Kasetsart J. (Nat. Sci.) 48 : 929 - 941 (2014)

Effect of Pre-Hydrolysis by Alcalase on Enzymatic Membrane Reactor Performance in Production of Low Molecular Weight Peptide from Nile Tilapia Skin Gelatin

Mantaka Tauntong[1,3], Nualpun Sirinupong[2] and Wirote Youravong[1,3,*]

ABSTRACT

Enzymatic hydrolysis, performed under batch processing has several disadvantages including low productivity. To overcome some limitations, an enzymatic membrane reactor (EMR) has been proposed. Pre-hydrolysis possibly plays an important role in EMR performance. Therefore, the effects of pre-hydrolysis were investigated in an enzymatic reactor on the membrane performance of a low molecular weight peptide produced from Nile tilapia skin gelatin containing 4 % weight per weight alcalase at 50 °C, pH 8.0. The gelatin solution was pre-hydrolyzed for 0, 15, 30 or 60 min before passing through the 1 kDa molecular weight cutoff ultrafiltration membrane. The permeate flux, protein conversion, capacity, productivity, fouling resistance and angiotensin-I converting enzyme inhibitory activity were investigated. The results showed that the pre-hydrolysis time had a significant effect on the gelatin solution viscosity permeate flux and fouling. The results also indicated that pre-hydrolysis could noticeably increase EMR performance. A pre-hydrolysis gelatin solution pre-hydrolyzed with alcalase for 60 min was required to enhance the EMR performance during the production of low molecular weight peptides from Nile tilapia skim gelatin.

Keywords: bioactive peptide, angiotensin-I converting enzyme (ACE) inhibitory, enzymatic membrane reactor

INTRODUCTION

Nile tilapia (*Oreochromis niloticus*) is one of the fish species cultured in Thailand in large numbers (Yi and Diana, 2008). Furthermore, during processing, a lot of waste such as bone, scale and skin is generated which is usually discarded or sold at a low value to feed mills (Zeng *et al*., 2009). However, many researchers have reported that fish skin contains an abundance of collagen and the collagen or gelatin hydrolysate is usually produced by enzymatic hydrolysis of collagen (Nishimoto *et al*., 2008; Woo *et al*., 2008; Heu *et al*., 2010). Currently, gelatin is one of the most popular food supplement products (Ge *et al*., 2011). Gelatin hydrolysate exhibits various bioactivities, as an antioxidant (Jia *et al*., 2010; Lin *et al*., 2010; Liu *et al*., 2010; Ding *et al*., 2011), in angiotensin-I converting enzyme (ACE) inhibition (Saiga *et al*., 2008; Gu *et al*., 2011) and chondroprotective

1 Department of Food Technology, Faculty of Agro-Industry, Prince of Songkla University, Songkhla 90112, Thailand.

2 Nutraceutical and Functional Foods Research and Development Center, Prince of Songkla University, Songkhla 90112, Thailand.

3 Membrane Science and Technology Research Center, Prince of Songkla University, Songkhla 90112, Thailand

* Corresponding author, e-mail: wirote.y@psu.ac.th

Received date : 01/04/14 Accepted date : 11/09/14

effects (Bello and Oesser, 2006; Benito-ruiz *et al*., 2009; Nakatani *et al*., 2009). However, enzymatic hydrolysis performed in a batch-type process has several disadvantages because it is time-consuming, labor-intensive, requires a large space, has a high cost of enzymes (that cannot be reused) and requires non homogeneous products of varying molecular weights; there is inhibition of enzyme activity by the end product, with low yields and productivity and inactivation of the enzyme by pH adjustment or heat treatment at the end of the reaction (Chiang *et al*., 1999, Lin *et al*., 2010). To overcome some of these problems, the enzymatic membrane reactor (EMR) has been developed. The major advantages of an EMR are that the enzyme can be reused and the molecular size of the product can be governed by appropriate selection of the ultrafltration membrane (Chiang *et al*., 1999). However, flux decline and fouling are the main, critical problems in membrane application. Fouling is a result of blocking of the membrane pores by feed and product compounds or both. If high-molecular weight compounds such as proteins are the cause of fouling, this can be possibly mitigated by providing a pre-hydrolysis step before passing the substrate through the membrane separation unit. Pre-hydrolysis may also reduce the viscosity of the mixture and enhance or stabilize the enzymatic reaction in the EMR (Kim and Senevirathne, 2011). Therefore, the current study investigated the effects of pre-hydrolysis on the permeate flux, protein conversion, capacity and productivity of low molecular weight peptide produced from Nile tilapia skin gelatin.

MATERIAL AND METHODS

Materials

Nile tilapia skin (*Oreochromis niloticus)* (500–600 g per fish) was supplied from a factory located in Nakhon Phanom province, Thailand and shipped by air to the laboratory.

Nile tilapia skin collagen preparation

The Nile tilapia skin was mechanically and manually separated from the residual adhering tissue. Then, the skin was washed thoroughly with water and cut into 0.5 × 0.5 cm pieces. To remove noncollagenous proteins, the cleaned skin was treated with 10 volumes (volume per weight) of 0.05 N NaOH for 5 hr at 4 °C. To extract the acid soluble collagen (ASC), the skin was soaked in 0.5 M acetic acid at 24 ± 1 °C for 5 hr with a solid-solvent ratio of 1: 40 (weight per volume). The ASC obtained was diluted with water to make a final concentration at 0.7g.L^{-1}. The insoluble skin was removed by filtering with a sieve and the acetic acid was removed using a hollow fiber membrane (300 kDa molecular weight cut off, MWCO) and concentrated to 8.0 g.L^{-1}.

Continuous enzymatic membrane reactor system

The continuous enzymatic hydrolysis was performed using the device shown in Figure 1. A sample of 0.5 g of collagen was dissolved in 1 L of 0.05 mM phosphate buffer (pH 8) at 50 °C in a beaker and then was introduced into the hydrolysis tank. After the temperature and pH had stabilized, 160 µL of enzyme was added to the tank. The gelatin was hydrolyzed for 0, 15, 30 or 60 min before being introduced to the EMR. During the process, the pH was adjusted to pH 8.0 with 2.0 M sodium hydroxide using a disposable pipette and phosphate buffer was continuously fed into the hydrolysis tank to maintain the reactor volume. The inlet pressure and flow rate were adjusted using a pressure regulating valve. The reaction mixture was pumped continuously to the membrane filter unit equipped with a 1.0 kDa molecular weight cutoff polysulfone membrane, where small fractions passing through it collected as permeate, while the rejected mixture recycled back to the hydrolysis tank. The level of the reaction mixture in the vessel was controlled by adjusting the flow rates of the gelatin solution fed to the EMR and of the permeate, removed from the EMR system.

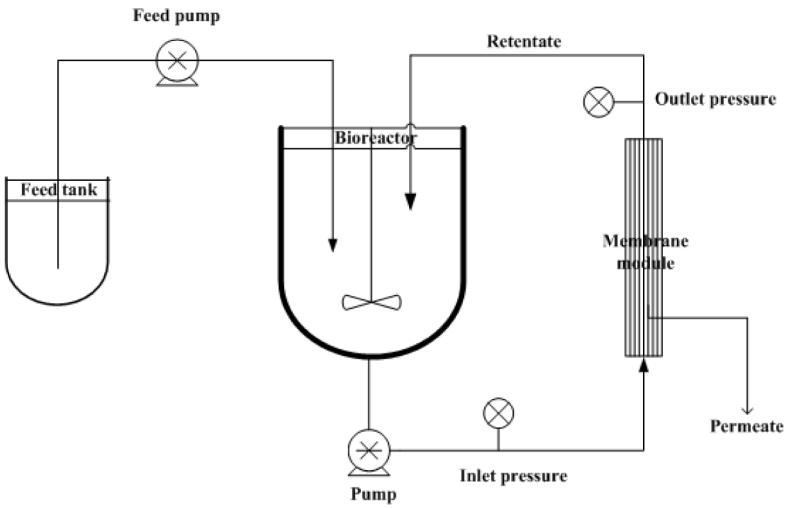

Figure 1 Schematic diagram of continuous enzymatic membrane reactor.

Analytical methods

Membrane performance

This procedure was undertaken according to Cui *et al*. (2010).

Conversion

The conversion index was used to illustrate the amount of protein recovered in permeate and was expressed as the ratio between the protein concentration in permeate to the concentration in the reactor. The total protein contents in the substrate and permeate were measured by the biuret method (Robinson and Hogden, 1940). Conversion (*X*) was expressed using Equation 1:

$$X(\%) = \frac{C_{permeate}}{C_{retentate}} \times 100 \quad (1)$$

where $C_{permeate}$ is the protein concentration in the permeate and $C_{retentate}$ is the protein concentration in the reactor, both measured in milligrams per milliliter.

Productivity

Productivity (P_{EMR}) was defined as the mass of protein produced per unit mass of enzyme or per unit volume of permeate. The productivity of the enzymatic membrane reactor (P_{EMR}) was expressed as Equation 2:

$$P_{EMR} = \frac{X.S_0.J_t.t}{E.V} \quad (2)$$

where S_0 is the total protein in the reactor tank measured in milligrams per milliliter, J_t is the permeate flow rate measured in milliliters per minute, T is the time period measured in minutes, E is the amount of enzyme measured in milligrams per milliliter and V is the substrate volume measured in milliliters.

Capacity

Capacity (*C*) was defined as the mass of protein produced per unit mass of enzyme per unit time and was expressed as Equation 3:

$$C = \frac{X.S_0.J}{E.V} \quad (3)$$

where S_0 is the total protein in the reactor tank measured in milligrams per milliliter, J_t is the permeate flow rate measured in milliliters per minute, E is the amount of enzyme measured in milligrams per milliliter and V is the substrate volume measured in milliliters.

Analysis of membrane fouling and resistance

The analysis followed Laorko *et al.* (2011). The permeate flux (J) was expressed by the

resistance-in-series model as shown in Equation 4:

$$J = \frac{TMP}{\mu R_t} \quad (4)$$

where J is the permeation flux measured in meters per second, TMP is the transmembrane pressure measured in bar, μ is the viscosity of the permeate measured in pascal seconds and R_t is the total resistance to the permeate measured per meter. R_t is classified by Equation 5 as follows;

$$R_t = R_m + R_f = R_m + R_{rf} + R_{if} \quad (5)$$

when $R_{if} = R_{if-in} + R_{if-ex}$

where R_m is the hydraulic resistance of cleaned membrane, R_f is the total (overall) fouling resistance, R_{if} is divided into two types: the external irreversible fouling resistance (R_{if-ex}) and the internal irreversible fouling resistance (R_{if-in}) where the latter is due to internal fouling inside the pores. In this study, R_{rf} was defined as the fouling removed by water flushing. The residual fouling after water flushing was R_{if} which was further removed by chemical cleaning. R_m was determined by measurement of the water flux of the clean membrane. R_t was calculated using Equation 4. After filtration of the gelatin solution, the water was flushed through the membrane surface to remove R_{rf} while the permeate valve was closed. Water flushing was operated with clean water at CFV of 1.4 $m.s^{-1}$ and TMP of 0.3 bar for 15 min. After the first water flushing, the permeate valve was opened and the water flux was measured to determine the residual fouling resistance. Then the permeate valve was closed again and 0.5 N NaOH solution was applied to clean the membrane at 50 °C, TMP 0.3 bar and a cross flow velocity (CFV) of 1.4 $m.s^{-1}$ for 40 min to remove external irreversible fouling. Then, the 0.5 N NaOH solution was removed by water flushing. After that, the water flux was measured to evaluate the residual resistance ($R_m + R_{if-in}$) and then R_{if-in} was removed by circulating 50 ppm of NaOCl at 50 °C with a TMP of 0.3 bar and a CFV of 1.4 $m.s^{-1}$ for 40 min. All types of resistance were calculated using Equations 5.

Chemical analysis

Residual enzyme activity

The residual enzyme activity ($A_{residual}$) was determined according to the method of Cheison *et al*. (2007) with slight modifications.

Measurement of angiotensin-I converting enzyme inhibitory activity

The ACE inhibitory activity was measured by the method of Cushman and Cheung (1971) with slight modifications. A sample of 50 μL of solution with 50 μL of ACE solution (2.5 $mU.mL^{-1}$) was pre-incubated at 37 °C for 10 min and the mixture was incubated with 50 mL of substrate (3 mM Hip-His-Leu (HHL)) in 100 mM borate buffer containing 0.3 M NaCl at pH 8.3) for 60 min at the same temperature. The reaction was terminated by the addition of 0.5 M HCl (200 μL). The resulting hippuric acid was extracted with 1.5 mL of ethyl acetate. After centrifugation (4,000×*g*, 15 min), 1 mL of the supernatant was transferred into a test tube and evaporated at room temperature for 4 hr under vacuum pressure. The hippuric acid was dissolved in 3.0 mL of distilled water and the absorbance was determined at 228 nm using a spectrophotometer (Genesys 10 UV-VIS Series; Thermo Scientific; West Palm Beach, FL, USA). The inhibition activity was calculated using Equation 6:

$$\text{Inhibition activity}(\%) = \frac{(A_a - A_b)}{A_a - A_c} \quad (8)$$

where A_a is the absorbance with ACE and HHL without the sample, A_b is the absorbance with ACE, HHL and the sample; and A_c is the absorbance with HHL and the sample. The IC_{50} (half maximal inhibitory concentration) value was defined as the concentration of inhibitor that could inhibit 50% of the ACE activity.

Statistical analysis

A factorial randomized complete block design was used for the statistical analysis. Data was subjected to analysis of variance. Mean comparisons were carried out using Duncan's multiple range test at a significance level of $P <$

0.05 using the statistical software package SPSS (SPSS 10.0 for Windows; SPSS Inc.; Chicago, IL, USA).

RESULTS

Effect of pre-hydrolysis on gelatin viscosity

The viscosity and degree of hydrolysis (DH) of gelatin solution at different hydrolysis times were measured. Figure 2 shows the viscosity and DH of 5 $g.L^{-1}$ gelatin solution at varying hydrolysis times using 4 % (w/w) alcalase at 50 °C and pH 8 in a batch reactor. The results illustrated that the DH sharply increased in a short initial period of hydrolysis and the viscosity also rapidly decreased with increasing DH. However, the viscosity tended to stabilize after 30 min of hydrolysis while the DH tended to increase with increasing hydrolysis time.

Effect of pre-hydrolysis on permeate flux

Measurements of the permeate flux (*J*) during the experiment (Figure 3) showed a slow decline in the flux rate for all pre-hydrolysis samples, excluding the sample with 15 min of pre-hydrolysis which showed a fast decline in the flux rate in the first 60 min of operation. It was also observed that the permeate flux of the sample with no pre-hydrolysis (0 min) was the lowest when compared with those of others samples with pre-hydrolysis times, while pre-hydrolysis for 15 min could increase the permeate flux by about two times. However, a long period of pre-hydrolysis (higher than 15 min) caused the permeate flux to decrease. In addition, the permeate flux reached a quasi steady-state value after an operating time of approximately 90 min.

Effect of pre-hydrolysis on membrane fouling

The effect of pre-hydrolysis on membrane fouling during the ultrafiltration of gelatin in an enzymatic membrane reactor at CFV of 0.5 $m.s^{-1}$ and TMP of 1.0 bar is shown in Table 1. It was observed that R_t, R_f and R_{if} values following pre-hydrolysis at 15 and 60 min were significantly decreased when compared with non pre-hydrolysis. However, pre hydrolysis for 30 min did not lead to a decrease in fouling. It was likely that pre-hydrolysis for 15 or 60 min was more effective in reducing the fouling than pre-hydrolysis for 30 min. In addition, pre-hydrolysis for 15 min caused a decrease in all fouling resistance levels.

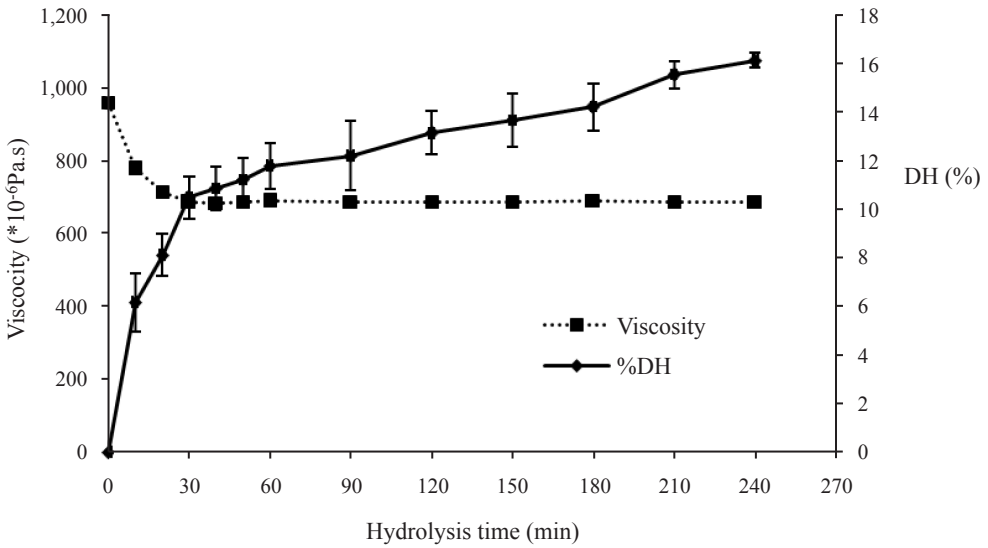

Figure 2 Viscosity and degree of hydrolysis (DH) of gelatin solution hydrolyzed with 4% alcalase at pH 8 and 50°C in a batch reactor. Vertical error bars show ± SE.

Effect of pre-hydrolysis on protein conversion

Protein conversion was used to illustrate the amount of protein recovered in permeate. Figure 4 shows the protein conversion at varying pre-hydrolysis times. It was observed that the protein conversion for all pre-hydrolysis time samples gradually increased over a short initial period of operation (0–90 min) then gradually decreased after 300 min of operation (for 60 min of pre-hydrolysis treatment) while it sharply decreased (for 0 and 30 min of pre-hydrolysis treatment). The results indicated that a long period of pre-hydrolysis (60 min) increased the protein conversion by about two times. The protein conversion of the sample pre-hydrolyzed for 15 min which had the highest permeate flux was lower than that pre-hydrolyzed for 60 min.

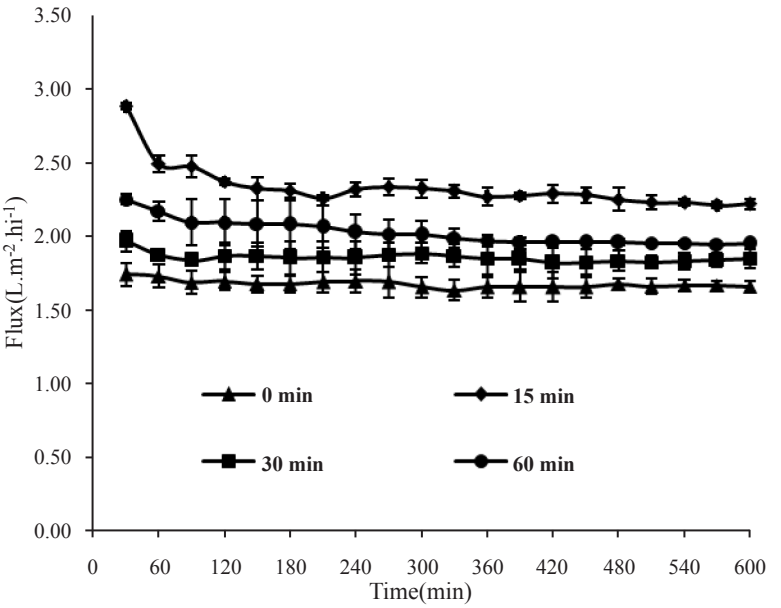

Figure 3 Effect of pre-hydrolysis on permeate flux of enzymatic membrane reactor (substrate concentrations, 5 g.L^{-1}; enzyme-substrate ratio, 4:100; transmembrane pressure, 1.0 bar; hydrolysis at pH 8 and 50 °C). . Vertical error bars show ± SE.

Table 1 Membrane fouling during continuous ultrafiltration membrane reactor. (Substrate concentrations, 5 g.L^{-1}; enzyme-substrate ratio, 4:100; transmembrane pressure, 1.0 bar; hydrolysis at pH 8 and 50 °C.)

Pre-hydrolysis time (min)	R_t ($\times 10^{13}$ m^{-1})	R_m ($\times 10^{13}$ m^{-1})	R_f ($\times 10^{13}$ m^{-1})	R_{rf} ($\times 10^{13}$ m^{-1})	R_{if} ($\times 10^{13}$ m^{-1})	$R_{if\text{-}ex}$ ($\times 10^{13}$ m^{-1})	$R_{if\text{-}in}$ ($\times 10^{13}$ m^{-1})
0	23.96±0.60^a	12.62	11.34±0.60^a	5.47±1.03^b	5.67±0.42^a	1.21±0.13^a	4.67±0.56^a
15	18.35±1.46^c	12.62	5.74±1.44^c	4.60±1.48^b	1.14±0.04^c	0.51±0.07^b	0.58±0.04^c
30	22.67±0.23^a	12.62	10.00±0.25^a	7.42±0.23^a	2.58±0.01^b	1.50±0.24^a	1.08±0.24^b
60	21.44±1.44^b	12.62	8.82±1.45^b	5.92±1.00^b	2.90±0.45^b	1.53±0.28^a	1.38±0.18^b

R_t = Total resistance; R_m = Hydraulic resistance of clean membrane; R_{rf} = Reversible fouling resistance; R_{if} = Irreversible fouling resistance; $R_{if\text{-}ex}$ = External irreversible fouling resistance; $R_{if\text{-}in}$ = Internal irreversible fouling.

Values are shown as mean±SD. Different lowercase superscript letters in a column show significant difference ($P < 0.05$). Three replicates were performed.

Effect of pre-hydrolysis on capacity

Capacity was defined as the mass of protein produced per unit mass of enzyme per unit time. Figure 5 illustrates the influence of pre-hydrolysis on the capacity of protein in an enzymatic membrane reactor. All capacities at all

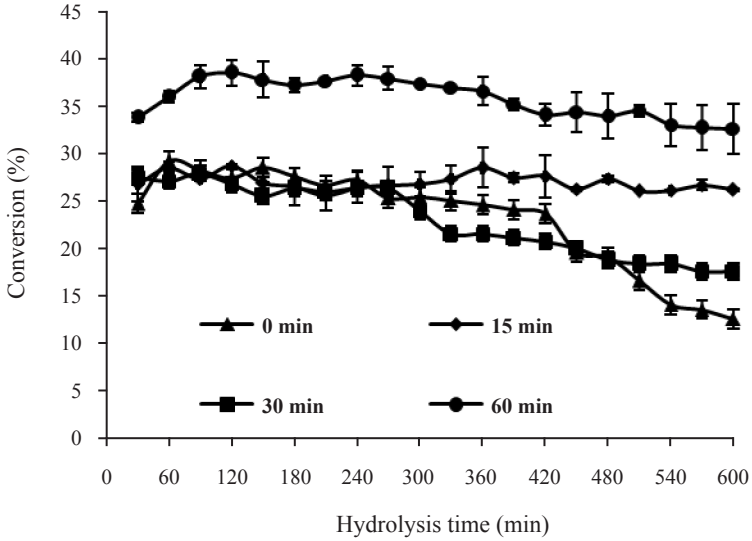

Figure 4 Effect of pre-hydrolysis on conversion of enzymatic membrane reactor (substrate concentrations, 5 $g.L^{-1}$; enzyme-substrate ratio, 4:100; transmembrane pressure, 1.0 bar; hydrolysis at pH 8 and 50 °C). . Vertical error bars show ± SE.

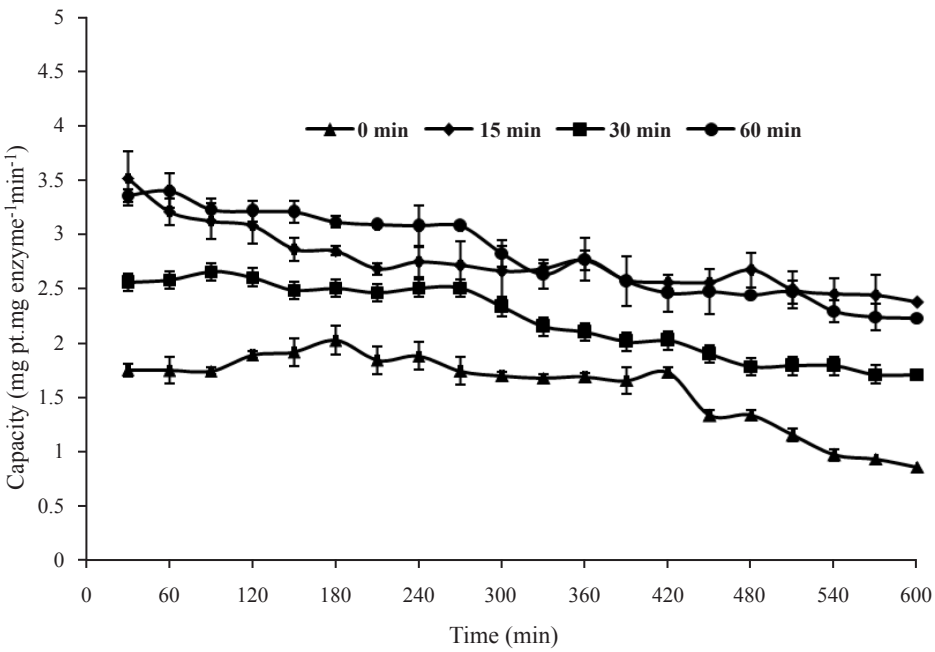

Figure 5 Effect of pre-hydrolysis on capacity of enzymatic membrane reactor (substrate concentrations, 5 $g.L^{-1}$; enzyme-substrate ratio, 4:100; transmembrane pressure, 1.0 bar; hydrolysis at pH 8 and 50 °C).

pre-hydrolysis times had a tendency to decrease with increasing operation time. However, the results indicated that pre-hydrolysis could increase the capacity of protein, and it was highest after 15 and 60 min of pre-hydrolysis.

Effect of pre-hydrolysis on productivity

In this study, the productivity was defined as the mass of protein produced per unit volume of permeate as shown in Figure 6. The results illustrate that in general the productivity of the EMR gradually increased with increasing EMR time. The lowest productivity was observed using the non pre-hydrolysis feed while the highest was observed using 60 min pre-hydrolysis. These results suggested that pre-hydrolysis could help to improve the productivity of the EMR.

Influence of pre-hydrolysis on the residual enzyme activity

During the operation, the residual enzyme activity of all feed samples pre-hydrolyzed with different times gradually decreased. Cui *et al*. (2010) found a similar trend with hydrolysis in the modification of wheat gluten using alcalase in a continuous EMR. However, in the current study, residual enzyme activity was not found in the permeate (data not shown). This result indicated that enzyme did not leak through the membrane into the permeate.

Influence of pre-hydrolysis on angiotensin-I converting enzyme inhibitory activity

After 10 hr of enzymatic membrane reactor operation, the total peptide in the permeate was subjected to measurement of the ACE inhibitory activities reported in terms of the IC_{50} defined as the concentration needed to inhibit 50 % of the original ACE activity under the conditions tested. The results are shown in Table 2. Pre-hydrolysis for 30 min provided the highest IC_{50} value which represented the lowest ACE inhibitory activity. Non pre-hydrolysis and pre-hydrolysis for 15 and 60 min showed similar IC_{50} values.

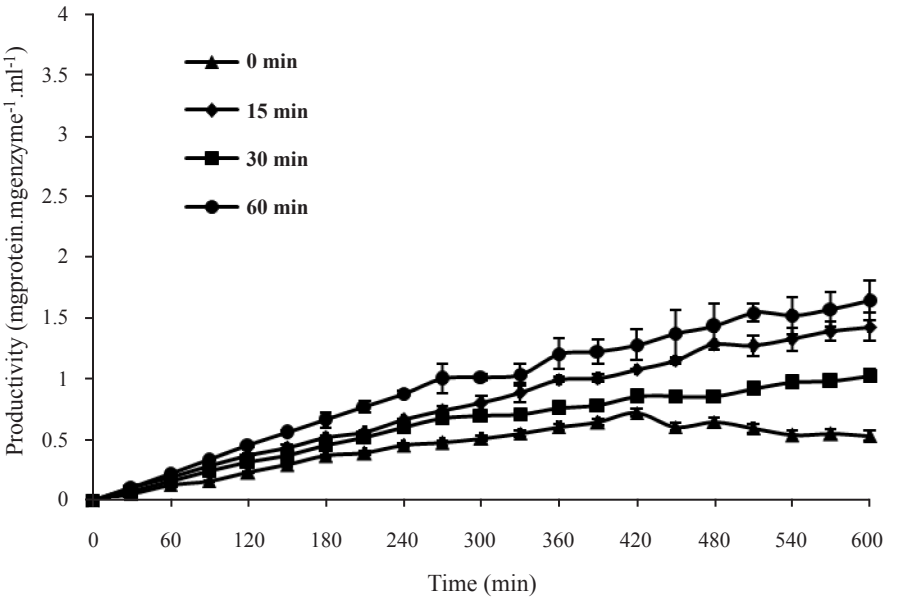

Figure 6 Effect of pre-hydrolysis on productivity of enzymatic membrane reactor (substrate concentrations, 5 $g.L^{-1}$; enzyme-substrate ratio, 4:100; transmembrane pressure, 1.0 bar; hydrolysis at pH 8 and 50 °C). . Vertical error bars show ± SE.

DISCUSSION

Enzymatic membrane reactors have been used to produce bioactive peptides. However, flux decline and membrane fouling are critical problem especially when complex structured, high molecular weight, compound mixtures such as collagen or gelatin are used as a liquid feed. Gelatin is a complex structure and a high molecular weight protein with around 100 kDa per chain and is a denatured form of collagen induced by heat or stress. In this study, the collagen was heated to 50 °C and thus it was transformed to gelatin. This gelatin was hydrolyzed with 4 % w/w alcalase at pH 8 and 50 °C. The viscosity of the gelatin decreased from 1,000 × 10^{-6} to 700 × 10^{-6} Pa.s within 30 min of hydrolysis as shown in Figure 2. The viscosity of gelatin is partially controlled by its molecular weight as a higher molecular weight will increase gelatin viscosity (Jamilah *et al*., 2011). During hydrolysis, low molecular weight peptides were generated leading to a decrease in gelatin viscosity. However, the viscosity result suggested that a long period of hydrolysis did not

Table 2 IC_{50} (half maximal inhibitory concentration) value of angiotensin-I converting enzyme - inhibitory activities of the permeate from different pre-hydrolysis times**.**

Pre-hydrolysis time (min)	IC_{50} ($mg.mL^{-1}$)
0	0.6047 ± 0.0547^{a}
15	0.6246 ± 0.0608^{a}
30	0.9295 ± 0.0727^{b}
60	0.6334 ± 0.0688^{a}

Six replicates were performed.

Values are shown as mean±SD. Different lowercase superscript letters in a column significant a significant difference ($P < 0.05$).

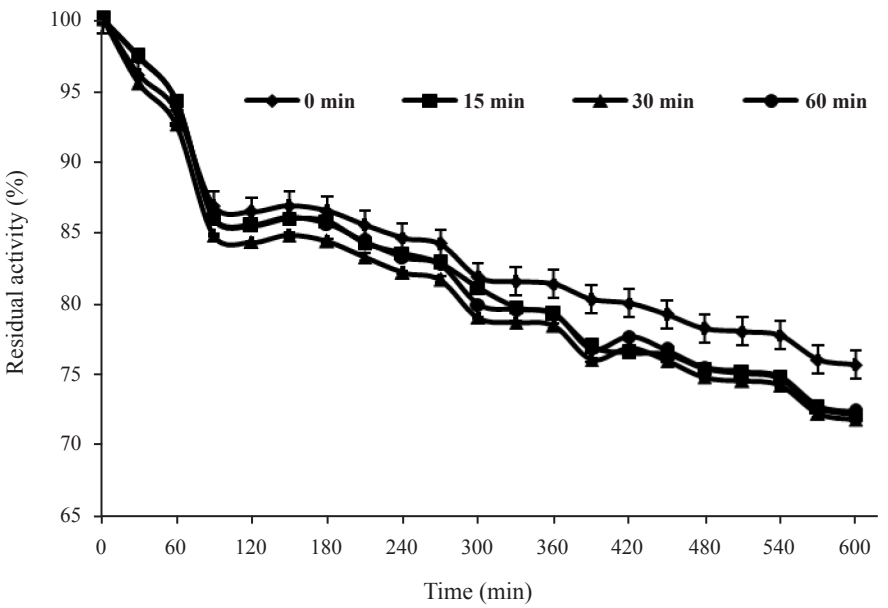

Figure 7 Effect of pre-hydrolysis on residual activity of enzymatic membrane reactor (substrate concentrations, 5 $g.L^{-1}$; enzyme-substrate ratio, 4:100; transmembrane pressure, 1.0 bar; hydrolysis at pH 8 and 50 °C). . Vertical error bars show ± SE.

affect the gelatin viscosity which was probably affected by the polydiversity of peptides depending on the molecular distribution (Gudmundsson and Hafsteinsson, 1997; Jamilah *et al*., 2011). The viscosity of the substrate probably influenced membrane fouling and EMR performance as well as the quality of the product. Therefore pre-hydrolysis in the range 0–60 min was chosen for further study.

Gelatin solution was pre-hydrolyzed for 0, 15, 30 and 60 min with 4 % w/w of 2.4 L alcalase enzyme at pH 8 and 50 °C in a stirred reactor tank before passing through the membrane unit. The presence of a pre-hydrolysis process before starting the EMR mitigated membrane fouling due to a decrease in the substrate viscosity (Rios *et al*., 2004), and an increase in the dissolvability of low molecular weight peptides (Wang *et al*., 2013) which were helpful in decreasing the blocking of membrane pores by high molecular weight peptides (Wang *et al*., 2013). The current results also showed that pre-hydrolysis could increase the permeate flux and reduce membrane fouling. Even though pre-hydrolysis for 15 min provided the highest permeate flux and the lowest fouling, prolongation of pre-hydrolysis did not increase the permeate flux when compared with pre-hydrolysis for 15 min. This phenomenon was probably explained by the effect of the molecular weight of the peptide rather than by the viscosity of the gelatin solution. During hydrolysis, low molecular weight peptides were generated and a long period of hydrolysis would reduce the molecular weight of the peptides which might be suitable to become absorbed on the membrane pore wall surface thus narrowing the pore and consequently reducing the permeate flux. Sman *et al*. (2012) reported that complete blocking occurs with particles that could fit the size of the pore, which completely impeded the pore mouth, while standard blocking occurs with particles much smaller than the pore size, which are absorbed on the walls of the pores. In addition, they also pointed out that standard blocking is thought to be irreversible fouling which is related to the results of high irreversible fouling resistance (R_{if}) after both 30 and 60 min of pre-hydrolysis as indicated in Table 1. While pre-hydrolysis for 15 min produced a high molecular weight peptide able to block the pore, it was probably less of a pore narrowing mechanism.

Conversion and capacity are good indicators of changes in EMR performance (Chiang *et al*., 1999). In the current study, protein conversion, capacity and productivity were used to indicate the EMR performance related to the protein content in permeate, permeate flux and enzyme concentration. Although pre-hydrolysis for 15 min provided the highest flux, it had the lowest membrane performance index because it had a low protein content in the permeate leading to low conversion, capacity and productivity while pre-hydrolysis for 60 min provided the highest conversion, capacity and productivity. These results suggested the effect of pre-hydrolysis time on the size of the derived peptides and their influence on the separation characteristics of the process. Increasing the pre-hydrolysis time decreased the size of the peptides which can permeate freely through the membrane and protein retention. Thus a higher concentration of peptides in the permeate led to increased protein conversion. In addition, the productivity in this study was slightly low when compared with others. Cui *et al*. (2010) hydrolyzed wheat gluten in an enzymatic reactor membrane coupling with 10 kDa MWCO using alcalase. The working conditions were substrate concentrations of 20 $g.L^{-1}$, an enzyme-substrate ratio of 0.03, an operating pressure of 0.4 bar and a temperature of 40 °C and pH of 9. They reported that the productivity gradually increased to a maximum value around 6 mg $N.mg^{-1}$ enzyme which was about twice as high as the productivity of the current study. However, it can be further improved by studying the optimum conditions of both separating factors and reaction factors such as the cross flow velocity, substrate concentration or the enzyme-substrate ratio.

The decrease in enzyme activity could

be explained by several reasons. First, the effect of the cross flow velocity was tied to enzyme leakage through the membrane and led to a lower residual activity (Cheison *et al*., 2006). Second, a higher flow rate may be attributed to the effect of shear forces and lead to the loss of enzyme activity (Harrington *et al*., 1991). Finally, the enzyme activity was reduced because of the use of high temperature (50 °C) for a long period. All these factors led to a decrease in the residual activity in the EMR.

Enzymatic hydrolysis can release bioactive peptides from proteins as bioactive peptides usually contain 2–20 amino acid residues per molecule (Pihlanto-Leppala, 2000). Angiotensin converting enzyme plays an important role in the regulation of blood pressure (Wijesekara *et al*., 2011). In addition, previous studies of ACE-inhibitory peptides indicated that low molecular weight peptides show a high ACE inhibitory activity such as tri-peptide (Val-Ala-Pro) in grass carp protein hydrolysate (Chen *et al*., 2012); penta-peptide (Glu-Asp-Pro-Gly-Ala) in sea cucumber galatin hydrolysate (Zhao *et al*., 2007); hexa and octa-peptide in seaweed pipefish muscle protein hydrolysate (Wijesekana *et al*., 2011) and tetra-peptide (Ala-His-Leu-Leu) in loach (Li *et al*., 2012). Thus, the current study used 1 kDa MWCO membrane in the EMR. The ACE inhibitory activity of the peptides in the permeate was investigated and reported as IC_{50} and the present results suggested that gelatin peptides prepared in an EMR will be useful in pharmaceutical industries owing to their bioactive properties.

CONCLUSION

The study showed that pre-hydrolysis for 60 min could increase the EMR performance as indicated by the protein conversion, capacity and productivity and did not affect the ACE inhibitory activity of the peptides. Pre-hydrolysis of the gelatin solution with alcalase for 60 min was required to improve the EMR performance during the production of low molecular weight peptides from Tilapia skin gelatin. In addition, the EMR was proven to be an effective process to produce low molecular weight peptides with ACE inhibitory activity properties. However, the EMR optimum conditions should be further studied to improve performance.

ACKNOWLEDGEMENT

This study was supported by the Higher Education Research Promotion and the National Research University Project of Thailand, Office of the Higher Education Commission, the Thailand Research Fund under the Royal Golden Jubilee Ph.D. Program (PHD/022/2552) and the Graduate School, Prince of Songkla University, Songkhla, Thailand.

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