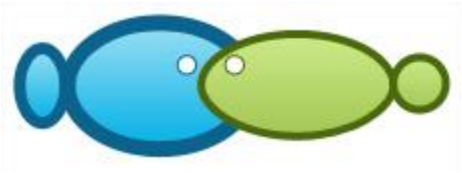

Chemical and amino acid composition of snapper scrap meat hydrolysate

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Abstract. Increased volume and high export value of frozen snapper products in Indonesia, can produce scrap meat that can be processed into high nutritional products. Fish protein hydrolysate (FPH) is produced from an enzymatic process using the alcalase enzyme, which contains peptides and amino acids that can be applied to other derived products. The aim of this study is to produce FPH on a scale up production, determine the characteristics of raw materials and FPH products. The method used is the enzymatic hydrolysis process, which uses alcalase enzyme at 55^0 C with 20.000 U/kg substrate of enzyme concentration for 7 hours. The parameters measured are the degree of hydrolysis (DH), yield, proximate analysis, and the amount of amino acids in raw materials and FPH. DH value was 71.88% ± 6.48 at the 7^{th} hour and based on the ANOVA results the P-value < 0.05, so that the measurement at 0 - 7 hours shows a significantly different DH value. The yield of FPH was 89.30%, the protein content of raw materials 81.93% ± 1.39 and FPH 95.30% ± 0.73. Snapper scrap meat and FPH contain glutamic acid as a non-essential amino acid and lysine as an essential amino acid. The conclusion of this study is that snapper meat has good potential as a raw material for FPH because it contains essential amino acids needed by the human body.

Key Words: alcalase, Lutjanidae, lysine, snapper scrap meat, degree of hydrolysis.

Introduction. The increasing volume and value of Indonesian exports for the fisheries and marine sector in 2018 by 11.06% indicates business sustainability, which is one of the pillars in managing marine and fisheries resources. Frozen fish is one of the commodities with the highest volume value after seaweed (Marine and Fisheries Ministry 2018). One of the dominant frozen fish products is the snapper fillet. Snapper is a type of sea fish that has white meat and commonly lives at a depth of 200-400 meters. The genus that is more commonly caught in Indonesia is *Lutjanus*, including *Lutjanus malabaricus, L. bohar, L. sebae, L. argentimaculatus, L. timorensis, L. johnii* and *Pristipomoides*, including *Pristipomoides multidens,* and *P. typus*. The processing of frozen snapper fillets produces skin, head, scales, stomach contents and scrap meat in the form of pieces of meat originating from the trimming process. Snapper heads are usually sold to Padang restaurants that provide snapper head fish curry, while the scrap meat is usually used in the local cuisine as raw material for nuggets, meatballs, sausages, and fish dumplings. Actually, the utilization of snapper scrap meat can be used in different ways, one of which is as a raw material for making Fish Protein Hydrolysate (FPH) because it contains many important elements such as protein and fat (Lepongbulan et al 2017). FPH is known to have a number of properties that are better than the original protein, caused by an increase in functional properties (He et al 2013) so it is good to add to food because it still has a high protein content (Peinado et al 2016). The population of children in developing countries raises concerns that malnutrition will affect children's growth and intelligence. The aim is to develop large amounts of FPH products, to target the malnutrition issue (UNICEF 2019). FPH is an alternative source of protein with good nutritional composition,

amino acid profile and antioxidant activity of bioactive components from fish protein and can be used in various industrial applications (Chalamaiah et al 2012).

FPH is a product that utilizes functional compounds in fish produced from the protein hydrolysis process by adding a protease enzyme which functions to break down muscle meat protein into peptides and amino acids to produce soluble and insoluble fractions in water (Kurozawa et al 2011). FPH contains peptides and amino acids and can be derived to other products, with high protein concentrations, low molecular weight, high solubility in water and essential amino acid content needed by humans (Ishak & Sarbon 2018). Peptides and amino acids derived from FPH have bioactive capabilities such as anti-hypertension, antioxidants, antithrombotic, immuno-modulators and antimicrobials (Chalamaiah et al 2012) and cryoprotectants (Jankelunas & Li-Chan 2018).

Research conducted by Joung et al (2018) explains that the process of making FPH using hydrolysis enzymatically provides a greater level of safety to the environment, because it uses commercial food grade protease enzymes, with alcalase being one of the best candidates for protease enzymes to hydrolyze at 50^0 - 60^0 C. The production of FPH on a pilot plant which was hydrolyzed for 1 hour using a temperature of 40^o C and the concentration of the enzyme papain 0.5 % (which is derived from the *Carica papaya* plant or fruit) produced a value of degree of hydrolysis (DH) of 71 - 86 %, yield of FPH 70 %, 12 % protein content, fat 1 %, ash 1 % and water 86 % (Himonides et al 2011). Martosuyono et al (2019) in their research on FPH production on a pilot plant scale using a temperature of 55^o C, the concentration of the alkalase enzyme 20,000 U/kg resulted in a FPH filtrate yield of 77.77%, containing 105.99 protein (% dry base), amino acids lysine 11.18 µg/mg, and leucine 8.12 µg/mg. Thus the use of alcalase enzyme in the enzymatic production process of FPH will be greatly influenced by control during the production process, because the enzyme activity at that time will be effective and provide the best performance if it meets the appropriate environmental conditions. Environmental conditions suitable for enzymes are optimal conditions in the hydrolysis process to produce a maximum DH value. Comparison of substrate and enzyme concentration, temperature, pH, and time are variable so that the quality of FPH products produced has a good yield, nutrient content, and amino acids (Nazeer & Kulandai 2012). According to research carried out by Guerard et al (2001), Kristinsson and Rasco (2010), and Muzaifa et al (2012), the ability level of the alcalase enzyme produced by the microbial *Bacillus licheniformis* has been proven as one of the best protease enzymes used in the process of making FPH.

The aim of this study was to produce FPH, determine the characteristics of raw materials and FPH products derived from snapper scrap meat.

Material and Method

Material. This research took place between August and December 2019. The material in this research is snapper scrap meat from the fish processing company of Perindo in Muara Baru, North Jakarta, Indonesia. The scrap meat is in the cutlet form of boneless meat and skin that resulted from the trimming process of fillets. The protease enzyme used is derived from the collection of Indonesian Research and Development Center for Marine and Fisheries Product Processing and Biotechnology, and is the result of *Bacillus* sp. fermentation process in media containing 0.7% ammonium sulfate ($(NH_4)_2SO_4$), 0.1% dipotassium phosphate (K_2PO_4), 0.1% natrium chloride (NaCl), 0.05% yeast extract, 0.01% magnesium sulfate ($MgSO_47H_2O$) and 0.6% skim milk (Martosuyono et al 2019). Other materials used for analyzing the degree of hydrolysis (DH) are disodium tetraborate dehydrate, o-phtaldialdehyde (OPA) 97%, sodium dodecyl sulfate (SDS), ethanol, dithiotreitol (DTT) and tricloroacetate 6.25% and the materials used for testing the proximate content is 0.02 N hydrocloric acid (HCl), potassium sulfate (K_2SO_4). sulfuric acid (H_2SO_4), 40% sodium hydroxide (NaOH) and diethyl ether.

Protein hydrolysis process. The process of making fish protein hydrolysate using the enzymatic hydrolysis method described by Martosuyono et al (2019) was used, with several adaptations. Snapper scrap meat is minced using a meat bone separator then put into a hydrolysis tank that has been filled with water in a ratio of 1:1 (weigth/volume) and

homogenized. The temperature is set between 55^0 - 60^0 C, if the temperature is too high or low then the fire in the stove must be adjusted, by looking at the temperature indicated by the thermostat. The alcalase enzyme is mixed when the temperature has reached 55° C, the concentration of the enzyme added is 20.000 U/kg of the substrate with a hydrolysis time of 7 hours (Martosuyono et al 2019). Next is the enzyme inactivation process carried out by raising the temperature to a temperature of 90° C for 20 minutes and then allowed to stand until the next day for precipitation to occur and produce two separate fractions. The filtrate fraction and the resulting residue are then separated, the resulting filtrate is filtrated using a spinner with a filtration bag equipped with a mesh, with pore distribution of 300 pores per inch and 600 pores per inch, to separate bones, skin, scales, and coarse residue. The next step is to filter the mixture using a micro and ultrafiltration machine with a pore size membrane of 0.5 and 0.1 μm to separate between clear liquid FPH filtrate and residue in the form of leftover meat that cannot be completely hydrolyzed. This clear liquid FPH will be used in the next process.

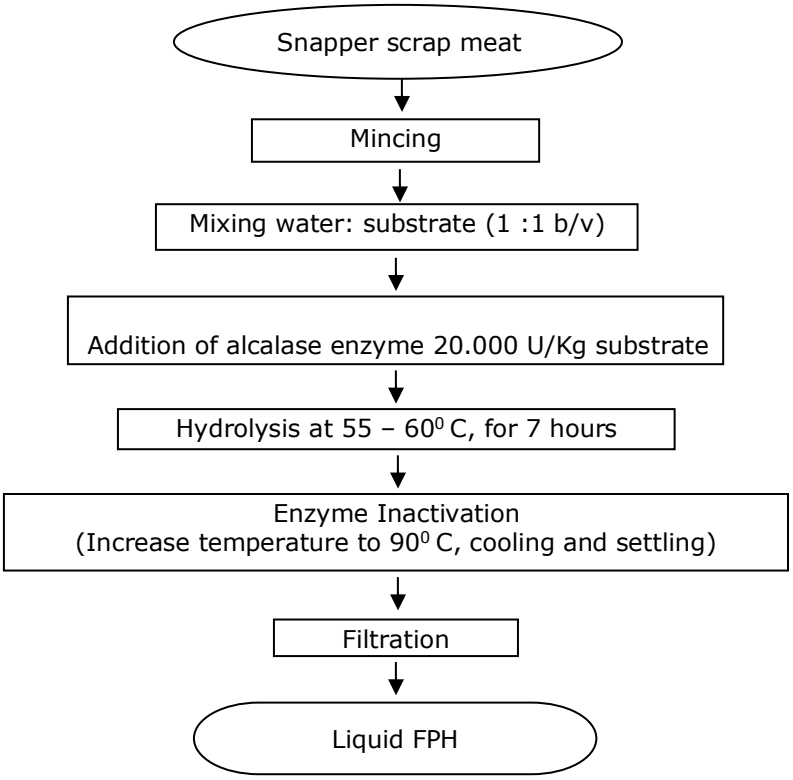

Figure 1. Martosuyono et al (2019) process of making liquid FPH, adapted to present study.

DH measurement as a process control. Measurement of the degree of hydrolysis (DH) is based on Auwal et al (2017) method adapted to the present study. Samples were divided into two types, namely the addition of 6.25 % TCA (trichloroacetic acid, which is a very effective protein-precipitating agent) and without the addition of TCA as a control sample used as a comparison. The sample with added TCA was incubated for 15 minutes and centrifuged at 8.000 x g (unit of relative centrifugal force) for 15 minutes, and the formed filtrate was used for testing. A total of 20 μL of sample was added with 150 μL of OPA solution, the mixture was homogenized using vortex and then analyzed using a spectrophotometer with a wavelenght of light 340 nm. DH value can be calculated by formula :

$$DH\ \% = \frac{\text{Dissolved nitrogen in TCA 6.25\%}}{\text{Total nitrogen in sample}} \text{x } 100\%$$

Calculation of yield. Yield is one of the parameters of succes of enzyme activity during the hydrolysis process. The results are calculated by comparison of the weight of the liquid protein hydrolysate product of fish with the weight of the raw material plus the water solvent.

Proximate analysis. The analysis was carried out in the chemical laboratory of Indonesian Research and Development Center for Marine and Fisheries Product Processing and Biotechnology. The test sample used was snapper scrap meat and products consisting of FPH filtrate and residue. The water content was analyzed by drying the sample at 105° C for 24 hours according to the Association of Officiating Analytical Chemists protocol (AOAC 2005). Ash content was analyzed by drying the sample at 600^0 C for 6 hours based on the protocol from AOAC (2005). The fat content was analyzed using the Soxhlet method based on AOAC (2005) protocol by extracting the sample for 4 - 6 hours, then heating it further in an oven at 60° C for 24 hours. Proteins were analyzed by destruction, distillation, and titration-based methods (AOAC 2005).

Amino acids analysis. Amino acid composition was determined at the Laboratory of Testing, Calibration and Certification Services Unit, Bogor Agricultural University. The sample used was snapper scrap meat and the products consisting of FPH filtrate and residue. Samples were analyzed using IK.LP-04.7-LT-1.0 (HPLC), the internal method of the Testing, Calibration and Certification Services Laboratory Unit, Bogor Agricultural University. Amino acid analysis using HPLC consists of 4 steps, that are making protein hydrolyzate, drying, derivatization, injection and amino acid analysis. Drying the hydrolysate sample used a rotary evaporator for 15-30 minutes to convert cysteine to cystine then added 5 mL of 0.01 N HCl filtered by milipore paper. The derivatization step is mixing 30 µl of the derivatization solution into the drying product, then filtered with Whatman paper. In the injection step, 5 µL of the filter results were injected into the HPLC. The separation of the amino acids takes about 25 minutes. Calculation of the concentration of the amino acid is carried out by making a standard chromatogram using ready-to-use amino acids which passed the same treatment as the sample.

Statistical analysis. Degree of hydrolysis measurements were carried out with two replications and the proximate content analysis, residue amount and FPH filtrate quantity determination were carried out with three replications. Data is calculated to find the average value and standard deviation. DH and proximate measurement data were analyzed by ANOVA Test and continued with Duncan Multiple Range Testing (DMRT) using the software SPSS v.25 X86-X64 from IBM.

Results and Discussion

FPH production process. A total of 32 kg of snapper scrap meat was minced using a meat-bone separator and a food processor so that the raw material becomes smoother and separates scales, veins and skin that are not needed in the hydrolysis process. The purpose of grinding raw materials is to facilitate the hydrolysis process, the basic principle is that proteins cell walls are destroyed, so that the protein contained in cells becomes more easily broken down by enzymes in the hydrolysis process. This is in accordance with the statement from Azhar (2016) about how mechanical movements such as grinding, beating and shaking can damage the weak interactions of peptide bonds that maintain the shape of proteins.

Minced snapper scrap meat is put into a 70 liters hydrolysis tank that has been filled with water to a volume of 32 liters, heated to a temperature of 55^0 C and stirred at a rate of 70 rpm. The hydrolysis tank unit is equipped with a temperature reader and a mixer which is operated by an electric motor. In the hydrolysis process water facilitates the stirring and homogenization between enzymes and available substrates, thereby affecting the rate of enzymatic reaction. The amount of water added will provide the appropriate level of protein breakdown under optimal conditions and eliminates additional costs for the product drying process (Petrova et al 2018). Research conducted by Himonides et al (2011)

also explained that water added to the hydrolysis process provides a good protein yield. Production costs are determined by the amount of residue deposition from hydrolysis, because a large amount of residue will result in low protein yields and expensive production costs. So, reducing the cost of drying is the right way to reduce the costs in the production stage.

After the temperature reaches 55° C, the alcalase enzyme is mixed into the hydrolysis tank and the hydrolysis process continues at a temperature of 55° – 60° C for 7 hours. Determining the optimum conditions for the hydrolysis process in this study refers to the modified method of Fawzya et al (2017). According to him, the hydrolysis process will produce a product with a relatively higher protein content when using the protease enzyme produced from *Bacillus licheniformis*. The concentration of addition of the enzyme was 20,000 U/kg of the substrate, and the hydrolysis process at 55° C for 6 hours could produce a DH value of 58.60%. The hydrolysis process at a temperature of 55° – 60° C for 7 hours is a condition where the reaction rate has reached the maximum limit, meaning that the optimal performance of the enzyme at that temperature and time has weakened because all substrates have undergone a process of degradation. The addition of the optimum enzyme concentration is 20,000 U/kg (Fawzya et al 2017), because excess enzyme concentration will cause saturation of the substrate and make the enzyme work not optimal. The addition of high enzyme concentrations will increase the DH value faster, but at certain hours the hydrolysis process will slow down because it enters the stationary phase and the substrate has become saturated (Fawzya et al 2017).

After 7 hours, the inactivation process is carried out by raising the temperature to 90° C. This aims to stop the enzyme activity and the hydrolysis process. If the optimum time of the hydrolysis process has been reached, the performance of the enzyme has begun to weaken and does not have a major effect on the dissolved protein content because all substrates have reacted during the hydrolysis process (Intarasirisawat et al 2014). The mixture of hydrolysate products is then left overnight to reach room temperature before filtering the next day. In the process of cooling and settling, three layers will form which must be separated before filtering. The first layer on the surface is a layer of fat that is separated from the hydrolysate product, the second layer is a liquid fraction called a protein hydrolysate mixture which will be filtered and used in the next research step. The last layer is a precipitate, which is a residue of the hydrolysis process that cannot dissolve and decompose made from bones, skin, and scales.

Separation of the fish protein hydrolysate mixture from the insoluble material was carried out using gradual filtration. The first step is to filter the mixture using a spinner with 300 pores per inch and 600 pores per inch filtration bags to separate bones, skin, scales, and rough residues. The next step is to filter the mixture using a micro and ultrafiltration machine equipped with a membrane with the pore size of 0.5 and 0.1 μm. At the end of this process a yellowish clear filtrate called liquid fish protein hydrolysate is produced. In addition to the hydrolysis process that produces amino acids and simple peptides, gradual filtration is an optimization of the hydrolysate yield by fractionating fish protein hydrolysate products to isolate and enrich peptide fractions with molecular weights (MW) measuring 1 - 4 kDa and to improve their functional properties (Suwal et al 2018). Through the diafiltration step on micro membranes and ultrafiltration, amino acids of a certain size in a solution undergo a process of transfer or separation to produce a pure solution with a peptide fraction size of 1 - 4 kDa (kilodalton) (Abejon et al 2018).

DH measurement as a process control. Kurozawa et al (2011) state that good FPH quality is influenced by controlling the production process so that the best enzyme activity can be fulfilled. The optimal conditions in the hydrolysis process can be known from the DH value which can be influenced by several variables such as addition of substrate, enzyme concentration, temperature, pH, and hydrolysis time. The optimum hydrolysis time is indicated by the highest DH value. DH value of FPH snapper scrap meat can be seen in Figure 2 below.

Figure 2 shows that the DH value from the 0^{th} hour to the 7^{th} hour has increased from 36.03% to 71.88%. So, if the hydrolysis time is more than 7 hours, it will show that the DH value is not significantly different. DH values have rose due to an increase in the

number of peptides and amino acids dissolved in TCA, caused by breaking of peptide bonds during the hydrolysis process (Tejpal et al 2017). Based on the results of the analysis of variance on the DH value of FPH snapper scrap meat obtained P-value < 0.05, thus at the alpha level = 0.05 hypothesis H_{zero} is rejected so it can be concluded that there is a very significant difference between DH measurements at 0 - 7 hours against DH value. Duncan's test results can be seen from the difference in the letters on the Figure 2. DH values at the 6th (71.24%) and 7th hours (71.88%) showed no significant difference. Thus, the hydrolysis time has reached its optimum point at 6 hours and the hydrolysis process at 7 hours onwards will only cause inefficiencies that can add to the process costs. The results of measurement of DH values in this study were confirmed by the results of Martosuyono et al (2019) which mentions the level of hydrolysis of fish protein on a maximum pilot pant scale after 6 hours and will enter a stationary phase thereafter.

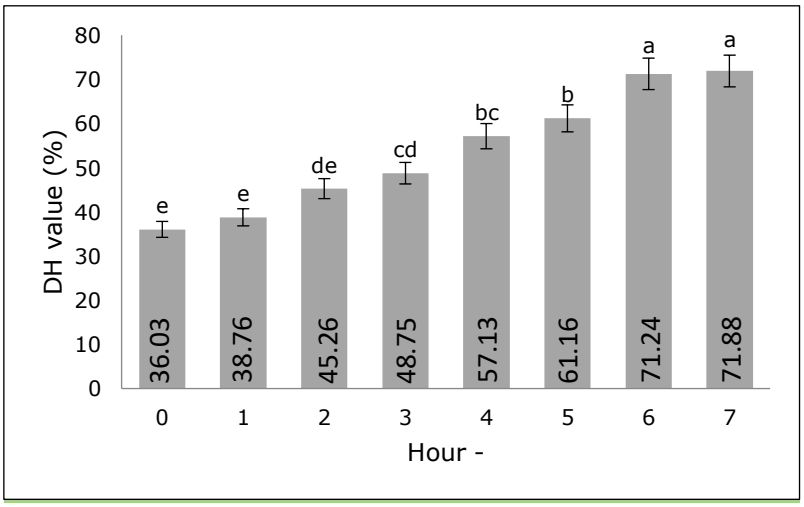

Figure 2. DH value of FPH snapper scrap meat at 55^0 C, for 7 hours determined by OPA method (Auwal et al 2017).

Abraha et al (2017) states that differences in hydrolysis results can be caused by differences in substrate type, type and concentration of enzymes, temperature, and time of hydrolysis. The difference in hydrolysis time can produce different sizes of peptides and free amino acids which can be determined from the percentage of DH value. The DH value in this study was higher than the results of research by Roslan et al (2014) which used the by-product of tilapia (*Oreochromis niloticus*) fillets as raw material. Different protein content is caused by the type of raw material, snapper fish has a higher protein content than the by-product of tilapia (*Oreochromis niloticus*) fillet processing. This statement was confirmed by Gajanan et al (2016) using yellowstripe fish (*Selaroides leptolepis*) and Vieira et al (2017) using sardines (*Sardinella lemuru*), all of which are seawater species. The different types of enzymes used also affect the optimum performance of enzymes at certain concentrations (Villamil et al 2017) and the required hydrolysis time (Intarasirisawat et al 2014).

Calculation of yield. The yield calculation is done to find out the efficiency in the process of making FPH. Table 1 shows the yield of FPH produced from the filtration process which is 89.30%. The yield value in this study is different from previous studies conducted by Prihanto et al (2019) concerning FPH from the heads of parrotfish hydrolyzed for 24 hours incubation resulted in a yield of FPH of 49.04 %, Himonides et al (2011) about the production of FPH on a pilot plant scale that was hydrolyzed for 1 hour using a temperature of 40^o C, the concentration of the papain enzyme was 0.5 % which resulted in a yield of 70 % liquid FPH, as well as Martosuyono et al (2019) research on FPH production on a pilot plant scale at a temperature of 55^o C, an enzyme concentration of 20.000 U/kg resulted in a liquid FPH yield of 77.77%. The research shows that the yield of FPH can also be influenced by the type of substrate, the ratio of raw materials and solvents, hydrolysis time

and temperature, as well as the concentration and type of enzyme. In the hydrolysis process using enzymes, the substrate used will be converted into hydrolysate products. Protein hydrolysis involves adding water so that the amount of water in the process is greater than the amount of substrate used. The use of water is also able to expand the contact area between the enzyme and the substrate, so that over a certain period a greater hydrolysate product can be produced. Dissolution of nutritional components such as fats, proteins and minerals during the hydrolysis process affects the yield of the hydrolysate produced.

Table 1

Mass balance from enzymatic hydrolysis of FPH snapper scrap meat

Material	*Quantity*
Frozen raw material	32 kg
Smooth raw material	30 kg
Water	30 L
Alcalase enzyme	1.2 L
Liquid FPH	60 L
Spinner residue	Bone and scale 0.8 Kg
Nano and ultrafiltration residue	Unhydrolyzed meat 5.2 Kg
FPH from filtration	53,58 Kg

Proximate analysis of raw materials and FPH products. Proximate content analysis such as protein, fat and water content are often needed to ensure that they meet regulatory requirements regarding food ingredients and commercial specifications. In this study, the analysis of the proximate content was carried out on raw materials, residues and FPH. The results of the analysis of the proximate content can be seen in Table 2.

Based on the results of the analysis of variance in the proximate content obtained P value (P-value) < 0.05, thus at alpha = 0.05 hypothesis H_{zero} is rejected so that it can be concluded that there is a very significant difference between the raw material, residue and FPH regarding the protein content, water, ash and fat. Duncan's further test results explained that the raw materials, residues and FPH significantly affected the composition of the protein, fat, water, and ash. FPH has a protein content that is significantly different from raw materials and residues, FPH has a water content that is significantly different from residues and raw materials, and residues have fat content that is significantly different than raw materials and FPH. Information on the results of Duncan's test can be seen from the difference in the letters on the Table 2.

The water content of the FPH has increased due to the hydrolysis process, the optimum processing time converts protein compounds into simpler compounds and are soluble in water so that the impact increases the liquid volume which ultimately increases the water content of the product. In this study, the water content of the raw material from snapper is higher than the results of Prihanto et al (2019) which used raw material from parrotfish (71.68%), Roslan et al (2014) used tilapia fish with a water content of 66.57%, Srikanya et al (2018) which used tilapia with a water content of 66.29%, but lower than the study of Abraha et al (2017) which used manyung fish with a water content of 79.15%. Differences in water content of fish raw materials can be caused by differences in species or they can even occur within the same species. According to research conducted by Jim et al (2017) the composition of nutrients in fish can be determined from differences in geographical, environmental and changes in biological conditions in fish. This statement was also reinforced by the results of research conducted by Zaman et al (2014) that the water content of freshwater fish is slightly lower when compared to fish originating from the sea, the composition of water content in freshwater fish ranges from 69 - 79%, while fish caught in seawater ranges between 68 - 87%. The condition of water activity in fish is also influenced by the type and activity of bonded and free water. In fish muscles, water is tightly bound to proteins in the structure of tissues, so that when they undergo

processing or handling these proteins are less able to hold all water and some of them dissolve and lose water (Dawson et al 2018). The water content in residues and FPH has a high value due to the hydrolysis process that uses water solvents so that nutritional components such as fats, proteins, and minerals are also dissolved during the hydrolysis process. The use of water is also able to expand the contact area between the enzyme and the substrate, so that in a certain period a hydrolysate product can be produced in liquid form. The water content will greatly affect the quality and shelf life of the product, so it is necessary to do the drying process. Drying can be done using freeze drying or spray drying in accordance with the desired final FPH properties (Hau et al 2018).

Table 2

Proximate content of snapper scrap meat as raw material, residue and FPH

Analysis	*A_w (%wb)*	*Ash (%db)*	*Protein*	*Fat (%db)*
Raw material	74.36 ± 0.46 ^{c}	2.69 ± 0.69 ^{b}	81.93 ± 1.39 ^{b}	8.85 ± 1.03 ^{b}
FPH	92.10 ± 0.49 ^{a}	4.30 ± 0.92 ^{a}	95.30 ± 0.73 ^{a}	4.05 ± 2.37 ^{c}
Residue	79.27 ± 0.29 ^{b}	2.60 ± 0.00 ^{bc}	81.53 ± 0.36 ^{c}	19.60 ± 0.31 ^{a}

Ash content shows the large amount of minerals contained in that material. The FPH ash content value is significantly different from the raw material and the residue. The value of ash content in FPH has the highest value of 4.30 ± 0.92% (% db) compared to raw materials and residues, this can be caused by the remaining solids in the form of scales, bones and meat are not hydrolyzed and filtered during the filtration process. Salt content in fish raw materials and enzymes during cultivation and medium fermentation using NaCl solution can increase sodium content, but the filtration stage cannot eliminate the salt molecules in FPH and its residues because the size of the salt molecules is much smaller than the pore size of the filtration membrane (Martosuyono et al 2019). However, the value of the ash content produced in this study is smaller than the results of Srikanya et al (2018) with an ash content of 11.06% (% db) on FPH products that use acidic and basic solutions in their hydrolysis process. Chemical solution was also able to cause the formation of salt compounds, thus increasing ash content in protein hydrolysate. This research resulted in a smaller ash content value because it did not use acid or alkaline solutions in the hydrolysis process.

Proteins are macromolecules that are formed from amino acids that bind to the peptide chain. The protein content of the raw material of snapper scrap meat is still having a large value, such as the content of fresh fish protein in general, ranging from 16 - 21% (Dawson et al 2018). This is because the snapper scrap meat is still a cutlet of fish meat (Ghaly et al 2013) and still contains protein derived from structural protein, sarcoplasmic protein, and connective tissue protein (Wang et al 2019). Another possible cause is the handling process in fish processing companies that follow the cold chain system so that the quality of the snapper scrap meat is still maintained. Handling in this way is done because snapper scrap meat still has economic value so that the washing, cooling, and freezing process is the best preservation process (Rostini 2013). Table 2 shows the amount of protein content of FPH has a high value, which is 95.30 ± 0.73 (% db), the increase in protein content is caused during the hydrolysis process with the conversion of insoluble proteins into soluble nitrogen compounds, which subsequently decompose in simpler compounds such as peptides, amino acids and ammonia (Ween et al 2017). Proteins break down easily at the hydrolysis stage due to preliminary treatments such as the grinding process, the process is known to aim to damage or break the cell wall of meat so that the protein contained in cells becomes more easily broken down by enzymes during the hydrolysis process. This is in accordance with the statement of Azhar (2016) about mechanical movements such as grinding, beating, and shaking can damage the weak interaction of peptide bonds that maintain the shape of proteins. Whereas the use of protease enzymes under optimum conditions will improve the performance of the enzyme function to break the peptide bond chain to produce amino acids. The increasing value of protein content in FPH means that the hydrolysis process that has been done is proven to

be able to improve the quality of fish protein content. Previous research on FPH states that the protein content ranges from 20 - 90% of the total composition of ingredients, and this is determined by the basis of calculations that use a wet or dry basis. Source and type of raw material, type and concentration of enzymes and hydrolysis time (Khantaphant et al 2011) and removal of non-hydrolyzed solid material using filtration methods are also known to affect the amount of protein content (Chalamaiah et al 2012). High protein content of FPH offers its potential as a protein supplement for human nutrition, especially for children and the elderly. Enzymatic hydrolysis products consist of small proteins or free peptides or amino acids. These products have advantages compared to protein from fresh fish raw materials in terms of digestion and allergic reactions. Small peptides and free amino acids in FPH can be digested easily and absorbed by cells in the human body. In addition, some people who have an allergic reaction after consuming protein derived from fresh fish will mediate their immune response to specific proteins in fish. This protein reacts in the human body to create allergic reactions (Kuehn et al 2014). By enzymatic hydrolysis, this protein can lose its allergic activity.

Fats contained in protein hydrolysate partly separate with proteins that are not dissolved, namely during the filtration process (Petrova et al 2018). Purbasari (2008) states that during the enzymatic hydrolysis process changes in the structure of the tissue are amazingly fast, causing a decrease in fat content. Cell membranes that are relatively resistant to damage form insoluble bubbles resulting in loss of the lipid membrane, which results in a decrease in fat content. Table 2 shows the residual fat content was significantly different compared to the raw material and FPH. FPH has the lowest fat content value which is 4.05 ± 2.37 (% db) because FPH undergoes a micro and ultrafiltration process that causes the separation of insoluble fat in water as a suspension, an effective filtering process will avoid free particles entering the membrane (Petrova et al 2018). The low-fat content in FPH can also be caused by the process of fat separation after hydrolysis, which is carried out by the precipitation method. A relatively low-fat content (<1%) is generally more stable and long-lasting when compared to hydrolysate products which have high fat content. Roslan et al (2014) stated that in several studies FPH had a fat content of <5%. FPH with a low fat content can be used as dietary food and as a supplement in making plain bread and baby food (Abraha et al 2017). The results of the FPH fat content in this study are still lower than that of Roslan et al (2014) and Srikanya et al (2018) who used tilapia with 1.83% (% wb) and 1.52% (% wb) fat content, Abraha et al (2017), who used manyung fish with a fat content of 0.54% (% wb), Amiza et al (2011) who used cobia fish with a fat content of 0.54% (% wb). Hafiluddin et al (2014) explained that the water content tends to have an inverse comparison pattern with the fat content, i.e. when the water content is high, the fat content tends to be lower. Different fish species and different fish habitats also influence fat content. Differences in fish species are internal factors, while differences in fish habitat are external factors. According to Hafiluddin et al (2014), the nutritional value of each fish will be different, which is very dependent on internal factors and external factors. Internal factors are the type or species of fish, sex, age, and reproductive phase of fish, while external factors include factors that exist in the fish's environment such as habitat, food availability, and the quality of the waters where the fish live. Fish habitat affects the chemical content in the meat such as proximate contents, amino acids, and fatty acids.

Overall, differences in nutrition in fish can be caused by differences in fish species and other species, even within the same species (Takama 1999). Nutrient composition in fish can also be determined from differences in geography, environment, changes in biological conditions in fish and season (Jim et al 2017). According to him, water quality is determined by the amount of dissolved oxygen, phosphate, nitrate, pH, and minerals in it to form the structure of meat in fish. This is caused by the way fish absorb and assimilate nutrients from components available in their habitat.

Amino acid analysis of raw materials and FPH. Amino acids that cannot be synthesized by the human body and need to be supplied from the consumed food are called essential amino acids. Meanwhile, those that can be produced by the body even though they are not obtained from food are called non-essential amino acids (Usydus et al 2009).

Analyzing the composition of amino acids aims to determine the type and amount of amino acids contained in raw materials and FPH. The quality is determined by the amino acid content of the protein breakdown in the enzymatic hydrolysis process. A good hydrolysis will produce a peptide with a smaller size with the amount of 2-20 kinds of amino acids. The composition of amino acids in protein hydrolysate is especially important because of nutrition and its effect on functional properties (Santos et al 2012). Amino acids identified in raw materials and FPH were 18 amino acids consisting of 10 essential amino acids and 8 non-essential amino acids. The ten types of essential amino acids are valine, lysine, leucine, isoleucine, threonine, tryptophan, phenylalanine, methionine, arginine, and histidine. While the eight types of non-essential amino acids are glycine, alanine, serine, tyrosine, cysteine, proline, aspartic acid, and glutamic acid. This indicates that the hydrolysis process carried out is nearing perfect. If the hydrolysis runs perfectly, a hydrolysate consisting of a mixture of 18 - 20 kinds of amino acids will be produced (Vandanjon et al 2009). Martosuyono et al (2019) added that the hydrolysis process can maintain the quality of the composition of amino acids, as an indication that the dominant amino acid content in the raw material is glutamic acid and all types of amino acids can be present in FPH.

Table 3

Amino acid composition of raw materials and FPH

Amino acid	*Snapper scrap meat (% w/w)*	*FPH (% w/w)*
Non-essential amino acids		
Aspartic acid	3.08	0.89
Tyrosine	0.96	0.12
Serine	1.14	0.17
Glutamic Acid	5.50	1.54
Proline	1.36	0.36
Glycine	2.37	0.93
Alanin	2.30	0.75
Cysteine	0.29	0.35
Essential amino acids		
Treonin	1.36	0.19
Valine	1.57	0.27
Methionine	0.97	0.14
Isoleucine	1.52	0.21
Leucine	2.60	0.45
Phenylalanine	1.26	0.20
Histidine	0.72	0.12
Lysine	2.99	0.74
Arginine	2.62	0.46
Tryptophan	0.10	0.02
Total amino acids (% w/w)	32.71	7.90

Table 3 shows that the highest non-essential amino acid content in raw materials and FPH is glutamic acid which is 5.50% and 1.54%. The highest essential amino acid content in raw materials and FPH is lysine which is 2.99% and 0.74%. Glutamic acid is the most abundant type of amino acid present in fishery products and acts as a shaper of savory and sweet flavors. Ovissipour et al (2010) stated that glutamic acid, aspartic acid, glycine, and alanine are amino acids that play a role in increasing the aroma of fishery products. Seeing these facts, snapper protein hydrolysate has a potential to be applied as a flavor enhancer product. Martosuyono et al (2019) added that glutamic acid was the most dominant amino acid in fish and in FPH products. Snapper scrap meat protein

hydrolysate also has potential to be developed as a source of essential amino acids in food products because it contains complete essential amino acids. The essential amino acid content of raw materials and liquid FPH products is lysine. Lysine is needed to optimize growth and prevent immune deficiency.

These results indicate that by looking at the amino acid content of raw materials, snapper scrap meat can be used as a raw material for making FPH. FPH contains essential amino acids that are needed by the human body because it has a high quality as a source of protein and cannot be produced by the body. FPH products can also be added to other products to enhance the nutritive capacity, bioactive content, and functional properties (Shavandi et al 2018). Among the various functional properties of proteins, solubility is one of the most influential characteristics that significantly influences other traits (Liu et al 2014). Perfect hydrolysis will produce hydrolysate consisting of a mixture of 18 - 20 kinds of amino acids with low molecular weight peptide content and a solubility with a wider pH range compared to the original protein (Taheri et al 2013). According to research on the solubility carried out by Liu et al (2014), the solubility of surimi proteins containing by-products increased from 10% to 60% after being hydrolyzed using protamex and alcalase.

Conclusion. Snapper scrap meat as raw material which is hydrolyzed under optimum conditions, temperature 55° – 60° C, enzyme concentration 20.000 U/kg, for 7 hours and using alcalase enzymes obtained 89.30% FPH yield with a protein content of 95.30% (% db) and amino acids produced amounted to 18 types, 10 types of essential amino acids and 8 types of non-essential amino acids. Snapper scrap meat and FPH contains a high number of amino acids, glutamic acid and lysine being the most dominant. Glutamic acid is the most abundant amino acid in fishery products and acts as a shaper of savory flavors and while the amino acid lysine is needed to optimize growth and deficiency which causes immune deficiency. By looking at the amino acid content of FPH, snapper scrap meat can be used in the production of FPH and their derivative products because it contains essential amino acids that are needed by the human body that has a need for protein.

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