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Skimmed milk microfiltration in diafiltration mode: Impact of solvent nature and concentration factor on spiral-wound membrane performance operated at low temperature

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ABSTRACT

Cold skimmed milk microfiltration using polymeric spiral-wound membranes has a growing interest to separate serum proteins from casein micelles. Diafiltration using reverse osmosis water (ROW-DF) is needed to increase the transmission of serum proteins. The objective of this study was to investigate the performance of skimmed milk microfiltration in ROW-DF mode when operating with single industrially sized standard SW modules at low temperature (~12 °C). As expected during ROW-DF, the permeation flux increased with the rate of diafiltration, due to both the decrease of permeate viscosity and decrease of ionic strength. It is shown for the first time, that the transmission of protein (mainly β-Lactoglobulin, β-LG, major serum protein of bovine milk) presents different scenarios (increase, decrease and 'parabolic' shape) as function of diafiltration rate and operating conditions. These performances are quite different from the ones obtained when operating diafiltration with permeate from milk ultrafiltration, PUF-DF, and can be explained by the modifications induced by ROW on the properties of the fluid (feed) and on the properties of the concentrated layers of casein micelles accumulated at the membrane. The quantity of β-LG which can be released into the permeate was linked to the concentration factor applied before ROW-DF and the ability of deposit to swell was linked to the transmembrane pressure applied as critical conditions could lead to a cohesive deposit unable to swell. If the increase of repulsive electrostatic interactions (induced by the increase of diafiltration rate) between casein micelles and β-LG supported the release of β-LG entrapped in the deposit, it impaired the crossing of β-LG remaining in the retentate. The result was a progressive decrease of β-LG transmission with the increasing diafiltration rate. In summary, transmission of β-LG was maximized for low value of transmembrane pressure (0.7 bar), a high value of concentration factor (3.2) and an intermediate diafiltration rate (2.1).

1. Introduction

Fractionation of skimmed milk proteins using crossflow microfiltration (MF) is widely used in the dairy industry [5,27,2]. The increasing number of MF equipment is explained by a large panel of applications using retentate and permeate fractions which have advantageous composition and functionality [28]. So far, separation of casein micelles and serum proteins (SP) by MF is commonly operated at a temperature above 50 °C using ceramic membranes with a 0.1 μm mean pore diameter. However, the number of MF plants equipped with spiral-wound (SW) membranes is aimed to increase in dairy industry due to the low prices of polymeric membranes (800 kDa – 0.1 μm) and their high compactness. These membranes are expected to be mainly conducted at low temperature (~10 °C) to limit bacterial growth in the casein micelles retentate, as it is the case in skimmed milk ultrafiltration performed with SW membranes.

To date, MF with SW membranes was reported to have poor performances (permeation flux (Jp) and serum protein transmission) compared to the ones obtained with ceramic membranes [31]. Lower performances can be partly explained by the higher value of transmembrane pressure (TMP) applied in MF with SW membranes compared to ceramic ones [11]. The low temperature used for MF with SW

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membranes could also be suspected to be responsible for a lower serum protein transmission but results obtained by Hartinger et al., (2019a) at 10 °C and 50 °C are not clear about the impact of temperature because TMP also varied during their experiments.

To increase serum protein transmission during MF operating with SW membranes, filtration can be carried out in diafiltration mode (DF). DF is a washing procedure where a solvent - typically reverse osmosis water (ROW) or permeate from milk ultrafiltration (PUF) - is added to the retentate, either in batch and intermitted mode or in continuous mode, to drag residual serum proteins through the membrane. In addition to the transmission of serum proteins, DF with ROW eliminate the minerals and the lactose from retentate whereas DF with PUF keep their concentrations constant. Lactose is known to be highly responsible for the viscosity of the soluble phase and minerals are partly responsible for the structure of the casein micelles, which accumulated at the membrane surface [25]. Then, different performances of filtration are expected when operating MF in DF mode with PUF or ROW.

Microfiltration carried out in diafiltration mode with PUF has already been shown to be efficient, both with ceramic and SW membranes. Heidebrecht and Kulozik [14] studied the transmission of serum proteins during continuous DF of skimmed milk retentate (concentration factor, CF = 2) with PUF, using Isoflux ceramic membranes (Tami industries, 0.14 μm, 50 °C) at various TMP (from 0.6 to 3.0 bar). They showed that, the transmissions of native serum proteins were stable and constant up to a DF rate of 7 with no dependence on the TMPs (except for the lower TMP of 0.6 bar). The transmissions of native serum proteins were satisfactory with an order of transmission depending on the size of serum proteins and electrostatic interactions between the individual proteins and the casein micelles accumulated at the membrane surface (the transmission of α-lactalbumin (55 %) > β-lactoglobulin (50 %) > IgG (47 %) > other minor proteins). However, during their experiments, the transmissions of the overall serum protein were impaired by the long-term process exposure at 50 °C, which was responsible for a denaturation of serum proteins. Moreover, this study does not bring data on permeation flux obtained during the experiments performed.

Hartinger and Kulozik [12] studied the performance of skimmed milk microfiltration in continuous PUF diafiltration mode performed with SW membrane (Synder V0.1) at low temperature (10 °C). They showed that regardless of the CF applied (1 – 4) the permeation flux was stable during DF up to the diafiltration rate of 7. During DF performed at CF 1 and TMP 0.7 bar, the transmission of β-lactoglobulin was stable up to DF rate of 7. According to the results obtained, it could be expected that protein transmission is dependent of the CF value chosen for the DF step (from 1 to 4). However, as described by Heidebrecht and Kulozik, [14], Hartinger and Kulozik, [12] operated the MF and the DF at constant axial pressure drop of 1.0 bar·m^{-1} meaning that the cross-flow velocity was not similar between the different concentration factors. In these conditions, it is not possible to conclude about the role of operating conditions (concentration factor and crossflow velocity) on protein transmission during DF operated with PUF.

Diafiltration mode with water is the most used mode in dairy industry. It has already been investigated with ceramic membranes [30] and SW membranes at high temperature (~50 °C) [19] and ambiant temperature (~24 °C) [18]. These studies carried out batch and intermittent DF (consisting in succession of dilutions and concentrations) with ROW during MF at 50 °C and CF 3 [30,19] or at 24 °C and CF 4 [18]. They confirmed the increase of permeation flux during DF with ROW as previously observed by Ng et al (2008) when operating ROW DF with ultrafiltration polymeric membranes. They also showed that the purity of retentate (casein/true proteins) and the transmission of serum proteins were higher when the rates of DF increased in the range from 0.7 to 2.0. However, it is difficult from these studies to understand the role of dilution and resulting physico-chemical modifications on structural changes of casein micelles deposit layer and on transmission of serum protein as DF was performed in intermittent mode resulting in valleys and peaks in the flux and protein removal graphs.

Reitmaier et al. [25,26] investigated the impact of different aqueous phases (including PUF and ROW) on casein micelles characteristics, deposited casein layers properties and separation properties in milk protein fractionation by MF. They showed that ROW induces a significant increase of deposited casein layer hydration, thereby increasing flux levels and separation process. However, they operated at 50 °C with a single ceramic membrane and the transferability of the observed effects to common application of SW membranes at ~ 10 °C has not been demonstrated so far. This information is crucial for dairy industry, as SW membranes at low temperature have a large potential.

The objective of this study was to investigate the performance of skimmed milk microfiltration in ROW-DF mode when operating with single industrially sized standard SW modules at low temperature (~12 °C). In order to identify optimal operating conditions and to maximize serum protein transmission from the retentate, the permeation flux and transmissions of the two major individual serum proteins (α-lactalbumin and β-lactoglobulin) were measured up to a DF rate higher than 5.0. DF was performed in continuous mode, on skimmed milk concentrated at CF ranging from 2.5 to 3.5 and at two TMP values. The role of structural changes in the deposit layer was examined by comparing results with those obtained with a diafiltration mode operating with PUF in similar operating conditions.

2. Materials and methods

2.1. Fluids

Thermized skimmed milk was provided by Entremont Alliance (Bretagne, France) (Table 1) and stored at 4 °C one day prior MF. Before MF, skimmed milk was maintained at 12 °C for 30 min to recover a stable mineral balance.

Diafiltration step was carried out with either ROW or PUF. ROW was water from network treated by reverse osmosis and electrodialysis (Pretreatment pack Progard TL1 CL2, Merck-Millipore) and by UV lamp at 25 °C to reach a resistivity of 15 MΩ·cm. PUF was obtained by ultrafiltration of skimmed milk at 45 °C. It was either directly prepared with a 5 kDa SW membrane (6338 HFK 328 VYT, Koch membrane systems, Massachusetts, USA) at TMP of 2.1 – 2.6 bar and permeation flux, Jp of 16 – 32 L·h^{-1}·m^{-2} (STLO Dairy Platform) or obtained after 1:4 dilution of concentrated (27 % of dry matter) PUF. The concentrated PUF, provided by Eurosérum (Grand Est, France) was initially obtained with 5 kDa SW and reverse osmosis membranes. Regardless of their origin, the two PUFs had similar compositions (Table 1). Compared to

Table 1
Average composition and standard deviation of skimmed milks and PUF (permeate of ultrafiltration).

	Skimmed milk at 12 °C	PUF produced at 45 °C
pH	6.72 ± 0.04[a]	6.64 ± 0.04[d]
Fat, g·kg^{-1}	1.5 ± 1.0[a]	0 [d]
Dry matter, g·kg^{-1}	91 ± 1[b]	58 ± 3 [d]
Ashes, g·kg^{-1}	7.6 ± 0.2[b]	4.9 ± 0.3 [d]
Soluble calcium, mg·L^{-1}	337 ± 16[c]	282 ± 1 [d]
Soluble phosphate, mg·L^{-1}	970 ± 41 [a]	813 ± 4 [d]
Lactose, g·L^{-1}	48 ± 2	52 ± 3
Total Nitrogen, C_{TN}, g·kg^{-1}	33.3 ± 1.4 [a]	1.6 ± 0.1 [d]
β-lactoglobulin, β-LG, g·L^{-1}	3.14 ± 0.13[b]	0 [d]
α-lactalbumin, α-LA, g·L^{-1}	0.94 ± 0.08[b]	0 [d]

[a] n = 6;
[b] n = 5;
[c] n = 4;
[d] n = 2.

the aqueous phase of skimmed milk at 12 °C, the PUFs showed a composition with lower soluble calcium and phosphate contents (Table 1). This difference is attributed to the lower solubility of phosphate and calcium at 45 °C (temperature of PUF preparation) compared to 12 °C resulting in lower release of calcium and phosphate from the casein micelles. Lactose was slightly higher for PUFs than for skimmed milk.

2.2. Microfiltration set-up and experimental protocol

Pilot scale MF was carried out using single industrially sized standard polymeric 800 kDa SW membrane (FR 3A 6338, spacer of 46 mil, PVDF, total membrane area of 15.9 m^2, Synder, California, USA). All the filtration experiments were performed with the same cleaned membrane. Membrane was first cleaned with sodium hydroxide (alkaline solution, NaOH 30 %, Quaron, France) at pH 11 and 45 °C, with a retentate recirculating flow rate of 18 $m^3 \cdot h^{-1}$ and two successive steps: the first 10 min were performed without permeation and the next 10 min with permeation. Another similar sequence was then performed with nitric acid (acid solution, HNO_3; 58 % purity, Quaron, France) at pH 2.5 and 45 °C with a recirculating flow rate of 18 $m^3 \cdot h^{-1}$. Water used to rinse and clean the filtration pilot before and after MF was water from network filtered sequentially on 5.0, 1.0 and 0.2 µm cartridges. The hydraulic resistance, calculated according to Darcy's law, was recovered after cleaning: $Rm = (3.1 \pm 0.5) \times 10^{11}\ m^{-1}$.

Before MF, water initially present into the retentate compartment was gently flushed with skimmed milk (three times the volume of the retentate compartment).

All the experiments were performed at constant temperature (12.0 ± 0.3 °C), mean retentate pressure (2.0 ± 0.1 bar), and retentate recirculating flow rate (18.0 ± 0.7 $m^3 \cdot h^{-1}$). They were carried out at CF ranging from 2.5 to 3.5 and at two constant TMP, 0.7 ± 0.1 and 0.9 ± 0.1 bar. TMP = 0.7 ± 0.1 bar corresponded to the lowest pressure applicable in the range of CF studied to avoid *retro*-filtration (TMP > ½ Retentate Pressure drop). TMP = 0.9 ± 0.1 bar was a value closer to critical conditions of filtration.

Each MF experiment was divided into three phases: a concentration phase, a stabilization phase, and a MF phase operated in DF mode. Concentration phase of skimmed milks was realized by concentrating the retentate either in the feed tank (by returning retentate into the feed tank while permeate is extracted) or in the retentate loop. During the stabilization phase (15 to 90 min), MF was performed at a constant CF and retentate was continuously extracted at a flowrate fixed by the targeted CF. During the diafiltration phase, known volume of retentate was continuously diafiltrated with ROW (ROW-DF) or PUF (PUF-DF). The chosen diafiltration solvent was hold at 12 °C for 30 min before the experiment. DF was carried out at constant flowrate, meaning that the DF flowrate was maintained equal to the permeation flowrate.

During the three phases of the experiment, Jp and SP transmission were evaluated as performance indicators.

2.3. Analyses

Skimmed milk, retentate and permeate were collected for analyses.

Fat content was measured by the Gerber method [21]. Dry matter was obtained after desiccation of the sample at 105 °C for 7 h. Ashes were measured after combustion of 5 g of sample at 550 °C for 5 h. Total and soluble calcium were determined by atomic absorption spectrophotometer (AA300, Varian France) after dilution of the samples in a solution containing 10 % (v/w) of 6 $g \cdot L^{-1}$ lanthanum chloride and 10 % (v/w) of N/50-hydrochloric acid [3]. The phosphate content was measured by chromatography method (Dionex DX 500 HPLC, Thermo Fisher Scientific, Les Ulis, France) as described by Gaucheron et al. [10]. Total cations contents were determined from ashes solubilized in 1 *N*-hydrochloric acid. Soluble ions were extracted from samples by ultrafiltration on 10 kDa membranes (Vivaspin VS2002, Sartorius, Aubagne, France). Conductivity was determined at 12 °C (samples were maintained 20 min at 12 °C before measurement) with a waterproof four-ring ATC conductivity meter (HI 9033, HANNA instruments, measurement error 1 %).

Lactose content was determined by high-performance liquid chromatography (Dionex HPLC) according to a protocol adapted from Thierry et al. [29]. Briefly, lactose was extracted from samples by ultrafiltration (12 °C, 1 800 *g*, 30 min) on 10 kDa membranes (Vivaspin VS2002, Sartorius) before dilution with 0.01 *N*-sulphuric acid. HPLC were realized using a column packed with an ion-exchange resin (Aminex A-6, Bio-Rad, France).

Total nitrogen (TN), non-casein nitrogen (NCN) and non-protein nitrogen (NPN) were determined by the Kjeldahl method according to the standard [20], (International Dairy Federation, 2014). For protein content calculation, 6.38 was used as conversion factor. For protein content calculation in NCN and NPN filtrates, a correction factor was calculated to consider the weight of precipitate. TN was determined with an experimental error of 1 % and NCN and NPN with an experimental error of 5 % (Experimental errors were calculated as the ratio between standard deviation and the mean value).

Concentrations of α-lactalbumin (α-LA) and β-lactoglobulin (β-LG) were determined in skimmed milks, retentates and permeates by reversed phase high-performance liquid chromatography (RP-HPLC) according to a protocol adapted from Resmini et al. (1989). RP-HPLC was performed with a PLRP-S column (PL1912-3801, 300 Å, 8 µm, 150 mm × 2.1 mm, Agilent Technologies) on an Alliance 2695 (Waters, Saint-Quentin-en-Yvelines, France). Contents of α-LA and β-LG in milks and retentates were determined from the NCN filtrates. In both skimmed milks and retentates, measured values correspond to content of α-LA and β-LG present in the aqueous phase either in native or partially denatured forms. In permeates, contents of α-LA and β-LG were measured directly on samples and measured values correspond to the total content of α-LA and β-LG. Contents of α-LA and β-LG were calculated with an experimental error of 3 %.

All the analyses were realized in duplicate, some have been done in triplicate or quadruplicate.

Viscosity of permeates was measured at 12 °C by shear stress with a viscometer (LS400, LAMY RHEOLOGY Instruments, Champagne au Mont d'Or, France). Samples were maintained at 12 °C during 5 min before measurement.

3. Calculations

The rate of diafiltration (Tx), was calculated as:

$$Tx = \frac{V_d}{V_{R_0}} = \frac{V_p}{V_{R_0}} \quad (1)$$

with V_d, V_{R0} and V_p the volumes (L) of solvent of diafiltration, of retentate to be diafiltered and of permeate extracted during the microfiltration in DF mode.

The transmission (Tr) of β-LG or α-LA in the aqueous phase was calculated according to:

$$Tr = \left(\frac{C_p}{C_r}\right) \times 100 \quad (2)$$

with C_p and C_r the concentrations of β-LG or α-LA measured in permeate and retentate respectively at different sampling times ($g \cdot L^{-1}$).

During diafiltration and in case of total transmission of component (e.g. case of lactose, soluble ions), the retentate concentration of the component can be calculated from the mass balance equation defined as [8,23]:

$$C_r(Tx) = C_{r_0}\, e^{-Tx} \quad (3)$$

with C_{r0} ($g \cdot kg^{-1}$) the concentration of the component in the retentate to be diafiltered and C_r its concentration in the retentate at Tx.

In skimmed milk, calcium and phosphate ions are distributed between the solvent and the colloidal phase (casein micelle). The concentration ($mg{\cdot}L^{-1}$) of a specific ion i in the colloidal phase, $C_{i;colloidal}$ was calculated as suggested by Ferrer et al. [7]:

$$C_{i;colloidal} = C_{i;total} - C_{i;soluble} \times VF \quad (4)$$

with $C_{i;total}$, the total concentration of the ion i; $C_{i;soluble}$ its concentration in the soluble phase; and VF, the volume factor that considers the effects of the dilution, the non solvent water and the volume occupied by the casein micelles. It was calculated as suggested by Ferrer et al. [7]:

$$VF = \left(1 - \left(1.01 \times C_{fat} + 0.03 \times C_{CN} + 0.2 \times C_{TP}\right)\right) \times \left(\frac{\rho_i}{\rho_s}\right) \quad (5)$$

where C_{fat}, C_{CN} and C_{TP} represent respectively the content of fat, casein (CN = TN-NCN) and true proteins (TP = TN-NPN) in $g{\cdot}kg^{-1}$. ρ_i and ρ_s are the densities of the sample and the soluble phase, respectively. They are estimated from the content of casein and lactose of the sample, based on a correlation established with data from Sodiaal (confidential).

The Concentration Factor, CF is defined by.

$$CF = \left(\frac{C_{CN-ret}}{C_{CN-Milk}}\right) \quad (6)$$

where $C_{CN\text{-}ret}$ and $C_{CN\text{-}Milk}$ represent respectively the content of casein in retentate and initial milk.

4. Results

4.1. Concentration and stabilization phases

As expected, during the concentration phase, Jp decreased with the increase of CF. Between CF 1.0 and 3.2 and for a TMP of 0.7 bar, Jp dropped by about 50 %, from 14.2 to 7.4 $L{\cdot}h^{-1}{\cdot}m^{-2}$. These results are consistent with the work of Hartinger and Kulozik, [12] who observed a drop of about 60 % of Jp when CF raised from 1.0 to 3.0 during MF carried out with SW membrane in similar conditions (T = 10 °C, TMP = 0.7 bar). At CF 2.8, Jp was equal to 7.5 and 10.7 $L{\cdot}h^{-1}{\cdot}m^{-2}$ with a TMP of 0.7 and 0.9 bar respectively, confirming that the limiting flux was not reached at TMP = 0.7 bar and CF = 2.8.

Transmission of β-LG ($Tr_{\beta\text{-}LG}$) and α-LA ($Tr_{\alpha\text{-}LA}$) remained constant with the increase of CF, regardless of the mode of concentration (concentration in feed tank or in the retentate loop). Despite a high variability of $Tr_{\alpha\text{-}LA}$ from one experiment to another ($Tr_{\alpha\text{-}LA}$ varied from 57 ± 1 % to 78 ± 2 % for a TMP of 0.7 bar) $Tr_{\alpha\text{-}LA}$ was always higher than $Tr_{\beta\text{-}LG}$, in accordance to the size of the proteins (α-LA: 14.2 $kg{\cdot}mol^{-1}$ and β-LG (dimer): 36 $kg{\cdot}mol^{-1}$). The lower the TMP, the higher the transmission of serum proteins: $Tr_{\beta\text{-}LG}$ was 36 ± 2 % (n_s = 14) and 30 ± 1 % (n_s = 4) at TMP 0.7 and 0.9 bar respectively, and $Tr_{\alpha\text{-}LA}$ was 69 ± 9 % and 58 ± 3 % at TMP 0.7 and 0.9 bar respectively (n_s is the number of samples considered for the calculation of the transmission. The samples used for the calculation of serum protein transmission are samples from the "concentration and stabilization phases" of experiments performed at a given applied TMP. The transmission values obtained at different CFs and time points were averaged because the transmission values of the serum proteins were constant at a given applied TMP over the CF range studied). These results are consistent with the works of Gésan-Guiziou et al. [9] and Hartinger et al. [13].

When CF increased and regardless of the TMP applied, the contents of total calcium and phosphate increased in the retentate compartment. At the same time, the contents of soluble calcium and phosphate remained constant (Results not shown) suggesting that increase in the total content was induced by the colloidal calcium and phosphate. During the concentration phase, the proportions of colloidal calcium and phosphate on casein content expressed as $C_{Ca;colloidal}/CN$ and $C_{phosphate;colloidal}/CN$ ratios (Eq. (4)) were steady: 0.87 ± 0.05 and 0.36 ± 0.02 $g{\cdot}mol^{-1}$ respectively. These results suggested that calcium phosphate present in casein micelles was not solubilized in the aqueous phase during concentration and that only the colloidal fraction of minerals associated to casein micelles was concentrated. These results are consistent with Ferrer et al. [7] who did not observe any solubilization of colloidal calcium phosphate during ultrafiltration of casein micelles at 40 °C and CF lower than 5.

During the stabilization phase, the permeation flux and the transmission of α-LA and β-LG were stable during 15 to 90 min, regardless of TMP and CF values used.

4.2. Diafiltration phase

4.2.1. Diafiltration with PUF (PUF-DF) at TMP 0.7 bar

Regardless of the CF, Jp remained stable during the PUF-DF carried out at TMP = 0.7 bar: Jp = 9.6 ± 0.2 and 7.9 ± 0.3 $L{\cdot}h^{-1}{\cdot}m^{-2}$ at CF 2.5 and 2.8 respectively (Fig. 1a).

During the PUF-DF, and regardless of CF, $Tr_{\beta\text{-}LG}$ and $Tr_{\alpha\text{-}LA}$, calculated from experimental retentate and permeate concentrations, decreased (Fig. 2a & b). Between Tx = 0.0 and 2.0, $Tr_{\beta\text{-}LG}$ decreased from 35 to 23 % for CF = 2.5 and from 34 to 31 % for CF = 2.8 while $Tr_{\alpha\text{-}LA}$ dropped from 75 to 51 % and from 57 to 39 % respectively.

During the PUF-DF, the permeate lactose concentration slightly increased (Results not shown) due to the difference of lactose content between the PUFs and the skimmed milk. Conductivity of retentate fractions remained stable at 3110 ± 78 $\mu S{\cdot}cm^{-1}$ (Fig. 1 b) and mineral contents of the retentate fractions remained stable. These results suggest that the slight differences of lactose and mineral contents observed between the soluble fractions of skimmed milk and PUFs (Table 1) have no consequence on the ionic environment of skimmed milk during PUF-DF nor on Jp values.

4.2.2. Diafiltration with ROW (ROW-DF) at TMP 0.7 and 0.9 bar

During ROW-DF, Jp increased progressively with the increase of Tx (Fig. 1c). The increase of Jp (ΔJp) was of the same order of magnitude for the experiments performed with the low CF values (from 2.6 to 3.2) (Table 2): during diafiltration operated between Tx = 0.0 and 2.0, Jp increased by 44 and 36 % at TMP 0.7 bar and CF 2.6 and 3.2 respectively and by 42 % during DF operated at TMP 0.9 bar and CF 3.0. However, ΔJp was much lower during DF carried out with the high values of CF (3.5) and TMP (0.9 bar): in these conditions, Jp increased by only 15 % between Tx = 0.0 and 2.0.

For higher rate of diafiltration (Tx > 2.0), ΔJp levelled off. For instance, Jp increased by only 28 % (from 42 to 54 $L.h^{-1}{\cdot}m^{-2}$) between Tx = 2.0 and 4.0 with TMP = 0.9 bar and CF 3.0.

During ROW-DF at TMP of 0.7 bar, $Tr_{\beta\text{-}LG}$ followed a "parabolic shape", which was more pronounced with higher CF (3.2compared to 2.6) (Fig. 2c): for ROW-DF operated at CF 3.2, $Tr_{\beta\text{-}LG}$ increased from 37 to 50 % when Tx ranged from 0.0 to 2.1 and then decreased to 26 % at Tx = 5.7. For ROW-DF operated at CF 2.6, $Tr_{\beta\text{-}LG}$ increased from 33 to 36 % when Tx ranged from 0.0 to 0.5 and then decreased to 31 % at Tx = 2.0. Simultaneously, $Tr_{\alpha\text{-}LA}$ regularly decreased during the ROW-DF at CF 3.2 (from 77 to 66 and 38 % at Tx = 0.0, 2.1 and 5.7 respectively) but remained stable during ROW-DF at CF 2.6 (64 ± 2 %) (Fig. 2d).

During ROW-DF at TMP of 0.9 bar, $Tr_{\beta\text{-}LG}$ slightly increased regardless of CF (from 30 ± 2 % to 37 ± 3 % between Tx = 0.0 and 4.0) (Fig. 2e). Simultaneously, $Tr_{\alpha\text{-}LA}$ decreased from 61 ± 5 % to 37 ± 3 % regardless of CF (Fig. 2f).

During ROW-DF, viscosity of the permeate dropped with the increase of Tx (Fig. 3b). The viscosity of permeate decreased from about 1.45–1.50 mPa·s at Tx = 0.0 to 1.25 mPa·s after Tx = 4.0. The permeate concentration of lactose decreased progressively when Tx increased (Fig. 3a): it dropped from 48 ± 2 $g.L^{-1}$ at Tx = 0.0 to 6 and 1 $g{\cdot}L^{-1}$ at Tx = 2.0 and 4.0, respectively. The drop of permeate concentration of lactose during ROW-DF was directly induced by the dilution of the retentate lactose concentration with ROW as the lactose (342 $g{\cdot}mol^{-1}$) is totally transmitted through the MF membrane (prediction according to Eq. (3)).

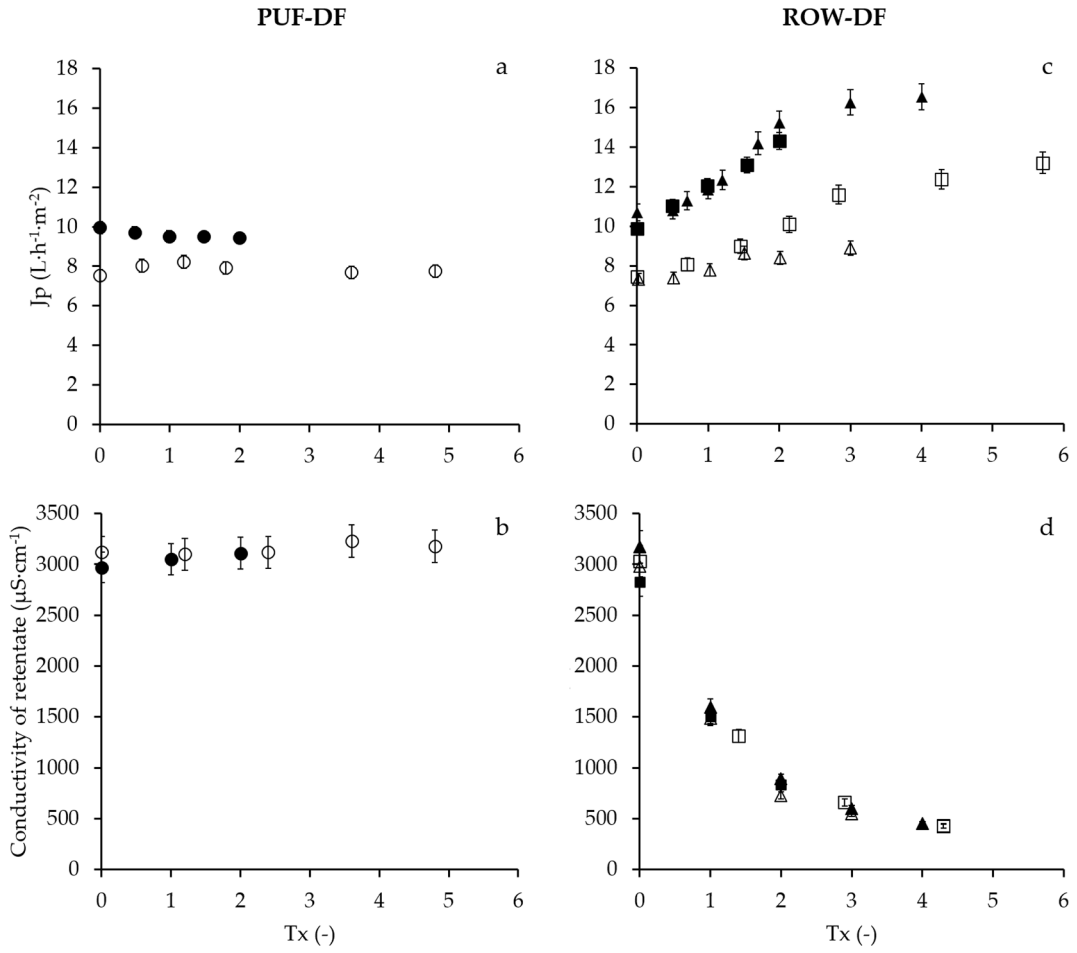

Fig. 1. Permeation flux (Jp) and conductivity of retentate during the diafiltration (DF) of skimmed milk retentate with either milk ultrafiltrate (PUF) (a & b) or reverse osmosis water (ROW) (c & d). CF 2.5 and TMP 0.7 bar (solid circles, ●); CF 2.8 and TMP 0.7 bar (open circles, ○); CF 2.6 and TMP 0.7 bar (solid squares, ■); CF 3.2 and TMP 0.7 bar (open squares, □); CF 3.0 and TMP 0.9 bar (solid triangles, ▲); CF 3.5 and TMP 0.9 (open triangles, Δ).

Conductivity of the retentate decreased when Tx of ROW-DF increased (Fig. 1d). Regardless of TMP and CF applied the decline of the conductivity was similar, from 3003 ± 140 µS.cm^{-1} to 449 µS.cm^{-1} for Tx = 0.0 and 4.0, respectively.

Simultaneously, total and soluble contents of phosphate in the retentate dropped when Tx increased (Table 3). However, the drop of total phosphate was higher than the drop of soluble phosphate. Between Tx = 0.0 and Tx = 2.0, total phosphate content dropped by 806 mg·L^{-1} whereas soluble phosphate content only dropped by 553 mg·L^{-1} at TMP = 0.7 bar and CF 2.6. Similar observations are done at TMP = 0.7 bar and CF = 3.2.

These results indicate a release of colloidal phosphate in the soluble phase induced by a partial solubilization of phosphate during ROW-DF: the ratio of $C_{phosphate\text{-}colloidal}$/CN calculated with Eq. (4) confirmed this hypothesis as it dropped from 0.37 to 0.28 mol·g^{-1} between Tx = 0.0 and 2.0 when DF is carried out at TMP = 0.7 bar and CF = 2.6.

5. Discussion

Realising dairy protein fractionation with SW membranes requires improving the performance of skimmed milk microfiltration equipped with these membranes. A solution is to carry out MF in diafiltration mode with reverse osmosis water (ROW-DF). This study provides elements to define the optimal operating conditions to conduct cold MF in ROW-DF mode with SW membrane. It gives some guidelines for the industry to maximize the removal of serum proteins and minimize the volume of ROW to be used.

This work shows that the performances (permeation flux and individual serum protein transmission) of skimmed milk MF operated in ROW-DF mode are quite different from the ones obtained when operating PUF-DF. The modifications observed can be explained by the modifications induced by ROW on the properties of the fluid (feed) including the characteristics of casein micelles, and on the properties of the concentrated layers of casein micelles accumulated at the membrane.

5.1. Permeation flux with ROW-DF

The increase of Jp with the increase of Tx during ROW-DF is in general agreement with previous studies. To our knowledge, this is the first time that an increase of Jp was observed when MF is operated in ROW-DF mode at 12 °C, but this result is very similar to the ones previously obtained by others when operating ROW-DF at ~ 50 °C [30,19,18].

5.1.1. Role of permeate viscosity

The increase of Jp observed during ROW-DF can partially be explained by the decrease of permeate viscosity. When the viscosity of the permeate is unchanged (case of PUF-DF), Jp is constant. In contrast, when the viscosity of the permeate decreases (case of ROW-DF), Jp increased. It can be assumed that the decrease in viscosity of the permeate is mainly attributed to the decrease in lactose content as it is largely accepted that the concentration of serum proteins has little impact on viscosity as their concentration is about 8 times lower than

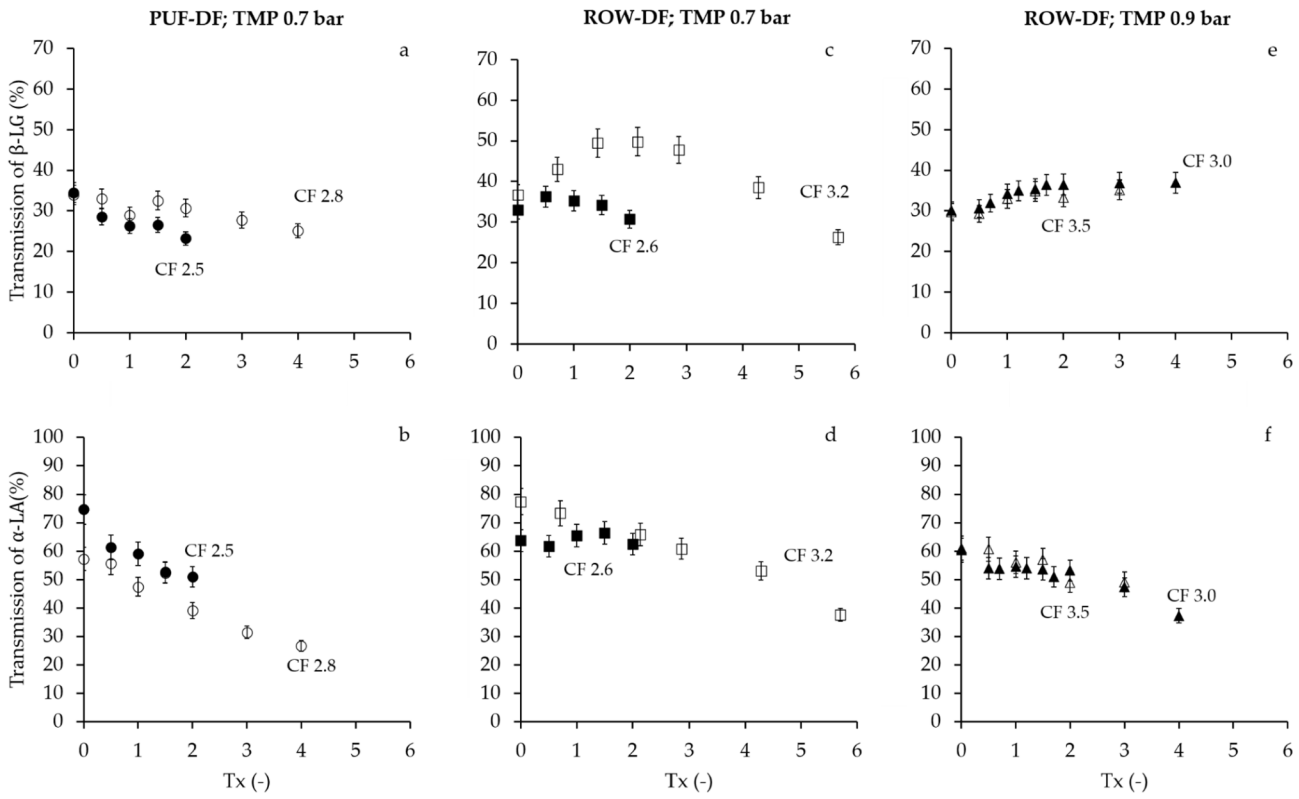

Fig. 2. Transmission of β-LG (a, c & e) and α-LA (b, d & f) during PUF-DF (a & b) or ROW-DF (c-f). Operation conditions. 800 kDa polymeric spiral-wound membrane; retentate recirculating flow, Qr = 18 $m^3 \cdot h^{-1}$; T = 12 °C. CF 2.5 and TMP 0.7 bar (solid circles, ●); CF 2.8 and TMP 0.7 bar (open circles, ○); CF 2.6 and TMP 0.7 bar (solid squares, ■); CF 3.2 and TMP 0.7 bar (open squares, □); CF 3.0 and TMP 0.9 bar (solid triangles, ▲); CF 3.5 and TMP 0.9 (open triangles, Δ).

Table 2
Increase of permeation flux (ΔJp) in % during diafiltration with reverse osmosis water (ROW-DF) at a transmembrane pressure value (TMP) of 0.7 or 0.9 bar and a concentration factor (CF) from 2.6 to 3.5.

TMP (bar)	CF (-)	Tx (-)	Jp ($L \cdot h^{-1} \cdot m^{-2}$)	ΔJp (%)
0.7	**2.6**	0.0	9.9	–
		2.0	14.3	44
0.7	**3.2**	0.0	7.4	–
		2.1	10.1	36
		5.7	13.2	78
0.9	**3.0**	0.0	10.7	–
		2.0	15.2	42
		4.0	16.5	54
0.9	**3.5**	0.0	7.3	–
		2.0	8.4	15
		3.0	8.9	22

Abbreviations. Tx, rate of diafiltration; Jp, permeation flux.

that of lactose. Taking this into account, the viscosity of permeate can be estimated as a function of lactose content (and then as a function of Tx) from our experimental data. An estimated permeation flux corrected by the viscosity of the permeate for each Tx (Jp_{corr}) can then be calculated (Fig. 4a & b):

$$Jp_{corr}(T_x) = Jp(T_x) \times \frac{\mu_p(T_x)}{\mu_{p\ (T_x=0)}} \quad (7)$$

with Jp the permeation flux ($L \cdot h^{-1} \cdot m^{-2}$) measured during the DF and μ_p (mPa·s) the viscosity of the permeate at a given Tx. Jp_{corr} during PUF-DF was calculated by considering the slight evolution of lactose content of permeate resulting from the slight differences in lactose content between skimmed milk and PUFs used for the PUF-DF. Finally, the percentage of increase of Jp induced by the decrease of viscosity (ε) can be calculated as follows:

$$\varepsilon = \left(\frac{(Jp_{ROW-DF} - Jp_{corr\ ROW-DF})}{(Jp_{ROW-DF} - Jp_{corr\ PUF-DF})} \right)_{T_x} \times 100 \quad (8)$$

The calculations of ε in two groups of operating conditions (at TMP 0.7 bar and CF = 2.5–2.6 in one hand and at TMP = 0.7 bar at CF = 2.8–3.0 in the other hand), shows that regardless of the CF, the decrease of lactose content in the permeate during ROW-DF explains about 40 % of the increase of Jp (comparison of Fig. 1 c and Fig. 4b). Exception has to be made for the ROW-DF on high CF (3.5) and high TMP (0.9 bar) (open triangle - Fig. 4b) for which 100 % of the Jp increase is explained by the reduction of the permeate viscosity. This is an interesting result which indicates that the permeability properties of casein micelles fouling formed in high TMP (0.9 bar) and CF (3.5) are not modified during ROW-DF whereas properties of accumulated layers formed at lower TMP or CF changed.

5.1.2. Role of ionic strength

As already mentioned in the literature (at 50 °C) (Ng et al. 2008, [25], the increase of Jp during ROW-DF may be attributed to the decrease in ionic strength of retentate (conductivity as indicator) that may affect the casein micelles properties and the features of accumulated layers at the membrane surface.

The decrease of ionic strength leads to modification of the internal properties of casein micelles [16]. In this work, the « surplus » of phosphate recovered in permeate during the ROW-DF was estimated as the difference between the content of phosphate measured in the permeate at Tx and the expected phosphate content in the permeate at Tx calculated from the soluble phosphate content in the retentate (at Tx) and the mass balance equation (Eq. (3)). Such a calculation shows a solubilization of phosphate from the casein micelle to the soluble phase (Fig. 5) that is similar for all investigated operating conditions of ROW-

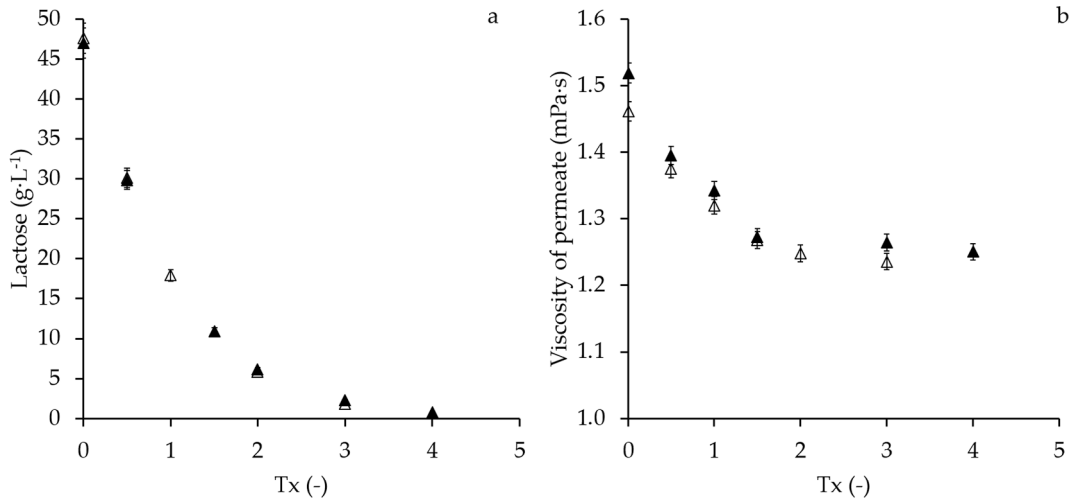

Fig. 3. Content of lactose and viscosity of permeate during diafiltration, DF with reverse osmosis water (ROW). Diafiltration is performed after concentration of skimmed milk at CF = 3.5 (solid triangles, ▲) or 3.0 (open tringles, Δ). Operating conditions. TMP = 0.9 bar.

Table 3
Evolution of total phosphate (ΔTotal phosphate) and of soluble phosphate (Δsoluble phosphate) in retentate during diafiltration with reverse osmosis water (ROW-DF) at a concentration factor (CF) of 2.6 and 3.2. Operating condition. TMP 0.7 bar.

TMP (bar)	CF (-)	Tx (-)	Total phosphate (mg·L^{-1})	Soluble phosphate (mg·L^{-1})	ΔTotal phosphate (mg·L^{-1})	ΔSoluble phosphate (mg·L^{-1})
0.7	**2.6**	0.0	2940	867	–	–
		2.0	2134	314	806	553
0.7	**3.2**	0.0	3749	888	–	–
		5.7	2305	151	1444	737

Abbreviation. Tx, rate of diafiltration.

DF. This result is in agreement with Li and Corredig [17] and Alexander et al. [1] who showed partial solubilization of micellar calcium during skimmed milk UF (CF = 2.5) operated in ROW-DF at Tx 2.5 to 5.0. This result was supported by Ferrer et al. [6] who additionally showed that the structure (e.g. higher turbidity due to lower refractive index of the casein micelles) and the physical properties of casein micelle (e.g. lower susceptibility to aggregation, lower storage modulus of rennet induced gels) were modified by the solubilization of calcium and individual caseins at high rate of DF with ROW (>50). Lazzaro [16] explained also that solubilization of colloidal minerals leads to a swelling of the casein micelles that might result in an increase of Jp.

However according to our results, it is unlikely that the solubilization of colloidal minerals affects Jp. Indeed, the solubilization of colloidal minerals was similar regardless of operating conditions used (Fig. 5) but at TMP 0.9 bar and CF 3.5, Jp_{corr} (Jp corrected by the permeate viscosity) remained constant during ROW-DF despite the increase of Tx and solubilization of colloidal minerals (Fig. 6).

The decrease in ionic strength of retentate may be more likely responsible for change in the properties of the protein accumulated layers, which themselves dependent on the operating conditions.

The decrease of ionic strength may result in an increase of electrostatic repulsions between charged proteins (casein micelles and entrapped serum proteins) and swelling of accumulated protein layers [15]. As a result, the resistance of the accumulated layers is reduced, and

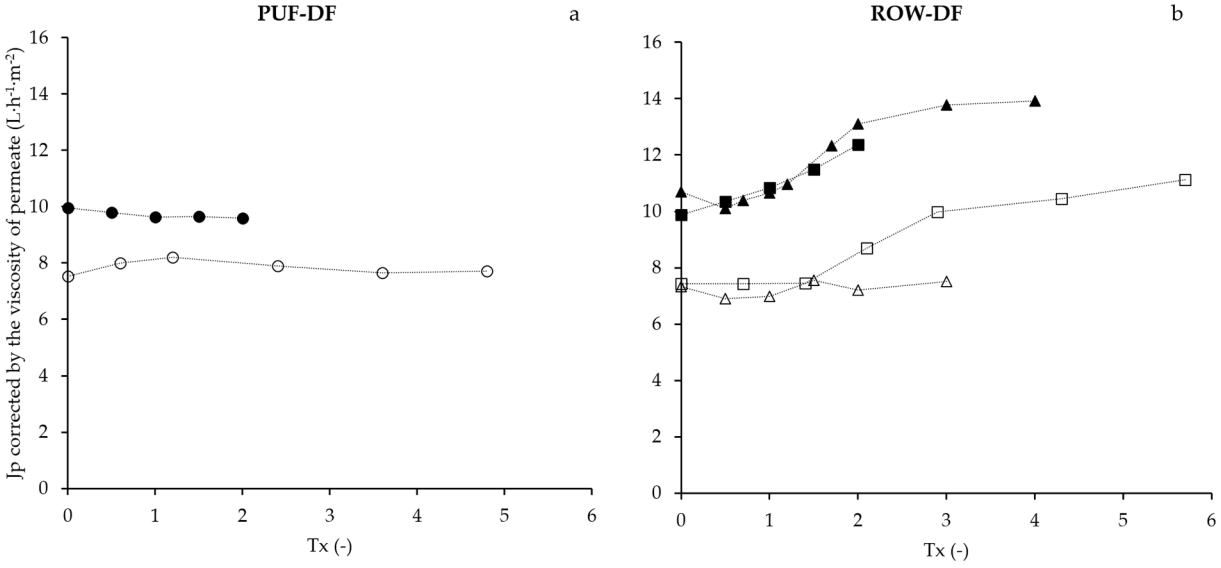

Fig. 4. Permeation flux corrected by the viscosity of the permeate during the diafiltration (DF) of microfiltration retentate with PUF (a) or ROW (b). Operating conditions. CF 2.5, TMP 0.7 bar (solid circle, ●); CF 2.8, TMP 0.7 bar (open circle, ○); CF 2.6, TMP 0.7 bar (solid square, ■); CF 3.2, TMP 0.7 bar (open square, □); CF 3.0, TMP 0.9 bar (solid triangle, ▲); CF 3.5, TMP 0.9 (open triangle, Δ).

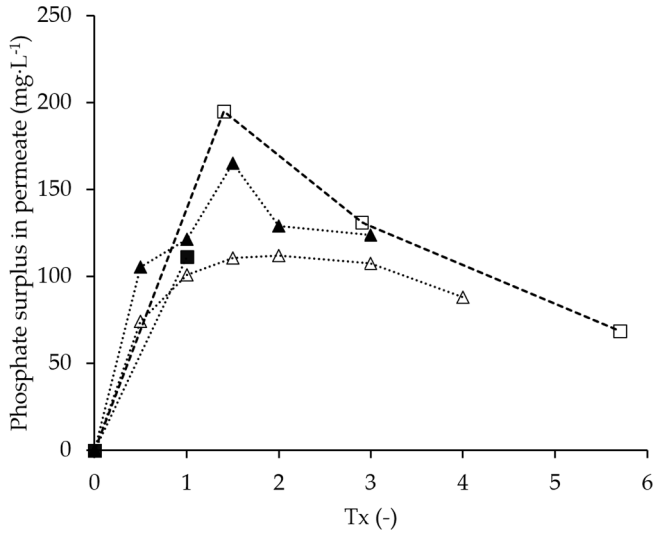

Fig. 5. "Surplus" of phosphate in the permeate ($mg \cdot L^{-1}$) as a function of diafiltration rate, Tx with reverse osmosis water (ROW) at TMP 0.7 bar and CF 2.5 (solid square, ■) or CF 3.2 (open square, □) or at TMP 0.9 bar and CF 3.0 (open triangle, Δ) or CF 3.5 (solid triangle, ▲).

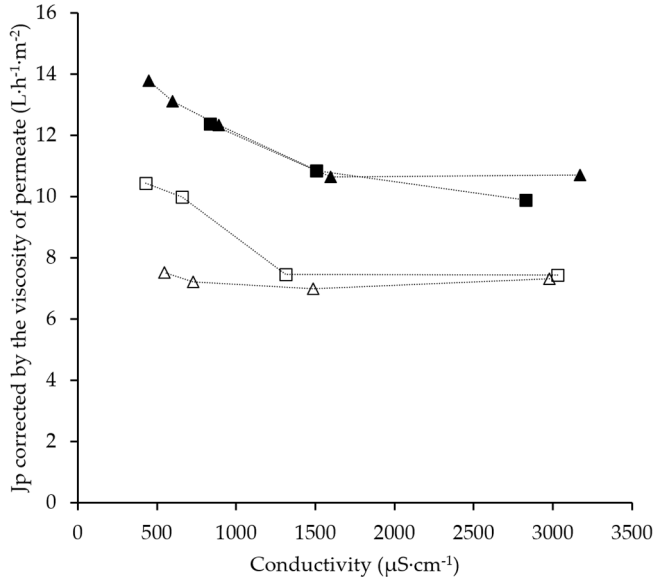

Fig. 6. Permeation flux corrected by the viscosity of the permeate as function of conductivity of retentate during the diafiltration (DF) with reverse osmosis water (ROW). Operation conditions. CF 2.6, TMP 0.7 bar (solid square, ■); CF 3.2, TMP 0.7 bar (open square, □); CF 3.0, TMP 0.9 bar (solid triangle, ▲); CF 3.5, TMP 0.9 (open triangle, Δ).

the permeation flux increases. The same conclusion was found by Ng et al. [22] during ultrafiltration of skimmed milk in ROW-DF mode. However, our work shows that the swelling of the accumulated layers depends of the operating conditions. Indeed, in high CF and TMP conditions (respectively 3.5 and 0.9 bar in our work), the permeability of protein accumulated layers was not modified during ROW-DF: the stable Jp_{corr} in these conditions could be explained by the cohesive interactions of the deposit. Qu et al., [24] demonstrated that the casein micelles deposit can shift from a solution state to a gel state under specific conditions of CF and critical TMP. We assumed that for high values of CF and TMP cohesive interactions of the casein micelles are high and modification of the ionic environment does not modify the structure of the deposit during ROW-DF.

Not surprisingly when the repulsive electrostatic charges are unmodified (case of PUF-DF), the structure of the deposit is unchanged and Jp_{corr} is unchanged. These results are convenient with the work of Hartinger and Kulozik [12].

5.2. Transmission of serum proteins

Transmissions of serum proteins have never been investigated in skimmed milk MF operated in ROW-DF mode at low temperature. Interestingly our study shows that the transmission of serum protein (mainly transmission of the major serum protein, β-LG) highlights various scenarios (decrease, increase, 'parabolic shape') versus Tx, depending on operating conditions (CF, TMP). These results indicate that the assumption of constant protein transmission classically considered in diafiltration [8,23] is not relevant in our conditions to establish a mass balance and predict the transmission and concentration of proteins in the permeate. The experimental values of β-LG protein transmission highlight the fact that the properties of casein micelles accumulated layers that depend on operating conditions, may have a crucial role in entrapment and/or release of β-LG during ROW-DF. We could reasonably assume that β-LG accumulates into the casein micelles deposit and that the swelling of the deposit and change of physico-chemical environment during ROW-DF leads to the progressive removal of this accumulated β-LG into the permeate.

In order to better understand the different situations encountered in the study (experiments performed at low (0.7 bar) or high (0.9 bar) TMP as well as low (2.5) and high (3.5) CF), we calculated the "surplus" of protein in the permeate during a variation of ΔTx ($\Delta Tx = Tx_n - Tx_{n-1}$; with n: sample number). The "surplus" of protein in the permeate during ΔTx was calculated by the difference between the quantity of protein recovered in the permeate during ΔTx and the quantity of β-LG released from the retentate (central portion of the pipe) during the same ΔTx. It was then expressed as:

$$\text{Surplus of protein in the permeate during } \Delta Tx = C_{pm}(\Delta Tx) \times Vp(\Delta Tx) - (C_r(Tx_{n-1}) - C_r(Tx_n)) \times Vr(\Delta Tx) \quad (9)$$

with C_{pm}, the average concentration of β-LG recovered in the permeate during ΔTx. C_{pm} was calculated from the instantaneous C_p measured values using the trapeze method; Vp (ΔTx) and Vr (ΔTx), the volumes recovered in the permeate and diafiltered in the retentate during ΔTx. As Vp (ΔTx) = Vr (ΔTx), the surplus is expressed in g per Liter of diafiltered retentate.

Two extreme scenarios can occur:

- When "surplus" > 0: More proteins than have disappeared from the retentate (pipe) are recovered in the permeate. This indicates that proteins accumulated in the surface layers are released into the permeate.
- When "surplus" < 0: Proteins that are removed from the retentate do not reappear in the permeate, indicating retention of proteins in the surface layers of the membrane.

Depending of the operating conditions, three cases can be distinguished during ROW-DF at Tx ranging from 0 to 2 (Fig. 7):

i. When the MF is carried out with a high CF and a low TMP (case of MF in DF mode with CF 3.2 and TMP 0.7 bar) (Fig. 7b), the "surplus" of β-LG in the permeate is high. In that case, it can be assumed that the accumulation of β-LG into the deposit before DF is high (high CF) and that the accumulated layers are "non-cohesive" (low TMP, low compression). The swelling of the deposit leads to the release of high quantity of β-LG into the permeate.
ii. When the MF is carried out with a low CF and a low TMP (case of MF in DF mode at CF 2.6 and TMP 0.7 bar) (Fig. 7b), only few

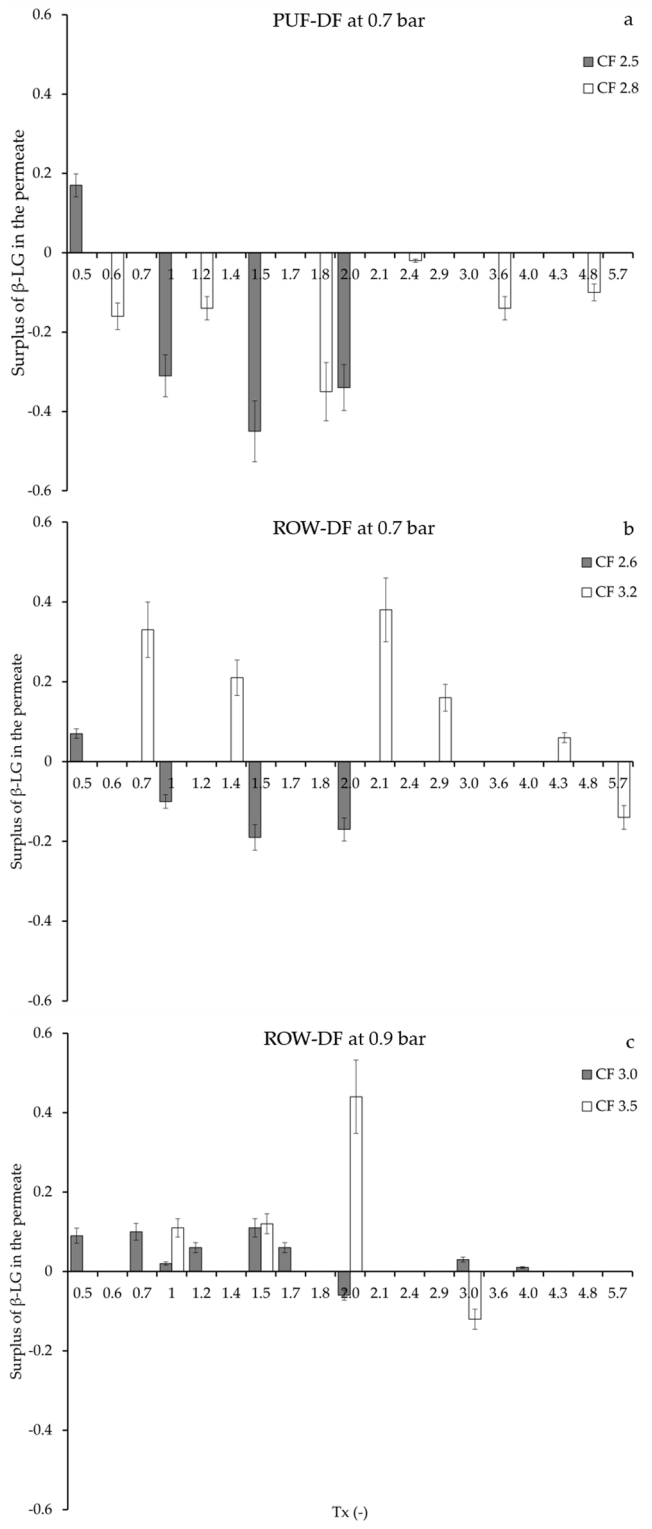

Fig. 7. Surplus of β-lactoglobulin (β-LG) in the permeate ($g{\cdot}L^{-1}$ of diafiltered retentate) as a function of rate of diafiltration (Tx) during PUF-DF (a) or ROW-DF either at TMP = 0.7 bar (b) or 0.9 bar (c).

β-LG is released into the permeate during the very first rate of diafiltration. In that case, the accumulation of β-LG into the deposit before DF is low (low CF) and the swelling of the accumulated layers leads to a release of a small quantity of β-LG.

iii. When the MF is carried out with a high CF and a high TMP (case of MF in DF mode at CF 3.5 and TMP 0.9 bar) (Fig. 7c), a release of β-LG is observed up to an intermediate value of the diafiltration rate, Tx = 2.0. In that case, it can be assumed that the accumulation of β-LG into the accumulated layers at the membrane surface before DF is high (high CF). In these conditions, the casein micelles layers did not swell, the deposit stayed cohesive and Jp_{corr} was unchanged despite the solubilization of colloidal minerals. Due to the decrease of ionic strength, repulsive electrostatic interactions increase between casein micelles and β-LG (both negatively charged at neutral pH) leading to the expulsion of β-LG out of the deposit. At high Tx, more extensive rate of ROW-DF is assumed to increase the repulsive electrostatic interactions between casein micelles and β-LG, resulting in reduction of β-LG accumulation into the deposit. When all the β-LG accumulated into the deposit before the ROW-DF is released, transmission β-LG starts to decrease.

Contrary to ROW-DF, when the structure of the deposit is unchanged and the repulsive electrostatic charges between proteins (casein micelles and serum proteins) are not modified (case of PUF-DF), β-LG accumulated into the deposit ("surplus" of β-LG in the permeate < 0; Fig. 7a) and transmission of β-LG slightly decreased with the increase in Tx (Fig. 2a). This result is different from those of Heidebrecht and Kulozik [14] and Hartinger and Kulozik [12] who observed constant transmission of serum proteins during PUF-DF. However, Heidebrecht and Kulozik [14] carried out MF at 50 °C instead of 12 °C in this study, and it was recently shown that such high temperature leads to low cohesive deposit compared to 12 °C [4], that could explain a higher transmission of β-LG. Moreover, Hartinger and Kulozik [12] only focus on the transmission of serum proteins (β-LG) for the PUF-DF operated at CF = 1. For higher CF (>1), Hartinger and Kulozik [12] assumed that transmission of β-LG was constant but we showed in this study that transmissions of serum proteins during diafiltration depend on CF.

Remarkbly, the behavior of α-LA is quite different from the one of β-LG. No 'parabolic shape' of transmission versus Tx can be observed at low TMP (0.7 bar) and high CF (3.2) during ROW-DF. This observation may be linked to the physicochemical properties of α-LA. As it is a metalloprotein and as it conformational structure depends on the presence of a calcium moiety, it can be assumed that changes of ionic strength and solubilization of calcium phosphate during ROW-DF do not only act on swelling of accumulated protein layers but also on the net charge and/or conformation of α-LA. As a consequence, electrostatic interactions between casein micelles and α-LA during ROW-DF could be different for the ones between β-LG and casein micelles.

Our work highlighted that cold MF performances in ROW-DF mode are impacted by the properties of the fluid and the properties of the accumulated layers of casein micelles. Critical conditions leading to cohesive properties of the deposit ('gel state', in our case TMP 0.9 and CF 3.5) impair the performances of the diafiltration. On the contrary, if the protein accumulated layers are not densely packed, the decrease of ionic strength increases the repulsive electrostatic interactions of casein micelles within the deposit that swells. As a result, both permeation flux and transmission of β-LG increased. The decrease of ionic strength also increases the repulsive electrostatic interactions between casein micelles and β-LG resulting in the release of entrapped β-LG in the deposit. However, when high rates of ROW-DF are applied, the repulsive electrostatic interactions between casein micelles and β-LG impairs the transmission of the β-LG into the permeate.

6. Conclusion

Performances (Jp and $Tr_{β\text{-}LG}$) of cold MF using spiral-wound membrane can be improved by DF mode using reverse osmosis water, ROW. In this work the effects of operating conditions (concentration factor, CF and transmembrane pressure, TMP) were studied. Regardless of the TMP, Jp increased when the rate of ROW-DF increased. On the contrary, Jp was constant with the rate of DF when DF was realized with permeate

of milk ultrafiltration, PUF. When MF in DF mode with ROW was carried out at low TMP (0.7 bar), $Tr_{\beta\text{-LG}}$ had a parabolic shape, all the more pronounced that the CF was high. When the ROW-DF was realized at high TMP value (0.9 bar), $Tr_{\beta\text{-LG}}$ slightly increased. On the contrary, when DF was run with PUF, $Tr_{\beta\text{-LG}}$ decreased with the rate of DF.

This work shows that the modifications of MF performances during DF mode were attributed to the modification of the properties of the fluid, of the properties of casein micelles and of the properties of the accumulated layers of casein micelles.

About 40 % of Jp increases are attributed to the drop of permeate viscosity induced by the removal of lactose. The drop of conductivity that leads to the increase of repulsive electrostatic interactions between casein micelles into the deposit, favors the swelling of deposit (if the MF operating conditions do not induce cohesive deposit), and consequently an increase of Jp. During ROW-DF, the β-LG accumulated into the deposit formed during MF, is released during the first rate of DF. If DF is extended, the increase of repulsive interactions between casein micelles and β-LG penalized its crossing in permeate. In case of cohesive deposit, the decrease of ionic strength has no impact on Jp, transmission of β-LG slightly increases before stabilizing due to the increase in repulsive interactions between casein micelles and entrapped β-LG. In that case, no more β-LG accumulated into the deposit and none are released into the soluble phase. When the deposit is unchanged because the ionic environment of milk is not modified (case of PUF-DF), the β-LG accumulated progressively into the deposit.

As a result, to increase $Tr_{\beta\text{-LG}}$ during ROW-DF, it is preferable to accumulate β-LG into the deposit before the ROW-DF (by applying high CF value) and promote conditions that limit cohesive deposit formation (low TMP). $Tr_{\beta\text{-LG}}$ will be optimal during diafiltration for a DF rate of 2. Extended diafiltration would impair the $Tr_{\beta\text{-LG}}$ and lead to a high volume of diluted permeate that will need to be further concentrated.

CRediT authorship contribution statement

Manon Granger-Delacroix: Conceptualization, Methodology, Data curation, Formal analysis, Visualization, Writing – original draft. **Nadine Leconte:** Conceptualization, Methodology, Formal analysis, Validation. **Cyril Grassin:** Methodology, Formal analysis, Validation. **Françoise Le Goff:** Methodology, Formal analysis, Validation. **Fabienne Garnier-Lambrouin:** Methodology, Formal analysis, Validation. **Marieke Van Audenhaege:** Conceptualization, Methodology, Validation, Supervision, Writing – review & editing, Project administration. **Geneviève Gésan-Guiziou:** Conceptualization, Methodology, Validation, Supervision, Writing – review & editing, Project administration, Funding acquisition.

Declaration of Competing Interest

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.

Data availability

The authors do not have permission to share data.

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