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Publication date	2020-04-02
Original Citation	Li, B., Waldron, D. S., Tobin, J. T., Subhir, S., Kelly, A. L. and McSweeney, P. L. H. (2020) 'Evaluation of production of Cheddar cheese from micellar casein concentrate', International Dairy Journal, 107,) 104711 (10pp). doi: 10.1016/j.idairyj.2020.104711
Type of publication	Article (peer-reviewed)
Link to publisher's version	http://www.sciencedirect.com/science/article/pii/S0958694620300819 - 10.1016/j.idairyj.2020.104711
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Download date	2026-11-05 22:16:54
Item downloaded from	https://hdl.handle.net/10468/10211

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Evaluation of production of Cheddar cheese from micellar casein concentrate

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ABSTRACT

The objective of this study was to evaluate the production of Cheddar cheese using micellar casein concentrate (MCC), a novel milk ingredient powder with a high casein content (~92%). Four types of Cheddar cheese were manufactured and ripened for 180 days from the following starting materials: standardized control milk (control), skim milk with cream (SC), reconstituted MCC with cream (MC) and reconstituted low-heat skim milk powder with cream (PC). Only minor differences were found in composition between treatments, but MC cheese showed higher levels of proteolysis compared to other treatments, linked to significantly higher plasmin and chymosin activities. No differences were observed in hardness between treatments (60, 120 and 180 days), but the springiness and cohesiveness of MC and PC cheeses were significantly higher than that of the control and SC cheeses at 60, 120 and 180 days. In conclusion, the use of casein-dominant dairy streams has the potential for production of Cheddar cheese with tailored functionality.

1. Introduction

Global milk production was approximately 843 million tonnes in 2018 (FAO, 2019); among dairy products (i.e., cheese, casein and butter), cheese production used the highest proportion of milk. Whey protein powder, production of which is traditionally associated with the manufacture of cheese, represents 57% of the market for global protein supplement for exercising and nutrition, while there is a huge market for whey protein powders in infant formula. Cheese output is increasing at a rate of 2% yearly while the demand for whey protein has been growing at 6-7% yearly (Hoogwegt Group, 2019). Nowadays, whey protein with high quality is required as it can be used to manufacture a range of food ingredients or products with nutrition and functional properties (Boland, 2011). Kelly (2019) reported that the ultrafiltration (UF) properties of sweet and acid whey are influenced by their high or low pH, respectively. However, microfiltration (MF) permeate made directly from skim milk is considered to be an ideal whey source for whey protein ingredients production. Therefore, recovering whey protein from milk rather than cheese whey could improve the whey quality and is an option for whey protein ingredients manufacture.

During membrane filtration of skim milk, native micellar casein is concentrated in the retentate, which could be recovered and concentrated to produce micellar casein concentrate (MCC). This is a novel dairy ingredient powder with a high casein fraction of 85-95% of total protein which may be used in functional and nutritional

applications (Crowley et al., 2018). As the traditional way to manufacture Cheddar cheese is followed by whey protein manufacture, the concept of recovering whey protein before Cheddar cheese manufacture is of interest.

In terms of the protein ingredients being used for Cheddar cheese manufacture, low heat skim milk powder (LHSMP) can be used to enhance cheese yield giving a constant cheese production throughout the year (Freeman et al., 1970). LHSMP is produced from skim milk using low temperatures during manufacture and is mostly used for condensed milk, UHT-treated fluid milk and ice-cream (Augustin & Margetts, 2003). Unlike reconstituted medium- and high-heat skim milk powder that have impaired rennet coagulation ability, the rennet coagulation properties of LHSMP are good as the whey protein is not highly denatured (Ménard et al., 2005). With only physical separation processing, the micellar casein in MCC may have better rennet coagulation properties compared to LHSMP. The rennet coagulation properties and cheese-making potential of MCC may be better than those of LHSMP.

Two main factors that influence cheese yield are lactation and seasonality. The gross composition of bovine milk varies with the stage of lactation. Kuchtík et al. (2008) reported that protein and casein content increase and lactose content decreases through the lactation. After 200 days lactation, cows are in the late lactation stage, which requires the udder tissue to repair and recover for next lactation. During late lactation, milk yield decreases dramatically, which may influence cheese yield, unless milk is standardised. Also, at specific times of the year, the milk volume decreases, and the composition and rennet coagulation properties of milk are

significantly influenced (Freeman et al., 1970; O'Brien et al., 1999). Both cheese yield and manufacturing efficiency are influenced by seasonal variation of milk protein and fat composition (Barbano & Sherbon, 1984).

To solve the problem of low cheese yield, one solution is adding LHSMP to low-protein milk to increase the protein composition (Freeman et al., 1970). Another possibility may be making Cheddar cheese with MCC that is manufactured as a co-product of high-quality whey protein.

This study aimed to evaluate the production of Cheddar cheese from micellar casein concentrate, with LHSMP used for comparison. The consequence of this manufacture was evaluated concerning composition, proteolysis, texture and functionality.

2 Materials and Methods

2.1 Preparation of cheese milk

Micellar casein concentrate (MCC) powder was obtained from Teagasc (Moorepark, Fermoy Co. Cork, Ireland); whereby pasteurized bovine skim milk was microfiltered at 50 ºC using 0.14-µm TAMI Isoflux ceramic membranes (TAMI, Lyons, France). MF was performed in a batch mode involving two diafiltration steps to produce a final 3X MF retentate (liquid MCC) which was evaporated at 65 ºC using a pilot plant single-effect falling-film evaporator (Anhydro F1 Laboratory;

Copenhagen, Denmark). This was followed by spray-drying of the evaporated liquid MCC in a single-stage spray dryer (Anhydro Laboratory Spray Dryer, SPX Flow Technology, Denmark) equipped with nozzle atomisation at inlet and outlet temperatures of 185 and 85°C, respectively. The protein content (91.6% casein) of MCC was determined by the Kjeldahl method (IDF, 1986). MCC powder and low heat skim milk powder (LHSMP; WPNI=6.0) (Uelzena, Uelzen, Germany) were rehydrated using a Silverson mixer at 55 ℃ for 2 h.

Raw milk was obtained from a local farm in Cork, Ireland. Skim milk and cream were separated from raw milk using a separator. Composition of milk samples was measured by MilkoScan™ Mars (Foss, Hilleroed, Denmark) and casein content was calculated by multiplying the casein percentage of protein of the milk sample (an estimated percentage of 78% for milk and LHSMP milk and 91.6% for MCC). Control milk was prepared by combining raw milk and skim milk to a casein: fat ratio of 0.7:1. Both reconstituted MCC and reconstituted LHSMP were made up to the same casein level as skim milk. Reconstituted MCC and LHSMP and skim milk were blended with cream and lactose according to the same standardization ratio (casein: fat of 0.70:1.00) and lactose content (~5%) as control milk to obtain three more vats: skim milk with cream (SC), reconstituted MCC milk with cream (MC) and reconstituted LHSMP milk with cream (PC). All milk samples were pasteurized at 72 ℃ for 15 s (Microthermics Inc., Raleigh, NC, USA) and stored at 4 ℃ until analysis and cheese-making.

112 *2.2 Rennet Coagulation Properties*

113 Dynamic oscillatory analysis (small amplitude oscillatory measurement) of
114 renneted control milk, SC milk, MC milk and PC milk was performed using a Peltier
115 concentric cylinder geometry, which comprised of an aluminium conical rotor [42.01
116 mm (h) by 28.02 mm (d)], on an AR-G2 controlled stress rheometer (Waters TA
117 Instruments, Leatherhead, Surrey, UK). Aliquots (25 mL) of each sample were pre-
118 warmed in a water bath at 32℃ for 15 min, and 80 µL of a 1:10 (v/v) dilution of
119 Maxiren™ (Chr Hansen A/S, Hørsholm, Denmark) was added. The sample was
120 placed immediately in the preheated cup (32 ℃), the frequency of oscillation was set
121 at 0.6283 rad s^{-1}, and the storage modulus, G' of the sample was recorded
122 continuously as a function of time at a low-amplitude shear strain (0.01) over 90 min.
123 Each sample was analysed in triplicate (Ibáñez et al., 2015).

124 *2.3 Cheese Manufacture*

125 Cheddar cheeses were made from 20 L control, SC, MC and PC milks which
126 were prepared, pasteurised and stored at 4 ℃ overnight before cheese-making.
127 Cheddar-type cheeses were manufactured according to a standard Cheddar cheese
128 manufacture protocol (Fox et al., 2000). An aliquot of 6 g (0.03% w/w) of Cheddar
129 cheese starter culture (R604Y Chr. Hansen Ltd., Little Island, Co. Cork, Ireland) was
130 added into each sample and allowed ripen for 30 min, followed by 0.09% (v/w) of 1
131 mol L^{-1} $CaCl_2$ and 60 IMCU L^{-1} rennet being added to all samples. Whey was drained
132 when the pH dropped to 6.2 and curd was cheddared. When the pH decreased to 5.4,

the curd was milled into small pieces and salted at a level of 2.5% (w/w) NaCl. The curds were then wrapped in cheesecloth and moved to 2-kg circular moulds, which were pressed at a pressure of 2.5 kg cm^{-2} for 14 h. The cheeses were then removed, vacuum-packed and ripened at 8 ℃ for 180 days.

2.4 Compositional analysis

Gross composition of cheeses was analysed at 14 days old. Moisture was determined by oven-drying method (IDF 1982), protein (%N×6.38) by the Kjeldahl method (IDF 1986) and salt was analysed by titration with $AgNO_3$ (Fox, 1963). The pH was measured at 14, 30, 60, 120 and 180 days of ripening by using a calibrated pH probe placed in contact with dry grated cheeses. All results were determined in triplicate.

2.5 Microbiological analysis

Enumeration of starter lactic acid bacteria (LAB) was performed using LM 17 agar plates (Terzaghi and Sandine 1975), incubated for 3 days at 30 ℃. Enumeration of non-starter lactic acid bacteria (NSLAB) was performed on Rogosa agar plates (Rogosa and Mitchell 1951), incubated anaerobically for 5 days at 30 ℃. Enumeration of LAB and NSLAB were performed in duplicate after 60 and 90 days of ripening.

153 *2.6 Analysis of proteolysis*

154 Urea-polyacrylamide gel electrophoresis (urea-PAGE) of cheese samples was
155 used to study the proteolysis of α_{S1}- and β-casein (CN) during ripening using the
156 method of Andrews (1983) with modifications of Shalabi and Fox (1987). The pH
157 4.6-soluble and insoluble fraction were prepared (Kuchroo & Fox, 1982), and peptide
158 profiles of pH 4.6-soluble fractions filtered through 0.22-μm cellulose acetate filters
159 (Sartorious GmbH, Gottingen, Germany) were analysed by reverse-phase high-
160 performance liquid chromatography (HPLC) using an ultra-performance liquid
161 chromatography (UPLC) Waters Acquity UPLC H-Class Core System (Waters,
162 Milford, MA, USA), with a Waters Acquity UPLC TUV Detector (dual-wavelength;
163 Waters) operated by Empower 3 software (Waters Corp., Milford, MA, USA),
164 following the method of Mane et al. (2019).

165 For determination of free amino acids (FAA), frozen pH 4.6-soluble fractions
166 were de-proteinised by mixing equal volumes of 24% (w v^{-1}) trichloroacetic acid
167 (TCA) and sample and following the method of Fenelon and Guinee (2000).

168 *2.7 Plasmin activity*

169 The plasmin activity of cheese samples at 180 days of ripening was measured
170 using the coumarin peptide method (Richardson & Pearce, 1981). A standard curve
171 of the emission intensity at 460 nm was constructed using 7-amido-4-methyl
172 coumarin (AMC), and results expressed in nmol AMC min^{-1} mL^{-1}, which was
173 defined as one unit of plasmin activity.

174 *2.8 Residual coagulant assay*

175 Grated cheese samples (50 mg) were extracted by dissolving cheese in 1 mL of
176 0.1 M trisodium citrate, followed by 30 minutes incubation at 37 °C. Fat was
177 separated by centrifugation at 1000 *g* (Sigma 1-16K, Harz, Germany) for 1 min, and
178 the aqueous layer was used for analysis. An aliquot of 70 μL citrate dispersion of
179 cheese was incubated with 30 μL of 1 mg mL^{-1} aqueous solution of a synthetic
180 heptapeptide substrate (Pro-Thr-Glu-Phe-[NO_2-Phe]-Arg-Leu) in 400 μL 0.1 M
181 sodium formate buffer, at 37 °C, pH 3.2, for 24 h. The mixture was heated at 70 °C
182 for 10 min to stop the reaction, followed by centrifugation at 16,000g for 10 min, and
183 the supernatant was used for UPLC analysis. Substrate and product levels were
184 determined using the reversed-phase HPLC system described above, following the
185 method of Hurley et al. (1999).

186 *2.9 Texture profile analysis*

187 Texture profile analysis was performed using a Texture Analyzer TA-XT2i
188 (Stable Micro Systems, Godalming, Surrey, UK) at 60, 120, and 180 days of ripening.
189 Cheese samples were cut into 20 mm height, 20 mm diameter cylinders and kept at
190 4 ℃ overnight. Cheese cylinders were compressed to 25% of the initial height in two
191 continuous compressions with a speed of 1 mm s^{-1}. Hardness, cohesiveness and
192 springiness were measured (Truong et al., 2002), and five cheese samples were
193 measured for each treatment.

194

195 *2.10 Meltability*

196 Meltability was analyzed using the Schreiber meltability test as described by
197 Altan et al. (2005). Cheese samples were heated at 232 ℃ for 5 min, and meltability
198 was measured as the percentage increase in diameter of the original samples.
199 Analyses were performed in triplicate at 60, 120, 180 days of ripening.

200 *2.11 Dynamic small amplitude oscillatory rheology*

201 The rheological properties of meltability of cheese after 180 days ripening were
202 analysed with a controlled stress AR-G2 rheometer (TA Instruments, Waters LLC,
203 Leatherhead, Surrey, UK) according to the method of Ibáñez et al. (2015). Storage
204 modulus (G'), loss modulus (G"), and loss tangent (LT) were measured during
205 heating. The maximum LT (LT_{max}) and the temperature where LT=1, which are
206 indicators of melting, were also recorded. Each sample was analysed in triplicate.

207 *2.12 Colour measurements*

208 Colour values were measured using a Konika-Minolta colourimeter CR400
209 (Konika-Minolta Optics Inc., Osaka, Japan) at 14, 30, 60, 120, 180 days of ripening.
210 The measurement used the CIELAB system based on illuminant D65 and a visual
211 angle of 2°. Five random readings were taken on fresh-cut cheese at 20°C. The
212 Eluclidean distance between the colour of control cheese and that for other treatments
213 was calculated by $\Delta E^*_{ab} = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$ where $\Delta L^* = L^*_{sample} -$
214 $L^*_{control}$, $\Delta a^* = a^*_{sample} - a^*_{control}$ and $\Delta b^* = b^*_{sample} - b^*_{control}$ (Mahy et al., 1994).

2.13 Statistical analysis

Control, SC, MC and PC cheeses were made in three independent trials, 4 × 3 blocks. Statistical analysis was carried out using one-way analysis of variance (ANOVA) with R project for Windows version i386 3.4.0 to establish significant differences between samples. The probability level used for statistical significance was $P < 0.05$.

3 Results and Discussion

3.1 Rennet coagulation properties

The rennet coagulation properties of milk samples are shown in Table 1. During the first 10 min, G' value of all the treatments was low and constant, after which the G' value of all samples increased, with the MC and PC samples exhibiting a slower rate of increase compared to other samples (Fig. 1). The gel strength (G' value) at 90 min of MC and PC samples was significantly ($P<0.05$) lower than that of other samples. The gel strength of MC and PC kept increasing and did not reach a constant level during the analysis, while control and SC reached stable values at 45 min. The loss tangent at 90 min of MC sample was significantly ($P<0.05$) higher than that of the other treatments.

Martin et al. (2010) reported that, during the manufacture of milk protein concentrate (MPC), casein micelles were not damaged; however, the minerals

235 removed through diafiltration and decreased calcium ionic strength could depress
236 rennet coagulation. The rennet coagulation ability of reconstituted MPC could be
237 restored by adding extra calcium ions. The production of MCC involves two
238 diafiltration steps, which may reduce soluble calcium levels; thus, 0.09% (v/w) of 1
239 mol L^{-1} $CaCl_2$ was added to all of the batches before cheese manufacture.

240 *3.2 Cheese composition and pH*

241 The composition of cheese at 14 days of ripening and pH of cheese samples at
242 14, 30, 60, 120 and 180days of ripening are shown in Table 2; the results presented
243 are means of data from the three trials. Guinee et al. (2007) stated that different casein
244 to fat ratio can influence the protein and fat content of cheese. As the casein: fat ratio
245 of milk was adjusted to 0.7 for all samples, no statistically significant differences
246 (P>0.05) were found in cheese protein and fat contents between all treatments, and
247 the use of MCC or LHSMP for Cheddar cheese manufacture did not affect these
248 parameters.
249 The moisture content of PC cheese was significantly (P<0.05) higher than that of SC
250 and MC cheese; no significant differences (P>0.05) were found for moisture content
251 between control, SC and MC cheese. The salt content of MC cheese was significantly
252 (P<0.05) higher than that of control and SC cheese. The pH of MC cheese was
253 significantly (P<0.05) higher than that of other treatments, the reason for which is
254 not clear. No significant differences (P>0.05) were found for moisture in non-fat
255 substances (MNFS) and fat in dry matter (FDM) for all treatments; MNFS is an

256 important parameter for Cheddar cheese quality, so it is important that all batches of
257 cheese sample had similar levels of MNFS (Fox et al., 2000; Bogenrief & Olson,
258 1995).

259 *3.3 Proteolysis*

260 Table 2 shows the level of pH 4.6-SN/TN (%) of control, SC, MC and PC cheeses at
261 180 days of ripening; pH 4.6-SN/TN is an index of proteolysis (Sousa et al., 2001).
262 Significantly (P<0.05) higher pH 4.6-SN/TN levels were found in MC cheese than in
263 SC and PC cheese. In addition, the levels of pH 4.6-SN/TN were significantly (P<0.05)
264 lower in PC than control cheese. The value (23%) found at 180 days of ripening in this
265 study was comparable with previous studies on Cheddar cheese (Lucey et al., 2005;
266 O'Mahony et al., 2005). The main agent producing pH 4.6-soluble nitrogen is the
267 coagulant (O'Keeffe et al., 1978), while Farkye & Fox (1992) reported that plasmin,
268 the principal indigenous milk proteinase, is also important for primary proteolysis in
269 Cheddar cheese during ripening.

270 Urea-PAGE electrophoretograms of cheese samples during ripening are shown in
271 Fig. 2. All cheese samples showed break-down of β- and α_{S1}-CN, but lower levels of
272 intact β-and α_{S1}-CN were apparent during ripening in MC cheese. Due to the action of
273 plasmin, β-CN is hydrolysed to β-CN (f29-209), β-CN (f106-209) and β-CN (f108-209)
274 (Eigel et al., 1984). The levels of β-CN (f106-209) and β-CN (f108-209) of MC cheese
275 were higher than other treatments during ripening, which suggests higher plasmin
276 activity. Also, in Fig. 2, the MC cheese showed the lowest level of intact α_{S1}-CN level,

277 followed by PC cheese, control and SC cheese from 60 to 180 days of ripening; at 180
278 days of ripening, no intact α_{S1}-CN was found in MC and PC samples. Residual
279 chymosin in cheese hydrolyses α_{S1}-CN to α_{S1}-CN (f24-199) and α_{S1}-CN (f102-199) and,
280 as the residual chymosin activity increases, the breakdown of α_{s1}-CN increases
281 (Sheehan et al., 2008). The faster breakdown of α_{S1}-CN of MC and PC samples may
282 indicate higher residual chymosin activity in these samples.

283 The peptide profiles of the pH 4.6-soluble extracts for control, SC, MC and PC
284 cheeses at 180 days of ripening were generated by ultra-performance liquid
285 chromatography (Fig. 3). The highest peak areas were observed at the retention time of
286 1 to 5 min for all treatments. The peak area of peptides that were eluted later (28-45
287 min retention time) was higher in MC sample compared to the other treatments.
288 Peptides in the pH 4.6-soluble extracts reflect the effect of proteinases and peptidases
289 of starter (Fox & McSweeney, 1997). The number of peptide peaks for all treatments
290 was similar, which suggests that the proteolysis for all treatments broadly followed
291 similar pathways, but differences in peak area indicate that there was more extensive
292 proteolysis in MC cheese compared to the other treatments.

293 The individual FAA levels of all treatments at 120 and 180 days of ripening are
294 shown in Fig. 4 (A and B). The major FAA determined in cheese were glutamic acid,
295 valine, leucine, phenylalanine, histidine and lysine; Bansal et al. (2009) also reported
296 that glutamic acid, valine, leucine, phenylalanine histidine and lysine are the principal
297 FAA in Cheddar cheese after 180 days of ripening. No significant differences (P>0.05)
298 were found between treatments at 120 days of ripening. At 180 days of ripening,

299 significantly (P<0.05) higher concentrations of threonine, serine, glycine, alanine,
300 valine, methionine, isoleucine, phenylalanine, histidine and proline were found in
301 control and SC cheese than that of MC and PC cheese. No significant differences
302 (P>0.05) were found between treatments in the levels of aspartic acid, glutamic acid,
303 leucine, tyrosine and arginine. From 120 to 180 days of ripening, the concentrations of
304 glutamic acid, alanine, leucine, tyrosine and phenylalanine increased significantly
305 (P<0.05) for all treatments, while only control and SC cheese had a significant increase
306 in the concentration of threonine, glycine, valine and isoleucine. The total FAA levels
307 of all treatments at 120 and 180 days of ripening are shown in Fig. 4 (C). No significant
308 differences (P>0.05) were found between all treatments at 120 days of ripening, but
309 significantly higher (P<0.05) levels of total FAA were found in control cheese than in
310 MC and PC cheeses at 180 days of ripening. No significant differences (P>0.05) were
311 found between control and SC cheese at 180 days of ripening.
312 Fox and McSweeney (1996) reported that peptidases of starter and non-starter lactic
313 acid bacteria are the principal agents releasing FAA in Cheddar cheese during ripening.
314 No significant (P>0.05) differences were found for the numbers of starter bacteria and
315 NSLAB between treatments in this study (result not shown). Therefore, the release of
316 the FAA by starter and NSLAB should not have been an influence in this regard, and
317 so the reasons for this difference is not clear.
318
319

320 *3.4 Plasmin and residual chymosin activities in cheese*

321 The plasmin activity of MC cheese was significantly (P<0.05) higher than that of the
322 other cheese batches (Table 3) which is consistent with the result of urea PAGE
323 electrophoresis. The plasmin activity of PC cheese was significantly (P<0.05) lower
324 than that of the other cheese samples. As the pH increases, the hydrolysis of β-CN
325 increases since plasmin has an alkaline optimum pH (Watkinson et al., 2001).

326 Aaltonen and Ollikainen (2011) reported that in diafiltration, with the removal of
327 whey protein, the concentration of inhibitors of both plasmin and plasminogen
328 activators decreases, which promotes the conversion from plasminogen to plasmin and
329 thus increases plasmin activity. In this study, MCC was made using microfiltration,
330 which may have enhanced the plasmin activity of retentate by removing inhibitors in
331 the permeate, resulting in the higher plasmin activity and more β-CN breakdown.MC
332 cheese showed significantly (P<0.05) higher residual chymosin activity compared to
333 the other treatments (Table 3), which is consistent with the faster breakdown of α_{S1}-CN
334 in that cheese. No significant differences (P>0.05) were found between the residual
335 chymosin activities of control and SC cheese, so the recombination of fat and skim milk
336 did not affect the retention of coagulant during cheese manufacture. The residual
337 chymosin activity of PC cheese was significantly (P<0.05) lower than that of control
338 cheese. The MC cheese apparently retained more coagulant compared to control, SC
339 and PC cheeses; the percentage of retained chymosin in cheese curd depends on the pH

at the curd-cutting stage, pH at whey drainage, cooking temperature and method (Hurley et al., 1999).

3.5 Texture profile analysis

No significant changes ($P>0.05$) were found in the hardness of MC and PC cheeses from 60 to 180 days of ripening (Fig. 5). The hardness of control cheese significantly ($P<0.05$) decreased between 60 and 120 days of ripening, while that of SC cheese significantly ($P<0.05$) increased. At 60 days of ripening, the hardness of control cheese was significantly ($P<0.05$) higher than that of SC and PC cheeses. No significant differences ($P>0.05$) were observed in the hardness of the cheeses made by different treatments at 120 and 180 days of ripening. Chevanan and Muthukumarappan (2007) reported that the contents of calcium, phosphate, and residual lactose affect the texture profile of Cheddar cheese. The cheese-milk of SC, MC and PC samples was reconstituted according to the composition of control cheese-milk, which would not influence lactose content. In this study, extra calcium chloride was added to all treatments, but the addition of calcium chloride does not cause significant changes to the texture of ripened Cheddar cheese (Soodam et al., 2015).

From 60 days to 180 days of ripening, the springiness and cohesiveness of MC cheese decreased, and the springiness and cohesiveness of MC and PC cheeses were significantly ($P<0.05$) higher than that of control and SC cheese at 60 and 180 days of ripening. At 120 days of ripening, the springiness of MC cheese was significantly

(P<0.05) higher than that of the other treatments and the cohesiveness of MC and PC cheeses was significantly (P<0.05) higher than that of control and SC cheese. Everard et al. (2006) found that higher pH of Cheddar cheese was associated with increased springiness and cohesiveness; the pH of MC cheese was significantly (P<0.05) higher than that for the other treatments (Table 2), which may explain the increases in springiness and cohesiveness. O'Mahony et al. (2005) reported that, as levels of secondary proteolysis increase, the charged groups released from peptides would associate with free water, which may lead to increased cohesiveness and springiness during ripening.

3.6 Meltability

Meltability (percentage increase in diameter) of Cheddar cheeses was determined by the Schreiber method; results are shown in Fig. 6. The four types of cheeses showed increases in meltability between 60 and 180 days. At 60 and 120 days of ripening, no significant differences (P>0.05) for meltability were found between control and SC cheese. Significantly (P<0.05) higher meltability was found in control cheese compared to SC cheese. At 60, 120 and 180 days of ripening, the meltability of MC cheese was significantly higher (P<0.05) than that of cheese from the other treatments. At 120 and 180 days of ripening, the meltability of PC cheese was significantly lower (P<0.05) than that of cheese from the other treatments.

The lower meltability of PC cheese may be caused by the changes of casein in protein during drying of milk powder. Moiseev et al. (2017) reported that Mozzarella

382 cheese made with reconstituted non-fat milk powder has lower meltability than the
383 control, due to the drying process of non-fat milk powder decreasing the stability and
384 dispersity of casein micelles and promoting demineralization of calcium salts. Dave
385 et al. (2003) found that the meltability of Mozzarella cheese is related to the
386 breakdown of (α_{S1}-CN and α_{S1}-CN (f24-199)) and breakdown of intact β-casein,
387 while Bogenrief and Olson (1995) reported that hydrolysis of β-casein increases the
388 meltability of Cheddar cheese. Hydrolysis of β-casein is primarily related to the
389 action of plasmin (Eigel et al., 1984) and, although both MC and PC cheeses were
390 made from powder, and showed a higher breakdown of α_{S1}-casein and β-casein, the
391 meltability of MC cheese was significantly higher than that of PC.

392 *3.7 Dynamic small amplitude oscillatory rheology*

393 The results of the rheology of meltability of all treatments at 180 days of
394 ripening are shown in Table 4. Higher LT value indicates a higher extent of melting
395 (Lucey et al., 2003). MC cheese showed significantly higher (P<0.05) LT_{max} values
396 than PC cheese, followed by control and SC cheese. The temperature at LT_{max} of MC
397 and PC cheeses was significantly higher (P<0.05) than that of control and SC cheese.
398 The LT_{max} is an indicator of cheese meltability (Mounsey & O'Riordan, 1999); the
399 more thermal energy needed to melt cheese, the higher the LT_{max} temperature of
400 cheese will be.

401 In the Schreiber test for cheese meltability, MC cheese also exhibited the
402 highest percentage increase in diameter (Fig. 4). There were differences between

403 Schreiber melting test and dynamic small amplitude rheology for control, SC and PC
404 cheese. Cooke et al. (2013) stated that differences between results generated by those
405 tests may be linked to the more complete fat melting during the higher temperature
406 of the Schreiber melting test. MC cheese exhibited the lowest temperature when
407 LT=1 compared to the rest of the treatments (P<0.05). At the point of LT=1, cheese
408 is considered to start transforming from a solid to a viscous form (Gunasekaran &
409 Ak, 2002). MC cheese thus commenced melting at the lowest temperature and had
410 the highest meltability compared to the other treatments, which may be linked to the
411 more extensive proteolysis of casein.

412 *3.8 Cheese colour*

413 The colour values of samples at 180 days of ripening are shown in Table 5.
414 Whiteness (L* values) of control, SC cheese was significantly higher (P<0.05) than
415 that of MC cheese at 180 days of ripening. The greenness of MC and PC cheeses was
416 significantly lower (higher a* values) (P<0.05) than that of control or SC cheese at
417 180 days of ripening. The yellowness (b* values) of MC cheese was significantly
418 lower (P<0.05) than that of SC cheese at 180 days of ripening. Ibáñez et al. (2015)
419 reported that the whiteness of cheese may be related to proteolysis; lower whiteness
420 of MC cheese may thus be attributed to higher proteolysis. The ΔE^*_{ab} values are also
421 shown in Table 5. Sharma (2003) reported that ΔE^*_{ab} of above 2.3 leads to a just
422 noticeable difference (JND) and, on this basis, overall, no JND was found between

423 control cheese and the other treatments. Overall, no visible difference was found
424 between the cheeses made from protein ingredient and control and SC cheese.
425

426 ## Conclusions

427 Cheddar cheeses were made from standardised cheese milk, skim milk with cream,
428 reconstituted MCC with cream and reconstituted LHSMP with cream according to the
429 same casein, fat and lactose content. Only minor differences in composition were found
430 with cheese made from reconstituted MCC with added cream compared to control
431 cheese. The cheese manufactured with MCC had significantly higher (P<0.05) levels
432 of pH 4.6-SN/TN than cheese manufactured with skim milk or LHSMP with cream.
433 The level of intact β-and α_{S1}-CN of MC cheese was lower than rest treatments,
434 consistent with significantly (P<0.05) higher plasmin and chymosin activity. No
435 significant difference (P>0.05) were found in hardness between all treatments.
436 Significantly higher (P<0.05) springiness and cohesiveness were found in cheese
437 manufactured from MCC and LHSMP powder, meltability and maximum loss tangent
438 in MC cheese were significantly (P<0.05) higher than that of the other treatments. No
439 overall differences of colour were found between all treatments. Use of novel casein-
440 dominant dairy streams such as MCC has potential for production of Cheddar cheese
441 with tailored functionality. The results of this study suggest that using reconstituted
442 LHSMP with cream for the manufacture of Cheddar cheese may result in changes in

443 functionality, while using reconstituted MCC with cream for the manufacture of
444 Cheddar cheese may be more feasible.
445
446
447 **Acknowledgements**
448 The authors gratefully acknowledge Department of Agriculture, Food and the
449 Marine, Ireland and Teagasc, Ireland for providing the samples of MCC for Cheddar
450 cheese manufacture.

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