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Pilot-scale ceramic membrane filtration of skim milk for the production of a protein base ingredient for use in infant milk formula

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ABSTRACT

The protein composition of bovine skim milk was modified using pilot scale membrane filtration to produce a whey protein-dominant ingredient with a casein profile closer to human milk. Bovine skim milk was processed at low (8.9 °C) or high (50 °C) temperature using ceramic microfiltration (MF) membranes (0.1 µm mean pore diameter). The resulting permeate stream was concentrated using polyethersulfone ultrafiltration (UF) membranes (10 kDa cut-off). The protein profile of MF and UF retentate streams were determined using reversed phase-high performance liquid chromatography and polyacrylamide gel electrophoresis. Permeate from the cold MF process (8.9 °C) had a casein:whey protein ratio of ~35:65 with no α_S- or κ-casein present, compared with a casein:whey protein ratio of ~10:90 at 50 °C. This study has demonstrated the application of cold membrane filtration (8.9 °C) at pilot scale to produce a dairy ingredient with a protein profile closer to that of human milk.

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1. Introduction

The design of membrane-based fractionation processes for the development of enriched protein ingredients can provide an enhanced nutritional profile in infant milk formulations (IMF). The compositional differences between human and bovine milk are of particular importance for infant food formulations as the majority of infant formula is based on bovine milk (O'Mahony & Fox, 2013). The use of unmodified bovine milk as the sole source of nutrients for infants is discouraged (Morgan, 2006). Most current-generation IMFs consist of milk-derived ingredients, vegetable oils and micronutrients blended together in proportions consistent with those found in human milk (Martin, Ling, & Blackburn, 2016). The protein component of IMFs is critical to support the growth and development of infants, but achieving the correct protein profile in IMFs is complicated by milk protein heterogeneity and inter-species variation.

As described by O'Mahony and Fox (2013), milk proteins can be divided into two principal families, caseins (CNs) and whey proteins (WPs), which can be further sub-divided into individual CNs (e.g., α_S-CN, β-CN, κ-CN) and WPs (e.g., α-lactalbumin, β-lactoglobulin, lactoferrin). The protein profile of IMFs intended for infants between 0–6 months ('first age') is designed to closely mimic that found in human milk. WPs account for ~60% of the total protein present in human milk, compared with only ~20% in bovine milk (de Wit, 1998); thus, IMFs are typically prepared by adjusting the CN:WP ratio of bovine milk (80:20) to that of human milk (40:60) by mixing skim milk with WP-based ingredients (e.g., whey protein concentrate and demineralised whey). The CN and WP fractions of human milk consist primarily of β-CN and α-lactalbumin, respectively, while α_S-CNs and β-

lactoglobulin predominate in bovine milk (Lönnerdal, 2003). Increasing the proportion of proteins such as β-CN and α-lactalbumin is an important objective in efforts to improve the nutritional properties of IMFs and can be achieved through the addition of enriched or purified protein ingredients (Fox, Uniacke-Lowe, McSweeney & O'Mahony, 2015).

The need to use multiple dairy ingredients to achieve a humanised protein profile places additional burdens on IMF manufacturers in terms of ingredient sourcing/transit and prediction of ingredient functionality/stability during reconstitution, thermal processing and re-drying. An alternative strategy is here proposed where the development of 'protein base' ingredients, in which several of the major humanisation targets (CN:WP ratio, CN profile) are achieved in a single processing operation. Such a strategy is feasible using a process based on small-pore (0.1 µm) microfiltration (MF), described in the present study.

Although there have been major developments in the manufacture of WP-based ingredients for IMFs (e.g., enriched α-lactalbumin fractions, purified lactoferrin), progress in CN ingredient development has been comparatively slow. 'Cold MF' (i.e., filtration temperature: 0 to 15 °C) of skim milk is a promising approach to generating β-CN-enriched WP-dominant ingredients for use in the production of IMFs. There are a number of studies that have demonstrated the enrichment of β-CN using MF (or sometimes UF) at temperatures < 10 °C (Crowley et al., 2015; Le Berre & Daufin, 1994; O'Mahony, Smith, & Lucey, 2014; Woychik, 1992). Crowley et al. (2015) recently demonstrated the concept at laboratory scale using polyvinylidene-difluoride (PVDF, 0.1 µm pore size) or polyethersulfone (PES, 1000 kDa cut-off) membranes at temperatures <5 °C. It is possible to effectively enrich β-CN from renneted milk gels (Huppertz et al., 2006), but MF has the

advantage of generating a soluble and highly-functional co-product, micellar casein concentrate (MCC). The enrichment of β-CN in the whey permeate during cold MF is based on the principle that an increasing proportion of β-CN is monomeric as temperature is decreased, with a concomitant migration of the monomers from micelles into to the serum phase (Dauphas et al., 2005; Rose, 1968). β-CN can be manufactured at relatively high purity (70–80% of total protein) by cold MF of liquid MCC (Christensen & Holst, 2014) or warm MF (>26 °C) of the β-casein-enriched whey permeate generated from cold MF of milk; however, for the production of WP-dominant IMFs pure β-casein is not an essential requirement, as the presence of WPs in the ingredient is likely preferable.

Most of the MF processes that have been described for β-CN enrichment have used cold temperatures and polymeric membranes, and have been associated with low permeate flux values, due to a combination of high feed viscosity and severe fouling of the membrane. Strategies to improve flux are primarily restricted to adjusting the type of membrane; for example, it has been shown that flux-enhancement in a cold MF process can be achieved by using PES rather than PVDF membranes (Crowley et al., 2015). However, it can be predicted that a switch to ceramic membranes would facilitate operation at much higher flux values. While the surface area of ceramic membrane configurations may not be as large as for spiral-wound polymeric systems, they have the advantage of being compatible with technology designed to ensure a consistent trans-membrane pressure (TMP) across the length of the membrane. An example of this technology is the Isoflux® membrane, in which the active layer decreases in thickness from inlet to outlet, so that the pressure:thickness ratio, and therefore flux, is theoretically constant along the hydraulic path (Adams & Barbano, 2013).

The objective of this study was to produce an ingredient from skim milk for application as a protein base during IMF manufacture. The target protein profile for the ingredient was a CN:WP ratio close to that of human milk, in which the CN fraction consisted primarily of β-CN. The ingredient was prepared using MF and DF of skim milk at low temperatures using ceramic Isoflux® membranes, followed by concentration using UF. A cold MF process was carried out and compared with the more traditional warm MF in terms of process performance (i.e., permeate flux) and nutrient partitioning (i.e., minerals, proteins, non-protein nitrogen and fat).

2. Materials and methods

2.1. Materials

Raw bovine whole milk was obtained from the Teagasc Grassland Research Centre, with the fat separated centrifugally at 50 °C using facilities at Moorepark Technology Limited (Fermoy, Co. Cork, Ireland). Urea, bis-tris propane, and 2-mercaptoethanol were obtained from Sigma (Wicklow, Ireland). The electrophoresis chemicals were obtained from Bio-Rad (Fannin, Dublin, Ireland). All other chemicals used were of analytical grade. Milli-Q water (Millipore, Ireland) was used for all solutions.

2.2. Compositional analysis

Total nitrogen, non-protein nitrogen and true protein were determined using the Kjeldahl method (ISO, 2001a,b,c), and a nitrogen-protein conversion factor of

144 6.38. Fat content was determined using the Gerber method (IDF, 1991). Mineral
145 analysis was determined by an Agilent 7700s inductively-coupled plasma mass
146 spectrometry (ICP-MS) (Agilent Technologies, Santa Clara, California, USA).
147
148 *2.3. Pilot-scale membrane filtration and powder manufacture*
149
150 Skim milk (300 kg) was diluted prior to filtration by adding 600 kg of reverse
151 osmosis (RO) water. In-house testing has shown that diluting unheated skim milk
152 prior to filtration increased the dissociation and solubilisation of β-casein. The diluted
153 skim milk was held at ~4 °C for 16 h, before holding at 8.9 °C or heating to 50 °C
154 prior to membrane filtration. The diluted skim milk was subject to MF using 0.14 μm
155 pore size Tami Isoflux® ceramic membranes (Tami Industries, Nyons Cedex, France)
156 on a GEA Model F filtration unit (GEA Process Engineering A/S, Skanderborg,
157 Denmark) with complete retentate recycling (i.e., retentate is returned to the feed).
158 Three ceramic membranes were used in parallel, each with an area of 0.35 m^{-2}. The
159 temperature throughout processing was maintained at 8.9 ± 1.2 °C or 50 ± 1.0 °C
160 using an in-line heat exchanger. The feed recirculation rate was adjusted to 1500 L h^{-1}
161 at a feed pressure of 1 bar and a membrane inlet pressure of 2.7 bar (0.9 bar per
162 membrane element). The permeate flux was measured gravimetrically throughout
163 filtration until a volume concentration factor (VCF) of 3 was reached. VCF was
164 calculated by dividing the feed volume (V_f) by the final retentate volume (V_r):

165 Volume concentration factor $VCF = \frac{V_f}{V_r}$ (1)

166 All permeate streams from MF were subsequently subjected to UF with two
167 10 kDa cut-off Synder spiral-wound membranes (Synder Filtration, CA, USA)

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168 arranged in series in the Model F filtration unit. UF was performed at a temperature of
169 50 ± 1.0 °C for all trials with a feed recirculation rate of 1600 L h^{-1} at 1 bar pressure
170 and a membrane inlet pressure of 1.8 bar. A final VCF of 14 was attained. Permeation
171 behaviour of individual proteins through MF membranes were characterised using the
172 sieving coefficient (S_o).

173 $$S_o = \frac{C_P}{C_R} \quad (2)$$

174 where C_p and C_R are the simultaneous concentration of the protein in the permeate and
175 retentate, respectively.

176 Membrane filtration performance was monitored using permeate flux
177 measurements taken during each MF concentration run (Fig. 1A); solids content of the
178 feed was also monitored (Fig. 2B); the starting feed material was skim milk (9.3%
179 solids) diluted to ~3.1% solids with RO water. The solids content and pH of the
180 original skim milk and rehydrated powders (protein content 3.3%, w/w) were
181 measured using a CEM Smart Trac moisture analyser (Damastown, Dublin, Ireland)
182 and a WTW 3310 pH meter (WTW, Weilheim, Germany), respectively.

183 Retentate streams obtained from MF and UF were heated to 30 °C prior to
184 evaporation using a Tetra Scheffers™ single-stage falling-film evaporator operated at
185 60 °C (Tetra Pak, Gorredijk, The Netherlands). The concentrate was spray-dried using
186 a pilot scale Anhydro Lab 3 spray dryer (SPX Flow Technology A/S, Soeborg,
187 Denmark), equipped with a wheel atomiser. Inlet and outlet temperatures were set at
188 178 °C and 88 °C, respectively. Powder samples were coded as follows: MFR_{warm},
189 dried retentate from MF of skim milk at 50 °C; MFR_{cold}, dried retentate from MF of
190 skim milk at 8.9 °C; UFR_{warm}, dried retentate from UF of warm (50 °C) MF permeate;
191 UFR_{cold}, dried retentate from UF of cold (8.9 °C) MF permeate.

Membrane filtration processes at 50 and 4 °C were carried out in triplicate from a total of six different batches of skim milk, producing three independent powder samples for each of MFR_{warm}, MFR_{cold}, UFR_{warm} and UFR_{cold}.

2.4. Protein profile analysis

Protein profile analysis of MF and UF retentate powder samples was carried out using reverse phase-high performance liquid chromatography (RP-HPLC) and sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) to quantify individual protein fractions and to observe the presence of minor whey protein components, respectively.

2.4.1. Reversed phase-high performance liquid chromatography

All MF retentate and UF retentate powder samples were dispersed in buffer containing 7 M urea, 20 mM bis-tris propane and 71.5 mM 2-mercaptoethanol (pH 7.5) in a 1:20 ratio (v/v) at room temperature. Each sample was incubated for 1 h at room temperature before being filtered (pore size of 0.22 µm). A 5 µL aliquot of each sample was injected three times into a Poroshell 300SB-C18 (Size: 2.1 × 7.5 mm, 5 µm; Agilent Technologies, Ireland) column equipped with a Zorbax poroshell guard column (Size: 1.0 × 17 mm, 5 µm; Agilent Technologies). The HPLC was equipped with a UV-vis detector (61365D MWD Agilent Technologies 1200 series). The column was operated at temperature 35 °C at a flow rate of 0.5 mL min^{-1} and was equilibrated in 74% solvent A (0.1% trifluoroacetic acid and 10% acetonitrile in MilliQ water) and 26% B (0.1% trifluoroacetic acid and 10% MilliQ water in acetonitrile). A series of linear gradients were then applied by raising the

concentration of solvent B to 37% over 10 min, then to 45% over 23 min, and to 100% over 3.5 min. Solvent B was held at 100% for 4.5 min before reducing its level over 34 min back to the initial level of 26% where it was held for 2.5 min. Detection was by absorbance at 214 nm and total run time was 36.5 min per sample. HPLC analysis was carried out in triplicate

2.4.2. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis

SDS-PAGE analysis was performed based on the methods of Laemmli (1970) and modified as described by Oldfield, Singh, Taylor, and Pearce (2000). Reducing SDS-PAGE was run with the addition of 2-mercaptoethanol, with heating at 95 °C for 5 min. Gels were run on a Mini-Protean III dual cell system (Bio-Rad Laboratories, Hercules, CA, USA). Mini-Protean TGX gels (anyKda, 10 wells; Bio-Rad) were used in the analyses. MF retentate and UF retentate powder samples were diluted to 0.13% (w/w, protein basis) with the appropriate sample buffer before loading. Subsequently, the corresponding sub-samples were loaded accurately (10 µL per well) into the sample wells using a micro-pipette. Electrophoresis was carried out at 200 V. The bands were stained with 0.1% (w/v) Coomassie Brilliant Blue G250 in 34% methanol for 24 h and destained in a solution containing 10% (v/v) glacial acetic acid and 10% (v/v) isopropanol. The destaining solution was changed every 4 h to obtain a light background on the gel. Destained gels were scanned on Epson Scan Perfection V700 Photo scanner (Epson, Hertfordshire, UK) and the protein bands were identified using the software program, ImageQuant (GEHealth care, Ireland).

2.5. Statistical analysis

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Fisher's one-way multiple-comparison test was used as a guide for pair comparisons of the mean permeate flux values and the powder compositional data produced from membrane filtration. The level of significance was determined at $P < 0.05$ using Minitab 17 (Minitab Ltd, Coventry, UK) statistical analysis package and the effects of treatment and replicates were estimated for each response variable.

3. Results and discussion

3.1. Membrane filtration process performance

The performance of the MF process under warm (50 °C) and cold (8.9 °C) conditions can be compared in Fig. 1A. Pre-dilution of the skim milk 16 h prior to MF was performed to promote β-CN dissociation. Low temperature increases serum-phase β-CN by reducing hydrophobic interactions, while both cold processing and milk dilution partially dissolve colloidal calcium phosphate (CCP), further increasing transmission of β-CN (Broyard & Gaucheron, 2015). Initial permeate flux (time 0) of ~380 L m^{-2} h^{-1} for warm MF rapidly decreased to ~307 L m^{-2} h^{-1} after 0.25 h; flux remained relatively constant at this value until ~1.7 h of processing and a solids content of ~4.5% when a sudden and dramatic decline in flux to <150 L m^{-2} h^{-1} occurred (Fig. 1A). Once the retentate reached ~75% protein (w/w, dry matter) the MF rig was shut down, coinciding with a total process time of ~3 h and a final solids content of 12.2%, w/w. Previous studies for the MF of skim milk at 50 °C have reported much lower permeate flux values than those measured in the current study; for example, Hurt, Zulewska, Newbold, and Barbano (2010) obtained a constant permeate flux for skim milk of ~54.0 L m^{-2} h^{-1} using ceramic membranes with a pore-

size of 0.1 µm, operated under uniform trans-membrane pressure (0.42 bar) at 50 °C. The high flux rates shown in Fig. 1 are probably due to a combination of a high retentate flow rate (1500 L h^{-1}) and the use of highly dilute skim milk as the feed. MF carried out at 8.9 °C had a significantly ($P < 0.05$) lower initial permeate flux (~98 L m^{-2} h^{-1}; Fig. 1A), compared with MF at 50 °C (~380 L m^{-2} h^{-1}; Fig. 1A). Permeate flux continued to decrease in a linear manner throughout filtration (Fig. 1A), reaching a value of 52 L m^{-2} h^{-1} after ~13 h.

Although the average permeate flux was much lower for cold MF compared with the warm MF process, the extent of flux decline was much less for the former, due to reduced fouling (Fig. 1), in agreement with other research demonstrating the benefits of cold membrane processing. Luo, Ramchandran, and Vasiljevic (2015) compared the performance of UF membranes during processing of milk at 15, 30 or 50 °C and found a more rapid flux decline at the highest UF temperature due to increased protein- and calcium-based fouling. Similarly, Méthot-Hains et al. (2016) found that flux decline during UF of skim milk was more rapid at 50 °C than at 10 °C as the VCF increased from 1 to 4. The flux values shown in the present work for cold MF were significantly higher than values reported in previous studies for cold MF processes; although, diafiltration water was added upfront to the skim milk in the current study, which would significantly increase permeate flux. For example, O'Mahony et al. (2014) obtained an initial permeate flux of ~10 L m^{-2} h^{-1} during the enrichment of β-CN from skim milk using polymeric membranes at 3–6 °C.

3.2. Composition and physicochemical properties of dried retentates

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291 The composition and physicochemical properties of MF and UF retentate
292 powders after spray drying are shown in Table 1. Fat content increased in the MF
293 retentates compared with the original skim milk, with very low levels of fat present in
294 the UF retentates. There was a significantly ($P < 0.05$) lower fat content in UFR_{cold},
295 indicating that milk fat had lower transmission at the low temperature during cold
296 MF.

297 The mineral composition of powders from MF and UF retentate streams are
298 given in Table 3. Calcium and phosphorous levels decreased in MFR_{cold} compared
299 with MFR_{warm}; in turn, UFR_{cold} had a higher calcium and phosphorous content
300 compared with the UFR_{warm} powder. Similarly, Méthot-Hains et al. (2016) found that
301 the permeation of calcium increased during UF of milk with 10 kDa PES spiral-
302 wound membranes at 10 °C compared with 50 °C, due to an increase in soluble
303 calcium at low temperatures. Magnesium, sodium and potassium were all
304 significantly ($P < 0.05$) higher in UF retentates compared with MF retentate powders,
305 while the temperature of MF processing had no significant effect ($P > 0.05$) on their
306 transmission (Table 3). Therefore, MF temperature can be said to have only had an
307 effect on the major components of CCP. The pH of MF and UF retentate streams were
308 significantly ($P < 0.05$) higher than that of the diluted skim milk, likely due to the loss
309 of these soluble minerals along with citric acid that would otherwise act as buffering
310 salts (Table 1). Hurt et al. (2010) found a similar result during the MF of skim milk at
311 50 °C, when the pH of the retentate fraction increased from pH 6.6 to pH 7.0.
312
313 *3.3. Protein profile of retentate streams from microfiltration and ultrafiltration*
314
315 *3.3.1. β-Casein transmission and casein:whey protein ratio*

316 MF of skim milk at 50 or 8.9 °C resulted in very different protein profiles
317 (Table 2). MF of skim milk at 50 °C facilitated extensive depletion of whey proteins
318 with almost complete retention of casein (Fig. 2; lane 3 MFR_{warm}). UFR_{warm} powders
319 contained primarily whey proteins (91.2% of protein) and a minor quantity of β-CN
320 (8.81% of protein). SDS-PAGE profiles (Fig. 2; lane 1 UFR_{warm}) and HPLC
321 chromatograms (Fig 3; profile E) indicated the presence of some β-CN in UFR_{warm}
322 powders, quantified at 8.81% of total protein, with the remaining 91.2% consisting of
323 WPs (Table 2). The average So of β-CN during MF at 50 °C was 0.07, which was
324 significantly lower than the value of 0.26 measured during MF at 8.9 °C (Table 2; Fig.
325 3E).

326 Due to the high So values reached for β-CN and whey proteins during cold
327 MF the UFR_{cold} powder had a CN:WP ratio of 35:65 with 100% of the CN comprised
328 of β-CN (Table 1); this protein profile is close to that found in human milk and
329 compares favourably in this respect with previous reports of MF processes involving
330 similar pore-size membranes. For example, Woychik (1992) enriched β-CN using 0.1
331 µm MF membranes at 4 °C; however, the β-CN purity was low (55% of total CN) and
332 the system was CN-dominant (55:45 CN:WP). Glas, te Biesebeke, Kromkamp and
333 Klarenbeek (2013) reported enriching β-CN from skim milk using MF with 0.15 µm
334 membranes at 10 °C, which resulted in a low CN:WP ratio of 14:86 and a high β-CN
335 purity of 95% (total CN basis). Crowley et al. (2015) measured a β-CN purity of
336 100% and a CN:WP ratio of 49:51 when a permeate generated at <4 °C using a 0.1
337 µm MF membrane was analysed. The process described in the present study achieves
338 a balance of appropriate CN:WP ratio and excellent β-CN purity (confirmed using
339 Maldi-TOF analysis; results not shown).
340

341 *3.3.2. Whey protein transmission and proteolysis products*

342 Cold MF resulted in greater retention of β-lactoglobulin (Fig. 2; lane 4),
343 compared with warm MF (Fig. 2; lane 3). Gésan-Guiziou, Daufin, and Boyaval
344 (2000) studied the factors affecting the transmission of β-lactoglobulin during MF of
345 skim milk at 50 °C and showed that above the critical flux (i.e., pressure independent
346 region) β-lactoglobulin transmission decreased significantly, due to modified fouling
347 layer characteristics. The hindered protein transmission observed for cold MF in this
348 study may also be caused by altered sieving characteristics due to an altered fouling
349 layer; the increased hydrodynamic volume of the micellar phase at low temperatures
350 (Liu, Weeks, Dunstan, & Martin, 2013) could be a possible explanation for this effect.

351 The detection of a band with a molecular mass of ~12 kDa in SDS-PAGE
352 analysis of MF retentate streams may be the result of casein proteolysis occurring
353 during membrane filtration (Fig. 2; lanes 3 and 4). Its absence from UF retentate
354 powders indicated that, while lower in molecular mass than α-lactalbumin, it may be
355 associated with the CN micelle and thus not be transmittable during MF. The band
356 appeared fainter in MFR_{cold} powders (Fig. 2; lane 4) than in MFR_{warm} powders (Fig. 2;
357 lane 3), indicating that proteolysis may have been more prevalent at 50 °C than at 8.9
358 °C. Hurt et al. (2010) found the presence of a similar band in SDS-PAGE gels of MF
359 retentate streams after filtration of skim milk at 50 °C using 0.1 µm pore sized UTP
360 ceramic membranes. Jost, Brandsma, and Rizvi (1999) identified the band as proteose
361 peptone component 5 (β-casein 1–105/1–107; Andrews, 1978) and suggested that the
362 phosphoprotein remains associated with the casein micelle during MF and therefore
363 does not permeate 0.1 µm MF membranes even though its molecular mass is much
364 less.

365

4. Conclusion

Cold MF and DF facilitated the formulation of a protein base with a casein profile close to human milk using a skim milk feed. Using this process it was demonstrated that two major formulation targets, a CN:WP ratio (40:60) and CN profile (β-CN), can be achieved. Other potential benefits of the cold MF and DF process were also identified, ranging from a reduction in β-lactoglobulin levels and reduced in-process proteolysis (lower levels of casein hydrolysis products). The manufacture of suitable infant formula protein base ingredients using integrated membrane systems will be an important development in next-generation IMF processing. Future work could also focus on the re-micellisation of β-CN through addition of κ-casein, to more closely mimic the protein profile of human milk while increasing heat stability.

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Figure legends

Fig. 1. Membrane filtration time of microfiltered diluted skim milk, measured as (A) a function of permeate flux (L m^{-2} h^{-1}) and (B) total solids at 50 °C (□) and 8.9 °C (■).

Fig. 2. Reducing SDS-PAGE patterns (4–20% acrylamide gel) of retentate powders from microfiltered and ultrafiltered skim milk. Lane 1, UF retentate produced after microfiltration at 50 °C (UFR_{warm}); lane 2, UF retentate produced after microfiltration at 8.9 °C (UFR_{cold}); lane 3, MF retentate produced by microfiltration at 50 °C (MFR_{warm}); lane 4, MF retentate produced by microfiltration at 8.9 °C (MFR_{cold}); lane 5, α-lactalbumin standard; lane 6, β-lactoglobulin standard; lane 7, lactoferrin standard.

Fig. 3. Reversed-phase HPLC profiles of (A) skim milk, (B) microfiltration retentate at 50 °C (MFR_{warm}), (C) microfiltration retentate at 8.9 °C (MFR_{cold}), (D) UF retentate produced after microfiltration at 8.9 °C (UFR_{cold}) and (E) UF retentate produced after microfiltration at 50 °C (UFR_{warm}); eluate was measured at 214 nm.

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Table 1

Composition of microfiltration and ultrafiltration retentates after processing at 50 or 8.9 °C.[a]

Parameter	Skim milk	MFR_{warm}	MFR_{cold}	UFR_{warm}	UFR_{cold}
Protein (%, w/w, dry basis)					
TPN	37.8 ± 2.1	74.3 ± 0.8	72.1 ± 2.2	44.2 ± 1.0	34.4 ± 2.2
NPN	0.03 ± 0.0	0.10 ± 0.0	0.01± 0.0	0.03 ± 0.0	0.03 ± 0.0
Fat (%, w/w, dry basis)	0.01 ± 0.0	0.81 ± 0.9	0.80 ± 0.6	0.40 ± 0.1	0.24 ± 0.2
Moisture content (%, w/w)		4.37 ± 0.8	4.69 ± 0.8	2.58 ± 0.5	2.21 ± 0.7
pH	6.64 ± 0.05	7.30 ± 0.1	7.22 ± 0.1	7.06 ± 0.0	7.08 ± 0.1

[a] Abbreviations are: MFR_{warm}, retentate of microfiltration performed at 50 °C; MFR_{cold}, retentate of microfiltration performed at 8.9 °C; UFR_{warm}, ultrafiltration retentate after warm (50 °C) microfiltration; UFR_{cold}, ultrafiltration retentate after cold (8.9 °C) microfiltration; TPN, true protein nitrogen; NPN, non-protein nitrogen.

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Table 2

Protein profile of ultrafiltered retentate powders after processing at 50 or 8.9 °C. [a]

Protein	Skim milk	UFR_{warm}	UFR_{cold}
Total casein (%, w/w, of TP)	85.9[a]	8.81[b]	35.5[c]
α_S-Casein (%, w/w, of TC)	50.1[a]	0.00[b]	0.00[c]
β-Casein (%, w/w, of TC)	39.6[a]	8.81[b]	35.5[c]
κ-Casein (%, w/w, of TC)	10.3[a]	0.00[b]	0.00[c]
Total whey protein (%, w/w, of TP)	15.6[a]	91.2[b]	64.5[c]
α-La (%, w/w, of TW)	17.7[a]	19.1[b]	17.9[c]
β-Lg (%, w/w, TW)	82.3[a]	72.0[b]	46.6[c]

[a] Abbreviations are: UFR_{warm}, ultrafiltration retentate after warm (50 °C) microfiltration; UFR_{cold}, ultrafiltration retentate after cold (8.9 °C) microfiltration; α-La, α-lactalbumin; β-Lg, β-lactoglobulin; TP, total protein; TC, total casein; TW, total whey protein. Values are the means of 3 replicates ± standard deviations; values within a row not sharing a common superscript letter differ significantly, $P < 0.05$.

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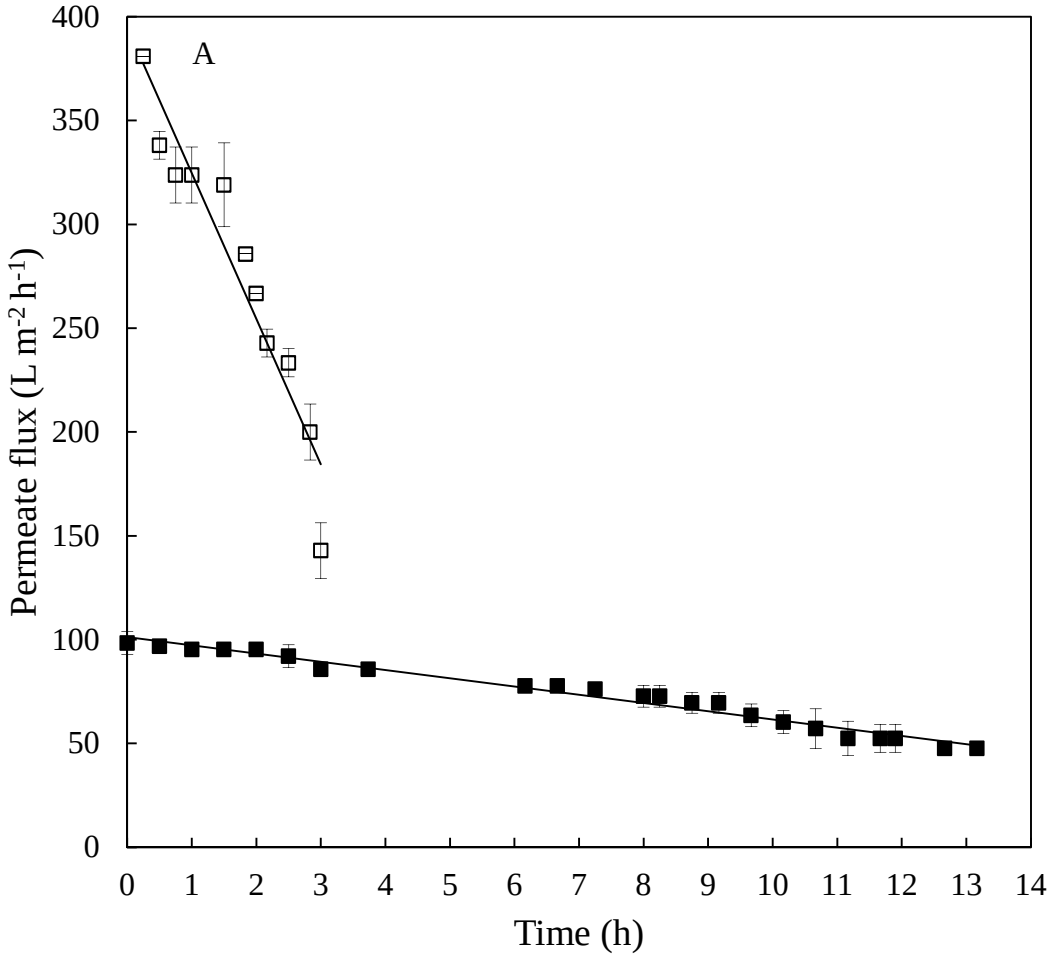
Table 3

Mineral profile of microfiltration and ultrafiltration retentate powders obtained at 50 or 8.9 °C. [a]

Process	Calcium	Magnesium	Sodium	Phosphorous	Potassium
MFR_{warm}	35.6[a]	1.65[a]	2.22[a]	24.2[a]	10.3[a]
UFR_{warm}	24.2[b]	4.34[b]	23.8[b]	20.6[b]	70.4[b]
MFR_{cold}	30.9[c]	1.81[a]	2.84[a]	22.7[a]	12.2[a]
UFR_{cold}	29.2[d]	4.44[b]	31.4[b]	27.5[c]	66.3[b]

[a] MFR_{warm}, microfiltration retentate performed at 50 °C; MFR_{cold}, microfiltration retentate performed at 8.9 °C; UFR_{warm}, ultrafiltration retentate after warm (50 °C) microfiltration; UFR_{cold}, ultrafiltration retentate after cold (8.9 °C) microfiltration. All ultrafiltration processing was performed at 50 °C. Values (mg g^{-1} of protein) are the means of 3 replicates ± standard deviations; values within a column not sharing a common superscript letter differ significantly, $P < 0.05$.

1


A
Permeate flux (L m-2 h-1)
Time (h)
0
50
100
150
200
250
300
350
400
0
1
2
3
4
5
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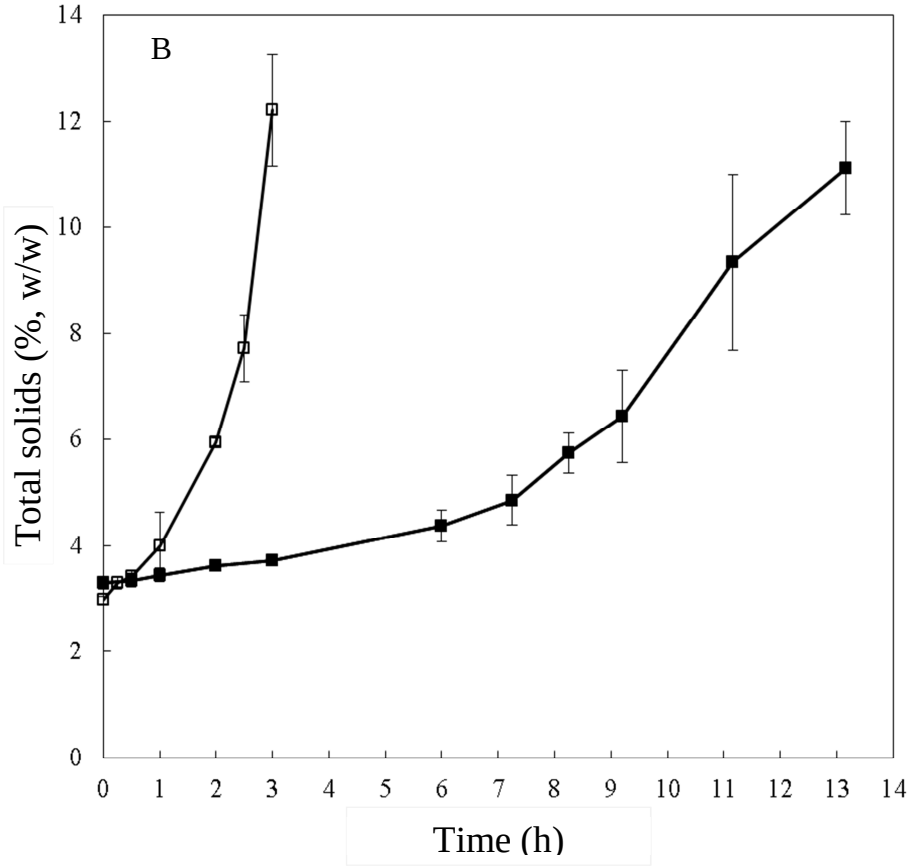


Fig. 1.

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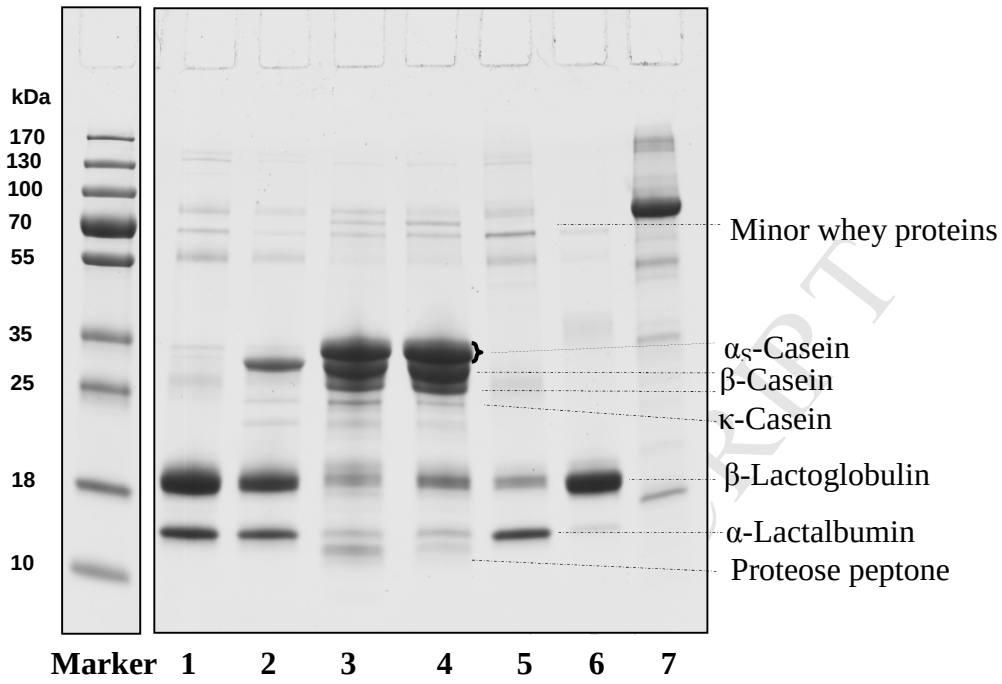


Fig. 2

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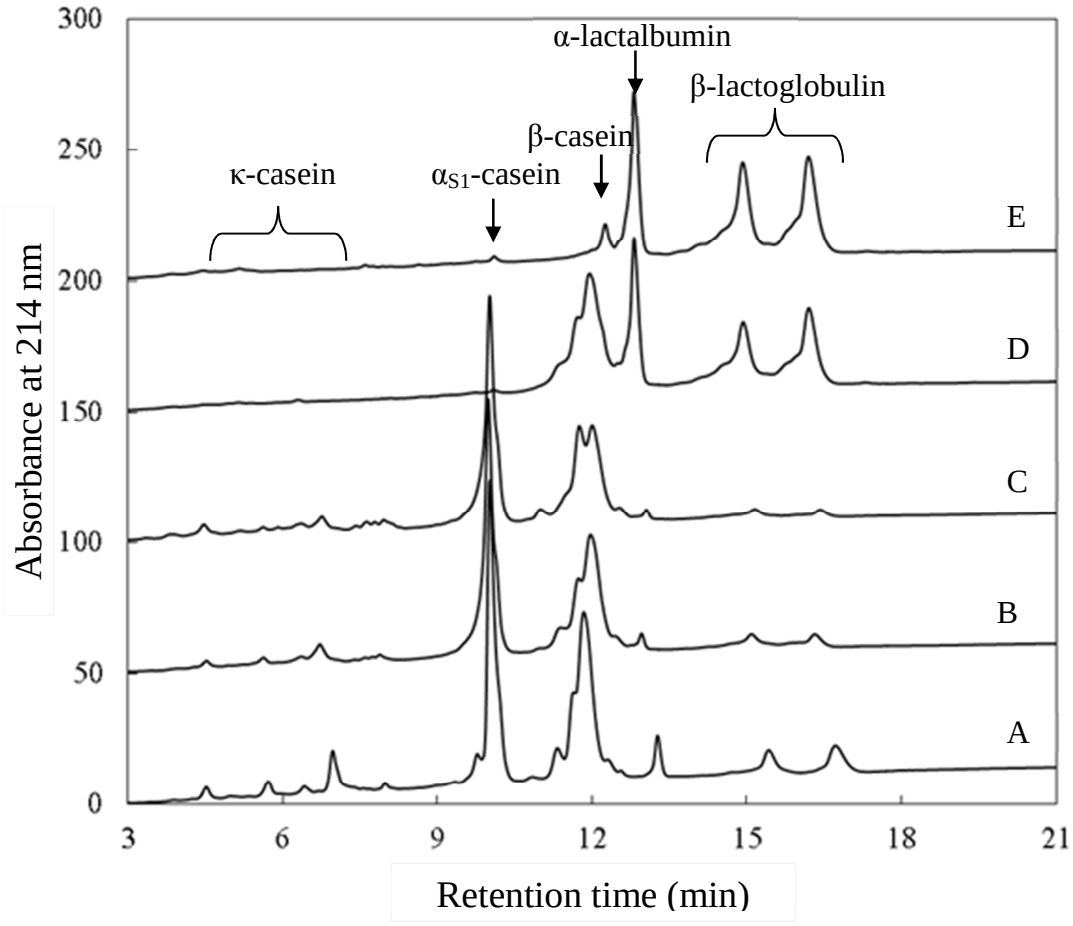


Fig. 3.

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