Advances in Biological Sciences Research, volume 16
6th International Conference of Food, Agriculture, and Natural Resource (IC-FANRES 2021)

Functional Properties of Protein Hydrolysate of Sea Fish and Low Economic Value Hydrolysis Results Using Biduri Protease

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ABSTRACT
In 2017, The Indonesian fishing productivity reached 6,424,114 tons from the sea and 467,821 tons from freshwaters. Some kinds of sea fish which contain high protein are Crocodile flathead fish (17.86%) and Cardinal fish (18.26%), while kinds of freshwater fish that contain high protein are Common barb (12.5%) and Java barb (19%). The high protein content of fish has the potential to be manufactured as a protein hydrolysis product. Enzymatic hydrolysis by using Biduri protease is one of the easier and more profitable methods. This study aimed to determine the functional properties of the protein hydrolyzate of Crocodile flathead fish, Cardinalfish, Common barb, and Java barb hydrolyzed using biduri protease under controlled conditions. This research used four kinds of fish (Crocodile flathead, Cardinalfish, Common barb, and Java barb) and was analyzed using descriptive information. The analysis includes soluble proteins, emulsion capacity and stability, foaming capacity and stability, water holding capacity, solubility, and K_m/V_{max}. The results showed that the highest water holding capacity, solubility, emulsion capacity, foaming capacity, foam stability were obtained on Cardinalfish of 9.01%; 0.0097 mg/ml; 32.37 m2/g; 5.73 ml, and 4 ml respectively. The highest emulsion stability and K_m/V_{max} of Common barb were 4.22 hours, K_m value was -2962.58g/ml and V_{max} was -2.370 unit/ml. The highest soluble protein was in crocodile flathead at 0.72g/ml.

Keywords: *Hydrolysis protein, 'biduri' protease, functional properties.*

1. INTRODUCTION

The productivity data of catch fisheries in Indonesia in 2017 reached 6,424,114 tons from maritime sources and 467,821 tons from mainland public water sources, according to the Ministry of Marine Affairs and Fisheries. Crocodile flathead and cardinal fish, which sell for roughly Rp 3,000 per kilogram, are examples of low-value sea fish. Crocodile flathead and cardinal fish, despite its modest cost, protein amounts of 17.86% and 18.26%. Fresh fish with high protein content, such as common barb and java barb, have 12.5 percent and 19 percent. (Wahyuningtyas, 2017) [1] The four fish have high protein, which could be used as a source of protein hydrolysis.

Hydrolyzing breakdown of proteins by acids, alkalise, or enzymes produces protein hydrolysate. Hydrolysis using protease enzymes is regarded to be more advantageous due to its simplicity of usage and lower costs. Exopeptidase enzymes like biduri protease work by cutting polypeptides at the end of proteins. Protein hydrolysis produces peptides and amino acids that have bioactive properties such as antioxidants, antihypertensives, antiproliferative, anticoagulant, antidiabetic, and antiobesity. The hydrolysis process can increase the functional characteristics of proteins in addition to producing bioactive peptides. (Taheri et al., 2012) [2]. The purpose of the research was to identify the functional properties of protein hydrolyzed crocodile flathead, cardinal fish, java barb, and common barb hydrolysis

results under controlled settings using the enzyme biduri protease.

2. MATERIALS AND METHOD

Crocodile flathead (*Platycephalidae cymbacephalus*), cardinal fish (*Apogon albimaculosus*), common barb (*Rasbora jacobsoni*), and java barb (*Rasbora jacobsoni*) were the main ingredients in this research. Biduri protease enzymes were extracted from the sap of biduri plants located in the coastal area of Watu Ulo, Jember, East Java, for this research. Material used for analysis is distilled water, vegetable oils, pH 7 phosphate buffers, lowry mix reagents, Follin reagent, and SDS (sodium dodecyl sulfate). Analytical balance (Ohaus), homogenizer, Freeze dryer, water bath GFL 1083, Centrifuse Yenaco model YC-1180, Shimadzu spectrophotometer, Oven dryer, pH meter and glass tool.

Making protein hydrolysis with fish meat that's been cleaned and crushed in a blender, with the distilled water that are 1:2 (weight / volume) of the weight of the fish meat. The pH of the produced fish meat suspension is then set to 7. After that, add 3 percent (b/v) biduri protease enzyme to the weight of the fish meat. The hydrolysis process is carried out in a water bath at 55°C for 3 hours, followed by an enzyme inactivation step at 85°C for 20 minutes. The suspension is centrifuged after chilling to separate supernutrients that comprise dissolved proteins, lipids, and insoluble components (pellets). After that, the supernatan is dried in a freeze dryer. The results of fish protein hydrolysis analyzed soluble protein (Sudarmadji et al., 1997) [3], emulsion capacity and stability (Zhang et al., 2013)[4], foaming capacity and stability, water holding capacity (Shahidi and Synowiecki, 1997)[5], solubility (Anderson et al., 1984) [6] and K_m/V_{max} (Putra, 2009) [7].

3. RESULTS AND DISCUSSION

3.1. Analyzed soluble protein

The capacity of proteins to be hydrolyzed into amino acids by protease enzymes is known as soluble protein or protein digestibility (Pellet and Young, 1980) [8]. The Lowry analysis is used to identify soluble protein levels. Figure 1 shows observations of soluble protein in crocodile flathead, cardinal fish, java barb, and common barb.

According to research, the crocodile flathead has the highest soluble protein levels, while the java barb has the lowest. The content of amino acids in fish can be used to determine soluble protein levels. It contains 20.719 percent amino acids in crocodile flathead and 17.675 percent amino acids in java barb. Temperature, pH, enzyme concentrations, and substrates are all parameters that influence the amount of soluble protein produced. The enzyme will denature when the pH is too high or too low. As a result, the correct pH of enzymes is needed during hydrolysis in order for the reaction to proceed successfully. (Koesoemawardani et al., 2011) [9].

3.2. Water Holding Capacity (WHC) with Meat System Model

The ability of meat to bind water or water added from outside forces such as heating, meat cutting, pressure, and milling affects its water holding capacity. Water holding capacity is one of the important features of protein, according to (Kristinsson and Rasco, 2000) [10], and it can affect texture and characteristics in the food system. Figure 2 shows data on water holding capacity using the meat system in crocodile flatheads, cardinal fish, java barbs, and common barbs.

According the data, cardinal fish have the maximum water holding capacity at 9.01 percent and common fish have the lowest at 1.58 percent. Because each fish has a different pH, the value of its water holding capacity varies. The pH of cardinal fish and crocodile flathead is similar, ranging from 8 to 8.5, but java barb and common barb have a pH of 6-7. The water holding capacity is affected by pH levels that are too high or too low. The capacity to bind water is reduced when the pH of the meat muscles is low. This is due to actuosine's enhanced contraction, which allows the liquid in the flesh to escape. (Balti et al., 2010)[11]. Fish with a low water holding capacity lose more fluid, resulting in a significant weight loss. In fish hydrolysate, (Jemil et al., 2014) [12] reported a water holding capacity of 7.7g/g. Salt is another component that influences water holding capacity. Salt can supply an electrical charge to proteins bound by Na and CI, resulting in decreased protein interaction and increased water-protein interaction.

3.3. Solubility

One of the important characteristics of proteins that affects other functional properties is their solubility. At the pH at the isoelectric point, protein

solubility in water reaches a low. Because the protein is not charged at isoelectric pH, there is no pull force between molecules. Isoelectric proteins become negatively or positively charged again at pH below or above, increasing solubility. Figure 3 shows the solubility of the protein hydrolysate of Crocodile flathead fish, Cardinalfish, Common barb, and Java barb.

According to the data, the cardinal fish protein hydrolysate has the highest solubility value of 0.0097 mg/ml and the common barb protein hydrolysate has the lowest solubility value of 0.0088 mg/ml. The pH of fish protein hydrolysate affects high low solubility. The solubility of hydrolysate increases as the pH increases. Protein hydrolysate has better solubility, is good at high pH, and maintains heat stability Li, Luo, Shen, and You (2012) [13]. Increasing the hydrolysis time, which results in peptides and decreased molecular weight, is another way to increase the solubility of protein hydrolysate. (de Castro & Sato, 2014; He, Franco, & Zhang, 2013) [14-15].

3.4. Emulsion capacity and stability

Emulsions are created when hydrophilic and hydrophobic components are balanced. Hydrophilic bonding is polar, whereas hydrophobic bonding is non-polar and binds to oil. Emulsion-forming components are hydrophilic and hydrophobic, and if one is destroyed, the power of the generated emulsion is reduced. Figure 4 shows the results of emulsion capacity of the protein hydrolysate of Crocodile flathead fish, Cardinalfish, Common barb, and Java barb.

According to the data, protein hydrolysate of Cardinal fish has the maximum emulsion power value of 2.37 m2/g, while hydrolysate proteins of java barb has the lowest at 1.97 m2/g. pH can impact the emulsion capacity of high and low emulsions. The ideal pH for emulsion capacity is pH 8, and proteins of Cardinal fish degradation occurs at the same pH, allowing the emulsion power to be controlled more effectively. (Taheri et al., 2013) [16] The molecular size and weight of peptides, as well as the surface characteristics of proteins, are other factors that influence emulsion capacity (Liu et al., 2014; Pires & Batista, 2013) [17, 18].

Emulsion stability is the ability of a material's emulsions to remain stable in the presence of other particles. The consistency of a good emulsion does not alter during storage, it does not change color, and it does not develop a layer. Figure 5 shows the results of evaluating the emulsion stability protein hydrolysate of Crocodile flathead fish, Cardinalfish, Common barb, and Java barb.

Figure 5 shows that protein hydrolysate of common barb has the best emulsion stability with a value of 4.22 hours and protein hydrolysate of java barb the the worst with a yield of 3.16 hours. The pH of the emulsion is one factor that influences its stability. The pH of protein hydrolysate of common barb ranges from 6-7, and the closer it reaches to 7, the greater the emulsion qualities. Emulsion stability is found in soy hydrolysis at DH 4 percent and pH 7 according to (Jung, Murphy, and Johnson, 2005) [19]. The greater the emulsion in hydrolysate's characteristics at alkaline pH, the better the unfolding conditions for proteins under alkaline conditions. The hydrophobic residues of the protein are exposed as the disease progresses, resulting in increased oil-water contact.

3.5. Foaming Capacity and Stability

Foaming is a dispersion of the gas phase in the liquid phase that forms when something is shaken. The creation of froth is caused by the open bonding of protein molecules, which allows air to enter between the molecules whose chains are open and held, causing them to expand. When a substance contains oil, its potential to gather it is increased (Raikos et al., 2006) [20]. Figure 6 shows the findings of the research on the foaming capacity and stability of the protein hydrolysate of Crocodile flathead fish, Cardinalfish, Common barb, and Java barb.

According to the research, protein hydrolysate of cardinal fish has the maximum foaming capacity of 5.73 ml and protein hydrolysate of crocodile flathead has the lowest foaming capacity of 3.60 ml. The topographical and chemical features of the protein surface have a significant impact on the protein surface. Furthermore, the success of functional qualities is determined by the properties of physomia, particularly protein molecules (Fennema, 1996) [21]. Because pH is linked to protein solubility, it has an impact on foaming capacity. Solubility will be near the minimum at pH near the isoelectric point, whereas maximal creases will occur at pH distant from the isoelectric point. If the protein solubility is at its highest, the protein will be diffused uniformly, allowing the froth shaper to spread equally and produce more froth. (Chayati and Ari, 2008).

The ability of a foam structure to last for a specific amount of time is known as foaming stability. The size

of the foam drain at a given moment contains indicators of froth stability, which are indicated in weight or volume. According to the findings, protein hydrolysate of cardinal fish has the highest foam stability at 4 ml and protein hydrolysate of crocodile flathead has the lowest at 1.33 ml. The amount of hydrophobic components in the foam will affect its stability, as well as the properties of proteins and their ability to minimize surface tension (Mutilangi, Panyam and Kilara, 1996) [22].

4. CONCLUSION

Soluble protein levels of hydrolysate crocodile flathead (0.72 mg/ml), WHC (5.54%), solubility (0.0094 mg/ml), emulsion capacity and stability (2.17m2/g; 3.18 hours), foam power and stability (3.60;1.33 ml). Soluble protein of hydrolysate cardinal fish (0.63 mg/ml), WHC (9.01%), solubility (0.0097 mg/ml), emulsion capacity and stability (2.37m2/g; 4.19 hours), foam capacity and stability (5.73;4 ml). Protein hydrolysate java barb results in soluble protein levels (0.60 mg/ml), WHC (3.99%), solubility (0.0095 mg/ml), emulsion capacity and stability (1.97m2/g; 3.16 hours), and foaming capacity and stability (4.60;2.33 ml). Protein hydrolysate if common barb has soluble protein levels (0.66 mg/ml), WHC (1.58 percent), solubility (0.0088 mg/ml), emulsion capacity and stability (2.05m2/g;4.22 hours), and foaming capacity and stability (4.27;2 ml). The highest K_m/V_{max} value is obtained in the protein hydrolysate of common barb with a value of K_m of -2962 g/ml and V_{max} of -2,730 units/ml while the lowest K_m/ V_{max} value is obtained in protein hydrolysate of crocodile flathead with a value of K_m of 3880,960 g/ml and a V_{max} value of 8,733 units/ml. Suggestions for this study, can be continued by making nanoparticles and tested in vivo.

ACKNOWLEDGMENTS

This research has been carried out by funding support from the IDB supporting research program of University of Jember.

REFERENCES

[1] Wahyuningtyas, W.S. 2017. Antioxidant Activity Hydrolysis Protein Fish Sea Water And Freshwater Fish. Jember : Department of Agricultural Products Technology Agricultural Technology Faculty of Jember University.

[2] S. Taheri, L. Lin, D. Austin, T. Young, and E. Mignot, “Short sleep duration is associated with reduced leptin, elevated ghrelin, and increased body mass index,” PLoS Medicine, vol. 1, no. 3, article e62, 2004.

[3] Sudarmadji, S., B. Haryono, dan Suhardi. 1997. Analytical Procedures for FoodStuffs and Agriculture. Yogyakarta: Liberty.

[4] Zhang, R.; Yan, Q.; Zhang, Z. S.; Jiang, D.; Otto-Bliesner, B. L.; Haywood, A. M.; Hill, D. J.; Dolan, A. M.; Stepanek, C.; Lohmann, G.; Contoux, C.; Bragg, F.; Chan, W.-L.; Chandler, M. A.; Jost, A.; Kamae, Y.; Abe-Ouchi, A.; Ramstein, G.; Rosenbloom, N. A.; Sohl, L.; Ueda, H. (2013). *Mid-Pliocene East Asian monsoon climate simulated in the PlioMIP. Climate of the Past, 9(5), 2085–2099.* doi:10.5194/cp-9-2085-2013

[5] F. Shahidi; J. Synowiecki; V. Venugopal; R.B. Pegg; J.R. Botta (1997). *Characteristics of chicken-seal salami., 45(4), 0–559.* doi:10.1016/s0309-1740(96)00038-1

[6] Anderson, D. M. ; Bowland, J. P., 1984. Evaluation of buckwheat (*Fagopyrum esculentum*) in diets of growing pigs. Can. J. Anim. Sci., 64 (4): 985–995

[7] Putra G.P., Ganda, 2009, Determination of Kinetics of Endogenous Polygalacturonase Enzymes (PG) from Cocoa Bean Pulp, *Jurnal Biologi* 13(1):21 -24.

[8] Pellet PL and VR Young (eds). 1980. Nutritional Evaluation of Protein Food. The Unted Nations University, Tokyo.

[9] Koesoemawardani, D, F. Nurainy, dan S. Hidayati. 2011. Process of Making Protein Hydrolysis Rucah Fish. Jurnal Natur Indonesia, 13 (3): 256-261.

[10] Kristinsson, H.G. & Rasco, B.A. 2000. Fish Protein Hydrolysates: Production, Biochemical, and Functional Properties. Crit Rev *Food Sci Nutr* 40(1): 43–81.

[11] Balti, R., Bougatef, A., Ali, N. E. H., Zekri, D., Barkia, A., & Nasri, M. (2010). Influence of degree of hydrolysis on functional properties and angiotensin I-converting enzyme inhibitory activity of protein hydrolysates from cuttlefish (Sepia officinalis) by-products. Journal of the Science of Food and Agriculture, 90(12), 2006–2014.

[12] Jemil, I., Jridi, M., Nasri, R., Ktari, N., Ben Slama-Ben Salem, R., Mehiri, M., et al. (2014). Functional, antioxidant and antibacterial properties of protein hydrolysates prepared from fish meat fermented by Bacillus subtilis A26. Process Biochemistry, 49(6), 963–972.

[13] Li, X., Luo, Y., Shen, H., & You, J. (2012). Antioxidant activities and functional properties of grass carp (Ctenopharyngodon idellus) protein hydrolysates. Journal of the Science of Food and Agriculture, 92(2), 292–298.
[14] de Castro, R. J. S., & Sato, H. H. (2014). Comparison and synergistic effects of intact proteins and their hydrolysates on the functional properties and antioxidant activities in a simultaneous process of enzymatic hydrolysis. Food and Bioproducts Processing, 92(1), 80–88.
[15] He, S., Franco, C., & Zhang, W. (2013). Functions, applications and production ofprotein hydrolysates from fish processing co-products (FPCP). Food Research International, 50(1), 289–297.
[16] Taheri, A., Anvar, S. A. A., Ahari, H., & 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(1), 154–169.
[17] Liu, Y., Li, X., Chen, Z., Yu, J., Wang, F., & Wang, J. (2014). Characterization of structural and functional properties of fish protein hydrolysates from surimi processing by-products. Food Chemistry, 151, 459–465.
[18] Pires, C., & Batista, I. (2013). Utilization of fish waste. CRC Press, pp. 59–75.
[19] Jung, S.; Murphy, P.A.; Johnson, L.A. Physicochemical and functional properties of soy protein substrates modified by low levels of protease hydrolysis. J. Food Sci. 2005, 70, C180–C187.
[20] Fennema, O.R. 1996. *Food Chemistry*. Marcel Dekker Inc. New York.
[21] Mutilangi, W. A. M., Panyam, D., & Kilara, A. (1996). Functional properties of hydrolysates from proteolysis of heat-denatured whey protein isolate. Journal of Food Science, 61(2), 270–275.
[22] Fox, P.F. (1991*). Food Enzymology*, *Elsevier Applied Science, vol 2*, London.
[23] Saropah Dyah Ayu, Akyunul Jannah, Anik Maunatin, 2012, Kinetics Of Enzyme Reactions Crude Extract Cellulase Enzyme Cellulolithic Bacteria Isolation Results From Bran, Alchemy 2(1):34-45.
[1] [24] Radzicka A, Wolfenden R., 1995, "*A Proficient Enzyme*". *Science* 6(267):90–931.