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Microfiltration of raw milk for production of high-purity milk fat globule membrane material

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ABSTRACT

Commercial ingredients containing milk fat globule membrane (MFGM) material are currently isolated from heavily processed dairy streams. The aim of this study was to achieve a more gentle isolation of MFGM material by means of ceramic dia-microfiltration of raw whole milk to separate fat globules from casein micelles and whey proteins prior to MFGM extraction. A pilot-scale experiment with 1.4 µm pore size (membrane surface area 1.05 m^2) resulted in an optimal outcome of low permeation of fat (2.5% permeation) and high permeation of proteins (97% permeation). This yielded an MFGM isolate with 7% w/w polar lipids and 30% w/w proteins, where contamination of non-MFGM proteins was only 25% of total protein content. Furthermore, mild pasteurization (72 °C, 15 s) introduced either before or after microfiltration had no impact on filtration efficiency or MFGM yield and composition. The work describes an industrially relevant production method for a less-processed MFGM material of high purity with potential for further separation and valorisation of protein-rich permeate streams.

INTRODUCTION

The milk fat globule membrane (MFGM) is a unique biological assembly, which surrounds and stabilises fat globules in milk. Its main constituents include proteins specific to the MFGM, most notably periodic acid Schiff base 6 and 7 (PAS 6/7, also known as lactadherin), xanthine oxidase, butyrophilin and proteose peptone 3 (PP3), and polar lipids, mainly sphingomyelin (SM) and phosphatidylcholine (PC), but also phosphatidylethanolamine (PE), phosphatidylserine (PS) and phosphatidylinositol (PI) (Dewettinck et al., 2008). The polar lipids form a tri-layered membrane around the milk fat globule, wherein the proteins are distributed with varying affinities to the membrane.

A wide range of bioactivities have been ascribed to components of MFGM, including anti-cancer, immunoregulatory, anti-adhesive properties towards gut bacteria, and stimulation of neural development (reviewed by Dewettinck et al., 2008). The latter bioactivity has been attributed to the nutritional value of the polar lipid content of MFGM and has spawned considerable interest in adding MFGM-containing material to infant formula in order to minimise differences in cognitive development between breast- and formula-fed babies (Anderson et al., 1999; Timby et al., 2014). Not only the total content of polar lipids, but also its specific composition, have shown to influence infant brain development. Deoni et al. (2017) compared three different infant formulas and found that a high content of SM and PC correlated with higher levels of de novo myelination in different brain regions as well as positive cognitive outcomes. The fact that SM is not present in lipids of vegetable origin (e.g. soy lecithin) has further elevated the valorisation potential of MFGM as an ingredient (Vanderghem et al., 2010).

In addition to its nutritional value, MFGM has potential in terms of functionality, especially its emulsifying capacity. Phan et al. (2016) separated MFGM polar lipids from MFGM proteins and tested the emulsifying capacity of both fractions separately and in combination in an oil-in-water emulsion. Interestingly, the researchers found that the MFGM proteins adsorbed more readily to the oil-water interphase than the polar lipid fraction. Moreover, emulsions formed by a combination of 0.3% polar lipids and 0.3% proteins did not exhibit less phase separation than one formed by 0.3% protein alone. A

similar study (He et al., 2017) demonstrated that MFGM polar lipids alone could not stabilize oil-in-water emulsions. Addition of MFGM proteins was shown to be necessary in order to achieve efficient emulsification and further that MFGM protein composition played an important role.

Both the polar lipid and protein content as well as the composition of the MFGM material influence its bioactivity and functionality. The protein profile of MFGM material is known to be affected by up-stream processing such as pasteurization or cream washing. Pasteurization can induce covalent cross-linking between MFGM proteins and β-lactoglobulin from the milk serum phase (Corredig and Dalgleish, 1996; Ye et al., 2002), whereas the shear stress induced during extensive cream washing results in the release of entire membrane fragments from the milk fat globule surface, including indigenous MFGM proteins and polar lipids (Holzmüller et al., 2016). Furthermore, mechanical stress during pumping, stirring or homogenization can induce association of casein micelles and whey proteins to the surface of fat globules in lieu of membrane fragments (Michalski et al., 2002). Nonetheless, most studies using microfiltration to obtain pure MFGM are based on heavily-processed dairy side-streams as starting materials, e.g. buttermilk from butter production (Astaire et al., 2003; Barry et al., 2017; Le et al., 2013; Morin et al., 2007) or butter serum from the production of anhydrous milk fat (Gassi et al., 2016; Rombaut et al., 2006). The isolation and purification of MFGM material from unprocessed raw milk using microfiltration has received relatively little research attention. Jukkola et al. (2016) subjected raw milk to 1.4 µm ceramic membrane filtration and separated 88% of all milk proteins from the fat phase, whilst losing only 3% of the fat. The same authors achieved 93% protein removal by a three-stage dilution and concentration of pasteurized cream (Jukkola et al., 2019), however the authors focused on butter production and not on MFGM, which was not analyzed and was discarded with the buttermilk.

Our research group has shown that diafiltration of raw milk using 0.65 µm polymeric membranes is a gentle processing approach for isolation of MFGM with low levels of contamination from serum proteins, if pasteurization occurred after, rather than before, filtration (Hansen et al., 2018). The main aim of this study was to obtain MFGM material by pilot-scale ceramic microfiltration of raw milk. Ceramic

membranes are much preferred due to their endurance and higher tolerance towards pressure, pH and cleaning agents (Crowley et al., 2015). We hypothesized that optimisation of pore size and process parameters during filtration would result in a high-purity MFGM material. First, fat globules were sseparated from skim milk proteins on a lab-scale filtration unit using two different membrane pore sizes. Secondly, the best-performing pore size was chosen for pilot-scale filtration. At this scale, the impact of pasteurization, before or after filtration, was evaluated based upon the purity of MFGM material in combination with both protein and polar lipid composition.

MATERIALS AND METHODS

Materials

Raw bovine milk was collected from the Teagasc dairy herd (Moorepark, Fermoy, Co. Cork, Ireland). Chemicals for gel electrophoresis were purchased from Biosciences Ltd (Dublin, Ireland). All other chemicals were obtained from Sigma Aldrich (Arklow, Ireland), except where noted. Reverse osmosis water was used as diafiltration medium, and MilliQ water was used for preparation of all other solutions.

Methods

Lab-scale experiment

Tubular ceramic membranes (TAMI Industries, Nyons Cedex, France; Ø = 10 mm, L = 600 mm, surface area = 0.02 m2) of two different pore sizes, 0.8 µm or 1.4 µm, were fitted vertically in a bespoke lab-scale microfiltration unit equipped with an inline heat exchanger and two variable speed, centrifugal pumps, capable of replicating flow conditions in subsequent larger scale trials. Clean-in-place (CIP) was carried out pre- and post-run by recirculating a 0.1% caustic solution (Ecolab Ltd., Wicklow, Ireland) for 20 min at 50 °C.

Fresh milk (3 L) was heated to 50 °C prior to microfiltration. Initially, milk was recirculated for 30 min with both retentate and permeate valves open to allow stabilization of the system to 50 ± 2 °C. The transmembrane pressure (TMP) was set at 1.2 bar for 0.8 µm, but was lowered to 0.5 bar for 1.4 µm microfiltration to avoid premature fouling of the membrane (Morin et al., 2004).

The diafiltration process was commenced by collecting permeate stream in a separate tank, whilst demineralised water was added to the feed tank maintaining a constant feed volume. Permeate flux was measured at regular intervals using a graduated cylinder. After permeation of 200% of the initial feed volume, i.e. two diafiltration steps, the retentate was pumped out of the system and stored over-night at 4°C.

Scale up of the filtration process

Pilot-scale trials were carried out on a Model F multi-membrane filtration plant (GEA Process Engineering A/S, Skanderborg, Denmark). Tubular ceramic membranes of pore size 1.4 µm with 1.05 m^2 surface area (TAMI Industries) were fitted, and the system was cleaned-in-place according to manufacturer's specification. A pasteurization step was implemented either before or after filtration, and 50 L of raw milk (in the case of pre-filtration pasteurization) or filtration retentate (in the case of post-filtration pasteurization) were subjected to a 72 °C, 15 s pasteurization using a UHT/HTST pilot scale plate heat exchanger (Microthermics, Raleigh, NC, USA). Diafiltration was run at 50 °C at a TMP of 2 bar, until four volumes (400%) of the original feed volume had been replaced by RO water.

MFGM material isolation

Further purification of MFGM materials was carried out using a modified version of the procedure described by Hansen et al. (2018). After filtration, retentate was separated into cream and serum phases by centrifugation using an Armfield disc bowl centrifuge (Armfield, Ringwood, UK) at 50 °C. This was also carried out on non-filtered raw milk, as a control. The serum phase was skimmed a second time to increase cream yield. The cream phase was stored overnight at 4 °C before churning using a domestic blender, during which most of the MFGM fragments were released from the fat globules (butter phase) and transferred to the buttermilk phase. Butter granules were melted at 60 °C in a water bath to expel any inherent aqueous phase (butter serum), which was collected after crystallization of the fat phase at 4 °C. Buttermilk and butter serum fractions were pooled and adjusted (by addition of 1 M HCl) to pH 4.8, the isolectric point of MFGM proteins (Kanno and Kim, 1990). MFGM material was separated at 17,900 x g for 80 min at using a Sorvall Lynx 6000 centrifuge operating at 4 °C (Fisher Scientific, Dublin, Ireland). The subsequent MFGM-containing pellet was freeze-dried (Labconco, Kansas City, MO, USA) and stored at -20 °C until further analysis.

Particle size analysis

Particle size distributions of raw milk, retentates and permeates were determined using a Mastersizer 3000 (Malvern Instruments, Malvern, UK) with particle diffraction index of 1.458 against 1.33 of water (Michalski et al., 2001). Samples were measured in triplicate and compared by their volume-weighted particle size distribution and mean diameter (d4,3).

Protein profile analysis

To evaluate the protein permeation behaviors of microfiltration streams, retentate and permeate were analysed by bis-tris sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Protein solutions (1% w/w) were mixed at a 1:1 volume ratio with Nu-PAGE LDS sample buffer with reducing agent, 50 mM dithiothreitol (Biosciences Ltd., Dublin, Ireland). Approximately 5 µg protein was loaded into each well on a 12% Nu-PAGE cassette (Biosciences), which was submerged in 5% Nu-PAGE MOPS (3-morpholinopropane-1-sulfonic acid) running buffer, and separated according to electrophoretic mobility for 50 min at 125 mA.

Freeze-dried MFGM preparations were dissolved and analyzed as previously described (Hansen et al., 2018). Four MFGM proteins (xanthine oxidase, butyrophilin, PAS 6/7 and proteose peptone 3) as well as individual caseins were identified based on their electrophoretic mobility compared to previously run in-house gel and published gels (Gassi et al., 2016; Hansen et al., 2018). Within each lane, the relative density of protein bands was measured under UV radiation using ImageLab software (Bio-Rad Laboratories, Hercules, CA, USA) and normalized to 100%. The percentage casein obtained was chosen to represent the impurity of MFGM material. Reported protein densities were averaged based on duplicate gels.

Lipid extraction, separation and quantification from MFGM fragments by HPLC-CAD

A modified Folch extraction procedure, described by Rodríguez-Alcalá and Fontecha (2010), was carried out, in triplicate, for lipid extraction of MFGM fragments. Extracts were dissolved in 1 mL chloroform/methanol (95:5, v/v) and aliquots (100 µL) were injected into an UltiMate 3000 HPLC system

coupled to a Corona Veo charged aerosol detector (CAD), (ThermoFisher Scientific, Waltham, MA, USA). A Eurospher ll 100-5Diol 250 x 4 mm column (KNAUER Wissenschaftliche Geräte GmbH, Berlin, Germany) was used for separation using the following solvents: A, chloroform/methanol/water (887.5:12:0.5, v/v/v); B, chloroform/methanol/water (28:60:12, v/v/v); C, isooctane/tetrahydrofuran (99:1, v/v), D, 100% isopropanol. The elution gradient program was as follows: C:D 97:3 at t=0 min, A:B 60:40 at t=51 min, 100% B at t=51.2 min, 100% A at t=51.3, A:D 5:95 at 52 min, C:D 97:3 at t=58.2 min until termination at 72 min. Prior to each sample run, a standard curve was created using serial dilutions of PC (1,2-dioleoyl-sn-glycero-3-phosphocholine), PE (1,2-dioleoyl-sn-glycero-3-phosphoethanolamine), PI (L-alpha-phosphatidylinosotiol from bovine liver) and PS (1,2-dioleoyl-sn-glycero-3-glycero-3-phospho-L-serine) from Avanti (Aalbaster, AL, USA), SM from egg yolk (Fluka, 85615) and cholesterol (C3292) from Sigma (St. Louis, MO, USA). Commercial corn oil was used as a triacylglyceride standard. Each lipid species was quantified by measuring the area under the curve in CAD chromatograms and expressed as % of total polar lipid or % w/w of dry matter MFGM isolate.

Compositional analysis

All samples were analysed for gross composition by standard ISO methods, including the Kjeldahl method for proteins (conversion factor 6.38; ISO, 2014), total solids by oven test (ISO, 2010) and Folch method (Folch et al., 1957) for fat content. Furthermore, the following calculations were made: true protein = (Total N-NPN)*6.38, casein = (Total N-NCN)*6.38 and whey protein = true protein - casein, where NPN is non-protein nitrogen and NCN non-casein nitrogen (Mercier-Bouchard et al., 2017).
For each species, the permeation yield was calculated as follows:

$$\text{Eq. 1} \quad Yield = \frac{m_{perm}}{m_{ret} + m_{perm}} * 100\%,$$

where m_{perm} and m_{ret} denote the mass of each component in the permeate and retentate streams, respectively.

Statistics

All experiments were carried out in duplicate, from which means and standard deviations were calculated. Means were analysed by one-way ANOVA using Duncan's multiple range test for statistical significance ($p<0.05$) in the software R (v. 3.1.1).

RESULTS AND DISCUSSION

To choose the right pore size for separation of proteins from milk fat globules, raw milk was subjected to dia-microfiltration using lab-scale ceramic membranes with pore sizes of 0.8 or 1.4 µm. Briefly summarized, 1.4 µm pore size provided higher flux (109 ± 9.90 L/m^2/h) than 0.8 µm (47.5 ± 17.7 L/m^2/h), as well as a higher permeation yield of skim milk proteins (75 ± 1.9% compared to 9.7 ± 9.5%) in combination with a low permeation yield of fat globules (5.2 ± 1.2% and 0.5 ± 1.2% for 1.4 and 0.8 µm, respectively). Moreover, the MFGM fraction obtained after 1.4 µm microfiltration was numerically less contaminated by caseins than after 0.8 µm, constituting 64 ± 24% and 80 ± 18% of the total MFGM protein composition, respectively. Thus, 1.4 µm was chosen as the most suitable pore size for the pilot-scale experiment.

Pilot-scale filtration of raw milk

Membranes of pore size 1.4 µm were fitted into a pilot-scale filtration plant (surface area 1.24 m2). A mild pasteurization (72 °C, 15 s) was introduced either before or after filtration, and its effects on filtration efficiency and MFGM material composition assessed. Previously, we observed that the order of these processing steps had a major impact on the protein composition of MFGM (Hansen et al., 2018). The extent of filtration was increased from two to four diafiltration steps in order to increase MFMG purity, i.e. lower non-MFGM protein and higher polar lipid contents. Flux was not monitored rigorously, but fluctuated between 500 and 700 L/m2/h, with the volume of diafiltration water in the system. Flux was not affected by position of the pasteurization step. This number was comparable to the 800 L/m2/h obtained by Jukkola et al. (2016) using a 1.4 µm membrane. Pasteurization did not affect fat globule integrity, which was not affected by process-induced destabilization (according to particle size distributions - data not shown).

Fig. 1A shows protein profiles of permeates and retentates following filtration. Permeates had the same protein composition of caseins, α-lactalbumin and β-lactoglobulin as their respective retentates, indicating

non-specific permeation behaviour. On the other hand, certain MFGM proteins, such as XO, were much lower in permeate samples, indicating retention of fat globules and their associated proteins. Moreover, pasteurization history did not affect permeation pattern (Fig. 1A), indicating that a mild pasteurization might be applied at any time in the filtration process without implications for the protein composition of retentates and permeates.

Permeation patterns, in terms of gross composition of permeates and retentates, revealed similar filtration behavior regardless of pasteurization history: high and non-selective permeation of proteins along with low permeation of fat (Fig. 2). Derived permeation yields for each component are given in Table 1, which shows no significant differences between the order of pasteurization before or after filtration. Losses of fat to permeate were 2.6 ± 0.1% and 2.7 ± 0.9% in samples pasteurized before or after filtration, respectively, while the permeation of total protein were 97 ± 0.9% and 97 ± 0.8%, respectively. Regardless of pasteurization history, permeation of proteins was non-selective for caseins and serum proteins (between 97 ± 0.8 and 97 ± 2.3%, and 96 ± 1.9 and 99 ± 1.0%, respectively). Total solids content was reduced by ~80%, with the remaining ~20% being made up of fat. As such, an effective filtration scenario with maximum fat retention (~97%) and protein permeation (~97%) was obtained with four diafiltration steps. Previous attempts to separate fat globules and proteins from raw milk resulted a similar fat retention (97%), but performed lower in terms of protein permeation at 88% (Jukkola et al., 2016). The use of two diafiltration steps only in that study might explain the lower protein permeation ratio.

MFGM material from pilot scale filtration

After filtration, retentate was retrieved from the filtration system and further processed (by skimming, churning, precipitation and freeze drying) into MFGM material before compositional analysis (Table 2). Yield was unaffected by pasteurization order. No significant differences in protein content were seen between different processing orders, with 30 % w/w of the isolate made up by proteins. Of the total protein, the proportion of caseins relative to MFGM proteins was significantly reduced to around 24% of MFGM protein composition compared to unfiltered milk (90% as of Table 2), regardless of pasteurization

history. Still, 24% was a surprisingly high amount considering that 97% of all caseins were transferred to permeate during the preceding filtration (Table 1). Jukkola et al. (2019) observed that even after three microfiltration cycles of cream, 34% of the cream proteins were still made up of caseins, and ascribed this to the pasteurisation step (75 °C, 20 s) preceding microfiltration. While this may be partly true due to slightly higher pasteurization temperature and time, the present results, where pasteurization order showed no effect, suggested an alternative explanation for the contamination of MFGM with caseins. It is possible that the residual 3% of retentate casein micelles had actual diameters above 1.4 μm as a result of micelle clustering (Zulewska et al., 2018), and/or the effective pore size was below 1.4 μm as a result of progressive fouling. Moreover, remaining casein subunits probably dissociated during filtration due to calcium permeation and, hence, could act more powerfully as surfactants on the MFGM surface due to their greater emulsifying capacity than in the micellar state (Ye, 2011). Either way, residual casein probably interacted with the MFGM during the filtration process to such extent that they resisted being washed off the fat globule surface during subsequent diafiltration steps.

Previous results from our group have shown that pasteurizing milk at temperatures above 80 °C before filtration lead to a higher content of β-lactoglobulin in the MFGM due to disulphide-mediated cross-linking to MFGM proteins (Hansen et al., 2018). Fig. 1B does not indicate the same tendency, since neither samples pasteurized before nor after filtration contained detectable amounts of β-lactoglobulin. The less intense heat treatment (72 °C, 15s) employed in this study was probably not sufficient to induce cross-linking between β-lactoglobulin and MFGM proteins. A previous study also found that only at temperatures above 80 °C did this interaction occur (Kim and Jiménez-Flores, 1995).

The order of pasteurization relative to filtration did not affect triglyceride or cholesterol contents of the final MFGM material (Table 2). Likewise, total polar lipid content was unchanged at around 7 % w/w regardless of pasteurization history. This content was comparable to the 6.2 % w/w obtained following ultrafiltration of buttermilk powder (Barry et al., 2017), and 7 % w/w prior to supercritical fluid extraction of whey buttermilk powder (Costa et al., 2010). Gassi et al. (2016) obtained MFGM material with 31.5 %

w/w polar lipids upon ultrafiltration of butter serum, which is a much richer, but also more processed, source of MFGM than raw milk (Dewettinck et al., 2008).

Pasteurizing before or after filtration did not affect polar lipid composition, which was similar to that of unfiltered raw milk (Table 2). Both polar lipid content and its composition are important aspects of MFGM material with regard to use in infant formulas. Recently it was shown that infant formulas with a higher content of SM and PC resulted in more extensive de novo myelination than those containing lower amounts of polar lipids (Deoni et al., 2018). Due to their high contents of SM, PC and PE, the MFGM fragments obtained by the methods described here can be considered suitable for inclusion into infant formula products. Microbial quality would have to be addressed first, since the long residence times typical of industrial filtration at 50 °C could create conditions ideal for bacterial growth. Pasteurization prior to filtration would reduce this risk, but would not inactivate bacterial spores or other thermophilic/thermoduric species, which could start growing during long processing times. Hence, the microbial quality of the final MFGM material remains a topic for future studies.

CONCLUSION

A gentle isolation method for MFGM material based on dia-microfiltration was developed. Pilot-scale filtration with a 1.4 µm membrane with four diafiltration steps resulted in a high-purity MFGM isolate composed of 7% polar lipids and 30% protein, of which 25% were non-MFGM proteins, mainly caseins. The filtration method employed provided a more gentle separation of fat globules compared to methods that use several cream washing steps (Brans et al., 2004). Furthermore, the method is more industrially relevant than cream washing, since it can be applied in-line with subsequent separation of the protein-rich permeate streams into milk protein concentrate or whey protein isolate upon further concentration. The results demonstrated that mild pasteurization (72 °C, 15s) can be applied either before or after filtration without changing the composition of MFGM material; however, assessment of the microbial quality is warranted for future studies.

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Conflict of interest

The authors have nothing to declare

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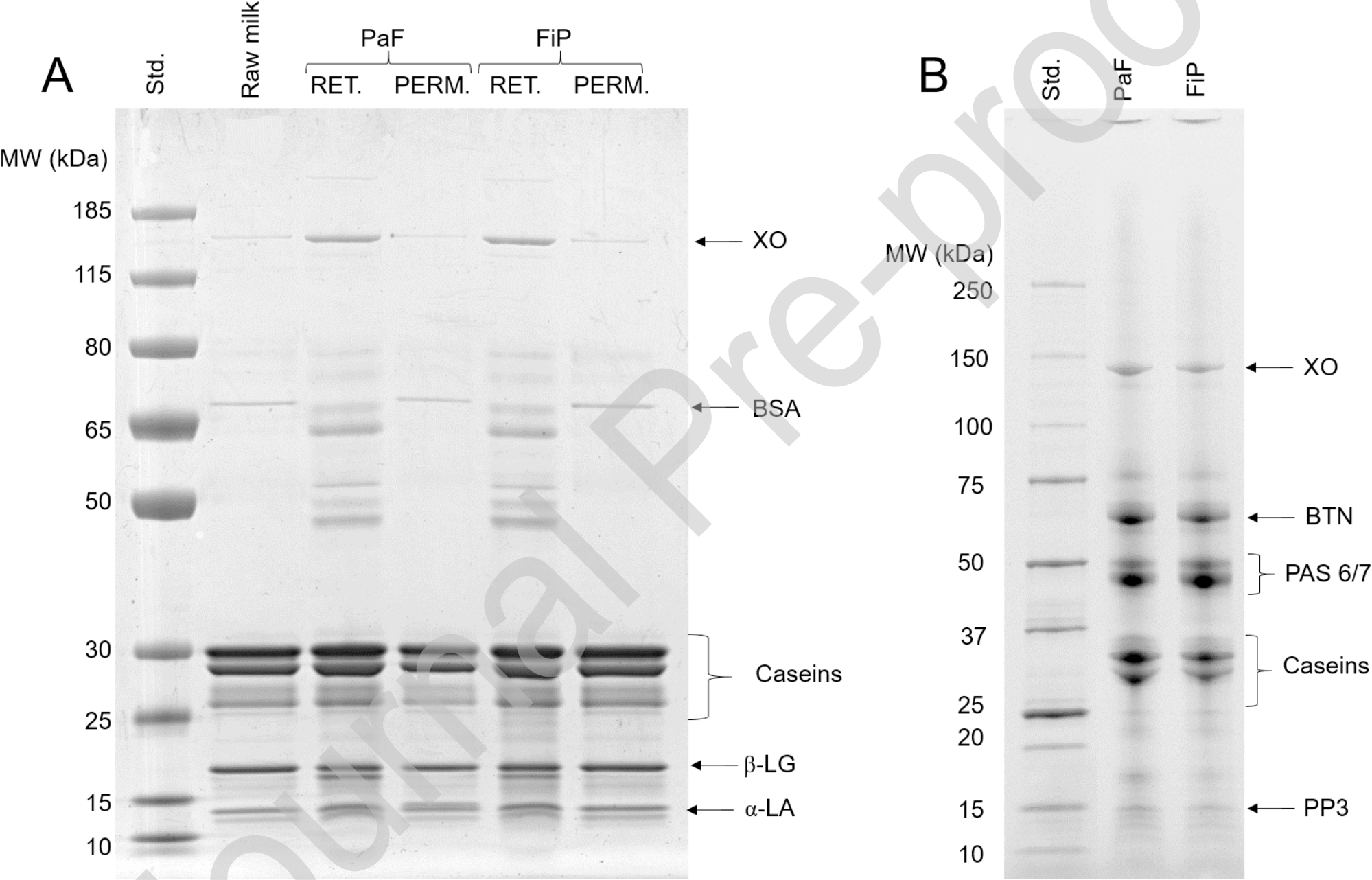
Fig. 1. Representative SDS-PAGE gels under reducing conditions of A) raw milk feed, permeates and retentates after pilot-scale filtration on 1.4 µm membrane for four diafiltration steps and B) MFGM fragments obtained from retentates. Samples were pasteurized at 72°C, 15s before (PaF) or after (FiP) filtration. Individual proteins were identified based on their electrophoretic mobility. MW, molecular weight; Std., molecular weight standard; RET., retentate; PERM., permeate; BSA, bovine serum albumin; β-LG, β-lactoglobulin; α-LA; α-lactalbumin; XO, xanthine oxidase; BTN, butyrophilin; PAS 6/7, periodic acid Schiff base 6 and 7; PP3, proteose peptone 3.

Fig. 2. Concentration values (g/L feed) of permeates and retentates following four diafiltration steps using a pilot-scale 1.4 µm ceramic microfiltration membrane. Samples were pasteurized at 72°C, 15 s, either before (PaF) or after (FiP) filtration. Protein is expressed as true protein, i.e. total protein minus non-nitrogen protein. Casein content is true protein minus non-casein nitrogen. Serum protein content is true protein minus casein content. Means of two experiments are shown, and standard deviations denoted with error bars. Sum of retentates and permeate amount for each component were analyzed by one way ANOVA, and different superscript letters indicate significantly different means ($p<0.05$).

Article highlights

- Microfiltration of raw milk was applied to obtain gently processed MFGM material
- At lab-scale, 1.4 μm ceramic membrane was found superior to 0.8 μm
- Pilot-scale filtration using 1.4 μm resulted in high-purity MFGM material
- Mild pasteurisation can be applied before or after filtration without implications


A
Std.
Raw milk
PaF
FiP
RET.
PERM.
RET.
PERM.
MW (kDa)
185
115
80
65
50
30
25
15
10
XO
BSA
Caseins
β-LG
α-LA
B
Std.
PaF
FiP
MW (kDa)
250
150
100
75
50
37
25
20
15
10
XO
BTN
PAS 6/7
Caseins
PP3

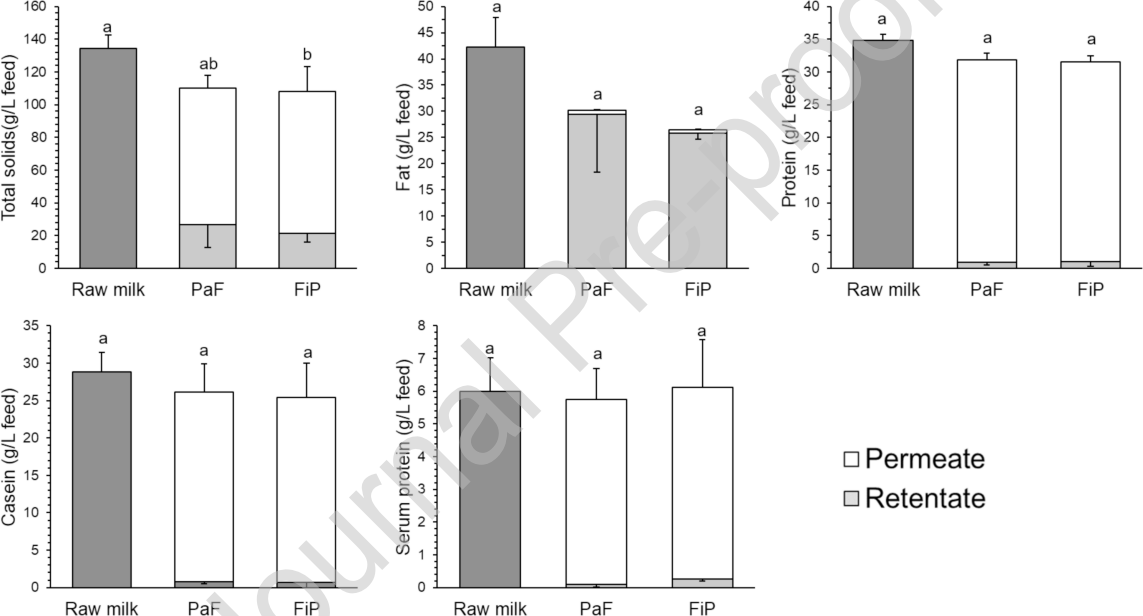


Table 1. Permeation percentages of samples pasteurized at 72°C, 15 s, either before (PaF) or after (FiP) pilot-scale diafiltration on 1.4 μm ceramic membranes[†]

	Permation yield (%)				
Sample	Fat	Protein	Casein	Serum protein	Total solids
PaF	2.7 ± 0.9[a]	97 ± 0.9[a]	97 ± 0.8[a]	99 ± 1.0[a]	76 ± 11 [a]
FiP	2.6 ± 0.1[a]	97 ± 1.5[a]	97 ± 2.3[a]	96 ± 1.9[a]	80 ± 6.8[a]

[†]Protein is expressed as true protein, i.e. total protein minus non-nitrogen protein. Casein content is true protein minus non-casein nitrogen. Serum protein content is true protein minus casein content. Means ± standard deviations of two replicates are shown. Values within each column with different superscript letters are significant different ($p<0.05$) according to one way ANOVA.

Table 2. Composition of MFGM material isolated from retentates pasteurized at 72°C, 15s before (PaF) or after (FiP) pilot-scale 1.4 µm microfiltration of raw milk.

Component	Raw milk	PaF	FiP
Yield (g/L)‡	2.6 ± 0.3[a]	0.4 ± 0.1[b]	0.4 ± 0.0[b]
Protein (g/100 g)	58 ± 1.4[a]	29 ± 12[b]	32 ± 5.9[b]
Casein (%)†	90 ± 1.3[a]	24 ± 18[b]	24 ± 9.0[b]
Triglyceride (% w/w)	26 ± 6.4[a]	44 ± 14[a]	45 ± 15[a]
Cholesterol (% w/w)	1.8 ± 0.1[a]	1.0 ± 0.1[b]	1.3 ± 0.1[b]
Total polar lipids (% w/w)	2.5 ± 0.1[a]	7.2 ± 3.5[a]	7.1 ± 0.1[a]
Individual polar lipids (%)*			
PI	8.3 ± 0.9[a]	11 ± 8.1[a]	5.6 ± 0.0[a]
PE	31 ± 1.0[a]	26 ± 10[a]	30 ± 1.0[a]
PS	14 ± 0.3[a]	13 ± 2.1[a]	14 ± 0.3 [a]
PC	24 ± 2.1[a]	27 ± 1.6[a]	28 ± 0.4[a]
SM	22 ± 2.0[a]	24 ± 1.6[a]	23 ± 0.9[a]

†Casein content as % of total protein based on densiometric measurements of SDS-PAGE gels.

*Expressed in percentage of total polar lipids. ‡Yield is in g per L feed milk. Means ± standard deviations of two replicates are shown. PI, phosphatidylinositol; PE, phosphatidylethanolamine; PS, phosphatidylserine; PC, phosphatidylcholine; SM, sphingomyelin. Values within each row with different superscript letters are significantly different ($p<0.05$) according to one-way ANOVA.