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Anti-diabetic and antihypertensive activities of two flaxseed protein hydrolysate fractions revealed following their simultaneous separation by electrodialysis with ultrafiltration membranes

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ABSTRACT

Flaxseed protein hydrolysate has been fractionated by electrodialysis with two ultrafiltration membranes (20 and 50 kDa) stacked in the system for the recovery of two specific cationic peptide fractions (KCl-F1 and KCl-F2). After 360 min of treatment, peptide migration increased as a function of time in KCl compartments. Moreover, the use of two different ultrafiltration membrane allowed concentration of the 300–400 and 400–500 Da molecular weight range peptides in the KCl-F1 and KCl-F2 fractions, respectively, compared to the initial hydrolysate. After mass spectrometry analysis, higher amounts of low molecular weight peptides were recovered in the KCl-F2 compartment while relatively higher molecular weight peptides were more detected in the KCl-F1 compartment. Amino acid analysis showed that His, Lys and Arg were especially concentrated in the KCl compartments. Finally, glucose-transport assay demonstrated that the KCl-F2 fraction increased glucose uptake while oral administration of KCl-F1 and final FPH decreased systolic blood pressure.

1. Introduction

Over the past two decades, consumer's demand for nutraceuticals and functional foods has grown markedly. Indeed, the functional food and nutraceutical markets represent $21.3 billion in the USA. In Europe this market is estimated to be $8 billion (Basu, Thomas, & Acharya, 2007). Consequently, to meet consumer demand, new bioactive molecules originating from various food matrices are being investigated especially bioactive peptides obtained and isolated after enzymatic hydrolysis (Korhonen & Pihlanto, 2003, 2006). These bioactive peptides exert physiological effects on the gastrointestinal, nervous, cardiovascular and immune system.

Recently, peptide fractions recovered from flaxseed protein after hydrolysis have demonstrated interesting bioactive properties (Omoni & Aluko, 2006). Indeed, flaxseed protein hydrolysate (FPH) fractions, obtained after alcalase digestion induced a change in the secondary and tertiary structures of calmodulin known to be involved indirectly in the appearance of neurodegenerative diseases (Omoni & Aluko, 2006). Udenigwe and Aluko (2010) recovered FPH fractions following protein digestion with thermolysin and pronase which exhibited *in vitro* antioxidant and antihypertensive properties. Moreover, Udenigwe, Lu, Han, Hou, & Aluko, (2009) obtained bioactive peptides with antioxidant and anti-inflammatory properties after enzymatic hydrolysis with various proteases. Marambe, Shand, and Wanasundara (2008) showed that flaxseed proteins treated with Flavourzyme® demonstrate angiotensin converting enzyme (ACE) inhibition and hydroxyl radical scavenging activities which suggests that FPH could act as antihypertensive and antioxidant agents. Finally, hydrolysis of flaxseed protein with pepsin, ficin, trypsin, papain, thermolysin,

Abbreviations: ACE, angiotensin converting enzyme; AEM, anion-exchange membrane; BSA, bovine serum albumin; CEM, cation-exchange membrane; DSA, dimensionally-stable anode; EDUF, electrodialysis with ultrafiltration membranes; FPH, flaxseed protein hydrolysate; HMWP, high molecular weight peptide; IEM, ion-exchange membrane; LMWP, low molecular weight peptide; MW, molecular weight; MWCO, molecular weight cut-off; SBP, systolic blood pressure; SHR, spontaneously hypertensive rats; UFM, ultrafiltration membrane.

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pancreatin and alcalase have also been shown to have *in vitro* inhibition against ACE and renin activities (Marambe et al., 2008).

However, as protein hydrolysates represent complex mixtures of similar molecular weight peptides, it is important to use an appropriate method of fractionation to obtain purified peptide fractions with higher functionality and bioactivity. Generally speaking, pressure-driven (Deeslie & Cheryan, 1992; Jeon, Byun, & Kim, 1999; Pouliot, Gauthier, & Bard, 1993; Pouliot, Gauthier, & L'Heureux, 2000) and chromatographic processes (Alpert, 1990; Schroeder, Jones, Cromick, & McCalla, 1962) are the most frequently used technologies to isolate bioactive fractions or molecules. However, pressure-driven processes have low selectivity due to fouling formation while chromatographic technology is too expensive (Bargeman, Houwing, Recio, Koops, & van der Horst, 2002) and not applicable for the fractionation of large sample volumes.

Hence, electrodialysis with ultrafiltration membranes (EDUF) which was recently developed and patented (Bazinet, Amiot, Poulin, Labbé, & Tremblay, 2012) represent an interesting alternative to conventional separation processes for the recovery of specific peptide fractions. Indeed, EDUF allows separation of molecules according to their electric charges and molecular masses (Poulin, Amiot, & Bazinet, 2006). The driving force is the electric field. Consequently, contrary to pressure-driven processes, no significant fouling at membrane interface was detected due to the absence of pressure (Aider, Brunet, & Bazinet, 2008; Doyen, Beaulieu, Saucier, Pouliot, & Bazinet, 2011b; Firdaous et al., 2009; Poulin et al., 2006). EDUF technology has already shown potential applications for the separation and the recovery of bioactive compounds from diverse food hydrolysates. Firdaous et al. (2009, 2010) isolated an ACE inhibitory peptide fraction from alfalfa white protein hydrolysate and more recently an anticancer and antimicrobial peptide fractions from hydrolysate of snow crab by-products were identified and isolated (Doyen, Beaulieu, Saucier, Pouliot, & Bazinet, 2011a, 2012). The EDUF configurations used for the recovery of bioactive fractions cited above consisted of stacking ion exchange-membranes (IEM) and one or more UFMs of the same MWCO. However, the staking of two UFMs with different MWCOs in the EDUF system to determine the impact on peptide migration and separation selectivity has never been studied.

The objectives of the present study were (1) to perform the fractionation of FPH by electrodialysis with two different MWCO UFMs, (2) to evaluate the impact of this double stacking on peptide migration and fractionation selectivity, (3) to characterise peptides present in the peptide fractions recovered and (4) to test the bioactivities of FPH and peptide fractions in terms of glucose capitation (anti-diabetic activity) and ACE inhibitory activity (antihypertensive activity).

2. Materials and methods

2.1. Materials

Porcine pancreas trypsin was purchased from Sigma–Aldrich (St. Louis, MO, USA) and pronase from *Streptomyces griseus* was purchased from Roche Diagnostics GmbH (Mannheim, Germany). HCl and NaOH 1.0 M 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).

2.2. Flaxseed protein hydrolysate

Flaxseed protein hydrolysis was carried-out using trypsin and pronase according to the protocol of Udenigwe, Adebiyi, Doyen, Bazinet, and Aluko (2011). Briefly, flaxseed protein isolate was suspended in distilled water (5% flaxseed protein, w/v) and the slurry was adjusted to pH 7.0 with 0.5 M NaOH and at a temperature of 37 °C. Trypsin was added at an E/S ratio of 1:100 to initiate protein hydrolysis, and the reaction mixture incubated for 5 h. During hydrolysis, the reaction was maintained at pH 7.0 with 0.5 M NaOH using a pH-Stat instrument (Metrohm Titrando, Herisau, Switzerland). The reaction was stopped by adjusting the mixture to pH 4.0 using 0.5 M HCl. The mixture was readjusted to pH 7.4 and temperature of 40 °C, and thereafter pronase at E/S ratio of 1:100 was added; hydrolysis was continued for 2 h and terminated with 0.5 M HCl (pH 4.0). The resulting mixture was cooled to room temperature and centrifuged at 7000 rpm for 1 h. The supernatant was collected as the FPH, freeze dried and stored at −20 °C. Afterwards, FPH was demineralized by conventional electrodialysis to decrease salt concentration (Bazinet & Firdaous, 2013).

2.3. 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). The anode, a dimensionally-stable anode (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 cell is composed 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 MWCOs of 20 and 50 kDa (Spectrum Laboratories Inc., Rancho Dominguez, CA, USA). The UFM placed near the anode with a MWCO of 50 kDa was named UFM1 while the one placed near the cathode with a MWCO of 20 kDa was named UFM2. The electrodialysis configuration, presented in Fig. 1, was divided into four compartments. Two of them contained 1.5 L of KCl solution (2 g/L) for the recovery and concentration of peptides: the KCl solution located between the UFM1 (50 kDa) and UFM2 (20 kDa) was named KCl-F1 and one located between the UFM2 (20 kDa) and the CEM was named KCl-F2 (Fig. 1). One compartment contained the 20 g/L Na_2SO_4 electrode rinsing solution (3 L) and another one the feed solution (FPH, 1.5 L). The solutions were circulated using four centrifugal pumps and the flow rates were controlled using flow metres. The KCl permeates (KCl-F1 and

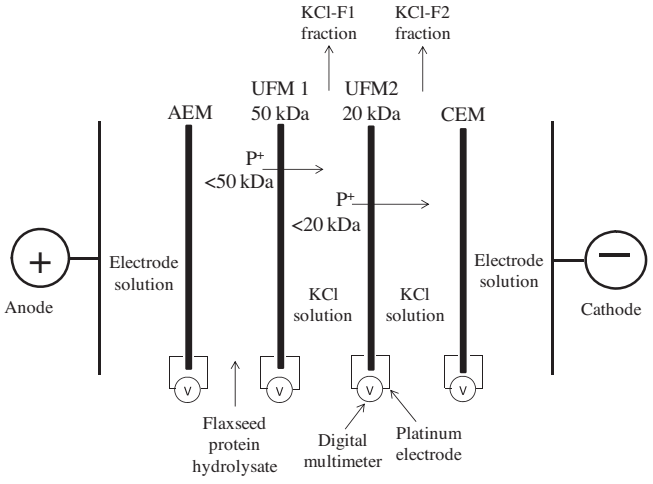

Fig. 1. EDUF cell configuration for the fractionation of FPH. AEM: anion-exchange membrane, UFM: ultrafiltration membrane, CEM: cation-exchange membrane, P^+: cationic peptide.