Effects of Preheating on Properties of Aggregates and of Cold-Set Gels of Whey Protein Isolate

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Various concentrations (1−9%) of whey protein (WP) isolate solutions were heat-denatured at 80 °C for 30 min. Size exclusion HPLC and dynamic light scattering revealed formation of soluble aggregates in 3−9% denatured WP solution. Size and content of the aggregates increased with increases in preheated WP concentration. The 4−9% denatured WP solutions were diluted to 3% WP with distilled water. Upon addition of $CaCl_2$ (20 mM) or glucono-δ-lactone (0.6%, w/v), all 3% denatured WP solutions formed gels at 37 °C. Hardness of the gels (3% WP) remarkably increased with WP concentration during preheating or with the aggregate size and content. The HPLC elution profiles showed that prolonging the heating (80 °C) time (2−30 min) for 8% WP solution also gradually increases aggregate size and concentration, which then led to increases in hardness of cold-set gels. The results may guide companies in how to manipulate aggregate size and content during developing WP products with capacity for cold gelation.

Keywords: *Whey proteins; denaturation; aggregates; cold-set gels*

INTRODUCTION

Thermally denatured whey protein (WP) solutions can gel at ambient temperature upon addition of salt or acid (Barbut and Foegeding, 1993; Nakamura et al., 1995). The cold-gelling ability was ascribed to formation of soluble aggregates after preheating of the protein solution (Sato et al., 1995; Ju and Kilara, 1998a). This technology has been utilized in the food industry, and a WP product used for cold gelation has been developed (Thomsen, 1995). However, knowledge of the effects of preheating on the formation and development of aggregates and on the properties of cold-set gels is still limited.

The denatured WP isolate (WPI) or β-lactoglobulin (β-Lg) solution can be obtained by heating the solution below the critical protein gelling concentration, above the denaturation temperature of the WP, at neutral pH and in the presence of little or no salt. The formed aggregates could be detected with size exclusion (SE) HPLC or gel electrophoresis (Ju et al., 1997; Shimada and Cheftel, 1989). Heating bovine serum albumin (BSA) or β-Lg resulted in single protein aggregates as evidenced by the HPLC (Matsudomi et al., 1992, 1994). Heating the β-Lg mixture with BSA or α-lactalbumin (α-La) led to formation of coaggregates. However, heating α-La alone did not lead to the formation of aggregates (Dalgleish et al., 1997; Matsudomi et al., 1992). The aggregates in the denatured WPI solution were considered also to be coaggregates of WP (Ju et al., 1997).

The hardness of cold-set gels formed by the addition of salt or acid was found to be strongly dependent on the temperature and hold time of preheating (Barbut and Foegeding, 1993; Nakamura et al., 1995). It is not clear, however, if these effects are due to changes in the properties of the aggregates upon the different preheating treatments. Information relating preheating conditions to properties of aggregates and gels would be valuable in the development of new WP products. The objective of this research was to investigate effects of preheating WP solutions at different protein concentrations and for various holding times on aggregate size and on hardness of cold-set gels.

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MATERIALS AND METHODS

Materials. Commercial WPI, BiPro, was from Davisco International Inc. (Le Sueur, MN). WPI solutions (1−9%, w/v, protein basis) were prepared with distilled water. The solutions were held overnight at 5 °C before use. Glucono-δ-lactone (GDL) and $CaCl_2$ were purchased from Sigma Chemical Co. (St. Louis, MO).

Heat Treatment. A 100-mL portion of 1−9% WP solutions (WPS; pH 7.0) was centrifuged for 1 h at 12000*g*. The supernatants were placed in 100-mL Erlenmeyer flasks and heated in an 80 °C water bath for 30 min. Immediately after heating, the samples were cooled with tap water (18 °C) and stored at 5 °C overnight. The 8% WPS were also heated for various times (0−30 min) in the 80 °C water bath.

Gel Preparations. The preheated 4−9% WPS were diluted to obtain a final protein concentration of 3% with distilled water. Thirty-milliliter samples were placed in 50-mL beakers and adjusted to 20 mM $CaCl_2$ with 2 M $CaCl_2$ solutions, or 0.6% GDL powder (w/v, resulting in pH 5.0) was added at 5 °C. The sample was then incubated at 37 °C for 1 h to induce gelation. The gel samples were cooled with tap water and stored at 5 °C overnight (∼16 h) before gel hardness was determined.

The same procedures for inducement of gelation were used for 8% WPS that were preheated to 80 °C for various times (0−30 min), except 0.8% GDL (w/v, resulting in pH 5.0) was added. Each experiment was replicated three times.

Gel Hardness. Gel hardness was determined by a texture analyzer (TA XT-2, Texture Technologies Corp., Scarsdale, NY), as described by Ju and Kilara (1998a). The gels formed in the beaker were penetrated with a cylinder probe of 12-mm diameter. A force−time curve was obtained at a crosshead

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speed of 60 mm/min for a 15-mm displacement, and gel hardness was expressed as the force (*g*) at the maximum peak of the force−time curve (Bourne, 1978).

SE-HPLC. SE-HPLC was performed using a Waters HPLC system (Waters, Division of Millipore, Milford, MA) mounted with a Progel-TSK G4000SW_{XL} column and an SWXL guard column, both of which were from Supelco (Bellefonte, PA). Samples of the denatured WPS (1−9%) were diluted to 0.5% protein with distilled water and filtered through a 0.45-μm filter (Millipore Corp., Bedford, MA), and 30 μL of sample was injected onto the column. The samples were eluted with a buffer (pH 6.8) consisting of 0.05 M Na_2HPO_4 and 0.05 M NaH_2PO_4 at a 0.6 mL/min flow rate. The components were detected at 280 nm. The α-La and β-Lg were identified by analysis of standard proteins. A calibration curve for the column was established with seven standard proteins from 29 $\times$ 10^3 (carbonic anhydrase) to 2000 $\times$ 10^3 (blue dextran) molecular weights (MW).

Dynamic Light Scattering. The particle or aggregate size in the native and denatured WPS was determined by dynamic light scattering (DLS) using a Microtrac ultrafine particle analyzer (Leeds & Northrup Instruments, St. Petersburg, FL) (Ju and Kilara, 1998b). Samples at different WP concentrations (1−9%) were diluted to 0.2% WP with distilled water. The particle size determinations were replicated two times.

Transmission Electron Microscopy. The samples of gels induced by $CaCl_2$ (20 mM) and GDL (0.8%, w/v) from the most-heated (80 °C, 30 min) 8% WPS were examined by transmission electron microscopy according to the method of Otte et al. (1996). Ultrathin sections (60−70 nm) were imaged and photographed using a JEOL 1200 EXII transmission electron microscope (Peabody, MA) under an accelerating voltage of 80 kV.

RESULTS AND DISCUSSION

Effect of Protein Concentration. *Gel Hardness.* The heating at 80 °C for 30 min was over the transition temperature (62−78 °C, at pH 6.7) of individual WP (Mulvihill and Donvan, 1987; Hollar et al., 1995) and should denature 1−9% WPS. However, visually, the 1−9% preheated WPS were still brown and transparent. In a preliminary test, the 1−2% preheated WPS did not form self-supported gels upon addition of $CaCl_2$ (20 mM) or GDL (0.6%, w/v), but the 3% preheated WPS did form a gel. Thus, the 4−9% preheated WPS were all diluted to 3% WP to examine the effect of the protein concentration during preheating on the hardness of cold-set gels.

The WP concentration during preheating had a remarkable effect on the gel hardness. The $CaCl_2$- and GDL-induced gels, at the same protein concentration (3%), linearly increased in hardness with increased protein concentration (3−9%) during preheating (Figure 1). For example, the gel (3% WP) from dilution of the 9% preheated WPS was >2 times stronger than the gel (3% WP) from the 3% preheated WPS. This suggests that the hardness of cold-set gels (at the same WP concentration) strongly depended on the protein concentration during the heating−denaturation.

The difference in gel hardness might be due to differences in properties of thermally induced aggregates in the parent denatured WPS. SE-HPLC and DLS were used to detect changes in the aggregate size in the 1−9% denatured WPS.

Aggregate Size. The SE-HPLC profile of the original WPI solution mainly shows peaks of β-Lg and α-La (Figure 2), which were eluted around 13.6 and 14.3 min, respectively. After 1−9% WPS were heated at 80 °C for 30 min, the areas of β-Lg and α-La peaks progressively decreased with increased WP concentration.

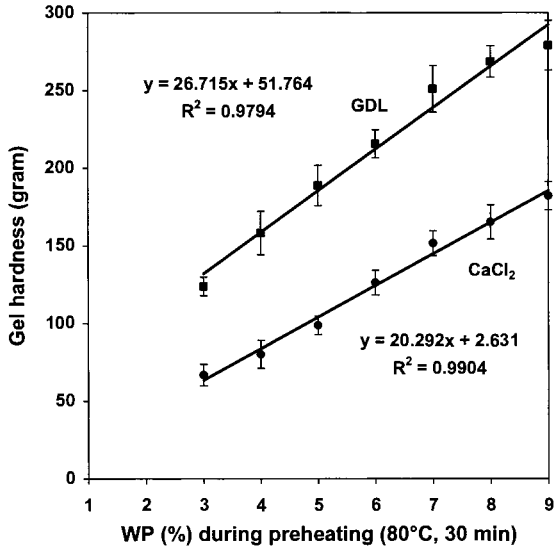

Figure 1. Hardness of 3% WP gels induced by addition of $CaCl_2$ (20 mM) or GDL (0.6% w/v) to 3% denatured WP solutions. Preheated (80 °C, 30 min) 4−9% WPS (*x*-axis) were diluted to the 3% denatured WPS.

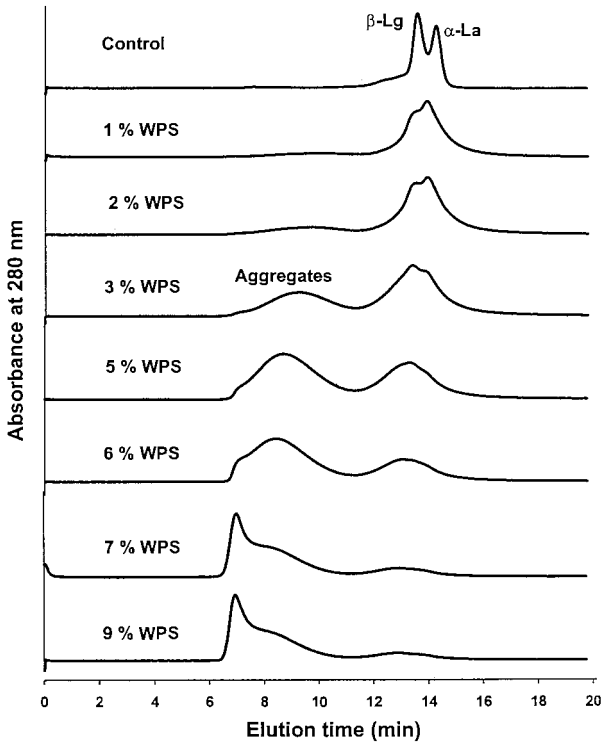

Figure 2. SE-HPLC elution profiles of original WPI solution and the denatured solutions that were preheated (80 °C, 30 min) at various protein concentrations (1−9%). The 1−9% preheated WPS were diluted to 0.5% protein with distilled water for running the HPLC.

Simultaneously, the area of a peak representing formed aggregates gradually increased, and, especially, elution time of the aggregate peak decreased with increased WP concentration (Figure 2). These indicate that both aggregate content and size increased with increased WP concentration. By calculating the percentage of aggregate peak area as a function of the total peak area, it was observed that 34% WP were aggregates in the 3% preheated WPS, compared to 56% in 5% WPS, 90% in 7% WPS, and 97% in 9% WPS. From the calibration curve for the column, the MWs of aggregates in the 3%

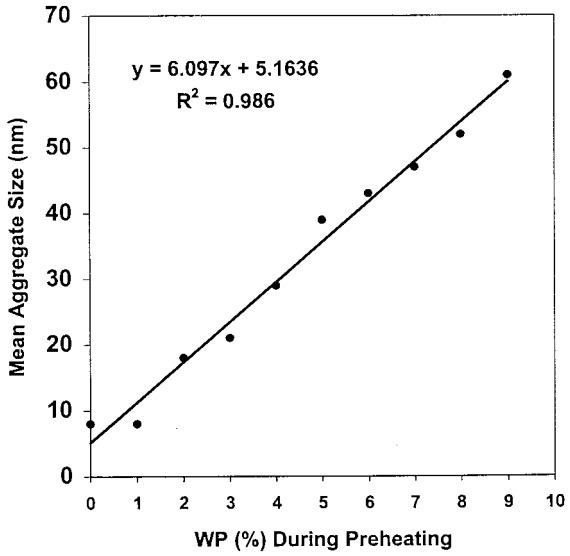

Figure 3. Mean aggregate sizes in denatured WPI solutions that were preheated (80 °C, 30 min) at various protein concentrations (1−9%).

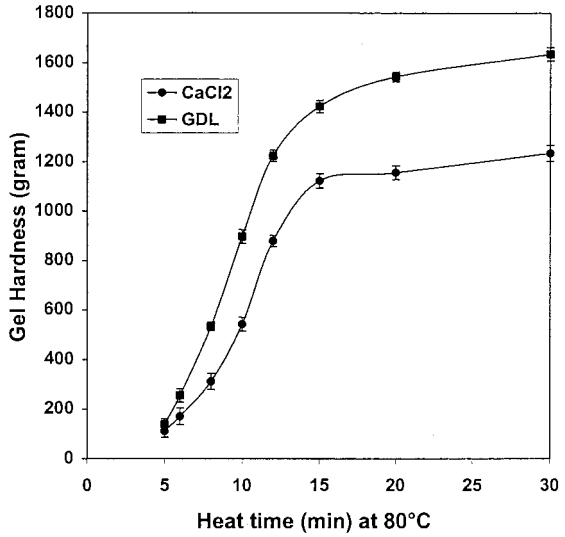

Figure 4. Hardness change of the gels induced by addition of $CaCl_2$ (20 mM) or GDL (0.8%, w/v) to 8% WPS preheated at 80 °C for various times (5−30 min).

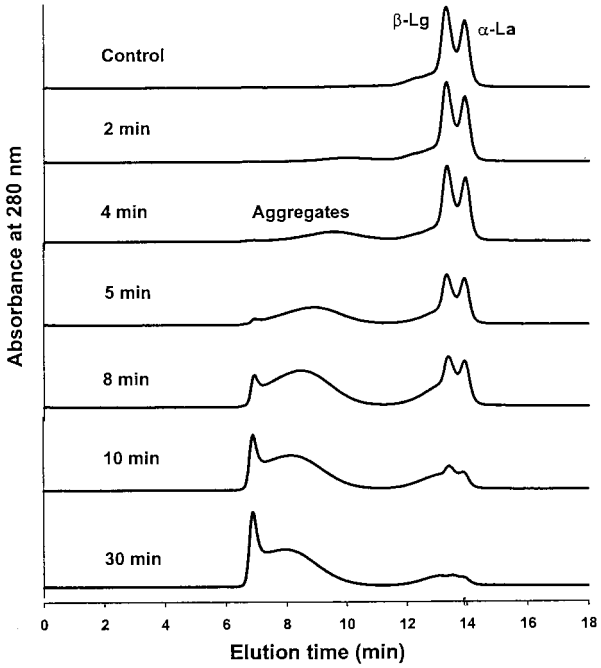

Figure 5. SE-HPLC elutions profiles of 8% WPI solutions preheated for various times (0−30 min) at 80 °C.

preheated WPS were calculated to be ~103×10^4, whereas MWs of aggregates in 5% WPS were ~125×10^4 and aggregates in 7−9% WPS were around or over 1000×10^4. Therefore, higher concentration of preheated WPS contained higher content and larger aggregates. Note that the 1−9% preheated WPS were diluted to the same protein concentration (0.5%) and then analyzed by SE-HPLC (Figure 2).

Except for the formation of the aggregates, after the preheating (80 °C, 30 min), peaks of β-Lg and α-La could not be resolved (Figure 2). This may suggest that parts of β-Lg and α-La were in an unfolded state.

Results from DLS (Figure 3) showed that aggregate size increased with increased WP concentration in a linear manner ($R^2 = 0.986$). Native WPS measured 8 nm of mean particle size, which coincides with the size (~7 nm) reported by Aguilera and Stanley (1993). Upon the same heat treatment (80 °C, 30 min), higher WP concentration resulted in larger sized aggregates. The 3% denatured WPS contained 21-nm mean aggregates, whereas 9% denatured WPS had 61-nm mean aggregates, reflecting a 7.6-fold increase in mean particle size from that of native WP, compared to 5−6-fold increases (5 nm to 25−30 nm) in thermal β-Lg aggregate size (Hoffmann et al., 1996). Heating (80 °C, 30 min) 12% WPS might lead to even larger and more aggregates, which could then form a gel upon the heating alone (Otte et al., 1996).

From above results (Figures 2 and 3), it could be concluded that the 3% WPS that was diluted from the higher concentration of denatured WPS prior to gelation were composed of bigger sized and greater numbers of aggregates, which then led to harder cold-set gels (Figure 1). The result suggests that to develop WP products for applications of cold gelation, the proteins should be heat-treated at a high protein concentration.

Effect of Heating Time. *Gel Hardness.* The 8% WPS preheated from 5 to 30 min in an 80 °C water bath formed self-supporting gels upon addition of $CaCl_2$ (20 mM) or GDL (0.8%, w/v). Less than 5 min of heating time did not result in gelation. The hardness of both kinds of gels significantly increased with heating time (Figure 4). Rapid increases occurred at heating times from 5 to 12 min. Further heating (15−30 min) led to limited increases in gel hardness. From the same denatured WPS, the gels resulting from acidification (to pH 5.0) by GDL were significantly harder than those from $CaCl_2$ addition (Figures 1 and 4). This agreed with previous studies (Nakamura et al., 1995; Ju and Kilara, 1998a).

Aggregate Size. Figure 5 shows SE-HPLC profiles of 8% WPS that were preheated at 80 °C for various times (2−30 min). As previously reported (Ju et al., 1997), native protein peaks decreased and the aggregate peak correspondingly increased upon prolonged heating time (Figure 5), suggesting that the native proteins gradually converted to aggregates. Meanwhile, the elution time of the aggregate peak gradually decreased, indicating growth of aggregate MW with heat time. Previous work (Ju et al., 1997; Matsudomi et al., 1992), however, did not show any change in the elution time of the aggregate peak. This might be due to the small elution particle

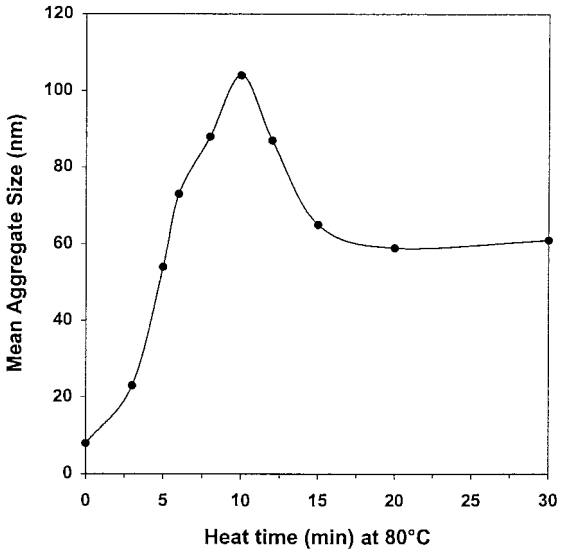

Figure 6. Mean aggregate sizes in WPI solutions preheated for various times (0−30 min) at 80 °C.

size [(5−150) × 10^3 or (10−500) × 10^3 MW] of previous SE-HPLC columns, whereas this research used a column capable of detecting a much higher range of particle size [(20−10000) × 10^3 MW]. Furthermore, prolonging heating time up to 8 min only decreased heights and areas of α-La and β-Lg peaks and did not change their resolution. This may suggest that these preheated WPS contained both denatured (formed aggregates) and native WP.

Heating 8% WPS for 4 min only resulted in 46% native proteins converting to aggregates, and the aggregates were ∼64 × 10^4 MW. This preheated WPS did not gel upon addition of $CaCl_2$ or GDL. After 5 min of heating, 62% of the native protein formed aggregates with a larger MW of 94 × 10^4. This preheated WPS gelled upon addition of $CaCl_2$ or GDL. This suggests a requirement of minimum aggregation level for cold gelation. The greatest preheating (80 °C, 30 min) resulted in 96% native proteins converting to aggregates (∼1000 × 10^4 MW), which led to formation of the hardest gel (Figure 4). With gel electrophoresis, Matsudomi et al. (1992) also showed considerable large aggregates in a heat-denatured BSA solution (250 × 10^4 MW) or in a heat-denatured mixture of BSA with α-La (160 × 10^4 MW).

Therefore, the longer time of heating could also lead to higher MW and higher content of aggregates. The enlarged and concentrated aggregates led to the formation of harder gels. Barbut and Foegeding (1993) and Nakamura et al. (1995) found that increasing heating temperature (70−100 °C) caused increases in the hardness of cold-set gels. This might also be due to enlarged and concentrated aggregates upon higher temperature treatment.

DLS, however, revealed a result different from that of SE-HPLC: mean aggregate size quickly increased at initial heat time (up to 10 min) and then slowly decreased upon further heating (12−30 min, Figure 6). The maximum aggregate size (102 nm), which occurred at a 10-min heating time, was nearly twice as large as those (62 nm) in the most-heated (30 min) WPS. By DLS, Hoffmann et al. (1996) also observed a continuous decrease in diameter of protein aggregates upon more time (∼1−22 h) of heating (68.5 °C) 3% β-Lg solution in situ. The explanation for this phenomenon was that

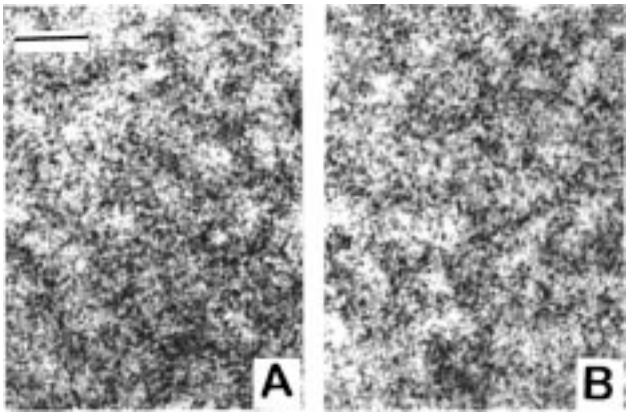

Figure 7. Transmission electron micrographs of the gels induced by $CaCl_2$ (A) or GDL (B) from 8% WPS preheated at 80 °C for 30 min. Bar represents 200 nm.

in colloidal systems with nonattractive or repulsive forces between the particles, increased particle concentration could cause a decrease of real particle size.

Microstructure. As in many previous papers (Barbut and Foegeding, 1993; Ju et al., 1997; Kawamura et al., 1993; Nakamura et al., 1995), cold-set gels from the denatured WPS had fine-strand microstructures (Figure 7). The networks of two gels induced by $CaCl_2$ and GDL were built with similar thicknesses of fine-stranded aggregates. This may indicate that salt and acid induced gelation by promoting interactions of the aggregates that originally existed in the denatured WPS. The result could be confirmed from the micrographs of Nakamura et al. (1995), which showed that NaCl- or GDL-induced gels were composed of the same shaped and sized aggregates as those in the denatured solution. Therefore, the aggregates in the denatured WPS were responsible for cold gelation and possibly for gel properties, too. Manipulating thermal conditions may control properties of aggregates and of cold-set gels.

Conclusions. The hardness of cold-set gels was dependent on the size and content of soluble aggregates in thermally denatured WPS. Preheating WPI solution at a higher protein concentration resulted in the formation of larger sized and more numbers of aggregates. At equivalent protein concentration, the larger sized and greater numbers of aggregates led to the formation of harder cold-set gels. Preheating (80 °C, 30 min) WPI at low protein concentration (<2%) unfolded or denatured the protein, but most of the denatured protein did not form the soluble aggregates.

The longer heating time (up to 30 min) also increased the hardness of the cold-set gels because of increased molecule weight and number of aggregates in the parent denatured WPS. The research results suggest that to develop WP products for application of cold-set gelation, companies may manipulate preheated protein concentration or amount of heat to control the size and concentration of formed soluble aggregates in their products.

ABBREVIATONS USED

β-Lg, β-lactoglobulin; BSA, bovine serum albumin; DLS, dynamic light scattering; GDL, glucono-δ-lactone; MW, molecular weight; α-La, α-lactalbumin; SE-HPLC, size exclusion HPLC; WP, whey protein; WPI, WP isolate; WPS, WP solutions.

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