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Deciphering the segregation of proteins in high-protein dairy powders after spray-drying

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ABSTRACT

High-protein dairy powders are ingredients mainly produced by spray-drying, then subjected to aging during transport and storage. They often undergo physicochemical changes at this stage, such as the development of the Maillard reaction, primarily because of their intrinsic chemical properties, but also as a result of nonoptimal storage conditions. Components present at the particle surface are the first to be targeted by moisture and other environmental disruptions. Consequently, the identification, control, and prediction of particle surface components are useful to anticipate the effect of powder aging on product quality. Here, a new diafiltration method is proposed which fractionates proteins from a binary colloidal dispersion of 80% casein micelles and 20% whey proteins, according to their presence at the surface or core of the particle. This method shows that whey proteins are strongly enriched at the particle surface, whereas casein micelles are located at the core of the particles. This protocol also allows the identification of the rehydration kinetics for each rehydrated protein layer of the particle, revealing that 2 distinct forms of swelling occur: (1) a rapid swelling and elution of whey proteins present at the particle surface, and (2) a swelling of casein micelles located below the whey proteins, associated with a slow elution of casein micelles from the particles being rehydrated.
Key words: dairy proteins, rehydration kinetics, protein location, particle surface

INTRODUCTION

Milk drying is commonly performed to reduce the weight and volume of dairy products for transport and storage, as well as to prevent microorganism growth and activity and other degradation kinetics. Drying also allows for the extended storage of perishable ingredients. Among the different dairy powder categories, high-protein dairy powders are basically used for cheese, yogurt, and other processed food manufacture, but also in nonfood industries such as pharmaceutical, plastic, textile, or paper manufacture (Audic et al., 2003). High-protein dairy powder ingredients are subdivided into different categories, depending on their protein concentration [e.g., protein concentrates (<90% proteins) or protein isolates (>90% proteins)] or depending on the type of proteins (e.g., whey protein concentrates or isolates, milk protein concentrates or isolates, or casein and caseinates powders).

Powder manufacture uses a sequence of different processing steps, including pasteurization, concentration, and drying. The heat treatments applied in each of these steps promote protein degradation, such as protein denaturation, aggregation, or modification, which could be linked to a deterioration in powder solubility and other functional properties (Thomas et al., 2004; Baldwin, 2010; Schuck et al., 2013; Rupp et al., 2018). In addition, recent data show that protein denaturation and modification that occur during powder manufacture could promote a deterioration in the product digestibility and, as a result, a loss of its physiological benefit (van Lieshout et al., 2020). As the particle surface component is the first part of the dairy powder to be subjected to environmental disturbance (moisture, particle-particle interaction, temperature disruption), the study of surface components is essential to understand and control changes that can occur during powder storage or transport.

Nowadays, different basic methods are used to explore the structure and shape of particle surfaces and their rheological properties, such as scanning electron microscopy, atomic force microscopy, and single droplet drying analyses. Using these techniques, it is possible to establish correlations between the surface properties and rehydration or powder solubility (Bur-

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gain et al., 2017; Lanotte et al., 2018; Murayama et al., 2021; Nair and Corredig, 2021; Yu et al., 2021). Also, using X-ray photoelectron spectrometry (**XPS**), and time-of-flight secondary ion mass spectrometry, it is possible to decipher chemical components at particles surfaces and detect protein, lactose, or lipid enrichment at the surface (Nikolova et al., 2015; Burgain et al., 2017). In addition to these techniques, the Kjeldahl method, HPLC, dynamic light scattering, and more recently mid-infrared spectroscopy are performed to study protein quality in high-protein dairy powder (Norwood et al., 2016, 2017; Saxton and McDougal, 2021). However, these techniques are performed on the whole powder, and specific data about protein quality at the surface (denaturation, aggregation, modification) are lost.

Here, a novel, fast, simple, and cheap method is proposed to fractionate proteins from a binary colloidal dispersion of 80% caseins and 20% whey proteins, depending on their location in the particle (either at the particle surface, or deep within the particles). This fractionation method is based on the diafiltration properties of free proteins and particles being rehydrated to cross over a membrane during powder rehydration. Surface proteins are isolated from particles being rehydrated, and subjected to downstream biochemical analyses such as quantification and identification of eluted proteins. As surface proteins are the first to be subjected to environmental disruption, this method can identify and isolate proteins at the surface of high-protein dairy particles, and this could be useful for predicting proteins that are first to be subjected to aging in high-protein dairy powder.

MATERIALS AND METHODS

No human or animal subjects were used, so this analysis did not require approval by an Institutional Animal Care and Use Committee or Institutional Review Board.

Dairy Powder Production

Fresh whey protein concentrate (confidential industrial source; 27% DM, 95% protein on DM) and micellar casein concentrate (confidential industrial source; 13% DM, 87% protein on DM) were standardized down to 10% wt/wt DM using reverse osmosis water (water basis) or ultrafiltration permeate (**UFP** basis) recombined at 6% wt/wt DM from UFP powder (Lactalis). The 2 protein dispersions were then mixed to obtain a dispersion with the same protein ratio as cow milk (i.e., 80/20: casein micelles/whey proteins) and a DM content of 10% (wt/wt). Protein dispersions were concentrated from 10 to 20% wt/wt DM using a 2-stage falling film vacuum evaporator (Laguilhare, GEA; evaporative capacity 180 kg/h). The inlet flow rate was set at 250 L/h. The concentrated protein dispersion temperature was about 51°C at the first stage outlet and 37°C at the evaporator outlet. Concentrated protein dispersions were spray-dried using a 3-stage drying pilot (Niro atomizer, GEA) at BIONOV. Spray-drying was carried out to obtain fine powders. The inlet temperature of the concentrate was 40°C and the feeding flow rate was set at 100 L/h. Inlet air humidity was set by a dehumidifier (Munters) at 1 g of water per kilogram of dry air. The inlet and outlet air temperatures were 218–223°C and 81–84°C, respectively, to obtain powders with water activity of about 0.2. Powders were then packed into aluminum tins and stored at 13°C before further analysis. Median particle diameter ($\mathbf{d_{50}}$) was measured using a laser diffraction granulometer (Mastersizer 3000, Malvern Instruments) equipped with an Aero S dry powder dispersion unit (see Particle Size Analysis During Rehydration section for more details on d_{50} measurements).

Sequential Powder Rehydration

Sequential powder rehydration was performed using a stirred cell 200-mL system (Millipore). For each experiment, 100 mg of powder was spread on a hydrophilic polyvinylidene fluoride (**PVDF**) membrane with a 0.45-μm pore size [i.e., 100× smaller than particle size (40 μm)]. Ultrapure water (30 mL) was added under agitation on a magnetic stirrer at 330 rpm. After 60 s, eluted proteins from the particles were collected under the membrane by tangential flow filtration at a 3.5 bar N_2 pressure. After sample collection, the volume was readjusted to 30 mL. Protein samples were collected after 1, 10, 20, 50, 80, 200 min, and 20 h and correspond to fractions 0–1 min, 1–10 min, 10–20 min, 20–50 min, 50–80 min, 80–200 min, 200 min–20 h, respectively.

Protein Content Analysis of Fractions

Protein concentrations in the collected fractions were measured from their absorbance properties at 280 nm using a Nanodrop 2000c system (Thermo Scientific). Fifteen micrograms of protein was loaded onto 4–15% Mini-PROTEAN TGX Precast Protein Gels (Bio-Rad) in the presence of dithiothreitol as a reducing agent in the samples. After 120 V running, SDS-PAGE gels were colored with InstantBlue Coomassie Protein Stain (Abcam). Protein content was estimated using the GelAnalyzer 19.1 software (www.gelanalyzer.com). The concentration of protein in each fraction was estimated from the global protein

quantity in the sample and its relative proportion in the fraction.

Identification of Physical Parameters of Rehydration

Particle Visualization by Scanning Electron Microscopy. A high-resolution field-emission scanning electron microscope type JEOL JSM-7100F, supplied with a hot (Schottky) electron gun (JEOL Ltd.), and having a resolution around 1 nm at 30 kV was used to investigate the surface and internal structure of dairy particles. The equipment was operated at 3 kV. Samples were mounted onto scanning electron microscope stubs by fixing them on a carbon double-sided adhesive tape. Finally, a coating of iridium was applied to the samples until reaching around 10 nm coating thickness. Scanning electron microscopy has been performed on whole particles, or after manual cutting of powder with a scalpel blade.

Particle Size Analysis During Rehydration. The initial particle size distribution was obtained by static light scattering using a laser diffraction granulometer (Mastersizer 3000, Malvern Instruments) equipped with an Aero S dry powder dispersion unit. The sample was dispersed at 1 bar air pressure. Feed rate and hopper length were adjusted to obtain a correct obscuration of between 1 and 15%. Particle size distribution was calculated from the Mie theory and provided as a volume density function. For particle size distributions during rehydration in water, data were collected every 5 s for 200 min with the Hydro MV module (Malvern Instruments). Three independent measurements were performed on each sample. The particle size estimator was the d_{50}, which means that 50% of particles are smaller in diameter.

Quantification of Calcium Content

ICP-OES Analyses. The dry-ashing method was used to digest the sample before mineral analysis. Two g of powder were placed in a porcelain crucible and heated in a muffle furnace (Nabertherm). The powders were ashed for 5 h at 550°C until a white or gray ash residue was obtained (Schuck et al., 2012). The residue was first dissolved in 5 mL of HNO_3 (2% vol/vol) then, the resulting solution was diluted by 5,000 with HNO_3 (2% vol/vol) before analysis.

Ion concentrations (calcium, magnesium, sodium, potassium, and phosphorus) were measured using an inductively coupled plasma-optical emission spectrometer (ICAP 7200, Thermo Fisher Scientific). Argon was used as the operating gas.

XPS Analyses. Atomic composition at the particle surface (depth of up to 5–6 nm) was measured by XPS. Spectra were obtained with a KRATOS Axis Ultra X-ray photoelectron spectrometer (Kratos Analytical) equipped with a monochromated Al Kα X-ray ($h\nu$ = 1,486.6 eV) operated at 150 W. Spectra were collected at a normal take-off angle (90°), and the analysis area was 700 × 300 μm^2. Calcium content is expressed as the proportion in percentage of calcium atoms identified for each 100 atoms.

Protein Separation by SEC-HPLC. Protein concentrations in each fraction were measured with a Nanodrop 2000c (Thermo Scientific), and samples were adjusted to 2 g/L. The HPLC system (Shimadzu Corporation) was composed of a DGU-20A3 Prominence 3-ways degasser, a SCL-10A VP system controller, a LC20-AD pump, a SIL-10AD VP auto-injector, and an SPD-M10A VP UV/VIS diode array detector, a regulator, and column oven (Croco-cil 100–040–220P, Cluzeau Info Labo). For SEC-HPLC analyses, 40 µg of protein at 2 g/L was injected into an Agilent Bio SEC-5, 150 Å, 7.8 × 300 mm, 5-µm column, preequilibrated in PBS 1X pH 6.8. Each run was performed at 25°C at 1 mL/min for 18 min, and all samples were performed in triplicates. Data and peak integration were obtained at 280 nm with the Shimadzu LC solution software. After data normalization (to total injected proteins, for fraction comparison), the area under the peak of dead volume, including casein micelles and aggregates (>150 kDa molecular weight protein aggregates or complexes), was integrated and expressed as

$$DV(\%) = \frac{A_{DV}}{A_{WC}} \times 100,$$

where DV (%) is the proportion of casein micelles and aggregates in the fraction; A_{DV} is the area under the peak of dead volume; and A_{WC} is the area under the whole curve.

Statistical Analyses

Data were reported as mean values plus standard error of the mean of at least 3 replicates. The significance level using a paired 2-tailed Student's t-test was set to the following P-values: nonsignificant >0.05, $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

RESULTS AND DISCUSSION

Whey Proteins Are First Released from Particles

Protein fractionation from the particles in the course of powder rehydration using the stirred cell equipped with a hydrophilic PVDF membrane is depicted in Figure 1A. After different periods of rehydration, a

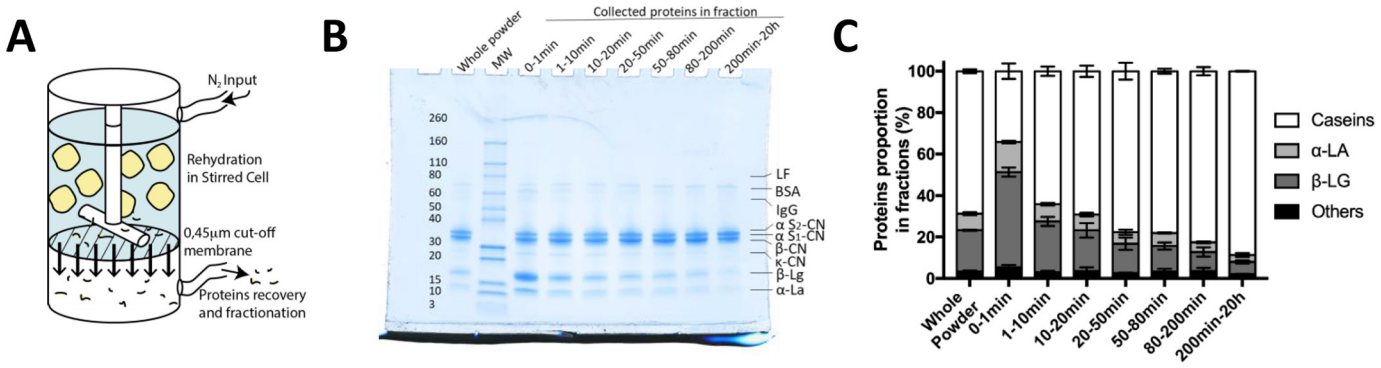

Figure 1. Whey proteins are quickly eluted from particles at the beginning of the rehydration process. (A) Diagram of a diafiltration method employed for protein fraction at the ion with the stirred cell system (Millipore). Seven fractions are collected and are recomposed by proteins eluted from particles between 0 and 1 min, 1–10 min, 10–20 min, 20–50 min, 50–80 min, 80–200 min, and 200 min–20 h. (B) Collected proteins are loaded on an SDS-PAGE and colored with Coomassie blue. (C) Semi-quantification of proteins in each lane in panel B using densitometry. Error bars indicate mean ± SD. Others = high molecular weight proteins (BSA, IgG, lactoferrin; n = 4). MW = molecular weight.

tangential pressure of nitrogen is applied from the top of the stirred cell, and proteins eluted from particles undergoing rehydration cross over the membrane at the bottom of the diafiltration system and are collected. Membrane cutoff is a critical factor. In this case, a 0.45-µm membrane was selected, as this is 100 times smaller than the particle size (39.9 µm ± 0.6 µm), thus ensuring that small whole particles or large particle pieces do not cross over the membrane. However, a 0.45-µm cutoff is far larger in size than free globular proteins (approximately 3 to 10 nm diameter for bovine milk proteins), or casein micelles in dispersion [around 200 nm (Nair and Corredig, 2021)]. At first glance, soluble proteins such as β-LG and α-LA are widely enriched in the first fractions collected (Figure 1B). These observations are in agreement with other studies, showing that whey proteins are the first to be eluted from casein or whey protein mix powder (Mimouni et al., 2010; Crowley et al., 2015; Li and Zhong, 2021), and that lower molecular weight whey proteins are overexpressed at the particle surface of dairy colloidal mixes (Lanotte et al., 2018). Semi-quantification of band intensity by densitometry (Figure 1C) clearly shows that whey proteins are mainly eluted from particles at the beginning of the rehydration process, whereas casein micelles are eluted in a second stage (t >20 min, Mimouni et al., 2010; Li and Zhong, 2021). High temperatures applied during spray-drying could promote the cross-linking of casein and whey proteins, mainly due to advanced Maillard reaction and lipid oxidation, such as glyoxal or methylglyoxal cross-linking with side chains of protein residues (Le et al., 2011). As shown in Figure 1B, aggregates formed by nondisulfide covalent bonds are not detectable by Coomassie staining (no smear of aggregates in the gel or in wells at the top of the gel, Paul et al., 2022), indicating that free caseins, casein micelles, and whey proteins are not degraded by powder production.

Kinetics of Protein Elution from Particles

To investigate changes in rehydration kinetics, the amount of protein collected was estimated using the total protein quantity recovered in each fraction. Normalized values for the rehydration of 100 mg of powder revealed that the rehydration kinetics were not homogeneous during the whole process (Figure 2). Around 14 mg (i.e., 14% wt/wt) of protein was eluted from the particle during the first minute of rehydration, while protein recovery between the first and the 10th minute was about 6 mg or, in other words, less than 1 mg of protein eluted per minute during this period. In the same way, rehydration kinetics drastically decreased during the following stages of the rehydration process. These fast and slow dissolving patterns have already been reported for milk protein concentrate powders (Mimouni et al., 2010; Crowley et al., 2015). In addition, 2 studies have suggested that spray-drying mixed casein micelles and whey protein concentrate results in a mobility of smaller whey proteins toward the droplet skin during spray-drying, and with the result that whey proteins are mainly located at particle surfaces (Lanotte et al., 2018; Yu et al., 2021). Conversely, based on the cross-linked appearances of casein micelles at particle surfaces after 5 min rehydration, also visualized by scanning electron microscopy, Li and Zhong (2021) proposed that cross-linked casein micelles might be enriched at the particle surface, above whey proteins. Nevertheless, as shown in Figure 2, and physically identified by Yu et al. (2021), whey proteins are identified in the upper particle surfaces. One hypothesis is that a surface skin composed of whey proteins forms a direct

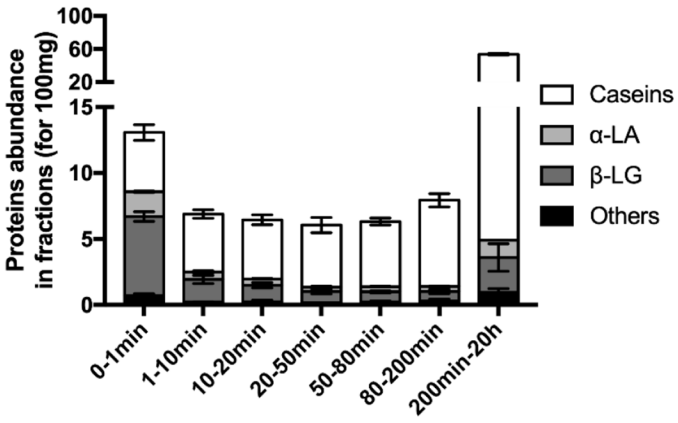

Figure 2. Rehydration kinetics of high-protein dairy powders. Protein abundance in fractions is expressed in milligrams of proteins eluted in each fraction for 100 mg of proteins in powder. Error bars indicate mean ± SD. Others = high molecular weight proteins (BSA, IgG, lactoferrin; n = 4).

bridge on a cross-linked layer of casein micelles below (Mimouni et al., 2010; Fyfe et al., 2011; Lanotte et al., 2018; Li and Zhong, 2021; Yu et al., 2021). The fast elution profile of whey proteins tends toward a quickly exposed cross-linked casein micelles skin (Mimouni et al., 2010; Li and Zhong, 2021).

Particle Morphology and Rehydration Characteristics

Particle Morphology. The scanning electron microscopy snapshots reveal that this high-protein dairy powder exhibits a typically smooth and bumpy structure with a large vacuole (Figure 3, Mimouni et al., 2009; Fyfe et al., 2011; Crowley et al., 2015; Li and Zhong, 2021). The particle wall thickness was approximatively 1–3 μm, corresponding to 5% of the overall particle diameter, with a dense and smooth appearance at the surface typical of whey protein structuration (Yu et al., 2021). At a larger magnification, it showed a nonporous rough structure in the core of the wall, which could indicate casein micelles, as recently illustrated by Nair and Corredig (2021) with field-emission scanning electron microscopy.

Granulometry During Powder Rehydration. Particle size distribution during rehydration was monitored during the first 200 min by granulometry with static light scattering. Results showed a slight decrease in size distribution during rehydration (Figure 4). The absence of a break point during the rehydration process, and the slow speed of particle disruption, indicated that particles were not subjected to fragmentation (no increase in peak width or decrease in peak intensity during rehydration, Figure 4A, Han et al., 2021). From their initial average diameter of 40 μm, particles first encountered a swelling stage, reaching an average diameter of 56 μm after few seconds. This swelling stage was followed by a quick decrease in particle size to an average diameter of 38 μm during the first minutes of the rehydration process (Figure 4B, number 1 circled). Considering the amount of proteins eluted from particles during the first minute of rehydration (Figure 2), this swelling and decrease suggest that the diameter of particles during the first minutes may be due to the release of whey proteins. The only slight difference between the 40-μm diameter of dry particles, and 38-μm diameter of particles after elution of the whey protein crust could be explained by the swelling of casein micelles as soon as they are exposed to water. After 10 min of rehydration, another gradual, low-amplitude swelling occurred (Figure 4B, number 2 circled). As shown by the principal proteins eluted from this step, this swelling point could be assigned to the casein micelle layers present under the whey protein layer, at the core of the particle wall. This 2-step rehydration process has already been observed with the rehydration of micellar casein powder containing residual whey proteins (Gaiani et al., 2006), reinforcing the idea that the second step could be assigned to the swelling of casein micelles.

Taken together, these morphological and size distribution observations suggest that the whey proteins are driven to the edge of the droplet during spray-drying and are located at the particle surface. The disruption of the whey proteins layer is rapid, and micellar casein elution could be considered slow compared with the speed of whey proteins elution. These observations also confirm the aforementioned studies showing that whey proteins form the skin of the droplet during particle drying (Lanotte et al., 2018; Yu et al., 2021).

Casein Micelles Are Under-Represented at Particle Surface

Calcium Distribution in the Particles. To confirm the under-representation of casein micelles at the particle surface, calcium was used as a tracer, given its preponderance in micellar caseins, where it is bound to phosphoserine or phosphate (Holt, 1997), whereas it is absent from whey proteins. The proportion of total calcium present within the bulk of the particle and at the particle surface was measured by an inductively coupled plasma-optical emission spectrometer and XPS, respectively. The whole powder was composed of 2.28% calcium, whereas the surface only showed 0.36% calcium (Figure 5A). This observation indicates that there are 6 times fewer calcium or casein micelles present at the particle surface than in the particle, reinforcing the

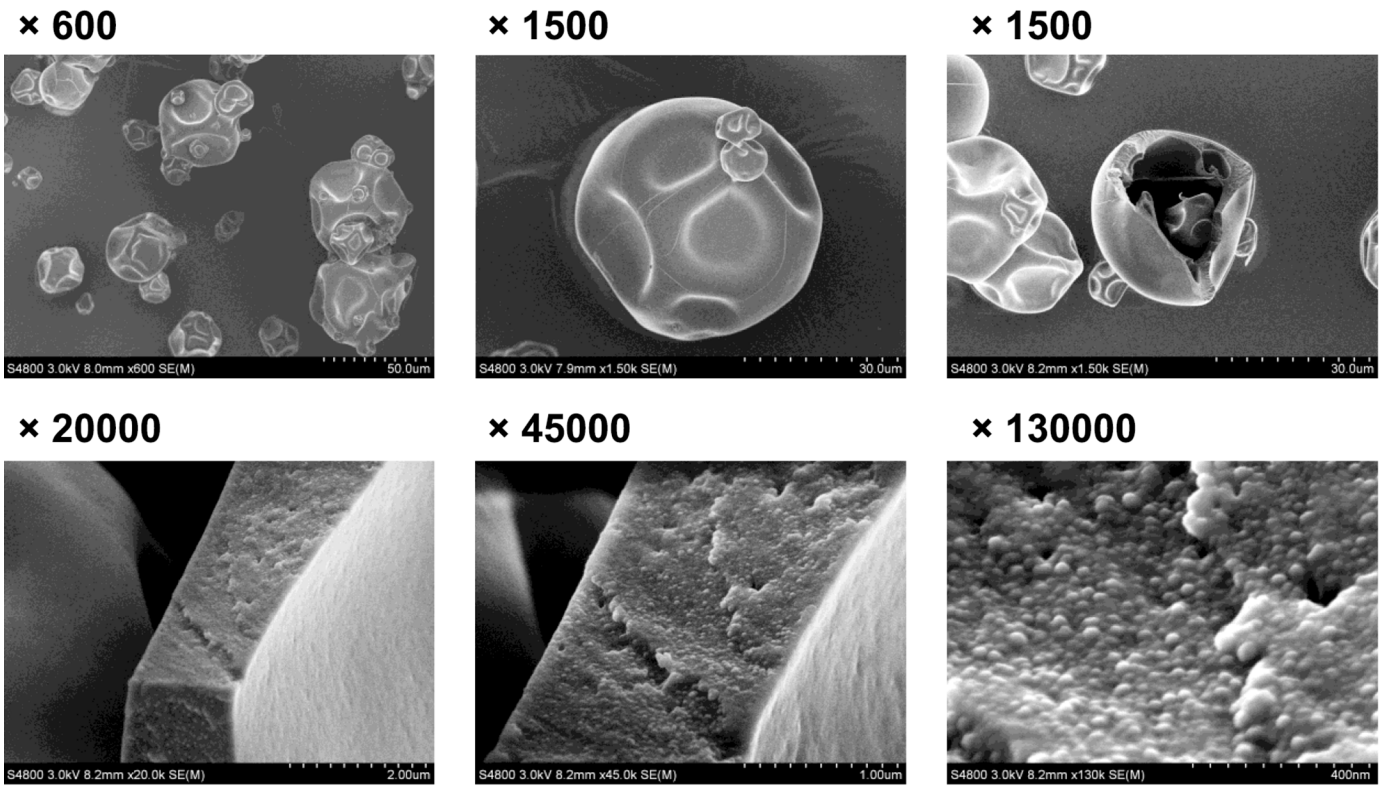

Figure 3. Particle morphology is visualized by scanning electron microscopy. Scanning electron microscopy images of intact or sliced particles are shown with different magnifications. Scales are indicated for each image.

idea that calcium and casein micelles are excluded from the particle edges.

Casein Micelles Distribution in the Fractions. To confirm the segregation of casein micelles in particles, proteins from each fraction were subject to size exclusion chromatography (**SEC**)-HPLC. After powder rehydration and fractionation, the same amount of protein was injected and proteins, protein complexes, aggregates, and casein micelles crossed over the column, depending on their hydrodynamic radius (i.e., molecular weight for globular proteins). The heaviest molecules or complexes passed through the column faster, whereas small and free proteins were collected subsequently. Analysis of chromatograms revealed that the more advanced the rehydration, the larger the dead volume peak containing casein micelles and protein complexes or aggregates (molecular weight >150 kDa; Figure 5B). We observed 2 to 3 times more casein micelles and other heavy protein complexes or aggregates on the particle surface compared with the internal particle wall, suggesting that they are enriched inside the particles. As shown in Figure 5B, a considerable amount of free casein is identified in the whole reconstructed powder. It has been suggested that this free casein fraction is enriched at the particle surface (Li and Zhong, 2021), and could contribute to the identified pool of eluted caseins in the first fraction of rehydration, along with whey proteins, on the particle edges (Figure 1B).

Effect on Protein Segregation and Rehydration Kinetics of UFP Use During Powder Manufacture

UFP Does Not Affect Protein Segregation. Milk protein concentrates are spray-dried after dilution of milk protein concentrates in water or in UFP. Calcium, phosphate, and other small molecules, enriched in UFP (Bastian et al., 1991), are linked to casein micellization (Schiffer et al., 2021). We hypothesize that casein micelles structuring could be modified according to the surrounding medium (water vs. UFP). As depicted in Figure 5B, micellar caseins are over-represented in the internal part of the particles when water is used for the preparation of dairy concentrates. Nevertheless, as shown in Figure 6, protein segregation in the particles was not drastically modified by the use of UFP (particle size = 34.9 μm ± 0.3 μm). Thus, it seems that the differences in the chemical environment (mineral content, lactose content) between UFP and water did not influence protein segregation. This result suggests that changes in the micellar phase or soluble phase

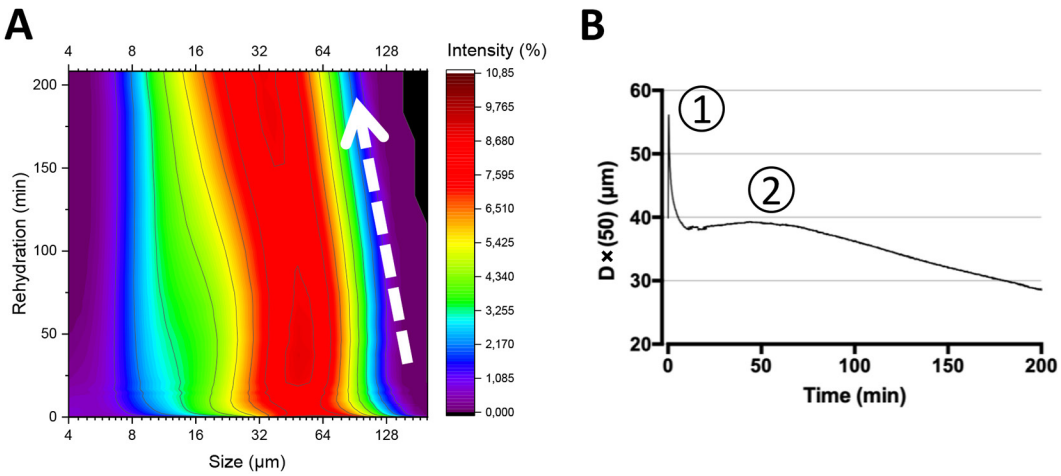

Figure 4. Particle size evolution during powder rehydration is shown. (A) Flat view from a 3-dimensional graph shows the particle size evolution during rehydration. Data are collected by granulometry every 5 s. White dashed arrow illustrates the overall trend of the particle size decrease and reflects the particle erosion phenomenon. (B) Median particle size D× (50) evolution during particle rehydration is shown. Encircled numbers 1 and 2 indicate the swelling steps of particles.

mineral equilibria (as a result of using UFP or water) do not result in micelle destabilization, insofar as they lead to differences in casein segregation in the particle (Schiffer et al., 2021).

Minerals Facilitate Casein Micelles Solvation and Powder Rehydration. Minerals are widely known to promote casein stability (Tsioulpas et al., 2007). To determine whether the additional mineral content could promote powder dissolution, the amount of proteins collected in each fraction was estimated from protein concentrations and fraction volumes (Figure 7). The first step corresponded to the elution of most of the whey proteins, and kinetics parameters were not statistically modified when using UFP, indicating that additional solvation with UFP had no effect on whey protein elution from particles. When the whey protein skin was removed, UFP promoted micellar casein solvation and powder rehydration: indeed, the quantity of eroded proteins doubled between 1 and 20 min (from 10 mg to >20 mg). Taken together, these experiments indicate that UFP does not disrupt protein localization in the particles, or casein

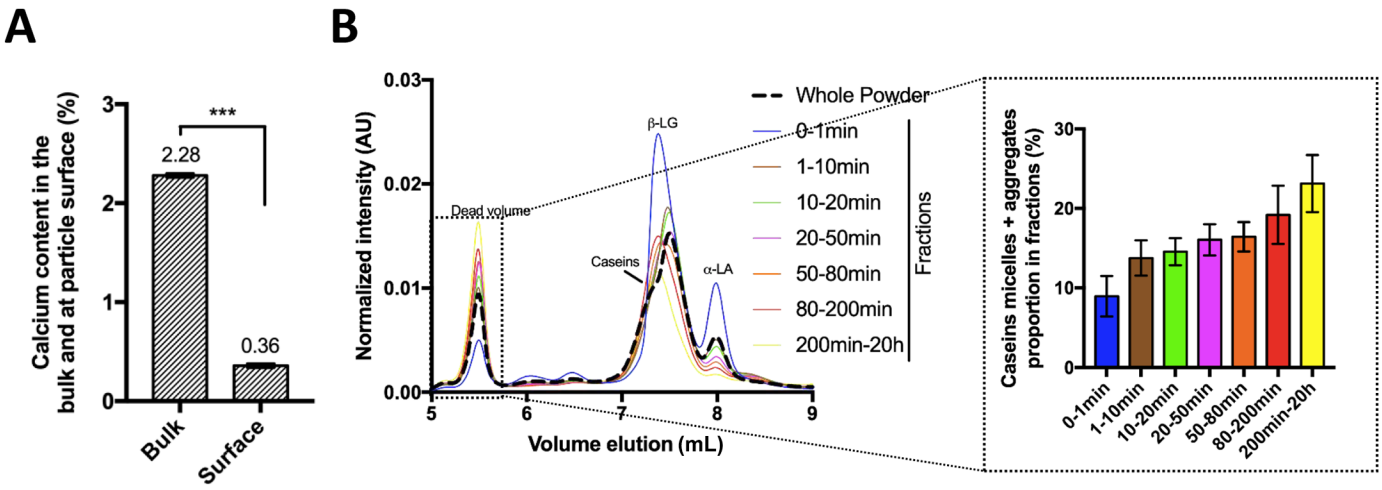

Figure 5. Identification of the location of casein micelles within particles. (A) Calcium proportion in the bulk and at the particle surface of milk powder dry in water. Calcium in the whole powder is quantified by inductively coupled plasma-optical emission spectroscopy (n = 3), and X-ray photo electron spectrometry (XPS) measurements were used for surface calcium detection (n = 3). (B) Native proteins, casein micelles, and aggregates are separated in the different fractions by size exclusion chromatography (in PBS at pH 6.8). The dead volume image includes casein micelles and protein complexes or aggregates (>150 kDa; left). Quantification of micelle or aggregate proportions in each fraction (100% indicates that whole proteins in fractions are aggregated or in micelles; n = 3; right). Error bars indicate mean ± SD.

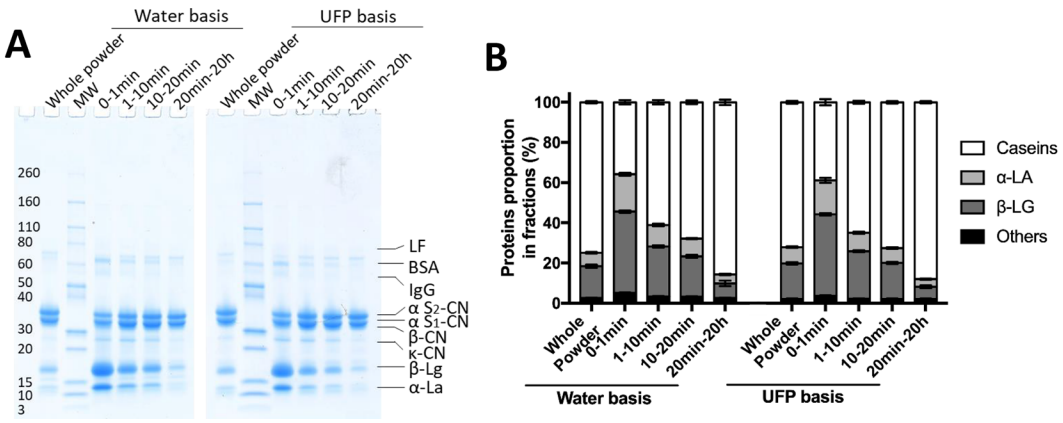

Figure 6. Protein segregation in particles after the drying of protein concentrates prepared in water or ultrafiltration permeate (UFP). (A) Proteins are collected by diafiltration after 1, 10, 20 min, and 20 h, and are loaded on an SDS-PAGE. (B) Semi-quantification of proteins in each lane is performed using densitometry (n = 4). Error bars indicate mean ± SD. Others = high molecular weight proteins (BSA, IgG, lactoferrin; n = 4).

micellization equilibrium (Figure 6), but promotes their rehydration (Figure 7).

CONCLUSIONS

This study provides an original approach, based on sequential rehydration with real-time sample collection. With this method, proteins can be separated according to their localization in the particles. A series of experiments confirms that the first eluted proteins are located at the particle surface. Scanning electron microscopy, particle size distribution monitoring during rehydration, ICP-EOS, XPS, and SEC-HPLC demonstrated with a high degree of confidence that particle rehydration occurs by erosion. For the high-protein dairy powders tested (80% casein micelles, 20% whey proteins), whey proteins were located at the particle surface, whereas micellar caseins were located inside the particle. The investigation of particle rehydration kinetics revealed that the whey proteins were quickly eluted (during the first 1–2 min of rehydration) after a rapid, strong swelling stage. In a second stage, a second swelling was associated with a slow release of casein micelles that were located under the surface whey protein layer.

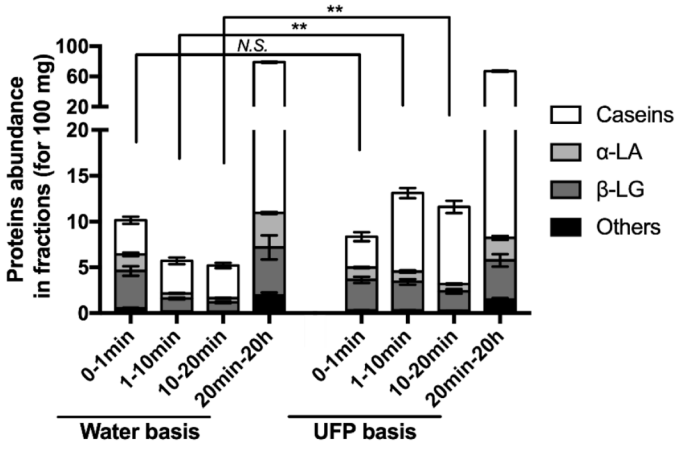

Figure 7. Rehydration kinetics of particles after drying of water- or ultrafiltration permeate (UFP)-basis high-protein concentrates. Protein abundance infractions are expressed in milligrams of proteins eluted in each fraction for 100 mg of proteins in powder, for powder dry in water, and for UFP (n = 3). Error bars indicate mean ± SD. Others = high molecular weight proteins (BSA, IgG, lactoferrin; n = 4).

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REFERENCES

Audic, J.-L., B. Chaufer, and G. Daufin. 2003. Non-food applications of milk components and dairy co-products: A review. Lait 83:417–438. https://doi.org/10.1051/lait:2003027.

Baldwin, A. J. 2010. Insolubility of milk powder products – A mini-review. Dairy Sci. Technol. 90:169–179. https://doi.org/10.1051/dst/2009056.

Bastian, E. D., S. K. Collinge, and C. A. Ernstrom. 1991. Ultrafiltration: Partitioning of milk constituents into permeate and retentate. J. Dairy Sci. 74:2423–2434. https://doi.org/10.3168/jds.S0022-0302(91)78417-8.

Burgain, J., J. Petit, J. Scher, R. Rasch, B. Bhandari, and C. Gaiani. 2017. Surface chemistry and microscopy of food powders. Prog. Surf. Sci. 92:409–429. https://doi.org/10.1016/j.progsurf.2017.07.002.

Crowley, S. V., B. Desautel, I. Gazi, A. L. Kelly, T. Huppertz, and J. A. O'Mahony. 2015. Rehydration characteristics of milk protein concentrate powders. J. Food Eng. 149:105–113. https://doi.org/10.1016/j.jfoodeng.2014.09.033.

Fyfe, K. N., O. Kravchuk, T. Le, H. C. Deeth, A. V. Nguyen, and B. Bhandari. 2011. Storage induced changes to high protein powders: Influence on surface properties and solubility. J. Sci. Food Agric. 91:2566–2575. https://doi.org/10.1002/jsfa.4461.

Gaiani, C., J. Scher, P. Schuck, J. Hardy, S. Desobry, and S. Banon. 2006. The dissolution behaviour of native phosphocaseinate as a function of concentration and temperature using a rheological approach. Int. Dairy J. 16:1427–1434. https://doi.org/10.1016/j.idairyj.2005.12.004.

Han, J., J. Fitzpatrick, K. Cronin, V. Maidannyk, and S. Miao. 2021. Particle size, powder properties, and the breakage behaviour of infant milk formula. J. Food Eng. 292:110367. https://doi.org/10.1016/j.jfoodeng.2020.110367.

Holt, C. 1997. The milk salts and their interaction with casein. Pages 233–256 in Advanced Dairy Chemistry Volume 3: Lactose, Water, Salts, and Vitamins. P. F. Fox, ed. Springer. https://doi.org/10.1007/978-1-4757-4409-5_6

Lanotte, L., F. Boissel, P. Schuck, R. Jeantet, and C. Le Floch-Fouéré. 2018. Drying-induced mechanisms of skin formation in mixtures of high protein dairy powders. Colloids Surf. A Physicochem. Eng. Asp. 553:20–27. https://doi.org/10.1016/j.colsurfa.2018.05.020.

Le, T. T., B. Bhandari, J. W. Holland, and H. C. Deeth. 2011. Maillard reaction and protein cross-linking in relation to the solubility of milk powders. J. Agric. Food Chem. 59:12473–12479. https://doi.org/10.1021/jf203460z

Li, N., and Q. Zhong. 2021. Improving rehydration properties of spray-dried milk protein isolates by supplementing soluble caseins. Food Res. Int. 150:110770. https://doi.org/10.1016/j.foodres.2021.110770.

Mimouni, A., H. C. Deeth, A. K. Whittaker, M. J. Gidley, and B. R. Bhandari. 2009. Rehydration process of milk protein concentrate powder monitored by static light scattering. Food Hydrocoll. 23:1958–1965. https://doi.org/10.1016/j.foodhyd.2009.01.010.

Mimouni, A., H. C. Deeth, A. K. Whittaker, M. J. Gidley, and B. R. Bhandari. 2010. Rehydration of high-protein-containing dairy powder: Slow- and fast-dissolving components and storage effects. Dairy Sci. Technol. 90:335–344. https://doi.org/10.1051/dst/2010002.

Murayama, D., Y. Zhu, and S. Ikeda. 2021. Correlations between the solubility and surface characteristics of milk protein concentrate powder particles. J. Dairy Sci. 104:3916–3926. https://doi.org/10.3168/jds.2020-18311.

Nair, P. K., and M. Corredig. 2021. Time-dependent aggregation of casein micelle concentrates. J. Dairy Sci. 104:92–101. https://doi.org/10.3168/jds.2020-18493.

Nikolova, Y., J. Petit, C. Sanders, A. Gianfrancesco, J. Scher, and C. Gaiani. 2015. Toward a better determination of dairy powders surface composition through XPS matrices development. Colloids Surf. B Biointerfaces 125:12–20. https://doi.org/10.1016/j.colsurfb.2014.11.009.

Norwood, E.-A., M. Chevallier, C. Le Floch-Fouéré, P. Schuck, R. Jeantet, and T. Croguennec. 2016. Heat-induced aggregation properties of whey proteins as affected by storage conditions of whey protein isolate powders. Food Bioprocess Technol. 9:993–1001. https://doi.org/10.1007/s11947-016-1686-1.

Norwood, E.-A., S. Pezennec, J. Burgain, V. Briard-Bion, P. Schuck, T. Croguennec, R. Jeantet, and C. Le Floch-Fouéré. 2017. Crucial role of remaining lactose in whey protein isolate powders during storage. J. Food Eng. 195:206–216. https://doi.org/10.1016/j.jfoodeng.2016.10.010.

Paul, A., C. Gaiani, L. Cvetkovska, C. Paris, M. Alexander, C. Ray, G. Francius, S. EL-Kirat-Chatel, and J. Burgain. 2022. Deciphering the impact of whey protein powder storage on protein state and powder stability. J. Food Eng. 326:111050. https://doi.org/10.1016/j.jfoodeng.2022.111050.

Rupp, L. S., M. S. Molitor, and J. A. Lucey. 2018. Effect of processing methods and protein content of the concentrate on the properties of milk protein concentrate with 80% protein. J. Dairy Sci. 101:7702–7713. https://doi.org/10.3168/jds.2018-14383.

Saxton, R., and O. M. McDougal. 2021. Whey protein powder analysis by mid-infrared spectroscopy. Foods 10:1033. https://doi.org/10.3390/foods10051033.

Schiffer, S., E. Scheidler, T. Kiefer, and U. Kulozik. 2021. Effect of temperature, added calcium, and pH on the equilibrium of caseins between micellar state and milk serum. Foods 10:822. https://doi.org/10.3390/foods10040822.

Schuck, P., A. Dolivet, and R. Jeantet. 2012. Determination of the ash content. Pages 113–118 in Analytical Methods for Food and Dairy Powders. John Wiley & Sons Ltd. https://doi.org/10.1002/9781118307397.ch6

Schuck, P., C. Le Floch-Fouéré, and R. Jeantet. 2013. Changes in functional properties of milk protein powders: Effects of vacuum concentration and drying. Dry. Technol. 31:1578–1591. https://doi.org/10.1080/07373937.2013.816316.

Thomas, M. E. C., J. Scher, S. Desobry-Banon, and S. Desobry. 2004. Milk powders ageing: Effect on physical and functional properties. Crit. Rev. Food Sci. Nutr. 44:297–322. https://doi.org/10.1080/10408690490464041.

Tsioulpas, A., M. J. Lewis, and A. S. Grandison. 2007. Effect of minerals on casein micelle stability of cows' milk. J. Dairy Res. 74:167–173. https://doi.org/10.1017/S0022029906002330.

van Lieshout, G. A. A., T. T. Lambers, M. C. E. Bragt, and K. A. Hettinga. 2020. How processing may affect milk protein digestion and overall physiological outcomes: A systematic review. Crit. Rev. Food Sci. Nutr. 60:2422–2445. https://doi.org/10.1080/10408398.2019.1646703.

Yu, M., C. Le Floch-Fouéré, L. Pauchard, F. Boissel, N. Fu, X. D. Chen, A. Saint-Jalmes, R. Jeantet, and L. Lanotte. 2021. Skin layer stratification in drying droplets of dairy colloids. Colloids Surf. A Physicochem. Eng. Asp. 620:126560. https://doi.org/10.1016/j.colsurfa.2021.126560.

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