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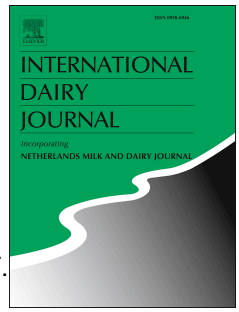

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Suitability of nitrogen fractions determination to assess serum protein separation efficiency from a mass balance perspective during microfiltration of skim milk

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ABSTRACT

Efficient separation of serum from colloidal proteins in bovine milk in their native form can be achieved by microfiltration (MF). This study assessed partitioning of serum proteins (SP) in skim milk by a MF process using 0.1 μm graded permeability ceramic membranes with two diafiltration (DF) steps, from a mass balance and energy utilisation perspective. A mass balance focused on dry matter and true protein yielded recoveries of 99.5 and 95.3%, respectively. However, an accurate mass balance relative to colloidal and SP contents in the retentate and permeate streams was not achieved, linked to errors surrounding quantification of nitrogen fractions in processed streams using standard methodologies designed for raw milk. Additionally the energy required for each MF/DF step was 13.1, 13.7 and 20.6 kW h kg^{-1} of SP removed, respectively, demonstrating the dynamic relationship between SP partition relative to diafiltrant utilisation and its impact on energy consumption.

1. Introduction

The introduction of membrane separation technology to the dairy industry can be traced back to the 1970s for applications such as treatment of acid whey and standardisation of milk protein concentration prior to cheese making to increase yield (Maubois & Mocquot, 1975). Dairy filtration processes have evolved over time and are utilised for fractionation and standardisation of whole/skim milk and a variety of whey streams. Of these, microfiltration (MF) as a technology has experienced a rapid growth in the dairy industry in the past two decades due to its potential to both retain and partition a wide range of milk components. In particular, fractionation of skim milk via MF has emerged as an innovative process solution for producing whey or serum protein and casein fractions in their native form (Saboyainsta & Maubois, 2000). Several studies have investigated the dynamics of casein and serum protein (SP) partition during MF, based on manipulation of filtration conditions and equipment configurations to optimise process efficiency relative to selectivity and flux performance (Nelson & Barbano, 2005; Tremblay-Marchand, Doyen, Britten, & Pouliot, 2016; Zulewska & Barbano, 2014; Zulewska, Newbold, & Barbano, 2009). Ceramic MF membranes are often the design of choice for dairy applications due to their long operational life and ability to withstand high processing temperatures and aggressive cleaning cycles (Baker, 2004). In recent years, ceramic graded permeability (GP) MF membranes, whereby the design of the membrane controls the pressure drop along the membrane length, have been widely adopted as a more energy efficient design compared with earlier ceramic MF systems that required recirculation of the permeate to create a uniform transmembrane pressure (UTP) (Saboyainsta & Maubois, 2000).

Depending on the MF equipment design, it is reported that up to 95% of the SP can be removed from skim milk, creating a retentate stream enriched in micellar casein, and a

permeate stream containing the partitioned SP and other soluble components (Nelson & Barbano, 2005). The study by Nelson and Barbano (2005) established a benchmark for assessment of MF based protein separation efficiency, which was later applied in several studies (Beckman, Zulewska, Newbold, & Barbano, 2010; Hurt, Zulewska, Newbold, & Barbano, 2010; Lawrence, Kentish, O'Connor, Barber, & Stevens, 2008; Zulewska et al., 2009). However, the reported SP retention or depletion efficiency depends on whether the permeate or retentate mass and compositional data are used for comparison with the feed stream. Often studies consider soluble whey proteins or SP, measured as non-casein nitrogen (NCN) by Kjeldahl, without considering the presence of soluble denatured whey proteins (Brodkorb, Croguennec, Bouhallab, & Kehoe, 2016) and serum casein (Rose, 1968), which may lead to inaccuracies in reported SP contents. The literature concerning SP quantification post MF only considers 'whey proteins' as those measured using standard methods designed for raw liquid milk. This classical definition of SP does not incorporate serum caseins that contribute to the soluble proteins in the serum phase of milk (Rose, 1968; Von Hippel & Waugh, 1955). Additionally, the feed milk and subsequent retentate and permeate streams are often subjected to extensive thermal and mechanical treatments which denature whey proteins (Hinrichs & Rademacher, 2005; Wijayanti, Bansal, & Deeth, 2014), leaving them susceptible to precipitation at pH 4.6 and interpretation as casein during nitrogen fractions determination.

Several studies provide data surrounding fractionation efficiency of SP in skim milk by MF, focused on the composition of the permeate stream, whereby partition of SP is expressed in terms of percentage removal relative to the skim milk based on determination of N fractions. However, there is a lack of a standardised mass balance approach in the literature relative to the separation of milk proteins by MF. This study assessed MF of skim milk with respect to protein partitioning on a mass/energy consumption basis to provide an overall

balance of milk components for a ceramic MF/DF process at pilot plant scale. In corollary, we address discrepancies reported in the literature relative to the separation efficiency of SP based on quantitative analysis of N fractions by Kjeldahl, supported by qualitative analysis of the protein profile to identify partition dynamics during MF.

2. Materials and methods

2.1. MF feed material

Pasteurised skim milk, heat-treated at 73 °C for 15 s, was collected from a local dairy processor 24 h prior to MF trials and was stored at 4 °C. On the day of processing the skim milk was heated to 50 °C prior to filtration. The trials were performed in triplicate with a discrete batch of pasteurised skim milk sourced for each trial.

2.2. Membrane system and process design

The MF process was performed using a pilot-scale membrane plant (GEA Process Technologies, Dublin, Ireland) operated in continuous mode, with the retentate and permeate collected in separate tanks. The processing parameters are reported in Table 1. The feed and recirculation pressures (307 kPa retentate pressure in and 100 kPa retentate pressure out, respectively) were kept constant throughout the filtration run, yielding a constant trans-membrane pressure (TMP). A permeate back pressure of 98 kPa was applied during the MF process. Under these set operational pressure conditions, the membrane experienced an overall pressure drop (ΔP) and TMP of 207 and 105.6 kPa, respectively. The plant and membranes were cleaned before and after filtration according to the standard cleaning-in-

114 place procedure (see Supplementary material). The membrane plant was equipped with three
115 0.1 µm ceramic graded permeability (GP) MF membranes (Membralox® model, EP 3730,
116 0.1 µm alumina; Pall Corp, California, CA, USA) with a total surface area of 1.05m^2. The
117 membrane plant was also equipped with a digital data logger (Endress + Hauser AG,
118 Reinach, Switzerland) that recorded performance data including, flow rates, pressures,
119 temperatures and energy consumption (each individual pump was monitored using an ABB
120 B23 direct kW h^{-1} meter (ABB Ltd, Zurich, Switzerland).

121 On the day of processing, pasteurised skim milk (~300 kg) was subjected to
122 microfiltration followed by two discrete diafiltration (DF) steps under a volume concentration
123 factor (VCF) of 3 at 50 °C. For the DF process, the collected MF retentate was mixed with
124 100 kg of RO water (50 °C) and re-introduced into the membrane plant as the feed material,
125 while maintaining continuous operation of the MF process. The volume reduction factor
126 (VRF) during the two DF steps was 2, i.e., 100 kg of additional permeate was collected
127 during each DF step, equivalent to the volume of added water. The entire filtration process
128 was maintained in a continuous steady state at a VCF of 3 across all three stages. The mass of
129 all streams was recorded including the dead/residual volume of the membrane plant, which
130 was collected as a final flush post processing to minimise solids loss, ensuring an accurate
131 mass balance. The final MF permeate was concentrated by reverse osmosis (RO) with the
132 same membrane plant used for the MF process. For RO concentration the membrane plant
133 was equipped with two spiral-wound composite polyamide RO membranes (Dairy
134 AF3838C30, David Kellet & Partners Ltd, Hereford, UK) connected in series with a total
135 surface area of 14 m^2, whereby MF permeates were concentrated to a VCF of 5 at ~49 °C.
136

137 *2.3. Sample preparation and compositional analysis*
138

The skim milk feed was sampled prior to MF, while retentates and permeates were sampled after MF, MF/DF1, MF/DF2 steps and post RO of MF permeates.. All samples were stored at 4 °C post sampling. The final MF/DF2 permeate sample was only used for dry matter (DM) determination to support the mass balance calculations. The remaining compositional analysis pertaining to the MF/DF2 permeate was performed on the RO retentate, to limit the analytical error associated with determination of N fractions in low DM content permeates. The composition of the RO retentate was used to calculate the composition of the MF permeate relative to the dry matter therein. Analysis of DM and nitrogen fractions was performed within 48 h, while for other analyses samples were stored for two weeks at 4 °C with a preservative added (Broad Spectrum Microtabs® II, D & F Control Systems, Inc., CA, USA).

DM was measured by oven drying (AOAC International method 925.23; AOAC, 2000). Total nitrogen (TN), non-protein nitrogen (NPN) and non-casein nitrogen (NCN) were determined by the Kjeldahl method (methods IDF 20-1:2014, IDF 20-4:2016 and IDF 29-1:2004; IDF, 2014, IDF, 2016 and IDF, 2004, respectively). To ensure further accuracy in NCN analysis and minimise overestimation of NCN, the pH was monitored during acetic acid and sodium acetate addition according to the procedure of Zhang and Metzger (2011). Calculation of crude protein (CP), true protein (TP), casein (CN) and serum protein (SP) contents were determined from N fraction analysis using a conversion factor of 6.38. Total CP was calculated as TN × 6.38, TP content was calculated as (TN – NPN) x 6.38, CN content was calculated as (TP – NCN) × 6.38 and SP (where SP is considered as non-sedimentable true protein) was calculated as (NCN – NPN) × 6.38.

2.4. Separation of serum and colloidal proteins

Separation of serum phase from colloidal proteins in skim milk and MF retentate samples was achieved by ultracentrifugation at 100,000 × *g* for 1 h at 25 °C (Sorvall Discovery 90SE ultracentrifuge, Kendro Laboratory Products, Asheville, NC, USA). Prior to ultracentrifugation, skim milk and retentate samples were heated to 50 °C.

2.5. Protein profile analysis by SDS-PAGE

The protein profiles of skim milk, RO and MF retentates, skim milk and MF retentate ultracentrifugates and filtrates from NCN analysis were determined using pre-cast sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) (Novex Technologies, ThermoFischer Scientific, Dublin, Ireland) using the method described by Buggy, McManus, Brodkorb, McCarthy, and Fenelon (2017). All samples were dissolved in a lithium dodecyl sulphate (LDS) buffer under reducing conditions at pH 8.4, with 10 µL of the sample loaded to the wells in a 12% Bis-Tris Gel. The electrophoresis was performed using an X-Cell Surelock electrophoresis unit (Novex Technologies). The samples were not standardised relative to their protein content with dilution factors and loading volumes (10 µL) kept constant for all samples. Post electrophoresis, the samples were stained in instant blue stain (Expedeon, Cambridge, UK) and gels were de-stained in MilliQ® water until a clear background was achieved. Proteins were identified using a standard molecular mass kit (PageRuler™ Unstained Low Range Protein Ladder, Thermo Fisher Scientific, Dublin. Ireland).

2.6. Reverse-phase high pressure liquid chromatography for casein and whey protein profiles

189 The RP-HPLC analysis for casein and whey protein profiles in skim milk, MF
190 retentates (and ultracentrifugates thereof) and RO retentates was performed as described by
191 McCarthy, Wijayanti, Crowley, O'Mahony, and Fenelon (2017). Samples were pre-treated in
192 7 M urea buffer and 2-mercaptoethanol followed by a 1 h incubation at room temperature
193 before being filtered (0.2 µm PES syringe filters, Agilent Technologies, Dublin, Ireland). A 5
194 µL aliquot of filtered sample was injected twice into a Poroshell 300SB-C18 (Size: 2.1 × 7.5
195 mm, 5 210 µm; Agilent Technologies) column. The HPLC system (Agilent 1200 Series,
196 Agilent Technologies) was equipped with a UV-vis detector (61365D MWD Agilent
197 Technologies 1200 series) with detection at an absorbance of 214 nm. Identification and
198 quantification of caseins and whey proteins was carried out using α-lactalbumin/β-
199 lactoglobulin and κ-, $\alpha_{S1/S2}$- and β-casein standards (Sigma, St. Louis, MO, USA).
200
201 *2.7. Mass and energy balance for serum protein removal*
202
203 To assess the separation performance of the MF process, mass balance calculations
204 were performed. The total mass (kg) of all microfiltration streams was recorded and the
205 corresponding mass (kg) of DM and N fractions (CP, TP, CN and SP) therein was calculated
206 to assess component partition dynamics.
207 After converting DM and protein contents into kg (Table 3), % recoveries for DM, CP,
208 TP, CN and SP were calculated as follows (equations 1 to 5):

209 $$1)\ \%\ \text{TS Recovery} = \frac{(\text{kg of TS in permeate}) + (\text{kg of TS in retentate})}{\text{kg of TS in skim milk}} \times 100$$

210 $$2)\ \%\ \text{CP Recovery} = \frac{(\text{kg of CP in permeate}) + (\text{kg of CP in retentate})}{\text{kg of CP in skim milk}} \times 100$$

211 $$3)\ \%\ \text{TP Recovery} = \frac{(\text{kg of TP in permeate}) + (\text{kg of TP in retentate})}{\text{kg of TP in skim milk}} \times 100$$

212 4) % CN Recovery $= \frac{(\text{kg of CN in permeate}) + (\text{kg of CN in retentate})}{\text{kg of CN in skim milk}} \times 100$

213 5) % SP Recovery $= \frac{(\text{kg of SP in permeate}) + (\text{kg of SP in retentate})}{\text{kg of SP in skim milk}} \times 100$

214

215 SP removal efficiency was calculated using either MF permeate or retentate composition

216 (equations 6 and 7):

217 6) % SP Removal (permeate composition) $= \frac{\text{kg of SP in permeate}}{\text{kg of SP in skim milk}} \times 100$

218 7) % SP Removal (retentate composition) $= \frac{(\text{kg of SP in skim milk}) - (\text{kg of SP in retentate + flush})}{\text{kg of SP in skim milk}} \times 100$

219

220 *2.8. Energy consumption*

221

222 The energy usage was calculated relative to kilowatt-hour (kWh) consumed by the

223 feed pump and recirculation pump. Energy consumption was calculated relative to kW $h.L^{-1}$

224 of permeate produced (Eqn 8) and kW h kg^{-1} of SP removed (Eqn 9) for the MF and DF

225 processes:

226 8) Energy kW h L^{-1} Permeate$= \frac{(\text{feed pump energy in kWh + recirculation pump energy in kWh})}{\text{Permeate flow rate in L } h^{-1}}$

227 9) Energy kW h kg^{-1} TP$= \frac{\left(\frac{\text{Total L permeate produced}}{\text{Permeate flow rate in L } h^{-1}}\right) \times (\text{feed pump energy in kWh + recirculation pump energy in kWh})}{\text{Total kg of SP removed}}$

228

229 *2.9. Statistical analysis*

230

231 Statistical analysis was carried out using SPSS software (SPSS v.24, IBM, New York,

232 NY, USA). Differences between triplicate trials were analysed for variance using one way

ANOVA and significant differences were tested with Tukey's honest significant difference (HSD) test at $p < 0.05$.

3. Results and discussion

The total processing time for the ceramic MF process was on average 8 h 20 min. The flux and processing time for individual processing stages of the MF process are shown in Fig. 1. The mean running temperature of the process was 50.1 °C and a TMP of ~105.6 kPa and VCF3 were maintained throughout the production cycle. The recommended TMP range for the GP membrane is 100 to 150 kPa. A value close to the lower end of this range was chosen to minimise the potential for fouling accumulation throughout the processing cycle. The average flux for the initial VCF 3 MF stage was 45.5 L m^{-2} h^{-1}, which is considerably lower than reported by Zulewska & Barbano (2014) and Zulewska et al. (2009) and at 71.8 and 72.5 L m^{-2} h^{-1}, respectively, for continuous MF processes. Tremblay-Marchand et al. (2016) also observed a higher flux of 79–90 L m^{-2} h^{-1} using a similar GP MF membrane, although the TMP was higher at 152 kPa. As their study involved a batch process, where the end of the filtration process was achieved once a VCF3 was reached, it is difficult to predict the flux evolution throughout a typical production cycle at higher TMP. In the current study, an initial flux of 326 L m^{-2} h^{-1} decreased to ~76 L m^{-2} h^{-1} in the first 13 min of production, after which the plant entered a steady state with a more gradual flux decline thereafter (Fig. 1). The rapid initial flux decline may be attributed to accumulation of foulants on the membrane surface increasing resistance (Carić, Milanović, Krstić, & Tekić, 2000; Savello, Caric, & Mahmoud, 1997), coupled with changes in DM content and viscosity as the plant stabilises and initial water within the recirculation loop is expelled. The addition of diafiltrant caused a slight increase in flux at 46.1 and 46.5 L m^{-2} h^{-1} for MF/DF1 and MF/DF2 stages respectively

(Table 1), which is significantly lower than the increases reported by Zulewska and Barbano (2014) and Tremblay-Marchand et al. (2016) for a similar MF/DF process albeit with half the diafiltrant on a volume basis used in the current study. The design of the pilot MF process in this study more closely reflects commercial operations in terms of process cycle duration and maintenance of a continuous steady state during production, as opposed to the batch processes more commonly reported in the literature. The sequential addition of lower volumes of DF water directly into the feed stream while maintaining steady state conditions within the plant simulates commercial practice. The processing cycles were not extended beyond 8 h 20 min, which could have been achieved by increasing the skim milk feed volume, due to concerns for the microbial quality of the MF retentate when operating at 50 °C for an extended time.

3.1. Determination of mass and SP partition efficiency during MF

The compositional analysis for DM and N fractions and associated mass balance for the skim feed and MF/DF retentates/permeates at each stage of the filtration process are presented in Tables 2 and 3. The overall mass balance focused on directly comparing the skim feed with the final MF/DF2 retentate and permeate relative to DM contents and the N fractions therein. The MF/DF2 retentate flush was also collected to limit any loss of milk solids, minimising errors associated with determination of component recovery. The composition of the DM within the MF retentate flush was equivalent to that of the MF/DF2 retentate. Recoveries were calculated for DM (99.52%), CP (94.87%) and TP (95.33%) according to equations 1, 2 and 3 respectively.The high DM recovery indicates minimal loss of milk solids, while the lower recoveries for CP/TP identified the standard methodologies for N determination as a potential source of error.

Based on the MF permeate mass/compositional data, the total SP removal decreased as the solvent phase was diluted by successive diafiltrant addition from 0.69 for MF, 0.34 (1.03–0.69) for DF1 and 0.25 (1.28–1.03) kg for DF2 (Table 3). Relative to the skim milk feed this equates to 48.6, 23.9 and 17.6% SP removal (Eqn 6) for each individual filtration step. These results are lower than those reported by Zulewska and Barbano (2014) who reported SP removal efficiencies of 56, 26.6 and 13.9%. respectively. with a cumulative value of 96.5%, for their 3 step ceramic MF/DF process. Zulewska et al. (2009) reported a SP removal efficiency of 61% for a single stage MF process. Similar to the current study a SP removal rate of 47% was reported by Tremblay-Marchand et al. (2016) for the initial MF step (3 step process), with 17 and 8.5% SP removal reported for subsequent DF steps. When comparing the cumulative SP removal in this study based on the SP content in the MF/DF2 permeate (1.28 kg) relative to the skim feed (1.42 kg) this equates to a 90.1% SP removal efficiency on a mass basis. It should be noted that although Zulewska and Barbano (2014) reported an improved SP removal efficiency compared with this study, these authors used twice the volume of diafiltrant, which would have significant implications relative to equipment sizing and subsequent water recovery requirements in commercial installations.

3.2. Determination of SP partition efficiency based on MF retentate composition

In contrast to determining SP removal efficiency based on the permeate mass/composition, when assessing cumulate SP removal based on the MF retentate mass/composition (Eqn 7) a higher rejection of SP was observed with an overall removal efficiency of 55.63% (Table 3) in line with the observations of Tremblay-Marchand et al. (2016). The study of Hurt et al. (2010) calculated SP removal using both approaches, MF permeate and MF retentate composition and reported it as 95.2 and 78.6% respectively. These

observations highlight the challenges in accurate determination of SP removal rates using the retentate composition, as the precipitation step which is part of the NCN analysis (IDF 29-1:2004) fails to precipitate all CN, leading to an overestimation of NCN (and hence SP) in MF retentates. For the current study, when directly assessing the total cumulative SP in the final MF retentate (0.63 kg) (0.51 + 0.12 kg) and MF permeate (1.28 kg) of 1.91 kg (1.28 + 0.63 kg) relative to the 1.42 kg of SP in the skim feed, this gives a recovery of 134.51% of the SP in the skim feed considering analysis of NCN content by Kjeldahl. In corollary, while the CN concentration in the final MF retentate was ~2.8 times that of the skim milk (Table 2), the cumulative CN recovery was only 89.4% indicating underestimation of CN and overestimation of SP according to the standard methodology for NCN determination (IDF 29-1:2004). The standard methodology for NCN determination by Kjeldahl requires precipitation of caseins at their isoelectric point and determination of the remaining proteins soluble at pH 4.6. High protein/casein contents in MF retentates are a particular challenge if they are not standardised to the protein content for which the test is designed, i.e., raw milk.

In this study the pH was monitored during NCN analysis of MF retentates to ensure that pH 4.6 was achieved and that casein micelles were fully precipitated (Southward, 2002). This pH monitoring step was also suggested by Zhang and Metzger (2011) to account for variation in casein solubility relative to the altered ionic environment in MF retentates.

Further investigation of the protein profile contained within the NCN filtrates derived from the MF/DF2 retentates by SDS-PAGE, identified the presence of serum caseins therein. These serum caseins are reported as SP according to the NCN test when in fact they are clearly CN (Fig. 3). Nelson and Barbano (2005) also detected intact casein in NCN filtrates and suggested that the standard NCN test (IDF 29-1:2004) may underestimate CN concentration in MF retentates. To address this challenge Di Marzo, Pranata, and Barbano (2021) compared the results from quantitative approaches for CN determination and found

that CN/TP% was overestimated by NCN analysis (IDF 29-1:2004) compared with SDS-PAGE, again highlighting the challenges of accurate determination of N fractions. In the current study, serum CN in NCN filtrates post isoelectric precipitation of colloidal caseins was not observed for the skim milk feed (Fig. 3), indicating significantly higher levels of serum CN relative to total protein contents in the MF/DF2 retentates.

To further clarify the relationship between serum to colloidal proteins, the skim milk feed and MF/DF2 retentates were ultracentrifuged to gravimetrically precipitate colloidal from serum components, followed by analysis of CP and TP contents in the ultracentrifugates (Table 4). Refrigerated samples were pre-heated to 50 °C prior to ultracentrifugation to reflect the temperature at which the MF process was performed and reverse any solubilisation of caseins during storage at lower temperatures (Schiffer, Scheidler, Kiefer, & Kulozik, 2021). Direct comparison of TP in the skim milk with that of the ultracentrifugate thereof indicated that 31% of the TP in the skim milk remained in the serum phase post ultracentrifugation. When performing the same calculation for the MF/DF2 retentates it was found that 27% of the TP remained in the serum phase after ultracentrifugation. The high SP/TP% in the MF/DF2 retentate indicates a relationship between casein concentration and changes in the ionic environment with respect to ratios of serum to colloidal casein.

Identification of the protein profile in the ultracentrifugates of the skim milk and MF/DF2 retentates by SDS PAGE indicated the presence of serum α_{S1}-, α_{S2}-, and β- and κ-caseins (Fig. 2). This was supported by subsequent HPLC analysis which identified serum caseins in the ultracentrifugates of the skim milk and MF/DF2 retentates with a large increase in serum κ-casein in particular observed in the latter (Fig. 4). Studies have shown that at elevated pH and temperatures, κ-casein tends to dissociate from the casein micelle (Anema & Klostermeyer, 1997; Anema & Li, 2000; Anema, Creamer, & Singh, 1993). The study by Anema (2007) reported that in heated skim milk samples at pH values above 6.7, the level of

serum κ-casein increases, with ~70% solubilisation at pH 7.1. In the current study the MF retentate had a pH of 7.06, which could explain the presence of elevated levels of serum κ-caseins in the ultracentrifugates (Figs. 2 and 4). Additionally the loss of soluble/serum ionic species to the permeate during MF/DF processes may increase surface charge of the micelles, thus increasing the solubility of surface κ-casein in particular. Analysis of the MF/DF2 permeates (Figs. 2 and 4) indicate that serum caseins are retained while whey proteins permeated through the membrane. According to Eigel et al. (1984) individual casein fractions have a molecular mass in the range of 19 to 24 kDa while that of α-lactalbumin and β-lactoglobulin is 14 and 18 kDa, respectively, thus serum caseins may have a higher rejection coefficient relative to their size and shape. This observation is reinforced by the fact that high molecular mass globular SP such as lactoferrin (~78 kDa) and bovine serum albumin (~69 kDa) permeated through the MF membrane while serum CN did not (Fig. 2).

Measurement of N fractions in skim milk and MF permeates (MF/DF1/DF2) also highlights areas for potential underestimation of SP. The skim milk itself had a CN/TP% value of 85.6%, which could be considered high given that expected average casein to whey proteins ratios of 80:20 are widely reported in the literature (Fox & Brodkorb, 2008; Fox, McSweeney, & Paul, 1998; Jenness & Patton, 1959). The high-temperature short-time (HTST) pasteurisation of the skim milk at 73 °C for 15s, may result in higher levels of whey protein denaturation compared with raw milk (Guinee et al., 1997) and unpasteurised skim milk (Svanborg et al., 2014). However, the CN/TP% results in this study are similar to those reported by Beckman et al. (2010), Mercier-Bouchard, Benoit, Doyen, Britten, and Pouliot (2017) and Tremblay-Marchand et al. (2016), which were reported at 83.6, 83.86 and 83.2%, respectively. The skim milk was subjected to pasteurisation and mechanical treatment (e.g., pumping) prior to and during MF, which could damage/partially unfold whey proteins (Brodkorb et al., 2016), affecting their solubility at pH 4.6 during subsequent NCN testing. In

this scenario damaged SP is measured as CN according to the test (IDF 29-1:2004) leading to an overestimation of CN/TP%. This hypothesis is supported by the high levels of CN/TP% reported in the MF permeates at each filtration stage at 10.8, 10.09 and 9.37% for MF, DF1 and DF2 steps respectively. It is therefore possible that all TP transmitted through the membrane is SP based on optical clarity of the permeate, supported by particle size measurements (results not shown). In addition, neither SDS PAGE (Fig. 2) nor HPLC (Fig. 4) identified the presence of serum casein in the MF/DF2 permeates. However, during NCN determination a precipitate wass formed which consisted of SP insoluble at pH 4.6, which almost certainly consists of whey proteins denatured during processing. Thus, the separation efficiencies for MF, based on determination of N fractions, reported in the literature (Hurt et al., 2010; Nelson & Barbano, 2005; Zulewska et al., 2009) are challenged as follows:

1. SP available for permeation in the starting pasteurised skim milk is underestimated based on NCN analysis which overestimates CN/TP% contents therein, due to the presence of pH 4.6 insoluble whey proteins.
2. Denatured whey proteins present in MF permeates are reported as CN without performing confirmatory tests of the protein profile therein.
3. Protein profiles determined by HPLC and gel electrophoresis indicate that ceramic MF permeates contain negligible amounts of casein.
4. SP present in MF retentates is overestimated as a proportion of serum CN is reported as SP according to NCN analysis.
5. Reliability of true SP partition efficiency reported in the literature is impacted by errors associated with determination of N fractions in processed dairy streams.

These observations clearly highlight challenges concerning accurate determination of N fractions and in particular SP in MF retentates/permeates by NCN analysis (IDF 29-1:2004). Hence, relying solely on N fractions analysis to determine SP partition efficiency

leads to compounding analytical errors, masking true SP separation behaviour during MF. Complementary qualitative SDS-PAGE and HPLC analysis performed in tandem with N fractions determination by Kjeldahl, may be a better approach to gauge SP removal efficiency for MF processes.

3.3. Energy consumption

The energy required per litre of permeate was calculated for each discrete MF/DF step at 0.043 (MF), 0.042 (MF/DF1) and 0.041 (MF/DF2) kW h L^{-1} (Table 5).There was no significant difference in energy usage per unit of permeate produced between the MF/DF1 and MF/DF2 filtration steps [based on energy consumption of feed and recirculation pumps (Eqn 8)], which is not unexpected considering the flux consistency across the process cycle (Fig. 1). Tremblay-Marchand et al. (2016) reported higher energy consumptions of 0.089, 0.083 and 0.077 kW h L^{-1} of permeate removed for their 3 step ceramic GP MF/DF process. They reported that the recirculation pump only accounted for 34% of the energy consumed during MF of skim, stating that their plant design utilised two feed pumps, as the membrane plant was a multipurpose system also capable of delivering the high pressures required for RO. It is likely that this membrane plant was not equipped with frequency inverters to control the speed of each pump, potentially overestimating the required kW h L^{-1} of permeate separated. In contrast, the pumps in the current study were equipped with frequency inverters to minimise the energy required to deliver a given cross flow velocity/TMP. Thus the recirculation pump, which provides the high cross flow velocities (~4.8 m s^{-1}), accounted for 93% of the energy used by the filtration plant.

Broadening the scope of the energy consumption calculation relative to the mass of SP separated within each MF/DF stage (Eqn 9) provides insights into the impact of diafiltrant

433 volume on the energy efficiency of SP removal. The initial MF step operating at VCF3
434 removed ~ 200 kg of permeate, with each sequential DF step adding 100 kg of diafiltrant and
435 hence generating an additional ~100 kg of a diluted permeate (Table 3). The initial MF (VCF
436 3) and subsequent diafiltration (DF1/DF2) steps had a corresponding energy usage of 13.1,
437 13.7 and 20.6 kW h kg^{-1} of SP removed respectively. In contrast, Tremblay-Marchand et al.
438 (2016) reported energy consumptions of 25.83, 63.99 and 124.85 kW h kg^{-1} of SP removed,
439 for their 3 stage MF/DF process, although these authors utilised a higher volume reduction
440 ratio (VRR) of 3 during DF compared with a volume reduction ratio of 2 applied in this
441 study. Operation of the plant at VCF3 while applying an overall VRR of 2 during the DF1
442 step had a clear benefit in terms of SP removal efficiency as there was no significant
443 difference observed in the energy consumption in kW h kg^{-1} SP removed between the initial
444 MF and DF1 steps (Table 5). Further diafiltrant addition during the DF2 step did not
445 significantly increase flux; however; energy consumption kW h kg^{-1} SP removed
446 significantly increased. Minimising DF volumes is beneficial in terms of energy usage per kg
447 of SP separated, as a point of diminishing returns is quickly reached due to dilution of soluble
448 phase components in the retentate when using large DF volumes. Further implications
449 concerning membrane area and associated capital/operational costs and water recovery/reuse
450 or discharge to effluent strategies, all require careful consideration in determining optimal DF
451 processes.
452

453 **4. Conclusion**

454

455 A comprehensive mass balance indicated overall recoveries on a dry matter basis for
456 DM (99.5%), CP (94.9%) and TP (95.3%). However, CN (89.4%) and SP (134.51%)
457 recoveries indicated analytical errors associated with determination of NCN and indirectly

458 CN according to standard methodologies for determination of N fractions. A cumulative SP
459 removal rate for a 3× MF/DF process of 90.14% on a mass basis was achieved, based on
460 comparative analysis of NCN contents in the feed and permeate streams. When the same
461 comparison was performed based on the SP content in the MF retentate the cumulative
462 removal rate was 55.63%. These observations highlight the challenges of determination of N
463 fractions for evaluation of the separation efficiency of SP by MF, based on analysis of the
464 retentate stream. Combining quantitative N fractions analysis of the feed/permeate streams by
465 Kjeldahl with qualitative analysis by HPLC/SDS PAGE provides better insights into the true
466 partition efficiency of SP during MF. In parallel, the relationship between energy
467 consumption in kW h kg^{-1} SP removed, diafiltrant volumes used and plant operational
468 conditions can enhance the energy efficiency of SP removal.
469

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471

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476

477 **References**

478

479 AOAC. (2000). *Method no. 925.23. Official methods of analysis of AOAC International* (17th
480 ed). Gaithersburg, MD, USA: AOAC International.

Anema, S. G. (2007). Role of κ-casein in the association of denatured whey proteins with caseinmicelles in heated reconstituted skim milk. *Journal of Agricultural and Food Chemistry, 55*, 3635–3642.

Anema, S. G., & Klostermeyer, H. (1997). Heat-induced, pH-dependent dissociation of casein micelles on heating reconstituted skim milk at temperatures below 100 °C. *Journal of Agricultural and Food Chemistry, 45*, 1108–1115.

Anema, S. G., & Li, Y. (2000). Further studies on the heat-induced, pH-dependent dissociation of casein from the micelles in reconstituted skim milk. *LWT-Food Science and Technology, 33*, 335–343.

Anema, S., Creamer, L., & Singh, H. (1993). The relative importance of protein and mineral concentrations on the dissociation of kappa-casein from the micelles in heated reconstituted skim milk. In *Protein and fat globule modifications by heat treatment, homogenization and other technological means for high quality dairy products. IDF Seminar, Munich (Germany), 25–28 Aug 1992*: FIL-IDF.

Baker, R. W. (2004). *Membrane technology and applications.* New York, NY, USA: John Wiley & Sons Ltd.

Beckman, S., Zulewska, J., Newbold, M., & Barbano, D. (2010). Production efficiency of micellar casein concentrate using polymeric spiral-wound microfiltration membranes. *Journal of Dairy Science, 93*, 4506–4517.

Brodkorb, A., Croguennec, T., Bouhallab, S., & Kehoe, J. J. (2016). Heat-induced denaturation, aggregation and gelation of whey proteins. In P. L. H. McSweeney, & J. A. O'Mahony (Eds.), *Advanced dairy chemistry, Vol. 1B: Proteins: Applied aspects* (pp. 155–178). San Diego, CA, USA: Springer.

Buggy, A. K., McManus, J. J., Brodkorb, A., Mc Carthy, N., & Fenelon, M. A. (2017). Stabilising effect of α-lactalbumin on concentrated infant milk formula emulsions heat treated pre-or post-homogenisation. *Dairy Science & Technology, 96*, 845–859.

Carić, M. Đ., Milanović, S. D., Krstić, D. M., & Tekić, M. N. (2000). Fouling of inorganic membranes by adsorption of whey proteins. *Journal of Membrane Science, 165*, 83–88.

Di Marzo, L., Pranata, J., & Barbano, D. M. (2021). Measurement of casein in milk by Kjeldahl and sodium dodecyl sulfate–polyacrylamide gel electrophoresis. *Journal of Dairy Science, 104*, 7448–7456

Eigel, W., Butler, J., Ernstrom, C., Farrell Jr, H., Harwalkar, V., Jenness, R., et al. (1984). Nomenclature of proteins of cow's milk: fifth revision. *Journal of Dairy Science, 67*, 1599–1631.

Fox, P., & Brodkorb, A. (2008). The casein micelle: Historical aspects, current concepts and significance. *International Dairy Journal, 18*, 677–684.

Fox, P., McSweeney, P. L., & Paul, L. (1998). *Dairy chemistry and biochemistry*. Cham, Switzerland: Springer International Publishing.

Hinrichs, J., & Rademacher, B. (2005). Kinetics of combined thermal and pressure-induced whey protein denaturation in bovine skim milk. *International Dairy Journal, 15*, 315–323.

Hurt, E., Zulewska, J., Newbold, M., & Barbano, D. (2010). Micellar casein concentrate production with a 3×, 3-stage, uniform transmembrane pressure ceramic membrane process at 50 °C. *Journal of Dairy Science, 93*, 5588–5600.

IDF (2004). *Milk. Determination of casein nitrogen content – (Reference Method) IDF 29-1:2004*. Brussels, Belgium: International Dairy Federation.

IDF. (2014). *Milk and milk products determination of nitrogen content—Part 1: Kjeldahl principle and crude protein calculation*. *20–1:2014*. Brussels, Belgium: International Dairy Federation.

IDF (2016). *Milk and milk products - Determination of nitrogen content - Part 4: Determination of protein and non-protein nitrogen content and true protein content calculation (Reference method). IDF 20-4:2016*. Brussels, Belgium: International Dairy Federation.

Jenness, R., & Patton, S. (1959). *Principles of dairy chemistry.* London, UK: Chapman & Hall Ltd.

Lawrence, N., Kentish, S., O'Connor, A., Barber, A., & Stevens, G. (2008). Microfiltration of skim milk using polymeric membranes for casein concentrate manufacture. *Separation and Purification Technology, 60,* 237–244.

Maubois, J., & Mocquot, G. (1975). Application of membrane ultrafiltration to preparation of various types of cheese. *Journal of Dairy Science, 58,* 1001–1007.

McCarthy, N. A., Wijayanti, H. B., Crowley, S. V., O'Mahony, J. A., & Fenelon, M. A. (2017). Pilot-scale ceramic membrane filtration of skim milk for the production of a protein base ingredient for use in infant milk formula. *International Dairy Journal, 73,* 57–62.

Mercier-Bouchard, D., Benoit, S., Doyen, A., Britten, M., & Pouliot, Y. (2017). Process efficiency of casein separation from milk using polymeric spiral-wound microfiltration membranes. *Journal of Dairy Science, 100,* 8838–8848.

Nelson, B., & Barbano, D. (2005). A microfiltration process to maximize removal of serum proteins from skim milk before cheese making. *Journal of Dairy Science, 88,* 1891–1900.

552 Rose, D. (1968). Relation between micellar and serum casein in bovine milk. *Journal of*
553 *Dairy Science, 51*, 1897–1902.
554 Saboyainsta, L., & Maubois, J. (2000). Current developments of microfiltration technology in
555 the dairy industry. *Lait, 80*, 541–553.
556 Savello, P., Caric, M., & Mahmoud, R. (1997). Fouling of ceramic membrane by milk
557 proteins during microfiltration. *Australian Journal of Dairy Technology, 52*, 60–62.
558 Schiffer, S., Scheidler, E., Kiefer, T., & Kulozik, U. (2021). Effect of temperature, added
559 calcium and pH on the equilibrium of caseins between micellar state and milk serum.
560 *Foods, 10*, 822.
561 Southward, C. (2002). Milk proteins| Caseins, industrial production and compositional
562 standards. In H. Roginski (Ed.), *Encyclopedia of dairy sciences* (pp. 1915–1924).
563 Amsterdam, The Netherlands: Elsevier Science Publishing Co Inc.
564 Tremblay-Marchand, D., Doyen, A., Britten, M., & Pouliot, Y. (2016). A process efficiency
565 assessment of serum protein removal from milk using ceramic graded permeability
566 microfiltration membrane. *Journal of Dairy Science, 99*, 5230–5243.
567 Von Hippel, P. H., & Waugh, D. F. (1955). Casein: Monomers and polymers. *Journal of the*
568 *American Chemical Society, 77*, 4311–4319.
569 Wijayanti, H. B., Bansal, N., & Deeth, H. C. (2014). Stability of whey proteins during
570 thermal processing: A review. *Comprehensive Reviews in Food Science and Food*
571 *Safety, 13*, 1235–1251.
572 Zhang, H., & Metzger, L. (2011). Noncasein nitrogen analysis of ultrafiltration and
573 microfiltration retentate. *Journal of Dairy Science, 94*, 2118–2125.
574 Zulewska, J., & Barbano, D. M. (2014). The effect of linear velocity and flux on performance
575 of ceramic graded permeability membranes when processing skim milk at 50 °C.
576 *Journal of Dairy Science, 97*, 2619–2632.

577 Zulewska, J., Newbold, M., & Barbano, D. (2009). Efficiency of serum protein removal from
578 skim milk with ceramic and polymeric membranes at 50 °C. *Journal of Dairy Science,*
579 *92*, 1361–1377.

Table 1

Operational parameters during 3× ceramic MF/DF process.

Parameter	Stage 1 MF	Stage 2 DF	Stage 3 DF	RO
Recirculation flow rate (L h^{-1})	13687	13602	13474	12700
Feed flow rate (L h^{-1})	73.87	74.52	73.5	
Retentate flow rate (L h^{-1})	25.1	25.13	24.7	2055
Permeate flow rate (L h^{-1})	47.73	48.38	48.8	345.7
Permeate flux (L m^{-2} h^{-1})	45.45	46.08	46.48	24.7
Feed pressure (kPa)	306.6	307	306.7	1741
Recirculation pressure (kPa)	100.2	100.6	100.6	1395
Back pressure (kPa)	98	98	98	1574
TMP (kPa)	105.4	105.8	105.7	
Processing temperature (ºC)	50.3	50.1	49.9	48.7

Table 2

Compositional analysis of skim milk, MF/DF retentates and permeates. [a]

Sample	DM (%, w/w)	CP (%, w/w)	TP (%, w/w)	NPN (%, w/w)	NCN (%, w/w)	CN (%, w/w)	SP (%, w/w)	CN/TP (%)
Stage 1: microfiltration								
Skim milk	8.65 ± 0.17	3.43 ± 0.07	3.24 ± 0.05	0.19 ± 0.02	0.66 ± 0.05	2.77 ± 0.01	0.47 ± 0.04	85.5
Permeate	4.8^{a} ± 0.13	0.53^{a} ± 0.01	0.37^{a} ± 0.01	0.16^{a} ± 0.01	0.49^{a} ± 0.01	0.04^{ab} ± 0.01	0.33^{a} ± 0.01	10.8
Retentate	12.42^{A} ± 0.3	7.58^{A} ± 0.2	7.4^{A} ± 0.2	0.18^{A} ± 0.01	0.94^{A} ± 0.07	6.64^{A} ± 0.13	0.76^{A} ± 0.06	89.72
Stage 2: after 1 diafiltration	4.14^{b} ± 0.18	0.49^{b} ± 0.03	0.34^{b} ± 0.02	0.15^{a} ± 0.01	0.46^{b} ± 0.01	0.03^{a} ± 0.01	0.32^{ab} ± 0.02	10.09
Permeate	13.71^{B} ± 0.2	8.55^{B} ± 0.3	8.36^{B} ± 0.3	0.19^{A} ± 0.01	1.02^{B} ± 0.02	7.53^{B} ± 0.2	0.81^{B} ± 0.04	90.1
Retentate								
Stage 3: after 2 diafiltrations								
Permeate	3.64^{c} ± 0.14	0.41^{c} ± 0.01	0.32^{b} ± 0.1	0.10^{b} ± 0.02	0.36^{c} ± 0.05	0.05^{b} ±0.02	0.3^{b} ± 0.02	9.37
Retentate	10.77^{C}± 0.7	8.61^{B} ± 0.7	8.53^{C} ± 0.6	0.11^{B} ± 0.02	0.79^{C} ± 0.15	7.82^{B} ± 0.6	0.68^{C}± 0.1	91.7
Flush	2.52 ± 0.4	1.81 ± 0.3	1.75 ± 0.3	0.06 ± 0.01	0.2 ± 0.02	1.6 ± 0.21	0.14 ± 0.1	91.4

[a] Abbreviations are: DM, dry matter; TP, total protein; CP, crude protein; NPN, non-protein nitrogen; CN, casein; SP, serum protein. Values calculated as: TP = CP–NPN; CN = CP–NCN; SP= NCN–NPN; CN/TP% = CN as a percent of TP = (CN/TP) × 100. Results are the means ± standard deviations of data from three independent trials. Permeate and retentate values in the same column without common superscript lowercase and uppercase letters, respectively, are significantly different ($p < 0.05$).

Table 3

Mass balance of fractionated skim milk proteins relative to dry matter and protein when subjected to 3× ceramic MF/DF process at 50 °C. [a]

Process stage	Mass collected (kg)	DM (kg)	CP (kg)	TP (kg)	CN (kg)	SP (kg)
Stage 1: microfiltration						
Skim milk	301.67 ± 2.89	26.08 ± 0.75	10.35 ± 0.31	9.78 ± 0.25	8.37 ± 0.11	1.42 ± 0.15
Permeate	209.17 ± 5.96	10.05 ± 0.44	1.11 ± 0.03	0.77 ± 0.02	0.08 ± 0.01	0.69 ± 0.04
Retentate	88 ± 6.24	10.93 ± 0.62	6.67 ± 0.5	6.51 ± 0.5	5.85 ± 0.45	0.66 ± 0.08
Stage 2: after 1 diafiltration						
Permeate*	319 ± 16.25	13.21 ± 0.59	1.58 ± 0.06	1.09 ± 0.04	0.11 ± 0.02	1.03 ± 0.05
Retentate	79.4 ± 7.3	10.89 ± 0.5	6.79 ± 0.3	6.64 ± 0.5	5.98 ± 0.4	0.64 ± 0.06
Stage 3:after 2 diafiltrations	443.67 ± 12.03	16.13 ± 1.44	1.8 ± 0.07	1.43 ± 0.05	0.24 ± 0.09	1.28 ± 0.3
Permeate*	75.67 ± 6.44	8.04 ± 0.7	6.44 ± 0.12	6.36 ± 0.08	5.85 ± 0.05	0.51 ± 0.1
Retentate	87 ± 7.2	2.19 ± 0.04	1.57 ± 0.02	1.52 ± 0.03	1.39 ± 0.02	0.12 ± 0.01
Flush						
% Recovery		99.52	94.87	95.33	89.4	134.5

[a] Abbreviations are: DM, dry matter; TP, total protein; CP, crude protein; NPN, non-protein nitrogen; CN, casein; SP, serum protein. Values calculated as: TP = CP–NPN; CN = CP–NCN; SP= NCN–NPN; CN/TP% = CN as a percent of TP = (CN/TP) × 100. Results are the means ± standard deviations of data from three independent trials. An asterisk indicates collected permeate masses are cumulative values representing 2 or more filtration stages.

Table 4

Mean (% by weight) CP and TP values of skim milk and MF retentate pre and post ultracentrifugation. [a]

Sample	% CP		% TP	
	Before ultracentrifugation	After ultracentrifugation	Before ultracentrifugation	After ultracentrifugation
Skim milk	3.43 ± 0.07	1.3 ± 0.09	3.24 ± 0.05	1.01 ± 0.06
MF Retentate	8.61 ± 0.2	2.85 ± 0.2	8.53 ± 0.6	2.31 ± 0.4

[a] After ultracentrifugation, serum phase layer collected was sampled and analysed. Results are the means ± standard deviations of data from three independent trials.

Table 5

Total energy consumption during the MF/DF process. [a]

Filtration stage	Feed pump (kW h)	Recirculation pump (kW h)	Permeate flow rate ($L\ h^{-1}$)	Energy consumption per unit of permeate ($kW\ h\ L^{-1}$)	Total permeate (L)	Total energy consumption (kW h)	Total SP removed (kg)	Energy consumption ($kW\ h\ kg^{-1}$ SP removed)
MF	0.14 ± 0.02	1.93 ± 0.03	47.7 ± 0.4	0.043^{a} ± 0.04	209.2 ± 6	9.07^{a} ± 0.03	0.69 ± 0.03	13.14^{a} ± 0.06
DF1	0.14 ± 0.01	1.91 ± 0.01	48.8 ± 0.3	0.042^{ab} ± 0.03	109.8 ± 16	4.66^{b} ± 0.03	0.34 ± 0.02	13.7^{a} ± 0.04
DF2	0.14 ± 0.01	1.87 ± 0.02	48.8 ± 0.3	0.041^{b} ± 0.01	124.7 ± 12	5.15^{c} ± 0.01	0.25 ± 0.02	20.6^{b} ± 0.05

[a] Results are the means ± standard deviations of data from three independent trials; values in the same column without a common superscript letterare significantly different ($p < 0.05$).

Figure legends

Fig. 1. Permeate flux as a function of time during step 1 microfiltration (MF), step 2 diafiltration (DF1) and step 3 diafiltration (DF2).

Fig. 2. SDS-PAGE protein profiles of (1) serum phase of skim milk, (2) skim milk, (3) RO retentate representing the MF permeate, (4) serum phase of MF retentate, (5) final MF retentate and (6) molecular mass standards. Samples of serum phase of skim milk and MF retentate were obtained by collecting supernatant post ultracentrifuge. Bands are identified on the gel as: α_{S1}-CN, α_{S1}-casein; α_{S2}-CN, α_{S2}-casein; κ-CN, κ-casein; β-CN, β-casein; β-LG, β-lactoglobulin; α-LA, α-lactalbumin; LF, lactoferrin; BSA, bovine serum albumin.

Fig. 3. SDS-PAGE profile of NCN filtrates of (1) skim milk, (2) MF retentate, (3) RO retentate obtained from the three stage 3× ceramic microfiltration process and (4) molecular mass standards. Bands are identified on the gel as: β-LG, β-lactoglobulin; α-LA, α-lactalbumin.

Fig. 4. HPLC profiles of (A) skim milk, (B) RO retentate, (C) Skim milk ultracentrifugate and (D) MF Retentate ultracentrifugate. Peaks identified as: 1, κ-casein; 2, α_{S2}-casein; 3, α_{S1}-casein; 4, β-casein; 5, α-lactalbumin; 6, β-lactoglobulin b; 7, β-lactoglobulin a.

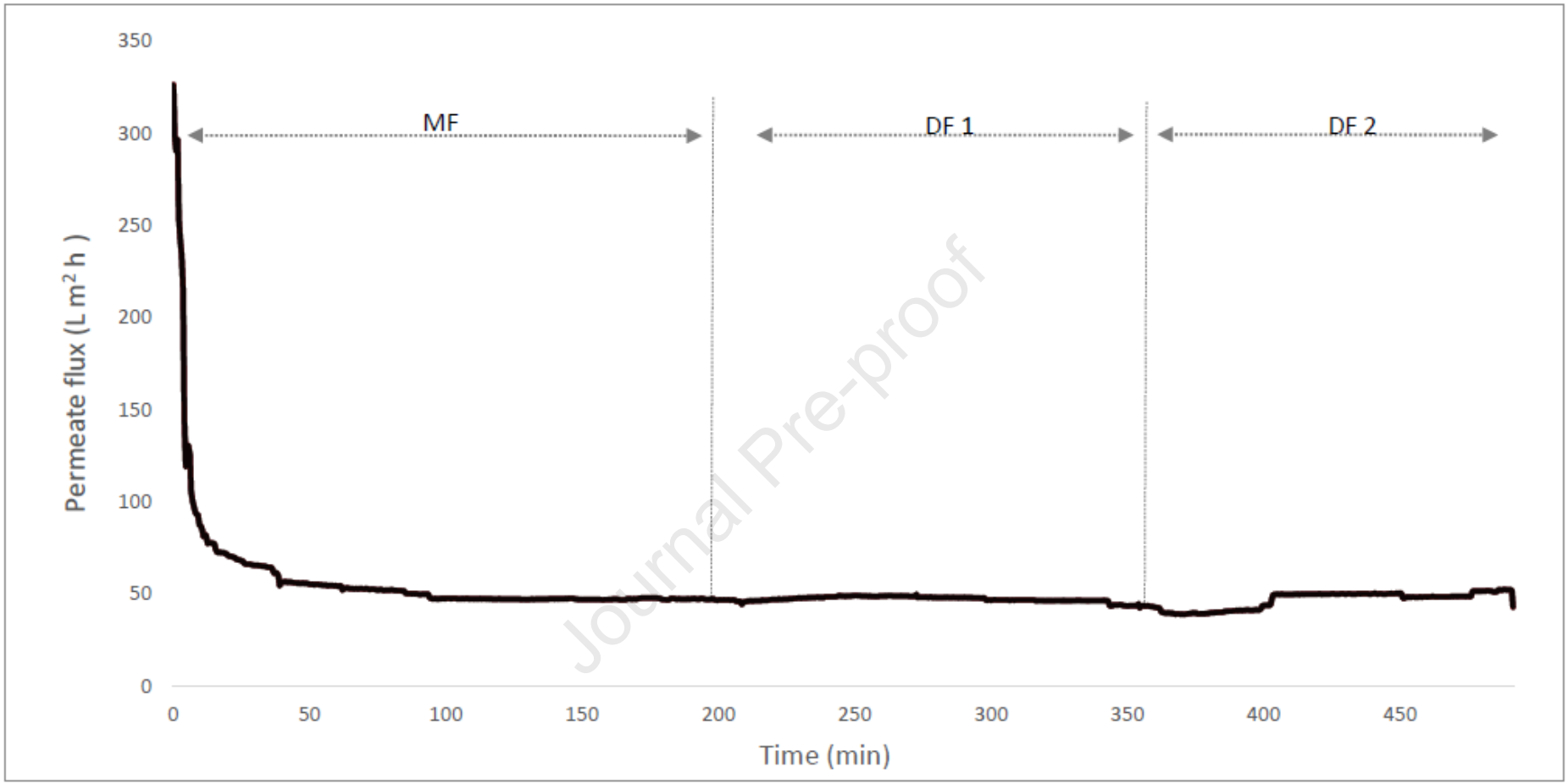

Figure 1

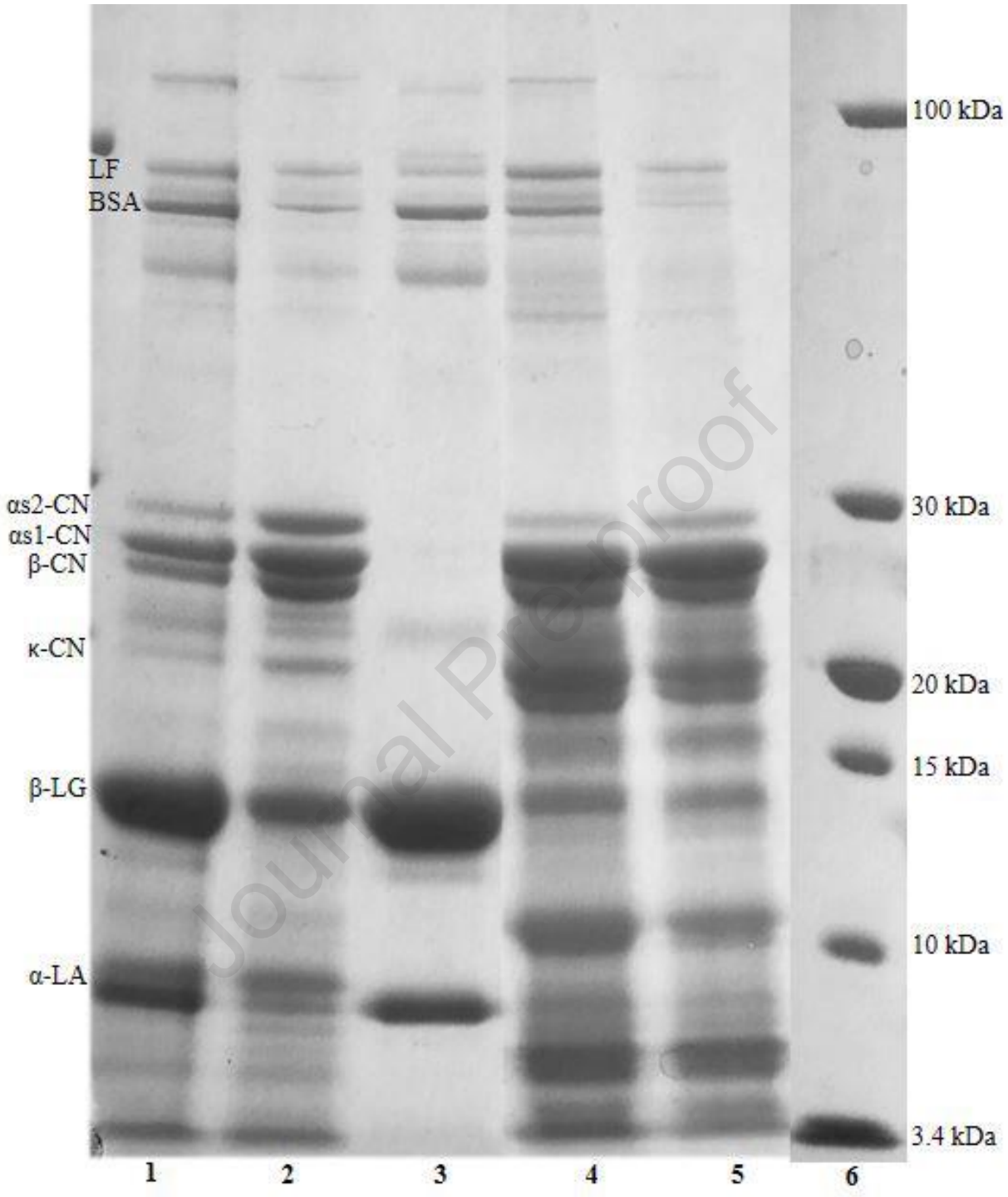

Figure 2

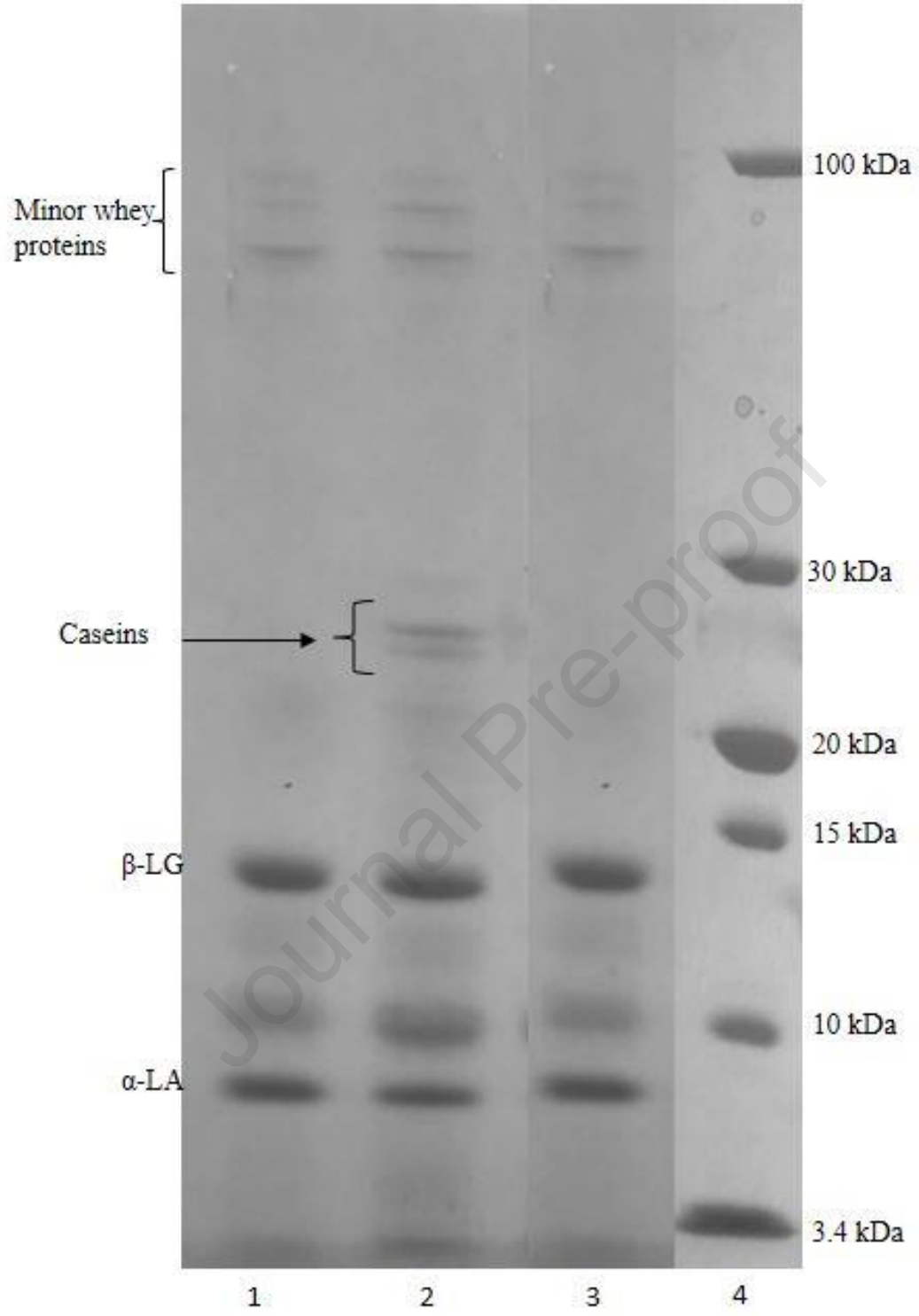

Figure 3

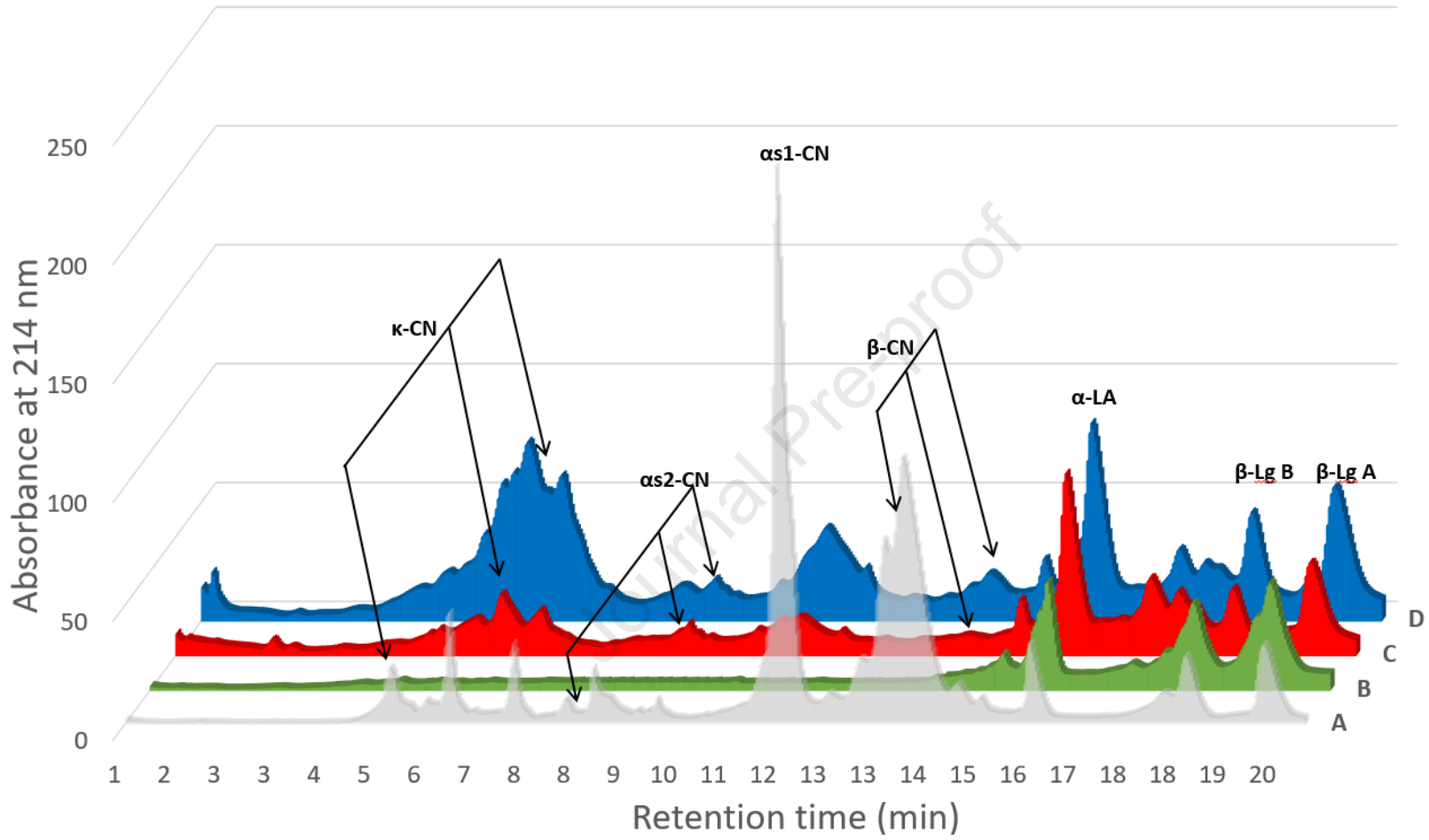
Absorbance at 214 nm
250
200
150
100
50
0
αs1-CN
κ-CN
β-CN
α-LA
αs2-CN
β-Lg B
β-Lg A
D
C
B
A
1 2 3 3 4 5 6 7 8 8 9 10 11 12 13 13 14 15 16 17 18 18 19 20
Retention time (min)

Figure 4

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: