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Impact of Ultrafiltration Membrane Material on Peptide Separation from a Snow Crab Byproduct Hydrolysate by Electrodialysis with Ultrafiltration Membranes

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ABSTRACT: Electrodialysis with ultrafiltration membrane (EDUF) is a technology based on the separation of molecules according to their charge and molecular mass. Some works have already successfully demonstrated the recovery of bioactive peptide fractions. However, the impact of ultrafiltration membrane (UFM) material, used in the EDUF system, on the peptide migration has never been studied. Consequently, the objectives of this work were (1) to evaluate the effect of two different UFM materials on the selective separation of peptides from a snow crab byproduct hydrolysate by electrodialysis with ultrafiltration membranes and (2) to determine the effect of UFM material on their potential fouling by peptides. It appeared that, after 6 h of EDUF separation using polyether sulfone (PES) and cellulose acetate (CA) UFM, peptides with low molecular weights ranging from 300 to 700 Da represented the most abundant population in the KCl1 (compartment located near the anode for the recovery of anionic/acid peptide fractions) and KCl2 (compartment located near the cathode for the recovery of cationic/basic peptide fractions) permeates. Peptides with molecular weights ranging from 700 to 900 Da did not migrate during the EDUF treatment. Moreover, only CA UFM allowed the recovery of high molecular weight molecules (900−20000 Da) in both KCl compartments. Peptides desorbed from PES and CA UFM after 6 h of EDUF separation had low molecular weights and belonged mainly to the 600−700 Da molecular weight range. These peptides represented a low proportion of the peptides initially present in the snow crab byproduct hydrolysate with individual molecular weight range proportions from 1.52 ± 0.31 to 10.2 ± 2.32%.

KEYWORDS: electrodialysis with ultrafiltration membranes, ultrafiltration membrane fouling, peptide migration, peptide separation, ultrafiltration membrane material

INTRODUCTION

Electrodialysis with ultrafiltration membrane (EDUF) has been developed and patented by Bazinet et al.[1] and consists of ultrafiltration and ion-exchange membranes stacked together in a conventional electrodialysis cell. The ultrafiltration membrane (UFM) molecular weight cutoff (MWCO) allows the concentration and purification of solutes according to their sizes, and the electrodialysis (ED) process allows a selective separation of molecules according to their electrical charges. However, during a separation by EDUF, no pressure is applied in the electrodialysis cell, and the electrical field is the only driving force of this technology.

EDUF technology showed several potential applications for the food industry, notably for the separation and recovery of bioactive compounds from diverse raw matrices. Labbé et al. performed the migration and selective recovery of catechins from green tea, which are antioxidant molecules.[2] Recently, a cranberry juice enriched with natural phenolic antioxidant compounds was obtained by Bazinet et al.[3] The fractionation of a β-lactoglobulin hydrolysate was performed by Poulin et al. and allowed the simultaneous separation of acid and basic bioactive peptides by stacking 20 kDa MWCO cellulose acetate (CA) UFMs.[4] Firdaous et al. isolated an angiotensin converting enzyme (ACE) inhibitor peptide fraction from alfalfa white protein hydrolysate by stacking a 10 kDa MWCO polyether sulfone (PES) UFM in the EDUF cell.[5,6] Doyen et al. identified an anticancer peptide fraction from snow crab byproduct hydrolysate after a selective separation by EDUF with 20 kDa MWCO CA UFM stacked in the system.[7] However, the impact of the UFM material with the same MWCO on the peptide migration during EDUF separation has never been studied. In addition, even if the fouling phenomenon is quite low during the separation by EDUF due to the absence of pressure in the EDUF cell,[3−6] some interactions between the PES or CA UFM and the peptides in solution may appear during the separation and could create a weak fouling on and inside the UFM, which could influence peptide migration. Indeed, PES and CA UFMs present some differences in their structures and performances. CA is obtained by acetylation of cellulose, which can be performed with different acetylating agents such as acetic anhydride, acetyl chloride, and ketene.[8] In terms of performance, CA membranes

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have excellent hydrophilicity, which is very important in minimizing fouling and for good resistance to chlorine and solvent.[9] However, CA material presents several drawbacks such as its low resistance to aggressive cleaning, its low oxidation and chemical resistance, and poor mechanical strength.[10] PES contains repeated ether and sulfone linkages alternating between aromatic rings.[11] PES material provides high rigidity, superior strength, and dimensional stability.[11] However, PES material is more hydrophobic than CA material; consequently, during pressure-driven filtration, PES membranes fouled more seriously than hydrophilic CA membranes.[12] Consequently, the differences between the PES and CA materials could have an impact on peptide migration, peptide−peptide aggregation, and peptide−membrane affinity.

The aim of the present work was (1) to compare the impact of PES and CA UFM materials on peptide selective migration from snow crab byproduct hydrolysate and (2) to evaluate the impact of the UFM material on membrane potential fouling during EDUF separation.

MATERIALS AND METHODS

Chemicals. NaCl and Na_2SO_4 were obtained from Laboratoire MAT (Québec City, QC, Canada). KCl was purchased from ACP Inc. (Montreal, QC, Canada), and 1.0 M HCl and 1.0 M NaOH solutions were obtained from Fisher Scientific (Montreal, QC, Canada).

Raw Material. The snow crab byproduct hydrolysate was obtained from the Aquatic Products Technology Centre (CTPA, MAPAQ, Gaspé, QC, Canada) and was prepared according to the procedure described previously.[13] Briefly, the snow crab byproduct consisted of cephalothorax, shells, and digestive systems, which were enzymatically hydrolyzed at pH 9.0 with the proteolytic enzymes blend Protamex. After the recovery of the peptide fractions, different steps of pressure-driven membrane filtration were performed for the purification and concentration of peptides (ultrafiltration and nanofiltration processes). The initial concentration of peptides in the snow crab byproduct hydrolysate was 100 g/L measured on a wet basis. The water content was 87%, no lipids were present, and the ash content represented 2.12% (w/v).

Electrodialysis Cell and Configuration. The electrodialysis cell used for this experiment was an MP type cell (100 cm^2 of effective surface area) manufactured by ElectroCell Systems AB Co. (Täby, Sweden) with one Neosepta CMX-SB cationic membrane (Tokoyuma Soda Ltd., Tokyo Japon), one Neosepta AMX-SB anionic membrane (Tokoyuma Soda Ltd.), and two ultrafiltration membranes with a MWCO of 20 kDa. The UFM materials were PES (GE, France) and CA (Spectrum Laboratories Inc., Rancho Dominguez, CA).

The UFM placed near the anode was named UFM1, and the UFM placed near the cathode was named UFM2. The electrodialysis configuration presented in Figure 1 was the same as the one used by Doyen et al.[7] The configuration was formed of four compartments. Two of them contained 1.5 L of KCl solution (2 g/L) for the recovery and concentration of peptides (the KCl1 and KCl2 compartments were located near the anode and the cathode, respectively), one compartment containing the electrode 20 g/L Na_2SO_4 rinsing solution (3 L) and the other the feed solution (snow crab hydrolysate, 3 L). The solutions were circulated using four centrifugal pumps, and the flow rates were controlled using flow meters. The permeate and feed solution flow rates were 2 L/min, whereas the flow rate of the electrode solution was 6 L/min.

Protocol. Electroseparation was performed in batch process using a constant voltage difference of 2 V/cm (voltage of 7 V applied and electrodialytic cell thickness of 3.5 cm). The duration of the treatment was fixed at 360 min to obtain a large electrodialytic migration and sufficient quantity of peptides. A 1:10 dilution ratio was achieved for the feeding compartment by mixing 1 L of the snow crab byproduct hydrolysate, at 10% (w/w) peptide concentration, to 9 L of distilled water. The anode, a dimensionally stable electrode (DSA), and the cathode, a 316 stainless steel electrode, were supplied with the MP cell. The anode/cathode voltage difference was supplied by a variable 0−100 V power source. The system was run in a cold room at a constant temperature of 4 °C to minimize microbial contamination and proliferation.

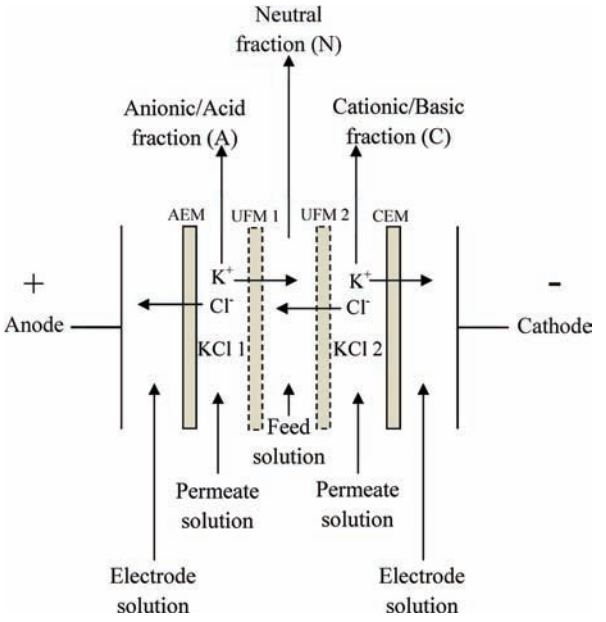

Figure 1. Configuration of the electrodialysis with ultrafiltration membrane cell according to Doyen et al. [7]. UFM, ultrafiltration membrane; AEM, anion-exchange membrane; CEM, cation-exchange membrane.

The EDUF treatment was performed at pH 9, a value that showed, in previous work, the highest peptide recovery yield.[7] The pH of the hydrolysate and permeate solutions (KCl1 and KCl2) was adjusted before each run with 1.0 M NaOH and controlled afterward to pH 9. Three replicates at pH 9 of each condition (separation with PES and CA UFM) were performed. Volume of 10 mL samples of hydrolysate and KCl solutions were collected before voltage was applied and every hour during the treatment. Conductivities of KCl and hydrolysate compartments were taken every hour during the 6 h of separation to follow the solution mineralization or demineralization process taking place during the separation. Following each 6 h EDUF treatment, the electrodialysis cell was dismantled and PES and CA UFM1 and UFM2 were soaked in a 0.17 M NaCl solution (10 g/L) overnight with agitation. Such higher ionic strength is used to decrease electrostatic attractions between charged proteins and oppositely charged material. Consequently, this soaking would allow the recovery of electrostatically adsorbed peptides at the UFM surface.[14,15] Solutions, which contained NaCl and peptides desorbed from the UFM surface, were freeze-dried to perform mass spectrometry analyses. After the UFM desorption by NaCl solution, the electrodialysis cell was reassembled and a cleaning-in-place with enzymatic solutions was performed according to the procedure of Doyen et al.[7] to ensure the recovery of the process performances.

pH Measurements. A pH-meter model SP20 (Thermo Orion, West Chester, PA) was used with a VWR Symphony epoxy gel combination pH electrode (Montreal, QC, Canada).

Solution Conductivity. Conductivity of KCl solutions and snow crab byproduct hydrolysate was measured using a YSI conductivity meter, model 3100, with a YSI immersion probe model 3252, cell constant K = 1 cm^{-1} (Yellow Springs Instrument Co., Yellow Springs, OH).

Total Peptide Determination in Liquid Samples. The peptide concentrations in the KCl1 and KCl2 solutions, recovered during and after 6 h EDUF treatments, were determined using BCA protein assay reagents (Pierce, Rockford, IL). Assays were conducted on microplates by mixing 25 μL of the sample with 200 μL of the working reagent followed by incubation at 37 °C during 30 min.[7] The microplate was then cooled to room temperature, and the absorbance was read at 562 nm on a microplate reader (THERMOmax, Molecular Devices, Sunnyvale, CA). Concentration was determined with a standard curve in a range of 25−2000 μg/mL of bovine serum albumin (BSA).

Total Peptide Determination in Freeze-Dried Samples. The KCl1 and KCl2 solutions were lyophilized to concentrate their contents. The final concentrations of peptides in KCl1, KCl2, and hydrolysate fractions after the 6 h EDUF treatment were obtained by total nitrogen determination. Total nitrogen was analyzed by combustion of a 150 mg sample of KCl and hydrolysate lyophylized powder using a LECO-FP528 carbon and nitrogen analyzer (LECO, St. Joseph, MI).[16,17] Nitrogen concentrations in the samples were converted into peptide percentage by multiplying the nitrogen result by a factor of 6.25, the value commonly used for crude proteins.[16,18]

Peptide Molecular Weight and Profiles. The molecular weights of peptides present in the KCl1 and KCl2 permeates and in the hydrolysate solution were analyzed by mass spectrometry as previously described by Firdaous et al.[6] The freeze-dried samples recovered as described above were dissolved in HPLC grade water and injected into an Agilent 1100 series at a final peptide concentration of 50 μg/mL. Peptides were analyzed with a Luna 5 μm C_{18} column (2 mm i.d. × 250 mm, Phenomenex, Torrance, CA). Solvent A, trifluoroacetic acid (TFA) at 0.11% (v/v) in water, and solvent B, acetonitrile/water/TFA (90%/10%/0.1% v/v), were used for elution at a flow rate of 0.2 mL/min. A linear gradient of solvent B, from 2 to 100% in 110 min, was used. Detection of the peptide bonds was performed with DAD G1315A detector at a wavelength of 214 nm.[5,19]

The peptide abundance in percent was determined with the MS results. For each peptide fraction (initial snow crab byproduct hydrolysate and KCl1 and KCl2 liquid fractions recovered after EDUF separations), the surface under the curve of each peak detected after MS analysis was summed. Afterward, peptides were separated according to their molecular weight and were classified in the different molecular weight ranges. The equation used follows, where Ab represents the abundance in percent of peptides in the molecular weight range $X-Y$ Da, $S_{X-Y\mathrm{Da}}$ the peak surface of peptides with molecular weight in the range $X-Y$ Da, and S_{total} the peak surface of all the peptides in the fraction (initial snow crab byproduct hydrolysate and KCl1 and KCl2 liquid fractions recovered after EDUF separations):

$$\mathrm{Ab}\ (\%) = (S_{X-Y\mathrm{Da}}/S_{\mathrm{total}}) \times 100$$

Peptide electromigration, in percent, was determined with the MS results. The surface under the curve of each peak detected with MS of the permeate solution was compared to the surface of the corresponding peak on the chromatogram of the snow crab byproduct hydrolysate solution. The equation used follows, where Tr represents peptide migration in percent, S_{permeate} the surface of a given peak in the permeate, and $S_{\mathrm{hydrolysate}}$ the surface of the corresponding peak in the feed solution at time 0:

$$\mathrm{Tr}\ (\%) = (S_{\mathrm{permeate}}/S_{\mathrm{hydrolysate}}) \times 100$$

Membrane Thickness Measurements. Membrane thickness was measured using a Mitutoyo Corp. digimatic indicator (model ID-110 ME, Japan) and a digimatic mini-processor (model DP-1HS, Japan) specially designed for plastic film thickness measurement. The resolution was of 1 μm and the range of 10 mm.

Membrane Electrical Conductivity. The membrane electrical conductivity was measured according to the method of Bazinet and Araya-Farias, using a specially designed clip from the Laboratoire des Matériaux Échangeurs d'Ions (Université Paris XII, Créteil, Val de Marne, France).[20]

Molecular Weight and Distribution of Peptides Desorbed from UFM. The peptides desorbed from the surface of the UFM after the membranes had been soaked in 0.17 M of NaCl were analyzed by mass spectrometry as described under Peptide Molecular Weight and Profiles. The freeze-dried samples recovered were dissolved in HPLC grade water and injected into the system as previously described in the same section, and abundance and peptide migration of peptide fractions recovered after desorption by NaCl solution were determined with equations described there also.

Statistical Analyses. All experiments were carried-out in triplicate. The electrical conductivity data of the UFM, the evaluation of peptides on the surface and inside the PES and CA UFM, and the peptide content in freeze-dried KCl1 and KCl2 samples were subjected to a Student t test using SAS software version 9.1 (SAS Institute Inc., Cary, NC). The Student t test is used for the comparison of two mean responses. The KCl1 and KCl2 compartments' conductivity data and the peptide migration data as a function of time were subjected to a repeated-measure analysis of variance (ANOVA) using SAS software version 9.1 (SAS Institute Inc.). Significant differences were declared at a probability (P) level of $\alpha = 0.05$.

RESULTS AND DISCUSSION

Solution Conductivity. During the separation, the evolutions of conductivity in the KCl1 and KCl2 compartments were similar for both UFM materials with linear decreases during the 6 h of separation (Figure 2a,b). These decreases corresponded to similar demineralization rates of 49.8% for PES UFM (from 2.72 ± 0.06 to 1.36 ± 0.09 mS/cm) and 48.5% for CA UFM (from 2.79 ± 0.06 to 1.35 ± 0.07 mS/cm). These results are in accordance with the cell configuration. Indeed, K^+ and Cl^- ions from the KCl1 solution migrated, respectively, into the hydrolysate and into the electrolyte solutions on the cathode side. K^+ and Cl^- ions from the KCl2 solution migrated, respectively, into the electrolyte solutions on the anode side and into the hydrolysate solution (Figure 1). The anions and cations present in the hydrolysate have migrated, respectively, to the electrode solutions on the anode and cathode sides.

This decrease in conductivity over time was similar to earlier observations by Poulin et al.[4] and by Doyen et al.[7] with snow crab byproduct hydrolysate separated by EDUF with CA UFM material for the same electrodialysis cell configuration. In contrast, the conductivity in the hydrolysate solution increased linearly over time during the 6 h of EDUF separation to reach a 21% mineralization (Figure 2c), irrespective of the UFM material used (PES or CA). As observed and explained in previous studies, the mineralization observed in the feeding solution could be due to the presence of K^+ ions migrating from the KCl1 compartment and Cl^- ions migrating from the KCl2 compartment.[4,7]

Total Peptide Determination in Liquid Samples. No significant difference was observed between the two types of UFM material studied during EDUF separations ($P \geq 0.5087$). The peptide concentration was dependent only on the duration of EDUF separation ($P < 0.0001$) and of the recovery compartment (KCl1 and KCl2) ($P \leq 0.0160$). Indeed, in the KCl1 compartments, similar peptide concentrations were recovered at 92.0 ± 30.0 and 105.0 ± 21.0 μg/mL for PES and CA UFM, respectively (Figure 3a). In the KCl2 compartments, whatever the UFM material used, no peptide was detected until 180 min of migration (Figure 3b). However, afterward, from 180 to 360 min of

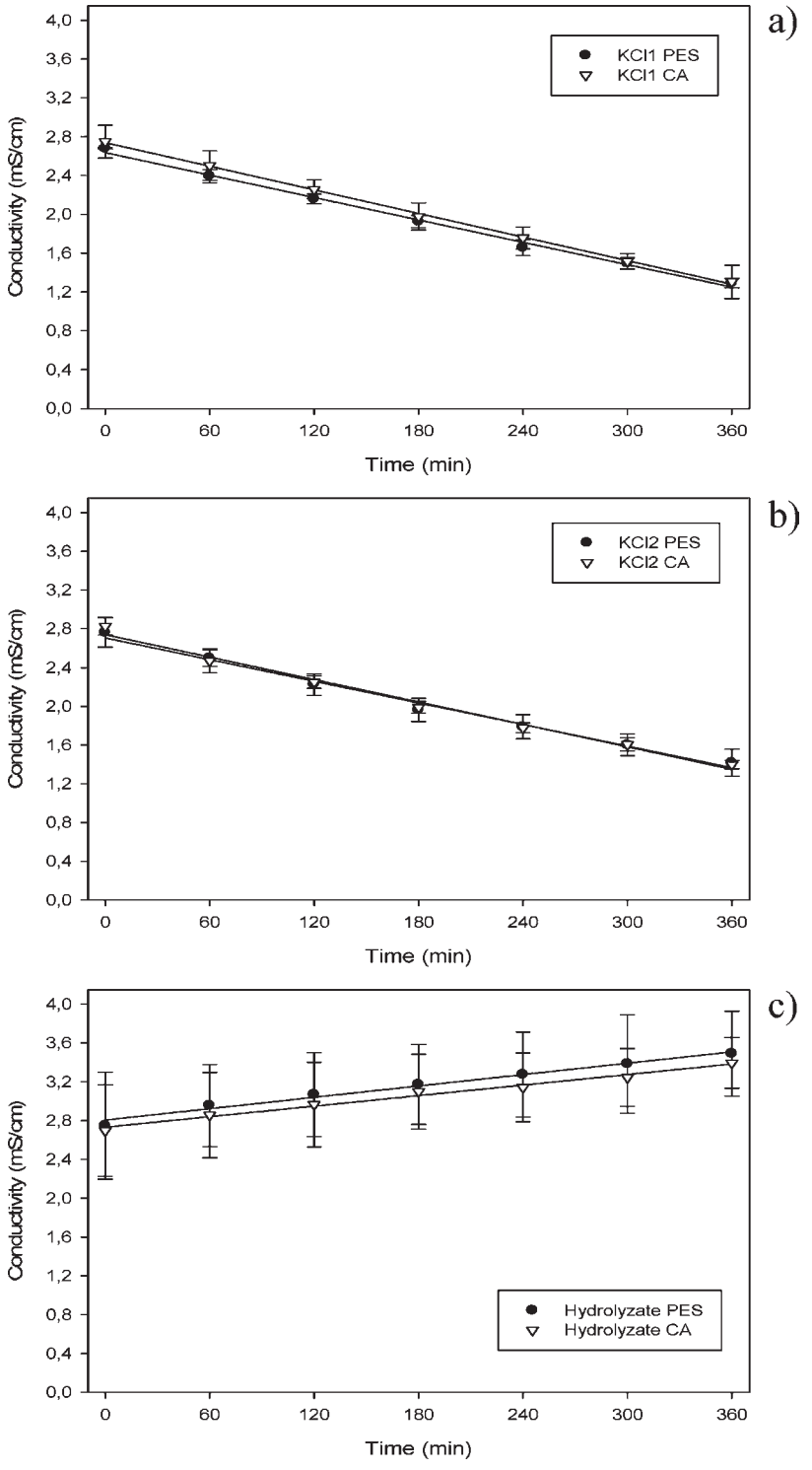

Figure 2. Evolution of conductivity of KCl1 and KCl2 permeates and snow crab byproduct hydrolysate at pH 9 during 6 h of EDUF with PES or CA ultrafiltration membrane material.

separation, the peptide concentration increased slowly, with highest peptide concentration recovery in the KCl2 PES from 300 min, which was probably due to the UFM material used. The results observed with the KCl1 and KCl2 compartments can be explained by the fact that the initial snow crab byproduct hydrolysate contained, at pH 9, more negatively charged peptides as observed by Doyen et al. for the same matrix.[7] Moreover, as the concentration of cationic/positively charged peptides was lower in comparison with anionic peptides, the duration of EDUF separation to obtain cationic peptide migration was longer than that needed for anionic peptides. Thus, the detection of cationic peptides in the KCl2 compartment appeared only after 180 min of separation. These authors obtained a peptide migration flux of 7.13 ± 1.10 g/m^2·h in the KCl1 compartment after 6 h of EDUF

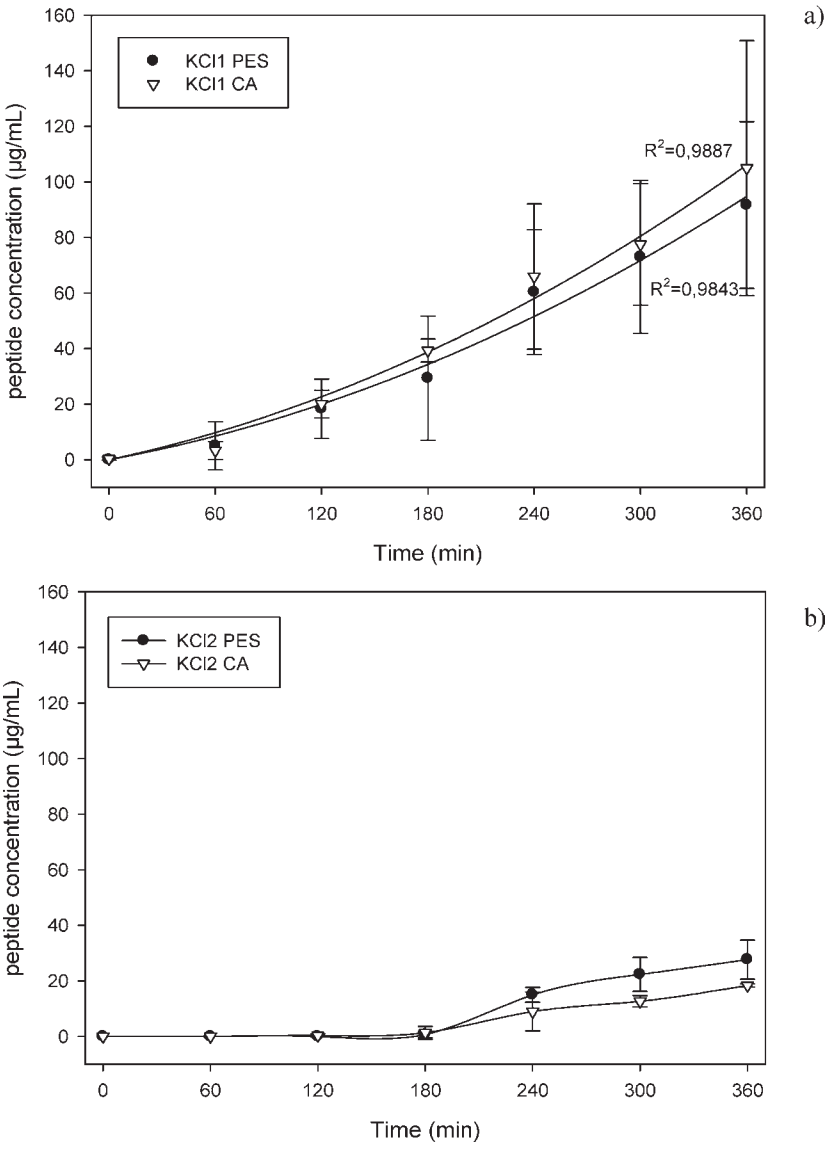

Figure 3. Evolution of the peptide concentration in the (**a**) KCl1 and (**b**) KCl2 compartments during EDUF treatment at pH 9 with PES or CA UFM material.

separation at room temperature, whereas in the present study peptide migration fluxes of only 2.29 ± 0.75 and 2.62 ± 0.05 g/m^2·h were reported in the KCl1 compartment with PES and CA UFMs with the same EDUF configuration and duration conditions. However, the separations were performed at a different temperature; 4 °C in the present study versus room temperature.[7] Consequently, as anticipated, a decrease in temperature during the separation has lowered the peptide migration by reducing its electrophoretic mobility due to the increase of solution viscosity.

With respect to the EDUF separation performed with β-lactoglobulin and alfalfa white hydrolysates, relatively similar peptide concentrations, recovered in the KCl compartments, were measured.[4,5]

Total Peptide Determination in Freeze-Dried Samples. The KCl1 samples recovered after EDUF separation with PES and CA UFMs contained, respectively, 17.88 ± 0.45 and 17.12 ± 0.47% of peptide on a dry basis. For the KCl2 samples recovered after EDUF separation with PES and CA UFM, the peptide proportions were 11.83 ± 0.56 and 10.53 ± 0.37%, respectively. These results confirm those obtained with BCA protein assay, for total peptide migration, performed on liquid samples after 6 h of treatment.

Peptide Profiles. Initially, peptides of low molecular weights in the 300−700 Da range were the most prevalent in the hydrolysate (64.3%). Peptides in the 700−1000 and 1000−20000 Da molecular weight ranges represented 3.97 and 31.7%, respectively, of the total peptide found in the snow crab byproduct hydrolysate, which had previously been obtained by nanofiltration on a 1 kDa MWCO (Figure 4). As the pore size distribution is expressed in terms of Gaussian normal distributions,[21] peptides with molecular weights higher than the MWCO could migrate through the membrane of nanofiltration and could therefore be recovered in the permeate in limited quantity.[22] Moreover, the recovery of high molecular weight peptides could be due to a peptide−peptide aggregation reaction.[23,24]

No significant difference in abundance for the peptides in the 300−700 Da molecular weight range and recovered in the KCl1 PES and CA and KCl2 PES and CA compartments was observed

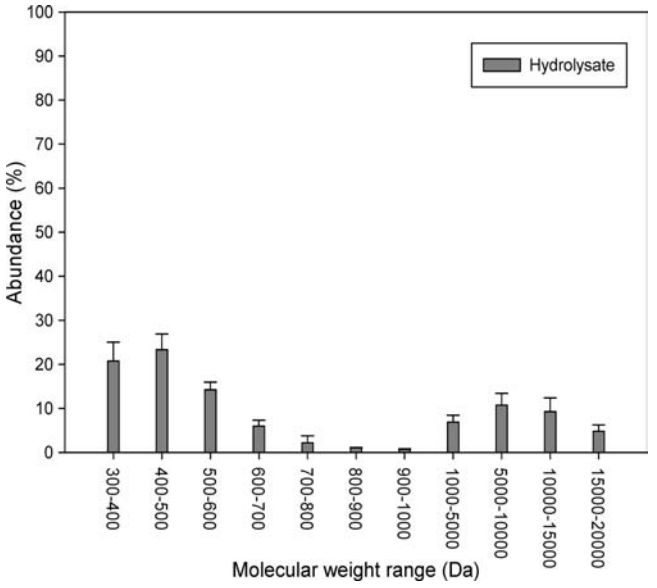

Figure 4. Distribution of peptide molecular masses initially present in the snow crab byproduct hydrolysate.

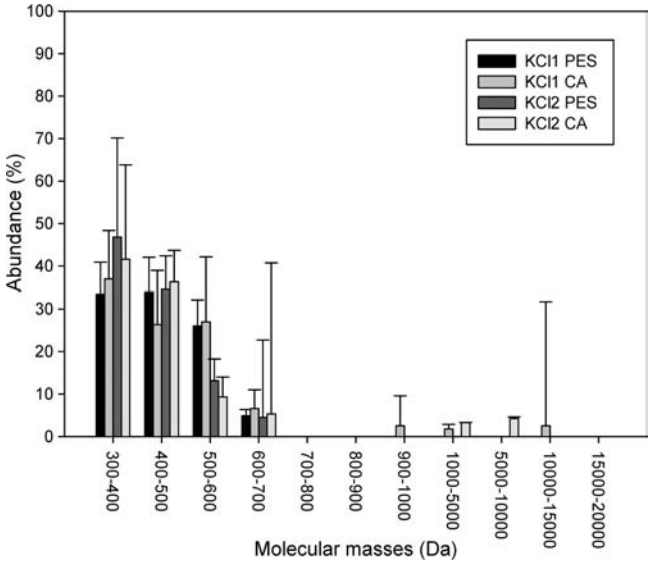

Figure 5. Distribution of peptide molecular masses recovered from the KCl1 PES and CA and KCl2 PES and CA compartments after 6 h of EDUF separation.

except for peptides in the 500−600 Da molecular weight range in the KCl2 CA compartment, which was lower than the other compartments (Figure 5). Moreover, peptides of the 300−700 Da molecular weight range were the most abundant in the KCl1 and KCl2 compartments, whatever the membrane material used (Figure 5), with an average of about 90%. This suggests that EDUF is a selective technology for the separation of peptides with different molecular weights. Indeed, peptides in the 300−700 Da molecular weight range were the ones with the highest migration rate (Table 1). The high peptide migration rates observed for peptides in the KCl1 PES and CA compartments compared to the KCl2 compartments (Table 1) confirmed results obtained with BCA protein assay for the total peptide concentration determination (Figure 3). However, the peptide migration rates in the KCl1 CA compartment were higher than in the KCl1 PES compartment. Similar peptide migration rates were observed for KCl2 PES and CA compartments.

The absence of migration for peptides in the 700−900 Da range after EDUF separation with PES and CA UFM material may perhaps be explained by the net peptide charges, which were not sufficient to allow their migration, or by peptide interactions with UFM material.

A small proportion of high molecular weight range peptides (900−15000 Da) was detected in the KCl1 and KCl2 CA compartments in comparison with respective PES compartments (Figure 5), with migration rates ranging from 3.11 ± 0.01% (5000−10000 Da) to 24.4 ± 11.8% (900−1000 Da) (Table 1). Peptide−peptide interactions may account for the recovery of high molecular weight peptides in the KCl compartment. These interactions may be caused by the fact that limiting current density value was reached and surpassed in the EDUF system. Indeed, when the limiting current density is exceeded, the water is dissociated into H^+ and OH^- ions at the AEM interface and with a lower intensity at the cation-exchange membrane (CEM) interface.[25] Consequently, even if the pH of KCl compartments was maintained at pH 9, the pH of the KCl1 solution at the AEM and CEM interfaces, where H^+ ions were in larger concentration, drastically decreases. Consequently, this acidic pH may induce peptide−peptide hydrophobic interactions.[26] Furthermore, the fact that peptides with high molecular weight were recovered only after separation with CA UFM could be explained by the difference in electrostatic repulsions due to the difference in UFM material.

Membrane Electrical Conductivity. The membrane electrical conductivity parameter is an indicator of membrane fouling because conductivity decreases as membrane fouling becomes more important.[20,27] Hence, a significant difference between the type of UFM used to perform EDUF separation and the controls (new PES and CA UFM materials) would indicate a fouling at the UFM surface and/or into the UFM.

Electrical conductivities of the PES and the CA UFM used during EDUF were measured at the end of the three treatment repetitions. For the CA UFM1 (0.93 ± 0.04 mS/cm) and UFM2 (1.03 ± 0.09 mS/cm), in comparison with the CA UFM control (0.92 ± 0.01 mS/cm), no significant difference was detected ($P \geq 0.841$ and $P = 1.000$, respectively). However, a significant difference in electrical conductivity was observed between the PES control (0.86 ± 0.02 mS/cm) and PES UFM1 (0.76 ± 0.03 mS/cm; $P < 0.001$) and PES UFM2 (0.80 ± 0.02 mS/cm; $P < 0.001$). This difference could be due to the presence of peptide fouling at the surface and/or into the pores of the PES material.

Profiles of Peptides Desorbed from UFM. Mass spectrometry analyses were performed on freeze-dried samples collected from PES and CA UFM1 and UFM2 after desorption by NaCl to confirm the presence of peptide fouling and to characterize these peptides in terms of molecular weight range (see Materials and Methods for explanations). The peptides desorbed from the membrane were composed mainly by peptides in the 600−700 Da molecular weight range with an abundance of 50% (Figure 6). In comparison with the 600−700 Da molecular weight range concentration in the hydrolysate, the 600−700 Da peptides desorbed from the membrane represented 10% of this initial concentration (Table 2). This trend was observed whatever the UFM material and position (UFM1 and UFM2 in the EDUF system). The peptides desorbed were also composed, but with lower abundances, by peptides from the 300−400, 400−500,

Table 1. Percentages of Peptide Migration in KCl1 and KCl2 Compartments after 6 h of EDUF Separation with PES UFM or CA UFM Material

	peptide migration[a] (%)					
mol wt range (Da)	KCl1 PES	KCl2 PES	total	KCl1 CA	KCl2 CA	total
300−400	19.0 ± 4.22	17.7 ± 8.84	36.7 ± 13.1	39.6 ± 12.2	16.1 ± 8.57	55.7 ± 20.8
400−500	17.2 ± 4.13	11.6 ± 1.24	28.8 ± 5.37	25.1 ± 4.46	12.5 ± 1.05	37.6 ± 5.51
500−600	21.5 ± 6.07	7.82 ± 1.13	29.32 ± 7.20	42.1 ± 6.64	5.25 ± 0.96	47.35 ± 7.60
600−700	9.60 ± 2.83	5.91 ± 3.37	15.51 ± 6.20	14.4 ± 4.39	7.13 ± 4.40	21.53 ± 8.79
700−800	nd[b]	nd		nd	nd	
800−900	nd	nd		nd	nd	
900−1000	nd	nd		24.4 ± 11.8	nd	24.4 ± 11.8
1000−5000	nd	nd		5.70 ± 0.13	3.86 ± 0.08	9.56 ± 0.21
5000−10000	nd	nd		nd	3.11 ± 0.01	3.11 ± 0.01
10000−15000	nd	nd		6.10 ± 0.74	nd	6.10 ± 0.74
15000−20000	nd	nd		nd	nd	nd

[a] See Material and Methods for the determination of peptide migration (%). [b] nd, not detected.

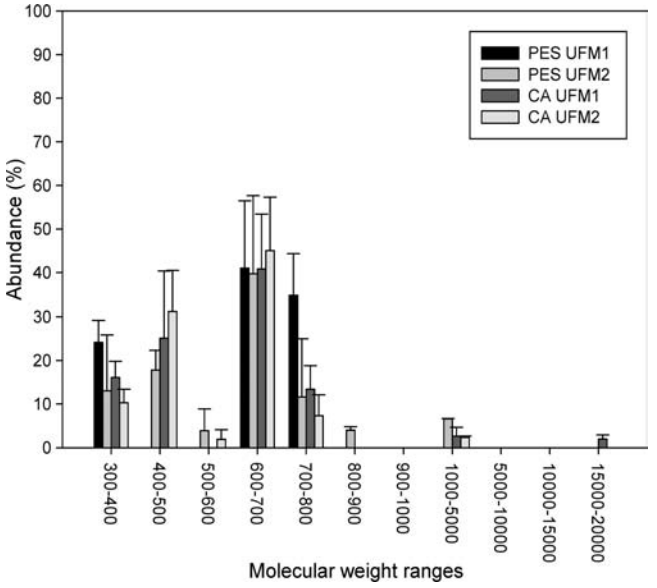

Figure 6. Distribution of peptide molecular masses desorbed from PES and CA UFM with 0.17 M of NaCl after 6 h of EDUF separation.

and 700−800 Da molecular weight ranges with respective abundance values of 15, 24, and 16%. They showed significant differences according to UFM material ($P \leq 0.023$ and $P \leq 0.031$) and position (UFM1 and UFM2) only in the case of PES material ($P \leq 0.017$). The same trends were observed for peptides in the 500−600, 800−900, 1000−5000, and 15000−20000 Da molecular weight ranges, but these peptides represented only a very small part of the peptide population desorbed, with an average abundance of 3%. Peptides in the 300−400 and 700−800 Da molecular weight ranges desorbed from the PES UFM1 and CA UFM1 represented 24.15 ± 5.03 and 16.08 ± 3.75% of their respective concentration in the hydrolysate. No peptides in the 400−500 and 1000−5000 Da molecular weight ranges were recovered after desorption from the PES UFM1; this means that no peptide from these molecular weight ranges was adsorbed on the membrane or that no peptide was effectively desorbed because they were not electrostatically linked. Finally, the peptides in the 800−900 and 15000−20000 Da molecular weight ranges were recovered only from the PES UFM2 and the CA UFM1, respectively. As mentioned previously, the difference in adsorption/recovery observed can possibly be explained by the (1) UFM net charge at pH 9, (2) their positions in the EDUF system, and/or (3) another type of membrane/peptide interaction. Indeed, the global UFM charge was generally determined by the zeta-potential measurement. Several studies have shown that the zeta-potential value of PES UFM and CA UFM at pH 7 is negative.[28,29] Burns and Zydney have determined that a PES UFM with nominal molecular cutoffs of 30, 100, and 300 kDa is negatively charged between pH values of 2.5 and 7.0.[30] Moreover, Susanto et al. have shown that PES UFM with MWCO of 50 kDa is negatively charged between pH values of 4 and 10.[31] As EDUF separations were performed at pH 9, the zeta-potential of the UFM is negative, and consequently the UFM environment was negatively charged. Thus, repulsion or attraction phenomena may occur. According to Figure 1, repulsion has occurred between the UFM1 and peptides, which allowed the peptide migration in the anionic compartment of recovery (KCl1) and negatively charged peptides. This could explain that a lower concentration of peptides desorbed was obtained at the surface of PES and CA UFM1 in comparison with PES and CA UFM2 (Table 2). On the contrary, the lowest adsorption of peptides or peptide recovery observed for PES and CA UFM2 was probably due to electrostatic interactions between the negatively charged membrane UFM2[32] and the positively charged peptides or to the presence of other interactions such as hydrophobic ones allowing peptide−membrane interactions.[33] However, it appeared that the peptide UFM fouling due to electrostatic interactions is not important in comparison with the total peptide present initially in the snow crab byproduct hydrolysate either because hydrophobic interactions allowed peptide−membrane links and/or because the electric field applied in the EDUF system was sufficient to allow peptide migration without leading to peptide−UFM interactions. In summary, EDUF appears to be a selective purification technology with a separation of low molecular weight compounds (300−700 Da) presenting an abundance of about 91% after treatment irrespective of the UFM material used. The specific separation of low molecular weight peptides by EDUF is interesting because most peptides with low molecular

Table 2. Molecular Weight Ranges and Proportions (Expressed as Percentage of Their Initial Concentrations in the Feed Hydrolysate) of Peptides Desorbed from PES and CA UFM with 0.17 M NaCl after 6 h of EDUF Separation

	peptide desorbed (% of initial concentration)					
mol wt range (Da)	PES UFM1	PES UFM2	total	CA UFM1	CA UFM2	total
300−400	5.51 ± 0.91	3.80 ± 0.33	9.31 ± 1.24	3.60 ± 0.45	2.70 ± 1.22	6.30 ± 1.67
400−500	nd[a]	1.78 ± 0.54	1.78 ± 0.54	3.17 ± 0.71	3.54 ± 1.07	6.71 ± 1.78
500−600	nd	3.81 ± 1.21	3.81 ± 1.21	nd	1.52 ± 0.31	1.52 ± 0.31
600−700	4.73 ± 1.22	4.82 ± 1.09	9.95 ± 2.31	4.85 ± 1.11	5.36 ± 1.21	10.2 ± 2.32
700−800	5.37 ± 1.77	1.11 ± 1.17	6.48 ± 2.94	2.69 ± 2.23	3.44 ± 0.98	6.13 ± 3.21
800−900	nd	3.76 ± 0.98	3.76 ± 0.98	nd	nd	
900−1000	nd	nd		nd	nd	
1000−5000	nd	8.52 ± 4.65	8.52 ± 4.65	4.33 ± 1.43	2.59 ± 0.77	6.92 ± 2.20
5000−10000	nd	nd		nd	nd	
10000−15000	nd	nd		nd	nd	
15000−20000	nd	nd		2.56 ± 0.76	nd	2.56 ± 0.76

[a] nd, not detected.

weight generally exhibit biological activities such as antimicrobial[34] and antihypertensive[35] properties. However, the peptides that composed this molecular weight range also represented the most adsorbed species at the UFM surface. Indeed, even if no pressure was applied in the system, contrary to pressure-driven technologies, electrostatic interactions between the UFM material and the peptides do also occur. Moreover, the fouling detected after EDUF separation was very low in comparison with pressure-driven technologies with which the formation of a strong gel layer was generally observed.[36,37] At this moment, EDUF technology has been tested on a pilot scale for biomolecule separation.[3−6] However, as the ED process was already used on an industrial scale, the scale-up of EDUF technology for industrial use could be developed by stacking the UFM membrane in the ED cell.[6]

ABBREVIATIONS USED

EDUF, electrodialysis with ultrafiltration membranes; ED, electrodialysis; UFM, ultrafiltration membrane; CA, cellulose acetate; PES, polyether sulfone; MWCO, molecular weight cutoff; ACE, angiotensin converting enzyme; BCA, bicinchoninic acid; CEM, cation-exchange membrane.

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