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Comparative functional properties of jellyfish (*Lobonema smithii*) protein hydrolysate as influenced by bromelain and hydrochloric acid

Somboonsak Silaprueng[1] Benjawan Thumthanaruk[1]*and Pisit Wongsa-ngasri[2]

[1]Department of Agro-Industrial, Food and Environmental Technology, Faculty of Applied Science, King Mongkut's University of Technology North Bangkok, 1518 Pracharat1 Road, Wongsawang, Bangsue, Bangkok 10800, Thailand

[2]Fishery Technological Development Division, Department of Fisheries, Ministry of Agriculture and Cooperatives Kasetklang.Chatuchak. Bangkok 10900, Thailand

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ABSTRACT

Proteins in jellyfish (*Lobonema smithii*) are mainly collagen. The research was to compare the physicochemical and functional properties of jellyfish (*Lobonema smithii*) protein hydrolysate produced using bromelain (eb-JPH) or hydrochloric acid (a-JPH) hydrolysis. The bromelain concentrations used were 5, 10 and 15% (w/w) and hydrolysis times of 3, 6, 12 and 18 h at 50°C. Hydrochloric acid (1 N or 6 N) and hydrolysis times of 12 and 24 h at 95°C were used to produce a-JPH. Results of acid hydrolysis showed increased degree of hydrolysis (%DH) of a-JPH depending on HCl concentration. The duration of hydrolysis (6 and 12 h) had no impact on %DH. The highest %DH obtained was at 6 N HCl for 24 h. Neither bromelain concentration nor hydrolysis time had impact on %DHs of eb-JPHs. Functional properties (foaming and emulsifying properties) of eb-JPHs were higher than those of a-JPHs. The foaming and emulsifying properties of eb-JPHs increased with increasing bromelain concentration. However, the increased hydrolysis time increased only foaming properties. The scavenging activity of 1,1-diphenyl-2-picrylhydrazyl (DPPH) of eb-JPHs had higher than that of a-JPHs. This can be concluded that eb-JPH produced better functional properties than a-JPHs.

* *Corresponding author*:
Email: btr@kmutnb.ac.th

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INTRODUCTION

Jellyfish have been found in the Gulf of Thailand and the Andaman Sea. The jellyfish are rich source of collagen as measured by hydroxyproline content (Wongsangasri *et al*., 2008; Hsieh *et al*., 2001). Enzymatic hydrolysis has been used to produce protein hydrolysates. The functional properties of small peptides derived from hydrolysis such as solubility, foaming, emulsifying, chelating and antioxidant activity have been reported by Galla, (2012); Pacheco-Aguilar *et al*., (2008); Tsumura *et al*., (2005); Nalinanon *et al*., (2011) and Mendis *et al*., (2005). The properties of protein hydrolysate depend on type of enzyme, type of food and hydrolysis condition. Marine protein hydrolysate were prepared from various sources such as oyster (Chen *et al*., 2013), yellow stripe treally (*Selaroides leptolepis*) (Klompong *et al*., 2007) and jellyfish (*Rhopilema esculentum*) (Liu *et al*., 2012) which could be potentially used to as functional food ingredients (Kristinsson and Rasco, 2000). Wasswa *et.al*., (2007) reported the protein hydrolysate from grass carp (*Ctenopharyngodon idella*) skin produce by Alcalase had better oil holding and emulsifying capacity at low DH.

The aim of research was to analyze the functional properties of jellyfish (*Lobonema smithii*) protein hydrolysate produced using bromelain (eb-JPH) or hydrochloric acid (a-JPH) hydrolysis.

MATERIALS AND METHODS

Chemicals

Bromelain were obtained from K-Much industry Co.,Ltd. (Bangkok, Thailand). 2,4,6-trinitrobenzenesulphonic acid (TNBS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma Chemical Co. (St. Louis MO, USA). All reagents used in this study were of analytical grade.

Dried jellyfish powder sample preparation

Jellyfish (*Lobonema smithii*) were obtained from Mahachai seafood Co.,Ltd. Samutsakorn province, Thailand. The jellyfish were commercially preserved with commercial salt (sodium chloride) alum (aluminium sulfate) and soda (sodium bicarbonate) as described by Wongsangasri *et.al*. (2008). The sample used was prepared by the method of Lueyot and Thumthanaruk (2014). The sample was cut into small pieces and washed with tap water until the washed water had <1°Brix measured by a hand salinometer (ATAGO, Japan). The washed jellyfish were dried at 50°C for 24 h in a tray dryer (Binder, USA). The dried jellyfish were ground, packed in plastic bag and kept in a dessicator at room temperature until used.

Production of eb-JPH and a-JPH hydrolysis

The eb-JPHs were prepared by dispersing dried jellyfish powder in distilled water 1:25 (w/v). The samples were pre-heated at 50°C for 15 min and then adjusted to a pH of 6.0 with 1N NaOH or 1N HCl. The samples were hydrolyzed by bromelain at the concentration of 5, 10. 15% (w/w). The hydrolysis times were 3, 6, 12 and 18 h at 50°C. The enzymatic reaction was stopped by heating at 95°C for 15 min. The dispersion was centrifuged at 6,000 rpm for 15 min using a high speed refrigerated centrifuge (Suprema 21, Tomy, Japan). The supernatant was kept at -20°C until used.

The a-JPHs were preparaed. Samples were dispersed in 1N or 6N HCl and heated to 95°C for 12 and 24 h. After hydrolysis, the samples were adjusted to pH 7.0 using pH meter (Cyberscan, Neitherland). The dispersion were centrifuged at 6,000 rpm for 15 min. The supernatants (a-JPHs) were collected and kept at -20°C until used.

Proximate analysis of dried jellyfish

Protein, moisture, ash and fat of dried jellyfish powder were analyzed by the method of AOAC (2000).

Color measurements

The color of the a-JPH and eb-JPH was evaluated using the Hunter Lab colorimeter (Color quest, USA), The apparent colour of sample was measured in terms of L* (Degree of lightness), a* (Degree of redness and greenness) and b* (Degree of yellowness and blueness). Chroma and hue angle were calculated used Equation 1 and Equation 2.

$$\text{Chroma} = (a^{*2} + b^{*2})^{1/2} ; \quad (1)$$

$$\text{Hue angle} = \tan^{-1}(b^{*2}/a^{*2}) \quad (2)$$

Determination of degree of hydrolysis (DH)

The DHs of a-JPHs and eb-JPHs were determined according to the method of Benjakul and Morrissey, (1997). Briefly, 125µL of JPHs (eb-JPHs and a-JPHs) were mixed with 2.0 mL of 0.2M phosphate buffer (pH8.2) and 1.0 mL of 0.01% TNBS solution. The solution was mixed and placed in a temperature controlled water bath (Memmert, WNB7, Germany) at 50°C for 30 min in the dark. The reaction was stopped by adding 2.0 mL of 0.1M sodium sulphite. The mixtures were cooled at room temperature for 15 min. The absorbance was measured at 420 nm. The percentage of DH was calculated using Equation 3:

$$\%DH=[(L-L_0)/L_{max}-L_0)]\times 100 \quad (3)$$

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

Determination of functional properties of a-JPH and eb-JPH

Emulsifying property (EP)

Emulsifying property was determined according to the method of Chandi and Sogi, (2007) and Cao *et al*., (2009) with a slight modification. An aliquot (30 mL) of sample mixed with soy bean oil (10 mL) homogenized at 20,000 rpm for 1 min and centrifuged at 2,000 rpm for 5 min. The percentage of emulsion was calculated according to the following Equation 4 :

$$\%EP= (A/B)\times 100 \quad (4)$$

where A is the volume of emulsion, B is the volume of control solution, all measured in milliters

Foaming property (FP)

Foaming was investigated according to the method of Sathe and Salunkhe (1981) with a slight modification. An aliquot (20 mL) of sample was homogenized at 16,000 rpm for 2 min. The whipped sample was immediately transferred into a cylinder and read the total volume. The percentage of foaming was calculated according to the following Equation 5

$$\%FP=(A-B)/B\times 100 \quad (5)$$

where A is the volume after whipping (mL), B is the volume before whipping (mL), all measured in milliters

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

DPPH radical scavenging activity was analyzed using the method of Yen and Wu (1999) with a slight modification. The 1.0 mL of sample solution and 2.0 mL of 0.1mM DPPH in 95% methanol were mixed and incubated at room temperature in the dark for 30 min. The absorbance was measured at 517 nm. The control was conducted in the same manner, except that distilled water was used instead of sample. DPPH radical scavenging activity was calculated according to the following Equation 6:

DPPH radical scavenging activity (%) = (1-[A_{517} of sample/A_{517} of control])×100 (6)

Statistical analysis

The data analyses were performed using a SPSS version 19 software program (SPSS Inc., Chicago, lll., USA). ANOVA was used to find differences between treatment and using Duncan's test with a confidence level of 95%.

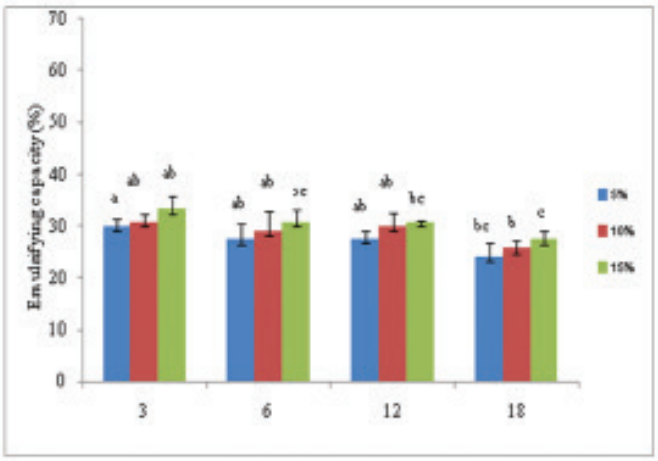
RESULTS AND DISCUSSION

Chemical composition of dried jellyfish powder and color of a-JPH and eb-JPH

The composition of dried jellyfish powder had protein, fat, moisture and ash of 75.09%, 5.16%, 9.66% and 8.56% respectively. The values were expressed on a dry weight basis. The dried jellyfish had high protein content and could be an excellent source of protein for producing protein hydrolysates. The color of a-JPHs had a brown color having hue angle ranging from 50.51 to 81.78. The color of a-JPHs had more intense dark brown color than that of eb-JPHs. The eb-JPHs yielded yellowish color which had a range of hue angle in values of 63.49 to 77.87.

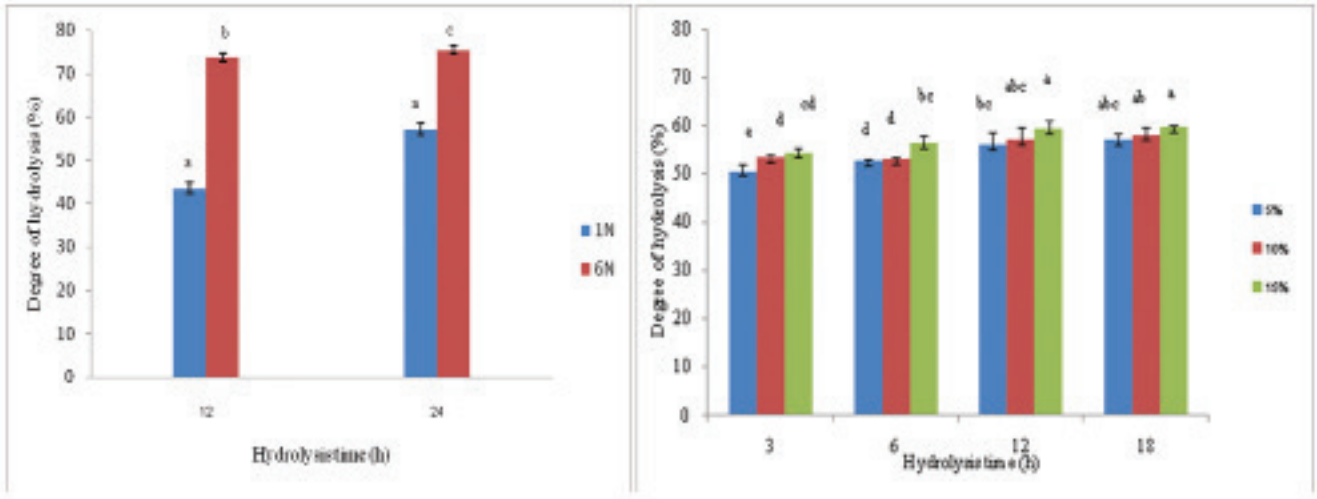
Degree of hydrolysis of a-JPH and eb-JPH

The a-JPHs showed increased of %DH depending on concentration of HCl. The duration of hydrolysis time (6 and 12 h) had no impact on %DH (data not shown). The a-JPHs produced by 6N HCl at hydrolysis time of 12 and 24 h gave high %DH of 73.70 and 75.40 (Figure 1-a). Neither bromelain concentration nor hydrolysis time greater than 3 hours had an impact on %DHs of eb-JPH. 15% bromelain with hydrolysis times of 12 or 18h produced %DH which were 59.40 and 59.60%, respectively (Figure 1-b). The %DH of jellyfish protein hydrolysate reported by Lueyot and Thumthanarak (2014) was 60.62% using 1.5% pepsin at 45°C for 9 h. These results suggested that different enzyme and condition used for hydrolysis gave different %DH. Klompong *et al*., (2007) reported that protein hydrolysates produced from yellow stripe trevally (*Selaroides leptolepis*) hydrolyzed by Alcalase gave a higher %DH than those hydrolyzed by Flavourzyme because alkaline protease showed higher proteolytic activity than acid hydrolysis or neutral protease. Therefore, the effect of raw material, enzyme, time and temperature could influence the degree of hydrolysis.



(2)

Figure 2 Emulsifying properties of eb-JPH (2)



(1-a) (1-b)

Figure 1 Degree of hydrolysis of a-JPH (1-a) and eb-JPH (1-b

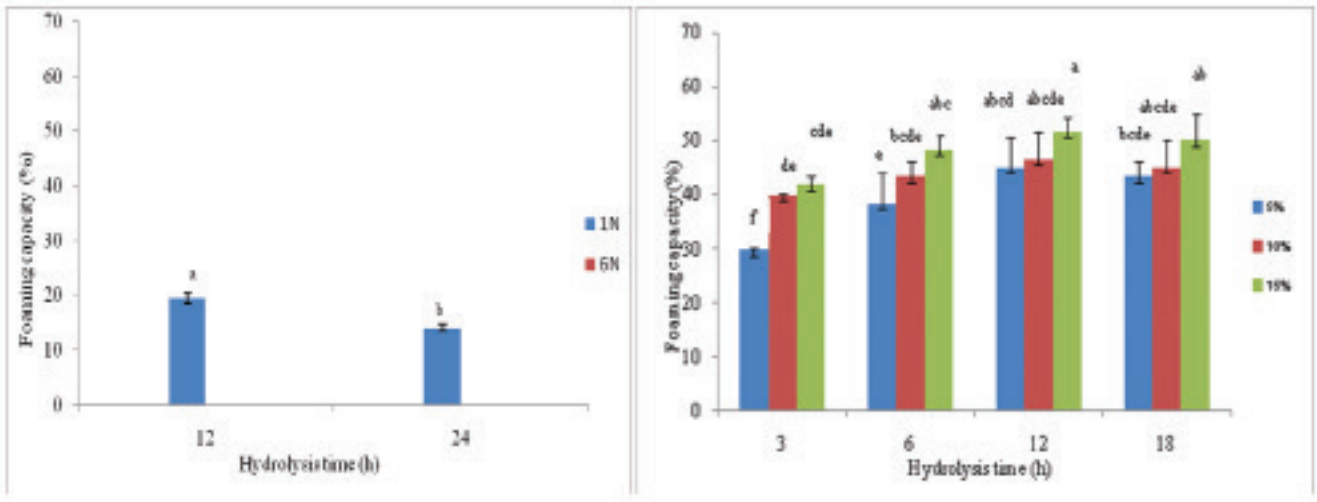
Functional properties of a-JPH and eb-JPH

Emulsifying capacity

Protein hydrolysates can be a surface-active material which helps to stabilize oil-in-water emulsion because of their hydrophilic and hydrophobic groups (Gbogouri *et al*., 2004 ; Kristinsson and Rasco, 2000). The emulsifying capacities of eb-JPH were higher than those of a-JPH. The eb-JPH hydrolyzed with 15% bromelain for 3h presented the highest (33.3%) emulsifying capacity (Figure 2). However, a-JPH did not show any emulsifying properties because of an extensive hydrolysis of jellyfish collagen (data not shown). The values of %DH is correlated to the emulsifying property. Gbogouri *et al*. (2004) reported hydrolysate from salmon byproduct with higher %DH had low emulsifying properties due to their small peptide size. The peptides with low molecular weight will have low amphiphilic properties, thereby reducing emulsifying property. In order to retain the emulsifying properties, the hydrolysate must have proper molecular weight and charge (Chobert *et al*., 1988). The emulsifying effect was also affected by pH. Lueyot and Thumthanaruk (2014) reported that the emulsifying activity index increased at increased pHs and the highest emulsion stability index of JPH found at pH8.

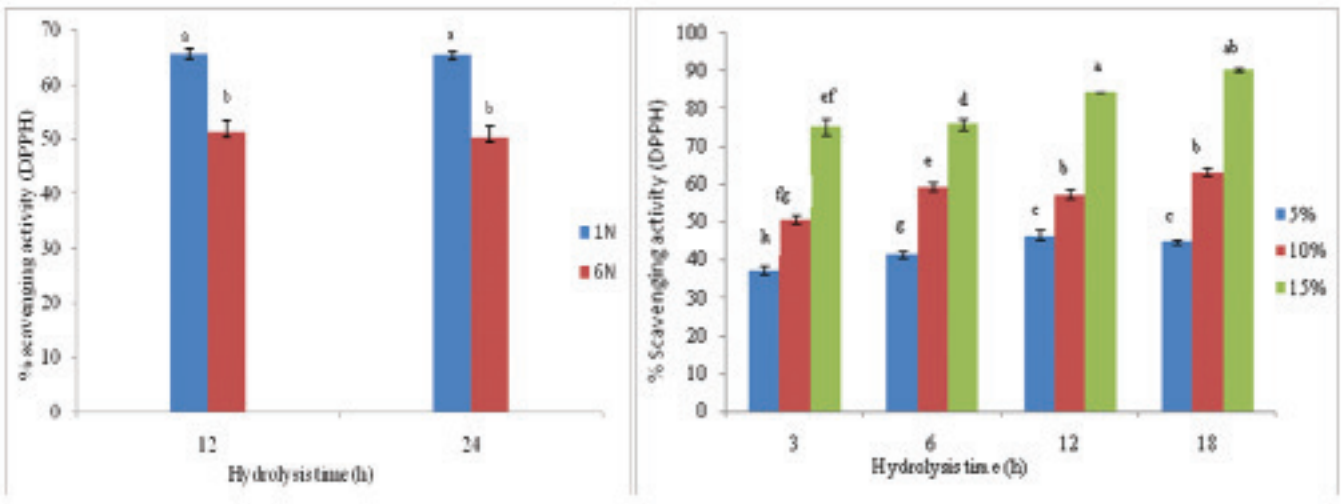
Foaming capacity

Foaming is formed by transportation, penetration and reorganization of molecules at the air-water interface. To obtain good foaming, any protein or peptide must be migrate rapidly to air-water interface, unfold and rearrange at the interface (Halling, 1981). The results showed foaming properties of eb-JPHs were higher than those of a-JPHs. The eb-JPH produced using 15% bromelain for 12h and a-JPH with 1N HCl for 24h gave foaming properties of 51.60 and 25.10% respectively (Figure 3-a, 3-b). The different conditions used for hydrolysis had substantial influence on JPH foaming. The value of DH is also related to foaming. Lueyot and Thumthanaruk (2014) showed the foam capacity of JPH at the DH of 60.62% decreased at pH4 (45%), increased at pH2 (80%). Chalamaiah *et al*., (2010) reported different foaming of protein hydrolysates produced from meriga (*Cirrhinus mrigala*) egg hydrolyzed by Alcalase and papain. The foaming capacity of Alcalase hydrolysate gave higher foaming than that of



(3-a) (3-b)

Figure 3 Foaming properties of a-JPH (3-a) and eb-JPH (3-b)



(4-a) (4-b)

Figure 4 % Scavenging properties of a-JPH (4-a) and eb-JPH (4-b).

papain assisted hydrolysate which was 70 and 25% respectively. The adsorption rate to the air-water interface may be influenced by the protein structure, molecular size and hydrophobicity (Martin *et al*., 2002).

2,2-dipheny l-1-picrylhydrazyl (DPPH) radical scavenging activity

DPPH is a stable free radical that showes maximal absorbance at 517 nm in ethanol, when DPPH encounters a protein-donating substance (Shimada *et.al*.,1992). The scavenging activity of eb-JPH were higher than a-JPH because the action of enzymatic hydrolysis was more specific cleavage than acid hydrolysis. This could result in more bioactive peptides. The eb-JPH with 15% bromelain for 18h and a-JPH with 1N HCl for 12h gave the highest %scavenging effect on DPPH of 90.30 and 65.70%, respectively (Figure 4-a, 4-b). Based on the results of this research. JPHs can donate electrons to DPPHº, thereby reducing the action of free radical. Protein hydrolysates obtained from different sources of protein and hydrolysis conditions also had different in DPPH scavenging activities. Lueyot and Thumthanaruk (2014) reported JPH (DH 60.62%) had DPPH scavenging activity of 66.67% and the reducing power of 0.582. Ktari *et.al*., (2012) reported the protein hydrolysate from muscle of zebra blenny (*Salaria basilisca*) hydrolyzed by crude alkaline protease exhibited the highest radical scavenging activity value of 76.56%. Antioxidant activity of protein hydrolysate is highly dependent on sequence and amino acid composition (Chen et al., 2013), hydrolysis condition (Jao and Ko, 2002) and protease enzyme (Jun *et al*., 2004).

CONCLUSION

Functional properties (foaming and emulsifying capacities) of eb-JPHs were higher than those of a-JPHs. The eb-JPHs had higher scavenging activity with 1,1-diphenyl-2-picrylhydrazyl (DPPH) than a-JPHs. The eb-JPHs had better functional properties than a-JPHs. Therefore, eb-JPH may be used in functional food applications due to its ability to quench the free radicals or used in development of food products if foaming and emulsifying are needed.

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