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Effect of spray drying and storage temperatures on whey protein concentrate rehydration determined using broadband acoustic resonance dissolution spectroscopy

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ABSTRACT

Understanding whey protein concentrate (WPC80) powder rehydration over time is important for those who incorporate powders into food products. Inhibited rehydration behavior and prolonged rehydration time can cause processing challenges and increased production costs. In this study, we investigated how spray-drying inlet, outlet, and storage temperatures influenced the rehydration behavior of WPC80 powders, elucidating the relationships between particle morphology, powder bulk density, protein modifications, and inhibited rehydration. Powder rehydration over time was measured using the novel broadband acoustic dissolution spectroscopy method. This method monitors gas release during rehydration, which occurs simultaneously with water penetration. Powder dried at higher temperatures released more gas and had a prolonged delay before complete dissolution (>990 s) compared with those dried at lower temperatures (730 s). These observed differences can be attributed to contributions from various factors, including alterations in particle size and shape, powder packing behavior, and protein modifications, such as protein denaturation and lactosylation. Our findings suggest that higher spray-drying inlet and outlet temperatures, as well as storage at 40°C for 2 mo may deteriorate rehydration properties. **Key words:** spray drying, whey protein concentrate, particle morphology, powder rehydration

INTRODUCTION

Rehydration properties of whey-based powders are influenced by temperature conditions during spray drying

and powder storage (Anandharamakrishnan et al., 2007; Gaiani et al., 2021; Paul et al., 2022). Rehydration properties are important selection criteria for end-users who incorporate whey protein powder ingredients into food systems. Whey protein powders typically have inhibited wettability, making it the key rate-limiting stage during rehydration for these powders (Gaiani et al., 2007; Crowley et al., 2016; Ji et al., 2016).

Dairy powders rehydrate through 5 overlapping and sequential stages: wetting, sinking below the surface, swelling, dispersion into smaller particles, and dissolution into a homogeneous solution. Defined treatments such as wettability, dispersibility, and insolubility index analyses (GEA, 2024a,b,c) can be used to distinguish rehydration stages. However, such analyses are not suitable to monitor powder rehydration over time (Vos et al., 2016; Roy and Amamcharla, 2025). A recent review gives an overview of methods that could possibly be used to meet the requirement for monitoring rehydration behavior in real time (Roy and Amamcharla, 2025), mentioning broadband acoustic resonance dissolution spectroscopy (BARDS).

The novel BARDS method monitors rehydration by tracking the release and elimination of compressible gases, with initial increase in gas volume showing the initial water penetration into the powder (Shoa et al., 2021). When the gas volume–time curve returns to pre-addition gas volume and plateaus, this indicates complete dissolution and complete elimination of the submerged gas from the solution. Thus, the steady state time point represents how long it takes for the powder to be dissolved in water, as indicated by return to the gas volume baseline (Pedapatla et al., 2018). Complete dissolution is necessary for releasing and activating the functional properties of the protein (Crowley et al., 2016; Barone et al., 2019; Ren et al., 2025). This rehydration time interval can be useful, in that inhibited or insufficient rehydration can

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The list of standard abbreviations for JDS is available at adsa.org/jds-abbreviations-26. Nonstandard abbreviations are available in the Notes.

result in loss of nutritional and functional properties in the formulation, as well as increased operating cost due to fouling (Roy and Amamcharla, 2025). The BARDS method offers a way to rapidly evaluate complete dissolution time, which can be used to adjust the time for routine hydration process steps.

Different food powders have distinctive product and process-specific chemical and structural characteristics, which influence water penetration and gas release during rehydration, necessitating powder-specific studies (Crowley et al., 2016; Vos et al., 2016; Ren et al., 2025). Few studies have applied BARDS to analyze the rehydration properties of milk protein powders, mostly focusing on casein-dominated powders (Vos et al., 2016; Wu et al., 2019; Wu et al., 2020). Whey protein powder BARDS studies remain very limited, with the exception of one study by Barone et al. (2019) which found that α -LA enriched whey powder had more rapid dissolution when the particles were porous, compared with more dense particles. The mechanisms involved in rehydration of casein- and whey protein-dominated powders differ. Whey protein powders typically lack both swelling and delayed particle surface erosion, having lower wettability and higher solubility than casein-dominated powders (Crowley et al., 2016; Roy and Amamcharla, 2025).

Spray drying is the most widely used method for producing whey protein concentrate powders with at least 80 % protein on DM basis (**WPC80**). Previous studies have shown that protein modifications before and during spray drying, as well as during storage, influence the rehydration properties of high-protein powders (Norwood et al., 2017; Andersson et al., 2020; Burgain et al., 2023). When whey proteins denature, their protein structure is modified, exposing hydrophobic AA residues, which are folded inward in the native protein molecule. As reported in literature (Schmid et al., 2014; Gaiani et al., 2021; Ren et al., 2025), this phenomenon can influence powder rehydration properties by increasing the hydrophobicity of the particle surfaces.

Compared with skim milk powder, WPC80 contains less lactose, which reduces its relative importance to the WPC80 powder properties. Although lactose is not generally considered to have a negative influence on powder rehydration (Crowley et al., 2016; Gaiani et al., 2021), protein modification resulting from the interaction between whey protein and lactose has been shown to influence powder rehydration negatively, with lactosylation possibly increasing the hydrophobicity of particles in high-protein whey powders (Sun et al., 2015; Norwood et al., 2017; Burgain et al., 2023). Lactosylation, a non-enzymatic browning process involving lactose and AA, is dependent on temperature-induced activation energy and the availability of moisture and reducing sugars (Norwood et al., 2017; Andersson et al., 2020; Paul et

al., 2022). Exposing whey protein-lactose systems to heat has been demonstrated to give a limited effect of protein cross-linking on powder wettability and dispersibility (Andersson et al., 2020). Furthermore, modification of particle surfaces has been reported to increase powder hydrophobicity, hindering wetting, sinking and dispersion via water-repellent films which may cause nonhydrating clumps (Ji et al., 2016; Fitzpatrick et al., 2019; Ren et al., 2025).

The browning index (**BI**) is an established method for investigating the effects of lactosylation in food products. This is achieved by quantifying the purity of the brown color (Le et al., 2011; Burgain et al., 2016; Gaiani et al., 2021). Although it is difficult to evaluate the browning of dairy powders chemically, the presence of free 5-hydroxymethylfurfural (**HMF**) indicates protein glycation via the Maillard reaction, the most common nonenzymatic browning reaction in dairy powders (Le et al., 2011; Burgain et al., 2016; Gaiani et al., 2021).

There is a research gap concerning the influence of spray-drying and storage temperatures on WPC80 powder rehydration over time. The present study aimed to investigate the effect of spray-drying inlet, outlet, as well as storage temperatures on the rehydration behavior of WPC80 powders. It was hypothesized that high spray-drying and storage temperatures would inhibit rehydration. The BARDS gas volume–time profiles were used to elucidate the relationships between inhibited rehydration, particle morphology and protein modifications, including denaturation and lactosylation. This study is of industrial importance as it links observed morphology (small, irregularly shaped particles) with wetting and sinking.

MATERIALS AND METHODS

Experimental Design

Spray-drying conditions and storage of the WPC80 powders are described by Gausemel et al. (2025). Industrially produced, fat-separated, pasteurized (73°C, 15 s), and ultrafiltered liquid whey protein concentrate (**LWPC**) from bovine cheese whey was spray dried using a 2-stage, co-current, pilot-scale spray dryer, with a water evaporation capacity of 10 to 15 kg h⁻¹ and fines return to the atomization nozzle (FSD-4.0 type, GEA Niro, Søborg, Denmark). As the feed was pumped to the atomizing nozzle (peristaltic pump NEMA 4X IP66, Watson-Marlow Pumps Group, Falmouth, UK), it was preheated to 60°C to 62°C. Spray drying at low, moderate, or high spray-drying inlet and outlet temperature conditions (**L**: 180°C/77°C, moderate, **M**: 197°C/94°C and high, **H**: 214°C/111°C, respectively) was followed by storage at 20°C, 30°C, or 40°C for 61 d (Table 1) in

dry, temperature-controlled chambers. The difference between inlet and outlet spray-drying air temperatures was kept at 103°C. Each spray-drying batch represented 9 to 10 kg of WPC80, collected from the internal fluidized bed. This scale of production ensured sufficient spray dryer run-time to minimize variability associated with start-up fluctuations in temperatures and feed flow rate. Representative samples were packaged in plastic bags (polyamide and polyethylene, 90-µm thickness, Maske Emballasjefabrikk, Trondheim, Norway) and double heat sealed without applying vacuum. After 61 d, the samples were stored in a climate-controlled chamber until time of analysis, to further minimize any risk of exposure to humid air or variable temperature (20°C, 20% relative humidity; HPP750 eco, Memmert GmbH, Schwabach, Germany). Representative powder fractions from the L, M, and H treatment blocks were recorded in spreadsheets. Powder fractions were randomly assigned to storage treatments using random number tables to avoid bias. Later, samples for analysis were randomly selected using the same method and given sample names only identifiable to the designer of the study, contributing to allocation concealment. It was not possible to blind the study, as the person responsible for implementing randomization also assessed the outcomes.

Compositional Analysis and Physical Properties

Gausemel et al. (2025) presented composition and viscosity results for the LWPC feed used in the present study, representing composition and viscosity across the 3 consecutive production days. Composition of LWPC was measured using a Fourier transform infrared method (MilkoScan FT1, Foss Analytical A/S, Hillerød, Denmark). The dynamic viscosity was measured using a rate/stress rheometer (Brookfield, Stuttgart, Germany) operated in shear rate mode, equipped with a CC45 concentric cylinder measuring system (Brookfield, Stuttgart, Germany). Measurements were conducted in accordance with DIN 35019/ISO 3219. This method was used to compare viscosity at 3 consecutive days using Tukey's honest significant difference (**HSD**) test ($n = 2$, 5% significance level). Gausemel et al. (2025) also presented composition, bulk density, and Carr Index results for the same 9 WPC80 samples as studied in the present study. Composition of WPC80 powders was analyzed on DM basis using Fourier transform near infrared (DairyQuant B3, Q-Interline, Tølløse, Denmark), except for moisture, which was measured using a standard method (GEA Niro, 2004). Bulk density and Carr Index were analyzed using rheometer methods (Physica MCR301, Paar Physica, Anton Paar, Stuttgart, Germany).

The nitrogen content of the 9 WPC80 powders was measured using the Kjeldahl method (Kjeltec 8400

Table 1. Experimental design for whey protein concentrate (WPC80) powder preparation; spray-drying combinations of inlet and outlet air temperatures as well as feed flow rates are categorized as low (L), moderate (M), or high (H), with storage temperatures of 20°C, 30°C, or 40°C

Item	Spray-drying temperature ¹		
	L	M	H
Inlet air temperature (°C)	180	197	214
Outlet air temperature (°C)	77	94	111
Feed flow rate (mL/min)	330	276	233
Storage temperature			
20°C, 61 d	L20	M20	H20
30°C, 61 d	L30	M30	H30
40°C, 61 d	L40	M40	H40

¹Highlighted in bold: 9 unique sample codes.

Analyzer Unit with Tecator Digester Auto 20, Foss, Höganäs, Sweden) and total protein content was calculated using a nitrogen-to-protein conversion factor of 6.38 (ISO, 2004, 2014).

Broadband Acoustic Resonance Dissolution Spectroscopy

Powder rehydration was acoustically monitored using BARDS, representing primary outcomes, tracking sound frequency changes due to gas release into solution. In contact with water, the water penetrates the powder, and the entrained gas is released as bubbles. Entrained gas includes interparticle gas and gas entrained within particles. The method quantifies gas volume via changes in solvent compressibility and consequent changes to speed of sound (Vos et al., 2016; Wu et al., 2019). The following equation was automatically applied to represent the relationship between sound velocity in water and the fractional bubble volume based on an approximation (Wood, 1930):

$$\frac{v_w}{v} = \sqrt{1 + 1.49 \times 10^4 \times f_a}, \quad [1]$$

where v_w is the velocity of sound in pure water, v is the velocity of sound in bubble-filled water, and f_a is the fractional volume occupied by air bubbles. The relationship between gas release and water penetration allows BARDS to track rehydration dynamically in real time (Vos et al., 2016; Barone et al., 2019; Wu et al., 2019). Powder rehydration was carried out in a lime glass vessel, with a magnetic stirrer producing sound (broadband acoustic excitation). A microphone (Sony ECM-CS10, Tokyo, Japan) recorded frequency modulations and BARDS spectra were registered after applying a fast Fourier transform. The fundamental curve frequency value was calculated using resonance frequencies for

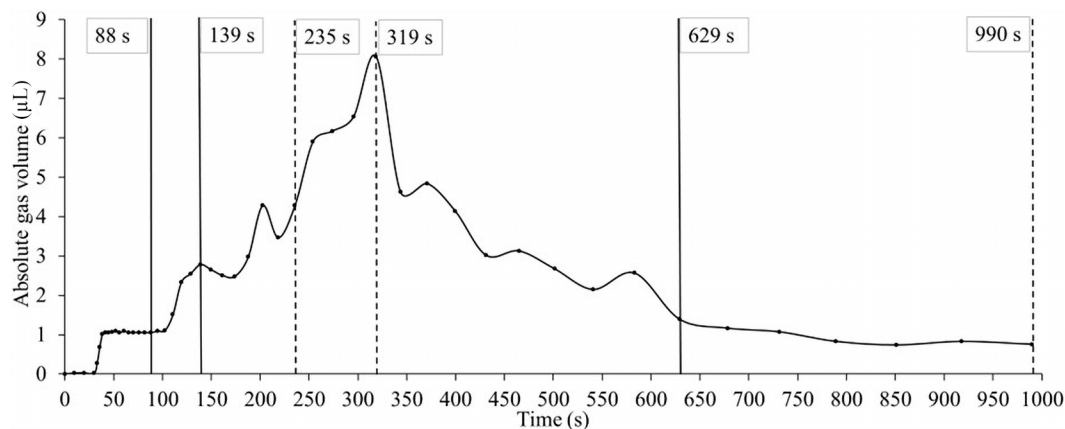


Figure 1. Example of a broadband acoustic resonance dissolution spectroscopy gas volume–time profile illustrating gas release and gas elimination (falling curve) during the rehydration of WPC80. The figure illustrates the time points compared in our study (vertical dashed lines = selected, vertical solid lines = randomly selected) with the 319 s time point representing the t_M time point of the example gas volume–time profile. The maximum of the curve represents the equilibrium between the release of gas into the solution and the elimination of gas through the water surface.

pure and bubble-filled water (Fitzpatrick et al., 2012; Peddapatla et al., 2018), and the gas volume in solution (V_g) was calculated for each time point, using Equation 2 (Wu et al., 2019). Details are provided by Fitzpatrick et al. (2012) and Crawford (1982):

$$V_g = f_a \times V_w, \quad [2]$$

where V_g is the volume of gas in the BARDS measuring cell, V_w is the volume of water used for rehydration during BARDS experiments, and f_a is the fractional volume occupied by air bubbles. The BARDS analysis was calibrated using deionized water and performed according to the method described by Peddapatla et al. (2018) with slight modifications. Resonances were recorded in the 0-to-16 kHz frequency band, and BARDS spectra were recorded over 990 s during the rehydration of 0.4% (wt/wt) WPC80 powder in water. Deionized water (24°C) was saturated with air by vigorous shaking for 60 s, followed by a 30 min rest to avoid gas oversaturation. The container was tapped gently on the table to remove any gas bubbles on its inner surface. Before the addition of 100 ± 0.2 mg WPC80, BARDS spectra were recorded for pure water over a period of 30 s. The frequency of 10 s was set as the steady state frequency, allowing the identification of the time point at which the curve plateaued and reached steady state, with the curve returning to baseline gas volume representing the end of the dissolution stage (Peddapatla et al., 2018).

Two rate constants were calculated for the L, M, and H gas volume–time profiles: gas release (k_r) and gas elimination (k_e). These were calculated using a modified kinetic analysis method (Vos et al., 2016). In gas volume–time profiles, the time point at which the maxi-

mum gas volume is reached is termed t_M (Wu et al., 2019). The gas elimination rate constant was fitted linearly from t_M to steady state, or 990 s. An example of a BARDS gas volume–time profile is provided in Figure 1. Gas volume values were compared at 6 time points, including the 2 time points representing t_M values for H samples (235 s) or for L and M samples (319 s). Furthermore, time points at the end of the BARDS analysis (990 s), and at 3 randomly selected times (88, 139, 629 s) were included. To avoid observer bias, randomly selected time points were generated using Excel's randbetween() function, which returned a random integer from a list of specified values, in this case time points between 35 and 750 s. Any time point less than 50 s from a previously listed time point was disregarded. Statistically comparing gas volume at selected time points is beneficial for monitoring rehydration over time and identifying differences in initial, maximum and late water penetration and gas release. Additionally, the total BARDS gas volume area under the mean curve (AUC, $\mu\text{L}\cdot\text{s}$) values, representing net gas volume (release minus elimination) for each sample, was calculated using trapezoidal integration. This value descriptively represents the presence of gas in solutions during BARDS analysis, not including any changes after 990 s.

Visible floating clumps, with an estimated diameter >1 mm, were manually counted after 600 s in the transparent BARDS measuring cell. Here, clumps refer to behavior in contact with water. Such clumps were relevant to the release of gas in solution as clump submersion continued after 600 s. This cut-off time was chosen as it was expected that the WPC80 powders would be fully submerged by this time, based on introductory BARDS tests. Clumping in spray-dried powders at increased (M

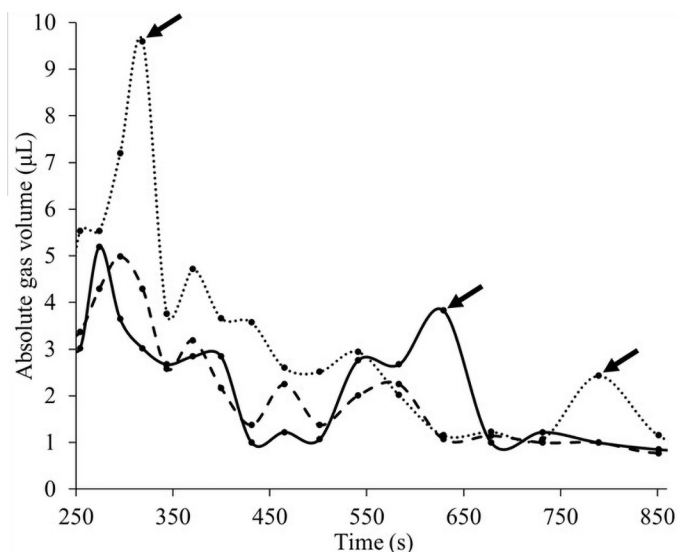


Figure 2. Illustration of the influence of periodical gas release from clumps leading to local increases in measured gas volume and local differences in gas volume values. The results are an excerpt from a comparison of WPC80 sample replicates that were spray dried at 197°C/94°C temperatures, and then stored at 20°C. The 3 replicate gas volume–time curves, from 250 to 850 s, are illustrated by dotted, dashed, and solid lines, respectively. Arrows indicate local gas volume peaks associated with submerging clumps.

and H) inlet and outlet temperatures possibly explained irregular gas volume values over time, due to episodic clump submersion resulting in local curve fluctuations. Divergence between sample replicate values caused by these fluctuations (Figure 2) resulted in high absolute gas volume SD between the 3 sample replicate measurements ($n = 3$). Therefore, an additional comparison was made in which sample replicate variation was compensated for by selecting the 2 most representative sample replicates ($n = 2$) and disregarding the sample replicate with the greatest deviation.

Wettability and Insolubility Index

Wettability and insolubility index was analyzed as secondary outcomes, using standard methods (GEA, 2024a,b,c). The standard method for wettability is primarily used for milk powders, monitoring the time required for powder to wet and sink through the water surface in still-standing water, with rapid wettability being indicated by short wetting times. This method was adapted to WPC80 samples: The time limit for what is considered “non-wetting” was extended to 990 s to facilitate comparisons of powders which are more slow-wetting than milk powders. The powder (0.5 ± 0.05 g) was dropped from a thin metal foil onto 250 mL of water at $25^\circ\text{C} \pm 1^\circ\text{C}$.

Particle Size and Shape

Volumetric particle size distribution (PSD) was analyzed using a Mastersizer 3000 (S.no. MAL1083189, Malvern, UK) with an Aero S dry disperser, following Gausemel et al. (2023). Particle size was calculated using Mie theory (Malvern Instruments Ltd., 2013) with absorption and refractive indices of 0.005 and 1.461, respectively. The median particle size (**Dv50**; μm) value represented the diameter at which 50% of the sample volume had a lower value. The span value described the PSD width (Malvern Instruments Ltd., 2013).

The median roundness shape descriptor was measured using dynamic image analysis. Powders were dispersed at 0.2 bar, 100% feed rate, with a 2.5-mm hopper gap (Qicpic/L, Oasisdry/L, Rodos/L, Vibri, M7 lens; Sym-patec GmbH, Clausthal-Zellerfeld, Germany). Roundness was calculated as the ratio of the averaged radius of curvature of all convex regions to the circumscribed circle of the particle (Equation 3). The circumscribed circle was automatically limited, touching the extreme edges of each silhouette:

$$\text{Roundness} = \frac{\sum_{i=1}^n \left(\frac{r_i}{R} \right)}{n}, \quad [3]$$

where R is the radius of the circumscribed circle and r_i is the radius of the inscribed circle at convex corner i .

Protein Profile Analysis

Capillary electrophoresis was conducted using an Agilent CE-system (model 7100, Waldbronn, Germany) with Agilent Openlab CDS software. A modified method following Jørgensen et al. (2016) involved overnight hydration of 3.0 g WPC80 in 27 mL of deionized water at $4^\circ\text{C} \pm 1^\circ\text{C}$. Protein profile peak areas were calculated by dividing the area of each identified peak (milli-absorbance unit [AU] seconds; $\text{mAU} \cdot \text{s}$) by the migration time (min). Relative content of whey proteins identified as BSA, caseinomacropeptide (CMP), α -LA, and β -LG was calculated, facilitating comparison across samples in terms of relative abundance.

Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) was conducted to compare thermal stability and heat flow during whey protein denaturation using a Micro DSC 3 S60/36135 (Setaram, Caluire, France). Heat flow was thermo-analytically monitored as a function of temperature and time. Powder (3.0 g) was dissolved in 27 mL of

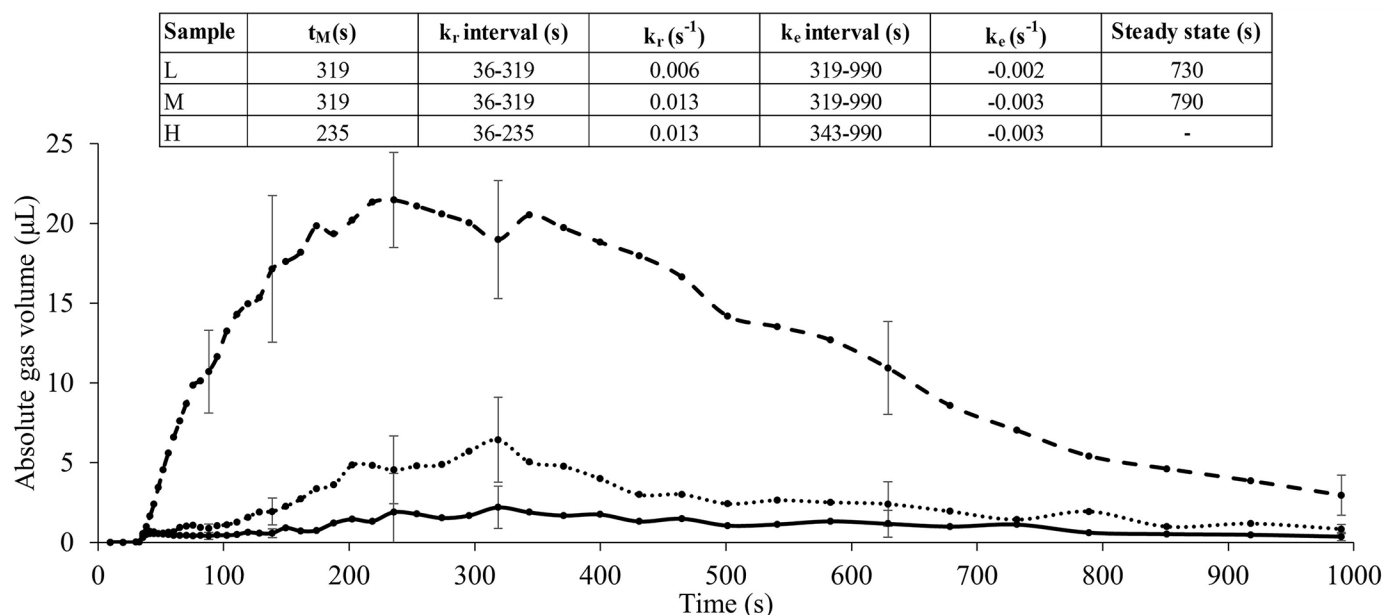


Figure 3. Comparison of mean value gas volume–time profiles measured using broadband acoustic resonance dissolution spectroscopy (BARDS) for whey protein concentrate (WPC80) powders spray dried at 3 temperature settings (see Table 1 for more specifications), including samples stored at differing temperatures: solid line = L samples, dotted line = M samples, dashed line = H samples ($n = 9$). Error bars represent $\pm$ SD for the samples, per time point. The table at the top of the figure describes the time points for maximum gas volume values (t_M) and steady state, as well as the kinetic gas release (k_r) and gas elimination (k_e) rate constants, as influenced by spray-drying temperature settings. The steady state values represented the time required to fully dissolve the samples.

deionized water ($4^\circ\text{C} \pm 1^\circ\text{C}$), and 0.760 g solution was sealed in a Hastelloy C276 vessel, using 0.760 g of deionized water as a reference. Samples were equilibrated to 30°C before analysis, heated from 30°C to 40°C and from 40°C to 100°C at $0.5^\circ\text{C}/\text{min}$, then cooled from 100°C to 30°C at $1^\circ\text{C}/\text{min}$. Data were processed using Calisto processing software v1.493, DB v1.4902 (Setaram, Caluire, France).

BI and 5-Hydroxymethylfurfural Analysis by Capillary Electrophoresis

Color measurements were performed using a chromameter (CR-400, Konica Minolta Sensing Inc., Tokyo, Japan) and BI was calculated following Burgain et al. (2016), based on dimensionless L^* , a^* , and b^* color coordinate values representing 3 axes: L^* (white to black), a^* (red to green), and b^* (yellow to blue). The BI was calculated as in Equation 4:

$$BI = \frac{100 \times (x - 0.31)}{0.17},$$

$$x = \frac{a^* + 1.75L^*}{5.645L^* + a^* - 3.012b^*}. \quad [4]$$

Color coordinates for the 3 LWPC samples from before spray drying were also measured to ensure that initial LWPC color did not influence powder color.

In addition to the prespecified focus on BI, color coordinate values and sensory attributes were also evaluated as part of the broader study. These analyses were considered exploratory and are not included in the reported results.

Maillard reaction activity was evaluated by measuring the content of HMF, with L samples stored at different temperatures being compared using an Agilent CE-system (model 7100, Waldbronn, Germany) with Agilent Openlab CDS software. The L samples were chosen for this comparison, as it was expected that storage would influence the HMF content more than the spray-drying temperatures would. Additionally, the M and H samples stored at 20°C were analyzed to represent the effect of spray drying. A modified method by Morales and Jiménez-Peréz (2001) involved stirring 2 g of WPC80 in 15 mL of deionized water for 1 h, ensuring complete dissolution. Electromigration was conducted using an Agilent G1600AX system, with the following settings: extended light path capillary 50 μm , 72 cm (part no. G1600-62232), conditioned for 1 h with 0.1 M NaOH. Running buffer (SDS, 0.1000 M, Merck, Darmstadt, Germany), sodium dihydrogen phosphate monohydrate ($\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, 0.0733 M, Merck, Darmstadt,

Table 2. Effects of spray-drying and storage temperatures on gas volume values (mean $\pm$ SD, $n = 2$) and BARDS gas volume area under the curve (AUC) values for whey protein concentrate (WPC80) powders measured using broadband acoustic resonance dissolution spectroscopy (BARDS).

Sample ¹	BARDS gas volume (μL)						BARDS gas volume AUC ($\mu\text{L}\cdot\text{s}$)
	88 s	139 s	235 s	319 s	629 s	990 s	
L20	0.2 ^a $\pm$ 0.1	0.2 ^a $\pm$ 0.1	0.8 ^a $\pm$ 0.3	1.0 ^a $\pm$ 0.2	0.2 ^a $\pm$ 0.0	0.1 ^a $\pm$ 0.0	735
L30	0.3 ^a $\pm$ 0.0	0.7 ^b $\pm$ 0.2	0.9 ^a $\pm$ 0.1	3.3 ^{ab} $\pm$ 0.0	0.7 ^a $\pm$ 0.2	0.3 ^{ab} $\pm$ 0.0	1,139
L40	0.7 ^a $\pm$ 0.1	0.9 ^{abc} $\pm$ 0.1	1.0 ^a $\pm$ 0.1	3.6 ^{ab} $\pm$ 0.9	2.4 ^a $\pm$ 0.6	0.8 ^{bc} $\pm$ 0.0	1,469
M20	1.0 ^a $\pm$ 0.1	1.1 ^{abc} $\pm$ 0.2	3.3 ^{ab} $\pm$ 0.1	3.7 ^{ab} $\pm$ 0.9	1.1 ^a $\pm$ 0.1	0.9 ^{bc} $\pm$ 0.1	1,999
M30	1.2 ^a $\pm$ 0.2	2.8 ^c $\pm$ 0.7	4.4 ^{ab} $\pm$ 0.6	6.5 ^{bc} $\pm$ 1.6	4.0 ^a $\pm$ 0.1	0.6 ^{abc} $\pm$ 0.1	2,388
M40	0.7 ^a $\pm$ 0.0	2.5 ^{cd} $\pm$ 0.3	5.1 ^b $\pm$ 1.5	9.1 ^c $\pm$ 1.1	1.5 ^a $\pm$ 0.1	1.2 ^c $\pm$ 0.3	3,031
H20	14.3 ^c $\pm$ 1.8	21.7 ^e $\pm$ 1.2	19.1 ^c $\pm$ 2.5	21.8 ^c $\pm$ 1.3	9.3 ^b $\pm$ 0.1	2.6 ^{dc} $\pm$ 0.1	12,015
H30	8.4 ^b $\pm$ 1.5	21.2 ^e $\pm$ 0.3	21.2 ^c $\pm$ 0.8	21.7 ^c $\pm$ 1.3	8.7 ^b $\pm$ 1.1	2.3 ^d $\pm$ 0.1	11,689
H40	8.8 ^b $\pm$ 0.8	15.4 ^d $\pm$ 0.4	20.6 ^c $\pm$ 0.3	17.7 ^d $\pm$ 0.7	13.8 ^c $\pm$ 2.6	3.0 ^e $\pm$ 0.4	11,384

^{a-e}Within a column, means with differing superscripts differ significantly ($P < 0.05$).

¹The WPC80 powders were spray dried at low (L), moderate (M), or high (H) inlet and outlet temperatures, then stored at 20°C, 30°C, or 40°C. See Table 1 for more specifications.

Germany) and sodium phosphate monobasic dihydrate ($\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, 0.0011 *M*, Merck, Darmstadt, Germany) in deionized water, with pH adjusted to 7.5 using 1 *M* NaOH, was sonicated for 20 min and filtered through a 0.45- μm polyethersulfone filter (Merck, Darmstadt, Germany). The system was preconditioned with 0.1 *M* NaOH and run buffer for 5 min, before each sample injection. After the injection, the cartridge temperature was 25°C. This method was validated by spiking control samples with 5-hydroxymethylfurfural (Merck, Darmstadt, Germany). The comparison between the samples was done using 2 sample replicates ($n = 2$).

Statistical Analysis

Sample size was determined based on interim studies and evaluations of sample replicate variation. Gas volume, particle morphology, CE, DSC, and BI data were analyzed in R (version 4.3.2) using one-way ANOVA and Tukey's HSD test (significance level $P < 0.05$), using either the spray-drying temperature conditions (L, M, H) or the 9 sample codes as factor levels. Pair-wise correlation coefficients of measurement variables were calculated using a correlation plot ($P < 0.01$; 2-way test). The main effects of spray-drying and storage temperature, and their interactions ($P < 0.01$), were calculated using 2-way ANOVA. Whether or not the protein content (Kjeldahl) remained consistent between WPC80 powders after storage was evaluated using Tukey's HSD test ($n = 3$, $P < 0.05$).

RESULTS

Gross Composition and Physical Properties

The 9 powder samples had a mean total protein content of $76.0\% \pm 0.4\%$ (as analyzed by Kjeldahl) and no significant differences were observed. The composition,

Carr Index and bulk density of the 9 powder samples in the present study were reported by Gausemel et al. (2025), which studied the same samples. A summary of the previously published results is included here: WPC80 powder fat and moisture content was 4.6% to 5.3% and 5.8% to 6.9% (wt/wt, $n = 3$), respectively. The moisture content was significantly ($P < 0.05$) higher for L samples than for M and H samples. Regarding physical properties, L samples had significantly higher bulk density (381 kg m^{-3}) than M samples (361 kg m^{-3}), which in turn had higher bulk density than H samples (342 kg m^{-3}). Following a similar trend, L samples had significantly lower Carr Index (38%) than M and H samples (44%). The same study reported the composition of the LWPC batch used to produce the following samples: TS (27.2%), protein (21.6%), fat (1.52%), and carbohydrates (1.38%). The dynamic viscosity of LWPC was in the range 0.046 to 0.047 Pa·s, showing no significant differences across the 3 production days.

BARDS for L, M, and H Samples

Higher and wider peaks in the BARDS gas volume–time profiles indicate more submerged and released gas. When the gas volume values are reduced over time it represents gas elimination during degassing. Differences in gas volume and gas elimination behavior were observed in our experiments (Figure 3, Table 2). The BARDS gas volume AUC of each sample showed a trend of low values for L samples, moderate values for M samples, and high values for H samples. The H samples achieved their maximum gas volume value (t_m) earlier and had higher gas volume values (maximum $20.3 \mu\text{L}$) compared with the 2.2- and $6.4\text{-}\mu\text{L}$ maximum values recorded for the L and M samples, respectively. Rate constants for gas release (k_r), derived from the logarithmic scale plots, showed that the initial gas volume increase for the L

samples was less steep, compared with the corresponding values for M and H samples. Although the L samples plateaued and reached complete dissolution (steady state) faster than the M samples, the H samples did not plateau within the analysis time (990 s; Figure 3). Only one of the L samples had a floating clump at 600 s, compared with 4 of the 9 M samples and 6 of the 9 H samples. The H samples had significantly higher gas volume values than the L and M samples at each of the first 5 time points (88, 139, 235, 319, and 629 s). At the end of the analysis (990 s), the gas volume values of sample H40 did not significantly differ from the L and M samples.

In terms of spray-drying inlet and outlet temperature effects (L, M, and H), reducing the number of sample replicates did not affect the influence of the spray-drying temperatures, as shown in Table 2, which displays the same trend as the summarized results for the full dataset ($n = 3$). Storage at different temperatures (20°C, 30°C, or 40°C) for 61 d had less effect on gas volume over time than the spray-drying temperatures. For the L samples, the gas volume values of samples stored at 40°C were higher than those stored at 20°C, suggesting a trend of increased gas volume with increased storage temperature. However, the BARDS results were found to be sample dependent: The M and H samples did not show a clear trend based on storage temperature.

Wettability Analysis and Insolubility Index

The L20, L30, and L40 samples (as defined in Table 1) were wetted within 700 s, 830 s, and 800 s, respectively ($n = 3$), whereas neither the M nor the H samples were wetted within 990 s. All WPC80 samples demonstrated good solubility, with insoluble sediments in the 0.5 to 0.8 mL range ($n = 2$).

Particle Size and Shape for L, M, and H Samples

The results for L, M, and H samples for particle size, span, and particle roundness reflect the influence of spray-drying inlet and outlet temperatures on particle morphology (Table 3). The particle size (Dv50) and roundness decreased significantly as the spray-drying temperature increased, whereas the PSD width (span) became significantly wider.

Protein Profile Analysis

The effect of protein modifications during spray drying and storage influenced the relative content of whey proteins (Table 4). The L samples stored at 20°C and 30°C had a significantly higher relative content of α -LA and significantly lower relative content of β -LG than the H samples. Samples spray dried at the same inlet and

Table 3. Effects of spray-drying temperature on the particle size distribution and roundness values (mean $\pm$ SD, $n = 24$) of whey protein concentrate (WPC80) powders¹

Sample	PSD value		Particle shape descriptor
	Dv50 (μ m)	Span (-) ²	Roundness (-) ²
L samples	76.3 ^c $\pm$ 1.0	2.0 ^a $\pm$ 0.0	0.51 ^c $\pm$ 0.01
M samples	52.8 ^b $\pm$ 0.7	2.5 ^b $\pm$ 0.1	0.48 ^b $\pm$ 0.01
H samples	48.5 ^a $\pm$ 0.5	2.7 ^c $\pm$ 0.2	0.46 ^a $\pm$ 0.01

^{a-c}Within a column, means with differing superscripts differ significantly ($P < 0.05$).

¹The WPC80 powders were spray dried at low (L), moderate (M), or high (H) inlet and outlet temperatures. See Table 1 for more specifications.

²Values marked with (-) are dimensionless.

outlet temperatures had lower relative content of α -LA and a higher relative content of β -LG when stored at 40°C compared with 20°C. However, the results show a different trend for the L, M, and H samples. The data demonstrate that increased thermal exposure during the process of drying and storage systematically modifies the protein profile, resulting in a decreased relative content of α -LA and an increased relative content of β -LG. In the L samples, the relative content of CMP decreased with higher storage temperatures.

Differential Scanning Calorimetry

During thermal protein denaturation, sample L40 had a peak maximum denaturation temperature at 75.3°C, significantly higher than for samples L20 and L30 (Table 5). This likely reflected the influence of storage temperature on thermal stability in L samples, suggesting greater heat stability for sample L40. When spray dried at the same inlet and outlet temperatures, samples stored at 20°C had significantly lower peak height values than those stored at 40°C (Figure 4, Table 5). The L samples stored at 20°C had significantly lower peak maximum and peak height values than all H samples. The DSC curves show similar trends of an endothermic decline in the heat flow down to the lowest registered value, likely representing the temperature at which denaturation was most apparent (Figure 4). The shoulder observed at the end of the return to baseline heat flow likely represents protein with higher denaturation temperatures, denaturing later. The results demonstrate that both higher spray-drying and storage temperatures induce a shift of the DSC results toward higher peak maximum temperatures and a higher heat flow curve.

BI and Hydroxymethylfurfural Content

The color coordinate values of the 3 LWPC samples from the same batch showed no significant differences. Figure 5 shows the BI values for the powders after storage.

Table 4. Effects of spray-drying and storage temperatures on the relative content of different whey proteins in whey protein concentrate (WPC80) powders as analyzed by capillary electrophoresis (mean $\pm$ SD, $n = 3$)

Sample ²	Relative content ¹			
	BSA (%)	CMP (%)	α -LA (%)	β -LG (%)
L20	3.8 ^{ab} $\pm$ 0.1	8.4 ^a $\pm$ 0.1	20.4 ^c $\pm$ 0.3	67.3 ^a $\pm$ 0.5
L30	4.0 ^b $\pm$ 0.2	8.0 ^a $\pm$ 1.1	19.8 ^c $\pm$ 0.2	68.3 ^a $\pm$ 1.0
L40	4.2 ^b $\pm$ 0.2	6.9 ^a $\pm$ 1.4	13.7 ^a $\pm$ 0.5	75.3 ^{de} $\pm$ 1.3
M20	4.1 ^b $\pm$ 0.4	7.2 ^a $\pm$ 0.5	18.8 ^{de} $\pm$ 0.2	69.9 ^{ab} $\pm$ 1.1
M30	3.7 ^{ab} $\pm$ 0.3	6.7 ^a $\pm$ 0.8	17.6 ^{cd} $\pm$ 0.5	72.0 ^{bc} $\pm$ 1.5
M40	3.7 ^{ab} $\pm$ 0.2	7.0 ^a $\pm$ 1.1	15.0 ^{ab} $\pm$ 0.9	74.2 ^{cde} $\pm$ 0.3
H20	3.5 ^{ab} $\pm$ 0.3	7.3 ^a $\pm$ 0.6	16.7 ^{bc} $\pm$ 1.0	72.5 ^{bcd} $\pm$ 1.0
H30	3.5 ^{ab} $\pm$ 0.1	6.7 ^a $\pm$ 0.3	16.6 ^{bc} $\pm$ 0.7	73.2 ^{cd} $\pm$ 0.4
H40	3.2 ^a $\pm$ 0.5	7.4 ^a $\pm$ 0.5	13.4 ^a $\pm$ 1.1	76.1 ^c $\pm$ 1.3

^{a-c}Within a column, means with differing superscripts differ significantly ($P < 0.05$).

¹Relative contents = peak area divided by total peak area. Profiles identified as BSA, caseinomacropeptide (CMP), α -LA, and β -LG.

²The WPC80 powders were spray dried at low (L), moderate (M), or high (H) inlet and outlet temperatures, then stored at 20°C, 30°C, or 40°C. See Table 1 for more specifications.

Increasing the storage temperature increased powder browning significantly, with the clearest trend observed in the L samples. The L40 and M20 samples had the highest (20.0) and the lowest (12.4) BI values, respectively (Figure 5). Sample L40 had a significantly greater BI than all the other samples, which highlights the pronounced effect of the storage temperature on browning. When samples were stored at the same storage temperature, the L and H samples showed significantly more browning than the M samples. Furthermore, the H20 and H30 samples had greater BI values than the L20 and L30 samples, respectively. This demonstrates that high (H samples) spray-drying inlet and outlet temperatures significantly increased browning for samples stored at 20 or 30°C.

The HMF results show the effects of storage temperature for the L samples corresponding to inlet and outlet temperatures at 180°C/77°C. After 61 d of storage, the peak area of HMF analyzed by capillary electrophoresis was significantly higher for the L40 sample (1.17 ± 0.01 mAU·s) than for the L20 (0.86 ± 0.02 mAU·s) and L30 (0.87 ± 0.01 mAU·s) samples. In addition, analysis of HMF for all samples stored at 20°C (at L, M, and H spray-drying conditions) showed that the M20 sample (0.72 ± 0.06 mAU·s) had lower HMF content than H20 (0.82 ± 0.05 mAU·s), but that both M20 and H20 had lower HMF than sample L20.

Correlation Plot and Analysis of Variance Interaction Tests

The correlation plot (Figure 6) and the results of the ANOVA interaction test provide further insight into the

Table 5. Mean values for peak maximum and peak height, analyzed using differential scanning calorimetry and comparing whey protein concentrate (WPC80) powders

Sample ¹	Peak maximum (°C)	Peak height (mW)
L20	74.3 $\pm$ 0.1 ^a	-0.58 $\pm$ 0.01 ^a
L30	74.6 $\pm$ 0.0 ^{ab}	-0.55 $\pm$ 0.01 ^a
L40	75.3 $\pm$ 0.2 ^d	-0.41 $\pm$ 0.02 ^{cd}
M20	74.6 $\pm$ 0.2 ^b	-0.54 $\pm$ 0.07 ^a
M30	74.7 $\pm$ 0.1 ^b	-0.51 $\pm$ 0.01 ^{ab}
M40	75.1 $\pm$ 0.1 ^{cd}	-0.43 $\pm$ 0.02 ^{cd}
H20	74.9 $\pm$ 0.2 ^{bc}	-0.46 $\pm$ 0.01 ^{bc}
H30	74.9 $\pm$ 0.1 ^{bc}	-0.44 $\pm$ 0.00 ^{bcd}
H40	75.1 $\pm$ 0.1 ^{cd}	-0.37 $\pm$ 0.01 ^d

^{a-d}Within a column, means with differing superscripts differ significantly ($P < 0.05$).

¹The WPC80 powders were spray dried at low (L), moderate (M), or high (H) inlet and outlet temperatures, then stored at 20°C, 30°C, or 40°C. See Table 1 for more specifications.

correlations between each response variable, as well as the interactions between spray-drying and storage factors. Correlations can be noted between BARDS gas volume and morphological responses. The gas volumes at 4 time points correlated positively with β -LG, whereas all 6 time points correlated negatively with BSA and positively with heat absorption (peak height). Furthermore, the correlations between α -LA, β -LG, calorimetric properties, and BI can be noted. In terms of interactions between spray-drying and storage factors, significant interactions were observed for the response variables BI, peak max, α -LA ($P < 0.001$), and β -LG ($P < 0.05$).

DISCUSSION

Elevated spray drying temperatures, corresponding to inlet and outlet temperatures at 197°C/94°C (M samples) and 214°C/111°C (H samples), were associated with pronounced delays in WPC80 rehydration most probably due to differences in particle morphology and protein modifications. As supported by Carr Index results, small and irregular particles and less dense packing behavior may have contributed to the observed negative effect on rehydration, including delayed wetting, water penetration, and sinking. Powder clumping and delays in early rehydration steps appeared to be associated with prolonged dissolution times. The H samples remained incompletely dissolved after 990 s. The insolubility index results indicated that solubility was not the rate-limiting step for these samples. The BARDS results suggested that there were sequential delays in the initial stages of rehydration. Wettability analysis showed faster wettability for samples spray dried at lower inlet and outlet temperatures at 180°C/77°C (L samples) than for M and H samples spray dried at higher inlet and outlet temperatures.

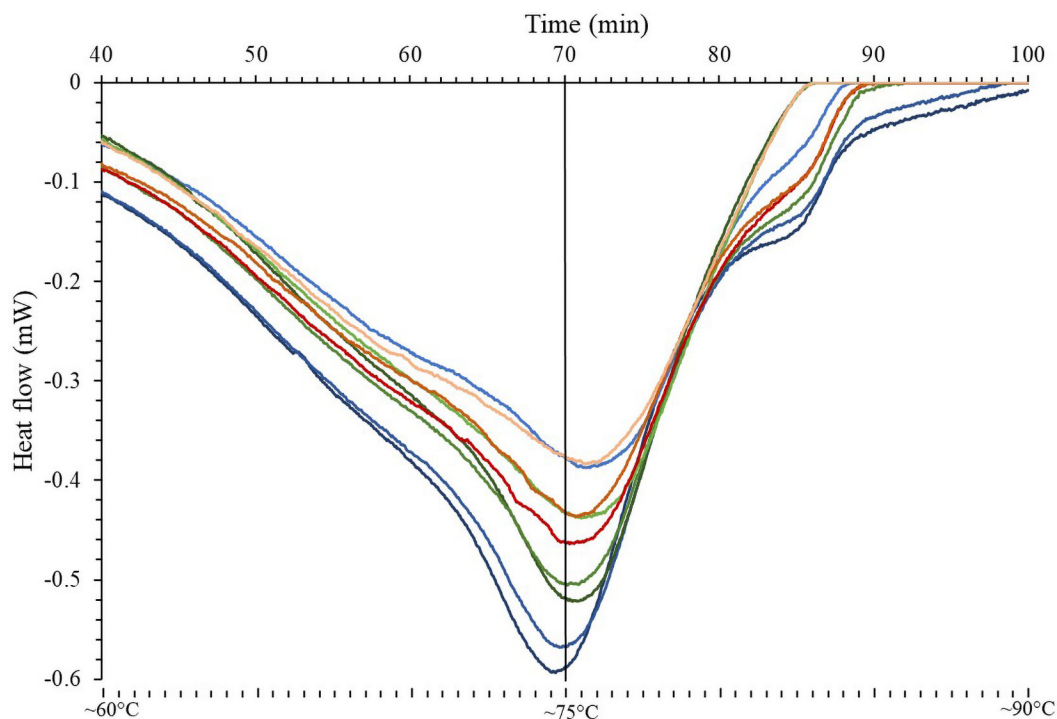


Figure 4. Nine representative differential scanning calorimetry curves from 40 to 100 min, comparing whey protein concentrate (WPC80) powder heat flow. Sample L20 = dark blue, L30 = blue, L40 = light blue, M20 = dark green, M30 = green, M40 = pale green, H20 = red, H30 = dark orange, and H40 = pale orange. The WPC80 powders were spray dried at low (L), moderate (M), or high (H) temperatures, and stored at 20°C, 30°C, or 40°C. See Table 1 for more specifications. Based on DSC analysis conditions across samples, the temperature of the sample was ~60°C at 40 min, ~75°C at 70 min, and ~90°C at 100 min.

Rehydration Properties: BARDS

During spray drying, gas vacuoles can form within particles as water evaporates causing pressure buildup and expanding the vacuole within the particle crust. Several authors have reported that this can result in particle breakage (Alamilla-Beltrán et al., 2005; Sadek et al., 2013; Sadek et al., 2014). The H samples had lower bulk density than the L and M samples, with the samples spray dried at high temperatures having small, irregularly shaped particles, consistent with particle breakage (Alamilla-Beltrán et al., 2005; Sadek et al., 2014; Han et al., 2022). Small and irregularly shaped dairy powder particles can also arise from other mechanisms, such as droplet size variation, particle agglomeration or shrinkage (Alamilla-Beltrán et al., 2005; Han et al., 2020, 2022). Han et al. (2020) demonstrated increased powder density with reduced particle size in infant formula powders. This contrasts with our findings of reduced powder density with reduced particle size. These results demonstrate that different trends can be observed for different materials.

Compared with the more regularly shaped particles in the L samples, the more irregularly shaped particles in the M and H samples had more concave regions, were

less rounded and were more elongated (Gausemel et al., 2025). Low bulk density values, which can indicate less densely packed particles (Fu et al., 2012), may help explain the high volumes of released gas and delayed wettability, sinking, and dissolution observed using BARDS and wettability analyses. These results were consistent with trends in the calculated BARDS gas volume AUC. The M and H samples released more gas compared with samples corresponding to lower inlet and outlet temperatures at 180°C/77°C (L samples). Although Barone et al. (2019) linked elevated gas release during BARDS analysis to the extensive presence of gas vacuoles in large particles, our findings demonstrate that small, irregularly shaped WPC80 particles also entrain notable gas volumes. This was consistent with the lower Carr Index values for the L samples, which are a proxy for less entrained air, compared with the M and H samples. Air entrained in powder can increase powder buoyancy and inhibit sinking due to lowered density (Ishak, 2015; Ren et al., 2025; Roy and Amamcharla, 2025).

The BARDS results in our study are consistent with previous reports (Vos et al., 2016; Barone et al., 2019; Wu et al., 2019). These findings emphasize the importance of particle morphology and entrained gas in interpreting BARDS analysis results. The H samples showed faster

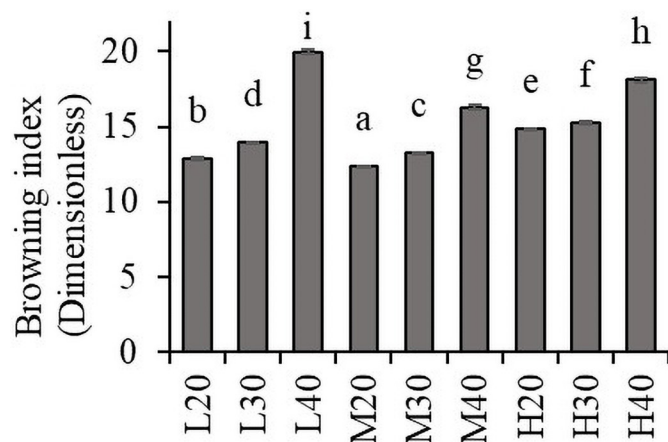


Figure 5. Mean values of the browning index ($n = 3$) for whey protein concentrate (WPC80) powders. The WPC80 powders were spray dried at low (L), moderate (M), or high (H) temperatures, and stored at 20°C, 30°C, or 40°C. See Table 1 for more specifications. Columns with different letters were significantly different (Tukey's HSD test, $P < 0.05$). Error bars represent $\pm$ SD.

initial gas release compared with the L and M samples. This was possibly due to greater availability of entrained air. However, delayed wetting and sinking were observed in the same samples, consistent with a negative influence of increased entrained air on rehydration.

Protein Modifications

Protein denaturation and cross-linking are known to inhibit powder rehydration (Schmid et al., 2014; Andersson et al., 2020; Ren et al., 2025). The differences in whey protein composition among the WPC80 samples in the present study were due to modifications that occurred during spray drying and storage, as all powders originated from the same batch of LWPC. Our results are consistent with the hypothesis that protein denaturation, possibly combined with protein cross-linking, contributes to inhibited rehydration of WPC80, although this was not directly measured. Outlet air temperatures were likely the driver of the observed heat-induced differences between spray-drying conditions, as outlet air temperature is the primary determinant of whey protein denaturation during spray drying (Anandharamakrishnan et al., 2007). Storage at elevated temperatures can result in further protein denaturation and protein modifications, as previously reported (Paul et al., 2022).

Whey protein denaturation is an important factor in determining the rehydration properties of whey protein powder (Ren et al., 2025). The composition of whey proteins reflects differences in sensitivity to thermal denaturation, with α -LA and BSA being less heat stable than β -LG (Bernal and Jelen, 1985; Ju et al., 1999).

In the present study, this difference in sensitivity may explain inversed patterns: Increased spray-drying and storage temperatures reduced the relative concentration of native α -LA and increased the relative concentration of β -LG, with the absolute content of native β -LG remaining less altered. In terms of relative concentration, this is shown as decreased levels of α -LA and increased β -LG, consistent with modification of the more heat-sensitive α -LA. The β -LG and BSA content was positively and negatively correlated, respectively, with gas volume (BARDS). In our results, higher relative concentrations of native α -LA and BSA, and lower relative concentrations of native β -LG, were associated with BARDS results indicating less inhibited rehydration. Elevated β -LG content correlated with increased thermal stability and reduced heat absorption, suggesting that modification of protein microstructure influenced calorimetric properties as previously reported by Ju et al. (1999). Considered together, the inverse patterns for α -LA and β -LG reflect their different thermal and chemical stabilities. Therefore, it is likely that whey protein modification could negatively affect rehydration behavior by altering properties of the protein DM.

The relative content of CMP in the L samples, corresponding to the lowest spray-drying temperatures (180°C/77°C inlet and outlet air), appeared to be more reduced during storage compared with M and H samples. It is hypothesized that this greater reduction may be related to these samples retaining a higher absolute content of CMP after spray drying. The content of CMP decreased more after 40°C storage than after 20°C or 30°C storage, indicating that 40°C storage was more effective in modifying CMP, although more research is needed to confirm this.

Lactosylation, as indicated by HMF and browning results, likely contributed to WPC80 protein modifications and possibly also inhibited rehydration. Compared with spray-drying temperatures, storage temperatures had a greater effect on browning (BI), especially for the L samples stored at 40°C. Greater protein denaturation and BI values for H samples compared with M samples were most likely due to more rapid water evaporation at the 214°C/111°C inlet and outlet air temperatures. Rapid evaporation was followed by higher particle surface temperatures in the later stages, during spray drying using high outlet air temperatures (Anandharamakrishnan et al., 2007). Powder browning (BI) and whey protein composition after storage were likely also influenced by moisture content and the presence of early Maillard reaction products after spray drying. For BI results, the interaction between spray drying and storage conditions likely reflects increased water availability. Because α -LA is more sensitive to lactosylation (Cardoso et al., 2018),

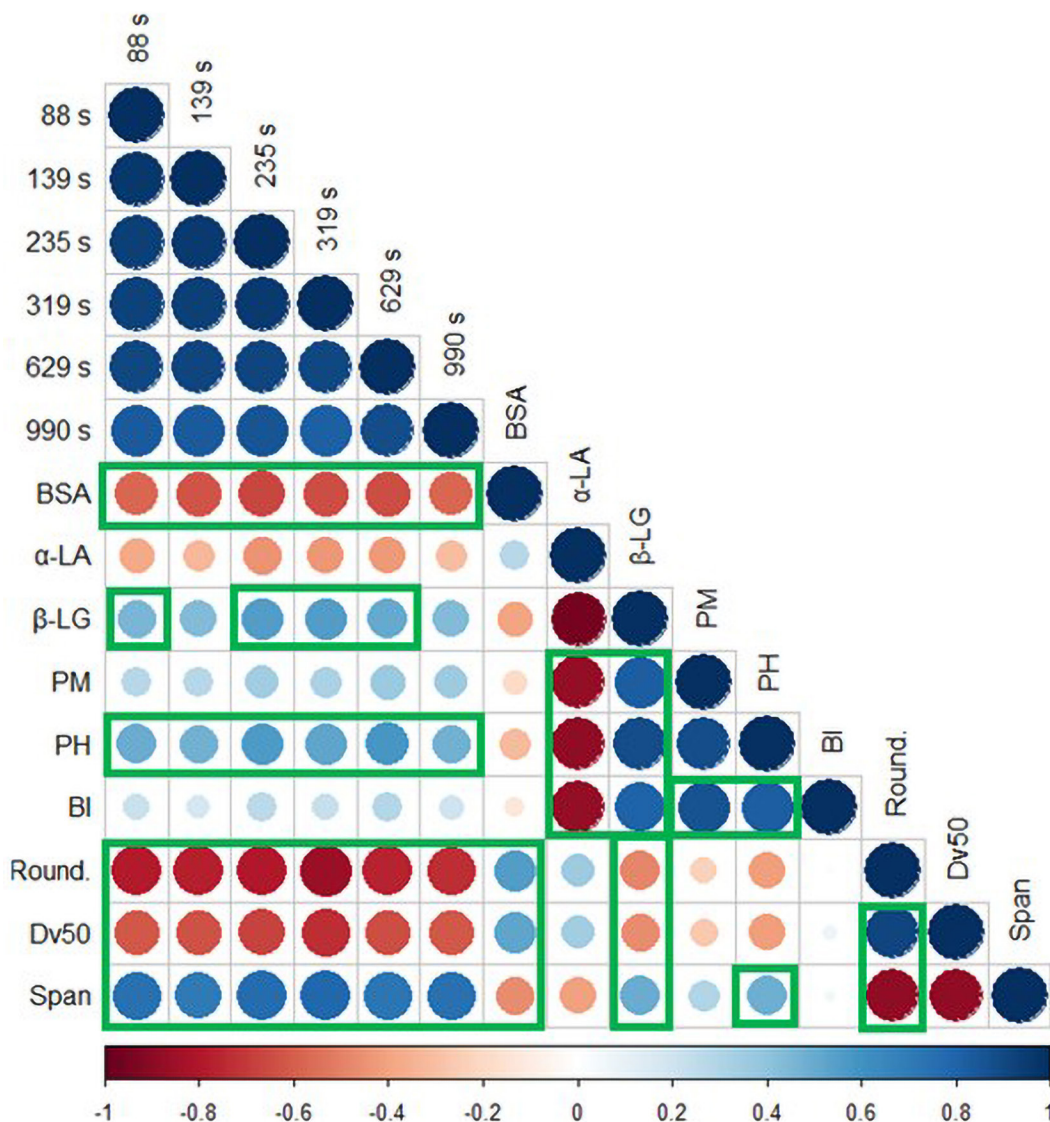


Figure 6. Correlation plot for measurement variables, excluding variables that did not show significant differences in a Tukey's HSD test ($P < 0.05$). Green frames indicate correlations between measurement variables with $P < 0.01$ and correlation coefficients of at least ± 0.458 , excluding correlations between values from the same measurement method. Codes: 88, 139, 235, 319, 629, and 990 s (gas volume at the indicated time). Capillary electrophoresis relative content values: BSA, α -LA, and β -LG. Calorimetric values: PM = peak maximum; PH = peak height. BI = browning index. Morphological responses: Round = roundness shape descriptor; Dv50 = median particle size. Span and Dv50 based on the laser diffraction particle size distribution.

this likely contributed to its reduction in relative content after storage at 40°C, and thus also the increase in relative content of β -LG. Correlations between BI, α -LA and β -LG therefore support that both denaturation and lactosylation contributed to the observed shifts in whey protein composition. Protein denaturation and lactosylation both likely contributed to reduced relative content of α -LA and CMP, whereas the relative β -LG content increased. The results are consistent with the hypothesized mechanistic link between modified whey protein composition and inhibited rehydration.

Several authors have linked lactosylation and browning with inhibited wettability and increased hydrophobicity of the surfaces of high-protein whey powder particles (Sun et al., 2015; Norwood et al., 2017; Burgain et al., 2023). Although the present study did not measure particle surface hydrophobicity, it was hypothesized that the reported mechanism of increased hydrophobicity of whey protein after heat exposure (Schmid et al., 2014; Andersson et al., 2020; Ren et al., 2025) would also apply to the present results. Based on this assumption, it was hypothesized that both protein denaturation and

lactosylation could contribute to the reduced wettability of WPC80 after storage at 40°C. The L samples retained more moisture and had higher water activity than M and H samples (Gausemel et al., 2025). Thus, lactosylation and browning were likely accelerated in samples spray dried at low temperatures due to more available water, especially when combined with elevated thermal activation energy during storage at 40°C (Norwood et al., 2017; Andersson et al., 2020; Paul et al., 2022). The elevated BI and HMF content for sample L40 strongly support Maillard-driven lactosylation and browning, consistent with the observed reduced relative content of CMP and α -LA. Maillard reactions likely also explain differences in browning and HMF values between samples spray dried at different temperatures and stored at 20°C. Sample L20 had the highest HMF of samples stored at 20°C, likely due to higher water content and accelerated Maillard reactions as described above. The H20 sample had more HMF than sample M20, likely due to higher thermal exposure during spray drying.

The DSC curves showed greater thermal stability and lower heat absorption for samples stored at 40°C, compared with 20°C. This is consistent with protein aggregation through cross-linking, which reduces the maximum energy required for denaturation (Ju et al., 1999). It is also consistent with the changes in protein composition discussed above, which suggests contributions of denaturation and lactosylation. Findings related to calorimetry and BI suggest protein cross-linking in L and M samples when stored at 40°C. Lactosylation-induced protein cross-linking could negatively affect rehydration, as cross-linking of whey protein may inhibit powder wettability and dispersibility (Andersson et al., 2020).

DM-Water Interaction

For powder to be wetted, the surface tension between water and DM must first be overcome, so that water can be absorbed onto particles. In the present study, it is hypothesized that the combined effects of protein denaturation and nonenzymatic browning on the hydrophobicity of the WPC80 particle surfaces may have inhibited dry matter-water interaction in WPC80, thus also inhibiting powder wetting, sinking and rehydration efficiency (Ren et al., 2025; Roy and Amamcharla, 2025).

The M and H samples showed persistent floating clumps during BARDS, consistent with reported film-like surface layers and water-repellent behavior in whey systems (Ji et al., 2016; Fitzpatrick et al., 2019; Ren et al., 2025). A recent review describes clumping as a challenge specific to whey protein powders (Ren et al., 2025), describing clumping as a limitation to powder dispersion, as hydrophobic films has been reported to form gel-like layers around nonhydrating domains. Such lay-

ers have been reported to remain intact even after sinking (Fitzpatrick et al., 2017; Wu et al., 2022; Ren et al., 2025). The BARDS profiles for M and H samples, corresponding to 197°C/94°C or 214°C/111°C inlet and outlet air, showed episodic clump submersion and gas release, delaying wetting and sinking. These samples had lower bulk density and more entrained air, explaining the higher released gas volumes. In contrast, L samples, corresponding to 180°C/77°C inlet and outlet air, were denser, clumped less and released less gas, which was eliminated more rapidly. Previous studies (Ji et al., 2016; Barone et al., 2019; Wu et al., 2019) support that larger particles contribute to enhanced wetting and water penetration, as well as reduced clumping. This effect is due to the lower surface area-to-volume ratio and the porous structure of intact agglomerated particles. The current study does not contradict that porous particles, such as agglomerated or instantized particles, can improve powder wettability by facilitating water penetration. This has been reported by Barone et al. (2019). Rather, our findings are consistent with the possibility that WPC80 powder rehydration is inhibited by combined negative effects of small particles, entrained air and impaired dry matter-water interaction.

Storage of L samples at 40°C inhibited rehydration after only 61 d, which is a relatively short storage time compared with the typical shelf life of WPC80, which is 1 to 2 yr. Sample H40 had the most inhibited rehydration properties of all samples. This was consistent with any negative contribution of the hypothesized increase in surface hydrophobicity after storage of samples at 40°C, possibly combined with any negative effect of small, irregularly shaped particles and entrained air.

Due to cost limitations, this study used only relatively high spray-drying temperatures and analyzed HMF only for a selection of treatments. A limitation of the study is the lack of process-level replication in the spray-drying step, which restricts the generalizability of the findings. In some cases, treatment effects were within the variation range, and these results are interpreted with caution. Minor seasonal variation in LWPC cannot be ruled out. These effects are expected to influence the absolute values more than the trends in the responses to spray-drying and storage conditions. Each spray-drying condition produced 9 to 10 kg WPC80 powder, making full replication infeasible within budgetary restrictions. All powder samples were derived from the same batch of homogeneous LWPC, and representative samples were obtained using a riffle splitter. Combined with a controlled spray-drying process, this ensured that properties of the baseline powder were largely determined by the 9 treatments rather than by uncontrolled variation.

Overall, the BARDS method proved effective in characterizing rehydration behavior of the powders, and the integrated, dynamic method provided a novel

framework for optimizing rehydration properties. The BARDS method can be used as a tool to evaluate the complete dissolution times for various purposes, such as avoiding insufficient or unnecessarily prolonged rehydration process steps.

CONCLUSIONS

Using BARDS, this study shows relationships between elevated spray-drying inlet and outlet and storage temperatures and inhibited WPC80 rehydration. This effect was associated with small and irregularly shaped particles, low bulk density, and protein modifications, which may have contributed to a hypothesized increase in particle surface water-repellent behavior. Storage at 40°C for 61 d was associated with further inhibition of rehydration, consistent with increased protein denaturation and lactosylation. Low spray-drying temperatures (180°C/77°C inlet and outlet) were associated with reduced browning and protein denaturation, and with larger, more regularly shaped particles. Combining low spray-drying and storage temperatures enhanced wetability and reduced sequential delays in rehydration stages. Future research should include spray drying at temperatures lower than 180°C/77°C.

NOTES

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Nonstandard abbreviation list: AU = absorbance unit; AUC = area under the mean curve; BARDS = broadband acoustic resonance dissolution spectroscopy; BI = browning index; CMP = caseinomacropeptide; DSC = differential scanning calorimetry; Dv50 = median particle size; HMF = 5-hydroxymethylfurfural; HSD = honest significant difference; k_e = rate constant for gas elimination; k_r = rate constant for gas release; L, M, and H = low, medium, and high spray-drying inlet/outlet temperatures, respectively; LWPC = liquid whey protein

concentrate; PH = peak height; PM = peak maximum; PSD = particle size distribution; tM = time point at which the maximum gas volume is reached; WPC80 = whey protein concentrate.

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