Enhanced Functionalities of Whey Proteins Treated with Supercritical Carbon Dioxide

Q. Zhong¹ and M. Jin

Department of Food Science and Technology, University of Tennessee, Knoxville 37996

ABSTRACT

The functionality of whey proteins can be modified by many approaches; for example, via complexation with carbohydrates, enzymatic cross-linking, or hydrolysis, and the objective of this work was to research the effects of supercritical carbon dioxide (scCO₂) treatments on the functionalities of commercial whey protein products including whey protein isolates (WPI) and whey protein concentrates (WPC). The WPI and WPC powders and a 10% (wt/vol) WPI solution were treated with scCO₂. The WPI solution was treated at 40°C and 10 MPa for 1 h, whereas WPI and WPC powders were treated with scCO₂ at 65°C and 10 or 30 MPa for 1 h. Dynamic rheological tests were used to characterize gelation properties before and after processing. Compared with the unprocessed samples and samples processed with N₂ under similar conditions, scCO₂-treated WPI, whether dispersed in water or in the powder form during treatments, formed a gel with increased strength. The improvement in gelling properties was more significant for the scCO₂-treated WPC. In addition, the scCO₂-processed WPI and WPC powders appeared to be fine and free-flowing, in contrast to the clumps in the unprocessed samples. Proximate compositional and surface hydrophobicity analyses indicated that both compositional and structural changes may have contributed to enhanced whey protein functionalities. The results suggest that functionalities of whey proteins can be improved by scCO₂ treatment to produce novel ingredients.

Key words: whey protein, supercritical carbon dioxide, functionality, rheology

INTRODUCTION

Whey proteins have been studied extensively because of their widespread use as nutritious, highly functional but affordable food ingredients. Depending on the sepa-

ration processes used to extract proteins, variations in product compositions can be obtained. Whey protein concentrates (WPC) are commercial products with a protein content of 25 to 80%, whereas whey protein isolates (WPI) usually have more than 90% protein (Foegeding et al., 2002). Variations in the composition and preparation methods significantly change protein functionality (Damodaran, 1997; van Vliet et al., 2002).

Protein functionality is a general term used to describe any function a protein provides: solubility, water-holding capacity, structure formation in dairy and meat products, the ability to form and stabilize oil-water (in emulsions) and air-water (in foams) interfaces, and the ability to remain in suspension during thermal processing among others (Foegeding et al., 2002). Functional properties of protein ingredients are affected by factors such as origin, preparation method (which leads to different compositions and structure changes), physicochemical properties of the systems in which proteins are incorporated, and storage conditions (van Vliet et al., 2002; Baier and McClements, 2005).

Approaches to modifying protein functionality include complexation with carbohydrates via physical (noncovalent) or chemical (covalent) bonds to enhance functionalities such as interfacial activity (Dickinson and Galazka, 1991; Dickinson and Semenova, 1992), cross-linking (via thermal aggregation or enzymatic reaction), and hydrolysis (Foegeding et al., 2002). When modifying protein functionality, however, attention must be paid to regulations that may limit the application of these modified products as ingredients in foods (McClements, 2005).

Modifying protein functionality with physical methods may encounter fewer regulatory hurdles. High hydrostatic or dynamic pressure has been investigated as a physical method to modify whey and soy protein functionality. The irreversible change in structure needed to alter protein functionality of α -LA, β -LG, and glycinin occurs above 400, 150, and 400 MPa, respectively (Tanaka et al., 1996a,b; Tanaka and Kunugi, 1996; Molina et al., 2001).

Supercritical carbon dioxide (scCO₂) may be a more practical and affordable approach to modifying protein

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¹Corresponding author: qzhong@utk.edu

functionalities, a fact that has been exemplified by the commercial production of decaffeinated coffee. Carbon dioxide has also been used as a weak acid to precipitate milk and soy proteins dispersed in aqueous solutions (Tomasula et al., 1997, 1998; Strange et al., 1998; Hofland et al., 1999; Thiering et al., 2001a,b; Tomasula and Yee, 2001). For the case of whey proteins, separation of WPC dispersions into α -albumin-rich or β -LG-rich fractions can be achieved by manipulating precipitation conditions (Tomasula et al., 1998; Tomasula and Yee, 2001). Compared with a reference WPC solution, the fractionated β -LG-rich fraction enhanced interfacial activity and thickening and gelling properties (Tomasula and Yee, 2001). Another technique of supercritical fluid extrusion has been developed to process foods with novel functionalities properties (Alavi et al., 1999; Gogoi et al., 2000).

Thermodynamically, scCO₂ has a liquid-like density and gas-like diffusivity and viscosity (McHugh and Krukonis, 1994). Supercritical conditions of CO₂ are relatively easy to achieve (critical temperature of 31.1°C and critical pressure of 7.38 MPa). Further, CO₂ is an environmentally friendly solvent that is ideal for food processing. It is inflammable, nontoxic, chemically inert, physiologically safe, and can be easily recycled and reused. Further, scCO₂ has a surface tension of zero (McHugh and Krukonis, 1994), which leads to complete and rapid wetting, thus allowing penetration of complex structures (Zhang et al., 2006). The scCO₂ can be used to extract lipids and other nonpolar and polar (with a cosolvent such as ethanol) compounds in commercial whey protein ingredients and cause direct changes in compositions. Protein structures may also change due to the depletion or redistribution of nonpolar compounds. These aspects may introduce novel protein functionalities.

The objective of this work was thus to evaluate the functionalities of whey proteins before and after scCO₂ processing, with a focus on gelation properties. Gelation of whey proteins has been studied extensively at various solvent conditions. pH values of 3.35 and 7.0 were selected as representative conditions below and above the isoelectric point of whey proteins, respectively; both pH values have been used to evaluate gelation properties of whey protein ingredients (Ikeda et al., 1999; Resch et al., 2005). The outcome of this work may lead to specialized ingredients of novel applications.

MATERIALS AND METHODS

Materials

A WPC 80% sample and a WPI sample were kindly supplied by Davisco Foods International Inc. (Eden Prairie, MN). Samples were stored at -20°C upon ar-

Figure 1. Schematic of the supercritical CO₂ system.

rival and used directly without further purification. All processed and unprocessed powder samples were analyzed for total protein (AOAC, 1990; method 984.13), lipids (AOAC, 1990; method 948.15), and moisture (AOAC, 1990; method 950.46) contents by ABC Research Corporation (Gainesville, FL). Duplicate tests were done for protein and moisture contents, but only one test was performed for the total lipids content because of the insufficiency of sample quantities from the same batch treatment.

Equipment and Processing Protocols

The scCO₂ system (model SAS50, Thar Technologies, Pittsburgh, PA) is illustrated schematically in Figure 1. Bone-dry grade CO₂ (Airgas, Chicago, IL) from a cylinder was cooled to 3°C by a heat exchanger and delivered by a CO₂ pump continuously at a controlled mass flow rate. The CO₂ was heated to a set temperature by another heat exchanger before entering the pressure vessel (500-mL volume). The vessel pressure was regulated by an automatic pressure regulator downstream, and the vessel temperature was controlled by an outer heat jacket.

In a typical experiment, 25 g of WPI or WPC powder was contained in a sample basket with a 5- μ m frit at the bottom, which was then placed into the pressure vessel. The pressure vessel cap was then closed, and the valve to the CO₂ cylinder was opened. All processing parameters were controlled by computer. A treatment time was defined as that after the system reached the set pressure and temperature (in about 5 min). After 1 h treatment with CO₂ at a mass flow rate of 50 g/

min, the vessel was depressurized in about 15 min and the sample was collected and stored at -20°C before rheological tests. In another set of experiments, the sample basket was replaced by an open-top stainless steel container with a similar diameter, filled with 100 mL of a 10% (wt/vol in deionized water without pH adjustment) WPI solution and processed with scCO_2 as above. A control was processed similarly with compressed nitrogen (N_2) for both WPI powders and a 10% (wt/vol) WPI solution.

Because BSA shows partial, reversible unfolding between 42 and 50°C (Lin and Koenig, 1976), a temperature of 40°C was chosen for the WPI solution treatments to single out the scCO_2 effects from the thermal denaturation of proteins. On the other hand, because a higher temperature facilitates the extraction of lipids from powder samples (Froning et al., 1990; Manganiello et al., 2000; Wolf et al., 2003), a temperature of 65°C was selected, while keeping the temperature below the denaturation temperatures of α -LA (67.1°C) and β -LG (76.3°C ; Ju et al., 1999), the 2 major whey proteins.

Rheological Tests

Sample Preparation. Five grams of WPC or WPI powder was hydrated in 50 mL of deionized water overnight at room temperature. Then, the pH was adjusted to 3.35 or 7.0 with 1 *N* HCl or 1 *N* NaOH. The final powder concentration was 9.9% (wt/vol) at pH 7.0 and 9.4% (wt/vol) at pH 3.35. The pH 7.0 samples also contained 100 mM NaCl. The scCO_2 -processed 10% WPI solution had a pH of 5.80 after opening the vessel (a time lag of approximately 30 min after stopping the CO_2 stream, much slower depressurization than processing powders to prevent extensive foaming) and some protein precipitated (due to acidification). The suspension was continuously stirred overnight at room temperature to deplete the dissolved CO_2 and dissolve the precipitate. The stirred solution recorded no difference in pH (pH = 6.75) from the unprocessed sample before pH adjustment and was then adjusted to pH 3.35 with 1 *N* HCl. The solubility of N_2 in water is low, and the WPI solution processed by N_2 did not have the foaming issue and recorded no pH change. The pH adjustment to 3.35 was therefore done shortly after processing. The amount of 1 *N* HCl used to adjust pH to 3.35 was identical for the unprocessed WPI solution and the solutions processed by CO_2 and N_2 .

Rheological Test Procedures. Dynamic rheological tests were performed with an AR2000 rheometer (TA Instruments, New Castle, DE) using a Searle set up (bob, outside diameter = 28 mm and cup, inside diameter = 30 mm). After positioning the bob and removing the excess sample, a layer of mineral oil was applied

on top of the sample, and a sealing cap was used to minimize moisture loss during measurements. A 1% strain amplitude (within the linear viscoelasticity regime) was used for the following steps: 1) a linear heating step from 20 to 90°C at $1^{\circ}\text{C}/\text{min}$, 2) an isothermal step at 90°C for 3 h, 3) a frequency sweep step from 0.01 to 10 Hz at 90°C , 4) a linear cooling step from 90 to 20°C at $1^{\circ}\text{C}/\text{min}$, and 5) a frequency sweep step from 0.01 to 10 Hz at 20°C . The oscillation frequency during steps 1, 2, and 4 was 1 Hz. Triplicate tests were performed for each sample.

Surface Hydrophobicity of Proteins

Salting-out properties of WPI were used as an indirect measurement of relative protein surface hydrophobicity (Lindahl et al., 1981). Different concentrations of ammonium sulfate, $(\text{NH}_4)_2\text{SO}_4$ (0 to 3.465 *M*, pH 3.35) were prepared by mixing different proportions of a $(\text{NH}_4)_2\text{SO}_4$ stock solution (3.465 *M* in 20 mM sodium phosphate, adjusted to pH 3.35) and a 20 mM sodium phosphate solution (pH 3.35). The $(\text{NH}_4)_2\text{SO}_4$ solution (990 μL) was mixed with 10 μL of the WPI pH 3.35 samples (the unheated leftover from rheological tests), and the mixture was continuously agitated for 30 min on an end-to-end shaker (Lab Industries Inc., Berkeley, CA) at room temperature. The suspension was then centrifuged at $14,500 \times g$ for 15 min (MiniSpin Personal, Eppendorf, Westbury, NY). The supernatant was analyzed for the total protein concentration by the Bradford method (Bradford, 1976) using an assay kit containing Coomassie (Bradford) reagent and BSA standard from Pierce Biotechnology (Rockford, IL). All samples were measured in triplicate; results, in averages and 95% confidence intervals, were reported by normalizing to the corresponding sample protein concentration at 0 *M* $(\text{NH}_4)_2\text{SO}_4$.

Scanning Electron Microscopy

The scanning electron microscopy tests were performed with a LEO 1525 SEM microscope (LEO Electron Microscopy, Oberkochen, Germany) for WPI and WPC powder samples before and after processing. The powder sample was glued directly onto an adhesive tab mounted on the specimen stub and sputter-coated with a thin layer of gold (~ 5 nm).

Statistical Analysis

An ANOVA was used to evaluate the compositional and rheological data; the Tukey test was performed with the assistance of JMP software (version 6.0, SAS Institute, Cary, NC). However, statistical analysis was

Table 1. Sample compositions of whey protein concentrate (WPC) and whey protein isolate (WPI) powders¹ unprocessed and processed with supercritical CO₂ (scCO₂) or N₂

Sample	Temperature (°C)	Pressure (MPa)	Composition		
			Protein ¹ (%)	Lipids ² (%)	Moisture ¹ (%)
Unprocessed WPI	—	—	91.21 ± 0.16 ^b	0.13	5.93 ± 0.17 ^a
scCO ₂ -processed WPI	65	10	92.27 ± 0.13 ^a	0.11	5.17 ± 0.08 ^{ab}
scCO ₂ -processed WPI	65	30	92.71 ± 0.01 ^a	0.05	4.41 ± 0.29 ^{bc}
N ₂ -processed WPI	65	10	90.48 ± 0.19 ^b	0.21	5.80 ± 0.23 ^a
Unprocessed WPC	—	—	75.46 ± 0.21 ^e	7.16	4.44 ± 0.46 ^{bc}
scCO ₂ -processed WPC	65	10	76.63 ± 0.15 ^d	7.74	3.51 ± 0.12 ^c
scCO ₂ -processed WPC	65	30	78.58 ± 0.44 ^c	6.41	3.53 ± 0.28 ^c

^{a-e}Means with a different letter in each column are statistically different.

¹Values are averages ± standard deviations from 2 tests.

²Only 1 replicate was performed, as explained in the Materials and Methods section.

not performed for the lipids content because only one test was done for each sample.

RESULTS AND DISCUSSION

Sample Compositions

Proximate composition analyses of WPI and WPC powders before and after processing are listed in Table 1. The measured compositions of the unprocessed WPI sample were similar to the product label that listed 92.7% protein, 0.3% fat, and 5.0% moisture. The moisture content of WPI powders decreased slightly after scCO₂ processing, and the decrease was more significant for the 30 MPa treatment than for the 10 MPa treatment. Also decreased was the lipid content, which was expected from the well-known capability of scCO₂ to extract lipids. The protein content per unit mass of powders was statistically greater for the scCO₂-processed samples. In contrast, there was no significant difference in moisture and protein contents between the unprocessed sample and that treated with N₂. These data indicate that scCO₂, because of zero surface tension, had much better penetration capability than N₂ at similar temperatures and pressures, which increased the rate of moisture and lipids transportation out of protein powder granules.

For WPC, the measured compositions of unprocessed WPC sample were also similar to the labeled 78.0% protein, 8.0% fat, and 4.5% moisture. In comparison, reduced moisture content was recorded for the scCO₂-treated samples. The lipids content after the 10 MPa treatment was similar to that in the unprocessed samples, possibly due to the inefficiency of the CO₂ extraction at this pressure. Conversely, the sample after the 30 MPa treatment had a lower lipid content. The protein content significantly increased after the scCO₂ extraction.

Sample Appearance and Notable Sensory Changes

Visual appearance of processed and unprocessed whey protein powder samples is presented in Figure 2. The unprocessed WPI (panel A) and that processed by N₂ (panel D) had loose clumps and did not move freely, whereas those processed by scCO₂ appeared as fine powders (panels B and C). Similarly, the unprocessed WPC had many clumps (panel E) that still existed after being processed by scCO₂ at 65°C and 10 MPa (panel F); but no more clumps were observed after processing with scCO₂ at 65°C and 30 MPa (panel G). The changes in lipid content (Table 1), although lacking statistical support, seemed to correlate with the sample appearance depicted in Figure 2: treatments that lowered the lipid content (panels B and C vs. panels A and D; panel G vs. panels E and F) may have also reduced the chance of sticking between powder granules. When observed with scanning electron microscopy, granules appeared irregular in shape and size, and no strong conclusions could be drawn for the samples before and after processing with scCO₂ or N₂ (Figure 3). Therefore, the advantages from scCO₂-processed powders include better flowability and a better visual appearance that may attract ingredient buyers and consumers.

In addition, WPC and WPI powders had a reduced creamy, sweet aroma when processed with scCO₂. This may have been due to the well-established fact that scCO₂ is capable of extracting small nonpolar molecules (Maheshwari et al., 1995). Sensory analyses and characterization of volatile compounds, however, are future research focuses. Nevertheless, whey proteins with a milder aroma and taste may be incorporated in products in which the aroma and flavor of protein ingredients are undesirable, while providing other functionalities of proteins.

Figure 2. Appearance of powders (5 g) of A) unprocessed whey protein isolate (WPI); B) WPI processed with CO₂ at 65°C and 10 MPa; C) WPI processed with CO₂ at 65°C and 30 MPa; D) WPI processed with N₂ at 65°C and 10 MPa; E) unprocessed whey protein concentrate (WPC); F) WPC processed with CO₂ at 65°C and 10 MPa; and G) WPC processed with CO₂ at 65°C and 30 MPa.

Rheological Data of WPI Solutions Processed by scCO₂ and N₂

For the WPI solution that interacted with scCO₂, the sample after resuspending precipitates and adjusting pH to 3.35 showed a steady development in storage modulus upon heating (Figure 4A), further strengthened during cooling (Figure 4B). Storage modulus (G') of the scCO₂-processed WPI solution was greater than that of the unprocessed sample and that of the sample processed with N₂ under the same conditions (Figure 4). This approach of modifying WPI suspended in solutions was not pursued further because similar results were obtained from scCO₂-processed powder samples (Table 2), an easier operation that does not present the foaming issue and is easier to scale up. Solutions processed by scCO₂ and N₂ were not analyzed for composition

with the assumption that no significant compositional changes would occur because proteins were dispersed in water. Water evaporation during processing was expected via the container opening due to the continuous gas stream inside the pressure vessel. However, it would be expected that the amount of water evaporated would be similar for scCO₂ and N₂ treatments. The apparent increase in gel strength for the scCO₂-treated solution compared with the controls (unprocessed and that processed by N₂) may have been caused by the dissolved scCO₂ that interacted with proteins, causing irreversible structure changes after CO₂ depletion.

Phase-angle changes of WPI solutions during the second step (isothermal at 90°C) where storage modulus developed steadily are plotted in Figure 5. The tangential function of a phase angle is defined as a ratio of

Figure 3. Scanning electron microscopy images for powders of A) unprocessed whey protein isolate (WPI); B) WPI processed with supercritical (sc)CO₂ at 65°C and 10 MPa; C) WPI processed with scCO₂ at 65°C and 30 MPa; D) WPI processed with N₂ at 65°C and 10 MPa; E) unprocessed whey protein concentrate (WPC); F) WPC processed with scCO₂ at 65°C and 10 MPa; and G) WPC processed with scCO₂ at 65°C and 30 MPa. Scale bar = 100 μm.

Figure 4. Storage modulus of 9.4% (wt/vol) whey protein isolate (WPI) solutions (pH 3.35) processed with supercritical CO₂ or N₂ and unprocessed during (A) heating from 20 to 90°C at 1°C/min and holding at 90°C for 3 h and (B) during cooling from 90 to 20°C at 1°C/min. Data are averages from 3 tests.

Figure 5. Phase angle of 9.4% (wt/vol) whey protein isolate (WPI) solutions (pH 3.35) processed with supercritical CO₂ or N₂ and unprocessed during holding at 90°C for 3 h after heating from 20 to 90°C at 1°C/min. Data are averages from 3 tests.

loss (G'') to storage (G') modulus. A phase angle $<45^\circ$ indicates a storage modulus that dominates the loss modulus, and a system behaves more elastic- or solid-like. Therefore, the phase angle is a convenient way of monitoring the gelation process and a system with a phase angle $<45^\circ$ is commonly referred to as a gel (Ross-Murphy, 1994). With the assumption that all 3 samples had identical composition, the WPI processed with scCO₂ reached a phase angle of 45° (i.e., gelation) faster than the unprocessed WPI and that processed with N₂ (insert in Figure 5). The latter 2 samples had an almost identical gelation time. The differences in gelation time further indicated that the dissolved CO₂ changed whey protein structures (CO₂ has a much greater solubility in water than does N₂), which enabled a faster gelation and a stronger gel. Both computer simulations (of lysozyme) and experiments (from films of albumin and lyso-

Table 2. Storage modulus (G')¹ of whey protein isolate (WPI) or whey protein concentrate (WPC) samples (9.9%, wt/vol, at pH 7.0; 9.4%, wt/vol, at pH 3.35) unprocessed and processed with supercritical CO₂ (scCO₂) or N₂

Sample	Temperature (°C)	Pressure (MPa)	G' (Pa) after step (2) at 90°C		G' (Pa) after step (4) at 20°C	
			pH 3.35	pH 7	pH 3.35	pH 7
Unprocessed WPI	—	—	449 ± 19 ^c	6,198 ± 66 ^a	1,279 ± 31 ^b	19,197 ± 138 ^a
scCO ₂ -processed WPI solution	65	10	586 ± 7 ^a	—	1,518 ± 15 ^a	—
N ₂ -processed WPI solution	65	30	421 ± 14 ^c	—	1,200 ± 16 ^b	—
scCO ₂ -processed WPI powder	65	10	526 ± 25 ^a	6,262 ± 11 ^a	1,518 ± 20 ^a	21,130 ± 132 ^a
scCO ₂ -processed WPI powder	65	30	508 ± 13 ^b	6,599 ± 47 ^a	1,519 ± 30 ^a	20,590 ± 355 ^a
N ₂ -processed WPI powder	65	10	419 ± 9 ^c	—	1,229 ± 8 ^b	—
Unprocessed WPC	—	—	55 ± 3 ^e	334 ± 17 ^c	190 ± 9 ^e	2,025 ± 42 ^c
scCO ₂ -processed WPC powder	65	10	75 ± 7 ^e	397 ± 7 ^{bc}	314 ± 14 ^d	2,253 ± 16 ^{bc}
scCO ₂ -processed WPC powder	65	30	113 ± 12 ^d	443 ± 15 ^b	405 ± 13 ^c	2,603 ± 37 ^b

^{a-e}Means with a different superscript letter within a column are statistically different.

¹Results are averages ± 95% confidence intervals from 3 tests.

Figure 6. Mechanical spectra of 9.4% (wt/vol) whey protein isolate (WPI) solutions (pH 3.35) processed with supercritical CO₂ or N₂ and unprocessed after cooling to 20°C. Data are averages from 3 tests and error bars are 95% confidence intervals. G' = storage modulus; G'' = loss modulus.

zyme) have shown that scCO₂ alternates the secondary structure (the relative amount of α -helix, β -sheet, and random coil) of proteins (Striolo et al., 2003; Liu et al., 2004). Further studies are needed to illustrate the exact mechanisms of whey protein molecular structure changes induced by CO₂ and how these changes correlate with rheological properties.

Frequency sweep tests during step 3 (at 90°C) and step 5 (at 20°C) showed that G' was much greater than G'', exemplified in Figure 6 from step 5, indicating that all samples formed weak gels. Data suggested that the gel formed from the CO₂-treated WPI was statistically stronger than the other 2 samples ($P < 0.05$).

Rheological Data of WPI Powders Processed by scCO₂ and N₂

Comparing thermal gelation properties, WPI powders processed by scCO₂ formed a stronger gel (higher G') than those unprocessed or processed by N₂ (Figure 7). During the heating and holding steps, the sample extracted by scCO₂ at 65°C and 10 MPa formed the strongest gel, whereas that extracted by scCO₂ at 65°C and 30 MPa was slightly weaker (Figure 7A). Upon cooling (Figure 7B), the 2 scCO₂-treated samples had an almost identical gel strength. Comparing the compositions of 2 scCO₂-processed samples (Table 1), there was no statistical difference in protein content, which might have led to a similar gel strength after cooling.

The sample processed by scCO₂ at 30 MPa showed a much faster gelation (a shorter time to reach a phase

Figure 7. Storage modulus (G') of 9.4% (wt/vol) whey protein isolate (WPI) solutions (pH 3.35) constituted from powders processed with supercritical CO₂ or N₂ and that unprocessed during (A) heating from 20 to 90°C at 1°C/min and holding at 90°C for 3 h and (B) cooling from 90 to 20°C at 1°C/min. Data are averages from 3 tests.

angle of 45°) than the other 3 samples (Figure 8). The gelation time for the unprocessed sample was similar to that of samples processed by scCO₂ at 10 MPa and shorter than for samples processed by N₂. The gelation times correlated well with protein content ($R^2 = 0.95$) and lipid content ($R^2 = 0.98$) but not with moisture content ($R^2 = 0.65$).

The time to reach a phase angle of 45° correlated with observations from the "salting out" of proteins (Figure 9). The WPI powder after scCO₂ processing at 65°C and 30 MPa had the greatest soluble protein content after precipitation by ammonium sulfate, indicating that the sample had the lowest surface hydrophobicity. This lowered surface hydrophobicity, possibly due to removal of bound lipids (Table 1), may have induced the faster gelation. There was no difference in surface hydrophobicity for the unprocessed sample and that

Figure 8. Phase angle of 9.4% (wt/vol) whey protein isolate (WPI) solutions (pH 3.35) constituted from powders processed with supercritical CO₂ or N₂ and that unprocessed during holding at 90°C for 3 h after heating from 20 to 90°C at 1°C/min. Data are averages from 3 tests.

processed by scCO₂ at 65°C and 10 MPa (Figure 9), which correlated with no difference in the gelation time for these 2 samples (Figure 8). Conversely, the sample processed by N₂ had the poorest protein solubility during precipitation (Figure 9), which is reflected by the longest gelation time of this sample (Figure 8). It seemed that surface hydrophobicity may have contributed to the observed differences in gelation kinetics. Eventually, if the sample is allowed enough time to

Figure 9. Salting-out of whey protein isolate (WPI) samples at pH 3.35 by different concentrations of ammonium sulfate. The remaining soluble protein concentrations are normalized to that at 0 M ammonium sulfate.

form chemical and physical bonds, the total protein concentration (Table 1) may be a critical factor determining the final gel strength (Table 2, Figure 7), because heating denatures whey proteins before formation of the bonds.

Storage moduli of samples following rheological test steps 2 and 4 are compiled in Table 2. When correlating compositions in Table 1 and G' data in Table 2, protein contents correlated much better with rheological data at pH 3.35 ($R^2 = 0.89$ for G' after step 2 and 0.94 for G' after step 4) than lipid and moisture contents (all $R^2 < 0.77$). The unprocessed sample and that processed by N₂ showed no statistical difference in G', whereas the scCO₂-processed samples formed gels with similar strength. It is also interesting to note that there was no statistical difference in gel strength for the scCO₂ treatment of WPI in either powder form or dispersed in water. However, to judge whether the same interactions between protein and scCO₂ occurred for the solution and powder treatments, detailed information of composition, surface hydrophobicity, secondary (possibly tertiary) structures of proteins, and interactions between proteins and lipids is needed.

Storage moduli of samples at pH 7.0 and 0.1 M NaCl were much greater than the corresponding sample at pH 3.35 (Table 2). Enhanced gel strength was also observed for scCO₂-processed WPI at pH 7.0, but the increase was not statistically significant. Ikeda et al. (1999) studied whey protein gelation with different NaCl concentrations at pH 7.0 and observed the strongest gel at 0.1 M NaCl. Therefore, the addition of 0.1 M NaCl to the WPI sample may have lessened the enhanced gel strength due to scCO₂ processing.

Rheological Data of WPC Powders Processed by scCO₂

Compared with the unprocessed sample, the WPC powders processed by scCO₂ formed a gel with a much greater storage modulus (Figure 10), with the 30 MPa treatment being more significant than the 10 MPa treatment. Table 1 shows statistical differences of protein content among all samples, but only the 30 MPa treatment lowered the lipids content. The enhanced gelling properties may have been induced by compositional changes and the interaction between scCO₂ and lipid-bound protein in WPC. Greater lipid content in WPC compared with WPI signified the enhancement in gelation properties by scCO₂ treatments.

When comparing phase-angle changes of WPC samples during step 2, the gelation time followed a decreasing order of 30 MPa treatment, 10 MPa treatment, and untreated sample (Figure 11). Storage modulus data after test steps 2 and 4 are summarized in Table 2. At

Figure 10. Storage modulus (G') of 9.4% (wt/vol) whey protein concentrate (WPC) solutions (pH 3.35) constituted from powders processed with supercritical CO₂ and that unprocessed during (A) heating from 20 to 90°C at 1°C/min and holding at 90°C for 3 h and (B) cooling from 90 to 20°C at 1°C/min. Data are averages from 3 tests.

pH 3.35, G' increased from 55 to 75 Pa for the 10 MPa treatment and to 113 Pa for the 30 MPa treatment after step 2; after step 4, G' increased from 190 to 314 Pa for the 10 MPa treatment and to 405 Pa for the 30 MPa treatment. Similar to WPI, protein contents correlated much better with rheological data at pH 3.35 ($R^2 = 1.00$ for G' after step 2 and 0.95 for G' after step 4) than did lipid and moisture contents (all $R^2 < 0.81$). The improvement in the relative gel strength after scCO₂ processing was less significant at pH 7.0: G' increased from 334 to 397 Pa for the 10 MPa treatment and to 443 Pa for the 30 MPa treatment after step 2; and G' increased from 2,025 to 2,253 Pa for the 10 MPa treatment and to 2,603 Pa for the 30 MPa treatment after step 4. Similar to pH 3.35, protein contents correlated much better with rheological data at pH 7.0 ($R^2 =$

Figure 11. Phase angle of 9.4% (wt/vol) whey protein concentrate (WPC) solutions (pH 3.35) constituted from powders processed with supercritical CO₂ and unprocessed during holding at 90°C for 3 h after heating from 20 to 90°C at 1°C/min. Data are averages from 3 tests.

0.95 for G' after step 2 and 1.00 for G' after step 4) compared with lipid and moisture contents (all $R^2 < 0.81$).

In summary, gelling properties of whey proteins, either dissolved in water or in powder form, can be improved after scCO₂ processing. Compositional and surface hydrophobicity analyses indicated that rheological properties were affected not only by composition (particularly protein content), but also by structural changes due to the scCO₂ treatment. Removal of lipids from WPC was incomplete and may be improved further under other conditions, making scCO₂ a low-cost purification technique compared with those based on membrane filtrations or chromatography. We expect that compositional and structural changes will also significantly change interfacial properties of proteins; this aspect will be studied in the near future. We are currently extending extraction conditions and characterizing the corresponding functionality changes over a broader range of testing conditions and techniques, with a goal of establishing correlations between extraction, composition, and structure and functionalities of whey proteins.

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