Functional properties of milk protein concentrates: Emulsifying properties, adsorption and stability of emulsions

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Abstract

Aggregation of casein in milk protein concentrates (MPCs) is altered by changes in calcium content. Emulsifying properties of MPCs and stabilities of emulsions formed with MPCs were investigated by examining emulsions formation, adsorption behaviours of proteins and emulsion microstructures. Compared with emulsions formed with higher calcium MPCs at a given protein concentration, emulsions formed with low calcium MPCs were finer, the total surface protein concentration was lower and the protein composition on the surface of the emulsion droplets was altered. Thus, the aggregation state of casein dominates the emulsifying capability of MPC products and the adsorption behaviours of proteins in MPCs. In low-calcium-MPC-stabilized emulsions, the stability of the emulsions decreased with an increase in the emulsion size at low protein concentrations and decreased with increasing protein concentration beyond a maximum level, suggesting that the protein state in low calcium MPCs may cause depletion flocculation in the emulsions.

1. Introduction

Milk proteins, because of their high nutritional value and unique physicochemical properties, are key functional components in many processed foods. Many different grades and types of protein-enriched products, e.g., caseins and caseinates, whey protein concentrates (WPCs) and whey protein isolates (WPIs), milk protein concentrates (MPCs) and specific protein blends designed especially for particular applications, are manufactured from milk.

MPCs are processed directly from skim milk by a combination of ultrafiltration/diafiltration (Mulvihill, 1992). MPC can have a range of protein contents from 56 to 82%; the casein is in a micellar form, similar to that found in milk, and the whey proteins are also in their native form. MPCs have been used as ingredients in many food applications such as milk extension in cheese, yoghurt manufacture and nutritional beverages.

The individual milk proteins, such as $\alpha_s$-1-, $\alpha_s$-2-, $\beta$- and $\kappa$-caseins, $\beta$-lactoglobulin ($\beta$-lg) and $\alpha$-lactalbumin ($\alpha$-la), have very good emulsifying properties (Dickinson, 1998), but their ability as emulsifying agents is compromised when they are present in an aggregated or micellar form (Mulvihill & Murphy, 1991). The emulsifying ability of a protein can be determined from the particle size of the emulsion droplets generated at a given protein concentration under defined homogenization conditions. The smaller the droplet size (i.e., the larger the surface area), the better is the protein as an emulsifying agent. The emulsifying ability of "aggregated" milk protein products, such as MPC and calcium caseinate, is much lower than that of whey protein and sodium caseinate (Euston & Hirst, 1999; Singh & Ye, 2008; Ye, Srinivasan, & Singh, 2000). Both sodium caseinate and whey protein products (WPC and WPI) show excellent emulsifying ability, and it is possible to make stable emulsions at a relatively low protein-to-oil ratio (about 1:60) (Ye & Singh, 2000, 2001). Conversely, much higher concentrations of MPC or calcium caseinate are required to make a stable emulsion and larger droplets are formed in these protein-stabilized emulsions under similar homogenization conditions. The relatively low emulsifying ability of MPCs has limited their applications in some food formulations.

New MPC products, in which the casein micelles have been dissociated to some extent by reducing the calcium content, are now being promoted for extensive applications. The objective of this work was to evaluate the emulsifying abilities and the adsorption behaviours of these MPC products with different calcium contents or extents of casein dissociation, as well as the stabilities of emulsions made with these MPCs. The relationship between the emulsifying properties and the dissociation of casein micelles in the MPCs is discussed.
2. Materials and methods

2.1. Materials

MPCs and sodium caseinate were supplied by the Fonterra Co-operative Group Limited, New Zealand. The protein, sodium and calcium contents in the MPC powders are shown in Table 1. The calcium-depleted MPCs were produced using cation exchange to replace divalent ions with monovalent ions and then ultrafiltration/diafiltration (Dybing, Bhaskar, Dunlop, Fayerman, & Whitten, 2002). Soya oil was purchased from Davis Trading Co., Palmerston North, New Zealand. All the chemicals used were of analytical grade and were obtained from either BDH Chemicals (BDH Ltd, Poole, England) or Sigma Chemical Co. (St. Louis, MO, USA) unless otherwise specified.

2.2. Characterization of particle size of protein dispersions

MPCs and sodium caseinate dispersions (1%, w/w) were prepared by adding MPC and sodium caseinate powders to Milli-Q water (Millipore Corp., Bedford, MA, USA) and then stirring for 60 min at room temperature to ensure complete dispersion. The pH of the dispersions was adjusted in the range 6.8–7.0.

Particle size (z-average hydrodynamic diameter) measurements were made by dynamic light scattering using a Nanosizer ZS (Malvern Instruments Ltd, Malvern, Worcestershire, UK) with a He/Ne laser emitting at 633 nm and a 4.0 mW power source (Anema & Li, 2003). The instrument used a backscattering configuration with detection at a scattering angle of 173° using an avalanche photodiode. The temperature of the cell was maintained at 20 ± 0.5 °C for the duration of the experiments. Average diffusion coefficients were determined by the method of cumulants and were translated into average particle diameters using the Stokes–Einstein relationship for spheres. The hydrodynamic diameter was calculated assuming that the diffusing particles were monodisperse spheres and was based on the average of 13 measurements each.

2.3. Preparation of emulsions

MPC or sodium caseinate-stabilized emulsions were prepared according to the procedure described by Ye (2008). MPC dispersions (0.3–5.0%, w/w) were prepared by adding MPC powder to Milli-Q water (Millipore Corp., Bedford, MA) and then stirring for 60 min at room temperature to ensure complete dispersion. The pH of the dispersions was adjusted in the range 6.8–7.0. Appropriate quantities of soya oil were then mixed with the protein solutions to give 20% oil in the oil phase. Emulsions were prepared in duplicate.

A Malvern MasterSizer 2000 (Malvern Instruments Ltd, Malvern, Worcestershire, UK) was used to determine the average diameter (d32) of the emulsion droplets (Ye & Singh, 2001). The relative refractive index (N), i.e., the ratio of the refractive index of the emulsion droplets (1.456) to that of the dispersion medium (1.33), was 1.095. The absorbance value of the emulsion droplets was 0.001. Droplet size measurements are reported as average diameters, d32, with d32 being defined as \( \sum n d_i^3 / \sum n d_i^2 \), where \( n_i \) is the number of particles with diameter \( d_i \). Mean particle diameters were calculated as the average of duplicate measurements.

Table 1

<table>
<thead>
<tr>
<th></th>
<th>Protein (g 100 g⁻¹)</th>
<th>Sodium (mg 100 g⁻¹)</th>
<th>Calcium (mg 100 g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPC I</td>
<td>8.15</td>
<td>70</td>
<td>2230</td>
</tr>
<tr>
<td>MPC II</td>
<td>8.00</td>
<td>1150</td>
<td>1340</td>
</tr>
<tr>
<td>MPC III</td>
<td>8.22</td>
<td>1300</td>
<td>1200</td>
</tr>
<tr>
<td>MPC IV</td>
<td>8.25</td>
<td>2300</td>
<td>300</td>
</tr>
</tbody>
</table>

2.4. Determination of average droplet size of emulsions

A Malvern MasterSizer 2000 (Malvern Instruments Ltd, Malvern, Worcestershire, UK) was used to determine the average diameter (d32) of the emulsion droplets (Ye & Singh, 2001). The relative refractive index (N), i.e., the ratio of the refractive index of the emulsion droplets (1.456) to that of the dispersion medium (1.33), was 1.095. The absorbance value of the emulsion droplets was 0.001. Droplet size measurements are reported as average diameters, d32, with d32 being defined as \( \sum n d_i^3 / \sum n d_i^2 \), where \( n_i \) is the number of particles with diameter \( d_i \). Mean particle diameters were calculated as the average of duplicate measurements.

2.5. Determination of surface protein concentration and composition

Determination of surface protein concentration and composition in the protein-stabilized emulsions was following the procedure described by Ye (2008). The emulsions were centrifuged at 45,000 × g for 40 min at 20 °C in a temperature-controlled centrifuge (Sorvall RCSC, DuPont Co., Wilmington, DE, USA). The subnatants were carefully removed using a syringe. The cream layer was dispersed in deionized water and re-centrifuged at 45,000 × g for 40 min. The subnatant was filtered sequentially through 0.45 and 0.22 μm filters (Millipore Corp., Bedford, MA, USA). The filtrates were analysed separately for total protein using the Kjaeldahl method (1026 Distilling Unit and 1007 Digestor Block, Tecator AB, Hoganas, Sweden). The surface protein concentration (mg m⁻²) was calculated from the surface area of the oil droplets.

Adsorbed protein (g)

\[
= \text{total protein (g) taken for making an emulsion} - \left[ \text{protein (g) present in the subnatant} + \text{protein (g) present in the sediment} \right]
\]

Total protein coverage (mg m⁻²)

\[
= \text{total protein adsorbed (mg)/total fat surface area (m²)}
\]

The compositions of the adsorbed protein at the surface of the emulsion droplets and the non-adsorbed protein in the aqueous phase were determined using sodium dodecyl-sulphate polyacrylamide gel electrophoresis (SDS-PAGE), as described by Ye and Singh (2001). A certain amount of cream was spread on to a filter paper and a known amount of cream was mixed with SDS buffer (0.5 % Tris, 2% SDS, 0.05% mercaptoethanol, pH 6.8). A portion (5 μl) of this dispersion or the subnatant (after filtration) was applied to SDS gels previously prepared on a MiniProtean II system (Bio-Rad Laboratories, Richmond, CA, USA). After destaining, the gels were scanned on a laser densitometer (LKB Ultrascan XL, LKB Produkter AB, Bromma, Sweden). The percentage composition of each sample was determined by scanning the areas for α-lactalbumin and β-lactoglobulin and expressing the individual protein peaks as a fraction of the sum total.

2.6. Confocal laser scanning microscopy

A Leica (Heidelberg, Germany) confocal laser scanning microscope with a chosen objective lens and an Ar/Kr laser with an excitation line of 488 nm (such that only the fluorescent wavelength band could reach the detector system) was used to...
determine the microstructures of the emulsions. Emulsions were made as described above; approximately 3 mL of sample was taken in a test tube. Nile blue (fluorescent dye) was mixed through and the mixture was placed on a microscope slide. The slide was covered with a coverslip and observed under the microscope.

2.7. Statistical analysis

The samples were prepared in duplicate. All tests were replicated twice. Experimental data were evaluated by running analysis of variance (ANOVA) tests, which determine whether there are any significant differences amongst the means at a 95.0% confidence level. These analyses were performed using the Minitab 14 (Minitab Pty Ltd, State College, PA, USA) for Windows package. Treatment means were considered to be significantly different at \( P \leq 0.05 \).

3. Results

3.1. Particle size of milk protein concentrate dispersions

The average particle sizes of 1% (w/w) MPC dispersions and sodium caseinate are shown in Fig. 1. The particle size of the MPC I dispersion was \( \sim 200 \) nm, which was similar to the size of casein micelles in milk (Anema & Klostermeyer, 1997), indicating that intact casein micelles remained in MPC I. The size decreased in the order MPC I > MPC II > MPC III > MPC IV, indicating that the casein micelles in the MPCs dissociated to some extent when the calcium content was reduced (Table 1). This suggests that some (or all) of the casein in the MPC II, MPC III and MPC IV dispersions, in which the caseins had dissociated from the casein micelles to some extent, may no longer have been present as micelles. However, it does not suggest molecular casein in the MPC dispersions. The casein in these MPCs may have formed sub-micelle-type particles, ranging in size from 20 to 200 nm. Sub-micelle-type particles have been observed in casein and sodium caseinate dispersions (HadjSadok, Pitkowski, Nicolai, Benyahia, & Moulai-Mostefa, 2008; Kumasinski, King, & Farrell, 1994; Lucey, Srinivasan, Singh, & Munro, 2000). The state of the casein in these MPC products may reflect their various functional properties, in particular their emulsifying properties.

3.2. Emulsifying capability of milk protein concentrate

The average particle size (\( d_{32} \)) of emulsions formed with different concentrations of the MPC dispersions is shown in Fig. 2; the size of emulsions formed with sodium caseinate is also shown as a control. The \( d_{32} \) values of the MPC-stabilized emulsions decreased with an increase in the protein concentration. At a given protein concentration, the \( d_{32} \) values of the emulsions were MPC I > MPC II > MPC III > MPC IV > sodium caseinate. For example, at a protein concentration of 0.3% (w/w), the \( d_{32} \) values were greater than 1 \( \mu \)m in the emulsions made with MPC I, MPC II and MPC III; only the \( d_{32} \) value of the emulsion formed with MPC IV was <1 \( \mu \)m, the same as that of the sodium caseinate-stabilized emulsion (Fig. 2). The protein concentrations that were required to form a fine emulsion (\( d_{32} < 1 \mu m \)) were 0.3% (w/w) for MPC IV, 1% (w/w) for MPC III, 2% (w/w) for MPC II and 5% (w/w) for MPC I. For MPC I, a fine emulsion was not formed at a protein concentration of <4% (w/w; \( d_{32} > 5 \mu \)m) and could be formed only at a protein concentration of 5% (w/w). It has been shown that the smaller the size of the oil droplets generated, the greater is the emulsifying capability of the protein (Euston & Hirst, 1999). Therefore, the present result indicates that the order of emulsifying capability was MPC IV > MPC III > MPC II > MPC I.

3.3. Adsorption behaviour of milk protein concentrate

Fig. 3 shows the total surface protein concentration (mg m\(^{-2}\)) of MPC in the emulsions formed with different protein concentrations. For all MPC products, the total surface protein concentration increased with increasing protein concentration up to 5% (w/w), except that the total surface protein concentration remained constant above 2% (w/w) protein in the emulsions formed with MPC I. It has been reported that the total surface protein concentration of MPC is in the range 8–15 mg m\(^{-2}\) and is much higher than that of sodium caseinate (Euston & Hirst, 1999; Singh & Ye, 2008). In the present work, the surface protein concentrations of MPC I were from 8 to 14 mg m\(^{-2}\) in emulsions formed with protein concentrations in the range from 0.3 to 5% (w/w). The surface protein concentrations of the other MPCs were between that of MPC I and that of sodium caseinate at a given protein concentration. The order was MPC I > MPC II > MPC III > MPC IV. Compared with the total surface protein concentrations of sodium caseinate, those of MPC IV...
were lower at low protein concentration (≤0.5% (w/w)), but were similar at protein concentrations ≥1% (w/w) (Fig. 3). This suggests that, at high protein concentrations, when the caseins were probably aggregated to some extent, the state of the protein and the properties of MPC IV and sodium caseinate were similar.

The surface protein compositions of MPC-stabilized emulsion droplets are shown in Fig. 4. At the surface of emulsion droplets formed by MPC I, compared with the proportions in milk, the proportions of αs-casein, β-casein, κ-casein at the surface were similar to that in casein micelles, but those of β-lg and αs-la were lower from 0.3% (w/w) to 5% (w/w) protein (Fig. 4A). This indicates that, in the emulsions stabilized using MPC I, the caseins adsorbed at the surface of emulsion droplets in the form of casein micelles and dominated the surface layer, probably because of their large size compared with whey protein molecules. For emulsions stabilized by MPC IV, compared with the proportions in milk, more αs1-casein and less β-casein were adsorbed at the droplet surface in the emulsions stabilized by protein concentrations from 0.3% (w/w) to 5% (w/w) (Fig. 4B). This suggests that there was competitive adsorption among the individual caseins during the formation of emulsions in which the caseins were no longer in the form of casein micelles.

### 3.4. Stability of emulsions

The creaming stabilities of emulsions stabilized with MPCs are shown in Fig. 5. The stabilities of the emulsions formed with MPC I were very low at protein concentrations ≤3% (w/w) and then increased at higher protein concentrations up to 5% (w/w). For the emulsions formed with the other MPCs, the stability increased with an increase in the protein concentration up to a maximum and then decreased with a further increase in the protein concentration. The protein concentrations for the maximum stabilities of the emulsions were 4, 3 and 2% (w/w) for MPC II, MPC III and MPC IV respectively. It should be noted that the maximum stability point of the sodium caseinate-stabilized emulsion was 1% (w/w) (Fig. 5) and that the maximum stabilities of the emulsions stabilized by these MPCs and by sodium caseinate were not significantly different (P > 0.05).

At protein concentrations <2% (w/w), the order of emulsion stability was sodium caseinate > MPC IV > MPC III > MPC II > MPC I, suggesting that the stability was dependent on the size of the emulsion droplets at low protein concentrations, which was in the order sodium caseinate > MPC IV > MPC III > MPC II > MPC I (Fig. 3). However, the reverse order of emulsion stability — MPC I > MPC II > MPC III > MPC IV > sodium caseinate — occurred at 5% (w/w) protein, when the sizes of the emulsion droplets were almost identical (Fig. 2).

### 3.5. Microstructures of emulsions

The confocal micrographs show the microstructures of the emulsions made with these MPCs (Fig. 6). At 0.5% (w/w) protein, large-sized droplets and flocculation of some small droplets were observed in the emulsions made with MPC I, MPC II and MPC III, whereas smaller droplets and no flocculation were observed in the emulsion made with MPC IV. At 1% (w/w) protein, the droplets were evenly distributed with some large-sized droplets in the
emulsions formed by MPC II, MPC III and MPC IV, whereas the droplets in the MPC I emulsion were flocculated and large. At 5% (w/w) protein, the droplets in the MPC I emulsion were small and evenly distributed, slight flocculation of small droplets was observed in the MPC II emulsion and a network formed from strong flocculated droplets occurred in the emulsions stabilized with MPC III and MPC IV (Fig. 6).

4. Discussion

The present results demonstrate that the emulsifying abilities of MPCs are related to the extent of casein aggregation. MPCs with lower calcium content and smaller casein particles can form more stable emulsions with smaller emulsion droplets. Euston and Hirst (1999) have reported that aggregated protein products such as skim milk powder and MPC have lower emulsifying abilities than sodium caseinate and WPC. Aggregation would decrease the number of protein molecules that are available for adsorption to the newly created oil droplet surfaces. In addition, after adsorption, aggregated protein particles at the surface may restrict effective spreading and rearrangement of the surface proteins. This will result in the formation of large-sized droplets due to coalescence during homogenization and in the formation of aggregated particles due to bridging flocculation as a result of protein sharing between droplets (Fig. 6).

The high surface protein concentration in the MPC I emulsions suggests that the micellar casein adsorbed to the interface as particles and formed a thick surface layer. Micellar casein may be less flexible than unfolded casein molecules on the interface, although casein micelle spreading at the interface of fat globules has been observed in homogenized milk (Walstra, 1995). It has been reported that emulsions formed with aggregated protein such as MPC and skim milk protein have a higher protein load at the droplet surface than emulsions formed with caseinate and whey protein (Euston & Hirst, 1999). This is supported by the surface protein compositions, which were similar to the casein composition in the casein micelle (Fig. 4). The results also show that whey protein adsorbed to the interface but that the proportions of whey proteins (β-lg and α-La) on the interface were low in the MPC I dispersion (Fig. 4), indicating that more casein than whey protein

Fig. 5. Changes in the stability rating (%) of MPC-stabilized emulsions (20% soya oil, pH 7.0) as a function of protein concentration: (■) MPC I emulsion; (●) MPC II emulsion; (▲) MPC III emulsion; (●) MPC IV emulsion; (●) sodium caseinate emulsion. Each data point is the average of two determinations on separate emulsions.

Fig. 6. Confocal micrographs of MPC-stabilized emulsions (20% soya oil, pH 7.0). Emulsions formed with protein concentrations of 0.5% (A), 1% (B) and 5% (C).
adsorbed at the interface from the aqueous phase. This is in agreement with Sharma, Singh, and Taylor (1996), who observed little whey protein and mainly casein micelles and spread micelles on the surface of milk fat globules after homogenization. However, these results do not prove that casein micelles adsorb preferentially to the interface, because we do not know how much area of surface is covered by casein micelles or whey proteins. This larger amount of casein or higher proportions of the caseins at the surface may be due to the larger size of the micelles at the surface and a thicker interface, which may cover less interfacial area.

The lower surface protein concentrations in the emulsions formed with the MPCs with lower calcium contents suggest that the dissociated casein (not aggregated) is adsorbed at the interface. This was also shown by the fact that the proportions of the individual caseins at the interface of emulsion droplets formed with low calcium content MPCs were not identical to the proportions in casein micelles (Fig. 4). It has been suggested that individual caseins adsorb competitively at the interface (Dickinson, Rolfe, & Dalgleish, 1988; Robson & Dalgleish, 1987). The greater proportion of αs1-casein at the interface may be attributed to the aggregation of β-casein to some extent, which reduced its adsorption (Hunt & Dalgleish, 1994). Also, the proportions of β-β and α-α increased at the interface in the low calcium MPC emulsions (Fig. 4), suggesting that they also compete to adsorb at the interface. This has been shown in emulsions made with mixtures of caseins and whey proteins (Ye, 2008).

The increase in the stability of the emulsions with increasing protein concentration in the low concentration range (Fig. 5) suggests that the stability was dependent mainly on the particle size of the emulsions. Emulsion stability was promoted by reducing the particle size through increasing the protein concentration used to make the emulsions or increasing the emulsifying ability of the protein through dissociating the casein micelles.

However, when the protein concentration was high enough to form a fine emulsion, i.e., the droplet size of the emulsion could not be decreased further by increasing the protein concentration, the stability of the emulsions decreased with a decrease in the calcium content of the MPCs (Fig. 5). This decrease in stability can be attributed to depletion flocculation induced by the protein particles in the aqueous phase (Dickinson & Golding, 1997). This suggests that the size of the protein particles in MPCs with low calcium content is in the right range to induce flocculation. Furthermore, the concentration of protein particles with this size increased with a decrease in the calcium content because less protein adsorbed to the surface (Fig. 3). It should be noted that these protein particles with a size range of 20–50 nm were probably formed from the self-assembly of caseins dissociated from the casein micelles.

It has been reported that the depletion flocculation in caseinate-stabilized emulsions can be attributed to protein particles with an appropriate particle size and an appropriate concentration in the aqueous phase. The size range of protein particles is 10–100 nm and the optimal value is 40 nm (Radford & Dickinson, 2004); the concentration in the aqueous phase should be higher than 2% (Dickinson & Golding, 1997). Once these two conditions (size and concentration) are met, flocculation takes place and the stability of the emulsion is reduced. This decrease in stability is enhanced by an increase in the concentration of the protein particles in the aqueous phase (Ye, 2008). However, the depletion effect will disappear when the particle size is out of range, e.g., through the aggregation of casein molecules induced by calcium or high ionic strength (Ye & Singh, 2001). Therefore, this phenomenon has not been observed in emulsions formed with other milk proteins such as whey protein, calcium caseinate, WPC and skim milk protein (Euston & Hirst, 1999; Ye & Singh, 2000; Ye et al., 2000).

5. Conclusions

The emulsifying properties of MPC and the stability of the emulsions formed with MPC were influenced by the state of the protein particles in the MPCs. When casein micelles in the MPCs dissociated because of a reduction in the calcium content, the emulsifying ability was improved through the formation of a fine emulsion with a smaller droplet size at a lower protein concentration. The fine emulsions demonstrated a high stability at low protein concentration. The surface protein concentration and the surface protein composition were also altered in the emulsions formed by these MPCs with different casein states. These alterations in the surface protein concentration and composition due to alteration in the aggregation of casein in MPC changed the size and the amount of unadsorbed casein in the aqueous phase. Thus, the stability of an emulsion may be decreased by the depletion flocculation induced by the unadsorbed protein at high protein concentration. This suggests that depletion flocculation may need to be considered when a protein emulsifying agent is chosen and a suitable protein concentration, taking into account the properties of the protein products, should be selected to obtain the best emulsion stability.

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References


