UNIVERSITI PUTRA MALAYSIA

FRACTIONATION OF CATFISH PROTEIN HYDROLYSATES USING ULTRAFILTRATION MEMBRANE

DONYA NOVIN

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By

DONYA NOVIN

Thesis Submitted to School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

July 2014

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DEDICATION

To my dearly beloved father and mother for their endless love, support, care and encouragement**.**

Abstract of thesis presented to the Senate of Universiti Putra Malaysia
in fulfilment of the requirement for the degree of Master of Science

FRACTIONATION OF CATFISH PROTEIN HYDROLYSATES USING ULTRAFILTRATION MEMBRANE

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July 2014

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Faculty: Engineering

Membrane filtration process of different protein solutions has attracted the interest of many recent researchers. By manipulating the operating parameters it can be an efficient process to concentrate, separate and purify with the purpose of increasing the biological activity of proteins. The objective of this study was to investigate the effect of operating parameters experimentally, such as transmembrane pressure (TMP), pH, ionic strength and feed concentration on permeate flux and transmission of protein hydrolysates. Regenerated cellulose (RC) membranes of 5kDa and 10 kDa were employed in dead-end ultrafiltration mode. The selection of best parameters was based on the highest transmission considering the appropriate amount of permeate flux. The protein hydrolysates were prepared by enzymatic hydrolysis of Catfish muscle. The enzymatic hydrolysis was performed at pH 9, 50˚C, 2.5% w/v of substrate to buffer and 1% enzyme. The degree of hydrolysis of two selected samples at time of 0.5 h and 5 h hydrolysates for filtration process were 44.88 % and 58.72 % respectively. The two selected samples of hydrolysates (0.5 h and 5 h) were used to compare the antioxidant activity as well as the separation efficiency of hydrolysates based on their molecular weights.

Screening for the optimum parameters showed that the highest transmission was achieved at pH 7, pressure of 1.5 bar, 0.15 M of NaCl and 1.5 mg/ml concentration of feed for 0.5 h hydrolysate. The transmission was found to range between 64.54%-88.89% for 5 kDa and 84.62%-93.14%for 10 kDa membrane. For 5 h hydrolysate, the highest transmission was achieved at pH 5.1, pressure of 2 bar, 0.15 M of NaCl and feed concentration of 1.5 mg/ml. The transmission ranged between 52.96% - 56.37%for 5 kDa and 74.70%-77.76% for 10 kDa. The best value of antioxidant activity from DPPH-scavenging assay was 69.04% for 10 kDa fraction; and from metal ion chelating assay was 74.06% for fraction of 10 kDa for 0.5 h hydrolysate. The highest absorbance of 0.44±0.011was obtained from reducing power assay for 5 h hydrolysate using 5 kDa membrane. The results of hydrolysis and ultrafiltration process showed that by manipulating and appropriate selection of the operating parameters, the degree of hydrolysis and the yield of filtration process (permeate flux and transmission of protein hydrolysates)can improve. Also, the results showed the

effectiveness of ultrafiltration to achieve higher antioxidant activity based on the molecular weight separation.

Abstrak tesis yang dikemukakan kepada Senat of Universiti Putra Malaysia
Sebagai memenuhi keperluan untuk Ijazah Master Sains

PEMISAHAN HIDROLISAT PROTEIN IKAN KELI MENGGUNAKAN MEMBRAN ULTRATURASAN

Oleh

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Proses penurasan membrane bagi larutan protein yang berbeza telah menarik minat ramai penyelidik. Dengan memanipulasikan operasi parameter, ia boleh menjadi satu proses yang efisien untuk pemekatkan, pemisahan dan penulenan dengan tujuan meningkatkan aktiviti biologi protein. Objektif kajian ini adalah untuk menyelidik kesan operasi parameter seperti tekanan, pH, kekuatan ion dan kepekatan larutan masuk ke atas fluks telap dan transmisi hidrolisat protein.Membran selulosa yang telah diperbaharui bersaiz 5kDa dan 10 kDa telah digunakan pada system ultraturasan hujungmati.Pemilihan parameter yang optimum adalah berdasarkan kepada transmisi tertinggi dengan mempertimbangkan kesesuaian fluks.Hidrolisat protein disediakan daripada tindak balas hidrolisis berenzim ke atas ikan keli. Hidrolisat protein telah disediakan oleh hidrolisis enzimatik otot Keli..Hidrolisis berenzim telah dijalankan pada pH 9, 50 ºC, 2.5% (w/v) kepekatan substrat dan kepekatan enzim 1%. Darjah hidrolisis bagi dua sampel yang dipilih untuk proses penurasan pada masa 0.5 jam dan 5 jam ialah 44.88% dan 58.72%. Dua sampel hidrolisat yang berbeza pada 0.5 jam dan 5 jam telah digunakan untuk membandingkan aktiviti ntioksida dan juga kecekapan pemisahan hidrolisat berdasarkan kepada berat molekul. Pemilihan parameter yang optimum menunjukkan transmisi tertinggi dicapai pada pH 7, tekanan 1.5 bar, 0.15 M NaCl dan 1.5mg/ml kepekatan larutan masuk bagi hidrolisat 0.5 jam. Bagi membrane saiz 5 kDA, transmisi yang diperolehi antara 64.54-88.89% dan 84.62-93.14% bagi membrane bersaiz 10 kDa.Bagi hidrolisat 5 jam, transmisi tertinggi dicapai pada pH 5.1, tekanan 2 bar, 0.15 M NaCl dan kepekatan larutan masuk 1.5 mg/ml. Nilai transmisi berada pada julat 52.96-56.37% bagi membrane bersaiz 5 kDa dan 74.70-77.76% bagi membrane bersaiz 10 kDa. Hasil terbaik dicapai bagiaktiviti antioksida daripada pencerakinan DPPH adalah 69.04% bagi membran 10 kDa dan kadar ujian ion metal bagi 10 kDa adalah 74.06% yang diperolehi selama 0.5 jam. Serapan tertinggi iaitu 0.44±0.011 diperolehi daripada pencerakinan kuasa penurunan yang diperolehi bagi hidrolisat 5 jam menggunakan membrane ber saiz 5 kDa. Keputusan bagi hidrolisis dan proses ultraturasan menunjukkan bahawa dengan me manipulasikan dan pemilihan operasi parameter yang sesuai, dapat memper baiki darjah hidrolisis dan hasil proses penurasan (ketelapan fluks dan transmisi protein hidrolisat). Selain itu, keputusan menunjukkan kecekapan ultraturasan

untukmencapai aktiviti antioksida yang tinggi berdasarkan kepada pemisahan berat molekul.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS AND SYMBOLS

2, 2-diphenyl-1-picryl-hydrazylradical	(DPPH)
Acrylamide + bisacrylamide	(AB)
Alkoxy	(RO°)
Alkyl	(R°)
Amino acid composition	(AAC)
Ammonium persulfate	(APS)
Angiotensin-converting-enzyme	(ACE)
Bovine Serum Albumin	(BSA)
Butylated Hydroxyanisole	(BHA)
Butylated Hydroxytoluene	(BHT)
Catfish protein hydrolysate	(CFPH)
Cellulose acetate	(CA)
Chitosan	(CHI)
Degree of hydrolysis	(DH)
Deionized water	(DI)
Ethylenediaminetetraacetic acid	(EDTA)
Fat adsorption capacity	(FAC)
Feed concentration	(C_f)
Ferric ion	(Fe^{3+})
Ferrous ion	(Fe^{2+})
Fish protein hydrolysate	(FPH)
Free amino acid	(FAA)
Gel buffer	(GB)

Hemoglobin	(Hb)
Hydrochloric acid	(HCl)
Iron (II) chloride	($FeCl_2$)
Isoelectric point	(IEP)
Microfiltration	(MF)
Molecular weight cut-off	(MWCO)
Molecular weight	(MW)
Nanofiltration	(NF)
O-phthaldialdehyde	(OPA)
Permeate concentration	(C_p)
Peroxide	(ROO°)
Polyamide	(PA)
Polyethersulfone	(PES)
Polypropylene	(PP)
Polystyrenesulfonate	(PSS)
Polysulfone	(PS)
Polyvinylidene fluoride	(PVDF)
Propyl Gallate	(PG)
Protein solubility	(PS)
Reactive oxygen species	(ROS)
Retention	(Ret)
Reverse osmosis	(RO)
Sodium chloride	(NaCl)
Sodium dodecyl sulphate	(SDS)
Sodium hydroxide	(NaOH)

Tert Butylhydroquinone	(TBHQ)
Tetramethylethylenediamine	(TEMED)
Transmembrane pressure	(TMP)
Transmission	(Tr)
Trichloroacetic acid	(TCA)
Trinitro-benzene-sulphonic acid	(TNBS)
Ultrafiltration	(UF)
Water holding capacity	(WHC)

CHAPTER 1

INTRODUCTION

The separation or purification of proteins is a crucial process in biotechnology due to its wide range of applications in biomedical and food industries. The common laboratory techniques for protein separation such as chromatography, electrophoresis, and affinity operations due to the difficulty in scale-up and high operating cost with low throughput are not useful in biomedical and food industries (Scopes, 1994; Sarfert and Etzel, 1997; Lin *et al.*, 2008). Besides, some methods like chromatography and electrophoresis require complex instrumentation support to run efficiently. Hence, the separation techniques that can yield high throughput of the products at a low cost are highly desired in biotechnological industries. Ultrafiltration (UF) has attracted a considerable amount of attention in recent years for the separation of proteins due to comparatively gentler towards the proteins than separation process on phase changes and more economical than gel chromatography (Lin *et al.*, 2008).

In fishery industry, membrane process is applied to purify or separate valuable marine molecules from effluents, by-products from seafood processing industries (Bourseau *et al.*, 2009), to recover marine flavours(Vandanjon *et al.*, 2002) and concentrate polysaccharides (Lignot *et al.*, 2003). In membrane process where an ultrafiltration membrane is considered for protein separation, it is desirable to have a reasonable transmission of particular proteins by controlling of fouling which is considered as a main limiting factor on transmission and rejection. This issue could be controlled by manipulating the operating parameters. The studied operating parameters in this work, included: transmembrane pressure (TMP), pH, ionic strength and feed concentration which are related to physicochemical properties of feed and membranes (Field *et al.*, 1995; Lin *et al.*, 2008).

Recently, membrane separation process specially, ultrafiltration (UF) of single protein and proteins mixture by varying operating parameters has been considered. The effectiveness of membrane separation and filtration were reported in many research works such as, fractionation of whey protein (Almécija *et al.*, 2007), recovery of proteins from casein whey (Sarkar *et al.*, 2009), fractionation of BSA and lysozyme (Ghosh and Cui, 1998), ultrafiltration of mixed protein solutions of lysozyme and lactoferrin (Rabiller *et al.*, 2001), fractionation of fish protein hydrolysates (Bourseau *et al.*, 2009).

Annually, more than 100 million tons of fish are harvested, of which 29.5% is converted into fish meal (FAO, 2006). However, more than 50% of remaining fish tissue is converted to non-edible by-product material(Kristinsson and Rasco, 2000; Ovissipour *et al.*, 2010). Recognition of the limited biological resources and increasing environmental pollution have emphasized the need for better and more value-added utilization of under-utilized fish and by-products from the fishing industries (Guerard *et al.*, 2002; Ovissipour *et al.*, 2010). Hence, enzymatic hydrolysis was carried out to produce Catfish protein hydrolysates in this research work condition. Hydrolysis can improve the functional properties of proteins

(Quaglia and Orban, 1990; Klompong *et al.*, 2007)due to the changes in molecular size, hydrophobicity and polar groups of the hydrolysates (Adler-Nissen, 1986; Kristinsson and Rasco, 2000; Klompong *et al.*, 2007). Different applications and properties of this type of fish protein hydrolysates (nutritional and pharmaceutical)were mentioned in many latest works such as: Antioxidant and antiradical activity (Zhou *et al.*, 2012), anti cancer (Picot *et al.*, 2006), preservatives in foods and cosmetics (Guerard, 2007)and as a nitrogenous substrates for microorganisms growth (Guerard *et al.*, 2001).

In this study alcalase enzyme was applied for enzymatic hydrolysis due to the high activity and high yield of hydrolysis among the other proteolytic enzymes (Shahidi *et al.*, 1995; Benjakul and Morrissey, 1997). Recently, the tendency to use the natural additives (due to their bio-properties like antioxidant activity) instead of synthetic ones specially in food industries is increasing. This desire has led to the production of protein hydrolysates from different animal and plant sources, such as Alaska Pollack frame (Je *et al.*, 2005),egg-yolk (Sakanaka *et al.*, 2004) and alfalfa leaf (Xie *et al.*, 2008).

In addition, the most reported results on bioactivity of these hydrolysates is related to molecular weight range of less than 10 kDa (Wu *et al.*, 2003; He *et al.*, 2012; Li *et al.*, 2013; Wang *et al.*, 2013). Therefore, the aim in this research should be finding suitable methods and tools to increase the concentration of these value added products. A few works have been reported about separation and fractionation of fish protein hydrolysates so far (Chabeaud *et al.*, 2009a; Chabeaud *et al.*, 2009b; Saidi *et al.*, 2013).

The overall objective of this research was to study the behaviour of flux and transmission through ultrafiltration (UF) of catfish protein hydrolysates by manipulating the operating parameters. The specific objectives of this work were:

- To investigate the best parameters of enzymatic hydrolysis based on the degree of hydrolysis to produce high yield of catfish protein hydrolysates.
- To evaluate the performance of ultrafiltration to enhance the antioxidant activity of protein hydrolysates based on the molecular weight.

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