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Nutritional profile of Rabbitfish (*Siganus* spp.) from the Kepulauan Seribu (Thousand Islands), Jakarta, Indonesia

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Abstract

Rabbitfish are an economically important coral reef fish in countries such as the Philippines, Hong Kong, Singapore, Taiwan, China, Malaysia, and Indonesia. Rabbitfish are herbivorous fish in the family Siganidae that are bred for farming. Understanding rabbitfish nutritional contents, such as amino acids, fatty acids, vitamins, and minerals, are important to optimizing the fishes use. We explored the nutritional content of rabbitfish obtained from the Kepulauan Seribu (Thousand Islands), Jakarta. Nine essential and seven non-essential amino acids were determined. Glutamic acid was most abundant amino acid with a level of 1.983 mg/100 g. The quantities of eicosapentaenoic acid, docosahexaenoic acid, and arachidonic acid were 0.54%, 6.45%, and 1.21%, respectively. Vitamin A content was 187.27 IU/100 g and vitamin B12 content was 1.40 µg/100 g.

Introduction

The value of Indonesian coral-reef fish has not been widely explored, particularly in the Java Sea. The Indonesian Ministry of Maritime Affairs and Fisheries reported that approximately 14.029 tons of coral-reef fish were caught for consumption in 2012. Coral-reef fish have high fisheries potential because they have unique characteristics and are clean water indicators. There are many species (from 56 families) of coral-reef fish but their economic value remains unknown.

Rabbitfish (*Siganus* spp.) are widespread in the Indo-Pacific region from the east coast of Africa to Polynesia and from southern Japan to northern Australia. Rabbitfish, in the family Siganidae,are economically important in the Philippines, Hong Kong, Singapore, Taiwan, China, Malaysia, and Indonesia. Species that inhabit Indonesian waters are *Siganus canaliculatus, S. guttatus, S. virgatus, S. spinus, S. punctatus*, and *S.fuscescens* (Jaikumar, 2012).

Rabbitfish are economical, herbivorous fish fed on algae or seaweed. Market demand for rabbitfish is developing, they have mainly been consumed locally but could be exported. According to Jaikumar (2012),

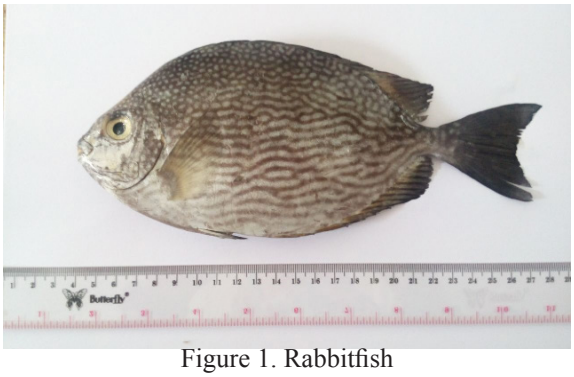

Figure 1. Rabbitfish

rabbitfish is one of the most potentially important fish. Increased demand for rabbitfish makes it difficult to rely only on wild stocks; thus, rabbitfish farming has proliferated. However, no study has investigated the nutritional content of rabbitfish.

A comprehensive study of rabbitfish nutritional content is important to optimize the fishes' use and determine their amino acid, fatty acid, vitamin, and mineral contents. Thus, our aim was to determine the nutritional value of rabbitfish (Figure 1) obtained from Kepulauan Seribu (Thousand Islands), in the Jakarta Bay, Indonesia.

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Materials and Methods

Sample preparation

Rabbitfish were obtained from the Kepulauan Seribu, in the Jakarta Bay (Thousand Islands), Indonesia, transported on ice for 4 hours, and stored at −20°C until used. We performed a proximate analysis and measured rabbitfish amino acid, fatty acid, vitamin, and mineral contents. Rabbitfish were measured and identified morphologically prior to analyses. Samples were filleted without skin and divided for the proximate amino acid, fatty acid, vitamin, and mineral analyses, which were conducted in triplicate. The size of fish sample was 200.67 gram. The sample was cought in Island of Pramuka and Panggang, part of Kepulauan Seribu (Thousand Islands). The fishing equipment was used to catch the fish. The Fish was caught on August 2014.

Proximate analysis

The proximate analysis was conducted using the AOAC (2005) method to measure protein, fat, moisture, and ash contents.

Amino acid analysis

Amino acid analysis was conducted according tothe AOAC (2005) method. The amino acid analysis was conducted using high performance liquid chromatography (HPLC)in four steps of protein-hydrolyzation, drying, derivatization, and injection. Sample as much as 0.1 g was homogenized, then added by 10 mL HCl 6 N, heated in an oven at 100°C for 24 hours. The sample was filtered with milipore filter paper. The result was taken as many as 30 µL and added with 30 µL of solution dryer. Solution of dryer was made from a mixture of methanol, pico-thiocyanate and triethylamine with a comparison of the 4:4: 3. Derivatization of solution as many as 30 µL was added to the result of drying, a solution for derivatization was made from a mixture of methanol, sodium acetate and triethyllamine with a comparison of 3:3:4. The process of derivatization was done so that the detector was easy to detect compounds that exist in the sample, further dilution was added by 20 mL acetonitrile 60% or 1 M sodium acetate buffer, and then left for 20 minutes. A 40 µl aliquot of the sample was pipette and injected into the HPLC. The amino acid concentrations were determined from a standard chromatogram using ready-to-use amino acids treated the same as the samples. Column was C18, detector was UV-Vis, with flow rate 1.0 mL/minute and mobile phase was acetonitrile.

Fatty acid analysis

Fatty acid analysis was conducted according to the AOAC (2005) method. The initial step involved extracting the fat using the Soxhlet method to obtain fat as oil. Methylation was conducted by refluxing the fat over a water bath with 0.5 N NaOH-methanol, BF_3 and isooctane. Oil (0.02 g) from the sample was introduced into a test tube and 1 ml of 0.5 N NaOH-methanol was added and heated in a water bath for 20 min at 80°C. The solution was cooled and 2 mL of BF_3 added to the test tube; the test tube was then re-heated in the water bath at 80°C for 20 min, cooled, and 2 mL of saturated NaCl added; finally, the mixture was shaken and 1 ml iso-octane added. A 1 µl aliquot of the sample was injected into a gas chromatograph. Fatty acids in the methyl ester were identified with a flame ionization detector. The instrument was GC-FID Shimadzu 17-A, capiler column length was 60 m, column diameter was 0.25 mm, and detector temperature was 230°C.The initial temperature of the injector was 190°C and the temperature rise was 10°C /min.

Quantification of fatty acid was calculated: $Cx = \frac{Ax.R.Cs}{As}$

Cx = concentration of component X
Cs = concentration of internal standard
Ax = peak area of component X
As = peak area of internal standar
R = Detector response

Vitamin A analysis

The vitamin A analysis was conducted according to the AOAC (2005) method. Five g of sample was added to 3 mL of distilled water, followed by 10 mL of 95% methanol. A 2.5-mL portion of 50% KOH was pipetted into an Erlenmeyer flask containing the sample and quickly placed over a water bath at 80°C, with a cooling condenser placed on the mouth of the Erlenmeyer flask. This solution was transferred to a 25-mL volumetric flask and calibrated with THF: ethanol (1:1)solution, filtered, and precipitated. A vitamin A standard solution that had saponified was injected, followed by the mobile phase to resolve the cis and trans forms. All trans-retinol was resolved. The cis-retinol was resolved as a small peak before the trans form. Standards and the sample were injected into small auto sampler bottles and run on the HPLC.

Vitamin B_{12} analysis

Vitamin B_{12} analysis was conducted according to the AOAC (2005) method. Five g of sample was added to a closed test tube and 20 mL acetate buffer

and 0.2 mL potassium cyanide were added. The mixture was homogenized for 5 min by ultrasound and allowed to stand at room temperature to cool. A 25 mL aliquot of methanol was added followed by2% acetic acid until the volume reached 50 mL. The samples were centrifuged at 4000 rpm for 30 min, and the supernatant was retained for HPLC.

Mineral analysis

Mineral analysis was conducted according to the AOAC (2005) method. The wet ashing process to analyze Ca, K, Na, Fe, Zn, and Se was conducted by weighing 1 g of sample. A 5 mL aliquot of HNO_3 was added to a flask and allowed to stand for 1 hour at room temperature. A 0.4 mL aliquot of concentrated H_2SO_4 was added and heated on a hotplate until the solution became more concentrated (± 1 hour). Then, two or three drops of $HClO_4$ and HNO_3 (2:1) were added, and the sample was left on the hotplate until its color changed from brown, to dark yellow, and finally to light yellow over 1 hour. The samples were transferred, cooled, and 2 mL distilled water and 0.6 mL concentrated HCl were added. The solution was re-heated to dissolve the sample (15 min) and then made up to 100 ml volume. The wet ash results were collected by atomic absorption spectrophotometer. Before mineral analysis all glassware and plastics must be pre-cleaned in acid bath to avoid metal contamination. Water should be ultrapure grade water. Blank sample must be carried out with samples to minimize error coming from solutions might contain some trace amount of metals.

Results and Discussion

Yield

The yield was calculated by comparing the weight of each body part with the weight of the whole rabbitfish. The rabbitfish were weighed and then divided into viscera, skin, head, and flesh for further weighing. The results are presented in Figure 2. Flesh was the highest (45.67%), indicating that this fish can be used as a raw material for further processing.

Proximate analysis

Proximate analysis was conducted to determine the crude nutritional content of samples, including moisture, protein, fat, ash, and carbohydrate contents. Fish generally consist of 70–84% moisture, 15–24% protein, 0.1–22% fat, and 1–2% mineral contents (Holma *et al*., 2013).

Rabbitfish from the Kepulauan Seribu (Thousand Islands),in the Jakarta Bay, Indonesia contained 77.95% moisture. Water is a basic component and its present as free water and bound water. Free water can be easily removed by evaporation or drying, whereas bound water is very difficult to remove, even by drying. According to Davies and Davies (2009), fish are highly perishable due to their high moisture content. Rabbitfish and snapper are both coral-reef fish and our results indicate that rabbitfish moisture content was similar to that of snapper. The moisture content of fish that live at the bottom differs from fish that live in the middle or surface of the water column.

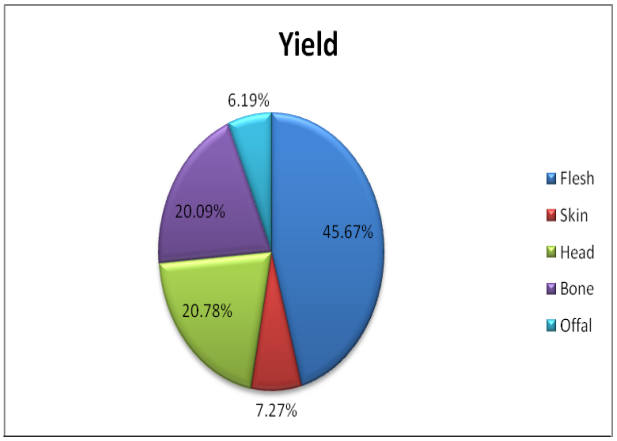

Figure 2. Rabbitfish yield

Rabbitfish protein content was 15.93%, whereas that of snapper is 20.00%. Rabbitfish protein content is similar to that of carp *(Cyprinus carpio)* 15.20–17.83% (Afkhami *et al.*, 2011). This difference might be caused by the feed consumed. According to Fellows (2000), differences in protein content can be caused by external factors such as the environment, season, catch method, storage, and processing.

The rabbitfish fat content was 0.93%, which was lower than that in snapper. Fat content in coral-reef fish is quite different from that in freshwater fish, such as carp *(C. carpio)*. The fat content measurement is influenced by the moisture content measured. The more moisture that escapes from the sample, the lower amount of fat measured in the proximate analysis. There is an inverse relationship between moisture and lipid content. Any fish having more moisture means having less lipid.

Amino acid analysis

Table 1 shows the amino acids in rabbitfish. Amino acid content consisted of nine essential and seven non-essential amino acids. These results indicate that rabbitfish have high quantities of essential amino acids required by the human body. Glutamic acid was the most prevalent amino acid in fresh rabbitfish (1.98 mg/100 g) (Table 1). Perkins (1992) reported that high glutamic acid content causesmarine fish flesh to taste savory and sweet. The rabbitfish glutamic acid content was higher than that

Table 1. Rabbitfish amino acids

Amino acid	mg/100 g
Essential amino acids	
Histidine	330±17.32
Threonine	733±20.82
Arginine	1,020±26.46
Methionine	517±20.82
Valine	757±5.77
Phenylalanine	653±25.17
l-Leucine	727±11.55
Leucine	1,113±11.55
Lysine	1,297±57.74
Non-essential amino acids	
Aspartic acid	1,203±15.28
Glutamic acid	1,983±25.17
Serine	647±30.55
Glycine	707±15.28
Alanine	940±36.06
Tyrosine	557±20.82
Taurine	94.411±4.45

Means ± SD (n=3)

in Northern red snapper *(Lutjanus campechanus)* at 0.9–1.4 mg/100 g (Antoinne *et al.*, 2001).

The rabbitfish histidine content was the lowest of the amino acids (330 mg/100 g). However, histidine content in *L. campechanus* is 2.55–5.14 mg/100g (Antoinne *et al.*, 2001). Amino acid contents differ according to each species' physiology. Differences in amino acid content could also be caused by age, catch season, and life cycle stage. Amino acid has an important function for human. A growing body of literature has led to the development of the concept of functional Amino acid (FAA), which are defined as those amino acid that regulate key metabolic pathways to improve health, survival, growth, development, lactation, and reproduction of organisms (Wu and Prentice, 2010). Millward (2015) reported that human amino acid requirements totally is 173 mg/g.

Fatty acid analysis

Rabbitfish fatty acids consisted of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) (Table 2). The SFA, MUFA, and PUFA contents were 34.33%, 11.18%, and 9.67%, respectively. The main fatty acids identified from rabbitfish were lauric acid (C12:0), myristic acid (C14:0), stearic acid (C18:0), palmitic acid (C16:0), oleic acid (C18:1 n9), palmitoleic acid (C16:1 n7), arachidonic acid (C20:4 n6, ARA), docosahexaenoic acid (C22:6 n3, DHA), and eicosapentaenoic acid (C20:5 n3, EPA).

SFAs were the most prevalent among the fatty acids, which may be due to feed affecting the digestive system. The Kepulauan Seribu (Thousand Island) rabbitfish palmitic acid (SFA group) content was the highest among the fatty acids at 19,326.67 mg/100 g. Oksuz *et al.* (2010) reported that *Siganus luridus* fatty acid contentis 28.20 % and in *Siganus rivulatus* is 32.64 %. Fatty acids from both species were higher than those from the Thousand Islands rabbitfish. Gehring *et al.* (2009) reported that intramuscular fat content of fish varies greatly and is highly dependent on species, age, spawning, feeding, and muscle type.

Table 2.Rabbitfish fatty acid composition

Composition		%
Saturated fatty acids (SFA)		
Lauric acid	C12:0	0.83±0.02
Myristic acid	C14:0	4.55±0.09
Pentadecanoic acid	C15:0	1.13±0.04
Palmitic acid	C16:0	19.32±0.38
Heptadecanoic acid	C17:0	1.17±0.03
Stearic acid	C18:0	5.85±0.12
Arachidic acid	C20:0	0.85±0.06
Heneicosanoic acid	C21:0	0.16±0.01
Behenic acid	C22:0	0.30±0.00
Tricosanoic acid	C23:0	0.06±0.00
Lignoceric acid	C24:0	0.17±00
Total SFA		**34.43**
Monounsaturated fatty acids (MUFA)		
Myristoleic acid	C14:1	0.04±0.01
Palmitoleic acid	C16:1	3.53±0.07
Cis-11-Eicosenoic acid	C20:1	0.35±0.01
Nervonic acid	C24:1	0.27±0.02
Elaidic acid	C18:1n9t	0.11±0.01
Oleic acid	C18:1n9c	6.50±0.14
Erucic acid	C22:1n9	0.35±0.05
Total MUFA		**11.18**
Polyunsaturated fatty acids (PUFA)		
Cis-11,14-Eicosatrienoic acid	C20:2	0.21±0.01
Linoleic acid	C18:2n6c	0.64±0.02
γ- Linolenic acid	C18:3n3	0.05±0.01
Linolenic acid	C18:3n3	0.23±0.01
Cis-11,14,17 Eicosatrienoic acid	C20:3n3	0.23±0.17
Cis-8,11,14-Eicosatrienoic acid	C20:3n6	0.09±0.00
Arachidonic acid	C20:4n6	1.21±0.02
Cis-5,8,11,14,17-Eicosapentaenoic acid	C20:5n3	0.54±0.01
Cis-4,7,10,13,16,19-Docosahexaenoic acid	C22:6n3	6.45±0.19
Total PUFA		**9.67**

The most abundant fatty acid was DHA, which is the most common PUFA. This result agrees with previous studies reporting that *S. rivulatus* has 8.45% DHA and that *Siganus luridus* has 7.94% DHA. Previous researcher reported that docosahexaenoic acid (DHA) content of pangasius from Jambi,

The rabbitfish MUFA content is shown in Table 2. Oleic and palmitoleic acids were most abundant, at 6.51% and 3.53%, respectively. The rabbitfish PUFAs were high in EPA, DHA, and ARA contents. The total rabbitfish EPA, DHA and ARA contents were 0.54%, 6.45%, and 1.21%, respectively. The rabbitfish ARA content was higher than the EPA content. These results accord with Oksuz *et al.* (2010), who reported that ARA contents in *S. rivulatus* and *S. luridus* exceed EPA contents in both species. Higher ARA than EPA content is a characteristic of the family Siganidae.

Indonesia was 0.76%, pangasius from Karawang, Indonesia was 1.55% and imported pangasius was 2.72% (Nurilmala *et al.,* 2015). Docosahexaenoic acid (DHA) of pangasius from Jambi, Karawang and imported are lower than rabbitfish. Saito *et al*. (1999) stated that the differences in fatty acid content in fish depend on dietary pattern and are also influenced by species, age, size, and water temperature.

Vitamins

Vitamins are organic nutrients required in small amounts for a variety of biochemical functions and are generally not synthesized in the body; thus, they must be supplied through the diet. Vitamins are chemical catalysts that help transform macronutrients. Vitamins are divided into fat-soluble vitamins, such as vitamins A, D, E, and K, and water-soluble vitamins, such as vitamins B_{12}, B_6, biotin, and niacin.

The rabbitfish vitamin A content was 187.27 IU/100 g. Chakraborty *et al.* (2014) reported that marine fish are rich in fat-soluble vitamins, including vitamins A, D, E, and K, which are required in human metabolism. Fish vitamin A content varies greatly among species, depending on age, sex, environment, and season (Alasalvar *et al*., 2011). The rabbitfish vitamin B_{12} content was 1.40 µ/100 g. Vitamin B_{12} maintains neuron and red blood cell health and participates in DNA replication.

Minerals

Minerals are organic compounds stored in food and are divided into major minerals and trace elements. Major minerals in the human body at > 5 g include Ca, F, K, S, Na, Cl, and Mg. Minerals contained in fish, such as K, Ca, Mg, and P, and micro minerals,such as Se, F, I, Co, and Mn, are 0.6–1.5% of wet weight. Fe, Zn, and Se are trace minerals that are abundant in fish.

Rabbitfish contained of Na 2,032.78 mg/Kg, Ca 251.81 mg/Kg, Fe 18.25 mg/Kg, Zn 13.16 mg/Kg, Se less than 0.002 mg/Kg. This result showed that potassium was most abundant, at 10,509.49 mg/Kg. Potassium is directly related to cellular energy-producing reactions (Belitz *et al.*, 2004). Potassium regulates the osmotic pressure within cells involved in membrane transport as well as several enzyme activities. Oksuz *et al*. (2010) reported that potassium content in *S. rivulatus* and *S. luridus* was the highest among minerals, demonstrating that rabbitfish has high potassium content. The rabbitfish contained less than 0.002 mg/Kg selenium, which was the lowest of the minerals analyzed. Mineral diversity is influenced by age, type, size, habitat, geographical location, and environmental conditions (Gokce *et al.* 2004).

Conclusions

Rabbitfish contained 77.79% moisture, 15.93% protein, 1.01% ash, and 0.93% fat. Rabbitfish contained 16 amino acids, including nine essential and seven non-essential amino acids. Glutamic acid was most abundant at 1.98 mg/100 g. The quantities of EPA, DHA, and ARA were 0.54%, 6.45%, and 1.21%, respectively. Vitamin A content was 187.27 IU/100 g and vitamin B_{12} content was 1.403 µg/100 g. Potassium was the most abundant mineral at 10,509.49 mg/Kg.

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