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Impact of variation in calcium level on the technofunctional properties of milk protein concentrate

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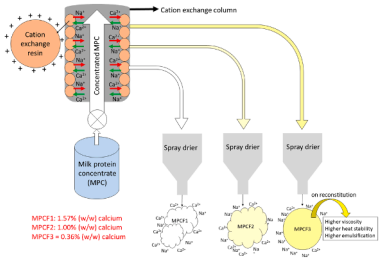
HIGHLIGHTS

- Calcium content impacts the technofunctional properties of milk protein concentrate (MPC).
- The reduced calcium MPC had high heat stability and high viscosity.
- The reduced calcium MPC had improved emulsion properties.
- MPC with the lowest calcium level had spherical shaped particles.

GRAPHICAL ABSTRACT



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ABSTRACT

The technofunctional properties of milk protein concentrate containing 80% protein (MPC80) with different calcium contents, i.e., MPCF1, MPCF2 and MPCF3 contained 1.57%, 1.00% and 0.36% calcium, respectively, were studied. The MPC samples with reduced calcium were produced using partial acidification followed by a cation exchange process thereby replacing calcium with sodium in an MPC80 concentrate. Scanning electron microscopy analysis of MPC80 powder particles showed that the MPCF3 powder particles were more spherical than the other samples. The MPCF3 sample had the highest emulsion stability, apparent viscosity (η_{app}) and thermal stability (during heating between 110 and 140 °C). The results showed that modification of the calcium content in MPC80 using cation exchange significantly altered its microstructure, particle size distribution, apparent viscosity, thermal stability, colour properties, oil binding capacity and emulsion stability.

1. Introduction

Population growth along with limitations in global protein resources highlights the role of the dairy industry in fulfilling the requirement of humans for high quality dietary protein. Milk proteins are the preferred protein source for the majority of consumers in the Western world. Bovine milk protein concentrate (MPC) contains both caseins (CNs) and whey proteins (WPs). MPC is a highly digestible protein ingredient having a high level of all essential amino acids, thus it is a suitable ingredient for the formulation of food and nutritional products [1]. The occurrence of micellar CN aggregates during MPC manufacture and storage as a consequence of the formation of CN crosslinks in the presence of calcium reduces the aqueous solubility of MPCs [2,3]. Therefore, reduction in the calcium content has been reported as an approach to enhance the solubility of MPC [4]. Calcium removal from MPC has been achieved via acidification of skim milk, the addition of calcium

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chelators, the addition of monovalent ions and the application of ion exchange to replace calcium with, e.g., sodium ions [2].

The specific assembly of CN micelles in the presence of calcium may impact their technofunctional properties. This in turn may limit some applications of MPCs in food products [5]. While low calcium MPCs have been reported to have improved solubility compared to high calcium MPCs, other technofunctional properties of MPCs may also be affected in low calcium MPCs. To date, apart from information on the impact of total calcium on the aqueous solubility of MPC, limited information appears to be available on the impact of modification of the mineral composition on other technofunctional properties of MPC.

Pandalaneni et al. [6] reported that MPC80 with a 20 – 30% reduction in calcium had a lower susceptibility to thermal instability. However, investigations on the thermal stability of MPC80s having different calcium contents over a range of temperatures may provide useful information for the selection of MPCs for different ingredient applications. The impact of different levels of calcium on the apparent viscosity (η_{app}), colour stability and the oil binding capacity (OBC) of MPC appears to be less studied. Pandalaneni et al. [6] showed that a 30% reduction in calcium level did not have a significant impact on the viscosity of MPC80. These authors also did not observe a significant difference in colour of MPC80 upon 30% reduction of calcium. In a study with MPCs containing 2–3% (w/w) calcium, the MPC with a lower level of calcium was reported to have a higher emulsification ability compared to the MPC with higher calcium [5]. However, the impact of a wider range of calcium reduction on the emulsification properties of MPC warrants further investigation.

The hypothesis of this study was that calcium depletion may alter the surface structure and technofunctional properties of MPCs. Therefore, in order to yield optimal technofunctional properties, the level of calcium reduction may need to be carefully controlled in order to achieve targeted functionality. The impact of calcium level on the microstructure, particle size distribution (PSD) on reconstitution, η_{app}, thermal stability, colour properties, OBC and emulsion stability of MPC80 samples were investigated. Three different MPC80 samples having different calcium levels produced using cation exchange method were compared in this study.

2. Materials and methods

2.1. Reagents

Hydrochloric acid (HCl) was from VWR (Dublin, Ireland). Sodium hydroxide (NaOH) was from Fisher Scientific Ireland (Dublin, Ireland). Sulphuric acid ($\geq$ 98%), Kjeldahl catalyst tablets (free of Hg and Se) and boric acid for Nitrogen analysis were from Sigma (Dublin, Ireland). All other reagents were of analytical grade. Corn oil was obtained from a local food store.

The control MPC80 (CMPC) powder was produced as described in Khalesi and FitzGerald [7] and Rafiee et al. [8]. MPC samples with different calcium contents were produced using partial acidification followed by a cation exchange process as described in Khalesi and FitzGerlad [4]. Therefore, at the end of the manufacturing process four MPC samples including CMPC, MPCF1, MPCF2 and MPCF3 having 2.87, 1.57%, 1.00% and 0.36% (w/w%) calcium, respectively, were produced. The calcium ion activity of CMPC, MPCF1, MPCF2 and MPCF3 was 2.64, 1.23, 0.88 and 0.21 mmol/L, respectively. Specific details on the gross composition of the MPC samples and the surface composition at a depth of 10 nm are available in Khalesi and FitzGerald [4].

2.2. Scanning electron microscopy

Scanning electron microscopy (SEM) using an Hitachi SU-70 SEM (Krefeld, Germany) was utilised for observation of the microstructure of the MPC powder particles [9].

2.3. Particle size (PS) analysis of reconstituted MPC aqueous suspensions

Samples of the reconstituted (5% w/v, protein) MPC were dissolved by heating at 50 °C for 30 min and were then cooled to room temperature. After subsequent standing at room temperature for 1 h, the particle size of the samples was analysed (n = 3) using a laser light scattering particle size analyser (Mastersizer 2000, Malvern Instruments, Worcestershire, England) equipped with an Hydro 2000S sample dispersion system (set at a stirring speed of 1000 rpm) interfaced with Mastersizer 2000 software (*version 5.61*; Malvern Instruments, Malvern, UK). The volume weighed mean ($D_{(4,3)}$), the surface weighed mean ($D_{(3,2)}$), the absolute deviation from the median (uniformity) as well as the average particle size ($d_{(0.5)}$), the sizes of particles below which 10% and 90% of the sample lie ($d_{(0.1)}$ and $d_{(0.9)}$, respectively) and the specific surface area (SSA) were determined as described by Cermeño et al. [10].

2.4. Apparent viscosity (η_{app}) measurement

The η_{app} of reconstituted MPC samples (5% (w/v) on a protein basis) was determined (n = 5) using a DV-II viscometer (Brookfield, Harlow, UK) at a shear rate of 100 s^{-1} according to Khalesi and FitzGerald [7].

2.5. Thermal behaviour

For thermal stability analysis, the heat coagulation time (HCT, min) and the heat induced gelling time (HIGT, min) were determined (n = 5) for all samples [7].

The MPC samples were reconstituted at 5% ((w/v) protein basis), adjusted to pH 7.0 and then stirred at 25 °C for 1 h followed by storage at 4 °C for 24 h to aid full hydration prior to the thermal stability assessments. Aliquots (2.3 mL) of the MPC samples were then transferred into glass tubes (length = 130 mm; external diameter = 10 mm; thickness = 2 mm) and sealed with rubber stoppers. The tubes were then placed in a metal rack and immersed in an oil bath (Elbanton BV, Kerkdriel, The Netherlands) at 110, 120, 130 and 140 °C with constant slow oscillation (8 swings/ min). The time of the first visible onset of coagulation (clots) was reported as the HCT. The time elapsed between putting the reconstituted MPC sample in the oil bath (at 140 °C) and the appearance of gelation and the cessation of liquid flow was considered as the HIGT.

2.6. Colour measurement

Colour analysis of the samples was carried out using a CR-600d spectrophotometer (Konica Minolta Inc., Japan). Additionally, the samples were heated at 95 °C in a vacuum oven (Gallenkamp, Gallenkamp Ltd, Loughborough, UK) for 6 h and the colour of the samples was re-analysed. The results were expressed according with LAB scale where L*, a* and b* represent lightness, redness and yellowness, respectively. The colour differences (ΔE) between the samples before and after heating were calculated according to Khalesi and FitzGerald [7].

2.7. OBC measurement

The OBC of the MPCs was measured according to Shilpashree et al. [11]. Briefly, MPC80 powder (1.0 g, n = 3) was added to a 15 mL centrifuge tube of known weight, to which 10 mL of corn oil was added. After stirring for 30 s and holding for 30 min, the tubes were centrifuged (3,000 *g*, 20 °C, 30 min) using an Hettich Zentrifugen Universal 320 R centrifuge (Andreas Heittich GmbH & Co., Tuttlingen, Germany). The supernatant was removed and the tubes were re-weighed. The OBC, expressed as g of oil retained per g of protein, was calculated according to Eq. (1) [9]:

$$OBC = \frac{m3 - m1}{m2} \times 100 \quad (1)$$

where m1, m2 and m3 represent the weight of MPC sample, the mass of protein in each MPC powder sample and the weight of the residue remaining after centrifugation following the removal of the supernatant, respectively.

2.8. Emulsion characterization

To generate oil in water (o/w) emulsions, first of all, the MPC80 samples were dissolved in dH_2O to reach a 0.05% (w/v, protein basis) reconstituted MPC80 [12,13]. The pH of the suspension was adjusted to pH 2.0, 4.0, 7.0 and 10.0 using 1 M HCl or NaOH. Corn oil (6.0 g containing 0.004% (w/v) Sudan Red III for subsequent visualisation purposes) was mixed with 14.0 g MPC80 suspensions. This mixture was then sheared using an Ultra Turrax T-25 (IKA ® Werke Gmbh, Staufan, Germany) at 16000 rpm for 60 s in 50 mL plastic screw lid containers. The short-term emulsion activity index (EAI) and emulsion stability (ES) of the MPC emulsions was determined (n = 3) according to Connolly et al. [13].

The longer term stability of the MPC80 emulsions was analysed according to Cermeño et al. [10], with some modifications. A suspension (1% (w/v) protein) of the different MPC80 samples was prepared in dH_2O (3.5 g per 350 mL). This suspension was then gently stirred at room temperature until completely hydrated. The pH of the solution was adjusted to pH 7.0 using 1 M NaOH. The oil phase was prepared by the addition of 0.004% (w/v) Sudan Red III and 0.02% (w/v) sodium azide (antimicrobial agent). Oil (150 g) was then added to the MPC suspension to obtain a total mixture of 500 g. The solution was then sheared at 13000 rpm for 30 s using an Ultra Turrax T-25 (IKA). The PSD of the emulsions was determined using a Mastersizer 2000 (Malvern). The analysis was performed in triplicate on days 4, 14 and 33 following quiescent storage of the samples at 4 °C. Droplet coalescence in the day-33 samples was assessed by calculating the coalescence index (CI) according to Eq. (2) [14]:

$$CI \quad (\%) = \left(\frac{D_{(3,2)d33}}{D_{(3,2)d1}} - 1\right) \times 100 \tag{2}$$

where $D_{(3,2)d33}$ and $D_{(3,2)d1}$ represent the surface weighed mean of the droplet diameters at day-33 and day-1, respectively.

2.9. Statistical analysis

All analyses were carried out at least in triplicate. Data values were presented as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) followed by the *Tukey* post hoc comparison test was carried out to test for significant differences using Minitab® Release 15 for Windows. A p value < 0.05 was considered statistically significant.

3. Results and discussion

3.1. Microstructural visualisation of MPC powder particles

SEM analysis of the MPC80s provides an overview on the shape and size of the powder particles. In general, the particles associated with MPCF1, MPCF2 and MPCF3 had a wide range of sizes (Fig. 1). This is in agreement with the observation for CMPC [9]. The main feature of the MPCF3 powder consisted of particles with highly globular structures and smooth surfaces, with larger particles compared to the other samples. In addition, MPCF3 contained a low amount of wrinkled particles, with minimal aggregation. On the other hand, MPCF2 contained large wrinkled particles and MPCF1 contained a high extent of aggregation which presented as highly uneven particles.

Generally, the MPCs with a higher calcium content has extensive cross linkages in their micellar CN compared to low calcium MPCs. This will lead to differences in the protein structure of the particles. This may be linked with differences in their technofunctional properties. Particles with spherical shapes appeared to have a tendency to retain water molecules, presumably via Van der Waals interactions, which may be relevant as previously outlined for the generation of MPC80s with enhanced aqueous interaction properties [9]. The MPC sample with a lower level of calcium was less susceptible to particle aggregation. This was attributed to the presence of calcium promoting aggregation of micellar CNs. The presence of calcium promotes cross-linkages between micellar casein which promotes the formation of more compact structures thereby enhancing the formation of irregular shaped particles. Such irregularly shaped MPC particles were recently observed using SEM analysis [15]. Therefore, it was not unexpected that the MPC samples with the lowest calcium level (MPCF3) had spherical shaped particles.

To our knowledge, this is the first report on the SEM analysis of MPCs having different levels of calcium when obtained using a cation exchange process.

3.2. PSD of reconstituted MPC80

The results of PSD analysis of 5% (w/v) aqueous reconstituted MPC samples are given in Table 1. The average particle diameter ($d_{(0.5)}$) for MPCF1, MPCF2 and MPCF3 were 0.14, 0.52 and 0.15 μm, respectively. The $d_{(0.5)}$ for CMPC was previously reported to be 0.13 μm [9]. The SSA showed higher values for MPCF1 (46.93 m^2/g) and MPCF3 (44.97 m^2/g) compared to MPCF2 (20.70 m^2/g) which reflects the particle size values obtained herein. The SSA for CMPC was previously reported to be 55.40 m^2/g [9]. A lower $d_{(0.9)}$ for MPCF1 (0.26 μm) indicated a lower distribution of particle sizes in this suspension. The value obtained for the MPCF1 suspension was in accordance with the $D_{(4.3)}$ value which was lower for MPCF1 in comparison with the two other MPCs. The lower uniformity index for MPCF1 indicated a lower deviation from the $d_{(0.5)}$. This further confirms the higher uniformity in

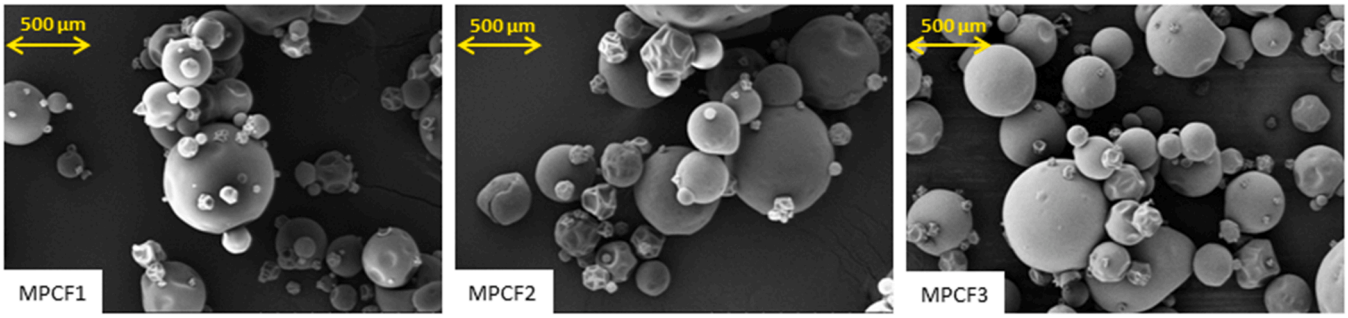

Fig. 1. Scanning electron microscopy (SEM) images of reduced calcium milk protein concentrate (MPC) samples MPCF1 (1.57% calcium), MPCF2 (1.00% calcium) and MPCF3 (0.36% calcium) taken at an acceleration voltage of 10 kV and a working distance of 11 mm. Image for CMPC was taken from Khalesi and FitzGerald [9].

Table 1
Specific surface area (SSA), uniformity and particle diameters of 5% (w/v, on a protein basis) reconstituted milk protein concentrate (MPC), control (CMPC, 2.87% calcium) and reduced calcium MPC samples MPCF1 (1.57% calcium), MPCF2 (1.00% calcium) and MPCF3 (0.36% calcium).

Parameters	CMPC*	MPCF1	MPCF2	MPCF3
SSA (m^2/g)	55.40 ± 0.20^{a**}	46.93 ± 1.20^{b}	20.70 ± 0.79^{c}	44.97 ± 2.00^{b}
Uniformity index	0.55 ± 0.01^{a}	0.75 ± 0.33^{a}	2.61 ± 1.45^{b}	6.19 ± 1.42^{c}
$D_{(4.3)}$ (μm)	0.15 ± 0.01^{a}	0.20 ± 0.05^{a}	1.83 ± 1.19^{b}	1.03 ± 0.25^{b}
$D_{(3,2)}$ (μm)	0.11 ± 0.01^{a}	0.13 ± 0.02^{a}	0.29 ± 0.01^{b}	0.13 ± 0.01^{a}
$d_{(0.1)}$ (μm)	0.06 ± 0.01^{a}	0.08 ± 0.01^{a}	0.12 ± 0.01^{b}	0.07 ± 0.01^{a}
$d_{(0.5)}$ (μm)	0.13 ± 0.01^{a}	0.14 ± 0.06^{a}	0.52 ± 0.01^{b}	0.15 ± 0.01^{a}
$d_{(0.9)}$ (μm)	0.25 ± 0.01^{a}	0.26 ± 0.06^{a}	2.15 ± 0.21^{b}	3.52 ± 1.49^{b}

* Data taken from Khalesi and FitzGerald [8]. ** Different letters in each row indicates a significant difference ($p < 0.05$), n = 3.

MPCF1 aqueous suspensions. The low uniformity in the MPCF2 and MPCF3 suspensions may be related to their multimodal size distributions (Fig. 2), while MPCF1 particles showed a monomodal particle distribution. A monomodal particle distribution for CMPC was previously observed by Khalesi and FitzGerald [9].

Overall, the results showed that lower levels of calcium were associated with reduced uniformity of the colloidal particles in the reconstituted MPC80. De-agglomeration occurred when MPC80 was exposed to the hydration process. This was more pronounced for MPC80 with a lower calcium content as its solubility was higher [4]. However, due to the cation exchange step employed herein, particles with different agglomerate sizes were generated. Variation in the sizes of the agglomerates led to the observation of multimodal particle size distributions (within a range of 100–1000 nm) for MPC particles in suspension having a low calcium content [16] as shown in Fig. 2. The low uniformity of MPCF3 observed herein may also be related to the higher proportion of the reduced calcium concentrated MPC (i.e., the concentrated MPC subjected to the cation exchange process) which was required when blending with the initial concentrated MPC for the production of the final MPC80 having a low calcium level.

Regenstein et al. [17] reported that removal of calcium resulted in dissociation of micellar CNs, thereby reducing the particle sizes of the reconstituted MPC. Their report is in agreement with the observations herein. Pandalaneni et al. [6] also showed that MPCs with lower calcium contents obtained by addition of 0.15% sodium hexametaphosphate resulted in a reduction in the particle sizes of micellar CNs from 192 to 90 nm due to dissociation of the CN micelles. Another study showed that reducing the calcium content using gaseous CO_2 injection during membrane filtration increased micellar CN particle size [18]. Differences in the methods used for reducing the calcium content of the MPCs may be the reason for the differences observed in the results. Furthermore, addition of citric acid to MPC was shown to lower calcium ion activity, consequently resulted in lower particle sizes in MPC [19].

This is the first report on PSD analysis of reconstituted MPCs having different calcium contents obtained using a cation exchange approach. The results of this study are in agreement with the literature which show that by reducing the calcium level, the more dissociated the CN micelles become.

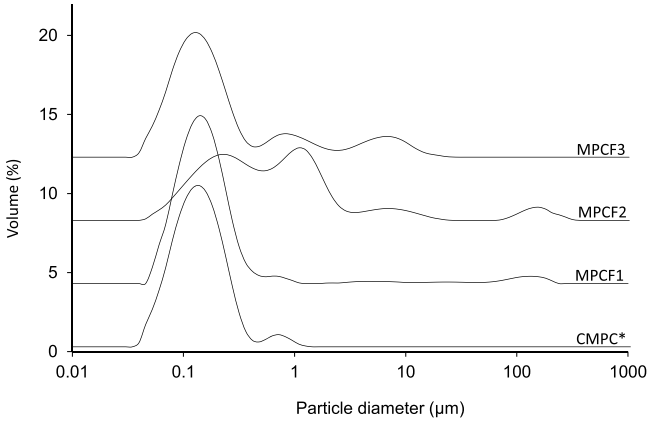

Fig. 2. Representative particle size distribution profiles of 5% (w/v, on a protein basis) reconstituted milk protein concentrate (MPC) samples, control (CMPC, 2.87% calcium) and reduced calcium MPC samples MPCF1 (1.57% calcium), MPCF2 (1.00% calcium) and MPCF3 (0.36% calcium). The measurement was performed 1 h after generation of the suspensions. * Data taken Khalesi and FitzGerald [9].

3.3. Apparent viscosity (η_{app}) analysis

Reconstituted MPC with a lower level of calcium had a higher η_{app} ($p < 0.05$). The η_{app} of MPCF1, MPCF2 and MPCF3 at a concentration of 5% (w/v) on a protein basis was observed to be 2.83 ± 0.05, 2.85 ± 04 and 3.13 ± 0.05 mPa.s, respectively. The η_{app} of CMPC under similar conditions was recently reported to be 2.75 ± 0.05 mPa.s [9].

The impact of different levels of calcium on the viscosity of MPC appears to be less studied in the literature. Previously, the viscosity of reconstituted MPC80 under similar experimental conditions was reported to be close to the values reported for CMPC, MPCF1 and MPCF2 herein [20]. A 30% reduction of calcium in MPC was reported to have no significant influence on the η_{app} of 8% (w/w) reconstituted MPC80 [6]. This is also in agreement with the η_{app} of the MPCF1 and MPCF2 samples herein, where MPCF2 had ~27% lower calcium compared to MPCF1 (and 65% lower calcium compared to CMPC) while the η_{app} of these two samples was not significantly different ($p > 0.05$). However, replacement of calcium with Na_2HPO_4 in a concentrated micellar CN solution was reported to result in a loosening of CN micelles and a reduction in calcium ion activity along with an increase in η_{app} [21]. Removal of the calcium from the network of CN micelles has also been reported to lead to an increase in η_{app} [22] of the MPC suspension due to the following sequence of events: a) removal of calcium increases electrostatic repulsion between CN micelles, b) this causes a loss in the extent of the crosslinks formed by colloidal calcium phosphate between the micelles, c) leading to a significant reduction in the structural integrity of CN micelles, d) this promotes CN micelle dissociation, e) thus more diffusion of CN into the serum phase occurs; f) accordingly, the CN micelles become more hydrated and voluminous during MPC dispersion, g) consequently, the viscosity of reconstituted MPCs with lower calcium is increased.

3.4. Heat stability of MPC

The heat stability of reconstituted MPC is an important factor for ingredient applications where high heat treatments are required. Heat stability measured in terms of HCT is usually examined using an oil bath heated at 120–140 °C. The results of the heat stability analysis of the MPC80 samples between 110 and 140 °C are shown in Fig. 3. The relationship between the reduction in HCT of MPCF1, MPCF2 and MPCF3 and increasing heating temperature (110–140 °C) was logarithmic. This is in agreement with previous studies by Dumpler et al. [23] and Khalesi and FitzGerald [7]. The results herein indicated that lower levels of calcium in MPC80 led to a higher heat stability at all temperatures tested. The gelling time at 140 °C for MPCF1 (29.5 ± 1.8 min) and MPCF2 (27.4 ± 0.9 min) was not significantly different ($p > 0.05$). The MPCF3 sample, however, did not show gelation during 5 h heating at 140 °C. The gelling time for CMPC was recently shown to be 19.7 min [7].

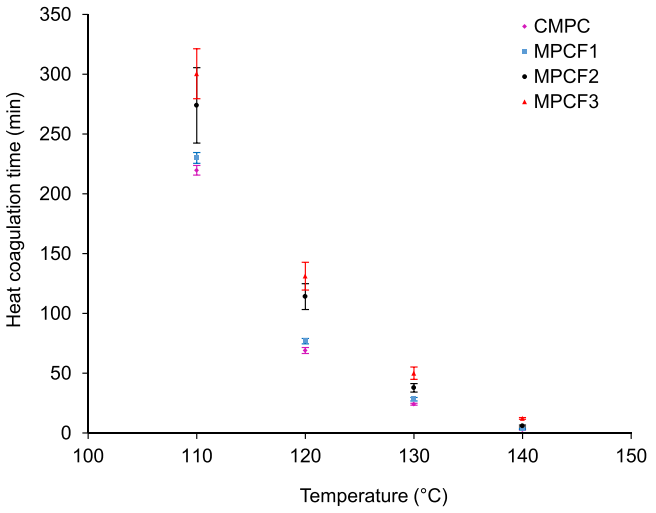

Fig. 3. Heat coagulation time (HCT, n = 5) in min for milk protein concentrate (MPC) suspensions, control MPC (CMPC, 2.87% calcium) and reduced calcium MPC samples MPCF1 (1.57% calcium), MPCF2 (1.00% calcium) and MPCF3 (0.36% calcium) as a function of heating temperature (110–140 °C) when tested at 5.0% protein (w/v). Data for CMPC was taken from Khalesi and FitzGerald [7].

Protein coagulation is dependent on several factors such as pH, concentration, temperature, processing conditions and mineral composition [24]. Eshpari et al. [25] reported that MPC with higher calcium contents may contain an extensive aggregation between the WPs and the CNs. Therefore, it is expected that a reduction in calcium concentration may result in an increase in the heat stability of MPC. Accordingly, a 40% reduction in the calcium content of MPC was shown to improve heat stability at neutral pH [26]. Furthermore, Singh et al. [27] showed that increasing the calcium concentration in a 7.5% reconstituted MPC suspension resulted in a reduction in heat stability. Pandalaneni et al. [6] reported that MPC80 with a 20 – 30% reduction in calcium had a lower susceptibility to thermal instability. Likewise, the use of calcium binding agents such as disodium uridine monophosphate and disodium hydrogen phosphate have been previously reported to improve the heat stability of concentrated micellar CN solutions [21]. However, Eshpari et al. [28] did not observe differences in the HCT for similar MPC samples having calcium contents in the range 1.6–1.8% (w/v).

It is hypothesised that the extent of cross-linking of the micellar CNs influences the heat stability of MPC. The higher thermal stability of MPCs having a lower calcium concentration may be attributed to the weakening of CN micelle structure as a result of having lower calcium. The results herein showed that MPC80s obtained using cation exchange resins with a lower level of calcium had higher thermal stability.

Herein, it was shown that replacement of calcium with sodium in MPC using cation exchange enhanced the heat stability probably due to a reduction in the capability to form cross-linked micellar CNs at a reduced level calcium. In addition, it was observed that MPCF3 with a lower level of calcium, thus a higher level of dissociated micellar CNs, had a higher level of denatured WPs [4]. This may also be related to the observed higher thermal stability for MPCF3. It has been demonstrated that WP denaturation in MPC enhanced its thermal stability [7,29].

This appears to be the first report on the impact of calcium level on the heat stability of MPC at different temperatures. It is also the first report on the impact of the employment of cation exchange for calcium removal on the thermal stability of MPC.

3.5. Colour measurement

The appearance and colour stability of MPCs following thermal treatment may be important for some MPC applications, e.g., in cheeses and confectionary products [30]. The visual appearance of the reconstituted MPCF1, MPCF2 and MPCF3 samples showed clear differences (Fig S1). While the colour of MPCF1 was white, a yellowness was dominant in the appearance of MPCF3 (Fig S1). Sample MPCF2 had a colour between MPCF1 and MPCF3 and was closer to MPCF1 in appearance. The lightness, redness and yellowness of MPCF1, MPCF2 and MPCF3 powder particles were compared (Fig. 4). The results showed that MPCF2 had the highest lightness (L*) index, while MPCF1 had the lowest L*. A recent study on MPC80 reported a negligible change in L* for MPC with a 20% and 30% lower calcium compared to control [31]. Differences in the method of generation of MPCs with

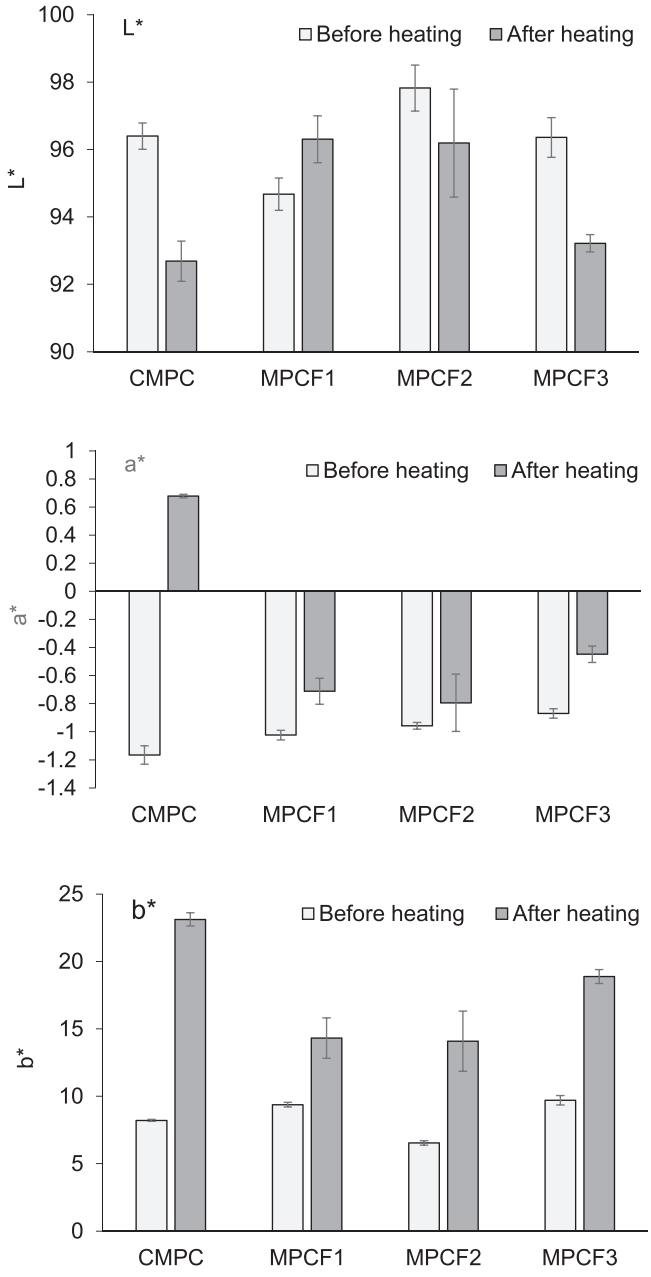

Fig. 4. The lightness (L*), redness (a*) and yellowness (b*) of milk protein concentrate powder (MPC) samples, control MPC (CMPC, 2.87% calcium) and reduced calcium MPC samples MPCF1 (1.57% calcium), MPCF2 (1.00% calcium) and MPCF3 (0.36% calcium) before and after oven heating (95 °C, 6 h) as determined using colorimetric. Different small letters above the columns represent significant differences ($p < 0.05$, n = 5) between the samples. Values plotted represent mean ± SD. Data for CMPC was taken from Khalesi and FitzGerald [7].

different calcium contents, i.e., CO_2 injection in the study by Pandalaneni et al. [31] vs using cation exchange herein, may be a reason for differences in the colour indexes observed between these two studies.

After oven heating (95 °C, 6 h) of the MPC samples, however, the lowest L* was associated with MPCF3. MPCF1 showed the lowest redness (a*), while MPCF3 showed the highest. After oven heating of the samples, MPCF3 showed the lowest a*. Minimum yellowness (b*) was associated with MPCF2. The maximum b* on the other hand was associated with oven dried MPCF3 (Fig. 4). Pandalaneni et al. [31] did not observe a significant difference in colour in MPC80 upon 30% reduction of calcium. The study herein is the first report on the colourimetric analysis of MPCs with different calcium levels when produced employing a cation exchange process.

The results for ΔE showed that MPCF3 displayed a significant colour change after oven heating ($\Delta E = 9.7$), while the values for MPCF1 and MPCF2 were lower (5.2 and 4.4, respectively). This showed that the appearance of MPCF3 was more affected by heating than MPCF1 and MPCF2. This may be due to higher dissociation of CN micelles in the low calcium MPC, which may provide a higher number of available amino acid groups to interact with other components, e.g., lactose, in turn resulting in more extensive chemical interactions, e.g., Maillard reactions. Accordingly, the MPC with lower calcium was more susceptible to colour change on heating. The colour stability of MPCF2 was higher than MPCF1 and MPCF3 following oven heating. According to Khalesi and FitzGerald [7], the ΔE for CMPC was 15.5 which is higher than the calcium depleted samples herein. In addition, it was previously shown that WP denaturation was associated with a reduced ΔE [7]. This is also in agreement with the observation herein where the minimum ΔE was associated with the MPC with the highest WP denaturation level, i.e., MPCF3. The observations herein are also in agreement with a previous report showing that the L* values of reconstituted MPCs having different calcium levels were different following heating (140 °C, 15 s) [31]. These authors showed that MPC with a higher calcium content (2.2%) had a higher L* value after heat treatment compared to a sample having 1.5% calcium.

As already mentioned, high colour stability may be an advantage for MPC powders for applications where high temperature processing is required, e.g., during use in recombined milk, and in enteral and clinical nutrition products. This appears to be the first report on the impact of calcium level on the colour changes in MPC powders following oven heating.

3.6. OBC of MPC80s with different levels of calcium

The impact of different levels of calcium on the OBC of MPC has not yet been well documented. The results herein showed no significant difference ($p > 0.05$) in OBC between MPC80s with different calcium contents. The OBC for MPCF1, MPCF2 and MPCF3 was 2.8 ± 0.1, 2.9 ± 0.1 and 2.8 ± 0.1 g oil/g protein, respectively. The OBC of the samples did not differ probably because the lipid content of all three MPCs was in the range of 1.0–1.5%. Previously, the OBC of CMPC was reported to be 2.8 ± 0.1 g/g protein [9]. Generally, MPCs with high OBC values may be suitable for emulsification applications. The OBC of MPC60 was previously reported to range between 3.3 and 4.7 g oil/g protein [32,33]. The higher lipid content of MPC60s (1.6–2.1%) compared to MPC80s (1.0–1.5%) is considered the main reason for the differences in the OBC results.

3.7. Emulsion properties

Study of the emulsification properties of MPCs is relevant as the demand for natural emulsifiers grows [34,35]. The absorbance of the MPC emulsion samples generated with CMPC, MPCF1, MPCF2 and MPCF3 at λ_{500} at different pH values was measured (Fig S2). A higher absorbance at λ_{500} is indicative of smaller droplet sizes and correlates with higher EAI values [10]. The results showed that the minimum EAI for the emulsion samples occurred at pH 4.0, which is near the isoelectric point (~pH 4.6) of the CNs (Table 2). The EAI significantly increased ($p > 0.05$) on increasing the pH above pH 4.0 for all samples. The maximum EAI for all samples occurred at pH 10.0. Among the three samples, MPCF3 showed the highest EAI at pH 7.0 (117 ± 21 m^2/g) and pH 10.0 (204 ± 50 m^2/g) (Table 2). Previously, the EAI of WP isolate (WPI) and a WPI:milk protein blend (2:1) was reported to be 148.20 ± 1.37 and 165.03 ± 1.47 m^2/g, respectively [36].

The short term (30 min) ES of all emulsions, at a protein content of 0.05% (w/v) in the pH range 2.0–10.0, was 100%. The long term (33 d) emulsion stability properties of MPCF1, MPCF2 and MPCF3 emulsions was studied using laser light scattering particle size analysis. The results showed that MPCF3 presented the most stable emulsion along with having particles with the lowest Sauter mean diameter ($D_{(4,3)}$: 2.39 ± 0.30 μm) in suspension following 33 days storage at 4 °C. The emulsion generated with the two other MPC samples (i.e., MPCF1 and MPCF2) became unstable after 4 days of manufacture (Fig. 5). It was previously shown that the CMPC emulsion became unstable following 4 days storage at 4 °C [7]. However, after 33 days storage at 4 °C only the emulsions generated with MPCF2 showed monomodal distributions while the MPCF1 and MPCF3 emulsions showed multimodal PSD's (Fig S3). After 33 days storage at 4 °C the uniformity index of the particles, which shows the absolute deviation from the median ($d_{0.5}$), was 4.39, 0.65 and 2.70 for the emulsions generated with MPC80 sample MPCF1, MPCF2 and MPCF3, respectively. As shown, the lowest $d_{(0.5)}$ was associated with MPCF2 which also had a monomodal particle distribution. The distribution ($d_{(0.9)} - d_{(0.1)}$) of particle sizes in the emulsion manufactured with MPC80 sample MPCF1 (1153.63 μm) was higher than sample MPCF2 (90.38 μm) and MPCF3 (136.32 μm). In addition, the CI of the emulsions generated with MPCF1, MPCF2 and MPCF3 was 57.4 ± 1.0, 29.8 ± 1.0 and 459.7 ± 30.5%, respectively. This showed that the rate of emulsion droplet size change was higher for the MPCF3 emulsion, however, this emulsion still had the lowest mean particle diameter after 33 days.

Many factors may alter the emulsion properties of MPCs, e.g., calcium and protein contents, pH, etc. Herein, it was shown that MPCs with lower calcium levels had smaller emulsion droplet sizes, thus the ES of low calcium MPC80 was higher than MPCs with a higher calcium level. This is in agreement with a report suggested that low calcium MPCs can be used in order to obtain more stable emulsions with smaller particle sizes [37]. The lower particle sizes are probably related to minimisation of the formation of CN micelle aggregates. The dissociation of CNs by removal of calcium resulted in the formation of non-micellar CN. A higher ratio of non-micellar CN:micellar CN has been linked with better emulsification properties [38]. In addition, a higher concentration of calcium promotes the formation of insoluble aggregates between denatured WPs, which contributes to a reduction in emulsification properties. This has been associated with impairment of the spread and rearrangement of proteins at the interface of oil droplets or by flocculation occurring between emulsion droplets at high calcium concentrations, resulting in the formation of large sized droplets [39]. Emulsions

Table 2
Emulsion activity index (EAI, m^2/g) of milk protein concentrate (MPC) samples, control MPC (CMPC, 2.87% calcium) and reduced calcium MPC samples MPCF1 (1.57% calcium), MPCF2 (1.00% calcium) and MPCF3 (0.36% calcium) at pH 2.0, 4.0, 7.0 and 10.0.

	EAI (m^2/g)			
Sample	pH 2.0	pH 4.0	pH 7.0	pH 10.0
CMPC	42 ± 10^{aAB}	33 ± 4^{bA}	47 ± 11^{aAB}	58 ± 8^{aB}
MPCF1	64 ± 10^{bBC}	31 ± 6^{abA}	51 ± 2^{aB}	79 ± 9^{bC}
MPCF2	38 ± 11^{aA}	45 ± 3^{cA}	64 ± 23^{aAB}	79 ± 7^{bB}
MPCF3	52 ± 6^{abB}	23 ± 3^{aA}	117 ± 21^{bC}	204 ± 50^{cD}

Values represent mean ± SD (n = 3). Small letters in each column and capital letters in each row show significant difference ($P < 0.05$), n = 3.

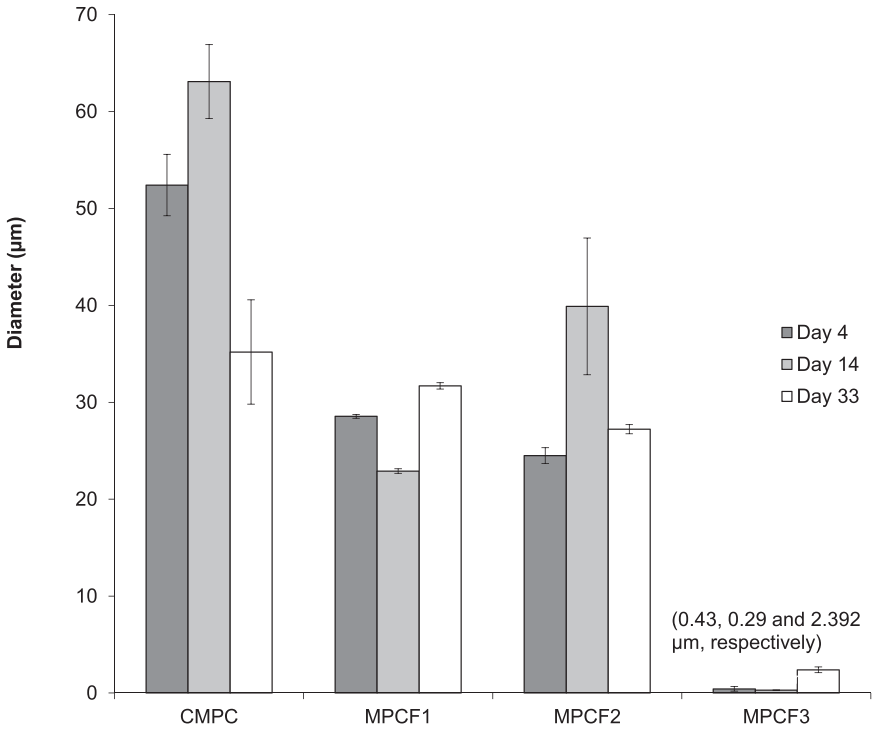

Fig. 5. The Sauter mean diameter of the droplets in emulsions generated from reconstituted milk protein concentrate (MPC, 1.0% (w/v) on a protein basis) samples control MPC (CMPC, 2.87% calcium) and reduced calcium MPC samples MPCF1 (1.57% calcium), MPCF2 (1.00% calcium) and MPCF3 (0.36% calcium):corn oil (70:30, w/w%) blend after 4, 14 and 33 d of storage at 4 °C.

formed with low calcium MPCs were reported to have less susceptibility to aggregation, thereby providing a higher number of available protein molecules for adsorption to oil droplet surfaces [37]. The results herein showed that MPCs generated using a cation exchange process (which replaced calcium with sodium) resulted in the formation of a highly stable emulsion in both the short and long term. This may be beneficial for the application of calcium reduced MPCs in emulsion formulations (such as in soups and sauces) and may be considered as an alternative for WPs in emulsification formula. The results herein showed that MPCs generated using a cation exchange process resulted in the formation of a highly stable emulsion in both the short and long term.

4. Conclusions

The functionality of MPCs including their emulsification behaviour and thermal stability are relevant for their applications in a range of food products. In this study, it was shown that modification of the calcium level in MPC using cation exchange was a promising strategy to improve some technofunctional properties of MPC80, e.g., the thermal stability, viscosity and emulsification properties. The heat stability of MPC is important for a number of applications e.g., in soups, sauces, recombined and evaporated milk and clinical nutrition products where high temperatures are employed during processing. However, the lower level of calcium in the ion exchange treated MPCs, i.e., 0.36%, delayed/prevented gelation which may restrict some applications of such low level calcium MPCs, e.g., in meringues, cakes, and where they are used as egg white substitutes, etc, which may be more applicable for MPCs with higher calcium levels. The good emulsification properties of MPCs with a low calcium content was probably attributed to dissociation of micellar CNs at lower calcium levels. The high viscosity in the MPCF3 suspension is due to a 'loosening' of the CN micelle structure resulting in less calcium ion binding and high aqueous interactions between CNs and water molecules. This may be desirable in some applications such as in soups and sauces and in the formulation of processed cheese. The information obtained herein on the technofunctional properties of MPCs with different calcium contents may be relevant in the targeted generation of specific MPC ingredients for different applications.

CRediT authorship contribution statement

MK designed the experiments, collected the data, carried out the data analysis, prepared the first draft and subsequent revisions, and contributed to funding acquisition. RJF contributed to experimental design, edited the draft manuscript and contributed to funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this manuscript.

Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.colsurfa.2022.128741.

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