Effect of NaCl addition during diafiltration on the solubility, hydrophobicity, and disulfide bonds of 80% milk protein concentrate powder

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ABSTRACT

We investigated the surface hydrophobicity index based on different fluorescence probes [1-anilinonaphthalene-8-sulfonic acid (ANS) and 6-propionyl-2-(*N,N*-dimethylamino)-naphthalene (PRODAN)], free sulfhydryl and disulfide bond contents, and particle size of 80% milk protein concentrate (MPC80) powders prepared by adding various amounts of NaCl (0, 50, 100, and 150 mM) during the diafiltration process. The solubility of MPC80 powder was not strictly related to surface hydrophobicity. The MPC80 powder obtained by addition of 150 mM NaCl during diafiltration had the highest solubility but also the highest ANS-based surface hydrophobicity, the lowest PRODAN-based surface hydrophobicity, and the least aggregate formation. Intermolecular disulfide bonds caused by sulfhydryl-disulfide interchange reactions and hydrophobic interactions may be responsible for the lower solubility of the control MPC80 powder. The enhanced solubility of MPC80 powder with addition of NaCl during diafiltration may result from the modified surface hydrophobicity, the reduced intermolecular disulfide bonds, and the associated decrease in mean particle size. Addition of NaCl during the diafiltration process can modify the strength of hydrophobic interactions and sulfhydryl-disulfide interchange reactions and thereby affect protein aggregation and the solubility of MPC powders.

Key words: milk protein concentrate, solubility, surface hydrophobicity, disulfide bond

INTRODUCTION

An 80% milk protein concentrate (MPC80) is a high-protein powder manufactured from skim milk by ultrafiltration plus diafiltration and spray-drying (Mulvihill, 1992; Novak, 1992); it can be used in the manu-

facture of cheese, yogurt, beverages, and confections. Water solubility (i.e., rehydration capacity) of MPC80 powder is an important prerequisite for its improved functional properties such as emulsification, gelation, and foaming. However, MPC powders with 40 to 90% (wt/wt) protein content can have poor solubility, which restricts their potential food applications (De Castro-Morel and Harper, 2002; Fang et al., 2011). Therefore, improving the solubility of MPC80 powder is essential for enhancing its functionality and use.

Intermolecular electrostatic interactions, hydrophobic interactions, and molar mass are the main factors determining protein solubility (Damodaran and Paraf, 1997; Walstra et al., 2006). Hydrophobicity is one of the important chemical properties of milk protein systems because of its contribution to stabilization of milk proteins (Cairolì et al., 1994; Nakai et al., 1996; Erdem, 2006). To a great extent, protein surface hydrophobicity determines the tendency of protein molecules to aggregate and therefore to lose solubility (Wagner et al., 2000). The exposure of buried hydrophobic regions of protein promotes aggregation and cross-linking of partially hydrolyzed proteins, thus decreasing the solubility of the resulting hydrolysate (Paraman et al., 2007). Both 1-anilinonaphthalene-8-sulfonic acid (ANS) and 6-propionyl-2-(*N,N*-dimethylamino)-naphthalene (PRODAN) are aromatic hydrophobic probes, and PRODAN is a neutral probe that can eliminate potential inclusion of electrostatic interaction contributions in the measurement of surface hydrophobicity. Therefore, the surface hydrophobicity of MPC80 was determined in the present study using both ANS and PRODAN probes.

In addition to hydrophobic interactions, sulfhydryl-disulfide (SH-SS) interactions also contribute to protein insolubility. Milk casein micelles are associated colloidal particles with a stabilizing hairy brush of calcium-insensitive κ -CN on their surfaces. Heat treatment of milk causes whey proteins to denature and interact with the fat globule membrane components, as well as with κ -CN, through sulfhydryl-disulfide bridge

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exchange reactions (Houlihan et al., 1992; Corredig and Dalgleish, 1999). These interactions may result in loss of solubility of dairy protein powders (Singh, 2007). The mechanisms of decreased solubility may direct formation of disulfide bonds or more complex interactions that still need to be clarified.

The effect of salts on protein solubility is fundamentally related to the hydrophilicity-hydrophobicity characteristics of the protein surface and to ionic strength (Damodaran and Paraf, 1997). Generally, the solubility of proteins with a large number of exposed hydrophobic patches decreases at low ionic strength ($<0.5\text{ M}$) and increases under high ionic strength. In addition to ionic strength, mineral type affects the solubility of milk protein powders. The addition of a monovalent salt to ultrafiltered retentate before drying improves MPC80 solubility (Carr, 2002). Replacement of approximately 30% of the calcium of the ultrafiltered skim milk retentate by sodium ions can result in a MPC80 powder with little insoluble material, because removal of calcium ions increases the electrostatic repulsive forces between the casein micelles, to improve hydration properties of MPC80 powder (Havea, 2006). Compared with that of MPC80 powder made without addition of NaCl, the solubility of MPC80 powder produced with NaCl addition during diafiltration increases significantly (Gualco, 2010). The mechanism behind this effect needs further elaboration.

Therefore, in the present work, we investigated the contribution of changes in surface hydrophobicity (as measured by different fluorescence probes) and the content of free sulfhydryl and disulfide bonds. The specific purposes of this study were (1) to determine the effect of added NaCl during diafiltration on the solubility, surface properties, and structural characteristics of the obtained MPC80 powder, and (2) to explore the relationships of surface hydrophobicity, free sulfhydryl and disulfide bond content, and particle size to MPC80 solubility.

MATERIALS AND METHODS

Reagents and Materials

Pasteurized skim milk was obtained from Producer's Dairy Foods Inc. (Fresno, CA). 5',5-Di,thio,bis, 2-nitrobenzoic acid (**DTNB**) and ANS were purchased from Sigma-Aldrich Co. (St. Louis, MO), and PRODAN was purchased from Invitrogen Life Technology Co. (Carlsbad, CA). The bicinchoninic acid (**BCA**) protein assay kit was obtained from Pierce (Rockford, IL). All chemicals used were reagent grade.

Preparation of MPC80 with NaCl Addition During Diafiltration

The MPC80 powders were manufactured in the pilot plant of Dairy Products Technology Center at California Polytechnic State University (San Luis Obispo) with an R12 cross-flow membrane pilot-plant unit (Niro Inc., Hudson, WI) equipped with dual 10-kDa cut-off, spiral-wound, polyethersulfone membranes (Snyder Filtration, Vacaville, CA). Diafiltration water with different concentrations of NaCl (0, 50, 100, and 150 mM) was used to produce MPC80 powders. The liquid MPC was spray-dried with a pilot Niro Filtermat Spray Dryer (Niro Inc.) to approximately 3.5% moisture, and the obtained MPC80 powders were immediately collected and sealed in airtight bags for further analysis. The protein content of the produced MPC80 powders was approximately 80%.

Solubility Determination

A 5% (wt/wt) solution of each MPC80 powder was prepared in distilled water. After mixing with a magnetic stirrer for a certain period (0, 10, 30, 60, and 90 min) at room temperature, aliquots of MPC80 solutions were withdrawn and the supernatants were obtained by centrifugation at $700 \times g$ for 10 min at 23°C. The protein content in both the supernatants and original MPC80 solutions was determined by using Elementar rapid N cube (Elementar Analysensysteme GmbH, Hanau, Germany). Solubility was calculated according to the following equation:

$$\text{Solubility (\%)} = \frac{\% \text{ protein in supernatant}}{\% \text{ protein in MPC80 solution}} \times 100.$$

Surface Hydrophobicity Determination

The surface hydrophobicity was measured by using ANS and PRODAN as fluorescent probes according to the method described by Hayakawa and Nakai (1985) and Haskard and Li-Chan (1998). The relative fluorescence intensity of the samples was measured by using a Jasco FP-6500 spectrofluorimeter (Jasco International Co. Ltd., Tokyo, Japan) with a quartz cuvette at an excitation wavelength of 390 nm and emission wavelength of 470 nm when ANS was used as fluorescence probe. When the PRODAN probe was used, the excitation and emission wavelengths were 365 and 465 nm, respectively. Protein concentration was determined by BCA assay (Smith et al., 1985).

Determination of Free Sulfhydryl and Disulfide Bond Content

Free sulfhydryls (SH) and disulfide bond (SS) contents in MPC solutions were determined using Ellman's reagent according to the method detailed by Beveridge et al. (1974), with some modifications. For SH, 5% (wt/wt) MPC80 solutions were made in a Tris-glycine buffer (0.086 M Tris, 0.09 M Gly, 4 mM EDTA, pH 8.0) containing 8 M urea and reacted with DTNB. For SS, 5% (wt/wt) MPC80 solutions were made in the above Tris-glycine buffer with 10 M urea and 0.02 mL β -mercaptoethanol (β ME), and were reacted with DTNB. Absorbance at 412 nm was measured with a spectrophotometer (Molecular Devices Spectra MAX Plus, Bio-Rad Laboratories, Hercules, CA). The SH and SS contents were calculated using the molar extinction coefficient of $13,600\text{ M}^{-1}\text{ cm}^{-1}$ (Ellman, 1959) and expressed as micromoles per gram of protein.

Particle Size Distribution Analysis of MPC80 Powder During Reconstitution

The particle size distribution of the 5% (wt/wt) MPC80 solutions was measured by using a Coulter LS230 Particle Size Analyzer equipped with Fluid Module (Beckman Coulter, Brea, CA) in the size range from 0.4 to 2,000 μm . The aqueous protein solution was added into the water chamber dropwise until the polarization intensity differential scattering reached the working range and stabilized. The measurement time was set to 60 s, and measurements were conducted in triplicate. The fluid module was flushed with water for 15 min between samples.

Effect of Denaturing and Reducing Reagents on the Solubility of MPC80 Powder

To better understand the role of disulfide bonds on MPC80 powder solubility, 5% (wt/wt) MPC80 solutions were treated with 10% SDS for 6 min at 70°C or with 5% β ME for 6 min at 85°C. The levels of SDS and β ME were based on the protein content of the MPC80 solutions. The resulting MPC80 solutions were evaluated for protein solubility and particle size distribution.

SDS-PAGE

The protein composition and level in the supernatants obtained from the solubility studies were determined by SDS-PAGE using a Mini-Protein 3 Cell electrophoresis system (Bio-Rad Laboratories) according to the method of Laemmli (1970). Samples were prepared

under reducing (with β ME) and nonreducing conditions (without β ME) to determine the role of disulfide bonds. Samples were electrophoresed with a 12% polyacrylamide resolving gel and a 5% acrylamide stacking gel. After electrophoresis, the gels were stained using Coomassie Brilliant Blue R-250 stain solution (Bio-Rad Laboratories). After staining overnight, the gels were destained until a clear background was achieved. The molecular weight of each band was determined using Precision Plus Protein Standard (Bio-Rad Laboratories) as molecular weight standards consisting of proteins in the range from 10 to 250 kDa.

Statistical Analysis

The sample treatments and chemical analyses in the present study were run in triplicate. Data were expressed as means $\pm$ standard deviations (SD). Statistical analysis was performed with SPSS package (SPSS 13.0 for Windows, SPSS Inc., Chicago, IL). The differences between the means of the treatments were compared by one-way ANOVA at a significance level of $P < 0.05$.

RESULTS

Change of Solubility of MPC80 Powder With Reconstitution Time

The solubility of all MPC80 powders was greater with longer reconstitution time. The solubility of MPC80 with NaCl addition during diafiltration was significantly higher than that of the control (without NaCl addition; $P < 0.05$, Figure 1). The data indicated that the dispersion of casein micelles increased with the addition of NaCl and with longer reconstitution time.

Change of Hydrophobicity in MPC80 Powder Obtained by Adding Different Concentrations of NaCl During Diafiltration

Compared with the control MPC80, relative fluorescence intensity increased with the addition of NaCl and exhibited a concentration-dependent effect for ANS-based (Figure 2A) and PRODAN-based (Figure 3A) probes. These observations indicate that the number of exposed ANS- and PRODAN-accessible hydrophobic regions increased upon addition of NaCl. Figure 2B displays the ANS-based surface hydrophobicity of MPC80 with and without addition of NaCl during diafiltration. It shows that the ANS-based surface hydrophobicity of NaCl-treated MPC80 powders increased significantly compared with that of the control MPC80, and the sur-

Figure 1. Solubility of 80% milk protein concentrate powder with and without addition of NaCl during the diafiltration process as the function of reconstitution time.

face hydrophobicity of MPC80 powder was enhanced with an increase in NaCl concentration. The 150 mM NaCl-treated MPC80 exhibited the highest ANS-based surface hydrophobicity ($P < 0.05$). The increase in ANS-hydrophobicity may be the result of unfolding of the milk protein, which leads to the exposure of the hydrophobic interior of the protein. The increase in surface hydrophobicity indicated that more hydrophobic groups in MPC80 were exposed by NaCl treatment.

The relative fluorescence intensity and surface hydrophobicity using the PRODAN probe are shown in Figures 3A and 3B. The relative fluorescence intensity of NaCl-treated MPC80 powders was significantly higher than that of the control MPC80. Further, the greater the concentration of added NaCl, the higher the relative fluorescence intensity of the MPC80. However, the PRODAN-based surface hydrophobicity of NaCl-treated MPC80 samples was significantly lower than that of the MPC80 without NaCl addition during diafiltration (Figure 3B). This differed from the result obtained using ANS as fluorescence probe.

Change of Free SH and SS Bond Content in MPC80 Powder Treated with Different Concentrations of NaCl During Diafiltration

Free SH and SS bond contents of MPC80 powders are shown in Figure 4. When the concentration of added NaCl was higher, the number of SS bonds formed was lower and the free SH content was higher. This trend suggests that NaCl addition during the diafiltration process attenuated the formation of SS bonds that lead to protein-protein interactions during the spray-drying

process. This may contribute to lesser aggregate formation and partially explain the relatively higher solubility of NaCl-treated MPC80 powders.

Changes in Free SH, SS Bond, and Particle Size in MPC80 Powder Treated with SDS or β ME

As depicted in Table 1, the solubility of MPC80 powder increased after being treated with β ME and SDS, and the greatest change occurred for control MPC80. This suggests that hydrophobic interactions and SS bonds exhibited much more detrimental effects on the solubility of MPC80 powder without addition of NaCl. These observations indicate that both hydrophobic and disulfide interactions are key factors in MPC80 insolubility. Moreover, the particle size of all MPC80 samples decreased with the addition of NaCl during diafiltration (Figure 5) and decreased further after β ME and SDS treatment (Table 1). Thus, the decrease in particle size was consistent with the increase in solubility, suggest-

Figure 2. The relative fluorescence intensity (A) and surface hydrophobicity (B) of 80% milk protein concentrate (MPC80) powder with and without addition of NaCl during the diafiltration process (using 1-anilinonaphthalene-8-sulfonic acid probe). The values are presented as means $\pm$ SD of 3 replications. The hydrophobicities of MPC80 samples with different letters are significantly different at $P < 0.05$.

Figure 3. The relative fluorescence intensity (A) and surface hydrophobicity (B) of 80% milk protein concentrate (MPC80) powder with and without addition of NaCl during the diafiltration process (using 6-propionyl-2-(*N*, *N*-dimethylamino)-naphthalene probe). The values are presented as means $\pm$ SD of 3 replications. The hydrophobicities of MPC80 samples with different letters are significantly different at $P < 0.05$.

ing that both NaCl addition and treatment with β ME or SDS can prevent the formation of aggregates, which may be a key reason for the differences in solubility between NaCl-treated and control MPC80.

SDS-PAGE

The SDS-PAGE patterns under nonreducing and reducing conditions for MPC80 supernatants are presented in Figure 6. The intensity of the major protein bands (α -CN, β -CN, κ -CN, BSA, and β -LG) in the NaCl-treated MPC80 samples was significantly different from those of the control MPC80 in nonreducing SDS-PAGE (Figure 6A). The native SDS-PAGE electrophoretic pattern (Figure 6A, lanes 1 to 4) showed that the formation of aggregates (high molecular weight bands, >200 kDa) decreased with addition of NaCl or aggregates became more dissociated under SDS-PAGE conditions. The aggregate intensity was lowest with ad-

Figure 4. Content of free sulfhydryl (A) and disulfide bonds (B) of 80% milk protein concentrate (MPC80) powder with and without addition of NaCl during the diafiltration process. The values are presented as means $\pm$ SD of 3 replications. Columns with different letters are significantly different at $P < 0.05$.

Table 1. Change of solubility and particle size distribution of 80% milk protein concentrate (MPC80) powder with and without β -mercaptoethanol (β ME) or SDS as reducing or denaturing reagents, respectively

Treatment	Solubility (%)	Average particle size (μ m)
Control	65.52 $\pm$ 3.65 ^d	125.40 $\pm$ 6.24 ^a
Control (+ β ME)	84.69 $\pm$ 3.33 ^{bc}	60.05 $\pm$ 3.21 ^b
Control (+ SDS)	90.23 $\pm$ 4.32 ^b	46.32 $\pm$ 3.02 ^d
50 mM NaCl	81.76 $\pm$ 4.36 ^c	61.68 $\pm$ 3.98 ^b
50 mM NaCl (+ β ME)	94.76 $\pm$ 3.55 ^{ab}	19.83 $\pm$ 2.24 ^f
50 mM NaCl (+ SDS)	95.32 $\pm$ 4.99 ^{ab}	35.24 $\pm$ 2.98 ^e
100 mM NaCl	90.76 $\pm$ 3.12 ^b	53.11 $\pm$ 2.36 ^c
100 mM NaCl (+ β ME)	97.51 $\pm$ 2.27 ^a	39.33 $\pm$ 3.09 ^e
100 mM NaCl (+ SDS)	97.55 $\pm$ 2.16 ^a	34.22 $\pm$ 3.14 ^e
150 mM NaCl	96.11 $\pm$ 3.43 ^a	41.61 $\pm$ 3.87 ^{de}
150 mM NaCl (+ β ME)	97.51 $\pm$ 2.15 ^a	7.961 $\pm$ 0.62 ^g
150 mM NaCl (+ SDS)	98.43 $\pm$ 1.05 ^a	38.55 $\pm$ 2.89 ^d

^{a-g}Means within a column followed by different superscript letters differ at $P < 0.05$ level.

¹The values are presented as means $\pm$ SD of 3 replications.

Figure 5. Particle size distribution of milk protein dispersions after reconstitution for 1 h of 80% milk protein concentrate (MPC80) powder with and without addition of NaCl during the diafiltration process.

dition of 150 mM NaCl during diafiltration. Integration showed that the intensity of the major casein bands was different among MPC80 powders with or without addition of NaCl during diafiltration. The intensity of κ -CN band was the highest and the β -LG band was stronger with 150 mM NaCl than in the control and the treatments with lower concentrations of NaCl added. These observations suggest that adding NaCl during diafiltration resulted in less complex formations. These results clearly indicate that the higher solubility of the NaCl-treated MPC80 is associated with the reduced formation of aggregates between κ -CN and β -LG. The aggregates were formed mainly between κ -CN and β -LG

and between BSA and β -LG, and gave rise to much more intense bands of κ -CN, β -LG, and BSA in the SDS gels. These aggregates predominantly involved disulfide bond interactions. Reducing SDS-PAGE (Figure 6B, lanes 5 to 8) showed that the aggregate in MPC80 supernatant could be diminished by β ME. The intensity of the major protein bands in all of the samples was similar, which suggests that the aggregates were dissociable under reducing SDS-PAGE and, therefore, minimal covalent cross-linking occurred in the MPC80 supernatants.

DISCUSSION

The solubility of MPC80 increased with the addition of NaCl during the diafiltration process. To better understand the mechanisms for this effect, the changes of surface hydrophobicity based on anionic and neutral fluorescence probes, sulfhydryl and disulfide contents, and particle size distribution were investigated. Our observations indicated that all of these factors play important roles in the solubility of MPC80. The present study showed that addition of NaCl during the diafiltration process modified the strength of hydrophobic interactions, which led to fewer sulfhydryl-disulfide interactions and thereby a reduction in particle size of MPC powders.

Salts (minerals) influence protein solubility through electrostatic and hydrophobic interactions (Melander and Horvath, 1977). We showed that the ANS-based surface hydrophobicity of MPC80 with and without

Figure 6. Sodium dodecyl sulfate-PAGE electrophoresis of 80% milk protein concentrate (MPC80) supernatant under reducing and nonreducing conditions. MW = molecular weight standard (kDa); lanes 1 to 4 (nonreducing) = control MPC80, 50 mM NaCl-treated MPC80, 100 mM NaCl-treated MPC80, 150 mM NaCl-treated MPC80, respectively; lanes 5 to 8 (reducing) = control MPC80, 50 mM NaCl-treated MPC80, 100 mM NaCl-treated MPC80, 150 mM NaCl-treated MPC80, respectively. The protein samples were treated with (+ β ME) or without (– β ME) 5% β -mercaptoethanol.

addition of NaCl during diafiltration exhibited a positive relationship with its solubility; that is, the higher the ANS-based surface hydrophobicity, the higher the solubility. Hayakawa and Nakai (1985) reported that ANS hydrophobicities correlate well to the protein insolubility determined at zero zeta-potential. Wagner et al. (2000) also reported that the higher the ANS-based surface hydrophobicity, the greater the solubility of soy protein isolates. The increase of ANS-based surface hydrophobicity reflects the existence of suitable hydrophobic "clefts" for ANS binding; that is, more exposed ANS-binding hydrophobic moieties. β -Lactoglobulin molecules are more hydrophobic and flexible when pH values are above 7.5 (Phillips et al., 1994). For NaCl-treated MPC80 dispersions in the present study, the pH values were 7.76. The nonreducing SDS-PAGE pattern in the present study also showed that more free β -LG was present in 150 mM NaCl-treated MPC80 powder compared with MPC80 with other levels of NaCl. All of these results can help to explain the relatively higher ANS-based hydrophobicity of the NaCl-treated MPC80 powders. Our results showed that the particle size of MPC80 was smaller with the addition of NaCl during diafiltration.

In addition to hydrophobicity, charge frequency also has a great influence on protein solubility (Bigelow, 1967). As an anionic probe, ANS cannot prevent the effect of electrostatic interaction on the solubility, whereas, as a neutral probe, PRODAN may circumvent this problem (Haskard and Li-Chan, 1998). In the present study, the PRODAN-based surface hydrophobicity of NaCl-treated MPC80 powders differed from the ANS-based surface hydrophobicity. The surface hydrophobicity of MPC80 powder treated with NaCl was significantly higher than that of control MPC80 when using ANS as probe, whereas it was substantially lower than that of the control when using PRODAN as probe. For an apolar solute, the higher the surface hydrophobicity, the lower the solubility. This was confirmed by the PRODAN-based surface hydrophobicity and solubility results of MPC80 powders in the present study.

Besides protein-protein hydrophobic interactions, sulfhydryl and disulfide interactions might also influence the surface hydrophobicity. To further investigate the reason for solubility improvement in NaCl-treated MPC80 powder, we investigated the effect of disulfide formation by a direct β ME treatment and reduced SDS-PAGE method. After reconstitution in the presence of β ME, the solubility of all MPC80 powders increased to varying degrees, whereas disulfide content and particle size decreased. The nonreducing SDS-PAGE pattern showed that as the amount of NaCl added increased, aggregates appearing in the high mo-

lecular weight bands (>200 kDa) gradually decreased and almost disappeared in the supernatant of 150 mM NaCl-treated MPC80. These observations suggest that these aggregates are more dissociable under SDS conditions, which can eliminate hydrophobic interactions. Moreover, the aggregates completely disappeared in reducing SDS-PAGE bands. In total, the observations indicated that the aggregates formed through intermolecular disulfide bonds and hydrophobic interactions in the supernatant were soluble. The disulfide-linked aggregates consist mainly of κ -CN, β -LG, and BSA in the supernatant (thus, they are soluble), which means that this kind of interaction may not always contribute to the insolubility of MPC80 powders. Havea (2006) also reported that disulfide-linked protein aggregates were not considered to play a major role in the formation of the insoluble material in MPC powders. Generally, protein molecule unfolding leads to an increase in surface hydrophobicity. However, unfolding may be followed by protein aggregation, through hydrophobic interactions or through SH-SS interchange reactions (Laligant et al., 1991). The intermolecular interactions could lead to an increase in surface hydrophobicity. Therefore, NaCl-treated MPC80 possessing high solubility and high ANS-based hydrophobicity may be a result of fewer disulfide interactions.

Some studies show that the addition of NaCl to milk can increase the hydration of casein micelles (Grufferty and Fox, 1985; Le Ray et al., 1998). The increased ionic strength induces a decrease in activity coefficients of the diffusible ions and consequently increases the dissociation of the ion pairs (Walstra and Jenness, 1984). Therefore, NaCl leads to an increase in calcium concentration in the diffusible phase. Ionic environment affects protein-protein aggregation. High ionic strength can increase protein solubility by increasing the hydration of proteins (Xiong, 1992). The difference between ANS-based and PRODAN-based surface hydrophobicity in the present study suggested that the ionic strength and corresponding electrostatic interaction in the MPC80 solution changed with addition of NaCl. The effects of NaCl on electric potential and mineral components of MPC80 dispersions, and thus on protein solubility, need further evaluation, and the effect of various ions on MPC80 solubility still needs to be elucidated.

CONCLUSIONS

The effects of NaCl treatment and changes in surface hydrophobicity on MPC80 solubility were investigated in the present study. The changes in disulfide content and particle size with solubility were also determined. Our results demonstrated that the beneficial effects of NaCl incorporation during diafiltration on MPC80

solubility may be attributable to the modification of hydrophobicity sites, decreased formation of disulfide bonds, and a reduction of particle size, all of which make MPC80 more water-accessible and more soluble. The exposure of buried ANS-binding hydrophobic sites, which may improve the hydrophilic-hydrophobic balance and casein micelle hydration, is associated with enhanced MPC80 solubility. Both hydrophobic interactions and protein-protein interactions play important roles in controlling the solubility attributes of MPC80 powders. Hence, NaCl-treated MPC80 powder with good solubility may possess potential applications for the production of milk beverages and other high-protein foods. Further research is needed to evaluate the physicochemical characteristics and functional attributes of the obtained MPC80 powders.

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