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Influence of Enzyme Concentration, Hydrolysis Duration, and Drying Temperature on the Production of Antioxidant-containing Peptide from Catfish (*Clarias* sp.) Gills

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Abstract

Catfish gills are a potential raw material for producing protein hydrolysate with antioxidant activity. Protein hydrolysate was produced using crude papain enzyme in two stages. The first stage was to obtain the optimum enzyme concentration (w/w) including 1%, 2%, 3%, 4%, 5% and 6%, with 0% as a control, followed by the second stage to obtain the optimum hydrolysis duration for 24, 48, 72, and 96 hours, with 0 hour as a control. The supernatant containing protein hydrolysate was then dried using an oven at various temperatures, including 60, 70, and 80°C, for 48 hours to obtain catfish gill protein hydrolysate (CGH) powder. CGH was tested for degree of hydrolysis (DH) and antioxidants, including DPPH and ABTS. The highest antioxidant activity was obtained from 3% papain with DPPH and ABTS values of 85% and 9.57 µM TEAC, respectively. Further stage on the hydrolysis duration gave 48 hours as the optimal one, with antioxidant activity of 85.25 % for DPPH and 4.29 µM TEAC for ABTS. The oven-drying temperature concluded that CGH has stable antioxidant activity. The IC_{50} on antioxidant activity based on DPPH ranged from 1799.85 mg/L to 1749.50 mg/L (IC_{50} of ascorbic acid was 2.61 mg/L) and was included as very weak. Even though based on IC_{50}, CGH has low antioxidant activity, the protein content was found to be high (56.86±1.51%), which could be a high-protein food additive.

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Introduction

Catfish (*Clarias* sp.) is a commercial freshwater fish that is easy to breed, has a high growth rate, and is tolerant to harsh water and environmental conditions (Shrestha and Pant, 2012). Yogyakarta is one of the centers of catfish aquaculture, with production reaching 40,825.6 tons in 2020 and increasing to 48,229.96 tons in 2023 (Agency for Regional Development Yogyakarta Special Province, 2020). In the processing of catfish, by-products are produced and still have the potential to be utilized. Those by-products have been widely used to make certain products, for example, catfish bones and heads, which can be a source of several minerals, especially calcium, which can be used as fishmeal (Mubarokah et al., 2021). Catfish viscera can be used as animal feed because they have a high protein content, so they are suitable for livestock growth (Rimalia, 2002). However, gills and arborescent, additional respiratory organs in catfish, have not been widely utilized. This organ still has nutritional content, which leads to its potential as a material for protein hydrolysate. Peptides produced from protein hydrolysis have been reported to possess functional properties such as anti-inflammatory, antioxidant, and antimicrobial (Rocha et al., 2018), anti-obesity (Mizushige et al., 2017), immunomodulatory effect, and anti-cancer (Chalamaiah et al., 2018), and also antihypertensive effects (Yathisha et al., 2019). Among all bioactivities mentioned, this study will focus on antioxidant activity by using DPPH and ABTS analysis. DPPH and ABTS assays are widely used because they are simple, rapid, and provide a reliable measurement of antioxidant activity through stable free radicals. Together, they offer complementary insights into hydrogen atom transfer and electron transfer mechanisms, allowing comprehensive evaluation across different types of antioxidants.

Protein hydrolysate, which contains peptides and amino acids, can be made via hydrolysis with the aid of

enzymes, acid or alkaline treatment, or fermentation (Sarmadi and Ismail, 2010). In terms of enzymatic hydrolysis, protein hydrolysate has been studied using various types of enzymes, such as papain (Annisa et al., 2017), pepsin (Prastika et al., 2019), bromelain (Darmawan, 2020), biduri protease (Witono et al., 2007), and calotropin (Witono et al., 2020). One of the enzymes that can be used is papain, which is naturally found in papaya fruit (*Carica papaya* L.) (Najafian and Babji, 2015). Witono et al. (2020) reported that the papain enzyme used in producing *Rasbora jacobsoni* protein hydrolysate showed a high antioxidant activity. Even though papain was broadly used, producing protein hydrolysate from catfish gill using papain has not been reported.

Many factors need to be optimized to produce protein hydrolysate with high antioxidant activity, including enzyme concentration, hydrolysis duration (Shahi et al., 2020), and drying temperature (Dong et al., 2005). Hydrolysis duration is crucial due to its effect on the size of oligopeptides produced, which may break down the protein into simpler peptides or amino acids, which leads to the loss of antioxidant activity (Najafian and Babji, 2015). Drying was also applied to produce protein hydrolysate powder. Many drying techniques with different temperatures have been reported, such as spray drying (Hoyle and Merritt, 1994), oven drying (Abraha et al., 2017), freeze drying (Elavarasan and Shamasundar, 2016), and foam-mat drying (Sukkhown et al., 2018).

Based on the facts above, research on the effects of enzyme concentration, hydrolysis duration, and drying temperature is required to produce protein hydrolysates from catfish gills and arborescent by-products. Numerous factors must be optimized to produce an antioxidant-containing protein hydrolysate.

Materials and Methods

Materials

The materials used in this research include catfish gills and arborescent (*Clarias* sp.), crude papain enzyme (PAYA 1.0593 Unit/gram), aquadest, 0.1 M HCl, 0.1 M NaOH, 10% TCA, 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Sigma Aldrich), 95% ethanol, ascorbic acid (vitamin C), methanol (Merck), TPTZ (Sigma-Aldrich), 40 mM HCl, 20 mM $FeCl_3.6H_2O$, 300 mM acetate buffer pH 3.6, and $FeSO_4.7H_2O$.

The equipment used includes a silicon tray, thermocouple, micropipette (Nichipet EX II), pH meter (Ohaus)/(ATC Pen Type PH-009), vortex (Barnstead M37610-33)/(OHAUS VXMNDG Part No. 30392124), refrigerator -30°C (Sanyo SR-D180F, Japan), Soxhlet (Hanon SOX606), Kjeldahl flask, blender, water bath shaker (Memmert), analytical balance (Ohaus Pioneer (PX) Internal Calibration PX224), UV-Vis spectrophotometer (Genesys 10s UV-Vis, United States), refrigerated centrifuge (Sorvall st16r centrifuge, United States), hot plate stirrer (Nuova Stir Plate, United States), magnetic stirrer, analytical balance, vacuum oven drying (Memmert UN110), moisture analyzer (MB120), and Muffle Furnace (Electric Muffle Furnace, Indonesia).

Research Flow

The research was divided into three stages, including optimizing enzyme concentration, optimizing hydrolysis duration, and optimizing drying temperature. At each stage, the analysis included proximate analysis, degree of hydrolysis, soluble protein, antioxidant activity, including DPPH and ABTS, and statistical analysis. The research flows can be seen in Figure 1.

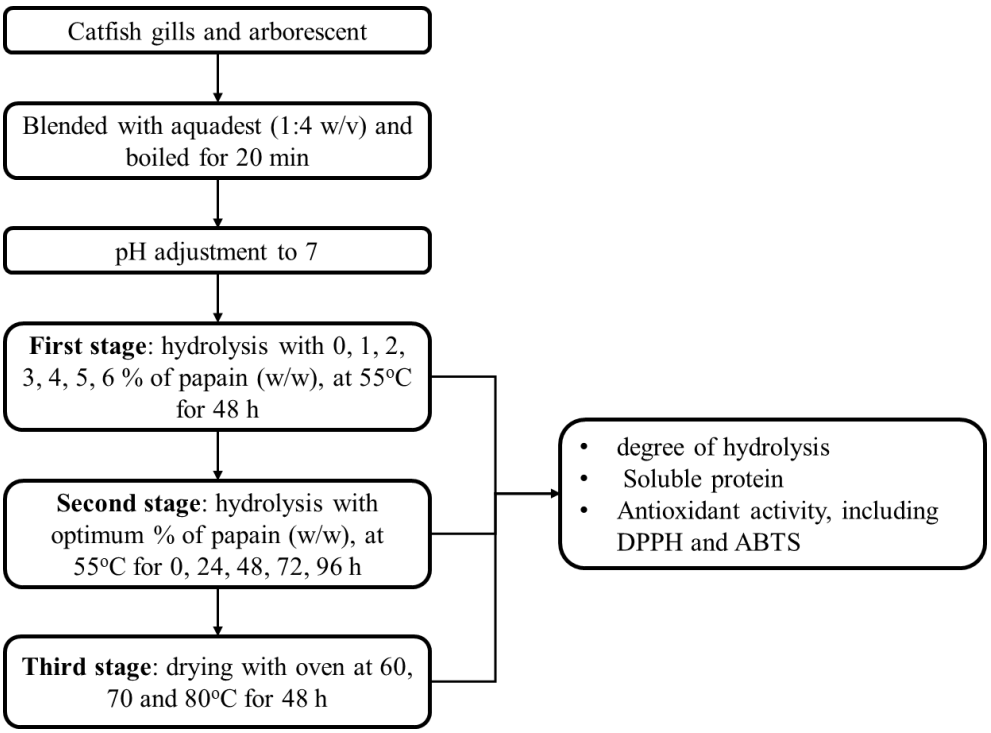

Figure 1. Research flow diagrams

Production of Fish Protein Hydrolysate

Catfish gill samples were obtained from a local restaurant. To maintain their freshness, the samples were placed in a cooler box and covered with ice. Furthermore, they were cleaned and stored at -18°C until used. The production of fish protein hydrolysate refers to (Salamah et al., 2012), which was conducted through enzymatic hydrolysis using papain. The gills and arborescent of catfish were homogenized with distilled water in a 1:4 ratio (w/v) using a blender for 2 minutes. Then, the mixture of catfish gills, arborescent, and distilled water was heated at 90°C for 20 minutes, aimed at removing fat and inactivating the endogenous enzymes present in the catfish gills. Next, the pH is adjusted by adding 1 M NaOH solution and/or 1 M HCl solution until pH 7. In the first step, the mixture was added with papain enzyme at various concentrations, namely 1%, 2%, 3%, 4%, 5%, and 6%, with 0% as the control. Hydrolysis was conducted for 48 h at 55°C in a water bath shaker. Boiling for 20 minutes was done afterward to stop the hydrolysis process. To obtain the dissolved fraction (supernatant), the sample was centrifuged at 5000 rpm for 20 min at 4°C. Subsequently, the supernatant was dried in an oven at 80 °C for 48 h. The hydrolyzed protein powder was then analyzed as mentioned above. For the second step, optimum enzyme concentration was used to hydrolyse the sample under the same conditions for 0, 24, 48, 72, and 96 h, followed by the same analysis. Finally, the catfish gill protein hydrolysate (CGH) produced in optimum enzyme concentration and hydrolysis duration was dried at various temperatures, including 60, 70, and 80°C for 48 h.

Analysis of Catfish Gill Protein Hydrolysate

Yield

Yield was described as the ratio between the weight of catfish gills and their hydrolysate. The yield value indicates the number of raw materials that can be converted into a product

Proximate Analysis

The proximate analysis of catfish gills was carried out based on the AOAC method (2005), which includes fat content (Soxhlet), ash content (muffle furnace), protein content (Kjeldahl), and water content (moisture analyzer).

Degree of Hydrolysis (DH)

The degree of hydrolysis was determined according to (Hoyle and Merritt, 1994) with slight modifications. First, 1 g of the sample was added with 10 ml of 10% (w/v) Trichloroacetic Acid (TCA). Then, the solution was left for 30 minutes and then centrifuged at 6700 x g at 10°C for 20 minutes in order to collect 10% of the material dissolved in TCA. Total nitrogen was measured using the Kjeldahl method with a conversion factor of 6.25. The DH was calculated as follows:

DH (%) = (% TCA soluble protein) / (% total protein (N)) × 100%

Antioxidant Analysis

DPPH (2,2-difenil-1-pikrilhidrazil)

DPPH analysis was conducted, which refers to Wu et al. (2003). A sample of 1.5 ml was added with 1.5 ml of DPPH from 0.1 mM in 95% ethanol. Furthermore, the mixture was left at room temperature in the dark for 30 minutes. The absorbance was then measured at 517 nm using a spectrophotometer. Blanks were made similarly, but the sample was replaced with distilled water. DPPH was calculated using the following equation:

%DPPH = (Blank absorbance - sample absorbance) / (Blank absorbance) × 100

The IC_{50} was calculated by preparing various sample concentrations (4000, 2000, 1000, 500, 250, 125 mg/mL) and plotting on y = a + bx. It was expressed as mg/ml of sample concentration with 50% inhibition. The results of the IC_{50} value of each treatment were compared to ascorbic acid as a control.

ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)

The ABTS antioxidant test method used in this study is a modification of the procedure reported by (Re et al., 1999). The ABTS reagent was made by mixing 7 mM ABTS with 2.45 mM potassium persulfate (1:1, v/v) and stored in a dark room for 8-12 hours until the reaction was complete. Then, the ABTS solution was dissolved in ethanol until the absorbance was 0.700 ± 0.020 at 734 nm (fresh before analysis). A total of 0.9 ml of ABTS solution and 0.1 ml (30 mg/ml) of hydrolysate dissolved in methanol were mixed. The mixture was vortexed for 45 s, and then the absorbance was measured at 734 nm after 1 minute. Blanks were made using distilled water with the same treatment, and the reducing power was expressed in µM TEAC (Trolox Equivalent) per mg of dry sample. The standard Trolox solution was made with concentrations of 0.84 µM, 1.56 µM, 3.12 µM, 4.69 µM, and 6.25 µM.

Statistical Analysis

All experiments were done using a completely randomized design with three replications. The data obtained were then analyzed using ANOVA; if the results were significant, a Tukey post hoc test was conducted to determine the significance between samples. The best treatment is determined using the DMRT post hoc test. Data analysis was performed using IBM SPSS Statistics 25 software.

Results and Discussion

Yield

The percentage of by-products from catfish processing is shown in Table 1. The gills and arborescent contribute as much as 7.51% of the total weight; thus, the availability of catfish gills as a by-product can be utilized as raw materials for protein hydrolisate. Based on our research, the proximate composition of catfish meat compared to gills and arborescent catfish was relatively equal. The fat content of catfish meat is 3%, while the gills and arborescent are 5%, while the protein content showed values of 16.08±0.07% and 15.41±0.33% for meat and gills, respectively. This shows that the gills and arborescent catfish possess relatively high protein.

Table 1. The Catfish Processing By-Products

Part	Weight (gram)	Percentage (%)
Catfish total body weight	122.67±3.51	100
Viscera	6.56±0.31	5.35
Fin	4.96±1.01	4.04
Gill and arborescent	7.51±0.8	6.12
Head	32.52±1.72	26.51
Skin	8.19±1.15	6.68
Bone	17.70±1.46	14.43
Meat	44.98±0.76	36.67

Further production of catfish gill protein hydrolysate resulted in a yield from 1.34% to 6.55%. The yield of CGH in this study showed a lower value when compared to the protein hydrolysate from catfish meat, which was reported to be 21.16% (Nurhayati et al., 2013), protein hydrolysate from Malong fish, which produced a yield of 50.07% (Ilham et al., 2019), and the protein hydrolysate from Mackerel fish, which achieved 59.89% (Nurdiani et al., 2022). These phenomena can be caused by variations in the weight and proximate composition of fish or by differences in substrate types, hydrolysis duration, enzyme concentrations, and drying methods used.

Protein Hydrolisate Characteristics

The color of catfish gill protein hydrolysate (CGH) was darker following the increase in enzyme concentration. The browning was influenced by non-enzymatic browning reactions (Maillard reactions) during the hydrolysis process due to the reaction of reducing sugars with free amino groups of amino acids. Because crude papain contains dextrose, the higher the concentration of crude papain, the darker the CGH color will be. The higher the enzyme concentration, the more hydroxyl groups of reducing sugars can be increased, which can then react with the amino groups of proteins to produce more Maillard products, resulting in a darker product (Barzana and Gracia, 1994).

The degree of hydrolysis of CGH was varied from 26,46±0,18% on the control treatment without enzyme addition to 92.65±0.46% on 3% papain enzyme addition. The drying temperature showed no significant difference in DH, ranging from 90.53±1.74% to 92.76±1,1%. The degree of hydrolysis (DH) is essential since it is a key parameter in monitoring hydrolysis reactions. The higher the DH, the more effective it is in breaking peptide bonds. The amount of oligopeptides and amino acids produced will affect the antioxidant activity. The amino acid side chains, such as tyrosine, serine, threonine, lysine, glycine, tryptophan, valine, histidine, isoleucine, proline, and phenylalanine, can reduce free radicals by donating hydrogen ions (Samaranayaka and Li-Chan, 2011).

Influence of Enzyme Concentration on CGH Antioxidant Activity

The effect of enzyme concentration on CGH antioxidant activity can be seen in Figure 2. The DPPH activity ranged from 55% to 85%. The treatment of papain enzyme 0% has the lowest percentage of inhibition, which is 55%, while the treatment of papain enzyme concentration 3% resulted in the highest antioxinant activity, which is 85% and is classified as strong. At 4%, there was a decrease in the percentage of inhibition activity. It was suspected that the peptide produced from the hydrolysate of catfish gill and arborescent proteins tends to decrease due to the change on peptide size and its solubility, amino acid composition, strands, and the free amino acids, which were the important factors of peptide-based antioxidant to scavange the DPPH radicals.

Based on ABTS activity, the antioxidant activity of CGH showed ABTS inhibition ranged from 1.66 to 9.57 µM TEAC. The highest results were shown at a concentration of 3% with a value of 9.57 µM TEAC, followed by 5% with a value of 7.70 µM TEAC, and the lowest value obtained was at 0% with a value of 2.66 µM TEAC. These results are relatively low compared to the study of Guo et al. (2019) on armoured catfish with the highest ABTS value of 17.47 µM TEAC. Based on DPPH and ABTS, we used 3% of papain enzyme to optimize the hydrolysis duration further.

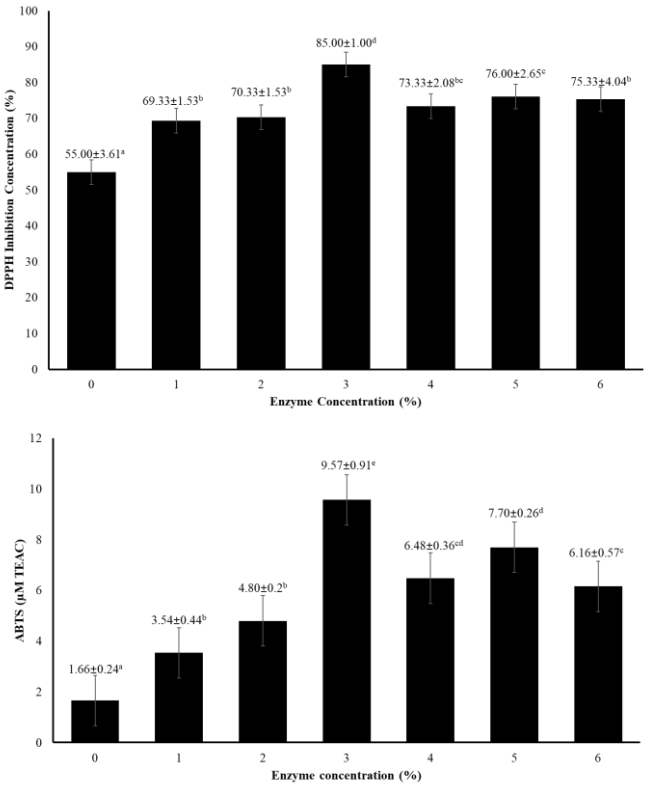

Figure 2. Effect of Enzyme Concentration on Antioxidant Activity of Catfish Gill Protein Hydrolisate (up: DPPH; down: ABTS)

Influence of Hydrolysis Duration on CGH Antioxidant Activity

The effect of hydrolisis duration resulted in antioxidant activity based on DPPH ranging from 57.87-81.25%, where the highest antioxidant activity occurs in the 48 h hydrolysis treatment with an inhibition value of 81.25% (Figure 3). The results on DPPH were higher when compared to previous studies conducted by Susanto et al. (2018) on chicken leg protein hydrolysate with a value of 41.65-55.10%, Puspawati et al. (2020) on chicken skin protein hydrolysate with a value of 16.75-58.35%, and Sampath et al. (2011) on horse mackerel viscera protein hydrolysate with a value of 49.8-57.8%.

Based on ABTS analysis, the results of the antioxidant activity of CGH showed the highest antioxidant activity also occurred at 48 h hydrolysis duration, with a value of 4.29 µM TEAC, and the lowest antioxidant activity occurred in the 0 h treatment, with a value of 2.32 µM TEAC. Based on antioxidant activity, 48 h was concluded as the optimum hydrolysis duration for producing antioxidant-containing peptides. The CGH produced by using 3% papain and a 48 h hydrolysis duration was then further used in the last stage, which was drying temperature.

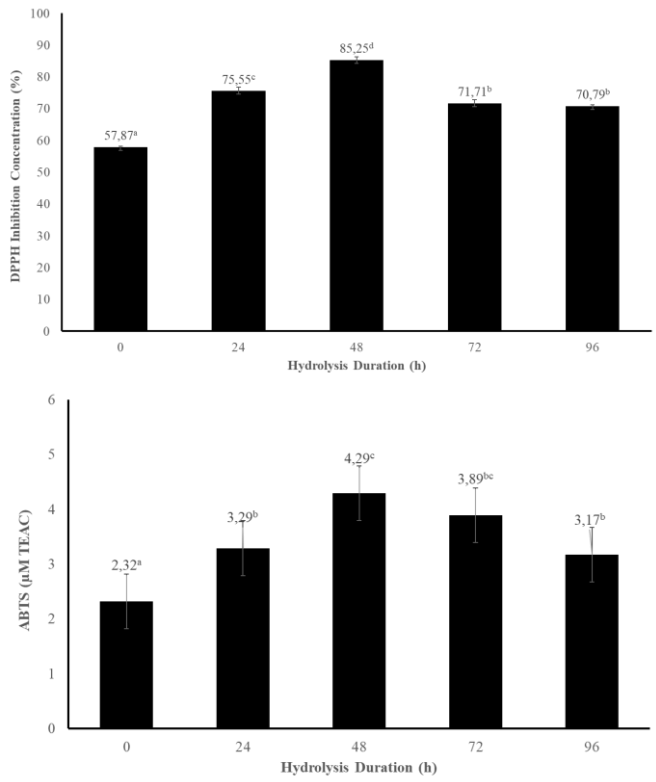

Figure 3. Effect of Hydrolysis Duration on Antioxidant Activity of Catfish Gill Protein Hydrolisate (up: DPPH; down: ABTS)

Influence of Drying Temperature on CGH Antioxidant Activity

Based on statistical analysis, the drying temperature did not significantly affect antioxidant activity, which ranged from 64.48-68.88% (Figure 4). These results were in line with the study of Elavarasan and Shamasundar (2016), which stated that different drying processes (freeze drying and oven drying) showed no significant effect on the protein hydrolysate of *Cirrhinus mrigala* fish. In addition, the protein hydrolysate of omate threadfin bream fish has good thermal stability, where the protein hydrolysate of omate threadfin bream can maintain more than 97% of its antioxidant properties when heated at a temperature of 100℃ for 180 minutes (Nalinanon et al., 2011). Other studies have stated that different temperature treatments (30 ℃ and 90 ℃) and different durations in the production of yellow stripe trevally fish protein hydrolysate do not affect its antioxidant activity (Klompong et al., 2008).

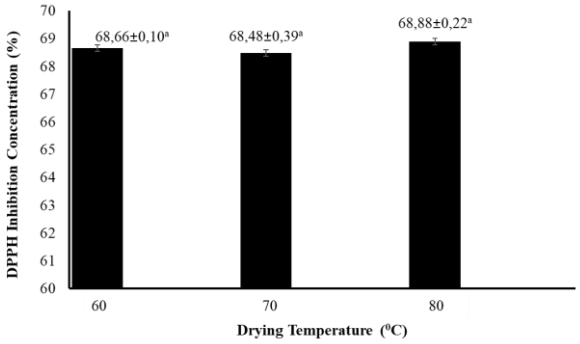

Figure 4. Effect of Drying Temperature on Antioxidant Activity of Catfish Gill Protein Hydrolisate

To evaluate the antioxidant level of CGH, we tested the IC_{50} (inhibitory concentration), as shown in Figure 5. The IC_{50} reported ranges from 1,749.50±127.86 mg/l to 1,839.18±64.94 mg/l, much greater if compared to ascorbic acid (2.61mg/l). This IC_{50} of CGH was classified as a very weak antioxidant. The IC_{50} in this study was greater compared to the IC_{50} value of white snapper offal protein hydrolysate, which was 1,048.40 mg/l (Nurjanah et al., 2021), but smaller than the IC_{50} value of shark (*Sphyrna lewini*) protein hydrolysate, which was 3,060 mg/l (Luo et al., 2012). Antioxidants with high IC_{50} values, indicating lower radical scavenging efficiency. This kind of material may still have valuable applications in food systems where a mild and sustained antioxidant effect is desirable. They can help maintain oxidative stability over longer periods without excessively altering the flavor, color, or sensory properties of foods. In some cases, low antioxidant activity is preferred to avoid interference with natural ripening processes or to balance pro-oxidant and antioxidant dynamics in complex food matrices.

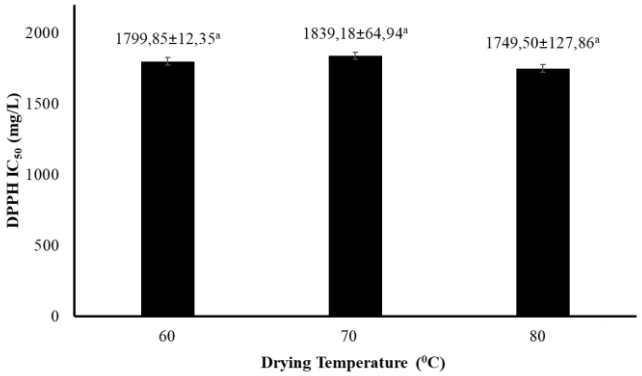

Figure 5. The IC_{50} of Catfish Gill Protein Hydrolisate Based on DPPH

Furthermore, such antioxidants can be strategically combined with more potent compounds to achieve

controlled preservation effects tailored to specific food products.

Although the antioxidant value of CGH was very weak according to the IC_{50} value, the protein content was reported to be high (Table 2).

Table 2. Proximate Analysis of Catfish Gill Protein Hydrolisate

Proximate	Percentage (% wb)
Moisture	2.16±0.67
Ash	26.09±1.81
Protein	56.86±1.51
Lipid	8.68±1.21
Carbohydrate	6.21*

*calculated by difference

The protein content of CGH is high (56.86±1.51%), indicating an effective hydrolysis method in maintaining protein even at high temperatures. Protein in hydrolysate is more easily digested because the hydrolysis process breaks down the protein into ready-to-absorb peptides and amino acids. This benefits groups with special protein needs, such as children, the elderly, and individuals with digestive disorders. Protein hydrolysate can be used in medical food products for patients with chronic diseases that require high nutrition, such as cancer and diabetes, because it is easily absorbed and provides efficient protein for recovery. Protein hydrolysate can also be added to various food and beverage products as a nutritional supplement to increase daily protein intake and benefit athletes' recovery and muscle growth after exercise. Protein hydrolysates from natural sources are safe for consumption and can be a healthier alternative to synthetic proteins, although their production and use must meet strict food safety standards.

Conclusion

Protein hydrolysate from catfish gills (CGH) had the highest antioxidant content, with 3% papain enzyme and a 48-hour hydrolysis duration, and was stable during drying at high temperatures. Although exhibiting low antioxidant activity, CGH powder with high protein content presents promising prospects in food systems, particularly as a functional ingredient for protein enrichment, nutritional fortification, and the development of high-protein formulations aimed at promoting health and wellness.

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References

Abraha, B., A. Mahmud, M. Samuel, W. Yhdego, S. Kibrom, & W. Habtom. 2017. Production of Fish Protein Hydrolysate from Silver Catfish (*Arius thalassinus*). MOJ Food Processing & Technology. 5(4): 328-335. https://doi.org/10.15406/mojfpt.2017.05.00132

Agency for Regional Development Yogyakarta Special Province. 2020. Data Dasar Perikanan Budidaya Yogyakarta. http://bappeda.jogjaprov.go.id/dataku/data_dasar/index.

Annisa, S., Y. S. Darmanto & U. Amalia. 2017. Pengaruh perbedaan spesies ikan terhadap hidrolisat protein ikan dengan penambahan enzim papain. Saintek perikanan: Indonesian Journal of Fisheries Science and Technology. 13(1): 24-30. https://doi.org/10.14710/ijfst.13.1.24-30

Barzana E, & G. N. Gracia. 1994. Production of fish protein concentrate. Martin, A.M. (ed) Fisheries Processing Biotechnology Application. London (UK): Chapman & Hall (207-222).

Benzie, I. F. & J. J. Strain. 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem. 239 (1): 70-76. https://doi.org/10.1006/abio.1996.0292

Chalamaiah M, Yu W., and Wu J. 2018. Immunomodulatory and anticancer protein hydrolysates (peptides) from food proteins: A review. Food Chem. 245: 205-222. https://doi.org/10.1016/j.foodchem.2017.10.087

Darmawan, E. 2020. Pengaruh Konsentrasi Enzim Bromelin Terhadap Sifat Kimia dan Organoleptik Hidrolisat Protein dari Ikan Lele Ikan Dumbo (*Clarias gariepinus*). Agrotech: Jurnal Ilmiah Teknologi Pertanian. 4 (1): 1-8. https://doi.org/10.37631/agrotech.v3i1.172

Dong, Y., G. Sheng, J. Fu, & K. Wen. 2005. Chemical Characterization and Anti-anaemia Activity of Fish Protein Hydrolysate from *Saurida elongata*. Journal of the Science of Food and Agriculture. 85: 2033-2039. https://doi.org/10.1002/jsfa.2219

Elavarasan, K. & B. A. Shamasundar. 2016. Effect of Oven drying and Freeze drying on The Antioxidant and Functional Properties of Protein Hydrolysates Derived from Freshwater Fish (*Cirrhinus mrigala*) Using Papain Enzyme. Journal of Food Science and Technology. 53: 1.303-1.311. https://doi.org/10.1007%2Fs13197-015-2084-9

Guo, Y., Michael, N., Fonseca Madrigal, J., Sosa Aguirre, C., & Jauregi, P. 2019. Protein hydrolysate from *Pterygoplichthys disjunctivus*, armoured catfish, with high antioxidant activity. *Molecules, 24*(8), 1628. https://doi.org/10.3390/molecules24081628

Hoyle, N. T., & J. H. Merritt. 1994. Quality Of Fish Protein Hydrolysates from Herring (*Clupea harengus*). Journal of food Science. 59(1): 76-79. https://doi.org/10.1111/j.1365-2621.1994.tb06901.x

Ilham D., Dewita dan Karnila. 2019. Karakteristik hidrolisat protein ikan malong (*Congresox talabon*) yang dihidrolisis dengan menggunakan enzim papain. Berkala Perikanan Terubuk. 47(2):186.

Luo, H. Y., B. Wang, Z. R. Li, C. F. Chi, Q. H. Zhang, & G. Y. He. 2012. Preparation and Evaluation of Antioxidant Peptide from Papain Hydrolysate of *Sphyrna lewini* Muscle Protein. Food Science Technology. 51: 281-288. https://doi.org/10.1016/j.lwt.2012.10.008

Klompong, V., S. Benjakul, D. Kantachote, K. D. Hayes, & F. Shahidi. 2008. Comparative Study on Antioxidative Activity of Yellow Stripe Trevally Protein Hydrolysate Produced from Alcalase and Flavourzyme. Int J Food Sci Technol. 43: 1019–1026. https://doi.org/10.1111/j.1365-2621.2007.01555.x

Mizushige, T., M. Komkiya, M. Onda, K. Uchida, K. Hayamizu, Y. Kabuyama. 2017. Fish protein hydrolysate exhibits anti-obesity activity and reduces hypothalamic neuropeptide Y and agouti-related protein mRNA expressions in rats. Biomedical Research 38(6): 351-357. https://doi.org/10.2220/biomedres.38.351

Mubarokah, U., A. Kriswantriyono, H. Horiq & R. Syarif. 2021. Inovasi Olahan Tulang dan Kepala Ikan Lele sebagai Upaya Pemberdayaan Masyarakat dalam Pengelolaan Limbah Ikan Lele Berbasis Zero Waste. Jurnal Resolusi Konflik, CSR dan Pemberdayaan. 6 (1): 49-62.

Najafian L, & Babji. 2015. Isolation, purification and identification of three novel antioxidative peptides from patin (*Pangasius sutchi*) myofibrillar protein hydrolysates. LWT - Food Sci Technol. 60 (1): 452-46. https://doi.org/10.1016/j.lwt.2014.07.046

Nalinanon, S., S. Benjakul, H. Kishimura, & F. Shahidi. 2011. Functionalities and antioxidant properties of protein hydrolysates from the muscle of ornate threadfin bream treated with pepsin from skipjack tuna. Food Chem. 124 (4): 1354-1362. https://doi.org/10.1016/j.foodchem.2010.07.089

Nurdiani, R., M. Ramadhan, A. A. Prihanto & M. Firdaus. 2022. Characteristics of fish protein hydrolysate from mackerel (*Scomber japonicus*) by-products. Journal of Hunan University Natural Sciences. 49 (1): 75-83. https://doi.org/10.55463/issn.1674-2974.49.1.10

Nurhayati, T., Nurjanah & C. N. Sanapi. 2013. Karakterisasi Protein hidrolisat Ikan Lele Dumbo (*Clarias gariepinus*). Jurnal Pengolahan Hasil Perikanan Indonesia. 16(3): 207-214. https://doi.org/10.17844/jphpi.v16i3.8058

Nurjanah, T. Nurhayati, A. Latifah, & T. Hidayat. 2021. Aktivitas Antioksidan dan Komponen Bioaktif Hidrolisat Protein Jeroan Ikan Kakap Putih (*Lates calcalifer*). Warta IHP. 38 (1): 70-78.

Puspawati N.M., Dewi P.P., Bogoriani N.W., & Ariati N.K. 2020. Produksi Hidrolisat Protein Antioksidan Melalui Hidrolisis Enzimatik Protein Kulit Ayam Broiler Dengan Enzim Papain. Journal of Chemistry 14(2): 206-212. https://doi.org/10.24843/JCHEM.2020.v14.i02.p16

Prastika, H. H., K. Ratnayani, N. M. Puspawati & A. A. I. A. M. Laksmiwati. 2019. Penggunaan enzim pepsin untuk produksi hidrolisat protein kacang gude (*Cajanus cajan* (l.) Millsp.) yang aktif antioksidan. Cakra Kimia (Indonesian E-Journal of Applied Chemistry). 7(2): 180-188. https://doi.org/10.24843/CK.2019.v07.i02

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology & Medicine, 26 (9–10): 1231–1237. https://doi.org/10.1016/S0891-5849(98)00315-3

Rimalia, A. 2002. Pengaruh Limbah Ikan Terhadap Pertumbuhan, Kualitas Darah dan Kandungan Protein Ikan Patin (*Pangasius hypothalamus* HB). UGM Press. Yogyakarta.

Rocha, M. D., A. Alemán, G. C. Baccan, M. E. López-Caballero, C. Gómez-Guillén, P. Montero & C. Prentice. 2018. Anti-Inflammatory, Antioxidant, and Antimicrobial Effects of Underutilized Fish Protein Hydrolysate. Journal of Aquatic Food Product Technology, 27:5, 592-608. https://doi.org/10.1080/10498850.2018.1461160

Salamah, E., T. Nurhayati, I. R. Widadi. 2012. Pembuatan dan karakterisasi hidrolisat protein dari ikan lele dumbo (*Clarias gariepinus*) menggunakan enzim papain. Jurnal Pengolahan Hasil Perikanan Indonesia. 15 (1): 9-16.

Samaranayaka, A.G.P. & Li-Chan, E.C.Y. 2011. Food-Derived Peptidic Antioxidants: A Review of Their Production, Assessment, and Potential Applications. Journal of Functional Foods, 3, 229-254. https://doi.org/10.1016/j.jff.2011.05.006

Sampath, K.N.S., Nazeer, R.A. & Jaiganesh, R. 2011. Purification and biochemical characterization of antioxidant peptide from horse mackerel (*Magalaspis cordyla*) viscera protein. Peptides 32(7): 1496–1501. https://doi.org/10.1016/j.peptides.2011.05.020

Sarmadi, B. H. & A. Ismail. 2010. Antioxidative peptides from food proteins: a review. Peptides 31(10): 1949-1956. https://doi.org/10.1016/j.peptides.2010.06.020

Shahi, Z., S. Z. Sayyed-Alangi, & L. Najafian. 2020. Effects of enzyme type and process time on hydrolysis degree, electrophoresis bands and antioxidant properties of hydrolyzed proteins derived from defatted *Bunium persicum* Bioss. press cake. Heliyon, 6 (2): 1-10. https://doi.org/10.1016/j.heliyon.2020.e03365

Shrestha M. K. & J. Pant. 2012. Small-scale aquaculture for rural livelihoods: Proceedings of the Symposium on Small-scale aquaculture for increasing resilience of Rural Livelihoods in Nepal. 5-6 Feb 2009. Kathmandu, Nepal, WorldFish.

Sukkhown, P., K. Jangchud, Y. Lorjaroenphon, & T. Pirak. 2018. Flavoured-Functional Protein Hydrolysates from Enzymatic Hydrolysis of Dried Squid By-Products: Effect of Drying Method. Food Hydrocolloids. 76: 103-112. https://doi.org/10.1016/j.foodhyd.2017.01.026

Susanto E., Djalal R., & Lilik E.R. 2018. Optimasi Aktivitas Antioksidan Peptida Aktif Dari Ceker Ayam Melalui Hidrolisis Enzim Papain. Jurnal Ilmu dan Teknologi Hasil Ternak. 13(1): 14-26. https://doi.org/10.21776/ub.jitek.2018.013.01.2

Witono, Y., Aulanni'am, A. Subagio & S. B. Widjanarko. 2007. Preliminary Study for Enzymatic Processung of Milkfish Hydrolysate by Using "Biduri" Protease. Prosiding Seminar Nasional Perhimpunan Ahli Teknologi Pangan Indonsesia (PATPI), Bandung 17-18 Juli 2007.

Witono Y., M. Maryanto, I. Taruna, A. D. Masahid & K. Cahyaningati. 2020. Aktivitas antioksidan hidrolisat protein ikan wader (*Rasbora jacobsoni*) dari hidrolisis oleh enzim calotropin dan papain. Jurnal Agroteknologi. 14(01): 44-57. https://doi.org/10.19184/j-agt.v14i01.14817

Wu, H., H. M. Chen, & C. Y. Shiau. 2003. Free Amino Acids and Peptides as Related to Antioxidant Properties in Protein Hydrolysates of Mackerel (*Scomber austriasicus*). Food Research. 36: 949-957. https://doi.org/10.1016/S0963-9969(03)00104-2

Yathisha, U. G., I. Bhat, I. Karunasagar, & Mamatha, B. S. 2019. Antihypertensive activity of fish protein hydrolysates and its peptides. Crit Rev Food Sci Nutr. 59 (15): 2363-2374. https://doi.org/10.1080/10408398.2018.1452182