

Protein and fatty acid profile of marine fishes from Java Sea, Indonesia

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Abstract. Priatni S, Ratnaningrum D, Kosasih W, Sriendah E, Srikandace Y, Rosmalina T, Pudjiraharti S. 2018. Protein and fatty acid profile of marine fishes from Java Sea, Indonesia. *Biodiversitas* 19: 1737-1742. Indonesia is the second largest producer of capture fisheries products in the world and the most capture fisheries production comes from marine fisheries. Marine fish is a source of protein, amino acid, saturated and unsaturated fatty acids, which are important components of diet. The objective of the study was to investigate the protein and fatty acids profile of nine marine fish samples from Java Sea of Indramayu West Java, Indonesia. The analysis data showed that the total protein content of fish samples ranged from 61.07% (*Pampus argenteus*) to 86.56% (Tetraodontidae). Meanwhile, total lipid content of fish samples ranged from 1.73% (Tetraodontidae) to 9.82% (*Leiognathus equulus*). The concentration of α -Amino Nitrogen (AN) of fish protein hydrolysate was ranging from 31 mM (*Nemipterus hexodon*) to 69 mM (*Mystacoleucus padangensis*) and % Degree of Hydrolysis (DH) was ranging from 9.33% to 20.39%. The molecular weight of protein fish samples had similar profiles primarily for almost all samples, which could be observed from a typical band with the weight around 49 kDa. The saturated fatty acid (Σ SFA) compositions of fish species ranged from 1094.03-4233.03 $\mu\text{g/g}$. Oleic acid (MUFA) content of all fish species ranged from 257.91-1216.06 $\mu\text{g/g}$. However, only three fish species contain of Poly Unsaturated Fatty Acid (PUFA) linoleic acid as the following; *Selaroides leptolepis* (171.36 $\mu\text{g/g}$), *Oxyeleotris marmorata* (249.40 $\mu\text{g/g}$) and Tetraodontidae (140.35 $\mu\text{g/g}$). The highest SFA content was found in *S. leptolepis* with palmitic acid (C16:0) as the dominant saturated fatty acid (2320.88 $\mu\text{g/g}$). *S. leptolepis* also contained high oleic acid (1216.06 $\mu\text{g/g}$) and linoleic acid (171.36 $\mu\text{g/g}$).

Keywords: marine fish, Indonesia, protein, fatty-acid

INTRODUCTION

The chemical composition of marine fish is important for basic information of the research and development in fish species study included physiology, biochemistry, ecology, and conservation. Indonesia has a lot of fisheries sources and the largest archipelago, with more than 17,500 islands, which extend between the Pacific and the Indian Oceans. Fishing area in Indonesia covers 5.8 million km^2 of marine waters. In 2011, fisheries production increased to 5.7 million tonnes. After China, Indonesia was the second largest producers of capture fisheries products and the majority comes from marine fisheries. These production were attributed to some of fishing areas in Indonesia (Stobutzki et al. 2013). Fisheries production has become the big issues on ecological impact to marine biodiversity. Java Sea is one of large slight water that contributed significantly on the fish production among of fisheries manufactures in Indonesia (Nugroho et al. 2016).

Fish is important food component in the diet, not only as a source of protein but also a significant supply to the need of polyunsaturated fatty acids or omega-3. These nutrition are very beneficial to human health and stamina. Fish is consumed at several place of the world because of its high contents of protein, amino acid and saturated fatty acid. Because of the nutrition content of fish, the utilization of marine fish and its products increased significantly (S. Suvitha et al. 2014). The chemical composition of fish

species is the basic importance to be applied in process production (Diniz et al. 2013). The information of the nutrient composition of some important foods is important to understand the correlation between food productions, access, nutrient intakes and innovation of production technologies to guarantee that food supply population fulfils nutrient requirements optimally (Bogard et al. 2015).

Generally, the lipid content of fish meat is lower than beef or chicken. The important nutritional components of Fish products can be used as source of energy for human life. However, the nutrition content is varied depending on species, size, sexual condition, feeding season and physical activity. Proximate analysis such as protein, lipids and moisture contents is necessary to ensure that this analysis is suitable with requirements of food regulation and commercial specification (Ondo-azi et al. 2013).

Protein from marine fish is potential as raw material of protein hydrolysate production, which can produce by acidic or enzymatic hydrolysis method. Protein hydrolysates of fish is obtained from hydrolysis reaction of peptide bonds in proteins. It causes peptides becoming shorter. It also causes amino acids could be absorbed easily by animal (Wisuthiphaet and Kongruang 2015). Peptone is one of protein hydrolysates product. Fish protein hydrolysates contain secondary protein including polypeptides, dipeptides and amino acids. These secondary protein are nitrogen source of microorganisms, which are water soluble, so that they are suitable for being used as

microbiological culture media (Al-Bahri et al. 2009).

The advantage of marine fish as dietary sources of polyunsaturated fatty acids (PUFA) and unsaturated fatty acid (HUFA), especially the omega-3 PUFA, eicosapentaenoic acid (EPA) and docosa-hexaenoic acid (DHA). The nutritional benefits of fish consumption containing omega-3 PUFA have been published well in the world (Dhaneesh et al. 2012). Omega-3 fatty acids such as EPA and DHA have the ability to reduce the blood serum triglycerides. Long chain PUFA can prevent the disease of human coronary artery, rheumatoid arthritis, retina improvement and brain development, asthma, inflammatory bowel, decrease the breast cancer incidence, and can regulate the prostaglandin synthesis (Suvitha et al. 2014; Bahurmiz et al. 2017).

The comparison study of total protein, fat and omega-3 fatty acids content of raw and pressurized fish of *P. pangasius* (yellowtail catfish) and *H. macrura* (long tail shad) has been reported by Asmah et al. (2014). This study concluded that the pressurized fish is a potential source of omega-3. The Java Sea of West Java has a great variety of fish species, which are potential for fish product industries especially for protein isolate production. The objective of the study was to investigate the protein and fatty acids profile of nine marine fish samples from Java Sea of Indramayu West Java, Indonesia.

MATERIALS AND METHODS

Collection of sample

Fresh samples of fish were collected from fish market at the Java Sea of Indramayu West Java, Indonesia (Figure 1). They were kept in cold iced box and transported to the laboratory and kept in a freezer before used.



Figure 1. Fish sampling location at the Java Sea of Indramayu off shore, West Java, Indonesia

Determination of total protein and total lipid

The protein content was determined by estimating the total Nitrogen using Kjeldahl method. The protein content was calculated by multiplying total nitrogen by 6.25 factor, while lipid content in pulp was extracted using hexane in a Soxhlet extractor as described by AOAC (2000).

Fish protein hydrolysates preparation

The preparation was carried out following the method of Fahraniyah et al. (2002) with slightly modification. The frozen marine fish was thawed and mixed with distilled water with a ratio 1:4. Samples were blended and adjusted to pH 6.0. The hydrolysis was carried out in a water bath using 0.1% of papain at 50°C for 7 h, which was then stopped by heating at 85°C. The hydrolysate was allowed to stand for 15 min prior to vacuum-filtrated, which was then stored at -20°C.

Analysis of soluble protein content

Soluble protein content was analyzed using a modification of Lowry method (Rahman et al. 2004). Absorbance was measured by a UV-Vis Spectrophotometer at 500 nm. A series concentration of bovine serum albumin (BSA) was used as protein standard curve.

α -AN assay was determined using formol titration

The assay was done according to the method described by Wang et al. (2012) with slight modification. 1 gram of dried fish sample was mixed with 25 mL of deionized water. The solution was adjusted to pH 8.2 with 0.1 M NaOH, and it was subsequently added with 10 mL of 35% (w/w) formalin (pH 8.2). The pH value of the solution went down due to the addition of formol. The titration using NaOH was done until the pH of the solution was 9.20. The concentration of α -AN was calculated using the following equation:

$$C_{\alpha-AN} \text{ (mM)} = \frac{\Delta V \times n \times 103}{V}$$

Where, ΔV is the volume in mL of NaOH used for the titration; n (mol/L) is the molar concentration of the NaOH solution; and V (mL) is the sample volume.

Determination of degree hydrolysis

Degree hydrolysis (DH) of the extract was calculated using (AOAC 1995) method. The DH was calculated using relationship between α -amino nitrogen (AN) and total nitrogen (TN) according to the following equation:

$$\%DH = \frac{\alpha\text{-amino Nitrogen (AN)}}{\text{Total Nitrogen (TN)}} \times 100$$

TN was determined by the Kjeldahl method.

Molecular weight analysis

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was done on all samples and protein marker on a discontinuous buffered system according to Bollag et al. (1996) method. 20 μ L of each sample was added with 40 μ L of sample buffer. Samples were placed in

a foam rack and heated in boiling water for 4 min. The preparation of polyacrylamide gel was placed in an electrophoresis unit. Running buffer was filled to the upper buffer chamber of the gel until the buffer reaches halfway between the tops of the short and long glass plates. 5 μ L of standard protein markers and 25 μ L of each samples were loaded to the polyacrylamide gel. Electrophoresis was conducted at a constant 200 V for 30 min. The gel was removed and placed in staining solution.

Methylation Preparation

During fatty acid methyl ester (FAME) preparation, pre-test was done to identify the suitable method for methylation process. The pre-test includes sodium methoxide method, potassium hydroxide method and boron trifluoride (BF₃) method. Finally boron trifluoride (BF₃) was chosen while comparing the peak. Firstly 0.125 g of fish oil was put in a test tube. Secondly, 0.5 ml of boron trifluoride (BF₃) in MeOH (14%) was added to the test tube. Afterward, the test tube containing the fish oil and boron trifluoride (BF₃) in MeOH (14%) was incubated in an incubator shaker at 55°C for 1.5 hour. 0.5 ml of saturated sodium hydrogen carbonate (NaHCO₃) and 0.75 ml of n-hexane was then added to the test tube. The mixture was mixed and shaken well using a vortex for about 30 second. The subsequent mixture was stored for 5 minutes under room temperature so that it will form two layers. Lastly, 0.5 ml of upper layer contain hexane was carefully transferred into a vial for Gas Chromatography (GC) analysis.

Gas Chromatography (GC) analysis

Fatty acids composition of fish hydrolysate samples were analyzed using gas chromatography (GC) (Agilent Chemstation Version 5) equipped with split-splitness injector, detector Hewlett-Packard EL-980 flame ionization detection (FID) system to separate and quantify each FAMES components. FAMES were separated using Ultra 1

column (25 m x 0.32 mm thickness 0.17 μ m methyl siloxan film). Chromatography data were recorded and integrated using Chemstations software (version 5.0). Oven temperature was held at 70°C for 3 min, which then increased to 200°C at 10°C/min and lastly increased to 260°C, held for 3 min. Temperatures for injector and detector were set at 280°C. 1 μ L of sample volume was injected with split ratio of 0:50 at column temperature 110°C. Carrier gases used for the system were helium gas, 1.0 ml/min controlled at 4.92 psi, hydrogen and air used for FID was held at 40 and 80 lbs/inc².

RESULTS AND DISCUSSION

Results

In this study, nine fish species had been collected freshly from a fish market at the Java Sea of Indramayu West Java, Indonesia. Fish samples are predominantly collected by the species with the small size fish group between 10-20 cm in length. The common and species names of fish was presented in Figure 2.

Table 1 presents the total protein and total lipid content of nine species of marine fish samples. The total protein content of fish samples ranged from 61.07% (*Pampus argenteus*) to 86.56% (Tetraodontidae). Meanwhile, total lipid content of fish samples ranged from 1.73% (Tetraodontidae) to 9.82% (*Leiognathus equulus*). This data showed that Tetraodontidae contained the highest protein and the lowest lipid content.

Total nitrogen (TN), soluble protein content, alpha amino content and degree hydrolysis of nine fish species was shown in Table 2. These data represents the protein profile of fish species, which was found in Java Sea of West Java. The data showed that the content of total nitrogen, ranging from 9.95% (*P. argenteus*) to 13.81% (Tetraodontidae) based on the dry weight.

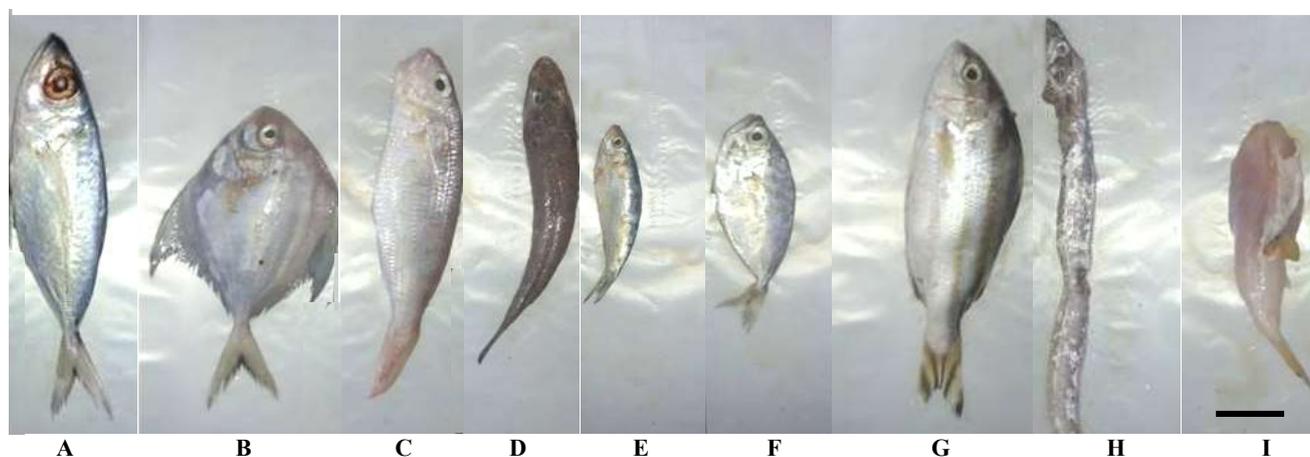


Figure 2. Fish samples; A. Selar (*Selaroides leptolepis*), B. Bawal (*Pampus argenteus*), C. Kurisi (*Nemipterus hexodon*), D. Boso (*Oxyeleotris marmorata*), E. Bilis (*Mystacoleucus padangensis*), F. Peperek (*Leiognathus equulus*), G. Kerong (*Terapon jarbua*), H. Layur (*Trichiurus lepturus*), I. Buntal (Tetraodontidae). Bar = 1 cm

Table 1. Protein and total lipid content of nine species of Marine Fishes from Java Sea of West Java, Indonesia

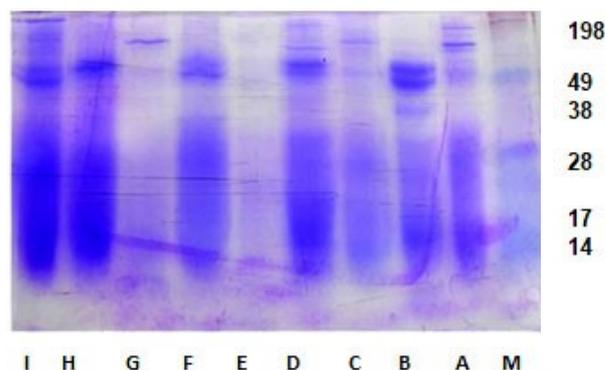
Species	Total protein (% w/w)	Total lipid (% w/w)
<i>Leiognathus equulus</i>	66.58	9.82
<i>Mystacoleucus padangensis</i>	74.53	6.88
<i>Nemipterus hexodon</i>	73.19	9.71
<i>Oxyeleotris marmorata</i>	78.53	4.66
<i>Pampus argenteus</i>	61.07	4.03
<i>Selaroides leptolepis</i>	73.26	5.45
<i>Terapon jarbua</i>	65.00	7.41
Tetraodontidae	86.56	1.73
<i>Trichiurus lepturus</i>	83.02	4.74

In this study, fish samples were hydrolyzed by papain enzyme. The concentration of α -AN of fish protein hydrolysate was ranging from 31 mM (*Nemipterus hexodon*) to 69 mM (*Mystacoleucus padangensis*) and the percentage (%) of DH was ranging from 9.33% to 20.39%. In our study, the hydrolysis was carried out using 0.1% of papain at 50°C for 7 hours.

Table 3 presents the fatty acid composition of nine fish samples. Gas chromatography analysis of fatty acid methyl esters from the lipids of those fish samples revealed the presence of five fatty acids. The saturated fatty acid (Σ SFA) compositions of fish species ranged from 1094.03-4233.03 μ g/g. Oleic acid (MUFA) content of all fish species ranged from 257.91-1216.06 μ g/g, except for the species *P. argenteus* was not detected. However, only three fish species contain linoleic acid (PUFA) as follow; *Selaroides leptolepis* (171.36 μ g/g), *Oxyeleotris*

marmorata (249.40 μ g/g) and Tetraodontidae (140.35 μ g/g). The highest SFA content was found in *Selaroides leptolepis* with palmitic acid (C16:0) as the dominant saturated fatty acid (2320.88 μ g/g). *S. leptolepis* also contain high oleic acid (1216.06 μ g/g) and linoleic acid (171.36 μ g/g). However, the highest linoleic acid was found in *O. marmorata* (249.40 μ g/g).

The soluble protein of marine fish samples was identified its molecular weight profile by SDS PAGE method. The result was presented on Figure 3.

**Figure 3.** SDS PAGE profile of fish samples; M: protein molecular weight marker (kDa), A. Selar (*Selaroides leptolepis*), B. Bawal (*Pampus argenteus*), C. Kurisi (*Nemipterus hexodon*), D. Boso (*Oxyeleotris marmorata*), E. Bilis (*Mystacoleucus padangensis*), F. Peperek (*Leiognathus equulus*), G. Kerong (*Terapon jarbua*), H. Layur (*Trichiurus lepturus*) and I. Buntal (Tetraodontidae)**Table 2.** Protein profile of nine species of marine fishes from Java Sea of West Java, Indonesia

Species	Total nitrogen (%)	Soluble protein (mg/g)	α -amino nitrogen (mM)	Degree hydrolysis (%)
<i>Leiognathus equulus</i>	11.03	286.68	53	16.82
<i>Mystacoleucus padangensis</i>	11.85	252.67	69	20.39
<i>Nemipterus hexodon</i>	11.63	134.29	31	9.33
<i>Oxyeleotris marmorata</i>	12.57	169.30	52	14.49
<i>Pampus argenteus</i>	9.95	203.03	54	19.00
<i>Selaroides leptolepis</i>	11.69	168.34	47	14.98
<i>Terapon jarbua</i>	10.39	196.53	53	17.86
Tetraodontidae	13.89	163.22	42	10.59
<i>Trichiurus lepturus</i>	13.34	191.74	38	9.97

Table 3. Fatty acid profiles of nine species of marine fishes from Java Sea of West Java, Indonesia (μ g/g)

Species	(C14:0)	(C16:0)	(C18:0)	Σ SFA	(C18:1)	(C18:2)
<i>Leiognathus equulus</i>	113.51	700.15	280.37	1094.03	257.91	nd
<i>Mystacoleucus padangensis</i>	534.69	1376.91	451.58	2363.18	362.73	nd
<i>Nemipterus hexodon</i>	61.16	812.16	469.70	1343.02	423.26	nd
<i>Oxyeleotris marmorata</i>	295.50	1750.22	679.86	2725.58	621.21	249.40
<i>Pampus argenteus</i>	37.33	737.13	572.15	1346.60	nd	nd
<i>Selaroides leptolepis</i>	250.68	2320.88	1661.52	4233.07	1216.06	171.36
<i>Terapon jarbua</i>	97.67	959.92	364.47	1422.05	628.82	nd
Tetraodontidae	65.69	1564.68	1131.00	2761.37	749.49	140.35
<i>Trichiurus lepturus</i>	122.31	652.63	338.51	1113.45	313.14	nd

Discussions

According to the statistic data from Noegroho et al. (2013), Indramayu was the highest fish producer in West Java, Indonesia. The total production was around 128,548 ton per year, in which the highest production \pm 16,664 ton was peperek fish (*L. equulus*), followed by selar fish (*S. leptolepis*) \pm 3,367 ton and layur fish (*Trichiurus lepturus*) \pm 2,601 ton. Nugroho et al. (2016) reported that the catching composition in inshore water was also predominantly composed of small size Leiognathida, included *L. equulus*. The sampling location was at Tegal City, the western part of the north coast of Central Java, Indonesia.

The results on Table 1 showed that Tetraodontidae contained the highest protein content. Nevertheless, Tetraodontidae was not recommended for human consumption. Hashiguchi et al. (2015) reported that family Tetraodontidae or pufferfish contained tetrodotoxin and saxitoxin in their organs and the degree of toxicity was highly variable even within each toxic species. Tetrodotoxin in the pufferfish localized in the ovary of its larvae as protection against predators (Itoi et al. 2014). From all samples study, *S. leptolepis* and *O. marmorata* were recommended as good edible fish due to its high protein content, no toxin and its taste.

Protein profile of nine fish samples from Java Sea of West Java Indonesia (Table 2) can represent the protein profile of fishes in this area. Diniz et al. (2013) reported that the total nitrogen of fish samples from coastal water of Brazil ranging from 11.6% (*M. argentiniae*) to 14.9% (*R. porosus*) of the dry weight. Taheri et al. (2016) also reported that the biochemical composition of *Sardinella gibbosa*, *Clupeonella engrauliformis* and *Stolephorus indicus* bones from the Oman Sea and Caspian Sea became an alternative of fish meal production with the adding value and high quality to fulfill the market demand. The concentration of α -amino nitrogen (α -AN) and degree hydrolysis (% DH) are often used as the quality indicator for fish protein hydrolysate product. This product can be used as condiments with unique flavors and aromas. The amount of amino acids formation is very important key to the flavor of sauces or flavoring. Wang et al. (2012) reported that fish sauce can be graded by α -AN concentration and the higher the α -AN concentration is the better of quality. Analysis of protein hydrolysate from the mixed marine fishes showed that the DH was in the range of 20-24% (Wisuthiphaet and Kongruang 2015). This product was obtained by hydrolysis the marine fish sample with 2-6% papain at 40°C for 15 hours. The condition of enzymatic hydrolysis is important for improvement the quality of protein hydrolysate product. The hydrolysis of protein is a conversion process from big molecule of protein to low molecular weight products, hydrolysis proteins yield proteomes, peptones, polypeptides, and finally the simpler amino acids (Al Bahri et al. 2009). During enzymatic hydrolysis, the exposures of peptide bonds lead to the increase of DH. However, the activity of enzyme can be reduced by high temperature and decreasing the hydrolysis rate (Salwanee et al. 2013). In our study, *M. padangensis* has the highest% of DH. This fish species can

be recommended for peptone or protein hydrolysate production.

The molecular weight of protein fish samples had a similar profile primarily for *S. leptolepis*, *P. argenteus*, *O. marmorata*, *L. equulus*, *T. lepturus* and Tetraodontidae with the molecule weight of typical band around 49 kDa. This data was indicated that marine fish has typical protein profile and could be explored as protein character of each fish species. Species identification, primarily using sarcoplasmic proteins as target proteins, has been studied using SDS-PAGE method. However, the quantification of fish myofibrillar protein (surimi) using SDS-PAGE has not been studied, primarily for the application of species identification (Reed and Park 2008).

The results on Table 3 show that *S. leptolepis* and *O. marmorata* contained high Oleic acid (MUFA) and also linoleic acid (PUFA). Fish oils and other marine animal oils are identified as a big group of fatty acids. These fatty acids are classified as saturated, monounsaturated and polyunsaturated groups (Immanuel and Palavesam 2010). Marine fishes had higher levels of PUFAs compared to freshwater fish. The differences of fatty acids content in marine and freshwater fish were influenced by its species and their natural diet (Dhaneesh et al. 2012). Fatty acids content of fishes were based on their feed and affected by its size, age, reproductive and environmental conditions, especially the temperature of water which could influence the lipid content and fatty acid composition. Marine fish and shellfish from warm water area contain a good composition of fatty acids and provided the health benefit if they were consumed regularly (Aziz et al. 2013). Fatty acid profiles in marine and freshwater fish were also studied by Łuczynska et al. (2014). In this study, the most abundant n-6 polyunsaturated fatty acids were linoleic (C18:2 n-6) and arachidonic (C20:4 n-6). Fatty acids in freshwater fish were on approximately similar to some of the marine fish examination.

We concluded that marine fishes are a source of high protein with low lipid content. Some of fish samples are potential as raw material for fish protein hydrolysate production due to its high% of DH. *S. leptolepis* and *O. marmorata* are recommended as potential sources of PUFA.

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