Relationship between whey protein nitrogen index of skim milk powder and the heat stability of recombined filled evaporated milk

Jianfeng Wu a,*, Chunxia Su a, Lorenz de Neve a, Ali Sedaghat Doost a, Karin De Grave b, Pieter Vermeir c, José C. Martins d, Paul Van der Meeren a

a Particle and Interfacial Technology Group (PaInT), Department of Green Chemistry and Technology, Ghent University, Ghent, Belgium
b Milcobol Dairy Corporation, Kallo, Belgium
c Laboratory for Chemical Analysis (LCA), Ghent University, Ghent, Belgium
d NMR-STRUCT Group, Ghent University, Ghent, Belgium

**A R T I C L E   I N F O**

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- Skim milk powder
- Whey protein nitrogen index
- Heat stability
- Recombined filled evaporated milk

**A B S T R A C T**

In this study, it was evaluated whether the whey protein nitrogen index (WPNI) of a range of skim milk powder (SMP) samples was correlated with the heat stability of the corresponding recombined filled evaporated milk (RFEM), as well as with the extent of lactosylation and changes in whey proteins (WPs). Pulsed field gradient NMR results showed that the diffusion coefficients of lactose in all samples varied only to a small extent, while the diffusion coefficients of the WP fraction ranged more widely. A lower heat stability of RFEM produced from SMP with a lower WPNI was found as compared with those with a higher WPNI: principal component analysis (PCA) and correlation analysis revealed that the decreased heat stability could be at least partly explained by the increased calcium solubilization induced by the lower pH of dispersions of SMP with a lower WPNI. Although it was claimed over the years that high-heat SMP (with a low WPNI) is beneficial to ensure a better heat stability of RFEM, it is clear from our results that a low WPNI of SMP alone was not sufficient to guarantee a better RFEM-heat stabilizing functionality.

1. Introduction

The manufacture of skim milk powder (SMP) typically involves a preheat treatment followed by evaporation and spray drying (Kelly & Fox, 2016). Among these, the preheat treatment is generally considered as the step most related to the functionalities of SMP, including its heat stability. The preheating may vary widely, ranging from 72 °C for 15 s, through 85 °C for 30 min to 120 °C for 2 min via direct or indirect heating using a variety of heat exchangers (Singh, 2007). More severe preheating (e.g. > 120 °C for >40 min) is even performed for some specific applications. One of the major effects of preheat treatment is the denaturation of whey proteins (WPs). The whey protein nitrogen index (WPNI), which is defined as the nitrogen quantity of native whey proteins per gram of powder, is widely used as an indicator for the severity of the heat treatment to which the SMP was subjected during its manufacture. Based on their WPNI, SMP samples can be classified into low-heat (LH, WPNI ≥6 mg/g), medium-heat (MH, 5.99 > WPNI ≥4.50 mg/g), medium-high-heat (MH-HH, 4.49 > WPNI ≥1.50 mg/g) and high-heat (HH, WPNI <1.50 mg/g) SMP (Wilcek, 1990). The heat classification based on the WPNI has been commercially used to provide general guidelines for various food applications. For example, HH SMP (WPNI <1.50 mg/g) with appropriate preheating was found to have an excellent thermal stability (Dumpler, Huppertz, & Kulozik, 2020; Newstead, Conaghan, & Baldwin, 1979; Singh & Tokley, 1990), and hence is considered suitable to produce heat stable recombined filled evaporated milk (RFEM) by homogenization of reconstituted SMP and fat. However, the validity of the SMP classification based on the WPNI has not been without controversy as the WPNI only reflects WP denaturation (Sharma, Jana, & Chavan, 2012; Williams, d’Ath, & Zisu, 2008). Besides WP denaturation, a large number of other reactions may occur during preheating, including the Maillard reaction between milk proteins and lactose, WP aggregation, association of whey proteins with casein micelles, dissociation of casein micelles and pH changes. Furthermore, many different heating regimes may be used to achieve a desired WPNI in the industry, such as various heating temperature-time profiles and residence time distributions (Murphy, Tobin, Roos, & Fenelon, 2013), which has a considerable impact on the heat-induced changes in milk. These complicated heat-induced changes via various
heating regimes during SMP manufacture could modify the heat stability of the SMP as well as of the derived RFEM to varying degrees.

It was claimed over the years that high-heat SMP, which is characterized by a low WPNI, is needed to improve the heat stability of (especially concentrated) reconstituted products made therefrom (Fox, 1981; Lin, Kelly, O’Mahony, & Guinee, 2018; Sikand, Tong, & Walker, 2010). However, how preheating of milk for powder manufacture enhances the heat stability of reconstituted powder produced from it has not been fully understood (Kelly & Fox, 2016). In this respect, Dumpler et al. (2020) mention that the used preheating conditions for milk are numbered in decreasing order of WPNI.

The aim of this study was to investigate the relationship between the WPNI of a range of commercial SMP samples and the heat stability of the derived RFEM emulsions. As protein glycation is known to improve their heat stability, the extent of the Maillard reaction and changes in the protein fraction in SMP with varying WPNI were investigated as well. To that end, commercial SMP samples with a wide range of WPNI (from 0.66 to 6.73 mg/g) were selected. Firstly, the native WP content and free lactose content of these SMP samples were measured. Moreover, the diffusion behavior of the lactose and of the whey protein fraction in the SMP was investigated using pulsed-field gradient NMR (pfg-NMR) in order to evaluate the degree of protein lactosylation and aggregation. Finally, the heat stability of RFEM produced from these SMP samples was determined based on particle size and viscosity measurements as a function of heating time. In order to evaluate the interrelationship between these various responses, principal component analysis (PCA) was performed.

2. Materials and methods

2.1. Materials

Ten commercial SMP samples with different WPNI (due to different pre-heating conditions during manufacture) were obtained from Milcobol Dairy Corporation, Kallo, Belgium. The chemical composition of the SMP samples provided by the supplier is shown in Table 1. The SMP samples are numbered according to their values of WPNI from high to low. BiPro (Davisco, Le Sueur, USA), containing 92.6% of protein, of which 85% was β-lactoglobulin, was used as whey proteins standard.

2.2. Methods

2.2.1. Determination of native whey proteins in SMP

As described previously (Manji & Kakuda, 1987), caseins and denatured whey proteins are precipitated from reconstituted SMP using NaCl, and then the native whey proteins content in the supernatant obtained upon ultracentrifugation was determined by a modified Lowry method (Schacterle & Pollack, 1973).

2.2.2. Measurement of free lactose content in SMP

SMP samples were dispersed in Milli-Q water to a final concentration of 10% (w/v). The SMP dispersion (1 ml) was transferred into a dialysis bag (Float-a-Lyzer G2, Cellulose Ester, molecular weight (Mw) cut-off 100–500 Da, Spectrum Europe, Breda, the Netherlands). The dialysis bag was placed into 100 ml Milli-Q water containing 0.02% NaN₃ as antimicrobial agent with a stirrer. In a preliminary experiment, the Float-a-Lyzer internal compartment was filled with 1 ml of 10% (w/v) lactose solution or 1 ml of 1% (w/v) whey protein isolate (WPI; with similar molecular weight as lactosylated proteins) solution, the Float-a-Lyzer was placed in 100 ml Milli-Q water, and the total carbon content of the outer solutions was measured at fixed time intervals until stable data were obtained. These results showed that lactose efficiently passed through this membrane (permeation efficiency = 99.8 ± 1.8%), while whey proteins were retained by the membrane (permeation efficiency = 1.6 ± 1.1%).

The free lactose content was determined using equation (1) via measuring the total carbon content of the external solution using a total organic carbon (TOC) analyzer (TOC-5000, Shimadzu, Japan) after 8 days of dialysis. All measurements were performed after 20 times dilution.

\[
\text{Content of free lactose} = \frac{C_{\text{lactose}} \times D \times V}{m_{\text{SMP}}} \times 100\%
\]

whereby \(C_{\text{lactose}}\) is the concentration of lactose calculated by the standard curve after 8 days of dialysis; \(D\) is the degree of dilution, i.e. 20 times; \(V\) is the volume of the outer solution, i.e. 100 ml; \(m_{\text{SMP}}\) is the mass of SMP, i.e. 100 mg.

2.2.3. Pfg NMR

As described previously (A’yun, Demicheli, de Neve, Wu, Balcaen, Setiowati et al., 2020), proton NMR measurements were performed on a Bruker Avance III 500 MHz spectrometer (Rheinstetten, Germany) equipped with a BBI probe with a maximum available magnetic field gradient strength calibrated at 50.2 G/cm. The measurements were performed at 25 °C. One dimensional (1D) proton (1H) spectra were measured using 16 transients with an interscan delay of 30 s and acquisition time of 4.37 s, and the spectral window was set as 15 ppm.

Diffusion measurements were conducted using a double stimulated echo pulse sequence with monopolar gradient pulses with a smoothed rectangular shape. The gradient pulse length (\(\delta\)) and diffusion delay (\(\Delta\)) was set at 3 ms and 300 ms, respectively. The diffusion coefficients were obtained by fitting the appropriate Stejskal-Tanner equation (mono-exponential function) to the peak integrals in function of gradient strength as below.

\[
I = I_0 e^{-\delta I - \gamma^2 D \Delta}
\]

I and \(I_0\) stand for the intensity attenuation at a given gradient strength (G) and zero gradient strength, respectively. D represents the diffusion coefficient, and \(\gamma\) the gyromagnetic ratio (2.68E8 s⁻¹ T⁻¹). The

<table>
<thead>
<tr>
<th>#SMP</th>
<th>Moisture (% w/w)</th>
<th>Fat (% w/w)</th>
<th>Protein (% w/w)</th>
<th>Total calcium (mg/100 g) *</th>
<th>WPNI (mg/g)</th>
<th>Heat Classification</th>
<th>Free lactose (% w/w) ±</th>
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<td>1164*</td>
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<td>LH</td>
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<td>LH</td>
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<tr>
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<td>1121*</td>
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<td>1063*</td>
<td>0.66</td>
<td>HH</td>
<td>49.6±</td>
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</table>

*aDifferent letters indicate significant differences between the values (P < 0.05).
diffusion delay $\Delta$' equals ($\Delta-0.602^\circ$s).

SMP samples were prepared by dispersing SMP in D$_2$O containing 2 mM sodium acetate (as internal standard) to obtain a concentration of 10 mg/ml.

2.2.4. Soluble and total calcium content in SMP dispersions

The supernatant of 20% (w/v) of reconstituted SMP was obtained by ultracentrifugation at 20 °C and 120,000 g for 1 h. An aliquot of SMP (0.25 g) and supernatant (2.5 ml) was digested in glass vials containing 3 ml nitric acid in a single reaction chamber (SRC) microwave oven (Ultrawave, Milestone Inc., USA), respectively. The SRC was pressurized with argon (40 bar), and the following microwave heating program was applied: 20 min of ramp and hold for 10 min at temperatures in the range from 190 to 250 °C. The digested samples were cooled and diluted to 50 ml with Milli-Q water. Finally, the digested samples from the SMP and supernatant of reconstituted SMP were analyzed for calcium using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES; IRIS Intrepid II XSP, Thermo Scientific, USA) to determine the total and soluble calcium content in SMP dispersions, respectively.

2.2.5. Heat stability of RFEM

2.2.5.1. RFEM preparation. SMP was dispersed in 0.02% NaN$_3$ solution, and sunflower oil was then added to prepare RFEM emulsions containing 16.5% (w/w) SMP and 6.5% (w/w) oil (Kasinos, Karbakhsh, & Van der Meeren, 2015). The mixtures were pre-homogenized by using an IKA Ultra-Turrax TV45 (Janke & Kunkel, Staufen, Germany) at 24000 rpm for 1 min at room temperature and subsequently were homogenized using a Microfluidizer 110S (Microfluidics Corporation, Newton, MA, USA) at 55 °C. The microfluidizer was operated at a compressed air pressure of 0.2 MPa, corresponding to a liquid pressure of 28 MPa, for 2 min (corresponding to about 12 passes through the interaction chamber). The pH of RFEM emulsions was measured at ambient temperature.

2.2.5.2. Heat stability test. The heat stability test of RFEM samples at their original pH was performed at 120 ± 4 °C for up to 30 min in an oil bath based on the method developed by Kasinos et al. (2015). The heat stability test of RFEM emulsions derived from SMP 4 and SMP 10 was also done after pH adjustment to 6.6 and 6.5 using a series of HCl or NaOH solution with concentration of 0.1 M. These samples after pH adjustment were stored overnight before heat treatment for ionic equilibrium.

2.2.5.3. Particle size and viscosity measurement. As described by Kasinos et al. (2015), the particle size of the RFEM before and after heating was determined using a Mastersizer 3000 (Malvern Instruments Ltd, Malvern, UK). Viscosity measurements of RFEM were performed using a Programmable LV-DV-II+ Viscometer (Brookfield, Stoughton, MA, USA) at a temperature of 20 °C at different shear rates. Spindle SC4-18 and SC4-34 were used to measure the viscosity of samples with a liquid and gelled consistency, respectively.

2.2.5.4. Principal component analysis (PCA). PCA was applied to the data of viscosity and particle size before and after heating to investigate the similarities between the heat stability of RFEM samples produced from SMPs with different WPNI. Similarity maps can be drawn by projection on the plane which is defined by each PC using SPSS 19.0.

2.2.6. Statistical analysis

The single factor variance analysis (one-way ANOVA) and the bivariate analysis of SPSS 19.0 was used to analyze the significance and Pearson’s correlation coefficients of the data.

3. Results and discussion

3.1. Native whey protein content in SMP

As shown in Fig. 1, the WPNI of SMP samples was strongly correlated to the content of native whey protein with a determination coefficient of 0.98 ($P < 0.01$), indicating that the WPNI of SMP samples could reliably reflect the native whey protein content in SMP. The WPNI of SMP is generally considered as an indicator of the denaturation of whey proteins. WPNI was previously reported to have a strong positive correlation with the native-like β-lg and total whey protein concentration (Patel, Anema, Holroyd, Singh, & Creamer, 2007).

3.2. Free lactose content in SMP

As protein glycation may mediate the heat stability of SMP, the residual amount of free lactose was determined in order to quantify the Maillard-induced lactosylation (Wu, Li, A Yun, Sedaghat Doost, De Meulenaer & Van der Meeren, 2021). Table 1 reveals that the difference in free lactose content between the SMP samples was much less distinct as compared to their difference in WPNI. Moreover, the reproducibility of the method was limited: the standard deviation of repeated measurements was more than 1%. As a further consequence, no significant difference was found among SMP1 to 3, SMP 5 to 8 and SMP 10 ($P > 0.05$). The relatively high standard deviation was most probably due to variations in the dialysis process between replicate experiments.

3.3. Pfg NMR

In pfg NMR, the diffusion behavior of lactose can be determined. As lactose is a small molecule, it will diffuse rapidly. On the other hand, upon covalent binding to proteins, the lactose is forced to move together with these proteins. As the latter are much bigger structures, they will diffuse much more slowly. Hence, an additional mode with slower diffusion coefficient is expected upon protein glycosylation. Similarly, whey protein aggregation will lead to a smaller diffusion coefficient of the proteins. The diffusion coefficient follows from the decay constant of the echo decay curve. Hence, the echo decay follows a mono-exponential decay if all molecules move with the same diffusion coefficient, whereas a bi-exponential decay will be obtained if some molecules move slowly whereas others move fast. Hence, the Maillard reaction and protein aggregation in SMP can be estimated via the determination of diffusion coefficient of lactose or WP fraction.

A typical 1D $^1$H spectrum of SMP is shown in Fig. 2. Based on previous studies (A’yun et al., 2020; Setiowati, Vermeir, Martins, De Meulenaer, & Van der Meeren, 2016) by comparing the 1D $^1$H spectra of a mixture of milk components (proteins and lactose) and individual components, the signal observed at 3.4–4.0 ppm is ascribed to lactose,
and the signal at 0.6–1.0 ppm belongs to the WP fraction. The echo decay of the lactose and the WP signal in SMP samples with varying WPNI (i.e., SMP 1, 7 and 10) is shown in Fig. 3. The echo decay of both lactose and WP in all SMP samples was characterized by a purely mono-exponential decay by fitting equation (2) with determination coefficients of at least 0.99 and 0.97 for lactose and WP, respectively. This implied that all lactose, as well as all WP molecules in each of the SMP samples had the same diffusion coefficient, and hence that both protein lactosylation and aggregation were of minor importance. The diffusion coefficients of lactose and of WP with 95% confidence interval were compared in Fig. 4. The difference between the experimentally determined diffusion coefficients of lactose in the different SMP samples was limited with the average values ranging from $3.80 \times 10^{-10}$ m$^2$/s, while the diffusion coefficients of the WP fraction ranged more widely from $4.74 \times 10^{-11}$ m$^2$/s. The diffusion coefficients of lactose among all SMP samples except SMP 8 exhibited no significant difference ($P > 0.05$). On the other hand, the diffusion coefficient of the WP fraction in SMP 4 was significantly higher than in the other samples ($P < 0.05$), while the diffusion coefficient of the WP fraction in SMP 10 was significantly lower than in SMP1 to 6 and SMP 8 ($P < 0.05$). It was previously reported that the diffusion coefficient of pure lactose and of whey proteins (WPI) was $3.88 \times 10^{-10}$ m$^2$/s and $7.17 \times 10^{-11}$ m$^2$/s, respectively (A’yun et al., 2020). Hence, the diffusion coefficients of all samples in this study were comparable with the value for free lactose, whereas the diffusion coefficients of the WP fraction in all samples were (slightly) lower than in WPI.

The results of the diffusion coefficient of lactose were generally in agreement with the free lactose content results in Table 1. It can be inferred that the extent of lactosylation of milk proteins during manufacturing SMP samples was limited, regardless of the severity of the heat treatment to which the milk was subjected during manufacturing. Van Boekel (1998) reported that the lactose content dropped in milk upon heating at 120 $^\circ$C for 5 min, and the lactose isomerization more importantly contributed to the decrease in lactose as compared to Maillard reaction (Van Boekel & Berg, 2005). In the study of Milkovska-Stamenova and Hoffmann (2016), the number of lactosylation sites did not follow the expected increasing trend from raw milk to UHT milk and infant formula. Considering that preheating is normally less severe than the conditions used in the above-mentioned studies (e.g., considering 120 $^\circ$C for 5 min or UHT treatment), the extent of binding between lactose and milk protein in SMP is thought to be limited.

Different populations of WP-containing structures that differed in their molecular weight and thus diffusion properties were present in different reconstituted SMP samples. The lower diffusion coefficient of the WP fraction in SMP 10 indicated the presence of large WP-structures upon more severe heat treatment during manufacturing which impaired the diffusion velocity. Upon heat treatment, the whey protein fraction may be involved in complicated interactions, such as self-aggregation or aggregation between whey proteins and casein micelles, which may lead

Fig. 2. 1D $^1$H spectrum of a dispersion containing 10 mg of SMP1 per ml of D$_2$O and 2 mM of Na-acetate.

Fig. 3. Diffusion echo decay of the proton signal between 3.4 and 4.0 ppm (lactose; A) and between 0.6 and 1.0 ppm (whey protein; B) for SMP1, 7 and 10 with WPNI of 6.73, 2.96 and 0.66 mg/g, respectively.

Fig. 4. Comparison of the diffusion coefficients of the proton signal between 3.4 and 4.0 ppm (lactose) and between 0.6 and 1.0 ppm (whey protein (WP)) between SMP samples with varying WPNI; different letters indicate significant differences between the values of each component (lactose or WP) ($P < 0.05$).
to larger WP-structures and thus adversely affect the diffusion velocity of the whey proteins. It was found that the residual concentration of various whey proteins in the serum was about 14–39% after preheating at 120 °C for 120 s (for HH SMP) as compared to the unheated milk, whereas >96% of whey proteins were present in the serum after heating at 72 °C for 15 s (for LH SMP) (Lin et al., 2018). This indicated that preheating for HH SMP induced much more formation of WP-casein complexes than LH SMP. Apart from WP-casein aggregation, whey protein aggregation may occur upon preheating; β-lg aggregation was found to increase with preheating temperature from 100 °C to 120 °C (Oldfield, Taylor, & Singh, 2005). The higher diffusion coefficient of WP fraction in SMP 4 compared with other LH SMP samples (i.e. SMP 1 to 3 and SMP 5) was possibly due to differences in heating regime during manufacturing, such as the temperature-time-profile and the residence time distribution during preheating, resulting in a different size distribution of WP-containing structures although a comparable WPNI was obtained.

3.4. pH of RFEM before heating and soluble calcium content in reconstituted SMP

As the pH plays an important role in the heat stability of RFEM according to the pH-HCT (heat coagulation time) profile of milk (Singh, 2004), the pH of the RFEM emulsions prior to heating was measured. As shown in Fig. 5, the pH values of RFEM derived from SMP 1 to 5 with a higher WPNI (i.e. pH 6.59–6.61) were significantly higher than those of RFEM produced from SMP 6 to 10 with a lower WPNI (i.e. pH 6.55–6.58) (P < 0.05). Moreover, the RFEM produced from SMP 10 with the lowest WPNI exhibited the lowest pH among all samples. It is not surprising that more severe heat treatment during manufacturing SMP for a lower desired WPNI induces a higher extent of pH decrease. The pH decrease could be due to the heat-induced degradation of lactose, and/or the precipitation of soluble calcium phosphate as Ca₃(PO₄)₂ with the release of H⁺ (Fox, Unlacey-Lowe, McSweeney, & O’Mahony, 2015).

Considering the well-known effect of the aqueous phase pH on the calcium solubilization from casein micelles, the soluble calcium content in the serum of aqueous SMP dispersions was measured as well. Fig. 6 shows the relationship between the soluble calcium content in aqueous dispersions of the different SMP samples and the pH of the corresponding RFEM emulsions. As expected, higher soluble calcium levels were found in SMP dispersions with lower pH; linear regression yielded a correlation coefficient of −0.56 (P < 0.1). This indicated that the lower pH of aqueous dispersions that were intensely heated was at least partly responsible for the increased soluble calcium content in the serum phase due to calcium solubilization from the colloidal calcium phosphate that is present within the casein micelles.

3.5. Heat stability of RFEM

3.5.1. Particle size and viscosity

The particle size analysis and viscosity results of RFEM emulsions stabilized by SMP samples with different WPNI before and after heating at 120 °C are shown in Fig. 7, respectively. It was observed that all RFEM emulsions had a comparable D₄,₃ and consistency coefficient before heating (i.e D₄,₃ < 0.4 μm; consistency coefficient <5 mPa s). The heat stability of the RFEM stabilized by SMP 1 to SMP 5 exhibited a similar trend: a significant increase in both D₄,₃ and consistency coefficient was found after heating for 20 min (P < 0.05), and a coagulum was observed visually after 30 min of heating (D₄,₃ > 10 μm; consistency coefficient >100 mPa s). The heat stability of RFEM samples stabilized by SMP 6 to SMP 10 was worse as compared to the RFEM stabilized by SMP1 to SMP5: 20 min of heating was sufficient to induce heat coagulation. Among these, the heat stability of the RFEM stabilized by SMP 10 was the worst with visually observed coagulation after 10 min of heating.

Generally, these results indicated that the RFEM emulsions stabilized by SMP with a higher WPNI (e.g. SMP 1 to 5) exhibited a better heat stability, while the heat stability of the RFEM emulsions stabilized by SMP with a lower WPNI (e.g. SMP 10) was less good. The heat stability of RFEM reflects the effects of the preheating history of the milk from which the SMP had been prepared, such as the temperature-time profile and the residence time distribution during preheating. It has been claimed in some literature that SMP with more severe pre-heat treatment and hence a lower WPNI under some empirical conditions is more favorable for applications where a higher heat stability is desired (Fox et al., 2015; Kelly & Fox, 2016; Sharma et al., 2012). However, the experimental evidence is limited so far. Lin et al. (2018) compared the heat stability of skim milk concentrates with different preheat treatments, i.e. 72 °C for 15 s (low heat) versus 120 °C for 120 s (high heat). It was reported that an improved heat stability of skim milk concentrates with high heat treatment as compared to that with low heat treatment was observed in a specific pH range, but it was observed that the heat coagulation time (HCT) of high-heat skim milk concentrate (total solid 15%) at pH 6.6 was shorter than that of low-heat skim milk concentrate. Furthermore, skim milk concentrates or reconstituted concentrated milk is discussed in most studies. However, the homogenization used to produce RFEM emulsions could mediate the pH-HCT profile and make the effects of preheating more complicated (Fox et al., 2015). McCrae and Muir (1991) found that the heat stability of recombined milk decreased with increasing severity of homogenization at pH 6.7, but weaker effects of homogenization were found at pH conditions far from 6.7.

The heat-induced destabilization of RFEM is a direct result of the
heat-induced unfolding and association of non-adsorbed globular whey proteins, such as β-lactoglobulin, with interfacial and other non-adsorbed proteins to initiate large flocculates and/or aggregates via non-covalent and/or disulfide bonds (Liang et al., 2017). It was found that an appropriate pre-heat treatment could impart sufficient denaturation of whey proteins to avoid pseudo-coagulation by crosslinking of casein micelles by denatured whey proteins in evaporated milk (Dumpler et al., 2020; Newstead et al., 1979; Singh & Tokley, 1990). However, preheating may be performed under different heating conditions to achieve a desired WPNI, such as various combinations of heating temperature and duration and using a range of heat exchange techniques, including plate heat exchangers, spiral heat exchangers wrapped around the tubes in the evaporator itself or direct steam injection (Murphy et al., 2013). Different heating regimes could have an important impact on the heat-induced interactions and the consequent heat stability. It was suggested that a worse heat stability upon severe preheating (a low WPNI) could be observed as a result of insufficient formation of WP-casein micelle complexes, but extensive WP denaturation during preheating (Early, 1998). Furthermore, in our previous study (Wu, Chen, Sedaghat Doost, A’yun & Van der Meeren, 2020), it was also found that prolonged heating of SMP had a negative effect on the corresponding RFEM emulsion, which probably was related to the complicated effects of κ-casein dissociation. Besides, the rate of increase in heating temperature might affect the protein interactions (Kelly & Fox, 2016). Indirect heating with a slower heating rate induced more WP-WP aggregates and a higher level of WP denaturation as compared to direct heating with a fast heating rate, which favors the formation of WP-casein micelle complexes.

Combining the results of the pH of the RFEM before heating and their heat stability, the worse heat stability of the RFEM derived from SMP with a lower WPNI could be related to the pH shift induced by more severe preheat treatment during manufacture. It was reported previously that the maximum heat stability of concentrated milk was around pH 6.6, and the HCT could drop dramatically if the pH increased or decreased by 0.05 (Fox et al., 2015; Singh, 2004). As observed in Fig. 5, the pH values of the RFEM emulsions produced from SMP with a lower WPNI (e.g. SMP 6 to 10) became lower than pH 6.6, which could have a detrimental impact on their heat stability.
3.5.2. Principal component analysis

The data of particle size and consistency coefficient before and after heating at 120 °C were subjected to PCA. The loading plot in Fig. 8 shows how strongly each characteristic (PS0 to PS30 and V0 to V30) influences the two major principal components (PC) through the projected values of a vector on each PC. The characteristics of particle size (PS10, PS20 and PS30) and viscosity (V10, V20 and V30) after heating all strongly influence PC1 which accounts for 65.46% of the total variation, whereas PC2 (21.77%) is characterized by the particle size and viscosity before heating (PS0 and V0). Hence, PC1 reflects the heat sensitivity. Fig. 8 reveals that SMP samples with different WPNI were well separated in the score plot (similarity map). According to the most important principal component (PC1), the SMP samples 1 to 5 with a higher WPNI presented negative score values from −0.32 to −0.20, whereas SMP 6 to 8 with an intermediate WPNI had a score close to zero. SMP 9 and 10 with a lower WPNI were characterized by a higher PC1 score than all others, i.e. 0.36 and 1.10 for SMP 9 and 10, respectively. Hence, it is possible to characterize the differences in heat stability between RFEM emulsions prepared using SMP samples with different WPNI by a PCA similarity map.

Table 2 shows the correlation between WPNI, PC1 and pH of the RFEM emulsions: the PC1 score and pH of the RFEM emulsions was highly negatively and positively correlated with the WPNI of the SMP used, respectively (P < 0.01), while PC1 was negatively correlated to the pH of the RFEM emulsions (P < 0.05). The latter correlation suggested that the decreased pH observed when using SMP with lower WPNI was (at least partly) responsible for the increased heat sensitivity of these emulsions.

3.5.3. Effect of pH and soluble calcium on the heat stability of RFEM

As PCA indicated a strong correlation between the pH prior to heating and the heat sensitivity of RFEM emulsions, the heat stability of RFEM emulsions derived from the SMP samples with the lowest and highest PC1 (heat sensitivity) at their original pH (i.e. SMP 4 and SMP 10) was compared at the same pH, i.e. pH 6.60 and 6.50. As shown in Fig. 9, after 20 min of heating at 120 °C, the particle size of the RFEM emulsions produced from SMP 4 and SMP 10 was significantly different (P < 0.05; 1.01 μm versus 79.9 μm) at their original pH (i.e. pH 6.61 and 6.55, respectively). With pH adjustment to a same level, the discrepancy in the particle size of emulsions derived from SMP 4 and SMP 10 after heating was less distinct, i.e. 2.23 μm versus 41.9 μm at pH 6.60, and 15.3 μm versus 91.0 μm at pH 6.50, although significant differences were still found (P < 0.05). Moreover, an increased particle size of emulsions produced from both SMP 4 and SMP 10 at a lower pH (i.e. pH 6.50) after heating was observed as compared to the original pH and pH 6.60. It was previously reported that concentrated milk with 20% (w/w) total solids exhibited the highest heat stability around pH 6.60 according to the pH-HCT profile, whereas a decreased heat stability was observed as the pH deviated from this optimal pH (Lin et al., 2018; Singh, 2004).

This result confirmed that the lower pH observed when using SMP with lower WPNI was responsible for the increased heat sensitivity of these emulsions to a certain extent. However, adjusting the pH to similar values when using different SMP samples could only partly reduce the differences in heat stability.

To better understand the mechanism behind the correlation between the pH prior to heating and the heat stability of RFEM emulsions, the soluble calcium content in SMP dispersions was considered. The latter is generally considered to be affected by the pH, and may have an important impact on the heat stability of milk (Lin et al., 2018). The results of a correlation analysis between the soluble calcium content and WPNI, PC1 and pH of the RFEM emulsions is shown in Table 2, whereby the pH of the RFEM emulsions was found to be negatively correlated with the soluble calcium content in SMP dispersions (P < 0.1). This indicated that a lower pH caused an increased soluble calcium content in SMP dispersions due to calcium solubilization. Hence, from a mechanistic point of view, the decreased heat stability of RFEM emulsions derived from SMP with lower WPNI was thought to be at least partly due to the increased calcium solubilization as a further consequence of their lower pH.

4. Conclusions

In this study, the relationship between the WPNI of a range of SMP samples and the heat stability of the corresponding RFEM emulsions was investigated. The results based on ten selected SMP samples (with a range of WPNI values from 0.66 to 6.73 mg/g) indicated that RFEM stabilized by SMP with a higher WPNI was more heat stable as compared to SMP with a lower WPNI under the conditions applied for RFEM preparation. PCA revealed that PC1 (heat sensitivity) was negatively correlated with the WPNI (R = −0.81) and the pH of the RFEM emulsions prior to heating (R = −0.71). The latter correlation suggested that the decreased pH of RFEM using SMP with lower WPNI was partly responsible for the increased heat sensitivity of these emulsions. Furthermore, correlation analysis revealed that the decreased heat stability can be at least partly explained by the increased calcium solubilization induced by the lower pH.

Whereas several authors claimed that high-heat SMP (characterized by a low WPNI) is needed to have a better heat stability of reconstituted products therefrom (Fox et al., 2015; Kelly & Fox, 2016; Sharma et al., 2012), our results clearly indicated that a low WPNI alone was not sufficient to guarantee a high RFEM heat stability. As several processes occur simultaneously during heating of (concentrated) dairy products, a full understanding of the underlying mechanisms of the effect of preheating on the heat stability of RFEM is still lacking (Dumpling et al., 2020). Hence, more research is needed to explore the fundamental processes that occur during preheating of milk and to develop alternative indices for SMP classification that can more accurately reflect the changes mediating the heat stability during its manufacturing.

CRediT authorship contribution statement

We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

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