*Lait* (1995) 75, 259-269


**Original article**

# Separation of casein hydrolysates using polysulfone ultrafiltration membranes with pH and EDTA treatments applied

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(Received 29 July 1994; accepted 20 February 1995)

**Summary** — The preparation of peptide fractions from casein hydrolysates can be achieved by the use of ultrafiltration for the removal of the enzyme from the reaction mixture and/or to obtain specific fractions of peptides. However, the ultrafiltration of casein hydrolysates is subjected to severe fouling phenomena which affect both the flux decline and the rejection properties of the membrane. Polysulfone membrane material used in previous work showed specific rejection properties towards charged or hydroxylated amino acids. Polysulfone and polyethersulfone flat sheet membranes were selected for their distinctive surface properties. The ultrafiltration of a tryptic hydrolysate from sodium caseinate was studied and physico-chemical variables were introduced, namely, pH of the hydrolysate (pH 6, 8, 10) and addition of a calcium sequestrant (EDTA 20 mmol $l^{-1}$). Flux decline measurements, total nitrogen rejection, molecular mass distribution profile, and amino acid composition of the permeates were determined. Although the polyethersulfone membranes showed greater permeability to water (+ 15%) at 25°C than polysulfone, no distinctive effect of membrane material was observed in the permeation flux (l $h^{-1}$ $m^{-2}$) during ultrafiltration of the hydrolysates. The rejection coefficients were similar for both materials. It was observed that the pH and the excess of EDTA had a much stronger effect than the material on the rejection properties (nitrogen, peptides, amino acids) and the flux decline. The two materials seemed to exhibit different surface reactivity towards charged molecules.

**casein hydrolysate / ultrafiltration / polysulfone membrane / fouling / adsorption / flux decline**

**Résumé** — *Séparation d'hydrolysats caséiques sur des membranes d'ultrafiltration en polysulfone avec variation de pH et addition de EDTA. La préparation de fractions peptidiques issues d'un hydrolysat caséique peut être réalisée par ultrafiltration afin de séparer l'enzyme du mélange réactionnel et/ou pour l'obtention de fractions peptidiques spécifiques. Toutefois, l'ultrafiltration d'hydrolysats caséiques conduit à des phénomènes d'encrassement sévères affectant à la fois la chute du flux de perméation et les propriétés de rejet de la membrane. Les membranes de polysulfone utilisées lors de travaux antérieurs présentaient des propriétés de rejet spécifiques face aux acides aminés chargés ou hydroxylés.Pour cette étude, des membranes planes de polysulfone et de polyéthersulfone ont été sélectionnées pour leurs propriétés de surface distinctes. L'ultrafiltration d'un hydrolysat trypsique de caséinate de sodium a été étudiée et des variables physico-chimiques ont été introduites, soient le pH de l'hydrolysat (pH 6, 8, 10) et l'ajout d'un agent séquestrant pour le calcium (20 mmol $l^{1}$ EDTA).*

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*L'évolution du flux de perméation, le coefficient de rejet de l'azote total, le profil de masse moléculaire et la composition en acides aminés dans les perméats ont été déterminés. Bien que les membranes de polyéthersulfone possédaient une perméabilité à l'eau à 25°C supérieure au polysulfone (+15%), des flux de perméation (l $h^{-1}$ $m^{-2}$) similaires ont été observés lors de l'ultrafiltration des hydrolysats. Les coefficients de rejet étaient similaires pour les deux matériaux. Il a été observé que le pH et l'excès de EDTA exerçaient un effet supérieur à celui du matériau sur les propriétés de rejet (azote, peptides, acides aminés) et sur la chute du flux de perméation. Les deux matériaux montrent un comportement différent face aux molécules chargées.*

**hydrolysat caséique / ultrafiltration / membrane polysulfone / encrassement / adsorption / chute du flux de perméation**

## INTRODUCTION

The enzymatic hydrolysis of milk caseins using pancreatic extracts has been extensively used for the preparation of therapeutical products designed for the nutrition of patients presenting disorders of protein digestion or following surgery of the digestive tract (Manson, 1980). Casein hydrolysates offer the advantage of providing the amino acid content of milk caseins in a predigested form which facilitates its intestinal absorption. However, since specific casein sequences have been identified as potential bioactive peptides such as opioids and mineral carriers (Meisel and Schlimme, 1990), downstream fractionation steps have been proposed (Touraine *et al*, 1987). Brulé *et al* (1980) have patented a process for the preparation of caseinophosphopeptides using ultrafiltration (UF) membranes under specific physico-chemical conditions, namely pH 6.2 and with 0.2% $Na_2HPO_4$ and 0.5% $CaCl_2$ added to the hydrolysate prior to UF. The retentate obtained showed an increased content in phosphoserine residues accompanied by a decrease in aromatic amino acids (Phe, Tyr, Trp). This shift in amino acid profile was explained by the sequestering action of $Ca^{2+}$ on phosphoserine residues which produced aggregated material that was rejected by the UF membrane. This development has succeeded in demonstrating the technological potential of a physico-chemical and membrane-based approach for the fractionation of casein hydrolysates. However, there remains a major limitation or drawback to such processes, namely the severe fouling phenomena by casein hydrolysates that strongly affect the permeability of UF-membranes.

Since the pioneer work of Brulé *et al* (1980), reports on the fractionation of casein hydrolysates using UF-membranes have been very scarce. Walsh *et al* (1989) studied the rejective properties of metallic ultrafiltration membranes for amino acids, calcium and casein hydrolysates. Their results illustrated the strong influence of the pH of the feed and of the surface charge of the membrane on the passage of charged amino acids and calcium. Nau (1991) reported on the membrane separation of tryptic hydrolysates from β-casein. It was found that pH and ionic strength of the feed, and electrostatic charge of the membrane material influenced the flux decline and the selectivity of the membrane. The results showed that the permeation flux was most affected at low pH (5.7 *vs* 7.5 or 12.0). Also, the net charge and the hydrophobicity of the peptides found in the hydrolysate could contribute to their retention behaviour. A low increase in ionic strenght could improve the passage of peptides initially retained while it increased the retention of the peptides concentrated in the permeate. A strong increase in ionic strength resulted in aggregation phenomena. However, modification of the ionic strength was not efficient for peptides of greater hydrophobicity. The nature and charge density of the membrane material played a role in the retention

of peptides even when the pore sizes were significantly higher than the size of the peptides.

Our first work (Pouliot and Gauthier, 1990) focussed on the effect of physico-chemical parameters such as pH on the flux decline during UF of casein hydrolysates using polysulfone membranes. It was observed that pH modification produced marked effect on both the flux decline upon UF and molecular mass profile of the permeates obtained. In another study (Pouliot *et al*, 1993), it was also shown that polysulfone exhibited specific rejective properties towards charged and hydroxylated amino acids.

The present study was led in order to investigate the effect of the compositional factors of the membrane materials and of the physico-chemical properties of the casein hydrolysates on their fractionation profile. Polyethersulfone (PES) was therefore chosen since: 1) it differs from polysulfone (PS) by its chemical composition, while being the same class of polymers (fig 1) (Kai *et al*, 1985); and 2) PES is known to possess greater permeability and chemical stability than PS which comprises one dimethyl group and one sulfonated group per repeating unit. PES only contains sulfonated groups per repeating unit. For both materials, molecular mass cut-offs (MMCO) of 5 kDa and 10 kDa were used for the experiment since no discrepancies in fractionation profiles were observed in a previous study (Pouliot and Gauthier, 1990) when ultrafiltering with 1 kDa, 2 kDa, or 5 kDa membranes. The specific physico-chemical conditions were also determined on the basis of a previous study; it was already known that changing the pH strongly affected the permeation flux; pH values of 6.0, 8.0 and 10.0 were chosen. Also the presence of EDTA (disodium-ethylenediamine-tetraacetate salt), a calcium chelating agent, had been identified as directly modifying the flux decline behaviour during the concentration of casein hydrolysates; a level of 20 mmol $l^{-1}$ was chosen for the experiment.

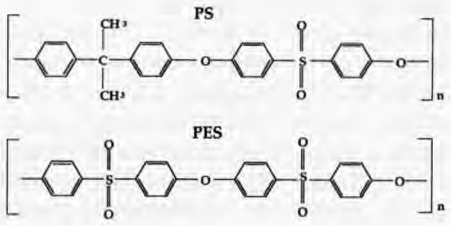


**Fig 1**. Comparison of chemical structure of polysulfone (PS) and polyethersulfone (PES) (adapted from Kai *et al*, 1985).
*Comparaison des structures chimiques du polysulfone (PS) et du polyéthersulfone (PES) (d'après Kai* et al, *1985).*

The effects of membrane material and MMCO, together with that of pH and calcium-chelating agent in the hydrolysate, on the flux decline, total nitrogen rejection, molecular mass distribution profile, and amino acid profile were studied.

## MATERIALS AND METHODS

### *Preparation of the total hydrolysate from casein*

Commercial sodium caseinate (ICN Chemicals, Cleveland, OH, USA) was reconstituted (100 l) to 3.5% (w/v, protein basis) and hydrolyzed using trypsin (Type III-S) from bovine pancreas (Sigma Chemical Co, St-Louis, MO, USA). Hydrolysis conditions were: pH 8.0, temperature 40°C, ratio enzyme:substrate 1:200 (mass of protein). The hydrolysis was carried out for 45 min and the pH was maintained by addition of 4 N NaOH, using a portable pH-meter (MDL 119 LCD, Fisher). The mixture was then pumped into a hollow fiber module equipped with two 30 kDa cut-off membranes (HF 1-43-PM30, Romicon, Woburn, MA, USA); the reaction mixture was concentrated 3 × and diafiltered 2 ×. The permeate was then freeze-dried, constituting the casein total hydrolysate (TH) used for the present study.

### *Ultrafiltration of the total hydrolysate from casein*

Polysulfone (Iris UF3026) and polyethersulfone (Iris UF3028) flat sheet membranes (0.02 $m^2$) were obtained from Tech-Sep (Rhône-Poulenc, Miribel, France). The membranes were conditioned at 50°C by a 10-min rinsing (one flow through), a 30-min recirculation of 0.3% NaOH, a 15-min rinsing, a 30-min recirculation of 0.6% $H_3PO_4$, and a final 15-min rinse. Distilled deionized rinsing water was used throughout the study. The membranes were cleaned after every filtration starting with a 15-min rinse and followed by a 20-min recirculation of the solutions described above. Whenever required, 250 ppm NaOCl was added to the caustic solution to restore the pure water flux. The membranes were stored in 25 ppm NaOCl. The pure water flux (Jw) was measured after each cleaning procedure and before every ultrafiltration, providing the reference value in order to compare the different conditions under study.

A plate-and-frame Rayflow 2 × 100 module (Tech-Sep, Rhône-Poulenc, Miribel, France) equipped with a variable rotary vane pump (model 7116, Cole-Parmer Instrument Company, Chicago, IL, USA) and a temperature-controlled water bath (± 1°C) was used for the experiments. The ultrafiltration runs were performed at 25°C under a constant transmembrane pressure of 160 kPa and an estimated tangential velocity of 1.6 m $s^{-1}$.

The total hydrolysate was solubilized in 1.2 l distilled deionized water at 1.65% total solids (w/v), and the solution was filtered on a Whatman no 4. Whenever required, 20 mmol $l^{-1}$ EDTA ([ethylenediamine]-tetraacetic acid disodium salt; Fisher Scientific, Ontario, Canada) was then solubilized. The pH of the solution was adjusted to 6.0, 8.0 or 10.0 using 4 N and/or 1 N HCl or NaOH.

The permeation rate (ml $min^{-1}$) was measured during the concentration of the total hydrolysate and further calculated as flux J (l $h^{-1}$ $m^{-2}$). Solutions were ultrafiltered to a volumic concentration factor (VCF) of 4 ×. Ultrafiltration experiments were performed in triplicate. For each experiment, 100 ml of permeate, designated as amino acids and small peptide fraction (AA), was collected and stored at –15°C for subsequent analysis.

### *Analyses*

Total nitrogen contents were determined in triplicate using the Kjeldahl method (IDF, 1986) performed on a Büchi block digestor 430/distillation unit Büchi 323 (Büchi, Flawil, Switzerland).

Molecular mass distribution profiles of the peptides (TH and AA) were determined by high performance size exclusion chromatography (HPSEC, LKB system) using a TSK-2000 SW column (Vijayalakshmi *et al*, 1986). The total surface of the chromatogram was separated in three ranges of molecular mass (< 2000 Da, 2000–5000 Da, and > 5000 Da) and expressed in percentage of the total surface.

The amino acid compositions of the fractions were determined with the Pico-Tag method using Pico-Tag work station and 3 μm column (Waters, Millipore) after acid hydrolysis (6 N HCl, 100°C, 24 h).

Titration curves were performed at 25°C on TH and AA fractions using an auto-titrator Model DL 21 (Mettler Instrumente AG, Greifensee, Switzerland), using the back titration method (Lucey *et al*, 1995). Solutions (50 ml) were titrated at a rate of 0.2 ml $min^{-1}$ with 0.5 N NaOH or 0.5 N HCl. The buffering index (dB/dpH) was calculated for TH solutions (1.5% w/v protein) and for the AA fractions obtained at pH 8.0, with and without added EDTA, for both materials.

## RESULTS

### *Flux decline during 4 × concentration*

The decreases in permeation flux with increasing VCF for the four membranes under study are shown in figure 2. The runs performed at pH 6.0 were stopped before reaching a VCF of 2 × because of the very low flux values obtained. Similar flux decline behaviours were generally observed for PS and PES. Although PES membranes showed greater permeability to water than PS (see fig 2), the initial fluxes were similar for the two materials. The MMCO of the membrane affected the initial permeation flux averaging 60 l $h^{-1}$ $m^{-2}$ for the 10 kDa and 35 l $h^{-1}$ $m^{-2}$ with the 5 kDa membranes. Ultrafiltering at pH 10.0 led to higher flux values than at pH

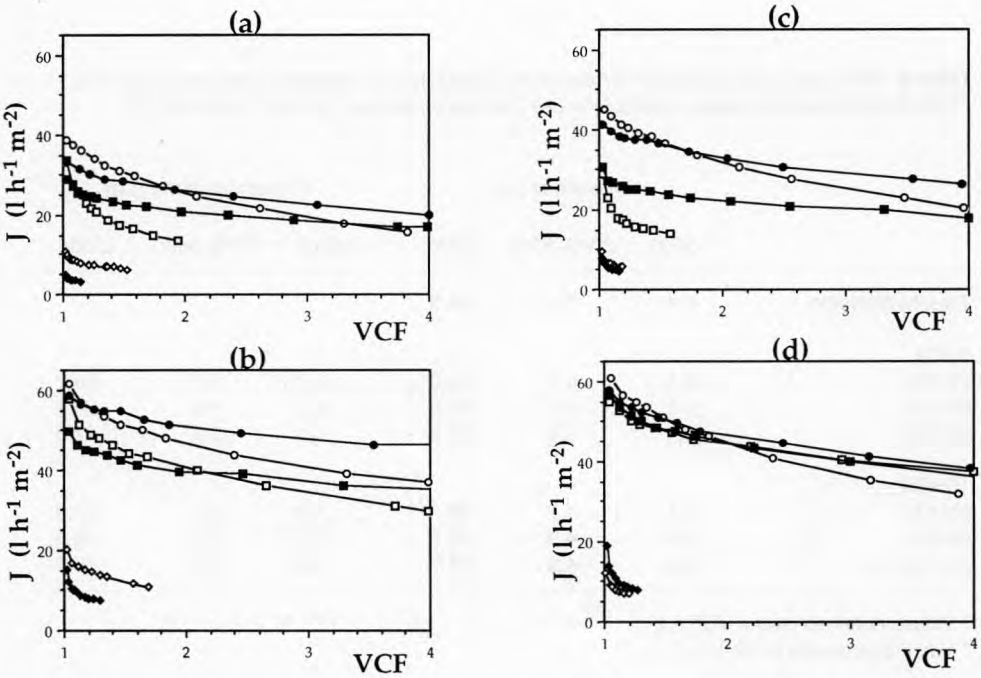


**Fig 2.** Permeation flux with increasing volumic concentration factor. **a**. PS 5 kDa (Jw: 173 l $h^{-1}$ $m^{-2}$). **b**. PS 10 kDa (Jw: 233 l $h^{-1}$ $m^{-2}$). **c**. PES 5 kDa (Jw: 184 l $h^{-1}$ $m^{-2}$). **d**. PES 10 kDa (Jw: 287 l $h^{-1}$ $m^{-2}$). ◊ pH 6.0; □ pH 8.0; o pH 10.0. Open symbols, 0 mmol $l^{-1}$ EDTA; closed symbols, 20 mmol $l^{-1}$ EDTA.
*Flux de perméation suivant l'augmentation du facteur de concentration volumique : **a**. PS 5 kDa (Jw : 173 l $h^{-1}$ $m^{-2}$). **b**. PS 10 kDa (Jw : 233 l $h^{-1}$ $m^{-2}$). **c**. PES 5 kDa (Jw : 184 l $h^{-1}$ $m^{-2}$). **d**. PES 10 kDa (Jw : 287 l $h^{-1}$ $m^{-2}$). ◊ pH 6,0 ; □ pH 8,0 ; o pH 10.0. Symboles ouverts : 0 mmol $l^{-1}$ EDTA; symboles fermés : 20 mmol $l^{-1}$ EDTA.*

**Table I**. Rejection coefficients (σ) for nitrogen over 4 × concentration of the total hydrolysate.
*Coefficients de rejet (σ) de l'azote après concentration de 4 × de l'hydrolysat total.*

| | | *Polysulfone* | | *Polyethersulfone* | |
|---|---|---|---|---|---|
| | *pH* | *no EDTA* | *EDTA* | *no EDTA* | *EDTA* |
| 5 kDa | 6.0[1] | 0.19 | 0.28 | 0.15 | 0.22 |
| | 8.0 | 0.00[1] | 0.17 | 0.02[1] | 0.14 |
| | 10.0 | 0.00 | 0.28 | 0.00 | 0.26 |
| 10 kDa | 6.0[1] | 0.14 | 0.19 | 0.12 | 0.22 |
| | 8.0 | 0.00 | 0.13 | 0.00 | 0.14 |
| | 10.0 | 0.00 | 0.23 | 0.00 | 0.26 |

[1] Values obtained over a VCF ≤ 2.
[1] *Valeurs obtenues au FCV ≤ 2.*

**Table II**. Molecular mass distribution profile of the permeates (%) obtained in absence of EDTA.
*Profil de distribution de masse moléculaire des perméats obtenus (%) sans l'ajout d'EDTA.*

| | *Polysulfone (Da)* | | | *Polyethersulfone (Da)* | | |
|---|---|---|---|---|---|---|
| | *>5000* | *2000–5000* | *<2000* | *>5000* | *2000–5000* | *<2000* |
| Total hydrolysate | 0.4 | 3.3 | 96.3 | | | |
| *5 kDa* | | | | | | |
| pH 6.0[1] | <0.1 | 0.9 | 99.0 | <0.1 | 1.7 | 98.2 |
| pH 8.0[1] | 0.1 | 2.4 | 97.5 | <0.1 | 1.8 | 98.1 |
| pH 10.0 | 0.1 | 2.0 | 97.9 | 0.1 | 2.3 | 97.6 |
| *10 kDA* | | | | | | |
| pH 6.0[1] | <0.1 | 1.7 | 98.3 | 0.1 | 2.0 | 97.9 |
| pH 8.0 | 0.3 | 4.9 | 94.8 | 0.2 | 3.3 | 96.5 |
| pH 10.0 | 0.3 | 4.0 | 95.7 | 0.2 | 3.6 | 96.2 |

[1] Values obtained over a VCF ≤ 2.
[1] *Valeurs obtenues au FCV ≤ 2.*

8.0; in all cases, filtrations at pH 6.0 resulted in very low initial fluxes (< 20 l $h^{-1}$ $m^{-2}$). Figure 2 also shows a marked effect of the presence of a chelating agent on the flux decline characteristics; the initial flux ($J_0$) and the total flux decline (TFD=100-[($J_0$/$J_f$)100]) were lowered when EDTA was added; for example, using a PS 10 kDa at pH 8.0 without EDTA $J_0$ = 58 l $h^{-1}$ $m^{-2}$ and TFD = 48% were obtained, while with added EDTA $J_0$ was 50 l $h^{-1}$ $m^{-2}$ and TFD = 29%. This phenomenon was observed at all pH values with the four membranes under study.

### *Nitrogen rejection*

The passage of peptides and amino acids was first characterized by determining the nitrogen rejection coefficient ($\sigma = 1 - C_P/C_R$, where $C_P$ and $C_R$ represent the nitrogen content in permeate and retentate). As seen from table I, low rejection values were obtained (< 0.28) for all the treatments under study. It was observed that pH and EDTA induced the most important changes in nitrogen rejection. Increasing pH from 6.0 to 10.0 decreased the rejection from 0.12–0.19 to 0 when no EDTA was added while oppositely, the rejection coefficient showed a minimum value at pH 8.0 ($\sigma$ = 0.13–0.17) compared to 0.19–0.28 at pH 6.0 and 10.0, when EDTA was added. Similar trends were observed for PS and PES, and for 5 kDa and 10 kDa membranes.

### *Molecular mass distribution profiles*

The molecular mass distribution profiles of the peptides found in permeates (see table II) were all characterized by a high content (>94.8%) in short peptides (< 2000 Da). The 2000–5000 Da fraction slightly increased as pH was shifted from 6.0 to 10.0 but always remained within the 0.95% to 4.90% range whereas the large peptides (> 5000 Da) content was always found to be lower than

0.30%. A greater proportion of large peptides was generally found in the permeates from the 10 kDa membranes. The use of PS and PES generally led to similar results. The molecular mass distribution profiles of the peptides found in permeates with 20 mmol $l^{-1}$ EDTA were all similar to those obtained without EDTA and therefore were not included in table II.

### *Amino acid profiles*

The amino acid profiles obtained for the permeates from 5 kDa and 10 kDa membranes of both PS and PES were generally similar. Table III shows the amino acid profiles obtained with the 10 kDa PS and PES membranes in the physico-chemical conditions under study. Some individual amino acids showed greater variations and were grouped as acidic (Asp, Glu) and basic (Arg, Lys, His) amino acids. The variations in amino acid profiles showed some discrepancies. For example, the content in acidic amino acids in presence of EDTA decreased from 24.98 to 21.91 as pH was adjusted from 6.0 to 10.0 with PS membrane whereas it increased from 21.66 to 27.01 with PES membrane. Such a phenomenon was not observed for basic amino acid content which slightly decreased for both PS and PES.

It was however observed that adjusting pH from 6.0 to 10.0 generally induced an increase in acidic amino acids and a decrease in basic amino acids. The addition of EDTA produced similar effects than pH on the acidic and basic amino acid profiles. A minor effect related to membrane material was found for the basic/acidic amino acids ratios which were generally higher (+10%) for PES compared to PS. Finally, lower basic/acidic ratios were obtained in the presence of EDTA.

### *Titration curves of total hydrolysate and permeates*

Further characterization of the hydrolysate and of the permeates obtained was accomplished by determining their titration curve between pH 3.0 to 11.0. Figure 3 shows the curves obtained with TH and with permeates obtained at pH 8.0 for PS and PES, with or without added EDTA. Two typical curve shapes were obtained, namely without EDTA (maximum dB/dpH $\cong$ 7.5) and with EDTA (maximum dB/dpH $\cong$ 6.2 and 9.8). Curves obtained at pH 6.0 and 10.0 (not shown) did show similar characteristics to those at pH 8.0.

## DISCUSSION

The results obtained in this study showed that physico-chemical parameters such as pH and presence of EDTA induced much stronger effects than membrane material and MMCO on the flux decline and on the rejection phenomena during ultrafiltration of total hydrolysate obtained from casein.

### *Effect of membrane materials and MMCO*

The similarity between the initial fluxes ($J_0$) obtained with PS and PES, despite the greater water permeability of PES, suggests that more important adsorption phenomena involving components from hydrolysates occur with PES. Contact angle measurements performed on PS and PES upon static fouling with casein hydrolysate (Gourley *et al*, 1994) support this view. Such similar flux behaviour between PES and sulfonated polysulfone was also observed by Millesime *et al* (1994) upon concentration of lysosyme.

PS and PES did not show distinctive differences in total nitrogen rejection and molecular mass distribution profiles in permeates, but variations in amino acid composition were observed. Since in all permeates,

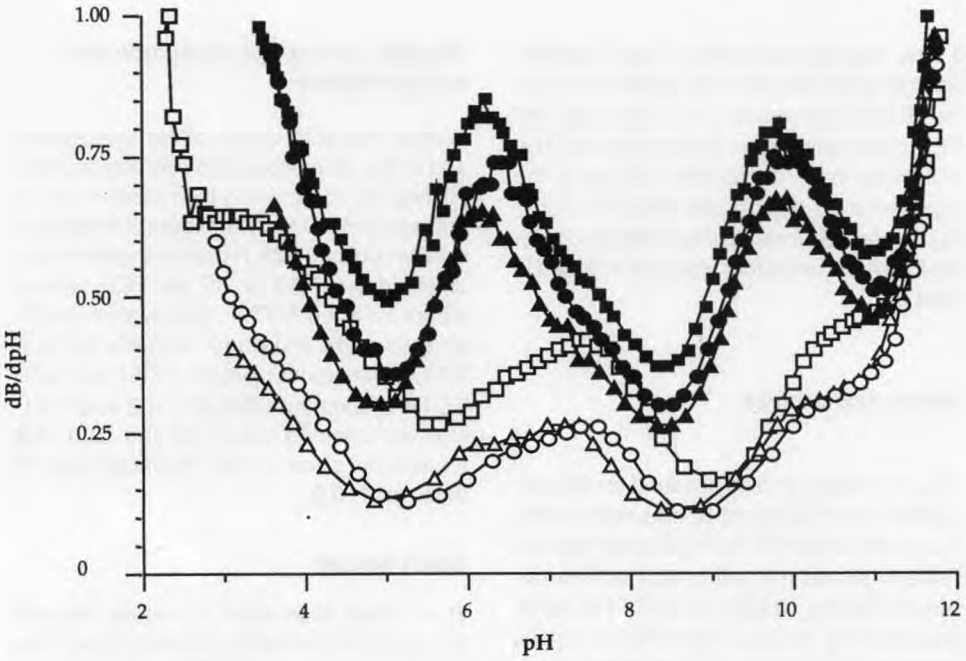


**Fig 3**. Titration curves of TH and permeates obtained at pH 8.0 with or without added EDTA, for PS and PES: □ TH; O PS; Δ PES. Open symbols, 0 mmol $l^{-1}$ EDTA; closed symbols, 20 mmol $l^{-1}$ EDTA. *Courbes de titration de l'hydrolysat total (TH) et des perméats obtenus à pH 8,0 avec et sans EDTA ajouté, pour les membranes PS et PES : □ TH ; O PS ; Δ PES. Symboles ouverts : 0 mmol $l^{-1}$ EDTA ; symboles fermés : 20 mmol $l^{-1}$ EDTA.*

> 94% of the peptides were smaller than 2000 Da, it can be argued that the differences induced by PS and PES could result from adsorption of peptides or amino acids onto the membrane surface and which did not modify its pore size. To some extent, this hypothesis would be controversal since it is known (Meireles *et al*, 1991; Mochizuki and Zydney, 1992; Brink *et al*, 1993) that protein adsorption modifies the pore size of sieving membranes. However, the surface properties of the adsorbed fouling layer on PS and PES would possess different characteristics and therefore affect the passage of amino acids. This is illustrated to some extent by the ratio basic/acidic amino acids which was generally lower for PS compared to PES.

The use of higher MMCO membrane (10 kDa *vs* 5 kDa) only slightly improved the permeation flux and the rejection of total nitrogen. The molecular mass distribution profile showed higher content of larger peptides (> 2000 Da) in permeates from 10 kDa membranes. These observations are in agreement with previous findings (Pouliot *et al*, 1993) indicating that the MMCO of polysulfone membranes did not influence to a great extent the separation characteristics of casein hydrolysates.

### *Effect of pH and EDTA*

The very low flux values obtained at pH 6.0 are in good agreement with the results obtained by Pouliot and Gauthier (1990) and by Nau (1991). It can be suggested that by changing the pH of the media from 6.0 to 10.0, the net negative charge of the peptides

**Table III**. Amino acid profiles of permeates obtained under specific pH conditions, with and without added EDTA, for PS and PES 10 kDa membranes.
*Profils d'acides aminés des perméats obtenus aux différents pH, avec et sans EDTA ajouté, pour les membranes de 10 kDa en PS et PES.*

| | | *Amino acid content (%)* | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *TH* | *Polysulfone (PS)* | | | | | | *Polyethersulfone (PES)* | | | | | |
| *Amino acids* | | 6.0 | | 8.0 | | 10.0 | | 6.0 | | 8.0 | | 10.0 | |
| | | NE | E | NE | E | NE | E | NE | E | NE | E | NE | E |
| Asp | 4.41 | 5.57 | 6.88 | 7.79 | 4.19 | 3.57 | 3.81 | 4.44 | 4.31 | 3.44 | 4.54 | 3.94 | 7.57 |
| Glu | 20.10 | 16.70 | 18.10 | 19.40 | 17.20 | 17.80 | 18.10 | 16.50 | 17.40 | 17.10 | 18.60 | 17.50 | 19.40 |
| Ser | 4.89 | 3.95 | 3.84 | 4.06 | 4.67 | 4.26 | 4.35 | 3.90 | 3.69 | 3.82 | 4.02 | 3.91 | 4.03 |
| Gly | 2.21 | 1.91 | 2.18 | 2.09 | 2.47 | 2.23 | 2.17 | 2.01 | 2.12 | 2.08 | 2.11 | 2.04 | 1.99 |
| His | 3.38 | 3.80 | 3.35 | 3.40 | 3.60 | 3.91 | 3.59 | 4.08 | 3.61 | 3.89 | 3.69 | 3.80 | 3.57 |
| Arg | 4.51 | 5.49 | 4.96 | 4.50 | 5.10 | 5.17 | 4.60 | 5.54 | 5.18 | 5.10 | 4.96 | 4.83 | 4.76 |
| Thr | 3.70 | 2.71 | 2.70 | 3.10 | 3.54 | 5.04 | 3.48 | 2.81 | 2.81 | 2.95 | 3.17 | 3.07 | 3.08 |
| Ala | 2.77 | 3.32 | 3.25 | 3.06 | 3.31 | 0.60 | 3.04 | 3.32 | 3.29 | 3.29 | 3.11 | 3.24 | 3.01 |
| Pro | 12.97 | 10.58 | 11.00 | 11.84 | 13.71 | 12.85 | 12.62 | 11.32 | 11.86 | 12.48 | 12.29 | 12.16 | 11.49 |
| Tyr | 5.65 | 6.83 | 6.37 | 5.19 | 5.78 | 6.12 | 5.83 | 6.48 | 6.63 | 6.10 | 5.64 | 5.70 | 5.27 |
| Val | 7.19 | 5.63 | 5.96 | 6.35 | 6.17 | 7.93 | 7.36 | 6.37 | 6.54 | 6.91 | 7.15 | 7.57 | 6.25 |
| Met | 1.14 | 0.21 | 0.58 | 0.62 | 0.85 | 0.74 | 0.84 | 0.19 | 0.58 | 0.74 | 0.39 | 0.93 | 0.76 |
| Ile | 4.60 | 5.30 | 4.90 | 4.50 | 4.60 | 5.00 | 4.90 | 5.30 | 5.10 | 5.10 | 4.90 | 5.10 | 4.50 |
| Leu | 8.69 | 8.53 | 8.75 | 8.73 | 9.53 | 9.22 | 9.52 | 8.78 | 9.10 | 9.17 | 9.20 | 9.18 | 8.69 |
| Phe | 5.67 | 6.06 | 6.47 | 6.02 | 6.39 | 6.12 | 6.36 | 6.03 | 6.57 | 6.42 | 6.28 | 6.36 | 5.91 |
| Lys | 8.12 | 13.40 | 10.70 | 9.29 | 8.86 | 9.46 | 9.44 | 12.90 | 11.30 | 11.40 | 10.00 | 10.70 | 9.68 |
| AA | 24.49 | 22.28 | 24.98 | 27.22 | 21.39 | 21.39 | 21.91 | 20.98 | 21.66 | 20.51 | 23.11 | 21.45 | 27.01 |
| BA | 16.00 | 22.69 | 19.05 | 17.19 | 17.56 | 18.54 | 17.63 | 22.48 | 20.09 | 20.38 | 18.67 | 19.30 | 18.01 |
| NPA | 43.03 | 39.63 | 40.91 | 41.12 | 44.56 | 42.46 | 44.64 | 41.31 | 43.04 | 44.11 | 43.32 | 44.54 | 40.61 |
| PA | 56.97 | 60.37 | 59.09 | 58.88 | 55.44 | 57.54 | 55.36 | 58.69 | 56.94 | 55.89 | 56.68 | 55.46 | 59.39 |
| B/A | 0.65 | 1.02 | 0.76 | 0.63 | 0.82 | 0.87 | 0.80 | 1.07 | 0.93 | 0.99 | 0.81 | 0.90 | 0.67 |

**NE**: no EDTA *(sans EDTA ajouté)*; **E**: EDTA *(avec EDTA ajouté)*. **AA**: acidic amino acids *(acides aminés acides)* : Asp, Glu. **BA**: basic amino acids *(acides aminés basiques)* : Arg, Lys, His. **NPA**: non-polar amino acids *(acides aminés non polaires)* : Ala, Leu, Ile, Phe, Pro, Met, Val. **PA**: polar amino acids *(acides aminés polaires)* : Arg, Lys, His, Asp, Glu, Ser, Thr, Tyr. **B/A**: basic/acid.

is increased, which favours repulsive effects (Nyström, 1990) with the membranes negatively charged over this pH-range. Increasing the pH in absence of EDTA decreased the total nitrogen rejection to $\sigma \cong 0$, which suggests that the repulsive forces between membranes and peptides would enhance their passage into the membranes. However,

the pH modifications also induced measurable changes in the amino acid profiles in the permeates. In accordance with previous findings (Pouliot *et al*, 1993), the UF-fractionation with PS and PES membranes affected the passage of polar amino acids to a greater extent than that of non-polar amino acids. Increasing pH could have affected both basic and acidic amino acids by modifying the ionization of the side chain residues and promoting peptide-peptide interactions. The effect of pH on the content in acidic amino acids must however be interpreted considering the fact that the analytical method used (Pico-Tag column) does not discriminate between Glu and Gln, and also between Asp and Asn. Therefore, since it is known (Eigel *et al*, 1984) that aproximately 50% of the Glx and Asx found in casein are present as Gln and Asn, the real extent of the phenomena observed for Asp and Glu may be greater.

The effect of EDTA on the flux decline profile and on the rejective properties of the membranes could be explained by the impact of this sequestering agent on charged peptides. From calcium determinations on the TH, an excess of 19 mmol $l^{-1}$ EDTA was found. Titration curves on the hydrolysate with and without EDTA revealed different pKa values of the TH when EDTA was added. As shown in figure 3, the two distinctive peaks at pH 6.2 and 9.8 on the dB/dpH curve correspond to the ionization of EDTA from $H_2Y^{2-}$ to $HY^{3-}$ and from $HY^{3-}$ to $Y^{4-}$ respectively. Considering this excess in EDTA over the pH range under study, the binding of positively charged peptides (or amino acids) by EDTA should be examined. As a consequence, the excess EDTA could have affected the passage of basic amino acids by the formation of peptide-EDTA complexes which would prevent their passage to the permeate. This would explain the lower proportion of basic amino acids (Arg, Lys, His) in the EDTA-permeates. The occurrence of interactions between EDTA and membrane materials should also be examined, but the present study did not provide experimental evidence for such phenomena.

## CONCLUSION

Although the PES showed greater permeability than PS to water, the two materials are similar, showing no distinctive differences in the fractionation; however, they seem to have a different surface reactivity towards charged molecules.

The flux was improved using a 10 kDa membrane but the increase in MMCO did not alter significantly the composition of the fractions obtained, as possible result of the concentration polarization.

Phenomena concerning the net charge of the peptides and/or the membrane are determining for the hydrolysates' fractionation. Ionization of the environment had a marked effect on the degree of association of the peptides and/or on the interactions membrane-peptides. Excess of EDTA in the hydrolysate modified the composition of the permeates obtained (nitrogen rejection, molecular mass distribution profile, and amino acid profile).

Further studies on specific interactions of membrane-peptides would lead to a better understanding of the fouling phenomena.

## ACKNOWLEDGMENTS

This research was funded by the FCAR and the Ministère de l'Enseignement Supérieur et de la Science du Québec.

## REFERENCES

Brink LES, Elbers SJG, Robbertsen T, Both P (1993) The anti-fouling action of polymers preadsorbed on ultrafiltration and microfiltration membranes. *J Membrane Sci* 76, 281–291

Brulé G, Roger L, Fauquant J, Piot M (1980) Procédé de traitement d'une matière à base de caséine contenant des phosphocaséinates

de cations monovalents ou leurs dérivés, produits obtenus et applications. Brevet Fr 2 474 829, INPI, Paris

Eigel WN, Butler JE, Ernstrom CA, Farrell Jr HM, Harwalkar VR, Jenness R, Whitney RMcL (1984) Nomenclature of proteins of cow's milk: Fifth revision. *J Dairy Sci* 67, 1599–1631

Gourley L, Britten M, Gauthier SF, Pouliot Y (1994) Characterization of adsorptive fouling on ultrafiltration membranes by peptides mixtures using contact angle measurements. *J Membrane Sci* 97, 283–289

International Dairy Federation (1986) Milk: Determination of nitrogen content (Kjeldahl method) and calculation of crude protein content. International Provisional IDF Standard 20A.

Kai M, Ishii K, Tsugaya H, Miyano T (1985) Development of polyether sulfone ultrafiltration membranes. *In: Reverse osmosis and ultrafiltration* (Sourirajan S, Matsuura T, eds) Am Chem Society, Washington, DC, 21–33

Lucey JA, Hanth B, Fox PF (1995) The buffering of milk. *J Dairy Res*, in press

Manson W (1980) The use of milk and milk constituents in pharmaceutical preparations. *Int Dairy Fed Bull* 125, 60–65

Meireles M, Aimar P, Sanchez V (1991) Effect of protein fouling on the apparent pore size distribution of sieving membranes. *J Membrane Sci* 56, 13–28

Meisel H, Schlimme E (1990) Milk proteins: precursors of bioactive peptides. *Trends Food Sci Technol* 1, 41–43

Millesime L, Amiel C, Chaufer B (1994) Ultrafiltration of lysosyme and bovine serum albumin with polysulfone membranes modified with quaternized polyvinylimidazole. *J Membrane Sci* 89, 223–234

Mochizuki S, Zydney AL (1992) Effect of protein adsorption on the transport characteristics of asymmetric ultrafiltration membranes. *Biotechnol Prog* 8, 553–561

Nau F (1991) *Production et séparation par ultrafiltration de peptides trypsiques issus de la caséine β bovine.* Thesis, ENSA, Rennes, France

Nyström M (1990) *Fouling prevention by modification of UF membranes.* Proc ICOM'90, August 20–24, Chicago, IL, 90

Pouliot Y, Gauthier SF (1990) *Effect of selected physico-chemical parameters on the flux decline of casein hydrolysate on polysulfone ultrafiltration membranes.* Proc ICOM'90, August 20–24, Chicago, IL, 273–275

Pouliot Y, Gauthier SF, Bard C (1993) Fractionation of casein hydrolysates using polysulfone ultrafiltration hollow fiber membranes. *J Membrane Sci* 80, 257–264

Touraine F, Brulé G, Maubois JL (1987) Séparation chromatographique de peptides issus de l'hydrolyse enzymatique de protéines de lactosérum et de caséines. *Lait* 67, 419–436

Vijayalakshmi M, Lemieux L, Amiot J (1986) HPLC analytical separation of peptides according to their molecular weight. *J Liq Chromatogr* 9, 3559–3576

Walsh CJ, Thomas RL, Kunkel ME (1989) The use of metallic ultrafiltration membranes to assess calcium availability *in vitro*. *Food Chem* 32, 307–317