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Production and characteristics of protein hydrolysate from tra fish (Pangasius hypophthalmus)

Produção e características do hidrolisado de proteínas de peixe-panga (Pangasius hypophthalmus)

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ABSTRACT

The goal of this study was investigating effects of pH and temperature on the production of tra fish protein hydrolysate (FPH) fish protein concentrate (FPC) using alcalase and characterization of FPHs. Temperature of 55 °C and pH 8.5 were the best condition for highest degree of hydrolysis (around 55%) after 60 min of reaction. Under hydrolysis, protein solubility was significantly increased from 4% to >90%. FPC contained fractions of 50, 40, 37, 15, 12, and 11×10^3 g mol^{-1}. After 15 or 120 min of enzymatic reaction, molecular weight was degraded to 4.86 and 4.31 (×10^3 g mol^{-1}) of FPH15 or FPH120 samples. Due to the increasing of protein solubility during the hydrolysis, water holding capacity reduced from 2.81 mL g^{-1} (FPC) to <0.3 mL g^{-1} of FPH15 and FPH120 samples. Besides, emulsion activity index and emulsion stability index of samples were ordered as FPH15>FPH120>FPC and FPH120>FPH15>FPC. Furthermore, foam activity index and foam stability index of samples were arranged as FPH15>FPH120> FPC. At pH 4.0, all these indexes were lowest. Generally, under enzymatic hydrolysis, FPH could be possessed of different molecular weights and physico-chemical properties. Thus, FPH could reach various demand of industrial applications.

Keywords: Enzyme. Emulsion. Molecular weight. Protein. Tra fish

RESUMO

O objetivo deste estudo foi investigar os efeitos do pH e da temperatura na produção e caracterização de hidrolisado de proteína de peixe-panga (FPH) concentrado protéico de peixe (FPC), utilizando alcalase. Temperatura de 55 °C e pH 8,5 foram as melhores condições para o maior grau de hidrólise (cerca de 55%) após 60 minutos de reação. Sob hidrólise, a solubilidade da proteína aumentou significativamente de 4% para> 90%. O FPC continha frações de 50, 40, 37, 15, 12 e 11 × 10^3 g mol^{-1}. Após 15 ou 120 min de reação enzimática, o peso molecular foi degradado para 4,86 e 4,31 (x 10^3 g mol^{-1}) de amostras de FPH15 ou FPH120. Devido ao aumento da solubilidade das proteínas durante a hidrólise, a capacidade de retenção de água reduziu de 2,81 mL g^{-1} (FPC) para <0,3 mL g^{-1} das amostras de FPH15 e FPH120. Além disso, o índice de atividade da emulsão e o índice de estabilidade da emulsão das amostras foram ordenados como FPH15> FPH120> FPC e FPH120> FPH15> FPC. Além disso, o índice de atividade da espuma e o índice de estabilidade da espuma das amostras foram organizados como FPH15> FPH120> FPC. Em pH 4,0, todos esses índices foram mais baixos. Geralmente, sob hidrólise enzimática, a FPH pode possuir diferentes pesos moleculares e propriedades físico-químicas. Assim, a FPH poderia atingir várias demandas de aplicações industriais.

Palavras-chave: Enzima. Emulsão. Peso molecular. Proteína. Peixe-panga

INTRODUCTION

Fish protein hydrolysate (FPH) could be produced by the enzymatic hydrolysis of fish products. Degree of hydrolysis, which depended on factors of the reaction (pH and temperature) significantly affected on characteristics of FPH (Clemente, 2000). Water-soluble FPH contained balanced amino acid components that have positive effects on the digestive system (Gbogouri et al., 2004). The hydrolysis could improve physico-chemical properties (such as solubility, emulsion activity index, emulsion stability index, foam activity index, foam stability index) of fish protein for food applications (AsbjørnGildberg, 1993; Chalamaiah et al., 2010). Fish is one of an important protein source for human diet. In 2010, around 16.7% of the global intake of animal protein was fish (Nurdiani et al., 2016). FPH was a bioactive nutrition that can be absorbed easily in the human body (Nesse et al., 2011). Nowadays, the advantages of natural and bioactive products attracted not only consumers but also food scientists. In many countries, fish protein hydrolysate was used as functional foods (Marchbank et al., 2008).

In this study, we produced tra fish protein hydrolysate using alcalase enzyme. Its molecular weight and physico-chemical properties were characterized. The results of this study can be useful for industrial production and food applications.

MATERIALS AND METHODS

a) Preparation of Fish Protein Concentrate (FPC) and Fish Protein Hydrolysate (FPH)

Tra fish fillet contained of 18.6% protein, 3.4% lipid, 1.1% ash and 76.5% moisture. FPC was prepared from tra fish fillet using isopropanol and ethanol following a previous study (Trinh et al., 2017). The FPC powder (10 g) was added 0.2 M phosphate buffer (1,000 mL, pH from 7.0 to 8.0) or 0.2 M borate buffer (1,000 mL, pH from 8.5 to 9.0). Enzyme alcalase 2.4 L FG (1.0 mL, 2.4 Anson unit g^{-1}, Novozyme) was added. For prevention of contaminants, sodium azide (0.2 g) was added to this mixture (Herman & Malcolm, 1944). Enzymatic reaction was conducted at 50-60 ^{o}C under continuous shaking (200 rpm). After 180 min, the reaction was stopped by boiling for 15 min. Then, the mixture was cooled down to room temperature. Next, FPH was collected by freeze-drying. FPH was ground, sieved (0.125 mm aperture) and kept in vacuum-PE plastic package at 4 ^{o}C until next experiments.

b) Degree of hydrolysis, protein solubility, and water holding capacity measurements

Degree of hydrolysis (DH, %) was determined by the percentage of soluble protein in 10 g% (w/v) trichloroacetic acid base on total protein of the sample (Hoyle & Merritt, 1994). Protein solubility (PS, %) was the percentage of soluble protein on total protein of the sample (Vojdani, 1996).

Water holding capacity (WHC, mL g^{-1}) was analyzed following a previous method (Rodríguez-Ambriz et al., 2005). Protein sample (100 mg) was mixed well in 1.0 mL of distilled water. Then, protein suspension was centrifuged at 1,800×g for 20 min at 30 ^{o}C. Supernatant was decanted and the tube was drained at a 45^{o} angle for 10 min. The volume of free liquid was measured and the remaining liquid was expressed as milliliter of water absorbed per gram of protein.

c) Emulsion activity index, emulsion stability index, foam activity index, and foam stability index measurements

Emulsion activity index (EAI) and emulsion stability index (ESI) were characterized following previous studies (Pearce & Kinsella, 1978; Klompong et al., 2007). Foam activity index (FAI) and foam stability index (FSI) were analyzed following a previous paper (Sze-Tao & Sathe, 2000).

d) Molecular weight measurement

Molecular weight of fish protein concentrate (FPC) and fish protein hydrolysate (FPH) were characterized using SDS-PAGE electrolysis and gel permeation chromatography (GPC). SDS-PAGE electrolysis was done following a published paper (Trudel & Asselin, 1989). GPC was done using HPLC1100 system (Agilent, USA), Ultrahydrogel 500 column (300 mm x 7.8 mm ID), and a Refractive Index detector. Column and detector temperatures are 40 and 35 ^{o}C, respectively. 0.5M KNO_3 was the mobile phase with the velocity of 1.0 mL min^{-1}. The injected volume of sample was 20 µl.

e) Statistical analysis

Experiments were conducted in triplicate, and the mean value was reported. Data were analyzed using analysis of variance (ANOVA), and the mean separations were determined by Duncan's test (p<0.05). All statistical analyses were carried out using SPSS software (Ver. 17.0, SPSS, Chicago, IL, USA).

RESULTS AND DISCUSSION

Fish protein concentrates powder has the component (%) of protein (88.7), lipid (<0.3), ash (0.58) and moisture (10.42). Obviously, this FPC reached A-type following FAO standard (Windsore, 2001). Comparing to the weight of fresh fillet, the yield of FPC preparation was around 13.0 %.

a) Effect of pH and temperature on degree of hydrolysis (DH) and solubility (PS) during enzymatic hydrolysis

Effects of pH on the hydrolysis were presented on Figure 1 and Figure 2. Actually, pH 7-8 were not suitable for enzymatic hydrolysis. pH 9 presented quickly increasing of DH at the first 45 min. However, pH 8.5 reached the DH_{max} (around 55%) and PS_{max} (around 90%) after 60 min. Thus, the best pH activity of alcalase in our reaction might be 8.5. Obviously, DH was positive correlated to PS. It indicated that the hydrolysis resulted in the elevation of protein solubility.

Effects of temperature on hydrolysis were present on Figure 3 and Figure 4. Temperature from 50-60 ^{o}C did not much affected on the degree of hydrolysis but significantly influenced the protein solubility (Bhaskar & Mahendrakar, 2008). At pH 8.5 and 55 ^{o}C, after 60 min of hydrolysis, both DH and PS reached maximum values.

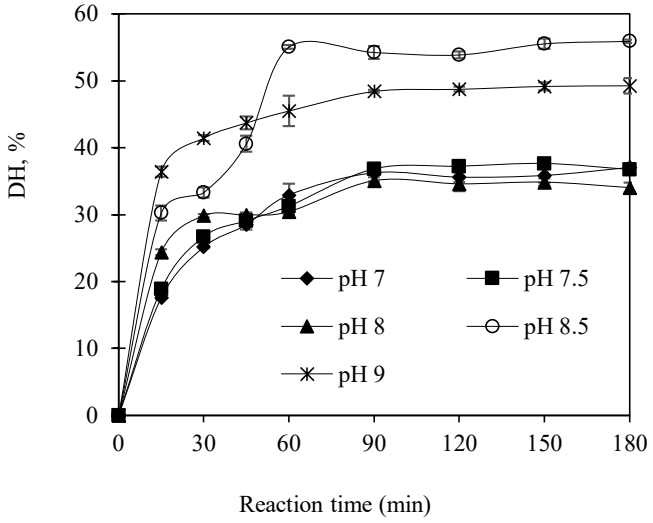

Figure 1. Effect of pH on degree of hydrolysis (DH, %) during enzymatic reaction at 55 °C

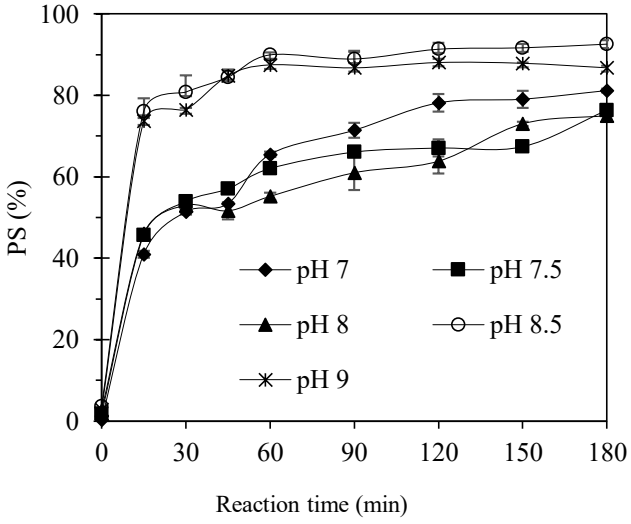

Figure 2. Effect of pH on protein solubility (PS, %) during enzymatic reaction at 55 °C

In general, both temperature and pH are the main factors affecting enzymatic hydrolysis. Alcalase was reported its best activity and stability at pH 8-10 and 55-75 °C (Novozymes, 2016). During the enzymatic reaction, pH and temperature altered reactants (such as structure and ionization properties) that affected the interaction of enzyme-substance complex (Boikess et al., 1986). At optimal temperature and pH, peptide linkages would be exposed for the attack of protease (Mukhin & Novikov, 2001; Nielsen, 1995). During the reaction, enzyme that continuously attacked on protein led to the breaking down of peptide bonds. Evidently, the increase of DH elevated more soluble peptides in tricloacetic solution resulting high value of PS (Montecalvo et al., 1984). Solubility was probably important functional property of protein hydrolysate and its potential applications.

Hydrophobic protein-protein interactions resulted in decrease solubility, whereas the increase solubility caused by ionic protein-protein interactions. Ionic residues that locate on the surface of protein or peptides introduced electrostatic repulsion between hydration shells around ionic groups and repulsion between peptide molecules. These mechanisms contributed to increase solubility of protein. Furthermore, during enzymatic hydrolysis, protein was gradually cleaved into smaller peptide units that shown the increase of protein solubility (Kristinsson & Rasco, 2000).

Following the results from this study, pH 8.5 and 55 °C were pH value and temperature for further sample preparation. FPH15 and FPH120 were sample that was taken at 15 and 120 min of hydrolysis. These samples represented to the intermediate and intense level of hydrolysis of FPC. Further analysis was conducted to characterize the effects of enzymatic hydrolysis on properties of tra fish protein hydrolysates.

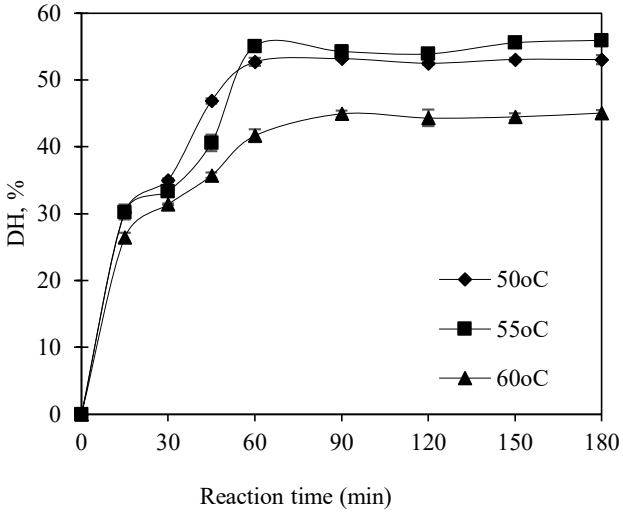

Figure 3. Effect of temperature on degree of hydrolysis (DH, %) during enzymatic reaction at pH 8.5

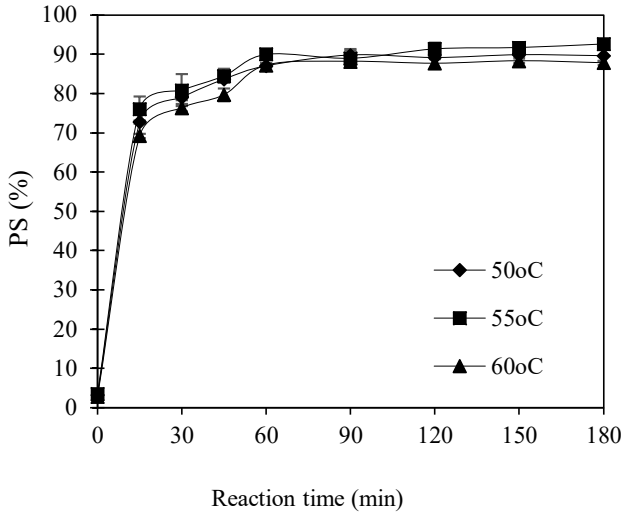

Figure 4. Effect of temperature on protein solubility (PS, %) during enzymatic reaction at pH 8.5

b) Water holding capacity (WHC)

Water holding capacity refered the water-binding ability of protein and it against centrifugal force. This was a useful property for certain food formulations. The formation of –COOH and $–NH_2$ during enzymatic hydrolysis had a substantial effect on the amount of adsorbed water. Normally, fish protein hydrolysate had excellent WHC (Rasco & Barbara, 2000). In this study (Figure 5), water holding capacity of FPC (2.81 mL g^{-1}) was highest comparing to FPH samples (<0.3 mL g^{-1}). Previous study (Taheri et al., 2013) reported a high WHC (5.1 mL g^{-1}) of poultry byproducts and rainbow trout viscera FPHs. Besides, these solubility of these samples was over 90% (at pH>5). Unfortunately, in our study, FPHs that had low molecular weight (Table 1) and highly water-soluble level could not be left on the tube under centrifugation (1,800×g, 20 min, 30 °C) resulting in their extremely low WHC (0.27 mL g^{-1}).

Table 1. Molecular weight[a] ($\times 10^3$ g mol^{-1}) of FPHs from GPC analysis

Sample	M_n	M_w	M_z	PI
FPH15	3.5194	4.8566	7.1423	1.3799
FPH120	3.3044	4.3053	6.0830	1.3029

[a] *M_n: Number-Average Molecular Weight; M_w: Weight-Average Molecular Weight; M_z: z-average molecular weight; PI: polydispersity index= M_w/M_n; 10^3 g mol^{-1} = 1 kDa*

c) Molecular weight

Following SDS-PAGE (Figure 6), FPC contained fractions with molecular weights: (a) >70 kDa (1 kDa=10^3 g mol^{-1}), (b) 50 kDa, (c) 40 kDa, (d) 37 kDa, (e) 15 kDa, (f) 11 and 12 kDa. However, there was no signal of the band of FPHs indicating that molecular weight of FPHs was lower than 11 kDa and could not be detected on SDS-PAGE.

Gel permeation chromatography was useful to determine the molecular weight of FPHs. In this study (Table 1), molecular weight was shown as $M_n<M_w<M_z$ (Agilent, 2015). The weight-average molecular weight (M_w). Actually, M_w of FPHs decrease during reaction. After 15 and 120 min of hydrolysis, M_w of FPH15 and FPH120 were 4.8566 and 4.3053 ($\times 10^3$ g mol^{-1}), respectively. Further hydrolysis (15 to 60 min of reaction), although DH increasing, M_w slowly decreased. Both FPH samples contained PI of 1.30-1.37 reflecting they were certain polymer distribution broadens (Agilent, 2015). Molar mass distribution of FPHs were shown on Figure 7 and Figure 8. Respectively, two samples contained peak (1) and peak (2) with the signal W (logM) as FPH15>FPH120 and FPH15<FPH120. Thus, the first 15 min, molecular weight of protein (from >70$\times 10^3$ g mol^{-1} to 11$\times 10^3$ g mol^{-1}) quickly degraded to smaller peptides (<4.9$\times 10^3$ g mol^{-1}). However, during 105 min of hydrolysis, large peptides (peaks 1) were slowly broken down into smaller molecules (peak 2). These molar masses correlated to their degree of hydrolysis (FPH15 was 30.25 and FPH120 was 53.89). High DH indicated the degradation of protein molecules to smaller peptides (Gbogouri et al., 2004; Sathivel et al., 2003).

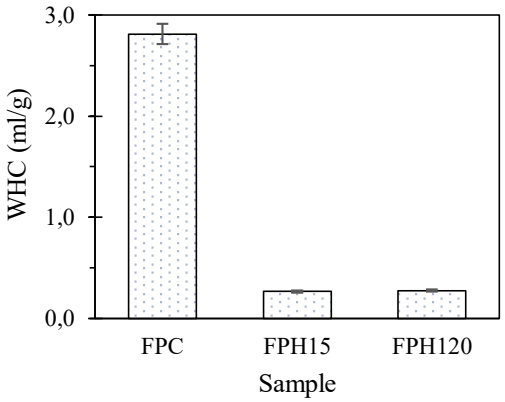

Figure 5. Water holding capacity (WHC, mL g^{-1}) of samples

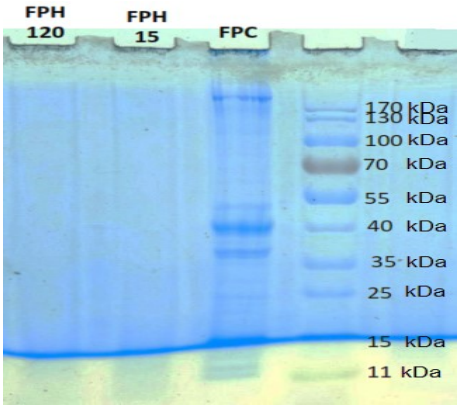

Figure 6. Molecular weight fractions from SDS-PAGE analysis

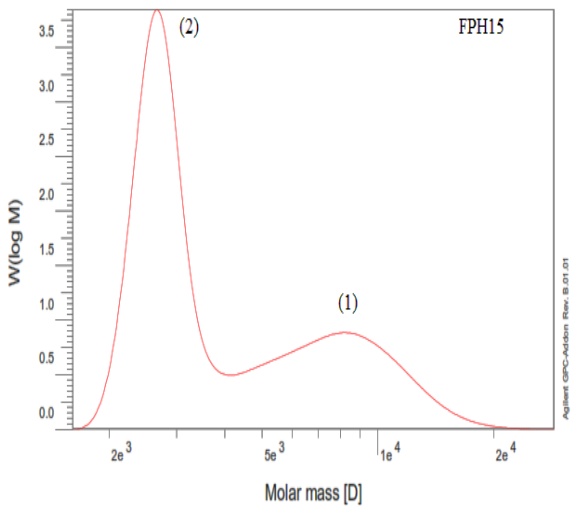

Figure 7. Molar mass distribution of FPH15

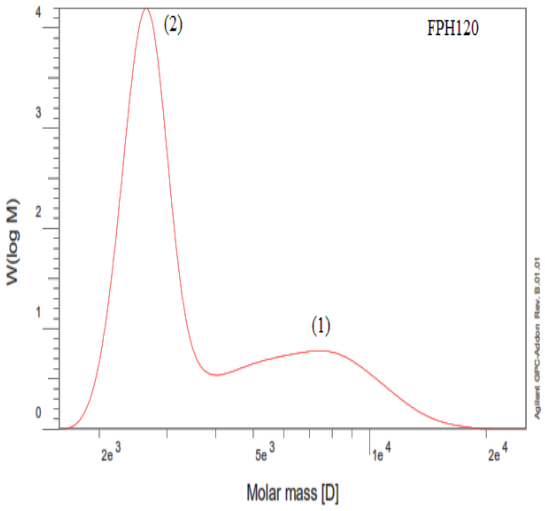

Figure 8. Molar mass distribution of FPH120

d) Emulsion activity index (EAI) and emulsion stability index (ESI)

Theoretically, emulsion properties indicated how effectively FPH reduces the interfacial tension between hydrophilic and hydrophobic parts in food (Kristinsson & Rasco, 2000). During homogenization, formed oil globules were coated by proteins or peptides resulting the protective membrane from coalescing (Demetriades et al., 1997). Emulsion indexes of samples were shown on Figure 9 and Figure 10. EAI and ESI of samples were respectively sorted as FPH15>FPH120>FPC and FPH120>FPH15>FPC. At pH 4.0, these indexes were lowest. At pH<4.0 or pH>4.0, both EAI and ESI dramatically increased.

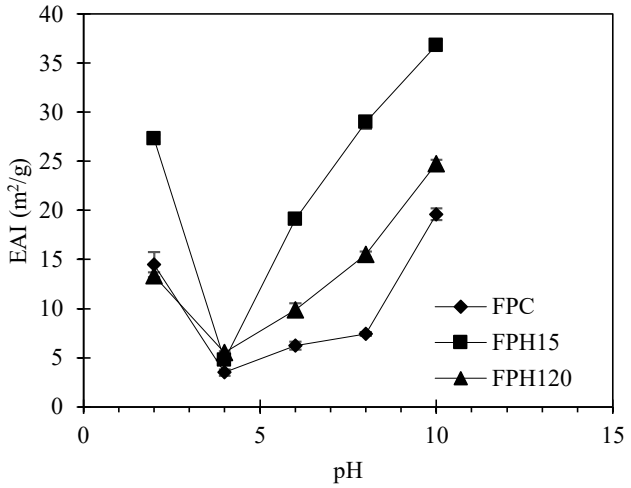

Figure 9. Effect of pH on emulsion activity index (EAI, $m^2 g^{-1}$) of samples

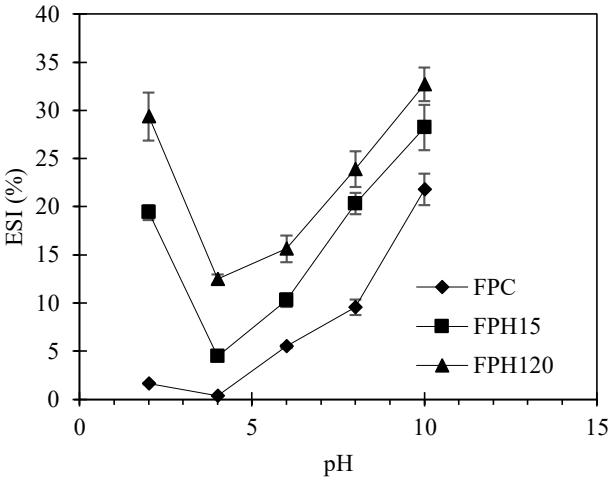

Figure 10. Effect of pH on emulsion stability index (ESI, %) of samples

The similar results were announced based on protein hydrolysates from pink perch muscle. EAI and ESI of this FPH were lowest at pH 4.0 and these increased at high pH values (Naqash & Nazeer, 2011). Low molecular weight peptides were not enough amphiphilic to exhibit good emulsifying activity (Chobert et al., 1988). Besides, limited hydrolyzed proteins showed better emulsifying and foaming than extensive hydrolysates (Kristinsson & Rasco, 2000). Generally, for good emulsifying and interfacial properties, peptides should be more than 20 amino acids in length (Lee & Yamauchi, 1987). Furthermore, amino acid composition, sequence and amphiphilic properties of peptides were major factors affecting on emulsion indexes.

In this study, not only molecular weight, but also environmental pH affected on both EAI and ESI of protein hydrolysate (Linder et al., 1996). Solubility, surface hydrophobicity of peptides and the charge of the protective layer surrounding the oil droplets were affected by pH (Sikorski, 2002). At isoelectric point (pH_i), the total charge of peptides was zero is resulting its lowest solubility. Comparing to pH_i, at lower or higher pH values, peptides could be unfolded due to positive or negative charges, respectively. These charges enhanced the repulsion between peptides and exposed the hydrophilic and hydrophobic residues of peptides promoting a major

interaction at the oil-water interface (Pacheco-Aguilar, 2008; Klompong et al., 2007). It was reported that solubility is well correlated to emulsion and foaming properties of FPH. At pH_i, both EAI, ESI and solubility were lowest. Thus, in this study, pH_i was 4.0 (Taheri et al., 2013).

e) Foaming activity index (FAI) and foaming stability index (FSI)

Foaming activity index (FAI) and foaming stability index (FSI) of samples were shown in Figure 11 and Figure 12. FPC contained lowest the indexes compared to that of others. FAI of FPH120 reached a minimum value at pH 6.0. At pH<6.0, FPA of FPH120 significantly increased. At pH>6.0, FAI of FPH120 slowly increased. In case of FPH15, at the range of pH2.0 to 10, FAI of FPH15 slightly decreased. Generally, foaming activity index of FPH15 was higher than that of FPH120. At pH 4.0, foaming stability index of all samples were zero. The reducing of pH from 4.0 to 2.0 resulting in the dramatically increasing of FSI of FPH15. Besides, the increasing of pH from 4.0 to 10 causing the gradual increase of FSI of FPHs.

Theoretically, the transportation, penetration and reorganization of molecules at the air-water interface affected on the foam formation (Wilde & Clark, 1996). To produce the best foaming properties, a protein must rapidly migrate to the air-water interface resulting the reduction of surface intension, unfold and reorganize its structure (Martin et al., 2002). A previous study said molecular size, structure and hydrophobicity of the protein hydrolysate influenced the absorption rate to the air-water interface (Martin et al., 2002). All these factors were dependent on the source of hydrolyzed parent protein (FPC). The hydrophobicity, net charge, and conformation of protein hydrolysate were changed during the hydrolysis in comparison to the native molecule. The FPH was more flexible, formed a stable interfacial layer and extended the rate of diffusion to the interface causing the improvement of forming ability (Wilde & Clark, 1996). Furthermore, the flexible FPH domains increased the viscosity of protein solution, protein concentration and film thickness (Phillips et al., 1994). The foaming capacity was affected by absorption rate, flexibility and hydrophobicity whilst the foaming stability depended on the viscoelastic nature of the film (Damodaran, 1996). Actually, the pH had a major effect on FAI and FSI values of FPH. The repulsion of peptides via ionic repulsion resulted in the decreased of these values (Klompong et al., 2007).

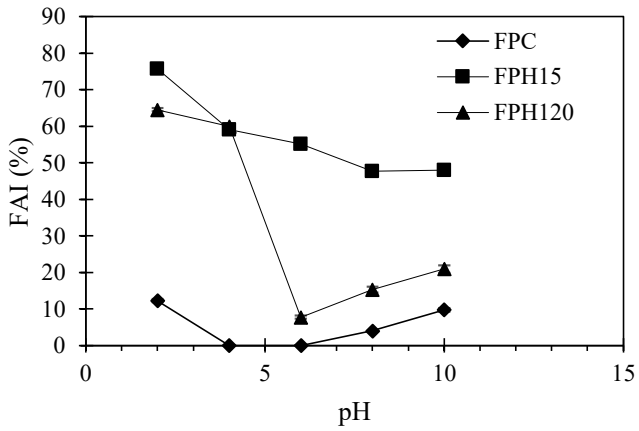

Figure 11. Foaming activity index (FAI, %) of FPH at various pH level

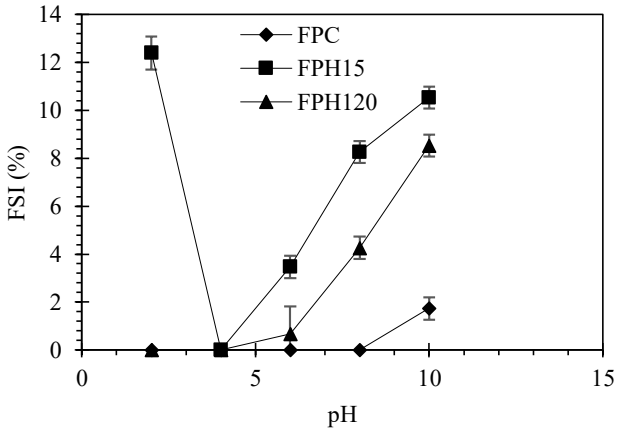

Figure 12. Foaming stability index (FSI, %) of FPH at various pH level

CONCLUSION

In this study, both pH and temperature strongly affected on the enzymatic hydrolysis of fish protein concentrate. Its products, the fish protein hydrolysates, showed different molecular weight depending on the condition of hydrolysis. FPHs had lower water holding capacity, higher solubility, and higher emulsion and foaming properties in comparison to FPC. These indicated that FPH contains improved ability that are useful for industrial applications.

CONTRIBUTION OF AUTHORS

The authors KST and TLN participated in laboratory research, writing and final revision of the manuscript.

COMPETING INTERESTS

The authors have declared that no competing interests exist.

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REFERENCES

Agilent, T. (2015). Polymer Molecular Weight Distribution and Definitions of MW Averages - Technical Overview. Retrieved from https://www.agilent.com/cs/library/technicaloverviews/public/5990-7890EN.pdf.

Asbjørngildberg, G. (1993). Enzymic processing of marine raw materials. Process Biochemistry, 28(1), 1-15. https://doi.org/10.1016/0032-9592(94)80030-8.

Bhaskar, N., & Mahendrakar, N. (2008). Protein hydrolysate from visceral waste proteins of Catla (Catla catla): Optimization of hydrolysis conditions for a commercial neutral protease. Bioresource Technology, 99, 4105-4111. https://doi.org/10.1016/j.biortech.2007.09.006

Boikess, R. K., Breslauer & Edelson, E. (1986). Elements of Chemistry - General organic and biological. Prentice-Hall, New Jersey.

Chalamaiah, M., Narsing Rao, G., Rao, D. G., & Jyothirmayi, T. (2010). Protein hydrolysates from meriga (Cirrhinus mrigala) egg and evaluation of their functional properties. Food Chemistry, 120, 652-657. https://doi.org/10.1016/j.foodchem.2009.10.057

Chobert, J., Bertrand-Harb, C., & Nicolas, M. (1988). Solubility and emulsifying properties of caseins and whey proteins modified enzymatically by trypsin. Journal of Agriculture and Food Chemistry, 36, 883-886. https://doi.org/10.1021/jf00083a002

Clemente, A. (2000). Enzymatic protein hydrolysates in human nutrition. Trends in Food Science and Technology, 11, 254-262. https://doi.org/10.1016/S0924-2244(01)00007-3

Damodaran, S. (1996). Amino acids, peptides and proteins. In O. R. Fennema (Ed.), Food chemistry (4th edition). Marcel Dekker, Inc., 321-429.

Demetriades, K., Coupland, J. N., & And Mcclements, D. J. (1997). Physical properties of whey protein stabilized emulsions as related to pH and NaCl. Journal of Food Science, 62(2), 342-347. https://doi.org/10.1111/j.1365-2621.1997.tb03997.x

Gbogouri, G., Linder, M., Fanni, J., & Parmentier, M. (2004). Influence of hydrolysis degree on the functional properties of salmon byproducts hydrolysates. Journal of Food Science, 69, 615-622. https://doi.org/10.1111/j.1365-2621.2004.tb09909.x

Herman, C., & Malcolm, H. (1944). Studies of the effect of sodium azide on microbic growth and respiration. Journal of Bacteriology, 47, 239-251.

Hoyle, N., & Merritt, J. (1994). Quality of fish protein hydrolysates from herring (Clupea harengus). Journal of Food Science, 59, 76-79. https://doi.org/10.1111/j.1365-2621.1994.tb06901.x

Klompong, V., Benjakul, S., Kantachote, D., & Shahidi, F. (2007). Antioxidative activity and functional properties of protein hydrolysate of yellow stripe trevally (Selaroides leptolepis) as influence by the degree of hydrolysis and enzyme type. Food Chemistry, 102, 1317-1327. https://doi.org/10.1016/j.foodchem.2006.07.016

Kristinsson, H. G., & Rasco, B. A. (2000). Fish Protein Hydrolysates: Production, Biochemical, and Functional Properties. Critical Reviews in Food Science and Nutrition, 40(1), 43-81. https://doi.org/10.1080/10408690091189266

Lee, S. M., & Yamauchi, K. (1987). Emulsifying properties of peptides obtained from the hydrolysates of ß-casein. Agricultural and Biological Chemistry, 51, 161-166. https://doi.org/10.1271/bbb1961.51.1535

Linder, M., Fanni, J., & Parmentier, M. (1996). Functional properties of veal bone hydrolysates. Journal of Food Science, 61, 712-716. https://doi.org/10.1111/j.1365-2621.1996.tb12187.x

Marchbank, T., Limdi, J. K., Mahmood, A., Elia, G., & Playford, R. J. (2008). Clinical trial: Protective effect of a commercial fish protein hydrolysate against indomethacin (NSAID)-induced small intestinal injury. Alimentary Pharmacology & Therapeutics, 28(6), 799-804. https://doi.org/10.1111/j.1365-2036.2008.03783.x

Martin, A., Grolle, K., Bos, M. A., Stuart, M., & Vanvliet, T. (2002). Network forming properties of various proteins adsorbed at the air/water interface in relation to foam stability. Journal of Colloid and Interface Science, 254, 175-183.

Montecalvo, J., Constantinides, S., & Yang, S. (1984). Enzymatic modification of fish frame protein isolate. Journal of Food Science, 49, 1305-1309. https://doi.org/10.1111/j.1365-2621.1984.tb14975.x

Mukhin, V., & Novikov, V. (2001). Enzymatic hydrolysis of proteins from Crustaceans of the Barents Sea. Applied Biochemistry and Microbiology, 37(5), 538-542. https://doi.org/10.1023/A:1010218712622

Naqash, S., & Nazeer, R. (2011). Antioxidant and functional properties of protein hydrolysates from pink perch (Nemipterus japonicus) muscle. Journal of Food Science and Technology, 50, 978-978. https://doi.org/10.1007/s13197-011-0416-y

Nesse, K. O., Nagalakshmi, A. P., Marimuthu, P., & Singh., M. (2011). Efficacy of a fish protein hydrolysate in malnourished children. Indian Journal of Clinical Biochemistry, 26(4), 360-365. https://doi.org/10.1007/s12291-011-0145-z

Nielsen, P. (1995). Enzyme technology for production. Denmark: Novo, 1-5.

Novozymes. (2016). Protease for biocatalysis for smarter chemical synthetic. Retrieved from https://www.novozymes.com/-/media/Project/Novozymes/Website/website/document-library/Advance-your-business/Pharma/Biocatalysis_brochure_Proteases.pdf.

Nurdiani, R., Nurdiani, R., Nurdiani, R., Donkor, O., Singh, T. K., & Vasiljevic, T. (2016). In vitro study of selected physiological and physicochemical properties of fsh protein hydrolysates from 4 Australian fsh species. International Food Research Journal, 23(5), 2029-2040.

Pacheco-Aguilar, R. M.-M.-S. (2008). Functional properties of fish protein hydrolysates from Pacific whiting (Merluccius productus) muscle produced by a commercial protease. Food Chemistry, 109, 782-789. https://doi.org/10.1016/j.foodchem.2008.01.047

Pearce, K., & Kinsella, J. (1978). Emulsifying properties of proteins: Evaluation of a turbidimetric technique. Journal of Agriculture and Food Chemistry, 26, 716-723. https://doi.org/10.1021/jf60217a041

Phillips, L., Whitehead, D., & Kinsella, J. (1994). Protein stabilized foams. In L. G. Phillips, D.M. Whitehead, & J. E. Kinsella (Eds.), Structure-function of food proteins. Academic Press, 131-152.

Rasco, H. G., & Barbara, A. (2000). Fish Protein Hydrolysates: Production, Biochemical, and Functional Properties. Critical Reviews in Food Science and Nutrition, 40(1), 43-81. https://doi.org/10.1080/10408690091189266

Rodríguez-Ambriz, S., Martínez-Ayala, A., & Millán, F. A.-O. (2005). Composition and functional properties of Lupinus campestris protein isolates. Plant Foods for Human Nutrition, 60, 99-107. https://doi.org/10.1007/s11130-005-6835-z

Sathivel, S., Bechtel, J. P., Babbitt, J., Smiley, S., Crapo, C., Reppond, K., & Al, E. (2003). Biochemical and functional properties of herring (Clupea harengus) byproduct hydrolysates. Journal of Food Science, 68, 2196-2200. https://doi.org/10.1111/j.1365-2621.2003.tb05746.x

Sikorski, Z. E. (2002). Chemical and Functional Properties of Food Components. Second Edition. CRC Press.

Sze-Tao, K., & Sathe, S. (2000). Functional properties and in vitro digestibility of almond (Prunus dulcis L.) protein isolate. Food Chemistry, 69(2), 153-160. https://doi.org/10.1016/S0308-8146(99)00244-7

Taheri, A., Anvar, S., H., A., & Fogliano, V. (2013). Comparison the functional properties of protein Hydrolysates from poultry byproducts and rainbow trout (Onchorhynchus mykiss) viscera. Iranian Journal of Fisheries Sciences, 12, 154-169.

Trinh, K. S., Nguyen, T. L., Le, T. T., & Le, T. N. (2017). Production of fish protein concentrate (FPC) from Pangasius Catfish and study on the effect of sodium chloride, sodium tripolyphosphate, sucrose and sorbitol to the protein solubility and water holding capacity of FPC. Journal of Science and Technology Development, 20(T1), 93-103.

Trudel, J., & Asselin, A. (1989). Detection of chitinase activity after polyacrylamide gel electrophoresis. Analytical Biochemistry, 178, 362-366. https://doi.org/10.1016/0003-2697(89)90653-2

Vojdani, F. (1996). Solubility. In: Methods of Testing Protein Functionality. Hall, G.M. (Ed.). Blackie Academic & Professional.

Wilde, P., & Clark, D. (1996). The competitive displacement of blactoglobulin by Tween 20 from oil–water and air–water interfaces. Journal of Colloid and Interface Science, 155, 48-54. https://doi.org/10.1006/jcis.1993.1008

Windsore, M. (2001). Fish Protein Concentrate. Torry Research Station, Torry Advisory Note No.39. FAO Corporate Document Repository.