Influence of Sucrose on the Thermal Denaturation, Gelation, and Emulsion Stabilization of Whey Proteins

Asylbek Kulmyrzaev, Cory Bryant, and D. Julian McClements*

Biopolymer and Colloids Research Laboratory, Department of Food Science, University of Massachusetts, Amherst, Massachusetts 01003

The influence of sucrose (0−40 wt %) on the thermal denaturation and functionality of whey protein isolate (WPI) solutions has been studied. The effect of sucrose on the heat denaturation of 0.2 wt % WPI solutions (pH 7.0) was measured using differential scanning calorimetry. Sucrose increased the temperature at which protein denaturation occurred, for example, by 6−8 °C for 40 wt % sucrose. The dynamic shear rheology of 10 wt % WPI solutions (pH 7.0, 100 mM NaCl) was monitored as they were heated from 30 to 90 °C and then cooled to 30 °C. Sucrose increased the gelation temperature and the final rigidity of the cooled gels. The degree of flocculation in 10 wt % oil-in-water emulsions stabilized by 1 wt % WPI (pH 7.0, 100 mM NaCl) was measured using a light scattering technique after they were heated at fixed temperatures from 30 to 90 °C for 15 min and then cooled to 30 °C. Sucrose increased the temperature at which maximum flocculation was observed and increased the extent of droplet flocculation. These results are interpreted in terms of the influence of sucrose on the thermal unfolding and aggregation of protein molecules.

Keywords: *Whey proteins; heat denaturation; functionality; emulsion stability; gelation; sucrose; osmotic stress*

INTRODUCTION

Whey proteins are widely used as ingredients in the food industry because of their ability to stabilize emulsions and form gels (Phillips et al., 1994; Dickinson, 1995; Huffman, 1996). These functional attributes are ultimately determined by their molecular structure and interactions and the way that these characteristics are altered by changes in environmental conditions, such as solvent composition, temperature and mechanical forces (Dickinson and McClements, 1995; Damodaran, 1996). A great deal of research has been carried out to establish the molecular basis for the functional properties of whey proteins (Phillips et al., 1994). Considerable progress has been made in this area by studying purified whey proteins under carefully controlled experimental conditions, for example, dissolved in distilled water at a particular pH, ionic strength, and temperature.

Commercial whey protein ingredients vary appreciably in their functional properties depending on their origin and the method used to isolate them (Huffman, 1996). This variability may be attributed to differences in the amount of protein, lactose, fat, and minerals, to differences in the ratio of the major whey proteins (e.g., β-lactoglobulin, α-lactalbumin, and bovine serum albumin), and to differences in the degree of protein denaturation and aggregation caused by processing (Swaisgood, 1996). Whey protein ingredient variability is one of the major challenges facing researchers trying to better understand the molecular basis of their functionality. Another major challenge is that whey protein ingredients are used in a wide variety of different food products, each with its own unique composition, structure, and processing requirements. It is well established that changes in pH, ionic strength, mineral type, and temperature have a major impact on the ability of whey proteins to act as emulsifiers and gelling agents (Mulvihill and Donovan, 1987; Mulvihill and Kinsella, 1987, 1988; Kinsella and Whitehead, 1989; Hunt and Dalgleish, 1995; Agboola and Dalgleish, 1996; Demetriades et al., 1997a,b; Demetriades and McClements, 1998). Nevertheless, there are a variety of other ingredients in food products that may interact with whey proteins and alter their functional characteristics, such as polysaccharides, sugars, vitamins, lipids, surfactants, and flavors.

A number of food and pharmaceutical products that contain whey proteins as functional ingredients also contain sugars. For this reason, we decided to study the influence of heating on the denaturation and functionality (emulsion stabilization and gel formation) of whey protein isolate (WPI) solutions containing sucrose. Previous studies have shown that sugars can increase the thermal denaturation temperature of β-lactoglobulin and various other types of globular proteins (Lee and Timasheff, 1981; Harwalker and Ma, 1989; Arntfield et al., 1990; Timasheff, 1993; Jou and Harper, 1996), and so we would expect them to have a similar effect on WPI. The proposed mechanism for the increased thermal stability of proteins is that sugars decrease the thermodynamic affinity of protein molecules for the solvent (Timasheff and Arakawa, 1989; Timasheff, 1993; Parsegian et al., 1995). For sucrose it is believed that this effect is mainly due to its ability to increase the surface free energy between oil and water (Timasheff, 1993; Taiwo et al., 1997). The thermal denaturation of a globular protein causes an increase in surface area exposed to the solvent, as well as an increase in surface hydrophobicity (Damodaran, 1996). Both of these factors oppose protein unfolding. The increase in surface free energy caused by sucrose therefore tends to oppose protein unfolding, which accounts for its ability to enhance the thermal stability of globular proteins. In

10.1021/jf9911949 CCC: $19.00
Published on Web 04/26/2000

addition, sucrose should also increase the attraction between whey protein molecules once they have unfolded, which should increase the degree of protein aggregation (Timasheff, 1993). The susceptibility of whey proteins to aggregate in the presence of sugars therefore depends on a balance between their ability to stabilize proteins against unfolding (which opposes aggregation) and their ability to enhance protein−protein interactions (which favors aggregation). Investigation of the effects of this balance on protein functionality in emulsions and gels is the major objective of the present study.

MATERIALS AND METHODS

Materials. WPI powder was obtained from New Zealand Milk Proteins (Santa Rosa, CA) and was used without further purification. The protein content, determined by Kjeldahl, was 93.4 wt %. The total solid content, determined after the powder had been heated to 102 °C for 5 h, was 96.0 wt %, that is, 4.0 wt % moisture. The ash content, determined by combustion at 550 °C for 12 h, was 1.6 wt %. The cation content of the powder, determined by atomic absorption, was as follows: Na, 0.45 wt %; Ca, 0.12 wt %; K, 0.1 wt %; and P, 0.05 wt %. Sodium chloride and sodium azide were purchased from Sigma Chemical Co. (St. Louis, MO). Soybean oil was obtained from a local supermarket and used without purification. Double-distilled water was used to prepare all the solutions, gels, and emulsions.

Differential Scanning Calorimetry (DSC). The influence of sucrose on the thermal denaturation of WPI solutions was measured using an ultrasensitive differential scanning calorimeter (VP-DSC, MicroCal, Northampton, MA). A sucrose solution containing 0.2 wt % WPI (pH 7) was placed in the sample cell, and a similar sucrose solution containing no protein was placed in the reference cell. The heat flow required to keep the two cells thermally balanced was then recorded as their temperature was increased from 10 to 110 °C at 90 °C/h. The cells were then held at 110 °C for 15 min, cooled to 10 °C, and rescanned. The first scan was therefore of native protein; the second scan was of heat-denatured protein. Measurements were carried out on two separate samples (replicates) and reported as the average. The thermal transition temperature was defined as the temperature at which a maximum occurred in the endothermic peaks. Measurements of the thermal transition temperatures of the proteins were reproducible to within 0.5 °C.

Gelation Experiments. The influence of sucrose on the dynamic viscoelastic properties of WPI solutions was measured using a constant stress rheometer (Bohlin CS10, Bohlin Instruments, Cranbury, NJ). The rheometer applied an oscillating stress of specified frequency to the sample and measured the resulting strain. The magnitude of the complex shear modulus G^* and the phase angle δ were calculated from the resulting stress−strain relationship. A concentric cylinder (C25) measurement system was used, which had a rotating inner cylinder of 25 mm diameter and a static outer cylinder of 27.5 mm diameter. Measurements were made at a frequency of 0.1 Hz and at a maximum strain of 0.005, which was well within the linear viscoelastic region of the material (as determined by a strain sweep).

Whey protein solutions were prepared by dispersing WPI powder (10 wt %) and crystalline sucrose (0−40 wt %) in an aqueous NaCl (100 mM) solution and stirring for 1 h to ensure complete dissolution. The pH of this solution was adjusted to pH 7.0 using 10% NaOH solution. These solutions were placed in the measurement cell of the rheometer and allowed to equilibrate to 30 °C for 10 min. The solutions were then heated from 30 to 90 °C at 90 °C/h, held for 20 min, and then cooled from 90 to 30 °C at 90 °C/h. Measurements were carried out on two protein solutions prepared at different times from the same whey protein ingredient (replicates) and reported as the average. The G^* and δ measurements of the two protein solutions followed the same patterns and were within 10% and 5° of each other, respectively.

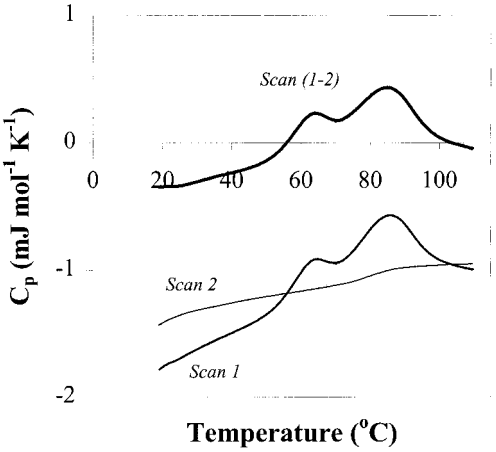

Figure 1. DSC scans of 0.2 wt % WPI solution in the absence of sucrose (pH 7.0). The difference (scan 1 − 2) between scans on "native" protein (scan 1) and "heat-denatured" protein (scan 2) was measured.

Emulsion Stability Experiments. Whey protein solutions were prepared by dispersing WPI (1 wt %) and NaCl (100 mM) in distilled water and stirring for 1 h to ensure complete dissolution of the protein. The pH of this solution was then adjusted to pH 7.0 using 10% NaOH solution. An oil-in-water emulsion was prepared by homogenizing 20 wt % soybean oil with 80% aqueous phase in a high-speed blender (homogenizer M133/1281-O, Biospec Products, Inc., Bartlesville, OK) followed by three passes at 3000 psi through a high-pressure valve homogenizer (APV-Gaulin, model Mini-Lab 8.30H, Wilmington, MA). This gave an emulsion with a mean droplet diameter of 1.8 μm. A series of 10 wt % emulsions with different sucrose concentrations were prepared by diluting the 20 wt % emulsion with distilled water and crystalline sucrose.

Droplet flocculation was monitored by measuring the increase in particle size of the emulsions after heating at temperatures between 50 and 90 °C for 15 min. The particle size distribution of the emulsions was measured using a laser light scattering instrument (Horiba LA-900, Irvine, CA). A refractive index ratio of 1.08 was used in the calculations of the particle size. Emulsions were diluted to a droplet concentration of <0.01 wt % prior to analysis to avoid multiple scattering effects. The mean particle size is reported as the surface-volume diameter, d_{32}. Measurements were carried out on two emulsions prepared at different times (replicates) and reported as the average. The measured particle sizes of the two emulsions followed the same pattern and were within 5% of each other.

RESULTS AND DISCUSSION

Influence of Sucrose on Thermal Denaturation. The thermographs of native (first scan) and heat-denatured (second scan) 0.2 wt % WPI solutions are shown in Figure 1. NaCl was not added to these solutions, as it was for the gelation and emulsion stability studies, because we did not want the proteins to aggregate extensively within the DSC cells. Previous DSC studies have shown that 100 mM NaCl has a negligible effect on the thermal denaturation of β-lactoglobulin (Harwalkar and Ma, 1989), and therefore we did not expect its omission to have a significant impact on the measurements made on the WPI samples. The scan of the native protein exhibited a broad endothermic transition between 60 and 90 °C, which had peaks at ∼63 and ∼85 °C, whereas the scan of the heat-denatured protein exhibited no thermal transitions (Figure 1). The peak at the lower temperature is

Table 1. Influence of Sucrose on Heat Denaturation of 0.2 wt % WPI Solutions (pH 7.0)[a]

sucrose (wt %)	T_{m1} (°C)	T_{m2} (°C)
0	63.2	84.6
10	64.5	85.6
20	66.4	88.5
30	68.1	90.5
40	69.2	92.6

[a] Thermal transition temperatures (T_{m1} and T_{m2}) were determined from the peaks in the DSC scans (see Figure 1).

probably due to unfolding of bovine serum albumin (T_m = 62 °C) and α-lactalbumin (T_m = 65 °C), whereas that at the higher temperature is due to unfolding of β-lactaglobulin (T_m = 71−73 °C) (Ruegg et al., 1977; Mulvihill and Donovan, 1987). In the following experiments we subtracted the scan for the heat-denatured protein from that of the native protein and then determined the thermal transition temperatures. The influence of sucrose on the thermal transition temperatures of 0.2 wt % WPI solutions is shown in Table 1. As the sucrose concentration was increased there was an increase in the thermal transition temperature for both peaks, there being an increase of ~6 °C for the lower peak and ~8 °C for the higher peak in the presence of 40 wt % sucrose (Table 1). These results are consistent with those found for β-lactoglobulin and other globular proteins (Lee and Timasheff, 1981; Harwalkar and Ma, 1989; Timasheff, 1993; Jou and Harper, 1996). The increase in thermal stability with sucrose concentration can be attributed to the decrease in thermodynamic affinity of the protein's surface for the solvent in the presence of sugar (Timasheff and Arakawa, 1989; Timasheff, 1993; Parsegian et al., 1995).

Influence of Sucrose on Gelation. The temperature dependence of the dynamic shear modulus (G^*) and phase angle (δ) of 10 wt % WPI solutions is shown in Figure 2. At the beginning of the experiment all of the solutions were liquid ($\delta \approx 90°$, $G^* \approx 0$). When the solution was heated above a particular temperature, the shear modulus increased and the phase angle decreased dramatically because the solution gelled. We define the apparent gelation temperature as the temperature at which the phase angle is 45°. The rigidity of the gels continued to increase when they were held at 90 °C and when they were cooled to room temperature (Figure 2a). After gelation had occurred, the phase angle of the gels remained relatively constant during holding at 90 °C and during cooling to 30 °C (Figure 2b). The increase in gel rigidity upon cooling suggests that decreasing the temperature either increases the attractive forces between protein molecules (e.g., van der Waals, hydrogen bonds, hydrophobic), decreases the repulsive forces (e.g., electrostatic and hydration), or decreases the entropy loss ($T\Delta S$) associated with trapping protein molecules within the gel network. The hydrophobic attraction decreases and the hydration repulsion increases with decreasing temperature, and therefore changes in these interactions cannot account for the increased gel rigidity upon cooling.

The addition of sucrose to the WPI solutions caused an increase in the temperature at which gelation occurred (Figures 2b and 3). It also caused an increase in the final rigidity of the gels once they had been cooled to 30 °C (Figures 2a and 4). The increase in the gelation temperature can be attributed to the fact that a higher temperature had to be reached before the globular protein molecules would unfold when sucrose was

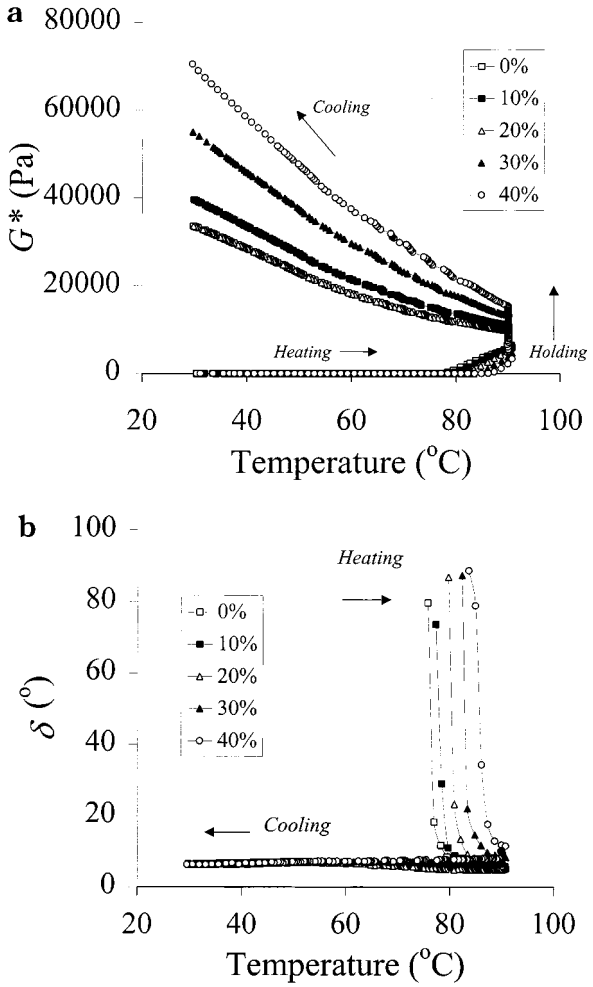

Figure 2. Temperature dependence of the dynamic shear rheology of 10 wt % WPI solutions (pH 7.0, 100 mM NaCl) heated and cooled in a rheometer: (a) magnitude of the complex shear modulus G^*; (b) phase angle, δ.

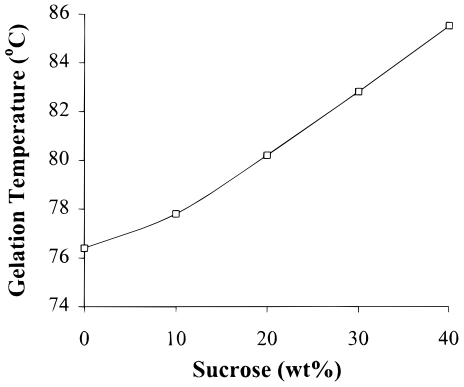

Figure 3. Dependence of gelation temperature on sucrose concentration for 10 wt % WPI solutions (pH 7.0, 100 mM NaCl).

present (Table 1). Protein unfolding is an integral part of the gelation process because it leads to the exposure of nonpolar amino acids that were originally located in the interior of the globular molecule (Mulvihill and Kinsella, 1987, 1988). Upon exposure, these nonpolar groups cause a strong hydrophobic attraction between neighboring whey protein molecules, which can lead to aggregation (Bryant and McClements, 1998). If the protein molecules do not unfold, then these groups are not exposed and the molecules are not able to form a gel. The increase in rigidity of whey protein gels cooled

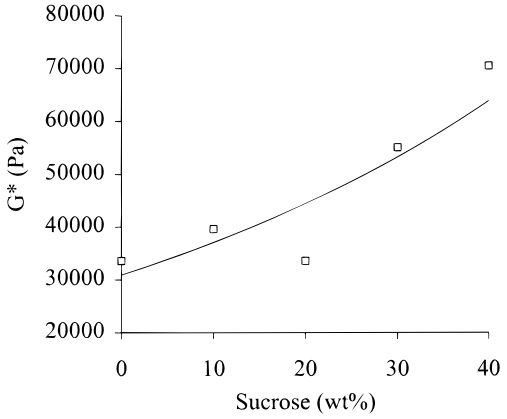

Figure 4. Dependence of final gel strength (*G**) on sucrose concentration for 10 wt % WPI solutions (pH 7.0) heated to 90 °C and then cooled to 30 °C.

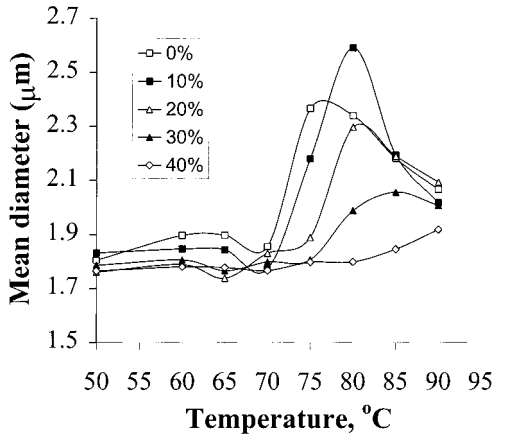

Figure 5. Influence of sucrose on droplet flocculation in 10 wt % soybean oil-in-water emulsions heated to different temperatures for 15 min and then cooled to 30 °C. Flocculation was monitored by measuring the increase in particle size determined by laser diffraction.

to 30 °C with increasing sucrose concentration can be attributed to the enhancement of protein−protein interactions in the presence of sugars.

Influence of Sucrose on Emulsion Stability. Whey protein stabilized 10 wt % soybean oil-in-water emulsions with different sucrose concentrations were heated to temperatures between 50 and 90 °C for 15 min, cooled to room temperature, and then stored for 24 h. The degree of flocculation in the emulsions was determined by measuring the increase in particle size after heating (Figure 5). In the absence of sucrose, droplet flocculation was first observed in the emulsions heated at temperatures >70 °C, exhibited a maximum around 75 °C, and then decreased at higher temperatures. A maximum in droplet flocculation around this temperature has been observed in previous studies (Monahan et al., 1993; Demetriades et al., 1997b; Demetriades and McClements, 1998). It has been shown that adsorbed whey proteins unfold at temperatures fairly similar to those at which nonadsorbed proteins unfold (Dalgleish, 1996). The extensive flocculation that occurred around 75 °C can therefore be attributed to the increase in hydrophobic attraction between emulsion droplets when the whey protein molecules unfold. The reason for the subsequent decrease in droplet flocculation at higher temperatures is still uncertain, but it may be due to the weakening of hydrophobic interactions that occur once the temperature exceeds a certain value (Creighton, 1993) or because protein molecules are able to change their orientation at the interface so that their nonpolar groups are less exposed to the aqueous phase (Demetriades et al., 1997b).

In the presence of 10 wt % sucrose the temperature at which maximum flocculation occurred increased, as well as the height of the maximum. At higher sucrose concentrations the temperature at which maximum flocculation occurred also increased with increasing sugar, but the height of the maximum decreased. The reason the emulsions had to be heated to a higher temperature before maximum flocculation was observed was because the temperature at which protein unfolding occurred increased with sucrose concentration (Table 1). The increased extent of flocculation in the emulsion containing 10 wt % sucrose can be attributed to the fact that once the protein molecules did unfold, the strength of the attraction between the droplets increased. As the sucrose concentration was increased further, the height of the flocculation maximum decreased, which can be attributed to the fact that not all of the protein molecules had unfolded, and therefore there were fewer nonpolar sites available for droplet−droplet interactions.

These results clearly demonstrate the two roles that sucrose plays in determining the thermal stability of whey proteins in emulsions and gels. First, its ability to stabilize the globular state of the protein means that it is necessary to heat the system to higher temperatures before the protein molecules unfold. Second, its ability to increase the strength of protein−protein interactions means that once the protein molecules have unfolded, there is an increased attraction between protein molecules, which leads to stronger gels and more droplet flocculation. The overall influence of sucrose therefore depends on the balance of these two different processes, which is determined by the thermal history of the system, that is, holding temperature and time.

LITERATURE CITED

Agboola, S. O.; Dalgleish, D. G. Kinetics of the Calcium-induced Instability of Oil-in-water Emulsions: Studies Under Quiescent and Shearing Conditions. *Lebensm.-Wiss. -Technol.* **1996**, *29*, 425−432.

Arntfield, S. D.; Ismond, M. A. H.; Murray, E. D. Thermal Analysis of Food Proteins. In *Thermal Analysis of Foods;* Harwalkar, V. R., Ma, C. Y., Eds.; Elsevier: London, U.K., 1990.

Bryant, C. M.; McClements, D. J. Molecular basis of protein functionality with special consideration of cold-set gels derived from heat-denatured whey. *Trends Food Sci. Technol.* **1998**, *9*, 143−151.

Creighton, T. E. *Proteins*, 2nd ed.; Freeman: New York, 1993.

Dalgleish, D. G. Food emulsions. In *Emulsions and Emulsion Stability*; Sjoblom, J., Ed.; Dekker: New York, 1996.

Damodaran, S. Amino acids, peptides and proteins. In *Food Chemistry*, 3rd ed.; Fennema, O. R., Ed.; Dekker: New York, 1996; p 321.

Demetriades, K.; Coupland, J. N.; McClements, D. J. Physical Properties of Whey Protein Stabilized Emulsions as Related to pH and Ionic Strength. *J. Food Sci.* **1997a**, *62*, 342−347.

Demetriades, K.; Coupland, J. N.; McClements, D. J. Physicochemical properties of whey protein stabilized emulsions as affected by heating and ionic strength. *J. Food Sci.* **1997b**, *62*, 462−467.

Demetriades, K.; McClements, D. J. Influence of pH and Heating on the Physicochemical Properties of Whey Protein Stabilized Emulsions Containing a Non-ionic Surfactant. *J. Agric. Food Chem.* **1998**, *46*, 3936−3942.

Dickinson, E. Properties of Emulsions Stabilized with Milk Proteins: Overview of some Recent Developments. *J. Dairy Sci.* **1995**, *80*, 2607−2619.

Dickinson, E.; McClements, D. J. *Advances in Food Colloids*; Blackie: Glasgow, U.K., 1995.

Harwalkar, V. R.; Ma, C.-Y. Effects of Medium Composition, Preheating and Chemical Modification upon Thermal Behavior of Oat Globulin and β-lactoglobulin. In *Food Proteins*; Kinsella, J. E., Soucie, W. G., Eds.; American Oil Chemists' Society: Champaign, IL, 1989; p 210.

Huffman, L. M. Processing Whey Protein for Use as a Food Ingredient. *Food Technol.* **1996**, *50*, 49−52.

Hunt, J. A.; Dalgleish, D. G. Heat Stability of Oil-in-Water Emulsions Containing Milk Proteins: Effect of Ionic Strength and pH. *J. Food Sci.* **1995**, *60*, 1120−1123.

Jou, K. D.; Harper, W. J. Effect of disaccharides on the thermal properties of whey proteins determined by differential scanning calorimetry (DSC). *Milchwissenschaft* **1996**, *51*, 509−512.

Kinsella, J. E.; Whitehead, D. M. Proteins in Whey: Chemical, Physical and Functional properties. *Adv. Food Nutr. Res.* **1989**, *33*, 343−438.

Lee, J. C.; Timasheff, S. N. The Stabilization of Proteins by Sucrose. *J. Biol. Chem.* **1981**, *256*, 7193−7201.

Monahan, F. J.; McClements, D. J.; Kinsella, J. E. Polymerization of Whey Proteins in Whey Protein Stabilized Emulsions. *J. Agric. Food Chem.* **1993**, *41*, 1826−1834.

Mulvihill, D. M.; Donovan, M. Whey Proteins and their Thermal Denaturation—A Review. *Irish J. Food Sci. Technol.* **1987**, *11*, 43−75.

Mulvihill, D. M.; Kinsella, J. E. Gelation Characteristics of Whey Proteins and β-lactoglobulin. *Food Technol.* **1987**, *41*, 102−111.

Mulvihill, D. M.; Kinsella, J. E. Gelation of β-lactoglobulin: Effects of Sodium Chloride and Calcium Chloride on the Rheological and Structural Properties of Gels. *J. Food Sci.* **1988**, *53*, 231−236.

Parsegian, V. A.; Rand, R. P.; Rau, D. C. Macromolecules and Water: Probing with Osmotic Stress. *Methods Enzymol.* **1995**, *259*, 43−94.

Phillips, L. G.; Whitehead, D. M.; Kinsella, J. E. *Structure−Function Properties of Food Proteins*; Academic Press: San Diego, CA, 1994.

Ruegg, M.; Moor, U.; Blanc, B. A calorimetric study of the thermal denaturation of whey proteins in simulated milk ultrafiltrates. *J. Dairy Res.* **1977**, *44*, 509−520.

Swaisgood, H. Characteristics of Milk. In *Food Chemistry*, 3rd ed.; Fennema, O. R., Ed.; Dekker: New York, 1996; p 841.

Taiwo, K.; Karbstein, H.; Schubert, H. Influence of Temperature and Additives on the Adsorption Kinetics of Food Emulsifiers. *J. Food Process. Eng.* **1997**, *20*, 1−16.

Timasheff, S. N. The Control of Protein Stability and Association by Weak Interactions with Water: How do Solvents Affect These Processes. *Annu. Rev. Biophys. Biomol. Struct.* **1993**, *22*, 67−97.

Timasheff, S. N.; Arakawa, T. Stability of Protein Structure by Solvents. In *Protein Structure: A Practical Approach*; Creighton, T. E., Ed.; Oxford University Press: Oxford, U.K., 1989.

Received for review November 3, 1999. Revised manuscript received March 20, 2000. Accepted March 20, 2000. We thank the Massachusetts Agricultural Experiment Station (Project MAS00745) and Dairy Management, Inc., for support of this project. A.K. thanks the Islamic Development bank for a scholarship to support his work on this project.

JF9911949