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Electrodialytic separation of peptides from snow crab by-product hydrolysate: Effect of cell configuration on peptide selectivity and local electric field

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ABSTRACT

Electrodialysis with ultrafiltration membrane (EDUF) has been successfully used to separate bioactive peptides from various food protein hydrolysates. Nevertheless, EDUF process was found to be affected by permeate and feed pHs, electric field strength and membrane materials, molecular weight cuf-off (MWCO) as well as surface area. In the present study, the effect of two EDUF cell configurations was examined on different electrodialytic parameters; first with one feed and two recovery compartments and second with two feed and one recovery compartments. The EDUF cell configurations had significant effect on peptide migration rate and selectivity such as amino acid composition and peptide molecular weight profiles of the permeate fractions obtained after 6 h of EDUF treatment. The configuration 1 led to the higher total peptide migration rate of 6.00 ± 0.12 g/(h m^2) in comparison to 4.41 ± 0.20 g/(h m^2) for configuration 2. However, in configuration 1, the local electric field in the hydrolysate compartment decreased linearly throughout EDUF process which limited peptide migration after about 2 h of EDUF treatment. Amino acid analysis of permeate fractions showed that anionic amino acids primarily Glu, Tau, Met and Phe were concentrated in both peptide recovery compartments of configuration 1, while cationic amino acids like Arg and Lys were mainly concentrated in peptide recovery compartment of configuration 2.

1. Introduction

Foods are now most intensively studied for added physiological benefits such as reducing risk of chronic diseases or optimizing health in addition to their nutritional values [1]. The potential use of bioactive peptides as a major component of functional food and nutraceutical has been extensively reviewed since last two decades [2–6]. Bioactive peptides derived from proteins of milk [7,8], whey [9], eggs [10], soybeans [11,12], alfalfa [13] and fish [14–17] have been most widely studied. Despite of many reported benefits of the food derived bioactive peptides such as antioxidant, antihypertensive, anticancer, antimicrobial, antiobesity, immunomodulatory and opioid-like, their separation and purification processes are critical in an industrial scale production system. Pressure driven membrane filtration techniques are most commonly used for bioactive peptide fractionation [18]. However, elevated membrane fouling as well as low selectivity are major limitations for the separation and/or concentration of bioactive peptides from a complex mixture of similar sized peptides by pressure driven membrane filtration techniques [19,20]. In addition, the use of chromatographic techniques appears to be too slow, highly expensive and only applicable for small volume. Therefore, continuous improvement in membrane based technology for obtaining high purity product is essential [21].

Eventually, an electromembrane filtration process called electrodialysis with ultrafiltration membrane (EDUF) was developed and patented by Bazinet et al. [22]. EDUF is basically a batch process in which one or more filtration membranes are stacked into a conventional electrodialytic cell and allows the separation of molecules according to their charge and molecular size in an electric field. Recently, antimicrobial [23] as well as anticancer [24]

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peptides from snow crab by product hydrolysate (SCBH) have been successfully fractionated by EDUF process. From the different studies carried-out separately, numerous parameters are found to affect EDUF treatment and its efficiency: the number of ultrafiltration membrane stacking [25], type of material of ultrafiltration membrane, pH [24], electric field strength and membrane pore size [23]. However, the effects of EDUF cell configurations i.e. placement of feed and permeate solutions on peptide migration rate and membrane selectivity have never been studied. Also, evolution of local electric field in each compartment of EDUF cell which is a major indicator of EDUF performance has not yet been measured.

The present study is a part of the optimization of EDUF process for separation of bioactive peptides from SCBH. The main objective of the present study was to compare the effect of two EDUF cell configurations using two ultrafiltration membranes of different molecular weight cut-off (MWCO) values (20 and 50 kDa) on electrodialytic parameters such as electrical conductivity, membrane resistance, local electric field, peptide migration rate, energy consumption, peptide molecular profile and amino acid composition of the fractions obtained by EDUF treatment of SCBH.

2. Materials and method

2.1. Materials and ED cell

2.1.1. Hydrolysate

A snow crab by-products hydrolysate (SCBH) was obtained from the Québec fisheries and aquaculture innovation center (Merinov, Gaspé, QC, Canada) which was produced according to the procedure described previously [23]. Briefly, the snow crab by-products were enzymatically hydrolyzed at pH 9.0. Pressure-driven filtration process (ultrafiltration and nanofiltration) were performed for the purification and separation of the fraction of interest containing the peptides. The SCBH used in this work was the fraction of permeate of ultrafiltration (1 kDa) and retentate of nanofiltration containing 82% water, 2.5% ash and 140 g/L (14%) peptides. This fraction was stored at −30 °C for further analyses and EDUF treatment.

2.1.2. Chemicals

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 ultrafiltration membranes (UFM) with MWCO values of 20 and 50 kDa, were purchased from Spectrum Laboratories Inc., (Rancho Dominguez, 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 and configurations

The electrodialysis cell used for the experiment was a MP type cell (effective surface area of 100 cm^2) manufactured by ElectroCell Systems AB Company (Täby, Sweden) with one AEM, one CEM and two UFMs as illustrated in Fig. 1. The UFM placed near the cathode with a MWCO of 50 kDa was named UFM1, while the one near the anode with a MWCO of 20 kDa was named UFM2. The cell consisted of the anode, a dimensionally-stable electrode (DSA), and the cathode, a 316 stainless steel electrode. The electric field was supplied between electrodes by a variable 0–100 V power source. Two different hydrolysate inlets, corresponding to the two different cell configurations were tested in this study:

- The first EDUF cell configuration, shown in Fig. 1(a), was divided into four closed loops. Two of them contained 3 L of KCl solution (2 g/L) for the recovery and concentration of peptides: the KCl solution circulating between the UFM1 and UFM2 was named KCl-F1 and the one circulating between the UFM2 and the AEM was named KCl-F2. The feed solution (SCBH, 3 L, 1%). was circulated in one compartment between UFM1 and CEM. The last loop contains the electrode rinsing solution (20 g/L Na_2SO_4, 3 L) which is split into two streams going to the two electrolyte compartments.
- The second EDUF cell configuration, as shown in Fig. 1(b), was divided into 3 closed loops. The KCl solution was circulated in the compartment between the UFM1 and UFM2 while the feed solution was circulated in two compartments, one in between UFM1 and CEM and the other one between UFM2 and AEM. The electrode rinsing solution was circulated into the both electrode compartments, similar to configuration 1. In configurations 1 and 2, the solutions were circulated using four and three centrifugal pumps respectively and the flow rates were controlled at 2 L/min using flow meters in each compartment.

The choice of the EDUF configurations was made following the results of a previous study [23]. In this study, the anionic peptides showing an antibacterial activity were separated by UF membrane of 50 kDa MWCO at pH 9 and electric field strength of 14 V/cm after simultaneous EDUF process. Therefore, the cell configuration used in the present study preferably tended to separate and concentrate anionic peptides from SCBH.

2.2. Electroseparation protocol

EDUF was performed in batch process in both cell configurations using constant electrical field strengths of 14 V/cm. The system was run in a cold room at a constant temperature of 4 °C to prevent growth of microorganisms [23]. The SCBH was diluted with de-mineralized water to the concentration of 1% (w/v) and the EDUF fractionation was performed for 6 h. The pH of SCBH and permeate solutions was adjusted to 9 before each run with 1.0 M NaOH and maintained constant during the process. The KCl fractions (KCl-F1 and KCl-F2 in configuration 1 and KCl in configuration 2), feed solution and electrode solution were maintained at a flow rate of 2 L/min/compartment in both configurations.

For each treatment 10 mL sample of SCBH and permeate were collected before applying voltage and each hour during the treatment to determine the peptide migration rate. Furthermore, conductivity of SCBH and KCl compartments was monitored, in order to follow their (de)mineralization kinetics. The current intensity, electrical potential differences of the AEM, CEM, UFM1, and UFM2 were recorded every 30 min during EDUF treatment for both configurations. Finally, 3 replicates of each condition were performed. At the end of each replicate, a cleaning-in-place was performed as mentioned elsewhere by Doyen et al. [24], and the cell was dismantled before to be reassembled.

2.3. Analyses

2.3.1. Solution conductivity

A YSI conductivity meter (Model 3100) equipped with a YSI immersion probe model 3252, cell constant $K = 1\ cm^{-1}$ (Yellow Springs Instrument Co., Yellowsprings, OH, USA) was used to measure the solution conductivities.