Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

Extraction and Separation of α-lactalbumin and β-Lactoglobulin from Skim Milk by Microfiltration and Ultrafiltration at High Shear Rates: A Feasibility Study

Valentina Espina[a]; Michel Y. Jaffrin[a]; Luhui Ding[a]
[a] Technological University of Compiègne, Compiègne, France

Online publication date: 09 December 2009

To cite this Article Espina, Valentina , Jaffrin, Michel Y. and Ding, Luhui(2009) 'Extraction and Separation of α-lactalbumin and β-Lactoglobulin from Skim Milk by Microfiltration and Ultrafiltration at High Shear Rates: A Feasibility Study', Separation Science and Technology, 44: 16, 3832 — 3853
To link to this Article: DOI: 10.1080/01496390903183238
URL: http://dx.doi.org/10.1080/01496390903183238

Separation Science and Technology, 44: 3832–3853, 2009

ISSN: 0149-6395 print/1520-5754 online
DOI: 10.1080/01496390903183238

Taylor & Francis
Taylor & Francis Group

Extraction and Separation of α-lactalbumin and β-Lactoglobulin from Skim Milk by Microfiltration and Ultrafiltration at High Shear Rates: A Feasibility Study

Valentina Espina, Michel Y. Jaffrin, and Luhui Ding
Technological University of Compiègne, Compiègne, France

Abstract: This paper presents a two-stage membrane filtration process for extracting and separating α-Lactalbumin (α-La) and β-Lactoglobulin (β-Lg), from UHT skim milk, using dynamic filtration. The 1st stage separates casein micelles in retentate from whey proteins in the permeate with rotating 0.2 μm pores ceramic membrane disks. Casein micelles rejection was excellent, while α-La and β-Lg transmissions remained between 80 and 90%. The permeate flux at 40°C ranged from 105 to 40 $Lh^{-1}m^{-2}$ at a volume reduction ratio of VRR = 4. 4. The 2nd stage consisted of ultrafiltration of the previous permeate with a metal disk rotating at 2000 rpm near a fixed 50 kDa PES membrane, in order to concentrate β-Lg in retentate, while collecting α-La in the permeate. The flux dropped from 270 $Lh^{-1}m^{-2}$ at VRR = 1, and remained nearly constant at 200 $Lh^{-1}m^{-2}$ until a VRR of 3.3. α-La transmission increased with VRR to reach 23% at VRR = 3.3, while β-Lg transmission decayed at increasing VRR to 3%, to give a maximum selectivity of 8.

Keywords: Dynamic filtration, α-lactalbumin and β-lactoglobulin separation, milk microfiltration, rotating ceramic membranes

Received 2 February 2009; accepted 16 June 2009.

Address correspondence to Michel Y. Jaffrin, UMR 6600, Technical University of Compiegne, BP 20529, Compiegne, Cedex 60205, France. Tel.: +33 3 44 23 43 98; Fax: +33 3 44 23 79 42. E-mail: michel.jaffrin@utc.fr

INTRODUCTION

The fractionation and purification of major whey proteins, such as α-Lactalbumin (α-La), β-Lactoglobulin (β-Lg), immunoglobulin (Ig), and bovine serum albumin (BSA), present an important commercial interest for the dairy industry as they can be used as food additives, or present nutritional or pharmaceutical applications (1,2). More specifically, α-La protein enhances the whippability in meringue formulations (3) and can be used in infant formula, while β-Lg have important emulsification and gelling properties (4). Since classical purification methods, such as ion exchange, affinity chromatography, selective precipitation (5), are time consuming, expensive, and not well suited to industrial production, there is a growing interest in developing membrane methods based on microfiltration (MF) and ultrafiltration (UF) for extracting and purifying specific whey proteins, as reviewed by Zydney (2) and Brans et al. (6). The task of separating α-La and β-Lg, which have close molecular weights, respectively 14 and 36 kDa in dimer form, is particularly challenging and requires a careful optimization of operating conditions, to be industrially attractive.

Separation of whey proteins from casein by MF requires a combination of high membrane shear rates and low uniform TMP, which can be achieved by high milk velocities and co-current recirculation of permeate (7,8). In a recent paper (9), we have used a MSD dynamic filtration system with ceramic membrane disks of 0.2 μm pores rotating around a shaft inside a steel housing. This system yielded permeate fluxes up to $120\,Lh^{-1}m^{-2}$ at a rotation speed of 1930 rpm with α-La and β-Lg transmissions of 70% and 20% respectively, with a casein rejection of about 99%. Gésan-Guiziou et al. (8), using 0.1 μm pores ceramic tubular membranes, reported permeate fluxes of $80\,Lh^{-1}m^{-2}$, a α-La transmission of 80%, but a 80% casein rejection.

Cheang and Zydney (10) separated α-La and β-Lg from a binary mixture of α-La and β-Lg in a NaCl solution with 1 mM Na_2HPO_4 prefiltered at 0.2 μm, using diafiltrations (DF) at 30 kDa. These DF were performed with a small Amicon stirred cell equipped with a 30 kDa cellulose or a 50 kDa PES membrane, at two pH of 5.5 and 7.2, adjusted by HCl addition. With the 30 kDa membrane, α-La transmission was 26% at a permeate flux of $14\,Lh^{-1}m^{-2}$ against only 0.5% for β-Lg. This transmission increased with ionic strength to reach a maximum of 60% for α-La at a ionic strength of 150 mM and pH = 5.5. Selectivity (ratio of α-La to β-Lg transmission) reached a maximum of 58 at a pH of 5.5 and an ionic strength of 50 mM, but decreased to 35 when the filtration velocity was doubled. With the 50 kDa membrane, the maximum selectivity was 10.5 at pH = 5.5 and a ionic strength of 150 mM. In a subsequent

paper (11), the same authors obtained purified α-La and β-Lg fractions from whey protein isolate. Their 1st process used a diafiltration (DF) at 100 kDa to separate α-La and β-Lg in permeate from BSA in retentate and another DF at 30 kDa to separate β-Lg from α-La in retentate. Initial concentrations were 1.5 gL^{-1} for α-La and 8 gL^{-1} for β-Lg. After 10 diavolumes, 75% of β-Lg and 100% of α-La was recovered in the 100 kDa permeate. After the first DF, a UF at 30 kDa reduced the volume from 500 mL to 100 mL containing 95% of α-La and 65% of β-Lg. The final selectivity was equal to 21 at the end of 2nd DF.

Muller et al. (12) proposed a pre-purification of α-La by UF of acid casein whey with limited transmission of β-Lg. UF steps were carried out with mineral membranes, a Carbosep of 150 kDa, and ceramic ones of 150, 200, and 300 kDa. α-La transmission at 150 kDa decayed from 80% at 0.5 bar to 58% at 3 bars with a permeate flux of 80 $Lh^{-1}m^{-2}$. Transmissions were lower for the 300 kDa membrane and decayed from 35% at VRR = 1.5 to 25% at VRR = 4.

Almécija et al. (13) investigated the effect of pH on the fractionation of whey proteins by diafiltration using a 300 kDa tubular ceramic membrane. A large fraction of α-La and β-Lg was collected in the permeate while the retentate was enriched in bovine serum albumin (BSA), immunoglobulins (Ig) and lactoferrins. They found that a pH of 8–10 maximized the permeate fluxes, while α-La and β-Lg permeate recovery yields were respectively 0.58 and 0.33.

Bhattacharjee et al. (14) studied the separation of β-Lg from casein using a complex process using a rotating membrane module with a stirrer rotating in opposite direction. Raw casein whey was first clarified by centrifugation followed by a MF at 0.45 μm. The permeate was then diafiltered at 5 kDa to remove lactose, and the retentate, after addition of hydrochloric acid to lower the pH to 2.8 or 5.6, was ultrafiltered at 30 kDa to remove BSA, lactoferrin and Ig in the retentate. The permeate was then ultrafiltered at 10 kDa and pH = 2.8 to concentrate proteins. The final separation between α-La and β-Lg was achieved by ion exchange chromatography.

This literature survey confirms the difficulty of extracting α-La and β-Lg with good selectivity and yield rate by membrane filtration alone. In addition most of these authors did not start from milk or whey, but from protein concentrates or isolates which requires a complex pre-treatment and increases initial protein purity.

Since skim fresh milk was not locally available, we have used skim UHT milk, which has a lower whey protein concentration than fresh milk, due to partial protein denaturation. But denaturated proteins were not included in concentration measurements by HPLC. We have used laboratory pilots of dynamic filtration operating at high shear rates, to

see if this improved the performance. Another advantage of dynamic filtration is that its performance is mainly governed by membrane shear rate and little by internal geometry, which facilitates the scale up from lab pilot to the industrial unit. In addition, the two dynamic filtration modules used for the MF and UF steps of our study are also available at industrial scale with a membrane area of 80 m^2 for the MSD system with rotating ceramic disks and up to 84 m^2 for rotating disks or stirrers with fixed polymeric membranes systems (Bokela or DMS, Germany) as that used for UF in this work.

MATERIAL AND METHODS

Dynamic Filtration Systems

MSD Pilot

The MSD pilot (Westfalia Separator, Aalen Germany), described in (9) consists normally of 12 ceramic membrane disks rotating on two parallel hollow shafts, and enclosed in a stainless steel housing. The maximum rotation speed of these disks is 1930 rpm. However, only six ceramic membranes on one shaft were used in these tests. TMP was calculated from measurements of operating pressure p_c at a pressure tap in the housing, close to disk periphery, using a Validyne DP 15 pressure transducer (Validyne Corp., Northridge, CA, USA) as,

$$TMP = p_c - \frac{\rho\omega^2(R_1^2 + R_2^2)}{4} \quad (1)$$

where $R_1 = 4.5$ cm and $R_2 = 1.02$ cm are, respectively, the outer and inner radius of the membrane disks.

Rotating Disk Module

The rotating disk module has been described previously by Bouzerar et al. (15). It is equipped with a single-polymeric membrane, of 188 cm^2 area (outer radius $R_1 = 7.75$ cm) fixed on the cover of a cylindrical housing in front of the disk. The disk was equipped with eight-6 mm-high vanes in order to increase the core fluid angular velocity $k\omega$, between the membrane and the disk, where ω is the disk angular velocity and k is the velocity factor. Its rotation speed can be adjusted between 500 and 2500 rpm. Peripheral pressure (p_c) and inlet pressures were measured as described in (15). Values of velocity factor k were obtained from

measurements of p_c at different speeds and found to be 0.89 for the disk equipped with 6-mm vanes. The pressure was adjusted by a valve on outlet tubing. The TMP was then determined as:

$$TMP = p_c - \frac{1}{4}\rho k^2 \omega^2 R^2 \quad (2)$$

where R is the inner housing radius.

Membranes and Cleaning Procedure

Ceramic membranes used in the MSD pilot were made from α-Al_3O_2 by Westfalia Separator and had a nominal pore size of 0.2 μm and a skin thickness of 10 μm. Their hydraulic permeability, determined by measuring the permeate flux with deionized water at various TMP and at 20°C, was found to be $886 \pm 227\,Lh^{-1}m^{-2}bar^{-1}$. For UF tests in the rotating disk module, a new 50 kDa cut-off PES membrane (Microdyn-Nadir, France) was used in its test. Its hydraulic permeability at 20°C was $126 \pm 19\,Lh^{-1}m^{-2}bar^{-1}$.

After each test ceramic membranes were rinsed with deionized water before cleaning and carried out with a P3 Ultrasil 10 (Ecolab) solution at 0.5% and 40°C for 1 h in closed circuit. Then, the system was drained and the membranes were rinsed with demineralized water until a pH of 7.0 was obtained. Initial permeabilities were recovered after cleaning for ceramic membranes.

Test Fluid

MF: The test fluid for MF was a commercial UHT skim milk (Lait de Montagne, Carrefour, France) with a pH of 6.8 and the following composition:, α-La:$0.456 \pm 0.128\,g\,L^{-1}$, β-Lg:$0.131 \pm 0.03\,g\,L^{-1}$. Concentrations of other proteins were not measured.

UF: Permeates of milk microfiltration were used as feed in two UF tests. Milk whey was also used in a 3rd UF test, in order to compare its filtration characteristics with those of MF permeate. This whey was obtained by precipitating caseins at pH = 4.6 through adding 1 L of 1 M nitric acid to 10 L of milk. After whey was separated from caseins, its pH was restored to 6.14 with 1 M sodium acetate. Although UHT milk has a smaller whey protein concentration than fresh milk, especially for β-Lg, its filtration characteristics in UF were shown in (16) to be identical to those of low heat milk with normal whey protein contents, until a VRR of 3.5.

Experimental Protocol

MF: The MSD module was fed from a stirred tank thermostated at 40°C by a volumetric diaphragm pump. The permeate was collected in a beaker placed on an electronic scale (Sartorius B3100 P, Gottingen, Germany) connected to a computer in order to measure the permeate flux. Tests were conducted without permeate recycling (concentration tests). Retentate and permeate samples were collected every 30 min for analysis.

UF: The rotating disk module was fed from the same tank and pump as the MSD. A disk with eight-6 mm-high vanes rotating at 2000 rpm was used in all tests. Tests were also conducted at 40°C. In order to investigate the effect of TMP, some tests were performed with permeate and retentate recycling. Permeate and retentate samples were collected every 15 min for further analysis, after flux stabilization in tests at variable TMP.

Analysis

α-La and β-Lg concentrations in the permeate and the retentate were measured by HPLC on samples collected at the permeate and retentate outlet of the module, according to the method of Jaubert and Martin (17) described in (9), using a Waters 510 chromatograph, a UV detector at 280 nm, and a Vydac-C4 column thermostated at 40°C. A calibration curve was made using pure α-La and β-Lg samples from bovine milk (Sigma Aldrich, Germany) of known concentrations.

Proteins concentrations of native proteins in g L^{-1} were calculated from

$$C_{\alpha-La} = 6.0 \times 10^{-7} A \tag{3}$$

$$C_{\beta-Lg} = 10^{-6} A \tag{4}$$

where A denotes the area in mm^2 under the corresponding peaks of the chromatogram. The measurement error was estimated to be 5%, which corresponds to a 10% error on transmissions given by Eq. (5).

Permeate turbidities, which characterize the transmission of casein micelles through the membrane (7), were measured with a Hach turbidimeter (Colorado, USA).

Calculated Parameters

α-La and β-Lg transmissions ($Tr_{\alpha-La}$ and $Tr_{\beta-Lg}$) were calculated by

$$Tr = \frac{C_p}{Cr} \tag{5}$$

where C_p denotes the permeate protein concentration and C_r the retentate one. The recovery yield in permeate (Y) was calculated as:

$$Y = \frac{M_p}{M_i} \tag{6}$$

where M_p denotes the protein mass in the permeate and M_i the initial one.

The selectivity S was calculated from:

$$S = Tr_{\alpha-La}/Tr_{\beta-Lg} \tag{7}$$

RESULTS

Concentration Tests by MF at 0.2 µm with the MSD

Three tests were conducted without permeate recycling in order to concentrate caseins in the retentate while recovering whey proteins in the permeate. The test 1 was performed at a rotation speed of 1044 rpm and tests 2 and 3 at a rotation speed of 1930 rpm. Their characteristics are listed in Table 1. They were all performed at a TMP slightly below 60 kPa to limit membrane fouling. The maximum VRR was limited by the size of the pilot dead volume.

The variations of the permeate flux with VRR in semi-log coordinates are shown in Fig. 3 for these tests. For VRR > 1.2, these fluxes decay linearly with ln(VRR) according to the polarization concentration theory of Blatt et al. (18), which shows that fluxes were mass transfer limited. As expected, the slope of the line representing the 1st test, equal to the mass transfer coefficient, is lower at 1044 rpm (23 $Lh^{-1}m^{-2}$) than in the other two tests at 1930 rpm (47 and 49 $Lh^{-1}m^{-2}$). Ratios of mass transfer coefficients are slightly higher than the rotation speed ratio.

Table 1. Characteristics of concentration tests. V_0:initial volume, V_p:permeate, V_R:retentate

	Test Nb	N (rpm)	TMP (kPa)	V_0 (L)	V_p (L)	V_R (L)	VRR_{max}	Duration (min)
MF 0.2 µm	1	1044	55.2 ± 5.8	11	8.0	2.87	3.8	202
	2	1930	59.3 ± 19	11	8.43	2.52	4.2	117
	3	1930	58.6 ± 4.7	9	6.20	2.78	3.2	80
UF 50 kDa	7	2000	501 ± 13.2	6	4.2	1.8	3.3	74

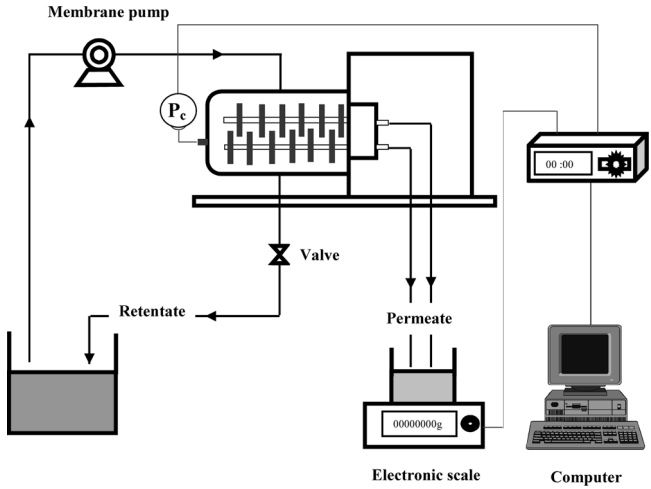

Figure 1. Schematic of MSD pilot and data acquisition system.

Values of fluxes for tests 2 and 3 were very close, indicating good reproducibility. The three lines representing the tests converge towards similar maximum VRR at zero flux of about 9. The drop in the permeate flux in tests 1 and 2 at VRR > 3.5 may be due to a rapid increase in milk viscosity at this VRR, as observed in (16). The variation of permeate turbidity with VRR, due in part to the presence of casein micelles, is given in Fig. 4 for the three tests. This turbidity drops rapidly as VRR increases to 1.5, due to the formation of a protein layer on the membrane which reduces micelles transmission. It is higher also at 1930 rpm than at 1044, as higher

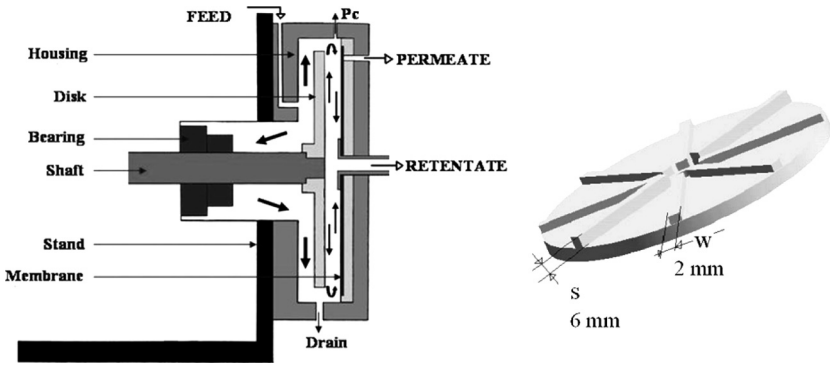

Figure 2. Schematic of the rotating disk module and of the disk with vanes.

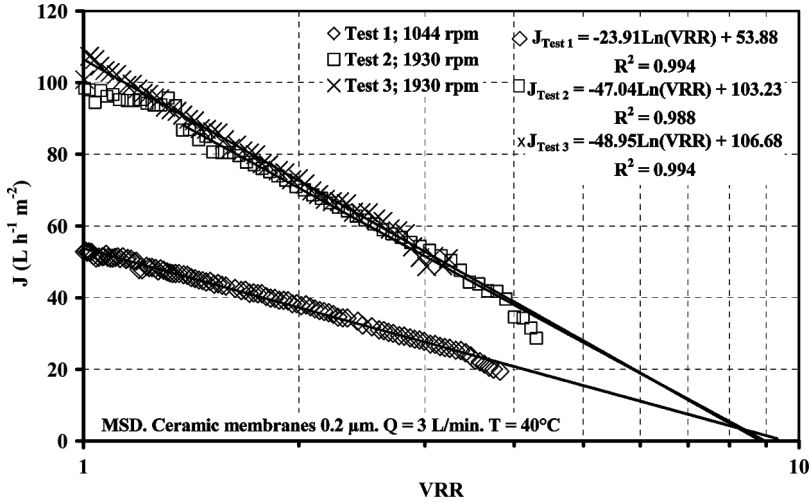

Figure 3. Variation of permeate flux with VRR (semi-log) in MF of skim milk with the MSD module for tests 1 to 3.

shear rates limit the growth of protein layer. Micelles concentration and rejection cannot be calculated accurately from turbidity, but a turbidity less than 50 NTU corresponds to almost complete micelle rejection (19).

The final and initial α-La and β-Lg concentrations in permeate and retentate measured by HPLC are listed in Table 2. Their initial values vary between tests, although all the milk was of the same type and purchased from the same company. This variation may be due to measurement errors, but also to raw milk composition which varies with season, feed, and between animals, or to differences in UHT treatment. Concentrations in the final retentate were lower than initial values except in test 1 at 1044 rpm which had the lowest transmission. The consistency of these concentrations can be verified in Table 3 which provides proteins masses in retentate and permeate and their balance. The total final α-La and β-Lg masses (sum of masses in permeate and retentate) are lower than initial masses, due to protein losses in collected samples and to proteins trapped in the layer deposited on the membrane. The difference was larger for β-Lg, which contains two intra-molecular disulphide bonds and one sulphydril group, inactive in the native protein, but reactive when the molecule is denatured, causing sulphydrylsulfide interactions with κ-casein (20). Thus, a fraction of native β-Lg proteins, bound to micelles deposited on the membrane, is not counted in the final mass. Variations of α-La concentrations in the retentate and the permeate with VRR are presented in Fig. 5. Permeate concentrations are highest for test 2, which

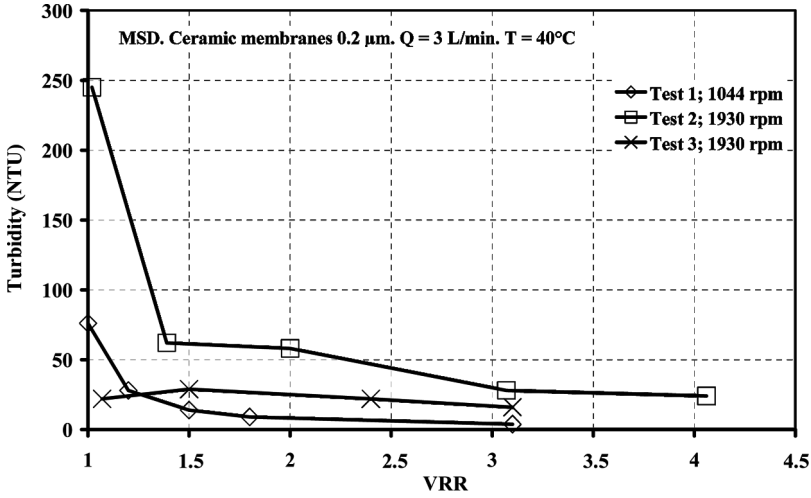

Figure 4. Variation of permeate turbidity with VRR for MF tests of Fig. 3.

Table 2. Initial and final protein concentrations in permeate and retentate for concentration tests

	Cα-La (gL^{-1})			Cβ-Lg (gL^{-1})		
Test Nb	Init R	Fin R	Fin P	Init R	Fin R	Fin P
1	0.432	0.661	0.336	0.116	0.157	0.078
2	0.594	1.023	0.456	0.169	0.356	0.113
3	0.341	0.390	0.314	0.122	0.089	0.137
7(UF)	0.264	0.516	0.082	0.057	0.170	0.006

Table 3. Initial and final protein masses in retentate and permeate and percent recovery in permeate of concentration tests

	Initial mass (g)		Final mass in retentate (g)		Final mass in permeate, (g)		Final total mass (g)		Permeate recovery yield	
Test Nb	α-La	β-Lg	α-La	β-Lg	α-La	β-Lg	α-La	β-Lg	α-La	β-Lg
1	4.75	1.28	1.917	0.455	2.688	0.623	4.60	1.08	0.57	0.49
2	6.54	1.86	2.578	0.898	3.876	0.952	6.46	1.85	0.59	0.51
3	3.07	1.10	1.084	0.248	1.947	0.850	3.03	1.10	0.63	0.77
7(UF)	1.58	0.34	0.929	0.306	0.344	0.025	1.30	0.33	0.22	0.04

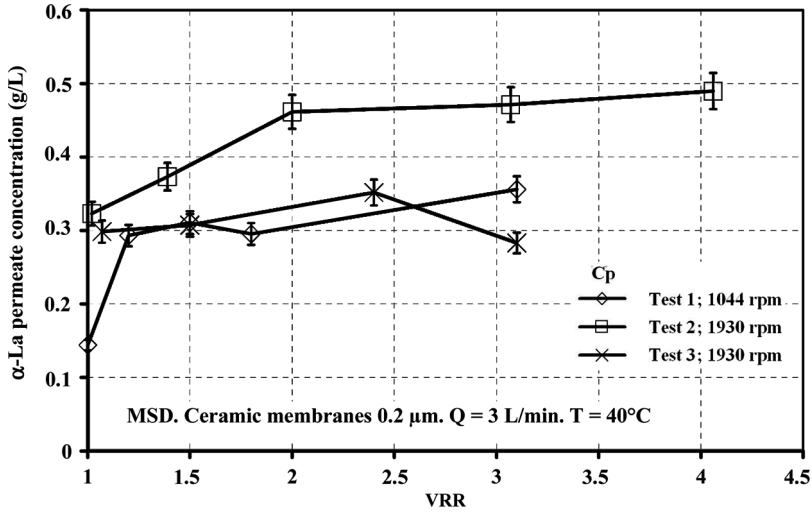

Figure 5. α-La concentrations in permeate (C_p) vs VRR for MF tests of Fig. 3.

also has the highest retentate concentration until VRR = 2.5 and they are lowest for test 3. These concentrations do not vary much when VRR exceeds 1.5. α-La transmissions, depicted in Fig. 6 as function of VRR, are higher at 1930 rpm, ranging from 80 to 90% than at 1044 rpm (test 1) in which it decayed from 70 to 50% at VRR = 3. β-Lg concentrations

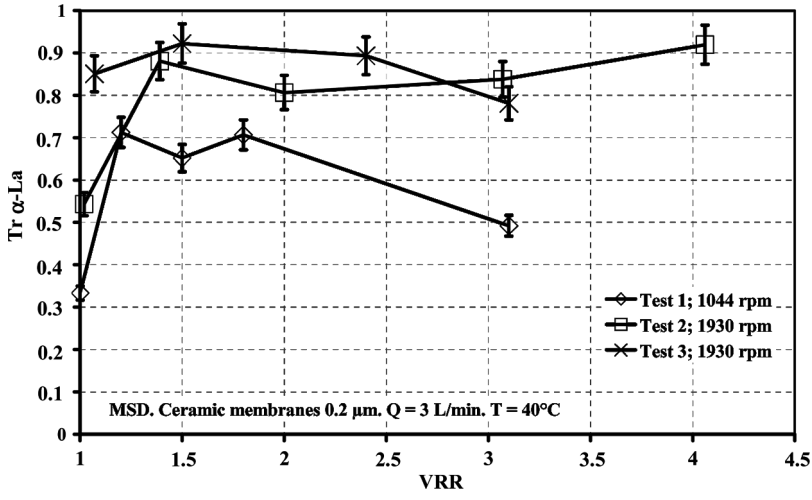

Figure 6. α-La transmission with VRR for MF tests of Fig. 3.

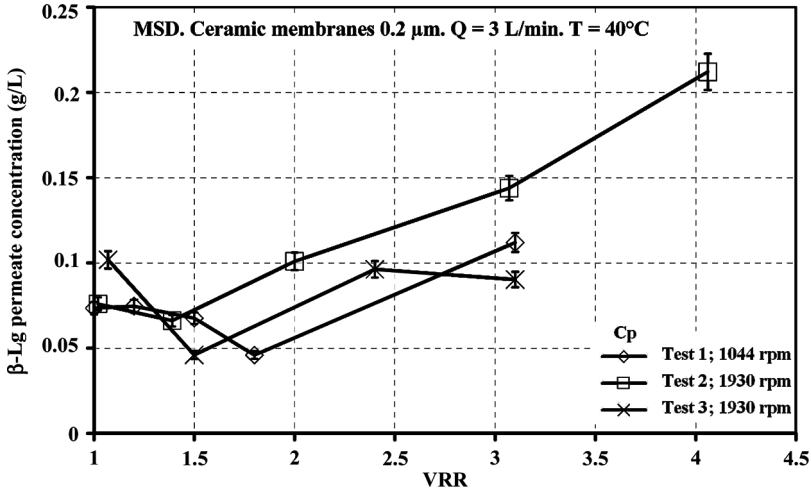

Figure 7. β-Lg concentrations in permeate vs VRR for MF tests of Fig. 3.

in retentate and permeate, shown in Fig. 7, reach a minimum around VRR = 1.5 and increase at larger VRR. β-Lg transmission, shown in Fig. 8, decayed with increasing VRR at 1044 rpm, due to membrane fouling, while, at 1930 rpm, it remained around 90%, like that of α-La at the same speed. Percentages of initial α-La and β-Lg masses recovered

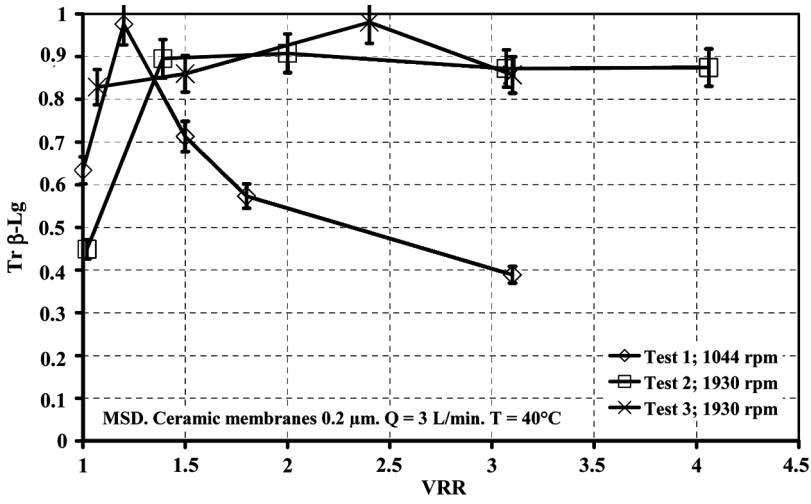

Figure 8. β-Lg transmission versus VRR for MF tests of Fig. 3.

in permeate (recovery yield Y) are listed in Table 3. This yield is lower for β-Lg than for α-La, in tests 1 and 2 due to β-Lg lower transmission, while β-Lg yield was larger in test 3 due to its high β-Lg transmission.

Ultrafiltration at 50 kDa of Whey Obtained by MF at 0.2 µm and 1930 RPM

These UF tests were carried out using PES polymeric membranes with the rotating disk module as ceramic membranes disks of 50 kDa cut-off were not available for the MSD.

Tests with Permeate and Retentate Recycling at VRR = 1

These tests were carried out in order to measure the effect of TMP on the permeate flux and the protein transmissions at initial concentration. The disk rotation speed was 2000 rpm. TMP was increased in steps of 200 kPa lasting about 20 min to obtain flux stabilization. The whey of test 4 was the permeate collected in test 2 and the whey of test 5 was the permeate of test 3. Test 6 was carried out on whey obtained from UHT skim milk, by removing caseins by precipitation after adding 1 L of nitric acid at 1 M to 10 L of milk in order to lower its pH to 4.6. As seen in Table 4, this procedure yielded a larger β-Lg concentration (0.147 gL^{-1}) than obtained in MF permeates used for tests 4 and 5 (0.043 and 0.056 gL^{-1} respectively), as less β-Lg proteins were lost by complexation with casein during precipitation than during MF.

Variations with TMP of permeate fluxes measured at the end of each pressure steps are displayed in Fig. 9 for tests 4, 5, and 6. In test 4, the flux keeps rising with TMP until the maximum pressure of 1265 kPa. For test 5, with lower protein concentrations, the permeate flux reached a plateau of 600 $Lh^{-1}m^{-2}$ at 1100 kPa, corresponding to a mass transfer limited regime, while in test 6, which had the highest total protein concentration, the flux reached 440 $Lh^{-1}m^{-2}$ at its maximum pressure of

Table 4. Characteristics of tests at VRR = 1 and variable TMP showing initial retentate concentrations and maximum transmissions

Test Nb	V_0 (L)	Duration (min)	N (rpm)	TMP max (kPa)	Max flux ($Lh^{-1}m^{-2}$)	Ca-La (gL^{-1})	Cp-Lg (gL^{-1})	Tr α max	Tr β max
4	8.4	110	2000	1265	345	0.456	0.113	0.16	0.11
5	6	88	2000	1500	590	0.264	0.057	0.34	0.16
6	6.6	160	2000	880	440	0.400	0.147	0.20	0

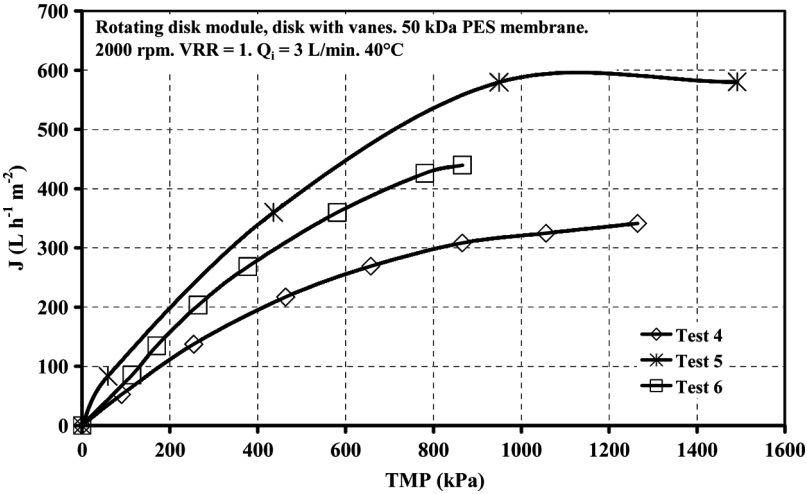

Figure 9. Permeate flux vs TMP in UF at 50 kDa of MF permeate of skim milk and whey (UF tests 4, 5, 6) using the rotating disk module at 2000 rpm and VRR = 1.

880 kPa and was not yet mass transfer limited. The permeate turbidity was only measured in tests 4 and 6, as it was very small, rising from 1 to 3.3 NTU at a TMP of 1250 kPa in test 4 and decaying from 3.8 NTU to 1.8 NTU in test 6, as seen in Fig. 10. This confirmed that the permeate contained very few casein micelles, although whey turbidity was 137 NTU. α-La transmissions, shown in Fig. 11 versus TMP, were higher in test 5 than in tests 4 and 6 by a factor of about 2. β-Lg transmission, displayed in Fig. 12, increased with increasing TMP in test 4, reaching 11% while it remained around 15% in test 5. No β-Lg was found in permeate of test 6.

Concentration of α-La and β-Lg by UF at 50 kDa

This test, denoted as test 7, was the direct continuation of test 5, after stopping permeate recycling, and was carried out at a TMP of 503 kPa. Its characteristics are given in Table 1.

The variation of flux J versus ln(VRR), plotted in Fig. 13, shows that, after an initial decline due to build-up of a protein layer, it remained nearly constant ($\sim$200 $Lh^{-1}m^{-2}$) until the maximum VRR, suggesting that it was pressure limited. Variations with VRR of α-La and β-Lg concentrations in permeate and retentate are presented in Fig. 14. The

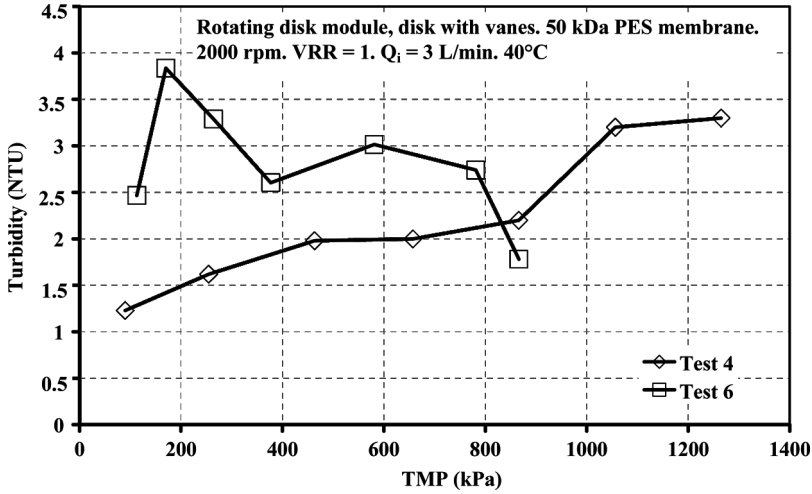

Figure 10. Variation of turbidity with TMP for UF tests 4 and 6.

retentate concentrations of both proteins increase with increasing VRR above VRR = 1.3, but, in the permeate, only the α-La concentration rose with VRR to a maximum of $0.14\,gL^{-1}$, while β-Lg concentration remained constant near zero. These observations are confirmed by the variation of transmissions displayed in Fig. 15. After an initial decay

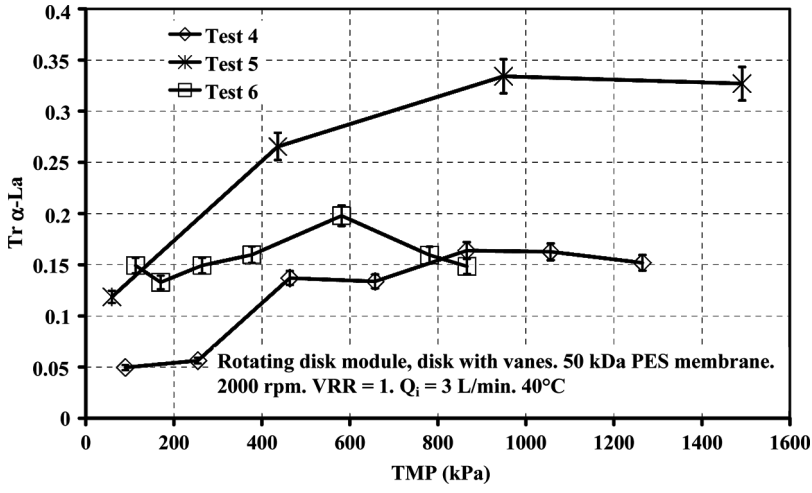

Figure 11. α-La transmission vs TMP for tests 4, 5, 6.

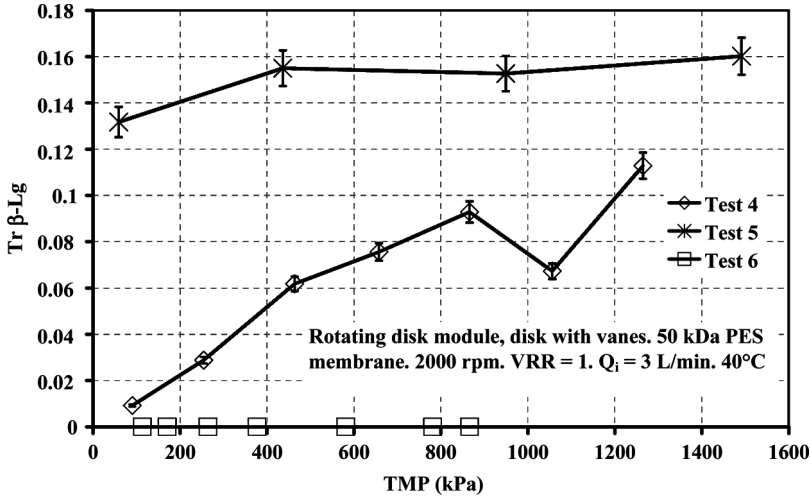

Figure 12. β-Lg transmission vs TMP for tests 4, 5, 6.

due to build–up of the protein layer on the membrane, the α-La transmission unexpectedly rises with increasing VRR while the β-Lg one keeps decreasing to about 0.02 at a VRR of 2 and remains constant at larger VRR.

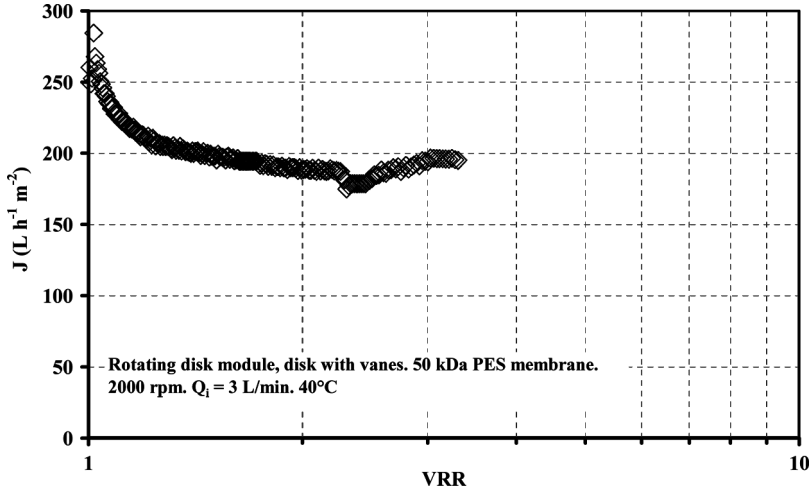

Figure 13. Variation of permeate flux with VRR for test 7 in UF at 50 kDa using the rotating disk module at 2000 rpm.

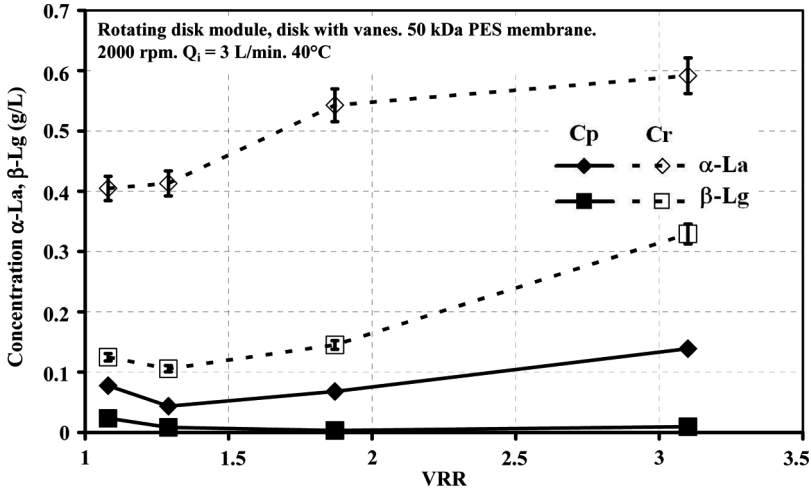

Figure 14. α-La and β-Lg permeate and retentate concentrations vs VRR in test 7.

Initial and final protein concentrations in the retentate and the permeate are presented in Table 2 and corresponding masses in Table 3 for this test. In contrast to MF tests, retentate concentrations were higher than initial values, because of lower transmission. The yield at the end of

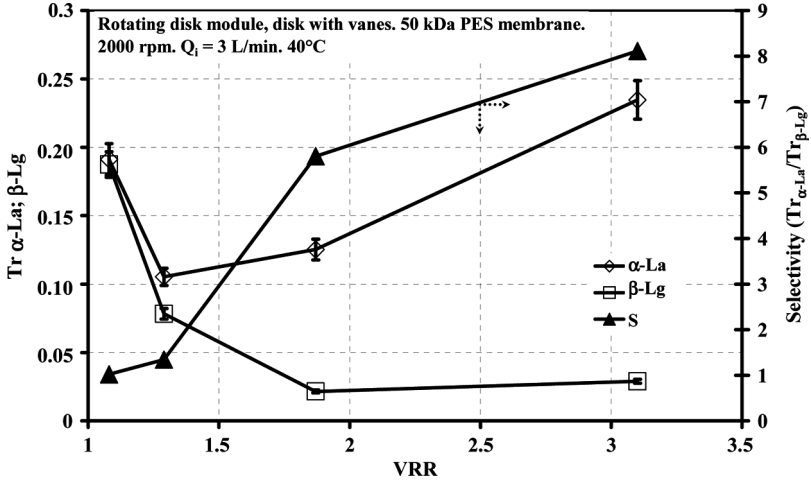

Figure 15. α-La and β-Lg transmissions, and variation of selectivity (Tr a-La)/(Tr p-Lg) vs VRR in test 7.

the concentration test was 0.22 for α-La and 0.04 for β-Lg, showing that the 50 kDA membrane can indeed separate these two proteins. The selectivity, from Eq. 7, also shown in Fig. 15, increases with increasing VRR up to a maximum of 8 at VRR = 3, and could be much higher at larger VRR, that could be obtained with larger initial volumes.

DISCUSSION

The purpose of this study was to evaluate the eventual benefits offered by dynamic filtration in α-La and β-Lg separation, when compared to cross flow filtration. We tested two steps of this process, a MF step to separate whey proteins from casein and a UF step at 50 kDa to separate β-Lg in retentate from α-La and smaller solutes in the permeate. For the MF step, the choice of rotating ceramic disks of 0.2 μm pores in a MSD module was found satisfactory. It gave, at a speed of 1930 rpm, a maximum flux of 105 $Lh^{-1}m^{-2}$, an excellent casein micelle rejection and α-La and β-Lg transmissions between 80% and 95%, up to a VRR of 4. By comparison, Le Berre and Daufin (7) obtained a α-La transmission ranging from 60% to 95% and a maximum steady flux of 75 $Lh^{-1}m^{-2}$ at 50°C, using a 0.1 μm Membralox ceramic membrane in uniform transmembrane pressure mode (UTP) with co-current permeate recirculation at 7.2 ms^{-1}. At the end of the run, α-La transmission had dropped to 30%. Using a similar filtration system with a 0.1 μm Kerasep ceramic membrane, Gésan-Guiziou et al. (8) reported transmissions of 60–90% for α-La and of 55–85% for β-Lg at a flux of 80 $Lh^{-1}m^{-2}$ at 50°C, with a permeate turbidity of 100–200 NTU at VRR = 1. Punidadas and Rizvi (21) used 0.2 and 0.05 μm ceramic membranes with a fluid velocity of 5.4 ms^{-1} and obtained a whey protein transmission of 70% and a permeate flux of 92 $Lh^{-1}m^{-2}$ at 50°C and VRR = 2.5.

The results of our MF tests 1 to 3 confirm the beneficial effect of high shear rates, as the flux at 1930 rpm was about twice that at 1044 rpm, while transmissions at VRR = 3 were 60% higher for α-La and 120% higher for β-Lg. Since the dead end volume of the MSD pilot was relatively important in comparison with the initial milk volume, the maximum VRR was only 4.2, which explains the modest yields obtained in the permeate, 0.60 for α-La and 0.51 for β-Lg in test 2 and less in tests 1 and 3.

Concerning the UF step, the 50 kDa membrane we used for separating α-La from β-Lg yielded a selectivity of 8 at VRR = 3.1, which according to Fig. 15, may increase further at higher VRR. This selectivity was close to the maximum one (10.5) reported by Cheang and Zydney (10) for a 50 kDa membrane after optimizing ionic force and pH. But their

selectivity fell to 4.5 and 2.8 respectively for ionic forces of 5 and 50 mM at a pH of 7.2. Figure 13 shows that our rotating disk module yielded a higher flux close to 200 $Lh^{-1}m^{-2}$ up to VRR = 3.3 than those reported in the literature (10), (13), (16) which were generally under 100 $Lh^{-1}m^{-2}$. Lucas et al. (22) have obtained α-La and β-Lg transmissions ranging respectively from 15 to 37% and from 5 to 10%, with a selectivity of 3, using a 50 kDa Carbosep membrane. With a chemically modified membrane of 40 kDa, the selectivity rose to about 10. Gésan-Guiziou et al. (23) obtained a transmission of 9% for α-La and of 6% for β-Lg during the ultrafiltration of redissolved precipitate from Gouda whey protein concentrate with a 50 kDa Carbosep membrane, at VRR = 10 and 50°C.

CONCLUSION

Rotating ceramic disks membranes of 0.2 μm pores in a MSD module were found to operate satisfactorily in concentration tests, giving both high whey protein transmissions and excellent casein rejection. The results of our UF step without ionic force or pH optimization are promising in terms of the permeate flux, α-La transmission and selectivity in comparison with the literature on whey protein fractionation. Since our HPLC method only measures concentrations of native α-La and β-Lg, their transmissions should be similar to those found in fresh milk without whey protein denaturation. This assumption is being confirmed by current tests made in our laboratory with skim pasteurized milk (24).

In future tests, it will also be important to use pasteurized skim milk with higher initial native proteins concentrations and to replace ultrafiltration by diafiltration with continuous dilution of retentate with water, in order to raise the a-La yield in permeate. Another UF step at around 5 kDa, will be carried out to isolate a-La in retentate from smaller solutes such as lactose.

ACKNOWLEDGMENTS

V. Espina has been supported by the Programme Alβan, the European Union Programme of High level Scholarships for Latin America, N° E06D101610CL. The authors thank Westfalia Separator for the loan of a MSD pilot, Dr. H. Adenier from our university for his assistance with HPLC, and Dr. G. Gésan-Guiziou from INRA of Rennes for information concerning micelle rejection.

NOMENCLATURE

C	Protein concentration (kg m^{-3})
k	Velocity factor
J	Permeate flux (L $h^{-1}m^{-2}$)
MF	Microfiltration,
Q_F, (Q_i)	Filtration (feed) flow rate (m^3 h^{-1})
r	Radial coordinate (m)
R, (R_d)	Housing, disk radius (m)
R_1, (R_2)	Outer (inner) membrane radius (m)
S	Selectivity (Eq .7)
T	Turbidity (NTU)
Tr	Transmission (Eq. 5)
TMP	Transmembrane pressure (kPa)
UF	Ultrafiltration
VRR	Volume reduction ratio
Y	Recovery yield (Eq. 6)

Greek Letters

γ (γ_{max})	maximum membrane shear rate at periphery (s^{-1})
ν	fluid kinematic viscosity (m^2 s^{-1})
ρ	density (kg m^{-3})
ω	angular velocity (rad s^{-1})

Subscript

p	permeate
r	retentate

REFERENCES

1. Daufin, G.; Pierre, A.; Garem, A.; Goudedranche, H.; Gésan-Guiziou, G. (1998) Industrie laitière. In: *Les Séparations Par Membrane Dans Les Procédés de L'industrie Alimentaire*, Daufin, G.; René, F.; Aimar, P., eds.; Tec Doc, Lavoisier: Paris, Chap. 7, 282–371.
2. Zydney, A.L. (1998) Protein separation using membrane filtration: new opportunities for whey fractionation. *Int. Dairy J.*, *8*: 243–250.
3. Pierce, R.J. (1983) Thermal separation of β-lactoglobulin and α-lactalbumin in bovine cheddar cheese whey. *NZJ. Dairy Sci. Technol.*, *15*: 13–22.
4. Cayot, P.; Lorient, D. (1997) "Structure-function relationships of whey proteins". In: *Food Proteins and Their Applications*, Damodaran, S.; Paraf, A., eds.; Marcel Dekker: New York, 225–256.
5. Bramaud, C.; Aimar, P.; Daufin, G. (1997) Optimization of a whey protein fractionation process based on the selective precipitation of α-lactalbumin. *Lait*, *77*: 411–423.

6. Brans, G.; Schroen, K.; Van der Sman, R.G.M.; Boom, R.M. (2004) Membrane fractionation of milk: state of art and challenges. *J. Membr. Sci.*, *243*: 263–272.
7. Le Berre, O.; Daufin, G. (1996) Skimmilk crossflow filtration performance versus permeation flux to wall shear stress ratio. *J. Membr. Sci.*, *117*: 261–270.
8. Gésan-Guiziou, G.; Boyaval, E.; Daufin, G. (1999) Critical stability conditions in crossflow microfiltration of skimmed milk: transition to irreversible deposition. *J. Membr. Sci.*, *158*: 211–232.
9. Espina, V.S.; Jaffrin, M.Y.; Frappart, M.; Ding, L.H. (2008) Separation of casein micelles from whey proteins by high shear microfiltration of skim milk using rotating ceramic membranes and organic membranes in a rotating disk module. *J. Membr. Sci.*, *325*: 872–879.
10. Cheang, B.; Zydney, A.L. (2003) Separation of a-lactalbumin and p-lactoglobulin using membrane ultrafiltration. *Biotech Bioeng.*, *83*: 201–209.
11. Cheang, B.; Zydney, A.L. (2004) A two-stage ultrafiltration process for fractionation of whey protein isolate. *J. Membr. Sci.*, *231*: 159–167.
12. Muller, A.; Chaufer, B.; Merin, U.; Daufin, G. (2003) Prepurification of a-lactalbumin with ultrafiltration ceramic membranes from casein whey: study of operating conditions. *Lait*, *83*: 111–129.
13. Almecija, M.C.; Ibanez, R.; Guadix, A.; Guadix, E.M. (2007) Effect of pH on the fractionation of whey proteins with a ceramic ultrafiltration membrane. *J. Membr. Sci.*, *288*: 28–35.
14. Bhattacharjee, S.; Bhattacharjee, C.; Datta, S. (2006) Studies on the fractionation of β-lactoglobulin from casein whey using ultrafiltration and ion-exchange membrane chromatography. *J. Membr. Sci.*, *275*: 151–150.
15. Bouzerar, R.; Jaffrin, M.Y.; Ding, L.; Paullier, P. (2000) Influence of geometry and angular velocity on performance of a rotating disk filter. *AIChE J.*, *46*: 257–265.
16. Akoum, O.; Chotard-Ghodsnia, R.; Ding, L.H.; Jaffrin, M.Y. (2003) Ultrafiltration of low-heat and UHT milks with a shear-enhanced vibrating filtration system. *Sep. Sci. and Technol.*, *38*: 571–589.
17. Jaubert, A.; Martin, P. (1992) Reverse phase analysis of goat caseins: Identification of α_{s1} and α_{s2} genetic variant. *Lait*, *72*: 235–247.
18. Blatt, W.F.; Dravid, A.; Michaels, A.S.; Nelson, L. (1970) "Solute polarization and cake formation in membrane ultrafiltration: Causes, consequences and control techniques". In: *Membr. Sci. and Tech.*, Flinn, J.E., ed.; Plenum Press: New York, N.Y.. 47–97.
19. Gésan-Guiziou, G. (2008) Personal communication.
20. Hui, Y.H. (2006) *Food Biochemistry & Food Processing*, 1st Ed.; Blackwell Publishing: Iowas.
21. Punidadas, P.; Rizvi, S.S.H. (1998) Separation of milk proteins into fractions rich in casein or whey proteins by cross flow filtration. *Food Research Intern.*, *31*: 265–272.
22. Lucas, D.; Rabiller-Baudry, M.; Millisime, L.; Chaufer, B.; Daufin, G. (1998) Extraction of α-lactalbumin from whey protein concentrate with modified inorganic membranes. *J. Membr. Sci.*, *148*: 1–12.

23. Gésan-Guiziou, G.; Daufin, G.; Timmer, M.; Allersma, D.; van der Horst, C. (1999) Process steps for the preparation of purified fractions of α-lactalbumin from whey protein concentrates. *J. Dairy. Res.*, *66*: 225–236.
24. Espina, V.S.; Jaffrin, M.Y.; Ding, L.H.; Frappart, M. (2009) Feasibility study of α-lactalbumin and β-lactoglobulin from pasteurized milk using dynamic ultrafiltration. 6th Nizo Dairy Conference, Papendal, NL, Sept 30-Oct 2.