Sulfitolysis of Whey Proteins: Effects on Emulsion Properties

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Whey protein isolate was modified by oxidative sulfitolysis to cleave 50%, 75%, and 100% of the total disulfide bonds. The volume fraction of oil emulsified and the emulsion activity index improved as the degree of sulfitolysis was increased from 0% to 100%. The volume of surface droplet diameter (d_{vs}) of these emulsions was observed to decrease with increasing sulfitolysis, and the coalescence rate decreased with the extent of sulfitolysis.

INTRODUCTION

The use of whey protein isolates and whey protein concentrates as functional ingredients in food systems, e.g., as stabilizers in low-fat emulsions, has increased in the past 5 years (Morr, 1989). A recent survey of commercially available whey protein concentrates and isolates showed considerable variability in the compositional and functional properties (Morr and Foegeding, 1990). Differences in functionality are attributable to the variability in whey source and processing parameters (Kinsella, 1984; Kinsella and Whitehead, 1989).

Previous studies have correlated the emulsifying properties of whey protein concentrates and isolates with net hydrophobicity (Elizalde et al., 1988), solubility (McWatters and Holmes, 1979), and composition (Kim et al., 1987). Several studies have examined the effects of heat treatment on the emulsifying properties of whey protein concentrates (Morr, 1987; Chen, 1989). The solubility and emulsifying properties of casein and whey protein concentrate were improved by limited trypsin hydrolysis. The emulsion activity index of hydrolyzed whey protein improved more than that of casein. This was attributed, in part, to the increased flexibility of the enzymatically modified whey protein concentrates (Chobert et al., 1988).

The emulsifying properties of bovine serum albumin (BSA), a component of whey protein isolate, were improved by the controlled oxidative sulfitolysis of disulfide bonds (Kella et al., 1989; Klemaszewski et al., 1990). It was speculated that the improved flexibility of the BSA molecule allowed for more rapid alignment of the hydrophobic and hydrophilic groups with the oil and aqueous phases, respectively. The enhanced flexibility of BSA may have facilitated the formation of a stronger interfacial film, and/or the increased net negative charge retarded the rate of coalescence (Klemaszewski et al., 1990).

The objectives of this study were to compare emulsion formation and coalescence properties of a commercially available whey protein isolate, following progressive oxidative sulfitolysis.

MATERIALS AND METHODS

Materials. Commercially available whey protein isolate (WPI) was obtained from LeSueur Co. (LeSueur, MN) and was dialyzed with distilled water to remove salts and impurities (Phillips et al., 1989). The WPI contained 95% protein, of which β-lactoglobulin contributed 75% of the nitrogen. EDTA and DTNB were obtainned from Sigma Chemical Co. (St. Louis, MO). Sodium sulfite, cupric sulfate, ammonium sulfate, and urea were purchased from Mallinkrodt (Paris, KY). Monobasic and dibasic potassium phosphate were obtained from Fisher Scientific (Pittsburgh, PA). Oxygen, oxidizer grade, was obtained from the Cornell General Chemical Store (Ithaca, NY).

The emulsions were made by using commercial pure peanut oil that did not contain added emulsifiers. The specific gravity of this oil was 0.91. All other chemicals used were of reagent grade, and distilled deionized water was used throughout this study.

Methods. *Oxidative Sulfitolysis of Whey Protein Isolate.* Increasing numbers of the disulfide bonds were cleaved with sodium sulfite and oxygen, in the presence of urea and catalytic cupric sulfate, according to the procedures of Kella and Kinsella (1985) and Kella et al. (1989). Reaction kinetics were determined, and the times required to cleave 50%, 75%, and 100% of the disulfide bonds in whey protein isolate were used. The disulfide bonds in whey protein isolate (10 mg/mL) were cleaved to the desired levels in phosphate buffer (pH 6.8), as described by Kella et al. (1989). The desired degree of sulfitolysis was obtained by stopping the reaction by addition of EDTA, to chelate catalytic copper. The numbers of intact disulfide bonds and free sulfhydryl groups were determined by using the methods of Kella and Kinsella (1985) and Ellman (1959).

Emulsion Preparation. Solutions of whey protein isolate, at 0.25% and 1.0% (w/w) protein, were made up in phosphate buffer (pH 6.8) with ionic strengths of 0.1 and 0.2. Phosphate buffer, 0.146 M, ionic strength 0.2, was prepared, and one-third of this buffer was equally diluted with distilled deionized water to give a buffer with an ionic strength of 0.1. The emulsions were prepared according to the methodology of Klemaszewski et al. (1989). The emulsion was transferred to a test tube placed on a Thermolyn Specimixer to accelerate coalescence. The temperature of the emulsions was held constant at 25 °C.

Determination of Emulsion Characteristics. The test tube containing the emulsion was removed from the shaker and held for 1 min. In some samples, separation into a small upper layer of coalesced oil and the lower emulsion was observed. The initial volume fraction of oil in the emulsion phase of all samples was determined from the specific gravity according to the methodology of Pearce and Kinsella (1978). The surface area of the emulsions and the emulsion activity index (EAI) were determined by using the methods of Walstra (1968) and Pearce and Kinsella (1978) as described by Klemaszewski et al. (1991). The d_{vs} of the emulsions was calculated by the method of Walstra (1968), and some of these data were confirmed by using the methodology of Klemaszewski et al. (1989).

RESULTS AND DISCUSSION

The emulsions formed in this study were composed of heterogeneous dispersions of spherical oil droplets, as determined by light microscopy. The volume fractions of oil and the droplet size distributions of the emulsions varied with the extent of whey protein sulfitolysis and protein concentration. The emulsions varied in stability and were observed to coalesce with time. Emulsion stability was

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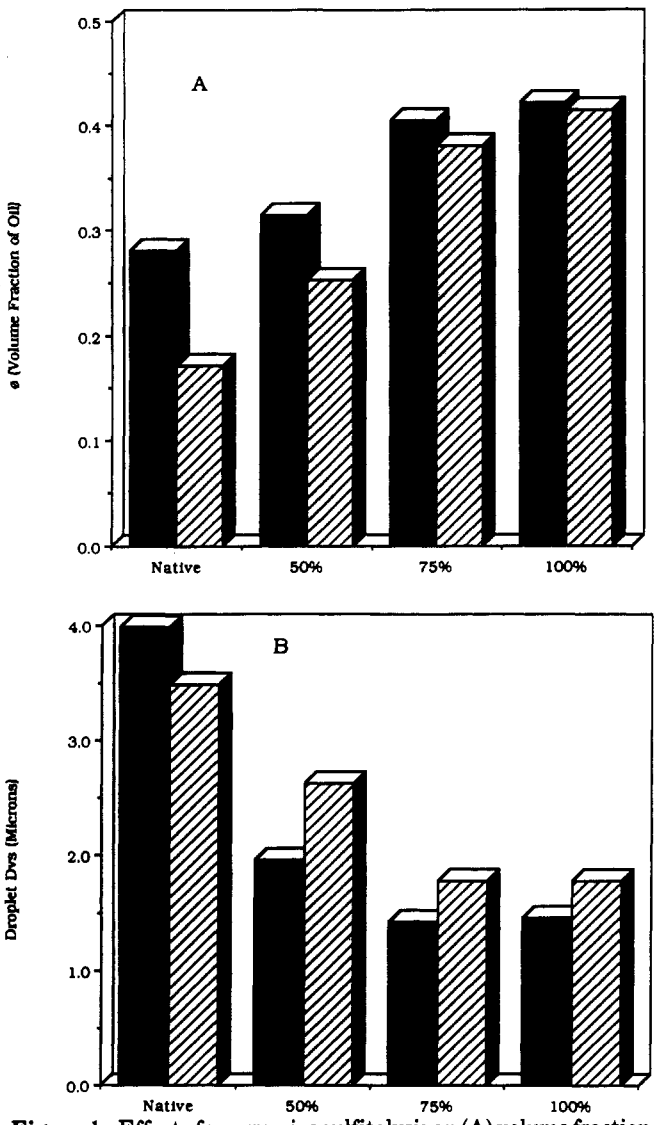

Figure 1. Effect of progressive sulfitolysis on (A) volume fraction of oil (ϕ) and (B) initial droplet diameter (d_{vs}) of emulsions stabilized by 1.0% (w/w) whey protein isolate. (Solid bars) μ = 0.1; (hatched bars) μ = 0.2 in phosphate buffer, pH 6.8.

affected by the number of disulfide bonds cleaved, protein concentration, and ionic strength.

The volume fraction of oil emulsified by whey protein isolate increased with increasing degrees of sulfitolysis at both protein concentrations and ionic strengths studied (Figure 1A). Most of the oil present was emulsified by 1% WPI with 75% and 100% of the disulfide bond cleaved. The maximum volume fraction of oil theoretically obtainable (i.e., ϕ = 0.42) was nearly emulsified by 1% WPI in which 75% of the disulfide bonds were cleaved, with volume fractions of 0.40 and 0.38 formed at μ = 0.1 and 0.2, respectively. The volume fractions of oil emulsified by 1% WPI with all of the disulfide bonds cleaved were 0.42 and 0.41 at μ = 0.1 and 0.2, respectively, while native WPI formed emulsions with volume fractions of 0.28 and 0.17 at ionic strengths of 0.1 and 0.2, respectively. At both protein concentrations and at all levels of sulfitolysis, increasing the ionic strength from 0.1 to 0.2 lowered the volume fraction of oil emulsified by WPI. However, this effect was less pronounced in emulsions formed with WPI with over 50% of the disulfide bonds cleaved (Figure 1A).

The average droplet size (d_{vs}) of the emulsion varied with protein concentration, ionic strength, and the number of disulfide bonds cleaved. The d_{vs} of emulsions formed with 1% protein decreased with increasing sulfitolysis of WPI (Figure 1B). The average droplet diameter decreased from approximately 4 μm, for emulsions stabilized by 1% unmodified whey protein isolate, to 2 μm in emulsions formed with 1% whey protein isolate with 75% or 100% of the disulfide bonds cleaved (Figure 1B). This indicated a marked improvement of the emulsifying properties of the modified proteins, because they effectively coated a much larger surface area of oil.

Molecular flexibility enhances the emulsion formation properties of proteins (Graham and Phillips, 1979; Klemaszewski et al., 1990), and sulfitolysis has been shown to improve the flexibility and foaming properties of whey proteins (Kella et al., 1989). Thus, the increased protein flexibility ostensibly enhanced unfolding at the interface and allowed more favorable alignment of hydrophilic and hydrophobic regions of the protein in the preferred phase (Phillips, 1981; Klemaszewski et al., 1990).

The droplet d_{vs} of emulsions formed at ionic strength 0.2 was approximately 0.5 μm larger than those formed at μ = 0.1, at 1% protein, for all proteins studied with the exception of unmodified whey protein isolate (Figure 1B). The phosphate ion may change the adsorption kinetics of the protein binding to the interface (Halling, 1981), alter the electrostatic interactions between proteins and/or proteins and the interface, or increase protein–protein interactions prior to interfacial binding. If the protein has less affinity for other proteins at 0.1 ionic strength than at 0.2 ionic strength (i.e., less protein–protein association), then the protein, at μ = 0.1, may be more available to unfold and coat the interface (Klemaszewski et al., 1991). Phosphate, at 0.2 M concentration, has been shown to increase protein–protein interactions of β-lactoglobulin by stabilizing the dimer form preferentially to the monomeric form (Kella and Kinsella, 1988). Klemaszewski et al. (1991) showed similar effects between phosphate concentration and emulsion surface area. It was speculated that the increased ionic strength altered the electrostatic interactions of the system components, and this was responsible for most of the effects of phosphate.

The sulfitolysis of disulfide bonds improved the emulsifying activity index (EAI, emulsion surface area per gram of protein) of whey protein isolate at protein concentrations of 0.25% and 1.0% (Figure 2). The magnitude of the improvement in emulsifying properties is dependent upon the protein to oil ratio (Phillips, 1981). The enhancement in the EA of modified whey protein isolate was more pronounced at 1.0% protein than at 0.25% protein. The low protein conncentration (0.25%) may be insufficient to stabilize the large newly formed interfacial area. Hence, at 0.25% concentration, the greater flexibility of the disulfide-cleaved WPI did not have a significant effect in increasing the interfacial area because there was insufficient protein to cover and stabilize the oil droplets as they were formed (Phillips, 1981).

The cleavage of disulfide bonds improved the stability of whey protein stabilized emulsions (Figure 3). Stability ranged from complete emulsion breakdown in approximately 48 h for emulsions stabilized by native or whey protein isolate with 50% of the disulfide bonds cleaved to 37% of the emulsion surface area remaining after 48 h in emulsions made with WPI with 100% of the disulfide bond cleaved (Figure 3). In emulsions stabilized by 0.25% protein, the emulsion was stable for 33 h when stabilized by whey protein with 75% and 100% of the disulfide bond cleaved, whereas emulsions made with 0.25% (w/w) native

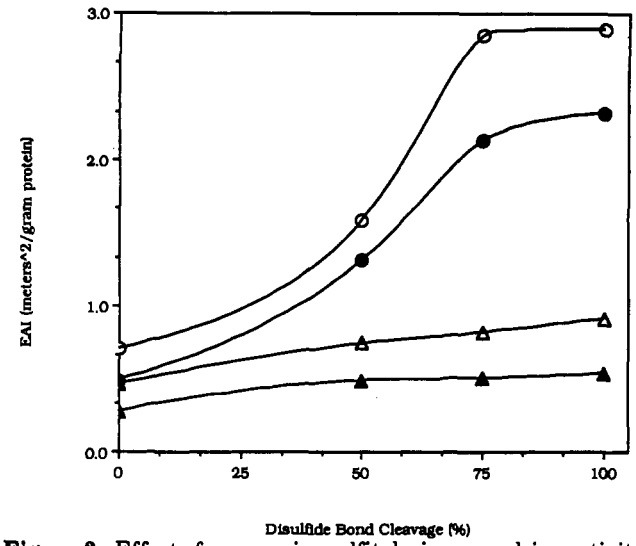

Figure 2. Effect of progressive sulfitolysis on emulsion activity index of emulsions formed with (○) 1.0% (w/w) whey protein isolate (WPI), $\mu = 0.1$; (●) 1.0% (w/w) WPI, $\mu = 0.2$; (△) 0.25% (w/w) WPI, $\mu = 0.1$; (▲) 0.25% (w/w) WPI, $\mu = 0.2$ in phosphate buffer, pH 6.8.

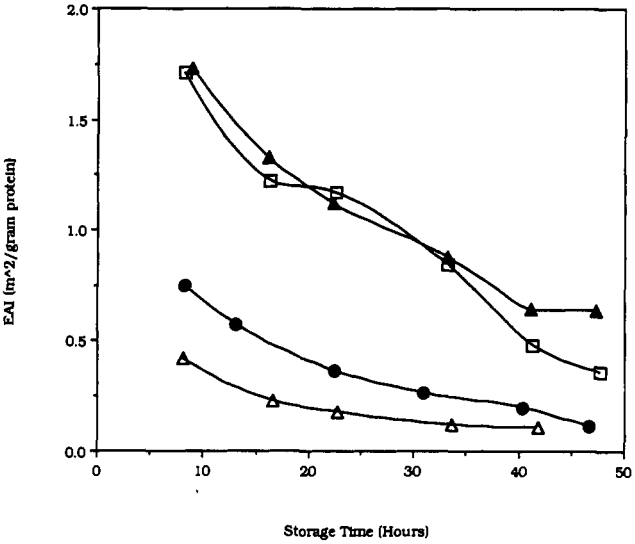

Figure 3. Effect of progressive sulfitolysis on the change in emulsion activity index, with increasing storage time, in emulsions stabilized by 1% whey protein isolate with (△) 0%, (●) 50%, (□) 75%, (▲) 100% of disulfide bonds cleaved, in phosphate buffer, pH 6.8, $\mu = 0.1$.

and 50% disulfide bond cleaved whey protein isolate were stable for only 16 h.

At pH 6.8, the net charge on whey proteins is negative, and following sulfitolysis the magnitude of the net charge is increased (Kella et al., 1989). The charged groups of interfacial proteins present a barrier to the close approach and coalescence of neighboring droplets (Parker, 1987). The higher number of negatively charged groups may be partly responsible for the stability of emulsions made with modified proteins. The folding of whey proteins after sulfitolysis is markedly different from that of native whey proteins (Kella et al., 1989). The unfolding and alignment of modified whey proteins at the interface may provide a stronger film and impart more steric repulsion, thereby retarding collisions between emulsion droplets (Das and Kinsella, 1990).

In conclusion, the cleavage of 75% or more of the disulfide bonds in whey protein isolate resulted in marked improvement of the emulsion formation and coalescence properties of whey protein isolate.

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LITERATURE CITED

Chen, S. I. The effect of thermal processing of milk on the functionality of whey protein concentrates from ultrafiltration. M.S. thesis, Ohio State University, Columbus, 1989.

Chobert, J.-M.; Bertrand-Harb, C.; Nicolas, M.-G. Solubility and emulsifying properties of caseins and whey proteins modified enzymatically by trypsin. *J. Agric. Food Chem.* **1988**, *36*, 883–892.

Das, K. P.; Kinsella, J. E. Stability of food emulsions: Physicochemical role of protein and non-protein emulsifiers. *Adv. Food Nutr. Res.* **1990**, *34*, 81–140.

Elizalde, B. E.; DeKanterewicz, R. J.; Pilsosf, A. M. R.; Bartholomati, G. B. Physicochemical properties of food proteins related to their ability to stabilized oil in water emulsions. *J. Food Sci.* **1988**, *53*, 845–848.

Ellman, G. L. Tissue sulfhydryl groups. *Arch. Biochem. Biophys.* **1959**, *82*, 70–77.

Graham, D. E.; Phillips, M. C. Proteins at interfaces, Parts 1-3. *J. Colloid Interface Sci.* **1979**, *70*, 403–439.

Halling, P. J. Protein-stabilized foams and emulsions. *CRC Crit. Rev. Food Sci. Nutr.* **1981**, *13*, 155–203.

Kella, N. K. D.; Kinsella, J. E. A method for the controlled cleavage of disulfide bonds in proteins in the absence of denaturants. *J. Biochem. Biophys. Methods* **1985**, *11*, 251–263.

Kella, N. K. D.; Kinnsella, J. E. Structural stability of β-lactoglobulin in the presence of kosmotropic salts: A kinetic and thermodynamic study. *Int. J. Pept. Protein Res.* **1988**, *32*, 396–405.

Kella, N. K. D.; Yang, S. T.; Kinsella, J. E. Effect of disulfide bond cleavage on structural and interfacial properties of whey proteins. *J. Agric. Food Chem.* **1989**, *37*, 1203–1210.

Kim, Y. A.; Chism, G. W., III; Mangino, M. E. Determination of the beta-lactoglobulin, alpha-lactalbumin and bovine serum albumin of whey protein concentrates and their relationship to protein functionality. *J. Food Sci.* **1987**, *52*, 124–127.

Kinsella, J. E. Milk proteins: Physicochemical and functional properties. *CRC Crit. Rev. Food Sci. Nutr.* **1984**, *21*, 197–262.

Kinsella, J. E.; Whitehead, D. M. Proteins in whey: Chemical, physical and functional properties. *Adv. Food Nutr. Res.* **1989**, *33*, 344–430.

Klemaszewski, J. L.; Haque, Z.; Kinsella, J. E. An electronic imaging system for determining droplet size and dynamic breakdown of protein stabilized emulsions. *J. Food Sci.* **1989**, *54*, 440–445.

Klemaszewski, J. L.; Das, K. P.; Kang, Y. J.; Kinsella, J. E. Effects of controlled sulfitolysis of bovine serum albumin on droplet size and surface area of emulsions. *J. Agric. Food Chem.* **1990**, *38*, 647–650.

Klemaszewski, J. L.; Das, K. P.; Kinsella, J. E. Factors affecting the formation and coalescence of emulsions stabilized by different milk proteins. *J. Food Sci.* **1991**, submitted for publication.

McWatters, K. H.; Holmes, M. R. Salt concentration, pH and flour concentration effects on nitrogen solubility and emulsifying properties of peanut flour. *J. Food Sci.* **1979**, *44*, 765–769.

Morr, C. V. Effect of HTST pasteurization of milk, cheese whey and cheese whey UF retentate upon the composition, physicochemical and functional properties of whey protein concentrates. *J. Food Sci.* **1987**, *52*, 312–317.

Morr, C. V. Whey proteins: Manufacture. In *Developments in Dairy Chemistry-4: Functional Milk Proteins*; Fox, P. F., Ed.; Elsevier Applied Science Publishers: London, 1989; pp 245–284.

Morr, C. V.; Foegeding, E. A. Composition and functionality of commercial whey and milk protein concentrates and isolates: A status report. *Food Technol.* **1990**, *44*, (4), 100–112.

Nakai, S. Structure-function relationships of food proteins with an emphasis on the importance of protein hydrophobicity. *J. Agric. Food Chem.* **1983**, *31*, 676–683.

Parker, N. S. Properties and functions of stabilizing agents in food emulsions. *CRC Crit. Rev. Food Sci. Nutr.* **1987**, *25*, 285–315.

Pearce, K. N.; Kinsella, J. E. Emulsifying properties of proteins: Evaluation of a turbidimetric technique. *J. Agric. Food Chem.* **1978**, *26*, 716–723.

Phillips, L. G.; Yang, S. T.; Schulman, W.; Kinsella, J. E. Effects of lysozyme, clupeine, and sucrose on the foaming properties of whey protein isolate and β-lactoglobulin. *J. Food Sci.* **1989**, *54*, 743–747.

Phillips, M. C. Protein conformation at liquid interfaces and its role in stabilizing emulsions and foams. *Food Technol.* **1981**, *35*, 50–55.

Walstra, P. Estimating globule size distribution of oil-in-water emulsion by spectroturbidimetry. *J. Colloid Interface Sci.* **1968**, *27*, 493–501.

Waniska, R. D.; Shetty, J. K.; Kinsella, J. E. Protein stabilized emulsions: Effects of modification on the emulsifying activity of bovine serum albumin in a model system. *J. Agric. Food Chem.* **1981**, *29*, 826–831.

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