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 0.10μm removed ～70.29% of serum proteins from milk and the resultant micellar casein concentrates (MCC) were subjected to: no heat treatment (control), pasteurization (72°C×15s) and high heat treatment (HHT, 90°C×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 ≥70°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°C×15s) 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 Kønigsfeldt, 2000, Heino et al., 2007), as well as having an absence of caseinomacropeptide, 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 0.35m^2, 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 δ at 40min after rennet addition (A_{40} and tan δ_{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:

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

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):

$$Y_{ma}=Y_a\times(\frac{100-Ma}{100-Mr})$$

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:

$$Y_{afcam}=Y_a\times(\frac{Frm+Crm}{Fcm+Ccm})$$

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 Yma 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 0.1μm, 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 at 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°C×15s) 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°C×15s) 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 δ value corresponded to improved syneresis in rennet induced gels (Van Vliet et al.,1991), similar tan δ_{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°C×15s) 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°C×15s) 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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Figure legends

Figure 1. Cascade filtration process applied in Cheddar cheese making[1]

[1]Abbreviation: 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- (□), pasteurised- (○), 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- (□), pasteurised- (○), 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

[1]Results are means of triplicate trials;

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

[3]Cheese 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).

[4]CON 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.

[5]UF 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]

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

[2]CON 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.

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

[4]NWP = 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}

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

[2]Cheese 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).

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

[4]N/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}

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

[2]Cheese 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).

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

[4]A_{40}: the storage modulus (G') of gel 40min after rennet addition.

[5]K_{35} or K_{70}: 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]

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

[2]Cheese 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).

[3]Total 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}

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

[2]Cheddar 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]FDM= fat in dry matter.

[4]MNFS=moisture in non-fat substance.

[5]S/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}

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

[2]Cheddar 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]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]Protein (% of milk protein) $= \frac{\text{protein content in cheese} \times \text{weight of cheese}}{\text{protein content in cheese milk} \times \text{weight of cheese milk}} \times 100$

[5]Y_a= actual yield (kg/100kg milk); Y_{ma}= moistrure-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.

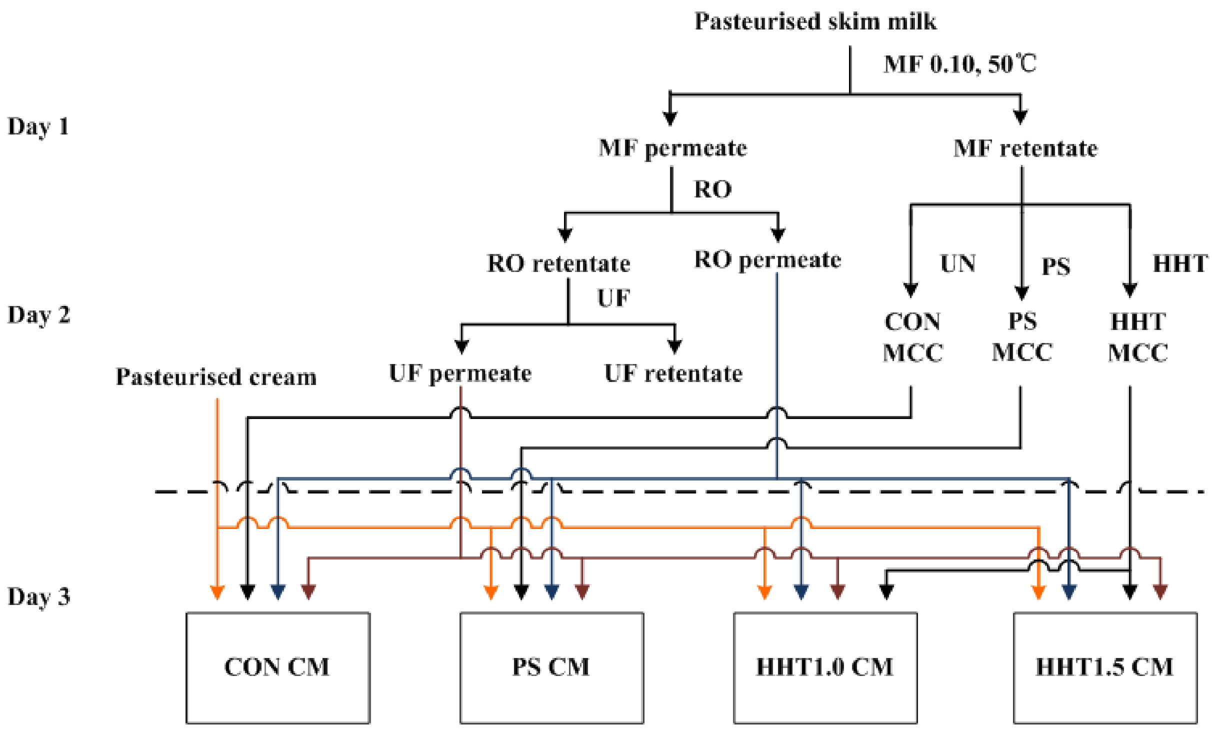
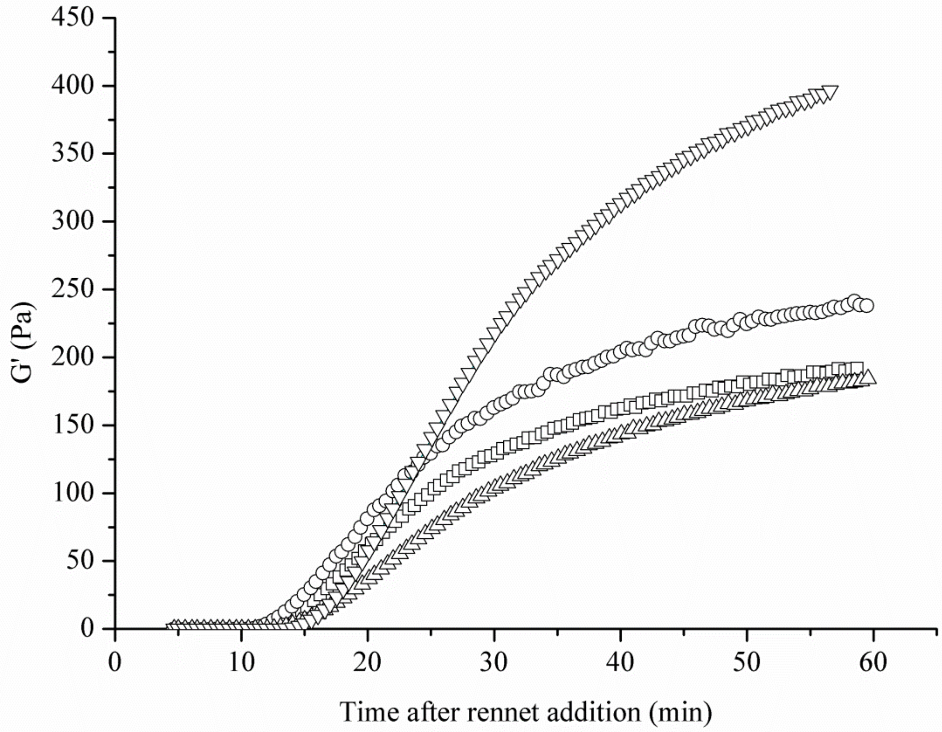

Pasteurised skim milk
MF 0.10, 50℃
Day 1
MF permeate
MF retentate
RO
RO retentate
RO permeate
UN
PS
HHT
Day 2
UF
CON MCC
PS MCC
HHT MCC
Pasteurised cream
UF permeate
UF retentate
Day 3
CON CM
PS CM
HHT1.0 CM
HHT1.5 CM

Figure 1.


450
400
350
300
250
200
150
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G' (Pa)
0
10
20
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60
Time after rennet addition (min)

Figure 2.

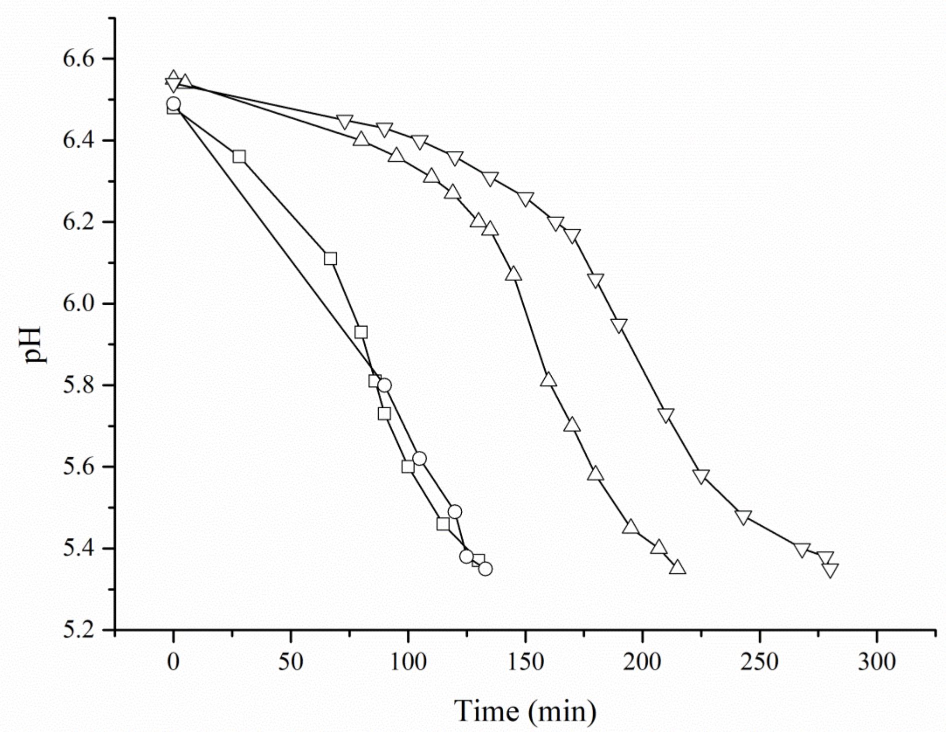

Figure 3.