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1 **Effect of thermal treatment on serum protein reduced micellar casein concentrate: An**
2 **evaluation of rennet coagulability, cheese composition and yield**

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ABSTRACT

Microfiltration at 0.10 μ m removed \sim 70.29% of serum proteins from milk and the resultant micellar casein concentrates (MCC) were subjected to: no heat treatment (control), pasteurization (72 $^{\circ}$ C \times 15s) and high heat treatment (HHT, 90 $^{\circ}$ C \times 15s) before formulation of cheese milk for Cheddar cheese manufacture. MCC showed good heat stability due to low serum protein content. For cheese milk of typical casein content, both pasteurization and HHT did not significantly influence pH, calcium distribution and rennet coagulability, or subsequent cheese composition and yield; although HHT elongated cheese make time significantly. On increasing casein content from 3.09% to 4.31%, there was no significant difference for rennet to cut time between cheeses made from milk with different thermal histories and casein contents. Overall, HHT of MCC had no significant impact on cheese make properties, cheese composition and yield of Cheddar cheese.

Key words: high heat treatment, microfiltration, rennet coagulability, cheese yield

INTRODUCTION

49 Heating of milk at temperatures $\geq 70^{\circ}\text{C}$ can cause serum protein denaturation; such
50 denatured serum proteins can form complexes with other denatured serum proteins or with κ -
51 casein (both on the surface of casein micelles or in milk serum phase) through thiol-
52 disulphide bond exchange reactions (Bulca et al., 2004). Since disulphide bonds formed
53 between serum proteins and casein micelles are located in the para- κ -casein region; the
54 denatured serum proteins will be attached to the para-casein micelles after rennet addition
55 and thus, incorporated into cheese curd (Anema et al., 2007). Partition of denatured serum
56 proteins from cheese milk to cheese provides a way to increase cheese yield (Banks et al.,
57 1987, Singh and Waungana, 2001). However, both serum protein/ para-casein micelle
58 complexes and serum protein/ soluble κ -casein complexes can impair the rennet coagulability
59 of cheese milk (Kethireddipalli et al., 2010). As a result, Guinee et al. (1997) and Fox et al.
60 (2017d) suggested that heat treatment above HTST pasteurization ($72^{\circ}\text{C}\times 15\text{s}$) conditions
61 should not be applied to cheese milk prior to cheese manufacture.

62 Native serum proteins can be separated from milk using microfiltration (MF) (Garem et
63 al., 2000) and are considered as 'ideal whey protein' due to their superior functional and
64 nutritional value over serum proteins recovered from cheese whey (Bacher and Kønigsfeldt,
65 2000, Heino et al., 2007), as well as having an absence of caseinomacropeptide, starter
66 bacteria, colorants, coagulant enzymes, cheese fines and fat in this stream. Serum protein
67 reduced or depleted milk called micellar casein concentrate (MCC) can be used in cheese
68 manufacture, either to fortify the casein content in cheese milk (St-Gelais et al., 1995,
69 Govindasamy-Lucey et al., 2007) or to be used for standardisation of cheese milk (Garem et
70 al., 2000, Neocleous et al., 2002, Heino, 2008). MCC produced by MF has an enhanced heat
71 stability compared to milk of typical casein and serum protein contents (Renhe and Corredig,

2018). Milk which is partially or completely reduced of serum protein content can be subjected to high heat treatment (above pasteurization conditions) with little or no impairment of rennet coagulation properties, and the lower the serum protein content, the higher the heat stability (Bulca et al., 2004). Thus it could be hypothesized that high heat treatment (more intensive than HTST, 72°C×15s) could be applied to denature and recover residual serum proteins in MCC to cheese curd leading to increased cheese yield without compromising rennet gelation and cheese making properties.

Previous research by this group (Xia et al., 2020) produced MCC of high casein number (casein content as a percentage of total protein, ~91%) using a cascade membrane filtration process, this MCC had a high pH (~7.0) as a result of diafiltration (DF) with RO water during the MF. Beliciu et al. (2012) suggested that aggregation or gelation in sterilised MCC can be prevented when the pH in unheated MCC is >6.7 and thus it is postulated that MCC produced by MF and DF with RO water might have a good heat stability. The objective of this study was to: 1) characterise the heat stability of MCC manufactured by MF and DF with RO water and; 2) evaluate the rennet coagulability, cheese making properties and cheese yield of cheese milks standardised from MCC of different thermal histories.

MATERIALS AND METHODS

Cascade filtration process

A pilot scale cascade membrane filtration process was carried out in triplicate (Figure 1) at Moorepark Technology Limited, Fermoy, Co Cork, Ireland:

Pasteurized skim milk was sourced from a local dairy company (Dairygold, Mitchelstown, Co Cork), stored at 4°C overnight, pre-heated to 50°C for 30min and then microfiltered at a membrane pore size of 0.1µm (Pall Corporation, New York, USA, model

95 no. EP 3730, surface area 0.35m², length 1020mm) on a GEA Model F filtration unit (GEA
96 Process Engineering A/S, Skanderborg, Denmark) at 50°C. The volume concentration factor
97 (VCF) was 3. Two steps of diafiltration with RO water (50°C) were also undertaken during
98 MF, with a dilution factor of 2. MF retentate was immediately chilled and stored at 4°C until
99 day 2. MF permeate was firstly subjected to reverse osmosis (VCF=5) and then ultrafiltration,
100 where RO permeate (water) and UF permeate (containing lactose and minerals) were
101 collected in sterilized containers, chilled in an ice bath and stored at 4°C until day 30.

102 On day 2 (Figure 1), MF retentate was divided into three portions and subjected to the
103 following treatments using a pilot-scale tubular heat-exchanger (MicroThermics®, Raleigh,
104 NC, USA): portion 1, unheated (control), denoted as CON MCC; portion 2, pasteurized at
105 72°C×15s, denoted as PS MCC; portion 3, high heat treatment: 90°C for 15s, denoted as
106 HHT MCC. The MCCs were stored separately in sterilized containers, cooled in an ice bath
107 and stored at 4°C until day 3.

108 *Preparation of cheese milk*

109 On day 3 (Figure 1), 4 cheese milks, namely CON- (control), PS-, HHT1.0-, and
110 HHT1.5 cheese milk (CM) were prepared from the following streams: pasteurized cream
111 (fat), CON-, PS-, and HHT MCC (casein), UF permeate (lactose and minerals) and RO
112 permeate (water) as described in Table 1. The protein, fat and lactose contents in the
113 pasteurised cream, MCCs and cheese milks were measured by FTIR (FOSS MilkoScan™
114 FT+, Hillerød, Denmark), the total solids in UF permeate was measured by microwave (CEM
115 Smart Trac moisture analyser, Damastown, Dublin, Ireland), while RO permeate is
116 essentially water and its composition was not determined. The lactose content, obtained by
117 multiplying total solids by 0.87 and expressed as a percentage of the total solids in UF
118 permeate, was estimated to be ~87%, in keeping with levels observed by previous studies

119 (unpublished data) undertaken by this research group. The casein contents for CON-, PS-,
120 and HHT1.0 CM were standardised to 2.72-2.74% and the casein content in HHT1.5 CM was
121 standardised to 1.5 times the casein content in HHT1.0 CM. The casein: fat ratio and lactose
122 content in the four cheese milks were standardised to 0.74 and 4.45-4.52% respectively.
123 HHT1.5 CM was formulated to mitigate any potential negative influence of high heat
124 treatment (90°C for 15s) on the rennet coagulability of cheese milk, by increasing casein
125 concentration.

126 *Preparation of cheese*

127 On the same day of cheese milk formulation, Cheddar cheese was manufactured as
128 described by Xia et al. (2020).

129 *Calcium in MCC and cheese milk*

130 Total calcium in MCCs, cheese milk and cheeses as well as colloidal- and soluble
131 calcium in cheese milk were determined with atomic absorption spectrometry (AA240,
132 VarianAA, Varian Inc., CA, USA) as described by Guinee et al. (2000), Gaucheron (2005)
133 and Lin et al. (2016). Colloidal- and soluble calcium were measured in fresh milk.

134 *Composition of liquid samples and cheese at 14 days*

135 Total solids and ash contents in the liquid samples (including MCCs, cheese milk and
136 cheese whey) and cheeses were determined by the methods described in IDF (2010) and IDF
137 (1964a) respectively. The fat content in liquid samples was measured by a gravimetric
138 method (IDF, 1996) and in cheese by NMR (CEM SMART Trac II, Damastown, Dublin,
139 Ireland). Total nitrogen, non-protein nitrogen and non-casein nitrogen contents were
140 determined using the Kjeldahl (IDF, 1964b, 1993) with a nitrogen-protein conversion factor
141 of 6.38. The casein number and native whey protein content (NWP, expressed as a

142 percentage of total protein) were calculated as described by Lin et al. (2018). The percentage
143 of whey protein denaturation (%WPD, as a percentage of total whey protein) in MCCs and
144 cheese milk was calculated with equations adapted from Lin et al. (2018):

$$145 \quad \% \text{WPD} = \frac{100 \times (NWP_{CON} - NWP_h)}{NWP_{CON}},$$

146 where NWP_{CON} represented the level of native whey protein in CON MCC or CON CM and
147 NWP_h represented the level of native whey protein in heated MCCs (PS MCC and HHT
148 MCC) or cheese milk prepared from heated MCCs (PS CM and HHT1.0 CM). The level of
149 serum protein denaturation arising due to pasteurisation of the feed milk was not considered
150 during the calculation of %WPD in MCCs to enable a comparison of the effects of the
151 various heat treatments on the MCCs.

152 ***Rennet coagulation characterisation***

153 A volume of 20mL of cheese milk was transferred from the cheese vat to a rheometer
154 (AR-G2 rheometer; TA Instruments, New Castle, DE, USA) 3min after rennet addition, and a
155 time sweep and frequency sweep were carried out as described by Xia et al. (2020). Rennet
156 coagulation time (RCT) (Sandra et al., 2011), storage modulus and $\tan \delta$ at 40min after rennet
157 addition (A_{40} and $\tan \delta_{40}$ respectively) and time to achieve storage modulus 35Pa (K_{35}) or
158 70Pa (K_{70}) (Panthi et al., 2019b) were recorded from the storage modulus-time curve. Since
159 the curds were cut on achieving gel firmness of 35Pa, K_{35} was used to represent set to cut
160 time (time from rennet addition to cutting). After a frequency sweep, the following equation
161 can be derived from the frequency (ω)-storage modulus (G') curve:

$$162 \quad \text{Log } G' = n * \log \omega + K;$$

163 Where n was defined as degree of frequency dependence (Tunick, 2010).

164 ***Cheese yield***

165 Actual and compositional adjusted cheese yield was calculated as described by Guinee
166 et al. (2006):

167 1. Y_a , actual cheese yield per 100kg of cheese milk (kg/100kg of cheese milk);

168 2. Y_{ma} , moisture adjusted (to 38.5%) cheese yield (kg/100kg of cheese milk):

169
$$Y_{ma} = Y_a \times \left(\frac{100 - M_a}{100 - M_r} \right)$$

170 Where M_a and M_r refer to the actual and reference (38.5%) cheese moisture content
171 respectively.

172 3. Y_{afcam} , actual cheese yield per 100kg of fat and casein adjusted milk:

173
$$Y_{afcam} = Y_a \times \left(\frac{F_{rm} + C_{rm}}{F_{cm} + C_{cm}} \right)$$

174 Where F_{cm} and C_{cm} refer to the actual fat and casein concentrations in cheese milk, and F_{rm}
175 (3.4%) and C_{rm} (2.53%) the concentrations in the reference milk.

176 4. Y_{mafcam} , moisture adjusted cheese yield per 100kg of fat and casein adjusted milk,
177 which was calculated from Y_{ma} with a similar formula to that described in formula 3.

178 *Statistical analysis*

179 The cascade filtration process and cheese manufacture trials were carried out in
180 triplicate. The effect of heat treatment on the composition of MCC, cheese milk, cheese
181 composition, yield, texture and gel properties were compared with least-squares difference
182 (LSD) at a 95% significance level in a one-way ANOVA using IBM SPSS statistics 24.0
183 (IBM Corp., 2016, Chicago, IL, USA).

184 **RESULTS AND DISCUSSION**

185 *Composition of MCC*

186 A level of 70.29% of serum protein originally present in pasteurised skim milk was
187 removed to permeate after microfiltration at 0.1 μ m, giving a serum protein reduced MCC
188 (casein number: 93.64%, Table 2). The total solids, total protein, ash and total calcium
189 contents in MCC were not affected by heat treatment (Table 2). As the intensity of heat
190 treatment increased, the native whey protein (NWP, as a percentage of total protein) content

191 in MCC decreased and % WPD increased (Table 2). There was no significant difference in the
192 NWP content and % WPD between CON MCC and PS MCC, which was not surprising, since
193 pasteurization only leads to 1% whey protein denaturation in skim milk (casein number:
194 75%) as reported by Guinee et al. (1996b). The % WPD in HHT MCC was significantly
195 higher than the CON- and PS MCC, corresponding to the significantly lower NWP content in
196 this stream (Table 2). The % WPD (15.97%, Table 2) in HHT MCC (90°C×15s) observed in
197 the current research was substantially lower than the % WPD (36.1%) reported in skim milk
198 (casein number: 74.2%) after high heat treatment (88°C×15s) by Guinee et al. (1997). The
199 enhanced heat stability in MCC (manifest by the lower % WPD in HHT MCC) was attributed
200 to its low serum protein content due to serum protein reduction (Bulca et al., 2004).

201 No significant difference was observed in pH levels between MCCs with different
202 thermal histories although the heated MCCs were lower in magnitude. It has been shown
203 previously that during heat treatment, soluble calcium content decreased, resulting in
204 increased colloidal calcium content and a pH drop in milk (Pouliot et al., 1989 a,b, c; On-
205 Nom et al., 2010). Both the soluble calcium content and pH levels in heated milk can be
206 almost or fully restored to their original level after cooling when the heating temperature is
207 less than 95°C (Kannan and Jenness, 1961, Pouliot et al., 1989d, Belicium et al., 2012). As the
208 pH values in heated MCC were similar to the control MCC, it is assumed that the soluble
209 calcium content and pH in heated MCC almost or totally returned to original levels after
210 cooling.

211 ***Composition of cheese milk***

212 Similar contents of total solids, total protein, casein, fat, ash, total calcium as well as
213 casein: fat ratio (Table 3) were achieved in CON-, PS- and HHT1.0 cheese milks as a result
214 of cheese milk standardization. There was no significant difference in the NWP content and
215 % WPD between CON- and PS cheese milk. The % WPD in HHT1.0 cheese milk was

216 significantly higher than the other cheese milks (Table 3), in line with the findings for MCC
217 (Table 2). The colloidal- and soluble calcium contents, %soluble calcium, colloidal calcium
218 per gram casein and the pH of cheese milk with different thermal histories were also similar
219 (Table 3), and comparable to the values reported by Gaucheron (2005). This suggests that a
220 similar calcium distribution between the colloidal and soluble phases in CON-, PS- and HHT
221 cheese milks was achieved as was a complete restoration of soluble calcium in the heated
222 MCC.

223 The total solids, total protein, casein, ash and total-, soluble-, colloidal calcium contents
224 in HHT1.5 cheese milk were significantly higher than those in HHT1.0 cheese milk, due to
225 the higher casein content in this HHT1.5 cheese milk. Similarly, the fat content was higher as
226 the milk had been standardised on a fat to casein basis. The casein: fat ratio, soluble calcium
227 as percentage of total calcium, colloidal calcium per gram casein and pH in the HHT1.5
228 cheese milk were similar to the other three milks (Table 3), reflecting an accurate
229 standardization of the HHT1.5 cheese milk.

230 ***Rennet coagulation property***

231 For cheese milks of typical casein content, the rennet coagulation properties were not
232 significantly affected by pasteurisation, with similar RCT, A_{40} , K_{35} and K_{70} between CON-
233 and PS cheese milks (Table 4). This was in keeping with the similar levels of %WPD in
234 CON- and PS cheese milks (Table 3) and reports of negligible effects of pasteurisation on the
235 coagulability of skim milk (Fox et al., 2017a). Even though the set to cut time (K_{35}) in
236 HHT1.0 CM increased by 21.84% compared to that in CON CM, it (22.42min) still ranged
237 between the value cheese makers usually use in cheese manufacture: 20 to 30min
238 (Govindasamy-Lucey et al., 2004, Heino, 2008, Panthi et al., 2019b). Guinee et al., (1997)
239 reported that the set to cut time at 20Pa in high heat treated ($88^{\circ}\text{C}\times 15\text{s}$) cheese milk (around
240 70min) is nearly twice to that in raw cheese milk (around 33.33min), leading to the

241 suggestion that cheese milk of typical casein number should not undergo high heat treatment
242 (i.e., $>72^{\circ}\text{C}\times 15\text{s}$) due to high levels of serum protein denaturation (Guinee et al., 1997, Fox et
243 al., 2017c). The lower level of serum protein denaturation as a result of serum protein
244 reduction was shown to mitigate this issue (Bulca et al., 2004, Renhe and Corredig, 2018).

245 Curd firming rate is improved by increasing the casein content in cheese milk (Guinee et
246 al., 1997, Panthi et al., 2019b), as a result, K_{35} and K_{70} decrease and A_{40} increases when the
247 casein content in cheese milk increase (Panthi et al., 2019b). For the cheese milks prepared
248 from high heat treated MCC, the significantly higher A_{40} value as well as lower K_{35} and K_{70}
249 value in HHT1.5 cheese milk suggested that the gel firming rate increased when the casein
250 content increased from 3.09% to 4.31% (Figure 2, Table 4). Interestingly, the set to cut time
251 (K_{35}) in HHT1.5 CM was similar to that of the control milk (CON CM) (Table 4). Suggesting
252 that there is no need to change the set to cut time when manufacturing Cheddar cheese from
253 HHT cheese milk with casein concentration as high as 4.31%, manufacture cheese from
254 cheese milk with high casein concentration can allow cheese makers to produce more cheese
255 with fixed facility and labour (Neocleous et al., 2002).

256 The degree of frequency dependence for storage modulus (n) is an indication of gel
257 structure (Chen et al., 1999), with $n=0$ for ideal covalent cross-linked gels and $n>0$ for non-
258 covalent cross-linked gels; an increased n value indicates an increased viscoelasticity (Zhou
259 and Mulvaney, 1998, Rosalina and Bhattacharya, 2002, Tunick, 2010). There was no
260 significant difference between the n value of the gels in this research (Table 4) arising from
261 the varying heat treatments and casein contents.

262 *Cheese manufacture time*

263 The rennet addition to drain time in HHT1.0 cheese was significantly higher than those
264 of CON- and PS cheeses, with no significant difference in drain to mill time between the
265 three cheeses (Table 5), as a result, the total make time for the HHT1.0 cheese was longer

266 than for the CON- ($p < 0.05$) and PS cheeses ($p = 0.055$) (Table 5). The pH and lactose content
267 in cheese milk, starter culture inoculum levels and cheese making procedures for the CON-,
268 PS-, and HHT1.0 cheeses were the same, as were the concentrations of casein, total protein,
269 ash, total-, soluble- and colloidal calcium contributing to the buffering capacity (Lucey et al.,
270 1993a, b) in milk (Table 3). It has been reported that heat treatment of milk can influence the
271 acid production capacity of lactic acid bacteria inoculated into the milk (Greene and Jezeski,
272 1957a, b; Singh et al., 1980; Stulova et al., 2011), and this capacity could be inhibited under
273 certain temperature-time heat combinations depending on levels of denatured serum protein
274 and the availability of $-SH$ groups. Given the significantly higher levels of serum protein
275 denaturation prior to HHT cheese manufacture, it is proposed that the acid production
276 capacity of the starter culture (*Lactococcus lactis*) in HHT 1.0 cheese milk may have been
277 reduced (Figure 3), leading to the extended cheese make time. However, further research is
278 required to definitively prove this.

279 A significant increase in the time from rennet addition to drain as well as for total make
280 time in HHT1.5 cheese compared to the CON- and PS cheeses (Table 5) may be due to a
281 greater buffering capacity resulting from higher casein and ash contents (Table 3), as reported
282 by St-Gelais et al. (1998). Extended manufacture times can result in lower cheese moisture
283 content (St-Gelais et al., 1997). Xia et al. (2020) reported loss of a large proportion of
284 minerals from MCC during microfiltration and diafiltration with water requiring addition of
285 milk salts to MCC to fortify the ash content in cheese milk. Future studies should determine
286 the possibility of decreasing the buffering capacity of cheese milk of high casein
287 concentration by adding less UF permeate (which was used to fortify lactose and milk salts in
288 cheese milk) to MCC during cheese milk preparation. Similarly, increasing the starter culture
289 addition in casein concentrated cheese milk to improve acid production during cheese
290 processing would also be an option (O'Keeffe et al., 1975, Guinee et al., 1996a).

291 ***Composition of 14 days cheese***

292 No significant difference was observed between CON-, PS and HHT1.0 cheeses (Table
293 6), for contents of protein, fat, moisture, salt, ash and total calcium as well as protein: fat
294 ratio, FDM, MNFS, S/M, Calcium/protein and pH, showing that the thermal treatments
295 applied and any subsequent serum protein denaturation did not affect the Cheddar cheese
296 composition. Guinee et al. (1995) and Rynne et al. (2004) reported that cheese made from
297 high heat treated (88°C×15s or 87°C×26s) cheese milk of typical serum protein content had
298 increased moisture contents due to reduced syneresis; it was suggested that the retarded
299 aggregation and fusion of denatured serum protein-para-casein complexes should be
300 responsible for the impaired syneresis in severely heat treated cheese milk (Rynne et al.,
301 2004). A higher $\tan \delta$ value corresponded to improved syneresis in rennet induced gels (Van
302 Vliet et al.,1991), similar $\tan \delta_{40}$ values (data not shown) for all coagula observed in the
303 current research suggests that the heat treatment applied (72°C×15s or 90°C×15s) did not
304 affect syneresis during rennet induced coagulation of serum protein depleted cheese milk,
305 with similar cheese moisture contents in CON-, PS- and HHT1.0 cheeses further supporting
306 this.

307 Similar cheese compositions and pH was observed between HHT1.5 cheese and the
308 other three cheeses (Table 6), although the moisture content and MNFS in the HHT1.5 cheese
309 were somewhat lower, if not statistically so. Panthi et al. (2019a) reported that for
310 concentrated cheese milk, lower quantities of cheese whey can lead to curd tearing and small
311 curd particles during cutting and stirring.

312 ***Cheese yield***

313 There was no significant difference in cheese yield between CON- and PS cheese
314 (Table 7), which was expected due to the negligible levels of serum protein denaturation in

315 the PS cheese milk. Similarly, high heat treatment of cheese milk did not affect the actual-
316 and moisture adjusted cheese yield (Table 7), contrary to earlier expectation. The moisture
317 adjusted actual yield (Y_{ma}) of HHT1.0 cheese, predicted to be 12.36kg if all the denatured
318 serum protein in HHT1.0 cheese milk was recovered to the resultant cheese, was achieved
319 (Table 7), suggesting that the low levels of serum protein present and in a denatured state due
320 to HHT had a negligible impact on the yield of Cheddar cheese.

321 The actual (Y_a) and moisture adjusted (Y_{ma}) cheese yield of cheeses made from the high
322 heat treated concentrated cheese milk (HHT1.5) was significantly higher than for cheeses
323 made from un-concentrated cheese milks in accordance with Xia et al. (2020) and was
324 attributed to the higher solids content in the HHT1.5 cheese milk as the casein and fat
325 adjusted cheese yields (Y_{afcam} and Y_{mafcam}) were similar between all four cheese types (Table
326 7).

327 CONCLUSION

328 Pasteurization ($72^{\circ}\text{C}\times 15\text{s}$) of MCC had no significant influence on the %WPD of
329 pasteurised MCC or on the %WPD, calcium distribution and pH in subsequent cheese milk.
330 Similarly, the rennet coagulation properties of cheese milk, cheese make time, cheese
331 composition and yield were not influenced by pasteurization of MCC compared to the
332 control. High heat treatment ($90^{\circ}\text{C}\times 15\text{s}$) of the MCC resulted in increased %WPD in both
333 MCC (15.97%) and the resultant cheese milk, although lower than that reported in studies on
334 milk and was attributed to the low serum protein content in MCC prior to heat treatment. The
335 calcium distribution and pH of serum protein reduced cheese milk were not affected by HHT,
336 nor were the cheese composition, pH and yield. HHT also elongated the cheese make time
337 significantly during Cheddar cheese manufacture compared to CON cheese, although it did
338 not result in a significant reduction in cheese moisture content.

339 After partial removal of serum protein, the curd firming rate for HHT cheese milk of
340 typical casein concentration (3.09%) was not significantly affected by denaturation of the
341 serum protein. The curd firming rate increased by increasing the casein concentration in
342 cheese milk from 3.09% to 4.31% with set to cut time decreased insignificantly.

343 Despite prior expectation, cheese yield was not significantly improved by HHT of MCC,
344 and similarly HHT did not significantly impact on the rennet coagulability, cheese making
345 properties and cheese composition from serum protein depleted cheese milk. As Amelia and
346 Barbano (2013) reported that pasteurised MCC had a long shelf life (>16weeks) at 4°C,
347 future research should determine whether the heat treatment of MCC (90°C×15s) would
348 result in an extended shelf life of MCC providing a commercial means of protein fortification
349 of cheese milk to mitigate seasonal variations in milk protein content. Overall, HHT of MCC
350 prior to cheese manufacture did not negatively influence the cheese manufacture process, or
351 composition and yield of resultant Cheddar cheese.

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532 **Figure legends**

533 **Figure 1.** Cascade filtration process applied in Cheddar cheese making¹

534 ¹Abbreviation: MF, microfiltration; RO, reverse osmosis; UF, ultrafiltration; UN, un-heated;
535 PS, pasteurisation (72°C×15s); HHT, high heat treatment (90°C×15s); MCC, micellar casein
536 concentrate; CON, control; CM, cheese milk.

537 **Figure 2.** Storage modulus (G') of rennet coagulations formed from cheese milks
538 standardised from control- (\square), pasteurised- (\circ), and high heat treated micellar casein
539 concentrate of typical (Δ) or 1.5 times typical casein content (∇).

540 **Figure 3.** Change of pH as a function of cheese manufacture time from cheese milks
541 standardised from control- (\square), pasteurised- (\circ), and high heat treated micellar casein
542 concentrate of typical (Δ) or 1.5 times typical casein content (∇).

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559 Table 1. Formulations on a weight basis for serum protein reduced cheese milk of different
 560 thermal history and casein content^{1, 2 and 3}

Weight of streams (kg)	CON CM	PS CM	HHT1.0 CM	HHT1.5 CM
Pasteurised cream	1.11	1.11	1.11	1.67
CON MCC ⁴	4.27	0	0	0
PS MCC ⁴	0	4.27	0	0
HHT MCC ⁴	0	0	4.27	6.41
UF permeate ⁵	5.06	5.06	5.06	4.72
RO permeate ⁵	1.57	1.57	1.57	0

561 ¹Results are means of triplicate trials;

562 ²Cheese milk formulations were calculated on a 12kg basis.

563 ³Cheese milk were standardised from control micellar casein concentrate (CON CM),
 564 pasteurised micellar casein concentrate (PS CM) or high heat treated micellar casein
 565 concentrate with typical or 1.5 times typical casein content (HHT1.0 CM, HHT1.5 CM).

566 ⁴CON MCC, control micellar casein concentrate; PS MCC, pasteurised micellar casein
 567 concentrate, 72°C×15s; HHT MCC, high heat treated micellar casein concentrate, 90°C×15s.

568 ⁵UF permeate or RO permeate refer to permeate from ultrafiltration or reverse osmosis
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582 Table 2. Composition and pH of control-, pasteurised-, and high heat treated micellar casein
 583 concentrate^{1, 2}

Compositional parameters	CON MCC	PS MCC	HHT MCC
Total solids (% , wt/wt)	11.09±1.30 ^a	10.96±1.16 ^a	10.99±1.16 ^a
Total protein (% , wt/wt)	8.80±1.05 ^a	8.79±1.06 ^a	8.76±1.08 ^a
Casein number ³	93.64±0.53 ^b	93.88±0.39 ^{ab}	94.59±0.35 ^a
Serum protein (% , wt/ wt)	0.52±0.08 ^a	0.52±0.08 ^a	0.52±0.08 ^a
Serum protein denaturation ⁴			
NWP (% of TP)	5.91±0.30 ^a	5.75±0.28 ^a	4.96±0.22 ^b
% WPD	0.00±0.00 ^b	2.69±0.64 ^b	15.97±2.96 ^a
Ash (% , wt/ wt)	0.94±0.11 ^a	0.92±0.11 ^a	0.93±0.10 ^a
Total Ca (m mol kg ⁻¹)	73.0±7.2 ^a	72.5±6.6 ^a	71.9±6.9 ^a
pH	6.90±0.10 ^a	6.82±0.12 ^a	6.83±0.12 ^a

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

586 ²CON MCC, control micellar casein concentrate; PS MCC, pasteurised micellar casein
 587 concentrate, 72°C×15s; HHT MCC, high heat treated micellar casein concentrate, 90°C×15s.

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

589 ⁴NWP = native whey protein, expressed as a percentage of total protein; % WPD = percentage
 590 of whey protein denaturation, expressed as a percentage of total whey protein.

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604 Table 3. Composition and pH of serum protein reduced cheese milk of different thermal
 605 histories and casein contents^{1,2}

Compositional parameters	CON CM	PS CM	HHT1.0 CM	HHT1.5 CM
Total solids (% , wt/wt)	12.50±0.49 ^b	12.62±0.53 ^b	12.81±0.81 ^b	15.51±0.33 ^a
Total protein (% , wt/wt)	3.45±0.39 ^b	3.44±0.41 ^b	3.44±0.40 ^b	4.74±0.32 ^a
Casein number	88.65±1.74 ^a	88.66±1.73 ^a	89.59±1.42 ^a	90.86±1.18 ^a
Serum protein denaturation ³				
NWP (% of TP)	7.49±2.51 ^a	7.23±2.80 ^a	6.75±2.19 ^a	6.42±2.53 ^a
% WPD	0.00±0.00 ^b	0.00±0.00 ^b	9.99±1.19 ^a	N/A ⁴
Casein content (% , wt/wt)	3.06±0.39 ^b	3.05±0.40 ^b	3.09±0.39 ^b	4.31±0.33 ^a
Fat content (% , wt/wt)	3.93±0.29 ^b	3.86±0.29 ^b	3.91±0.37 ^b	5.58±0.29 ^a
Casein: fat ratio	0.78±0.06 ^a	0.79±0.05 ^a	0.79±0.04 ^a	0.77±0.02 ^a
Ash (% , wt/ wt)	0.73±0.05 ^a	0.75±0.06 ^a	0.76±0.09 ^a	0.86±0.06 ^a
Calcium				
Total Ca (m mol kg ⁻¹)	32.34±1.78 ^b	32.22±1.32 ^b	31.28±3.46 ^b	41.42±1.51 ^a
Colloidal Ca (m mol kg ⁻¹)	21.90±1.30 ^b	21.63±1.87 ^b	20.44±0.51 ^b	28.67±2.23 ^a
Colloidal Ca /casein (m mol/g casein)	0.73±0.13 ^a	0.72±0.14 ^a	0.68±0.07 ^a	0.68±0.12 ^a
Soluble Ca (m mol kg ⁻¹)	10.44±3.03 ^a	10.59±2.92 ^a	10.84±2.95 ^a	12.75±3.66 ^a
%soluble Ca	32.01±7.56 ^a	32.69±7.85 ^a	34.22±5.94 ^a	30.60±7.90 ^a
pH	6.49±0.04 ^a	6.49±0.06 ^a	6.52±0.07 ^a	6.53±0.07 ^a

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

608 ²Cheese milk were standardised from control micellar casein concentrate (CON CM),
 609 pasteurised micellar casein concentrate (PS CM) or high heat treated micellar casein
 610 concentrate with typical or 1.5 times typical casein content (HHT1.0 CM, HHT1.5 CM).

611 ³NWP = native whey protein, expressed as a percentage of total protein; %WPD = percentage
 612 of whey protein denaturation, expressed as a percentage of total whey protein.

613 ⁴N/A: not available.

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618 Table 4. Gel forming properties of serum protein reduced cheese milk of different thermal
 619 histories and casein contents ^{1,2}

Parameters	CON CM	PS CM	HHT1.0 CM	HHT1.5 CM
Degree of frequency dependence, n	0.16±0.01 ^a	0.16±0.00 ^a	0.16±0.00 ^a	0.17±0.00 ^a
RCT (min) ³	11.83±2.59 ^a	13.63±3.71 ^a	13.79±3.23 ^a	15.07±1.77 ^a
A ₄₀ (Pa) ⁴	164.13±44.43 ^b	162.10±58.97 ^b	132.15±16.05 ^b	288.63±78.90 ^a
K ₃₅ (min) ⁵	18.40±1.44 ^a	20.46±6.43 ^a	22.42±3.66 ^a	19.17±0.76 ^a
K ₇₀ (min) ⁵	22.83±1.63 ^a	24.87±7.99 ^a	27.54±4.23 ^a	22.44±2.01 ^a

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

622 ²Cheese milk were standardised from control micellar casein concentrate (CON CM),
 623 pasteurised micellar casein concentrate (PS CM) or high heat treated micellar casein
 624 concentrate with typical or 1.5 times typical casein content (HHT1.0 CM, HHT1.5 CM).

625 ³RCT: the time required for the G' to reach the value of 0.1 Pa after rennet addition.

626 ⁴A₄₀: the storage modulus (G') of gel 40min after rennet addition.

627 ⁵K₃₅ or K₇₀: the time it take for the G' to reach the value of 35 or 70Pa respectively after
 628 rennet addition.

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642 Table 5. Manufacture times for Cheddar cheeses made from serum protein reduced cheese
 643 milk of different thermal histories and casein contents ^{1,2}

Manufacture time (min)	CON CM	PS CM	HHT1.0 CM	HHT1.5 CM
Rennet addition to drain	97.73±37.02 ^b	104.64±17.01 ^b	151.28±21.44 ^a	165.83±7.78 ^a
Drain to mill	65.67±21.13 ^a	72.00±25.94 ^a	78.33±19.86 ^a	97.33±6.43 ^a
Total make time ³	163.40±35.17 ^c	176.64±42.54 ^{bc}	229.61±16.99 ^{ab}	263.17±4.31 ^a

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

646 ²Cheese milk were standardised from control micellar casein concentrate (CON CM),
 647 pasteurised micellar casein concentrate (PS CM) or high heat treated micellar casein
 648 concentrate with typical or 1.5 times typical casein content (HHT1.0 CM, HHT1.5 CM).

649 ³Total make time: from rennet addition to drain.

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666 Table 6. Composition and pH of Cheddar cheeses manufactured from serum protein reduced
 667 cheese milk of different thermal histories and casein contents at 14 days^{1, 2}

Compositional parameters	CON cheese	PS cheese	HHT1.0 cheese	HHT1.5 cheese
Protein content (%)	26.23±1.50 ^a	25.90±2.60 ^a	26.63±1.69 ^a	27.56±1.35 ^a
Fat content (%)	30.42±0.81 ^a	30.44±1.11 ^a	30.47±0.97 ^a	31.40±0.62 ^a
Pro: fat ratio	0.86±0.07 ^a	0.85±0.07 ^a	0.88±0.07 ^a	0.88±0.06 ^a
Moisture content (%)	36.27±1.59 ^a	36.33±4.22 ^a	35.62±1.91 ^a	33.43±0.82 ^a
FDM (%) ³	47.75±1.77 ^a	47.90±2.15 ^a	47.36±1.74 ^a	47.21±1.40 ^a
MNFS (%) ⁴	52.13±2.37 ^a	52.19±5.40 ^a	51.24±2.62 ^a	48.74±1.54 ^a
Salt content (%)	1.76±0.10 ^a	1.73±0.09 ^a	1.82±0.07 ^a	1.80±0.18 ^a
S/M (%) ⁵	4.87±0.47 ^a	4.79±0.45 ^a	5.12±0.44 ^a	5.40±0.57 ^a
Ash content (%)	3.99±0.24 ^a	3.88±0.32 ^a	4.11±0.18 ^a	4.22±0.13 ^a
Ca (mg/100g)	775.45±25.03 ^a	713.02±108.66 ^a	756.40±47.35 ^a	782.81±64.61 ^a
Calcium/protein (mg/g of protein)	29.66±2.64 ^a	27.43±1.59 ^a	28.41±0.14 ^a	28.37±1.09 ^a
pH	5.29±0.10 ^a	5.23±0.09 ^a	5.26±0.06 ^a	5.33±0.09 ^a

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

670 ²Cheddar cheeses were manufactured from cheese milk standardised from control micellar
 671 casein concentrate (CON cheese), pasteurised micellar casein concentrate (PS cheese) or high
 672 heat treated micellar casein concentrate with typical or 1.5 times typical casein content
 673 (HHT1.0 cheese, HHT1.5 cheese).

674 ³FDM= fat in dry matter.

675 ⁴MNFS=moisture in non-fat substance.

676 ⁵S/M=salt in moisture.

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684 Table 7. Recoveries of components and yields of Cheddar cheese made from serum protein
 685 reduced cheese milk with different thermal histories and casein contents ^{1, 2}

Parameters	CON cheese	PS cheese	HHT1.0 cheese	HHT1.5 cheese
Recovery to cheese				
Fat (% total milk fat) ³	91.90±1.89 ^a	94.83±1.56 ^a	92.21±3.25 ^a	90.58±4.94 ^a
Protein (% total milk protein) ⁴	90.50±1.32 ^a	90.51±1.24 ^a	91.52±0.49 ^a	93.73±0.79 ^b
Cheese yield ⁵				
Y _a (kg/100kg)	11.87±0.61 ^b	12.00±0.35 ^b	11.80±0.69 ^b	16.07±0.46 ^a
Y _{ma} (kg/100kg)	12.31±0.94 ^b	12.44±1.17 ^b	12.36±1.06 ^b	17.39±0.48 ^a
Y _{afcam} (kg/100kg)	10.10±0.45 ^a	10.36±0.78 ^a	10.05±0.53 ^a	9.65±0.46 ^a
Y _{mafcam} (kg/100kg)	10.45±0.21 ^a	10.68±0.17 ^a	10.50±0.24 ^a	10.44±0.40 ^a

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

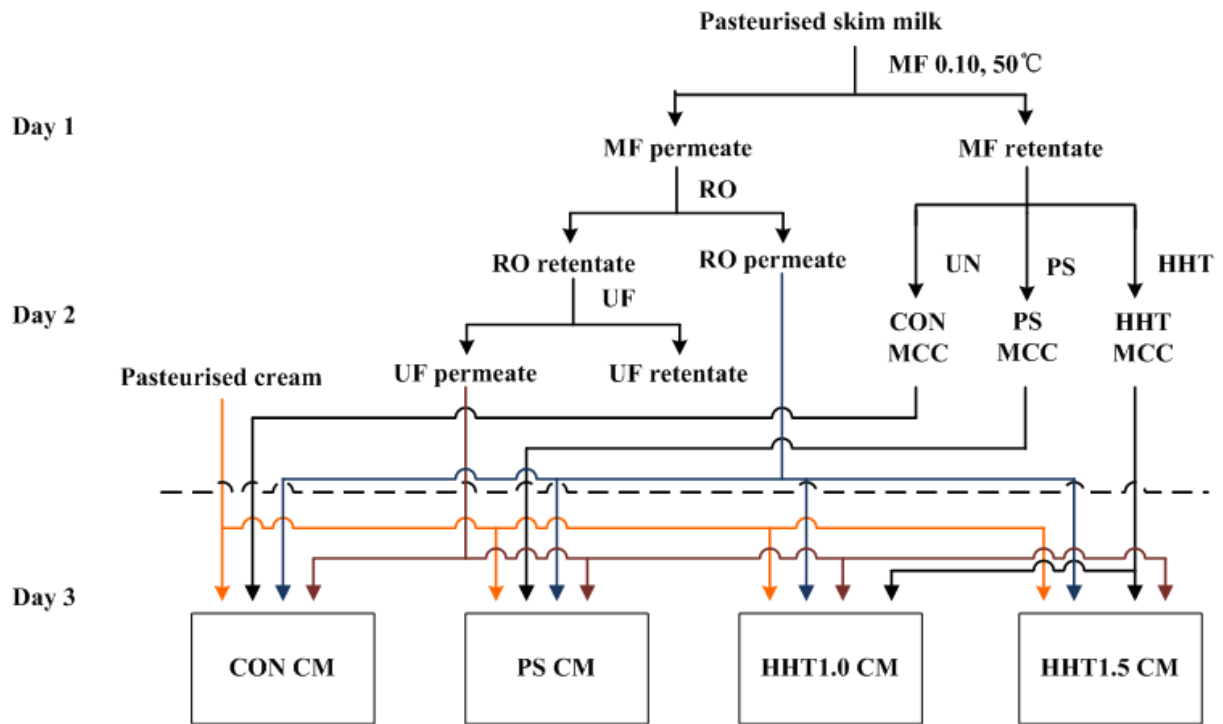
688 ²Cheddar cheeses were manufactured from cheese milk standardised from control micellar
 689 casein concentrate (CON cheese), pasteurised micellar casein concentrate (PS cheese) or high
 690 heat treated micellar casein concentrate with typical or 1.5 times typical casein content
 691 (HHT1.0 cheese, HHT1.5 cheese).

692 ³Fat (% of total milk fat) = $\frac{\text{fat content in cheese} \times \text{weight of cheese}}{\text{fat content in cheese milk} \times \text{weight of cheese milk}} \times 100$

693 ⁴Protein (% of milk protein) = $\frac{\text{protein content in cheese} \times \text{weight of cheese}}{\text{protein content in cheese milk} \times \text{weight of cheese milk}} \times 100$

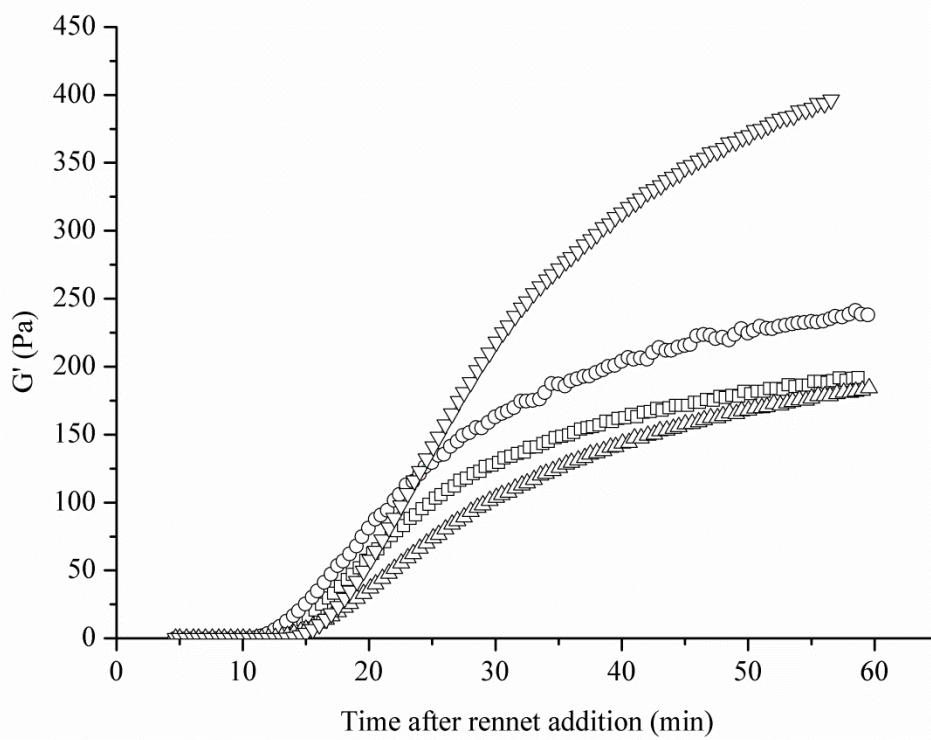
694 ⁵Y_a= actual yield (kg/100kg milk); Y_{ma}= moisture-adjusted yield; Y_{afcam}= yield per 100kg of
 695 milk normalized to reference fat (3.4%, w/w) and casein (2.53%, w/w) levels; Y_{mafcam}=
 696 moisture-adjusted yield per 100kg of milk normalized to reference fat (3.4%, w/w) and casein
 697 (2.53%, w/w) levels.

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Figure 1.



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713 Figure 2.

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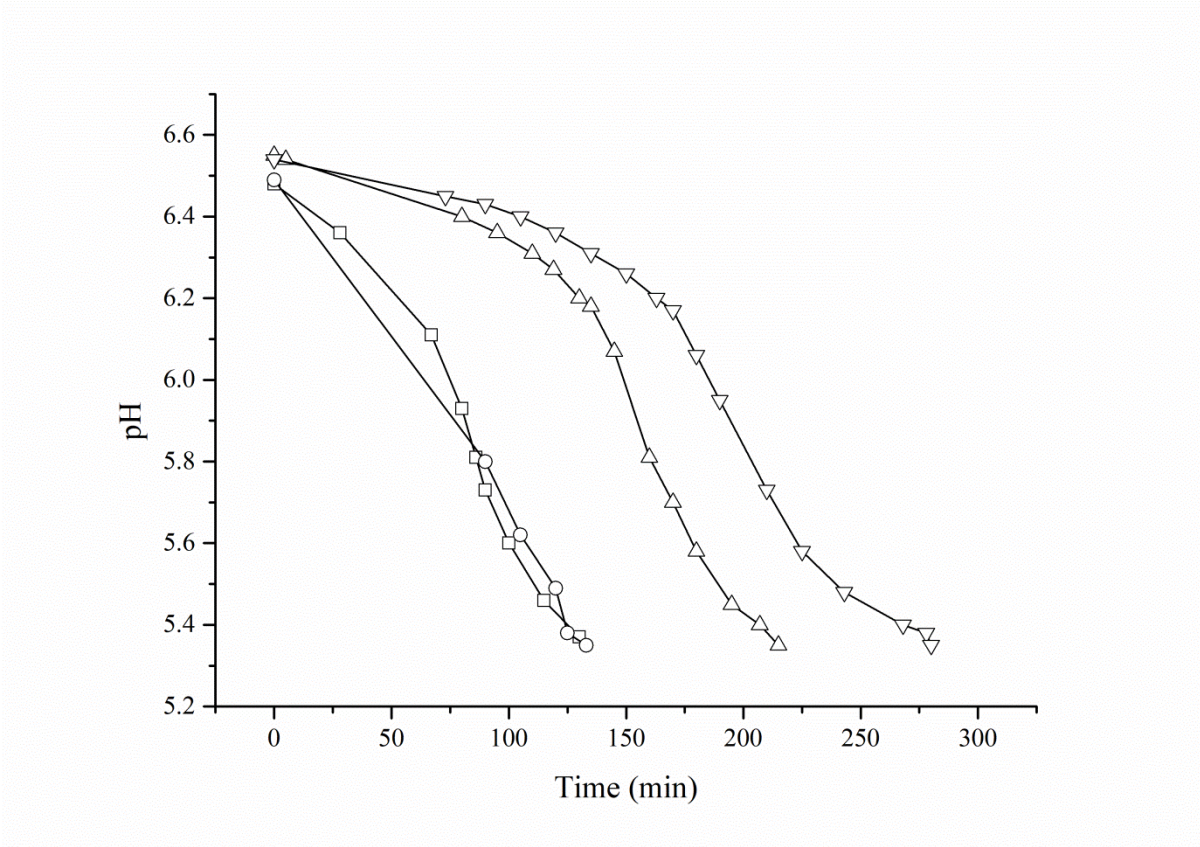
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730 Figure 3.

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