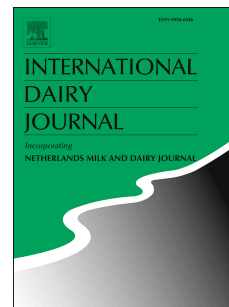


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Rehydration behaviour of spray-dried micellar casein concentrates produced using microfiltration of skim milk at cold or warm temperatures

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

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22

23

24 ABSTRACT

25

26 Microfiltration (MF) of skim milk, when combined with diafiltration (DF), facilitates
27 the manufacture of liquid micellar casein concentrate (MCC), which can be spray-
28 dried into high-protein ($\geq 80\%$ protein, dry-basis) powders. MCC powders rehydrate
29 slowly, which is typically considered a defect by end-users. This study compared the
30 impact of cold ($< 10\text{ }^{\circ}\text{C}$) or warm ($50\text{ }^{\circ}\text{C}$) MF/DF on the rehydration characteristics
31 of MCC powders (MCC_{cold} and MCC_{warm} , respectively). The wetting properties of
32 the MCC powders, measured using optical tensiometry, were found to be equivalent.
33 Pronounced differences in dispersion characteristics were measured, and, after 90
34 min rehydration at $50\text{ }^{\circ}\text{C}$, liberated casein micelles accounted for only 7.5% of
35 particle volume in MCC_{warm} compared with 48% in MCC_{cold} . Due to its superior
36 dispersion characteristics, MCC_{cold} yielded 50–60% less sediment during analytical
37 centrifugation experiments. Cold MF/DF may improve the solubility of MCCs by
38 accelerating the release of casein micelles from powder particles during rehydration.
39

40 1. Introduction

41

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

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

65 (i.e., reconstitution of dried blended powders for consumption), it is typically a
66 requirement that the powders rehydrate quickly and completely. For milk powders,
67 the rehydration process is characterised by multiple stages, including wetting of the
68 powder when added to the liquid and subsequent dispersion of the powder particles;
69 the duration of these stages, wetting and dispersion, have been reported to be
70 protracted for high-protein powders (Crowley, Jeantet, Schuck, Kelly, & O'Mahony,
71 2016). For MCCs, the wetting stage, and in particular the dispersion stage, are slow.
72 Poor dispersion characteristics can negatively affect powder handling in
73 manufacturing facilities, due to blockages in process lines, and impair consumer
74 acceptability of final products, due to the presence of lumps or sediment (Mitchell et
75 al., 2015). Although MCC powders are soluble, they exhibit exceptionally long
76 rehydration times in comparison with other milk-derived powders (e.g., skim milk
77 powder, whey protein concentrate, sodium caseinate); milk protein concentrate
78 powders (MPCs) have a similar challenge, although it is less pronounced than for
79 MCCs (Crowley et al., 2016) due to the higher proportion of the more soluble whey
80 proteins in the former (Mimouni, Deeth, Whittaker, Gidley, & Bhandari, 2010).

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

90 solubility enhancement (Udabage, Puvanenthiran, Yoo, Versteeg, & Augustin,
91 2012). Bouvier, Collado, Gardiner, Scott, and Schuck, (2013) investigated an
92 alternative drying technology, extrusion-porosification, which was found to produce
93 a more soluble powder than spray drying. Others have developed strategies that can
94 be applied during powder reconstitution itself, with various researchers reporting that
95 high-shear and ultrasonication technologies were effective when applied during
96 reconstitution of MCCs and MPCs (Augustin, Sanguansri, Williams, & Andrews,
97 2012; Chandrapala, Martin, Kentish, & Ashokumarr, 2014a; McCarthy, Kelly,
98 Maher, & Fenelon, 2013). Increasing either temperature or the number of stirrer
99 revolutions during rehydration improved the rehydration properties of an MCC
100 (Jeantet, Schuck, Six, Andre, & Delaplace, 2010). An elevated reconstitution
101 temperature, combined with the addition of monovalent salts (KCl), was more
102 effective in promoting the dispersion of MPCs than either method alone (Crowley et
103 al., 2015).

104 There is a lack of options available for processors who wish to improve the
105 rehydration performance of MCCs without potentially: (i) incurring significant
106 capital expenditure (to procure solubility-enhancing equipment); (ii) modifying
107 ingredient techno-functionality (by replacing or dissociating micellar casein); or (iii)
108 altering ingredient listings (through the use of additives). It is perhaps surprising
109 therefore that the temperature at which MF is carried out during MCC manufacture
110 has not received more attention. MF in the dairy industry has traditionally been
111 performed at ~50 °C, which is optimal for high permeate flux and efficient removal
112 of whey proteins (Hurt, Adams, & Barbano, 2015); however, MF (and also
113 ultrafiltration) at temperatures <15 °C is becoming more common in the dairy
114 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

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

143

144 **2. Materials and methods**

145

146 *2.1. Manufacture of micellar casein concentrate powders*

147

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

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

170

171 2.2. *Composition of powders and colloidal properties of reconstituted solutions*

172

173 Protein content of the MCC powders was measured by the Kjeldahl method
174 using a nitrogen-to-protein conversion factor of 6.38 (IDF, 2001). Mineral profiling
175 was carried out using inductively-coupled plasma mass spectrometry (Herwig,
176 Stephan, Panne, Pritzkow, & Vogl, 2011). The size and charge of casein micelles in
177 reconstituted solutions was assessed using a Zetasizer Nano ZS (Malvern
178 Instruments, Malvern, UK) according to McCarthy, Kelly, O’Mahony, and Fenelon
179 (2014). Protein profile was determined by reversed phase-high performance liquid
180 chromatography (RP-HPLC), as detailed by McCarthy et al. (2017).

181

182 2.3. *Distribution of protein and fat in powder particles*

183

184 The distribution of protein and fat in MCC powder particles was determined
185 using a Leica TCS SP5 confocal laser scanning microscope (CLSM; Leica
186 Microsystems CMS GmbH, Wetzlar, Germany). Dual labelling using Nile Red
187 (0.1%, w/v, in propanediol) and Fast Green FCF (0.01%, w/v, in water) was carried
188 out to visualise the protein and fat phases, respectively, in the powder particles. The
189 dye solutions were mixed in a ratio that allowed diffusion of the dyes into the

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

196

197 2.4. *Wetting behaviour: Optical tensiometry*

198

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

205

206 2.5. *Ion release: Conductimetry and calcium-ion concentration*

207

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

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

222

223 2.6. *Dispersion: Particle size distribution and analytical centrifugation*

224

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

232 To measure the sedimentation behaviour in MCC suspensions rehydrated for
233 90 min, an analytical centrifuge (LUMISizer[®], L.U.M. GmbH, Berlin, Germany)
234 was used according to the method of Crowley et al. (2015), in which the intensity of
235 transmitted NIR light (880 nm) was measured as a function of time and position over
236 the length of a polycarbonate cell held horizontally over the light path during
237 centrifugation. The height of initial sediments formed after centrifugation at $36 \times g$
238 for 10 min, and the compressed sediments formed during subsequent centrifugation
239 at $168 \times g$ for 10 min, were measured by subtracting the position of the

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

247

248 **3. Results**

249

250 *3.1. Composition and physicochemical properties of powders*

251

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

260

261 *3.2. Component distribution in MCC powder particles*

262

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

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

269

270 3.3. *Wetting behaviour of MCC powders*

271

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

280

281 3.4. *Ion release from MCC powders during rehydration*

282

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

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

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

298

299 3.5. Dispersion behaviour of MCC powders

300

301 The progression of dispersion for a casein-dominant powder such as MCC
302 can be tracked by measuring the PSD after a period of rehydration (Crowley et al.,
303 2015). The dispersion process of a MCC powder can be considered as comprising
304 primarily of the disappearance of micron-sized primary powder particles (after
305 wetting and submersion) and the release of nanometer-sized casein micelles; when
306 this process is complete, the powder can be considered dissolved. In Fig. 4A, it can
307 be seen that primary particles dominated the PSD after 90 min rehydration at 25 °C;
308 this does not necessarily mean that casein micelles have not been released, but only
309 that they are contributing little to the overall particle volume. Under these conditions,
310 the particles in the MCC_{warm} were notably larger, indicating that dispersion was less
311 advanced. When dispersion was promoted by increasing temperature of
312 reconstitution to 50 °C, a casein micelle population was apparent for both powders
313 (Fig. 4B). The MCC_{cold}, however, contained a much higher proportion of casein
314 micelles after 90 min rehydration at 50 °C compared with the MCC_{warm}, indicating

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

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

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

340 dispersion of both MCCs was promoted by the higher rehydration temperature (i.e.,
341 reduced sediment height), although the reduction in sediment height associated with
342 an increase in rehydration temperature from 25 to 50 °C was greater for the MCC_{warm}
343 (20% reduction) than the MCC_{cold} (14% reduction). The larger sediments formed in
344 the MCC_{warm} were also more susceptible to compression when subjected to a second
345 centrifugation step at $168 \times g$ for 10 min. Based on the data in Fig. 6, MCC_{warm}
346 sediments compressed by 22 and 33% after rehydration at 25 and 50 °C,
347 respectively, while the equivalent values for MCC_{cold} sediments were 16 and 14%.

348

349 **4. Discussion**

350

351 In this study, the influence of MF temperature on the rehydration
352 performance of MCC powders was investigated. Cold MF was found to have a
353 positive impact on the rehydration characteristics of MCC, due to modifications in
354 the composition, and, perhaps, the colloidal properties of the reconstituted powder
355 (Table 1). Milk-derived powders which contain >70% protein, of which 80–95% is
356 micellar casein, are known to have poor rehydration properties; in particular, the
357 release of discrete casein micelles from powder particles in MCCs and MPCs is slow
358 due to the poor dispersion characteristics of the powder particles (Crowley et al.,
359 2015; Gaiani, Schuck, Scher, Desobry, & Banon, 2007), which has been linked with
360 inhibited transfer of water into the powder (Richard et al., 2012; Vos et al., 2016).

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

365 dominant powders. Indeed, Richard et al. (2013) showed that increasing the level of
366 whey proteins in MCC powders improved their dispersibility, while Bhaskar et al.
367 (2001) developed a method of improving the dispersion of MPCs based on removal
368 of Ca. The lower casein:whey protein ratio in the MCC_{cold} was due to a reduced
369 efficiency of whey protein removal at lower filtration temperatures (Karasu et al.,
370 2010; O'Mahony et al., 2014), while the lower Ca level was caused by dissolution of
371 calcium phosphate from the micellar phase at the low temperature (Luo et al., 2015).
372 It is possible that other modifications to the casein fraction caused by the lower MF
373 temperature, such as the reduced β : α -casein ratio and the increased zeta-potential of
374 the casein micelles (Table 1), may have influenced the rehydration characteristics of
375 the MCCs. β -Casein is the most hydrophobic of the caseins, and its cold-induced
376 dissociation from micelles and removal in the MF permeate (McCarthy et al., 2017)
377 may make the MCC_{cold} better at absorbing water; however, this is not supported by
378 contact angle data (Fig. 2) and the differences in β : α -casein ratio are small.
379 Alternatively, the tendency for casein micelles to become inter-linked, resulting in
380 the formation of a poorly-dispersible 'skin' (Crowley et al., 2016), may be inhibited
381 by alterations in micellar structure (due to decreased β -casein: α -casein ratio) or
382 increased electrostatic repulsion (caused by increased zeta-potential). It is, however,
383 difficult to ascertain the influence of these factors compared with factors such as
384 mineral and whey protein, which are known to strongly affect milk protein powder
385 rehydration.

386 Gaiani et al. (2009) has previously shown that fat migration to the surface of
387 powder particles during storage is an important factor influencing the rehydration
388 behaviour of MCCs, in particular the wetting behaviour. In this study, there were
389 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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ACCEPTED MANUSCRIPT

1 **Figure legends**

2

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

7

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

13

14 **Fig. 3.** Conductivity (A) and calcium-ion concentration (B) over time during the
15 rehydration of micellar casein concentrate (MCC) manufactured by microfiltration at
16 <10 °C (○, ●) or 50 °C (□, ■); open and closed symbols represent powders
17 rehydrated at 25 and 50 °C, respectively. Data points are the means ± standard
18 deviations of data from duplicate experiments.

19

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

24

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

35

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

45

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.

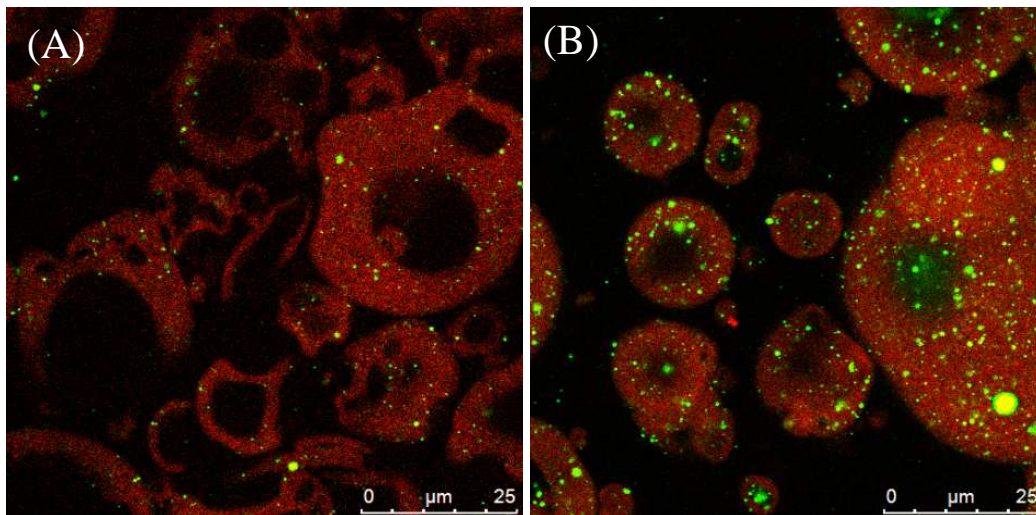


Fig. 1.

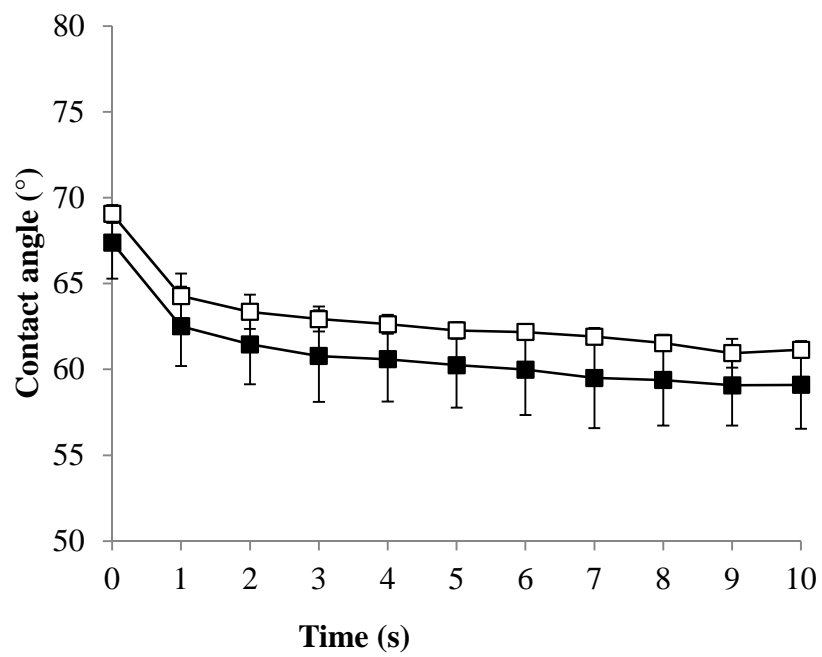


Fig. 2.

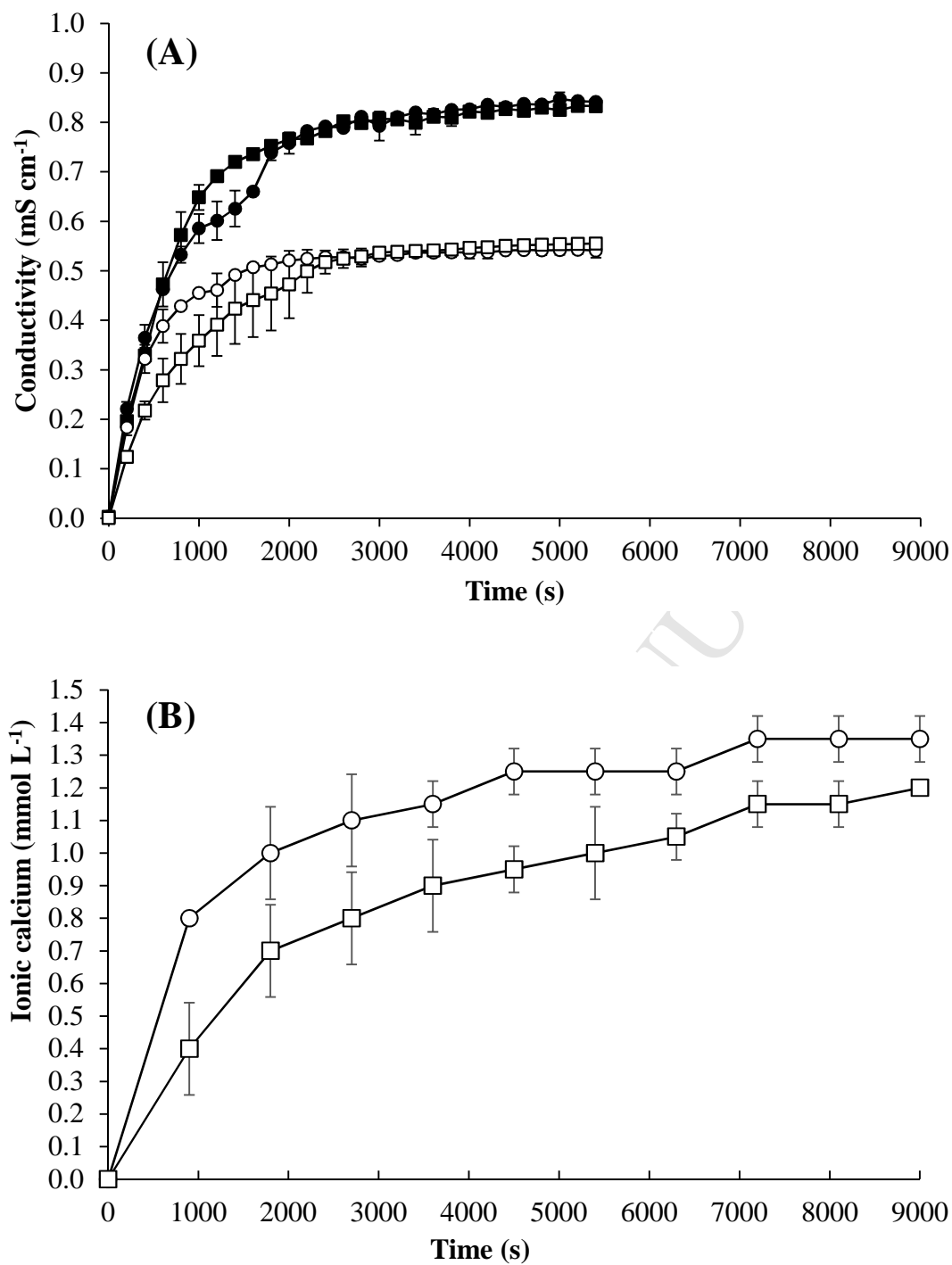


Fig. 3.

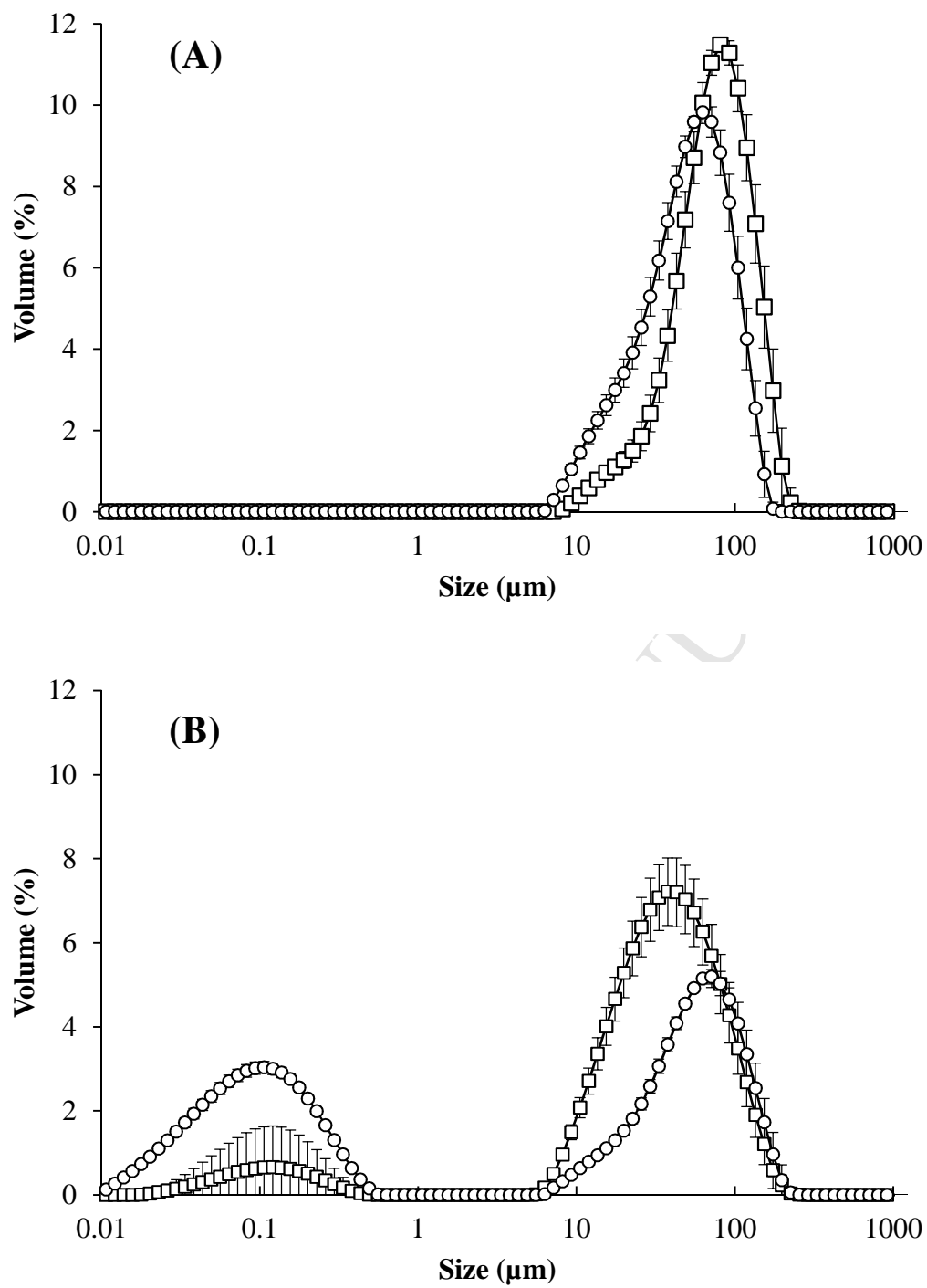
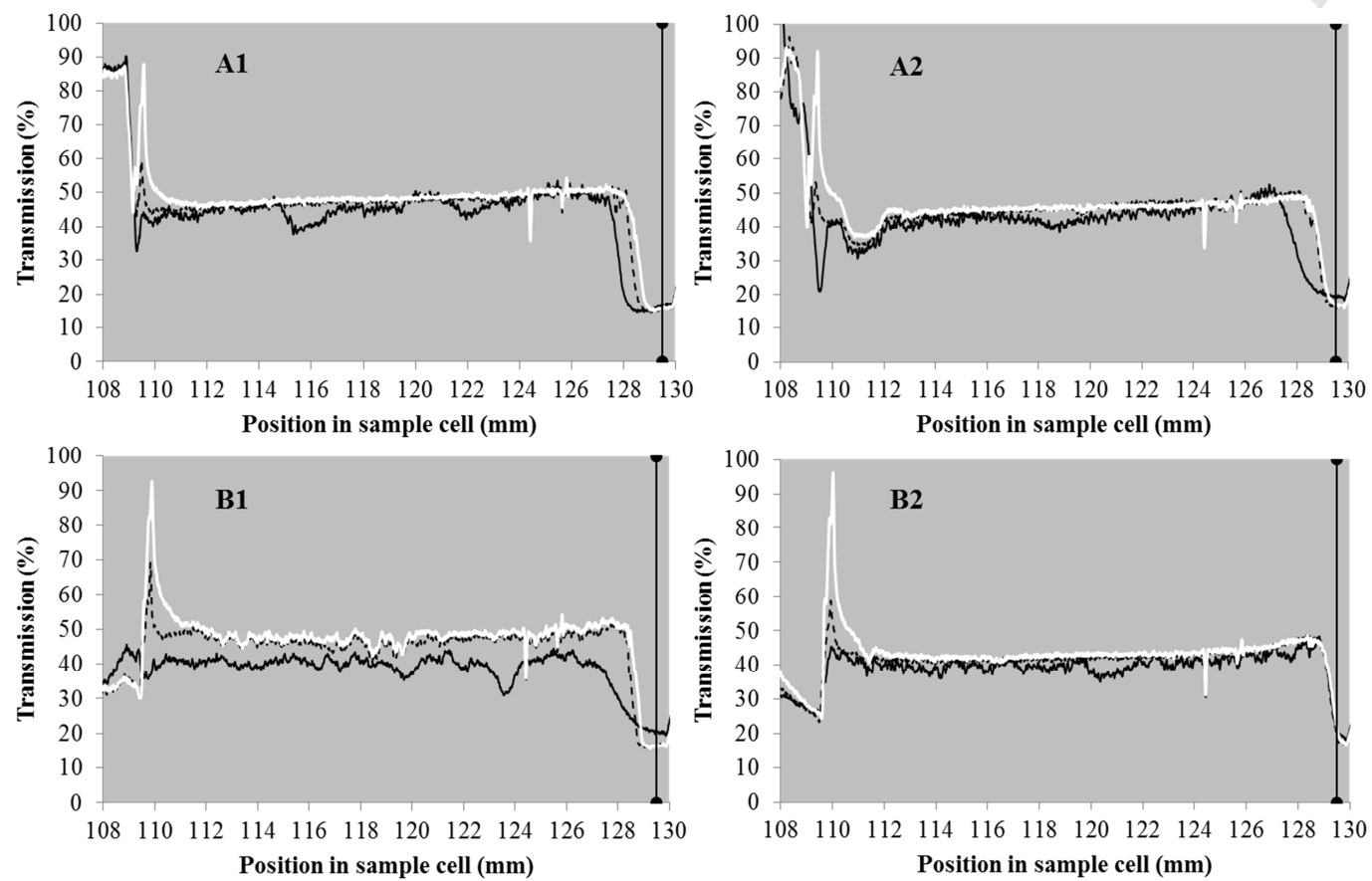


Fig. 4.

**Fig. 5.**

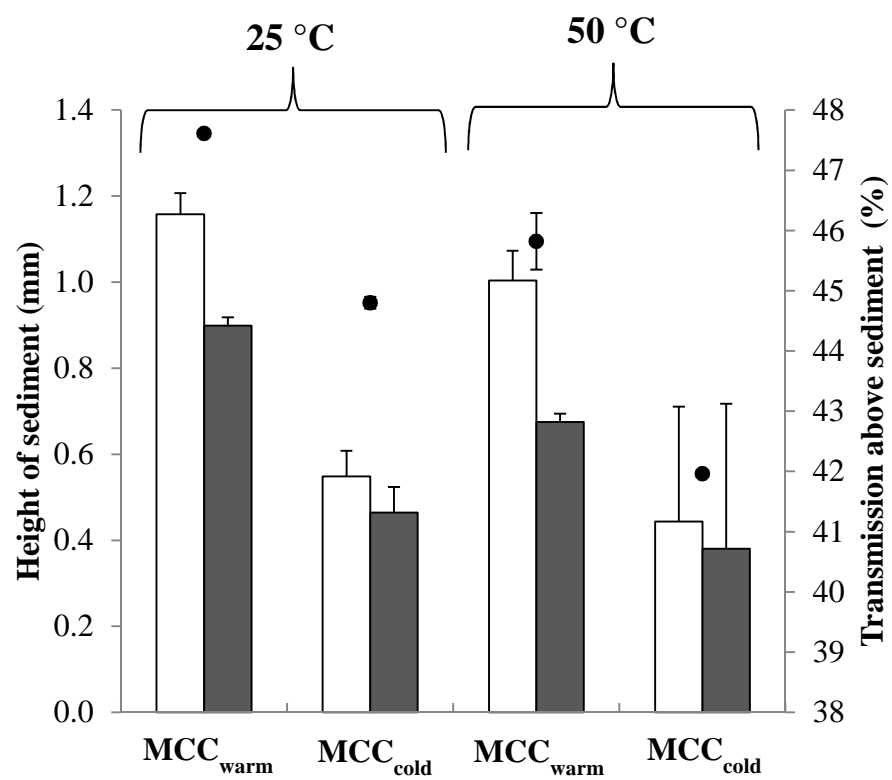


Fig. 6.