# PRODUCTION OF FISH HYDROLYSATES PROTEIN FROM WASTE OF FISH CARP (CYPRINUS CARPIO) BY ENZYMATIC HYDROLYSIS

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## ABSTRACT

*Fish Protein Hydrolysates (FPH) is the mixed products of polypeptide, dipeptides, and amino acid. It can be produced from materials that contained of protein by acid reaction, base reaction or enzymatic hydrolysis. The objectives of this study were to study the production of FPH from fish carp meat at post rigor phase and viscera by enzymatic hydrolysis, to determine the specific activity of papain enzyme, and to determine the solubility of FPH. Capacity of fish hydrolyzing can be identified by analyzing the content of dissolved total nitrogen (NTT) compared with nitrogen total ingredient (NTB) in order to get the value of total soluble nitrogen/total nitrogen material (NTT/NTB). The hydrolysis processes were carried out in 0,26% (w/v) papain, 60 $^oC$ for 3 hours. The result showed that the specific activity of papain enzyme was about 3.28 U/mg. Solubility of FPH by comparing NTT/NTB was about 0.29% (fish meat) and 0.40% (fish viscera). Proximate test of protein content of fish meat was 18.34 ± 0.04 (g/100 g); while viscera was about 0.95±0.04 (g/100 g). The result indicated that product waste of fish carp had potential as a major of source of FPH.*

***Keywords:*** *enzymes, papain, protein, hydrolysates*

## INTRODUCTION

More than 60% by-products as waste is produced by fish processing industry which includes skin, head, viscera, trimmings, liver, frames, bones, and roes. These by-product wastes contain good amount of protein rich material that are normally processed into low market-value products, such as animal feed, fish meal and fertilizer (Chalamaiah, Kumar, Hemalatha, and Jyothirmayi, 2012).

By-product as waste also produced by processing of carp fish. Carp fish (*Cyprinus carpio*) is a freshwater fish that has been cultivated and has important economic value in world trade and has a positive effect to Indonesian economy, especially in food sector. The growth of production of carp showed a good performance with an increase in average production from 2010 -2014 year amounted to 14.44 % as well as the numerical value of production during the same period showed a pretty good improvement with the average increase per year of 18.67% (KKP, 2014). Carp fish is a source of animal protein. More fish processing industries produces about 50-60% of total fish weight as waste (Bagus, Theresia, & Herbert 2014). In view of utilizing of waste products from fish processing industries can be done for increasing the value to several under-utilized fish species such as Fish Protein Hydrolysates (FPH).

Chalamaiah *et al*. (2012) stated that FPH are breakdown products of enzymatic conversion of Fish proteins into smaller peptides, which normally contain 2–20 amino acids. Nowadays FPH have

attracted much attention of food biotechnologists due to presence of high protein content with good amino acid balance and bioactive peptides (antioxidant, antihypertensive, immunomodulatory and antimicrobial peptides).

FPH is a protein derivative which is soluble in water and does not undergo the process of coagulation in hot water (Dufossë *et al*., 2001). Protein hydrolysate is a product of a mixture of polypeptides, dipeptides, and amino acids, can be produced from materials containing protein through acid hydrolysis or enzymatic reaction. Protein hydrolysate as the main nitrogen source in a commercial medium for bacterial growth is still imported at high prices (Fachraniah *et al.*, 2002). Therefore, an alternative is needed to produce FPH from by-product as waste such viscera or fish meat at post rigor phase. The objectives of this study were to study the production of FPH from fish carp meat at post rigor phase and viscera by enzymatic hydrolysis, to determine the specific activity of papain enzyme, and to determine the solubility of FPH.

# METHODS

## Material

The raw materials were used in this study were fish carp (*Cyprinus carpio*) by-products; meat at post rigor phase and viscera. The enzyme used was commercial local crude papain enzyme. Chemical and biochemical test materials were $H_2SO_4$, HCl, KOH, NaOH, $Na_2S_2O_3$, $H_3BO_3$, asetonitril. The tools that are used, hot shaker bath (B- Braunc)-, spectrophotometer, oven (Yamato) and a set of glassware (PYREX), (DURAN), and other standard analytical equipment.

## Fish Hydrolysates Protein Procedure (Saputra and Nurhayati, 2013)

Fish carp waste (meat at post rigor phase and viscera) respectively about 100 g, were firstly minced and then mixed with water with ratio of 1:2 by w/v, the mixture was blended and homogenized to improve the hydrolysis reaction for 2-5 minutes. The papain enzyme concentration about 0,26% was added to homogenate, divided into two parts and then adjusted to pH 7 with HCl. The hydrolysis processes were carried out in 0.26% (w/v) papain, 60 $^oC$ for 3 hours, then the papain activity was stopped by increase the hot shaker bath temperature to 85 $^oC$ and stirred for 15 minutes. The end product was filtered by nylon mesh size of 150 mesh. The liquid phase is taken, then deposited at 4 $^oC$ for 24 hours to separate the fat contained in the fluid, the next stage of analysis measurements of dissolved nitrogen.

## Specific Activity of Papain (Suhandana, 2010)

Specific activity of papain can be measured by using Bergmeyer's method which has been modified by reacting 1 ml casein (2%concentrate) with HCl 0.05 mol/L and buffer phosphate 1 mol/L (pH 7.5) and 0.2 enzyme solution. Then the mixture was incubated at 37 $^oC$ for 10 minutes. The tyrosine solution was used as the standard enzyme solution and distilled water is used as a blank solution. About 2 ml TCA (0.3 mol/L) was added and also $CaCl_2$ (2 mmol/L). Then the mixture was incubated at 37 $^oC$ for 10 minutes then filter through Whatman No. 42. The filtrate about 1.5 mL was added with $Na_2CO_3$ (0.5 mol/L) about 5 ml with folin ratio (1:2) as much as 1 mL. Then it was incubated at 37 $^oC$ for 20 minutes. Absorbance of filtrates at 578 nm was read against respective blanks with a spectrophotometer. The potency of test portion in United States Pharmacopeia (USP) units of papain can be calculated by the following formula:

$$UA = \frac{Asp - Abl}{Ast - Abl} \; x\, P\, x \; \frac{1}{T} \quad (1)$$

Where:
UA : The amount of enzyme that causes changes 1 μmol substrate per minute
Asp : Sample absorbance value
Abl : Blank absorbance value
Ast : Standard absorbance value o
P : Dilution factor
T : Incubation time

## Chemical Composition Analysis of Fish Carp (Association of Official Analytical Chemyst, 1995; 2005)

The composition of fish carp was determined using proximate analysis. The moisture content was determined by spreading about 2 g of sample on an aluminum dish that has been pre-weighted. Dried in an oven at 100 $^{o}$C for 5 hours then placed in a desiccator until reaching a constant weight. Total weight loss during drying process represents moisture content in the sample. The protein content in raw material was determined by using Kjeldhal method with nitrogen factor equal to 6.25. The total lipid was determined by Soxhlet extraction. The ash content was analyzed by incineration of 2 g of sample in a furnace at 550 $^{o}$C for 5 hours or until the white ash was formed.

## Fish Protein Hydrolysate Solubility (Saputra and Nurhayati, 2013)

Capacity of fish hydrolyzing can be identified by analyzing the content of dissolved total nitrogen (NTT) compared with nitrogen total ingredient (NTB) in order to get the value of FHP solubility degree

## Statistical Analysis

The results were presented as group means ± standard of deviation (SD) and statistically significant differences between mean values were determined by Descriptive Statistics Analysis using SPSS 20.0 software.

# RESULTS AND DISCUSSIONS

## Specific Activity of Papain

Papain (EC 3.4.22.2) is an endolithic plant cysteine protease enzyme which is isolated from papaya (Carica papaya L.) latex. Papain is obtained by cutting the skin of the unripe papaya and then collecting and drying the latex which flows from the cut (Amri and Mamboya, 2012). The greener the fruit, the more active the papain. Papain enzyme belongs to the papain super family, as a proteolytic enzyme, papain is a crucial importance in many vital biological processes in all living organisms (Tsuge *et al.*, 1999).

Activity of papain can be increased by the addition of cysteine and NaCl activator. A compound that was found in papain enzyme include more than 50 amino acids including aspartic acid,

threonine, serine, glutamic acid, proline, glycine, alanine, valine, isoleucine, leucine, tyrosine, phenylalanine, histidine, lysine, arginine, and cysteine (Pakki *et al*., 2009).

Papain enzyme is used in the hydrolysis of fish carp (meat at post rigor phase and also viscera), this enzyme has the specific activity. Papain activity was affected by the concentration of enzyme that is added and also the temperature used during hydrolysis. Fish carp waste (meat and viscera) which were hydrolyzed with 0,26% papain enzyme concentration at 60 $^{o}$C temperature for 3 hours. The specific activity of papain enzyme was about 3,28 U/mg. These results were different from Dongoran (2004), specific activity of papain enzyme was about 0.279 U/mg protein. This difference may be due to the differences in pH, temperature, enzyme concentration and the materials used. Suhandana (2010) reported that the specific activity of papain enzyme to hydrolysis of viscera of swordfish was about 3.2770 U/mg. The specific activity of papain enzyme implied that there were enzyme activities in 1 mg of protein enzymes that could perform hydrolysis resulting in a change 3.2770 µmoles of substrat per minute.

## Chemical Composition Analysis of Fish Carp

Part of Carp's bodies used in the research were fish meat at post rigor and fish viscera. Fish meat and viscera of fish carp has a different chemical composition. It was because the chemical composition of fish can be varied between species, individuals in one species and also across parts of the body of one individual. As for the comparison of chemical composition of meat and viscera fish, carp is presented in Figure 1 and 2.

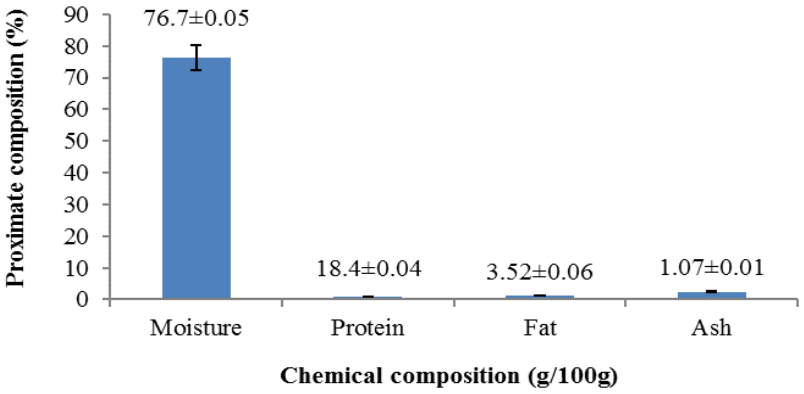


Figure 1 Chemical Composition of Fish Meat

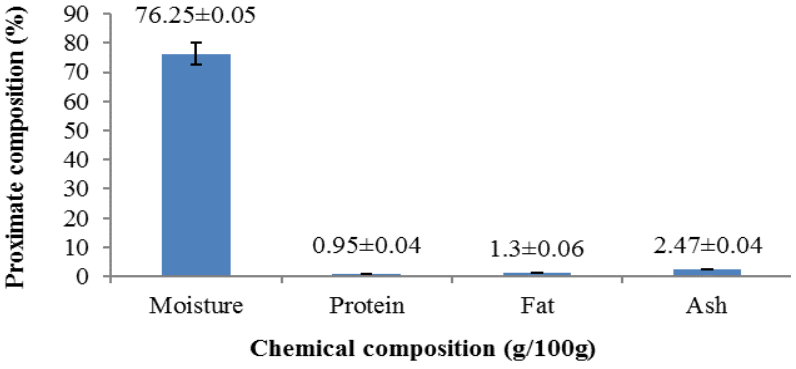


Figure 2 Chemical Composition of Fish Viscera

Based on Figure 1 and 2, it can be seen that the analysis of raw material (fish meat and viscera) was conducted to identify the chemical composition of fish carp waste including moisture, ash, fat and protein content. Results of chemical analysis of fish carp is shown in Figure 1 and 2 implies that protein content of the fish viscera was lower than protein content of meat of fish carp. The protein content of fisch carp meat in this result was higher than *Pangasius* sp fillet waste used by Bagus *et al.,* (2014); Chaijana *et al.* (2010) for their study reported that the protein content was about 12.51±10.5% and 16.88±4.45% (giant catfish muscle), while viscera protein content was lower than fish meat content of fish carp in this study. The fat content of meat and viscera of fish carp was much lower than *Pangasius* sp fillet waste used by Bagus *et al.*, (2014) was about 18.00±1.00%. The chemical composition of the fish was affected by species, age, sex, fishing season, the availability of feed in the water, habitat and environmental conditions. Protein content in the fish meat is relatively constant, but the ash content and fat content fluctuates (Bhaskar *et al*., 2008). The greater fat content in fish meat, the smaller the water content on meat fish. This can be seen from the result of this study that fat content on meat fish was about 3.52±0.06 g/100g with the protein content of the fish meat was about 18.40±0.04 g/100g. In addition, it can be seen that the composition of fat content of fish viscera was about 1.30±0.06 g/100g, with protein content 0.95±0.04 g/100g. The composition of fat and protein levels in fish viscera is smaller than the composition of the fat content in fish meat of fish carp. Similar results were also reported by Suhandana (2010), which states that the protein content was higher about 50.18 g/100g with a fat content was 0.47 g/100g.

## Fish Protein Hydrolysate Solubility

At the time the fish is entering the post rigor phase, amino acids and free nitrogen will be increased as a result of hydrolysis of fish meat. Hydrolysis of fish meat and viscera is strongly influenced by the ability of protease enzymes such as papain. Burges and Shaw (1986) in Sari (2008) reported that papain enzyme can break the peptide bond in the residue asparagine-glutamine, glutamate-alanine, valine and leucine-phenylalanine-tyrosine. The ability of papain enzyme to hydrolysis fish meat and fish viscera can be seen through the test of total dissolved nitrogen content (NTT) and compared with a total nitrogen content of materials (NTB) to obtain the value of total dissolved nitrogen/total nitrogen materials known as (NTT/NTB) shown in Table 1.

Table 1 Solubility of FPH from Fish Meat and Viscera of Fish Carp

| Material | Sample weigh (mg) | Vol. HCl (ml) | Protein (%) | NTT (%) | NTB (%) | NTT/NTB |
|---|---|---|---|---|---|---|
| Fish meat | 1024 | 6.43 | 5.2032 | 0.8325 | 2.88 | 0.29 |
| Fish viscera | 9910 | 4.10 | 3.4282 | 0.5485 | 1.36 | 0.40 |

The value of NTT/NTB on fish viscera was about 0.40 and NTT/NTB from fish meat was about 0.29. It is not much different from the value of NTT/NTB that reported by Saputra (2008); Saputra and Nurhayati (2013) on yellow stripe fish meat of that ranged from 0.21 to 0.34. The value of NTT/NTB on fish viscera is greater than the value of NTT/NTB on fish meat. This is because many fish viscera contain proteolytic enzymes that can be degradable of substrates, which are proteins. High concentration of enzymes can increase the activity of this enzyme so that the results of hydrolysis in the form of amino acids and dissolved peptides also become much higher.

Saputra (2008); Saputra & Nurhayati (2013), reported that the higher value of NTT/NTB is produced is influenced by the activity of enzyme, the concentration enzyme was added, then the specific activity of the enzyme to catalyze the protein and break down the bond will be faster, so it will be increase the amount of amino acids and polypeptides are dissolved. Safari *et al*. (2009) also reported that the head of the hydrolysis using alkalase enzyme for tuna can produce hte total nitrogen

was about 12.84% where the value is not much different from the amount of total nitrogen obtained in the hydrolysis of meat and viscera carp ranged in 5.4 to 8.3%.

Fish in the post-rigor condition also affects the value of NTT/NTB. The more rotten the fish, the value of NTT/NTB will increase along with the higher number of amino acids and dissolved nitrogen as a result of hydrolysis of proteins. It can be used as an indicator that the higher NTT/NTB value, indicates that the fish is in post rigor phase. Perfect hydrolysis process will produce about 18-20 amino acids (Ariyani *et al.,* 2003).

An enzyme used in hydrolyzes of meat and fish viscera also affects hydrolysates of meat and viscera. Each enzyme has a different optimum values that affect the resulting hydrolysate. The composition of the protein hydrolysate is affected by the type of enzyme used (Shahidi, Xiao-Qing, & Synowiecki, 1995). In addition, the enzyme substrate used also affects the protein hydrolysate. Kahar (1995) stated that the enzyme is ampholytic with different substrate structure resulting enzyme substrate complex ionization adaptation to different environments. FPH is a product that produced from the decomposition of fish protein into short-chain compounds for their good hydrolysis by enzymes, acids and bases.

## CONCLUSIONS

Fish Protein Hydrolysate (FPH) can be produced from the waste of fishery products such as meat at post rigor phase and viscera. Hydrolysis process of fish carp was carried out using enzymatic methods with papain concentration was about 0.26 % (w/v) for 3 hours at 60 °C, and the inactivation of papain activity can be carried out at 85 $^{o}$C. The result showed that the specific activity of papain enzyme was about 3.28 U/mg. Chemical composition of fish carp was conducted by proximate analysis including protein and fat content analysis, result showed that 18.34 ± 0.04 (g/100g) (protein content); 0.95 ± 0.04 (g/100 g) (fat content). Based on the results, meat of fish carp in post rigor phase and also viscera were potential as a major of source of FPH. Solubility of FPH by comparing NTT/NTB was about 0.29% (fish meat) and 0.40% (fish viscera).

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