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Rehydration behaviour of spray-dried micellar casein concentrates produced using microfiltration of skim milk at cold or warm temperatures

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ABSTRACT

Microfiltration (MF) of skim milk, when combined with diafiltration (DF), facilitates the manufacture of liquid micellar casein concentrate (MCC), which can be spray-dried into high-protein (≥80% protein, dry-basis) powders. MCC powders rehydrate slowly, which is typically considered a defect by end-users. This study compared the impact of cold (<10 °C) or warm (50 °C) MF/DF on the rehydration characteristics of MCC powders (MCC_{cold} and MCC_{warm}, respectively). The wetting properties of the MCC powders, measured using optical tensiometry, were found to be equivalent. Pronounced differences in dispersion characteristics were measured, and, after 90 min rehydration at 50 °C, liberated casein micelles accounted for only 7.5% of particle volume in MCC_{warm} compared with 48% in MCC_{cold}. Due to its superior dispersion characteristics, MCC_{cold} yielded 50–60% less sediment during analytical centrifugation experiments. Cold MF/DF may improve the solubility of MCCs by accelerating the release of casein micelles from powder particles during rehydration.

1. Introduction

The protein content of bovine milk is ~35 g L^{-1}, with caseins accounting for approximately 80% of protein and the remaining 20% consisting of whey proteins (Fox & McSweeney, 1998). This casein exists primarily as casein micelles, which are large colloidal assemblies of four individual phosphoproteins, α_{S1}-, α_{S2}-, β-, and κ-casein (Farrell et al., 2004), that bind ~ 69% of the calcium and ~ 46% of inorganic phosphate in milk as colloidal calcium phosphate (Gaucheron, 2005). Casein has long been fractionated from milk and converted into powders for use in both non-food, e.g., paint, glue (Audic, Chaufer, & Daufin, 2003) and food-based, e.g., analogue cheese, cream liqueur (O'Mahony & Fox, 2013) applications. Two traditional methods of purifying casein are isoelectric precipitation through acidification ('acid casein') or enzymatic hydrolysis with chymosin ('rennet casein'). Both of these approaches yield a solid curd, which can be mechanically disrupted and dried into powders that are insoluble. Additional processing steps are required to generate a soluble material for drying; for example, acid casein can be converted into sodium caseinate through alkalisation (Carr & Golding, 2016).

A more recent technology to manufacture casein in its 'native' (micellar) form is microfiltration (MF), a pressure-driven separation process incorporating semi-permeable membranes with a pore-size of ~0.1 µm (Pierre, Fauquant, Le Graet, & Maubois, 1992; Saboya & Maubois, 2000). Diafiltration (DF) with deionised water during MF facilitates the production of MCCs in which the protein fraction is usually 85–95% casein. MCCs are often used as ingredients in protein supplements and clinical nutrition products. As these applications can require reconstitution of MCCs by the manufacturer (i.e., wet mixing of dry ingredients) and/or the consumer

(i.e., reconstitution of dried blended powders for consumption), it is typically a requirement that the powders rehydrate quickly and completely. For milk powders, the rehydration process is characterised by multiple stages, including wetting of the powder when added to the liquid and subsequent dispersion of the powder particles; the duration of these stages, wetting and dispersion, have been reported to be protracted for high-protein powders (Crowley, Jeantet, Schuck, Kelly, & O'Mahony, 2016). For MCCs, the wetting stage, and in particular the dispersion stage, are slow. Poor dispersion characteristics can negatively affect powder handling in manufacturing facilities, due to blockages in process lines, and impair consumer acceptability of final products, due to the presence of lumps or sediment (Mitchell et al., 2015). Although MCC powders are soluble, they exhibit exceptionally long rehydration times in comparison with other milk-derived powders (e.g., skim milk powder, whey protein concentrate, sodium caseinate); milk protein concentrate powders (MPCs) have a similar challenge, although it is less pronounced than for MCCs (Crowley et al., 2016) due to the higher proportion of the more soluble whey proteins in the former (Mimouni, Deeth, Whittaker, Gidley, & Bhandari, 2010).

Various strategies have been developed to improve the rehydration of MCCs and MPCs, and have typically been targeted towards improving dispersion characteristics. Many successful approaches have been based on pre-treatments applied to concentrated protein fractions from milk prior to drying, such as that of Bhaskar, Singh, and Blazey (2001), in which calcium was removed from ultrafiltered milk by ion-exchange before mixing with untreated concentrate and drying into an MPC powder. Incorporation of sodium caseinate into the concentrate before the drying of MCC increased its solubility (Schokker et al., 2011), while the application of high-pressure treatments to the concentrate before drying of MPCs also resulted in

solubility enhancement (Udabage, Puvanenthiran, Yoo, Versteeg, & Augustin, 2012). Bouvier, Collado, Gardiner, Scott, and Schuck, (2013) investigated an alternative drying technology, extrusion-porosification, which was found to produce a more soluble powder than spray drying. Others have developed strategies that can be applied during powder reconstitution itself, with various researchers reporting that high-shear and ultrasonication technologies were effective when applied during reconstitution of MCCs and MPCs (Augustin, Sanguansri, Williams, & Andrews, 2012; Chandrapala, Martin, Kentish, & Ashokumarr, 2014a; McCarthy, Kelly, Maher, & Fenelon, 2013). Increasing either temperature or the number of stirrer revolutions during rehydration improved the rehydration properties of an MCC (Jeantet, Schuck, Six, Andre, & Delaplace, 2010). An elevated reconstitution temperature, combined with the addition of monovalent salts (KCl), was more effective in promoting the dispersion of MPCs than either method alone (Crowley et al., 2015).

There is a lack of options available for processors who wish to improve the rehydration performance of MCCs without potentially: (i) incurring significant capital expenditure (to procure solubility-enhancing equipment); (ii) modifying ingredient techno-functionality (by replacing or dissociating micellar casein); or (iii) altering ingredient listings (through the use of additives). It is perhaps surprising therefore that the temperature at which MF is carried out during MCC manufacture has not received more attention. MF in the dairy industry has traditionally been performed at ~50 °C, which is optimal for high permeate flux and efficient removal of whey proteins (Hurt, Adams, & Barbano, 2015); however, MF (and also ultrafiltration) at temperatures <15 °C is becoming more common in the dairy industry (Lawrence, Kentish, O'Connor, Barber, & Stevens, 2008), and studies have

115 shown that this may facilitate operation with lower fouling (Luo, Ramchandran, &
116 Vasiljevic, 2015), and enrichment of β-casein in the whey protein stream (Coppola,
117 Molitor, Rankin, & Lucey, 2014; O'Mahony, Smith, & Lucey, 2014). Modifications
118 in the functional properties of MCCs, such as gelation and melting, due to β-casein
119 depletion during cold MF have also been reported (O'Mahony, McSweeney, &
120 Lucey, 2008, 2009). However, the influence of MF temperature on the rehydration
121 characteristics of MCC powders has not been evaluated in detail and this study aims
122 to address this gap in current knowledge. Depletion of calcium (Bhaskar et al., 2001)
123 and increased levels of whey protein (Richard et al., 2013) have been shown to
124 improve the dispersibility of casein-dominant powders; as cold filtration of milk can
125 cause both effects (Karasu et al., 2010; Luo et al., 2015; O'Mahony et al., 2014) it is
126 possible that this process also improves powder solubility.

127 Two spray-dried retentate (casein) streams were analysed, one generated
128 using traditional warm MF (50 °C, MCC_{warm}) and another using cold MF (<10 °C,
129 MCC_{cold}). The rehydration characteristics of these powders were compared using a
130 range of analytical techniques to understand the impact of MF temperature on
131 rehydration characteristics of MCC powders. The present study builds on previous
132 work (McCarthy, Wijayanti, Crowley, O'Mahony, & Fenelon, 2017) that focused
133 primarily on the impact of different temperatures on filtration performance and
134 protein and mineral composition of the permeate (whey) stream generated during MF
135 of milk. McCarthy et al. (2017) demonstrated that MF of milk at low temperatures
136 decreased the calcium content of the micellar casein (MF retentate) fraction, which
137 was consistent with the results of Luo et al. (2015), who demonstrated a similar
138 effect for ultrafiltered milk. It has also been shown that the use of cold filtration
139 temperatures results in a higher whey protein:casein ratio in MF retentates (Karasu et

al., 2010; O'Mahony et al., 2014). This study investigates whether such alterations to the protein and mineral profile of the retentate during cold MF result in modified powder solubility after spray drying.

2. Materials and methods

2.1. Manufacture of micellar casein concentrate powders

The MCC powders used in the present study were two of several produced in a previous study; detailed descriptions of the processes used can be found in that report (McCarthy et al., 2017). The processes can be briefly summarised as follows; skim milk was batch-diafiltered 1:2 with reverse osmosis (RO) water and held overnight at ~4 °C, before holding at <10 °C or 50 °C prior to membrane filtration. MF/DF of the milk was performed with 0.14 µm Tami Isoflux® ceramic membranes (Tami Industries, Nyons Cedex, France) on a GEA Model F filtration unit (GEA Process Engineering A/S, Skanderbog, Denmark) operated in retentate recirculation mode. The temperature throughout processing was maintained at <10 °C or 50 °C using an in-line heat exchanger. MF was performed until the volume of the milk/RO water was reduced by a factor of 9. Liquid MCCs were then evaporated using a Tetra Scheffers® falling-film single-stage evaporator (Tetra Pak, Gorredijk, The Netherlands) and spray drying was carried out using a pilot-scale Anhydro Lab 3 spray dryer (SPX Flow Technology A/S, Soeborg, Denmark) with a wheel atomiser operating with inlet and outlet temperatures of 178 °C and 88 °C, respectively. The total solids levels of the liquid feeds for the drier were 15.6 ± 1.2% and 14.8 ± 2.1%, respectively, for MCCs generated using warm and cold MF. The MCC powders

contained 4.4–4.7% moisture across repeat process trials (McCarthy et al., 2017). The powders were stored in air-tight bags in the dark at 20 °C prior to analysis. All subsequent analyses and experiments (Sections 2.2–2.6) on the two MCC powders were performed in at least duplicate, with results presented as the means of at least two independent measurements on freshly prepared samples.

2.2. Composition of powders and colloidal properties of reconstitutes solutions

Protein content of the MCC powders was measured by the Kjeldahl method using a nitrogen-to-protein conversion factor of 6.38 (IDF, 2001). Mineral profiling was carried out using inductively-coupled plasma mass spectrometry (Herwig, Stephan, Panne, Pritzkow, & Vogl, 2011). The size and charge of casein micelles in reconstituted solutions was assessed using a Zetasizer Nano ZS (Malvern Instruments, Malvern, UK) according to McCarthy, Kelly, O'Mahony, and Fenelon (2014). Protein profile was determined by reversed phase-high performance liquid chromatography (RP-HPLC), as detailed by McCarthy et al. (2017).

2.3. Distribution of protein and fat in powder particles

The distribution of protein and fat in MCC powder particles was determined using a Leica TCS SP5 confocal laser scanning microscope (CLSM; Leica Microsystems CMS GmbH, Wetzlar, Germany). Dual labelling using Nile Red (0.1%, w/v, in propanediol) and Fast Green FCF (0.01%, w/v, in water) was carried out to visualise the protein and fat phases, respectively, in the powder particles. The dye solutions were mixed in a ratio that allowed diffusion of the dyes into the

powder particles whilst preventing their solubilisation, as proposed by Maher, Auty, Roos, Zychowski, and Fenelon (2015). The observations were performed using 63× oil immersion objective (numerical aperture = 1.4) at excitation wavelengths of 488 nm and 633 nm provided by Ar and He/Ne lasers. Images of 512 × 512 pixels were acquired using zoom factor of 3. At least three specimens of each sample were examined to obtain representative images.

2.4. Wetting behaviour: Optical tensiometry

Measurements of contact angle were carried out as described by Silva and O'Mahony (2017) using an optical tensiometer (Attension Theta, Biolin Scientific Ltd., Espoo, Finland). Contact angles were measured at 20 °C after a droplet of deionised water (5 µL) was placed on discs (d ≈ 13 mm, h ≈ 1.5 mm) of MCC powders prepared by compression using a Specac® manual hydraulic press (Perkin Elmer, Buckinghamshire, UK).

2.5. Ion release: Conductimetry and calcium-ion concentration

Monitoring of ion release during the rehydration of MCC powders was carried out using a Titrando autotitrator and accompanying Tiamo v2.3 software equipped with either a five-ring conductivity measuring cell or a calcium (Ca)-ion-selective electrode (Metrohm Ireland Ltd, Athy Road, Co. Carlow, Ireland). The probes were calibrated at 25 °C or 50 °C (depending on the rehydration experiment temperature) with buffer solutions of known conductivity and Ca-ion concentration (where applicable). A period of 1 min was allowed to elapse for establishment of a

baseline before the powder was added, over a period of 2 min, with continuous measurement throughout. For monitoring the release of ions during rehydration, powders were added to beakers containing deionised water to attain 1.5% protein suspensions. Beakers were placed in water baths equilibrated at 25 or 50 °C and the contents mixed using overhead stirrers with four impeller blades. If some wetted powder adhered to the vessel wall, it was removed by gentle washing with a Pasteur pipette filled with a small volume of the solution studied.

2.6. Dispersion: Particle size distribution and analytical centrifugation

A Malvern Mastersizer 3000 (Malvern Instruments Ltd., Malvern, UK) was used to measure the particle size distribution (PSD) in MCC suspensions after rehydration for 90 min. Analysis of PSD was performed using a particle refractive index of 1.46, absorption of 0.1 and dispersant refractive index of 1.33. MCC suspensions were introduced into the dispersing unit of the instrument with deionised water as dispersant until a laser obscuration of 12.5 ± 1% was achieved. Data are presented as volume-based PSDs.

To measure the sedimentation behaviour in MCC suspensions rehydrated for 90 min, an analytical centrifuge (LUMISizer®, L.U.M. GmbH, Berlin, Germany) was used according to the method of Crowley et al. (2015), in which the intensity of transmitted NIR light (880 nm) was measured as a function of time and position over the length of a polycarbonate cell held horizontally over the light path during centrifugation. The height of initial sediments formed after centrifugation at 36 × *g* for 10 min, and the compressed sediments formed during subsequent centrifugation at 168 × *g* for 10 min, were measured by subtracting the position of the

supernatant/sediment boundary from the position of the cell bottom. Mean transmission values were also calculated for the region above the (compressed) sediment and below the meniscus (114–124 mm, common for all experimental runs). For the PSD and sedimentation analyses, powders were added to beakers containing deionised water to attain 1.5% protein suspensions. Beakers were placed in water baths equilibrated at 25 or 50 °C and the contents mixed using overhead stirrers with four impeller blades.

3. Results

3.1. Composition and physicochemical properties of powders

Operation of the MF/DF process at <10 °C or 50 °C resulted in several differences between the MCC_{cold} and MCC_{warm} powders. The proportion of whey protein was higher in the former (indicated by lower casein content), the β-casein:α-casein ratio was lower, and there was a decrease in the level of both calcium and phosphorus (Table 1). Levels of the monovalent ions measured were, however, relatively unchanged. The influence of processing temperature on colloidal properties of reconstituted MCCs was also comparatively minor, but the size and net negative charge of the casein micelles was slightly higher for the MCC_{cold} (Table 1).

3.2. Component distribution in MCC powder particles

Representative CLSM images of the MCC powders are shown in Fig. 1. The particles in both MCC powders were characterised by large protein-dense regions

interspersed with a minor proportion of fat globules, which surrounded internal air vacuoles (black regions). The main difference observed between the two MCC powders in CLSM profiles appeared to be the size of the fat globules, which were larger in the MCC_{warm}.

3.3. Wetting behaviour of MCC powders

Wetting behaviour was analysed by measuring the contact angle formed between a droplet of water and a compressed disc of each MCC powder. Assuming that interference from topological differences was negligible, a high value for contact angle indicates that a powder is less wettable (i.e., more hydrophobic), while a reduction in contact angle over time is caused by spreading at the surface (Mitchell et al., 2015). The data from these experiments showed that there were no apparent differences in the initial wetting behaviour of the powders on initial contact with the droplet or over time (Fig. 2).

3.4. Ion release from MCC powders during rehydration

Conductivity was measured continuously during the rehydration of the MCC powders. There was an initial sharp increase in conductivity as ions were released from the powder on introduction to water, and an eventual steady-state condition was reached as the release of ions was completed (Fig. 3A). For both powders, rehydrated to 1.5% protein, the time to reach steady-state was ~3000 s (Fig. 3A). Rehydration at 50 °C resulted in a higher conductivity reading throughout the experiment compared with rehydration at 25 °C, due to increased

diffusion/dissociation of ions at the higher temperature; however, no trends for the effect of rehydration temperature on the time to reach steady-state conductivity were observed.

The final conductivity was directly proportional to the amount of powder added to the water (data not shown). More pronounced differences in ion release were detected when ionic Ca was measured in isolation (Fig. 3B). The MCC_{cold} powder exhibited a faster release of Ca, a quicker return to steady-state, and a higher total Ca level throughout.

3.5. Dispersion behaviour of MCC powders

The progression of dispersion for a casein-dominant powder such as MCC can be tracked by measuring the PSD after a period of rehydration (Crowley et al., 2015). The dispersion process of a MCC powder can be considered as comprising primarily of the disappearance of micron-sized primary powder particles (after wetting and submersion) and the release of nanometer-sized casein micelles; when this process is complete, the powder can be considered dissolved. In Fig. 4A, it can be seen that primary particles dominated the PSD after 90 min rehydration at 25 °C; this does not necessarily mean that casein micelles have not been released, but only that they are contributing little to the overall particle volume. Under these conditions, the particles in the MCC_{warm} were notably larger, indicating that dispersion was less advanced. When dispersion was promoted by increasing temperature of reconstitution to 50 °C, a casein micelle population was apparent for both powders (Fig. 4B). The MCC_{cold}, however, contained a much higher proportion of casein micelles after 90 min rehydration at 50 °C compared with the MCC_{warm}, indicating

that the former powder had far better dispersion characteristics; from Table 2, it can be seen that these rehydration conditions led to almost 50% of the particle volume in the MCC_{cold} being comprised of casein micelles (<1 μm), while this proportion was only <10% for the MCC_{warm}.

To investigate the influence of these differences in dispersion state (Fig. 4, Table 2) on the sedimentation behaviour of the MCC suspensions on rehydration, an analytical centrifuge was used. In Fig. 5, representative sedimentation profiles are shown for each MCC after rehydration at 25 or 50 °C, indicating the transmission (T%) through the length of the sample cell during centrifugation. Reading from left to right, these profiles represent an increasing distance from the rotor of the centrifuge, and can be characterised by an initial high T% region (air), a boundary region (meniscus), and an extended low T% region (suspension) leading finally to a sharp reduction in T% (sediment). The low T% in the region above the meniscus for MCCs rehydrated at 50 °C, which was also observed in a previous study on MPCs (Crowley et al., 2015), is noted, and is likely due to condensation effects. In addition, it was observed that T% data in the suspension region were noisier for the initial profiles, which was attributed to the presence of a non-uniformly dispersed population of wetted powder particles in suspension prior to their sedimentation.

A larger sediment was observed for the MCC_{cold} compared with the MCC_{warm} on rehydration at 25 °C, both of which became compressed at the second (higher) centrifugation speed. Increasing rehydration temperature to 50 °C appeared to reduce the amount of sediment formed (Fig. 5). These profiles were used to calculate sediment heights and mean T% values for the MCC powders rehydrated at different temperatures (Fig. 6). After 10 min at 36 × *g*, the height of sediments formed from MCC_{cold} suspensions were 53–56% smaller than sediments from the MCC_{warm}. The

dispersion of both MCCs was promoted by the higher rehydration temperature (i.e., reduced sediment height), although the reduction in sediment height associated with an increase in rehydration temperature from 25 to 50 °C was greater for the MCC_{warm} (20% reduction) than the MCC_{cold} (14% reduction). The larger sediments formed in the MCC_{warm} were also more susceptible to compression when subjected to a second centrifugation step at 168 × *g* for 10 min. Based on the data in Fig. 6, MCC_{warm} sediments compressed by 22 and 33% after rehydration at 25 and 50 °C, respectively, while the equivalent values for MCC_{cold} sediments were 16 and 14%.

4. Discussion

In this study, the influence of MF temperature on the rehydration performance of MCC powders was investigated. Cold MF was found to have a positive impact on the rehydration characteristics of MCC, due to modifications in the composition, and, perhaps, the colloidal properties of the reconstituted powder (Table 1). Milk-derived powders which contain >70% protein, of which 80–95% is micellar casein, are known to have poor rehydration properties; in particular, the release of discrete casein micelles from powder particles in MCCs and MPCs is slow due to the poor dispersion characteristics of the powder particles (Crowley et al., 2015; Gaiani, Schuck, Scher, Desobry, & Banon, 2007), which has been linked with inhibited transfer of water into the powder (Richard et al., 2012; Vos et al., 2016).

Some of the changes in MCC composition caused by the lower MF temperature (<10 °C) compared with the higher MF temperature (50 °C), including a higher proportion of whey proteins and a reduction in Ca content (Table 1), have been demonstrated by previous researchers to improve the rehydration of casein-

dominant powders. Indeed, Richard et al. (2013) showed that increasing the level of whey proteins in MCC powders improved their dispersibility, while Bhaskar et al. (2001) developed a method of improving the dispersion of MPCs based on removal of Ca. The lower casein:whey protein ratio in the MCC_{cold} was due to a reduced efficiency of whey protein removal at lower filtration temperatures (Karasu et al., 2010; O'Mahony et al., 2014), while the lower Ca level was caused by dissolution of calcium phosphate from the micellar phase at the low temperature (Luo et al., 2015). It is possible that other modifications to the casein fraction caused by the lower MF temperature, such as the reduced β:α-casein ratio and the increased zeta-potential of the casein micelles (Table 1), may have influenced the rehydration characteristics of the MCCs. β-Casein is the most hydrophobic of the caseins, and its cold-induced dissociation from micelles and removal in the MF permeate (McCarthy et al., 2017) may make the MCC_{cold} better at absorbing water; however, this is not supported by contact angle data (Fig. 2) and the differences in β:α-casein ratio are small. Alternatively, the tendency for casein micelles to become inter-linked, resulting in the formation of a poorly-dispersible 'skin' (Crowley et al., 2016), may be inhibited by alterations in micellar structure (due to decreased β-casein:α-casein ratio) or increased electrostatic repulsion (caused by increased zeta-potential). It is, however, difficult to ascertain the influence of these factors compared with factors such as mineral and whey protein, which are known to strongly affect milk protein powder rehydration.

Gaiani et al. (2009) has previously shown that fat migration to the surface of powder particles during storage is an important factor influencing the rehydration behaviour of MCCs, in particular the wetting behaviour. In this study, there were apparent differences in the size of fat globules in the powders (Fig. 1), although this

390 did not influence the wetting behaviour of the MCCs, which were equivalent (Fig.
391 2). Trends in conductivity during the rehydration of the MCC powders were also
392 essentially the same for the two MCC powders (Fig. 3A), likely due to domination of
393 conductivity changes by ions such as Na^+ and K^+ which are released quickly during
394 rehydration (Mimouni et al., 2010). However, the release of ionic Ca was faster and
395 progressed to a greater degree during the rehydration of MCC_{cold} compared with
396 MCC_{warm} (Fig. 3B). As a large proportion of Ca is associated with casein micelles in
397 casein-dominant powders, a delay in its ionisation may be due to a slow release of
398 micelles during rehydration, which would reduce the rate at which Ca re-equilibrates
399 from the micellar to the serum phase (Mimouni et al., 2010).

400 Measurement of particle size after 90 min rehydration confirmed that the
401 dispersibility of the two MCC powders was different (Fig. 4). Dispersion of primary
402 powder particles was far more advanced in the MCC_{cold} powder after this period of
403 rehydration, which resulted in a greater proportion of discrete casein micelles being
404 released, most notably after rehydration at 50 °C (Table 2). Increasing rehydration
405 temperature above ambient is commonly used to promote the dispersion of these
406 powders (Jeantet et al., 2010), and these results indicate that MCC_{cold} is more
407 susceptible to the positive influence of this approach compared with MCC_{warm}. The
408 dispersion tests (PSD, sedimentation) applied in this study were applied only after a
409 90 min rehydration time and the observed effects may be more pronounced after
410 shorter rehydration times.

411 As a result of the lower levels of primary powder particles in suspension after
412 rehydration (Fig. 4), MCC_{cold} yielded approximately half of the sediment that
413 MCC_{warm} produced during centrifugation (Figs. 5 and 6). The turbidity of the
414 supernatant after sedimentation was higher for MCC_{cold} (Fig. 6), as more casein

415 micelles had been released into a stable suspension and were capable of scattering
416 light. The highest turbidity and lowest sediment were measured in the MCC_{cold}
417 rehydrated at 50 °C, indicating it had the fastest and most complete dispersion
418 properties. In addition to a greater degree of sedimentation, the sediment yielded
419 during centrifugation of MCC_{warm} was more compressible (Fig. 6). Although the
420 higher rehydration temperature (50 °C) reduced the sediment generated by both
421 MCC suspensions, the compressibility of the MCC_{warm} sediments was higher
422 compared with those formed at 25 °C. This higher compressibility of sediment may
423 be due to a greater degree of water transfer into the powder particles at the higher
424 temperature, which was not sufficient to disperse the sedimentable particles, but
425 resulted in a material that was more mechanically pliable. Thus, the strategy of
426 increasing mixing temperature to promote dispersion of these powders may create a
427 sedimentable phase in MCC_{warm} that is more susceptible to consolidation during
428 storage, which may in turn make it more difficult to re-suspend this material by
429 actions such as shaking and stirring.
430

431 **5. Conclusions**

432

433 This study demonstrated that the dispersion characteristics of MCCs are
434 improved when the MF/DF step is operated at a cold temperature. From this study,
435 the improvement in rehydration performance by the use of cold MF/DF cannot be
436 attributed to a single factor, but it is proposed that partial, limited demineralisation of
437 the micellar phase and/or the presence of a higher proportion of whey proteins in the
438 final MCC are responsible. Cold membrane filtration of milk is increasingly
439 practiced due to associated benefits including reduced membrane fouling, better

440 microbial control and possibilities for β-casein enrichment; based on the results of
441 this study, increased solubility of MCC may be an additional benefit of this approach
442 to membrane filtration. In addition, cold MF/DF may present an alternative to
443 methods for solubility-enhancement that necessitate extra equipment or additive use.
444 A study on the rehydration of MCCs prepared using MF/DF at a broader range of
445 temperatures between 0–50 °C would provide further insights into the influence of
446 MF temperature on powder rehydration. In addition, the compositional changes in
447 MCC caused by cold MF may affect functional properties other than solubility (e.g.,
448 gelation, heat stability, foaming) and this will need to be considered in future
449 evaluation of the potential of cold MF in MCC production.
450

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452

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459

460 **References**

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462 Audic, J. -L., Chaufer, B., & Daufin, G. (2003). Non-food applications of milk
463 components and dairy co-products: A review. *Lait, 83*, 417–438.

Augustin, M. A., Sanguansri, P., Williams, R., & Andrews, H. (2012). High shear treatment of concentrates and drying conditions influence the solubility of milk protein concentrate powders. *Journal of Dairy Research, 79*, 459–468.

Bhaskar, G. V., Singh, H., & Blazey, N. D. (2001). Milk protein products and process. *International Patent Specification WO 2001/41578*.

Bouvier, J. -M., Collado, M., Gardiner, D., Scott, M., & Schuck, P. (2013). Physical and rehydration properties of milk protein concentrates: comparison of spray-dried and extrusion-porosified powders. *Dairy Science and Technology, 93*, 387–399.

Carr, A., & Golding, M. (2016). Functional milk proteins production and utilisation: casein-based ingredients. In P. L. H. McSweeney, & J. A. O'Mahony (Eds.), *Advanced dairy chemistry. Vol. 1. Part B, Proteins. Applied aspects* (pp. 35–66), New York, NY, USA: Springer.

Chandrapala, J., Martin, G. J. O., Kentish, S. E., & Ashokumarr, M. (2014a). Dissolution and reconstitution of casein micelle containing dairy powders by high shear using ultrasonic and physical methods. *Ultrasonic Sonochemistry, 21*, 1658–1665.

Coppola, L. E., Molitor, M. S., Rankin, S. A., & Lucey, J. A. (2014). Comparison of milk-derived whey protein concentrates containing various levels of casein. *International Journal of Dairy Technology, 67*, 467–473.

Crowley, S. V., Desautel, B., Gazi, I., Kelly, A. L., Huppertz, T., & O'Mahony, J. A. (2015). Rehydration characteristics of milk protein concentrate powders. *Journal of Food Engineering, 149*, 105–113.

Crowley, S. V., Jeantet, R., Schuck, P., Kelly, A. L., & O'Mahony, J. A. (2016). Rehydration and solubility characteristics of high-protein dairy powders. In

P. L. H. McSweeney, & J. A. O'Mahony (Eds.), *Advanced dairy chemistry: Vol. 1. Part B. Proteins* (pp 99–131). New York, NY, USA: Springer.

Farrell, Jr., H. M., Jimenez-Flores, R., Bleck, G. T. Brown, E. M., Butler, J. E., Creamer, L. K., et al. (2004). Nomenclature of the proteins of cows' milk – Sixth revision. *Journal of Dairy Science, 87,* 1641–1674.

Fox, P. F., & McSweeney, P. L. H. (1998). *Dairy chemistry and biochemistry*. London, UK: Blackie Academic & Professional.

Gaiani, C., Schuck, P., Scher, J., Desobry, S., & Banon, S. (2007). Dairy powder rehydration: influence of protein state, incorporation mode, and agglomeration. *Journal of Dairy Science, 90,* 570–581.

Gaiani, C., Schuck, P., Scher, J., Ehrhardt, J. J., Arab-Tehrany, E., Jacquot, M., et al. (2009). Native phosphocaseinate powder during storage: lipids released onto the surface. *Journal of Food Engineering, 94***,** 130–134.

Gaucheron, F. (2005). The minerals of milk. *Reproduction Nutrition Development, 45,* 473–483.

Herwig, N., Stephan, K., Panne, U., Pritzkow, W., & Vogl, J. (2011). Multi-element screening in milk and feed by SF-ICP-MS. *Food Chemistry, 124,* 1233–1230.

Hurt, E. E., Adams, M. C., & Barbano, D. M. (2015). Microfiltration of skim milk and modified skim milk using a 0.1-μm ceramic uniform transmembrane pressure system at temperatures of 50, 55, 60, and 65 °C. *Journal of Dairy Science, 98,* 765–780.

IDF. (2001). *Milk. Determination of nitrogen content, ISO 8969-2. IDF standard 20-2.* Brussels, Belgium: International Dairy Federation.

512 Jeantet, R., Schuck, P., Six, T., Andre, C., & Delaplace, G. (2010). The influence of
513 stirring speed, temperature and solid concentration on the rehydration time of
514 micellar casein powder. *Dairy Science and Technology*, *90*, 225–236.
515 Karasu, K., Glennon, N., Lawrence, N. D., Stevens, G. W., O'Connor, A. J., Barber,
516 A. R., et al. (2010). A comparison between ceramic and polymeric membrane
517 systems for casein concentrate manufacture. *International Journal of Dairy*
518 *Technology*, *63*, 284–289.
519 Lawrence, N. D., Kentish, S. E., O'Connor, A. J., Barber, A. R., & Stevens, G. W.
520 (2008). Microfiltration of skim milk using polymeric membranes for casein
521 concentrate manufacture. *Separation and Purification Technology*, *60*, 237–
522 244.
523 Luo, X., Ramchandran, L., & Vasiljevic, T. (2015). Lower ultrafiltration temperature
524 improves membrane performance and emulsifying properties of milk protein
525 concentrates. *Dairy Science and Technology*, *95*, 15–31.
526 Maher, P. G., Auty, M. A. E., Roos, Y. H., Zychowski, L. M., & Fenelon, M. A.
527 (2015). Microstructure and lactose crystallisation properties in spray dried
528 nanoemulsions. *Food Structure*, *3*, 1–11.
529 McCarthy, N. A., Kelly, P. M., Maher, P. G., & Fenelon, M. A. (2013). Dissolution
530 of milk protein concentrate (MPC) powders by ultrasonication. *Journal of*
531 *Food Engineering*, *126*, 142–148.
532 McCarthy, N. A., Kelly, A. L., O'Mahony, J. A., & Fenelon, M. A. (2014).
533 Sensitivity of emulsions stabilised by bovine β-casein and lactoferrin to heat
534 and $CaCl_2$. *Food Hydrocolloids*, *35*, 420–428.
535 McCarthy, N. A., Wijayanti, H. B., Crowley, S. V., O'Mahony, J. A., & Fenelon, M.
536 A. (2017). Pilot-scale ceramic membrane filtration of skim milk for the

production of a protein base ingredient for use in infant milk formula. *International Dairy Journal*, *73*, 57–62.

Mimouni, A., Deeth, H. C., Whittaker, A. K., Gidley, M. J., & Bhandari, B. R. (2010). Rehydration of high-protein-containing dairy powder: slow- and fast-dissolving components and storage effects. *Dairy Science and Technology*, *90*, 335–344.

Mitchell, W. R., Forny, L., Althaus, T. O., Niederreiter, G., Palzar, S., Hounslow, M. J., et al. (2015). Mapping the rate-limiting regimes of food powder reconstitution in a standard mixing vessel. *Powder Technology*, *270*, 520–527.

O'Mahony, J. A., & Fox, P. F. (2013). Milk proteins: introduction and historical aspects. In P. L. H. McSweeney & P. F. Fox (Eds.), *Advanced dairy chemistry*. Vol. 1A. *Proteins: Basic aspects* (4th edn., pp. 43–85). New York, NY, USA: Springer.

O'Mahony, J. A., McSweeney, P. L. H., & Lucey, J. A. (2008). Observations on the rheological and functional properties of model cheeses made using milk protein concentrate solutions with different ratios of α_{S1}-:β-casein. *Milchwissenschaft*, *63*, 145–148.

O'Mahony, J. A., McSweeney, P. L. H., & Lucey, J. A. (2009). Rheological properties of rennet-induced skim milk gels made from milk protein concentrate solutions with different ratios of α_S-:β-casein. *Milchwissenschaft*, *64*, 135–138.

O'Mahony, J. A., Smith, K. E., & Lucey, J. A. (2014). *Purification of beta casein from milk*. Patent 11/272,331 (US 2014/8889208 B2).

561 Pierre, A., Fauquant, J., Le Graet, Y., & Maubois, J. L. (1992). Préparation de
562 phosphocaséinate natif par microfiltration sur membrane. *Lait*, *72*, 461–474.
563 Richard, B., Le Page, J. F., Schuck, P., Andre, C., Jeantet, R., & Delaplace, G.
564 (2013). Towards a better control of dairy powder rehydration processes.
565 *International Dairy Journal*, *31*, 18–28.
566 Richard, B., Toubal, M., Le Page, J.-F., Nassar, G., Radziszewski, E., Nongaillard,
567 B., et al. (2012). Ultrasound tests in a stirred vessel to evaluate the
568 reconstitution ability of dairy powders. *Innovative Food Science and*
569 *Emerging Technologies*, *16***,** 233–242.
570 Saboya, L. V., & Maubois, J.-L. (2000). Current developments of microfiltration
571 technology in the dairy industry. *Lait*, *80*, 541–553.
572 Schokker, E. P., Church, J. S., Mata, J. P., Gilbert, E. P., Puvanenthiran, A., &
573 Udabage, P. (2011). Reconstitution properties of micellar casein powder:
574 Effects of composition and storage. *International Dairy Journal*, *11*, 877–
575 886.
576 Silva, J. V. C., & O'Mahony, J. A. (2017). Flowability and wetting behaviour of
577 milk protein ingredients as influenced by powder composition, particle size
578 and microstructure. *International Journal of Dairy Technology*, *70*, 277–286.
579 Udabage, P., Puvanenthiran, A., Yoo, J. A., Versteeg, C., & Augustin, M. A. (2012).
580 Modified water solubility of milk protein concentrate powders through the
581 application of static high pressure treatment. *Journal of Dairy Research*, *79*,
582 76–83.
583 Vos, B., Crowley, S. V., O'Sullivan, J., Evans-Hurson, J., McSweeney, S., Krüse, J.,
584 et al. (2016). New insights into the mechanism of rehydration of milk protein

concentrate powders determined by Broadband Acoustic Resonance Dissolution Spectroscopy (BARDS). *Food Hydrocolloids*, *61*, 933–945.

Figure legends

Fig. 1. Representative confocal laser scanning microscopy images of micellar casein concentrate (MCC) powders manufactured by microfiltration at <10 °C (A) or 50 °C (B), followed by evaporation and spray drying. Green indicates fat and red indicates protein, while black regions within particles are air vacuoles.

Fig. 2. Contact angle values over time for a droplet of deionised water deposited on compressed discs of micellar casein concentrate (MCC) powder manufactured by microfiltration at <10 °C (□) or 50 °C (■) followed by evaporation and spray drying. Results are the means ± standard deviations of data from triplicate experiments.

Fig. 3. Conductivity (A) and calcium-ion concentration (B) over time during the rehydration of micellar casein concentrate (MCC) manufactured by microfiltration at <10 °C (○, ●) or 50 °C (□, ■); open and closed symbols represent powders rehydrated at 25 and 50 °C, respectively. Data points are the means ± standard deviations of data from duplicate experiments.

Fig. 4. Particle size distributions for micellar casein concentrate (MCC) powder manufactured by microfiltration at <10 °C (○) or 50 °C (□), followed by evaporation and spray drying, and rehydrated at 25 °C (A) or 50 °C (B) for 90 min. Results are the means ± standard deviations of data from triplicate experiments.

Fig. 5. Sediment formation and compression during the centrifugation of suspensions of micellar casein concentrate (MCC) manufactured by microfiltration at <10°C (MCC_{cold}) or 50 °C (MCC_{warm}), followed by evaporation and spray drying, and rehydrated at different temperatures for 90 min. (A1) MCC_{warm} rehydrated at 25 °C, (A2) MCC_{cold} rehydrated at 25 °C, (B1) MCC_{warm} rehydrated at 50 °C and (B2) MCC_{cold} rehydrated at 50 °C. Three profiles are shown for each: the first profile (black line), the profile after the first centrifugation step of 36 × *g* for 10 min (broken black line) and the profile after the second centrifugation step of 168 × *g* for 10 min (white line). The cell bottom (129.5 mm) is indicated by a vertical black line to guide the eye.

Fig. 6. Height of sediment (bars) and transmission of near-infrared light above the sediment (closed markers) during analytical centrifugation of micellar casein concentrate (MCC) manufactured by microfiltration at <10 °C (MCC_{cold}) or 50 °C (MCC_{warm}). Rehydration was performed at 25 or 50 °C. White bars indicate the initial height of sediment after 10 min at 36 × *g* and grey bars represent compressed sediments after an additional 10 min at 168 × *g*. Transmission values (●) were taken for each MCC at both rehydration temperatures in the region of the sample above the sediment after the full 20 min centrifugation cycle. Results are the means ± standard deviations of data from duplicate experiments.

Table 1

Composition of micellar casein concentrate (MCC) powders, and colloidal properties of reconstituted MCCs, manufactured using microfiltration at 50 °C (warm) or < 10 °C (cold). [a]

Property	MCC	
	Warm	Cold
Protein		
Total (%, w/w)	75.3 ± 0.8	75.0 ± 0.9
Casein (% protein)	91.0 ± 0.35	86.2 ± 1.02
β-CN:α-CN ratio	1.0:1.0	0.9:1.0
Minerals (mg g^{-1})		
Sodium	2.33 ± 0.11	2.13 ± 0.16
Potassium	8.17 ± 0.49	8.71 ± 1.01
Calcium	28.8 ± 1.89	25.0 ± 1.43
Phosphorus	19.1 ± 0.96	17.7 ± 1.62
Micellar phase		
Size (nm)	147 ± 2	153 ± 3
Zeta potential (mV)	-25.5 ± 0.5	-26.9 ± 0.5

[a] Results are the means ± standard deviations of data from experiments performed in at least duplicate.

Table 2

Proportion of particles in specific size classes after rehydration of micellar casein concentrate (MCC) powders at 25 or 50 °C for 90 min. [a]

Size class (µm)	Rehydration temperature			
	25 °C		50 °C	
	MCC_{warm}	MCC_{cold}	MCC_{warm}	MCC_{cold}
0–1	0.00	0.00	7.50	48.2
1–10	0.24	0.07	2.50	0.91
10–50	26.1	49.8	58.3	25.5
50–100	43.8	38.5	23.3	22.7
100–1000	29.8	11.7	8.33	2.73

[a] Data (% of total particle volume) for all size classes were calculated using the means of data from triplicate particle size experiments on each MCC.

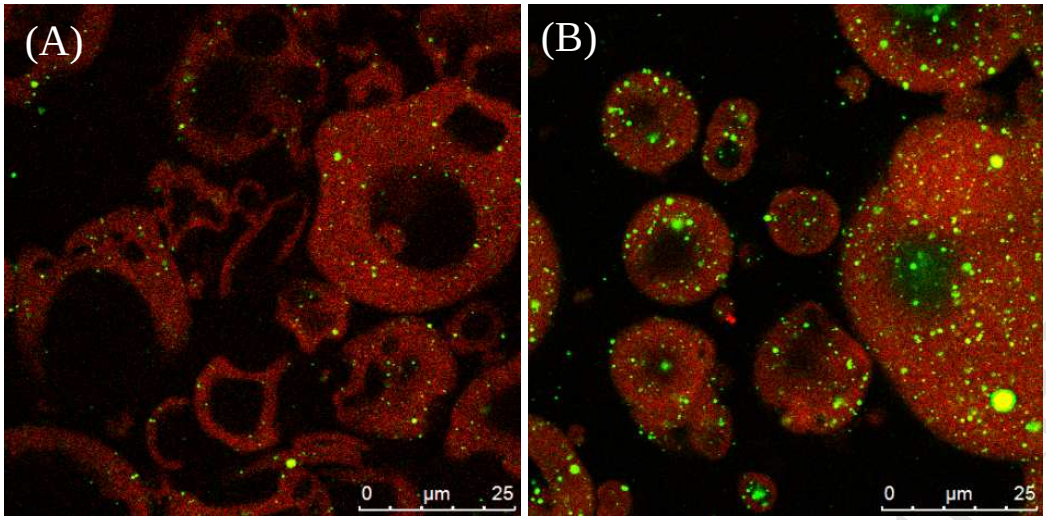

Fig. 1.

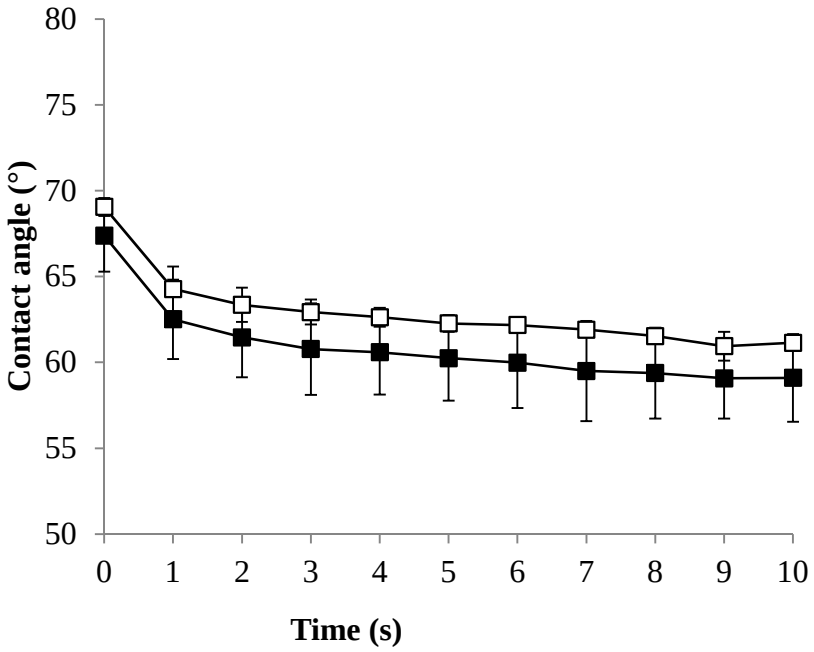

Fig. 2.

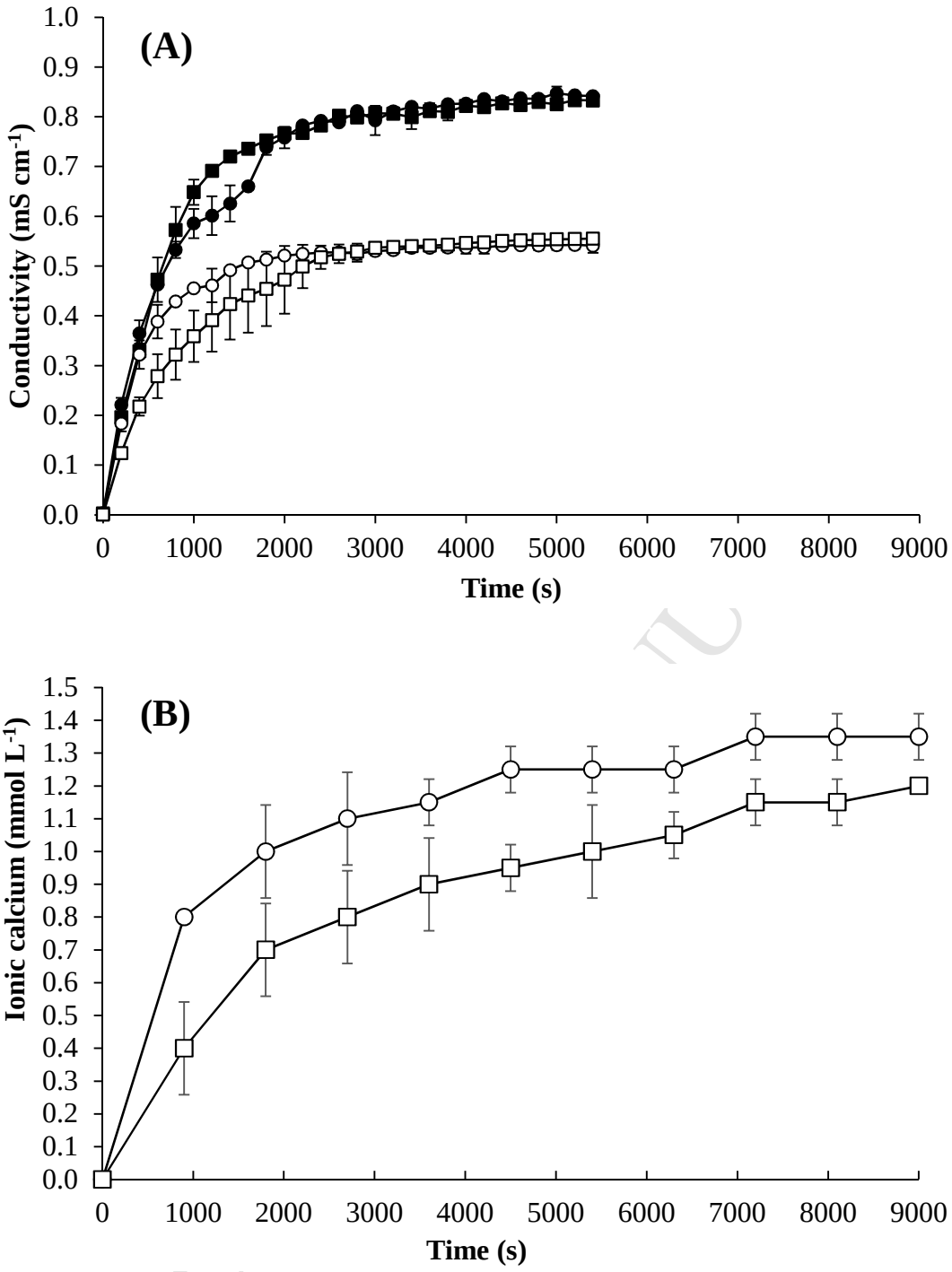
(A)
Conductivity (mS cm-1)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
1000
2000
3000
4000
5000
6000
7000
8000
9000
Time (s)
(B)
Ionic calcium (mmol L-1)
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
1000
2000
3000
4000
5000
6000
7000
8000
9000
Time (s)

Fig. 3.

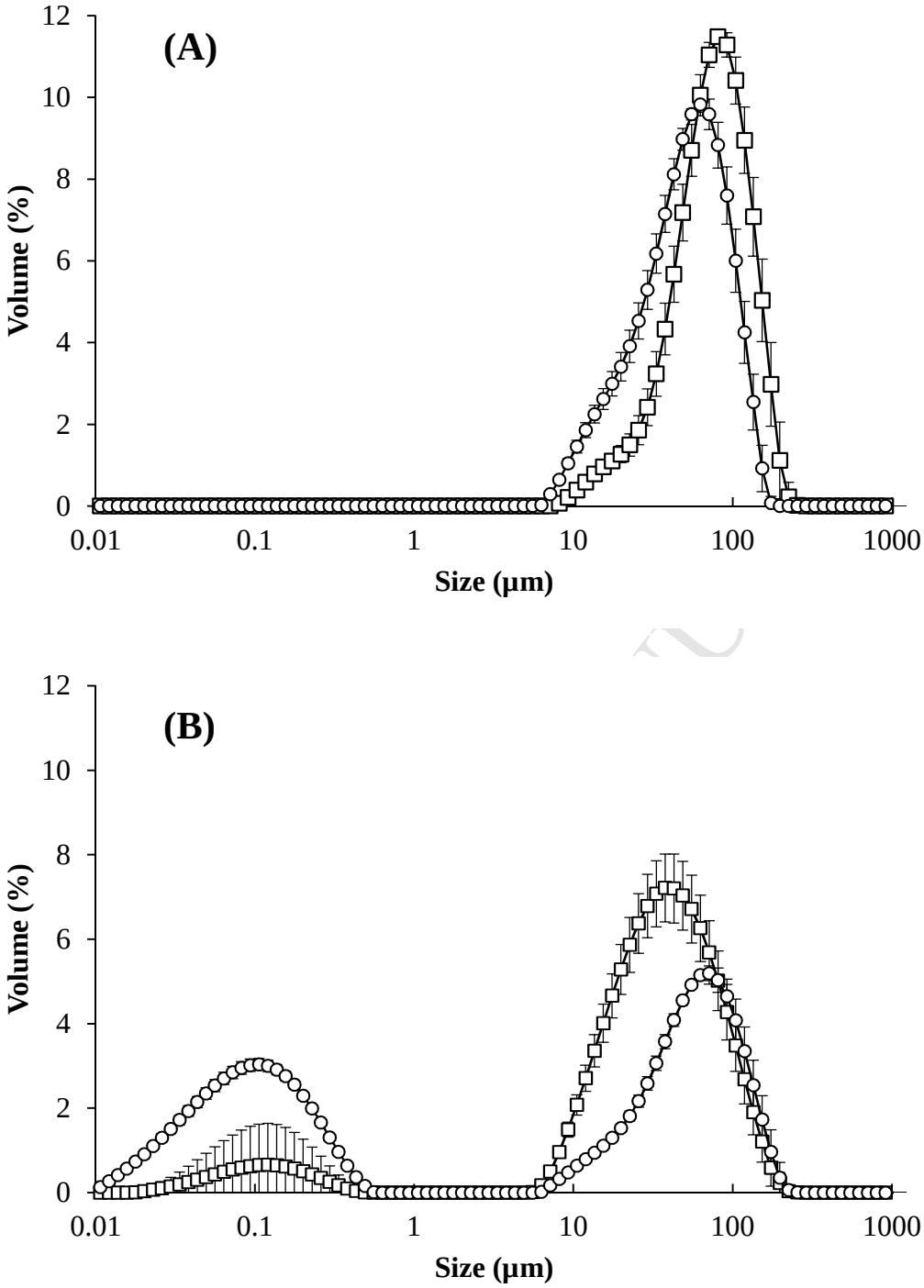
(A)
Volume (%)
Size (µm)
0
2
4
6
8
10
12
0.01
0.1
1
10
100
1000
(B)
Volume (%)
Size (µm)

Fig. 4.

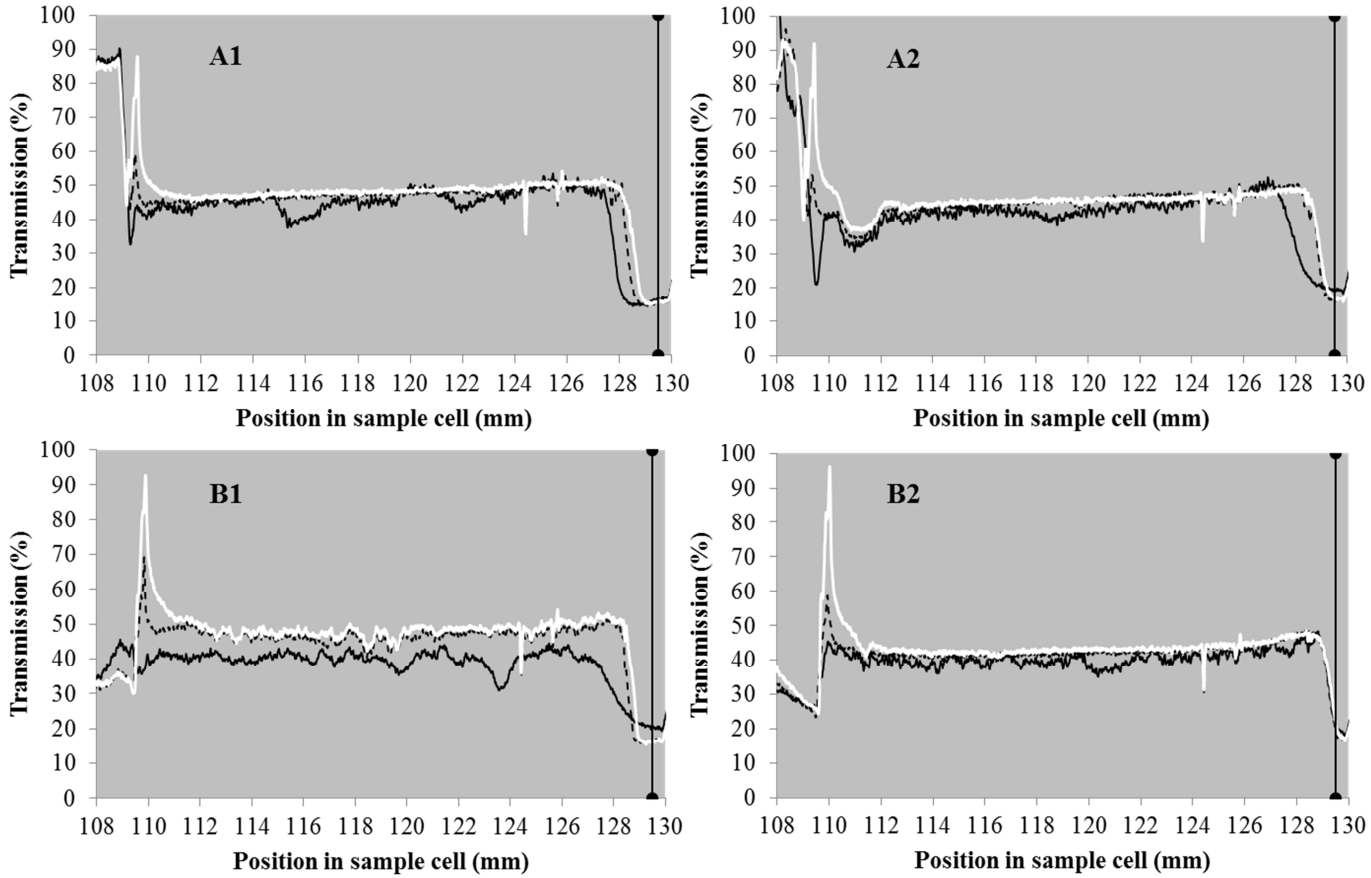
A1
A2
B1
B2
Transmission (%)
Position in sample cell (mm)
0
10
20
30
40
50
60
70
80
90
100
108
110
112
114
116
118
120
122
124
126
128
130

Fig. 5.

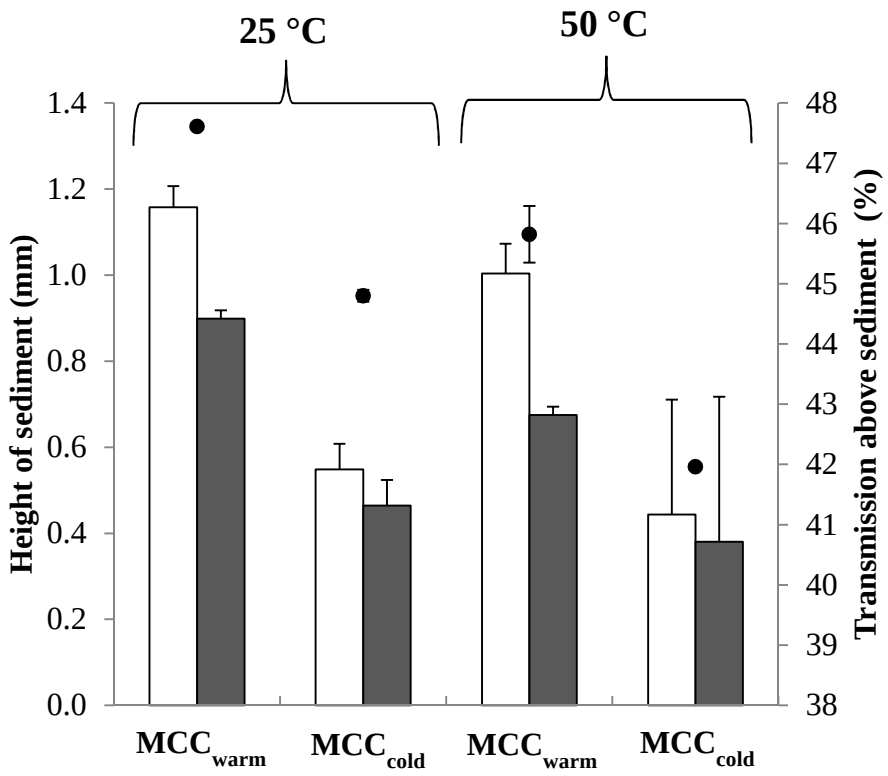

Fig. 6.