Effect of pepsin hydrolysis on antioxidant activity of jellyfish protein hydrolysate

Pratchaya Muangrod[1], *Wiriya* Charoenchokpanich[1], *Vilai* Rungsardthong[1], *Savitri* Vatanyoopaisarn[1], *Benjamaporn* Wonganu[2], *Sittiruk* Roytrakul[3] and *Benjawan* Thumthanaruk[1,*]

[1]Department of Agro-Industrial Food and Environmental Technology, Faculty of Applied Science, King Mongkut's University of Technology North Bangkok, Bangkok, Thailand
[2]Department of Biotechnology, Faculty of Applied Science, King Mongkut's University of Technology North Bangkok, Bangkok, Thailand
[3]National Center for Genetic Engineering and Biotechnology, National Science and Technology Development Agency (NSTDA), Pathum Thani, Thailand

Abstract. Edible jellyfish have been consumed as food for more than a century with offering high protein and crunchy texture. The pepsin hydrolysis of jellyfish protein yields jellyfish protein hydrolysate (ep-JPH), reported for potential bioactivities such as antioxidant activity or antihypertensive activities. Due to the substantial number of by-products generated from jellyfish processing, the by-products were then selected as a raw material of JPH production. This research aimed to evaluate the effect of the hydrolysis time of pepsin on the antioxidant activity of ep-JPH. The dried desalted jellyfish by-products powder was enzymatically hydrolysed by 5% (w/w) pepsin, and the hydrolysis time was varied from 6, 12, 18, and 24 h at 37°C. Results showed that increased hydrolysis time increased the degree of hydrolysis (DH) and inhibition of DPPH radical. The 24 h ep-JPH possessed the highest DH and the highest inhibitory effect of DPPH radical. The results demonstrated that, in this experiment, all ep-JPHs were DPPH radical scavengers, exhibiting different inhibition activities depending on DH values.

Keyword. Salted jellyfish by-products, Protein hydrolysate, DPPH, Degree of hydrolysis

1 Introduction

Protein hydrolysates are products of protein degradation that yield various sizes of peptides and free amino acids [1, 2]. There are several methods of protein hydrolysate production, including acids or alkali hydrolysis and enzymatic hydrolysis [1-3]. Different methods of protein hydrolysate production result in different bioactivity of protein hydrolysate due to its amino acid composition and sequence [1, 4, 5].

Enzymatic hydrolysis is one of the practical protein hydrolysate productions via protease enzymes that cleave specific peptide bonds and provide short peptides with small molecular weights [1]. Most low molecular weight enzymatic hydrolysate peptides containing approximately 2-20 amino acid residues are considered bioactive peptides [5, 6]. Commercial proteases are practically used for protein hydrolyses, such as trypsin, pepsin, collagenase, alcalase, papain, and bromelain [7, 8]. Different types of protease enzymes also provided different bioactive peptides.

To date, the different sources of food proteins for producing bioactive peptides are meat [9, 10], milk [11-13], eggs [14, 15], nuts [16], cocoa bean [17], beef [18], pork [18], chicken [18], turkey [18], and fish [19]. Factors of type of protease enzymes, hydrolysis time, and raw material factors used for peptide production yield different amino acid compositions and amino acid sequences [20-23], which offer different bioactive activities such as antioxidant, anticancer, antimicrobial, and antihypertensive [3, 24, 25]. Antioxidant activity, the ability to reduce or scavenge free radicals, is one of the major biological activities of protein hydrolysates that have mostly been reported [3]. If the number of free radicals exceeds normal, free radicals can cause diseases such as arthritis, diabetes, atherosclerosis, ageing, and cancer [8, 26]. Then, the novel protein sources of bioactive peptides having antioxidant activities are currently of interest for reducing the risk of various diseases [26]. The marine source has become attractive with various organisms that have potentially been reported for biological activities. Edible salted jellyfish, the oldest marine plankton consumed for more than a century by Chinese people, is selected to produce jellyfish protein hydrolysate (JPH). Apart from water content in the body, jellyfish are considered high protein food, mainly collagen [27, 28]. Few jellyfish species used for producing JPH have been reported for antioxidant activities [6, 8, 24]. No antioxidant activity of protein hydrolysate from jellyfish (*Lobonema smithii*) hydrolysed with pepsin has not been studied. Therefore, the by-products of salted jellyfish from salted jellyfish processing were selected for producing hydrolysate by pepsin hydrolysis (ep-JPH). The research aimed to

* Corresponding author: benjawan.t@sci.kmutnb.ac.th

evaluate the effect of the hydrolysis time of pepsin on the antioxidant activity of JPH.

2 Materials and methods

2.1 Preparation of salted jellyfish by-products

The broken pieces or irregular shapes of jellyfish (*Lobonema smithii*) sold as by-products were kindly received from Chockdee Sea Products Co., Ltd., Samut Songkhram, Thailand. Due to the high salt in jellyfish by-products, washing and drying were used to prepare the raw material as previously described [27, 29]. First, the washing of salted jellyfish by-products with tap water at a ratio of 1:40 (w/v) was performed by a rotational jellyfish washing machine for 2 cycles, and 15 min per cycle and drained for 30 min. The desalted jellyfish by-products were dried in a tray dryer (ED 400, Binder, USA) at 60°C for 24 h and then ground into fine particles and filtered through a sieve of size 100 mesh. The jellyfish protein powder (JPP) was analyzed for the chemical compositions (moisture, protein, fat, and ash content) before use (Fig. 1). The powder was packed in sealed PE bags and kept at room temperature until use.

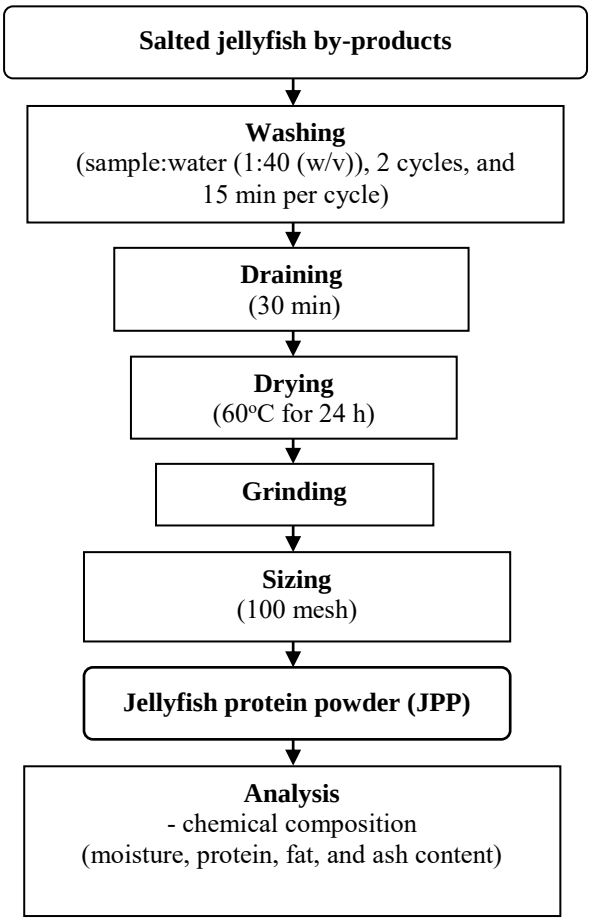

Fig. 1. Preparation of salted jellyfish by-products (JPP).

2.2 Preparation of jellyfish protein hydrolysate (JPH)

JPH was prepared as previously described [30, 31] with a slight modification (Fig. 2). Briefly, JPP was mixed in 0.05 M sodium acetate buffer at the ratio of 1: 25 w/v (4g/100mL) at 95°C for 10 min to inactivate the endogenous enzymes and then equilibrated to the optimum temperature conditions. Next, the sample was dissolved in 0.05 M sodium acetate buffer at the ratio of enzyme: substrate (1: 20 g: g; w/w). Then, the mixture was hydrolysed by pepsin at 37°C for 6, 12, 18, and 24 h using a shaking incubator (WIS-20R, WiseCube, Korea) at 150 rpm. After that, the enzyme was inactivated by heating at 95°C for 10 min in a temperature-controlled water bath shaker (Memmert, Schwabach, Germany) at 100 rpm. Next, the hydrolysate was centrifuged at 9,500 × g for 10 min. Finally, the supernatant of ep-JPH was filtered by Whatman filter paper No1. The samples were stored at −18°C until further analysis.

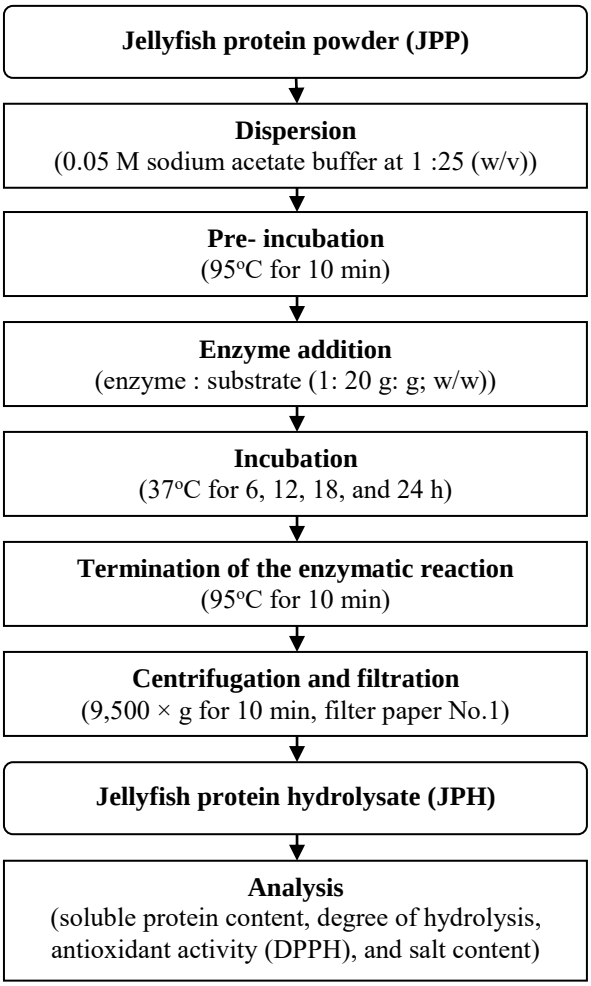

Fig. 2. Preparation of jellyfish protein hydrolysate (JPH).

2.3 Analysis

2.3.1 Chemical composition

The proximate composition of JPP including, moisture, protein, fat, and ash content, was measured according to

the AOAC standard methods [32]. The protein content was measured using the Kjeldahl method. A factor of 5.55 was used to convert the nitrogen value to protein [33].

2.3.2 Soluble protein content

The soluble protein content of JPH was determined according to the Lowry method [34]. Bovine serum albumin was used as a standard.

2.3.3 Degree of hydrolysis

The analysis of DH was slightly modified by the method of trinitrobenzyl sulfonic acid (TNBS) [35]. Briefly, a 125 µL of jellyfish protein hydrolysate was mixed with 2 mL of phosphate buffer (pH 8.2) and 1 mL of 0.01% TNBS reagent. Then, the mixture solution was incubated at 50°C for 30 min in the dark using a temperature-controlled water bath (WNB 45, Memmert, Germany). The reaction was stopped by adding 2 mL of 0.1 M sodium sulfite and incubated at room temperature for 15 min. The absorbance was measured at 420 nm using a spectrophotometer (SP 830 plus, Metertech, Taiwan). The DH was calculated according to the following equation (1):

$$DH\ (\%) = [(L - L_0) / (L_{max} - L_0)] \times 100 \qquad (1)$$

where L = The amount of α-amino groups of hydrolysate sample, L_0 = The amount of α-amino groups in the original substrate (blank), and L_{max} = The total α-amino groups in the sample hydrolysed by 6 N HCl at 100°C for 24h.

2.3.4 Antioxidant activity (DPPH radical scavenging assay)

DPPH radical scavenging activity was analyzed as previously described [26, 36] with slight modifications. A 0.1 mL of JPH was mixed with 1.9 mL of 0.15 mmol/L DPPH in 70% ethanol. The mixture was then incubated in the dark for 30 min at room temperature. The absorbance was measured at 517 nm using a spectrophotometer (SP 830 plus, Metertech, Taiwan). In this measurement, ascorbic acid was a positive control. The inhibition of DPPH radical scavenging activity was calculated by the following equation (2):

$$\%inhibition = [(A_{control} - A_{sample}) / A_{control}] \times 100 \qquad (2)$$

where $A_{control}$ is the absorbance of the control that contained all reagents except the test samples. A_{sample} is the absorbance of the JPH with reagents added.

2.3.5 Salt content

The salt content of jellyfish protein hydrolysate referred to the percentage of sodium chloride (NaCl) in JPH was determined using a conductivity meter (TDS Meter 308, Systronics, India). The percentage of sodium chloride in JPH was predicted from the standard curve.

2.4 Statistical analysis

All experiments were performed in triplicate. Results were shown as mean±standard deviations. The data were subjected to analysis of variance (ANOVA), and Duncan's multiple range test was used to measure the significant difference ($p<0.05$) using the statistical package for social science (SPSS) 22.0.

3 Results and discussions

3.1 Quality of jellyfish protein powder

The jellyfish protein powder (JPP) had moisture, protein, fat, and ash content of 7.42±0.20%, 76.59±0.11%, 1.32±0.05%, and 15.54±0.36%, respectively. Results of proximate composition of JPP were similar to the previous study that JPP had 7.69%, 76.41%, 1.35%, and 15.76%, respectively [27], but slightly different from the work of Emrerk et al. (2021) [37], who reported that JPP had 5.27%, 68.8%, 3.18%, and 9.11%, respectively. Therefore, according to the high protein content, the JPP was selected for producing jellyfish protein hydrolysate.

3.2 Effect of hydrolysis time of pepsin on soluble protein content, degree of hydrolysis, antioxidant activity (DPPH), and salt content of jellyfish protein hydrolysate (JPH)

Pepsin is an aspartic protease that has an optimum pH of 1.8. Pepsin hydrolyzes peptide bonds between large hydrophobic amino acid residues and yields peptides. Table 1 shows the soluble protein content of enzymatic pepsin hydrolysed JPH (ep-JPH) for different hydrolysis times (6, 12, 18, and 24 h) at 37°C. Increased hydrolysis time of pepsin increased soluble protein content of ep-JPH. The soluble protein contents of ep-JPH derived from 6, 12, 18, and 24 h were 1,428.47, 1,461.25, 1,491.80, and 1,589.30 mg/L, respectively. During the hydrolysis process, jellyfish collagen protein was denatured by pepsin and acidic conditions. As a result, the jellyfish protein was hydrolysed, thus generating the soluble proteins.

DH is a parameter used for determining how well the pepsin hydrolysed jellyfish protein. In this experiment, increased hydrolysis time of pepsin increased the values of DH. The hydrolysis condition of ep-JPH for 6, 12, 18, and 24 h showed the DH values of 52.20%, 54.01%, 54.50%, and 55.41% (Table 1). During the pepsin hydrolysis, the peptide bonds of a jellyfish protein were hydrolysed and provided a short-chain peptide and free amino acid [38], depending on hydrolysis factors, including pepsin concentration, hydrolysis time, and temperature. In this study, the pepsin hydrolysed jellyfish sample at the hydrolysis time of 12, 18, 24 h showed similar DH values, and these values were not significantly different. The DH results were similar to the previous studies that hydrolysed jellyfish protein with bromelain at 50°C for 18 h and trypsin at 50°C for 9 h [8, 37]. Thus, the soluble protein and DH values are affected by pepsin hydrolysis. In this

study, the pepsin hydrolysis for 24 h at 37°C was sufficient for producing ep-JPH.

Table 1. Soluble protein content and degree of hydrolysis of enzymatic pepsin-jellyfish protein hydrolysate (ep-JPH) at different hydrolysis times.

Sample	Soluble protein content* (mg/mL)	Degree of hydrolysis* (%)
ep-JPH 6 h	1,428.47±21.75^{c}	52.20±0.53^{d}
ep-JPH 12 h	1,461.25±12.27^{bc}	54.01±0.07^{c}
ep-JPH 18 h	1,491.80±23.48^{b}	54.50±0.10^{b}
ep-JPH 24 h	1,589.30±51.14^{a}	55.41±0.10^{a}

*Different superscripts (a, b, c, d) in the same column mean significant difference in value ($p<0.05$)

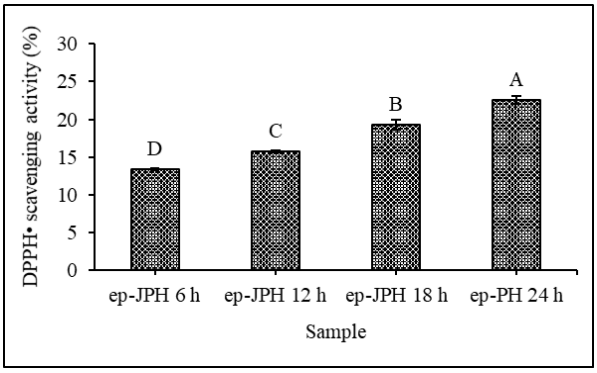

Fig. 3. DPPH radical scavenging activity (%) of jellyfish protein hydrolysate (JPH) at different duration times of hydrolysis (6, 12, 18, and 24 h); bars with the different superscripts were significantly different ($p<0.05$).

DPPH is an organic nitrogen radical that shows maximal absorbance at 517 nm in ethanol when DPPH encounters a protein donating substance that is an indicator of the antioxidant capacity of the sample [6, 39]. Results showed increased pepsin hydrolysis time of JPH increased % inhibition of DPPH radical scavenging activity. The inhibition values of DPPH radical scavenging activity by ep-JPHs obtained from 6, 12, 18, and 24 h of hydrolysis were 13.38±0.07%, 15.73±0.13%, 19.31±0.66%, and 22.56±0.51%, respectively (Fig. 3). Results of DPPH radical scavenging activity of ep-JPH were slightly different from that value of trypsin hydrolysed ribbon jellyfish (*Chrysaora* sp.) for 7 h, approximately 25% [8]. The factors that affected the antioxidant activity of protein hydrolysate are highly dependent on hydrolysis condition (temperature and duration time), sequence and amino acid composition, and protease enzyme (types and concentration) [6]. Thus, in this study, all ep-JPHs were able to scavenge DPPH radicals, and the 24 h ep-JPH showed the highest inhibition effect on DPPH radicals.

Results of DPPH radical scavenging activity were correlated with the results of DH. The small peptides generated by pepsin hydrolysis may donate electrons to DPPH radical, thereby reducing free radical action [6]. Thus, the highest DH value showed the highest inhibition of the DPPH radical. Previous research has reported that short-chain peptides containing 2 to 20 amino acids may positively impact health [17]. Therefore, the amino acid sequence of ep-JPHs is needed for further analysis.

The conductivity values measuring the salt ion content of ep-JPH increased as pepsin hydrolysis time increased, as shown in Table 2. The conductivity values of ep-JPH at different hydrolysis times were 1,853.33, 1,894.67, 1,960.67, and 1,997.00 µs/cm, respectively. All JPH samples presented a salt content of approximately 0.09%. Results also related to the previous work that the salt content of enzymatic bromelain-JPH at 50°C for 6-18 h were 1.50-2.22% [37]. Despite desalted jellyfish used, the salt content that remained in the solution must be removed to prevent the interferences of electrophoresis or LC-MS/MS analysis in the future.

Table 2. Salt content of enzymatic pepsin-jellyfish protein hydrolysate (ep-JPH) at different hydrolysis times.

Sample	Conductivity* (µs/cm)	Salt content* (%)
ep-JPH 6 h	1,853.33±1.52^{d}	0.090±0.00^{d}
ep-JPH 12 h	1,894.66±1.52^{c}	0.092±0.00^{c}
ep-JPH 18 h	1,960.66±7.02^{b}	0.096±0.00^{b}
ep-JPH 24 h	1,997.00±2.64^{a}	0.098±0.00^{a}

*Different superscripts (a, b, c, d) in the same column mean significant difference in value ($p<0.05$)

4 Conclusion

The 24 h ep-JPH at 37°C showed the highest soluble protein content, %DH, and %inhibition of DPPH radical. All ep-JPHs were able to scavenge DPPH radicals. Thus, jellyfish protein hydrolysate may be used in functional food applications due to its antioxidant ability. However, %inhibition of DPPH radical of ep-JPH was lower when compared with other studies. Therefore, further studies on ep-JPH conditions, such as an increase enzyme concentration, different hydrolysis times, and temperature are needed to offer the higher bioactivity of ep-JPH. The results of this study lead to an added value of salted jellyfish by-products and reduce waste from the salted jellyfish industry.

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