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Electroseparation of an antibacterial peptide fraction from snow crab by-products hydrolysate by electrodialysis with ultrafiltration membranes

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ABSTRACT

Recently, a snow crab by-products hydrolysate has demonstrated antibacterial properties due to a peptide with a molecular weight of about 800 Da, but only at high concentration. Consequently, peptide hydrolysate has been fractionated to obtain peptides in a more purified form. The aim of this work was to separate a snow crab by-products hydrolysate by electrodialysis with ultrafiltration membranes (EDUF). EDUF, which allows separation of molecules according to their charges and molecular weights, was used to recover and concentrate the active antibacterial fraction. Two different ultrafiltration membranes (20 and 50 kDa) and two electrical field strengths (2 and 14 V/cm) were used as separation parameters. After EDUF separation, the 300–600 Da peptide molecular weight range was the most recovered with an abundance of 94%. Moreover, fractionation at 14 V/cm with ultrafiltration membranes of 50 kDa allowed the recovery of an anionic fraction which showed antibacterial properties on *Escherichia coli* ATCC 25922 and *Listeria innocua* HPB 13.

1. Introduction

By-products and surpluses from food industries could generate peptides with great interest for food formulation. Hence, the separation and concentration of bioactive peptides from natural sources become increasingly attractive to the food industry (Korhonen & Pihlanto, 2003). Hydrolysates of different marine sources are used for the recovery of bioactive peptides. Hence, antioxidant peptides were purified from fish skin gelatine (Mendis, Rajapakse, & Se-Kwon, 2004), mackerel (Wu, Chen, & Shiau, 2003), capelin (Amarowicz & Shahidi, 1997) and Alaska Pollack hydrolysate (Je, Park, & Kim, 2005; Kim et al., 2001). An antiproliferative activity of fish protein hydrolysates on human breast cancer cell lines was recently demonstrated (Picot et al., 2006). Finally, several angiotensin I converting enzyme inhibitory peptides were purified from fishes (Byun & Kim, 2001; Fujita & Yoshikawa, 1999).

However, the isolation of bioactive peptides from hydrolysates, which represent complex polypeptide mixtures of similar molecular weight peptides, is difficult by membrane filtration since these techniques had too low selectivities whereas chromatography is too expensive and not applicable for the separation of important volume of feed solution (Bargeman et al., 2002). Hence, electrodialysis with ultrafiltration membranes (EDUF), which was recently developed and patented (Bazinet, Amiot, Poulin, Tremblay, & Labbé, 2005) represents a great alternative to conventional separation technologies for the recovery of specific peptide fractions. EDUF is a hybrid technology where ultrafiltration membranes are stacked in a conventional electrodialysis module without any pressure application. This technology allows the separation of molecules according to their charge and molecular weight. EDUF technology showed several potential applications for the food industry notably for the separation and the recovery of bioactive compounds from diverse food hydrolysates (Firdaous et al., 2010; Poulin, Amiot, & Bazinet, 2006).

Recently, a snow crab by-products hydrolysate showed antibacterial activity, due to a specific 800 Da molecular weight peptide, on several Gram negative and Gram positive bacteria (Beaulieu et al., 2010). However, the antibacterial activity was only detected at high peptide concentration. Recently, an anticancer peptide

Abbreviations: AEM, anion-exchange membrane; BHI, brain heart infusion; BSA, bovine serum albumin; CEM, cation-exchange membrane; EDUF, electrodialysis with ultrafiltration membranes; LC–MSD QUAD, liquid chromatography–mass spectrometry detector quadrupole; MWCO, molecular weight cut-off; MS, mass-spectrometry; UFM, ultrafiltration membrane.

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fraction was identified from snow crab by-products hydrolysate after a selective separation by EDUF (Doyen, Beaulieu, Saucier, Pouliot, & Bazinet, 2011a). However, no EDUF separation aiming specifically at the recovery of an antibacterial fraction was performed. Consequently, in this work, the objectives were (1) to perform the separation of snow crab by-products hydrolysate by EDUF with two different ultrafiltration membrane (UFM) molecular weight cut-offs (MWCO) (20 and 50 kDa) and electric field strengths (2 and 14 V/cm), (2) to compare the impact of this parameter on peptide selective migration from snow-crab by-products hydrolysate and (3) to test the anionic and cationic peptide fractions, named KCl1 and KCl2, respectively, recovered after each separation, for their antibacterial activity.

2. Materials and methods

2.1. Materials

2.1.1. Raw material

A snow crab by-products hydrolysate was obtained from the Merinov Centre (Merinov, MAPAQ, Gaspé, QC, Canada) which was produced according to a procedure described previously (Beaulieu, Thibodeau, Bryl, & Carbonneau, 2009). Briefly, the snow crab by-products hydrolysate was enzymatically hydrolysed at pH 9.0 and, after the recovery of the peptide fractions, different steps of membrane filtration pressure-driven process (ultrafiltration and nanofiltration) were performed for the purification and the recuperation of peptides. The snow crab by-products hydrolysate in this work was the nanofiltered fraction with peptide molecular weights ranging from 200 to 1000 Da. The initial peptide concentration in the snow crab by-products hydrolysate was 100 g/L. The water content was 87% and the ash was 2.12%.

2.1.2. Chemicals

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

2.1.3. Membranes

Two cellulose acetate UFMs with different MWCO, 20 and 50 kDa, were purchased from Spectrum Laboratories Inc. (Rancho Dominguez, CA, USA).

2.1.4. Electrodialysis cells and configuration

The electrodialysis cell used for this experiment was a MP type cell (100 cm^2 of effective surface area) manufactured by ElectroCell Systems AB Company (Täby, Sweden) with one Neosepta CMX-SB cationic membrane (Tokuyama Soda Ltd., Tokyo, Japan), one Neosepta AMX-SB anionic membrane (Tokuyama Soda Ltd., Tokyo, Japan) and two cellulose acetate UFMs with MWCO of 20 or 50 kDa. The UF membrane placed near the anode was named UFM1 and the one placed near the cathode was named UFM2. The electrodialysis configuration presented in Fig. 1 was the same used in previous work (Doyen et al., 2011a; Poulin et al., 2006). The electrodialysis cell was divided into four compartments. Two of them containing 1.5 L of KCl solution (2 g/L) for the recovery and concentration of peptides (the KCl1 and KCl2 compartments were located, respectively, near the anode and the cathode), one compartment containing the electrode 20 g/L Na_2SO_4 rinsing solution (3 L) and another one for the feed solution (snow crab by-products hydrolysate, 1.5 L). The solutions were circulated using four centrifugal pumps and the flow rates were controlled using flowmeters. The permeate and feed solution flow rates were 2 L/min while the flow rate of the electrode solution was 4 L/min.

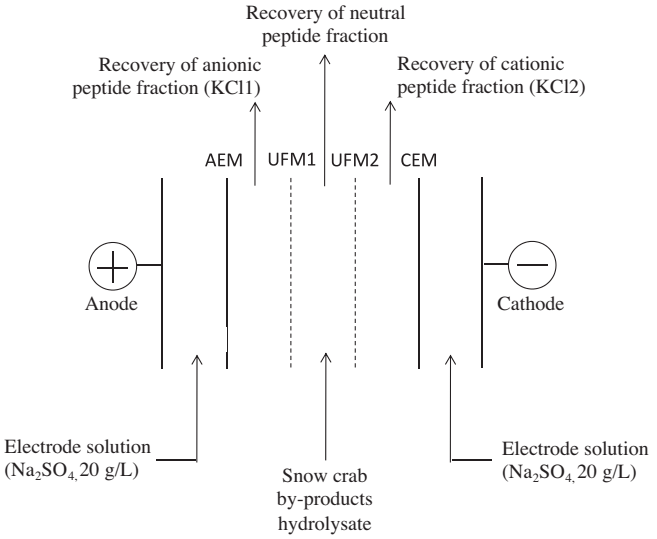

Fig. 1. Configuration of the electrodialysis with ultrafiltration membranes cell. UFM, ultrafiltration membrane; AEM, anion-exchange membrane; CEM, cation-exchange membrane.

2.2. Methods

2.2.1. Protocol

Electroseparation was performed in batch process using two electrical field strengths and UFM MWCO combinations, 2 V/cm with UFM MWCO of 50 kDa and 14 V/cm with UFM MWCO of 20 and 50 kDa. Results obtained with the three combinations were compared to those previously obtained by Doyen, Beaulieu, Saucier, Pouliot, and Bazinet (2011b) with electrical field strength of 2 V/cm with UFM MWCO of 20 kDa. 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 0.15 L of the snow crab by-products hydrolysate, at the protein concentration 10% (w/w), to 1.35 L of distilled water. This dilution allowed decreasing the viscosity of pure hydrolysate. 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 avoid as far as possible contamination by microorganisms. The EDUF treatments were performed at pH 9 which showed the highest peptide recovery in a previous study (Doyen et al., 2011a). In addition, at this pH value, the snow crab by-products hydrolysate demonstrated antibacterial activity at 500 g/L since initial snow crab by-product at peptide concentration of 100 g/L was concentrated 5-fold by evaporation (Beaulieu et al., 2010). The pH value of snow crab by-products hydrolysate and permeate solutions (KCl1 and KCl2) was adjusted before each run with 1.0 M NaOH or HCl and adjusted afterwards to the pH of the hydrolysate solution. Three replicates of each condition were performed. A volume of 10 ml-samples of snow crab by-products hydrolysate and KCl solutions were collected before applying voltage and every hour during the treatment. Conductivity values of snow crab by-products hydrolysate and KCl compartments were also measured every 1 h of treatment. After the 6 h of electroseparation, a cleaning-in-place developed by Doyen et al. (2011a) was performed to ensure the recovering of process performances.

2.2.2. Relative energy consumption of the EDUF process

The energy consumption, determined by multiplying the voltage by the intensity of the current, was integrated using the following equation (Bazinet, Lamarche, Labrecque, & Ippersiel, 1997):