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Food Chemistry 98 (2006) 725–732
Food Chemistry
www.elsevier.com/locate/foodchem

Angiotensin I-converting enzyme inhibitor derived from soy protein hydrolysate and produced by using membrane reactor

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Received 28 February 2005; received in revised form 28 June 2005; accepted 28 June 2005

Abstract

Five different proteolytic enzymes, including Alcalase, Flavourzyme, trypsin, chymotrypsin and pepsin were employed to hydrolyze isolated soy protein (ISP) to produce the hydrolysates, respectively. The result indicated that hydrolysis of ISP for 0.5–6 h with Alcalase produced the highest ACE inhibitory activity. Therefore, Alcalase was selected for further study on optimization of hydrolysis conditions. The optimum conditions for Alcalase to hydrolyze ISP to produce the lowest IC_{50} value were: $E/S = 0.01$, hydrolysis temperature = 50 °C, pH 9.0 and hydrolysis time = 6 h. Under these conditions, the IC_{50} value of ISP was significantly reduced from 66.4 to 0.67 mg protein/ml. The lower IC_{50} value represented the higher the ACE inhibitory activity. Moreover, several membranes with molecular weight cut-offs (MWCFs) of 1000–30,000Da were used to filter the hydrolysate. The 10 kDa permeate obtained from the treatment of the hydrolysate by 10,000 Da MWCF membrane could further reduce its IC_{50} value from 0.668 to 0.078 mg protein/ml with a peptide recovery of 67.5%. An operation stability study showed that the membrane reactor system could maintain a steady production of ISP hydrolysate for over 8 h. The in vitro effect of gastrointestinal protease on ACE inhibitory activity of 10 kDa permeate was also investigated. The results suggested that gastrointestinal proteases have very little effect on the ACE inhibitory activity of 10 kDa permeate.

Keywords: Isolated soy protein; Protein hydrolysate; ACE inhibitor; Peptide; Membrane reactor

1. Introduction

Hypertension is related to the incidence of coronary heart disease and treatment of hypertension is effective in reducing the risk of the disease (Collins et al., 1990; MacMahon et al., 1990). The angiotensin I-converting enzyme (ACE) (EC 3.4.15.1) is physiologically important in the regulation of blood pressure. ACE raises blood pressure by converting the inactive decapeptide angiotensin I to the potent vasoconstrictor octapeptide angiotensin II, as well as inactivating the vasodilating nonapeptide bradykinin (Erdos, 1975). Therefore, inhibition of ACE reduces the activity of angiotensin-II but increases bradykinin levels, and thus can result in a lowing of blood pressure (Koike, Ito, Miyamoto, & Nishino, 1980).

Since the discovery of a peptide ACE inhibitor in snake venom, many ACE inhibitors have been synthesized and various synthetic ACE inhibitors are currently in use as antihypertensive drugs, such as captopril and enalapril. ACE-inhibitory peptides derived from food proteins may prevent hypertensive diseases. For this purpose, many peptide derivatives of ACE inhibitors mainly from animal protein, such as casein, tuna muscle

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doi:10.1016/j.foodchem.2005.06.038

and bonito, have been developed (Yamamoto, 1997). Several fermented soy products have also been reported with soy-protein-derived ACE inhibitory peptides during fermentation, such as soy sauce, soy paste, natto, tempe (Cho et al., 2000; Okamoto, Hanagata, Matsumoto, Kawamura, & Koizumi, 1995; Shin et al., 2001).

Enzymatic hydrolysis is very costly in conventional batch-type operations because large quantities of enzyme are required and the enzyme cannot be reused (Adler-Nissen, 1986; Cheftel, Ahern, Wang, & Tannenbaum, 1971). Other disadvantages of the batch process have also been reported, such as time-consumtion, labour-intensity, low yield, low productivity and inconsistent quality (Deeslie & Cheryan, 1988; Lin, Chiang, Cordle, & Thomas, 1997; Prendergast, 1974; Xavier, Goncalves, Moreira, & Carrondo, 1995). In contract, the development and application of an ultrafiltration membrane reactor for the hydrolysis of proteins have been applied to overcome those problems (Deeslie & Cheryan, 1981). Several studies have concluded that production of protein hydrolysates in a continuous membrane reactor results in higher productivities (Chiang, Chih, & Chu, 1999; Iacobucci, Myers, Emi, & Myers, 1974) and more uniform products (Chiang, Cordle, & Thomas, 1995; Deeslie & Cheryan, 1981) than batch-type reactors.

The objectives of this present research were to screen both the proper enzyme and ultrafiltration membrane to apply in a continuous membrane reactor for production of soy protein hydrolysate with ACE inhibitory activity, to evaluate the performance of the membrane reactor for production of the hydrolysate by measuring its productivity and operation stability and to determine product quality by measuring its ACE inhibitory activity, IC_{50}, during processing and after hydrolyzing by gastric enzymes.

2. Materials and methods

2.1. Materials

POMP® Isolated soy protein (ISP) was purchased from Gemfont Company (Taipei, Taiwan). Commercial enzymes, Flavourzyme® Type A and Alcalase® 2.4 L, were purchased from NOVO Industri A/S (Copenhagen, Denmark). Trypsin, chymotrypsin and pepsin were purchased from Sigma Chemical Co. (St. Louis, MO). HPLC standards including cytochrome *c*, aprotinin, gastrin I, substance P, vitamin B12, and glycine were purchased from Merck (Damdstadt, Germany) or Sigma Chemical Co. (St. Louis, MO). Polyethersulfone spiral-wound membranes, including GE 1 kDa, PW 10 kDa and ER 30 kDa, were purchased from Osmonics Inc., USA.

2.2. Effects of different protease hydrolyses on ACE inhibitory activity

The 3.0% (w/v) ISP and 1% (w/w of ISP) protease were employed to produce and compare each hydrolysate with ACE inhibitory activity. Proteases employed in the study included Alcalase, Flavourzyme, trypsin, chymotrypsin and pepsin. The optimum reaction conditions of temperature and pH for each enzyme were suggested by the internal document of the supplier. The temperature and pH were adjusted to 55 °C and 7.5 for Alcalase, 45 °C and 6.0 for Flavourzyme, 37 °C and 7.5 for trypsin and chymotrypsin and 37 °C and 2.0 for pepsin, respectively. Samples, withdrawn at 0.5, 1, 2, 3, 4, 6 h from each proteolytic mixture, were immediately heated in a boiling water bath for 10 min, followed by centrifugation at 3000 rpm in a microcentrifuge for 10 min. The supernatants were used to determine their degree of hydrolysis (DH) and ACE inhibitory activity. The DH at any time was measured by the orthophthaldialdehyde (OPA) method described by Frister, Meisel, and Schlimme (1998).

2.3. Assay for ACE inhibitory activity

ACE inhibitory activity was analyzed by spectrophotometrically using hippury-L-histidyl-L-leucine (HHL) as substrate, according to the method of Cushman and Cheung (1971). Five millimolar HHL was prepared with 0.1 M sodium borate buffer (pH 8.3) containing 0.4 M NaCl. ACE from rabbit lung was dissolved in the same buffer at a concentration of 60 mU/ml. A mixture containing 225 μl of HHL solution and 25 μl of protein hydrolysate or the buffer (control) was incubated at 37 °C for 5 min, 75 μl of ACE solution was then added and the mixture incubated for 30 min. The reaction was stopped with 20 μl of 0.1% trifluoroacetic acid (TFA). Hippuric acid (HA) liberated by ACE was determined by RP-HPLC on a LiChrospher C18 column (4 × 250 mm, Merck, Germany). The mobile phase was 0.1% TFA in 50% methanol with a flow-rate of 0.8 ml/min. The effluent was monitored with an ultraviolet detector (Shimadzu, Tokyo, Japan) at 228 nm. The IC_{50} value was defined as the concentration of ACE inhibitor or protein hydrolysate needed to reduce 50% of the height of the HA peak, and determined by regression analysis of ACE inhibitory activity (%) versus protein concentration. The IC_{50} value was expressed as mg protein/ml. ACE inhibitory activity (%) was expressed as

$$\text{ACE Inhibitory Activity } (\%) = \frac{H_0 - H_p}{H_0} \times 100, \quad (1)$$

where H_0 is the height of HA peak without protein hydrolysate; H_p is the height of HA peak with protein hydrolysate.

2.4. Effect of different ultrafiltration membranes on ACE inhibitory activity

Hydrolysates generated by enzymatic hydrolysis of isolated soy protein were subjected to ultrafiltration using spirally-wound membranes with 30, 10 and 1 kDa molecular weight cut-offs, individually. Each permeate was collected to determine its protein concentration, profile of molecular weight distribution and IC50 for ACE inhibitory activity.

2.5. Batch reactor

The batch reactor consisted of a reaction vessel (1 l) with hot water jacket and stirring arm to provide uniform heating and mixing. Five to ten hundred millilitres of 3% ISP solution were introduced into the reaction vessel and the mixture heated. On reaching the desired temperature, a 2.5% (w/w) solution of enzyme was added.

2.6. Continuous spiral-wound (SW) membrane reactor system

A 3% ISP solution, prepared by vigorously stirring and heating, was passed through a 100-mesh sieve to remove large particles. The reaction vessel (Fig. 1) was filled with the desired volume of 3% of ISP filtrate and the temperature of the tank was maintained at 50 °C. 2.5% enzyme/substrate (w/w) was added to the soy protein filtrate. Inlet pressure and flow rate were properly adjusted. The reaction mixture was pumped to the spiral-wound membrane where the large particles, such as intact proteins or enzymes which could not penetrate the pores of ultrafiltration membrane, would recycle to the reaction vessel. The permeate, containing particles small enough to penetrate the membrane was collected and lyophilized. The level of reaction mixture in the vessel was controlled by adjusting the flow rates between the ISP solution from the feed tank and the permeate from the membrane.

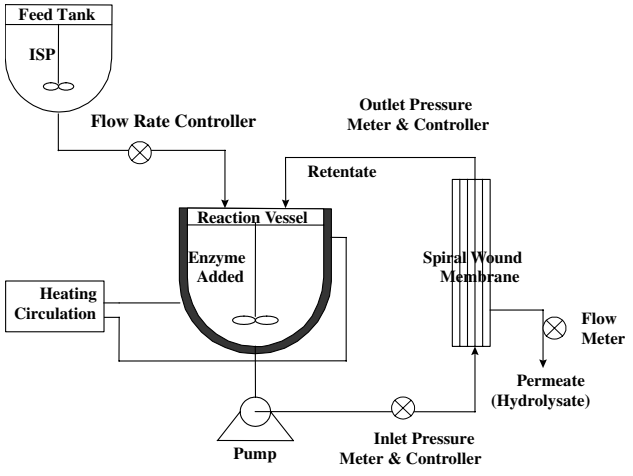

Fig. 1. Schematic diagram of continuous spiral-wound membrane system.

2.7. Total nitrogen

Total nitrogen was measured by the micro-Kjeldahl method (AOCS, 1989), using a KjelTec system 1026 with rapid distillation unit (Tecator, Sweden). All data were corrected for non-protein nitrogen (NPNi), which was determined as the initial nitrogen of ISP soluble in 10% trichloroacetic acid (TCA). The TCA-soluble nitrogen of the product (NPNp) was also determined. The % TCA soluble nitrogen or % conversion, X, was expressed as

$$X(\%) = \frac{\mathrm{NPNp} - \mathrm{NPNi}}{\mathrm{TN} - \mathrm{NPNi}} \times 100, \tag{2}$$

where TN is the total nitrogen in the product.

2.8. Productivity study

For the batch reactor, productivity was defined as

$$P_{\mathrm{batch}} = \frac{X\mathrm{TN}_i}{E}, \tag{3}$$

where E is the enzyme concentration (mg/ml); TN_i is the total nitrogen in unhydrolyzed substrate corrected for NPNi.

For the continuous HF membrane reactor, productivity was expressed as

$$P_{\mathrm{I}} = \frac{PJt}{EV} \tag{4}$$

or

$$P_{\mathrm{c}} = \Sigma P_{\mathrm{I}}, \tag{5}$$

where P_{I} is the instantaneous productivity; P is the average product output (mg N/ml) in a time period t (min); J is the flow rate (ml/min); E is the enyzme concentration (mg/ml); V is the substrate volume (l); P_{c} is the cumulative productivity. P_{c} was calculated over all time periods studied. The unit of productivity was mass hydrolysate/mass enzyme (mg N/mg enzyme). A more complete description of productivity was given by Cheryan and Deeslie (1983).

2.9. Molecular weight distribution

The molecular weight distribution of the hydrolysate was analyzed by high-performance size-exclusion chromatography (HPSEC). The HPSEC consisted of a

Superdex HR 10/30 column, connected to a UV detector set at 214 nm. The mobile phase was 0.02 M phosphate buffer (pH 7.2) containing 0.25 M NaCl and flow rate was set at 0.5 ml/min.

2.10. Stability of ISP derived ACE inhibitory peptides

The stability against in vitro gastric proteases was assessed as described by Wu and Ding (2002). One percent (w/v) of hydrolysate solution was treated, in 0.1 M KCl–HCl (pH 2.0) buffer, with pepsin for 4 h in a water bath at 37 °C, stopped by boiling in a water bath for 15 min and neutralized to pH 7.0 with addition of 2 N NaOH solution. One millilitre of neutralized suspension was centrifuged (10,000*g*, 40 min) and the supernatant was used for ACE inhibitory activity determination. The remaining neutralized suspension was digested further by 2% (w/w) pancreatin at 37 °C for 4 h. The enzyme was inactivated by boiling for 15 min followed by centrifugation, and the supernatant was used for ACE inhibitory activity determination.

3. Results and discussion

3.1. Effect of different protease hydrolyses on ACE inhibitory activity

Isolated soy protein (ISP) was hydrolyzed by Alcalase, Flavourzyme, chymotrypsin, pepsin and trypsin for 0.5–6 h, respectively. Fig. 2(a) and (b) show the comparison of ACE inhibitory activities and degrees of hydrolysis (DH), respectively, within five enzyme hydrolysates as a function of hydrolysis time. The DH and ACE inhibitory activity increased with the increase of hydrolysis time for each enzyme. These graphs clearly showed that hydrolysis was required to release ACE inhibitory peptides from the inactive form of intact ISP. After 2 h of hydrolysis Flavourzyme showed the highest DH but had the lowest ACE inhibitory activity. The results indicate that higher DH dose not guarantee a higher ACE inhibitory activity. This may be due to the fact that Flavourzyme contains both endoprotease and exoprotease activities, which offer the ability to achieve higher DH of ISP than the other enzymes (Pommer, 1995), but at the same time, the exoprotease may also involve the inactivation of the active peptide sequence by cutting one or more amino acids from N-terminal or C-terminal positions.

However, regardless of Flavourzyme, the increase of DH of ISP had a positive effect on the increase of ACE inhibitory activity by using the other four enzymes. All of the enzymes could release most of the ACE inhibition activity during the 30–60 min of the hydrolysis time. Among the four enzymes, Alcalase showed the highest DH and highest ACE inhibitory activity at any hydrolysis time. Therefore, Alcalase was selected for further study on enzymatic production of ACE inhibitor from ISP. Alcalase is a commercialized alkaline protease for industrial use and has been studied to hydrolyze plasma, albumin, casein, gelatin and some vegetable proteins to produce ACE inhibitor (Hyun & Shin, 2000; Yust et al., 2003).

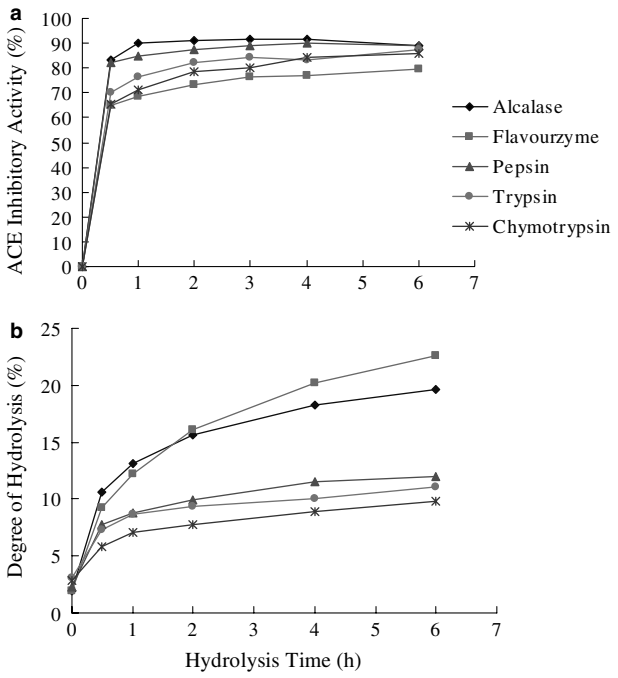

Fig. 2. Comparison of: (a) ACE inhibitory activity; and (b) degree of hydrolysis (DH) within five enzyme hydrolysates as a function of hydrolysis time. Results are mean values of at least two replicates. Standard deviation did not exceed 3% of the recorded values. The reaction conditions of temperature and pH were adjusted to 55 °C and 7.5 for Alcalase, 45 °C and 6.0 for Flavourzyme, 37 °C and 7.5 for trypsin and chymotrypsin, 37 °C and 2.0 for pepsin.

3.2. Hydrolysis conditions for Alcalase to produce ACE inhibitor

A batch reactor was used to evaluate the optimum hydrolysis conditions for Alcalase to hydrolyze ISP for 1 h to produce ACE inhibitory peptides. One hour of hydrolysis time was selected, based on the result of Fig. 2(a) for Alcalase. The optimum enzyme to substrate (*E*/*S*) ratio was determined by hydrolysis of 3.0% ISP with an *E*/*S* ratio from 0.025 to 2.25 for 1 h at 50 °C under pH 9.0. Fig. 3 shows the ACE inhibitory activity of hydrolysate as a function of *E*/*S* ratio. The ACE inhibitory had a tendency to increase with increased of *E*/*S* ratio. As the *E*/*S* ratio employed was 0.01 and above, the ACE inhibitory activity of hydrolysate could reached maximum at 85%.

Fig. 4 illustrates ACE inhibitory activity of hydrolysate as a function of temperature. The inhibitory activity of the hydrolysate gradually increased from 30 to 50 °C.

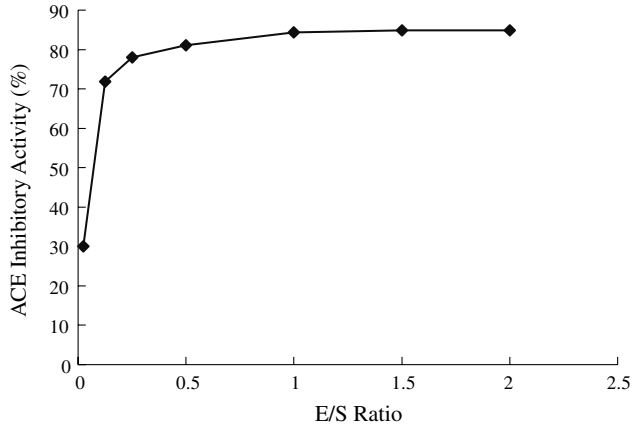

Fig. 3. ACE inhibitory activity of hydrolysate as a function of E/S ratio. Results are mean values of at least two replicates. Standard deviation did not exceed 2% of the recorded values.

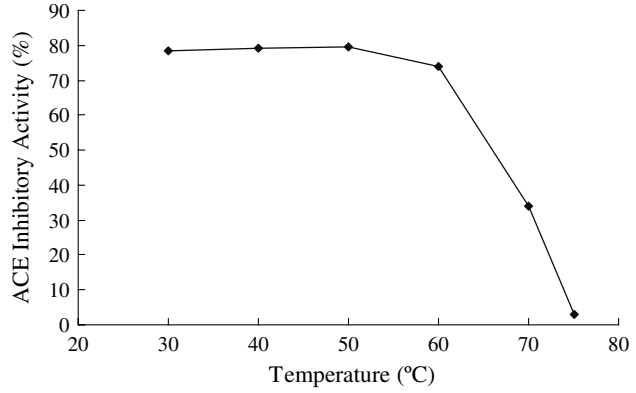

Fig. 4. ACE inhibitory activity of hydrolysate as a function of temperature. Results are mean values of at least two replicates. Standard deviation did not exceed 2% of the recorded values.

At temperatures above 60 °C, ACE inhibitory activity would dramatically decrease. The effect of pH on ACE inhibitory activity was also determined. The hydrolysate showed 75% to 82% inhibitory activity from 5 to 10 (Fig. 5). As the pH reached 9.0, the maximum ACE inhibitory activity could be achieved but there was very low activity both at pH < 5 and pH > 10. These results indicate that the optimum working conditions for Alcalase to hydrolyze ISP for 1 h to produce ACE inhibitory peptides were: $E/S = 0.01$, temperature = 50 °C, pH 9.0.

The effect of hydrolysis time on the ACE inhibitory activity, in terms of IC_{50}, was further evaluated under the optimum hydrolysis conditions for Alcalase (Fig. 6). A lower IC_{50} represents a higher ACE inhibitory activity. The IC_{50} of intact ISP was notably decrease from 66.4 to 1.79 mg protein/ml in 1 h of hydrolysis. As the hydrolysis time increased, the IC_{50} of the Alcalase-ISP hydrolysate also had a tendency to decrease. However, the IC_{50} levelled off after 6 h of hydrolysis.

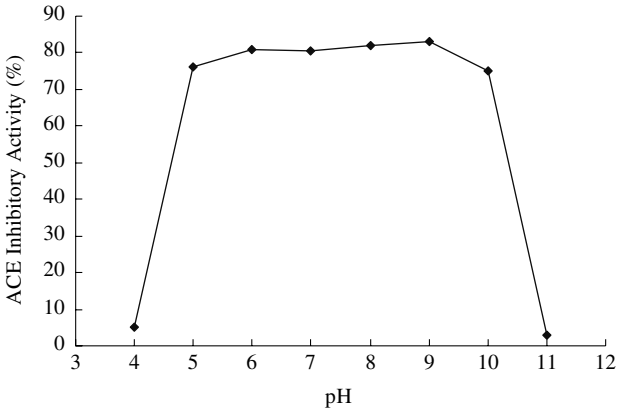

Fig. 5. ACE inhibitory activity of hydrolysate as a function of pH. Results are mean values of at least two replicates. Standard deviation did not exceed 2% of the recorded values.

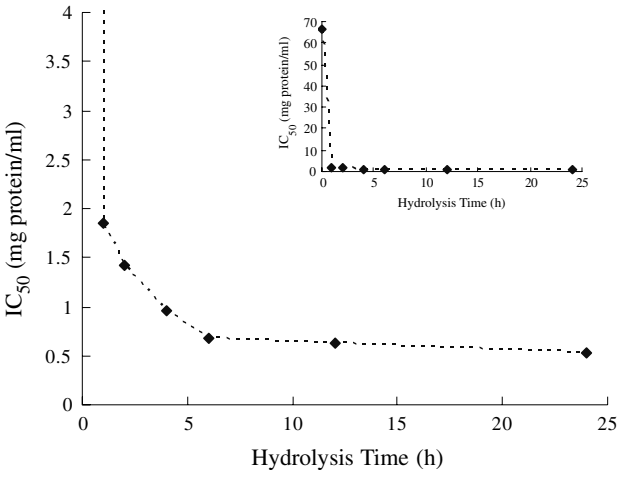

Fig. 6. The effect of hydrolysis time on the ACE inhibitory activity in terms of IC_{50}, further evaluated under the optimum hydrolysis conditions for Alcalase. Results are mean values of at least two replicates. Standard deviation did not exceed 2% of the recorded values.

3.3. Effect of different ultrafiltration membranes on ACE inhibitory activity

ISP hydrolysate (ISPH) was prepared by hydrolysis of 3.0% ISP with 1% Alcalase at 50 °C at pH 9.0 for 6 h and heated in a boiling water bath for 10 min, followed by centrifugation at 3000 rpm for 10 min. The ISPH was collected for further study on fractionation of ACE inhibitory peptides by spiral-wound membranes.

Table 1 shows the peptide content, peptide yield and ACE inhibitory activitiy (IC_{50}) of each permeate obtained by ultrafiltering ISPH with 1, 10 and 30 kilo daltons (kDa) molecular weight cut-off (MWCF) membranes, respectively. The ACE inhibitory activities of the permeates increased with decrease of MWCF and peptide yield. However, by comparison of IC_{50}

Table 1
The peptide content yield and IC_{50} value of the ISP hydrolysate (ISPH) and its permeates, respectively, filtered by different molecular weight cut-off membranes in a membrane reactor system

Sample[A]	Peptide content (mg protein/ml)	Peptide yield (%)	IC_{50} (mg protein/ml)[B]
ISPH	21.1	100	0.668[a]
30 kDa permeate	15.8	74.9	0.129[b]
10 kDa permeate	14.3	67.5	0.078[c]
1 kDa permeate	10.6	50.1	0.080[c]

[A] ISPH filtered by a membrane module with 30, 10 or 1 kDa MWCO, respectively, was denoted as 30, 10 or 1 kDa permeate.
[B] It is not significantly different ($p < 0.01$) with same letter.

value of 1 kDa permeate and 10 kDa permeate, both IC_{50} values were not significantly different ($p < 0.01$), but recovery yield of 10 kDa permeate was much higher than that of 1 kDa permeate. Therefore, a membrane module with 10 kDa MWCF was further applied for continuous production of ACE inhibitor.

Table 2 shows the molecular weight distribution of ISPH and its permeates. All permeates had the same profile (data not show) and were primarily composed of amino acids and small peptides with molecular weight <2000. The result also indicated that peptides with molecular weight >2000 in ISPH were effectively removed, up to 99%, by both 1 and 10 kDa MWCF membranes. As a matter of fact, ISPH was fractionated or ultrafiltered by either 1 or 10 kDa MWCF membrane, resulting in a decrease (about 8–9 times) of IC_{50} value (Table 1).

Karaki et al. (1990) reported that a tryptic casein hydrolyaste having an IC_{50} value of 0.166 mg protein/ml had an antihypertensive effect in spontaneously hypertensive rats. Mullally, Meisel, and Fitzgerald (1996) also concluded that the IC_{50} values (obtained from ultrafiltered tryptic digests of milk protein) ranging from 0.130 to 0.201 mg/ml had potential application as nutraceuticals in prevention of hypertension. Matsui, Yukiyoshi, Doi, Sugimoto, and Yamada (2002) indicated that single oral administration of royal jelly protein hydrolysates with IC_{50} value of 0.099 mg protein/ml in 10-week spontaneously hypertensive rats resulted in a significant reduction of systolic blood pressure. The IC_{50} values for ultrafiltered ISPH reported herein are therefore within and even below the concentration range likely to mediate an antihypertensive effect (Table 1). Wu and Ding (2002) characterized the soy protein-derived ACE inhibitor peptides. They concluded that the most economic way to market the peptides was as contained in the crude protein hydrolysate for consumption on a long-term basis for desired therapeutic effects.

3.4. Continuous spiral-wound (SW) membrane reactor

Productivity and operation stability of continuous SW membrane reactor were evaluated using a 10 kDa MWCO membrane. Fig. 7 illustrates the comparison of productivity between batch reactor and continuous reactors as a function of volume replacement. The productivity of the batch reactor was independent of volume replacement, whereas that of the continuous SW membrane reactor showed a linear increase as volume replacement increased. This was due to the fact that, in the batch reactor, each volume replacement (1 l) required the same amount of enzyme. However, in the continuous SW membrane reactor the enzyme was

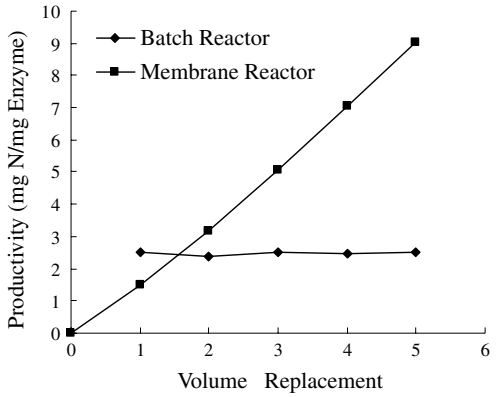

Fig. 7. Comparison of productivity between batch reactor and membrane reactor. Results are mean values of at least two replicates. Standard deviation did not exceed 3% of the recorded values.

Table 2
The molecular weight distribution of isolated soy protein hydrolysate (ISPH) and its permeate produced in a membrane reactor system

Sample[a] molecular weight	ISPH (%)	30 kDa permeate (%)	10 kDa permeate (%)	1 kDa permeate (%)
<500	8.9	14.4	12.8	12.4
500–1000	14.1	24.8	24.4	27.2
1000–2000	57.3	60.3	62.7	60.3
>2000	20.7	0.5	0.1	0.1

[a] ISPH filtered by a membrane module with 30, 10 or 1 kDa MWCO, respectively, was denoted as 30, 10 or 1 kDa permeate.

introduced only once, at the beginning of the run. After two volume replacement the continuous membrane reactor had a higher productivity than the batch reactor. The result indicates that reusing the enzyme has the advantage of higher productivity in the continuous membrane reactor. This result also agreed with the findings of Mannhein and Cheryan (1990) and Chiang et al. (1999). They produced protein hydrolysates by a continuous membrane reactor and concluded that the reuse of enzyme resulted in a higher productivity than with a batch reactor.

Fig. 8 shows the operation stability of the continuous membrane reactor system in terms of product output and IC_{50}. The continuous SW membrane reactor showed a steady production of protein hydrolysates for at least 8 h. Slight decrease in the product output, after a 6 h hydrolysis, reflected leakage of the enzyme. Enzyme leakage was confirmed by detection of residue activity in the permeate (data not shown). However, the leakage showed little effect on the ACE inhibitory activity of all the permeates within 8 h of hydrolysis. At any hydrolysis time, the IC_{50} of each permeate from a continuous SW membrane reactor maintained its inhibitory activity below 0.09 mg protein/ml.

Since some food-protein-derived ACE inhibitor failed to express hypotensive activity after oral administration in vivo (Fujita, Yokoyama, & Yoshikawa, 2000), the mixture of 10 kDa permeates collected from the continuous membrane reactor was investigated for its ACE inhibitory activity in vitro by treating with gastrointestinal proteases, including pepsin and the combination of pepsin and pancreatin, respectively. Gastric in vitro incubation has been reported to provide a practical and easy process, imitating the fate of these peptides after oral administration (Wu & Ding, 2002). Table 3 shows the effects of gastrointestinal proteases on ACE inhibitory activity of 10 kDa permeate in vitro. The result indicated that gastrointestinal protease had very

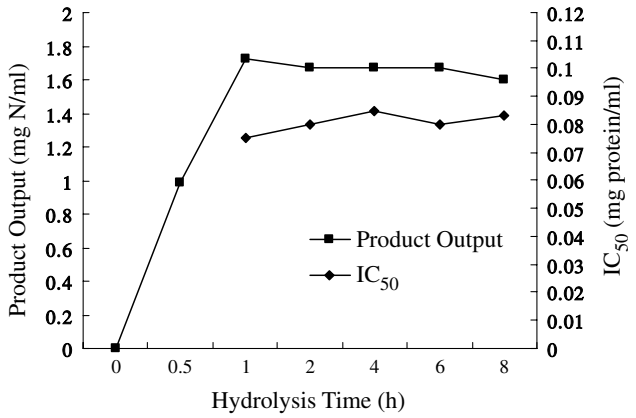

Fig. 8. Operation stability of the continuous membrane reactor system in terms of product output and IC50. Results are mean values of at least two replicates. Standard deviation did not exceed 2% of the recorded values.

Table 3
ACE inhibitory activity of 10 kDa permeate following digestion by gastrointestinal proteases

Enzyme	IC_{50} (mg protein/ml)[a]
Control (10 kDa permeate)	0.078
Pepsin	0.079
Pepsin + pancreatin	0.081

[a] Not significantly different ($p < 0.01$).

little effect ($p < 0.01$) on the ACE inhibitory activity of the mixture of 10 kDa permeates. The result also suggested that peptides in 10kDa permeate may be resistant to digestion in the gastrointestinal tract. Several reports have also concluded that small peptides have low susceptibility to hydrolysis by gastric proteases (Grimble et al., 1987; Wu & Ding, 2002).

4. Conclusion

When Alcalase, Flavourzyme, chymotrypsin, pepsin or trypsin were used as the enzyme source to produce protein hydrolysate with ACE inhibitory activity, the most active hydrolysate was obtained by Alcalase hydrolysis of ISP. Further ultrafiltration of Alcalase-ISP hydroylsate with different MWCO membranes resulted in a significant increase of the inhibitory activity. By using a 10 kDa MWCO membrane, the ACE inhibitory activity (IC_{50}) of the hydrolysate decreased from 0.688 to 0.078 mg protein/ml. The lower the IC_{50} represents the higher the ACE inhibitory activity. Based on the comparison of productivities between batch reactor and continuous membrane reactor, the result suggested that reusing the enzyme has the advantage of higher productivity in the continuous membrane reactor. Therefore, both commercialized Alcalase and 10 kDa MWCF spiral-wound (SW) membrane were selected to equip the continuous membrane reactor for hydrolysis of ISP and production of 10 kDa permeates with ACE inhibitory activity. According to the study of operation stability, the results indicated that the continuous SW membrane reactor could provide a steady production of 10 kDa permeates and the permeates could maintain their inhibitory activity (IC_{50}) below 0.09 mg protein/ml over 8 h. Moreover, the 10 kDa permeates also showed little change after in vitro incubation with gastric enzymes, suggesting that the permeates may be resistant to digestion in the gastrointestinal tract.

Acknowledgements

The authors thank the National Science Council, Taiwan, Republic of China, for financial support under Grant No. NSC-91-2626-E-264-001.

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