

1 **Application of a cascade membrane filtration process to standardise serum protein**
2 **depleted cheese milk for Cheddar cheese manufacture**

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ABSTRACT

A cascade membrane filtration process including microfiltration (MF), ultrafiltration (UF) and reverse osmosis (RO) was used to fractionate skim milk into different streams. Significant quantities of lactose and minerals were removed to permeate after MF at 0.14 μm . Cheese milk, of similar casein content to the raw milk, was standardized simultaneously for casein, lactose, ash and total calcium from the membrane streams without requiring CaCl_2 and lactose addition. Serum protein depleted cheese milk of typical casein content had similar rennet coaguability, cheese composition, texture and yield to the control; while milk of 1.5 times casein content had a faster coagulation rate and resulted in cheese of lower moisture content. On a dry matter basis, the serum protein content of MF permeate concentrated by UF was significantly higher than that in cheese whey (51.54% Vs 5.63-9.45%), with significantly lower contents of ash (0.95% Vs 7.11-7.53%) and lactose (9.50% Vs 61.98-70.35%) respectively.

Key words: microfiltration, diafiltration, cheese milk, standardisation

INTRODUCTION

56

57 Microfiltration (MF) with a membrane pore size of 0.08-0.20 μm is commonly used to
58 selectively partition soluble and colloidal components in milk. Dependent upon the
59 **membrane pore size for MF**, casein micelles remain in the retentate, and serum proteins,
60 lactose, minerals and other minor components permeate through the membrane (Jost et al.,
61 1999; Nelson and Barbano, 2005; Govindasamy-Lucey et al., 2007; Seibel et al., 2015). MF
62 retentate can be used for cheese milk standardisation (Brandsma and Rizvi, 1999) or for the
63 production of liquid or powdered micellar casein concentrates and isolates (Schuck et al.,
64 1994). MF permeate often termed native, virgin or 'ideal' whey provides a serum protein
65 stream free from starter culture, cheese colorants, caseinomacropetide (CMP), fat, cheese
66 fines, rennet and derivatives of microbial activity compared to conventional cheese whey
67 (Bacher and Kønigsfeldt, 2000). Process efficiencies are also achieved due to the higher
68 purity of MF permeate, **as** the process speed for ultrafiltration (UF) of MF permeate is much
69 faster than for that of cheese whey when separating and concentrating serum protein (Nelson
70 and Barbano, 2005). Because of the negligible fat content and lower heat treatments applied
71 to MF permeate, whey protein powders derived from MF permeate have superior functional
72 properties compared to those manufactured from cheese whey (Bacher and Kønigsfeldt,
73 2000). **In fact, Papadatos et al. (2003) suggested that serum protein products produced from**
74 **MF permeate could be sold at a higher price than those produced from cheese whey.**
75 Furthermore, MF retentate (i.e., casein micelle concentrate) is more heat stable than skim
76 milk as there is less serum protein present (Renhe and Corredig, 2018). Thus, optimal
77 recovery of serum protein from skim milk to permeate during microfiltration is desired
78 (Nelson and Barbano, 2005).

79 To maximise the serum protein removal from MF retentate, diafiltration (DF) with water
80 is applied (Amelia et al., 2013), **which results in a significant reduction in levels of lactose**

81 (Amelia, 2013; St-Gelais, 1995; Sauer, 2012; Outinen, 2008), calcium (Lu, 2016) and soluble
82 milk minerals (Boiani, 2017) in MF retentate. Thus, to ensure an acceptable set to cut time
83 during cheese manufacture, it is necessary to add CaCl_2 to the cheese milk prepared from MF
84 retentate (Heino, 2008; Zulewska et al., 2018). Similarly, low lactose content in cheese milk
85 caused by lactose depletion during MF and DF results in cheese with high pH (Heino, 2008).
86 Thus, an opportunity exists to develop a membrane filtration process providing good
87 separation of serum protein, and in parallel, facilitating the standardisation of cheese milk to a
88 target composition for casein, lactose and calcium contents as well as achieving a desired
89 casein/ fat ratio. To optimise such a process, it is suggested that small molecules (serum
90 protein, lactose and calcium) removed from the retentate after each microfiltration and
91 diafiltration step should be quantified, so as to inform the process of standardisation of cheese
92 milk from MF retentate based on individual components and similarly, to optimise the
93 membrane filtration process to produce a MF retentate which is suitable for cheese milk
94 standardisation.

95 In this study a cascade membrane filtration process was developed, where skim milk
96 was subjected to microfiltration at $1.4 \mu\text{m}$ to remove bacterial and other cells followed by MF
97 (pore size $0.14 \mu\text{m}$, with 2 steps of DF with RO water, 50°C), UF and reverse osmosis (RO)
98 to fractionate skim milk into different streams, i.e., micellar casein concentrate (MCC; casein
99 micelles), RO retentate (lactose and minerals), RO permeate (water) and UF retentate (whey
100 protein). The first objective was to determine the effect of MF at $0.14 \mu\text{m}$ and DF on the
101 composition of the MF retentates. The second objective was to develop and validate a process
102 for the simultaneous standardisation of the casein, fat, lactose, ash and total calcium contents
103 of cheese milk using pasteurized cream, MCC, RO retentate and RO permeate. The third
104 objective was to manufacture Cheddar cheese from cheese milk standardized from membrane

105 streams and evaluate the coagulation properties, composition, texture and yield. The
106 composition of UF retentate and subsequent cheese wheys were also considered.

107 **MATERIALS AND METHODS**

108 *Cascade filtration process*

109 Triplicate trials were undertaken over a five month period on a cascade filtration
110 process (Figure 1) with each trial conducted over three days at Moorepark Technology
111 Limited, Co Cork, Ireland.

112 On day 1, raw whole milk sourced from the Teagasc Animal & Grassland Research and
113 Innovation Centre (AGRIC), Moorepark, Co Cork, Ireland or from a local dairy company
114 (Dairygold, Mogeely, Co. Cork, Ireland) was separated into raw cream and raw skim milk
115 with a cream separator (GEA Westfalia, Oelde, Germany). Immediately after separation, a
116 quantity of raw cream (20 kg, fat content 25-40%) and raw skim milk (20 kg, fat content <0.1
117 %) were pasteurised separately (cream, 85°C for 20s; skim, 72°C for 15s) using a pilot-scale
118 tubular heat-exchanger (MicroThermics®, Raleigh, NC, USA), collected in sterilized
119 containers (Thermo Scientific™ Nalgene™ Products, NY, USA) and stored at 4°C until day
120 4. In parallel, 400 kg of raw skim milk was microfiltered at a membrane pore size of 1.4 µm
121 (Tami Isoflux® ceramic membranes, Tami Industries, Nyons, France) on a pilot filtration unit
122 (Model F, GEA Process Engineering A/S, Skanderborg, Denmark), where bacteria and spores
123 were retained in the MF 1.4 retentate, and the bacteria-free skim milk partitioned to MF 1.4
124 permeate (Mistry, 2013). A quantity of 20 kg MF 1.4 permeate was transferred to two 10 L
125 sterilized containers, cooled in an ice bath and stored at 4°C until day 4; the remainder of the
126 MF 1.4 permeate (350 kg) was collected in a double jacket tank and immediately cooled to 4
127 °C for use on day 2.

128 On day 2 (Figure 2), MF 1.4 permeate was heated to 50°C and then subjected to
129 microfiltration using three ceramic 0.14 µm membranes in parallel, each with a surface area
130 of 0.35 m² (Tami Isoflux® ceramic membranes, Tami Industries, Nyons, France). For
131 diafiltration, when the weight of the MF 0.14 permeate reached 250 kg (for diafiltration 1) or
132 400 kg (for diafiltration 2) respectively, 150 kg or 100 kg of RO water (50°C) were added to
133 the MF 0.14 retentate immediately. The retentate and permeate obtained after each MF or DF
134 step are referred to as MF 0.14 retentate 1, 2, 3 or MF 0.14 permeate 1, 2, 3 respectively
135 (Figure 2). The temperature of MF 0.14 was maintained at 50±3°C with chilled water, both
136 MF 0.14 permeate 3 and retentate 3 were immediately cooled to 4°C after processing and
137 stored until day 3.

138 On day 3 (Figure 1), the MF 0.14 retentate was evaporated at 65°C using a single-stage
139 falling-film evaporator (Tetra Scheffers™, Tetra Pak, Gorredijk, The Netherlands) until a brix
140 level of 21-22 (determined by a hand held refractometer, Bellingham + Stanley Ltd, Kent,
141 UK) was achieved in MCC. In parallel, MF 0.14 permeate was ultrafiltered using two spiral-
142 wound membranes (Synder Filtration, Vacaville , CA, USA) with a molecular weight (MW)
143 cut-off of 10 kDa. To partition all lactose and minerals to the UF permeate, diafiltration with
144 RO water was carried out until the brix level of the UF permeate became 0. The UF permeate
145 was concentrated by reverse osmosis (Hydranautics RO3840/30 membranes, Nitto,
146 Oceanside, CA, USA) to a total solids content of 15 % in the RO retentate, containing lactose
147 and minerals, with water removed to the RO permeate. The MCC, RO retentate and RO
148 permeate were then transferred to sterilized containers separately, cooled in an ice bath and
149 stored at 4°C until day 4. All membrane filtration processes were carried out on the same
150 filtration unit.

151 *Preparation of cheese milk*

152 On day 4 (Figure 1), 4 cheese milks (namely, PC PS, PC MF1.4P, MCC1.0 and
153 MCC1.5) were prepared from the following streams: pasteurized cream, pasteurized skim
154 milk, MF 1.4 permeate, MCC (micellar casein), RO retentate (lactose and minerals) and RO
155 permeate (water), as described in Table 1. The compositional parameters (protein, fat and
156 lactose contents) of pasteurised raw skim milk, raw cream, MCC and cheese milks were
157 measured by FTIR (FOSS MilkoScan™ FT+, Hillerød, Denmark). The total solids in RO
158 retentate was analysed with a rapid moisture analyser (CEM Smart Trac, Dublin, Ireland) and
159 the lactose content in the RO retentate was calculated as: $0.87 \times$ total solids in RO retentate.
160 RO permeate was considered as pure water. The casein content for PC PS, PC MF 1.4P and
161 MCC1.0 were standardised to the same level as the raw skim milk and the casein content for
162 MCC1.5 was standardised to $1.5 \times$ MCC1.0. The target casein: fat ratio for all cheese milks
163 was 0.74, the lactose contents in MCC1.0 and MCC1.5 cheese milks were standardised to the
164 same level with those in PC PS and PC MF1.4P cheese milk. Since MCC, RO retentate and
165 RO permeate all originated from the MF 1.4 permeate, and the MF 1.4 permeate may be
166 considered to be bacteria free (Mistry, 2013), a cheese milk designated PC PS was prepared
167 from pasteurized skim milk and cream, to act as control for the PC MF 1.4P, MCC1.0 and
168 MCC1.5 cheese milks. The purpose of PC MF 1.4P was to compare microbial removal using
169 MF 1.4µm to pasteurization (PC PS), a more conventional step for reduction of bacterial load
170 and for pathogen inactivation.

171 ***Preparation of cheese***

172 Each cheese milk was formulated to 10 kg in a model cheese vat (Type CAL 10L;
173 Pierre Guerin Technologies, Mauze, France) and heated to 32 °C with a re-circulating water
174 bath (Grant Y28; Grant Instrument Ltd., Cambridge, UK). The pH of the cheese milk was
175 standardised to 6.55 with a 4 % lactic acid solution. Starter culture (2 g per vat; R604, Chr.

176 Hansen Ireland Ltd., Co. Cork, Ireland) was added to the cheese milk immediately after pH
177 standardization. After a pre-ripening period of 30 min, rennet (1.8 mL Chymax-plus (Chr.
178 Hansen Ireland Ltd., Co. Cork, Ireland) mixed with 20 mL milli-Q water) was added to the
179 cheese milk. The curd was cut as described by Panthi et al. (2019b) at a gel firmness of 35 Pa
180 (determined by AR-G2 rheometer; TA Instruments, New Castle, DE, USA). Subsequently the
181 curds were cooked to 38°C at a rate of 0.25 °C/min, drained at pH 6.15, milled at pH 5.35,
182 salted at 2.7 % (w/w), mellowed for 25 min, moulded and then pressed at 44.23 kPa
183 overnight. Cheeses were vacuum packed and stored in 4 °C for 7 days.

184 ***Compositional analysis of membrane streams, cheese milks and cheese wheys***

185 ***Total solids, ash, total protein, NPN, NCN, fat***

186 Total solids and ash contents were determined as described by IDF (1964a, 2010). Total
187 nitrogen, non-protein nitrogen (NPN) and non-casein nitrogen (NCN) were determined using
188 the Kjeldahl method (IDF, 1964b, 1993), and a nitrogen-protein conversion factor of 6.38
189 was applied. MF 0.14 retentate 1, 2 and 3 and MCC were diluted with Milli-Q water to a
190 protein concentration similar to that in skim milk during sample preparation for NCN and
191 NPN analysis. Fat content was determined using a Gravimetric method (IDF, 1996).

192 ***Total calcium***

193 A volume of 1 mL of sample was ashed, dissolved in 3 mL 10% HCl, and diluted to
194 100 mL in volumetric flasks with milli-Q water. The solutions were further diluted (MCC: 1
195 in 50; MF 0.14 retentate 1, 2, and 3: 1 in 25 dilution; all the other liquid samples: 1 in 10)
196 prior to calcium determination using an Atomic Absorption Spectrometer (AA240, Varian
197 AA, Varian Inc., Palo Alto, CA, USA) (Gaucheron, 2005; Lin et al., 2016).

198 ***Lactose***

199 All liquid samples were diluted 1 in 100 with Milli-Q water, filtered with a 0.2 μm
200 nylon membrane filter (Chromacol20-SF-02(N), Thermo Scientific, Waltham, Massachusetts,
201 United States), and analysed as described by Pirisino (1983) and Hou et al. (2014b) .

202 ***Rheological properties of curds***

203 The rheological properties of coagula were monitored using a rheometer (AR-G2
204 rheometer; TA Instruments, New Castle, DE, USA) equipped with a conical concentric
205 cylinder geometry as described by Sandra et al. (2011). Cheese milk was mixed for 3 min
206 after rennet addition, and a volume of 20 mL milk was transferred to the rheometer, where a
207 time sweep test was subsequently carried out. Conditions for the time sweep test were 32 °C
208 with a gap distance 5920 mm, strain 0.02, and oscillation frequency 1 Hz as described by
209 Panthi et al. (2019b), the test continued for 90 min. Rennet addition time was defined as the
210 starting time and the following parameters was recorded or calculated from the $G' / \tan \delta$ -time
211 curve as described by Panthi et al. (2019b): MCFR (maximum curd-firming rate), A_{40} and \tan
212 δ_{40} (the value of G' and $\tan \delta$ after 40 min of rennet addition), K_{35} and K_{70} (time for the curds
213 to obtain gel firmness of 35 or 70 Pa respectively after rennet addition) and CW (cutting
214 window, calculated from K_{35} and K_{70}).

215 ***Compositional analysis of cheese***

216 Cheese samples were ground prior to analysis with measurements of moisture and fat
217 contents and pH conducted on fresh samples; with the remainder frozen at -20 °C until
218 analysis. Frozen cheese was defrosted at 4 °C overnight prior to analysis. Moisture, protein,
219 salt, ash and total calcium contents as well as pH in cheese were measured as described by
220 Fenelon and Guinee (1999), fat content was determined by NMR (SMART Trac II Moisture
221 and fat Analyzer, CEM Smart Trac, Damastown, Dublin, Ireland).

222 ***Textural properties of cheese***

223 After storage at 4 °C for 7 days, the cheeses were sampled for texture and cheese
224 composition analysis respectively. Cheese were prepared into 25 mm³ cubes (six cubes per
225 treatment), wrapped with foil paper and stored at 4°C overnight. Texture profile analysis
226 (TPA) was conducted on each cube with a P75 probe and 50 kg load cell (TA-XT plus, Stable
227 Micro Systems, Godalming, Surrey, UK), the cubes were compressed to 70% of original
228 height at a testing speed of 1.00 mm/s. The fracture force, fracture strain and firmness were
229 recorded and calculated as described in Hou et al. (2014a).

230 ***Statistical analysis***

231 Triplicate trials were undertaken for the cascade filtration process, cheese milk
232 preparation and Cheddar cheese manufacture. The effect of MF 0.14 and diafiltration on
233 retentate composition, cheese milk composition, rheological properties of curd as well as
234 cheese composition, textural properties and yield were compared with least-squares
235 difference (LSD) at 95% significance level by one-way ANOVA using SPSS 24.0 (IBM
236 Corp., 2016, Chicago, IL, USA).

237 **RESULTS AND DISCUSSION**

238 ***Effect of MF 0.14 and diafiltration on milk composition***

239 As a result of MF and DF, casein micelles were separated and concentrated in MF 0.14
240 retentates, while small molecules including serum protein, lactose and minerals were depleted
241 (Table 2). As MF and DF progressed and the casein content in MF 0.14 retentates increased,
242 specific ratios were determined (serum protein:casein, ash:casein, total calcium:casein and
243 lactose:casein ratios) to compare the relative loss of serum protein, ash, total calcium and
244 lactose compared to casein in these streams during the process. After MF but without a DF

245 step (Fig 2), the serum:casein, ash:casein, total calcium:casein and lactose:casein ratios in MF
246 0.14 retentate 1 decreased by 39.50%, 21.40%, 18.54% and 67.68% respectively compared to
247 the MF 1.4 permeate; after two diafiltration steps (i.e., MF with DF \times 1 and 2, Fig 2), the
248 serum:casein, ash:casein, total calcium:casein and lactose:casein ratios in MF 0.14 retentate 3
249 decreased by 20.45%, 35.32%, 11.45%, 26.46% respectively when compared to MF 0.14
250 retentate 1. It is clear that less serum protein, minerals, total calcium and lactose were lost
251 during MF with DF than MF without a DF step, suggesting that more small molecules were
252 removed to the MF 0.14 permeate during MF without a DF step. It is suggested that dairy
253 processors should consider whether the increased process costs of diafiltration would be
254 offset by the value of increased serum protein before the application of DF or even multi-step
255 DF with MF.

256 After MF together with two steps of DF, the total calcium:casein and lactose:casein
257 ratios in MF 0.14 retentate 3 decreased by 29.99% and 94.14% respectively compared to MF
258 1.4 permeate, suggesting that calcium and lactose contents may need to be supplemented
259 when standardising cheese milk from MF 0.14 retentate 3. Reduced lactose content in cheese
260 milk can lead to increased hardness and pH in cheese (Moynihan, 2016; Hou et al. 2012,
261 2014a), thus it may be of benefit to apply MF to reduce or standardise lactose levels in cheese
262 milk as a way to control cheese pH or texture. Similarly, demineralisation of cheese milk can
263 decrease the buffering capacity of cheese milk, decreasing the cheese make time (St-Gelais et
264 al., 1997) and resulting in increased cheese moisture content (Govindasamy-Lucey et al.,
265 2007). Thus, the demineralisation effect of MF could be beneficial to increase the moisture or
266 moisture in non-fat substance contents in low fat cheese or in cheeses made from
267 concentrated cheese milk, providing sufficient milk minerals are present to ensure good
268 rennet coaguability.

269 In addition, lactose was removed from the MF 1.4 permeate at a much faster rate than
270 serum protein and minerals (Figure 3), probably due to the smaller molecular size of lactose
271 compared to that of serum proteins. Although milk salts are also small molecules, they are
272 present in large quantities in the casein micelle in the form of colloidal calcium phosphate
273 (Gaucheron, 2005), and thus were depleted at a slower rate than lactose. Under
274 microfiltration, both with and without diafiltration, total calcium levels were depleted at a
275 lower rate than for ash (Figure 3). This was attributed to the fact that only 31 % of total
276 calcium is present in the serum phase, while more than 50% of the potassium, sodium,
277 chloride, inorganic phosphate, magnesium and citrate are present in the milk serum
278 (Gaucheron, 2005); thus minerals dissolved in the serum phase are more likely to partition in
279 the permeate during MF and DF.

280 Gaucheron (2005) reported that soluble calcium amounts to 31% of total calcium, and
281 in the current study the total Ca: casein ratio in MF 0.14 retentate 3 was 70% of that in MF
282 1.4 permeate (Table 2), suggesting that all the soluble calcium originally present in MF 1.4
283 permeate partitioned to MF 0.14 permeate 3 during MF and DF. Thus, to maintain the
284 calcium equilibrium, we presume that a certain amount of colloidal calcium phosphate (CCP)
285 dissociated and dissolved in the serum phase of MF 0.14 retentate 3, leading to a lower
286 colloidal calcium:casein ratio in MF 0.14 retentate 3 compared to the original skim milk,
287 although further research is required to prove this assumption. During diafiltration, the
288 addition of RO water will dilute the serum phase of the MF 0.14 retentate, which may disrupt
289 the calcium equilibrium between casein micelles (CM) and the serum phase. As a result, part
290 of the colloidal calcium phosphate (CCP) within the CM may be dissolved in the diluted
291 serum phase and ultimately removed to MF 0.14 permeate during diafiltration. Alexander et
292 al. (2011) and Li et al. (2014) reported that part of the CCP inside CM was washed away
293 during ultrafiltration (UF) and DF (with RO water) of milk. Both Boiani (2017, 2018) and Lu

294 et al. (2016) suggested that part of the CCP might be removed during MF and DF with water,
295 although this assumption was not proven in their research. CCP is very important for rennet
296 induced gelation of milk in cheese manufacture; when the colloidal calcium:casein ratio is
297 lower than 70% of the original level, a rennet induced gel cannot be formed (Shalabi and Fox,
298 1982, Choi et al., 2007). CCP loss from CM can also cause weak gels (Udabage et al., 2001)
299 and it becomes difficult to reverse or fortify CCP loss when a large amount of CCP is lost
300 through membrane filtration (Ferrer et al., 2014). Thus, when water is used as diafiltrant
301 during microfiltration, and especially when multiple DF steps are carried out, the colloidal
302 calcium:casein ratio in MF retentate should be monitored when the retentate is used to
303 prepare cheese milk directly.

304 A significant increase in pH was observed between MF 1.4 permeate and MF 0.14
305 retentate 3, and the pH of MF 0.14 retentate 1, 2 and 3 increased significantly after each
306 diafiltration step (Table 2). Boiani (2017) also observed a pH increase in MF retentate after
307 microfiltration and diafiltration with water, i.e., from 6.55 in skim milk to 7.02 in MF
308 retentate. We suggest that partial solubilization of CCP from casein micelles might have led
309 to the increased retentate pH (Fox et al., 2015).

310 *Cheese milk composition*

311 The streams generated (pasteurised cream, pasteurised skim milk, MF 1.4 permeate,
312 MCC, RO retentate and RO permeate) were combined to formulate four cheese milks (Table
313 1). For cheese milks of the same casein content, i.e., PC PS, PC MF1.4P, and MCC1.0, there
314 was no significant difference between their contents of total solids, total protein, casein, total
315 calcium and lactose (Table 3). Similarly no significant difference between PC MF 1.4P and
316 MCC1.0 was observed for ash content. The lactose content in MCC 1.5 cheese milk was
317 similar to those of the other three cheese milks as a result of lactose standardisation. The ash

318 and total calcium contents in MCC1.5 cheese milk were significantly higher ($p < 0.05$) than
319 those in the other cheese milk samples, and was attributed to the significantly higher casein
320 content in the former. The ash: casein ratio and total calcium: casein ratio in the MCC1.5
321 cheese milk were also significantly lower, although similar in magnitude, to the other three
322 cheese milks (Table 3).

323 Although only the casein and lactose contents as well as casein: fat ratio in MCC 1.0 and
324 MCC 1.5 cheese milks were deliberately standardised during cheese milk preparation, it was
325 observed that the ash and total calcium contents in the MCC1.0 cheese milk also achieved
326 standardisation, while the ash: casein, total calcium: casein ratios in MCC1.5 cheese milk
327 were lower, although similar in magnitude. This was attributed to the fact that the cascade
328 membrane filtration process resulted in all casein micelles originally present in skim milk
329 being separated and concentrated in the MCC, while the lactose and minerals were either
330 retained in the MCC or concentrated in the RO retentate.

331 The pH of the four cheese milks were approximately 6.63 (Table 3) which were in the
332 range of natural milk pH as suggested by Fox et al. (2017). The PC PS and PC MF1.4P
333 cheese milks were prepared from pasteurised cream (pH 6.61-6.65), pasteurised skim milk
334 (pH 6.72-6.74) and MF 1.4 permeate (pH 6.76). The MCC1.0 and MCC1.5 cheese milks
335 were prepared from pasteurised cream, MCC (pH 6.85), RO retentate (pH 6.19) and RO
336 permeate (6.43). Although the pH of the RO retentate and RO permeate were low, this was
337 offset by the high pH and high buffering capacity of MCC (casein micelles and milk serum)
338 resulting in a cheese milk pH of 6.63.

339 ***Curd rheology***

340 The Maximum Curd Firming Rate (MCFR) during coagulation of the MCC1.5 cheese
341 was significantly higher than for the other cheeses, corresponding with a significantly higher

342 gel firmness at 40 min (A_{40}) and significantly reduced time to obtain gel firmness of 35 and
343 70 Pa (K_{35} and K_{70}) (Table 4). Cheese milk pH in all vats was standardized to 6.55, however
344 the rennet was added on a volume basis, and in milk of a higher casein content (MCC1.5), the
345 para-caseins had a greater chance of collision, thus forming a more dense 3-D network,
346 resulting in a higher curd firming rate and gel firmness at any given time (Guinee et al., 1996,
347 Sandra et al., 2011, Panthi et al., 2019b). Due to the faster curd firming rate for the MCC1.5,
348 the time for the gel's elastic modulus (G') to reach 35 Pa (K_{35}) and 70 Pa (K_{70}) (used to
349 calculate cutting window; Panthi et al.; 2019b) were significantly lower than the other curds,
350 and as a result, the cutting window (CW) in MCC1.5 was significantly narrower than for the
351 other cheeses. The reduced cutting window would result in problems for cheese makers
352 during cutting, e.g., curd tearing and shattering and increased fat loss in cheese whey (Guinee
353 et al., 1994). This may be avoided by application of a lower set temperature to reduce gel
354 firming rate (Guinee et al., 1996, Panthi et al., 2019b), cutting of the curds when softer (a
355 lower G') (Govindasamy-Lucey et al., 2007) or overlay of the curds with UF permeate before
356 and after cutting (Panthi et al., 2019a). The tendency for all curds to synerese was not
357 influenced by their differing casein contents, as suggested by their similar $\tan \delta$ value at 40
358 min in agreement with Panthi et al. (2019b).

359 For cheese milk of similar casein and total calcium contents, no significant difference
360 was observed for curd firming rates, suggesting that methods to decrease the bacteria load
361 (pasteurization Vs MF1.4) as well as milk serum protein content did not have a significant
362 impact on their rennet induced gelation properties.

363 ***Cheese composition***

364 The moisture and MNFS contents in the MCC1.5 cheese were significantly lower than
365 those in the PC PS cheese and were lower in magnitude (although not significantly) than the
366 PC MF1.4P and MCC1.0 cheeses (Table 5). It has previously been reported that cheese curds

367 manufactured from milk of higher casein content have lower moisture contents than those
368 originated from milks of lower casein content, due to the lower moisture content in cheese
369 milk of higher casein content (Panthi et al., 2019a); in addition, such curds are more prone to
370 syneresis due to the higher casein concentration and higher pressure created by more frequent
371 curd particles collisions (Guinee et al., 2006). Since the casein content and ash content in
372 MCC1.5 cheese were significantly higher than the other cheeses (Table 5), it is expected that
373 the buffering capacity in this cheese would be higher thus resulting in the significantly higher
374 pH (Table 5).

375 There was no significant difference in all other compositional parameters between PC
376 PS, PC MF1.4P and MCC1.0 cheeses (Table 5). It was concluded that use of MF to remove
377 bacteria and serum protein content in cheese milk had no significant impact on the cheese
378 composition.

379 ***Cheese texture***

380 The fracture stress and firmness of the MCC1.5 cheese were significantly higher than
381 those of PC MF1.4P cheese and were higher in magnitude, although not significantly so than
382 PC PS and MCC1.0 cheeses at day 7 of ripening (Table 4). The firmer texture obtained by
383 MCC1.5 cheese is attributed to the combined effect of its higher gel-forming protein content
384 (Guinee, 2016) and its lower gel-filler moisture content (Neocleous et al., 2002). Similarly,
385 higher (although not significantly so) levels of S/M in MCC1.5 cheese could also enhance the
386 hydration and swelling of para-casein strands in gel network, making the gel more resistant to
387 deformation (Pastorino et al., 2003, McCarthy et al., 2016). Neocleous et al. (2002) also
388 reported that fresh cheese produced from concentrated cheese milk had increased hardness
389 due to higher protein and lower moisture contents compared to control cheeses (made from
390 typical cheese milk); however increasing the moisture content in cheese manufactured from
391 concentrated milk through adjustment of cheese making procedures can result in cheese with

392 a comparable texture to the control. No significant difference was observed for fracture strain
393 between the four cheeses (Table 4).

394 ***Cheese yield***

395 The actual yield (Y_a) and moisture adjusted cheese yield (Y_{ma} ; target moisture content:
396 38.5%), as defined by Guinee et al (2006) were significantly higher for the MCC1.5 cheese
397 compared to the other cheeses (table 4). This was attributed to significantly higher casein
398 content in the MCC1.5 cheese milk. It reflects the ability to produce more curd per vat when
399 utilizing concentrated cheese milk as reported by Neocleous et al. (2002b) and St-Gelais et al.
400 (1995). The difference for Y_{ma} between MCC1.5 cheese and the other cheeses was more
401 pronounced than for Y_a , reflected by the significantly lower moisture content in MCC1.5
402 cheese (Neocleous et al., 2002, Guinee et al., 2006). To eliminate the effect of different fat
403 and casein concentrations in the cheese milks between the vats, both Y_a and Y_{ma} per 100 kg
404 of cheese milk were adjusted to arbitrary levels of fat (3.4%, wt/wt) and casein (2.53%,
405 wt/wt) contents as described by Guinee et al (2006), i.e., yield of cheese per 100 kg fat- and
406 casein- adjusted milk (Y_{afcam}) and moisture adjusted yield of cheese per 100 kg fat- and
407 casein- adjusted milk (Y_{mafcam}). No significant difference was found for Y_{afcam} and
408 Y_{mafcam} between four cheeses, supporting the conclusion that the significantly higher Y_a
409 and Y_{ma} for the MCC1.5 cheese was due only to the significantly higher casein content in
410 the cheese milk (Guinee et al., 2006).

411 ***Composition of cheese whey and UF retentate***

412 The weight of MCC1.5 cheese whey was significantly lower than the other three
413 cheese wheys (Table 6), in accordance with the findings of Outinen et al. (2010) and Daviau
414 et al. (2000), which could be due to the lower moisture content (reflected by higher total

415 solids content) in MCC1.5 cheese milk than the other cheese milks (Table 3) (Daviau et al.,
416 2000).

417 The UF retentate produced in the cascade filtration process has a much higher purity of
418 serum protein compared to cheese whey. Even though the total solids in the UF retentate
419 (3.78%) was much lower than those in cheese whey (6.03-6.76%, Table 6), the serum protein
420 content and serum protein as a percentage of total solids in the UF retentate (1.94%, 51.54%)
421 were significantly higher than those in cheese whey (0.34-0.62%, 5.63-9.45) respectively
422 (Table 6). The high purity of serum protein in UF retentate is mainly attributed to the low or
423 negligible amount of lactose and minerals as well as the absence of curd fines in this stream
424 (Table 6). Similarly, starter bacteria, enzymes and colorants added during cheese manufacture
425 will also be absent. The high purity and concentration of serum protein and the absence of
426 thermal history confers better functionality (gelation and foaming properties, solubility,
427 Bacher, 2000; Heino et al, 2007) to the UF retentate, making it a source of serum protein of
428 higher value compared to cheese whey. Furthermore, the significantly lower ash content
429 (0.95%) calculated on dry matter basis in UF retentate than that in cheese whey (7.11-7.53%)
430 makes the serum protein products produced from UF retentate significantly more valuable
431 particularly for applications in infant milk formula (Bylund, 2015) (Table 6), where it is
432 necessary to undertake demineralisation of standard cheese whey, as well as applications in
433 ice cream and bakery products.

434 CONCLUSION

435 Large amounts of serum protein, lactose and minerals were depleted from the retentate
436 by microfiltration at pore size 0.14 μm without diafiltration; while lower amounts of serum
437 proteins, lactose and minerals were removed during MF0.14 with diafiltration when RO

438 water was used as a diafiltrant. The comparable depletion level for small molecules during
439 MF and DF was: lactose> serum protein> ash> total calcium.

440 It was shown that serum protein depleted cheese milk can be accurately standardised
441 from pasteurized cream, MCC, RO retentate and RO permeate as, in particular when
442 standardising the lactose content in cheese milk with RO retentate, the mineral content and
443 total calcium content were also standardised simultaneously. The serum protein depleted
444 cheese milk also had a comparable pH to the control.

445 Cheese milk standardised from membrane streams of typical casein content had
446 comparable rennet coagulation properties, cheese composition, yield and texture to the
447 control. Cheese milk with an elevated casein content had a faster curd firming rate, narrower
448 cutting window, decreased cheese moisture as well as increased pH, hardness and actual
449 cheese yield.

450 The serum protein stream removed from milk by MF and concentrated by UF retaining
451 its globular structure had significantly higher serum protein purity, lower ash and lactose
452 contents as well as an absence of starter culture, cheese fines, fat and rennet in comparison to
453 cheese whey.

454 In this cascade filtration process, all streams originating from the whole milk can be
455 utilized: cream, MCC, RO retentate and RO permeate for cheese production; UF retentate
456 and cheese wheys can be used to produce serum protein products. Overall, this research
457 showed that the cascade membrane filtration process utilised in this research can produce
458 serum protein depleted cheese milk of target composition, resulting in Cheddar cheese of
459 standard quality and a native serum protein stream of high purity.

460

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629 **Figure legends**

630 **Figure 1.** Cascade filtration process applied in preparation of milk fraction streams and in
631 preparation of cheese milks

632 **Figure 2.** Microfiltration process with pore size 0.14 µm incorporating two diafiltration steps

633 **Figure 3.** Relative lactose:casein, serum:casein, ash:casein, and total calcium:casein ratios in
634 MF 1.4 permeate and MF 0.14 retentate 1, 2, and 3¹ streams respectively²

635 ¹Relative lactose:casein ratio was determined as: $\frac{\text{lactose:casein ratio in sample}}{\text{lactose:casein ratio in MF 1.4 permeate}}$; relative
636 lactose:casein, ash:casein and total calcium:casein ratios were calculated in similar way;

637 ²Figure 3 is derived from data in Table 2.

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657 Table 1. Component stream formulations for PC PS, PC MF1.4P, MCC1.0 and MCC1.5
 658 cheese milk^{1, 2, and 3}

Weight of streams (kg)	PC PS	PC MF1.4P	MCC1.0	MCC1.5
Pasteurised cream	2.04	1.85	2.02	3.03
Pasteurised skim milk	10.16	0	0	0
MF 1.4 permeate	0	10.15	0	0
MCC	0	0	2.27	3.41
RO retentate	0	0	2.86	2.49
RO permeate	0	0	4.85	3.08

659 ¹Abbreviations: PC PS, cheese milk prepared from pasteurized cream and pasteurized skim
 660 milk; PC MF1.4P, cheese milk prepared from pasteurized cream and MF 1.4 permeate; MCC
 661 1.0, cheese milk prepared from pasteurized cream, MCC, RO retentate, RO permeate, and of
 662 the same casein content in raw skim milk; MCC 1.5, cheese milk prepared from pasteurized
 663 cream, MCC, RO retentate, RO permeate, casein content 1.5 times that of the MCC1.0 (3.75-
 664 4.5%);

665 ²Results are means of triplicate trials;

666 ³Cheese milk formulations were calculated on a 12 kg basis.

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681 **Table 2.** Effect of microfiltration at 0.14 µm and diafiltration on the composition of resultant
 682 streams¹

Compositional parameters	MF 1.4 permeate	MF 0.14 retentate 1	MF 0.14 retentate 2	MF 0.14 retentate 3
Total solids (% , wt/wt)	8.74 ^c	14.58 ^a	11.40 ^b	11.38 ^b
Total protein (% , wt/wt)	3.52 ^b	9.06 ^a	8.56 ^a	9.32 ^a
Casein number (%) ²	78.95 ^c	86.98 ^b	89.76 ^a	91.83 ^a
Casein content (% , wt/wt)	2.78 ^b	7.90 ^a	7.69 ^a	8.56 ^a
Serum protein content (% , wt/wt)	0.58 ^c	0.97 ^a	0.76 ^b	0.70 ^{bc}
Ash content (% , wt/wt)	0.65 ^b	1.23 ^a	0.95 ^{ab}	0.87 ^b
Total calcium (m mol/ kg)	31.26 ^b	72.06 ^a	66.82 ^a	67.12 ^a
Lactose content (% , wt/wt)	4.51 ^a	4.07 ^a	1.61 ^b	0.77 ^b
Serum protein:casein ratio	0.21 ^a	0.12 ^b	0.10 ^c	0.08 ^c
Relative serum protein:casein ratio ³	100.00 ^a	60.50 ^b	48.59 ^{b, c}	40.05 ^c
Ash: casein ratio	0.24 ^a	0.16 ^b	0.12 ^c	0.10 ^c
Relative ash: casein ratio	100.00 ^a	78.60 ^b	52.22 ^c	43.28 ^c
Total calcium:casein ratio (m mol/g)	1.12 ^a	0.91 ^b	0.87 ^b	0.79 ^c
Relative total calcium:casein ratio	100.00 ^a	81.46 ^b	77.72 ^{b, c}	70.01 ^c
Lactose:casein ratio	1.48 ^a	0.47 ^b	0.19 ^c	0.09 ^c
Relative lactose:casein ratio (%)	100.00 ^a	32.32 ^b	13.34 ^c	5.86 ^d
pH	6.76 ^{b, c}	6.68 ^c	6.82 ^b	6.96 ^a

683 ¹ Results are means of triplicate trials, values within a row not sharing the same superscript
 684 differ significantly (p<0.05).

685 ²Casein number (%) = $\frac{\text{Casein content}}{\text{Total protein}} \times 100$.

686 ³Relative serum protein:casein ratio = $\frac{\text{serum protein:casein ratio in sample}}{\text{serum protein:casein ratio in MF 1.4 permeate}}$; relative lactose:casein,
 687 ash:casein and total calcium:casein ratios were calculated in similar way.

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694 Table 3. Compositional ratios of cheese milks formulated from streams produced by the
 695 cascade filtration process^{1,2}

Compositional parameters	PC PS	PC MF1.4P	MCC1.0	MCC1.5
Total solids (% , wt/wt)	12.53 ^b	12.53 ^b	12.09 ^b	15.72 ^a
Total protein (% , wt/wt)	3.55 ^b	3.40 ^b	3.34 ^b	4.96 ^b
Casein number ³	80.79 ^b	79.55 ^b	85.90 ^a	87.03 ^a
Casein content (% , wt/wt)	2.87 ^b	2.71 ^b	2.87 ^b	4.32 ^a
Serum protein content (% , wt/wt)	0.49 ^a	0.51 ^a	0.30 ^b	0.45 ^a
Fat content (%)	4.05 ^b	3.99 ^b	4.18 ^b	6.02 ^a
Casein: fat ratio	0.71 ^a	0.68 ^a	0.69 ^a	0.73 ^a
Ash content (% , wt/wt)	0.72 ^b	0.65 ^c	0.66 ^c	0.83 ^a
Total calcium (m mol/ kg)	29.17 ^b	28.19 ^b	29.04 ^b	40.79 ^a
Lactose content (% , wt/wt)	4.32 ^a	4.14 ^a	4.11 ^a	4.45 ^a
Ash:casein ratio	0.25 ^a	0.24 ^a	0.23 ^a	0.19 ^b
Total calcium:casein ratio	1.02 ^a	1.03 ^a	1.07 ^a	0.96 ^b
Lactose:casein ratio	1.56 ^a	1.61 ^a	1.45 ^a	0.95 ^b
pH	6.62 ^a	6.63 ^a	6.63 ^a	6.63 ^a

696 ¹Abbreviations: PC PS, cheese milk prepared from pasteurized cream and pasteurized skim
 697 milk; PC MF1.4P, cheese milk prepared from pasteurized cream and MF 1.4 permeate; MCC
 698 1.0, cheese milk prepared from pasteurized cream, MCC, RO retentate, RO permeate, and of
 699 the same casein content in raw skim milk; MCC 1.5, cheese milk prepared from pasteurized
 700 cream, MCC, RO retentate, RO permeate, casein content 1.5 times that of the MCC1.0 (3.75-
 701 4.5%);

702 ² Results are means of triplicate trials, values within a row not sharing the same superscript
 703 differ significantly (p<0.05).

704 ³Casein number (%) = $\frac{\text{Casein content}}{\text{Total protein}} \times 100$.

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712 Table 4 Coagulation properties, cheese yield and texture of cheese manufactured from PC
 713 PS, PC MF1.4P, MCC1.0 and MCC1.5 cheese milks^{1, 2}

Parameters	PC PS	PC MF1.4P	MCC1.0	MCC1.5
Curd coagulation				
MCFR (Pa/min) ³	2.69 ^b	2.45 ^b	3.88 ^b	18.49 ^a
A ₄₀ (Pa) ⁴	36.76 ^b	39.02 ^b	70.20 ^b	310.95 ^a
Tan δ_{40} ⁴	0.28 ^a	0.26 ^a	0.28 ^a	0.28 ^a
K ₃₅ (min) ⁵	40.67 ^a	38.28 ^a	31.16 ^a	18.00 ^b
K ₇₀ (min) ⁵	56.00 ^a	58.54 ^a	41.89 ^a	20.49 ^b
CW (min) ⁶	15.33 ^{a,b}	20.26 ^a	10.46 ^b	2.50 ^c
Cheese yield⁷				
Ya (kg/100 kg)	10.89 ^b	10.55 ^b	11.33 ^b	16.01 ^a
Yma	11.22 ^b	11.21 ^b	11.98 ^b	17.31 ^a
Yafcam	9.36 ^a	9.38 ^a	9.53 ^a	9.21 ^a
Ymafcam	9.62 ^a	9.96 ^a	10.07 ^a	9.96 ^a
Texture				
Fracture stress (kPa)	501.35 ^{a,b}	447.58 ^b	516.05 ^{a,b}	627.34 ^a
Fracture strain	0.69 ^a	0.72 ^a	0.71 ^a	0.70 ^a
Firmness (N)	306.24 ^{a,b}	266.69 ^b	310.49 ^{a,b}	380.27 ^a

714 ¹ Results are means of triplicate trials, values within a row not sharing the same superscript
 715 differ significantly (p<0.05).

716 ² Abbreviations: PC PS, cheese milk prepared from pasteurized cream and pasteurized skim
 717 milk; PC MF1.4P, cheese milk prepared from pasteurized cream and MF 1.4 permeate; MCC
 718 1.0, cheese milk prepared from pasteurized cream, MCC, RO retentate, RO permeate, and of
 719 the same casein content in raw skim milk; MCC 1.5, cheese milk prepared from pasteurized
 720 cream, MCC, RO retentate, RO permeate, casein content 1.5 times that of the MCC1.0 (3.75-
 721 4.5%);. .

722 ³ MCFR: maximum curd firming rate, calculated from $\Delta G' / \Delta t$ curve.

723 ⁴ A₄₀ and tan δ_{40} : the value of G' or tan δ after 40 min of rennet addition in respective.

724 ⁵ K₃₅ and K₇₀: the value of G' after 35 or 70 min of rennet addition separately.

725 ⁶ CW: cutting window, K₇₀-K₃₅.

726 ⁷Ya= actual yield (kg/ 100 kg milk); Yma= moisture-adjusted yield; Yafcam= yield per 100
 727 kg of milk normalized to reference fat (3.4%, w/w) and casein (2.53%, w/w) levels;

728 Ymafcam= moisture-adjusted yield per 100 kg of milk normalized to reference fat (3.4%,
729 w/w) and casein (2.53%, w/w) levels.

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760 Table 5. Composition at 7 days of cheeses manufactured from PC PS, PC MF1.4P, MCC1.0
 761 and MCC1.5 cheese milks^{1, 2}

Compositional parameters	PC PS	PC MF1.4P	MCC1.0	MCC1.5
Protein content (%)	24.61 ^a	24.11 ^a	24.42 ^a	25.96 ^a
Fat content (%)	32.27 ^a	34.07 ^a	33.91 ^a	33.65 ^a
Pro: fat ratio	0.76 ^a	0.71 ^a	0.73 ^a	0.78 ^a
Moisture content (%)	36.71 ^a	34.69 ^{a,b}	34.98 ^{a,b}	33.50 ^b
FDM (%) ³	50.96 ^a	52.14 ^a	52.12 ^a	50.59 ^a
MNFS (%) ⁴	54.18 ^a	52.6 ^{a,b}	52.92 ^{a,b}	50.53 ^b
Salt content (%)	1.39 ^a	1.34 ^a	1.32 ^a	1.53 ^a
S/M (%) ⁵	3.82 ^a	3.86 ^a	3.79 ^a	4.57 ^a
Ash content (%)	3.28 ^b	3.30 ^b	3.34 ^b	3.89 ^a
Total calcium (mg/ 100 g cheese)	711.21 ^b	716.37 ^b	732.87 ^b	809.50 ^a
Calcium to protein (mg/ g)	28.92 ^a	29.73 ^a	29.99 ^a	31.16 ^a
pH	5.09 ^b	5.08 ^b	5.15 ^b	5.33 ^a

762 ¹Results are means of triplicate trials, values within a row not sharing the same superscript
 763 differ significantly (p<0.05).

764 ² Abbreviations: PC PS, cheese milk prepared from pasteurized cream and pasteurized skim
 765 milk; PC MF1.4P, cheese milk prepared from pasteurized cream and MF 1.4 permeate; MCC
 766 1.0, cheese milk prepared from pasteurized cream, MCC, RO retentate, RO permeate, and of
 767 the same casein content in raw skim milk; MCC 1.5, cheese milk prepared from pasteurized
 768 cream, MCC, RO retentate, RO permeate, casein content 1.5 times that of the MCC1.0 (3.75-
 769 4.5%);

770 ³ FDM: fat in dry matter.

771 ⁴ MNFS: moisture in non-fat substance.

772 ⁵ S/M: salt in moisture.

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780 Table 6. Composition of UF retentate and cheese whey manufactured from PC PS, PC
 781 MF1.4P, MCC1.0 and MCC1.5 cheese milks^{1,2}

Compositional parameters	UF retentate	Cheese whey			
		PC PS	PC MF1.4P	MCC1.0	MCC1.5
Weight (kg/10 kg of cheese milk)	N/A ³	8.61 ^a	8.56 ^a	8.47 ^a	7.96 ^b
Total solids (% , wt/wt)	3.78 ^c	6.75 ^a	6.60 ^a	6.03 ^b	6.76 ^a
Fat (% , wt/wt)	N/A ⁴	0.39 ^b	0.42 ^b	0.34 ^b	0.63 ^a
Protein (% , wt/wt)	3.13 ^a	0.93 ^b	0.95 ^b	0.62 ^b	0.86 ^b
Serum protein content (% , wt/wt)	1.94 ^a	0.60 ^b	0.62 ^b	0.34 ^c	0.48 ^{b,c}
Serum protein (% of total solids)	51.54 ^a	8.85 ^b	9.45 ^b	5.63 ^b	7.13 ^b
Ash content (% , wt/wt)	0.04 ^b	0.51 ^a	0.50 ^a	0.45 ^a	0.48 ^a
Ash content (% of total solids)	0.95 ^b	7.51 ^a	7.53 ^a	7.47 ^a	7.11 ^a
Lactose content (% , wt/wt)	0.35 ^b	4.37 ^a	4.24 ^a	4.24 ^a	4.19 ^a
Lactose content (% of total solids)	9.50 ^b	64.77 ^a	64.21 ^a	70.35 ^a	61.98 ^a
pH	6.75 ^a	5.78 ^b	5.68 ^b	5.69 ^b	5.79 ^b

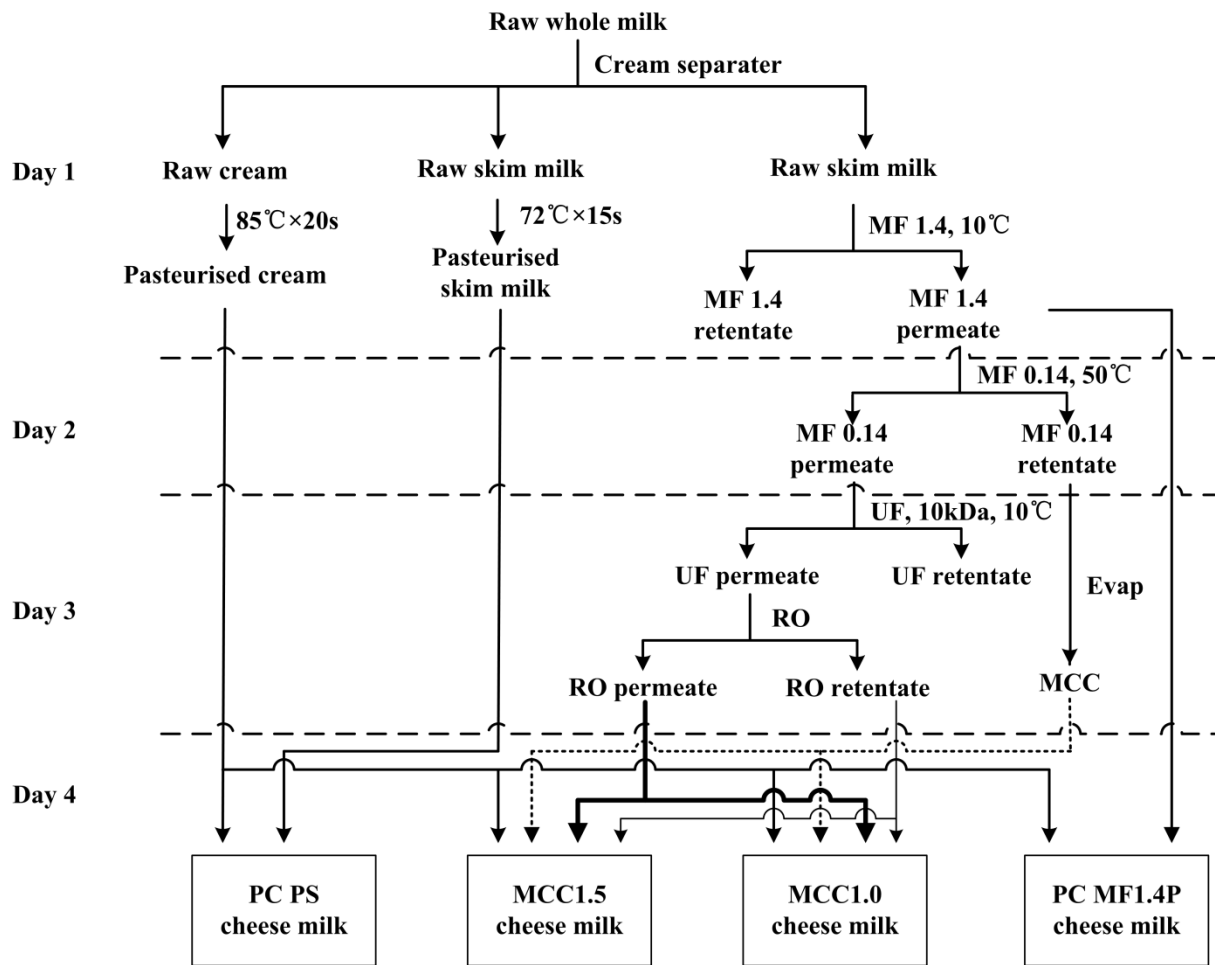
782 ¹ Results are means of triplicate trials, values within a row not sharing the same superscript
 783 differ significantly (p<0.05).

784 ² Abbreviations: PC PS, cheese milk prepared from pasteurized cream and pasteurized skim
 785 milk; PC MF1.4P, cheese milk prepared from pasteurized cream and MF 1.4 permeate; MCC
 786 1.0, cheese milk prepared from pasteurized cream, MCC, RO retentate, RO permeate, and of
 787 the same casein content in raw skim milk; MCC 1.5, cheese milk prepared from pasteurized
 788 cream, MCC, RO retentate, RO permeate, casein content 1.5 times that of the MCC1.0 (3.75-
 789 4.5%);

790 ³N/A: Not applicable;

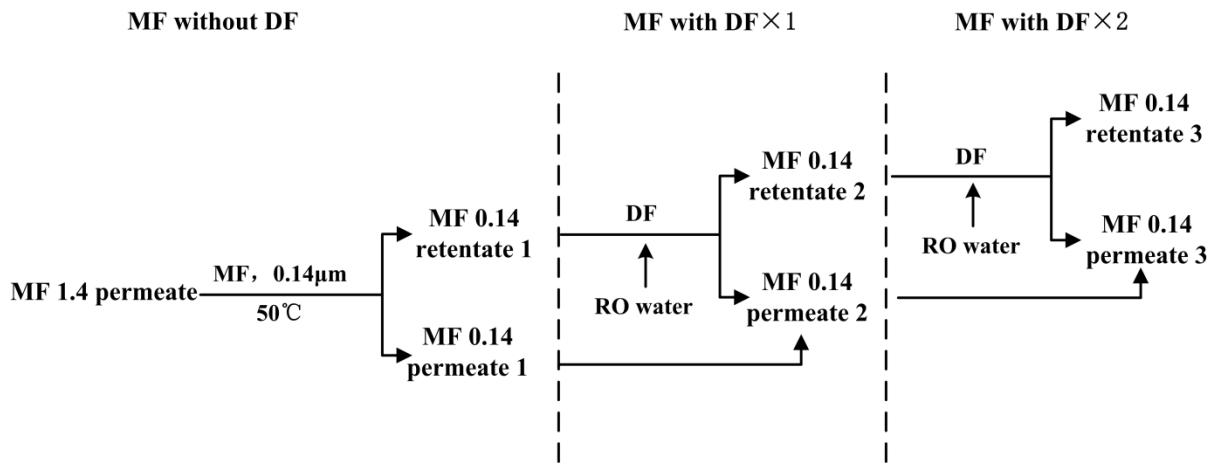
791 ⁴N/A: Not available.

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Figure 1.



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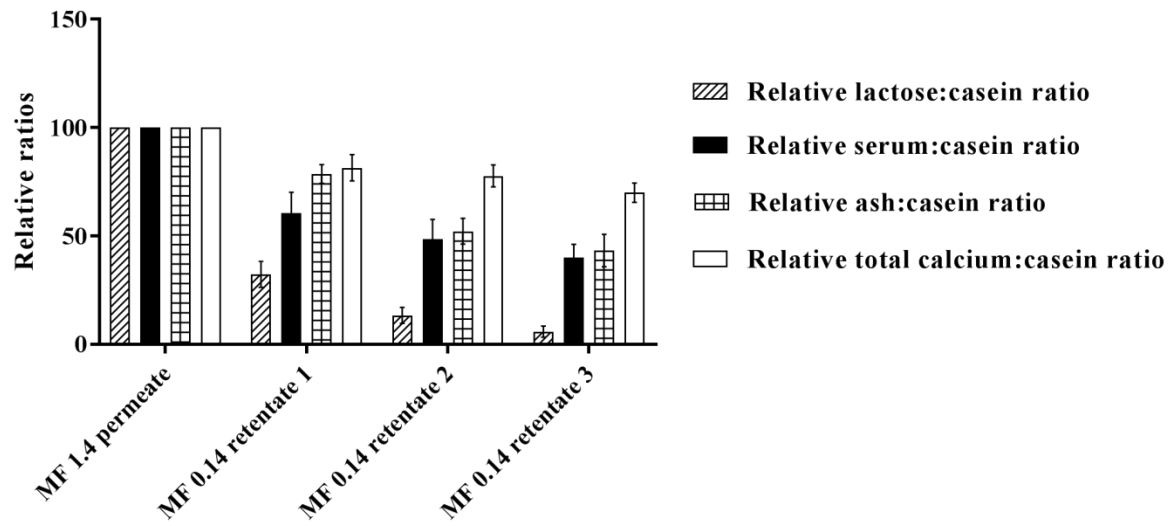
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834 **Figure 3**

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