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

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ABSTRACT

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

Key words: microfiltration, diafiltration, cheese milk, standardisation

INTRODUCTION

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

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

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

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

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

MATERIALS AND METHODS

Cascade filtration process

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

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

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

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

Preparation of cheese milk

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

Preparation of cheese

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

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

Compositional analysis of membrane streams, cheese milks and cheese wheys

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

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

Total calcium

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

Lactose

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

Rheological properties of curds

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

Compositional analysis of cheese

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

222 ***Textural properties of cheese***

223 After storage at 4 °C for 7 days, the cheeses were sampled for texture and cheese
224 composition analysis respectively. Cheese were prepared into 25 mm^3 cubes (six cubes per
225 treatment), wrapped with foil paper and stored at 4°C overnight. Texture profile analysis
226 (TPA) was conducted on each cube with a P75 probe and 50 kg load cell (TA-XT plus, Stable
227 Micro Systems, Godalming, Surrey, UK), the cubes were compressed to 70% of original
228 height at a testing speed of 1.00 mm/s. The fracture force, fracture strain and firmness were
229 recorded and calculated as described in Hou et al. (2014a).

230 ***Statistical analysis***

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

237 **RESULTS AND DISCUSSION**

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

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

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

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

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

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

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

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

Cheese milk composition

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

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

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

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

Curd rheology

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

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

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

Cheese composition

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

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

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

Cheese texture

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

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

394 ***Cheese yield***

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

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

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

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

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

434 **CONCLUSION**

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

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

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

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

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

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

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Figure legends

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

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

Figure 3. Relative lactose:casein, serum:casein, ash:casein, and total calcium:casein ratios in MF 1.4 permeate and MF 0.14 retentate 1, 2, and 3[1] streams respectively[2]

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

[2]Figure 3 is derived from data in Table 2.

Table 1. Component stream formulations for PC PS, PC MF1.4P, MCC1.0 and MCC1.5 cheese milk[1, 2, and 3]

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

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

[2]Results are means of triplicate trials;

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

Table 2. Effect of microfiltration at 0.14 μm and diafiltration on the composition of resultant streams[1]

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

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

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

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

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

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

696 [1]Abbreviations: PC PS, cheese milk prepared from pasteurized cream and pasteurized skim
697 milk; PC MF1.4P, cheese milk prepared from pasteurized cream and MF 1.4 permeate; MCC
698 1.0, cheese milk prepared from pasteurized cream, MCC, RO retentate, RO permeate, and of
699 the same casein content in raw skim milk; MCC 1.5, cheese milk prepared from pasteurized
700 cream, MCC, RO retentate, RO permeate, casein content 1.5 times that of the MCC1.0 (3.75-
701 4.5%);
702 [2] Results are means of triplicate trials, values within a row not sharing the same superscript
703 differ significantly ($p<0.05$).
704 [3]Casein number (%) = $\frac{\text{Casein content}}{\text{Total protein}} \times 100$.
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712 Table 4 Coagulation properties, cheese yield and texture of cheese manufactured from PC
713 PS, PC MF1.4P, MCC1.0 and MCC1.5 cheese milks[1, 2]

Parameters	PC PS	PC MF1.4P	MCC1.0	MCC1.5
Curd coagulation				
MCFR (Pa/min)[3]	2.69[b]	2.45[b]	3.88[b]	18.49[a]
A_{40} (Pa)[4]	36.76[b]	39.02[b]	70.20[b]	310.95[a]
Tan δ_{40}[4]	0.28[a]	0.26[a]	0.28[a]	0.28[a]
K_{35} (min)[5]	40.67[a]	38.28[a]	31.16[a]	18.00[b]
K_{70} (min)[5]	56.00[a]	58.54[a]	41.89[a]	20.49[b]
CW (min)[6]	15.33[a,b]	20.26[a]	10.46[b]	2.50[c]
Cheese yield[7]				
Ya (kg/100 kg)	10.89[b]	10.55[b]	11.33[b]	16.01[a]
Yma	11.22[b]	11.21[b]	11.98[b]	17.31[a]
Yafcam	9.36[a]	9.38[a]	9.53[a]	9.21[a]
Ymafcam	9.62[a]	9.96[a]	10.07[a]	9.96[a]
Texture				
Fracture stress (kPa)	501.35[a,b]	447.58[b]	516.05[a,b]	627.34[a]
Fracture strain	0.69[a]	0.72[a]	0.71[a]	0.70[a]
Firmness (N)	306.24[a,b]	266.69[b]	310.49[a,b]	380.27[a]

714 [1] Results are means of triplicate trials, values within a row not sharing the same superscript
715 differ significantly ($p<0.05$).
716 [2] Abbreviations: PC PS, cheese milk prepared from pasteurized cream and pasteurized skim
717 milk; PC MF1.4P, cheese milk prepared from pasteurized cream and MF 1.4 permeate; MCC
718 1.0, cheese milk prepared from pasteurized cream, MCC, RO retentate, RO permeate, and of
719 the same casein content in raw skim milk; MCC 1.5, cheese milk prepared from pasteurized
720 cream, MCC, RO retentate, RO permeate, casein content 1.5 times that of the MCC1.0 (3.75-
721 4.5%);. .
722 [3] MCFR: maximum curd firming rate, calculated from $\Delta G'/\Delta t$ curve.
723 [4] A_{40} and tan δ_{40}: the value of G' or tan δ after 40 min of rennet addition in respective.
724 [5] K_{35} and K_{70}: the value of G' after 35 or 70 min of rennet addition separately.
725 [6] CW: cutting window, K_{70}-K_{35}.
726 [7]Ya= actual yield (kg/ 100 kg milk); Yma= moisture-adjusted yield; Yafcam= yield per 100
727 kg of milk normalized to reference fat (3.4%, w/w) and casein (2.53%, w/w) levels;

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

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

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

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

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

770 [3] FDM: fat in dry matter.

771 [4] MNFS: moisture in non-fat substance.

772 [5] S/M: salt in moisture.

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

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

782 [1] Results are means of triplicate trials, values within a row not sharing the same superscript
783 differ significantly ($p<0.05$).
784 [2] Abbreviations: PC PS, cheese milk prepared from pasteurized cream and pasteurized skim
785 milk; PC MF1.4P, cheese milk prepared from pasteurized cream and MF 1.4 permeate; MCC
786 1.0, cheese milk prepared from pasteurized cream, MCC, RO retentate, RO permeate, and of
787 the same casein content in raw skim milk; MCC 1.5, cheese milk prepared from pasteurized
788 cream, MCC, RO retentate, RO permeate, casein content 1.5 times that of the MCC1.0 (3.75-
789 4.5%);
790 [3]N/A: Not applicable;
791 [4]N/A: Not available.
792

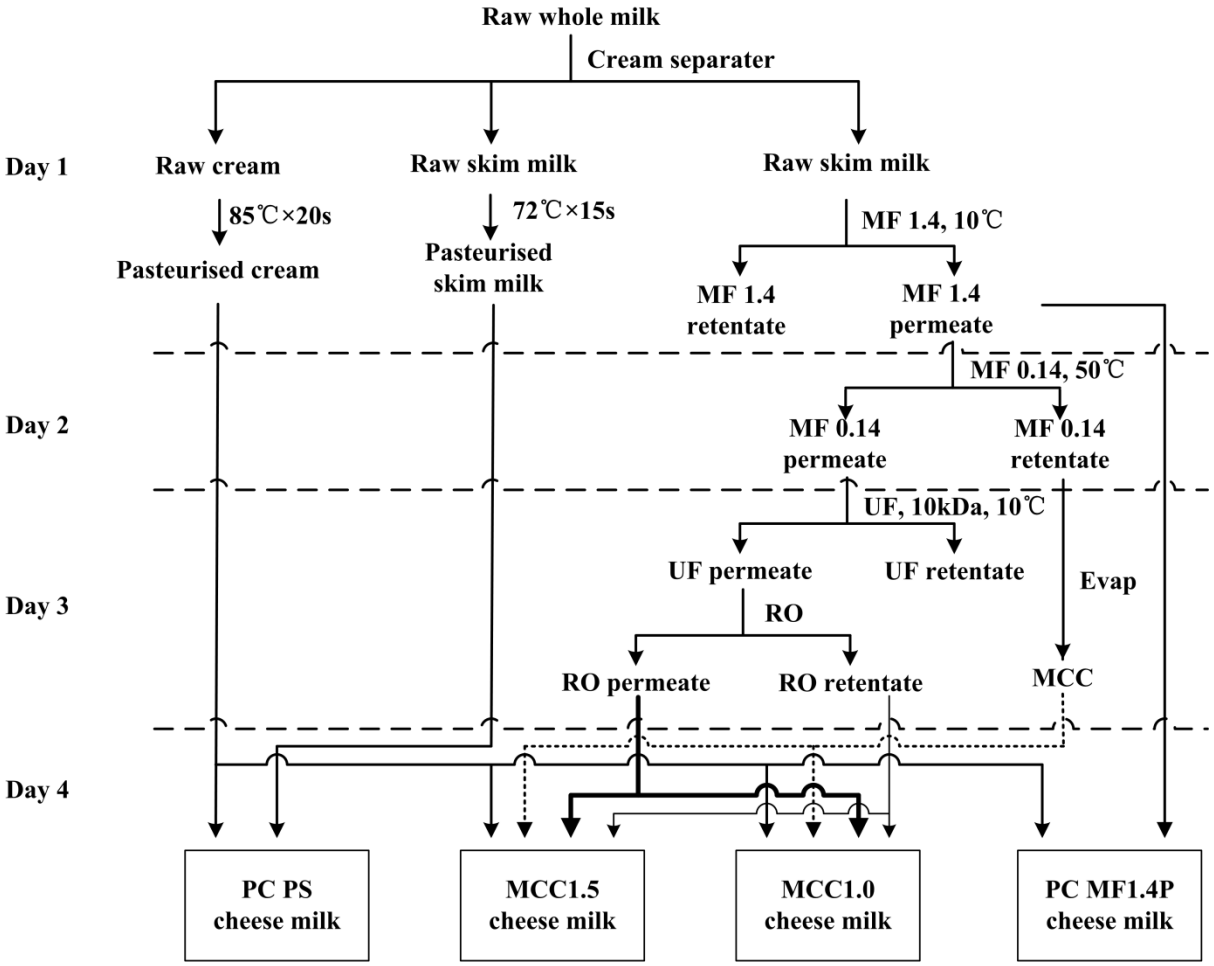

Raw whole milk
Cream separater
Day 1
Raw cream
Raw skim milk
Raw skim milk
85℃×20s
72℃×15s
MF 1.4, 10℃
Pasteurised cream
Pasteurised skim milk
MF 1.4 retentate
MF 1.4 permeate
MF 0.14, 50℃
Day 2
MF 0.14 permeate
MF 0.14 retentate
UF, 10kDa, 10℃
UF permeate
UF retentate
Evap
Day 3
RO
RO permeate
RO retentate
MCC
Day 4
PC PS cheese milk
MCC1.5 cheese milk
MCC1.0 cheese milk
PC MF1.4P cheese milk

Figure 1.

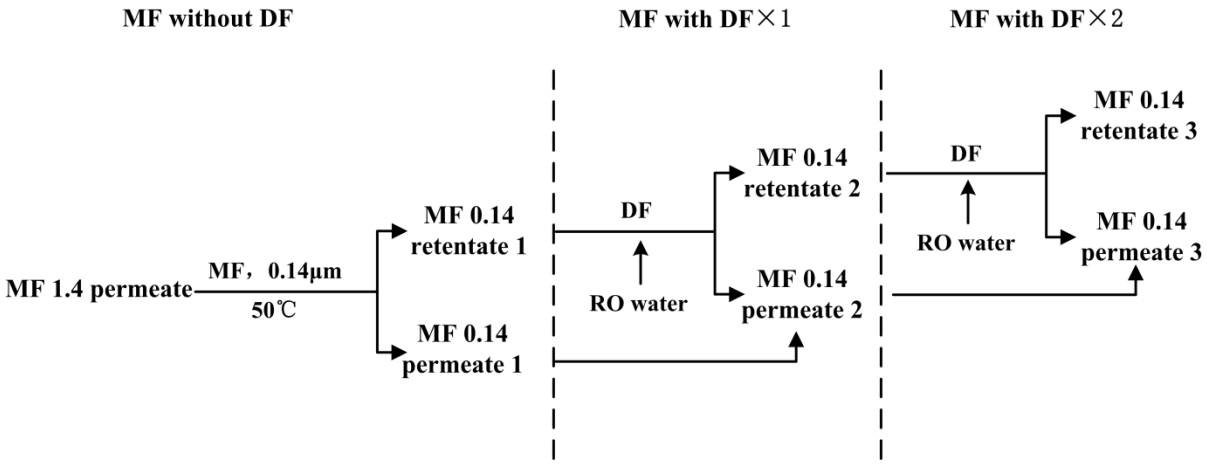
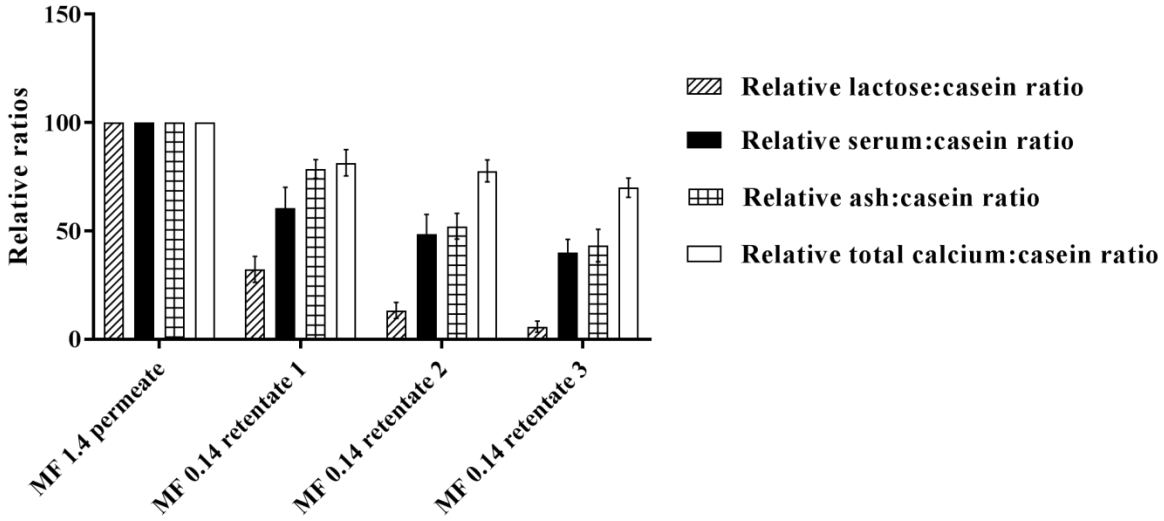

MF without DF
MF with DF×1
MF with DF×2
MF 1.4 permeate
MF，0.14μm
50℃
MF 0.14
retentate 1
MF 0.14
permeate 1
DF
RO water
MF 0.14
retentate 2
MF 0.14
permeate 2
DF
RO water
MF 0.14
retentate 3
MF 0.14
permeate 3

Figure 2.



833

834 Figure 3

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