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Fractionation of a tuna dark muscle hydrolysate by a two-step membrane process

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ABSTRACT

The aim of this work is to develop a two-step membrane separation process enabling the fractionation of protein hydrolysates obtained from enzymatic hydrolysis of tuna by-products. A screening of the more appropriate UF and NF membranes was first performed in dead-end and tangential filtration cells with a model solution prepared from a commercial fish hydrolysate (Prolastin®). Optimization of UF and NF steps was then investigated through the impact of operating parameters including pressure, peptide concentration and pH of the solution on performance of two selected membranes. Finally, the coupling of UF and NF steps was investigated for the fractionation of a raw hydrolysate prepared from tuna by-products. Three different operation modes coupling UF and NF steps in batch and diafiltration configurations were tested. A fraction rich in peptides of molar mass between 1 and 4 kg mol^{-1} was isolated and the positive effect of diafiltration on peptide purification process was underlined.

1. Introduction

Considering the limitation of marine bioresources, the need for better management of by-products in fishing industries is a current issue in countries where fishing is an important activity. In particular, these by-products often contain high concentration of proteins that can be transformed into valuable products through enzymatic hydrolysis. The complex mixtures of amino acids and peptides obtained can be used as food ingredients in order to improve the technological and nutritional properties of food products [1] or as a source of bioactive peptides. Actually, many recent studies have demonstrated the presence of peptides exhibiting functional properties and biological activities in fish protein hydrolysates [1–9]. A relationship between the physicochemical characteristics of peptides as their molar mass (MM) and the biological activity has been established. In particular, fractions with MM between 1 and 4 kg mol^{-1} would be the most interesting for nutritional and/or pharmaceutical uses. The extraction of this fraction from the hydrolysate mixture is thus a key issue.

Membrane technology which is an effective technology for concentration, extraction, and fractionation of molecules offers a good alternative to traditional separation techniques to achieve environmentally friendly and cost effective process [10]. This technology has been successfully used for the separation and fractionation of high value molecules from waste or by-products in food industries [11]. Studies dealing with protein and peptide fractionation have been generally devoted to the investigation of the operating conditions (pH, temperature, ionic strength, etc.) [12] and of the interactions between individual components or between these components and the membranes employed [13–15]. On the other hand, only few reports are related to the use of membrane technology for fractioning fish hydrolysates [16–19]. Actually, most studies involve UF membranes as a practical tool for splitting [20,21] or the NF membrane for peptide or amino acid concentration [13,22]. It has to be noted that the influence of the operating mode on the effectiveness of the membrane separation has been seldom taken into account.

In this paper, a two-step membrane process was investigated for the fractionation of a protein hydrolysate produced by enzymatic hydrolysis of tuna dark muscle. This raw material is a solid by-product of the tuna processing industry. This work aimed at obtaining a fraction enriched with peptides of MM between 1–4 kg mol^{-1}. In the first part, different UF membranes were tested using a model solution prepared from a commercial fish protein hydrolysate. Then the influence of operating parameters such as transmembrane pressure, concentration and pH of the feed solution on UF and NF performance were studied with the same model solution. Finally the fractionation of the tuna dark muscle hydrolysate was carried out using three different operating modes coupling UF and NF steps in batch and diafiltration configurations.

2. Materials and methods

2.1. Raw material (fish protein hydrolysates)

The tuna protein hydrolysate was produced at pilot-scale in a 5 L batch reactor using Alcalase® 2.4 L (LYVEN International,

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France). The tuna black muscle was obtained from TUNA EL SULTON Foods Industry Ltd. (Sfax, Tunisia). During the processing of canned tuna, the dark muscle was recovered after the steam-cooking of the whole fish. It was vacuum-sealed in 400-ml polyethylene bags and then transferred on ice to the laboratory where it stored at −20 °C until being used.

The tuna black muscle (1.5 kg) was suspended in 3 L of de-ionized water and the pH was adjusted at 8.5 with a 2 M NaOH solution. 30 g L^{-1} of Alcalase® were added and the reaction was carried out at 55 °C for 1 h. Afterward, the hydrolysate was heated at 90 °C in a boiling water bath for 20 min in order to stop the reaction. Several hydrolyses were carried out in the same conditions and all the hydrolysates were mixed together and then stored in 1-L bottles at −20 °C.

The physicochemical characteristics of the raw hydrolysate are given in Table 1 and the MM distribution obtained by Steric Exclusion Chromatography (SEC–HPLC) is given in Fig. 1. In this figure, is also given the MM distribution of the commercial hydrolysate (Prolastin, Copalis, France) obtained by enzymatic hydrolysis of the connective tissues of specific species of fish [23]. Both hydrolysates present a similar molar mass distribution of peptides. However the hydrolysate prepared from tuna dark muscle also contains high MM proteins which are eluted at the void volume of the column.

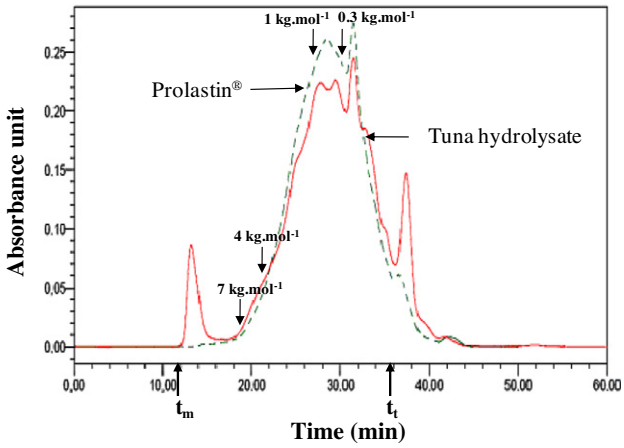

Fig. 1. Molar mass distribution of the commercial hydrolysate (Prolastin®) and the tuna hydrolysate (Superdex-Peptide HR 10/300 – Eluant: Water/TFA/ACN: 70/0.1/30 – t_m and t_t elution times corresponding respectively to void and total column volumes).

2.2. Membrane processes

2.2.1. Membranes

Six different flat polymeric UF membranes (three polyethersulfone (PES) membranes with a cut-off (CO) of 10 kg mol^{-1} (membranes A–C) purchased respectively from Alpha-Laval, Koch and Orelis, a 5 kg mol^{-1} PES membrane from Omega Filter (membrane D), an 8.5 kg mol^{-1} polyamide membrane (PA) from Osmonics (membrane E), a 10 kg mol^{-1} regenerated cellulose membrane (RC) from Millipore (membrane F)) and a 3 channel tubular ceramic membrane (hydraulic diameter: 3.5 mm, membrane length and surface area: 23 cm and 155 cm^2, CO 8 kg mol^{-1}) purchased from Tami industries (membrane G) were tested. The NF experiments were carried out with a 1 kg mol^{-1} polyethersulfone membrane (membrane NP010, Microdyn Nadir).

2.2.2. Filtration experiments

2.2.2.1. Membrane selection. The filtration experiments were carried out in frontal filtration mode with a dead-end stirred cell (Amicon 8050, surface area 15.9 cm^2) or in tangential filtration mode with a versatile lab-scale pilot plant (Fig. 2) which can be equipped with either tubular or flat sheet membranes. All UF membranes were characterized according to the following procedure. Firstly, pure water was filtered in order to determine the water flux of clean membrane ($J_{w,0}$), then the water was replaced by the peptide solution (a 10 g L^{-1} Prolastin® solution prepared in pure water) and the permeate flux (Js) was monitored versus time. The transmembrane pressure and the temperature were fixed at 2 bar and 20 °C, the stirring or the tangential velocities were respectively 350 rpm or 3 m s^{-1}. The membrane performance in terms of peptide rejection was evaluated by withdrawing permeate samples for analysis. Finally, the installation was rinsing with pure water and the water flux ($J_{w,1}$) was measured in the same conditions. The index of irreversible fouling (IF) was estimated according to the following equation:

$$\mathrm{IF}(\%) = \frac{(J_{w,0} - J_{w,1})}{J_{w,0}} \times 100 \quad (1)$$

2.2.2.2. Peptide fractionation. In order to improve the fractionation of peptide hydrolysate, three different operating modes were tested. The first one (configuration A) simply involved two successive membrane filtration steps carried out in batch mode (the retentate was recycled while the permeate was collected). At the end of the first step (UF step), the permeate recovered was used as feed solution in the second filtration step (NF step). In the second operation mode (configuration B), the UF step was carried out in diafiltration mode. As fast as the permeate was collected, an equal input of de-ionized water was added into the feed tank. It thus permitted to recover a larger amount of small molar mass species without observing an excessive concentration of the retentate. Like in configuration A, the permeate obtained at the end of the diafiltration step was used as feed solution in the NF step. The last operating mode (configuration C) consisted in a three-step process. The first step was similar to the UF step of the configuration A. At the end of this step, the UF retentate was diafiltrated on the same UF membrane. Finally, both permeates obtained at the end of these two steps were mixed in order to be used as the feed solution in the NF step. The flow-sheets of these three different operation modes are schematically presented in Fig. 3.

The filtrations were carried out at constant temperature (25 °C) under a transmembrane pressure of 2 bar for UF and 10 bar for NF experiments. The feed flow was fixed at 300 L h^{-1} which corresponds to a tangential velocity equals to 3 m s^{-1} and 1.25 m s^{-1} for UF and NF respectively.

Table 1
Composition of Prolastin® and tuna dark muscle hydrolysate.

			Peptide fractions (%)				
	Dry mater	Protein[a]	<0.3 kg mol^{-1}	0.3–1 kg mol^{-1}	1–4 kg mol^{-1}	4–7 kg mol^{-1}	>7 kg mol^{-1}
Prolastin® (commercial powder)	97.2% (w/w)	65% (w/w)	48	30.5	17.5	2	2
Tuna dark muscle hydrolysate	145 g L^{-1}	72 g L^{-1}	38	28.5	19	3	11.5

[a] According to Biuret method.