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Abstract: Nanofiltration (NF) membranes were previously used to fractionate peptides in β-lactoglobulin tryptic hydrolysates. It was shown that G-10 NF polyamide membrane coupons with a molecular weight cut-off of 2.5 kg · mol^{-1} retained all acidic (negatively charged) peptides, making it possible to separate them from basic and neutral peptides. The objective of the work described here was to characterize the ability of G-10 and G-50 NF membranes, which differ by their MWCO, mounted in spiral wound modules to fractionate acid, neutral, and basic peptides at pH 9 and at different peptide concentrations (0.1%, 0.5%, and 1.0%). The selectivity of separation of the peptide was influenced by the Donnan and size exclusion effect for both spiral wound NF membranes. The size exclusion effect was more important with the NF G-50 while Donnan effect was dominant with the NF G-10 membrane. Acidic peptides were completely retained at pH 9 and the transmission of basic and neutral peptides was optimal on the G-10 membrane at the lowest peptide concentration (0.1%).

Keywords: Nanofiltration, β-lactoglobulin, tryptic hydrolysates, peptite fractionation, Donnan exclusion

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INTRODUCTION

Peptides from whey protein hydrolysates, especially β-lactoglobulin (β-LG), have a wide range of functional, nutritional, and biological properties depending on the enzymes used to produce them. Proteinases like trypsin can release bioactive peptides such as the ACE inhibitory peptides β-LG f15–20, β-LG f102–105 and β-LG f142–148 (1, 2), the bactericidal peptides β-LG f15–20 and β-LG f92–100 (3), the hypocholesterolemic peptide β-LG f71–75 (4), and the opioid peptide β-LG f102–105 (5). However, protein hydrolysates are complex mixtures of amino acids and peptides with similar physicochemical characteristics such as charge and molecular weight (MW). Chromatography-based processes can be used to separate them, but because of their high operating cost and low productivity and the fact that some processes use organic solvents, they are not widely used industrially. Ultrafiltration (UF) membrane technologies have been used to remove enzymes after hydrolysis (6) or to fractionate protein hydrolysates (7, 8). However, UF separation depends mainly on the molecular weight cut-off (MWCO) of the membrane and cannot fractionate peptide mixtures with a narrow MW distribution spectrum (e.g., $0.2–1.0\,kg \cdot mol^{-1}$). Nanofiltration (NF) membranes have also been used to separate complex mixtures from milk protein hydrolysates (9–12). NF membranes are particularly useful for separating charged peptides because of their net negative charge. NF separation of peptides and amino acids (AA) is very dependent on physicochemical parameters such as pH and ionic strength. Solute charge plays a critical role in NF separations. For example, acidic AA are retained at pHs < 3 whereas basic AA are retained at pHs > 9 (13, 14). These observations emphasize the critical role of solute charge in NF separations.

The fractionation of β-casein peptide mixtures using NF membranes (11) is governed by attractive or repulsive interactions that depend on whether the peptides are positively or negatively charged at the membrane/solution interface. Moreover, the accumulation or adsorption of charged species on the membrane surface is thought to influence the transmission (Tr) of peptides. Lapointe et al. (15) showed that G-10 NF polyamide membrane coupons with a molecular weight cut-off of $2.5\,kg \cdot mol^{-1}$ retained all acidic peptides, making it possible to separate them from basic and neutral peptides. Adjusting the operating conditions and filtration times to enhance the adsorption of the weakly attached layer can modify the transmission behaviour of peptides in β-LG tryptic hydrolysates, possibly due to peptide-peptide interactions in the weakly attached layer. These findings were obtained using small membrane coupons installed on a plate and frame lab-scale module that provided laminar flow conditions ($Re \cong 1000$) at the membrane surface. Lapointe et al. (15) hypothesized that NF separations of β-LG tryptic peptides under turbulent flow conditions minimizes the concentration polarization layer (CP) and thus modifies the selectivity of the NF membrane. The objective of the present work was to investigate the

fractionation of whey protein tryptic hydrolysates using spiral wound NF membranes. The fractionation was performed at pH 9 and at peptide concentrations of 0.1%, 0.5% and 1% using two membranes with different molecular weight cut-offs.

EXPERIMENTAL

Preparation of the Tryptic Whey Proteins Hydrolysate

The tryptic hydrolysate was prepared as described by Lapointe et al. (15) from a 10% (w/v) solution of commercial whey protein isolate (BiPRO™, Davisco Foods international Inc., Le Sueur, MN, USA) using commercial trypsin (TrypsinVI, Inovatech, Abbotsford, BC, Canada). Trypsin previously solubilized in 110 ml of 0.001 N HCl was added to the reaction mixture to give a final enzyme:substrate ratio of 1:1,200. The trypsin preparation contained 2,400 Units $\cdot$ mg^{-1} of trypsin and 400 Units $\cdot$ mg^{-1} of chymotrypsin activity. Trypsin cleaves after the Arg and Lys residues at the C terminus, while chymotrypsin requires an aromatic or bulky nonpolar side chain on the carboxyl side of the scissile bond. During the hydrolysis, the reaction mixture was held at 40°C and maintained at pH 8.0 by pH-stat titration using 2 N NaOH. Once 5.6% degree of hydrolysis (DH) was attained, the reaction was stopped by transferring the mixture to an UF membrane system (GEA/Niro Inc., Hudson, WI, USA) to remove the enzyme and unhydrolyzed protein. The UF polysulfone membrane had a MWCO of 10.0 kg $\cdot$ mol^{-1} and an effective surface area of 2.09 m^2. The permeate was concentrated by reverse osmosis and freeze dried. The hydrolysate was stored at −20°C.

NF Membranes and System

G-10 and G-50 spiral wound cartridge membranes from Osmonics (Minnetonka, MN, USA) with 2.5 and 8.0 kg $\cdot$ mol^{-1} MWCOs, respectively, were used. The active layer of the membranes was composed of polyamide with anionic characteristics. The filtering area was 0.32 m^2 for both membranes and the maximum operating pressure was 2.76 MPa. Cross-flow filtration experiments were performed using a pilot scale module Lab Unit 1812 (Filtration Engineering Inc., Champlin, MN,) equipped with two pressures gauges (at the inlet and outlet of the retentate). The temperature was maintained at 25°C $\pm$ 2°C. All filtration cycles were operated in full re-circulation mode (permeate and retentate) to ensure a constant feed concentration.

Permeation Experiments

The pure water permeation flux (J_w) was determined volumetrically at 25°C for both membranes at transmembrane pressures (P_T) ranging from 0.69 to

2.41 MPa. Each pressure value was maintained for 10 min after which J_w was determined volumetrically using the following equation:

$$J_w = \frac{1}{Am} * \frac{\Delta V}{\Delta t} \quad (1)$$

where A_m is the effective membrane area (0.32 m^2) and $\Delta V/\Delta t$ is the permeate volume ΔV collected over time Δt ($L \cdot h^{-1}$).

A feed phase solution (18 L) was prepared by dissolving the hydrolysate in distilled water to a protein concentration (N × 6.38) of 1% (w/v). This solution was diluted to obtain the 0.1% and 0.5% hydrolysate solutions. The solutions were stirred overnight. The pH was then adjusted to 9.0 using 2 N NaOH. This pH was selected because it corresponds to the average isoelectric point of basic peptides in a typical tryptic hydrolysate of whey proteins. The feed solution was circulated for 10 min at a P_T of 0.69 MPa and the volumetric permeation flux was systematically measured during the NF runs as previously described. Feed phase and permeate samples were collected at each pressure value for peptide quantification. The Tr of each peptide was calculated using the following equation:

$$Tr = \frac{Cp}{Cr} \quad (2)$$

where Cp and Cr are the peptide concentrations in the permeate and retentate, respectively. The separation factor (S), which is defined as the ratio of mean Tr values (%) of two given groups of peptides, was estimated based on the identity of the peaks in an β-LG tryptic hydrolysate:

$$Sx/y = \frac{\sum_{i=1}^{n} Trxi/n}{\sum_{j=1}^{n} Tryj/m} \quad (3)$$

where x and y refer to acid, neutral or basic peptides.

Total Nitrogen Analysis

Total nitrogen was determined by pyro-chemiluminescence (Antek Nitrogen Analyzer 7000^{TM}, Antek, Houston, TX, USA) using a glycine (3, 6, 9, 12, and 15 ppm) calibration curve. A conversion factor of 6.38 commonly used for milk proteins was used to estimate the protein content.

Characterization of Peptide Mixtures

A liquid chromatography-mass spectrometry system (LC-MS QUAD, Agilent Technologies, Series 1100, Palo Alto, CA, USA) was used to separate and identify the peptides contained in the initial feed solutions and the permeate solutions. The reverse-phase high-pressure liquid chromatography (RP-HPLC) system consisted of an autosampler (G1329A), two pumps (bin G1323A) and a diode array detector (DAD G1315A) adjusted to 214 nm. Peptide solutions were separated on a Luna 5 μ C18 column (2 i.d. $\times$ 250 mm, Phenomenex, Torrance, CA, USA) using two solvents: (A) 0.11% (v/v) trifluoroacetic acid (TFA) in water, and (B) 90% (v/v) acetonitrile:0.1% (v/v) TFA in water. The column was operated at a flow rate of 0.2 mL $\cdot$ min^{-1} at 40°C. The column was equilibrated in solvent A, then the sample was injected and eluted using a series of linear gradients of solvent B as follows: 1% B from 0 to 63 min, 50% B over 7 min, 100% B over 5 min, then reducing the initial level of 1% B over 5 min, for the re-equilibration of the column.

To reduce the effect of TFA, the peaks eluted from the RP-HPLC column were collected and mixed with 12% (v/v) propionic acid and 12% (v/v) isopropanol being directly infused through the electrospray interface at a flow rate of 10 μL $\cdot$ min^{-1} for the mass determination. Mass spectra were acquired in positive ion mode using a 90 V fragmentation with a scan range of 250–3,000 m/z. Nitrogen was used both as the drying gas at a flow rate of 13 L $\cdot$ min^{-1} and a temperature of 350°C and as the nebulizer gas at a pressure of 0.241 MPa. The capillary voltage was set at 4,000 V. The instrument was calibrated using an ES tuning mix (G2431A, Agilent). MSD ChemStation software version A.08.04 (Hewlett Packard) was used for data acquisition and analysis. Peptides were identified based on mass values using Peptide Tools software version 8.03 (Agilent Technologies).

RESULTS AND DISCUSSION

Characterization of Membrane Permeability

Figure 1 illustrates the effect of operating pressure (0.69–2.41 MPa) on the permeation flux (L $\cdot$ h^{-1} $\cdot$ m^{-2}) at pH 9 of 0.1%, 0.5% and 1.0% tryptic hydrolysate solutions. The J_w values (broken lines) correspond to the water flux of the G-10 and G-50 membranes measured before the NF experiment. The J_w values of both membranes were linear over the entire P_T range with a R^2 of 1.0 and 0.99, respectively. The J_w value of the G-50 membrane was consistently twice as high as that of the G-10 membrane (75 L $\cdot$ h^{-1} $\cdot$ m^{-2} vs. 37 L $\cdot$ h^{-1} $\cdot$ m^{-2} at 0.69 MPa and 240 L $\cdot$ h^{-1} $\cdot$ m^{-2} vs. 112 L $\cdot$ h^{-1} $\cdot$ m^{-2} at 2.41 MPa, respectively). The permeation fluxes of the hydrolysate solutions were systematically lower than J_w for the G-10 membrane, whereas they

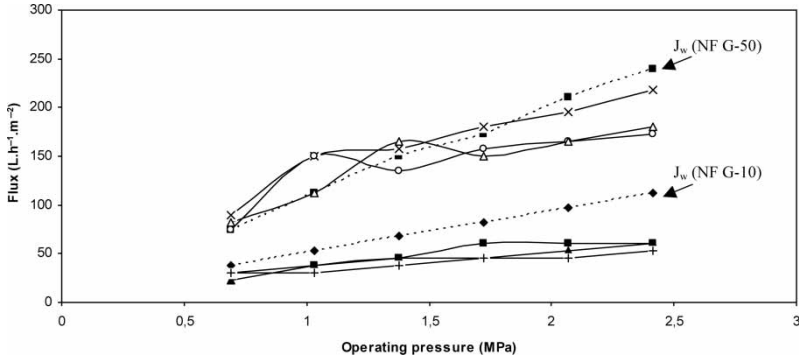

Figure 1. Effect of operating pressure (MPa) on permeation flux ($L \cdot h^{-1} \cdot m^{-2}$) during nanofiltration at pH 9 of a tryptic hydrolysate solution at different concentrations using a G-10 membrane (■, 0.1%; ▲, 0.5%; +, 1%) and a G-50 membrane (×, 0.1%; ○, 0.5%; △, 1%). Broken lines indicated J_w values for both membranes before NF experiments.

were close to J_w for the G-50 membrane. Permeation flux values increased proportionally with P_T at all concentrations, suggesting that changes due to concentration polarization and/or fouling were negligible. While Lapointe et al. (12) used different NF coupon membranes, they reported a linear flux-P_T relationship at pH 9 and a $P_T < 0.600$ MPa with the 0.1% and 0.5% feed phases.

Figure 2 illustrates the nitrogen transmissions observed for the three hydrolysate solutions at different concentrations (0.1%, 0.5% and 1.0%) for operating pressures ranging from 0.69 to 2.41 MPa. As can be seen, the nitrogen transmission values of the G-50 membrane were higher than those of the G-10 membrane. Total nitrogen transmission (Tr_N) with the 0.1% hydrolysate solution was 11–13% for the G-10 membrane and 22–25% for the G-50 membrane and decreased to 6–8% for the G-10 and 15–19% for the G-50 with the 1.0% hydrolysate. Figure 2 also shows that P_T had a limited effect on Tr_N at all hydrolysate concentrations. This finding is in good agreement with Garem et al. (16), who showed that the selectivity of NF membranes is highly dependent on the pH but to a much lesser extent on P_T.

Characterization of the Reference Whey Proteins Tryptic Hydrolysate and the NF Permeates

The RP-HPLC chromatographic profile of the whey proteins tryptic hydrolysate adjusted to pH 9 is presented in Figure 3. Mass spectrometry analyses allowed the identification of 21 different peptides in the hydrolysate solution (Fig. 3). The numbers in Fig. 3 refer to the peptides identified by LC/MS and was reported in Table 1, which presented their sequences,

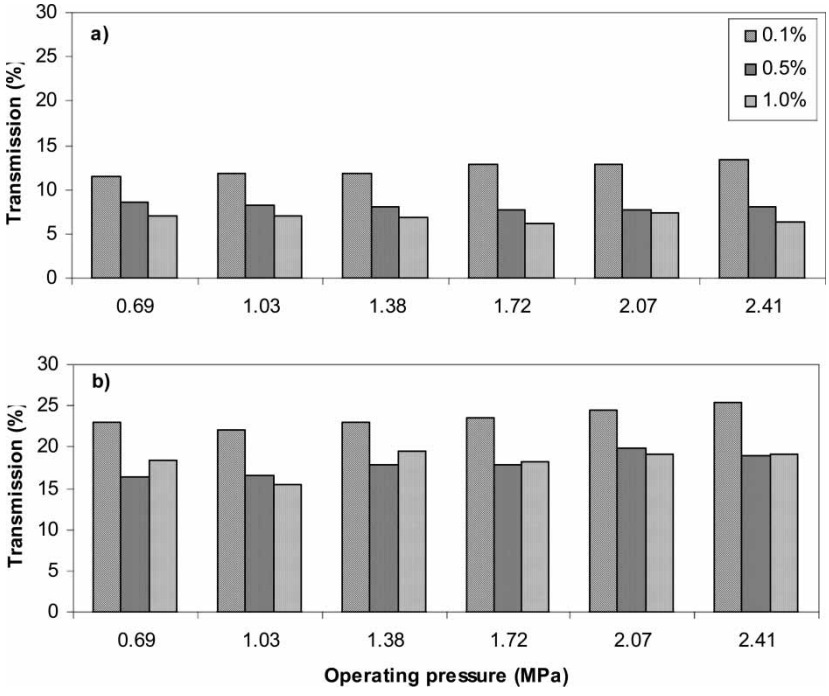

Figure 2. Total nitrogen transmission (%) of permeate samples taken during nanofiltration at pH 9 of a tryptic hydrolysate solution at different concentrations for each operating pressure (MPa) using G-10 (a) and G-50 (b) membranes.

physico-chemical characteristics, and classification as acid (A), basic (B) or neutral (N) peptides. Neutral peptides ($5 < pI < 8$) made up 24% of the total integrated area of the initial feed solution chromatogram and had molecular weights (MWs) ranging from 0.4 to 1.194 $kg \cdot mol^{-1}$. Acidic peptides ($pI < 5$) made up 31% of the total integrated area of the initial feed solution chromatogram and had MWs over 1.190 $kg \cdot mol^{-1}$. Basic peptides, mainly four with MWs between 0.4 and 1.0 $kg \cdot mol^{-1}$, made up 13% of the initial feed solution chromatogram. All of the peptides detected in the G-10 permeates in the present study have also been reported by Lapointe et al. (15) as being present in the permeates of G-10 flat sheet coupons.

The chromatograms of the permeates produced by nanofiltration of the tryptic hydrolysate solutions at different concentrations using the G-10 and G-50 membranes are presented in Fig. 4. The total areas under the peaks indicate that the amount of peptide material in the permeates was greater for the G-50 membrane than for the G-10 membrane, confirming that the G-50 membrane has a higher MWCO.

For both membranes, the total areas of the chromatograms increased with hydrolysate concentration. Theoretically, if size exclusion was the only

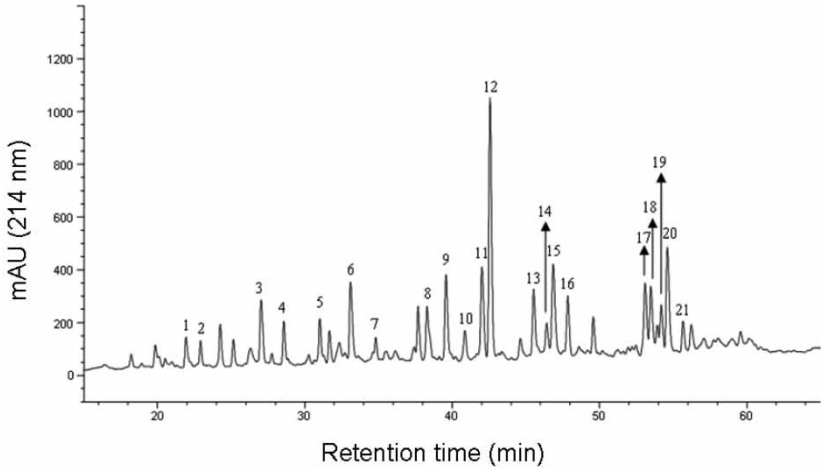

Figure 3. RP-HPLC peptide profile of the 0.5% initial whey proteins hydrolysate feed solution at pH 9.

separation criteria, most of the peptides with a MW under 2.5 $kg \cdot mol^{-1}$ should pass through the two membranes and therefore be present in all the permeates. However, no peptides with MWs over 0.1 $kg \cdot mol^{-1}$ permeated either membrane. Moreover, the acidic peptides in the tryptic hydrolysate were completely retained by the spiral wound G-10 membrane (Fig. 4). At pH 9, acidic peptides carry a net charge that varies from −1 to −4 (Table 1). Since both the peptides and the membrane are negatively charged at this pH, it is probable that electrostatic repulsion between the membrane and the acidic peptides together with the Donnan exclusion effect (17) prevented the peptides from permeating the membrane. The spiral wound G-10 membrane used in the present study clearly acted like a negatively charged membrane. These results are in accordance with those obtained by Lapointe et al. (15) who demonstrated a complete retention of acidic peptides in a β-LG tryptic hydrolysate at pH 9 by a G-10 polyamide coupon membrane. With the G-50 membrane, a small amount of the acidic peptide β-LG f84–91 (peak 5 on Fig. 4) was detected (1.1–1.2% of the total) in the permeates of the 0.5% and 1.0% hydrolysate solutions. The Tr of acidic peptides is generally lower than that of the neutral and basic peptides (9, 11, 12). The lower MW and net charge of β-LG f84–91 may explain its Tr in the G-50 permeate. The basic peptides (peaks 1, 8, 9, and 15), which were positively charged at this pH, were present in all the permeates.

Peptide Transmission

The Tr values (%) calculated for the basic and neutral peptides in the G-10 and G-50 permeates of the 0.1%, 0.5% and 1.0% tryptic hydrolysates are shown in

Table 1. Physico-chemical characteristics[a] of the peptide peaks identified by LC/MS in the tryptic hydrolysate of whey proteins (Fig. 3)

Peak number	β-LG peptide sequence	Amino acid sequence[b]	MW ($g \cdot mol^{-1}$)	pI	Net charge at pH 9	Peptide group[c]
1	146–148	HIR	424.3	9.76	0.6	B
2	136–138	FDK	408.3	5.84	−0.39	N
3	71–75	IIAEK	572.4	6	−0.2	N
4	33–40	DAQSAPLR	856.5	5.84	−0.13	N
5	84–91	IDALNENK	915.5	4.37	−1.2	A
6	9–14	GLDIK	672.4	5.84	−0.17	N
7	125–135	TPEVDDEALEK	1246.6	3.83	−4.22	A
8	1–8	LIVTQTMK	932.5	8.75	0.78	B
9	142–148	ALPMHIR	836.5	9.8	0.83	B
10	92–101	VLVLDTDYKK	1193.2	5.93	−0.33	N
11	125–138	TPEVDDEALEKFDK	1635.4	4.02	−4.25	A
12	15–20	VAGTWY	695.2	5.49	−0.27	N
13	$61–70_{A,B} + 149–162$	WEN(D_A,G_B)ECAQKK + LSFNPTQLEEQCHI	$2906.7_A/2849_B$	4.57/4.91	−2.35	A
14	$61–69_{A,B} + 149–162$	WEN(D_A,G_B)ECAQK + LSFNPTQLEEQCHI	$2778.6_A/2720.5_B$	4.25/4.48	−4.32/−3.32	A
15	76–82	TKIPAVF	774.4	8.41	0.78	B
16	78–82	IPAVF	545.3	5.52	−0.17	N
17	43–60	VEELKPTPEGDLEILLQK	2052.6	4.25	−3.25	A
18	21–40	SLAMAASDISLLDAQSAPLR	2031.6	4.21	−1.41	A
19	149–162 + 149–162	LSFNPTQLEEQCHI + LSFNPTQLEEQCHI	3313.6	4.47	−4.18	A
20	41–60	VYVEELKPTPEGDLEILLQK	2315.2	4.25	−2.33	A
21	21–32	SLAMAASDISLL	1190.5	3.8	−1.41	A

[a]Data from Lapointe et al. (15).
[b]Amino acids substitution for variant A and B of β-LG is shown in parenthesis ().
[c]Acid peptide (A); basic peptide (B); neutral peptide (N).

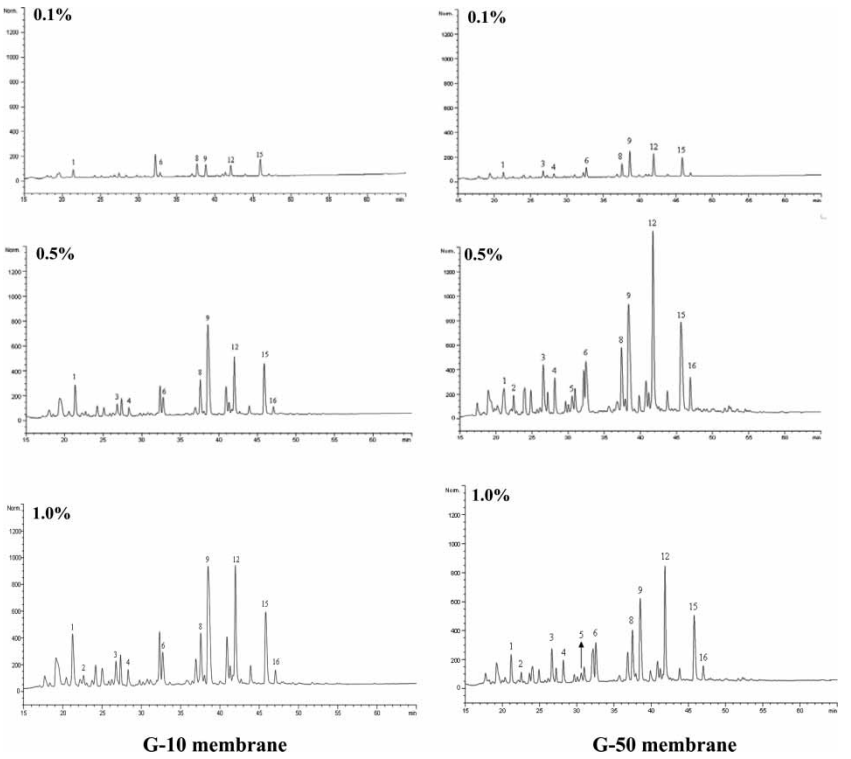

Figure 4. RP-HPLC peptide profiles of the permeates obtained by nanofiltration at a P_T of 1.72 MPa of the tryptic hydrolysate solutions at different concentrations (0.1%, 0.5% and 1.0%) using the G-10 and G-50 membranes.

Table 2. Influence of the tryptic hydrolysate concentration on β-LG peptide transmission (%) using two spiral wound NF membranes at a P_T of 1.72 MPa

	G-10			G-50		
	0.1%	0.5%	1.0%	0.1%	0.5%	1.0%
Basic peptides						
f146–148	573	558	453	297	322	213
f1–8	569	220	233	503	259	239
f142–148	200	750	363	349	396	240
f76–82	359	287	195	300	250	226
Neutral peptides						
f15–20	73	149	98	128	201	130
f9–14	63	74	63	114	125	81
f71–75	nd[a]	96	75	138	180	126
f78–82	nd	45	126	nd	56	231
f136–138	nd	nd	67	nd	100	153
f33–40	nd	89	66	116	147	110

[a]nd: peptide not detected in permeate.

Table 2. Others have reported that basic peptides have higher Tr than acidic and neutral peptides in the NF permeates of milk protein hydrolysates (9, 12, 15). However, in the present study, the Tr values of the basic peptides all exceeded 100% and were generally highest with the G-10 membrane, irrespective of the hydrolysate concentration. Lapointe et al. (12) obtained Tr values close to 100% for β-LG f142–148 using an SG-13 cellulose acetate flat sheet membrane coupon and specific hydrodynamic conditions and filtration times. At pH 9, positively charged peptides are the counter ions of the membrane and are thus electrostatically attracted to the membrane. In the case of charged peptides, fractionation is mainly explained by the Donnan effect. More recently, Lapointe et al. (15) observed higher Tr values for cationic peptides than neutral peptides in G-10 permeates. However, the Tr values are still much lower than those reported in the present study. A major difference between the NF-module used by Lapointe et al. (15) and ours are the hydrodynamic conditions at the retentate side that were indicative of a laminar flow. A possible explanation for the high Tr values of basic peptides we observed is that the turbulent flow generated by the membrane spacers of the spiral wound module minimizes the thickness of the CP layer, thus maximizing the charge effects in the vicinity of the membrane surface. Lapointe et al. (15) hypothesized that the Tr of bioactive peptides β-LG f142–148 and f102–105 decrease because they interact with high molecular weight molecules in the polarized layer.

The higher Tr of the positively charged basic peptides shows that charge plays an important role in peptide fractionation. This was evident in the

discrepancy observed between the Tr of the peptide β-LG f76–82, a basic peptide with a lysine in its sequence, and the neutral peptide β-LG f78–82. These peptides differ only by two amino acids and have similar molecular weights (0.7744 vs. 0.5453 $kg \cdot mol^{-1}$, respectively), but have very different pIs (8.41 vs. 5.52).

Moreover, it is recognized that amino acids (16, 18) and peptides (19) have higher Tr near their pIs. Unlike basic peptides, whose Tr were higher with the G-10 membrane than the G-50 membrane, the Tr of neutral peptides were higher with the G-50 membrane. This reflects the differences in MWCO of the two membranes (2.5 vs. 8.0 $kg \cdot mol^{-1}$), and also suggests that steric or size effects predominate in the Tr of neutral peptides (14, 16, 18, 19). Some β-LG neutral peptides such as f71–75 (peak 3), f78–82 (peak 16), f136–138 (peak 2), and f33–40 (peak 4) were not detected in the G-10 permeates of the 0.1% hydrolysate solution (Fig. 4). This observation was somewhat unexpected considering their low molecular weight (Table 1). However, this may be due to the fact that the concentrations of neutral peptides in the 0.1% hydrolysate solution were below the detection limit of the HPLC method. Neutral peptides such as β-LG f92–101 (peak 10) were not detected in the permeates of either NF membrane (Fig. 4), irrespective of the concentration of hydrolysate solutions. Once again, this may be because their concentration in the total hydrolysate is too low for them to be detected.

Table 3 lists the $S_{B/N}$ values of the basic and neutral peptides for each concentration of the tryptic hydrolysate and for the two membranes. In all cases, the $S_{B/N}$ values were higher with the G-10 membrane than the NF G-50 membrane, irrespective of the hydrolysate concentration. The highest $S_{B/N}$ value (18.83) was obtained with the G-10 membrane with the 0.1% hydrolysate solution. This may be due to the fact that the Tr of neutral peptides are lower at the lowest (0.1%) hydrolysate concentrations (Table 2). At pH 9, $S_{B/N}$ decreased with increasing hydrolysate concentration (Table 3). A similar trend was observed by Lapointe et al. (15), who explained this finding by suggesting that CP or fouling occurs at higher feed concentrations.

The lower selectivity of the G-50 membrane is probably related to its higher MWCO (8.0 $kg \cdot mol^{-1}$), which allows a greater transmission of neutral peptides (Table 2), thus decreasing apparent $S_{B/N}$ values (Table 3).

Table 3. Influence of hydrolysate concentration on the separation of basic and neutral peptides ($S_{B/N}$ values) during nanofiltration at a P_T of 1.72 MPa

Membrane	0.1%	0.5%	1.0%
G-10	18.83	6.01	3.78
G-50	4.38	2.28	1.66

CONCLUSION

The present study confirmed that the G-10 membrane has the best potential for separating the acidic, neutral, and basic peptides in whey proteins hydrolysates composed mainly by β-LG peptides. Acidic peptides were completely retained at pH 9, and the Tr of the basic and neutral peptides were optimal when the G-10 membrane was used to filter a dilute hydrolysate solution (0.1%) and, as a consequence, when the Donnan effect was more pronounced. The use of a spiral wound configuration significantly improved the separation of the basic peptides, possibly as a result of the Donnan exclusion effect. More work will be needed to assess the impact of recirculation velocity and/or flow turbulence at the membrane surface on the separation of peptide mixtures.

APPENDIX

List of abbreviations

A	Ala	Alanine
C	Cys	Cysteine
D	Asp	Aspartic acid
E	Glu	Glutamic acid
F	Phe	Phenylalanine
G	Gly	Glycine
H	His	Histidine
I	Ile	Isoleucine
K	Lys	Lysine
L	Leu	Leucine
M	Met	Methionine
N	Asn	Asparagine
P	Pro	Proline
Q	Gln	Glutamine
R	Arg	Arginine
S	Ser	Serine
T	Thr	Threonine
V	Val	Valine
W	Trp	Tryptophan
Y	Tyr	Tyrosine

(Note) 1 and 3 letters are amino acids codes

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REFERENCES

1. Mullaly, M.M., Meisel, H., and Fitzgerald, R.J. (1997) Angiotensin -I- converting enzyme inhibitory activities of gastric and pancreatic proteinase digests of whey proteins. *International Dairy J.*, 7: 299.
2. Fitzgerald, R.J. and Meisel, H. (2000) Milk derived peptide inhibitors of angiotensin -I- converting enzyme. *British J. of Nutrition*, 2000: 33.
3. Pellegrini, A., Detling, C., Thomas, U., and Hunziker, P. (2001) Isolation and characterization of four bactericidal domains in the bovine β-lactoglobulin. *Biochimica and Physica Acta.*, 1526: 131.
4. Nagaoka, S., Futamara, Y., Miwa, K., Awano, T., Kanamura, Y., Tadashi, K., and Kuwata, T. (2001) Identification of novel hypocholesterolemic peptides derived from bovine β-lactoglobulin. *Biochemical and Biophysical Research Communication*, 281: 11.
5. Meisel, H. and Schlimme, E. (1995) Bioactive peptides derived from milk proteins. Structural, physiological and analytical aspects. *Die Nahrung*, 1: 1.
6. Turgeon, S.L., Gauthier, S.F., and Paquin, P. (1991) Interfacial and emulsifying properties of whey peptide fractions obtained by a two-step ultrafiltration process. *J. of Agricultural and Food Chemistry*, 39: 673.
7. Gourley, L., Gauthier, S.F., and Pouliot, Y. (1995) Separation of casein hydrolysates using polysulfone ultrafiltration membranes with pH and EDTA treatments applied. *Lait.*, 75: 258.
8. Nau, F., Kerhervé, F.L., Leonil, J., and Daufin, G. (1995) Selective separation of tryptic β casein peptides through ultrafiltration membranes: Influence of ionic interactions. *Biotechnology and Bioengineering*, 46: 246.
9. Pouliot, Y., Wijers, M.C., Gauthier, S.F., and Nadeau, L. (1999) Fractionation of whey protein hydrolysates using charged UF/NF membranes. *J. Membr. Sci.*, 158: 105.
10. Pouliot, Y., Gauthier, S.F., and L'heureux, J. (2000) Effect of peptide distribution on the fractionation of whey protein hydrolysates by nanofiltration membranes. *Lait.*, 80: 113.
11. Garem, A., Daufin, G., Maubois, J.L.; Chaufer B., and Léonil, J. (1998) Ionic interactions in nanofiltration of β casein peptides. *Biotechnology and Bioengineering*, 57: 109.
12. Lapointe, J.F., Gauthier, S.F.; Pouliot Y., and Bouchard, C. (2003) Effect of hydrodynamic conditions on fractionation of β-lactoglobulin tryptic peptides using nanofiltration membranes. *J. Membr. Sci.*, 212: 55.
13. Garem, A., Daufin, G., Maubois, J.L., and Léonil, J. (1997) Selective separation of amino acids with a charged inorganic nanofiltration membrane: Effect of physicochemical parameters on selectivity. *Biotechnology and Bioengineering*, 54: 291.
14. Martin-Orue, C., Bouhallab, S., and Garem, A. (1998) Nanofiltration of amino acid and peptide solutions: Mechanism of separation. *J. Membr. Sci.*, 142: 225.
15. Lapointe, J.F., Gauthier, S.F., Pouliot, Y., and Bouchard, C. (2005) Fouling of a nanofiltration membrane by a β-lactoglobulin tryptic hydrolysate: Impact on the membrane sieving and electrostatic properties. *J. Membr. Sci.*, 253: 89.
16. Garem, A., Léonil, J., Daufin, G., and Maubois, J.L. (1996) Nanofiltration d'acides aminés sur membranes organiques: Influence des paramètres physico-chimiques et de la pression transmembranaire sur la sélectivité. *Lait.*, 76: 267.
17. Donnan, F.G. (1995) Theory of membrane equilibria and membrane potentials in the presence of non-dialysing electrolytes. A contribution to physical-chemical physiology. *J. Membr. Sci.*, 100: 45.

18. Timmer, J.M. K., Speelmans, M.P. J., and Van Der Horst, H.C. (1998) Separation of amino acids by nanofiltration and ultrafiltration membranes. *Separation and Purification Technology*, 14: 133.
19. Tsuru, T., Shutou, T., Nakao, S.I., and Kimura, S. (1994) Peptide and amino acid separation with nanofiltration membranes. *Separation Science and Technology*, 29 (8): 971.