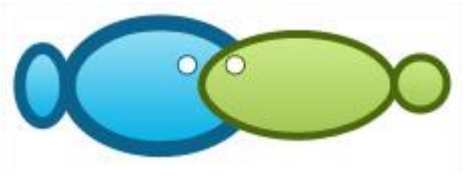

Utilization of papain enzymes in the production of protein hydrolysates of yellow pike conger (*Congresox talabon*)

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Abstract. Yellow pike conger (*Congresox talabon*) has a high protein content that can be used as a source of protein hydrolysate. The aims of this research were to determine the optimum conditions for the hydrolysis process and to characterize the produced protein hydrolysates. Protein hydrolysate from yellow pike conger was produced by enzymatic hydrolysis with papain enzymes. There were 3 treatments applied (0.125%; 0.150%; 0.175% papain enzyme). The experimental design was a completely randomized design, and the data were analyzed by variance analysis (ANOVA), continued with Duncan's test. Results showed that the protein hydrolysate from yellow pike conger contains 92.67% crude protein and the protein hydrolysate has a 50.07% yield. Protein hydrolysates from yellow pike conger present 9 essential amino acids (leucine had the highest value - 5.50%) and 8 nonessential amino acids (glutamic acid had the highest value - 10.53%). The optimum concentration of papain enzyme for hydrolyzing protein from yellow pike conger at 55^0C, pH 8 in 4 hours is 0.15%.
Key Words: amino acid, hydrolysis, proteolytic enzyme.

Introduction. Indonesia is an archipelago with high marine potential. The capture fisheries production of Indonesia amounted to 6.4 million tons in 2014, consisting of 6 million tons of marine fisheries production and 447000 tons of inland water production (rivers, lakes, ponds). The marine fisheries sector experienced an increase from 4.8 million tons in 2009 to 6 million tons in 2014 (Central Statistics Agency 2016).

Riau Province contributed with 107000 tons in 2014, from the total production of Indonesian marine fisheries, of which 1480 tons came from the Bengkalis Regency (Central Statistics Agency 2016). This shows that the Indonesian sea has a great potential for meeting the market demand. One of the fish that can be better utilized in Bengkalis Regency is the yellow pike conger (*Congresox talabon*). yellow pike conger production in 2014 was 4.4 tons (Bengkalis District Marine and Fisheries Service 2014).

Yellow pike conger caught by fishermen are not usually sold intact, because the swim bladder is collected, dried and sold separately. The price of dried fish swim bladder was approximately 368.74 USD per kg in 2018, while yellow pike conger without the swim bladder was between 1.48 USD and 2.22 USD per kg. Kartika et al (2016) stated that the swim bladder of the fish contains high levels of collagen, with a high content of glycine, proline and alanine. The high content of these three amino acids is one of the causes of the expensive price of the swim bladder. The meat has a relatively low economic value, but there are some potential utilizations, like raw material for producing protein hydrolysates, which is expected to increase the added value of yellow pike conger. Dewita & Syahrul (2015) stated that the fish contains high-quality proteins, so it could be used as a base for protein hydrolysate.

Protein hydrolysate is a product of protein hydrolysis, produced by breaking peptide bonds in proteins using enzymes (Karnila 2012). Protein hydrolysates can be liquid, paste or flour, which is hygroscopic. The liquid hydrolysate protein contains 30% solids, while the paste contains 65% solids. The typical flavor of the hydrolysate depends on the amino acid profile of the initial ingredient; for example, the hydrolysate produced

from gelatin is relatively sweeter because of its higher glycine content. The use of enzymes in the hydrolysis process is more advantageous than the use of other hydrolysis methods, such as the use of isoelectric points. Shu et al (2018) stated that enzymatic hydrolysis has several advantages consisting of low cost due to little damage to the nutritional value of proteins, easy to control hydrolysis process, and hydrolyzing proteins in a specific position to produce a specific type of peptides. The papain enzyme is one of the protease enzymes that catalyzes the breakdown of polypeptide chains in proteins by hydrolyzing peptide bonds into simpler compounds, such as peptides and amino acids. The hydrolysis process using the papain enzyme can run well under the following conditions: 4 hours in a temperature of 55°C, and pH 8.0 (Nurhayati et al 2014).

Numerous scientific findings have associated health benefits with fish protein hydrolysates. Protein hydrolysate has demonstrated effects against diabetes. The oral administration of protein hydrolysates from sandfish (*Holothuria scabra*) to rats reduced the blood glucose level (53.9% for 28 days), thus confirming that protein hydrolysate could be an anti-diabetic agent (Karnila 2012). Nurhayati et al (2014) stated that the protein hydrolysates of barramundi (*Lates calcarifer*) innards have quality characteristics and an amino acid profile that is appropriate for application as a protein source for animal feeds. Based on the description above, it is necessary to study the characteristics of yellow pike conger protein hydrolysate. Yellow pike conger as hydrolysate raw material is widely available for use. The availability of hydrolysis materials, namely the enzyme papain is also high. The aims of this research were to determine the optimum conditions for the hydrolysis process and to characterize the produced protein hydrolysates from yellow pike conger.

Material and Method

Methods. Yellow pike conger (*C. talabon*) was obtained from the Bengkalis Regency, Riau, Indonesia, in August 2018. This research was carried out in four stages: (1) preparation of the yellow pike conger and analysis of chemical composition; (2) the production of protein hydrolysates; (3) determination of optimum conditions for the protein hydrolysis process; and (4) the characteristics of yellow pike conger protein hydrolysates produced at optimum conditions. The experimental design used was completely randomized design with differences in enzyme concentrations. Each treatment was carried out three replications.

Yellow pike conger preparation. The initial stage is that of preparing the yellow pike conger (7.5 kg). The fresh yellow pike conger without swim bladders were transported to the Fish Processing Laboratory of Faculty of Fisheries and Marine Science, Riau University, Indonesia. The yellow pike conger meat was separated from other body parts (head, skin, bones, gills, innards). The meat was washed and chopped to reduce size and the proximate analysis was conducted (AOAC 2005).

Producing protein hydrolysates. The hydrolysate production was carried out using the method used by Karnila (2012) and Nurhayati et al (2014), modified. In the early stages, small pieces of yellow pike conger meat were dried (40°C) for 48 hours, then finely sieved (sieve eye diameter - 177 microns). Furthermore, yellow pike conger flour was mixed with distilled water (ratio 1:10) and homogenized for 2 minutes. The mix was heated at 85°C for 15 minutes and cooled to room temperature. Papain enzyme was added to the homogenate in different concentrations, in the 3 treatments: M_1=0.125% (w/v), M_2=0.15% (w/v), and M_3=0.175% (w/v). The optimum use of hydrolysis is at 55°C and pH 8.0 for 4 hours (Nurhayati et al 2014). The mix is then heated at 85°C for 15 minutes to activate the enzyme. After the hydrolysis process is complete, it is followed by the separation of the supernatant from the precipitate using centrifugation (8200 rpm for 15 minutes). The supernatant was evaporated using a rotary vacuum evaporator until all the solvents evaporated. The evaporation results obtained at this stage represent the fish hydrolysates and then freeze drying was carried out. The hydrolysate obtained was packed in sample containers (5 g each) and aluminum foil and stored in a cool room at 4°C until it was ready for use.

Proximate analysis. The proximate analysis of the samples were determined using AOAC methods (AOAC 2005). Moisture content was determined by drying the sample at 105°C. Ash content was measured using the dry ashing procedure in a muffle furnace at 550°C. Crude protein content was measured by converting the nitrogen content obtained by the Kjedhal method. Total lipid was measured by Soxhlet extraction method using petroleum ether as the extract agent. Carbohydrate content was determined by subtracting the combined percent of moisture, ash, protein, and lipid from 100. Data obtained for ash, protein, lipid, and carbohydrate were presented on a dry weight basis.

Degree of hydrolysis. The degree of hydrolysis was measured using the Hasnaliza et al (2010) method. The degree of hydrolysis is calculated based on the percentage ratio of trichloroacetic acid (TCA). About 20 mL of protein hydrolysate were mixed with 20 mL of 20% (w/v) TCA. The mixture is then allowed to rest for 30 minutes for precipitation to occur, then centrifuged (9000 rpm for 15 minutes). The crude protein is determined based on its nitrogen content using the Kjeldahl method (AOAC 2005).

Amino acid profile. The content of free amino acids in fish protein hydrolysate was determined according to AOAC (2005) with a slight modification, using the high-performance liquid chromatography (HPLC) method. This method is based on the reading of the fluorescence detector by derivatization with o-phthaldialdehyde (OPA) reagent. 3 mg of protein hydrolysate were dissolved in 2 mL of 6 N hydrochloric acid. The sample was then hydrolyzed under nitrogen at 110°C for 24 hours and dried using a rotary evaporator with adding 25 mL of 0.01 N hydrochloric acid. The hydrolyzed samples were dissolved in potassium borate buffer (pH 10.4). OPA reagent was added to the mixture (ratio 1:6) and injected into HPLC column.

Yield. The yield was determined by calculating the ratio of the weight of the protein hydrolysate to the weight of fishmeal of yellow pike conger:

Yield (%) = (Protein hydrolysate/fishmeal of yellow pike conger) x 100%

Statistical analysis. The data obtained were analyzed by variance analysis (ANOVA), with a 95% confidence interval, and continued with Duncan's test to discover the differences between treatments.

Results and Discussion

Chemical composition of muscle and fishmeal from yellow pike conger. The results of the chemical composition of the muscle and fishmeal are presented in Table 1.

Table 1

Chemical composition of yellow pike conger (*Congresox talabon*) muscle and fishmeal

Chemical composition (%)	*Muscle*	*Fishmeal*
Moisture	73.40	10.20
Ash (db)	10.19	9.25
Crude protein (db)	84.40	85.10
Crude fat (db)	0.34	0.85
Carbohydrates (db)	5.08	4.80

Note: db - dry matter basis.

The protein content of yellow pike conger muscle is 84.4%, and it has the potential to be used in human nutrition. The use of lower temperatures can reduce damage to proteins. The protein content produced after the drying and milling is 85.1%. Fishmeal samples are expected to be more homogeneous in the process of producing protein hydrolysates.

Vaclavik & Christian (2008) state that proteins consist of chains of amino acids that are connected by peptide bonds to form various complex structures. Amino acids are

very useful in protein synthesis and muscle formation. Protein cycles can occur in cells, tissues or other body parts and involve the digestive tract (Karnila 2012).

Degree of hydrolysis. The determination of the concentration of papain enzymes in the hydrolysis process of yellow pike conger proteins was made by calculating the degree of hydrolysis. The average hydrolysis rate of yellow pike conger protein with different concentrations of papain is presented in Figure 1. The results of the variance analysis showed that the concentration of the papain enzyme had a very significant effect on the degree of hydrolysis.

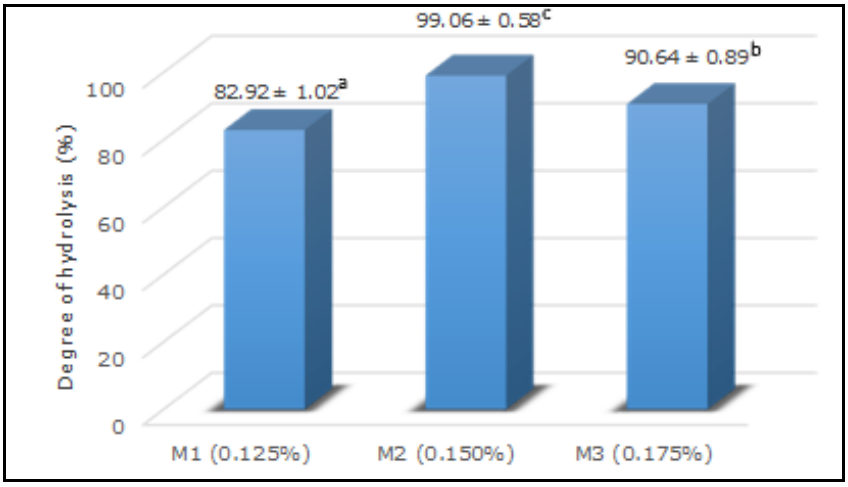

Figure 1. The mean protein hydrolysis value of yellow pike conger (*Congresox talabon*) with papain enzyme. M1 - treatment with 0.125% enzyme; M2 - treatment with 0.15% enzyme; M3 - treatment with 0.175% enzyme. Numbers followed by different superscript letters show very significant differences ($P<0.01$).

The maximum degree of hydrolysis is obtained in M_2, 99.06%, and is the optimum concentration in the process of protein hydrolysis of yellow pike conger. In M_2, the hydrolysis degree increased from M_1. In M_3, the hydrolysis degree decreased, compared to M_2. Even though some other results suggest that the greater the concentration of the papain enzyme added is, the higher is the hydrolysate degree of protein, after certain concentrations the value of the hydrolysis degree tends to remain or not experience significant changes (Nurhayati et al 2013). The results showed that the degree of hydrolysis can even decrease significantly. Lehninger (1993) states that this condition causes the enzyme to become saturated by its substrate and cannot function faster.

Proximate analysis of protein hydrolysate. The proximate analysis of the protein hydrolysate from yellow pike conger is presented in Table 2.

Table 2

Proximate analysis of protein hydrolysate of yellow pike conger (*Congresox talabon*)

Composition	*Protein hydrolysate (%)*
Moisture	10.38
Ash (db)	6.45
Crude protein (db)	92.47
Crude fat (db)	0.35
Carbohydrates (db)	0.54

Note: db - dry matter basis.

The moisture content of the yellow pike conger protein hydrolysate is not much different from that of snakehead (*Channa striata*) protein hydrolysate, due to the use of the same

drying method, namely freeze drying (Prastari et al 2017; Wijayanti et al 2016). Protein hydrolysate of yellow pike conger has an ash content of 6.45%. The enzymatic hydrolysis could be more effective in the breakdown of peptide bonds than acid and alkaline hydrolysis.

The protein hydrolysate of the yellow pike conger has a high protein content of 92.67%. This shows that the use of the papain enzyme runs optimally in the process of protein hydrolysis. The papain enzyme belongs to the group of endopeptidase enzymes that play a role in breaking peptide bonds, specifically in the middle part of the protein chain (Grzonka et al 2007).

Protein levels increase with increasing concentration of enzymes added. With increasing concentration of enzymes the speed of the hydrolysis reaction also increases, but at a certain level the addition of excess enzymes will result in a constant amount of hydrolysate because the addition of enzymes is no longer active (Wijayanti et al 2016).

Hydrolysate protein in yellow pike conger has a protein content of 92.67%, higher than snakehead (*Channa striata*) protein level, which is 90.43% (Prastari et al 2017) and the protein hydrolysate content of milkfish (*Chanos chanos*), of 85% (Wijayanti et al 2016). This was due to differences in the use of enzymes. Hydrolysis of milkfish protein and snakehead protein were conducted with bromelin enzyme (Prastari et al 2017).

Lipid content of yellow pike conger protein hydrolysate is 0.35%. Some of the lipids contained in the protein hydrolysate are thought to remain along proteins that are not dissolved when centrifuged. Protein hydrolysates that have low fat content are generally more stable against fat oxidation reactions than fish protein hydrolysates with high lipid content (Nilsang et al 2005). Shahidi et al (1995) explained that when the hydrolysis reaction takes place, the cell membrane unites and forms structures that are not dissolved, which causes the release of fat in the membrane structures. Changes in fish tissue structures that are very fast cause fat levels to decrease. Dewita et al (2017) stated that a higher solvent extraction power against moisture and lipids concentrates the protein and reduces the fat content. The lipid content of yellow pike conger protein hydrolysate is lower than the fat content of snakehead protein hydrolysate (1.48%) (Prastari et al 2017), and than the fat content of milkfish protein hydrolysate (0.48%) (Wijayanti et al 2016).

Amino acid profile of protein hydrolysate. The protein hydrolysate of yellow pike conger contains 9 essential amino acids and 8 non-essential amino acids (Table 3). The highest essential amino acid content in the protein hydrolysate of yellow pike conger is leucine (5.50%), while the highest non-essential amino acid is glutamic acid (10.53%). Prastari et al (2017) state that high-quality proteins are proteins that contain all types of amino acids in proportions that are suitable for growth.

Based on Table 3, it can be seen that the total essential amino acid content is dominated by leucine and valine, with 5.50% and 3.17%, respectively. Whereas for non-essential amino acids, glutamic acid and aspartic acid have the highest concentrations, of 10.53% and 3.88%, respectively.

Yield. The yield of yellow pike conger protein hydrolysate is 50.07%. The enzyme concentration, temperature, pH, and hydrolysis time affect the amount of yield produced. Karnila (2012) states that the use of enzymes in the hydrolysis process can produce a higher yield.

The yield of the hydrolysate product is the percentage of the amount of hydrolysate products produced from the weight of the raw material before being hydrolyzed (Wijayanti et al 2016). The dissolution of nutritional components such as lipids, proteins and minerals during the hydrolysis process affects the yield of the hydrolysate produced (Shahidi et al 1995). The use of water is also able to expand the contact area between the enzyme and the substrate, so that in a certain period of time more hydrolysate can be produced (Wijayanti et al 2016). The yield of the protein hydrolysate of the yellow pike conger was higher than that of snakehead protein hydrolysate, which was equal to 50.01% (Prastari et al 2017).

Table 3

Amino acid profile of protein hydrolysate from yellow pike conger (*Congresox talabon*)

Amino acids	*% in protein hydrolysate db*
Essential amino acids	
Arginine	1.35
Phenylalanine	1.45
Histidine	1.77
Isoleucine	2.25
Leucine	5.50
Lysine	3.21
Methionine	1.95
Threonine	2.21
Valine	3.17
Sum	22.86
Non-essential amino acids	
Alanine	1.13
Aspartate	3.88
Glutamate	10.53
Glycine	3.21
Proline	3.48
Serine	1.48
Cysteine	1.07
Tyrosine	1.12
Sum	25.90
Total	48.76

Note: db - dry matter basis.

Conclusions. The protein hydrolysates produced using papain enzyme present a protein content of 92.67% and a yield of 50.07%. The protein hydrolysate of yellow pike conger contains 9 essential amino acids and 8 non-essential amino acids. Leucine has the highest content in the protein hydrolysate of yellow pike conger out of the essential amino acids, with 5.50% db, while the non-essential amino acid with the highest concentration is glutamic acid, with 10.53% db. The optimum papain enzyme concentration for protein hydrolysate of yellow pike conger is 0.15% (w/v), under a temperature of 55°C, pH 8, and a hydrolysis time of 4 hours.

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