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Structural changes of milk proteins during heating of concentrated skim milk determined using FTIR

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ABSTRACT

This work aimed to establish the impact of total solids (TS) concentration (9, 17 and 25%) of raw skim milk on the conformational properties of proteins during heating up to 121 °C. Conformational changes were determined using Fourier transform infrared spectroscopy and correlated to physicochemical properties of the system. The results showed high total solids dependence in rearrangement of β-sheet of β-lactoglobulin. Heat denaturation of whey proteins was concentration and temperature dependant. The micelle appeared to undergo temperature dependant dissociation that induced redistribution of helical and loop structures. The conformational changes were further evaluated with principal component analysis and normalising for a concentration effect for all samples. The most affected structures were located in the region 1620–1655 cm^{-1} including intra- and inter-molecular β-sheets, α-helix/loop structures and randomly distributed structures. These findings may assist in predicting the heat stability of concentrates.

1. Introduction

Unconcentrated milk can withstand high temperatures applied during a sterilisation process. On the other hand, concentrating milk often results in several system modifications which may reduce heat stability of milk proteins, leading to partial or complete coagulation during sterilisation, sediment formation and age gelation (Huppertz, 2016). Therefore, to predict the stability of concentrated milk, it is important to know how the concentrated systems transition and behave during concentration with regard to their structural and physicochemical changes. In other work we specifically observed how increase in total solids affected particular components of the system (Markoska, Huppertz, Grewal, & Vasiljevic, 2018). An increase in total solids was accompanied by a decrease in pH, increase in ionic strength with a concomitant slight redistribution of ionic species between the serum and micellar phases, which also affected conformational properties of the proteins. Though an extensive body of literature covers heat stability of unconcentrated milk (Fox & Morrissey, 1977; Huppertz, 2016; McCrae & Muir, 1995; Singh, 2004), less is known about the concentrated milk systems.

Heat treatment, depending on its intensity, can denature whey proteins and destabilise casein micelles. In concentrated milk, denaturation of whey proteins is retarded at elevated temperatures due to greater solids concentration in the system hindering mobility of the particles and obstructing the space (Anema, 2000; McKenna & O'Sullivan, 1971). In addition, the casein micelle in concentrated pre-treated milk is destabilised during heat treatment mainly due to dissociation of κ-casein from the surface (Anema & Klostermeyer, 1996; Anema & Li, 2003; Anema, 1998; Dumpler, Wohlschläger, & Kulozik, 2017; Nieuwenhuijse, Sjollema, Van Boekel, Van Vliet, & Walstra, 1991), which is responsible for steric and electrostatic stabilisation of the micelle (Huppertz, 2013). However, a fundamental understanding of heat-induced changes of milk proteins is needed as they appear instrumental in all instabilities during downstream processing. Moreover, to predict stability of concentrated milk during processing and storage, it is also important to have a complete picture of the changes from the first processing step that is concentration.

In our previous study, FTIR was successfully used as a tool for evaluating conformational rearrangements of milk proteins during a concentration process (Markoska et al., 2018) and prediction of

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stability during processing and storage of UHT milk (Grewal, Huppertz, & Vasiljevic, 2018; Grewal et al., 2017a). In this study, we extended our investigations in the application of FTIR technique to map heat-induced conformational changes of proteins in concentrated milk systems. The fingerprinting would not only provide greater understanding of how proteins and other milk constituents rearrange during heating potentially leading to aggregation and gelation but also ascertain applicability of this method for in-line process monitoring. Hence, the current work aimed to establish major conformational changes of milk proteins during heating of raw concentrated skim milk under commercially applicable conditions and relate them to physicochemical properties of the concentrated milk, thereby forming a basis for developing predictive models in terms of storage stability of heat treated concentrated milk systems.

2. Materials and methods

2.1. Sample preparation

Fresh raw milk was collected from Murray Goulburn Cooperative Co. Ltd (Laverton North, Victoria, Australia). Raw skim milk was prepared for experimental purposes by fat separation and addition of sodium azide (0.01%) for prevention of bacterial growth as described by Markoska et al. (2018). Three types of samples, containing 9, 17 or 25% (w/w) TS, were prepared using a rotary evaporator (Rotavapor® R100, John Morris Scientific, Deepdene, Victoria, Australia) at 55 °C under vacuum. The required concentration was reached within 2 h (Markoska et al., 2018).

Each sample was divided into six 10 mL aliquots for heat treatment and seventh aliquot was kept as a control. The samples were placed in an oil bath set at 121 °C and one sample was removed when the temperature reached 75, 85, 95, 100 or 110 °C, i.e., with 0 s holding time, whereas the final sample was kept at 121 °C for 2.6 min. The calculated average heating rate was 18.5 °C min^{-1}. Every sample after heating was immediately submerged in an ice bath. Separation of the micellar and serum phase of the milk was performed as described by Grewal et al. (2018) by centrifugation at 21 °C for 1 h at 100,000× *g* using a Beckman Ultra L-70 centrifuge (Beckman Coulter, Australia Pty. Ltd, Gladesville, NSW, Australia).

2.2. Particle size and zeta potential (ζ)

The size distribution and zeta potential of unheated and heated samples were measured immediately after heating using a Zetasizer-Nano ZS (Malvern Instruments, Malvern, UK). Samples were diluted in simulated milk ultrafiltrate (SMUF) (Jenness, 1962) in a ratio of 1:100. The refractive index for casein micelles and SMUF was set up at 1.57 and 1.34, respectively. Three measurements from two different batches of milk were conducted for each sample.

2.3. Determination of mineral content

Calcium (Ca), magnesium (Mg), phosphorus (P) and sodium (Na) were determined in whole samples and serum phase using an inductively coupled plasma emission spectrometer (ICP-AES) sequential plasma spectrometer (ICP-9000 system, Shimadzu Corporation, Kyoto, Japan), following the method of Grewal, Chandrapala, Donkor, Apostolopoulos, and Vasiljevic (2017b).

2.4. Sodium dodecyl sulphate polyacrylamide gel electrophoresis

Whole samples and serum phase were analysed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) to study protein partioning between the serum and micellar phases following a previously described method (Grewal et al., 2017b). The analysis was performed under non-reducing and reducing conditions using β-mercaptoethanol as a reducing reagent. Every analysis was carried out in duplicate from two different batches of milk.

2.5. Fourier transform infrared spectroscopy

Immediately after cooling of heated samples, they were analysed for the effects of the treatments on the secondary structure of proteins using a PerkinElmer Fourier transform infrared (FTIR) spectrometer (Frontier, PerkinElmer, Boston, MA, USA) in the range of 4000 to 600 cm^{-1} with a resolution of 4 cm^{-1} and averaging 16 scans for each spectrum as described by Grewal et al. (2017a). Spectragryph software (version 1.2.7, Oberstdorf, Germany) was used for calculation of second derivative spectra in the region 1700–1600 cm^{-1} (Amide I) for greater resolution of the peaks. Spectra were smoothed to remove the vibrations resulting from the noise of derivation and for better identification of the effects. They were normalised for protein concentration as previously described by Militello et al. (2004).

2.6. Statistical analysis

To evaluate that changes in the FTIR spectra were significant to result in classification of samples based on concentration factor and heat treatment, the Principal Component Analysis (PCA) was applied. This technique presented an overview of all information in the data by generating a set of principal components (PCs) as coordinated axes with minimum loss of information. The score plots in PCA presented groupings of different samples while the loading plots identified the wavenumbers were identified which had high loading i.e. contributed the most in classifying the samples into the different groups. For the analysis, all the FTIR spectra were exported to Unscrambler software (version 9.8; CAMO AS, Trondheim, Norway) and processed as described elsewhere (Grewal et al., 2017a).

General linear model (GLM) and Tukey's Studentised Range (HDS) test was also used for data analysis. All the results were arranged in a split block design with the concentration as the main plot and the heat treatment as the subplot. The overall design was replicated on two occasions, which served as a block, using Statistical Analysis System (SAS). $P < 0.05$ was considered as the level of significance.

3. Results

3.1. FTIR analysis of conformational changes in milk proteins during heating

This study observed structural changes in milk proteins as a function of heat treatment and total solids concentration. The conformational properties of the proteins were observed using FTIR in the spectral region between 1700 and 1600 cm^{-1} (amide I), which mainly corresponds to C=O stretching of proteins (Kher, Udabage, McKinnon, McNaughton, & Augustin, 2007). The obtained spectra of milk samples at all concentrations clearly indicated the impact of heating on protein conformation resulting in variations of peak intensities observed at 1700–1695 (aggregated β-sheets), 1663 (β-turns), 1653 (α-helix/loops), 1645 (random structure), 1632 (intramolecular β-sheets) and 1620 (intermolecular β-sheets) and 1609 cm^{-1} (side chains) (Grewal et al., 2017a).

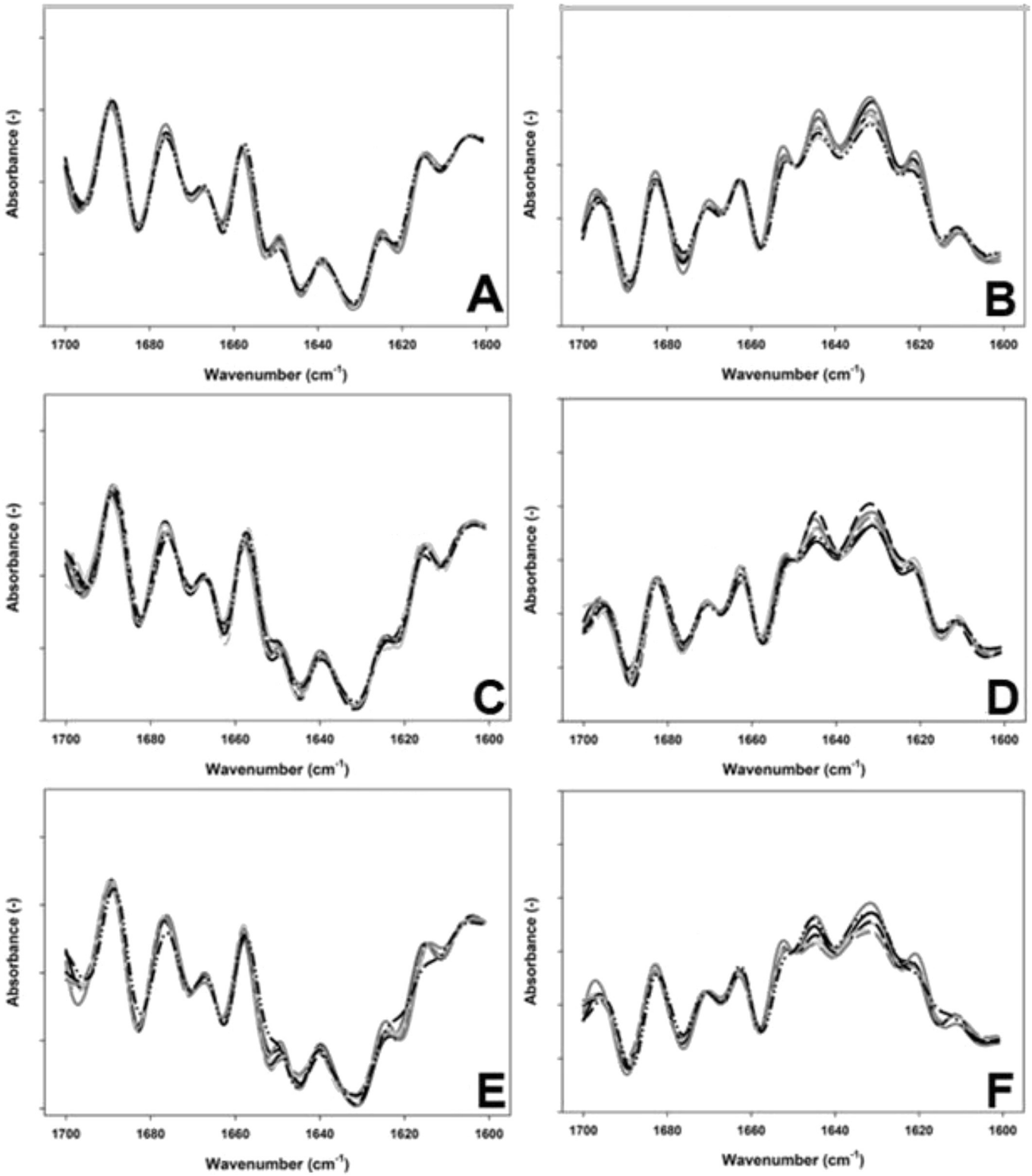

Fig. 1. Second derivative of the FTIR spectra (A,C,E) and concentration-normalised spectra (B,D,F) of samples containing 9% (A,B), 17% (C,D) or 25% (w/w) TS (E,F) heated up to 121 °C. The samples were assessed at: ——, 75 °C; ——, 85 °C; – – –, 95 °C; – – –, 100 °C; – – –, 110 °C; — · ·, 121 °C.——, control.

The pattern of change in secondary structure varied with the total solids concentration of milk.

In unconcentrated milk, heating at 75 and 85 °C resulted mainly in slight variations in β-sheets (Fig. 1A). More intense variations started at 95 °C with increase in the intensity of intermolecular β-sheets (1620 cm^{-1} and 1698 cm^{-1}). After prolonged heating at 121 °C, intermolecular β-sheets (1620 cm^{-1}) decreased, followed by slight increase in β-turns (peak 1663 cm^{-1}) and random structure (1645 cm^{-1}). In the concentration-normalised spectra the effect of temperature was even more pronounced (Fig. 1B). Hence, it was

confirmed that β-sheets (1620–1635 and 1680 cm^{-1}), random coil and α-helix (1645 and 1653 cm^{-1}) underwent substantial structural rearrangements when temperature rose from 95 to 121 °C. This was further confirmed by PCA analysis which separated samples based on heat treatment with PC1 and PC2 explaining 82 and 10% of the variance, respectively (Fig. 2A,B). The samples heated above 95 °C were separated from the other samples along the direction of PC1 with high loading for region 1618–1640 cm^{-1} indicating rearrangement of β-sheets at the assessed temperatures.

Heating 17% TS samples up to 121 °C was characterised by large variations in the peak intensity assigned to α-helical structure (1653 cm^{-1}) (Fig. 1C). When temperature increased from 75 to 100 °C, the peak intensity declined persistently. At 100 °C, this peak completely diminished, however, it reappeared after heating at 121 °C. In a similar fashion, the intensity of a peak assigned to

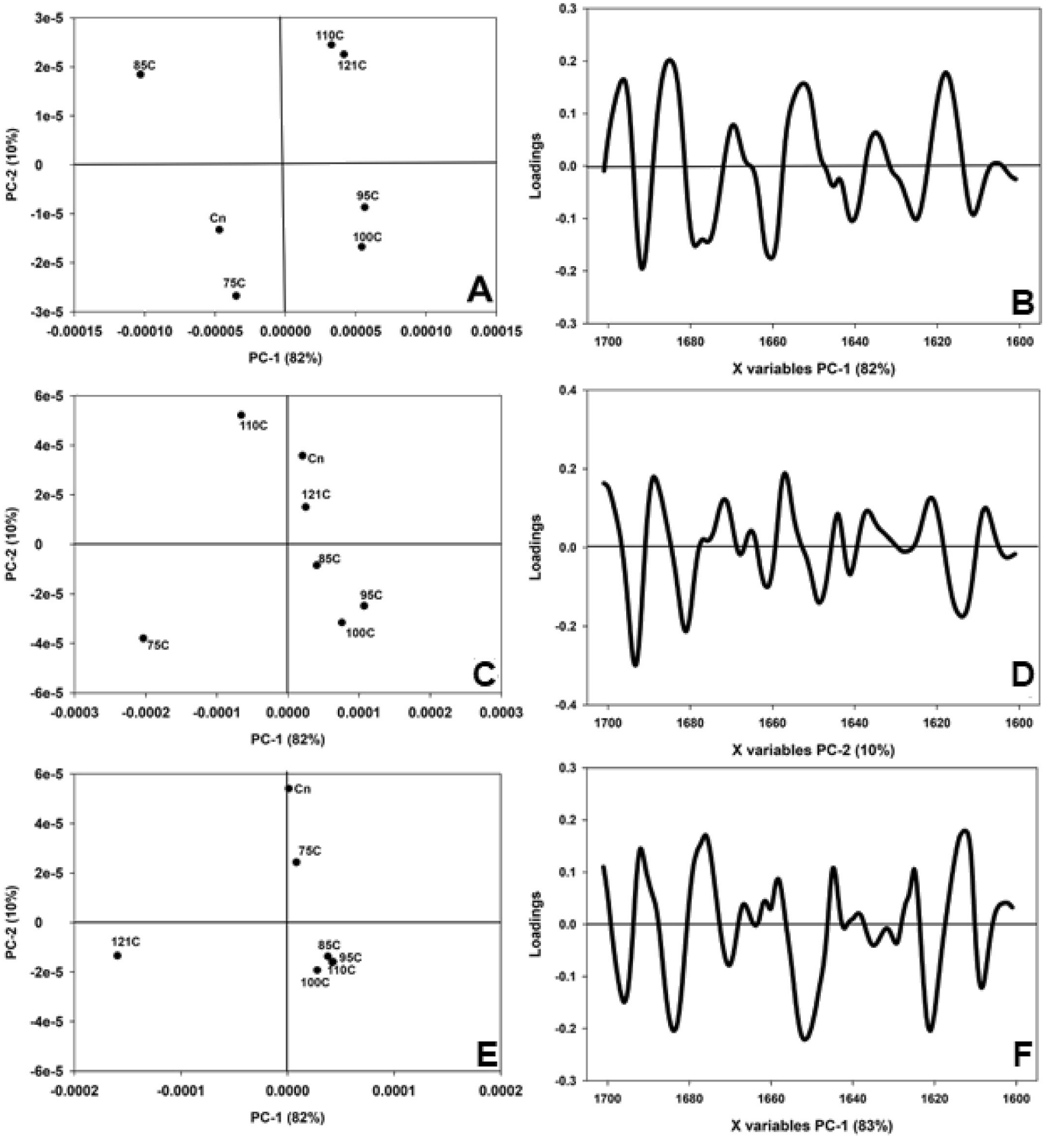

Fig. 2. Principal component scores (A,C,E) and loading plots (B,D,F) for Amide I region for heated skim milk with 9 (A,B), 17 (C,D) or 25% (w/w) TS (E,F).

intermolecular β-sheets (1620 cm^{-1}) gradually decreased as temperature increased up to 100 °C and then it reappeared after 121 °C, to almost original state. Decrease in intermolecular β-sheet and helical structure at 100 °C was concomitant with increased formation of turns (1663 cm^{-1}) and unordered structures (1645 cm^{-1}). Thus, in contrast with unconcentrated samples, 17% TS samples exhibited increase in unordered structure when heated up to 100 °C. At temperatures above 100 °C, the trend was reversed with reappearance of α-helical and β-sheet structures. The observations were further confirmed by PCA analysis which separated the samples based on heat treatment with PC1 and PC2 explaining 85 and 10% of the variance, respectively (Fig. 2C,D). The loading plot indicated that samples heated from 75 to 100 °C were separated along PC2 with lower loading in the region 1620–1640 cm^{-1} and intense loading of a peak at 1695 cm^{-1} attributed to aggregates containing intermolecular β-sheets. The unheated samples and the samples heated at 110 and 121 °C showed a high loading for α-helix, confirming the earlier observation of reappearance of this structure. In addition, the observed trend was further confirmed by concentration-normalised spectra exhibiting maximum structural changes appeared at 95 and 100 °C (Fig. 1D).

The spectra obtained from the most concentrated milk samples (25% TS) indicated that heating affected the secondary structure of proteins more extensively in comparison with other samples (Fig. 1E). Upon heating up to 121 °C, peak intensities shifted to more random structures (1645 cm^{-1}) at the expense of defined structures; i.e., a rearrangement in intermolecular β-sheets (1620 cm^{-1}) and α-helix (1653 cm^{-1}). PCA also separated samples treated at 121 °C along PC1 (explaining 83% of the variance) with high loadings associated with intermolecular β-sheets and loop structures (Fig. 2E,F). In addition, in concentration-normalised spectra the temperature effect was more pronounced when temperature rose above 85 °C resulting in intense deviations in a region of 1610–1653 cm^{-1} (Fig. 1F), especially with shifting of intermolecular β-sheets at high temperatures.

3.2. Physiochemical changes in heated milk with altered solids level

Heating of skim milk resulted in a significant ($P < 0.05$) increase of particle diameter only in samples with greater total solids content and at particular temperatures (Table 1). In unconcentrated samples, particle size did not show significant change and it was within a previously reported range (McMahon & Oommen, 2013). At 17% TS, the particle size remained fairly unaltered up to 110 °C, but beyond this temperature it increased significantly ($P < 0.05$) indicating extensive aggregation at 121 °C. More concentrated samples (25% TS) resulted in an increase in casein micelle size noticeable around 85 °C, but major increase in the particle size ($P < 0.05$) took place at and above 110 °C.

Table 1
Particle size diameter (nm) of concentrated raw milk samples containing 9, 17 or 25% total solids (TS) during heating and after sampling at indicated temperatures and holding time.[a]

TS (w/w; %)	Average particle size (nm)						
	Control	Temperature/holding time (°C min^{-1})					
		75/0	85/0	95/0	100/0	110/0	121/2.6
9	150 ^{aA}	149 ^{aA}	160 ^{aA}	158 ^{aA}	167 ^{aA}	159 ^{aA}	146 ^{aA}
17	164 ^{aA}	171 ^{aA}	169 ^{aA}	175 ^{aA}	177 ^{aA}	187 ^{aA}	414 ^{bB}
25	166 ^{aA}	165 ^{aA}	182 ^{aA}	217 ^{aA}	204 ^{bA}	392 ^{bB}	358 ^{bB}

[a] The results are means of six measurements from two different batches of milk; means in a row with different superscript lowercase letters and in a column with different superscript uppercase letters differ significantly ($P < 0.05$; SEM, 21.7).

In our previous work we showed that ζ-potential does not change significantly with increase in TS (Markoska et al., 2018). However, during heating, ζ-potential did change (Fig. 3). Surface potential of particles in the unconcentrated sample initially became more negative, from −15.6 to −17.4 mV at 95 °C ($P < 0.05$), followed by a significant decrease to −14.4 mV at 110 °C, remaining relatively constant until the end of the heating protocol. Heating concentrated samples exhibited different trends, which could be attributed to different protein interactions and changes in mineral balance leading to modifications of surface charge. For example, ζ-potential of the 17% concentrated raw skim milk during heat treatment initially decreased ($P < 0.05$) from −16.7 mV at 75 °C to −14.9 mV at 100 °C. Further heating apparently resulted in creation of particles with a significantly ($P < 0.05$) more negative ζ potential, almost equal to that at the start of the heating. On the other hand, ζ-potential of particles in the highly concentrated system (25% TS) experienced a significant increase from its initial value of −15.5 mV down to −17.6 mV at 110 °C. Prolonged heating at 121 °C did not result in a substantial change of the surface potential.

The mineral balance in milk represents equilibrium between minerals in the soluble and colloidal phases. The concentration of minerals (Ca, Mg, Na and P) was measured in the supernatant of the treated samples. As shown in Fig. 4, Ca and Mg were affected the most by heating, followed by P, while Na appeared unaffected (Fig. 4D). As an obvious trend, serum Ca, Mg and P gradually decreased ($P < 0.05$) with increasing temperature of heat treatment for samples at 9 and 17% TS. However, at 25% TS, a decrease was observed with increasing temperature up to 100 °C, above which a slight increase was observed, which could be related to minerals associated with increased levels of non-sedimentable casein, as discussed later.

Non-reducing SDS-PAGE of the whole samples revealed the presence of aggregates on the top of every gel (Fig. 5). The intensity of these bands increased with rise in both temperature and total solids concentration. Under reducing conditions, these aggregates were not observed. The main proteins involved in these aggregates were β-lactoglobulin (β-LG) and α-lactalbumin (α-LA), as well as κ-casein (κ-CN), which is already present in S-S linked oligomers in unheated milk (Fig. 5B,D,F).

The distribution of individual caseins varied between the serum and micelle (Fig. 6). Heating of unconcentrated milk clearly showed that whey proteins (α-LA and β-LG) were the most affected as their concentration decreased substantially with a rise in temperature (Fig. 6A,B). This reduction appeared to be mainly due to interactions with the casein micelle and subsequent separation by ultracentrifugation. After heating, some of these proteins still remained in the serum as part of aggregated whey protein-κ-CN complexes, which were soluble but not large enough to sediment in the pallet (Fig. 6A). Concentrations of κ-, α_S- and β-CNs appeared to remain constant in the serum when the temperature increased from 75 °C to 110 °C (Fig. 6B). Prolonged heating at 121 °C resulted in reduction of their concentration in the supernatant (Fig. 6B).

In comparison with unconcentrated milk, heat stability of whey proteins in the sample with 17% TS appeared greater as indicated by almost unchanged band intensity in the SDS patterns as the temperature increased up to 100 °C (Fig. 6C). Above this temperature, these proteins were more involved in disulphide-linked aggregation, which resulted in their complete incorporation into large aggregates when the temperature reached 121 °C (Fig. 6C,D). In addition, at 75 °C, the concentration of all caseins decreased in the serum (Fig. 6D). Further temperature rise to 85 °C produced an intense increase of the bands assigned to κ-CN in the supernatant, which can be related to its heat induced dissociation from the casein micelle (Anema & Klostermeyer, 1997). An additional rise in temperature minimised the dissociation of β- and α_S-CNs as they

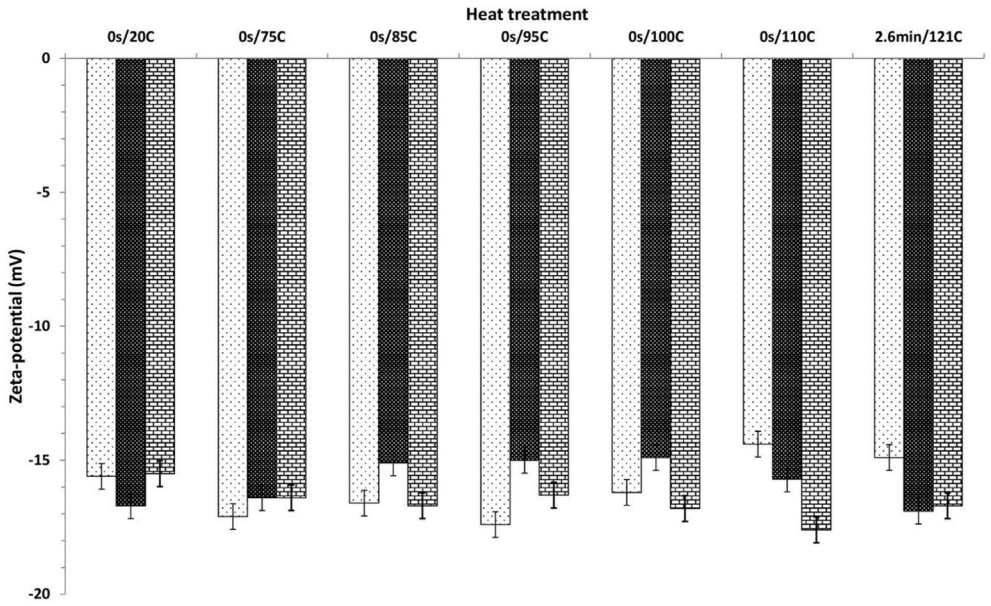

Fig. 3. Zeta potential (mV) of concentrated raw milk samples containing (▨) 9% (▩), 17% or (▤) 25% (w/w) total solids during heating and after sampling at indicated temperatures. The results are means of six measurements from two different batches of milk.

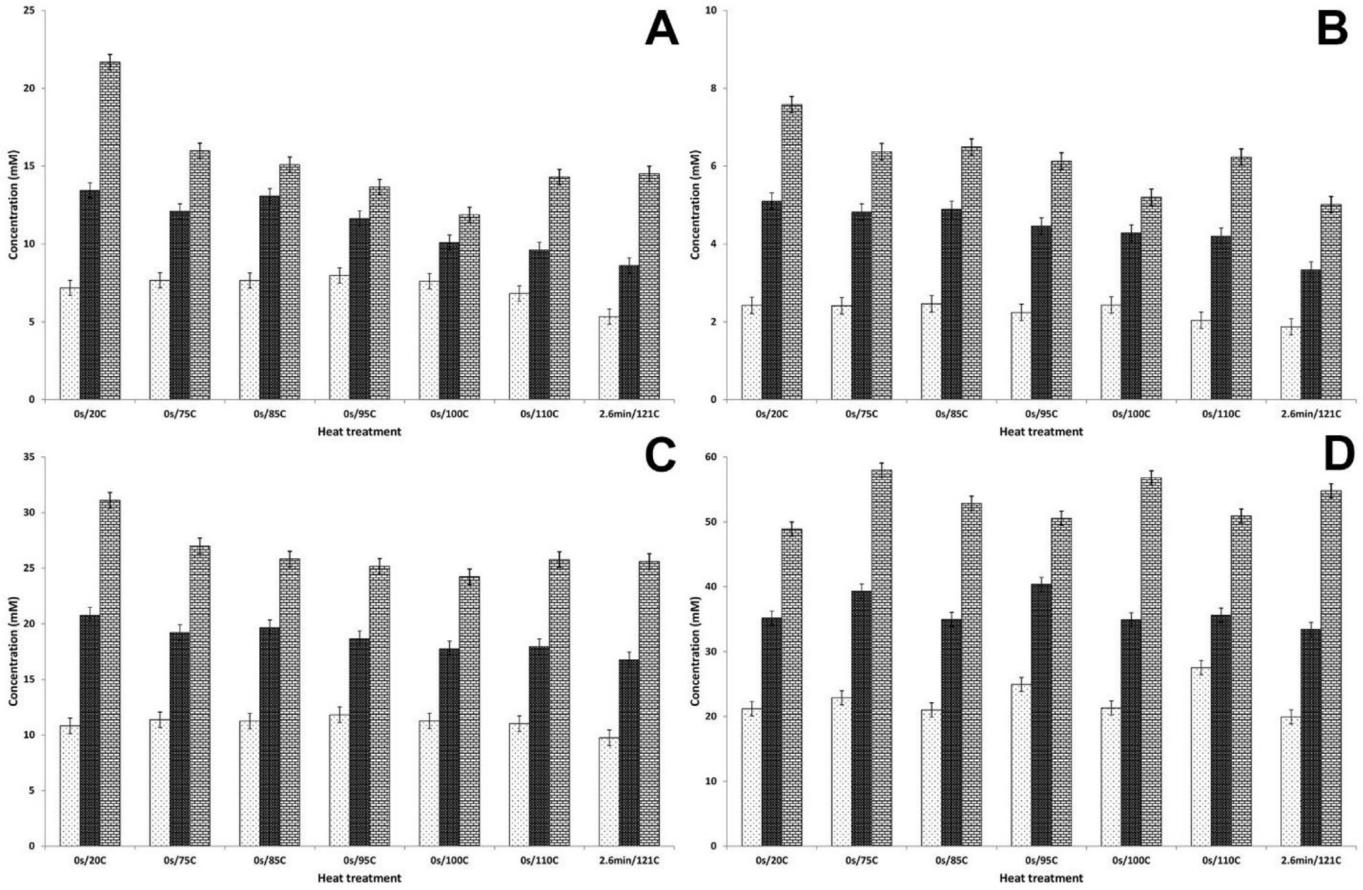

Fig. 4. Mineral concentration in the serum phase (mM) of three samples containing (▨) 9% (▩). 17% or (▤) 25% (w/w) TS determined upon heat treatment under indicated conditions. The results presenting calcium (A); magnesium (B); phosphate (C); and sodium (D) are means of four measurements from two batches of milk.

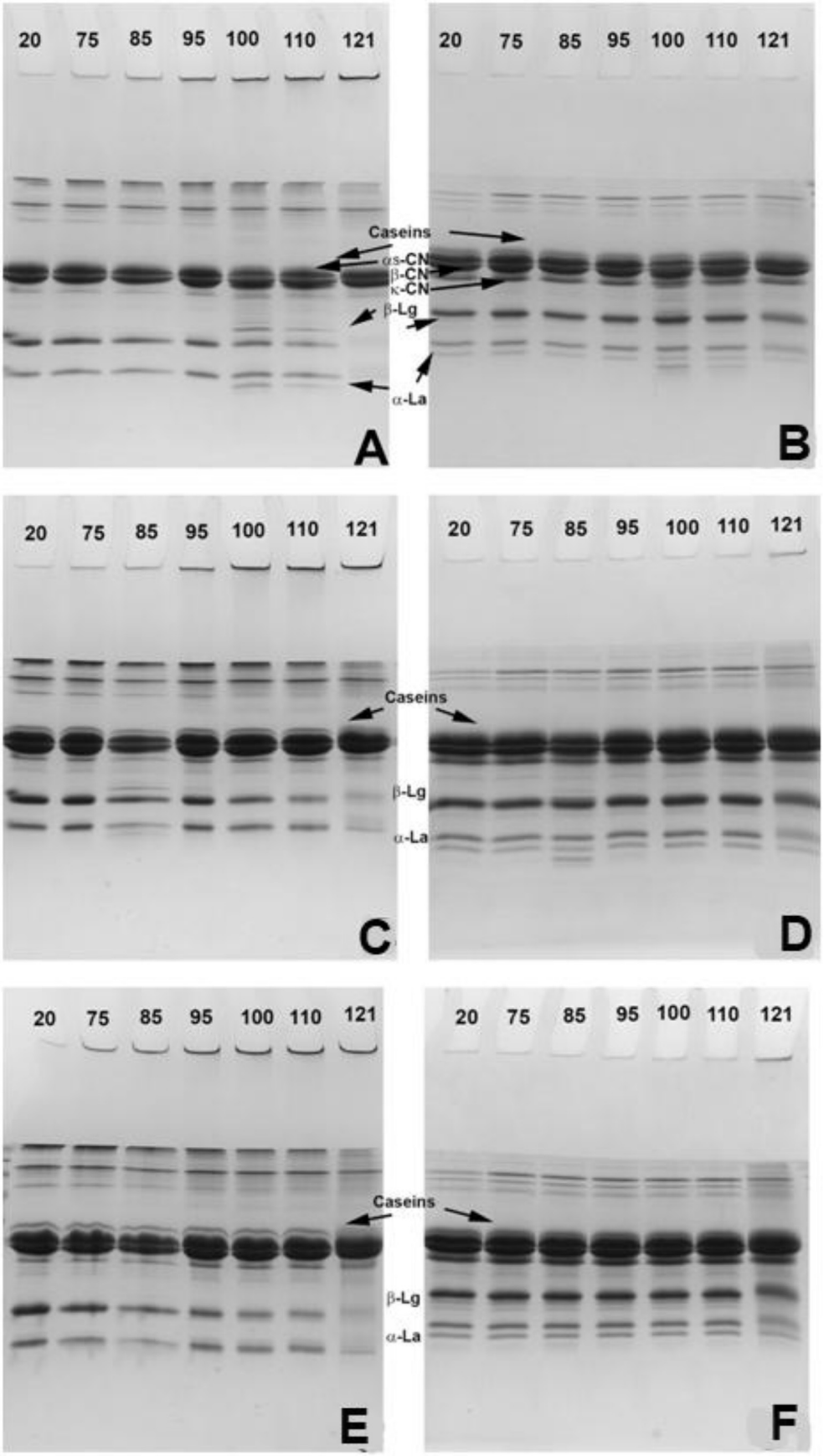

Fig. 5. SDS PAGE of bulk milk samples heated to 121 °C and sampled at 10 °C increments from 75 °C containing 9% TS (A, B), 17% TS (C, D), and 25% (w/w) TS (E, F). Where A,C,E are non-reduced gels and B,D,F are reduced gels. The first six lanes contain control and samples obtained during heating with 0 s holding time at a particular temperature (75–110 °C). The last lane (121 °C) contains the sample heated to and held at this temperature for 2.6 min.

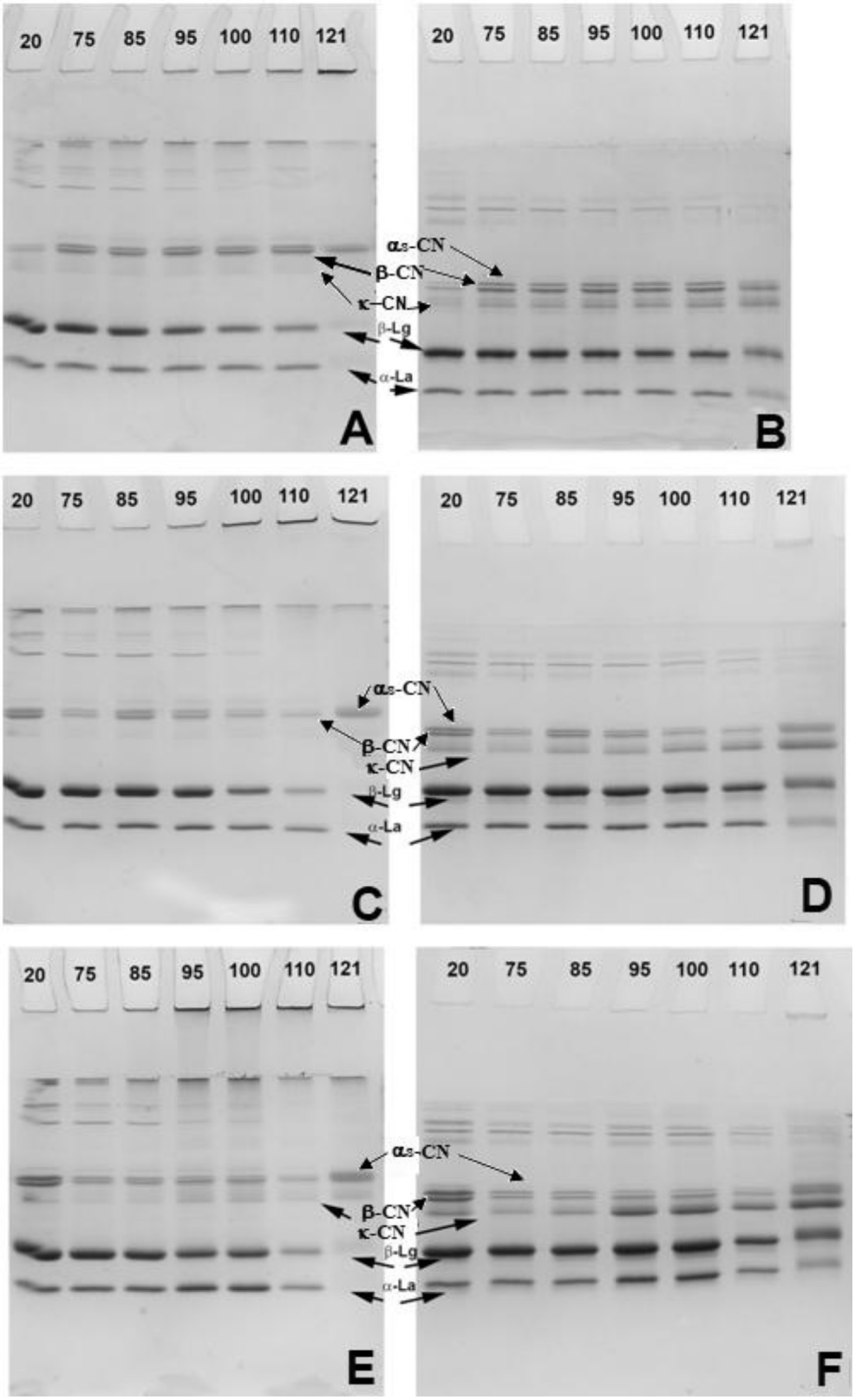

Fig. 6. SDS PAGE of supernatant obtained by ultracentrifugation of milk samples after heating to 121 °C containing 9 (A, B), 17 (C,D), and 25% (w/w) TS (E,F). Where A,C,E are non-reduced gels and B,D,F are reduced gels. The first six lines contain control and heated samples (75–110 °C) with 0 s holding time and the last line (121 °C) have sample heat treated for 2.6 min.

appeared to reassociate with the micelle up to 121 °C when their concentration in the serum again increased. In contrast to other caseins, κ-CN concentration concomitantly increased in the serum with rise in temperature (Fig. 6D). Moreover, κ-CN was involved in interactions with whey proteins resulting in formation of aggregates.

During heating of 25% TS milk, whey proteins showed greater stability in comparison to samples with lower TS (Fig. 6E,F). Slight denaturation of α-LA and β-LG started at lower temperatures; however, it was not as intense up to 110 °C. At 121 °C all of α-LA and β-LG were incorporated into disulphide-linked aggregates (Mediwaththe, Chandrapala, & Vasiljevic, 2018). Concentration of α_S-CNs in the serum initially decreased following the temperature rise from 75 °C up to 110 °C. However, after completion of the treatment at 121 °C these proteins appeared to dissociate from the micelle and become more prominent in the supernatant. This was confirmed with increase in serum minerals at this level indicating weakening of CCP nanoclusters and dissociation of proteins and minerals into the serum. On the other hand, concentration of κ-casein in the serum remained consistent up to 95 °C. After this temperature, further increase induced this protein to separate from the micelle. A slight reduction of the serum κ-casein can be noticed at 110 °C. Even concentration of β-casein declined at this temperature, which may indicate that it was fairly strongly associated with the micelle.

4. Discussion

FTIR was confirmed as a useful tool to fingerprint conformational changes in the secondary structure of the proteins which could be linked to various physiochemical changes during heating at different total solids concentrations. Similar to a previous report by Grewal et al. (2018), in the current study the most affected structure during heat treatment was intermolecular β-sheets of β-LG due to their involvement in denaturation and aggregation. FTIR was capable of identifying and characterising progression and extent of the heat denaturation of β-LG that was clearly concentration dependent (Anema, 2000; Anema & McKenna, 1996; McKenna & O'Sullivan, 1971). As depicted in the FTIR spectra and confirmed by PCA in concentrated samples (17 and 25% TS), intense variations of peak intensities associated with intermolecular β-sheets took place upon reaching a certain temperature point. Intermolecular β sheets loadings identified at 1620 and 1683 cm^{-1} (Fig. 2D,F) decreased with concomitant rise in loadings indicating formation of turns (1663 cm^{-1}). These rearrangements in the structure were previously reported to reflect the structural rearrangement of β-LG during its denaturation (Grewal et al., 2018; Qi, Ren, Xiao, & Tomasula, 2015). Interestingly, as evidenced in the PCA loading plots for 17 and 25% concentrated milk samples, these conformational changes occurred up to 100 and 110 °C, respectively. However, in unconcentrated milk the rearrangements in the intermolecular β-LG sheets were observed at lower heating temperature with unfolding and denaturation taking place up to 85 °C after which aggregation took place. These findings confirmed retardation of the β-LG aggregation in a concentrated system previously reported by Anema (2000). Furthermore, the same study reported that heating whey proteins at 100 °C resulted in denaturation independent of the solids concentration. However, in the current study, a delay in whey protein denaturation appears to be concentration dependant since rise in solids concentration to 17% or 25% elevated a denaturation temperature to 100 °C or 110 °C, respectively. Beyond these temperatures, extensive aggregation has taken place. At 121 °C, intermolecular β-sheets reappeared along with new turns (1660–1670 cm^{-1}) and aggregated β sheets (1695 cm^{-1}), which could be related to intense aggregation of β-LG with κ-CN (Dalgleish & Corredig, 2012) in the serum and interactions of β-LG with the casein micelle. These observations were confirmed by a greater band intensity of aggregates and reduction of band attributed to β-LG in SDS patterns. Furthermore, Anema and Li (2003) observed that the rate of denaturation of the whey proteins was greater than the level of association with the casein micelle. This may be correlated with our results at 100 °C in 17% TS sample as intense denaturation of β-LG and α-LA took place without an impact on the particle size.

From the concentration-normalised spectra in all concentrations, most heat affected region was 1618–1655 cm^{-1}, which in addition to intermolecular β sheets, involves variations in intramolecular β sheets, random and α helix/loop structures. During heating below temperatures at which denaturation was completed, i.e., up to 85, 100 and 110 °C for 9, 17 and 25% TS, respectively, higher loadings at 1645 cm^{-1} indicated increase in random coil structures. This may be related to greater involvement of caseins in interactions with the casein micelle. The SDS-PAGE analysis confirmed a slight concentration reduction of α_S- and β-CN in the concentrated samples in the serum. On the other hand, concentration of κ-CN increased in the serum resulting in complexation with whey proteins. Thus, additional increase of turns and loop structures could be attributed to greater dissociation of κ-CN from the micelle and formation of new stronger hydrogen bonds between α_S- and β-CN with or within the micelle (Deeth & Lewis, 2016; Farrell, Wickham, Unruh, Qi, & Hoagland, 2001). Prolonged heating at 121 °C resulted in a reformation of a band at 1653 cm^{-1}, which may be related to conformational restructuring of α-helical structure of α-LA. This proteins is known to refold back into its native state upon heating (Fang & Dalgleish, 1998). On the other hand, the SDS gels showed that concentration of α-LA in the serum was reduced (Fig. 6) predominantly due to disulphide interchange reactions with the casein micelle (Mediwaththe, 2017). This protein also appeared to engage in creation of soluble aggregates through disulphide interchange reactions with β-LG and κ-CN, and to a less extent with α_{S1}-CN. The reaction apparently started when the temperature exceeded 100 °C (Fig. 6).

5. Conclusions

FTIR for the first time identified the changes in the protein conformation in concentrated milk systems heated at different temperatures. The most notable changes involved reduction of intermolecular β-sheets of β-LG supported with more turns and random structures at a higher temperature in more concentrated samples; disappearance of α-helical structure at 100 °C and its reappearance upon conclusion of heating at 121 °C likely associated with behaviour of α-LA; and intensification of unordered, random and aggregated structures at these temperatures associated with a greater presence of individual caseins and protein aggregates in the serum. While κ-CN appeared constantly affected by concomitant concentration and temperature effects, other caseins seemed to frequent between two phases in a dynamic manner which could also be attributed to a shifting of a mineral balance towards the micellar phase at greater total solids concentrations and elevated temperatures. These findings confirmed that β-LG plays the major role in these extensive changes in the system and likely starts the chain reaction involving all other serum proteins, first α-LA and then dissociated κ-CN from micelle. For this reason, pre-warming of milk to deactivate β-LG seems as one of the strategies to stabilise condensed milk prior to heating, although the role of other proteins and especially maintaining the integrity of the casein micelle should be further examined.

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