BIOACTIVE PEPTIDES ISOLATED FROM ENZYMATIC HYDROLYSATE OF CATFISH (*Pangasius sutchi*) SKIN GELATIN

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Abstract- In the present study, gelatin extract from catfish (*Pangasius sutchi*) skin was hydrolyzed by alcalase under optimum conditions. The hydrolysis was performed for 0, 0.5, 1, 2 and 3 h. Skin gelatin had highest degree of hydrolysis of 64.87% after 2 h of incubation. The freeze dried gelatin hydrolysate with the highest degree of hydrolysis was characterized with respect to amino acid composition and molecular weight distribution. The result of SDS-PAGE showed that the proteolytic degradation of *P. sutchi* skin gelatin by alcalase exhibited lower molecular weight bands compared to parent gelatin. The result of amino acid composition exhibited that alcalase cleaved hydrophobic amino acid peptide bonds such as Trp, Try, Phe and Pro. Both hydrolyzed and unhydrolyzed gelatins were assayed for Angiotensin converting enzyme inhibitory activity. Inhibition potencies of gelatin hydrolysate (IC_{50} = 0.77 mg/ml) was significantly higher than unhydrolyzed gelatin (IC_{50} = 1.95 mg/ml). It was concluded that gelatin hydrolysate form *P. sutchi* catfish skin may have a wide application in functional food ingredients for high blood pressure treatment.

Key Words – Alcalase, Angiotensin-I-converting enzyme, High blood pressure

I. INTRODUCTION

Pangasius sutchi, known locally as "patin", is one of the most popular freshwater catfish sources in Malaysia [1]. Large amounts of by-product materials including skin are disposed from *P. sutchi* processing industry, which are sources of high quality protein. One of the tools for effective protein recovery from these protein-rich by-products is production of protein hydrolysates through enzymatic hydrolysis with desirable functional properties [2].

Alcalase, an alkaline enzyme produced from *Bacillus licheniformis*, has mostly been studied for protein hydrolysis and has continually shown the best efficiency in various hydrolysis processes [3]. Consumer interest in the relationship between diet and health has increased substantially in recent years. Therefore, functional foods have been the centre of attention for the last few decades by food producers as well as consumers. Over the past decade, a large number of studies have investigated enzymatic hydrolysis of protein to produce bioactive peptides with specific physiological properties such as antioxidative, antimicrobial and angiotensin-I-converting enzyme (ACE) inhibitory activity, which can be marketed as functional food ingredients [4]. Angiotensin converting enzyme (ACE) plays a crucial role in the regulation of blood pressure and hypertension. It catalyzes the conversion of inactive angiotensin-I into a potent vasoconstrictor, angiotensin-II [5]. Inhibitions of ACE activity by synthesized chemical drugs are reported to have side effects. Therefore, there is an increasing interest in exploring naturally-occurring ACE inhibitors [6].

In the present study, the extracted gelatin from *P. sutchi* catfish skin was hydrolyzed with alcalase. Additionally, the amino acid composition and molecular weight distribution of the gelatin hydrolysate was determined. The gelatin and its hydrolysate were also assayed for ACE-inhibityory activity.

II. MATERIALS AND METHODS

P. sutchi, 1.2 to 1.5 kg, was obtained from a farm fish located in Penang, Malaysia. Gelatin was extracted from fish skin according to the method described by [7]. For enzymatic

hydrolysis, the freeze-dried skin gelatin was adjusted to optimal pH and temperature for alcalase (pH 7.0; 50 ˚C). Gelatin was mixed with sodium phosphate (0.1 M) with a ratio of 1:100 (w/v) and the hydrolysis was performed for 0, 0.5, 1, 2 and 3 h in a shaking water-bath incubator. At the end of the reaction, the enzyme was inactivated at the temperature of 90 °C for 10 min. The samples were then centrifuged at 6000 x *g* for 20 min and the soluble fraction decanted and freeze-dried. The ratio of enzyme to fish skin protein was 1:100 (v/w).

Degree of hydrolysis (DH) was calculated according to percent of trichloroacetic acid (TCA) method as described by [8]. The percent DH is expressed as follows:
% DH = (10% TCA-soluble N in sample/ total N in sample) × 100

The amino acid composition was examined by a high performance liquid chromatography (HPLC), equipped with a Waters 410 Scanning Fluorescence and AccQ Tag column (3.9 x 150 mm).

Protein pattern of *P. sutchi* skin gelatin hydrolysate was analyzed using (SDS-PAGE), according to the method of Schagger and von Jagow [9].

The ACE inhibitory activity was assayed with RP-HPLC technique modified from the spectrophotometric method described by [10]. ACE inhibition rate was calculated as follows:

ACE inhibition (%) = (B - A/ B - C) × 100

Where A is the relative area of HA peak generated in the presence of ACE inhibitor component, B the relative area of HA peak generated without ACE inhibitors and C is the relative area of HA peak generated without ACE.

Experiments were performed in triplicates and data were analyzed using a one-way analysis of variance (ANOVA) using SPSS, version 14. The differences in means between the samples were determined at 5% confidence level ($P < 0.05$).

III. RESULTS AND DISCUSSION

Degree of hydrolysis (DH) is a measure of the extent of hydrolytic degradation of a protein. The hydrolysis of *P. sutchi* skin was characterized by a high rate of hydrolysis during the first 1-2 h (Figure 1). The rate of enzymatic hydrolysis was subsequently decreased, until reaching a stationary phase when no apparent hydrolysis took place. The DH of alcalase gelatin hydrolysate from skin after 2 h incubation was 64.87±4.6 % (Figure 1).

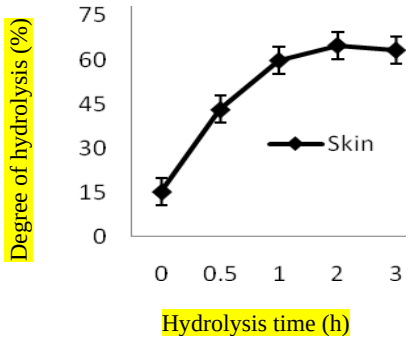

Figure 1. Degree of hydrolysis of *P.sutchi* skin gelatin with alcalase. for 0, 0.5, 1, 2 and 3 h.

SDS-PAGE electrophoregrams of *P. sutchi* skin gelatin hydrolysates is shown in Figure 2. As shown in Figure 2, *P. sutchi* skin gelatin contained at least 2 different α-chains (α_1 and α_2) and cross-linked β chain. The α_1, α_2 and β chains of skin gelatin were digested by alcalase to produce lower molecular weight fragments. The gelatin hydrolysate contained two low molecular weight bands close to 45 kDa and one single peptide fragment of 66 kDa (Figure 2). The presence of the low molecular weight bands in gelatin hydrolysate in this study may result in production of bioactive peptides with potent physiological properties.

It was reported that ACE inhibitory activity largely depends on the amino acid composition of the substrates or competitive inhibitors. High inhibition activity of these peptides is due to the interaction between C-terminal sequences of inhibitors with three subsites of active sites of ACE. The presence of tyrosine (Tyr), phenylalanine (Phe), tryptophan (Trp) and

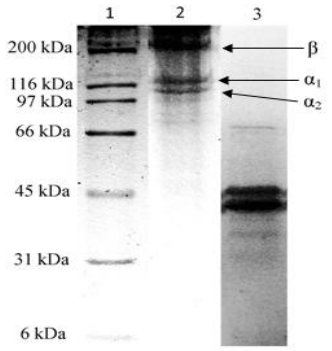

Figure 2 SDS-PAGE patterns of *P. sutchi* skin gelatin and its hydrolysate. Lane 1 represents protein marker; lane 2, control (untreated gelatin); lane 3, alcalase gelatin hydrolysate with the highest DH.

proline (Pro) at the C-terminus part of a peptide contributes to its higher activity [11]. The amino acid compositions of *P. sutchi* skin gelatin and its corresponding hydrolysate fraction are summarized in Table 1. The major constituent amino acids were Gly, Pro, Ala and Met. However, some differences were observed. In addition, there were significant differences in the contents of hydrophobic (particularly aromatic) amino acids such as Phe, Tyr, Trp, which increased in gelatin hydrolysate compared to unhydrolyzed gelatin (Table 1).

Table 1 Amino acid composition of *P. sutchi* skin gelatin and its hydrolysate (g/100 g protein)

Amino Acid	Gelatin	Gelatin hydrolysate
Asp	5.28±0.13*	4.34±0.09
Ser	3.79±0.07*	3.24±0.11
Glu	7.83±0.1*	5.61±0.05
Gly	31.23±0.17	32.33±0.2*
His	0.71±0.04	0.61±0.04
Arg	5.08±0.17	6.14±0.1*
Thr	3.4±0.14*	3.08±0.05
Ala	9.45±0.1*	9.01±0.15
Pro	11.98±0.12	12.82±0.09*
Tyr	0.61±0.04	0.72±0.05*
Val	2.76±0.09	2.84±0.1
Met	2.63±0.18	4.74±0.29*
Lys	3.45±0.17*	2.65±0.1
Ile	1.64±0.05*	1.25±0.07
Leu	2.74±0.06	2.6±0.14
Phe	1.17±0.02	1.98±0.05*
Cys	0.02±0.005*	0.01±0.006
Trp	0.01±0.004	0.03±0.005*
Hyp	6.31±0.09	6±0.08

*$P \leq 0.05$

Values are mean ± standard deviation from triplicate determinations

The ACE-inhibitory activity of gelatin and gelatin hydrolysate were assayed and calculated as IC_{50} (amount of peptide required to inhibit 50% of the ACE activity). Table 2 shows effect of hydrolysis time on degree of hydrolysis and IC_{50} value of *P. sutchi* skin gelatin hydrolysate. There were statistically significant differences of DH found in all incubation time ($P<0.05$). Moreover, there were significant differences of IC_{50} values in untreated and hydrolyzed gelatin fractions. Inhibition potencies of alcalase digest of skin gelatin (IC_{50} = 0.77±0.03 mg/ml) was significantly higher than untreated skin gelatin (IC_{50} = 1.95±0.03 mg/ml) (Table 2). The ACE inhibition activity of skin gelatin hydrolysate in the present study was almost similar with that of alaska pollack skin (IC_{50} = 0.63 mg/ml) [12], skate skin (IC_{50} = 0.68 mg/ml) [13] and sardinella by-product (IC_{50} = 0.81 mg/ml) [14] protein hydrolysates. Based on the results of the previous studies, ACE-inhibitory activity of peptides increased with prolonged incubation with enzyme. However, longer hydrolysis time led to the peptides lost their ability to inhibit ACE [15].

Table 2 Effect of hydrolysis time on degree of hydrolysis and IC_{50} value of *P.sutchi* skin gelatin hydrolysate.

Samples	**Time of incubation (h)**	**DH (%)**	**IC_{50}[a] (µg/mL)**
Untreated gelatin			1.95±0.03[a]
Gelatin hydrolysate	0.5	43.82±3.2[c]	1.14±0.05[b]
	1	58.65±4.3[b]	0.97±0.04[c]
	2	64.87±4.6[a]	0.77±0.03[d]

Values are mean ± standard deviation from triplicate determinations. Values in the same column with different superscript letters are significantly different ($P \leq 0.05$)

IV. CONCLUSION

In this study, functional bioactive peptides with *in vitro* ACE-inhibitory activity have been isolated from skin gelatin hydrolysate of *P. sutchi* catfish. Hydrolysis of gelatin with alcalase for 2 h showed an IC_{50} value of 0.77 mg/ml. Skin gelatin and its hydrolysate were tested for amino acid composition. It was concluded that alcalase cleaved hydrophobic amino acid peptide bonds of gelatin hydrolysate such as Trp, Try, Phe and Pro to favor the

interaction between peptides and ACE and therefore, decrease or inhibit the activity of enzyme. The present study recommended that potent ACE-inhibitory peptides extracted from catfish *P. sutchi* by-products gelatin can be investigated to synthesis the ACE-inhibitory peptides as health promoting ingredient in the functional foods to prevent hypertension.

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