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Physicochemical Characteristics and Antioxidant Activity in the Protein Hydrolysate of Common Dolphinfish (*Coryphaena hippurus*) Roe

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Abstract : Byproducts of the fisheries industry can be a source of nutritional and functional food ingredients, such as fish roe, which contains a high protein content and some important amino acids. This study aimed to explore the functional characteristics, amino acid profile, and antioxidant activity of the protein hydrolysate from the rose of the common dolphinfish (*Coryphaena hippurus*). The roe was prepared and hydrolyzed using a crude papain extract (CPE) and pure papain enzyme (PPE). Analysis of the chemical characteristics of the hydrolysate showed that PPE had a higher protein content and degree of hydrolysis than CPE (63.82% vs. 50.76%), but PPE had a lower fat content than CPE (0.26%vs. 0.71%). Based on the amino acid profile, the percentage of glutamate was lower in PPE than in CPE (8.39% vs. 10.335%). Analysis of the physical characteristics showed that the water absorption, fat absorption, and froth potency of CPE (1.35 mL/g, 2.50 mL/g, and 42%, respectively) were higher than for PPE (1.17 mL/g, 2.24 mL/g, and 39.50%, respectively), but the emulsion capacity of PPE was lower than that of CPE (39.50% vs 42.00%). The antioxidant activity (IC_{50}) of CPE was lower than that of PPE (0.059 mg/mL vs. 0.204 mg/mL). The chemical characteristics of the hydrolysate showed that PPE had a higher protein content and hydrolysis degree than CPE, but PPE had a lower fat content than CPE.
Keywords : antioxidant activity, enzymatic, protein hydrolysate.

Introduction

The fisheries industry produces about 60% byproducts by weight, including the head, bones, fins, skin, viscera, and roe of fish, leaving only 40% by weight of products that can be consumed by humans[1]. Most of these byproducts are used as a nutrition source for the food processing industry, such as the production of fish oil, fish flour, and fish meal; alternatively, these products can be used in the drug industry [2,3,4]. The byproducts are mostly sourced from large pelagic fish, such as tuna[4], skipjack tuna[5], and the common dolphinfish.

Statistical data of fisheries, Ministry of maritime Affairs and Fisheries Indonesia (2012) showed that the common dolphinfish, a large pelagic and economically important fish, is a highly caught marine fish in Indonesia (9,160 tons/year). Recently, the demand for the common dolphinfish has been high as it is both consumed locally and exported. The increasing amount of byproducts is a serious problem related to environmental pollution. Fish roe as a byproduct of the fisheries industry contains high amounts of protein and

amino acids [6,7]. Fish roe contains all the standard amino acids, and some studies have stated that bioactive peptides from fish roe can be obtained by enzymatic hydrolysis.

Enzyme utilization is an efficient way to avoid damage to amino acids in protein hydrolysates, particularly regarding tryptophan and glutamine. Protein hydrolysates also have antioxidant activity and can prevent food rancidity [8,9]. There have been many studies on the enzymatic hydrolysis of fish roe [10,11,12,13,14], but in Indonesia, this method is only applied to fish meat. This study aimed to explore the functional characteristics, amino acid profile, and antioxidant activity of the protein hydrolysate of common dolphinfish (*Coryphaena hippurus*) roe.

Experimental

Material and Sample Preparation

Common dolphinfish (*C. hippurus*) roe was obtained from a fishmonger at the Malang Traditional Central Market. It was placed in a cool box (roe:ice = 2:1). The roe was homogenized using a mixer then isopropyl alcohol was added 1:3 (w/v) to remove fat. After 3 hours, the precipitate was filtered through a sieve, dried in a 45ºC oven for 4 hours, and filtered again through a 60 mesh sieve.

Protein Hydrolysate

The protein hydrolysate was obtained by an enzymatic hydrolysis reaction using <1 U/mg of a crude papain extract (CPE; Paya) and pure papain enzyme (PPE; Sigma) by a modified method[15]. The defatted roe was homogenized with aqua dest (1:10) using a homogenizer for 2 minutes. The pH of the solution was adjusted to pH 7 by adding 1 M NaOH and/or 1 M HCl. The solution was supplemented with crude papain enzyme (CPE) (3%, 5%, and 7% (w/w)) and pure protein enzyme (PPE) (0.3%, 0.5%, and 0.7%). Hydrolysis was performed in a 55°C water bath shaker for 4, 6, or 8 hours for CPE and 1.5 hours for PPE. After the hydrolysis process, the sample was centrifuged at 500 rpm for 20 minutes at 4°C, then dried in a vacuum at 50°C.

Physicochemical Characteristics and Antioxidant Activity Analysis

Physicochemical characters including the amino acid profile, protein amount, hydrolysis degree, water and fat absorption ability, emulsion capacity, froth potency, and antioxidant activity were assessed. Proximate protein and amino acid analysis were based on the method of the Association of Official Analytical Chemists (2005)[16]. Dissolved protein was analyzed by the Biuret method[17]. Hydrolysis degree was analyzed by the Kjeldahl method to measure the nitrogen content[18]. Water and fat absorption ability were assessed by the unabsorbed water and oil-free volume[19]. Emulsion capacity was measured using the method of Yasumatsu et al. (1972) [20]. Froth potency was measured by assessing the froth potency [18]. Antioxidant activity was analyzed by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method and calculated by the formula below:

$$\text{Detention activity (\%)} = \frac{(A_{blank} - A_{sample})}{A_{blank}} \times 100$$

Statistical Analysis

Data from all parameters were analyzed using t-tests to compare the results between CPE and PPE treatment.

Results

Protein Hydrolysate Yield

The statistical analysis showed that the protein hydrolysate yield of the roe following CPE treatment was significantly lower than after PPE treatment (Table 1). This was caused by the high degree of activity and protein solubility of PPE and the different concentrations of the enzyme. The level of protein solubility in the hydrolysate depends on the enzyme, because the enzyme plays an important role in the proteolytic process.

Table 1. Protein hydrolysate yield of *C. hippurus* roe

Treatment	Yield (%)
CPE	10.92 ± 0.07
PPE	13.25 ± 0.07

Table 2. Hydrolysis degree of protein hydrolysate yield of *C. hippurus* roe

Treatment	Hydrolysis degree (%)
CPE	50.68 ± 0.68
PPE	80.64 ± 0.57

Degree of Hydrolysis

The statistical analysis showed that degree of hydrolysis in the roe after CPE treatment was significantly lower than after PPE treatment (Table 2). The peptide bonds in a protein molecule are broken by the proteolytic enzyme during the enzymatic hydrolysis process.

Chemical Characteristics

Proximate

Table 3 shows that the protein content of the hydrolysate after PPE treatment was higher than after CPE treatment.

The fat content of the protein hydrolysate after PPE treatment was lower than after CPE treatment (Table 3). This may have been influenced by the defatting process (before hydrolysis) that involved a consecutive filtration process at 4ºC for 24 hours in separating the fat from the protein hydrolysate.

The water content of the protein hydrolysate following CPE was lower than with PPE treatment (Table 3). The water content was higher than in the viscera of *Catla catla* and other commercial fish species (3.85% and 3.0-5.0%, respectively). This could be caused by differences in the filtration process, as the protein hydrolysates of *C. catla* and other commercial species are prepared using a spray drying method. The carbohydrate content of the protein hydrolysate following CPE was higher than after PPE treatment.

Table 3. Proximate analysis of protein hydrolysate of *C. hippurus* roe

Proximate	Treatment	
	CPE	PPE
Protein (%)	63.82 ± 2.02	75.42 ± 6.19
Fat (%)	0.71 ± 0.37	0.26 ± 0.08
Water (%)	7.84 ± 0.37	6.25 ± 3.12
Ash (%)	12.23 ± 3.05	7.35 ± 2.01
Carbohydrate (%)	15.4 ± 1.03	10.72 ± 0.97

Table 4. Amino acids profile of *C. hippurus* roe protein hydrolysate

Amino acids profile	Percentage (%)	
	CPE	**PPE**
Essential Amino acids		
Valine (np)	3.08	3.89
Metionine (np)	0.97	1.61
Isoleucine (np)	2.06	2.49
Leucine (np)	3.21	4.18
Phenylalanine (np)	1.36	3.12
Lisine (p)	**9.22**	**6.72**
Arginine (p)	4.31	4.93
Histidine (p)	2.06	2.61
Cystine (np)	0.07	0.24
Threonine	3.08	4.34
Non-essential amino acids		
Aspartate (p)	5.03	6.32
Glutamate (p)	**8.39**	**10.33**
Serine (p)	4.27	5.06
Glycine (np)	2.70	3.53
Tyrosine (p)	1.30	2.75
Alanine (np)	2.92	3.49
Proline (np)	5.82	6.47
TAAE	**25.42**	**34.13**
Total	**59.85**	**72.08**

Amino acids

Protein hydrolysates consist of free amino acids and short chain peptides. Table 4 shows that there were 17 amino acids in the protein hydrolysate products. The protein hydrolysates from CPE and PPE treatment were dominated by lysine, an essential amino. There were also some non-essential amino acids, such as glutamate, proline, and aspartate. Those amino acids were higher with PPE (10.33%, 6.47%, and 6.32%) than with CPE treatment (8.39%, 5.82%, and 5.03%); this was caused by differences in the protein content. The amino acid profile of a protein hydrolysate plays an important role in determining its nutritional value and functional properties.

Physical Characteristics

The statistical analysis showed that the water absorption ability of the roe following CPE treatment was significantly higher than after PPE treatment (Table 5). This ability depends on the protein concentration, i.e. a higher protein concentration provides a higher water absorption ability. It is also influenced by the amino acid profile, particularly amino acids with polar side chains. This is clearly shown by the amino acid profile in Table 4.

The emulsion capacity of the protein hydrolysate after PPE was higher than after CPE treatment (Table 5). Emulsion capacity is the ability of a solution or protein suspension to emulsify the oil. Proteins with a high non-polar amino acid content (more than 30% of all amino acids) have high emulsifying activity.

Table 5. Hydrolysate Protein Characteristics of *C. hippurus* roe

Physical characters	CPE	PPE
Water absorption (mL/g)	1.35 ± 0.14	1.17 ± 0.03
Fat absorption (mL/g)	2.50 ± 0.14	2.24 ± 0.21
Emulsion capacity (%)	42.87 ± 3.71	53.87 ± 1.94
Froth potency (%)	42.00 ± 2.83	39.50 ± 0.71

Table 6. IC50 value of protein hydrolysate Protein

Treatment	IC_{50} (mg/ml)
papain enzyme (CPE)	0,059 ± 0,001
pure papain enzyme (PPE).	0,204 ± 0,001

Antioxidant Activity

Evaluation of the antioxidant activity by DPPH analysis was used to identify the inhibitory concentration (IC_{50}). The statistical analysis (t-test) showed that the IC_{50} values of the two samples were not statistically significant (Table 5).

The results show that the IC_{50} value following CPE treatment was higher than after PPE treatment. This indicates that the protein hydrolysate from dolphinfish roe treated with CPE had high antioxidant activity with an IC_{50} value of 0.059 mg/ml (59 ppm), while dolphinfish roe treated with CPP had lower antioxidant activity with an IC_{50} value of 0.204 mg/ml (204 ppm).

Discussion

The result of protein hydrolysate was not statistically significantly higher than PPE treatment. According to Chalamiah et al., (2010) the dried protein hydrolysate of mrigal (*Cirrhinus mrigala*) roe, following alcalase and papain enzyme hydrolysis, showed a high yield (41.2% and 9.7%, respectively)[10]. Moreover, the hydrolysis degree of the roe after CPE treatment was low. Others studies have shown that the hydrolysis degree of African catfish (*Clarias gariepinus*) roe under optimum conditions is 35.37%[22]. Differences in enzyme-substrate concentrations and hydrolysis time influence the degree of hydrolysis[23,24].

The protein content in the protein hydrolysate of the roe of *C. mrigala*, *C. gariepinus*, and other commercial fish species is 80% and 70%; 53.29%; and 75.26-78.95%, respectively[10,22,25]. A higher protein content in a hydrolysate is caused by the availability of nitrogen and other simple compounds, such as peptides and amino acids[24]. The fat content of the protein hydrolysate was lower following PPE than after CPE treatment, and was lower than the fat content of the protein hydrolysate of carp (*Catla catla*) and other commercial fish species (1.94% and 19-22%, respectively[12,25]. The nutritional value of a protein hydrolysate is influenced by the protein quality. The amino acid profile is influenced by a number of factors, such as the source, enzyme used, and hydrolysis conditions[17].

The physical characteristics shown in Tabel 5 indicate that the absorption ability of dolphinfish roe following CPE treatment was higher that of cobia fish (*Rachycentron canadum*), i.e. 0.8-1.1 mL/g[27]. The statistical analysis showed that the fat absorption ability of dolphinfish roe was higher with CPE than with PPE. This is correlated with the hydrophobicity of the protein[27]. Differences in fat absorption ability are caused by extensive hydrolysis, which contributes to the degradation of the hydrolytic structure of the protein and decreases hydrophobicity[28]. A similar result was reported by Diniz and Martin (1977), in that better fat absorption ability (6.8-4.8 mL/g) was obtained from shark muscle protein along with an increasing degree of hydrolysis[28]. The fat absorption ability of cobia fish protein hydrolysate is similar to that found in this study (2.4-2.8 mL/g)[26], but the fat absorption ability of salmon (2.86-7.0 mL/g) and herring (3.7-7.3 mL/g) is higher than the value obtained in this study[29].

Additionally, the emulsion capacity of the protein hydrolysate after PPE treatment (Table 5) was influenced by factors that influence emulsifying characteristics, such as the of degree hydrolysis, enzyme, and pH[26]. The statistical analysis showed that the froth potency of the protein with PPE treatment was not significantly different from CPE treatment. This potency is also influenced by protein surface characteristics, hydrophobicity, and protein solubility. Townsend et al., (1983) stated that the amount of hydrophobic amino acids has a significant correlation with the ability produce froth[30]. Thus, based on the antioxidant activity, CPE can hydrolyze faster than PPE, which is one of the reasons for the differences in antioxidant activity. A previous study by You et al.(2010) showed that the protein hydrolysate from *Misgurnus anguillicaudatus* prepared with papain had an IC_{50} value of 1.86 mg/ml[27], and in another study on the protein hydrolysate from *Ctenopharyngodon idellus* treated with papain, the IC_{50} value was 2.86 g/ml[31,32]. Taken together, these results show that the hydrolysate from dolphinfish roe has excellent antioxidant activity. The hydrolysate of dolphinfish roe contains amino acids such as tyrosine, methionine, lysine, cysteine, histidine, and proline (Table 4). These amino acids can act as proton donors and react with free radicals; however, the detailed mechanism is still unclear[33]. Beside that, amino acid also have role as tissue elasticity, fissured skin, ligament and tendon healing, growth and rejuvenation of skin and also connective tissue [34]. Selecting the appropriate proteolytic enzyme is crucial for producing an antioxidant product from fish protein. Proteolytic enzymes (alkalase, α-chymotrypsin, pepsin, trypsin, neutrase, papain, bromelain, and pronase E) can be used for antioxidant peptide production from fish protein[35].

Conclusion

It can be concluded that the chemical characteristics of the protein hydrolysates show that PPE treatment provided a higher protein content and degree of hydrolysis than CPE (63.82% vs. 50.76%), but PPE provided a lower fat content than CPE (0.26% vs. 0.71%). Based on the amino acid profile, the percentage of glutamate in PPE was lower than in CPE (8.39% vs 0.335%). Analysis of the physical characteristics showed that the water absorption, fat absorption, and froth potency following CPE treatment (1.35 mL/g, 2.50 mL/g, and 42%, respectively) were higher than with PPE treatment (1.17 mL/g, 2.24 mL/g, and 39.50%, respectively), but the emulsion capacity with PPE was lower than with CPE (39.50% vs 42.00%). The antioxidant activity (IC_{50}) of the protein hydrolysate prepared with CPE was lower (0.059 mg/mL) than that prepared with PPE (0.204 mg/mL).

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