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# Effect of temperature and long storage against profile amino acid and value protein carbonyls fish snapper (*lutjanus sp*)

# Effect of temperature and long storage against profile amino acid and value protein carbonyls fish snapper (*lutjanus sp*)

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**Abstract**. Fish protein has high economic value to the fishing industry to improve the stability and function of fish products. This study aims to determine the effect of temperature and duration of storage of the protein amino acids snapper (Lutjanus sp) and damages resulting from the storage process. The results of the analysis of amino acid protein snapper (Lutjanus sp) for storage of 0 °C - 40 °C, respectively amino acid aspartate, glutamate, serine, glycine, alanine, histidine, arginine, tyrosine, methionine, valine, phenylalanine, isoleucine, leucine, and threonine amino acid with a total number of 88.21%, 69.00%, 67.70%, and 61.20%. Value carbonyl increases with the increase in temperature of 0°C - 40 °C by 5.2 times mol/mg/protein snapper.

## 1. Introduction

Fish protein has high economic value to the fishing industry to improve the stability and function of fish products. Fish protein in the form of concentrate has been used as a food supplement for low protein malnutrition group [1,2]. Protein is also a component of the most important food fish. The basic unit of protein is an amino acid that is produced when protein is hydrolyzed using acid, alkalis or enzymes.

Processing by freezing the fish has been used for thousands of years because of the quality and high product. The concept of storage by freezing depends on the product temperature decrease to slow decay so that when the fish melted, freshness can be maintained. However, fish and fishery products may undergo undesirable changes during storage and can damage the storage time limit.

The profile amino acid is hung off the characteristics that differ between species. There is always a free amino acid present in fish flesh and blood, resulting in the transport process, anabolism, and catabolism. This study aims to determine the effect of temperature and duration of storage of the protein amino acids snapper (*Lutjanus sp*) and damages resulting from the storage process.

## 2. Methods

### *2.1. Raw material*

Raw materials snapper (*Lutjanus sp*) obtained from the fish auction place (TPI) kobong, Village Kaligawe, Semarang, Central Java.

*2.2. Fish protein sample preparation*

500 g of meat snapper (*Lutjanus sp*) was homogenized with cold water as much as 3.5 liters. After it was added HCl for extracting the amount of protein contained in the fish meat. Fish meat that has been homogenized by HCl at pH 2.5 and then centrifuged (phase one) at 4 ° C at a speed of 4000 rpm. Furthermore, the precipitate obtained. The precipitate was then given NaOH until the pH reached 5.5 isoelectric points. In this isoelectric pH, then centrifuged (stage two) to obtain a second precipitate. After a second precipitate obtained, the sediment in the freeze dryer for three days to get the fish protein or fish protein concentrate.

*2.3. Proximate analysis*

Chemical analysis: Proximate moisture content, ash content, protein, and fat by AOAC.

*2.4. Amino acid analysis procedures*

Fish protein extract samples of 0.06 g were added 4 ml of 6 N HCl and blended for 2 minutes. Do neutralization with 1 N NaOH solution to pH 7. centrifugation at 20000g for 20 minutes at a temperature 4°C and filtered. From the filtrate taken 50 microliters and mixed with 250 microliters of OPA, stirring for 1 minute.

Taken 20 micro-liters of the mixture and injected into the HPLC. Specifications HPLC: Shimadzu LC Type 10 Column: Licrospher 100RP 18 (5µm). 125 ml column length x 4mm. Mobile phase: A = CH 3 OH: 50 mM Na-acetate: THF (2: 96: 2) pH 6.8. B = 65% CH 3 OH. Flow rate: 1 ml / min. Detector: Fluorescent Shimadzu RF-138. EX Wavelength: 360 EM Wavelength: 460

## 3. Results and discussion

*3.1. Proximate protein fish*

Based on table 1, protein levels snapper (*Lutjanus sp*) in this study was (18.77%; BB) than reported by Gooch et al. (1987) respectively 20.45%, 19.7%, and 19.30% [3]. Crude protein content in fresh fish (18.77%; B) and on the fish protein (88.92%; BK), it indicates that 95.83% of fish protein is in pure protein fractions (isolates).

**Table 1**. Proximate, protein fish and Fe content of fish snapper (*Lutjanus sp*)*.

| Parameter | Fresh fish | | Fish Proteins | Gooch e.l., (1987) [3] |
|---|---|---|---|---|
| | Content (BB) | Content (BK) | | |
| Moisture (%; BB) | 78.39 | - | 7.20 | 78.9 |
| Ash (%; BK) | 1.58 | 4.11 | 1.46 | 1.1 |
| Protein (%; BK) | 18.77 | 88.92 | 89.1 | 19.70 |
| Fat (%; BK) | 1.95 | 4.81 | 0.9 | 1.10 |
| Carbohydrates *by difference* (%; BK) | 0.30 | 2.16 | 0 | 0.10 |
| Fe (ppm) | 121.47 | - | 108.95 | - |

* Description: Data is derived from repeat

The freshness of the fish strongly influenced differences in protein content of fish is used as a critical factor in result isolates, methods of isolation/extraction is used, homogenization of meat processing, the ratio of fish and solvents (viscosity) used, length of descent, time and temperature processing and dissolution protein [4].

Table 1 shows Fe containing fish protein as much as 89.69% of total fresh fish meat Fe 121.47 ppm). The [5] illustrates the color of meat because the Fe content in meat is very high because it is rich in hemoprotein (80%), especially myoglobin and hemoglobin. Based on [5] the content of hemoprotein snapper meat (Lutjanus sp) is low, so the meat is white. It was assumed [5] that white

snapper meat is not susceptible to oxidation caused by small prooxidants, although Fe in fish protein is still high (89.96% of total Fe fish).

### *3.2. Protein amino acid composition of fish snapper (Lutjanus sp)*

**Table 2.** Composition amino acid protein snapper (*Lutjanus sp*) during storage.

| | During storage /day | | | | |
|---|---|---|---|---|---|
| Amino acid profile (%) | 0 °C/90 | 10 °C/45 | 20 °C/27 | 30 °C/18 | 40 °C/9 |
| Asam aspartate (Asp) | 21.96±0.20 | 5.09±0.03 | 7.24±0.20 | 6.55±0.15 | 4.00±0.05 |
| Asam glutamat (Glu) | 29.74±0.25 | 11.45±0.20 | 14.3±0.25 | 12.59±0.20 | 7.12±0.10 |
| Serin (S) | 2.17±0.03 | 3.09±0.03 | 3.26±0.02 | 3.02±0.04 | 2.59±0.03 |
| Glisin (Gly) | 3.08±0.04 | 3.70±0.04 | 3.46±0.03 | 2.89±0.03 | 2.91±0.02 |
| Alanin (Ala) | 4.65±0.09 | 5.58±0.05 | 5.68±0.08 | 5.80±0.10 | 4.69±0.09 |
| Histidin (His) | 1.54±0.01 | 1.50±0.01 | 1.34±0.01 | 1.62±0.01 | 8.22±0.10 |
| Arginin (Arg) | 4.72±0.09 | 6.42±0.08 | 7.09±0.08 | 6.73±0.10 | 5.67±0.06 |
| Tirosin (Tyr) | 1.40±0.01 | 4.32±0.10 | 4.42±0.08 | 4.27±0.06 | 3.55±0.03 |
| Metionin (Met) | 1.00±0.01 | 1.62±0.03 | 0.99±0.01 | 0.73±0.01 | 0.79±0.01 |
| Valin (Val) | 1.41±0.01 | 2.73±0.05 | 3.29±0.04 | 2.93±0.02 | 2.51± |
| Phenylalanine (Phe) | 1.25±0.01 | 2.34±0.05 | 2.88±0.03 | 2.83±0.02 | 2.04±0.01 |
| Isoleusin (Ile) | 1.33±0.01 | 2.64±0.06 | 3.40±0.04 | 3.05±0.03 | 2.46±0.01 |
| Leusin (Leu) | 6.47±0.08 | 5.84±0.10 | 6.84±0.10 | 6.29±0.10 | 5.21±0.10 |
| Lisin (Lys) | 7.45±0.10 | 21.33±0.20 | 6.29±0.10 | 5.73±0.09 | 6.83±0.10 |
| Treonin (Thr) | 0.09±0.01 | 2.88±0.03 | 3.25±0.04 | 3.14±0.05 | 2.60±0.03 |

According to table 2, the percentage of amino acids decreased respectively, i.e., aspartic acid, glutamic acid, glycine, methionine, leucine, and lysine during the storage of it is due to delamination of the amino acids contained in fish protein. At 40 °C storage and also increased the percentage of each amino acid that is a serine, alanine, histidine, arginine, tyrosine, valine, phenylalanine, isoleucine, and threonine. The increase in the percentage of amino acids during storage due to hydrophobic amino acids when the protein fish are kept in controlling the temperature or atmosphere.

Table 2 also seen that the protein snapper (*Lutjanus sp*) contains amino acids that are susceptible to oxidative damage such as histidine, alanine, tyrosine, methionine, valine, and phenylalanine. The fish protein was containing 18 amino acids, both essential and non-essential [6,7]. Amino acids are aspartic acid, glutamate acid, serine, histidine, arginine, glycine, threonine, alanine, tyrosine, tryptophan, cysteine, methionine, valine, phenylalanine, isoleucine, leucine, lysine, proline [8].

Based on the analysis performed in this study, only identified 15 amino acids, while three amino acid tryptophan, cysteine , and proline, were not identified due to the tryptophan will be damaged if done with the amino acid hydrolysis using acid (HCl). Cysteine-rich electrons are susceptible to oxidative damage by the attack of radical and non-radical oxidants.

### *3.3. Effect of temperature and time on fish protein carbonyls value*

Protein carbonyls are one of the 'biomarker' oxidation proteins and used as one indicator.

**Table 3.** Data analysis of protein carbonyls number snapper (*Lutjanus sp*) during storage.

| Temperature 0 °C | |
|---|---|
| Storage time (day) | Average (nmol/mg proteins) |
| 0 | 1.55±0.07 |
| 10 | 2.37±0.08 |
| 20 | 3.52±0.08 |
| 30 | 4.08±0.09 |
| 40 | 4.83±0.09 |
| 50 | 5.27±0.10 |
| 60 | 6.80±0.10 |
| 70 | 7.22±0.12 |
| 80 | 7.69±0.12 |
| 90 | 8.10±0.12 |
| Temperature 10 °C | |
| Storage time (day) | Average (nmol/mg proteins) |
| 0 | 1.55±0.07 |
| 5 | 2.52±0.08 |
| 10 | 3.74±0.08 |
| 15 | 4.76±0.10 |
| 20 | 5.34±0.10 |
| 25 | 6.79±0.15 |
| 30 | 7.24±0.15 |
| 35 | 8.48±0.18 |
| 40 | 9.08±0.18 |
| 45 | 9.84±0.20 |
| Temperature 20 °C | |
| Storage time (day) | Average (nmol/mg proteins) |
| 0 | 1.55±0.07 |
| 3 | 2.62±0.08 |
| 6 | 3.71±0.08 |
| 9 | 4.20±0.09 |
| 12 | 6.77±0.10 |
| 15 | 7.67±0.10 |
| 18 | 8.22±0.14 |
| 21 | 8.89±0.15 |
| 24 | 9.69±0.16 |
| 27 | 10.32±0.20 |
| Temperature 30 °C | |
| Storage time (day) | Average (nmol/mg proteins) |
| 0 | 1.55±0.07 |
| 2 | 3.00±0.09 |
| 4 | 3.04±0.10 |
| 6 | 3.88±0.11 |
| 8 | 4.65±0.15 |
| 10 | 5.74±0.15 |
| 12 | 5.93±0.18 |
| 14 | 7.12±0.18 |
| 16 | 7.66±0.20 |
| 18 | 10.93±0.20 |
| Temperature 40 °C | |

| Storage time (day) | Average (nmol/mg proteins) |
|---|---|
| 0 | 1.55±0.07 |
| 1 | 3.20±0.08 |
| 2 | 4.19±0.09 |
| 3 | 4.71±0.10 |
| 4 | 5.87±0.12 |
| 5 | 6.25±0.13 |
| 6 | 7.21±0.16 |
| 7 | 10.37±0.17 |
| 8 | 11.55±0.20 |
| 9 | 13.85±0.21 |

Value carbonyl increased 4.3 times at a temperature of 0 °C, while at 10, 20, 30, and 40 °C carbonyl numbers increased respectively to 4.8, 5.0, 7.2, and 9.2 times nmol/mg samples. Increased protein resulting in protein carbonyl snapper fish in this study naturally still contains iron (Fe) that can lead to oxidative damage.

Although assuming the levels of fatty acids in the protein isolate is very low so that it can be ignored. lipid oxidation catalyzed by natural Fe can still occur. The treatment temperature of 20-40 °C (heat radiation) increases the oxidation damage. This was evident in the results of this research primarily on storage temperature of 30 °C and 40 °C.

## 4. Conclusion

Protein is also a component of the most important food fish. The basic unit of protein is an amino acid that is produced when protein is hydrolyzed using acid, alkalis, or enzymes. Peptide relationship into polymers to produce peptides and proteins linking amino acid components. The percentage of amino acids decreased, respectively, i.e., aspartic acid, glutamic acid, glycine, methionine, leucine, and lysine. This is due to the deamination of the amino acids contained in fish protein.

The increase in the percentage of amino acids during storage is due to hydrophobic amino acids when fish protein is received. Figures carbonyl increased 4.3 times at a temperature of 0 °C, while at 10, 20, 30, and 40° C carbonyl numbers increased respectively to 4.8, 5.0, 7.2, and 9.2 times nmol/mg samples. Increased protein resulting in protein carbonyl snapper fish in this study naturally still contains iron (Fe) that can lead to oxidative damage.

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