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Effect of Disulfide Bond Cleavage on Structural and Interfacial Properties of Whey Proteins

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The effect of cleavage of the S–S bonds on the structural properties of whey proteins was studied in order to relate the structural changes with air–water interfacial behavior, i.e. surface adsorption, foaming capacity, and foam stability. Fluorescence, surface hydrophobicity, and viscosity measurements suggested that with up to 50% disulfide bond cleavage whey proteins unfold and aggregate with a concomitant increase in surface hydrophobicity. Beyond this cleavage, proteins disaggregate and unfold and a decrease in surface hydrophobicity was observed. S–S bond cleavage also resulted in a gradual loss of solubility in the isoelectric region. An increase in unfolding ability of the proteins in a chemical denaturing environment was observed with S–S bond cleavage. Adsorption at the air–water interface increased with S–S bond cleavage up to 50% and decreased again, and this property correlated well with the change in surface hydrophobicity. Foaming capacity increased gradually with S–S bond cleavage while foam stability increased up to 75% S–S bond cleavage and decreased slightly. The increase in foaming properties was correlated with the increase in the unfolding ability of S–S bond cleavage protein samples. The decrease in foam stability in the completely S–S bond cleaved proteins may reflect increased surface charge due to the incorporation of $-SO_3^-$ groups during chemical modification.

Foam-forming ability is an important functional property of a food protein (Kinsella, 1976, 1981; Halling, 1981; Stainsby, 1986). Physicochemically, protein foams are colloid systems that contain protein solution as the dispersion medium and air as the disperse phase (Bikermann, 1953). During foam formation, a layer of surface protein (i.e., a film) is adsorbed at the interface of the colloidal mixture trapping air bubbles (Cumper and Alexander, 1950; Cumper, 1953; Horbett and Brash, 1987). The ability of a protein to adsorb at and lower the intrinsic free energy of air–water interfaces make them highly effective in the formation and stabilization of foams. Several molecular properties such as size, net charge, amphipathicity, solubility, and flexibility and other structural properties affect the air–water surface activity (Cherry and McWatters, 1981; Halling, 1981; Kinsella, 1981). The structural properties, which are important in controlling the surface activity of proteins, are not well understood (Graham and Phillips, 1979; Kinsella and Phillips, 1989). Proteins likely to unfold to a greater degree should be more surface active because more contacts per protein molecule can be formed at the interface and conformational entropy gain favors adsorption (Dickinson and Stainsby, 1982; Waniska and Kinsella, 1989). If this prediction is true, disulfide (S–S) bond cross-linked proteins would be less likely to unfold completely at the interface and therefore be less surface-active (Graham and Phillips, 1979; Kim and Kinsella, 1987). This hypothesis can be tested experimentally if the reduction and blocking of disulfide bonds can be done specifically and if the end products of modifications are water-soluble.

Protein S–S bonds can be reduced with use of reducing agents such as β-mercaptoethanol, DTT, etc., followed by blocking the liberated free sulfhydryl groups by iodoacetamide (Means and Feeney, 1971). Because these modification methods are unacceptable for food proteins, we developed a S–S bond cleavage method that may be more practical for food proteins, using sulfite, molecular oxygen, and catalytic amounts of cupric ion (Kella and Kinsella, 1985). In this method the protein S–S bonds are unsymmetrically cleaved by sulfite and S-unsulfonated simultaneously in a cyclic fashion:

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$$PS\text{-}SP + SO_3^{2-} \xrightarrow[Cu^{2+}]{\text{oxygen}} PS\text{-}SO_3^- + PS^- \quad (1)$$

This reaction can be kinetically controlled (Kella and Kinsella, 1985), and hence protein fractions with varying degrees of S–S bond cleavage may be prepared (Kella et al., 1986a, 1988). This method has been used successfully for the cleavage of the S–S bonds of many proteins (Kella and Kinsella, 1985, 1986; Kella et al., 1986a,b, 1988; Reddy et al., 1988; Klemacezwski et al., 1989; Haque et al., 1989). Unlike DTT cleavage and iodoacetamide blocking, the end products of oxidative sulfitolysis are soluble near neutral pHs.

Whey protein isolate is a good source of proteins such as β-lactoglobulin (βLg), α-lactalbumin (αLa), and serum albumin. All these proteins have S–S bonds: β-lactoglobulin has 2, α-lactalbumin 4, and serum albumin 17 (McKenzie, 1970). These proteins have limited foaming properties primarily attributed to the presence of S–S bonds in these proteins (Cherry and McWaters, 1981; Halling, 1981; Dickinson and Stainsby, 1982; Stainsby, 1986).

In this paper we describe the effects of S–S bond cleavage of whey proteins by oxidative sulfitolysis on protein structure, fluorescence, surface polarity, solubility, viscosity, and the associated changes in air–water interfacial behavior (surface adsorption, foaming capacity, foam stability).

MATERIALS AND METHODS

Materials. Whey protein isolate 187 (BiPRO) was obtained from LeSuer Isolates Co., LeSuer, MN. Sodium sulfite, cupric sulfate, and urea were purchased from Mallinkrodt, St. Louis, MO. BSA, ANS, Tris, GuSCN, EDTA, and DTNB were obtained from Sigma, St. Louis, MO. ANS was recrystallized three times from warm water and used. Oxidizer grade oxygen was purchased from General Chemical Stores, Cornell University, Ithaca, NY.

Methods. *High-Performance Liquid Chromatography (HPLC).* HPLC of whey protein isolate was performed in a Zorbax GF-250 Bioseries (Du Pont, Wilmington, DE) hydrophilic gel filtration column (0.95 × 25 cm) with 0.1 M phosphate buffer of pH 7.0 as the eluant and a Waters (Milford, MA) pumping and detection system. Individual protein fractions were recorded as peaks on a Hewlett-Packard 3390A reporting integrator (Avondale, PA) interfaced with the instrument.

Synthesis of NTSB. NTSB was synthesized from DTNB and sodium sulfite with use of cupric ions and molecular oxygen as described in a previous paper (Kella and Kinsella, 1985). The stock solution (approximately 50 mM) was stored in small aliquots at −20 °C and used for the disulfide bond assay. NTSB assay solution was freshly prepared by diluting NTSB stock 40-fold with 6 M GuSCN, 0.4 M Tris, 0.2 M sodium sulfite, and 20 mM EDTA (Kella and Kinsella, 1985).

S–S Bond Cleavage. Disulfide bonds were cleaved essentially according to the method of Kella and Kinsella (1985) using 10 mg/mL protein, 0.1 M sulfite, 800 mM cupric sulfate, and oxygen in the presence of 4 M urea in 0.02 M phosphate buffer (pH 7.0) at 25 °C. Kinetics of sulfitolysis were followed by arresting the reaction by adding EDTA at 20 mM level and estimating the remaining S–S bonds. When the desired cleavage of S–S bonds was achieved, the reaction was stopped by adding 100 mL of 0.2 M EDTA with stirring and the protein was precipitated by adding solid ammonium sulfate to 50% saturation. The precipitate was recovered by centrifugation at 5000 rpm for 20 min in a Sorvall RC-5 (Du Pont, Boston, MA) centrifuge and dispersed in 1.5 L of water and the pH was adjusted to 10 to dissolve the protein and dialyzed extensively and lyophilized. Thus, fractions with 0, 25, 50, 75, and 100% S–S bond cleavage were prepared and stored in cold under desiccation.

S–S Bond, Free SH, and Protein Estimations. S–S bonds were estimated spectrophotometrically on a Cary 219 (Varian Instrument Co., Sunnyvale, CA) by the NTSB method (Kella and Kinsella, 1985). Free SH content was estimated by the method of Ellman (1959) using DTNB. Protein content of the samples was estimated by the microburet method (Bailey, 1961) using crystalline BSA as the standard. pH measurements were done with a Corning pH meter (Model 120).

Fluorescence and Surface Polarity. Ratiometric fluorescence spectra were recorded with a Perkin-Elmer (Model 650-40) fluorescence spectrophotometer (Norwalk, CT) using a 1 × 1 cm fused quartz cuvette (Uvonic Inc, Plainview, NY) thermostated at 25 °C. For intrinsic fluorescence measurements, protein samples (0.1 mg/mL) were excited at 280 nm and emission was measured in the range 300–440 nm. For extrinsic fluorescence measurements, protein and ANS (1.0 mg/mL protein plus 20 μM ANS) conjugates were excited at 350 nm and the emission was recorded in the range 400–640 nm. A 5-nm band width for both monochromators was used. The extrinsic fluorescence spectra were corrected for the emission of ANS blank, and ν_F, the reciprocal of the emission maximum, was calculated in each case. Using these values, surface polarity on Kosower's Z scale was calculated from a standard curve of ANS emission in dioxane (Turner and Brand, 1968).

Solubility. Solubility in the range pH 2–8 was determined by measuring the turbidity of 1 mg/mL protein solution (Kella et al., 1986b). In all the cases pH was first adjusted to 8.0 by 0.1 HCl. Transmittance of the protein solution at 500 nm as a function of pH was noted with use of a 1-cm path length cuvette and a Spectronic 700 spectrophotometer.

Viscosity. Viscosity measurements were made with an Ostwald capillary viscometer having a flow time of 209 s with distilled water at 30 ± 0.1 °C. Flow times of protein solution (5 mg/mL in 0.02 M phosphate buffer, pH 7.0) were measured to within ±0.2 s at 30 ± 0.1 °C. Specific viscosity (η_{sp}) of the protein samples was calculated from (Bradbury, 1970)

$$\eta_{sp} = (t_s - t_o)/t_o \quad (2)$$

where t_o is the flow time of the buffer and t_s that of the protein solution.

Unfolding Ability. The capacity of both unmodified and S–S bond cleaved protein to unfold in a denaturing environment was assessed by a UV difference spectroscopic technique (Donovan, 1969; McKenzie, 1970) with 6 M GuHCl as the denaturant. The different spectra of protein samples in 6 M GuHCl were recorded with a pair of matched 1-cm path length self-masking quartz cuvettes (Uvonic Inc., Plainview, NY) on a Cary 219 double-beam spectrophotometer. A protein concentration of 1 mg/mL and 1.0-nm band width were used. Corrections for the absorption of GuHCl in the UV range were made.

Surface Adsorption. Surface adsorption of both unmofidied and S–S bond cleaved protein at the air–water interface was monitored by Wilhelmy plate method (Bull, 1964; Graham and Phillips, 1979) using a Sartorius balance (Gottingen, West Germany). A thin glass plate of 6-cm length, suspended from the arm of the balance, was used as the sensor. A stock protein solution of 0.1% was prepared in 0.02 M phosphate buffer, pH 7.0. The solution was filtered through a 0.45-μm filter (Millipore Co., Bedford, MA). A 10-mL aliquot was diluted to 100 mL with the buffer, and 40 mL of this solution was poured gently into the trough. The glass sensor plate was then lowered into position and the weight recorded after 1 h was used to calculate the surface tension (γ) as

$$\gamma = gW/2L \quad (3)$$

where g is the acceleration due to gravity ($cm \cdot s^{-2}$), W is the weight (g), and L is the length of the sensor (cm). Then the surface pressure (π) is calculated as

$$\pi = \lambda^0 - \gamma \quad (4)$$

where γ^0 is the surface tension of the buffer and γ is that of the protein solution (Bull, 1964). The surface of the glass plate was cleaned as described by Kim and Kinsella (1987).

Foam Capacity and Stability. Foam capacity of the unmodified and the S–S bond cleaved proteins was measured on a Sunbeam Power Plus 350-W Selectric Mixmaster (Phillips et al., 1987). The protein solution (75 mL, 5%) was poured into a 1.9-L bowl and whipped with double beaters at a speed of 78 rpm for a total of 20 min. At 5-min intervals, the weights of 100-mL samples of foam were determined and returned to the bowl and

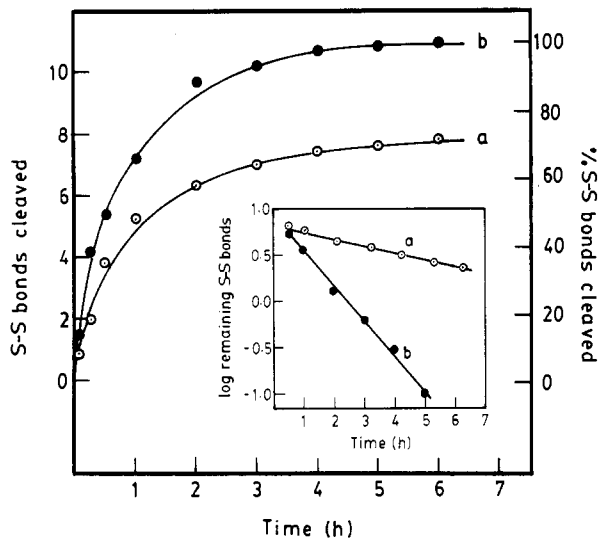

Figure 1. Kinetics of sulfitolysis of S–S bonds of whey proteins. A protein concentration of 10 mg/mL was used. Sulfitolysis was conducted (a) in 0.02 M phosphate buffer of pH 7.0 and (b) in the same buffer containing 4 M urea.

whipping was resumed. Foam capacity was calculated in terms of percent overrun (Phillips et al., 1987).

$$\% \text{ overrun} = \frac{\text{wt of 100 mL protein} - \text{wt of 100 mL foam}}{\text{wt of 100 mL foam}} \times 100 \quad (5)$$

After the samples were whipped for 20 min, the mixer was stopped and the rate of moisture drainage from the undisturbed foam was determined as described by Phillips et al. (1987). The drained liquid was collected in a tared container on a balance pan. The weight of the drained liquid was continuously recorded on a Sartorius balance interfaced with an Apple IIe computer. The time taken for half the liquid to drain from the foam ($t_{1/2}$), a reliable index of foam stability, was noted (Mita et al., 1977).

RESULTS AND DISCUSSION

Kinetics of Sulfitolysis. The whey protein isolate contained 90% β-lactoglobulin, serum albumin (6%), and α-lactalbumin (4%) as determined by HPLC. All these proteins contain S–S bonds, and the dialyzed unmodified whey protein isolate gave a total of 11 disulfide bonds/100 000 g of protein as estimated by NTSB assay. No free sulfhydryl could be detected by the method of Ellman (1959) using DTNB. When whey protein was subjected to sulfitolysis in 0.02 M phosphate buffer, the reaction resulted in a cleavage of about 70% of the total S–S bonds after 6 h (Figure 1). Cleavage of the disulfide bonds followed pseudo-first-order kinetics since a plot of logarithm of remaining S–S bonds versus time gave a straight line (Figure 1, inset). From the slope, the rate constant (k) and the time taken for the cleavage of all the S–S bonds ($t_{99\%}$) were calculated. The free energy of activation (ΔG^*) for sulfitolysis of S–S bonds of whey proteins was calculated by the Eyring equation

$$\Delta G^* = \frac{-RT(\ln k - \ln R)}{TNh} \quad (6)$$

where R and h are gas and Planck constants, respectively, N is Avogadro's number, and T is the temperature on the Kelvin scale. The calculated values of k, $t_{99\%}$, and sulfitolysis of whey protein are compiled in Table I. The time required to completely cleave the S–S bonds of whey proteins was about 20.5 h (Table I). The slow reaction could result from unavailability of the protein S–S bonds to the reagent owing to the aggregation of some of the S–S bond cleaved components (Kella et al., 1986a).

This problem may be avoided by including 4.0 M urea, which does not completely denature the protein (Kella and Kinsella, 1986, 1988) but facilitates the sulfitolysis reaction by preventing the aggregation of the partially S–S bond cleaved components during sulfitolysis (Kella et al., 1986a). In the presence of 4 M urea, the sulfitolysis reaction followed a pseudo-first-order reaction (Figure 1, inset). As compared with the reaction in the absence of urea, k increased 51.6-fold, the time taken for the complete cleavage decreased by 15 h, and the activation free energy decreased by 2330 cal/mol in the presence of 4 M urea (Table I). Hence, urea was included in the sulfitolysis reaction for preparing the S–S bond cleaved whey protein samples already described in Materials and Methods.

Table I. Important Parameters for Disulfide Bond Cleavage of Whey Protein

sample	k (25 °C), s^{-1}	ΔG^*, cal/mol	$t_{99\%}$, h
as such	6.4×10^{-5}	23 100	20.5
with 4.0 M urea	3.31×10^{-3}	20 770	5.5

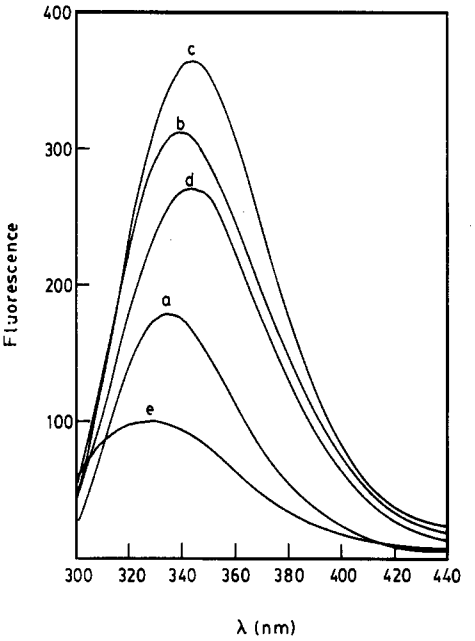

Figure 2. Fluorescence emission spectra of S–S bond cleaved whey proteins. A protein concentration of 0.1 mg/mL (in 0.02 M phosphate, pH 7.0) was used. Key: (a) unmodified; (b–e) 25, 50, 75, and 100% S–S bond cleaved fractions, respectively.

Conformational Changes. The conformational changes induced by the S–S bond cleavage per se of the whey protein were followed by measuring the changes in the intrinsic fluorescence spectrum of the protein. The unmodified whey protein had an emission maximum at 333 nm, indicating that it is mainly caused by tryptophan fluorescence (Figure 2). In the 25 and 50% S–S bond cleaved whey protein samples, the fluorescence emission increased and the maximum shifted to 340 and 345 nm, respectively. However, with further S–S bond cleavage, the emission decreased and the maximum shifted to lower wavelengths (Figure 2). The protein with complete cleavage of disulfide bonds had the least emission instability compared to all the other samples (Figure 2).

Increase in fluorescence with a blue-shift is usually ascribed to the transfer of tryptophan groups from a polar medium to the nonpolar medium (Teale, 1960) that may result from protein refolding or aggregation. However, in the present study the emission increase, in the case of 25 and 50% disulfide bond cleavage samples, is followed by

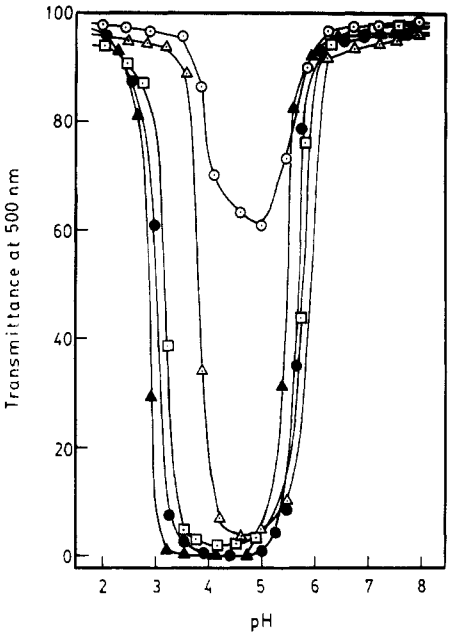

Figure 3. Solubility patterns of S–S bond cleaved whey proteins. A 1 mg/mL protein concentration was used. Key: (⊙) unmodified; (△) 25%, (□) 50%, (●) 75%, and (▲) 100% S–S bond cleaved fractions.

a red-shift. This may be because of an "exiplex" complex formation of tryptophan with any polar residue inside the protein interior (Walker et al., 1966, 1967) following either protein refolding or reaggregation. A decrease in the fluorescence intensity of proteins with 75 and 100% S–S bond cleaved could be because of the exposure of tryptophans to the surrounding aqueous polar plane from the protein interior. This may result from the increase in flexibility of the protein induced by S–S bond cleavage. However, it is rather difficult to explain the blue-shift in these two samples. It could be speculated that this results from an increased introduction of negatively charged sulfonate groups (Kella et al., 1988). A similar observation was made by other workers. For example, the addition of negatively charged iodide ion to lysozyme quenched and shifted the fluorescence spectrum of tryptophan to lower wavelengths (Lehrer, 1975).

Solubility Profiles. Solubility as a function of pH in the isoelectric region is an important property of proteins (Cohen and Ferry, 1943), and this property is an indication of the unfolding of the protein (Joly, 1965). The solubility (as measured by the turbidity technique) of the unmodified whey protein changed with pH in the range 6.2–3.4, showing one solubility minimum (with 40% insolubility) around pH 5.0 (Figure 3). This is in good agreement with the solubility profile reported for β-Lg (p*I* 5.2). All the S–S bond cleaved protein samples exhibited 95–100% insolubility in the range pH 6.0–2.0 with varying insolubility minima (Figure 3). The samples with 25, 50, 75, and 100% disulfide bond cleavage exhibited insolubility minima at pH 4.75, 4.38, 4.2, and 4.0, respectively. Solubility is the result of the balance of the attraction of the protein molecules for each other and the solvent molecules for the solute. At the isoelectric point, the attraction of the protein molecules for each other (through mostly hydrophobic interactions) is maximal because of least repulsive charge on the surface owing to zero net charge. Consequently, the solubility of the protein tends to be a minimum at the isoelectric point. Thus, the solubility minima can be taken as the isoelectric points. If the pH is shifted on either side of the isoelectric point, the protein acquires a net charge.

Table II. Surface Polarity of Disulfide Bond Cleaved Whey Protein As Estimated from the Emission Maxima of the Bound ANS

whey protein	$\nu_F \times 10^4$, cm^{-1}	Z
unmodified	2.083	91.3
25% S–S bond cleaved	2.100	90.1
50% S–S bond cleaved	2.132	87.2
75% S–S bond cleaved	2.123	87.9
100% S–S bond cleaved	2.110	88.6

This decreases the attraction of the protein molecules for each other, thus increasing the solubility. During the cleavage of S–S bonds the introduced negatively charged sulfonate groups apparently increase the surface charge of the protein as indicated by a shift in the pH of the protein (Figure 3). This should increase the solubility; however, the opposite effect was observed. Protein solubility depends not only on the surface charge but on the ratio of surface charge to surface hydrophobicity (Melander and Horvath, 1977). It is conceivable that the cleavage of disulfide bonds brings about structural changes that expose the nonpolar groups previously buried inside the protein interior, thereby increasing the surface hydrophobicity. This probably decreases the surface polarity to hydrophobicity ratio, facilitating the protein–protein association and ultimately resulting in a lower solubility.

Surface Hydrophobicity. Surface hydrophobicity plays an important role in the manifestation of many other functional properties of proteins (Kella et al., 1986b). Although there are several methods to measure the surface hydrophobicity, the method of Turner and Brand (1986), which utilizes binding of ANS to the protein, offers a clearly defined scale and has fewer limitations compared to other methods. Hence, in the present study, this method was employed to study the surface hydrophobicity of whey protein as a function of S–S bond cleavage. The extrinsic fluorescence emission spectra of the ANS conjugates of the unmodified whey protein (corrected for the fluorescence of the ANS blank) showed emission maximum at 480 nm. After the S–S bond cleavage the emission maximum changed. From the emission maxima, ν_F (which is a measure of transition energy) and Z (surface polarity) values were calculated. Unmodified whey protein gave a Z value of 91.25, and this decreased with S–S bond cleavage of up to 50%, indicating an increase in surface hydrophobicity, and increased again, suggesting a decrease in surface hydrophobicity (Table II). During protein folding, most nonpolar residues occupy the protein interior to minimize contact with water while the polar (charged) residues occupy the protein surface and favorably interact with water (Anfinsen and Scheraga, 1975). However, some nonpolar residues also appear on the protein surface in the form of hydrophobic patches (Schulze and Schirmer, 1979), thus contributing to the surface hydrophobicity of the native protein. Covalent cross-links such as S–S bonds maintain the structural integrity of the protein (Thornton, 1981). Cleavage of these S–S bonds (up to 50%) in whey protein may cause unfolding of the protein and may increase the number of nonpolar patches on the surface owing to the exposure of many hydrophobic residues from the protein interior. The decrease in the surface hydrophobicity in the 75 and 100% S–S bond cleaved samples could result from increased incorporation of charged sulfonate groups on the surface due to chemical modification of S–S bonds of the protein.

Viscosity Measurements. Unmodified whey protein had an η_{sp} value of 0.05 at 0.5% concentration. This value increased with S–S bond cleavage up to 50% and again decreased with further cleavage (Figure 4). Specific vis-

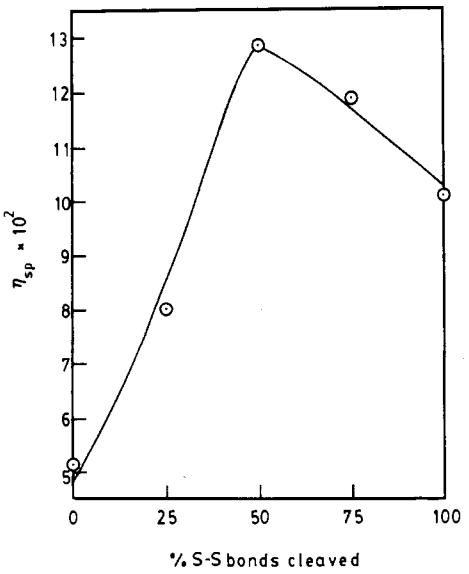

Figure 4. Specific viscosity of whey proteins as a function of S–S bond cleavage. A protein concentration of 5 mg/mL (in 0.02 M phosphate buffer, pH 7.0) was used.

cosity is related to the shape and hydrodynamic volumes of protein by

$$\eta_{sp} = \beta c(\nu_2 + \delta_1\nu_1) \quad (7)$$

where β is the shape factor, c is the concentration (by weight) of the protein, ν_2 and ν_1 are the specific volumes of the protein and the associated solvent, respectively, and δ_1 is the weight of the solvent associated per unit weight of the protein. The observed increase in viscosity may result from any one or a combination of change in shape of the protein or increased hydration which, in turn, could arise from aggregation and/or unfolding of the protein with cleavage of 25 and 50% S–S bonds. On the contrary, a decrease in specific viscosity in the samples with 75 and 100% S–S bond cleavage could arise from a partial dissociation and/or refolding of the unfolded proteins (Joly, 1965).

Surface Adsorption. The changes in the surface pressure (π) of the unmodified and S–S bond cleaved protein samples were measured for 1 h, and the final values were plotted as a function of S–S bond cleavage (Figure 5). The unmodified whey protein gave a π value of 16.25 $mN \cdot m^{-1}$. This value increased with cleavage up to 50% of S–S bonds and decreased with further S–S bond cleavage (Figure 5). Protein molecules diffuse to and adsorb at interfaces, thereby reducing interfacial tension (MacRitchie, 1978). The rate of adsorption (dn/dt) is related to the rate of diffusion as described by

$$dn/dt = C_0(D/\pi)^{1/2}t^{-1/2} \quad (8)$$

where C_0 is the bulk concentratin of the protein, D is the diffusion coefficient, t is the time, and π equals 3.14. In classical terms, D is related to the frictional coefficient, f, by the Stokes–Einstein equation

$$D = k_BT/f \quad (9)$$

where k_B is the Boltzmann constant and T the temperature. Since cleavage of S–S bonds may loosen the tightly folded native structure and increase the frictional coefficient of the molecule with up to 50% cleavage (as indicated by viscosity measurements), it should decrease the diffusion coefficient and hence surface pressure values. But, on the contrary, the opposite behavior was observed. Though the surface pressure of 75 and 100% S–S bond cleaved samples decreased as compared to proteins with

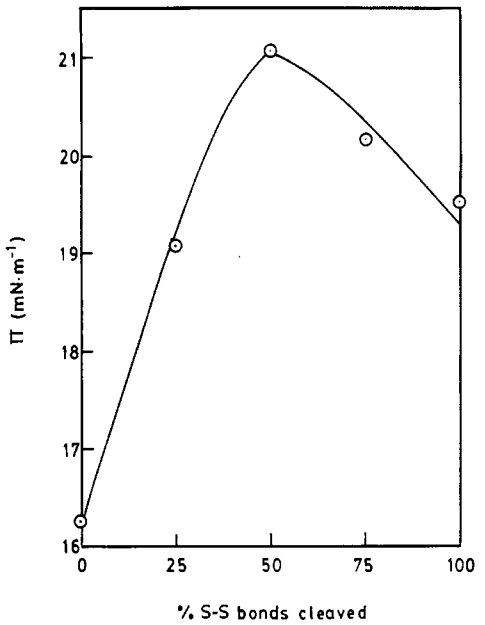

Figure 5. Surface pressure (π) of S–S bond cleaved whey protein fractions. A protein concentration of 0.1 mg/mL (in 0.02 M phosphate buffer of pH 7.0) was used.

50% S–S bonds cleaved, the π values of these two samples were greater than in the unmodified sample.

When a protein adsorbs from a solution in which the pH is kept close to its isoelectric point, the rate of adsorption at the interface is controlled primarily by the rate of diffusion to the interface (MacRitchie, 1978). However, when the protein molecule takes on a net electrical charge (as in the present case), an additional barrier to adsorption appears, owing to the electrical potential set up at the interface by the adsorbed protein. The rate of adsorption is then given by

$$dn/dt = k_aC_0 \exp[-(\pi\Delta A + \eta\Psi)/k_BT] \quad (10)$$

where k_a is the rate of adsorption, ΔA is the area to be cleared in order to adsorb, η is the net charge, and Ψ the electrical potential. In the present study, whey protein, as such, is negatively charged at pH 7.0 (since the pI is approximately 5.0). With the cleavage of S–S bonds the negative charge further increases and hence should lead to a decrease in surface pressure. However, this is not the case. It may be concluded that the S–S bond cleaved protein samples simply do not follow either simple laws of diffusion or the electrical potential barrier principle, and we need new theories to explain the adsorption behavior of these (Song and Damodaran, 1987). The molecular flexibility and the varying capacities of different domains or polypeptide segments of proteins to occupy the interface need to be incorporated into current concepts of protein foaming (Kinsella and Phillips, 1989; Damodaran, 1989).

Foam Capacity. There are at least three ways of forming a protein foam: by sparging air through or by shaking or whipping a protein solution (Halling, 1981). The third method is closer to food application and effectively incorporates all the protein solution into foam (Cherry and McWatters, 1981; Stainsby, 1986). Foam capacity can be measured as percent overrun, which is equivalent to the overrun volume increase in foam during whipping (Halling, 1981). The percent overrun of the unmodified and S–S bond cleaved whey protein fractions as a function of the whipping time is shown in Figure 6a. In all the cases, there was only a slight increase in percent overrun between 5 and 10 min whipping, considerable increase between 10 and 15 min, and almost constancy

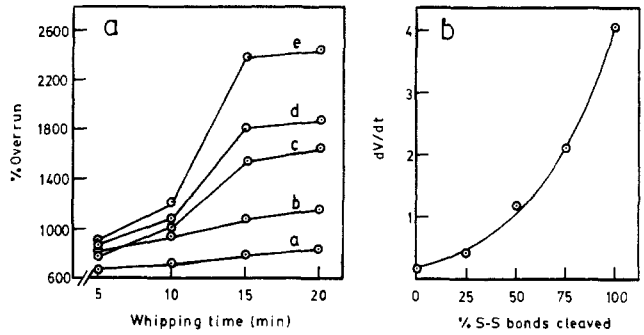

Figure 6. (a) Foaming capacity of S–S bond cleaved whey proteins as a function of whipping time. A 5% protein concentration was used. Curves a–e represent the unmodified and 25, 50, 75, and 100% S–S bond cleaved whey proteins. (b) Plot of first derivative of volume increase with time (between 10 and 15 min whipping) and percent S–S bond cleavage.

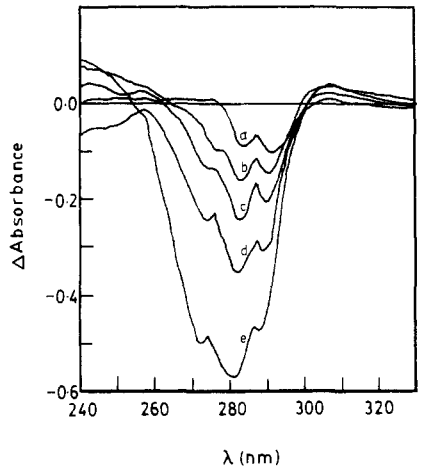

Figure 7. Unfolding ability of whey proteins as a function of S–S bond cleavage. A protein concentration of 1 mg/mL was used. Difference spectra were recorded against unfolded protein in 20 mM phosphate buffer. Curves a–e represent unmodified and 25, 50, 75, and 100% S–S bond cleaved whey proteins in 6 M GuHCl.

between 15 and 20 min whipping (Figure 6a). Since increase in percent overrun is a measure of the volume (V) increase in the system, the change in percent overrun with time or dV/dt between 10 and 15 min whipping time was plotted as a function of S–S bond cleavage (Figure 6b). dV/dt exponentially increased with S–S bond cleavage, indicating that S–S bond cleavage facilitates adsorption at the interface. In commercial mixers, the shear caused by whipper blades can cause mechanical unfolding of the proteins and facilitate the adsorption of protein at the interface (Kinsella, 1981).

Unfolding Ability. Determination of the shear-induced unfolding of the S–S bond cleaved protein fractions is difficult because unfolding is irreversible and results in insolubility. Hence, to examine the unfolding ability of the S–S bond cleaved fractions, an alternate approach was adopted. Both unmodified and S–S cleaved fractions were dissolved in 6 M GuHCl, which is a powerful chemical denaturant, and the UV difference spectra of these were recorded against the unmodified protein in buffer without any denaturant (Figure 7). The unmodified protein in the presence of 6 M GuHCl exhibited a blue-shift with two troughs at 282 and 288 nm. All the S–S bond cleaved samples exhibited an additional peak at 272 nm. There was a gradual increase in the magnitude of the blue-shift with an increase in the S–S bond cleavage (Figure 7). In general, the blue-shift in the UV spectrum of the protein at 282 and 288 nm is attributed to the exposure of tyrosines

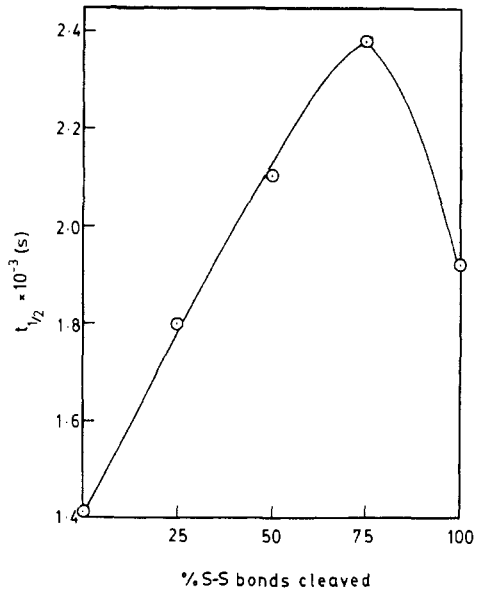

Figure 8. Foam stability as measured by drainage method, of the S–S bond cleaved whey proteins. A 5% protein concentration was used.

from the hydrophobic protein interior to the polar exterior as a result of protein unfolding (Leach and Scheraga, 1962; Kronman and Robbins, 1975). The peak at 272 nm could result from the exposure of phenylalanine. These results suggest that S–S bond cleavage increases the susceptibility of whey protein to unfold. S–S bonds in proteins are known to stabilize them against denaturation by decreasing the entropy of unfolding (Anfinsen and Scheraga, 1975). Increasing cleavage of S–S bonds may make whey protein more susceptible to unfolding by increasing the entropy of the system. Thus, the dV/dt plot in Figure 6b may correspond to the unfolding tendencies of the protein.

Foam Stability. While the ability of a protein solution to form a foam is a necessary requirement for certain functional applications, the stability of the foam is more important in ultimately determining its potential usage in foods. Foam stability of the unmodified and S–S bond cleaved whey proteins was assessed by determining $t_{1/2}$, the time required for the drainage of half of the total liquid in the foam (Mita et al., 1977). The $t_{1/2}$ values increased rather linearly with S–S bond cleavage of up to 75% and decreased again (Figure 8), suggesting that cleavage of up to 75% increases the stability of the foam and extensive cleavage destabilizes it. The stability of foams reflects their capacity to retain moisture and the mechanical and viscoelectric properties of the protein film (Halling, 1981; Graham and Phillips, 1979; Kinsella and Phillips, 1989). Increasing the thickness of the film and the extent of proteins to protein interaction enhances stability. Cleavage of the S–S bonds of whey proteins up to 75%, by increasing molecular flexibility, may have facilitated protein–protein association in the film, e.g. via hydrophobic interactions. In addition, the net negatively charged SO_3^- groups may have improved water retention and accentuated disjoining pressures between adjacent films, thereby enhancing stability. Halling (1981) reported that increased hydrophobicity in acetylated caseins led to increased foam stability whereas more hydrophilic derivatives caused decreased stabilities. It was speculated that changes in cohesive interactions in the adsorbed film provided the changes in stability. Similarly in the present study, although cleavage of S–S bonds might lead to an increase in surface hydrophobicity via unfolding in the 100% S–S bond cleaved sample, the increased incorporation of the polar $-SO_3^-$

groups during modification may have caused repulsion of the adsorbed protein molecules in the films. This may have decreased the cohesive properties of the adsorbed films, thus decreasing the foam stability. There is also some evidence that an appreciable degree of tertiary structure is required for the optimal foam stability (Graham and Phillips, 1979; Mita et al., 1978). Thus, it is possible that the required tertiary structure in whey protein may be lost by complete cleavage of S–S bonds, resulting in a decrease in the foam stability.

This study indicates that S–S bonds do contribute to the native structure of whey proteins and that their cleavage leads to changes in conformation, surface hydrophobicity, solubility, viscosity, and the ability to unfold. Fluorescence and viscosity measurements suggested that whey proteins unfold and tend to aggregate with S–S bond cleavage of up to 50%, whereas with cleavage beyond 50% they dissociate and unfold. This is consistent with the surface hydrophobicity measurements. Solubility in the isoelectric region decreased in all the S–S bond cleaved samples, with a concomitant decrease in the isoelectric points. S–S bond cleavage progressively raised the unfolding ability of these proteins in a chemical denaturant environment, suggesting that these can unfold more at the air–water interface.

From the results presented here, it may be possible to draw some correlations between some structural changes of whey proteins and certain interfacial phenomenon. Foaming capacity depends on unfolding of the proteins by shear, adsorption of these as monolayers at the interface, and entrapment of the air by encapsulation of air bubbles with protein (Halling, 1981). A good correlation was found between foaming capacity and unfolding ability of the S–S bond cleaved fractions. Adsorption at the interface correlated well with the surface hydrophobicity. Foam stability of S–S bond cleaved proteins could be related to the unfolding ability up to 75% of cleavage but not beyond. The decrease in the foam stability of the completely S–S bond cleaved whey proteins could not be attributed to aggregation. The 100% S–S bond cleaved protein had the highest negative charge density at pH 7.0 (due to incorporation of $-SO_3^-$ groups during cleavage of S–S bonds), which might lead to repulsion of the polypeptides constituting the film and facilitate the drainage of the liquid, thus leading to less stability. This study confirms the importance of disulfide bonds (Kim and Kinsella, 1987) in influencing the film forming and foaming properties of food proteins. The sulfitolysis method used in this study may be of practical use in improving functional proteins of food proteins.

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ABBREVIATIONS USED

ANS, 1-anilino-8-naphthalenesulfonate; BSA, bovine serum albumin; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); DTT, 1,4-dithiothreitol; EDTA, ethylenediaminetetraacetic acid; GuHCl, guanidinium hydrochloride; GuSCN, guanidinium thiocyanate; HPLC, high-performance liquid chromatography; NTSB, 2-nitro-5-mercaptosulfobenzoate; SH, sulfhydryl; S–S, disulfide; Tris, tris(hydroxymethyl)aminomethane.

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Changes in Physical and Chemical Parameters Associated with Quality and Postharvest Ripening of Harvester Peaches

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Changes in the quality characteristics of Harvester peaches related to ripening for 1, 3, 6, and 7 days and at different maturity stages as determined by the use of color chips (grades) were correlated. Hunter values L and b had only slight change while a values increased and hue angle (θ) decreased with ripening as chip maturity increased. Firmness and titratable acid decreased as ripening progressed. As maturity (chip 3 and above) and days of ripening increased, soluble solids/acid ratio increased. No change was found in the total phenolics with increasing chip maturity; however, with increasing days, ripening (day 6 and above) increases were found. As chip maturity increased, malic acid increased, citric acid decreased, and succinic acid remained the same. Glucose (1.21 mg/100 g) and fructose (1.04 mg/100 g) remained the same as maturity increased. Interactions with chip maturity and days ripening were found with sucrose and sorbitol. The sensory evaluation panel rated the peaches acceptable or better for unripened peaches at maturity chip 6 and maturity chip 4 and above for ripened peaches.

An estimated 1163.3 million pounds of peaches were produced in the United States in 1985 (USDA, 1986). Major production centers include the Southeastern states, California, New Jersey, Pennsylvania, and Washington.

Appearance of skin color, flavor, volatiles, texture, sugar, and acid content are the key components that contribute to a high-quality fresh peach. Studies on these parameters that define quality in a peach have been reported in the literature (Robertson et al., 1988; Shewfelt et al., 1987; Delwiche and Baumgardner, 1983; Kader et al., 1982; Watada et al., 1979; Spencer et al., 1978). These reports provide only a partial description of chemical and physical changes occurring in relation to quality. Because of this, it has not been possible to adequately define peach quality in relation to consumer acceptance. A better understanding of chemical changes in the peach that have positive and negative effects on organoleptic acceptance would facilitate the development of improved cultivars and enhance the postharvest preservation of quality (Dull and Hulme, 1971).

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