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**Effect of thermal treatment on serum protein reduced micellar casein concentrate: An
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°C $\times$ 15s) and high heat treatment (HHT, 90°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

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

Native serum proteins can be separated from milk using microfiltration (MF) (Garem et al., 2000) and are considered as ‘ideal whey protein’ due to their superior functional and nutritional value over serum proteins recovered from cheese whey (Bacher and Königsfeldt, 2000, Heino et al., 2007), as well as having an absence of caseinomacropptide, starter bacteria, colorants, coagulant enzymes, cheese fines and fat in this stream. Serum protein reduced or depleted milk called micellar casein concentrate (MCC) can be used in cheese manufacture, either to fortify the casein content in cheese milk (St-Gelais et al., 1995, Govindasamy-Lucey et al., 2007) or to be used for standardisation of cheese milk (Garem et al., 2000, Neocleous et al., 2002, Heino, 2008). MCC produced by MF has an enhanced heat 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

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

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

Preparation of cheese milk

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

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

Preparation of cheese

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

Calcium in MCC and cheese milk

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

Composition of liquid samples and cheese at 14 days

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

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

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

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

Rennet coagulation characterisation

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

$$\log G' = n \cdot \log \omega + K;$$

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

Cheese yield

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

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

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

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

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

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

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

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

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

Statistical analysis

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

RESULTS AND DISCUSSION

Composition of MCC

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

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

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

Composition of cheese milk

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

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

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

Rennet coagulation property

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

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

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

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

Cheese manufacture time

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

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

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

Composition of 14 days cheese

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

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

Cheese yield

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

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

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

CONCLUSION

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

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

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

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REFERENCE

Amelia, I. and D. M. Barbano. 2013. Production of an 18% protein liquid micellar casein concentrate with a long refrigerated shelf life. *Journal of Dairy Science* 96(5):3340-3349.

363 Anema, S. G., S. K. Lee, and H. Klostermeyer. 2007. Effect of pH at heat treatment on the
 364 hydrolysis of κ -casein and the gelation of skim milk by chymosin. *LWT-Food Science and*
 365 *Technology* 40(1):99-106.

366 Bacher, T. and P. K nigsfeldt. 2000. WPI by microfiltration of skim milk. *Scandinavian*
 367 *Dairy Information* (3):18-20.

368 Banks, J. M., G. Stewart, D. D. Muir, and I. G. West. 1987. Increasing the yield of Cheddar
 369 cheese by the acidification of milk containing heat-denatured whey protein.
 370 *Milchwissenschaft* 42(4):212-215.

371 Beliciu, C. M., A. Sauer, and C. I. Moraru. 2012. The effect of commercial sterilization
 372 regimens on micellar casein concentrates. *Journal of Dairy Science* 95(10):5510-5526.

373 Bulca, S., J. Leder, and U. Kulozik. 2004. Impact of UHT or high heat treatment on the
 374 rennet gel formation of skim milk with various whey protein contents. *Milchwissenschaft*
 375 59(11-12):590-593.

376 Chen, J., E. Dickinson, and M. Edwards. 1999. Rheology of acid-induced sodium caseinate
 377 stabilized emulsion gels. *Journal of Texture Studies* 30(4):377-396.

378 Fox, P. F., T. P. Guinee, T. M. Cogan, and P. L. H. McSweeney. 2017a. Bacteriology of
 379 Cheese Milk. Pages 105-120 in *Fundamentals of Cheese Science*. Springer US, Boston, MA.

380 Fox, P. F., T. P. Guinee, T. M. Cogan, and P. L. H. McSweeney. 2017b. Cheese yield. Pages
 381 279-331 in *Fundamentals of cheese science*. Springer.

382 Fox, P. F., T. P. Guinee, T. M. Cogan, and P. L. H. McSweeney. 2017c. Enzymatic
 383 Coagulation of Milk. Pages 185-229 in *Fundamentals of Cheese Science*. Springer US,
 384 Boston, MA.

385 Fox, P. F., T. P. Guinee, T. M. Cogan, and P. L. H. McSweeney. 2017d. Overview of Cheese
 386 Manufacture. Pages 11-25 in *Fundamentals of Cheese Science*. Springer US, Boston, MA.

387 Gareem, A., P. Schuck, and J.-L. Maubois. 2000. Cheesemaking properties of a new dairy-
 388 based powder made by a combination of microfiltration and ultrafiltration. *Le Lait* 80(1):25-
 389 32.

390 Gaucheron, F. 2005. The minerals of milk. *Reproduction Nutrition Development* 45(4):473-
 391 483.

392 Govindasamy-Lucey, S., J. Jaeggi, A. Bostley, M. Johnson, and J. Lucey. 2004.
 393 Standardization of milk using cold ultrafiltration retentates for the manufacture of Parmesan
 394 cheese. *Journal of Dairy Science* 87(9):2789-2799.

395 Govindasamy-Lucey, S., J. Jaeggi, M. Johnson, T. Wang, and J. Lucey. 2007. Use of cold
 396 microfiltration retentates produced with polymeric membranes for standardization of milks
 397 for manufacture of pizza cheese. *Journal of Dairy Science* 90(10):4552-4568.

398 Greene, V. W. and J. J. Jezeski. 1957a. Studies on starter metabolism. II. The influence of
 399 heating milk on the subsequent response of starter cultures. *Journal of Dairy Science*
 400 40(9):1053-1061.

401 Greene, V. W. and J. J. Jezbski. 1957b. Studies on starter metabolism. III. Studies on
 402 cysteine-induced stimulation and inhibition of starter cultures in milk. *Journal of Dairy*
 403 *Science* 40(9):1062-1071.

404 Guinee, T. P., M. A. E. Auty, and M. A. Fenelon. 2000. The effect of fat content on the
 405 rheology, microstructure and heat-induced functional characteristics of Cheddar cheese.
 406 *International Dairy Journal* 10(4):277-288.

407 Guinee, T. P., C. B. Gorry, D. J. O'Callaghan, B. T. O'Kennedy, N. O'Brie, and M. A.
 408 Fenelon. 1997. The effects of composition and some processing treatments on the rennet
 409 coagulation properties of milk. *International Journal of Dairy Technology* 50(3):99-106.

410 Guinee, T. P., D. J. O'Callaghan, E. O. Mulholland, and D. Harrington. 1996a. Milk protein
 411 standardization by ultrafiltration for Cheddar cheese manufacture. *Journal of Dairy Research*
 412 63(2):281-293.

413 Guinee, T. P., D. J. O'Callaghan, P. D. Pudja, and N. O'Brien. 1996b. Rennet coagulation
 414 properties of retentates obtained by ultrafiltration of skim milks heated to different
 415 temperatures. *International Dairy Journal* 6(6):581-596.

416 Guinee, T. P., B. T. O'Kennedy, and P. M. Kelly. 2006. Effect of milk protein
 417 standardization using different methods on the composition and yields of Cheddar cheese.
 418 *Journal of Dairy Science* 89(2):468-482.

419 Guinee, T. P., P. D. Pudja, W. J. Reville, D. Harrington, E. O. Mulholland, M. Cotter, and T.
 420 M. Cogan. 1995. Composition, microstructure and maturation of semi-hard cheeses from
 421 high protein ultrafiltered milk retentates with different levels of denatured whey protein.
 422 *International Dairy Journal* 5(6):543-568.

423 Heino, A. 2008. Microfiltration of milk I: Cheese milk modification by micro-and
 424 ultrafiltration and the effect on Emmental cheese quality. *Milchwissenschaft* 63(3):279.

425 Heino, A. T., J. O. Uusi-Rauva, P. R. Rantamäki, and O. Tossavainen. 2007. Functional
 426 properties of native and cheese whey protein concentrate powders. *International Journal of*
 427 *Dairy Technology* 60(4):277-285.

428 IDF. 1964a. Determination of the ash content of processed cheese products. *International*
 429 *Dairy Federation*, Brussels.

430 International Dairy Federation 1964b. Determination of casein content of milk. *IDF Standard*
 431 29. *Int. Dairy Fed*, Brussels, Belgium.

432 International Dairy Federation 1993. Milk: determination of the nitrogen content (Kjeldahl
 433 method) and calculation of crude protein content. *IDF Standard 20B. Int. Dairy Fed.*,
 434 *Brussels, Belgium*.

435 International Dairy Federation 1996. Milk. Determination of fat content (Röse-Gottlieb
 436 gravimetric method). IDF Standard 1D. Int. Dairy Fed., Brussels, Belgium.

437 IDF. 2010. Milk, cream and evaporated milk-Determination of total solids content (Reference
 438 method). ISO 6731:2010 (IDF 21:2010).

439 Kannan, A. and R. Jenness. 1961. Relation of milk serum proteins and milk salts to the
 440 effects of heat treatment on rennet clotting. *Journal of Dairy Science* 44(5):808-822.

441 Kethireddipalli, P., A. R. Hill, and D. G. Dalgleish. 2010. Protein interactions in heat-treated
 442 milk and effect on rennet coagulation. *International Dairy Journal* 20(12):838-843.

443 Lin, Y., A. L. Kelly, J. A. O'Mahony, and T. P. Guinee. 2016. Fortification of milk protein
 444 content with different dairy protein powders alters its compositional, rennet gelation, heat
 445 stability and ethanol stability characteristics. *International Dairy Journal* 61:220-227.

446 Lin, Y., A. L. Kelly, J. A. O'Mahony, and T. P. Guinee. 2018. Effects of milk heat treatment
 447 and solvent composition on physicochemical and selected functional characteristics of milk
 448 protein concentrate. *Journal of Dairy Science* 101(8):6799-6813.

449 Lucey, J., C. Gorry, and P. Fox. 1993a. Acid-base buffering properties of heated milk.
 450 *Milchwissenschaft* 48(8):438-441.

451 Lucey, J., B. Hauth, C. Gorry, and P. Fox. 1993b. The acid-base buffering properties of milk.
 452 *Milchwissenschaft* 48(5):268-272.

453 Neocleous, M., D. Barbano, and M. Rudan. 2002. Impact of Low Concentration Factor
 454 Microfiltration on Milk Component Recovery and Cheddar Cheese Yield. *Journal of Dairy*
 455 *Science* 85(10):2415-2424.

456 O'Keeffe, R. B., P. F. Fox, and C. Daly. 1975. Proteolysis in Cheddar cheese: influence of the
 457 rate of acid production during manufacture. *Journal of Dairy Research* 42(1):111-122.

458 On-Nom, N., A. S. Grandison, and M. J. Lewis. 2010. Measurement of ionic calcium, pH,
 459 and soluble divalent cations in milk at high temperature. *Journal of Dairy Science* 93(2):515-
 460 523.

461 Pandey, P. K., H. S. Ramaswamy, and D. St-Gelais. 2003. Evaluation of pH change kinetics
 462 during various stages of Cheddar cheese-making from raw, pasteurized, micro-filtered and
 463 high-pressure-treated milk. *LWT-Food Science and Technology* 36(5):497-506.

464 Panthi, R. R., A. L. Kelly, D. J. McMahon, X. Dai, A. H. Vollmer, and J. J. Sheehan. 2019a.
 465 Response surface methodology modeling of protein concentration, coagulum cut size, and set
 466 temperature on curd moisture loss kinetics during curd stirring. *Journal of Dairy Science*
 467 102(6):4989-5004.

468 Panthi, R. R., A. L. Kelly, J. J. Sheehan, K. Bulbul, A. H. Vollmer, and D. J. McMahon.
 469 2019b. Influence of protein concentration and coagulation temperature on rennet-induced
 470 gelation characteristics and curd microstructure. *Journal of Dairy Science* 102(1):177-189.

471 Pirisino, J. F. 1983. High Performance Liquid Chromatographic Determination of Lactose,
 472 Glucose, and Galactose in Lactose-Reduced Milk. *Journal of Food Science* 48(3):742-744.

473 Pouliot, Y., M. Boulet, and P. Paquin. 1989a. An experimental technique for the study of
 474 milk salt balance. *Journal of Dairy Science* 72(1):36-40.

475 Pouliot, Y., M. Boulet, and P. Paquin. 1989b. Experiments on the heat-induced salt balance
 476 changes in cow's milk. *Journal of Dairy Research* 56(3):513-519.

477 Pouliot, Y., M. Boulet, and P. Paquin. 1989c. Observations on the heat-induced salt balance
 478 changes in milk I. Effect of heating time between 4 and 90 C. *Journal of Dairy Research*
 479 56(2):185-192.

480 Pouliot, Y., M. Boulet, and P. Paquin. 1989d. Observations on the heat-induced salt balance
 481 changes in milk II. Reversibility on cooling. *Journal of Dairy Research* 56(2):193-199.

482 Renhe, I. R. T. and M. Corredig. 2018. Effect of partial whey protein depletion during
 483 membrane filtration on thermal stability of milk concentrates. *Journal of Dairy Science*
 484 101(10):8757-8766.

485 Rosalina, I. and M. Bhattacharya. 2002. Dynamic rheological measurements and analysis of
 486 starch gels. *Carbohydrate Polymers* 48(2):191-202.

487 Rynne, N. M., T. P. Beresford, A. L. Kelly, and T. P. Guinee. 2004. Effect of milk
 488 pasteurization temperature and in situ whey protein denaturation on the composition, texture
 489 and heat-induced functionality of half-fat Cheddar cheese. *International Dairy Journal*
 490 14(11):989-1001.

491 Sandra, S., C. Cooper, M. Alexander, and M. Corredig. 2011. Coagulation properties of
 492 ultrafiltered milk retentates measured using rheology and diffusing wave spectroscopy. *Food*
 493 *Research International* 44(4):951-956.

494 Sauer, A. and C. I. Moraru. 2012. Heat stability of micellar casein concentrates as affected by
 495 temperature and pH. *Journal of Dairy Science* 95(11):6339-6350.

496 Singh, J., A. Khanna, and H. Chander. 1980. Effect of incubation temperature and heat
 497 treatments of milk from cow and buffalo on acid and flavor production by *Streptococcus*
 498 *thermophilus* and *Lactobacillus bulgaricus*. *Journal of food protection* 43(5):399-400.

499 Singh, H. and A. Waungana. 2001. Influence of heat treatment of milk on cheesemaking
 500 properties. *International Dairy Journal* 11(4-7):543-551.

501 St-Gelais, D., C. A. Passey, S. Haché, and P. Roy. 1997. Production of low-fat Cheddar
 502 cheese from low and high mineral retentate powders and different fractions of milkfat
 503 globules. *International Dairy Journal* 7(11):733-741.

504 St-Gelais, D., M. Piette, and G. Belanger. 1995. Production of Cheddar cheese using milk
 505 enriched with microfiltered milk retentate. A preliminary study. *Milchwissenschaft*
 506 50(11):614-619.

St-Gelais, D., D. Roy, and P. Audet. 1998. Manufacture and composition of low fat Cheddar cheese from milk enriched with different protein concentrate powders. *Food Research International* 31(2):137-145.

Stulova, I., N. Kabanova, T. Kriščiunaite, T. M. Laht, and R. Vilu. 2011. The effect of milk heat treatment on the growth characteristics of lactic acid bacteria. *Agron. Res* 9:473-478.

Tunick, M. H. 2010. Small-strain dynamic rheology of food protein networks. *Journal of Agricultural and Food Chemistry* 59(5):1481-1486.

Van Vliet, T., H. J. M. Van Dijk, P. Zoon, and P. Walstra. 1991. Relation between syneresis and rheological properties of particle gels. *Colloid and Polymer Science* 269(6):620-627.

Xia, X., J. T. Tobin, P. Sharma, M. Fenelon, P. L. H. McSweeney, and J. J. Sheehan. 2020. Application of a cascade membrane filtration process to standardise serum protein depleted cheese milk for Cheddar cheese manufacture. *International Dairy Journal*:104796.

Zhou, N. and S. J. Mulvaney. 1998. The effect of milk fat, the ratio of casein to water, and temperature on the viscoelastic properties of rennet casein gels. *Journal of Dairy Science* 81(10):2561-2571.

Figure legends

Figure 1. Cascade filtration process applied in Cheddar cheese making¹

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

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

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

Table 1. Formulations on a weight basis for serum protein reduced cheese milk of different 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

¹Results are means of triplicate trials;

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

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

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

⁵UF permeate or RO permeate refer to permeate from ultrafiltration or reverse osmosis respectively.

Table 2. Composition and pH of control-, pasteurised-, and high heat treated micellar casein 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

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

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

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

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

Table 3. Composition and pH of serum protein reduced cheese milk of different thermal 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

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

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

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

⁴N/A: not available.

Table 4. Gel forming properties of serum protein reduced cheese milk of different thermal 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

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

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

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

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

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

Table 5. Manufacture times for Cheddar cheeses made from serum protein reduced cheese 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

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

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

³Total make time: from rennet addition to drain.

Table 6. Composition and pH of Cheddar cheeses manufactured from serum protein reduced 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

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

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

³FDM= fat in dry matter.

⁴MNFS=moisture in non-fat substance.

⁵S/M=salt in moisture.

Table 7. Recoveries of components and yields of Cheddar cheese made from serum protein 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

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

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

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

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

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

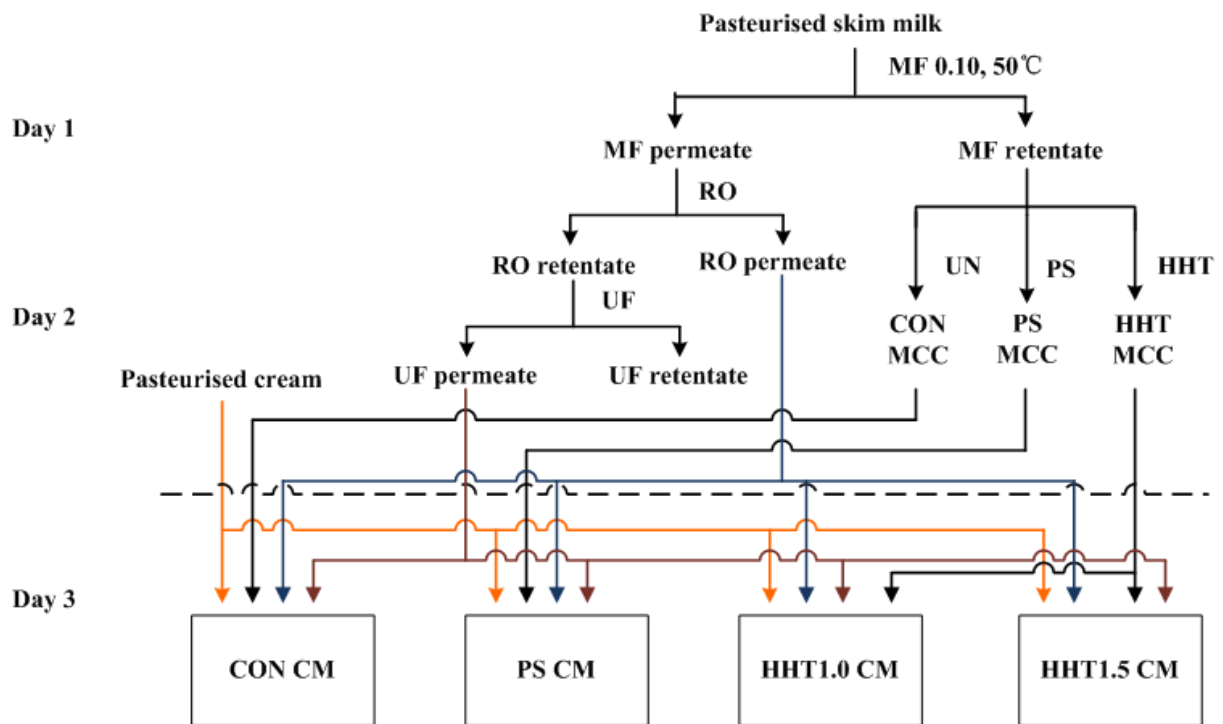


Figure 1.

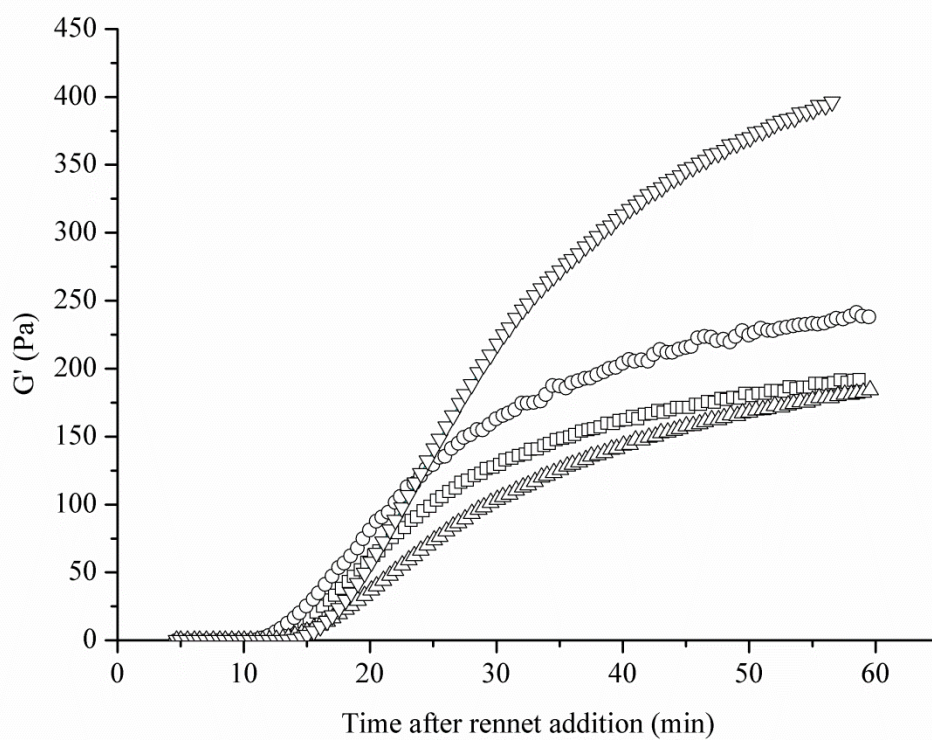


Figure 2.

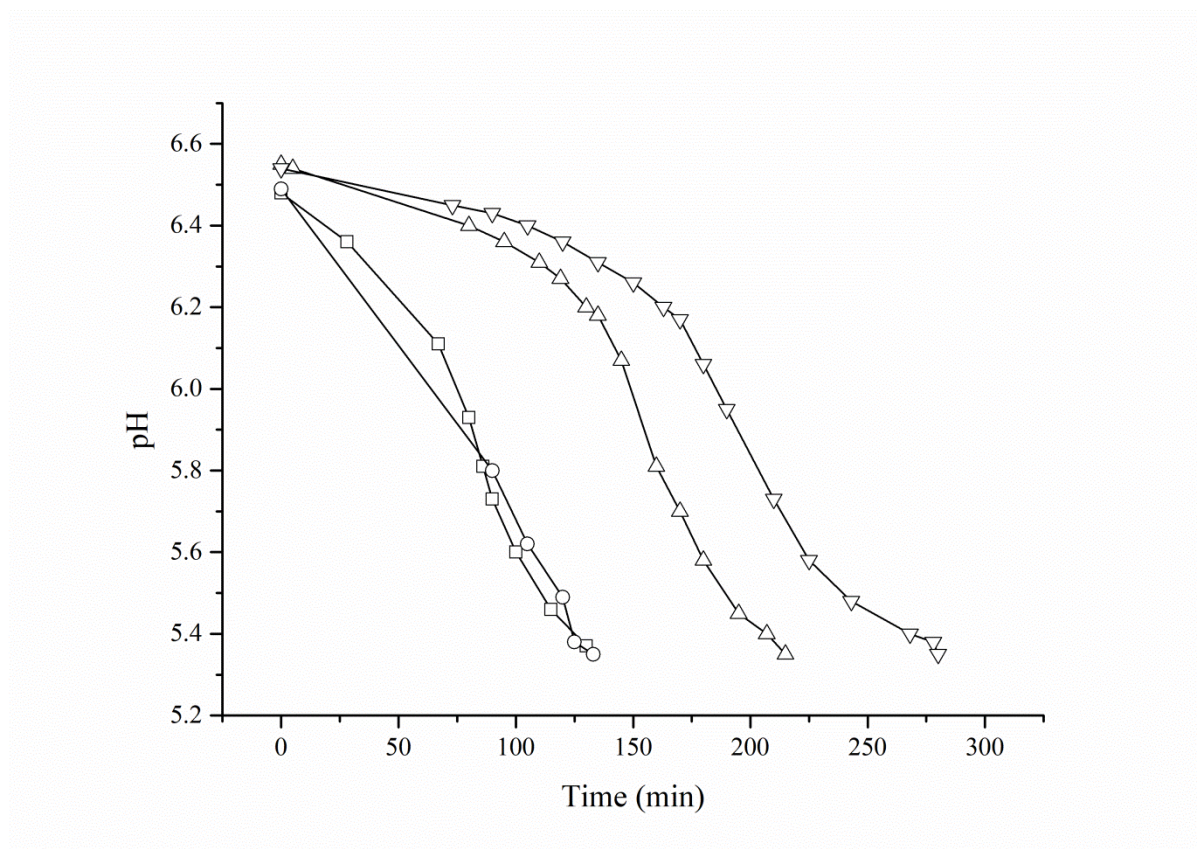


Figure 3.