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APPLICATION OF POLYMER MEMBRANE TO RECOVER CYSTEINE PROTEINASE INHIBITOR FROM CHICKEN EGG WHITE SOLUTION

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Key words: cystatin, ultrafiltration, regenerated cellulose, polyethersulphone

The objective of the study was to investigate the influence of membrane material and membrane *cut off* on transport and separation properties of ultrafiltration membranes, as well as verification of the possibility of membrane techniques application for the recovery of bioactive components isolated from chicken eggs. Egg white solutions contain cysteine proteinase inhibitor – cystatin, which can be utilized, in the convenient state in liquid form, in some food and pharmaceutical industries. Commercial membranes made of polyethersulfone (PES) and regenerated cellulose (RC) were used in our experiments. Applied transmembrane pressure (TMP) varied from 0.05 to 0.30 MPa. Membrane filtration process led two step: first – microfiltration (MF) and second – ultrafiltration (UF). Microfiltration, using cross flow technique membranes made from polypropylene with 0.2 μm pores, was used as the first step of cystatin isolation from egg white (initial fractionation of egg white). Liquid products obtained by fractionation of egg white can be used as a partial feeding in the ultrafiltration installation. During ultrafiltration asymmetric membranes were used that were made of polyethersulfone and regenerated cellulose, with *cut off* of a molecular weight equal to 10, 30, 50 and 100 kDa. Polyethersulfone membrane with *cut off* 30 kDa was characterised by the highest yield of fractionated products with a high level of remaining specific biological activity. When membrane *cut off* point was increased specific activity of cystatin was lowered. Liquid fractions of egg white were characterised by high inhibitory activity of cystatin. The efficiency of cysteine proteinase inhibitor – cystatin – separation from egg white varied from 22 to 87%.

INTRODUCTION

The rapid growth in the field of biotechnology led to an increase in the demand of efficient, large-scale protein purification processes. Techniques used in research laboratories for protein purification (*e.g*. chromatography, electrophoresis, affinity purification) are excellent for producing small quantities of protein [Guérin-Dubiard *et al.,* 2005; Konopska *et al.,* 2007]. Indeed, there are countless papers describing protein purification methods which can yield from a few micrograms to a few hundred milligrams of highly pure protein products. However, these processes are very difficult to scale-up, which limits protein production levels. In addition to scale up problems, techniques such as chromatography and electrophoresis require complex instrumentation support to run efficiently, and yield low throughput of product at extremely high cost. The major advantage of ultrafiltration processes over conventional bioseparation processes is high throughput of product [Ghosh & Cui, 2000].

Nowadays, many studies are carried out on cystatin (cysteine proteinases inhibitor with molecular weight of 12.8 kDa, present in egg white app. of 0.05% proteins) isolation from hen's egg white using membrane techniques. Implementation of well known process based on immobilized enzymes to the food factories (industrial scale) is quite difficult and expensive. Thus, membrane techniques are more competitive. Purified cystatin preparations can be utilized in pharmaceutical applications (paradontosis). Moreover, some possibilities of medical use of cystatin preparation exist, due to its ability to slowing down development of cancer tissue *in vivo* [Saleh *et al.,* 2005].

MATERIALS AND METHODS

The study was carried out on fresh eggs purchased on a local market. Eggs were separated on white and yolk. Following, the egg white was filtered through the Schott's funnel with pores of 1 mm and diluted with 150 mmol/L NaCl at a ratio 1:4 (v/v) and pH of the solution was adjusted to 8.5. Diluted egg white was then processed using microfiltration *cross flow* technique (MF) on polypropylene pipes filter. Nominal size of the membrane pores was 0.2-0.6 μm and working surface of the membrane equaled app. 3×10^{-2} m^2. Filter was supplied with feeding solution by peristaltic pomp BIO FLOW. Obtained microfiltrates were then ultrafiltrated (UF) in a inert gas stream using *dead end* technique in ultrafiltration chamber of AMICON 8200 with volume 200 mL and effective membrane surface of 2.8×10^{-3} m^2. Selectivity of the membranes produced from PES with MWCO 10, 30, 50, 100 kDa and made from CR with NWCO 30 and 100 kDa was evaluated at the early stage of the experiment.

Activity of cystatin was determined as the ability to inhibit enzymatic activity of papain using BANA (N-benzoil-DL arginyl-b-naphtylamide hydrochloride) as a substrate after

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incubation of the samples at 37°C. The reaction was stopped by the addition of DMBA (p-dimethyloaminobenzaldehyde) and the decrease of the absorbance was measured spectrophotometrically at a wavelength of 450 nm [Siewinski, 1991].

Permeate volume flux was calculated as follows: $J = m/A \cdot t$; where J is the permeate volume flux (kg/m^{2}h), m denotes mass of the permeate sample collected within time t (h), and A is the effective membrane surface (m^2) [Kowalska *et al.,* 2004]. Selectivity test was done using stream of distilled water. The results were calculated according to formula cited above and graphically showed in Figure 1.

RESULTS AND DISCUSSION

Use of membrane made of polyetherosulphone (PES) and regenerated cellulose (CR) with different molecular weight cut off (MWCO) allowed for partial purification of the solutions enriched with cystatin inhibitor.

In the ultrafiltration process (UF) use was made of the *dead end* technique through membrane with an effective surface area 2.8×10^{-3} m^2. The applied large spectrum MWCO of membranes particularly made from PES enabled affirming that it is advisable to use membranes with *cut off* of about 10 and 30 kDa (Figure 1). The above mentioned membranes permit the receipt of solutions enriched in cystatin suitably after side of concentrate for membranes with *cut off* of about 10 kDa and after side the permeates for membranes with *cut off* of about 30 kDa. During filtration by membranes of higher molecular weight *cut off* (50 and 100 kDa) no growth of specific activity of inhibitor was shown both in permeates and in the concentrate. The membranes with *cut off* 10 kDa were determined for an increase in the specific activity of cystatin in relation to feed solutions at the level intensify factor about to 2.50 on retentate side. The membranes with MWCO of about 10 kDa are subject to rapid polarization (fouling), which results in the growth of filtration cake [Güell & Davis 1996] rapid fall of the volume of permeate flux (Figure 2).

In the composition of cake structure come mainly: ovoalbumin, ovomucoid and ovotranferin that is main proteines of the egg white. UF through membrane with MWCO of about 30 kDa enables obtaining increased specific activity in permeates and increased for cystatin intensify factor amount about to 4.1 after side the filtrates. The above-mentioned membrane with the *cut off* of about 30 kDa undergoes polarization to a smaller extent, which was observed after smaller fall of stream of the volume permeate flux (Figure 2). During ultrafiltration any growth of specific activity was observed when subjection polymer was applied to this membrane (Table 1). Received preparations can be further cleaned from use chromatographic techniques with immobilization enzymes, and cleaned inhibitors can find use parafarmaceutical or in laboratory diagnostics.

CONCLUSIONS

1. Selection of membranes and operational conditions is the key to success for partial separation of proteins show a biologically activity *e.g.* cystatin.

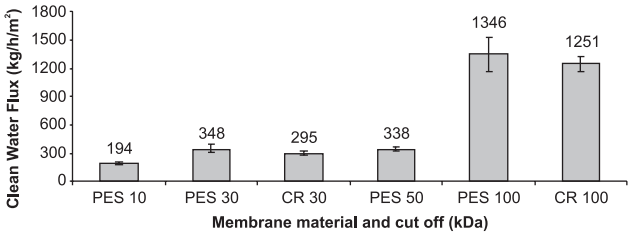

FIGURE 1. Water volume flux for membranes made of PES and CR.

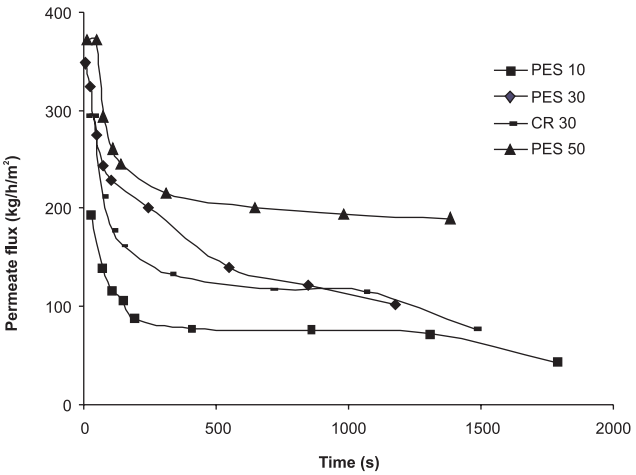

FIGURE 2. The dependence of the volume permeate flux *vs*. time.

TABLE 1. Recovery and increase in the specific activity of cysteine proteinase inhibitor.

Membrane material	Membrane MWCO*	Recovery of cysteine proteinase inhibitor		Increase in the specific activity of cystatin			
				Permeate		Retentate	
		(%)	σ		σ		σ
Polyethersulfone (PES)	10	4.95^a	0.65	1.27^a	0.07	2.41	0.14
	30	21.16^b	0.52	4.12^c	0.86	**	
	50	27.71^c	1.69	1.23^a	0.39	**	
	100	66.36^d	0.91	0.99^a	0.02	**	
Regenerated cellulose (RC)	30	17.58^b	0.63	3.42^b	0.89	**	
	100	73.54^e	0.92	0.94^a	0.03	**	

*NWCO – molecular weight *cut off*; ** lack of increase of specific activity of inhibitor in the concentrate; a, b – the same letters indicate no statistical significant difference at p = 0.05

2. The application of the membrane techniques to separate cysteine proteinase inhibitor – cystatin from chicken egg white is technically feasible with observed high product recovery and efficiency purity.

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ZASTOSOWANIE MEMBRAN POLIMEROWYCH DO POZYSKIWANIA INHIBITORA PROTEINAZ CYSTEINOWYCH Z BIAŁKA JAJA KURZEGO

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Celem pracy była ocena mozliwości wykorzystania technik membranowych do pozyskiwania biologicznie aktywnych protein białka jaja kurzego z uwzglednieniem materiału, z którego została wykonana membrana oraz jej punktu odcięcia molekularnego. Roztwory białka jaja zawierające inhibitor proteinaz cysteinowych – cystatynę mogą być wykorzystywane przez niektóre gałęzie przemysłu spożywczego i farmaceutycznego. W doświadczeniach wykorzystano komercyjne membrany z polieterosulfonu (PES) i regenerowanej celulozy (RC). Zastosowane ciśnienie transmembranowe zmieniało się od 0,05 do 0,30 MPa. Proces filtracji membranowej podzielona na dwa stopnie: pierwszy – mikrofiltracja (MF) i drugi ultrafiltracja (UF). Proces mikrofiltracji prowadzono techniką cross flow z wykorzystaniem filtra o konstrukcji rurkowej o nominalnym rozmiarze porów 0,2 μm wykonanego z polipropylenu do wstępnego frakcjonowania białka jaja w celu izolacji cystatyny. Filtrat po procesie mikrofiltracji stanowił nadawę w procesie ultrafiltracji. Podczas ultrafiltracji wykorzystano membrany wykonane z polieterosulfonu i regenerowanej celulozy o punkcie odcięcia molekularnego 10, 30, 50 i 100 kDa. Filtraty po procesie ultrafiltracji przez membranę wykonaną z polieterosulfonu o *cut off* 30 kDa charakteryzowały się najwyższym stopniem odzysku cystatyny i krotnością wzrostu aktywności specyficznej. Wraz ze wzrostem punktu odcięcia molekularnego membran obserwowano obniżanie krotności wzmocnienia aktywności specyficznej. Stopień odzysku aktywnej cystatyny z białka jaja kurzego zmieniał się od 22 do 87%.