Separation and Purification Technology 147 (2015) 227–236

Contents lists available at ScienceDirect

Separation and Purification Technology

journal homepage: www.elsevier.com/locate/seppur

Presence of free amino acids in protein hydrolysate during electroseparation of peptides: Impact on system efficiency and membrane physicochemical properties

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ARTICLE INFO

Article history:
Received 26 February 2015
Received in revised form 10 April 2015
Accepted 11 April 2015
Available online 27 April 2015

Keywords:
Peptide separation
Membrane fouling
Characterization
Ion exchange membrane
Electrodialysis with ultrafiltration membrane

ABSTRACT

Membranes, more particularly ion-exchange membranes (IEMs), are vulnerable to fouling by peptides and amino acids present in protein hydrolysate. In this context, IEM and ultrafiltration membranes (UFMs) staked in an electrodialysis with filtration membrane (EDFM) system were characterized during and after 6 successive peptide fractionations. The peptide concentration in the recovery compartment decreased by more than 22% and 32% for fifth and sixth batches, respectively. In addition, analysis of total nitrogen content of used membranes and FTIR confirmed that AEM and both cation-exchange membranes (CEMs) were fouled by peptides and/or free amino acids. Consequently, their electrical conductivity, IEC and water content decreased. In addition to the fouling, the cathode (diluate) side of AEM, where the dissociation of water molecules occurs, was deteriorated radically, possibly due to chemical reactions with OH^- ions produced. Consequently, the surface roughness of both IEMs was found to increase considerably. The IEM fouling noticed in the present study was never observed before in an EDFM treatment. This fouling would be mainly due to the presence of amino acids residues (100% free) in the SCBH, and able to migrate through the AEM (Phe) and the CEM (Arg and Lys).

1. Introduction

Electrodialysis with filtration membrane (EDFM) is an emerging technology for the selective separation and concentration of electrically charged molecules such as bioactive peptides. In this process, the peptides are separated according to their charge and their molecular size (weight) through a filtration membrane. EDFM process has been applied in the fractionation of antioxidant, antihypertensive, antidiabetic bioactive peptides from complex mixtures of different protein hydrolysates.

The EDFM technique has been claimed to increase the selectivity and to decrease fouling of filtration membrane in contrast to pressure-driven membrane processes. In fact, electromigration is the principal transport mechanism in an electrodialytic fractionation process as the driving force applied is an external electrical field. However, the membranes used in electrodialytic process such as anion-exchange (AEM) and cation-exchange membranes (CEM) are vulnerable to fouling. On the other hand, the lifespan of ion-exchange membrane (IEM) is considered to be an important factor in an ED process because the loss of their physicochemical properties increases energy consumption and decreases productivity and permselectivity, consequently, increasing operation and membrane replacement cost [1]. CEM was found to be poisoned by amino acids particularly by arginine (Arg) [2]. Ren et al. [3,4] demonstrated a significant fouling of CEM by amino acids during consecutive batches of bipolar membrane electrodialysis. In addition, AEM was found to be fouled by phenylalanine (Phe) during electrodialysis of aromatic amino acids [5,6]. It is well understood that the amino acids have greater affinity for oppositely charged membrane as compared to that of inorganic ion [2]. Recently, Langevin and Bazinet [7] demonstrated that peptides from soya protein hydrolysate strongly interact with IEMs, subsequently resulting in fouling even in the absence of external electric field. Moreover, IEMs used in electrodialysis process especially at

Abbreviations: IEM, ion exchange membrane; ED, electrodialysis; EDFM, electrodialysis with filtration membrane; EDUF, electrodialysis with ultrafiltration membrane; ATR–FTIR, attenuated total reflection–Fourier transform infrared; IEC, ion-exchange capacity; SCBH, snow crab by products hydrolysate; LEF, local electric field; AEM, anion-exchange membrane; UFM, ultrafiltration membrane; CEM, cation-exchange membrane; MWCO, molecular weight cut-off; CIP, cleaning-in-place; PES, polyether sulfone; Glu, glutamic acid; Phe, phenylalanine; Arg, arginine; Lys, lysine.

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http://dx.doi.org/10.1016/j.seppur.2015.04.014
1383-5866/

limiting current density have been found to be affected adversely causing significant loss in their structural, mechanical and physicochemical properties [1,8–10]. However, peptide and amino acid fouling on IEM during peptide fractionation using EDFM and their impact on system performance, membrane electrochemical and physicochemical properties have never been demonstrated yet.

In this context, consecutive runs of electrodialysis with ultrafiltration membrane (EDUF) of a snow crab by-product hydrolysate (SCBH), excessively hydrolyzed that contains considerable amount of free amino acids, were carried out to (1) evaluate the impact of long-term usage of EDUF on system performance (peptide migration rate), (2) assess the integrity of used membranes (membrane electrical resistance, conductivity, thickness, water content, ion exchange capacity and surface roughness) and (3) quantify and characterize fouling on used membranes (total membrane nitrogen content and membrane surface spectroscopy).

2. Materials and method

2.1. Materials and electrodialytic 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 by Beaulieu et al. [11]. Briefly, the snow crab by-products were enzymatically hydrolyzed at pH 9.0; pressure-driven filtration process (ultrafiltration and nanofiltration) were performed for the separation and purification of the fraction of interest containing the peptides. The SCBH used in this work was the permeate of ultrafiltration and the retentate of nanofiltration (1 kDa); therefore it contained mainly free amino acids (Table 1) and peptides of molecular weights ranging from 200 to 1000 Da [11]. This fraction was stored at −30 °C for further analyses and EDUF treatment. The total nitrogen content (peptides and free amino acids) in the SCBH was 140 g/L. The water and ash contents were 82% and 2.5%, respectively. The SCBH was diluted with distilled water to 2% before EDUF fractionations.

2.1.2. Chemicals

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

Table 1
Composition of total and free amino acids in initial snow crab byproduct hydrolysate.

	Amount of amino acids (g/100 g of dry mass)	
	Total	Free
Aspartic acid	4.42 ± 0.44	0.89 ± 0.01
Serine	1.87 ± 0.17	1.29 ± 0.04
Glutamic acid	6.56 ± 0.61	2.39 ± 0.01
Glycine	2.50 ± 0.24	0.81 ± 0.01
Histidine	1.39 ± 0.13	1.48 ± 0.01
Taurine	1.25 ± 0.03	1.01 ± 0.01
Arginine	4.44 ± 0.31	4.79 ± 0.01
Threonine	2.16 ± 0.22	1.09 ± 0.00
Alanine	2.87 ± 0.14	2.01 ± 0.01
Proline	3.07 ± 0.13	0.90 ± 0.02
Cystine	N.D[a]	2.45 ± 0.00
Tyrosine	1.38 ± .013	1.37 ± 0.13
Valine	3.36 ± 0.29	2.92 ± 0.04
Methionine	0.84 ± 0.07	1.13 ± 0.01
Lysine	3.58 ± 0.50	4.28 ± 0.06
Isoleucine	2.63 ± 0.20	2.43 ± 0.13
Leucine	3.68 ± 0.29	3.10 ± 0.54
Phenylalanine	2.10 ± 0.19	1.91 ± 0.07

[a] N.D: not detected.

2.1.3. Membranes

Polyether sulfone (PES) UFM with MWCO value of 20 kDa, were purchased from Synder filtration (Vacaville, CA, USA). Neosepta® CMX-SB cation-exchange membrane (CEM) and AMX-SB anion-exchange membrane (AEM) were purchased from Tokuyama Soda Ltd. (Tokyo, Japan). Neosepta® membranes were manufactured from powder of poly(vinyl chloride) (PVC) and two co-monomers: styrene (St) and divinylbenzene (DVB). The mixture was coated on a polymer cloth and polymerized to the co-monomers by heating followed by the introduction of the ion-exchange sites: quaternary ammonium and sulfonic acid groups for AEM and CEM respectively. The detail synthesis procedure and structural units of these membranes are depicted elsewhere [1].

2.1.4. Electrodialysis cell and configuration

The electrodialysis cell used for the experiment was a MP type cell (effective surface area of 100 cm^2) manufactured by Electro Cell Systems AB Company (Täby, Sweden) with one AEM, two CEMs (CEM1 and CEM2) and one UFM with a MWCO of 20 kDa as illustrated in Fig. 1. The CEM placed near the anode was named CEM1 and that near the cathode was named CEM2. The cell consisted of an anode, a dimensionally-stable electrode (DSA), and a cathode, a 316 stainless steel electrode. The electric field was supplied between electrodes by a variable 0–100 V power source.

EDUF cell configuration, as shown in Fig. 1, was divided into 3 closed loops. The 3 L of feed (SCBH, 2% w/v) solution was circulated in two compartments, between AEM and UFM and between CEM1 and CEM2 from a single external reservoir. The feed solutions were mixed at the exit of the EDUF cell and returned back to the reservoir. The 3 L of KCl solution at three different concentrations (1, 3 and 5 g/L) were circulated in the compartment between the UFM and CEM1 for the recovery and concentration of peptides. The electrode rinsing solution (20 g/L Na_2SO_4, 3 L) was circulated into the compartments adjacent to anode and cathode from a single external reservoir. The solutions were circulated using three centrifugal pumps and the flow rates were controlled at 2 L/min/compartment with flow meters. Experimental conditions used in this study were previously optimized in order to obtain the highest peptide migration rate [12].

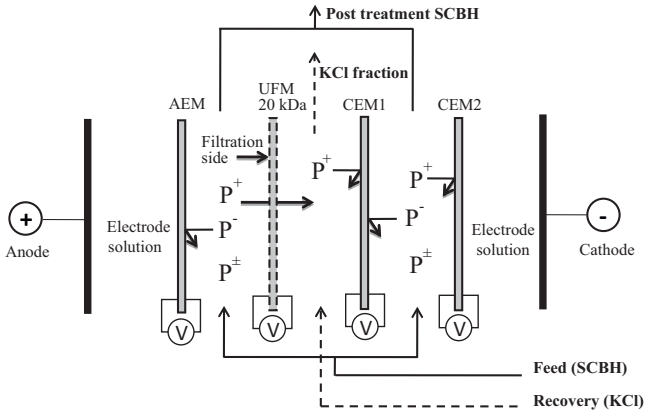

Fig. 1. EDUF cell configuration for the fractionation of SCBH. AEM: anion-exchange membrane, UFM: ultrafiltration membrane, CEM: cation-exchange membrane, P^+: cationic peptides, P^-: anionic peptides, $P^\pm$: neutral peptides and V: voltmeter connected to silver coated platinum electrode placed at the interface of membrane.