ABSTRACT

Micro- and nano-bubbles (MNB) have unique properties and have attracted great attention in the past 2 decades, offering prospective applications in various disciplines. The first objective of this study was to investigate whether venturi-style MNB generation is capable of producing sufficient bulk MNB. A nanoparticle tracking system was used to measure the bubble concentration and particle size of MNB-treated deionized water. The MNB-treated deionized water had a bubble concentration of $3.76 \times 10^8$ particles/mL ($\sim350$ million bubbles/mL more compared with control) and a mean particle size of 249.8 nm. The second objective of this study was to investigate the effects of MNB treatment on the microstructure and functional properties of milk protein concentrate (MPC) dispersions. Reconstituted MPC dispersions (21%, wt/wt) without air injection were considered as control (C-MPC), and MPC dispersions passed through the MNB system were considered as MNB-treated (MNB-MPC) dispersions. Control and MNB-MPC dispersions were evaluated in terms of rheological behavior and microstructure. The microscopic observations of MNB-MPC dispersions showed less aggregated microstructures and greater structural differences compared with C-MPC dispersions, therefore lowering the viscosity. The viscosity of MNB-MPC at a shear rate of 100 s$^{-1}$ significantly decreased to 57.58 mPa·s (C-MPC: 162.40 mPa·s), a net decrease in viscosity by $\sim65\%$ after MNB treatment. Additionally, MPC dispersions were spray dried after the MNB treatment, and the resultant MNB-MPC powders were characterized and compared with the control MPC in terms of rehydration characteristics and microstructure. Focused beam reflectance measurement of the MNB-MPC powders indicated lower counts of large particles (150–300 μm) during dissolution, signifying that MNB-MPC powders exhibited better rehydration properties than the C-MPC powders. This study, therefore, recommends the possibility of using MNB treatment for more efficient drying while improving the functional properties of the resultant MPC powders.

Key words: milk protein concentrate powder, micro- and nano-bubbles, viscosity, rehydration

INTRODUCTION

Applications of micro- and nano-scaled materials are constantly evolving, and they have great potential to bring significant advantages in various manufacturing sectors. Nanostructured materials are defined to be of diameters in the range of 1 to 100 nm. However, a single internationally accepted definition for nano-materials does not exist (Jeevanandam et al., 2018). The last 2 decades have seen substantial academic and industrial interest in investigations on the unique properties of both micro-bubbles and ultrafine or nano-bubbles (NB). Generally, micro-bubbles range from 10 to 50 μm, and bubbles with a particle size of <200 nm are typically referred to as NB (Temesgen et al., 2017). Previously, Agarwal et al. (2011) presented the name micro- and nano-bubbles (MNB), although distinct categories for NB and MNB are still unclear. Tiny bubbles with diameters ranging from hundreds of nanometers to several tens of micrometers, MNB are gaining interest due to their wide range of applications in many fields of science and technology. Micro- and nano-bubbles exhibit high internal pressures, high gas solubility, and large surface-to-volume ratios. The physical properties of MNB are different from those of milli-scaled bubbles, and MNB are stable for considerably long periods, having shown stability for as long as 2 wk; clusters of NB could further increase their stability (Weijs et al., 2012; Azevedo et al., 2016). The core application areas of MNB include wastewater treatment, agriculture, aquaculture, and medical applications. Recently, Amamcharla et al. (2017) and Phan et al. (2020) have outlined applications of MNB in various food and dairy processing applications.

Various techniques are available for generation of MNB, including the venturi-style MNB generator. The venturi-style generator has attracted much attention...
industrially as well as in research fields due to its simplicity, high efficiency, low energy consumption, and ease of scale up (Fan et al., 2010; Agarwal et al., 2011; Ahmadi et al., 2014). Overall, only limited literature is available on the effects of MNB on the performance and structural properties of different food and dairy systems.

Spray drying is a widely used unit operation for the manufacture of dairy powders; however, it is a very energy-intensive process. Therefore, technological approaches to better optimize the process are critical from a sustainability standpoint. Chamberland et al. (2020) reported that pre-concentrating cheese whey and milk ultrafiltered permeate to 20% and 22% wt/wt dry matter, respectively, by reverse osmosis before evaporation reduced natural gas and electricity consumption by 36% and 10%, respectively. Remarkably, Fox et al. (2010) reported that an increase of just 2% dry matter could achieve 6% energy reduction. However, viscosity is a significant concern when increasing dry matter content in spray drying feed concentrate. Therefore, an innovative approach to reduce the viscosity of high-solid milk concentrates could be a promising step toward greater energy savings.

Milk protein concentrate (MPC) powders are ideal ingredients for a wide range of applications, such as in beverages, yogurt, cheeses, nutritional formulations, and protein bars, due to their high protein content, low lactose content, high buffering capacity, pleasant milk flavor profile, and other functional properties (Agarwal et al., 2015). However, rehydration characteristics in MPC powders are influenced by protein contents (Babu and Amamcharla, 2021) and powder properties, such as surface and bulk composition (Crowley et al., 2014), rehydration conditions (Crowley et al., 2015), particle morphology (Babu et al., 2018), and storage (Babu and Amamcharla, 2018). Several studies have demonstrated that MPC powders exhibit poor solubility over extended storage time due to changes in the physicochemical interactions of the proteins (Anema et al., 2006; Havea, 2006). Numerous studies have investigated ways to improve rehydration behavior via tailoring the behavior of milk components, through alteration of the ionic environment of MPC dispersions (Marella et al., 2015), addition of whey proteins (Gaiani et al., 2007), and use of lecithin nanovesicles (Bansal et al., 2017). Additionally, various engineering techniques have also been shown to improve the solubility of MPC powders: static high-pressure treatment (Udabage et al., 2012), high-intensity ultrasonication (McCarthy et al., 2014), high-shear treatment (Augustin et al., 2012), extrusion porosification (Bouvier et al., 2013), hydrodynamic cavitation (Li et al., 2018), and nitrogen gas injection (McSweeney et al., 2021). Kosasih et al. (2016) studied the influence of CO₂ on the physical and functional properties of whole milk powder and reported increased dispersibility, powder porosity, and occluded air content.

In the present work, we are exploring the possibility of MNB treatment to improve the processability and rehydration properties of MPC. This study aims to (1) characterize the MNB generated by the venturi-based air injection method in deionized (DI) water in terms of bubble concentration and mean diameter; (2) evaluate the effects of MNB on rheology and microstructure of MPC dispersions; and (3) investigate the rehydration and microstructure of MNB-treated MPC powders.

MATERIALS AND METHODS

Development of MNB Generation System

A laboratory-scale MNB generation system was designed and assembled at Kansas State University (Manhattan, KS), and a schematic diagram of the experimental set-up is shown in Figure 1. A venturi injector (Hydra-Flex) was used to generate bulk MNB. The custom-built MNB system was connected to a Flowjet duplex diaphragm pump (Xylem, model no. D3835H5011A) with a flow rate of 7.57 L/min. The size of the bubbles was controlled by adjusting the airflow through the MNB injector using a TSI 4140 air flow meter.

Characterization of Bulk MNB in Water

The existence of MNB remains a puzzling and trending topic, mainly due to their exceptional longevity and other extraordinary properties, which are being investigated by many research groups worldwide (Jadhav and Barigou, 2020). In this study, a nanoparticle tracking analyzer (NTA) was used to measure MNB concentration and MNB mean diameter. The NTA measurements were carried out using a Malvern NanoSight LM10 equipped with a sample chamber with a 640-nm laser. Before experimentation, control DI water was initially examined for any nanoscale entities using the NTA. The control and MNB-incorporated DI water were characterized in terms of size and concentration using the NTA system. The samples were then injected into the chamber until the sample reached the tip of the nozzle, using sterile syringes (BD Discardit II). All measurements were taken at room temperature. The NTA software tracks the particles individually and uses the Stokes-Einstein equation to calculate the hydrodynamic diameter of the particles. The NTA software also
records a video file to visualize the differences in the dispersion of particles and simultaneously identifies and tracks each particle on a frame-by-frame basis.

**Characterization of Liquid MPC Dispersions Containing Bulk MNB**

A flow diagram of the experimental approach is provided in Figure 2. The MPC dispersions were prepared in the laboratory using 2 lots of MPC powder with 85% protein content (MPC85) obtained from a commercial dairy ingredient supplier in the United States. The average protein, moisture, fat, lactose, and ash contents of the MPC85 used for this study were 86.85%, 5.25%, 0.93%, 4.79%, and 6.68% (all on wt/wt basis), respectively. Dispersions of 21% (wt/wt) total solids were prepared by gradually adding 420 g of MPC powder to 1,580 g of DI water maintained at 45°C using a water bath (Fisher Scientific). Subsequently, the MPC dispersions were stored overnight at 4°C to ensure complete rehydration. After overnight hydration, the MPC dispersions were taken out, and all treatments were performed at room temperature. Control MPC dispersions (C-MPC; pumped through the diaphragm pump without MNB treatment) and MNB-treated MPC dispersions (MNB-MPC; treated with MNB using the custom-built MNB system with air injection at 0.008 L/min) were characterized using the NTA, rheological measurements, and microstructure. The C-MPC and MNB-MPC dispersions contained 21.58 ± 1.07 and 21.61 ± 0.20% (wt/wt) total solids content, respectively. Additionally, the C-MPC and MNB-MPC dispersions were spray dried and characterized by rehydration behavior and microstructure.

**NTA Analysis.** Casein micelles in MPC dispersions can interfere with measurement, as they are similar in size to MNB, making the quantification of MNB in the MPC system in terms of size and concentration
less realistic. The C-MPC and MNB-MPC dispersions were characterized in terms of concentration and size using the NTA system. Because greater dilution was required to test them using the NanoSight, C-MPC and MNB-MPC dispersions were serially diluted to achieve 1:50,000 dilution.

**Rheological Measurements.** The viscosities of C-MPC and MNB-MPC dispersions were measured at 20°C using a stress-strain-controlled rheometer (MCR-92, Anton Paar) equipped with a 50-mm-diameter stainless-steel cone with angle 1° and 101-μm gap. Flow curves were analyzed at shear rates between 0.1 and 100 s⁻¹. The data obtained were fitted to the power-law model to obtain the consistency coefficient and flow behavior index.

**Microstructure C-MPC and MNB-MPC Dispersions Before Spray Drying.** The microstructures of C-MPC and MNB-MPC dispersions were studied using confocal laser scanning microscopy (CLSM), following the method described by Gandhi et al. (2017). Samples were prepared for CLSM by diluting 1:100 using DI water before measurements. Proteins were stained using the Fast Green FCF stain (Sigma-Aldrich). Stock solution of Fast Green (5 mg of dye in 5 mL of water) was applied to the sample for 5 to 10 min. The stained samples were analyzed in a laser scanning microscope, 5 Pa (Zeiss). Three-dimensional images were obtained by scanning the sample across a defined section along the z-axis.

Transmission electron microscopy (TEM) was also used to image C-MPC and MNB-MPC dispersions, to illustrate the differences in microstructure. Samples were analyzed using the negative-staining technique. The dispersions were diluted at 1:100 with DI water before measurements. Proteins were stained using the Fast Green FCF stain (Sigma-Aldrich). Stock solution of Fast Green (5 mg of dye in 5 mL of water) was applied to the sample for 5 to 10 min. The stained samples were analyzed in a laser scanning microscope, 5 Pa (Zeiss). Three-dimensional images were obtained by scanning the sample across a defined section along the z-axis.

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**Characterization of C-MPC and MNB-MPC Powders**

The prepared C-MPC and MNB-MPC dispersions were spray dried in a laboratory-scale spray dryer (YC-015; Shanghai Pilotech Instrument & Equipment Co. Ltd.). The inlet temperature was set at 180°C, and the outlet temperature ranged between 60 and 65°C. The spray pressure was maintained at 206.84 kPa, and the relative humidity of the room was periodically recorded using a digital humidity meter (Traceable Humidity Meter, Fisher Scientific). The spray-dried MPC powders were collected and sealed in Whirl-Pak bags (Nasco) for further analysis.

**Rehydration Behavior.** The C-MPC and MNB-MPC powders were reconstituted at 5% (wt/wt) powder concentration in DI water, and solubilities of the powders were estimated based on the TS in the supernatant obtained by centrifugation at 700 × g for 10 min at 25°C, as described by Anema et al. (2006). The amount of soluble material (σ) in the MPC was calculated using the following equation:

\[
σ = \frac{\text{weight of dry material}}{\text{weight of solution}} \times 100.
\]

The dissolution characteristics of the C-MPC and MNB-MPC powders were evaluated using the focused beam reflectance measurement method (Babu and Amamcharla, 2018).

**Particle Size Analysis.** The average particle sizes of reconstituted C-MPC and MNB-MPC powders were measured by dynamic laser light scattering using a particle size analyzer (DelsaMax Pro, Beckman Coulter). The C-MPC and MNB-MPC powders were reconstituted to 5% solution using DI water and were kept overnight for complete rehydration before the analysis. Samples diluted with DI water (1:100, vol/vol) were used to measure the particle size. Samples were equilibrated inside the instrument for 2 min, and then analysis was carried out at 20°C. Zeta potential was measured by the phase analysis light scattering method in the DelsaMax Pro.

**Microstructure of C-MPC and MNB-MPC Powders.** The microstructures of the C-MPC and MNB-MPC powders were examined using a scanning electron micrograph according to the method described by Mimouni et al. (2010). The MPC powders were directly mounted onto a carbon double-sided adhesive tape on microscopy stubs and sputter-coated with palladium using a Denton Vacuum Desk II sputter coater (Denton Vacuum) for 15 min to avoid charge buildup under the electron beam. Imaging was performed using a S-3500N (Hitachi Science Systems Ltd.) and was examined by a secondary electron detector operating at 10 kV.

**Microstructure of Reconstituted C-MPC and MNB-MPC Powders.** For imaging of reconstituted C-MPC and MNB-MPC powders, 5% solutions were prepared, and TEM analysis was performed following the same method as that used for MPC dispersions.
Statistical Analysis

The MPC85 powders were procured form 2 independent lots and were treated as independent replicates. Both control and MNB treatments were carried out in duplicate for each lot. Statistical analysis of the C-MPC and MNB-MPC dispersions and resultant C-MPC and MNB-MPC powders were analyzed using PROC GLM-MIX of SAS (version 9.4, SAS Institute Inc.). Statistical differences among means were determined with a significance level of $\alpha = 0.05$.

RESULTS AND DISCUSSION

Characterization of Bulk MNB in Water

An NTA was used to test the efficiency of the custom-built MNB generation system. Figure 3 shows the NTA results obtained from control (not treated) and MNB-treated DI water. The NanoSight showed a significant increase in particle concentration upon MNB treatment, suggesting that the venturi injector was efficient in generating MNB. The concentration-weighted size distribution of DI water from NTA was observed with a mode of 94.7 nm, a mean of 174.4 nm, and standard deviation (SD) of 127 nm. The particle size distribution was D10: 86.5 nm; D50: 135.6 nm; D90: 321.7 nm. The MNB-treated DI water had a mode of 170.1 nm, a mean of 249.8 nm, and SD of 115.8 nm. The particle size distribution was D10: 149.2 nm; D50: 220.7 nm; D90: 388.3 nm. Concentrations were found to be $1.72 \times 10^5$ and $3.76 \times 10^5$ particles/mL for DI water and MNB-treated DI water, respectively, suggesting that MNB-treated DI water had $\sim$350 million more bubbles per milliliter compared with control. Ahmadi and Khodadadi Darban (2013) used a venturi-type MNB generation system. Figure 3 shows the NTA provided not only quantitative information but also visual information about the C-MPC and MNB-MPC dispersions. Within the field of view, the particles were seen moving under Brownian motion, visualized using the microscope oculars or via the camera.

Rheological Measurements. Viscosity is of great concern, as it influences the processability of dairy concentrates, process performance, and product quality (Bista et al., 2021; Patil et al., 2021). As seen in Figure 5, a significant decrease in viscosity occurred after the MNB treatment. For concentrated protein systems, it is well known that the presence of aggregates can result in a marked increase in viscosity (Nicoud et al., 2015). Based on the results observed, it is suggested that the MNB treatment-assisted decrease in viscosity is due to disruption of aggregates, possibly by reducing protein-protein interactions, which is also evident from the microstructures (Figures 5 and 6). The flow curves were measured at a shear rate of 0.1 to 100 s$^{-1}$ for all samples in this study. The viscosity of C-MPC and MNB-MPC dispersions at a shear rate of 100 s$^{-1}$ were 162.40 and 57.58 mPa·s, respectively. This represents a net decrease of $\sim$65% for the MNB-MPC dispersion. Previously, Amamcharla et al. (2017) noted that injecting MNB with an average diameter in the range of 100 nm to 30 μm helped in viscosity reduction in liquid dairy products. Similarly, Khaira et al. (2020) reported lower apparent viscosity (150 mPa·s; $\sim$30% decrease) of ice cream made with NB. A net decrease of MPC (23.47% total solids) viscosity of up to $\sim$55% by hydrodynamic cavitation was previously demonstrated by Li et al. (2018); however, the initial viscosity was higher ($\sim$200 mPa·s) than in the present study. This is also comparable to the reduction in viscosity reported by Zisu et al. (2013) following the acoustic cavitation of concentrated skim milk with an initial viscosity in the

Characterization of Liquid MPC Dispersions Containing MNB

NTA Analysis. Particle size distributions in the C-MPC and MNB-MPC dispersions were also investigated by NTA, and the obtained concentration and size profiles are shown in Figure 4A and 4B. The MNB treatment resulted in a significant ($P < 0.05$) decrease in particle mean diameters ($211.7 \pm 2.0$ nm and $202 \pm 2.3$ nm for the C-MPC and MNB-MPC dispersions, respectively), suggesting disruption of aggregates. The particle size distribution was D10–154.3; D50–201.3; D90–285.2 nm for C-MPC dispersions. By contrast, MNB-MPC dispersions had a D10–148.5; D50–190.2; D90–268.3 nm. This is in line with findings from a study by Gregersen et al. (2019). They used hydrodynamic cavitation treatments on whey protein concentrate (31% DM) and observed a significant decrease in D10, D50, D90, and mean diameter. However, the extent of particle size reduction is associated with the initial particle size (Yanjun et al., 2014). Additionally, the particles per frame were also significantly higher ($P < 0.05$) for the MNB-MPC dispersions. The concentration of MNB-MPC was $3.45 \times 10^9$ particles per milliliter, compared with $3.18 \times 10^9$ for the C-MPC. The NTA provided not only quantitative information but also visual information about the C-MPC and MNB-MPC dispersions. Within the field of view, the particles were seen moving under Brownian motion, visualized using the microscope oculars or via the camera.
Figure 3. Nanoparticle tracking analysis results shown as smoothed histogram presenting average concentration and size of control (A) and micro- and nano-bubble-treated (B) deionized water with the corresponding video frame.
same range as samples analyzed in the current study. Analysis of the viscosity profiles with the power-law model also highlighted the differences in the flow behavior index, $n$, and the consistency coefficient, $\kappa$, for the C-MPC and MNB-MPC dispersions. The $n$ and $\kappa$ values were 0.44 and 2.30 Pa S$^n$ for C-MPC, whereas it was 0.48 and 0.59 Pa S$^n$ for the MNB-MPC dispersion. A similar trend was observed by mechanical treatment to modify protein structure using acoustic cavitation in studies conducted by Yanjun et al. (2014) and by

**Figure 4.** Nanoparticle tracking analysis results shown as smoothed histogram presenting average concentration and size of the control (A) and micro- and nano-bubble-treated (B) milk protein concentrate dispersions with their corresponding video frame.
Meletharayil et al. (2016) on MPC and Greek-style yogurts. Marella et al. (2015) used CO₂ injection before and during ultrafiltration on MPC at 17% total solids and observed a reduction in viscosity of CO₂-injected samples (1.99 mPa s) compared with control (23.6 mPa s). The authors attributed the reduction in viscosity to a lower calcium content of the treated MPC dispersions. However, it has long been thought that the injected CO₂ bubbles collapse and vanish. We believe that the bulk MNB might have persisted (assisting in lowering the viscosity) but been previously unnoticed, as MNB were not a part of the study. In a recent study, the concentrations of bulk NB (generated by gas injection via a membrane) were reported to be stable for about a week (in DI water) before gradually decreasing over storage of 1 to 2 mo (Shi et al., 2021). Therefore, the viscosity reduction as reported by Marella et al. (2015) can also be attributed in part to the presence of bulk MNB in
the MPC dispersion. Recently, Phan et al. (2021) also reported a significant viscosity reduction in apple juice concentrate and canola oil due to incorporation of CO2.

This decrease in viscosity is attributed to the lesser volume occupied by new aggregates compared with the C-MPC dispersions. However, the exact mechanism is still unclear. The greater voluminosity occurred because of the inclusion of entrapped water in the structure during the aggregation process (Erabit et al., 2013). A high protein-to-lactose ratio in protein-rich concentrates can result in higher viscosity and can be attributed to the higher voluminosity of milk proteins. The voluminosity and volume fractions were calculated and found to be 3.47 mL/g and 0.79 for C-MPC, and 3.27 mL/g and 0.68 for the MNB-MPC dispersions. The higher viscosity can be attributed primarily to the higher voluminosity of the C-MPC dispersions (Sutariya et al., 2017), and the volume fraction (i.e., the place occupied by the particles in solution) is also a function of the viscosity of the dispersion. In fact, the altered microstructure (Figures 4 and 5) resulted in the decreased volume fraction that can be further linked to decreased product viscosity (Körzendörfer et al., 2019). Very recently, Tian et al. (2021) noted that the motion of NB is limited in high-viscosity (~100 mPa·s) samples, keeping the bubble size more stable. The size stability of MNB is thus dependent upon the system viscosity as well, which can directly affect coalescence of NB (Ritzoulis, 2013). However, the prolonged stability of the bulk MNB was not a concern for the present study, as the feed was spray dried immediately after MNB incorporation. It was previously proven that MNB can form stable bulk colloidal suspensions of particle/MNB complexes and mitigate aggregation due to their high longevity (Calgaroto et al., 2015; Zhang and Seddon, 2016). Indeed, the bulk MNB play a significant role as a buffer between milk protein particles, which prevents protein aggregation (Amamcharla et al., 2017). Keeping the viscosity low also allows the spray drying plant to operate continuously for long hours due to lower occurrences of nozzle blockage and fouling (Westergaard, 2004). Additionally, feeds at higher viscosity have a large droplet size resulting from reduced water mobility, leading to improper drying of the final product (Anandharamakrishnan, 2017). Furthermore, viscosity has a direct influence on the size of the droplets formed during atomization. Indeed, reducing viscosity also results in improved flowability (data not shown). Undeniably, viscosity reduction by MNB treatment is consequently beneficial for improving the efficacies and performance of downstream process technologies, including enhanced flux rate during membrane processing (Amamcharla et al., 2017), reduced fouling in evaporators and heat exchangers, and enhanced powder performance with improved powder properties of the dried powder particles. Overall, MNB treatment aids in reducing the viscosity of the feed, allowing higher feed solid content and thus more efficient and economical spray drying.

Microstructure C-MPC and MNB-MPC Dispersions Before Drying. The CLSM images are shown in Figure 6A and 6B. The green areas in the images correspond to milk protein, and the dark areas correspond to the localized water. From a microscopic point of view, MNB-MPC dispersions exhibited less aggregated structures with minor structural elements compared with C-MPC dispersions. Indeed, it can be seen from the images that C-MPC was present as larger, discrete, coarse, and non-uniform protein aggregates. However, an evenly dispersed and comparatively more homogeneous protein matrix was observed in MNB-MPC dispersions, visibly indicating the influence of MNB treatment. Overall, the CLSM images exhibited reduced particle size and relatively evenly distributed and smaller particles compared with the C-MPC dispersions. Previously, Körzendörfer et al. (2019) noted that acoustically cavitated Greek yogurt showed a “torn-apart” structure. The microstructure images agree with the viscosity result that MNB treatment aided viscosity reductions in the MPC systems.

Representative TEM images are shown in Figure 7. The electron-dense areas in the TEM micrographs are casein micelles (Xu et al., 2016). Overall, CLSM and TEM showed the presence of relatively large particle structures in the C-MPC dispersions, which could be attributed to the presence of larger protein aggregates. Figure 7A shows a continuous matrix of protein clusters against the more distributed structures of MNB-MPC dispersions (Figure 7B). With MNB treatment, the distribution of protein molecules is more diffuse, and the gap between the structures of the protein network (the white part of the micrograph) is larger. Large aggregated structures were noticeably reduced in the MNB-MPC. This may be explained by the structural breakdown of the protein aggregates due to bubble implosion during the hydrodynamic cavitation process. Indeed, the bulk NB may have aided in the prevention of protein aggregation by acting as a spatial buffer between particles in the milk system and resulting in lower viscosity. Although CLSM and TEM showed a more dispersed structure with MNB treatment of the MPC system, further investigations are needed to confirm whether this is a temporary phenomenon or whether it causes a permanent viscosity reduction. Overall, based on analysis of the structure, the influence of MNB on the rheological properties of C-MPC and MNB-MPC dispersions was clearly demonstrated.
Characterization of C-MPC and MNB-MPC Powders

Rehydration Behavior. The rehydration of MPC powders is a multistage process, and MPC powders with higher protein contents typically show poor wettability and solubility (Crowley et al., 2015). Kher et al. (2007) reported that conformational modifications of the protein during processing could be responsible for the loss of solubility. The solubility index of the C-MPC and MNB-MPC powders were 83 ± 1% and 94 ± 2%, respectively. Several pre-treatments (e.g., N₂/CO₂ air injection, microfluidization, acoustic cavitation) before spray drying have previously been studied, to improve the solubility of MPC powders. McSweeney et al. (2021) previously reported an improved dissolution of N₂-injected MPC powders (control: 83.6%; N₂-treated: 96.2%). The more porous structure of MNB-MPC powder particles and the presence of large air voids between these powder particles possibly assisted enhanced water transfer while also improving the physical space between casein micelles and reducing protein-protein interactions (McSweeney et al., 2021). Udabage et al. (2012) studied the application of static high-pressure treatment on MPC and reported an increase in solubility for treated samples compared with control MPC powders. Previously, Yanjun et al. (2014) noted increased solubility (35.78% to 88.30%) of power ultrasound pre-treated MPC powders after 5 min of ultrasound treatment.

Focused beam reflectance measurement has proven to be a suitable technique for studying the rehydration of MPC powders (Hauser and Amamcharla, 2016). Figures 7A and 7B show the changes in the counts for fine (<10 μm) and large (150–300 μm) particles for the C-MPC and MNB-MPC powders. It was observed that fine particle counts (Figure 8A) increased at a higher rate for MNB-MPC powders compared with the C-MPC powders. As can be observed, the large particle counts for MNB-MPC decreased rapidly, whereas the large particle counts for C-MPC had a gradual reduction. The time required for the primary particles to disintegrate was lower in the MNB-MPC, further confirming the positive effect of the MNB treatment on rehydration. The fine particle counts for both C-MPC and MNB-MPC powders were similar for the first 300 s, but then the rate of increase in fine counts were higher for MNB-MPC than for C-MPC. At 0 s, C-MPC and MNB-MPC had fine particle counts of 17,000 and 25,000, respectively. The MNB-MPC was able to reach a fine count of 86,000 by 500 s and maintained this count for the remainder of the experiment; however, C-MPC only attained a count of 76,000 by 500 s. Hauser and Amamcharla (2016) noted a particle count of 62,000 by 900 s for fresh MPC powders and reported that this count was maintained for the remainder of the experiment. Similar results were also obtained in a study by Gandhi et al. (2017), and it was noted that the rate of increase in fine counts was higher for more soluble MPC powders. The counts of large particles (Figure 8B) increased for the first 300 s for C-MPC and MNB-MPC powders, although it was significantly higher for the C-MPC. The increase in the large particle counts can be attributed to the initial aggregation of the powder particles. Larger particle counts decreased more rapidly for MNB-MPC, which indicated the faster disintegration of larger particles into smaller ones for MNB-MPC. The MNB-MPC attained the lowest large particle count around 500 s, and C-MPC reached the lowest large particle count around 1,500 s. The counts for large particles at 500 s were found to be 125 and 20

Figure 7. Representative transmission electron microscopy images of control (A) and micro- and nano-bubble-treated (B) milk protein concentrate dispersions before spray drying. Magnification = 34,000×; scale bar = 200 nm.
Interpretation of focused beam reflectance measurement data matches the overall trend reported by Crowley et al. (2015) and Hauser and Amamcharla (2016). Bouvier et al. (2013) reported that extrusion-porosified MPC powder particles had higher porosity and improved rehydration than conventionally spray-dried MPC powder. The presence of void structures might have promoted the structural collapse of powder particles, whereas rehydration and interactions between poorly dispersible micellar casein at the particle surface are considered to hinder the rehydration of MPC (Anema et al., 2006; Mimouni et al., 2009, 2010). Previously, researchers at Kansas State University have injected MNB before spray drying and reported that nonfat dry milk and MPC powders exhibited a highly porous structure.

Figure 8. Changes in fine (<10 μm, A) and large (150–300 μm, B) counts of powder particles were obtained from data collected using focused beam reflectance measurement for spray-dried control (solid lines) and micro- and nano-bubble-treated (dashed lines) milk protein concentrate powders at a dissolution temperature of 40°C.
(Amamcharla, et al., 2017), and indeed these pores act as water-communicating channels during MPC powder rehydration (Bouvier et al., 2013). Furthermore, as the protein powder particles rapidly disperse and solubilize by disintegrating into smaller particles, fouling of process equipment can be minimized (Gandhi et al., 2017).

**Particle Size Distribution.** The particle size distribution results indicated differences in the particle size distribution of reconstituted C-MPC and MNB-MPC dispersions (data not shown). The MNB treatment resulted in a significant ($P < 0.05$) decrease in the average particle size diameter (from 237.2 nm to 221.7 nm). The particle size and zeta potential measurements in this study were measured only after a 24-h rehydration time, and the observed effects may be more noticeable after shorter rehydration times. After MNB treatment, size distributions were observed to be monomodal, with a complete absence of secondary peaks associated with dispersed powder particles, suggesting that complete dissolution was achieved. McSweeney et al. (2021) reported that N$_2$-injected MPC powders exhibited bimodal volume-based distributions on stirring for 1 h in water at 23°C, suggesting that casein micelles were not released from primary powder particles. However, mean particle size values of 13.6 μm for N$_2$-injected MPC were reported, compared with 83.7 μm for regular MPC powders, suggesting that air injection techniques can help in improving rehydration rates of MPC powders. Previously, Mimouni et al. (2009) reported that 8 h of stirring at 24°C was required to fully solubilize an MPC powder (85%, wt/wt, protein). The net charge of the sample is a good indicator of the stability of the system, as it is evidence of electrostatic interaction. The zeta potentials of the MPC dispersions were found to be 22.02 and 27.94 mV for C-MPC and MNB-MPC samples, respectively. High absolute values of zeta potentials indicate improved stability against aggregation. These results are similar to those reported in the literature (Crowley et al., 2018).

**Microstructure of C-MPC and MNB-MPC Powders.** Representative scanning electron micrography images of the C-MPC and MNB-MPC powders are presented in Figure 9. The powder particles of C-MPC and MNB-MPC powders were relatively smooth, although particles are less round-shaped in C-MPC, which agrees with the circularity results from morphology studies (our unpublished data). Differences in morphology were observed between C-MPC and MNB-MPC powders; only minor dents are distinguishable at the surface of the MNB-MPC particles. In comparison, the C-MPC displayed deeper dents at the particle surface. Compared with C-MPC (Figure 9A), MNB-MPC had fewer small particles trapped in dents of large particles. A similar surface appearance (in MPC with 85% protein content) was demonstrated in a previous study (Fang et al., 2012) and for acoustically cavitated MPC powders (Yanjun et al., 2014), and such surfaces indicated shrinkage of the protein material (Mimouni et al., 2010).

**Microstructure of Reconstituted C-MPC and MNB-MPC Powders.** Representative TEM images of the reconstituted C-MPC and MNB-MPC powders are shown in Figure 10. The MNB-MPC powder particles were observed as smaller aggregates; however, C-MPC showed a distribution with relatively large aggregates. The observed microstructure was similar to that previously reported using TEM by Lie-Piang et al. (2021). They noted casein micelle clusters initially within the diameter size range of 600 to 800 nm in reconstituted studied skim milk powder, but size decreased considerably with reconstitution time. The MNB treatment aided in weakening the aggregate structures, leading to fragmentation and complete hydration of the powder, which is relatable to the particle size distribution.
results. Our TEM images (Figure 10B) reveal that the size of MNB-MPC particles decreased with MNB treatment.

**CONCLUSIONS**

In the present study, we investigated a method for generating MNB using a venturi-type cavitation technique. The NTA measurements revealed that bubbles produced in DI water had a mean diameter of 249.8 ± 115.8 nm. The MNB treatment was used as a mechanical pre-treatment to improve the rheological characteristics of MPC dispersions. Treatment of MPC dispersions using venturi-style air injection at 0.008 L/min reduced the viscosity by ~65% at 100 s⁻¹ shear rate. Notable differences in microstructure were also observed between the C-MPC and MNB-MPC dispersions subjected to the MNB treatment. Furthermore, the use of MNB treatment improved the rehydration behavior of the MPC powder particles. Indeed, controlling the viscosity of the MPC dispersion promotes high-solids drying, and therefore MNB treatment could help improve the spray drying plant’s overall performance. Additionally, MNB generation using the venturi-type generator is a scalable process technology suitable for industrial-scale production of MPC.

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**REFERENCES**


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Figure 10. Representative transmission electron microscopy images of the reconstituted spray-dried control (A) and micro- and nano-bubble-treated (B) milk protein concentrate powders. Magnification = 5,800×; scale bar = 1 μm.


