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Extrusion modifies some physicochemical properties of milk protein concentrate for improved performance in high-protein nutrition bars

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Abstract

BACKGROUND Extruded and ground milk protein concentrate powders, specifically those with 800 g kg–1 protein (i.e. MPC80), imparted softness, cohesion and textural stability to high-protein nutrition (HPN) bars. The present study evaluated some physicochemical properties of extruded and conventionally produced (i.e. spray-dried) MPC80 to explain these improvements. Protein chemical changes and aggregations within MPC80-formulated HPN bars during storage were characterized.

RESULTS Extruded MPC80 powders had broader particle size distribution ($P < 0.05$) and smaller volume-weighted mean diameter ($P < 0.05$) than the spray-dried control. Loose, tapped and particle densities increased ($P < 0.05$) and correspondingly occluded and interstitial air volumes decreased ($P < 0.05$) after extruding and milling MPC80. Extrusion decreased water holding capacity ($P < 0.05$) and solubility ($P < 0.05$), yet improved the wettability ($P < 0.05$) of MPC80. MPC80 free sulfhydryl ($P < 0.05$) and free amine ($P < 0.05$) concentrations decreased after extrusion. Sulfhydryl and amine concentrations changed ($P < 0.05$) and disulfide-linked and, more prominently, Maillard-induced aggregates developed during HPN bar storage.

CONCLUSION Extrusion and milling together changed the physicochemical properties of MPC80. Chemical changes and protein aggregations occurred in HPN bars prepared with either type of MPC80. Thus, the physicochemical properties of the formulating powder require consideration for desired HPN bar texture and stability.

Keywords

Free sulfhydryl, free amine, contact angle, water holding capacity, protein bar

Comments

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Extrusion Modifies Some Physicochemical Properties of Milk Protein Concentrate for Improved Performance in High-protein Nutrition Bars

Running title: Extrusion improves MPC for protein bars

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CONCLUSIONS: Extrusion and milling together changed the physicochemical properties of MPC80. Chemical changes and protein aggregations occurred in HPN bars prepared with either type of MPC80. Thus, the physicochemical properties of the formulating powder require consideration for desired HPN bar texture and stability.

KEYWORDS

Free sulfhydryl, free amine, contact angle, water holding capacity, protein bar

INTRODUCTION

Starchy matrices easily extrude to produce puffed snacks of low nutritional value. Adding protein is appealing from a nutritional standpoint, but decreases processability and negatively impacts extrudate textural quality.[1] Hence, literature has focused extensively on protein-starch interactions that develop during extrusion by processing different proteins with different starches, and then reporting extrudate properties (e.g., expansion index, hardness). Direct food applications here include expanded snacks, textural crisps, and meat analogs, but are limited overall.[2, 3] A lesser-studied use of extrusion is to simultaneously apply heat, shear force, and pressure to modify the physiochemical properties of protein concentrates and isolates to produce novel protein ingredients.

Extrusion modified the physicochemical properties of milk protein concentrate (MPC), pea protein isolate (PPI), whey protein concentrate (WPC), and soy protein isolate (SPI) for improved functionality in target applications.[4-7] Nutritional and other product qualities of puffed cornmeal were improved by adding extrusion-modified whey protein isolate (WPI).[8] Extruded MPC produced non-baked high-protein nutrition (HPN) bars (e.g, 200 to 500 g kg^{-1} protein) that were softer, more cohesive, and less prone to texture changes during storage than bars formulated with the spray-dried control.[9, 10] In both examples, extrusion modified each ingredient's physicochemical properties; not just the protein's structure-function relationships. The summation of change improved performance in each application. However, specific physical and chemical properties altered by extruding and milling MPC with protein content of 800 g kg^{-1} (i.e., MPC80) remain unknown. Further investigation is required to understand why, from both a chemical and physical standpoint, extruded MPC80 powders, in comparison with a spray-dried control, produce softer, less crumbly, and more texturally stable HPN bars.

HPN bars are complex systems to study because their texture deteriorates with time and is not attributable to a single mechanism. Formulation (e.g., macronutrient composition, ingredients) and processing (e.g., baked versus formed, mixing times) are two factorial categories that influence product texture even before storage at different environmental conditions (e.g., temperature, packaging).[11] Many scientific studies have looked at the effect of protein source on HPN bar texture and its time-dependent changes assuming that different proteins are chemically and structurally disposed to perform better or worse in these applications.[11-15] A common conclusion is that food protein hydrolysates produce soft, texturally stable HPN bars.[14, 16] Extruded and ground MPC80s resulted in HPN bars with similar texture attributes, yet, food protein extrusion is not known to hydrolyze proteins.[5, 10] While protein ingredients do perform differently in HPN bars, these differences are not only due to the molecular differences between proteins, but also due partially to the unapparent physical differences between the powders.

Conventional MPCs are derived from fluid skim milk through sequential ultrafiltration, concentration, and spray drying, which yields powder that maintains the casein to whey protein ratio (i.e., 800 g casein and 200 g whey kg^{-1} protein). Regardless, this production sequence, especially spray drying, is common to many protein concentrates and isolates, and partially dictates the resultant physicochemical properties (e.g., particle size distribution, particle and bulk densities, occluded and interstitial air volumes, wettability, surface hydrophobicity, dispersibility, solubility, water holding capacity). Subsequent extruding, drying, and milling a conventionally produced MPC80 will alter its physicochemical properties, several of which have been identified as variables that affect protein ingredient performance in HPN bars.[11] For example, soy protein ingredients with intermediate solubility (30% < soluble solids index <

55%) produced HPN bars that balanced hardness, e.g., not too hard, and cohesiveness, e.g., not too crumbly.[11] Compared to the spray-dried control, extruded MPC80 was less soluble.[4] Despite having an opposing effect on solubility than hydrolysis,[17] these extruded powders performed better than the control in model HPN bars.[9, 10] Moreover, extruded MPC80 also had lower water holding capacity (WHC) than spray-dried MPC80.[4] Reducing WHC may limit moisture migration between HPN bar constituents, a commonly proposed mechanism of texture change, during storage.[12, 18] The effect of extrusion on MPC80's other physiochemical properties, including particle size distribution, particle and bulk densities, occluded and interstitial air volumes, wettability, and surface hydrophobicity are not well characterized.

The following study characterizes the physicochemical properties of conventionally produced and extruded MPC80. Free sulfhydryl and free amine concentration of each MPC80 is considered since preceding work, albeit mostly done using whey protein derived ingredients, has strongly suggested that time-dependent texture changes of intermediate moisture foods occur as disulfide-bonded and Maillard-induced protein aggregates form.[15, 19] From a free sulfhydryl standpoint, MPC80 is less likely to participate in the formation of new disulfide bonds compared to whey-based ingredients since casein, the main protein fraction in MPC80, contains no free sulfhydryl groups whereas beta-lactoglobulin (β-lg), the predominant whey protein, contains 1 free sulfhydryl per macromolecule (i.e., C121). However, protein bars formulated with spray-dried MPCs were more susceptible to texture changes during storage than those formulated with whey proteins.[12, 13] Extruding MPC80 can decrease free sulfhydryl and free amine concentration via protein-protein disulfide bond formation and the Maillard reaction with residual lactose.[20, 21] These chemical changes may limit progressive and texture-changing protein aggregation within HPN bars and the latter part of this study seeks further understanding of such changes. The

aforementioned properties, including particle sizes, densities, interstitial and occluded air volumes, solubility, WHC, surface hydrophobicity, and wettability of conventional and extruded MPC80, were measured and used in combination with the chemical changes brought out by extrusion to explain why extruded MPC80s texturally outperform their spray-dried counterparts in HPN bars.

MATERIALS AND METHODS

Materials

Ultrafiltered and spray-dried MPC80 powder (protein: 785 g kg^{-1}, fat: 43 g kg^{-1}, ash: 67 g kg^{-1}, moisture: 49 g kg^{-1}, lactose: 56 g kg^{-1}; Milk Specialties Global, Eden Prairie, MN) was previously extruded at die-end melt temperatures of 95, 105, and 116°C. Extrudates were dried at 40°C for 26 h and jet-milled into three respective powders: E95 (protein: 740 g kg^{-1}, moisture: 76 g kg^{-1}), E105 (protein: 743 g kg^{-1}, moisture: 75 g kg^{-1}), and E116 (protein: 744 g kg^{-1}, moisture: 74 g kg^{-1}).[10] E105, E116, and MPC80 were each independently used to make (n = 2) model HPN bars with 300 g kg^{-1} protein that were kept at 22°C or 32°C for 0, 6, or 29 weeks prior to quick freezing in liquid nitrogen and storage at -80°C.[10]

The Pierce™ BCA protein assay, 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), urea, EDTA, SDS, boric acid, sodium chloride, sodium tetraborate decahydrate, isopropanol, and β-mercaptoethanol were purchased from Fisher Scientific (Waltham, MA). Dithiothreitol (DTT), O-phthalaldehyde (OPA), N_{α}-acetyl-L-lysine, L-cysteine hydrochloride monohydrate, and bovine serum albumin (BSA) were purchased from Sigma-Aldrich (St. Louis, MO). AnyKD™ Mini-Protean® TGX™ precast gels, 2x Laemmli sample buffer, Precision Plus Protein™ Standard, Bio-Safe™ Coomassie Stain, and 10x tris/glycine/SDS running buffer were from Bio-Rad, Inc. (Hercules, CA). Millipore water had resistivity of 18.2 MΩ·cm at 25°C.

Powder physical properties: Size, density, and interstitial & occluded air volumes

Powder particle size was measured (n = 2) by laser diffraction (Mastersizer 2000, Malvern Inc., Worcestershire, United Kingdom) while dispersed in isopropanol.[10] Particle density ($\rho_{particle}$) was measured (n = 2) using a helium pycnometer (G-DenPyc 2900, Gold APP Instruments Corporation, Beijing, China). Powder (30 g ± 0.1 g) volume in a 100-mL glass cylinder after 0, 100, and 1,250 taps (Autotap™, Quantachrome Instruments, Boynton Beach, FL) was used to calculate (n = 3) loose (ρ_{loose}), tapped (ρ_{100X}), and extremely tapped (ρ_{1250X}) densities, respectively. Solids density (ρ_{solids}) for MPC80 was 1380 g L^{-1}.[22, 23] Occluded ($V_{oa} = 100/\rho_{particle} - 100/\rho_{solids}$) and interstitial ($V_{ia} = 100/\rho_{100X} - 100/\rho_{particle}$) air volumes were reported for each powder.

Protein solubility

Powder was dispersed in Millipore water at protein content of 8 g kg^{-1} and stir bar speed of 650 rpm. Dispersion pH was adjusted, and readjusted if needed after 15, 45, and 75 min, to 2.0, 3.5, 4.6, 5.5, 6.8, 8.0, 9.5, or 11.0 using hydrochloric acid (2 to 8 mol L^{-1}) or sodium hydroxide (2 to 8 mol L^{-1}) (n = 3). After 90 minutes of dissolution, dispersions were centrifuged at 15,000 × g for 15 min and supernatant filtered through Whatman No. 1 paper. If needed, filtrate was diluted with Millipore water and protein concentration was calculated (n = 2) from the linear region of the BSA standard curve (0.125-1.5 g L^{-1}) that developed during the BCA assay. Reported solubility was the ratio of filtrate protein concentration to initial dispersion protein concentration (i.e., 8 g L^{-1}).

Water holding capacity

Preliminary estimation found that 5.1, 5.2, 5.3, and 3.6 g of E95, E105, E116, and MPC80 powders, respectively, were required for each WHC test.[24] Eight, 9, 10, and 11 g

Millipore water were added to separate 50-mL centrifuge tubes containing the indicated weight of each extruded powder. Nine and a half, 10.5, 11.5, and 12.5 g Millipore water were each added to 50-mL centrifuge tubes containing 3.6 g control MPC80. Powder and water was hand mixed with a spatula for 2 min. Resultant pastes were centrifuged at 3,900 × g for 10 min. After decanting the supernatant when present, water mass retained by the initial powder mass was calculated by difference. Native water mass in each powder sample, approximately 390 and 180 mg for extruded and control MPC80s, respectively, was added to the retained mass of water. Total water mass was divided by solids mass and reported WHC of each powder was the average between the tube with the lowest volume supernatant and the supernatant-less tube tested at 1 g less water addition. The assay was triplicated.

Dynamic contact angle analysis

Powder (10 mg) was loaded into a 13-mm pellet die and was pressed (Model 4350, Carver, Inc., Wabash, IN) to and maintained at 8,000 kg_f for 2 min.[25] A 4-µL Millipore water droplet was dispensed (Gilmont GS-1200 Micrometer Syringe, Cole-Parmer, Vernon Hills, IL) onto pressed surfaces (n = 4). Profile images were immediately acquired using a goniometer (Model 250, Ramé-hart Instrument Co., Succasunna, NJ) at 5 images s^{-1}, which was slowed to 1 image s^{-1} after 20 s. Mean contact angle at 0 s (i.e., θ_{0s}) and 25 s (i.e., θ_{25s}) and mean droplet volume at the same time points (i.e., V_{0s}, V_{25s}) were extracted from processed images (DROPimage®, version 2.8.02, University of Oslo, Norway). For the first 25 s, droplet contact angle and volume rates of change (i.e., $d\theta/dt$, dV/dt) on each surface were determined.

Free sulfhydryl concentration

Free sulfhydryl buffer (pH 8.5) contained 8 mol L^{-1} urea, 4.1 mmol L^{-1} EDTA, and 20 g L^{-1} SDS in borate buffer (100 mmol L^{-1} boric acid, 75 mmol L^{-1} sodium chloride, and 25 mmol

L^{-1} sodium tetraborate decahydrate). Powder (0.8 g) plus free sulfhydryl buffer (8 mL) and separately free sulfhydryl buffer sans SDS (8 mL) were mixed for 2 h at 900 rpm prior to diluting to 10 mL (n = 3). HPN bars (1.6 g) were mixed with free sulfhydryl buffer (14.4 g) containing SDS for 2 h at 750 rpm (n = 2). After centrifuging powder and HPN bar dispersions for 20 min at 15,000 × g, supernatant free sulfhydryl concentration was immediately measured (n = 2) by Ellman's assay and calculated in µmol L^{-1} using a cysteine standard curve (R^2 > 0.998) that encompassed measurement net absorbance.[21, 26] Results were divided by BCA assayed soluble protein (g L^{-1}) to report free sulfhydryl concentrations in µmole g^{-1} protein.

Reduced and non-reduced SDS-PAGE

HPN bar supernatants from the free sulfhydryl assay were standardized at 4 g L^{-1} protein and were diluted 1 volume to 2 volume with either non-reducing or reducing 2x Laemmli sample buffer. Three µL of each sample and 10 µL of the molecular marker were electrophoresed on precast gels at 150 V for 45 min. SDS-PAGE details, specific to HPN bars, are provided elsewhere.[21]

Free amine concentration

Free amine buffer (pH 9.0) contained 50 mmol L^{-1} boric acid, 37.5 mmol L^{-1} sodium chloride, 12.5 mmol L^{-1} sodium tetraborate decahydrate, 10 g L^{-1} SDS, and 1 g L^{-1} DTT. Protein powder (0.16-0.17 g) dispersed in free amine buffer (23 mL) was stirred at 900 rpm for 2 h before diluting to 25 mL. In 25-mL flasks, HPN bars (0.31 g) and free amine buffer (10 mL) mixed for 2 h at 650 rpm. After centrifuging 20 min at 15,000 × g, sample supernatants were filtered through Whatman No. 4 filter paper. The BCA assay measured filtrate protein concentration. One-hundred µL protein standardized supernatant (i.e., 1 g L^{-1}) was mixed with 900 µL OPA reagent (0.8 g L^{-1}). Absorbance at 335 nm was measured and OPA reagent

absorbance subtracted.[9, 12] Linear (R^2 > 0.9999) 3-point (500-1500 µmol L^{-1}) and 4-point (100-1000 µmol L^{-1}) N_α-acetyl-L-lysine standard curves were used to calculate powder and HPN bar free amine concentrations, respectively. After dividing by protein concentration (i.e., 1 g L^{-1}), free amine concentration was reported in µmole g^{-1} protein.

Statistical analyses

Powder physicochemical property mean values were differentiated using the generalized linear mixed model (GLMM) (SAS® version 9.4, SAS Institute Inc., Cary, NC). Protein powder was the only independent variable in the analysis of densities, volumes, particle sizes, WHC, and free amine concentration. pH, SDS, and time, each of which were set as categorical independent variables, were added to the models analyzing protein solubility, free sulfhydryl concentration, and water droplet contact angle and volume, respectively. Random error terms accounted for assay replication and replicated measure of each powder. Water droplet contact angle and volume were also modeled with time as a continuous variable and, using those models, average rate of change for each (i.e., $d\theta/dt$, dV/dt) were determined after correcting for multiplicity with the simulate adjustment ($\alpha = 0.05$). HPN bar free amine and free sulfhydryl concentrations were analyzed using the GLMM. Independent variables were formulating powder, time, temperature, and all interactions. Assay replicate and replicate nested preparation of each HPN bar were the random terms. Statistical contrasts were significant if the adjusted *P*-value was less than 0.05.

RESULTS AND DISCUSSION

Powder physical properties: Particle sizes, densities, and occluded & interstitial air volumes

Control MPC80 had larger particle size than the extruded powders ($P < 0.05$). Diameters (i.e., D_{10}, D_{50}, D_{90}, $D_{4,3}$) decreased in the order of E116, E105, and E95 (Table 1). Although $D_{4,3}$

values were separated by only 18 microns, span values revealed that the extruded powders had much broader particle size distribution than the control (Table 1). Particle size and its distribution affect ingredient functionality. Smaller milk protein isolate (MPI) powder particles were less absorptive of and less wettable by water than larger, agglomerated MPI particles.[27, 28] Particle size distribution of E105, E116, and MPC80 were previously discussed as factors affecting HPN bar texture,[10] but the specific functionalities of MPC80 altered by both particle size reduction and extrusion have not been discussed.

Mean ρ_{loose}, ρ_{100X}, ρ_{1250X}, and $\rho_{particle}$ for the extruded MPC80s were 520, 600, 640, and 1320 g L^{-1}, respectively, and each was greater ($P < 0.05$) than those measured for the control (Table 1). Extruded powders contained, on average, 0.91 and 0.033 L kg^{-1} interstitial and occluded air, respectively, and both V_{ia} and V_{oa} (Table 1) were lower than the control ($P < 0.05$). Smaller, more disperse powder particles in extruded MPC80 fill voids occupied by air in the control powder, which decreased its V_{ia}. Extruding and milling conventionally produced MPC80 reduced its occluded and interstitial air volumes and increased powder and particle densities.

While less relevant after complete dissolution, MPC80 particle structure (e.g., size, distribution, densities, air volumes) is partially maintained within HPN bars,[12] influenecing texture and stability. HPN bars made from E105 or E116 were denser and more cohesive than the one formulated with control MPC80.[10] Control HPN bar density was 170 g L^{-1} lower than those formulated with extruded MPC80, which was due to more occluded and interstitial air in the conventionally produced MPC80 and lower bulk density. The larger, more uniformly distributed particles found in the control powder offer less surface area for particle-particle interactions and, along with the powder introducing more air into the product, partly explain why HPN bars made from this powder are more crumbly than the ones made with extruded MPC80.

Powder solubility

Extrusion reduced soluble protein at each pH ($P < 0.05$), which is related to overall solubility for these high protein powders (Figure S1). The increase in insolubility indicates partial protein denaturation by extrusion. Extruder SME while processing at 95, 105, and 116 °C was 216, 238, and 253 Wh kg^{-1}, respectively. Increasing SME coincided with increasing melt temperature, but did not adversely affect extrudate solubility ($P > 0.05$), except at pH 9.5 where E116 was less soluble than E95, E105, and the control ($P < 0.05$). Protein denaturation becomes less dependent on temperature as processing concentration increases and so the 21°C melt temperature increase between E95 and E116 did not lead to more denaturation during extrusion.[29] MPC80 was 14% soluble at pH 4.6, casein's isoelectric point (pI), where complete whey protein dissolution or 20% protein solubility was expected. At the same pH, extruded MPC80 protein solubility decreased to 3%, which suggested whey protein denaturation and was consistent with solubility values reported for whey proteins extruded at temperatures greater than 90°C.[6, 30] Extruded MPC80 in the present work was slightly less soluble at each pH than MPC80 extruded on smaller equipment due to more shear being imparted.[4]

Model HPN bar pH ranged from 6.0 to 6.8.[10] In the encompassing pH range of 5.5 to 6.8, conventionally produced MPC80 protein solubility fell between 28% and 35%. The HPN bar made using this MPC80 may have lacked cohesion due to the powder's ability to resist dissolution, an attribute required in some degree to hold the system together. However, extruded MPC80 solubility at pH 5.5 and pH 6.8 revealed that these powders were about 24% and 19% less soluble, respectively, than the control, and still produced a cohesive HPN bar.[10] An alternative hypothesis is that soluble proteins possess stronger ability to pull water away from other HPN bar constituents, which dehydrates them and contributes to changing texture by way

of internal moisture migration.[11] Extrusion decreased the solubility of MPC80, and in doing so produced a more physically and chemically inert protein ingredient suitable for the production of texturally stable HPN bars.[9, 10]

Powder-water interaction

Extrusion decreased MPC80's WHC by 42% ($P < 0.05$). The WHC of E95, E105, and E116 did not differ significantly among themselves ($P > 0.05$) (Table S1). Occluded and interstitial air volume of powders serve as a reservoir for water within and between particles, respectively. Since extrusion reduced V_{oa} and V_{ia} (Table 1), it also reduced WHC by eliminating spaces that potentially entrap water in sponge-like fashion. The extruded powders possessed statistically equivalent V_{oa} and V_{ia} ($P > 0.05$) and this contributed to their statistically equivalent WHC ($P > 0.05$).

Initial water droplet contact angle (i.e., θ_{0s}) on each extruded powder surface was larger ($P < 0.05$) than that on the control (Table S1). However, droplet profiles on the former quickly changed as water spread and imbibed (Figure 1). After 25 s, droplet contact angles (i.e., θ_{25s}) on each powder were statistically equivalent ($P > 0.05$). During the first 25 s of dynamic contact angle analysis, contact angle on the control decreased at a slower rate ($P < 0.05$) compared to extruded MPC80, as suggested by $d\theta/dt$ values (Table S1). E95, E105, and E116 absorbed a significant ($P < 0.05$) portion of the initial water droplet after 25 s whereas water droplet volume on the control did not change ($P > 0.05$) during the same timeframe (Table S1). Water droplets on extruded powders collapsed, that is lost convex shape, after 60 s (Figure 1A). After the same amount of time, a stable, semi-spherical droplet remained on the conventionally produced MPC80 (Figure 1A).

Limitations of the contact angle analysis, e.g., particle structure changes during compaction, pellet surface roughness affects results, droplet evaporation occurs, solids dissolve into the droplet, are well known and assay results are qualitative. However, the simplicity and reproducibility of the assay make it a go-to method for comparing hydrophobicity and wettability of different powders. Larger θ_{0s} for the extruded MPC80s indicated a more hydrophobic surface. Rapid reduction in contact angle (Figure 1B) suggested improved wettability. Extruding MPC80 broke the water-impermeable crusts known to encapsulate these spray-dried powders and exposed hydrophobic components once relegated to the particle interior.[31] Water droplet spread and absorption on E95, E105, and E116 was similar to that observed on low-protein MPCs, [25] which interact better with water than high-protein varieties. Droplet stability on MPC80 (Figure 1A) reaffirms that spray-dried high-protein MPCs possess poor wettability. Though initial hydrophobicity increased, extruding MPC80 improved wettability and overall ability to rehydrate, but not necessarily dissolve, when exposed to water.

Improved wettability, lower solubility, and reduced WHC make extruded MPC80s a better choice for use in HPN bars than conventionally produced MPC80. During HPN bar production, extruded varieties hydrate rapidly which lowers powder glass-rubber transition temperature and contributes to particle structure loss and system plasticization.[32] Increased plasticization of E105 and E116 translated into HPN bars that were softer, more cohesive, and less crumbly than the one formulated with control MPC80, in which particle structure and properties were noticeably maintained.[10] In the HPN bar made with control MPC80, structurally intact particles absorbed moisture from other components just as MPC80 powder slowly absorbed a droplet of water (Figure 1B). Under right conditions, extrusion plasticize MPC80 powder particles to similar to hydrolysates during HPN bar production. Also, with lower WHC

to drive moisture migration and lower V_{oa} to entrap water molecules within structurally intact particles, extruded MPC80s are less able to pull water from other HPN bar constituents during storage, one of the main reasons for increase in bar hardness when MPCs are used in bars. Textural preservation of the extruded MPC80 formulated HPN bars was partially by reduction of internal moisture migration.[10] Extruded MPC80 interacted more favorably with water, which improved its ability to produce soft, cohesive, and texturally stable HPN bars.

Protein powder and HPN bar free sulfhydryl concentration

To increase DTNB's accessibility to buried free sulfhydryl groups in MPC80 and elicit a higher response during Ellman's assay, SDS was included in the assay buffer despite previous exclusion.[21] Inclusion increased solubility of E116 ($P < 0.05$), but had no significant effect on the solubility of the other powders ($P > 0.05$). Soluble protein, with and without SDS, ranged from 29.9 to 32.8 g L^{-1} and with similar solubility, differences in free sulfhydryl concentration were attributable to chemically induced changes (e.g., oxidation, disulfide bond formation). Extruded MPC80s had lower free sulfhydryl content than the control ($P < 0.05$); melt temperature did not have a significant effect ($P > 0.05$) (Table 2). The free sulfhydryl concentration of E116 was numerically lower than E95 and E105, which suggested more protein denaturation and disulfide bond formation and/or free sulfhydryl oxidations at higher processing temperatures.[9, 21, 33] SDS inclusion in the free sulfhydryl assay buffer did not affect ($P > 0.05$) measureable free sulfhydryl concentration of the powders (Table 2). Resultantly, HPN bar free sulfhydryl evaluation required only the SDS containing buffer. Initial HPN bar free sulfhydryl concentration (i.e., HPNB-0W-22) was comparable to that of the formulating protein powder (Table 2).

Protein powder and HPN bar free amine concentration

Even though OPA registers both ε- and α-amino groups, this method is favored for measuring reactive or nutritionally active lysine over the total lysine technique, which includes nutritionally unavailable lysine.[34-36] Protein solubility in the free amine buffer ranged from 3.1 to 4.2 g L^{-1} and, with similar solubility, differences in free amine concentrations were attributable to the different processing conditions. Extruded MPC80 free amine concentration was lower than the control and increasing melt temperature from 95°C to 116°C led to a larger decrease ($P < 0.05$) (Table 3). Initial HPN bar free amine concentration (i.e., HPNB-0W-22) was similar to yet slightly lower than that of the formulating powder (Table 3).

Chemical changes and protein aggregations during HPN bar storage

Storage of HPN bars at 32°C for 6 weeks simulated 52 weeks at 22°C,[14, 18] the minimum industry-required shelf life for such products. Evaluations made after 6 at 32°C or 29 weeks at 22°C served as intermediate time points only. Twenty-nine weeks at 32°C was an extreme treatment combination that accelerated storage for much longer than 1 year. Changes in sulfhydryl and amine concentrations were evaluated over simulated time (i.e., across rows in Tables 2 and 3).

HPN bar protein soluble in the free sulfhydryl buffer was between 22.9-23.4, 22.8-23.2, and 26.1-27.2 g L^{-1} when formulated with E105, E116, and MPC80, respectively, except after 29 weeks of storage at 32°C. Under the latter conditions, protein solubility decreased to 6.9, 7.5, and 14.6 g L^{-1} for the same respective HPN bars. After equivalent storage, soluble protein obtained from the control HPN bar was always greater than solutions derived from HPN bars made with extruded MPC80 ($P < 0.05$). Excluding samples kept for 29 weeks at 32°C, free sulfhydryl concentration in the HPN bars (Table 2) did not change significantly during storage

when formulated with MPC80 or E116 ($P > 0.05$). Free sulfhydryl concentration in the HPN bar formulated with E105 decreased, relative to the starting value, after 29 weeks at 22°C and after 6 weeks at 32°C ($P < 0.05$). Free sulfhydryl groups in the control HPN bar always maintained higher concentration than the ones prepared with extruded MPC80 ($P < 0.05$). Oddly, after 29 weeks at 32°C, sulfhydryl concentration (μmol L^{-1}) increased while soluble protein (g L^{-1}) decreased, which increased measureable free sulfhydryl concentration in each HPN bar ($P < 0.05$). If disulfide bonds formed internally and contributed to time-dependent HPN bar texture changes, then free sulfhydryl content should decrease. However, reshuffling disulfide bonds, driven by reactive thiol groups present in β-lg (C121) and less prevalent BSA (C34), amongst milk proteins could lead to aggregate formation without noticeable decrease. Sulfhydryl concentration, which can also decrease via oxidation, changed little over the course of ~7 months at 22°C even though HPN bar texture changed considerably during that time.[10] These results corroborated those from a shorter storage study, which also found limited change in free sulfhydryl concentration even as HPN bar texture changed substantially.[21]

HPN bar protein soluble in the free amine buffer was between 7.5 and 8.0 g L^{-1}, except after 29 weeks at 32°C. At that time, protein solubility decreased ($P < 0.05$) to 2.1, 1.9, and 3.6 g L^{-1} when formulated with E105, E116, and MPC80, respectively. Despite reduction, HPN bar free amine concentrations (Table 3) consistently decreased throughout storage ($P < 0.05$) due to glycation of the lysine residues with glucose, fructose, and lactose and by Maillard-induced protein aggregation previously related to worsening of model HPN bar texture.[9, 12, 13, 19] While there remains discrepancy about Maillard browning's effect on HPN bar texture change,[14] glycation of lysine decreases its nutritional value.[35] Sulfur containing amino acids are limiting in

MPC and initial lysine glycation by extrusion and that seen during HPN bar storage are unlikely a major nutritional concern.[37]

Decreasing protein solubility during HPN bar storage indicates aggregate formation.[19] Insoluble aggregate formation in the present study was not strictly due to disulfide bond formation, since DTT addition to the free amine buffer did not fully solubilize proteins or restore solubility in the HPN bars stored for 29 weeks at 32°C. Soluble protein aggregates (PA), including Maillard-induced and disulfide-linked (DLPA), formed within the HPN bars as storage time progressed (Figures 2 and 3). Extruding MPC80 led to DLPA formation. These high molecular weight (> 250 kDa) aggregates, which were too large to permeate into the non-reduced gels, were initially present within HPN bars formulated with extruded MPC80 and they persisted through week 6 at 32°C (Figure 2). These DLPA, along with some of those with molecular weight between 75 and 250 kDa that developed during HPN bar storage (Figure 2), were broken by the reducing agent and did not appear on the reduced gel (Figure 3). β-lg participated in initial DLPA and their formation during storage, which was confirmed by the reappearance or intensification of this protein band on the reduced gels (Figure 3). β-lg's inability to solubilize from extruded MPC80 without a reducing agent led to lower measurable free sulfhydryl concentrations in those HPN bars compared to the one made with conventionally produced MPC80 (Table 2). PA that remained on the reduced gels (Figure 3), indicated by in-lane vertical smearing, were due to covalently cross-linked, Maillard-induced aggregations. After 29 weeks at 32°C, there were more non-disulfide linked PA in the control HPN bar than in those prepared with extruded MPC80. In the latter samples, vertical lane smearing subsided and a single band appeared near the top of the lane, which indicated formation of a high molecular weight, non-reducible PA after extreme storage of these HPN bars. Similar PA formed during MPC80

powder storage as advanced Maillard browning products (e.g., glutaraldehyde) and di-carbonyls (e.g., glyoxal) cross-linked proteins.[38-40] HPN bars formulated with extruded MPC80s were less prone to internal glycations and induced protein aggregations because free amine concentration was significantly lower in the base material (Table 3). Since β-lg was not pre-aggregated in the control powder, it was more prone to glycation and increasing molecular weight during HPN bar storage, which was why the band for this protein dispersed and migrated a shorter distance on each gel (Figures 2 and 3). HPN bar texture changed slower when formulated with extruded MPC80 partly as β-lg was pre-aggregated into DLPA and less likely to participate in internal disulfide bond formations, and partly due to the inability of the protein to cause or participate in Maillard-induced aggregations.[10]

CONCLUSIONS

Extrusion followed by drying and milling altered some physicochemical properties of conventionally produced MPC80. Processing MPC80 increased its bulk and particle densities, and decreased interstitial and occluded air volumes. Solubility and WHC of extruded MPC80 were both lower than the spray dried control. Extrusion improved MPC80 wettability by disrupting the hydrophobic barrier commonly found on MPC powder particles. Chemically, extrusion decreased MPC80 free sulfhydryl and free amine concentrations by inducing protein-protein disulfide bond formation and protein glycation. Increasing powder density and lowering occluded air volume led to denser, more cohesive HPN bars. With improved wettability, extruded MPC80 readily hydrates during HPN bar production, which, in addition to cohesion, contributes to partial particle collapse and prevents intact particles from absorbing moisture from other constituents during storage. Maillard-induced aggregation was more prevalent than disulfide-induced aggregation in all the HPN bars. With lower free sulfhydryl concentration,

free amine concentration, and protein solubility, extruded MPC80 was less reactive in the HPN bars. Chemical changes occurred by extruding MPC80, yet texture and stability of the model HPN bars were influenced by the physical properties. Thus, the physicochemical properties of a protein powder need consideration when formulating HPN bars or other solid-like intermediate moisture foods.

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CONFLICT OF INTEREST

Erie Foods International, Inc. employed Author Banach at the time of submission. Completion of the study occurred while solely affiliated with Iowa State University.

SUPPORTING INFORMATION

Supplementary data associated with this article can be found, in the online version, at… .

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TABLES

Table 1. Protein powder particle size diameters[1], span values[2], densities[3], and air volumes[4]

Powder[5]	D_{10}	D_{50}	D_{90}	$D_{4,3}$	S	ρ_{loose}	ρ_{100X}	ρ_{1250X}	$\rho_{particle}$	V_{oa}	V_{ia}
MPC80	16^{a}	49^{a}	121^{c}	61^{a}	2.1^{d}	310^{b}	360^{c}	390^{c}	1110^{b}	0.178^{a}	1.89^{a}
E95	2^{c}	25^{c}	103^{d}	43^{d}	4.0^{b}	520^{a}	600^{ab}	650^{a}	1320^{a}	0.035^{b}	0.89^{c}
E105	2^{c}	25^{c}	147^{a}	52^{c}	5.7^{a}	510^{a}	590^{b}	630^{b}	1330^{a}	0.029^{b}	0.95^{b}
E116	3^{b}	38^{b}	132^{b}	57^{b}	3.3^{c}	530^{a}	610^{a}	650^{a}	1320^{a}	0.034^{b}	0.88^{c}

[1] D_{10}, D_{50}, and D_{90} are diameters (µm) where 10%, 50%, and 90% of all powder particles, respectively, have smaller size. $D_{4,3}$ is the volume-weighted mean diameter (µm). Particle size diameters for MPC80, E105, and E116 were previously reported.[10]

[2] S represents particle size distribution span, a unit less value that describes particle size distribution width.

[3] ρ_{loose}, ρ_{100X}, ρ_{1250X}, and $\rho_{particle}$ are loose, tapped, extremely tapped, and particle densities (g L^{-1}), respectively.

[4] V_{oa} and V_{ia} are occluded and interstitial air volumes (L kg^{-1}), respectively.

[5] MPC80, conventionally produced milk protein concentrate with 800 g kg^{-1} protein. E95, E105, and E116, MPC80 extruded at die-end melt temperature of 95°C, 105°C, and 116°C, respectively.

[a-d] Least squares means are significantly different ($P < 0.05$) if they do not share a common superscript within the same column.

Table 2. Protein free sulfhydryl concentration[1] for each powder[2] and within the high-protein nutrition bar formulated with that powder after storage[3]

Powder[4]	-SDS	+SDS	HPNB-0W-22	HPNB-6W-22	HPNB-29W-22	HPNB-6W-32	HPNB-29W-32
MPC80	$5.2^{a,z}$	$6.0^{a,z}$	$5.8^{a,y}$	$5.8^{a,y}$	$5.5^{a,y}$	$5.3^{a,y}$	$13.7^{a,z}$
E95	$2.9^{b,z}$	$2.9^{b,z}$	-	-	-	-	-
E105	$2.8^{b,z}$	$2.1^{b,z}$	$3.0^{b,z}$	$1.0^{b,yz}$	$0.4^{b,y}$	$0.6^{b,y}$	$3.1^{b,z}$
E116	$1.4^{b,z}$	$1.5^{b,z}$	$1.2^{b,y}$	$1.1^{b,y}$	$0.4^{b,y}$	$0.4^{b,y}$	$4.0^{b,z}$

[1] µmole g^{-1}

[2] Protein powder free sulfhydryl concentration was measured with (i.e., + SDS) and without (i.e., -SDS) in the assay buffer.

[3] High-protein nutrition bars (i.e., HPNB) were prepared using the indicated protein powder and were stored for 0, 6, or 29 weeks (i.e., 0W, 6W, or 29W) at 22°C or 32°C (i.e., 22 or 32).[10]

[4] MPC80, conventionally produced milk protein concentrate with 800 g kg^{-1} protein. E95, E105, and E116, MPC80 extruded at die-end melt temperature of 95°C, 105°C, and 116°C, respectively.

[a-b] Least squares means are significantly different ($P < 0.05$) if they do not share a common superscript within the same column.

[y,z] Protein powder or HPN bar least squares means are significantly different ($P < 0.05$) if they do not share a common superscript within the same row.

Table 3. Protein free amine concentration[1] for each powder and within the high-protein nutrition bar formulated with that powder after storage[2]

Powder[3]	R-NH_2	HPNB-0W-22	HPNB-6W-22	HPNB-29W-22	HPNB-6W-32	HPNB-29W-32
MPC80	877^{a}	$828^{a,z}$	$615^{a,y}$	$367^{a,x}$	$380^{a,x}$	$264^{a,w}$
E95	775^{b}	-	-	-	-	-
E105	748^{c}	$713^{b,z}$	$585^{b,y}$	$358^{a,x}$	$355^{ab,x}$	$229^{b,w}$
E116	695^{d}	$667^{c,z}$	$560^{b,y}$	$348^{a,x}$	$346^{b,x}$	$242^{ab,w}$

[1] µmole g^{-1}

[2] High-protein nutrition bars (i.e., HPNB) were prepared with the indicated protein powder and were stored for 0, 6, or 29 weeks (i.e., 0W, 6W, or 29W) at 22°C or 32°C (i.e., 22 or 32).[10]

[3] MPC80, conventionally produced milk protein concentrate with 800 g kg^{-1} protein. E95, E105, and E116, MPC80 extruded at die-end melt temperature of 95°C, 105°C, and 116°C, respectively.

$^{a-d}$ Least squares means are significantly different ($P < 0.05$) if they do not share a common superscript within the same column.

$^{w-z}$ HPN bar least squares means are significantly different ($P < 0.05$) if they do not share a common superscript within the same row.

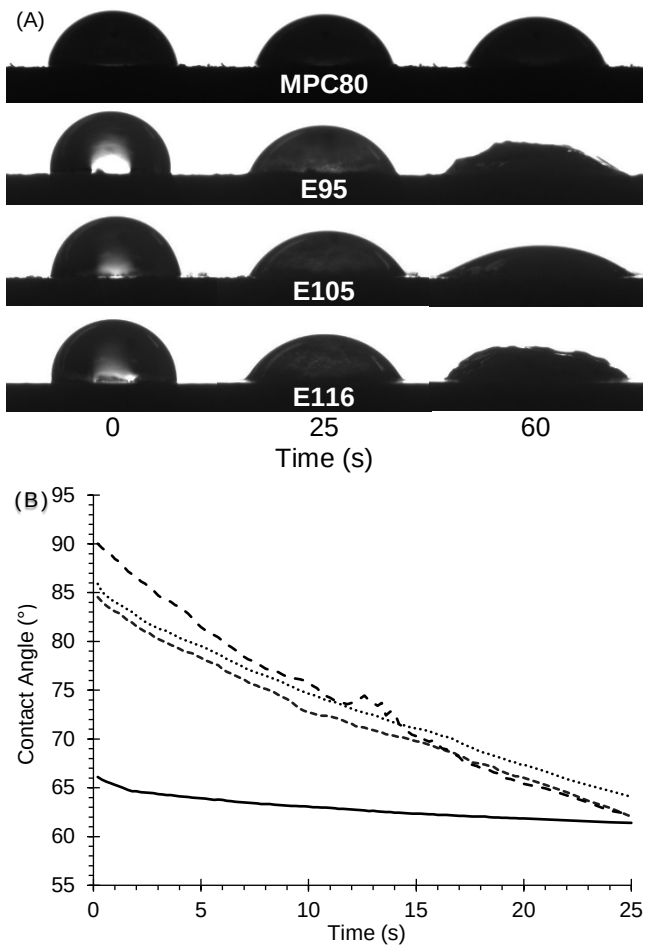

Figure 1. Representative side view (A) and apparent contact angle (B) of a water droplet on each protein powder during dynamic contact angle analysis. MPC80 (—), conventionally produced milk protein concentrate with 800 g kg^{-1} protein. E95 (···), E105 (---), and E116 (– – –), MPC80 extruded at die-end melt temperature of 95°C, 105°C, and 116°C, respectively.

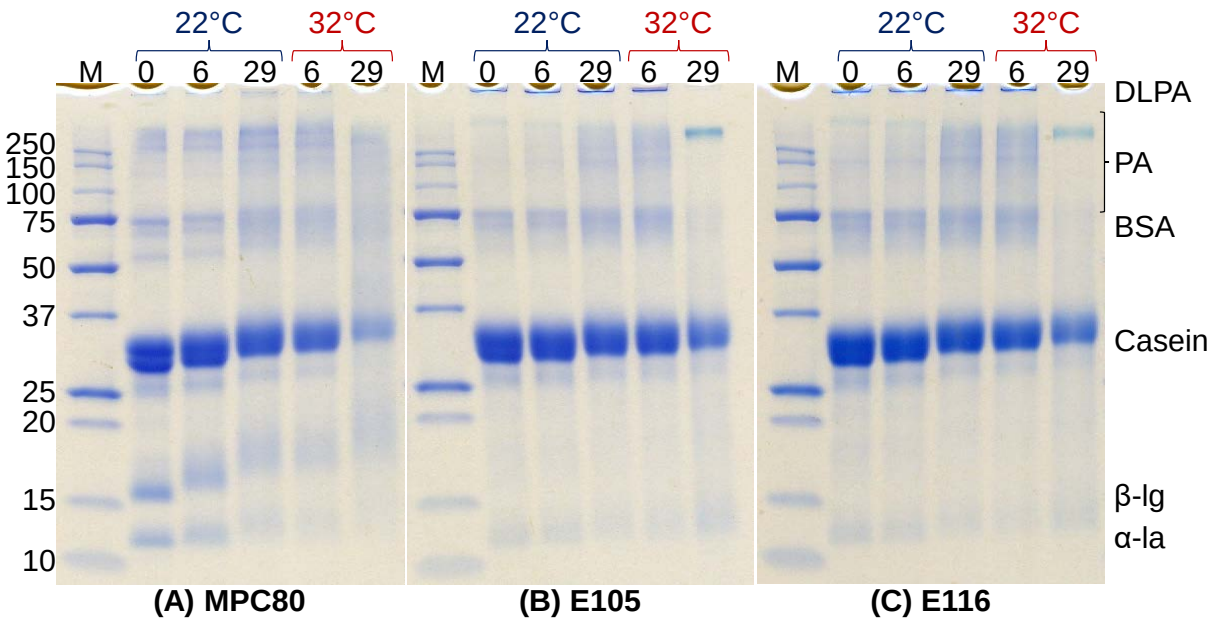

Figure 2. Non-reduced extraction/non-reduced SDS-PAGE of the proteins in model high-protein nutrition bars formulated with MPC80 (A), E105 (B), or E116 (C) after storage for 0, 6, and 29 weeks at 22°C or 32°C. MPC80, conventionally produced milk protein concentrate with 800 g kg^{-1} protein. E105 and E116, MPC80 extruded at die-end melt temperature of 105°C and 116°C, respectively. M, a molecular weight marker (kDa). DLPA and PA, disulfide-linked protein aggregates and protein aggregates, respectively. BSA, bovine serum albumin. Caseins, from high to low molecular weight, include: α_{S2}, α_{S1}, β, and κ. β-lg, beta-lactoglobulin. α-la, alpha-lactalbumin.

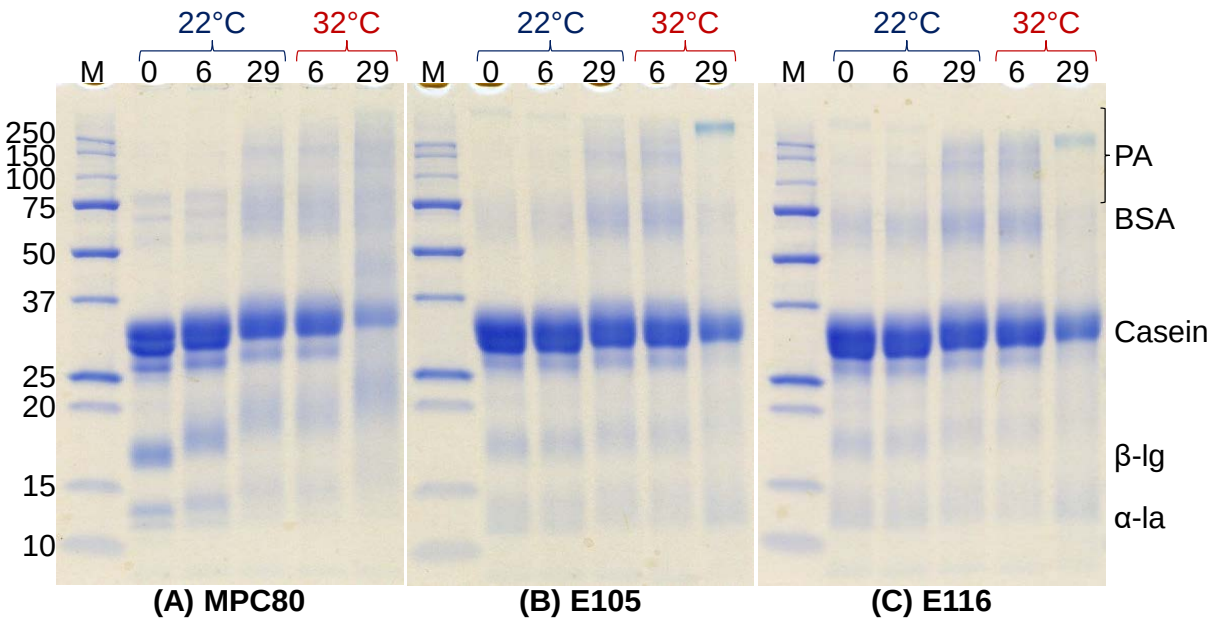

Figure 3. Non-reduced extraction/reduced SDS-PAGE of the proteins in model high-protein nutrition bars formulated with MPC80 (A), E105 (B), or E116 (C) after storage for 0, 6, and 29 weeks at 22°C or 32°C. MPC80, conventionally produced milk protein concentrate with 800 g kg^{-1} protein. E105 and E116, MPC80 extruded at die-end melt temperature of 105°C and 116°C, respectively. M, a molecular weight marker (kDa). PA, protein aggregates. BSA, bovine serum albumin. Caseins, from high to low molecular weight, include: α_{S2}, α_{S1}, β, and κ. β-lg, beta-lactoglobulin. α-la, alpha-lactalbumin.

SUPPORTING INFORMATION

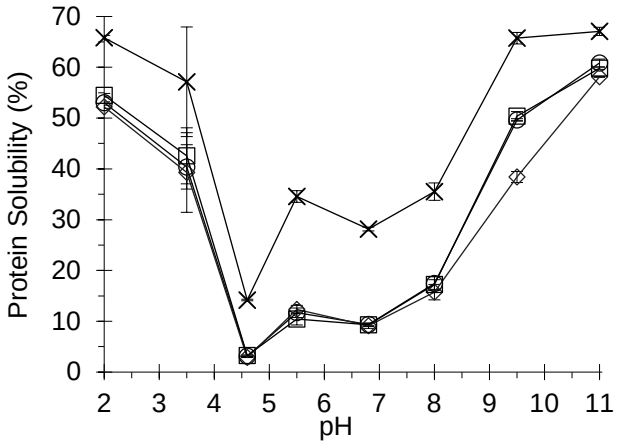

Figure S1. Protein solubility at different pH for extruded and conventionally produced MPC80. Average solubility (n = 2) was expressed as the soluble protein to total protein ratio. MPC80 (×), conventionally produced milk protein concentrate with 800 g kg^{-1} protein. E95 (□), E105 (○), and E116 (◊), MPC80 extruded at die-end melt temperature of 95°C, 105°C, and 116°C, respectively. Error bars indicate ± 1 SD.

Table S1. Protein powder water holding capacity[1], and water droplet contact angle[2], volume[3], and angular and volumetric rates of change[4] during dynamic contact angle analysis

Powder[5]	WHC	θ_{0s}	θ_{25s}	$d\theta/dt$	V_{0s}	V_{25s}	dV/dt
MPC80	3.3^{a}	$66^{b,z}$	$61^{a,y}$	-0.19^{b}	$3.11^{a,z}$	$3.08^{a,z}$	-1.31^{a}
E95	1.9^{b}	$86^{a,z}$	$64^{a,y}$	-0.88^{a}	$3.49^{a,z}$	$3.34^{a,y}$	-6.24^{a}
E105	1.9^{b}	$85^{a,z}$	$62^{a,y}$	-0.91^{a}	$3.45^{a,z}$	$3.28^{a,y}$	-6.81^{a}
E116	1.8^{b}	$90^{a,z}$	$62^{a,y}$	-1.12^{a}	$3.64^{a,z}$	$3.56^{a,y}$	-3.26^{a}

[1] WHC, water held per solid mass (kg kg^{-1})

[2] θ_{0s} and θ_{25s}, contact angle (°) after 0 and 25 s, respectively.

[3] V_{0s} and V_{25s}, volume (µL) after 0 and 25 s, respectively.

[4] $d\theta/dt$ and dV/dt, angular (° s^{-1}) and volumetric (nL s^{-1}) rates of change, respectively.

[5] MPC80, conventionally produced milk protein concentrate with 800 g kg^{-1} protein. E95, E105, and E116, MPC80 extruded at die-end melt temperature of 95°C, 105°C, and 116°C, respectively.

$^{a\text{-}b}$ Least squares means are significantly different ($P < 0.05$) if they do not share a common superscript within the same column.

^{y,z} Water droplet contact angle or volume least squares means are significantly different ($P < 0.05$) if they do not share a common superscript within the same row.