earlier (Cooke et al., 1995) to resolve possible differences in topographical features of the different whey samples.

Table 1. Viscosities of microparticulated slurries and gels of whey protein concentrate-sucrose composites.¹

Time of Shear (Minutes)	Slurry		Gel	
	Viscosity (Cp)	Particle size* (nm)	Moisture (%)	Viscosity (Cp)
0	1.15	331	38.8	662
2	1.22	266	42.2	316
4	1.15	188	39.3	454
6	1.26	220	44.6	213

¹Pooled standard deviations: slurry viscosity (0.03); particle size (9); moisture (1.4); gel viscosity (12). *Volume-weighted mean diameter (nm).

RESULTS

WPC-Sucrose

The viscosities and particle sizes of WPC-sucrose slurries and WPC-sucrose composite gels are presented in Table 1. Microparticulation of the slurries resulted in significant ($P > 0.05$) increases in viscosity at 2 and 6 min, respectively. Particle sizes of WPC-sucrose decreased from 331 nm to 266 and 188 nm as the time of microparticulation increased from 0 up to 4 min, reflecting shear breakage of the protein-sucrose slurry particles; but at 6 min reaggregation occurred as evidenced by increased particle size. The reduction in the number-weighted mean particle sizes is shown in Figure 1, as a shift to the left for the particles sheared at 2 and 4 min. Clearly, there is reduction in particle size with shear at 2 and 4 min, but this trend reversed as microparticulation continued longer than 4 min, and particle sizes increased at 6 min. Particle size of the WPC-sucrose slurry does not correlate with the viscosity.

Figure 1. Particle size distribution of whey protein concentrate-sucrose slurry. Non microparticulated control (0 min); samples microparticulated at 2, 4, and 6 min, respectively.

Composite gels made from microparticulated slurries of WPC-sucrose show higher moisture values after 2 and 6 min of microparticulation. Microparticulated gels were higher in moisture content and lower in viscosity than the untreated composite gels. The reduction in gel viscosity with size of slurry is significant ($P < 0.05$), indicating that changes in particle sizes induced by microparticulation affects the gel properties.

Viscoelastic response of the WPC-sucrose gels (data not shown) shows a reduction in shear storage modulus (G'), loss modulus (G''), and complex viscosity (η^*) with microparticulation. The microparticulated gels were more liquid-like ($G' > G''$) than gels from untreated material.

Scanning electron microscope images of fractured faces prepared from the composite gels containing whey protein are illustrated in Figure 2 (A-D). The images of the composites gels show closely packed granules or short threads, around 100 to 200 nm in diameter, separated by pores, ranging from 200 to 500 nm in diameter. The smallest pores are found after 2 and 4 min of microparticulation (Figure 2B and C), and the largest are found after 6 min (Figure 2D). Analysis of the images by Fast Fourier Transformation reveals greater intensity distributions at reciprocal spacings between 400 and 100 nm⁻¹ after 0 and 6 min of microparticulation; whereas at 2 and 4 min of treatment, the intensity distribution is similar and lower over this range of dimensions (Figure 3). Below 100 nm, all samples have a similar intensity distribution.

NaCN-Sugar Microparticulation

The physical properties of NaCN-sucrose slurries and NaCN-sucrose composite gels are presented in Table 2. Microparticulation of the slurries did not change the viscosity after shearing up to 6 min. Particle sizes decreased sharply after 2 min of microparticulation, but as the time of microparticulation increased, the particle sizes increased, suggesting that shearing and reaggregation of the slurry particles was occurring simultaneously. This phenomenon, simultaneous shearing and reaggregation is seen clearly in the number-weighted means of the particle sizes (Figure 4). There was a significant shift to smaller particle sizes at 2 min, and increase in sizes with continued microparticulation. The moisture content of NaCN-sucrose composite gels did not change with shearing time up to 6 min. Gel viscosity decreased after 2 min, corresponding to the sharp decline in particle size, but viscosity increased as microparticulation continued. Gel particle sizes indicated a corresponding pattern of the slurry reducing and increasing with shear.

Figure 2. Scanning electron micrographs of whey protein concentrate-sucrose gels showing protein matrix and pore distribution: (A) nonmicroparticulated control; (B) microparticulation, 2 min; (C) microparticulation, 4 min; (D) microparticulation, 6 min.

Viscoelastic response of the NaCN-sucrose gels (data not shown) show a reduction in shear storage modulus (G'), but a slight increase of loss modulus (G'') after 2 min and similar pattern of behavior with complex viscosity (η^*). Microparticulated gels were more liquid like ($G'' > G'$) than the nonmicroparticulated control sample.

Scanning electron microscopy of microparticulated gels (Figure 5) shows a distribution of pores in the fine

structures in the nonmicroparticulated gels. Phase structures were irregular, the meshing expanded as microparticulation time increased.

DISCUSSION

In typical mixed slurries, microparticulation reduces the particle size, the average particle diameters, and increases the specific-surface mean of particles.

Figure 3. Radial distribution profiles of reciprocal spacings of topographical features in Fast Fourier transforms of protein matrix images of whey protein concentrate-sucrose gels: (A) nonmicroparticulated control; (B) microparticulation, 2 min; (C) microparticulation, 4 min; (D) microparticulation, 6 min.

Microparticulating both WPC-sucrose and NaCN-sucrose slurries reduced their particle sizes but did not affect their viscosities. There seems to be no relation between microparticulation and the viscosity of slurries. Taylor and Fryer (1994) reported that gelation of static (nonsheared) slurries are controlled by chemical reactions. This may be due to thiol-disulfide linkages. It is known that disulfide bonding is important in gelation and that whey proteins and calcium enhance gel strength, but the strength of our WPC-sucrose gels were not enhanced. Taylor and Fryer (1994), have also shown that there was no correlation between particle size and rheological properties of gels. In heated gels, made from microparticulated slurries, superfine structures and interpenetrating networks are formed. The ability of heated milk proteins to interact and bind water was not disrupted by microparticulation, and may have been enhanced due to increased surface area. WPC-sucrose and NaCN-sucrose composite gels held more water than their nonmicroparticulated con-

Figure 4. Particle size distribution of NaCN-sucrose slurry. Non-microparticulated control (0 min); samples microparticulated at 2, 4, and 6 min, respectively.

trols. This result agrees with Hayakawa et al. (1993), who showed that microparticulated casein exhibited superfine structures, and Morr (1989) who reported substantial increases in viscosity due to aggregation of protein in heated slurries. This agrees with increases in particle diameter observed at 2 and 4 min for WPC-sucrose and at 6 min for NaCN-sucrose slurries. Microparticulation did not seem to alter the water binding capacity of the WPC or NaCN system, and did not result in increased viscosity, although fine mesh networks were formed.

Microparticulation induced a competing mechanism on the sheared protein: particle aggregation and breakage. When the particles are being sheared, aggregation rate increases as collision rate increases, and some aggregates disintegrate by shearing. The effect of this opposing dynamic is that microparticulated milk protein slurries can form strong gels upon heating if breakage is favored due to increased surface area, but, if aggregation is favored, the amount of protein available to form networks is reduced, leading to a weaker gel (Taylor and Fryer, 1994). WPC-sucrose gel strength decreased with increased microparticulation shear time, and we surmise that particle aggregation caused a weakened gel network. Harwalker (1979) showed that changes in molecular properties of protein, after heat treatment, cause loss of solubility, an indicator of protein denaturation. Aggregation behavior and dimension had a major effect on the strength of the whey protein gel network. The presence of sugars or polysaccharides with milk proteins intensifies aggregation of denatured molecules and promotes gelation (Walkenstrom et al., 1998; Samant et al., 1993; Steventon et al., 1994).

Table 2. Viscosities of microparticulated slurries and gels of NaCN-Sucrose composites.¹

Time of shear (min)	Slurry		Gel	
	Viscosity (Cp)	Particle size ² (nm)	Moisture (%)	Viscosity (Cp)
0	1.69	270	56.0	593
2	1.74	35	58.0	435
4	1.66	139	55.7	638
6	1.70	244	58.3	411

¹Pooled standard deviations: slurry viscosity (0.3); particle size (7.5); moisture (1.6); gel viscosity (14.5).

²Volume-weighted mean diameter (nm).

Figure 5. Scanning electron micrographs of sodium caseinate-sucrose gels showing protein network: (A) nonmicroparticulated control; (B) microparticulation, 2 min; (C) microparticulation, 4 min; (D) microparticulation, 6 min.

The pore size distribution within the gel matrices as imaged with SEM revealed evidence of the competing dynamics. Effectively microparticulated samples—WPC-sucrose at 2 and 4 min and NaCN-sucrose at 2 min—showed a field of closely spaced pores. Samples representing the longer duration shearing exhibited more heterogeneity and larger pores. This shows that

the network structure can be controlled or at least predicted, which will allow for a predetermined gel viscosity achieved by varying the time of shear. The results indicate that increasing shear microparticulation reduces particle size, and that the aggregation rate increases with heating; that the mechanism of microparticulation shear process is the competing dy-

namics of aggregate breakage and reaggregation and knowing when one mechanism is predominant, the viscosity of the resulting gel can be determined.

CONCLUSIONS

Microparticulation of milk proteins and sucrose slurries produces heated gels with reduced strength, but the quality of the gels are dependent on the time of shearing. Viscosity of a desired composite mixture of whey protein and sucrose can be achieved through controlled aggregate-size reduction. This information could aid the dairy industry in developing a new market niche through new products, increase milk component utilization, and bring more return to milk producers.

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