Saintek Perikanan: Indonesian Journal of Fisheries Science and Technology
Available at http://ejournal.undip.ac.id/index.php/saintek
Vol. 17 No. 3 : 180-187, October 2021

CHARACTERISTIC OF BLUE SWIM CRAB MUSTARD (*Portunus Pelagicus*) PROTEIN HYDROLYSATE WITH DIFFERENT PAPAIN ENZYME CONCENTRATIONS

Nur Fadilah, Eko Nurcahya Dewi*, Romadhon, Lukita Purnamayati
Fishery Products Technology Department, Faculty of Fisheries and Marine Science, Universitas Diponegoro
Jl. Prof. H. Soedarto, SH Tembalang, Semarang, Jawa Tengah – 50275, Phone/Fax. +6224 7474698
Email: nurdewisatsmoko@gmail.com

Received : 26 June 2021, Accepted : 10 September 2021

ABSTRACT

Mustard is a waste contain protein produced from the shell stripping process of blue swimming crab. It has a great potential to be processed as protein hydrolysate. Papain enzyme could be a hydrolysis agent due to its specific function and didn't cause protein damage. The liquid product of the mustard hydrolysate protein requires a drying process to become a powder product. The drying process method is known as foam-mat drying. The research aims to determine the effect of papain enzyme addition on the characteristics of mustard protein hydrolysate from blue swimming crab and the best enzyme concentration. The research method was used a completely randomized design (CRD) with different enzyme concentration treatments (0%, 5%, 7.5% and 10%). The data obtained were tested using ANOVA test, in order to determine differences between treatment a Honestly Significant Difference (HSD) test were applied. The results showed that the addition of different papain enzyme concentrations had a significantly different effect (P<5%) on all test parameters (yield, hydrolysis degree, protein content, moisture content, fat content, ash content, protein digestibility, and amino acid profile). Based on the results of the study, the best mustard hydrolysate protein were the addition of 10% papain enzyme concentration with a yield value of 19.14±0.29%, hydrolysis degrees of 40.19±0.24%, protein content 49.21±0.83% (dw), the moisture content 7.20±0.20%, fat content 1.49±0.29%, ash content 7.22±0.20%, protein digestibility 88.35% and the highest amino acid level was glutamic acid (3.746%).

Keywords: Papain enzyme; protein hydrolysate; swimming crab mustard

INTRODUCTION

Blue swimming crab (*Portunus pelagicus*) is a sea crab widely found in Indonesian waters. Sea crabs are in great demand by the public, both at domestic and abroad. In Indonesia, crab is a fishery commodity that has high economic value. The export value of Indonesian crab meat in 2017 was in the 3rd largest position after tuna and shrimp, with a value of almost USD 411 million. The largest export destination countries are the United States (71%), followed by Japan (9%), Malaysia (7%), and other countries (Ministry of Marine and Fisheries, 2018). Crab export products are generally in the form of frozen crab or packaged meat in cans. The crab meat processing industry produced solid wastes in the form of mustard, shells, and gills.

One small crab produces process waste consisting of 4.3% mustard, 50.8% shells, 6.03% gills, and 22.6% boiled water (Syahbuddin *et al.*, 2014). The abundant production of crab waste has an unfavorable impact on the surrounding environment because it causes pollution. According to Mudaningrat *et al.*, (2019), crab mustard is a yellowish product containing a high protein located under the surface of the shell. The mustard is perishable, so it requires handling to reduce pollution as well as to provide added value. One form of utilization of mustard with great potential is protein hydrolyzate.

Protein hydrolyzate is a by-product of protein breakdown into simple peptides and amino acids through a chemical or enzymatic hydrolysis process. Chemical hydrolysis of proteins has a drawback in the breaking process of peptide chain which unspecifically, hence the quality of the final product is not good. For example, the breakdown of essential amino acids (tryptophan, cysteine , or serine) and changing structure of L-amino acids into D-amino acids, cannot be consumed by humans. The by-products produced unwanted peptides that can cause a bitter taste (Restiani, 2016). Meanwhile, in enzymatic protein hydrolysis, the resulting amino acid composition and sequence are specific according to the type of protease used. The use of papain enzymes, which are an endoprotease group, has a fairly broad cutting specificity for the amino acid residues that make up the substrate, including arginine, lysine, tyrosine, and phenylalanine to produce a high degree of hydrolysis (Aledo & Jimenez-Riveres, 2010). Therefore, enzymatic hydrolysis is an effective method when compared to chemical hydrolysis. This enzymatic hydrolysis process also has the advantage of good quality hydrolyzate products due to the concentration of hydrolyzing agents, pH, temperature conditions, and controlled hydrolysis time (Palupi *et al.*, 2010). In the hydrolysis process, protease enzymes generally used are bromelain, ficin, and papain enzymes.

Papain enzyme is a proteolytic enzyme in papaya. It is relatively cheap and has the potential to be used as an enzyme in protein hydrolyzate processing. The speed of hydrolysis of protease enzymes influenced by temperature, hydrolysis time, pH, and the addition of enzyme concentration. The optimum

temperature for the papain enzyme to work optimally in the hydrolysis process is 55°C, with hydrolysis times in 6 hours, and the optimal pH is 7. During the hydrolysis process, enzyme inactivation has to be at a temperature of 85-90°C for the enzyme to stop working in the hydrolysis process (Salamah *et al.,* 2012). The research of Simanjorang *et al.,* (2012) explained that the amount of papain enzyme added was very influential on the hydrolysis process. Increasing the enzyme concentration can increase the volume of insoluble protein hydrolyzate into soluble nitrogen compounds. The higher the enzyme concentration added, the higher the enzyme activity would be. However, to a certain extent, the addition of enzyme concentration no longer affects its activity. According to Wijayanti *et al.,* (2016), the addition of excess enzymes will result in a constant amount of hydrolyzate due to the addition of enzymes that are no longer active. Salamah *et al.,* (2012) stated that the optimum concentration of papain enzyme used for protein hydrolysis of African catfish was 5%. Wijayanti *et al.,* (2015) also explained that the papain enzyme concentration of 5% could produce the best milkfish protein hydrolyzate. Based on the previous studies, the papain enzyme concentration is expected to affect the characteristics of the protein hydrolyzate.

The protein hydrolysis in the form of a liquid requires a drying process to turn into a powder. Generally, the drying process uses a spray drying method. However, it is less effective in maintaining product quality due to the high inlet temperature of 160°C and an outlet temperature of 80°C as the drying temperature (Annisa *et al.,* 2017). Therefore, the liquid requires a drying method with low drying temperature to maintain the quality of the protein hydrolyzate product, namely the foam-mat drying method. According to Asiah *et al.,* (2012), the foam-mat drying method accelerates the evaporation process with low temperatures, so it would not damage cell tissue and maintain nutritional value. The study aims to determine the effect of different papain enzyme concentrations on the characteristics of the mustard protein hydrolyzate and the best papain enzyme concentration by foam-mat drying method.

RESEARCH METHODS

Materials

The sample used in this study was a mustard blue swimming crab obtained from the Putra Mandiri Mini plan in Rembang, Central Java, Indonesia. Commercial papain enzymes were purchased online under the brand "PAYA" with a weight of 70 grams with an enzyme activity of 1.0593 U/g.

The Production of Mustard Protein Hydrolysate

The production of mustard protein hydrolysate from blue swimming crab was carried out based on Nurhayati *et al.,* (2007) with modifications. The mustard was homogenized with distilled water in a ratio of 1:4 using a blender. Papain enzyme was added with concentrations of 0%, 5%, 7.5%, 10%. The mixture was then hydrolyzed at 55ºC for 6 hours at neutral pH. The papain enzyme was inactivated by heating at 90ºC for 20 minutes. The mixture was then centrifuged at 4ºC at a speed of 5000 rpm for 20 minutes to obtain a solution fraction in the form of mustard protein hydrolyzate. The mustard protein hydrolyzate was dried using the foam-mat drying method by adding 2% egg white foam and 5% maltodextrin filler. Drying carried out using an oven at 58ºC for 48 hours. The powdered mustard protein hydrolyzate product was then stored in polypropylene plastic, coated with aluminum foil, and put in an airtight jar for later analysis.

Analysis

Yield

The protein hydrolysate yield was obtained by dividing the weight of the mustard protein hydrolysis obtained by the weight of the mustard multiplied by 100% (Anwar and Rosmawati, 2013).

Degree of Hydrolysis

The degree of hydrolysis calculated was based on the percentage ratio of trichloroacetic acid (TCA). A total of 20 mL of protein hydrolysate was added with 20% (w/v) TCA. The mixture was then allowed to stand for 30 minutes for precipitation to occur, then centrifuged (7,800 rpm for 15 minutes). The supernatant analyzed for nitrogen content using the Kjeldahl method. The degree of hydrolysis was calculated by dividing the amount of dissolved nitrogen in 20% TCA by the total nitrogen in the sample (Baehaki *et al.,* 2015).

Proximate Analysis

Proximate analysis consists of moisture content, protein content, fat content and ash content (AOAC, 2005).

Amino Acids Profile

Amino acid profile was carried out using Ultra Performance Liquid Chromatography (UPLC) method. It consists of several stages, namely the sample is weighed as much as 0.1 g is crushed and put into a 20 ml vial headspace. The sample solution was added with 5-10 mL of 6 N HCl, hydrolyzed in an oven at 110°C for 22 hours, then cooled at room temperature and transferred to a 50 mL volumetric flask. Added aqua bidest to the mark and filtered with a syringe filter GHP 0.2 m and 500 L pipette, added 40 L of 2.5 mM AABA internal standard, 400 L of distilled water and vortexed, then added 20 L of Flour Adan reagent and vortexed, allowed to stand for 1 minute and then vortexed. The sample was incubated for 10 minutes at 60°C. 1 L of the solution was injected into the UPLC system. Chromatographic conditions using AccQ-Tag Ultra C18 column, flow rate 0.5 mL/min, gradient pump system, injection volume 1µL, temperature 49°C, and using a 260 nm PDA detector (Meussen *et al.,* 2014).

Data Analysis

The experimental design used was a Completely Randomized Design (CRD). The data obtained were analyzed with ANOVA, Tukey's HSD to determine the differences in each treatment. The data were collected from three replications.

RESULTS AND DISCUSSION

The Proximate Analysis of Mustard Blue Swimming Crab

Mustard crab were analyzed for moisture, ash, fat and protein content. The results of the analysis are presented in Table 1.

Table 1. Proximate Analysis of Mustard

Analysis	Value (%)
Moisture content	72.44 ± 1.02
Ash content	2.83 ± 0.05
Protein content	17.07 ± 0.65
Fat content	3.85 ± 0.63

Note : Data were the average result of 3 replications ± standard deviation

The moisture content of the raw material has the highest content compared to other proximate contents. It is because fish and other types of marine biota have very high moisture content. The moisture content of the mustard crab reached 72.44%. According to Dewantoro *et al.,* (2019), the moisture content of mustard crab reached 73.025%. Kasaai (2014) stated that water is an essential component in food because it can affect appearance, texture, and taste. In addition, the water content will determine the other component that affects the quality of the food.

The protein content of the mustard crab reached 17.07% (Bb). This showed that mustard is rich in protein. Mustard crab is one of the wastes that can be utilized and classified as high protein. According to Listyarini *et al.*, (2018), high protein content has a value of 15-20%. The protein content of mustard from crab is almost similar like squid ink from Permatasari *et al.,* (2017) study, which is a by-product that has good nutritional value, especially protein and amino acids, with a protein content of 17.9%. One of the amino acids contained is glutamate. The amino acid glutamate provides the umami taste in food. The protein contained has a role as a building material in the body and regulates metabolism in living cells.

The fat content in mustard crab reached 3.85% (bb). According to Sasongko *et al.,* (2017), the fat content in mustard is 3.32% and it can be categorized has a moderate fat content. According to Adawiyah (2011), based on their fat content, fish are classified into three, namely fish with a high-fat content (>8%), medium fat content (2.5-8.0%), and low-fat content (0.5-2.5 %). The protein content is closely related to fat and water content. Fish that contain low fat on average have large amounts of protein, while high-fat fish have the opposite.

The ash content in the mustard crab reached 2.83% (Bb). According to Ellouze *et al.,* (2014), the ash content of squid ink of 3.47% is higher than the ash content of mustard crab. The ash content in a food material is related to the mineral content in a food material. Hadiwiyoto (2009) explained that the mineral salt content in marine animals could be in the form of phosphate, calcium, sodium, magnesium, sulfur, and chlorine salts. The salt content comes from the fish itself. The minerals contained in marine animals are more than those from lands.

Characteristics of Mustard Protein Hydrolysate

Yield

Yield value of mustard crab protein hydrolysate was carried out by comparing the materials used with the final product produced. The average yield values are presented in Table 2.

Table 2. Yield Value of Mustard

Papain Enzyme (%)	Yield (%)
0	$10{,}94 \pm 0{,}15^{a}$
5	$15{,}69 \pm 0{,}25^{b}$
7,5	$17{,}10 \pm 0{,}23^{c}$
10	$19{,}14 \pm 0{,}29^{d}$

Note :
- Data were the average result of 3 replications ± standard deviation
- Data followed by different lowercase letter indicate a significant difference (α<5%)

Table 2 shows that the addition of papain enzyme affects the yield of protein hydrolysate. It proves that an increase in the concentration of papain enzymes affected the enzyme activity of mustard protein hydrolysate. According to Kurniawan *et al.,* (2012), the protein hydrolysis process is heavily influenced by the concentration of the hydrolyzing agent, temperature, pH, and hydrolysis time. Increased concentration of the enzyme used would increase the volume of insoluble fish protein hydrolyzate into soluble nitrogen compounds, which later affected the yield produced.

Substrate hydrolysis in the control treatment has a lower yield of hydrolyzate products when compared to other treatments. The highest yield in this study was the addition of 10% papain enzyme concentration, which had a yield of 19.14% while the lowest was in the control of 10.94%. This indicated that higher papain enzyme added would make a higher yield. Permatasari *et al.,* (2017) regarding the protein hydrolyzate of squid ink with 10% enzyme, has the yield value of 10.15%.

Based on Table 2, there was an increase in the yield value of the mustard protein hydrolyzate product along with the papain enzyme concentration. It was due to the higher the papain enzyme concentration will produce more protein liquid. The drying process would create more protein hydrolyzate powder. The purpose of calculating the yield of the mustard protein hydrolyzate is needed to determine the productivity of HPI produced by the method used. The centrifugation process would separate the dissolved protein layer, which then dried into protein hydrolyzate. According to Briani *et al.,* (2014), the increase in yield value is due to the activity of the added papain enzyme where it can accelerate the water release process. This difference occurred because the added enzymes accelerate the process of releasing water from the fish meat tissue. The higher the concentration added, the faster the release of water, resulting in a higher volume of the supernatant as well. Drying using the foam-mat drying method using an oven is a relatively inexpensive drying method compared to the spray drying and freeze-drying methods. According to Asiah *et al.,* (2012), the foam mat drying method uses egg white foam and maltodextrin. The foaming process expands the interface area, thereby reducing drying time and accelerating the evaporation process, while the addition of a filler (maltodextrin) aims to accelerate drying and prevent heat damage, coat flavor components, increase total solids, and increase volume.

Annisa *et al.,* (2017) stated that the yield is the percentage amount of hydrolysate to the volume of the raw material. Yield is one of the essential parameters in the processing of fishery products which aim to estimate the number of parts of the raw material that could be utilized. The

yield value can describe the economic value of a material. A high yield value indicates a high economic value because of the amount that could be utilized from the material. The high and low yields are also due to the influence of drying. The drying product is free from water (liquid) material or contains low amounts of water. According to Wijayanti *et al.,* (2015), protein hydrolysis involves giving water so the amount of water in the process becomes greater than the amount of substrate used. It was also able to expand the contact area between the enzyme and the substrate, hence at a certain time, a larger hydrolyzate product can be produced, which affects the final product, namely, yield.

Degree of Hydrolysis

Degree of hydrolysis result presented in table 3. It showed that the addition of papain enzymes with different concentrations affected the degree of hydrolysis of the product. In this study, the degree of hydrolysis produced with papain enzyme of 10% has the hydrolyzate of mustard protein hydrolysate by 40.19%. The results in this study were higher than the protein hydrolyzate of squid ink in the study of Kurniawan *et al.,* (2012) at 34.51% but lower than Nurimala *et al.,* (2018), which was 53.54%.

Tabel 3. Degree of Hydrolysis of Mustard

Papain Enzyme (%)	Degree of Hydrolysis (%)
0	$7,08 \pm 0,36^{a}$
5	$34,84 \pm 0,38^{b}$
7,5	$37,77 \pm 0,34^{c}$
10	$40,19 \pm 0,24^{d}$

Note :
- Data were the average result of 3 replications ± standard deviation
- Data followed by different lowercase letter indicate a significant difference (α<5%)

The degree of hydrolysis was determined by the soluble nitrogen after trichloroacid precipitation (SN-TCA) method. Rutherfurd (2010) stated that the value of the ratio of -amino free nitrogen to total nitrogen is called the degree of hydrolysis. Free-amino nitrogen is also known as the dissolved nitrogen content in the TCA solution. Determination of the degree of hydrolysis using the SN-TCA analysis method is the measurement of dissolved nitrogen levels in a trichloro acid (TCA) solution after the insoluble components are deposited by the centrifugation process. The SN-TCA method has the advantage that the analysis process is relatively fast and practical. The degree of hydrolysis can be an indicator of the protein hydrolysis process. The value of the degree of hydrolysis is determined by the ratio of -amino nitrogen and total nitrogen. The low content of -amino nitrogen in the control treatment caused by the absence of enzymes that hydrolyze protein in the sample. The higher the enzyme concentration, the higher the ratio of -amino nitrogen and total nitrogen. Haslaniza *et al.,* (2014) explained that the higher the concentration of enzymes used in the hydrolysis process would cause an increase in dissolved nitrogen in TCA due to the breaking of peptide bonds. This condition causes an increase in the degree of hydrolysis.

The degree of hydrolysis might be influenced by several factors, namely hydrolysis time, enzyme concentration, and the type of enzyme used. Research by Kurniawan *et al.,* (2012) showed that the optimum hydrolysis time for the hydrolysis of squid ink as indicated by the value of total dissolved nitrogen compared to the increasing total nitrogen of the material. Time is one of the most significant factors for enzyme performance. The optimum hydrolysis time for the papain enzyme to work is 6 hours. Research by Wijayanti *et al.,* (2015) showed that the difference in enzyme concentration between papain and substrate enzymes caused differences in the degree of hydrolysis produced. Research by Ovissipur *et al.,* (2010) also explained that the different types of enzymes used (alkalize and protamex) could cause a difference in the value of the degree of hydrolysis in the protein hydrolysis process of yellowfin tuna fish head. The optimum enzyme at alkaline pH has greater peptide bond-breaking activity during the hydrolysis process, compared to the optimum enzyme at acidic or neutral pH, while the papain enzyme is included in the optimum enzyme at neutral pH so that there is a difference in the degree of hydrolysis between fish protein hydrolysates using papain enzymes, bromelain enzymes as well as alkalize and protamex enzymes.

Protein Content

The results of protein content analysis in mustard protein hydrolyzate with different concentrations of papain enzymes are presented in Table 4.

Table 4. Kadar Protein Hidrolisat Protein Lemi Rajungan

Papain Enzyme (%)	Protein Content (%wb)	Protein Content (% db)
0	$18,04 \pm 0,60$	$19,15 \pm 0,52^{a}$
5	$36,27 \pm 0,89$	$38,68 \pm 0,55^{b}$
7,5	$40,12 \pm 0,21$	$42,69 \pm 0,39^{c}$
10	$45,46 \pm 0,65$	$49,21 \pm 0,83^{d}$

Note :
- Data were the average result of 3 replications ± standard deviation
- Data followed by different lowercase letter indicate a significant difference (α<5%)

The results revealed that the level of protein hydrolyzate of the mustard was influenced by the concentration of the added papain enzyme. The value of the protein hydrolyzate with the papain enzyme 10% was higher than the research conducted by Permatasari *et al.,* (2017) on the hydrolyzate of squid ink protein with the 10% bromelain enzyme, which showed 12.43% (db). This showed that the sample and concentration heavily affect the hydrolysis product. Wijayanti *et al.,* (2016) stated that the protein content in fish protein hydrolyzate grew with the increasing concentration of added enzymes. The increase in protein value indicated a significant total amount of nitrogen in HPI. It was because the analytical method used is the Kjeldahl, which used the amount of nitrogen as a conversion in the calculation of protein content. It showed that with increasing enzyme concentration, the speed of the hydrolysis reaction also increased.

Protein is an essential molecule in the preparation of the structure and functional processes of living organisms. Proteins are composed of long-chain amino acids strung together with many peptide bonds (Suprayitno and

Sulistiyawati, 2017). According to Thaddee and Lyraz (1990), the protein content of fish protein hydrolyzate for flavor enhancers is 45%. In this study, the best protein concentration value is 10%, which is 45.46%. The product from mustard protein hydrolyzate is categorized as a flavor enhancer. The use of papain enzymes in the hydrolysis process of mustard protein hydrolyzate has a low specific activity, which is 1.0593 U/g. It indicated that every 1 gram of papain enzyme protein could catalyze hydrolysis reactions to convert 1.0593 mol of protein substrate per minute. The number of peptide bonds in mustard protein was successfully hydrolyzed by the papain enzyme, that only a few dissolved nitrogen compounds were produced, and the measured protein content was also low. According to Salamah *et al.,* (2012), the protein content measured in fish protein hydrolyzate is a soluble protein molecule. Some of the dissolved protein in catfish protein hydrolyzate is still in the form of peptides which causes low levels of amino acids in fish protein hydrolyzate.

The addition of papain enzyme to mustard protein hydrolyzate that was carried out in this study showed a difference. The protein levels of mustard protein hydrolyzate increased along with the increase in the concentration of the added papain enzyme. The papain enzyme used in the hydrolysis process could break the peptide bonds in the protein chain to increase the dissolved protein content and improve product quality. With the increase in the concentration of the papain enzyme used in the hydrolysis process, more peptide chains were broken from the raw material, hence the protein content of protein hydrolyzate increased (Simanjorang *et al.,* 2012).

Moisture Content

Moisture content of mustard protein hydrolysate are presented in Table 5.

Table 5. Moisture Content of Mustard

Papain Enzyme (%)	Moisture content (%)
0	$5{,}76 \pm 0{,}36^{a}$
5	$6{,}32 \pm 0{,}30^{ab}$
7,5	$6{,}59 \pm 0{,}21^{ab}$
10	$7{,}20 \pm 0{,}20^{b}$

Note :
- Data were the average result of 3 replications ± standard deviation
- Data followed by different lowercase letter indicate a significant difference (α<5%)

The addition of papain enzymes to the mustard protein hydrolyzate showed that the difference in papain enzymes affected the moisture content. The water content increased from the control treatment to a concentration of 10%. This indicated that the moisture content of the moisture protein hydrolyzate in this study increased along with the concentration of papain enzyme added. According to Wijayanti *et al.,* (2016), it is known that the hydrolysis reaction of protein compounds would turn into simpler and more soluble compounds affect the increase in the volume of the liquid.

The moisture content of the mustard protein hydrolyzate with different concentrations of papain enzymes showed values in the range of 5.76% to 7.20%. The quality standard of moisture content value in fish protein hydrolyzate as a flavor enhancer according to Thadde and Lyraz (1990), is 5.00%. The moisture content in all concentrations is low compared to research by Permatasari *et al.,* (2017) on the hydrolyzate of squid ink protein using the papain enzyme 10%, which is 17.52%. The difference in moisture content might be caused by different drying methods, namely the hydrolyzate of squid ink protein using the spray drying method whereas, the drying method used in this study is the foam-mat drying method..

The initial moisture content contained in the raw material would affect the protein hydrolyzate produced. Most of the water contained in the hydrolyzate would be evaporated in the foam mat drying method. The higher the moisture content, the more water must be evaporated by the oven, so the moisture content of the final product tends to be higher. According to Asiah *et al.,* (2012), foam mat drying is a drying method that is heat-sensitive materials through a foaming technique by adding a foaming agent. Drying in the form of foam could accelerate the evaporation of water, and is happening at a low temperature, thus minimizing damage to the cell tissue and the nutritional value can be maintained. The foam-mat drying method can expand the interface area, therefore reducing drying time and accelerating the evaporation process.

The production of mustard protein hydrolyzate in the form of powder could facilitate the storage process and also extend the shelf life. Drying and storage in low temperatures are generally used to extend the shelf life of food products containing high water content, one of which is the liquid product of mustard protein hydrolyzate into powder products. According to Djaafar *et al.,* (2020), the liquid hydrolyzate product that dried into a powder product with a moisture content of less than 10% to minimize fungal contamination and extend the shelf life.

Fat Content

Fat content obtained from blue swimming crab mustard protein hydrolysate presented in Table 6. The fat content of mustard protein hydrolysate from blue swimming crab showed that the fat content decreased along with enzyme concentration. It was happening because the fat contained in the protein hydrolysate partly separated from the insoluble protein during centrifugation. The centrifugation process has formed a liquid in four layers, namely, oil, fat, dissolved protein, and the remaining part of the mustard. When the oil and fat layers separated, it will cause fat levels to decrease. According to Wang and Shahidi (2018), during the enzymatic hydrolysis process, the structure of fish tissue was quick to change and cause fat levels to decrease. Electron microscopy observations of thin sections of fish muscle showed that myofibril protein was highly reduced during the hydrolysis process. The muscle cell membrane system appeared to be relatively resistant to damage. During the hydrolysis process, these membranes tended to formed into insoluble bubbles, resulting in loss of the lipid membrane.

The lowest fat content of mustard protein hydrolysate was 1.49% at a 10% concentration of the papain enzyme. This result was higher than the preliminary research of Nurhayati *et al.,* (2014) protein hydrolyzate of white snapper offal with 10% papain enzyme concentration obtained fat content of 0.84%.

Table 6. Fat Content of Swimming Crab Mustard Protein Hydrolysate Value

Papain Enzyme (%)	Fat content (%db)
0	5,97 ± 0,63[a]
5	2,45 ± 0,19[b]
7,5	2,03 ± 0,34[c]
10	1,49 ± 0,29[d]

Note :
- Data were the average result of 3 replications ± standard deviation
- Data followed by different lowercase letter indicate a significant difference (α<5%)

Fat content was expected to be lower in protein hydrolysate products. Protein hydrolysate with low-fat content was generally more stable against oxidation so that it has long-lasting durability. According to Purbasari (2008), hydrolyzed products with low-fat content are generally more stable and durable compared to products that have high-fat content. In addition, low levels of fat in hydrolyzate products could be used as dietary food ingredients, namely foods with a fat content of less than 5%, and as a supplement in the manufacture of white bread and baby food.

Ash Content

Ash content obtained from swimming crab mustard protein hydrolysate presented in Table 7.

Table 7. Ash Content of Swimming Crab Mustard Protein Hydrolysate Value.

Papain Enzyme (%)	Ash Content (%)
0	6,51 ± 0,57[a]
5	6,58 ± 0,48[a]
7,5	7,06 ± 0,17[a]
10	7,22 ± 0,20[a]

Note :
- Data were the average result of 3 replications ± standard deviation
- Data followed by different lowercase letter indicate a significant difference (α<5%)

Based on Table 7, the value of mustard protein hydrolysate from blue swimming crab showed no significant difference and ranged from 6.52-7.22%. Ash content in mustard protein hydrolysate from blue swimming crab is higher than white snapper offal (Nurhayati *et al.*, 2014). A mixture of acid and alkaline compounds in protein hydrolysate caused the formation of a salt compound and increase the ash content on protein hydrolysates. Hadiwiyoto (2009) stated that the increase in ash content was caused by the addition of compounds where salt formed during the hydrolysis process. The addition of NaOH and HCl compounds to adjust the optimum pH conditions caused the formation of mineral salts.

Amino Acid Profile

Analysis of the amino acid profile presented at Table 8. Mustard protein hydrolysate from blue swimming crab with 10% papain enzyme concentration showed higher amino acids than the control treatment. On the contrary, it was lower than the preliminary research of Permatasari *et al.*, (2017) with squid ink protein hydrolysate. The mustard protein hydrolyzate contains 15 types of amino acids consisting of 8 essential amino acids and 7 non-essential amino acids. Amino acids in the mustard protein hydrolysate consisted of several essential amino acids and essential amino acids.

Table 8. The Amino Acid Content in Swimming Crab Mustard Protein Hydrolysate /100 g sample

Amino Acids	Amount (%)		
	0% papain enzyme	10% papain enzyme	squid ink with 10% enzyme
L-Isoleusin	0,501	1,537	1,355
L-Valin	0,631	1,842	1,530
L-Alanin	0,531	1,451	2,532
L-Serin	0,593	1,699	1,582
L-Asam Aspartat	0,671	1,959	4,144
L-Arginin	0,619	2,131	1,159
Glisin	0,603	1,651	1,509
L-Threonin	0,483	1,436	-
L-Fenilalanin	0,614	2,063	2,037
L-Leusin	0,781	2,410	3,264
L-Prolin	0,426	1,247	-
L-Histidin	0,273	0,864	0,466
L-Lisin	0,466	1,332	1,565
L-Asam glutamat	1,099	3,746	7,702
L-Tirosin	0,284	0,924	2,369

Note: * = Squid ink amino acid protein hydrolysate (Permatasari *et al.*, 2017).

The essential amino acids that cannot be produced naturally but highly needed for the body are contained in the mustard protein hydrolysate. The essential amino acid included isoleucine, valine, arginine, threonine, phenylalanine, leucine, histidine, and lysine. While the non-essential amino acids that could be produced by the body were alanine, serine, aspartic acid, glycine, proline, glutamic acid, and tyrosine. In this study, the highest type of amino acid was glutamic acid, while other amino acids had a smaller amount. According to Kurniawan *et al.*, (2012), a complete hydrolysis process would produce a hydrolysate consisting of a mixture of 18-20 types of amino acids. All hydrolyzed proteins generate amino acids, but some proteins also generate other related protein molecules.

Based on Table 8, the highest amino acid was the glutamic acid in 1.099% for control and 3.746% for 10% papain enzyme concentration. Permatasari *et al.*, (2017) has a glutamic amino acid content of 7.702%. Glutamic acid was the most abundant type of amino acid in fishery products which has a role as a flavor enhancer.

The lowest amino acid content in mustard protein hydrolysate was histidine in 0.273% for control and 0.864% in 10% papain enzyme concentration. Permatasari *et al.*, (2017) has a 0.466% amino acid content. The amino acid histidine was an essential amino acid as a precursor of histamine for physical growth and tackling rheumatic diseases. According to Nurhayati *et al.*, (2014), complete hydrolysis would produce a hydrolysate consisting of a mixture of 15 kinds of amino acids. Table 8 shows that mustard protein hydrolysate from blue swimming crab has almost all types of amino acids, except for tryptophan, cysteine, asparagine, methionine, and

glutamine, which in this study were not analyzed. Some of the amino acid levels in the swimming crab mustard protein hydrolysate were lower than the amino acid levels in the squid ink protein hydrolysate.

CONCLUSION

The addition of different concentration of papain enzyme in mustard protein hydrolysate from blue swimming crab have a significant different effect (P<5%) on the values of yield, hydrolysis degrees, protein content, water content, fat content, ash content, protein digestibility and amino acid profile. The best product with the addition of 10% papain enzyme concentration, has a yield value 19.14%, hydrolysis degrees of 40.19%, protein content 49.21%, water content: 7.20%, fat content 1.49%, ash content 7.22%, protein digestibility 88.35%. The highest type of amino acid was glutamic acid at 3.746%.

REFERENCES

Adawyah, R. 2011. Pengolahan dan Pengawetan Ikan. Bumi Aksara. Jakarta.

AOAC, 2005. Official Methods of Analysis. Association of Official. Analytical Chemists. Benjamin Franklin Station, Washington.

Aledo, J. C., & Jimenez-Riveres, S. (2010). The effect of temperature on the enzyme-catalyzed reaction: insights from thermodynamics. *Journal of Chemical Education*, *87*(3), 296–298. https://doi.org/10.1007/BF02658056

Annisa, S., Darmanto, Y. S., & Amalia, U. (2017). The effect of various fish species on fish protein hydrolysate with the addition of papain enzyme. *Indonesian Journal of Fisheries Science and Technology*, *13*(1), 24–30. https://doi.org/10.14710/ijfst.13.1.24-30

Anwar, L. O., & Rosmawati. (2013). Karakteristik hidrolisat protein tambelo (Bactronophorus sp.) yang dihidrolisis menggunakan enzim papain. *Biogenesis: Jurnal Ilmiah Biologi*, *1*(2), 133–140. https://doi.org/10.24252/bio.v1i2.459

Asiah, N., Sembodo, R., & Prasetyaningrum, A. (2012). Aplikasi metode foam-mat drying pada proses pengeringan Spirulina. *Jurnal Teknologi Kimia Dan Industri*, *1*(1), 461–467. http://ejournal-s1.undip.ac.id/index.php/jtki

Baehaki, A., Lestari, S. D., & Romadhoni, A. R. (2015). Protein hydrolysis from catfish prepared by papain enzyme and antioxidant activity of hydrolyzate. *Jurnal Pengolahan Hasil Perikanan Indonesia*, *18*(3), 230–239. https://doi.org/10.17844/jphpi.2015.18.3.230

Briani, A. S., Darmanto, Y. S., & Rianingsih, L. (2014). The effect of papain enzyme concentration and fermentation period to the quality of fish sauce from by catch fish. *Jurnal Pengolahan Dan Bioteknologi Hasil Perikanan*, *3*(3), 121–128.

Dewantoro, A. A., Pratama, B. A., & Kurniasih, R. A. (2019). Biogas production from crab picking (Portunus pelagius) wastes. *IOP Conference Series: Earth and Environmental Science*, *246*, 1–6. https://doi.org/10.1088/1755-1315/246/1/012085

Djaafar, T. F., Monika, D. C., Marwati, T., Triwitono, P., & Rahayu, E. S. (2020). Microbiology, chemical, and sensory characteristics of cocoa powder: the effect of Lactobacillus plantarum HL-15 as culture starter and fermentation box variation. *Digital Press Life Sciences*, *2*(8), 1–12. https://doi.org/10.29037/digitalpress.22332

Ellouze, S. C., Benmabrouk, H., Baroudi, O., Ayed, N., & Marrakchi, N. (2014). Mediterranien cuttlefish sepia officinalis squid ink is cytotoxic but does not inhibit Glioblastoma U87 tumor cells proliferation, with high nutritional values of edible viscera. *International Journal of Basic and Applied Sciences*, *3*(2), 146–154. https://doi.org/10.14419/ijbas.v3i2.2387

Hadiwiyoto, S. 2009. Teknologi Pengolahan Hasil Perikanan. Jilid 1. Liberty. Yogyakarta.

Haslaniza, H., Maskat, M. Y., Wan Aida, W. M., & Mamot, S. (2014). Process development for the production of protein hydrolysate from cockle (Anadara granosa) meat wash water. *Sains Malaysiana*, *43*(1), 53–63.

Kasaai, M. R. (2014). Use of water properties in food technology: A global view. *International Journal of Food Properties*, *17*, 1034–1054. https://doi.org/10.1080/10942912.2011.650339

Kurniawan, Lestari, S., & Hanggita, S. (2012). Hidrolisis protein tinta cumi-cumi (Loligo sp) dengan enzim papain. *Fishtech*, *1*(1), 41–54.

Listyarini, S., Asriani, & Santoso, J. (2018). Konsentrat protein ikan lele dumbo (Clarias gariepenus) afkir dalam kerupuk melarat untuk mencapai sustainable development goals. *Jurnal Matematika Sains Dan Teknologi*, *19*(2), 106–113. https://doi.org/10.33830/jmst.v19i2.113.2018

Meussen, B. J., van Zeeland, A. N. T., Bruins, M. E., & Sanders, J. P. M. (2014). A fast and accurate UPLC method for analysis of proteinogenic amino acids. *Food Analytical Methods*, *7*, 1047–1055. https://doi.org/10.1007/s12161-013-9712-7

Ministry of Marine and Fisheries. 2018. Encourage Crab Business Growth with Combination of Catch and Cultivation. https://news.kkp.go.id/index.php/dorong-pertumbuhan-bisnis-rajungan-dengan-kombinasi-hasil-tangkapan-dan-budidaya/. Diakses 22 Juni 2021.

Mudaningrat, A., Ramdan, K., Salsabila, M., Aisyah, S., & Umami, M. (2020). Kerupuk lemi Portunus pelagicus sebagai solusi pengelolaan limbah rajungan di wilayah Cirebon. *Prosiding Seminar Nasional Pendidikan Biologi*, 35–42.

Nurhayati, T.-, Salamah, E.-, Cholifah, - -, & Nugraha, R.-. (2014). Optimaization process production hydrolysates of protein barramudi viscera. *Jurnal Pengolahan Hasil Perikanan Indonesia*, *17*(1), 42–52. https://doi.org/10.17844/jphpi.v17i1.8136

Nurhayati, T., Salamah, E., & Hidayat, T. (2007). Karakteristik hidrolisat protein ikan selar (Caranx leptolepis) yang diproses secara enzimatis. *Buletin Teknologi Hasil Perikanan*, *10*(1), 23–34.

Nurilmala, M., Nurhayati, T., & Roskananda, R. (2018). Limbah industri filet ikan patin untuk hidrolisat protein. *Jurnal Pengolahan Hasil Perikanan Indonesia*, *21*(2), 287–294.

Ovissipour, M., Benjakul, S., Safari, R., & Motamedzadegan, A. (2010). Fish protein hydrolysates production from yellowfin tuna Thunnus albacares head using Alcalase and Protamex. *International Aquatic Research*, *2*, 87–95.

Palupi, N. W., Windrati, W. S., & Tamtarini. (2010). The

effect of enzymatic hydrolysis on the properties of protein hydrolysate from paddy mushroom. *MAKARA of Technology Series*, *14*(2), 73–76. https://doi.org/10.7454/mst.v14i2.695

Permatasari, J., Sumardianto, & Romadhon. (2017). Pengaruh konsentrasi enzim bromelin pada kualitas hidrolisat protein tinta cumi-cumi (Loligo sp.) kering. *Prosiding Seminar Nasional Hasil-Hasil Penelitian Perikanan Dan Kelautan Ke-VI*, 344–356.

Restiani, R. (2016). Enzymatic hydrolysis of protein from nyamplung (Calophyllum inophyllum) oilseed press cake using bromelain. *Biota*, *1*(3), 103–110. https://doi.org/10.24002/biota.v1i3.1226

Rutherfurd, S. M. (2010). Methodology for determining degree of hydrolysis of proteins in hydrolysates: A review. *Journal of AOAC International*, *93*(5), 1515–1522. https://academic.oup.com/jaoac/article-abstract/93/5/1515/5655787

Salamah, E., Nurhayati, T., & Widadi, I. R. (2012). Production and characterization of protein hydrolysates from african catfish (Clarias gariepinus) using papain. *Jurnal Pengolahan Hasil Perikanan Indonesia*, *15*(1), 9–16. https://doi.org/10.17844/jphpi.v15i1.5328

Sasongko, A. Y., Dewi, E. N., & Amalia, U. (2018). The utilization of blue swimming crab (Portunus pelagicus) waste product, lemi, as a food flavor. *IOP Conference Series: Earth and Environmental Science*, *102*, 1–7. https://doi.org/10.1088/1755-1315/102/1/012030

Simanjorang, E., Kurniawati, Ni., & Hasan, Z. (2012). Pengaruh penggunaan enzim papain dengan konsentrasi yang berbeda terhadap karakteristik kimia kecap tutut. *Jurnal Perikanan Dan Kelautan*, *3*(4), 209–220.

Suprayitno, E. dan T.D. Sulistiyawati. 2017. Metabolisme Protein. UB Press. Malang.

Syahbuddin, S. A., Riyadi, P. H., & Romadhon. (2014). The effect of adding swimming crab egg (Portunus pelagicus) with different concentrations on the quality of wet noodle. *Jurnal Pengolahan Dan Bioteknologi Hasil Perikanan*, *3*(4), 65–70. http://www.ejournal-s1.undip.ac.id/index.php/jpbhp

Wang, D., & Shahidi, F. (2018). Protein hydrolysate from Turkey meat and optimization of its antioxidant potential by response surface methodology. *Poultry Science*, *97*(5), 1824–1831. https://doi.org/10.3382/ps/pex457

Wijayanti, I., Romadhon, & Rianingsih, L. (2015). Pengaruh konsentrasi enzim papain terhadap kadar proksimat dan nilai rendemen hidrolisat protein ikan bandeng (Chanos chanos Forsskal). *PENA Akuatika*, *12*(1), 13–23.

Wijayanti, I., Romadhon, & Rianingsih, L. (2016). Caracteristic of milkfish (Chanos chanos Forsk) protein hydrolysate as effect of different bromelin enzyme concentration. *Indonesian Journal of Fisheries Science and Technology*, *11*(2), 129–133. https://doi.org/10.14710/ijfst.11.2.129-133