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Original article

Whey microfiltration performance: influence of protein concentration by ultrafiltration and of physicochemical pretreatment

A Pierre, Y Le Graet, G Daufin, F Michel, G Gésan

Laboratoire de Recherches de Technologie Laitière, INRA, 65, rue de St-Brieuc, 35042 Rennes, France

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Summary — Microfiltration with membranes of 0.2 μm pore size was used to clarify and defat whey previously ultrafiltered at various volume concentration ratios (VCR, 1–5.5). Physicochemical pretreatment of whey combining calcium addition (in order to reach a final concentration in the range 1.3–3.3 $g \cdot kg^{-1}$), pH increase to 7.3 and a heat treatment (60°C, 10 min) were carried out. The efficiency of microfiltration, performed at a constant permeation flux, was compared with centrifugation. Pretreatment of retentate concentrated to a VCR of 5.5, led to poor hydraulic performance: a continuous increase in transmembrane pressure (TP) over the whole run (60 min) from 0.05 to 0.15 MPa brought evidence of rapid membrane fouling. A low total nitrogen matter recovery (TNMr) of 0.41 was obtained. Pretreatment of VCR 2.5 and VCR 4.0 retentates allowed higher TNMr (0.65) and better hydraulic performance, *ie* slower TP increase to be obtained. With no physicochemical pretreatment, VCR 2.5–4.0–5.5 retentates led to improved hydraulic performance and higher TNMr (0.90).

whey / microfiltration / pretreatment / permeation / performance

Résumé — Optimisation de la microfiltration du lactosérum. Influence de la concentration par ultrafiltration et des prétraitements physicochimiques de clarification. *Un lactosérum préalablement ultrafiltré jusqu'à des facteurs de concentration volumiques (FCV) de 2,5 ; 4,0 ; 5,5 a été microfiltré avec une membrane ayant une taille moyenne de pores de 0,2 μm. Un prétraitement physicochimique de clarification était effectué dans certains essais (addition du calcium jusqu'à une teneur finale de 1,3 à 3,3 $g \cdot kg^{-1}$, augmentation du pH jusqu'à 7,3 suivie d'un traitement thermique à 60°C pendant 10 min). Les performances hydrauliques de microfiltration et les taux de perméation des constituants du lactosérum ont été étudiés sur une installation fonctionnant à flux de perméation constant. Le prétraitement appliqué à un lactosérum à FCV 5,5 conduit à des performances hydrauliques faibles : une augmentation continue de la pression transmembranaire pendant toute la durée de l'essai (60 min) de 0,05 à 0,15 MPa est un indice du colmatage rapide de la membrane. Le rendement en matière azotée totale (MAT) est également bas : 0,41. Le prétraitement appliqué au lactosérum à FCV 2,5 ou 4,0 conduit à des rendements en MAT plus élevés (0,65) et à de meilleures performances hydrauliques. C'est en l'absence de prétraitement que les 3 types de lactosérum (FCV*

2,5 ; 4,0 ; 5,5) ont donné les meilleures performances hydrauliques et les rendements en MAT les plus élevés (0,90).

lactosérum / microfiltration / prétraitement / perméation / performance

INTRODUCTION

Clarification of whey is currently performed in order to remove constituents involved in membrane fouling and susceptible to interfere with subsequent purification of proteins. Clarification includes 2 steps: precipitation and separation of the formed precipitate.

Precipitation is achieved by manipulating the physicochemical equilibria between calcium and phosphate, either by reducing ionic strength (de Wit *et al,* 1978), or by adding calcium in association with a heat treatment. This process, also called thermal Ca-treatment, allows the formation of insoluble complexes between Ca and biological material (Lee and Merson, 1976). The feasibility of this process to all kinds of wheys was demonstrated by Fauquant *et al* (1985a, b) and Maubois *et al* (1987).

Three technologies have been proposed for separating the precipitate: decantation (de Wit *et al,* 1978); centrifugation (CF) (Wasen and Lehmann, 1989); and microfiltration (MF) (Maubois *et al,* 1987). The feasibility of the third was demonstrated by Baumy *et al* (1990) and by Maugas and Daufin (1992). Retention of precipitate by different pore-size MF membranes was compared and found suitable when the pore size was lower than 0.6 μm in average diameter (Rinn *et al,* 1990; Pearce *et al,* 1992). Large daily variations in the MF fluxes of an industrial plant were reported by Gésan *et al* (1993a), who were able to correlate them with efficiency of the membrane cleaning, daily variations of whey physicochemical characteristics and the MF operating parameters.

In a previous study (Pierre *et al,* 1992), the influence on protein and calcium contents of whey, temperature, time and pH of the applied heat treatment was tested in order to optimize clarification but in these experiments only CF was used for separating the precipitated fraction.

The objective of the current work was to compare how the efficiency of whey fractionation is influenced by the separation technology used, CF or MF, with regards to the physicochemical pretreatment (Ca concentration, pH and heating time/ temperature) applied to whey previously concentrated by ultrafiltration (UF).

MATERIALS AND METHODS

Experimental rig

The MF rig used was described by Daufin *et al* (1993). It was equipped with M14 Carbosep membranes (Techsep, Miribel, France) with an average pore diameter of 0.14 μm and a membrane area of 2.26 x 10^{-2} m^2. MF of the feeds described below was performed for 60 min at constant tangential flow velocity (6.00 ± 0.05 $m \cdot s^{-1}$), temperature (50.0 ± 0.1°C) and flux (60.0 ± 0.5 $l \cdot h^{-1} \cdot m^{-2}$).

Feeds

The chemical composition of the feeds studied in this work is given in table I. These were issued from sweet whey skimmed and filtered (20 μm) produced by an emmental cheese plant (ULN, Montauban-de-Bretagne, France). UF until a VCR of 2.5, 4.0 and 5.5 was performed as

Table I. Microfiltration of retentates. Composition of initial retentates (R) and microfiltrates (MF). *Microfiltration des rétentats. Composition des rétentats initiaux (R) et des microfiltrats (MF).*

Run N°	*Retentate*	*Product*	*OD*	*pH*	*Ca*	*P*	*TNM*	*TNMr*
					g·kg⁻¹			
	$R_{2.5}$	R	1.300	6.7	0.392	0.455	18.7	1.00
1	$R_{2.5}$	MF	0.010	6.8	0.302	0.393	16.5	0.88
2–3	$R_{2.5-1.3}$	MF*	0.013	7.3	0.621	0.130	12.3	0.66
4	$R_{2.5-3.3}$	MF	0.012	7.0	2.408	0.102	12.1	0.65
	$R_{4.0}$	R	1.900	6.7	0.453	0.507	28.9	1.00
5–6–7	$R_{4.0-2.3}$	MF**	0.010	7.1	1.386	0.118	18.7	0.65
	$R_{5.5}$	R	2.200	6.7	0.498	0.548	38.9	1.00
8	$R_{5.5}$	MF	0.034	6.7	0.450	0.469	32.4	0.83
9	$R_{5.5-1.3}$	MF	0.048	7.2	0.615	0.191	15.8	0.41
10	$R_{5.5-3.3}$	MF	0.041	6.9	2.280	0.129	16.3	0.42
SE			–	–	0.003	0.003	0.1	0.01

* Mean of 2; ** mean of 3; SE: standard error; TNM: total nitrogen matter; TNMr: total nitrogen matter recovery; OD: optical density.
SE = erreur expérimentale.

described by Pierre *et al* (1992). UF retentates were frozen until experimentation.

Pretreatment of the UF retentates was carried out according to Pierre *et al* (1992). Calcium content was adjusted to the range 1.3–3.3 $g \cdot kg^{-1}$ through the addition of a 4 $mol \cdot l^{-1}$ $CaCl_2$ solution in UF retentates at 16°C. The pH was then increased to 7.3 with 5 $mol \cdot l^{-1}$ NaOH; the temperature was raised to 60°C over 3 min and held for 10 min. When no calcium was added, the pH was left at its original value. The precipitate was separated by either CF (2000 g, 15 min, 30°C) with a Heraeus equipment (Osterode, Germany) or MF. Supernatant and microfiltrate samples were collected for analysis.

The nomenclature of feeds R_{x-y} was as follows. R is the UF whey retentate; *x* is its VCR achieved by UF; and *y* is the calcium level used for its pretreatment, if any. For example, $R_{2.5-1.3}$ was a retentate at VCR 2.5, pretreated with calcium to a level 1.3 $g \cdot kg^{-1}$.

Ten MF experiments were performed, combining VCR and the amount of calcium as shown in table I. One combination was tested in 3 replicates for the assessment of experimental error (standard error = SE). The order of performing trials was randomized to minimize experimental bias.

Calculations

Flux and transmembrane pressure (TP) measured during the MF runs, and pure water flux, before and after MF, allowed the hydraulic resistances to be calculated (membrane R_m; total fouling R_f; reversible fouling R_{rf}; irreversible fouling R_{if}) (Gésan *et al*, 1993b). The relative error for each hydraulic resistance ($\Delta R/R$) was assessed as < 5%. Results were reported as normalized fouling resistance: R/R_m.

The mean pore size estimation (r_t) after a whey retentate MF run of time *t*, was calculated from Poiseuille's law as follows:

$$\frac{TP_t}{TP_w} = \left(\frac{r_m}{r_t} \right)^4$$

where TP_t and TP_w are the transmembrane pressure, after t min of whey retentate MF and during water flux (J_w), respectively, assessed at the same flux, J = 60 $l \cdot h^{-1} \cdot m^{-2}$; r_m the clean membrane pore radius is 100 nm (nominal supplier figure). The size of particles in whey retentate was calculated from experimental retention TR according to Ferry's law:

$$TR = [1 - (1 - \frac{a}{r})^2]^2$$

where a is the particle radius to be determined.

TR was obtained from concentration of the constituents in initial whey retentate (C_r) and in whey permeate (C_p):

$$TR = \frac{C_r - C_p}{C_r}$$

Operating procedure

Before MF of the feeds, membrane cleaning was carried out with solutions at 50°C and retentate pressure 0.2 MPa: i) HNO_3 0.06 $mol \cdot l^{-1}$ for 45 min; ii) filtered water rinsing; iii) $NaOCl_3$ 1000 $mg \cdot l^{-1}$ Cl_2, pH 11, for 30 min; iv) filtered water rinsing. Pure water flux was measured at constant transmembrane pressure TP_w = 0.1 MPa, before, J_w, and after, J'_w, the 60-min MF run to assess the hydraulic resistance of clean membrane and fouled membrane. For the MF trials, the flux was gradually increased over 6 min at a rate of 10 $l \cdot h^{-1} \cdot min^{-1}$ until a steady-state flux of 60 $l \cdot h^{-1} \cdot m^{-2}$ was achieved. In all experiments, thermal equilibrium was first achieved with the rig full of water (2 $m \cdot s^{-1}$, 0.2 MPa). Water was then pushed out of the rig by the fluid to be microfiltered. Tangential flow rate and then flux were set to their steady-state values.

Chemical analysis

The qualitative composition of the proteinic fraction of samples was determined by electrophoresis on gradient polyacrylamide gels, T = 4–30% (Pharmacia, Uppsala, Sweden) under denaturing and native conditions. The electrophoresis buffer and sample preparation were as described by Andrews (1983).

Lactose and total nitrogen matter (TNM) were measured by IR analysis (Dairy Lab, Multispec, York, UK). Non-protein nitrogen (NPN) was according to Rowland (1938). Individual proteins, *ie* α-lactalbumin (αla), β-lactoglobulin (βlg) and caseinomacropeptide (CMP) were simultaneously quantified by gel filtration HPLC (SP 8800, Spectra Physics Analytical, San Jose, CA, USA) using a 30-cm TSK SW 2000 DX column (Supelco, Bellefonte, USA) and 30% acetonitrile in water (v/v), adjusted to 0.1% in trifluoroacetic acid, as a solvent (Efstathiu T, personal communication). The detection was at 215 nm.

The experimental TNM recovery (TNMr) was estimated as the ratio of TNM in the microfiltrate, at 60 min MF or in the supernatant, to the TNM of the initial whey retentate. The maximum TNM in microfiltrate and supernatant was estimated from initial whey retentate TNM minus the content of the constituents wholly retained by the membrane as determined by electrophoresis. The level of these retained constituents was quantified from mean values reported for milk by Walstra and Jenness (1984). This calculation accounted for neither membrane fouling nor sieving mechanisms related to relative size of molecules and pores.

Calcium was determined by atomic absorption spectrophotometry (Varian, Palo Alto, CA, USA); phosphorus according to FIL–IDF n°33B (1982) and chloride by conductivity with an Ag electrode (chloride analyzer 926–Ciba Corning Diagnostics, Halstead, UK) so as to determine dilution and allow correction for permeate concentration.

The total lipids were extracted with 80 ml methanol/chloroform (2:1 v/v) according to Folch *et al* (1957), applied on a glass column containing the freeze-dried sample (corresponding to 50 ml liquid whey), mixed with 10 g celite. Chloroform was added to the collected fraction at 33% (v/v), and then, water, at 60% of the actual volume. After mixing and standing overnight, the lower solvent phase was collected, evaporated and total lipids estimated by weighing. These lipids, solubilized in chloroform, were analyzed by HPLC for their content in phospholipids (Leseigneur *et al*, 1989; Surel, 1993). The optical density (OD) of whey was measured at 600 nm, as an estimation of turbidity (Spectrophotometer DU 62, Beckman I, Fullerton, USA).

The aggregate size in pretreated retentates was measured using an optical microscope (Wild Instruments, Heerbrugg, Switzerland) equipped with an ocular micrometer (magnification x 400). Colloidal particles in retentates, supernatants and microfiltrates (< 2 μm in size), were measured by quasi-elastic light diffusion on a Coulter N4D apparatus (Coultronics, Amherst, MA, USA), with a 90° angle for the incident light. The samples were diluted in water. The populations are expressed in weight percentage.

Rheological measurements were performed at 50°C, with a Rheomat 30 viscosimeter, equipped with an O-type coaxial cylindrical chamber (Contraves, Zurich, Switzerland). Viscosities were registered at shear rates (γ) from 12.9–953.6 s^{-1}.

RESULTS

Physical properties of whey retentates

Volume of precipitated fraction

Microscope observation of pretreated $R_{2.5}$ and $R_{4.0}$ showed large aggregates surrounded by a translucent liquid phase. In pretreated $R_{5.5}$, the aggregates coalesced and filled the whole volume. There was no translucent phase. The aggregates in pretreated $R_{2.5}$ and $R_{4.0}$ tended to sediment leading to a clear supernatant. The sedimentation was complete after 1 h standing. The volume fraction occupied by the precipitate increased with concentration of retentate: 0.86 for $R_{2.5}$, and 0.96 for $R_{4.0}$. Extrapolation of the results gives 1.00 for $R_{4.5}$. This means no more sedimentation of the aggregated matter would occur at VCR > 4.5.

Particle size

The aggregates in pretreated $R_{2.5}$ were 7–10 μm in size, as determined by microscope observation. These appeared to be constituted of smaller granulated material < 0.5–1 μm.

The detection of smaller particles in wheys and estimation of their mean diameter by quasi-elastic light diffusion is reported in table II. Initial UF whey retentate, its supernatant and pretreated UF whey retentate before any separation all showed particles of 206–209 nm, whereas in a second group of particles the size was different in initial retentate (1 460 nm), its supernatant (1 050 nm) and the non-separated pretreated retentates (726 nm). These particles were found in neither the 2 microfiltrates nor the supernatant of pretreated retentate, where only smaller particles of 4, 6 and 12 nm were detected.

Rheological measurements

The viscosity of whey retentates *versus* shear rate is shown in figure 1. The viscosity of non-pretreated retentates (Ca = 0.4 $g \cdot kg^{-1}$) was only slightly affected by VCR: μ = 0.94 mPa•s for $R_{2.5}$ and μ = 1.01 mPa•s (γ = 953.6 s^1) for $R_{5.5}$. Pretreated retentates showed a viscosity increase compared to non-pretreated ones. The increase was related to retentate VCR and to Ca level, up to a maximum (results not shown). Moreover, a shear thinning behavior was observed with these samples (fig 1). Supernatants of pretreated retentates had a viscosity close to that of the non-pretreated initial ones. As an example, the viscosity of $R_{2.5}$ was μ = 0.89 mPa•s and that of pretreated supernatant μ = 0.85 mPa•s (γ = 953.6 s^{-1}).

Fractionation by centrifugation and microfiltration

Biochemical studies

The fractionation obtained with microfiltration was extensively studied with 1 of the

Table II. Size of small particles (< 2 000 nm) in $R_{2.5}$. Incidence of separation process, centrifugation (CF) or microfiltration (MF), and of calcium pretreatment (non-pretreated $R_{2.5}$ or pretreated $R_{2.5-1.3}$)*. *Taille des petites particules (< 2000 nm) dans le $R_{2.5}$. Influence du procédé de séparation (centrifugation, CF, ou microfiltration MF) et du prétraitement du rétentat (non prétraité, $R_{2.5}$, prétraité, $R_{2.5-1.3}$).*

	Initial		*Supernatant CF*		*Microfiltrate MF*	
	$R_{2.5}$	$R_{2.5-1.3}$	$R_{2.5}$	$R_{2.5-1.3}$	$R_{2.5}$	$R_{2.5-1.3}$
Avd	209	206	208	12	6	4
SD	100	100	93	3	2	2
%	26	35	51	95	99	100
Avd	1 460	726	1 050			
SD	200	98	200			
%	72	47	49			

Average diameter (Av d) in nm; SD: standard deviation; % percentage by weight.
Av d : diamètre moyen en nm. SD: écart type; %: pourcentage en poids.

retentates, $R_{2.5}$. Comparison was made between centrifugation and microfiltration applied to either non-pretreated ($R_{2.5}$) or pretreated retentate ($R_{2.5-1.3}$). The amount of the main constituents in initial retentates and their supernatants and microfiltrates are reported in table III.

Centrifugation of the initial retentate had little effect on the OD (1.400/1.100). When combined with pretreatment, an OD decrease to 0.320 was obtained; this value is in good agreement with previous results (Pierre *et al*, 1992). Microfiltration resulted in a more drastic OD decrease, to 0.006–0.010, with pretreated as well as with non-pretreated retentates.

Centrifugation of non-pretreated $R_{2.5}$ resulted in little or no alteration of component contents. Any of the 3 other procedures (CF of pretreated $R_{2.5-1.3}$, MF of $R_{2.5}$ and $R_{2.5-1.3}$) led to a highly significant decrease of component concentration. TNM in centrifuged pretreated retentates and in microfiltered non-treated retentates was 0.88–0.89 times its initial value. A lower proportion (0.68) was obtained in microfiltrate of $R_{2.5-1.3}$. On the other hand, the best removal of lipids and phospholipids was performed in this particular case (0.06 $g \cdot kg^{-1}$ and non-detectable content).

The concentration of proteinic constituents is reported in figure 2. Compared with

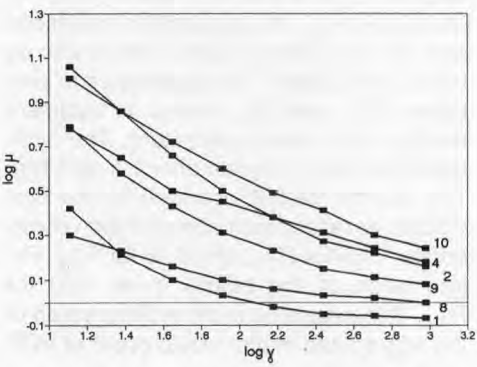

Fig 1. Whey UF retentates viscosity (μ) *versus* shear rate (g): $R_{2.5}$ (1); $R_{2.5\text{-}1.3}$ (2); $R_{2.5\text{-}3.3}$ (4); $R_{5.5}$ (8); $R_{5.5\text{-}1.3}$ (9); $R_{5.5\text{-}3.3}$ (10).
Viscosité des rétentats UF de lactosérum (μ) en fonction de la vitesse de cisaillements (g): $R_{2.5}$ (1); $R_{2.5\text{-}1.3}$ (2); $R_{2.5\text{-}3.3}$ (4); $R_{5.5}$ (8); $R_{5.5\text{-}1.3}$ (9); $R_{5.5\text{-}3.3}$ (10).

Table III. Composition of initial $R_{2.5}$ and its supernatants and microfiltrates obtained by centrifugation (CF) or microfiltration (MF) or pretreated ($R_{2.5-1.3}$) or non-pretreated ($R_{2.5}$) retentate $g \cdot kg^{-1}$.
Composition du rétentat initial $R_{2.5}$ et des surnageants et microfiltrats obtenus par centrifugation (CF) ou microfiltration (MF) du rétentat prétraité ($R_{2.5-1.3}$) ou non prétraité ($R_{2.5}$) $g \cdot kg^{-1}$.

	Initial $R_{2.5}$	*Supernatant CF*		*Microfiltrate MF*	
		$R_{2.5}$	*$R_{2.5-1.3}$*	*$R_{2.5}$*	*$R_{2.5-1.3}$*
OD	1.400	1.100	0.320	0.010	0.006
Lactose	48.6	48.7	48.2	43.7	40.7
P	0.459	0.425	0.175	0.393	0.115
Ca	0.393	0.337	0.667	0.302	0.564
Total lipids	1.14	1.14	0.25	0.11	0.06
Phospholipids	0.22	0.22	0.10	0.02	0.00
TNM	18.8	18.8	16.7	16.5	12.8
Experimental TNMr	1.00	1.00	0.89	0.88	0.68

OD: optical density; TNM: total nitrogen matter; TNMr: total nitrogen matter recovery.

initial $R_{2.5}$, there were few differences in the NPN, CMP and αla levels in supernatants and microfiltrates. On the contrary, the βlg content was greatly reduced, particularly in the microfiltrate of $R_{2.5-1.3}$: 5.8 $g \cdot kg^{-1}$ instead of 8.2 $g \cdot kg^{-1}$ in initial $R_{2.5}$ (fig 2), *ie* a retention equal to 0.29. On the other hand, the other non-specifically analyzed components were reduced from 4.40 to 2.6 $g \cdot kg^{-1}$ in supernatants of $R_{2.5-1.3}$ and to 1.5 $g \cdot kg^{-1}$ in microfiltrates of $R_{2.5-1.3}$. It is likely that this fraction mainly contained the high molecular mass proteins, mainly immunoglobulins, bovine serum albumin and lactoferrin.

The composition of samples was further assessed by electrophoresis which showed:

i) a lower level of high molecular mass proteins in both microfiltrates of $R_{2.5}$ and $R_{2.5-1.3}$;

ii) no proteose peptone PP_3 or PP_5 in both microfiltrates and the supernatant of $R_{2.5-1.3}$ (PP_3 = milk fat globule membrane fraction and PP_5 = β-casein 1–106 fraction);

iii) no PP_8 (PP_8 = β-casein 26–106 fraction) in the microfiltrate or supernatant of $R_{2.5-1.3}$. Presence of PP_8 in microfiltrate or supernatant of non-pretreated $R_{2.5}$.

Maximum TNMr calculated from these results, as described in *Materials and Methods,* were in the same range, 0.92–

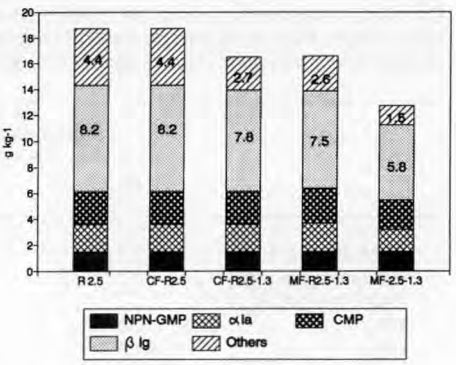

Fig 2. Proteinic constituents in microfiltrates (MF) and supernatants (CF) of $R_{2.5}$ and $R_{2.5-1.3}$.
Constitutants protéiques des microfiltrats (MF) et de surnageants (CF) du $R_{2,5}$ et $R_{2,5-1,3}$.

0.88, with the 2 microfiltrates and the supernatant of $R_{2.5-1.3}$ (table IV).

Incidence of protein concentration and calcium pretreatment on microfiltrate composition

The composition of microfiltrates obtained from retentates with various volume concentration factors and pretreated with different calcium levels was reported in table I.

OD values of microfiltrates ($0.010 < OD < 0.048$) showed no incidence of whey pretreatment. A slight but significant effect of protein concentration on OD was shown: average OD was 0.041 with $R_{5.5}$ and 0.011 with $R_{2.5}$ and $R_{4.0}$.

TNMr also depended on protein concentration. With no calcium pretreatment, TNMr values of 0.88 and 0.83 were obtained with $R_{2.5}$ and $R_{5.5}$, respectively, showing a slight transmission decrease at higher protein concentration.

Pretreating whey UF retentate resulted in a TNMr decrease: 0.65–0.66 with $R_{2.5}$ and $R_{4.0}$ and 0.42–0.41 with $R_{5.5}$. Other constituents (calcium, phosphorus) and pH were at the same level in the various microfiltrates.

Hydraulic performance

The recording of hydraulic parameters (R_f, TP) *versus* time of microfiltration allowed the calculation of R_f/R_m for all runs. As an example, figure 3 reports the results for $R_{2.5}$ and $R_{5.5}$. With $R_{5.5-1.3}$ and $R_{5.5-3.3}$, a progressive increase in R_f/R_m with time was observed.

The corresponding R_f/R_m were calculated from values of R_f and TP collected after 60 min microfiltration (table V). When plotted against R_m (fig 4), significantly higher values appeared for runs 9 and 10 ($R_{5.5-1.3}$ and $R_{5.5-3.3}$), all other values being lower and showing neither treatment effect nor residual incidence of R_m after correction. The higher values of R_f/R_m in runs 9 and 10 were related to retentate concentration (VCR), as shown in figure 5. Calcium level had no effect (fig 5).

Table IV. Calculation of maximum TNMr ($TNMr_{max}$) according to the fractionation observed for proteinic constituents in $R_{2.5}$ and to their quantification in $g \cdot kg^{-1}$ from Walstra and Jenness (1984).
Calcul du rendement maximal en matière azotée totale ($TNMr_{max}$) pour les différents procédés étudiés, compte tenu d'une part du fractionnement observé pour les constituants protéiques, d'autre part, de leur teneur moyenne dans le lactosérum ($g \cdot kg^{-1}$) estimée d'après Walstra et Jenness (1984).

		Supernatant CF		*Microfiltrate MF*	
		$R_{2.5}$	*$R_{2.5-1.3}$*	*$R_{2.5}$*	*$R_{2.5-1.3}$*
1	TNM of initial $R_{2.5}$	18.8	18.8	18.8	18.8
	Retained material (level in initial $R_{2.5}$)				
	PP_3	0	0.6	0.6	0.6
	PP_8	0	0.3	0	0.3
	PP_5	0	0.6	0.6	0.6
	βlg	0	0	0.8	0.8
2	Total retained	0	1.5	2.0	2.3
3	$TNM_{max} g \cdot kg^{-1}$ 1–2	18.8	17.3	16.8	16.5
4	$TNMr_{max}$ 3/1	1.00	0.92	0.89	0.88

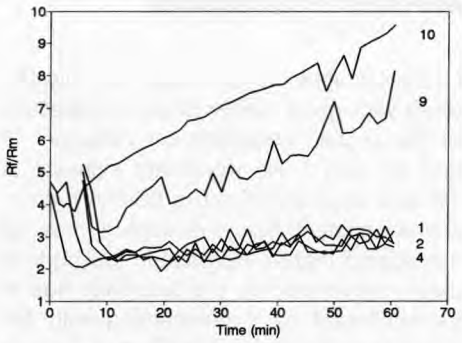

Fig 3. Variation of R_f/R_m *versus* time of microfiltration. Incidence of volume concentration ratio and pretreatment: $R_{2.5}$ (1); $R_{2.5\text{-}1.3}$ (2); $R_{2.5\text{-}3.3}$ (4); $R_{5.5\text{-}1.3}$ (9); $R_{5.5\text{-}3.3}$ (10).
Évolution de R_f/R_m en fonction du temps de microfiltration. Influence du facteur de concentration volumique (VCR) du rétentat et du prétraitement : $R_{2,5}$ (1); $R_{2,5\text{-}1,3}$ (2); $R_{2,5\text{-}3,3}$ (4); $R_{5,5\text{-}1,3}$ (9); $R_{5,5\text{-}3,3}$ (10).

DISCUSSION

Clarification efficiency

Microfiltration was more efficient than centrifugation for whey clarification. Very low OD values were observed in microfiltrates, even with no Ca pretreatment (0.010–0.041), while centrifugation, even combined with a pretreatment gave higher OD values; for supernatants produced under optimal conditions with $R_{2.5}$ and $R_{5.5}$ typical OD values would be 0.180–0.380. The difference in efficiency between the 2 processes may be explained by the particle sizes measured in retentates. Particles ranging from 200 to 1 500 nm in diameter were present in initial retentates. Conditions applied during centrifugation were not

Table V. Microfiltration of whey ultrafiltration (UF) retentates. Initial membrane hydraulic resistance R_m and fouling characteristics R_f, R_{rf} and R_{if} (see *Materials and Methods*)*.
Microfiltration des rétentats UF de lactosérum. Résistance hydraulique initiale de la membrane propre, R_m, et caractéristiques du colmatage, R_f, R_{rf} et R_{if} (Material and Methods)*.

Run	*Retentate*	*Microfiltration of water*		*Microfiltration of whey UF retentate*			
N°		J_w $l \cdot h^{-1} \cdot m^{-2}$	R_m $10^{12}\ m^{-1}$	*TP60* *MPa*	R_f $10^{12}\ m^{-1}$	R_{rf} $10^{12}\ m^{-1}$	R_{if} $10^{12}\ m^{-1}$
1	$R_{2.5}$	428	1.57	0.09	5.9	0.6	5.3
2	$R_{2.5-1.3}$	483	1.39	0.07	4.5	3.2	1.3
3	$R_{2.5-1.3}$	439	1.53	0.06	4.0	0.7	3.3
4	$R_{2.5-3.3}$	487	1.37	0.06	3.6	2.4	1.2
5	$R_{4.0-2.3}$	455	1.47	0.06	3.8	1.5	2.3
6	$R_{4.0-2.3}$	433	1.55	0.05	4.3	1.8	2.5
7	$R_{4.0-2.3}$	667	1.00	0.03	2.7	1.4	1.3
8	$R_{5.5}$	512	1.31	0.05	4.4	1.8	2.6
9	$R_{5.5-1.3}$	473	1.41	0.15	10.4	9.4	1.0
10	$R_{5.5-3.3}$	534	1.25	0.15	10.8	7.6	3.2
	$\overline{m}$	491	1.38				
	SD	71	0.17				
	SE			0.02	1.4	0.1	0.9

* TP: transmembrane pressure. SD: standard deviation; $\overline{m}$: mean; SE: standard error.

efficient enough to allow their sedimentation. Sedimentation was achieved after Ca pretreatment, which formed aggregates sized 7–10 µm. Using a microfiltration membrane with 200 nm mean pore size, total retention of the native individual particles (> 200 nm) took place, even with no pretreatment.

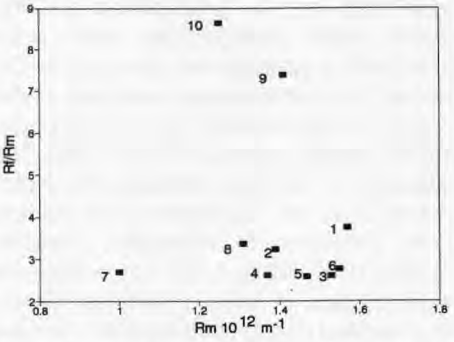

Fig 4. Overall fouling resistance R_f/R_m after 60 min MF run (J = 60 l•h^{-1}•m^{-2}) *versus* initial hydraulic resistance, R_m with retentate-treated feeds and numbered as in tables I and V.
Résistance de colmatage total R_f/R_m après 60 min de microfiltration des rétentats (J = 60 l•h^{-1}•m^{-2}) en fonction de la résistance initiale de la membrane propre, R_m. Pour la composition et le prétraitement des rétentats, les chiffres renvoient aux tableaux I et V.

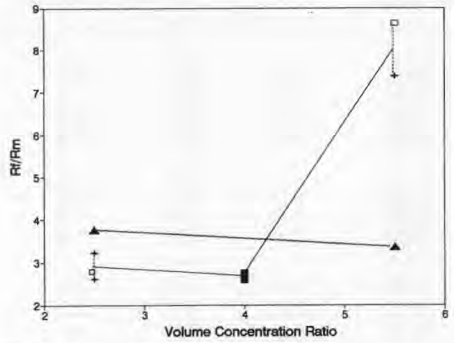

Fig 5. R_f/R_m *versus* volume concentration ratio of retentate: Rx (▲); Rx-$_{1.3}$ (+); Rx-$_{2.3}$ (■); Rx-$_{3.3}$ (□).
R_f/R_m en fonction du facteur de concentration volumique (VCR) du rétentat : Rx (▲) ; Rx-$_{1,3}$ (+) ; Rx-$_{2,3}$ (■) ; Rx-$_{3,3}$ (□).

Microfiltration performance

Non-pretreated wheys could be microfiltered with good performance, irrespective of the protein concentration between 18 and 40 g•kg^{-1}. No significant increase in TP was observed during a 60-min run under a constant flux, therefore it may be considered that no increase in fouling took place. Nevertheless, the feasibility has to be confirmed by experimenting with MF runs of several hours. No effect of Ca concentration was observed. Low amplitude effects of some parameters (VCR, calcium addition) might have been masked by the high standard deviation in R_m values (table V: 0.17 x 10^{12} m^{-1}). Nevertheless, R_f/R_m calculated after 60 min of microfiltration did not depend significantly on R_m values (fig 5). The threshold shape effect of VCR (between 4.0–5.5) on R_f, could be explained according to 2 hypotheses.

Pretreatment requires a pH increase from 6.7 to 7.3. This obviously might favor the formation of insoluble Ca phosphate from the soluble phase, and its precipitation on or in the membrane. If so, low performance would have been obtained with pretreated $R_{2.5}$ and $R_{4.0}$ and not only with pretreated $R_{5.5}$, as observed. In addition, the 60-min run is likely to be too short compared with the requested lag time for salt precipitation in our MF conditions. This hypothesis must also be validated with longer runs.

On the other hand, we had to question the effect of physical properties of pretreated $R_{5.5}$ on MF performance. In this product a stable continuous network was observed throughout the whole volume, arising from aggregated colloidal material concentrated enough to form interparticulate binding of low strength. This structure may be the reason why shear thinning properties were observed. The viscosity of these products was consequently dependent on flow rate.

In microfiltration, with tubular M14 membrane, the tangential flow rate was 6 $m \cdot s^{-1}$, which corresponds to a turbulent flow, owing to the membrane configuration. Close to the membrane, the flow velocity drastically decreased and a higher viscosity for the shear thinning product has to be considered. This might promote enhanced material deposition on and/or in the membrane. Higher values for R_{rf} (7.6 and 9.4 x 10^{12} m^{-1}, table V) were effectively observed in this case, corresponding to higher membrane surface fouling.

From a hydraulic point of view, the increase in membrane resistance to microfiltrate transfer can be assimilated to a pore size reduction and the correponding mean pore diameter can be calculated from Poiseuille's law. In the early stages of the runs, *ie* at 6 min, mean pore diameter was found to be 190 nm. After a 60-min run, it was found to be 182 nm for TP_{60} = 0.058 MPa and 113 nm for TP_{60} = 0.15 MPa (pretreated $R_{5.5}$), compared with 200 nm for the clean membrane on water.

Protein recovery

Microfiltration of non-pretreated wheys was as efficient as centrifugation for proteinic recovery (TNMr) and allowed $TNMr_{max}$ to be reached. Microfiltration of pretreated retentates showed lower TNMr. An assessment of nitrogen matter retention was given by the difference: $TNMr_{max}$ –TNMr. This was 0.89–0.65 = 0.24 for $R_{2.5}$ or $R_{4.0}$, and 0.89–0.41 = 0.48 for $R_{5.5}$ from data in table I.

The individual protein quantification in $R_{2.5}$ microfiltrates (fig 2) showed that low TNMr are due to retention of not only high molecular mass proteins, but also αla and especially βlg (0.29) although its molecular mass is low (βlg is at pH 7.0 as a dimer, *ie* 36 kDa). As βlg is the main protein in whey and its retention (0.29) was in the same range as TNM retention in pretreated $R_{2.5}$ (0.24), we used it as a model for explaining whey protein permeation.

Theoretical retention of βlg can be calculated according to Ferry's law. The diameter of the βlg dimer is estimated as 3.9 nm (McKenzie, 1971) with 2 nm more because of hydration and electrical double layer (Hiemenz, 1986) leading to 5.9 nm size. From fouled membrane resistance observations, the apparent mean pore size of the membrane after 60 min microfiltration is 182 nm (TP_{60} = 0.058 MPa) and 113 nm (TP_{60} = 0.15 MPa) with $R_{5.5–1.3}$ and $R_{5.5–3.3}$, respectively. These pore sizes give theoretical retention values of around 0.01. The experimental retention is of the same order of magnitude when microfiltering whey UF retentates with no pretreatment, but much higher values were assessed with pretreated feeds. Consequently, in the latter case, either βlg particle size or apparent pore size is different.

To get βlg retention of 0.24 ($R_{2.5}$) and 0.48 ($R_{5.5}$) we use Ferry's law to calculate that βlg particles would be 50 nm in size. But particle size determination in whey retentates did not show such values.

On the other hand, assuming βlg remained at its native size, experimental retention and Ferry's law enable calculation of a mean membrane pore size of 22 and 14 nm, when microfiltering pretreated $R_{2.5}$ and $R_{5.5}$, respectively. This result does not fit previously discussed hydraulic results.

There is obviously a discrepancy between protein permeation results and hydraulic results. Whey premeation performance remained good, as shown through low TP, while protein recovery was poor, as with a fouled membrane. We can assume that microfiltering pretreated wheys, colloidal aggregates block the larger pores of the membrane, dividing the initial pores into smaller sized pores. The smooth retic-

ulum obtained still remains highly hydrophilic, and consequently, permeable to water and microsolutes. This preserves the hydraulic properties of the membrane. In contrast, protein macromolecules would be slowed down in the flux by the reticulum and eventually build up inside the MF membrane pores.

CONCLUSION

Microfiltration was a useful tool to clarify non-pretreated whey UF retentates (VCR < 5.5). Pretreatment of UF whey retentates appears detrimental. It reduces TNM recovery of all the retentates and decreases hydraulic performance of VCR 5.5 retentates. The reason for this was not elucidated.

Clarification efficiency, as measured by OD of the resulting product, was higher with MF (0.010 < OD < 0.050) than with CF associated with pretreatment (0.180 < OD < 0.380). However, TNM recoveries obtained by MF of non-treated retentates were identical to those obtained by CF of treated retentates (TNMr = 0.89).

Present results were obtained from 1-h MF runs. It would be interesting to test if they remain valid for longer runs, in the range of several hours, to approach plant working conditions.

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