High Hydrostatic Pressure Modification of Whey Protein Concentrate for Improved Functional Properties

S.-Y. Lim, B. G. Swanson, and S. Clark¹

Department of Food Science and Human Nutrition, Washington State University, Pullman 99164-6376

ABSTRACT

Whey protein concentrate (WPC) has many applications in the food industry. Previous research demonstrated that treatment of whey proteins with high hydrostatic pressure (HHP) can enhance solubility and foaming properties of whey proteins. The objective of this study was to use HHP to improve functional properties of fresh WPC, compared with functional properties of reconstituted commercial whey protein concentrate 35 (WPC 35) powder. Fluid whey was ultrafiltered to concentrate proteins and reconstituted to equivalent total solids (8.23%) as reconstituted commercial WPC 35 powder. Solutions of WPC were treated with 300 and 400 MPa (0- and 15-min holding time) and 600 MPa (0-min holding time) pressure. After HHP, the solubility of the WPC was determined at both pH 4.6 and 7.0 using UDY and BioRad protein assay methods. Overrun and foam stability were determined after protein dispersions were whipped for 15 min. The protein solubility was greater at pH 7.0 than at pH 4.6, but there were no significant differences at different HHP treatment conditions. The maintenance of protein solubility after HHP indicates that HHP-treated WPC might be appropriate for applications to food systems. Untreated WPC exhibited the smallest overrun percentage, whereas the largest percentage for overrun and foam stability was obtained for WPC treated at 300 MPa for 15 min. Additionally, HHP-WPC treated at 300 MPa for 15 min acquired larger overrun than commercial WPC 35. The HHP treatment of 300 MPa for 0 min did not improve foam stability of WPC. However, WPC treated at 300 or 400 MPa for 15 min and 600 MPa for 0 min exhibited significantly greater foam stability than commercial WPC 35. The HHP treatment was beneficial to enhance overrun and foam stability of WPC, showing promise for ice cream and whipping cream applications.

Key words: whey protein concentrate, high hydrostatic pressure, protein solubility, foam stability

INTRODUCTION

Whey and whey products have been used successfully in the food industry for years. Whey proteins increase milk solids nonfat, are highly soluble, and improve nutritional value, foaming, and emulsification properties of ice cream and frozen desserts (Morr and Ha, 1993; Hoffman, 1996; Jayaprakasha and Brueckner, 1999; Young, 1999).

Traditional food processing methods rely on high temperatures to ensure food safety and prolonged shelf life. However, heat treatment at high temperatures can result in detrimental changes in the processed products (Martin et al., 2002). The changes result in products that are far from similar to original fresh products. These undesirable changes affect nutritional attributes as well as protein denaturation, which decreases protein solubility and foaming properties of whey proteins (Parris and Baginski, 1991; Martin et al., 2002). Moreover, severe thermal treatments (above 70°C) result in protein denaturation, accompanied by loss of aqueous solubility and foaming properties (Kester and Richardson, 1984). Whey protein concentrate (**WPC**) is available commercially in a variety of forms. Commercial whey protein concentrate 35 (**WPC 35**) contains a minimum of 35% protein and undergoes a spray-drying process to produce a powder. Proteins in WPC 35 powder typically experience temperatures greater than 75°C and are denatured. Heat denaturation at high temperature, greater than 75°C, generally results in negative effects on the functional properties of the protein: emulsifying capacity and foaming properties are commonly reduced (Pittia et al., 1996). Improvements in functional properties may be achieved by modifying the protein structure using physical treatments instead of heat (Kato et al., 1983).

High hydrostatic pressure (**HHP**) is receiving attention as an alternative to thermal processing. Small protein concentrations and pressures up to 200 to 300 MPa usually result in reversible pressure-induced partial denaturation. High pressures greater than 500 MPa

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¹Corresponding author: stephclark@wsu.edu

result in irreversible and extensive effects on proteins, including denaturation due to unfolding of monomers, aggregation, and formation of gels (Balny et al., 1989). Relatively high-pressure treatment (greater than 300 MPa for more than 30 min) of the primary whey protein, β -LG, induces irreversible denaturation, which results in increased hydrophobicity and formation of protein aggregates (Pittia et al., 1996). Simultaneously, the exposure of previously buried hydrophobic and SH groups by pressure treatment results in structural changes of proteins and increases flexibility more than heat treatment.

Yang et al. (2001) stated that HHP treatment at 600 MPa at 50°C induced β -LG to form a stable molten globule state, intermediate between the native state and the completely denatured state. The molten globule state of β -LG exhibits high affinity with hydrophobic probes when compared with the native state of β -LG. Because HHP treatment results in increased accessibility of the buried SH groups, SH oxidation occurs during or after high-pressure treatment of β -LG (Yang et al., 2001). The HHP-induced β -LG dimers tend to be surrounded by hydrophobic AA residues, resulting in an increase of hydrophobic affinity of β -LG at the surface hydrophobic sites (Yang et al., 2003). The pressure treatment probably induces partially reversible unfolding of the β -LG, resulting in the unmasking of buried hydrophobic groups and an increase in the hydrophobicity of the protein. Thus, pressure treatment results in an increase in hydrophobicity of β -LG, an expected enhancement of some functionality in food systems.

If functional properties of whey proteins are enhanced, whey proteins will provide desirable functionality to a wide range of food products, including improved appearance, body, texture, and consistency. One of the most important physicochemical and functional properties of whey proteins is solubility (Morr and Ha, 1993). Protein solubility depends on various physicochemical properties, including molecular weight, secondary and tertiary structure, hydrophobicity, and electrostatic charge (Morr and Ha, 1993). Processing treatments used to manufacture WPC may result in heat-induced protein denaturation, which then reduces whey protein solubility. Native whey proteins remain soluble at around pH 7; however, heat-induced denaturation renders whey proteins less soluble than native whey proteins (Morr and Ha, 1993). Thus, protein solubility of WPC is useful for estimating protein denaturation (Morr and Foegeding, 1990). Protein solubility did not decrease in 6% whey protein isolate (WPI) solution after HHP at 400 MPa for 10 min compared with untreated 6% WPI solution (Kanno et al., 1998). However, Lee et al. (2006) reported that the solubility of 1% WPC

decreased as HHP treatment time increased from 5 to 30 min at 690 MPa. Because protein solubility depends on HHP treatment conditions, careful selection of HHP treatment conditions are important to maintain protein solubility in potential food product formulations.

Enhancement of foaming properties can potentially reduce the cost of production, because less protein is necessary to provide desired functionality. Studies were conducted with reconstituted WPI or WPC to understand the effect of HHP on emulsifying and foaming properties of whey proteins. Ibanoglu and Karatas (2001) reported that WPI treated with HHP at 300 MPa exhibited greater foam stability than untreated WPI, but foam stability of WPI decreased at pressures greater than 300 MPa. Foaming properties of β -LG were enhanced at 300 MPa for 10 min, which was attributed to an increase of hydrophobicity (Pittia et al., 1996). However, the reduction in foam stability at greater than 300 MPa may be explained as the detrimental effect of unfolding due to the increase in hydrophobicity of β -LG upon high-pressure treatment (Pittia et al., 1996). Unfolding of proteins lessens viscoelasticity of a film with a reduced number of interactions between molecules (Ibanoglu and Karatas, 2001). The HHP-treated WPC contributes to an increase in emulsion stability of model oil-in-water emulsions; WPC treated at 690 MPa for 5 min exhibited increased emulsifying activity (Lee et al., 2006). The HHP treatment of WPC results in an increase of the surface hydrophobicity, attributed to the molten globule state, resulting in partial unfolding of whey proteins (Liu et al., 2005; Lee et al., 2006). The molten globule state may be attributed to hydrophobic patch stabilization at air-water interfaces of whey proteins to increase the foam stability of whey proteins. The molten globule state resulting from HHP treatment retains protein solubility and increases hydrophobicity to enhance foam stability of whey proteins. The HHP-treated whey proteins may have a potential for formulated food products requiring protein solubility and functional properties.

Fat content plays an important role in food products requiring foaming properties. Fat may form a matrix of partially coalesced fat that contains air bubbles. Foods with high fat content create foams. As fat content decreases, foams become soft and unstable (Hercules Technical Information, 2006). Thus, reduced-fat foods make unstable foams compared with regular-fat foods, but reducing the fat content in foods may offer a variety of benefits, including cost and calorie savings. An HHP-treated WPC may be appropriate to serve as a fat replacer if the solubility of WPC is maintained. Moreover, HHP is expected to increase surface hydrophobicity and contribute to enhance foaming properties (Ibanoglu and Karatas, 2001; Liu et al., 2005; Lee et al., 2006). How-

Table 1. Composition of diluted fresh WSU-WPC compared with reconstituted commercial WPC 35

Composition	WSU-WPC ¹ (%)	WPC 35 ² (%)
Total solids	8.23	8.23
Protein	3.02	3.06
Fat	0.28	0.26
Lactose	4.24	4.18

¹Washington State University-whey protein concentrate.²Commercial whey protein concentrate 35.

ever, previous studies utilized reconstituted WPC or WPI powders that could have been completely denatured by spray-drying. Little research is available regarding the effect of HHP on functional properties of fresh whey proteins.

Fresh sweet whey ultrafiltration (UF) retentate, called Washington State University WPC (WSU-WPC), was utilized in this research, because it was hypothesized that WPC prepared from fresh fluid whey will have better functionality than reconstituted spray-dried WPC 35. Ultimately, this study was designed to evaluate protein solubility and foaming properties of WSU-WPC after HHP, a step toward determining if HHP treatment of fresh WPC is appropriate for reduced-fat foods such as ice cream and whipping cream.

MATERIALS AND METHODS

Sweet whey from Cheddar cheese-making at the Washington State University (WSU) Creamery (Pullman) was ultrafiltered and diluted to about 8.23% total solids and 3.02% protein content (Table 1). The end-product is identified as WSU-WPC. Commercial WPC 35 powder from Foremost Farms (Baraboo, WI) was reported to contain 35.7% protein, 3.2% fat, 51.1% lactose, and 96% total solids, based on powder (Table 1). The WPC 35 powder was reported to have been pasteurized at a temperature above 75°C for at least 15 s, followed by spray-drying below 100°C. The WPC 35 was reconstituted to 8.23% total solids and 3.06% protein (Table 1). The WPC 35 with the same lot number was used throughout experiments of functional properties, whereas triplicate batches of WSU-WPC were produced by UF to maintain freshness. Standard proteins for SDS-PAGE were obtained from BioRad Laboratories (Hercules, CA).

UF of Whey

Before UF, the whey was pasteurized at 63°C for 30 min and cooled to 40.5°C in a jacketed kettle (Groen Div/Dover Corporation, Elf Grove Village, IL). The UF was conducted in the pilot plant of the WSU Food Sci-

ence and Human Nutrition Department using a UF unit (Romicon Inc., Woburn, MA) with a 50.8-mm, 5- μ m PM-10 hollow fiber (Koch Membrane Systems Inc., Wilmington, MA). Ultrafiltration was carried out at 40.5°C and at pressures of 172.37 kPa inlet and 34.47 kPa outlet. Based on the protein content of the whey (0.75 to 0.80%), the WPC was concentrated to an equivalent protein content (about 3%) as the WPC 35. The UF was stopped when the volume of permeate collected was 75% of the original volume. Ultrafiltered fresh WPC (WSU-WPC) was pasteurized at 68°C for 30 min and cooled to 10°C in the kettle with intermediate occasional stirring. The WSU-WPC is different from WPC 35 powder in that WPC 35 was heated during spray-drying to greater than 75°C, which yields a fully denatured product. The total solids were determined by drying samples of the UF whey at 105°C for 12 h. The total solids of the WPC were standardized to 8.23% by adding distilled water, cooled to less than 10°C. The WSU-WPC was studied immediately or stored at 4°C for less than 2 wk.

Pressurization of WSU-WPC

Portions of WSU-WPC were volumetrically poured into separate Lay-Flat Poly Tubing (Consolidated Plastics Company Inc., Twinsburg, OH), 5.3-cm wide, 4-mm thick, cut to desired length, and heat-sealed at both ends. Bags of WSU-WPC were treated with 300 and 400 MPa (0- or 15-min holding time) and 600 MPa (0-min holding time), at an initial temperature of 25°C in a warm isostatic press (Engineered Pressure Systems Inc., Haverhill, MA) with a cylindrical pressure chamber (height = 0.25 m, diameter = 0.10 m). Untreated WSU-WPC and WPC 35 solutions served as controls. The zero holding time indicates the come-up time, the compression time required to reach a pressure of 300, 400, or 600 MPa. After exposure to HHP, the solutions were studied immediately or stored at 4°C for less than 2 wk.

Protein Solubility Test

Protein solubility is used to identify the extent of whey protein denaturation. Morr et al. (1985) developed a reliable procedure for determining the solubility of food proteins. The pH of untreated WSU-WPC, WPC 35, and HHP-treated WSU-WPC solutions were adjusted to pH 4.6 or 7.0 with 0.1 N HCl or 0.1 N NaOH solutions, respectively. An aliquot of the solutions was centrifuged for 30 min at 20,000 $\times$ g, and the resulting supernatant fraction was filtered through Whatman No. 1 filter paper (Morr et al., 1985). The protein content of the filtrate was determined by both the UDY method (AOAC,

1990), modified for whey analysis, and the BioRad protein assay method (Bradford, 1976). Protein content, using the BioRad protein assay method, was determined after calibration with 5 dilutions of protein standards, including BSA as the external standard. The linear range of the assay was 0.1 to 1.0 mg/mL. Protein solubility (PS) is expressed as a percentage of the total protein content of the dispersion before centrifugation (Lee et al., 1992). Each analysis was performed in triplicate: $PS = \% \text{ protein, supernatant} / \% \text{ protein, total} \times 100$.

Overrun

The HHP-treated WSU-WPC, untreated WSU-WPC, and WPC 35 dispersions were adjusted to pH 7.0 with 0.1 *N* NaOH. A pH of 7.0 was selected, because the proteins are soluble and consistently form acceptable foams at this pH, suitable for determination of overrun and foam stability. Foams were formed by whipping the protein solutions in a household-type mixer (KitchenAid mixer, St. Joseph, MI) at cold temperature (from 4 to 10°C). The WPC dispersion (200 mL) was weighed and whipped for 15 min for overrun determinations. During foam formation, the mixer was stopped at 5-min intervals to determine overrun at each time. The mixer head was carefully lifted to minimize destruction of the foam, and the weight of 200 mL was noted (Phillips et al., 1990). Each analysis was performed in triplicate. The overrun was calculated by the following equation: $\% \text{ overrun} = (\text{wt 200-mL dispersion}) - (\text{wt 200-mL foam}) / (\text{wt 200 of mL foam}) \times 100$.

Foam Stability

A plastic whipping bowl was modified by drilling a 0.33-cm hole in the side of the bowl, 5.23 cm from the bowl bottom ridge. The hole was sealed before whipping by placing tape over the hole on the outside of the bowl. Whipping was started at the calibrated setting for a specified time (e.g., 5, 10, or 15 min). After whipping, the tape was quickly removed at time zero, and a timer was started. The drained liquid was collected by tilting the bowl to the hole above a tared container on a balance pan, and the time at which 50% drainage was observed was recorded. The time to attain 50% drainage of the initial weight was used as an index of foam stability (Phillips et al., 1990).

SDS-PAGE

Sodium dodecyl sulfate-PAGE (10 and 4 to 20%), without and with β -mercaptoethanol (β -ME), was used for identifying effects of HHP treatment on each constituent protein of WPC according to the instruction man-

ual of Ready Gel Precast Gels (catalog number 161-0993, BioRad Laboratories). Because a gradient gel (4 to 20%) presents a wide range of molecular weights (MW; 10 to 200 kDa), and a single-percentage gel (10%) produces the greatest resolution of a narrow range of MW (30 to 200 kDa), the 10% SDS-PAGE gel allowed observance of more clear whey protein bands than the gradient gel (4 to 20%).

One milliliter of the untreated WSU-WPC, WPC 35, or HHP-treated WSU-WPC solutions was diluted with 5 mL of 0.5 *M* Tris-HCl (pH 6.8), containing 20% glycerol, 0.01% bromophenol blue, and 10% SDS. Before analysis, solutions were heated for 3 min in a 100°C water bath, followed by cooling to room temperature with running tap water. Whey proteins of 1:5 dilution (20 μ L), without β -ME, were loaded onto a 10% Ready Gel. Four lanes (1 standard, 3 whey proteins) of electrophoresis were run at ambient temperature for 40 min at 200 V. Duplicate whey proteins of equivalent dilution, with β -ME, were loaded onto a different 10% Ready Gel. The equivalent samples, without and with β -ME, were also loaded onto 4 to 20% Ready Gels. Seven lanes (1 standard, 3 whey proteins, with and without β -ME) of electrophoresis were run under the equivalent conditions to the 10% Ready Gel. The gels were stained with a Coomassie Brilliant Blue solution containing 40% methanol, 7% acetic acid, and 0.05% Coomassie Brilliant Blue R-250 and were then destained with a 40% methanol and 7% acetic acid solution. Molecular weights of the protein bands were estimated by comparison to prestained SDS-PAGE standards (catalog number 161-0318, BioRad Laboratories). The protein standards included myosin (204 kDa), β -galactosidase (119 kDa), BSA (100 kDa), ovalbumin (52.0 kDa), carbonic anhydrase (37.4 kDa), soybean trypsin inhibitor (29.1 kDa), lysozyme (19.5 kDa), and aprotinin (7.0 kDa).

Statistical Analysis

Fresh WSU-WPC was processed in 3 different batches, and each analysis was conducted in triplicate. The ANOVA test for significant effects of treatments and assays was determined using the GLM procedure (PROC GLM) in SAS (SAS Institute, 1999). Main effect differences were considered significant at the $P \leq 0.05$ level. Mean separations were determined by Tukey's procedure for multiple comparisons.

RESULTS AND DISCUSSION

Protein Solubility

In general, the solubility of WPC 35 and WSU-WPC was significantly greater at pH 7.0 than at pH 4.6, as indicated by both UDY protein and BioRad protein

Figure 1. Protein solubility of commercial whey protein concentrate 35 [WPC 35; no high hydrostatic pressure (HHP)] or Washington State University-whey protein concentrate (No = no HHP; 300-0 = 300 MPa for 0 min; 300-15 = 300 MPa for 15 min; 400-0 = 400 MPa for 0 min; 400-15 = 400 MPa for 15 min; 600-0 = 600 MPa for 0 min) after selected HHP-time combinations at pH 4.6 and 7.0, determined with the UDY method. ^{a,b}Different letters denote significant differences across different treatments ($P \leq 0.05$). Vertical lines correspond to standard deviation.

Figure 2. Protein solubility of commercial whey protein concentrate 35 [WPC 35; no high hydrostatic pressure (HHP)] or Washington State University-whey protein concentrate (No = no HHP; 300-0 = 300 MPa for 0 min; 300-15 = 300 MPa for 15 min; 400-0 = 400 MPa for 0 min; 400-15 = 400 MPa for 15 min; 600-0 = 600 MPa for 0 min) after selected HHP-time combinations at pH 4.6 and 7.0, determined with the BioRad protein assay method. ^{a,b}Different letters denote significant differences across different treatments ($P \leq 0.05$). Vertical lines correspond to standard deviation.

assay methods ($P \leq 0.05$, Figures 1 and 2). This is not surprising, because the pH of the solvent determines the nature and the distribution of net charge of proteins. A protein usually exhibits the least solubility at the isoelectric point (Pelegrine and Gasparetto, 2005). At pH 4.6, near the isoelectric point of WPC, the net charge is minimized, and, consequently, less water interacts with the protein molecules. Decrease of water-protein binding results in aggregation of protein molecules, possibly precipitation, and poor solubility at pH 4.6. Although solubility of WPC was significantly smaller at pH 4.6 than 7.0, WPC is still used regularly in low-pH applications.

Although Lee et al. (2006) reported that HHP decreased the solubility of whey proteins, the treatment conditions (690 MPa for greater than 5 min) were more severe than our treatment conditions (300 MPa for 15 min). In the present study, the solubility of WPC did not change significantly under selected pressure and time treatments ($P > 0.05$) at either pH. Also, protein solubility of native or HHP-treated WSU-WPC did not significantly differ from WPC 35. These results confirm Bouaouina et al. (2006), who reported that dynamic high-pressure treatment (50 to 300 MPa) did not affect whey protein solubility ($88\% \pm 1\%$) for either native or treated protein solutions. Solubility of protein is a critical factor in the acceptability of dairy beverages, ice cream mix, and whipping cream. If the solubility

is not decreased after HHP, applications to the food industry are promising. Thus, it is appropriate to use HHP-treated WSU-WPC in similar applications as the commercial WPC 35.

Foaming Properties at Selected Pressure-Time Combinations

High pressure and treatment time are 2 important parameters affecting the foaming properties of food proteins (Halling, 1981). According to Ibanoglu and Karatas (2001), increase in both HHP and treatment times improved the foaming ability of WPI at pH 7. They suggested that HHP increased protein molecule flexibility; partial unfolding increased foam stability of whey proteins such that high pressure stabilized the interface between air and water in foam formation. For practical applications, foams prepared with whey proteins help to increase the volume and stability of ice cream foams. High overrun values (close to 100%) in low-fat ice cream provide soft texture and may yield a mouthfeel similar to regular-fat ice cream.

In the present study, the highest percentage of overrun for WSU-WPC was obtained at 300 MPa for 15 min of HHP treatment at the selected whipping times (Figure 3). However, the 3 treatments (300 and 400 MPa for 15 min, 600 MPa for 0 min) were statistically equivalent to each other. The lowest overrun occurred with the 2 treatments at 300 and 400 MPa, with come-

Figure 3. Whipping time and overrun for commercial whey protein concentrate 35 [WPC 35; no high hydrostatic pressure (HHP)] or Washington State University-whey protein concentrate (No = no HHP; 300-0 = 300 MPa for 0 min; 300-15 = 300 MPa for 15 min; 400-0 = 400 MPa for 0 min; 400-15 = 400 MPa for 15 min; 600-0 = 600 MPa for 0 min) treated with selected HHP-time combinations. ^{a-h}Different letters denote significant differences for individual whipping times ($P \leq 0.05$). Vertical lines correspond to standard deviation.

Figure 4. Whipping time and foam stability for commercial whey protein concentrate 35 [WPC 35; no high hydrostatic pressure (HHP)] or Washington State University-whey protein concentrate (No = no HHP; 300-0 = 300 MPa for 0 min; 300-15 = 300 MPa for 15 min; 400-0 = 400 MPa for 0 min; 400-15 = 400 MPa for 15 min; 600-0 = 600 MPa for 0 min) treated with selected HHP-time combinations. ^{a-o}Different letters denote significant differences for individual whipping times ($P \leq 0.05$). Vertical lines correspond to standard deviation.

up time, which was statistically equivalent to untreated WSU-WPC for overrun at the selected whipping time (Figure 3). The WSU-WPC that was treated at 300 MPa for 15 min exhibited significantly higher percentage of overrun than untreated WPC 35 when whipped for 10 or 15 min (Figure 3). Untreated WPC 35 exhibited higher percentage of overrun than untreated WSU-WPC (Figure 3). This result is evidence that WPC 35 was fully denatured by heat treatment, which increased foaming capacity. Untreated WSU-WPC was nearly in the native state, which made it less receptive to foaming than partially or profoundly denatured WPC (Kester and Richardson, 1984; Pittia et al., 1996).

Benefits of enhanced overrun are small, however, if the foam cannot be maintained in foods requiring a stable foam, such as ice cream and whipping cream. High-pressure treatment of the whey protein ingredient not only enhanced foaming ability but also foam stability. The largest foam stability was obtained after treatment of WSU-WPC at 300 MPa for 15 min. The shortest stability was observed for untreated WPC 35 (Figure 4). Foam stability of WSU-WPC treated at 300 and 400 MPa for 15 min was significantly larger than commercial WPC 35 at selected whipping times ($P \leq 0.05$, Figure 4). The HHP treatment likely resulted in partial denaturation of the whey proteins, inducing the molten glob-

ule state and increasing the hydrophobicity on the surface (Yang et al., 2003; Liu et al., 2005; Lee et al., 2006). Because hydrophobic patches in the open molecule structure stabilize interfaces of proteins between air and water, improved foam stability is attributed to increased hydrophobicity (Pittia et al., 1996). In contrast, WPC 35 powder was drastically denatured by heat treatment, which reduced the patches of hydrophobic affinity. Although WPC 35 yielded large overrun, it did not maintain foam stability. Foam stability of untreated WSU-WPC was inferior to HHP-treated WSU-WPC at the selected whipping times. Untreated WSU-WPC, in the native state, exists in the fully folded form. The native form does not have hydrophobic sites on the surface to maintain foam stability; thus, the foam stability was significantly smaller than HHP-treated WSU-WPC (Figure 4). The foam stability of WPC 35 was also significantly shorter than HHP-treated WSU-WPC (Figure 4), likely because WPC 35 proteins were fully denatured, and hydrophobic sites were not available to contribute to foam stability. The WSU-WPC treated at 300 MPa for 15 min also exhibited greater foam stability than WSU-WPC at 400 MPa for 15 min (Figure 4); the higher pressure more fully denatured the protein and decreased foam stability. Partial dena-

Figure 5. The SDS-PAGE patterns without and with β -mercaptoethanol (β -ME using 10% gel loaded with standard solution (S), untreated Washington State University-whey protein concentrate (WSU-WPC; A), high hydrostatic pressure-treated WSU-WPC (B), and untreated whey protein concentrate 35 (C) solutions. I = dimer-trimer α -LA and β -LG; II = intermediate aggregates; III = monomeric BSA; IV = large aggregates.

turation is better than complete denaturation, regardless of heat or HHP treatment, for foaming properties. It should be noted that temperature is more difficult to control than pressure during processing.

Overrun and foam stability results suggest foaming properties of WSU-WPC were improved by HHP treatment with 300 MPa for 15 min or 400 MPa for 15 min. Thus, use of HHP-treated UF fluid whey may potentially improve body and texture of whipping cream and ice cream. If the WSU-WPC had been subjected to a drying process, solubility would be similar to commercial WPC 35, but the high temperature concentrations of spray-drying reduce foaming properties. Although WPC 35 may be appropriate for some applications, HHP-treated UF WPC may have a place in the dairy industry. Such an application would be appropriate for plants that produce cheese and liquid products, frozen desserts, or both, in the same factory.

SDS-PAGE

The SDS-PAGE results further help to explain improvement in foaming properties of HHP-treated WSU-WPC. The 10% SDS-PAGE gels of untreated WSU-WPC, HHP-treated WSU-WPC (300 MPa for 15 min), and untreated WPC 35 exhibited 4 major regions. From the bottom to top in Figure 5, according to MW, are exhibited dimers-trimers of α -LA and β -LG (region I), intermediate-sized aggregates (region II), BSA (band III), and large aggregates (region IV, with ~200 kDa of MW; Havea et al., 2002; Liu et al. 2005). Electrophoresis

under nonreducing conditions obtained a result wherein large-sized proteins did not enter the stacking gel (Figure 5, 6). The protein bands representing dimer-trimer α -LA and β -LG (Figure 5, region I) were presented at a smaller concentration for untreated WPC 35 than for HHP-treated WSU-WPC, suggesting a greater number of small MW proteins were present in HHP-treated WSU-WPC than untreated WPC 35. In addition, several protein bands were noted within the intermediate-sized aggregates region (Figure 5, region II), and a unique protein band corresponding to a molecular weight of about 55,000, not present in the untreated WSU-WPC and WPC 35, was prominent for the HHP-treated WSU-WPC in the 10% gel. Because the MW of protein bands is the sum of MW of α -LA (12,400) and β -LG (18,600), the unique protein band present in HHP-treated WSU-WPC may indicate α - β dimer-trimers formed during HHP treatment. The observed differences in concentrations of large aggregates (Figure 5, region IV) among the 3 WPC treatments were small.

In the presence of β -ME, left lanes in Figure 5, most large aggregates of the 3 WPC solutions (region IV) observed without β -ME were dissociated into intermediate forms (Figure 5, region II and III), indicating that large aggregates of WPC were stabilized by disulfide bonds. The unique protein band in the intermediate region of HHP-treated WSU-WPC without β -ME disappeared with β -ME (Figure 5, region II). Moreover, a strong band in region I decreased in intensity under reducing conditions (Figure 5, region I). The decrease of intensity in protein bands under reducing conditions means that protein bands included disulfide bonds. The HHP treatment may disrupt large protein aggregates to form smaller proteins that are stabilized by disulfide bonds. Due to HHP treatment, large aggregates of β -LG, stabilized by disulfide bonds (arising from SH oxidation), were disrupted to form small proteins (Yang et al., 2001). Whey protein aggregates are disrupted by HHP treatment; HHP results in aggregate disruption into small proteins rather than aggregation. Particle size reduction may affect whey protein functional properties. The particle size reduction and enhancement of protein surface hydrophobicity will contribute to fostering protein adsorption to air-water interfaces, supported by greater foam stability of HHP-treated WSU-WPC compared with foam stability of native WSU-WPC or heated-dried WPC 35. Particle size reduction of proteins could be a positive consequence of increased hydrophobic interactions, supporting the formation of a more viscoelastic adsorbed layer between air and water interfaces than large aggregates. Increases in the elasticity of the air-water interface improve the strength of films and enhance foam stability (Bouaouina et al., 2006). Although untreated WPC 35 exhibited almost

Figure 6. The SDS-PAGE patterns without and with β -mercaptoethanol (β -ME using 4 to 20% gradient gel loaded with standard solution (S), untreated Washington State University-whey protein concentrate (WSU-WPC; A), high hydrostatic pressure-treated WSU-WPC (B), and untreated whey protein concentrate 35 (C) solutions. α -LA (12.4 kDa); β -LG (18.6 kDa); I = dimer-trimer α -LA and β -LG; II = intermediate aggregates; III = monomeric BSA; IV = large aggregates.

equivalent protein bands as HHP-treated WSU-WPC and untreated WSU-WPC in large and intermediate aggregate regions, the protein band in region I almost disappeared under reducing conditions (Figure 5). Disappearance of the protein band indicates that WPC 35 contained more disulfide bonds than the HHP-treated WSU-WPC or the untreated WSU-WPC. Thermal treatments likely induce disulfide bonds; total disulfide bonds of WPC 35 were greater than present in fresh WSU-WPC, as shown by 10% SDS-PAGE gels.

When the same 3 samples were separated with a 4 to 20% gradient gel in the absence and presence of β -ME (Figure 6), protein bands without β -ME exhibited similarities among the 3 WPC. However, the intensity of the β -LG band was stronger in reducing conditions than in nonreducing conditions (Figure 6). Additionally, protein bands of dimer-trimer α -LA and β -LG, observed in the absence of β -ME, almost disappeared under reducing conditions (Figure 6, region I). Disruption of dimer-trimer α -LA and β -LG by reducing agent resulted in the formation of monomers of β -LG and α -LA (based on MW of 18.6 and 12.4 kDa, respectively, Figure 6). Funtenberger et al. (1997) reported that aggregates of β -LG are stabilized by disulfide bonds arising from disulfide interchange, which was confirmed by the present findings. Compared with the 10% Ready Gel without β -ME (Figure 5, region I), equivalent patterns of protein bands were exhibited in region I (Figure 6) in the 4 to 20% Ready Gel, where protein band intensity of HHP-treated WSU-WPC exhibited greater concen-

tration than untreated WPC 35. However, a unique protein band was observed in the HHP-treated WSU-WPC in the 10% gel (Figure 5, region II), which was not observed in the 4 to 20% gel (Figure 6, region II), because the 10% gel exhibited clearer protein bands in the narrow MW range than protein bands in the 4 to 20% gel. Although protein bands in the 10% gels did not allow distinction among the smallest fractions, protein bands in the 4 to 20% gel enabled visualization of smaller MW fractions of the peptides, especially monomers of α -LA and β -LG, the major whey proteins. The fact that the protein bands exhibited smaller concentrations in the untreated WPC 35 lane than in the HHP-treated WSU-WPC lane with β -ME (Figure 6) suggests that smaller fractions were present in HHP-treated WSU-WPC than in untreated WPC 35. Overall, more disulfide bonds appeared to be present in WPC 35, based on the results of SDS-PAGE in 4 to 20% Ready Gel (Figure 6), supporting greater extent of denaturation of untreated WPC 35 compared with untreated WSU-WPC and HHP-treated WSU-WPC. Because WPC 35 was irreversibly denatured by the spray-drying process, subjecting it to HHP is not prudent.

CONCLUSIONS

The HHP treatment did not significantly reduce fresh WSU-WPC protein solubility at pH 4.6 or 7.0, suggesting that HHP treatment of WPC is appropriate for applications in selected dairy products. The HHP treatment conditions that promoted functionality of proteins, including large overrun and foam stability, were observed after 300 MPa for 15 min. The SDS-PAGE provided evidence that dissociation of protein aggregates occurred and more dimers-trimers were present in HHP-treated WSU-WPC compared with dimers-trimers of untreated WPC 35, which contained large aggregates held together by disulfide bonds. The particle size reduction occurring during HHP treatments of whey proteins partially explains improved foaming properties of WSU-WPC. Because HHP treatment increased overrun and foam stability of fluid WSU-WPC, improvements may potentially enhance body and texture of whipping cream or ice cream.

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