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บทความวิจัย

คุณสมบัติทางเคมีกายภาพโปรตีนไข่ขาวไฮโดรไลเซตแห้งแช่เยือกแข็งที่ย่อยด้วยเอนไซม์นิวเทรส

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บทคัดย่อ

การคัดเลือกโปรตีนไข่ขาวไฮโดรไลเซตไปประยุกต์ใช้ในอุตสาหกรรมอาหารนั้น สมบัติทางเคมีกายภาพด้านอุณหภูมิและค่า pH ส่งผลต่อสมบัติเชิงหน้าที่ทางด้านความสามารถในการละลาย การเกิดโฟม และอิมัลซิไฟเออร์ ถือว่าเป็นปัจจัยสำคัญในการคัดเลือกไปใช้ในอุตสาหกรรม การวิจัยนี้เพื่อศึกษาผลของการย่อยสลายโปรตีนไข่ขาวด้วยเอนไซม์นิวเทรสที่ความเข้มข้นเอมไซม์ร้อยละ 1 (v/v) ที่อุณหภูมิ 45, 50 และ 55 องศาเซลเซียส และ pH 5, 6 และ 7 ในช่วงระยะเวลา 30 นาที ถึง 6 ชั่วโมง ผลการศึกษาพบว่าเอนไซม์นิวเทรสสามารถย่อยสลายเพิ่มขึ้นเมื่อระยะเวลาเพิ่มขึ้น โดยมีระดับการย่อยสลายเหมาะสมสูงสุดเท่ากับ 51 เปอร์เซ็นต์ ที่อุณหภูมิ 50 องศาเซลเซียส และ pH 7.0 ซึ่งระดับการย่อยสลายขึ้นอยู่กับความเป็นกรด-ด่าง อุณหภูมิ และระยะเวลาของการย่อยสลายอย่างมีนัยสำคัญ การวิเคราะห์การกระจายน้ำหนักโมเลกุลของโปรตีนไฮโดรไลเซตในช่วง 1–100 กิโลดาลตัน พบว่ามีสัดส่วนของโปรตีนขนาดเล็กสูงกว่าโปรตีนไข่ขาวที่ไม่ผ่านการย่อยสลายด้วยเอนไซม์ มีสัดส่วนขนาด 30–100, 10–30, 5–10, 3–5, 1–3 และน้อยกว่า 1 กิโลดาลตัน 11.60, 9.90, 12.04, 8.54 และ 8.81 เปอร์เซ็นต์ ตามลำดับ การศึกษาสมบัติการละลาย การเกิดโฟม ความเสถียรของโฟม และอิมัลซิไฟเออร์ที่ระดับ pH 3.6, 7.6 และ 9.0 ของโปรตีนไข่ขาวไฮโดรไลเซตด้วยเอนไซม์นิวเทรสที่อุณหภูมิ 50 องศาเซลเซียส pH 7 ที่ผ่านการทำแห้งแบบแช่เยือกแข็ง พบว่าสมบัติในการเกิดโฟม ความเสถียรของโฟม และสมบัติอิมัลซิไฟเออร์ขึ้นอยู่กับค่าความเป็นกรด-ด่าง โปรตีนไข่ขาวไฮโดรไลเซตที่มีโปรตีนขนาดเล็กเพิ่มขึ้นส่งผลให้ความสามารถในการละลายและเกิดโฟมเพิ่มขึ้น แต่ความสามารถในการเป็นอิมัลซิไฟเออร์ลดลง

คำสำคัญ: โปรตีนไข่ขาวไฮโดรไลเซต นิวเทรส การกระจายตัวของขนาดโมเลกุล การเกิดโฟม อิมัลซิไฟเออร์

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Research Article

Physicochemical Characteristics of Freeze Dried Egg White Protein Hydrolyzed by Neutrase

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Abstract

The effects of temperature and pH on the physicochemical attributes as solubility, foaming and emulsifying properties of egg white protein hydrolysate are important functional quantities for selecting applications in food industries. In this research, 1% (v/v) Neutrase was used to hydrolyze egg white protein under various temperatures (45, 50 and 55°C) and pHs (5, 6 and 7) over increasing hydrolysis times (30 min to 6 hours). The effects of Neutrase hydrolysis showed that degree of hydrolysis (DH) increased with increasing hydrolysis times resulting in 51% DH at the optimum temperature of 50°C, pH 7.0. Significantly differences of DH were detected in different pH levels, temperatures and hydrolysis times. The molecular distributions of protein hydrolysate in the ranges of 1–100 kDa at optimum hydrolysis condition were reported. Result showed the smaller size proteins in range of 30–100, 10–30, 5–10, 3–5, 1–3 and <1 kDa of 11.60, 9.90, 12.04, 8.54 and 8.81%, respectively. The freeze dried yield of egg white protein hydrolysate at 50°C, pH 7.0 was further investigated for solubility in pH values, 3.6, 7.6 and 9.0 with the foaming capacity and stability associated to those pH values. The emulsifying activity index of various pHs was also explained. These results indicated that the foaming of egg white hydrolysate increased as increasing solubility; however, emulsifying properties were decreased as decreasing solubility.

Keywords: Egg White Protein Hydrolysate, Neutrase, Molecular Distribution, Foaming, Emulsifying Properties

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1. Introduction

Egg white protein hydrolysate (EWPH) products have attracted many interests in functional food especially for health and wellness society in 21st century because of high bioavailability and great content of essential amino acids [1], [2]. The most abundant protein in egg white is ovalbumin, comprising 54% of the protein content. Other proteins include 12–13.6% ovotransferrin, 11% ovomucoid, 3.5% ovomucin, 3.4–3.5% lysozyme, 0.8% ovoflavoprotein, 0.5% ovomacroglobulin, 1.5% ovoinhibitor, 1% ovoglycoprotein, 0.5% avidin, 8% ovoglobulins G2 and G3, 0.05% cystatin (ficin-papain inhibitor) and some other enzymes [3]. This source of protein also contains many useful vitamins (A, B2, B6) and minerals (Mg, Ca, Se, Zn and Fe). In order to produce the healthy protein hydrolysate products, commercially available hydrolyzing enzymes have investigated including Papain, Pepsin, Trypsin, Flavourzyme, Alcalase and Neutrase [4]–[7]. For our interest, under the considerations of a reasonable and easy to operation, a commercially available enzyme, Neutrase® (EC.3.4.24.28) is chosen because it is a neutral endopeptidase produced from *Bacillus amyloliquefaciens*. This endopeptidase has optimal ranges of pH and temperature of 5.5–7.5 and 45–55°C; therefore, it is easy to operate under the processing condition. Moreover, previously reports [8] , [9] that Neutrase® was the most appropriate enzyme to hydrolysate egg white protein to yield antioxidant properties and other bioactive compounds. The consequence of proteolytic action of this enzyme is a change in molecular conformation of native protein to produce protein hydrolysates with function peptides. Therefore, the chosen enzyme type and degree of hydrolysis are considered to be crucial factor affecting the hydrolysate resulting in different free amino acid distributions and variety of functional peptides which lead to different in their bioactivity. Presently, the producing functional peptides and bioactive products are widely used in food systems as additives for beverage and infant formula [3]. They also used as food texture enhancers and pharmaceutical ingredients [10]. Consequently, the appropriate biological activity of EWPH will provide further design of a low-cost process of direct food supplement and food ingredient. Not only EWPH has significantly increasing in consumption in food industry but also other different sources of protein hydrolysates such as milk, egg, meat muscle and marine have widely application [11]–[14].

Therefore, this study was to produce EWPH from egg white protein using 1% (v/v) commercial Neutrase® under the chosen hydrolytic temperatures and pHs. Evaluations of degree of hydrolysis on various temperatures and pHs were reported. Molecular size distribution of protein hydrolysate under the best condition was identified and reported. Moreover, physicochemical and functional properties of freeze dried EWPH powder in terms of solubility, emulsion stability and foaming capacity were also included.

2. Materials and Methods

2.1 Materials and chemicals

Fresh chicken eggs (No.3) were purchased from local supermarket (Makro, Thailand). Neutrase®, EC.3.4.24.28 (≥0.8 U/g Anson Units) from *Bacillus amyloliquefaciens* was obtained from Sigma-Aldrich, USA. One Anson Unit is defined as the amount of enzyme which, under specified conditions, digests

urea-denatured hemoglobin at an initial rate such that there is liberated an amount of TCA-soluble product per minute which gives the same color with the Folin–Ciocalteu phenol reagent as one milliequivalent of tyrosine at 25°C and pH 7.50. L-leucine and 2, 4, 6-trinitrobenzenesulphonic acid were purchased from Sigma-Aldrich, USA. Other chemicals used in this experiment including $Na_2HPO_4.12H_2O$, $NaH_2PO_4.2H_2O$, Tris (hydroxymethyl) aminomethane and $C_6H_8O_7H_2O$ were brought from Ajax Finechem Pty Ltd, New Zealand. $Na_3C_6H_2O_72H_2O$ was acquired from VWR Chemicals PROLABO, Belgium and sodium dodecyl sulfate were obtained from Sigma-Aldrich, USA.

2.2 Compositional analysis

Egg white solution was subjected to preliminary proximate analysis and reported as protein, fat, carbohydrate, ash and moisture. The method was determined according to using the protocols as described in AOAC [15].

The composition of classified egg size 3 is shown in Table 1.

Table 1 Composition of EWP

Composition	Content (g/100 g sample)
Protein	10.8
Fat	0.5
Ash	0.9
Moisture	87.8

2.3 Preparation of egg white protein hydrolysate

Egg white solution was manually separated from the egg yolk before further experimented. Series of 100 mL of egg white protein in 250 mL Erlenmeyer flasks were set up without dilution. After pH levels and temperatures were adjusted to (pH 5, 6, 7 and T 45, 50, 55°C), the experiments were run by adding 1% (v/v) Neutrase to liquid egg white. The reactions were placed in shaking incubator at 100 rpm. The hydrolyzed solutions were then sampling at interval 0.5, 1, 2, 3, 4, 5 and 6 h. The enzymatic hydrolysis of egg white solution was carried out by Box-Behnken Design. The reactions were stopped by placing in 4°C refrigerator to inactivate the enzyme. The hydrolysate solutions were collected and then subjected to freeze-drying in Freeze dryer (LABCONCO, USA) at –48°C, 0.014 mBar for 2 days. Freeze dried powder was finally milled into a geometric diameter of 10 μm and stored at 4°C before further experiments.

2.4 Degree of hydrolysis of enzymatic reaction

Degree of hydrolysis was determined according to the method of Benjakul and Morrissey [16]. To the diluted protein hydrolysate samples (125 μL), 2.0 mL of 0.2125 mM phosphate buffer, pH 8.2 and 1.0 mL of 0.01% TNBS solution were added. The solutions were mixed thoroughly and placed in a 50°C water bath (WNB14, Memmert, Germany) for 30 min in the dark. The reactions were terminated by the addition of 2.0 mL of 0.1 M sodium sulfite. The mixtures were cooled at room temperature for 15 min. The absorbance was measured at 420 nm and α-amino acid content was determined using L-leucine standard curve. The DH was then calculated as follows Equation (1).

$$DH = [(L_t - L_0)/(L_{max} - L_0)] \times 100 \quad (1)$$

where L_t is the amount of α-amino acid released

at time. L_0 is the α-amino acid content in the initial egg white protein hydrolysate. L_{max} is total α-amino acid content in the original egg white protein hydrolysate obtained after acid hydrolysis with 8 M HCl at 100°C for 24 h.

2.5 Molecular distribution of EPWH

Five mL of hydrolyzed egg white protein solution was loaded to membrane filtration to separate large to small molecular weight protein fractions by a series of molecular weight cut-off ultrafiltration membrane (Centrifugal Devices, PALL Corporation, USA) from 100 kDa followed by 30, 10, 5, 3 and 1 kDa, respectively. Fractions of protein separation were centrifuged at 2,000 g for 5 min before both permeate and retentate solutions were collected. The concentrations of each fractions were measured protein concentration by Nanodrop (OPTIZEN NANO Q, Mecasys Co., Ltd., Korea).

2.6 Solubility of EWPH

The solubility of EWPH powder was measured using the procedure of Morr [17] with slight modification. Each of 0.5 g of EWPH was dissolved in 50 mL of three buffers: 50 mM citrate-NaOH (pH 3.6), phosphate (pH 7.6) and Tris-HCl (pH 9.0). The mixtures were stirred at 2,000 rpm in room temperature for 1 h and then centrifuged at 2,800 g for 30 min using a Eppendorf 5804R Centrifuge, Germany. The protein content of the supernatant was quantified by Miller method using bovine serum albumin as a standard. Total protein content in the sample was determined after solubilized sample in 0.5 N NaOH. Protein solubility was then calculated as follows Equation (2).

$$\text{Solubility}(\%) = \frac{\text{protein content in supernatant}}{\text{total protein content in sample}} \times 100 \quad (2)$$

2.7 Foaming capacity and foaming stability

Foaming capacity (FC) and foaming stability (FS) of the samples were measured according to method described by Sathe and Salunkhe [18]. To represent to actual acidity, neutral and alkalinity based food products, 50 mM citrate-NaOH buffer (pH 3.6), phosphate buffer (pH 7.6) and Tris-HCl buffer (pH 9.0) were used as buffer solutions. A 1.0% (w/v) sample was dissolved in those buffers and then whipped for 10 min at 2,000 rpm in a blender (Blender, yellow line OTS 20 digital, Netherland). The whipped sample was immediately transferred into a graduated cylinder and the total volume was measured after 30s. The foaming capacity was calculated according to the following Equation (3).

$$\text{Foaming capacity (\%)} = \frac{(\text{Vol. after whipping 30 s.} - \text{Vol. before whipping})\,(\text{mL})}{(\text{Vol. before whipping mL})} \times 100 \quad (3)$$

The whipped sample was allowed to stand at 20°C for 3 min, and then the volume of whipped sample was recorded. Foam stability was calculated as follows Equation (4).

$$\text{Foaming stability (\%)} = \frac{(\text{Vol. after whipping 3 min.} - \text{Vol. before whipping})\,(\text{mL})}{(\text{Vol. before whipping mL})} \times 100 \quad (4)$$

2.8 Emulsifying activity index

The emulsifying activity index (EAI) at various pH values was measured by the turbidimetric method

described by Pearce and Kinsella [19]. To form an emulsion, a 1.0% (w/v) sample was dissolved in 50 mM citrate-NaOH buffer (pH 3.6), phosphate buffer (pH 7.6) and Tris-HCl buffer (pH 9.0). 12 mL of the dissolved sample and 4 mL corn oil were homogenized in a blender (Blender, yellow line OTS 20 digital, Netherland) at 12,000 rpm for 1 min. A 50-µL aliquot of the emulsion was taken from the bottom container at different time intervals and diluted in 5 mL of 0.1% sodium dodecyl sulfate. The absorbance of the diluted emulsion was measured at 500 nm. All the experiments were conducted at room temperature. EAI was than calculated according to the following Equation (5).

$$EAI\ (m^2/g) = (2T \times D)/(\varphi \times C \times 10^4) = (4.606 \times A \times D)/(\varphi \times C \times L \times 10^4) \quad (5)$$

Where T is the turbidity, D is the dilution factor, φ is the volume fraction of the dispersed phase (oil), C is protein weight per volume of aqueous phase before the emulsion, A is the observed absorbance and L is the path length of the cuvette.

2.9 Statistical analysis

All experiments were carried out in triplicate. Data were analyzed using analysis of variance (ANOVA), and mean comparisons were performed using Least Significant Difference (LSD) tests. The difference in DH, solubility, foaming capacity, foaming stability and emulsion activity index before and after digestion were tested for statistical significance by ANOVA. Analysis statistical significance was assessed at the level of $p<0.05$. Analysis was performed using SPSS software (SPSS 16.0 for windows, SPSS Inc., USA).

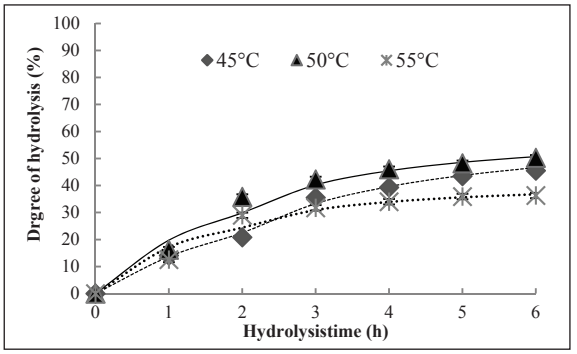

Figure 1 Progress curve of hydrolysis of egg white protein with different hydrolysis temperature.

All reported values were expressed as the mean of three replicate determinations and standard deviation.

3. Results and Discussion

3.1 Effects of temperature and pH on DH

Hydrolysis of egg white protein was carried out using 1% (v/v) Neutrase. Rapid hydrolysis was observed in Figure 1 during 1 h and then the hydrolytic reaction was slower from 2 h until the end at 6 h. The overall result showed non-linear relationship of DHs to hydrolysis times as all curve profiles illustrated in Figure 1.

Results found that DH increased with hydrolysis time, resulting in a gradual release of peptide fragments during protein hydrolysate and prolonging the hydrolysis time did not produce significantly DH after 2 h. The maximum peptide cleavage reported in DH yielded equivalent to 51% at 50°C at 6 h hydrolysis time while the DH reached 49% and 36% at 45°C and 55°C. As shown, DH at 50°C of 6 h hydrolysis were detected and found significantly higher (p=0.0001) once compared with other hydrolysis temperatures, 45 and 55°C. Based on these results, three effect

of temperatures on degree of hydrolysis from the different hydrolysis time were fitted with a pseudo first order rate (saturated enzyme kinetic model via Michaelis-Menten equation) of reaction showing the overall rate of enzymatic hydrolysis of EWP as shown in Table 2.

Table 2 Initial rate of egg white protein hydrolysis by Neutrase

Hydrolysis Temperatures (°C)	Hydrolysis Rate (h^{-1})	R^2
45	63.781±2.47	0.995
50	61.400±2.30	0.991
55	42.127±2.67	0.964

In the hydrolysis experiment, the decreasing of hydrolysis rate for the longer hydrolysis time was occurred due to the reduction in the specific peptide bond availability. Rate of hydrolysis in kinetic model result showed there was relatively the same rate of hydrolytic action of enzyme on EWP between 45 and 50°C. Since the higher temperature means the higher energy cost for production, is it worth using 50°C rather than 45°C to gain a little bit more %DH after 6 h of hydrolysis. Therefore, 50°C was the most effective temperature for this neutral protease to produce EWPH. Effect of pH values (5–7) over hydrolysis time were further experimented under 50°C and showed in Figure 2. All the enzymatic hydrolysis in different pH levels were compared. There are no significant differences in DHs over 1–4 h hydrolysis time. However, at 5 h hydrolysis time, the significantly higher DHs about 1.12 and 1.0 times found in pH 7 over other two pHs, 6 and 5, with p-values of 0.003 and 0.0001, respectively.

The DHs of 6 h hydrolysis time of EWPH were

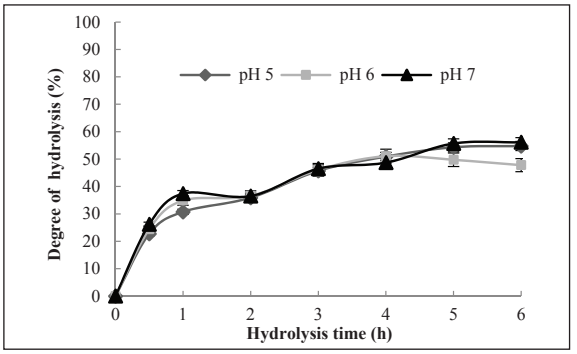

Figure 2 Effect of pH levels on DHs of Neutrase hydrolyzed egg white protein.

54%, 49% and 55% at pH 5, 6 and 7, respectively. Neutrase is classified as a neutral, metallo endo-protease from *Bacillus amyloliquefaciens* that it's hydrolytic action randomly on internal peptide bonds. At the initial period, the reaction of enzyme and insoluble egg white protein was occurred rapidly leading to the polypeptide chains bound to the surface were hydrolyzed. The slower reaction took place because the core protein was became dense resulting in slowly cleaved. These results were similar to Benjakul and Morrissey [17] observation. However, our results of Neutrase hydrolysis on egg white protein at 50°C, pH 7.0 over 1 h hydrolysis time gained higher DH (37%) than previously reports [20], [8]. Ai *et al*. [20] reported that DH of EWPH induced by 1%(v/v) Neutrase were the highest at 25.5% after being hydrolysed for 4 h under the optimum condition (pH 7.0, 37°C). Generally, the effect of pH on the affinity of EWP hydrolysis can be overcame by using a high substrate (EWP) concentration. It is worth after observed from the results (Figure 2) that a drift of pH from 5 to 7 was not affect to substantial changes in DH since the practical processing not run at fixed pH values.

Cho *et al.* [8] were also used Neutrase treatment and reported the highest DH of 23.4% of egg white protein. As known, the DH depends not only on the protein substrate and the specificity of the enzyme but also on the conditions used during hydrolysis. The DH is considered as an important parameter in enzymatic modification of proteins and a crucial factor controlling the composition and properties of products [21]–[23]. Hydrolysis effects as a consequence of DH on the protein substrate were represented by the changes in the molecular size, hydrophobicity and polar-group content. Therefore, the hydrolysate's characteristics from this enzymatic action directly affect its functional properties for using as a food ingredient [24].

3.2 Molecular distribution

Normally, EWP contained the larger size proteins as 45 kDa ovalbumin, 76 kDa ovotransferrin, 14.4 kDa lysozyme, 8.3 kDa ovomucin and 28 kDa ovomucoid [25]–[29]. The Neutrase EWPH obtained from hydrolysis were subjected to ultrafiltration using series of membranes with 30, 10, 5 and 1 kDa molecular weight cut off. After Neutrase hydrolysis, the action of proteolytic cleavage yielded at least six fractions of smaller size proteins were detected. Results showed seven fractions of obtained protein separation through membrane filtration were presented in Figure 3. According to calculated molecular weight distribution, only 36.44% of >100 kDa fraction were remained in the solution while 13.21% in the range of 30–100 kDa followed by 11.06% of 10–30 kDa, 9.90% of 5–10 kDa, 12.04% of 3–5 kDa, 8.54% of 1–3 kDa and 8.81% of <1 kDa protein were found.

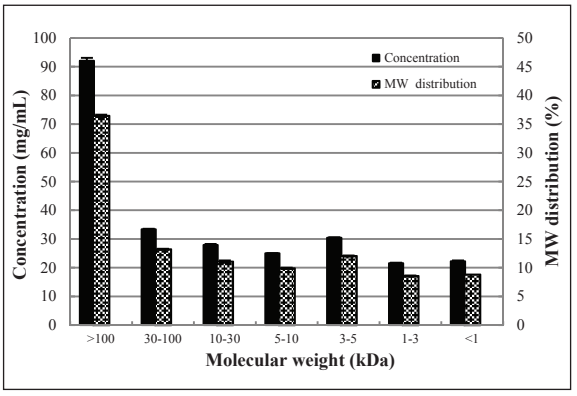

Figure 3 Molecular distribution of egg white protein hydrolysate.

It is apparent that the hydrolysis generated more peptides ≤ 3 kDa (17%) as well as the proportion of peptides 5–10 kDa were higher than control (21.94%). Under this hydrolysis condition, the generation of smaller peptide were produced. In future studies, the antioxidant and bioactive properties of each fraction will be tested since it has been an interest of food application and previously reported that the molecular weight ranges from 500 to 1800 Da [30], [31] showed the majority of the antioxidant activity. Our fraction <1 kDa from this experiment was further identified by Matrix-assisted laser desorption/ ionization and found at least 6 peptide sequences that potentially act as Angiotensin-Converting Enzyme (ACE) Inhibitor (data is not included in this report).

Results showed that enzymatic hydrolysis yielded high proportion of smaller peptides and confirmed the transformation of large molecular weight egg white proteins. The low molecular weight proteins (<10 kDa) obtained from this study may contribute to the usefulness of this protein hydrolysate as a source of bioactive peptides.

The possibility to apply peptides gained from our experiment as a functional peptide for food and pharmaceutical application may be consistent with earlier reports as following. Dávalos *et al.* [32] reported that hydrolyzed peptides from crude egg white proteins showed a strong antioxidant activity. For, Chiang *et al.* [33], they used Thermolysin to hydrolyze egg white and produced bioactive peptides that can inhibit the activity of ACE. Nevertheless, Miguel and Aleixandre [34] produced peptides by hydrolyzing egg white with Pepsin. Among these peptides previously reported, amino acid sequences of YREERYPIL, RAADHPFL and IVF showed strong ACE-inhibitory activities. Moreover, Fujita *et al*. [35] hydrolyzed ovalbumin with Pepsin, Trypsin, and α-Chymotrypsin and produced 7 ACE-inhibitory peptides, LKA, LKP, LAP, IKW, FQKPKR, FKGRYYP and IVGRPRHQG. Some of these peptides produced from ovalbumin not only showed strong ACE-inhibitory effects but also lowered blood lipid content [36]. Feeding these peptides to spontaneously hypertensive rats reduced blood pressure to the rats [34].

3.3 Solubility

In order to determine a key for the improvement of the appearance and the test of final product, the solubility of freeze-dried EWPH was evaluated in three pH levels. As represented in Figure 4, the increase of solubility of EWPH in all pH values were significant higher than those of unhydrolyzed EWP samples. Approximately 1.20, 1.33 and 1.35 times at pH 3.6, 7.6 and 9.0, respectively. The solubility of EWPH was highest at pH 9.0 of 90% solubility as shown in Figure 4 and gradually decreased with

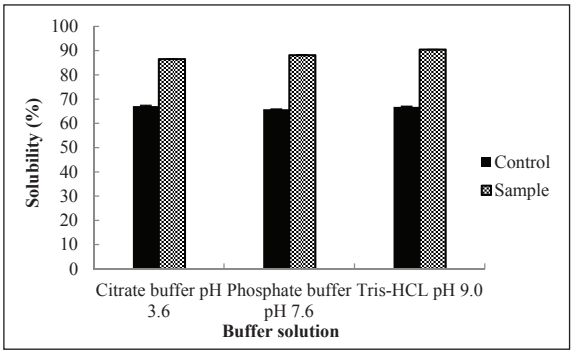

Figure 4 Solubility of hydrolysate in different pH values.

decreasing pH. This suggested that there was a high degree of aggregation at pH 3.6 because the protein would lead to denaturation.

EWPH solubility resulted from smaller peptides from hydrolysate which were contained more polar residues which could enhance the quality of hydrogen bonds with water resulting in the increment of protein solubility [37]. The enhanced solubility of the hydrolysate might be attributable to their smaller molecular size and the newly exposed ionizable amino and carboxyl groups of the amino acids, that increase the hydrolysate hydrophilicity [24]. The solubility profile obtained in this study is similar to the findings of Cho *et al*. [8] who reported that protein hydrolysates from egg white protein powders had an excellent solubility at pH 9.0 (80%). The high solubility of egg white protein hydrolysate could be useful in preparation of various foods and nutraceutical formulations.

3.4 Foaming capacity and foaming stability

The foaming properties of EWPH were affected by pH values as illustrated in Figure 5 (a), (b). Foaming capacity tended to decrease at pH 7.6 and 9.0

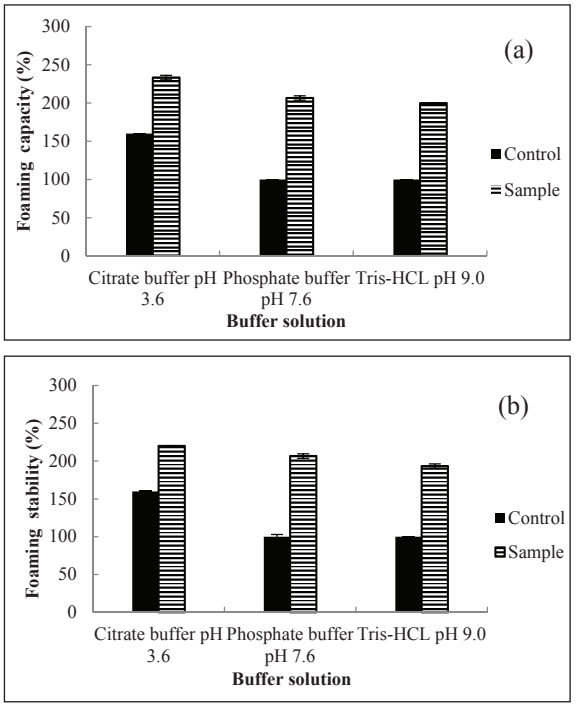

Figure 5 Effect of foaming capacity (a) and foaming stability (b) of EWPH in different pH values.

when compared with pH 3.6. The foaming capacity of EWPH reached a maximum at pH 3.6 at 233.33% and decreased at alkaline pH by 1.13 and 1.16 times. Neutrase hydrolysis also showed a significantly increase in foam capacity and foam stability compared with unhydrolyzed egg white (p<0.0001).

The lowest foaming stability levels seen might be the proteins coincided once the solubility was high and close to their isoelectric pH. Foam stability was lowest at pH 9.0 with was lower than that of stability at pH 3.6 and 7.6 by 6.74% and 13.99%. This finding was consistent with investigation of Cho *et al*. [8] which reported the effected of pH to the foaming properties of EWPH. They revealed that foaming capacity tended to decrease at pH 7.6 and 9.0. The foaming capacity of EWPH reached a maximum at pH 3.6 and decreased at alkaline pH. Molecularly, smaller peptide from hydrolysate were showed to have more smaller size distribution which considered to have more polar residues which could magnify the number of hydrogen bonds with water resulting in more solvated polypeptides units. The smaller size distribution of proteins would generate low surface tension at water-air interface leading to more foaming capacity and foaming stability. The protein with excellent foam capacity needs was first rapidly adsorbed onto the gas-liquid interface and then promptly molecule-transposed. Finally, sticky film was formed by intermolecular interaction [38]. Therefore, the increase of foam capacity and stability after hydrolysis found in this experiment may be possible to further applications of dried EWPH in food processing.

3.5 Emulsifying activity index

Emulsion stability is expressed as emulsifying activity index as showed in Figure 6. The stability of the emulsions made of EWPH in all pH values were significantly lower than those prepared from the unhydrolyzed one (p<0.0001). The highest ESI were observed for emulsions prepared with EWPH at pH 3.6, 7.6 and 9.0 possibly due to an increase in repulsion by the electrostatic charge of the proteins. Normally the net charge at pH 5 (near to the isoelectric point of egg white protein) is minimized, and the repulsion between the fat globules emulsified by the proteins is reduced resulting in lower emulsion stability.

Emulsion stability profile obtained in this study is in the same trend of the findings of Ai *et al*, [20] which investigated the effects of six proteases

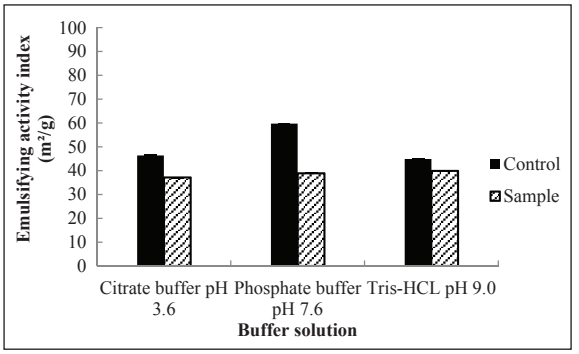

Figure 6 Emulsifying activity index in different pH values of EWPH.

(Flavourzyme, Neutrase, Alcalase, Papain, Pepsin, and Trypsin) on the properties of preserved egg white gel, including emulsifying capacity. They reported that the emulsifying stability of EWPH increased after the hydrolysis of Neutrase at pH 9.0. However, results in this study as seen in Figure 5 EAI of EWPH lower than the control EWP. Generally, hydrolysates with a higher DH exhibits lower EAI and ESI due to their small peptide size. Though small peptides diffuse to, and absorb fast at the interface, they are less efficient in reducing the interfacial tension due to lack of unfolding and reorientation at the interface [24]. Moreover, ions in the buffer were used mix with samples. Therefore, these elements would affect the emulsifying properties of the hydrolysates [20].

4. Conclusion

The study demonstrated that Neutrase hydrolysate of EWP yielded EWPH. The highest 51% DH of EWPH at 50°C, pH 7.0 was found resulting in 90.41% solubility at pH 9.0, 233% foaming capacity at pH 3.6, 220% foaming stability at pH 3.6 and 39.87% ESI at pH 9.0. However, this freeze dried EWPH at 51% DH was not applicable to use as emulsifier when compared with unhydrolyzed EWP. Functional properties of hydrolysate improve remarkably that could open the new channel for selecting applications in food industries and great possibility for developing pharmaceutical products.

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