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Simultaneous electroseparation of anionic and cationic peptides: Impact of feed peptide concentration on migration rate, selectivity and relative energy consumption

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ABSTRACT

The present work aimed to determine the effect of initial peptide concentration (0.5%, 1%, 2% and 4% (w/v) in feed solution during electrodialysis with ultrafiltration membranes (EDUF) on migration rate and selectivity of both anionic and cationic peptides as well as on electrodialytic parameters. The results showed that increasing the peptide concentration in the feed solution from 0.5% to 4% increased, in a linear way, the total separation rate and affected the selectivity of free amino acids in both recovery compartments RC_A- (Asp, Glu and His) and RC_C+ (Arg mainly) and/or peptides containing these amino acids. The highest migration rates observed at 4% were 16.2 g/m^2 h and 7.8 g/m^2 h for the cationic and anionic compartments respectively. Furthermore, the relative energy consumption decreased with increasing feed solution concentration from 17.4 Wh/g at 0.5% concentration to 3.53 Wh/g at 4% concentration. In addition, membrane integrity and physicochemical properties were not affected in the range of peptide concentration tested. To the best of our knowledge, it was the first time that the impact of feed peptide concentration on migration, selectivity and relative energy consumption is demonstrated in EDUF.

1. Introduction

The snow crab (*Chionoecetes opilio*) industry is one of the Canada's most successful fisheries, with a landing volume of 98,065 metric tons in 2013. However, it generates thousands of tons of byproducts [1]. Eventually, the valorization of these byproducts into value added protein-based products, such as functional food and nutraceuticals, has economical as well as environmental importance. Indeed, the market for functional food and nutraceutical products containing bioactive peptides from natural sources is increasing very rapidly [2]. Recent studies have demonstrated anticancer activities of peptides fractions from snow crab byproduct hydrolysate [3]. The production of such bioactive peptides requires the development of an energetically, economically and environmentally sound fractionation technique. The methods used most often are based on pressure-driven membrane filtration whose application is limited due to relatively lower selectivity and higher degree of membrane fouling [4]. Furthermore, chromatographic techniques are long and expensive; they are mostly used for analytical purposes. Thus, other alternatives like electromembrane filtration (EMF), electro-ultrafiltration (EUF) [5] and elec tro-ultrafiltration–electrodialysis (EUF–ED) [6] have been studied and are claimed to have better selectivity and no or reduced membrane fouling [7]. However EMF and EUF have the disadvantage to use a specially designed filtration system with only one ultrafiltration membrane, which reduces its application on large scale, and also to use pressure which limits the peptides or molecules selectivity due to membrane fouling. To overcome these limitations, electrodialysis with membrane filtration (EDMF) was developed and patented by Bazinet et al. [8]. Indeed, in EDMF, peptides are separated according to their electrophoretic mobilities which are mostly related to their molecular weights (sizes) and charges. EDMF technique has already been used for the separation of various bioactive peptides from different food protein hydrolysates such as soybean, flaxseed, β-lactoglobulin, alfalfa and snow crab byproduct hydrolysates.

Previous studies have demonstrated that several parameters affect EDMF efficiency such as: electric field strength; membrane material, effective surface area and molecular weight cut off (MWCO); solution pH, ionic strength and flow rate; and configuration of the module [9,10]. Recently, Suwal et al. [10] have shown that electrical conductivity of feed and recovery solution must be maintained for a linear peptide migration during EDMF process. In addition, an increase in feed protein concentration from 0.01

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to 0.05 w/v in an electrophoretic membrane contactor was previously found to increase proportionally the migration rate without impeding the final purity and yield [11]. However, the effect of peptide concentration at much higher range in the feed solution of protein hydrolysate during EDMF has not been yet studied.

In this context, the objective of this study was to determine the impact of increasing the feed peptide concentration of snow crab by-products hydrolysate on peptide migration rate, selectivity (total amino acid composition of peptide fraction), electrodialytic parameters (migration rate, relative energy consumption) and *in situ* membrane resistance.

2. Materials and method

2.1. Materials and ED cell

2.1.1. Hydrolysate of snow crab by products

Snow crab by-products hydrolysate (SCBH) was obtained from Québec fisheries and aquaculture innovation center (Merinov, MAPAQ, Gaspé, Quebec, Canada) and produced as described previously [1]. Snow crab by-products were enzymatically hydrolyzed by Protamex® (Novozymes, Bagsvaerd, Denmark; 1 g/kg of by-products) at pH 9 and then separated and purified by consecutives ultrafiltration and nanofiltration treatments. The snow crab byproducts hydrolysate used in this work was the permeate of ultrafiltration (1 kDa) and retentate of nanofiltration. The SCBH contained 72% of protein determined according to the Dumas method for total nitrogen by combusting a sample of known mass with oxygen at a temperature close to 900 °C using FP-428 LECO apparatus (LECO, St. Joseph, MI, USA).

2.1.2. Chemicals

Na_2SO_4 was obtained from MAT Laboratory (Quebec, QC, Canada). Solutions of NaOH and HCl (1.0 M) were obtained from Fisher scientific (Montreal, Qc, Canada) and KCl was purchased from ACP Inc. (Montreal, Qc, Canada).

2.1.3. Membranes

Polyether sulfone (PES) ultrafiltration membranes with MWCO of 100 kDa were purchased from Synder filtration (Vacaville, CA, USA). Neosepta CMX-SB cation-exchange membrane (CEM) and Neosepta AMX-SB anion-exchange membrane (AEM) were obtained from Tokuyama Soda Ltd. (Tokyo, Japan).

2.1.4. Electrodialysis cell configuration

The electrodialysis cell used in this experiment was a MP type cell manufactured by Electrocell Systems AB Company (Taby, Sweden) with an effective surface area of 100 cm^2. The electrodialysis stack contained one anion-exchange membrane (AEM), one cation-exchange membrane (CEM), and two ultrafiltration membranes. One UF membrane was placed near the cathode (UFM2) and the other one positioned near the anode (UFM1). The electrodialysis configuration shown in Fig. 1 is the same as that used previously [9] for simultaneous fractionation of anionic and cationic peptides from a tryptic digest of β-lactoglobulin. The EDUF configuration was divided into five compartments: two interconnected compartments containing electrode rinsing solution of Na_2SO_4 (20 g/L; 1.5 L); two recovery compartments (RC): one for anionic peptides called RC_A- (between AEM and UFM1) and another for cationic peptides called RC_C+ (between UFM2 and CEM), both containing KCl solution (5 g/L, 1.5 L); a feed compartment (between UFM1 and UFM2) containing SCBH (1.5 L) at four peptides concentrations of 0.5%, 1%, 2% and 4% (w/v). These solutions were circulated using four centrifugal pumps and the flow rates were controlled at 2 L/min in each compartment and 4 L/min in the compartments containing electrode rinsing solution. The power was supplied between a DSA anode and a stainless steel cathode by a variable 0–100 power supply.

2.2. Protocol

SCBH was first demineralised up to 52% by electrodialysis to reduce mineral salt content, using EUR-2C type cell (Eurodia, Rungis, France). This final demineralised SCHB used for the fractionation by EDUF contained 79% of protein on dry weight basis as determined by FP-428 LECO apparatus (LECO Corporation, Saint Joseph, MI, USA) using the Dumas method. The electroseparations were carried out in batch process with a constant potential difference of 20 volts (4.8 V/cm) for a period of 4 h. The ED system was equipped with cooling coils that maintained a constant temperature of 14 °C in each compartment. The electrodialytic separation was carried out at four peptides concentrations (0.5%, 1%, 2% and 4% w/v) of SCBH. The pH of feed and permeate (RC_A- and RC_C+) solutions was adjusted to 6 at the beginning and was maintained constant all along the treatment with the addition of HCl or KOH (1.0 M). Similarly, the electrical conductivities of feed and permeate solutions were monitored and maintained constant by the addition of NaCl (100 g/L) and KCl (100 g/L) respectively. Before each treatment, 10 mL samples of SCBH and permeate (RC_A- and RC_C+) were collected in each compartment before the application of voltage and then every 30 min after. Samples were frozen and kept at −30 °C until analyzed for protein concentration and amino acids. The electrical potential differences of each membrane and current intensity were recorded every 30 min during treatment. Three randomized repetitions were carried out for each condition of SCBH concentration and the membranes were changed after each block of repetitions. After each treatment, a cleaning in place (CIP) was performed according to the membrane manufacturer procedure. After cleaning, the cell was dismantled and the thickness and conductivity of each membrane were measured to evaluate membranes integrity used for EDUF treatment. The cell was then reassembled for the next treatment.

2.3. Analytical methods

2.3.1. Peptide concentration

The peptide concentrations in each solution sample were determined using BCA™ protein assay reagents (Pierce Biotechnology Inc., Rockford, IL, USA). Assays were conducted as recommended by the manufacturer. Analyses were performed on microplates with 25 μL of sample mixed with 200 μL of working reagents and then incubated at 37 °C for 30 min. The microplates were cooled at room temperature for 5 min and the absorbance of the samples was read at 562 nm with a microplate reader (Thermomax, Molecular Devices, Sunnyvale, CA, USA). The protein concentration was determined using BSA calibration curve in the range of 25–2000 μg/mL.

2.3.2. Peptide migration rate

The migration rate (g of peptides/m^2 h) was calculated at the end of the process with the amount of peptide (g) migrated determined previously by BCA, effective surface area of ultrafiltration membrane (m^2) and time (h) using Eq. (1).

$$\text{Migration rate} = \frac{\text{Amount of peptide (g)}}{\text{Area (m}^2\text{)} * \text{Time (h)}} \quad (1)$$

2.3.3. Total amino acid analysis

Total amino acid composition in the initial snow crab byproduct hydrolysate (SCBH) and recovery fractions (RC) after EDUF