

## **THE NUTRIENT AND STEROID CONTENT OF SOME DEEP SEA FISH SPECIES FROM WEST SUMATERA OCEAN**

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### **ABSTRACT**

The Purpose of this research is to observe the content of nutrient and steroid of some deep sea fish from west Sumatera ocean. The result of the research shows that 11 kinds of deep sea fish such as: *Dietmoides pauciradiatus*, *Benthodesmus tenuis*, *Beryx splendens*, *Haplathethus craaapinus*, *Haplathethus sp*, *Ophidiidae*, *Ostrucoberyx doryge*, *Godamus colletinis*, *Heteroglype japonica* contain protein 23,0-24,8 %, fat 1,9-4,1 %, carbohydrate 0-1,75 %, ash 1,7-2,4 %, water 70.1-72,1 %. In amino acid test, it can be identified 17 amino acid (9 essential amino acid and 8 non essential amino acid) Meanwhile in steroid test using Libermann Burchad, it can be identified 8 kinds of deep sea fish containing steroid and the greatest concentration is in *Dietmoides pauciradiatus*, *Benthodesmus tenuis*, *Beryx splendens*, *Haplathethus craaapinus*.

### **I. INTRODUCTION**

Based on the total prediction of Indonesian fishery ocean potential which is amount to 6.6 million tons/year, consist of 5 million ton in Indonesian ocean and 2.1 million tons in Zee ocean. The potential prediction comes from some kinds of ocean fish, such as the small pelagic fish 3.5 tons, coral fish 0.048 million tons/year (Anonymous 2000).

According to the potential estimation, production and utilization of pelagic fish in Indonesia in 2001, Malaka Street and Java Sea are in the state of over fishing (BRKP 2001) therefore it needs to look for new fishing ground area instead of area in coastal area and pelagic area. Part of ocean environment which is predicted as alternative area is deep sea area.

Deep sea area is located under shining dept area in the open ocean and deeper than continental shelf (>200m). The habitat is the widest part in the world where seldom organisms live in; its water volume is predicted of amount up to 85% of 70% world surface (Nybakken 1992).

The seldom fishes however, are important food source and often looked for by some people in the markets. In Europe, deep sea fish (lung lip) is marketed as cusk eel. In New Zealand called Hung, South America called Cangrio and in Japan called Kingu. This fish is marketed by retail and seldom appears in restaurant, because of the good quality and unique meat texture. Gold king lip, red and black

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are marketed internationally, but USA preferably the gold and red king lip (Perkins 1992). In Australia, deep sea fish (Beryx aptendens) was exploited, even occurred over fishing Anonymous (2004), in addition Soselia & Rustam (1993) reported that Cubiceps whiteleggi is one of important economical fish in the future. even thought in Indonesia deep sea fish is not utilized optimally yet.

The result of Baruna Jaya IV expedition leaded by The Agency for Marine and Fisheries. Research Ministry Marine Affairs and Fisheries showed about 529 kinds of deep sea fish. Some of those fishes are presumed to be containing aphrodisiac matter. Based on this information, the current work tried to analyze the content of bio substance of some fish collected from Indian Ocean of west Sumatra.

**II. MATERIALS AND METHODS**

Fish materials used in this work consisted of 11 species of deep sea fish, such as Dietmoides pauciradiatus, Benthodesmus tenuis, Beryx splendens, Haplosetheus craaapinus, Hoplosetheus sp, Hyteroglype japonica. The work is divided by 3 parts, as well; 1) proximate test, 2) Amino acid test and 3) Steroid test.

**1. Proximate Test**

Proximate analysis was done including protein, fat, water, ash and carbohydrate.

**Water level (Apriantono et al. 1989)**

Porcelain cup was dried in temperature of 102-105 °C, for approximately 10-12 hours. Then cup was put in desiccators (± 30 minutes), measured (A gram), the cup was measured by homogenated sample (B gram), the amount 5 gram, placed in the cup was measured (C gram).

Measurement:

$$\% \text{ water content} = \frac{B-C}{B-A} \times 100 \% \dots\dots\dots (1)$$

- Where:     A = Cup (grain)
- B = Cup and wet sample (gram)
- C = Dried cup (grain)

**Ask level**

Ask level was analyzed according to Apriantono et al. (1989). Porcelain cup was burned in 650 °C for 1 hour, after the temperature fallen about 200 °C. The cup was cold in desiccators for 30 minutes and measured (A gram). Then the

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cup was measured by homogenate sample (B gram), at 5 gram. The cup and the sample are put in fireplace under temperature increasing gradually until 650 °C. The white ash produced was get out. After that, the fireplace was fallen in 200 °C. The cup was cold for 30 minutes and measured the weight (C gram).

$$\% \text{ Ash} = \frac{C-A}{B-A} \times 100\% \dots\dots\dots (2)$$

Where; A = Cup (gram)  
B = Cup and wet sample (gram)  
C = Dried cup (gram)

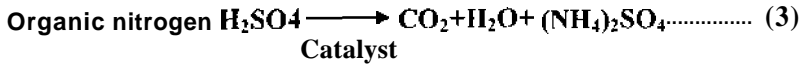
**Protein level**

Protein level was analyzed according to Apriantono *et al.* (1989)

a. Destruction

The sample was measured at 0.3 gram and put in a kjeltec tube. A kjeltec tablet was put in the tube; the tablet was used to catalisator.

**The reaction:**



Mercury (Hg); Ag and Selenium (Se) were usually used to catalyst matter kjeltec tablet consist of K<sub>2</sub>SO<sub>4</sub> and Se, after that destruction was done until the color of liquid changes to clear.

b. Distillation

The cup of result of destruction was cold and put to the filter cup, than it was diluted with 200 ml of water, not contain nitrogen, added some boiled stones and 100 ml of NaOH in order that the solution became base. The filter cup was put above filter fast. The process went on until nitrogen was caught by H<sub>2</sub>SO<sub>4</sub>, which is in the erlenmeyer or if 2/3 part of the liquid in the cup steamed.

HCl was added to a buret, done titration until the color of liquid in erlenmeyer changes to reddish, noted the volume of HCl

$$\% \text{ N} = \frac{14.01 \times (A-B) \times C}{D} \dots\dots\dots (4)$$

% Protein = % Nitrogen x conversion factor (6.25),  
Where A = titration (ml) C = Molarities of standard acid  
B = Blanko titration (ml) D = Sample (mg)

### **Fat level**

Fat level was analyzed according to Apriantono *et al.* (1989). The sample was measured at 3 gram (W1), than covered with filter paper in the up part while down part was given free fat cotton then prepared fat cup which was know the weight (W2) and attached with soxhlet tube. The tube was put in the extractor of soxhlet tube, then **sprayed** with fat dissolved (petroleum benzene), after that **extraction** was done for 16 hours in 40 °C. **After the** extraction had fished, the tube brought out. The fat dissolver, who is in fat cup, was **distilled** until all of the fat dissolver steamed. and then the fat cup **was dried** in oven at 105 °C for 3-5 hours. The cold fat cup was measured in desiccators until constant weight (W3).

$$\% \text{ Fat} = \frac{W3-W2}{W1} \times 100 \% \dots\dots\dots (5)$$

### **Carbohydrate level**

Carbohydrate level was analyzed according to Winamo, (1992) Carbohydrate level was reached **by** calculating the difference of 4 components water level, protein, fat and ask. The measurement is;

$$\% \text{ Carbohydrate} = 100\% - (\% \text{Water} + \% \text{Fat} + \% \text{Protein} + \% \text{Ask})$$

## **2. Amino and Analyses**

Amino acid level was analyzed according to Adijuna (1984). The composition of amino acid was **examined** by using HPLC. The kind of HPLC used in this research is HPLC water with **the principle of amino acid separation** based on acid base, moreover it used pico tag amino acids water color. The **process** used Na-acetate: asetonitril (60:40) as movement phase and trimetil xylena as **quite** phase. The completing explanations are:

### **a. Acid hydrolysis**

The sample at ± 0.25 gr was measured in closed tube reaction then added 5 ml HCl 6N and blown by N2 gas and closed. After that, the **sample was put** in oven at 100 °C for 18-24 hours, the liquid sample was filtered by using fitter paper.

### **b. Drying**

Sample liquid, resulted for hydrolysis process, was **taken** at 10 l to reaction tube and added at 30 l drying liquid (methanol: Na-acetate : tri etil acetate = 2:2:1). After that, the matter by vacuum **pump** having pressure 50 torr (3x)

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### c. Derivatisation

Derivat liquid (metanol: trimetil asetat, penilisati asianat = 7:1:1). The dried sample was added at 30  $\mu$ l, and then it was ignored for about 20 minutes, dried by vacuum pump, 50 torr pressure. After that, the dried sample was diluted by 200  $\mu$ l diluter liquid (Na-acetate 1 M) and gained sample liquid which is ready to be analyzed.

### d. Amino Acid analysis by HPLC

The condition of HPLC when analysis occurred is:

1. Color temperature : 38°C
2. Color : picco tag 3.9 x 150
3. Water speed : 1.5 ml/minutes
4. Pressure : 3000 psi
5. Program : Gradient
6. Movement face : - Asetonitril 60%  
- Buffer N
7. Detector : UV
8. The length of wave : 254 nm

The percentage of amino acid in 100 grams deep sea can be measured by

$$\% \text{ Amino acid} = \frac{\text{The width of sample area} \times \text{standard concentration} \times 5 \text{ ml} \times \text{BMA} \times 100 \dots (6)}{\text{Standard area} \times \text{weight sample}}$$

BMA = the weight of amino acid molecule

### 3. Steroid Hormone Test

Steroid extraction was done based on a method, reported by Touchtone and Kasparov (1970) referred by Riris (1994). The amount of deep sea at 20 grams, homogenized by blender was added by 45 ml of cold acetone, and then saved for 24 hours in cold room at 40 °C, centrifuged in 500 rpm for 10 minutes. The obtained deposit was separated from the liquid phase. The liquid phase was steamed in water heater at 40 °C. The obtained residue was estated 2 times in etil acetate, chloroform and water (1:1:1) by using separation tube so at makes 2 layers, extraction liquid solution down layer is chloroform and up layer is etil acetate was steamed in water heater at 4 °C until dry. The extract was used to identify steroid.

Steroid identification was done by Liebennan Burchard Test-Addition of some acetate anhydrate acid and 0,5 ml chloroform to a little extract of deep sea- then stirred it was added a drop of sulfate. The green color showed that the extract contained steroid (Cook (1958) referred by Riris (1994).

**111. RESULTS AND DISCUSSION**

**1. Proximate Test**

Result of proximate analysis of deep sea fish shows that there are differences of nutrient for each deep sea fish. It depends on kind of biota species, age and the conduction at living area (Zaitsev *et al.* 1969 cited by Septarina 1999).

**Table 1.** The result of proximate analysis

No	Sample code	Analysis Result				
		Water	Ash	Fat	Protein	Carbohydrate
1	A1	70,4	1,9	2,9	23,1	1,7
2	A2	70,9	2,0	2,6	24,5	0
3	A3	72,0	1,9	2,1	23,0	1
4	A4	70,4	2,1	4,1	23,4	0
5	A5	70,1	2,4	2,7	24,8	0
6	A6	71,2	2,1	2,9	23,2	0
7	A7	70,6	2,2	3,6	23,6	0
8	A9	71,9	1,7	2,1	23,1	1,2
9	A10	72,1	2,1	1,9	23,4	0,5
10	A11	72,1	2,2	2,4	23,3	0

**Explanations.**

A1 : *Dietmoides pauciradiatus*

A2 : *Benthodesmus tenuis*

A3 : *Beryx splendens*

A4 : *Hoplostethus crassipinus*

A5 : *Hoplostethus* sp

A6 : *Ophidiidae*

A7 : *Ostracoberyu dorygenis*

A8 : *Godamus colleti*

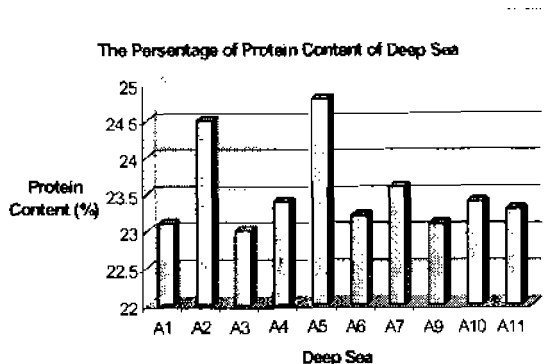
A10 : *Myctophidae* sp

A11 : *Hyteroglypne japonica*

**Protein level**

Figure 1 shows that the content of deep sea fish's protein varies from 23.0 to 24.8%. Based on Stanby & Olcott 1963 cited by Santoso 1998, deep sea fish's protein is in high level at protein and low at fat, it comparing with pelagic fish, the content of deep sea fish's protein is higher than that at pelagic fish,

It shows in Table 2 that *Hoplostethus crassipinus* contain the highest at protein of 27.4% compared with other researched deep sea fish.



**Explanations.**

- |                                      |                                    |
|--------------------------------------|------------------------------------|
| A1 : <i>Dietmoides pauciradiatus</i> | A6 : <i>Ophidiidae</i>             |
| A2 : <i>Benthodesmus tenuis</i>      | A7 : <i>Ostracoberyu dorygenis</i> |
| A3 : <i>Beryx splendens</i>          | A9 : <i>Godamus colleti</i>        |
| A4 : <i>Hoplostethus crassipinus</i> | A10 : <i>Myctophidae sp</i>        |
| A5 : <i>Hoplostethus sp</i>          | A11 : <i>Hyteroglypne japonica</i> |

**Figure 1.** Percentage Histogram of Deep Sea protein

**Table 2.** Composition nutrient some pelagic fish in 100 g RDD

Name of Fish	Protein Content (%)	Fat Content (%)	Ash Content (%)	Water Content (%)	Carbohydrate Content (%)
Lemuru <sup>a</sup>	20.00	3.00	1.00	76.00	0
Teri <sup>a</sup>	16.00	1.00	3.00	80.00	0
	19.90	2.70	1.20	76.20	0
Ten giri	18.50	2.70	1.40	77.40	5.13
Tuna <sup>c</sup>	22.00	1.01	1.30	70.56	0.27
Hiu <sup>c</sup>	20.98	4.51	1.39	72.85	0

Sources : a. Hardiansyah and D Brawan (1994)

b. FAO (1972)      c. Riana (2000)

**Fat level**

Fat level of some deep sea fish was in average of 1.9-4.1%, it is relatively higher than that of both pelagic fish and fresh water fish. However this difference in fat level is not clearly significant since fat level of deep sea fish was categorized low one (Stanby & Olcott 1963).

The content of deep sea fish's fat level, was shown in figure 2.

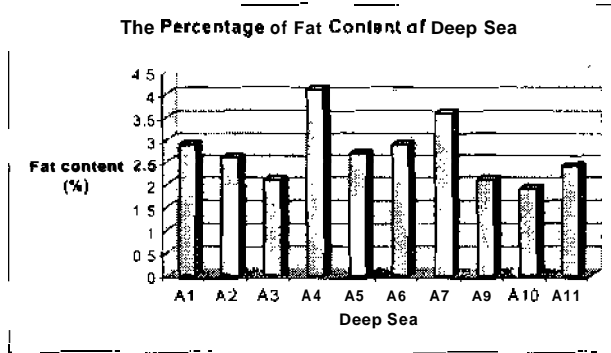


Figure 2. Histogram of percentage of deep sea fish's fat level

### Water level

Deep sea has high salinity with value 31.2 ‰ (Karleskint, 1998). The high rate of inorganic salt cause hypertonic. To maintain the condition of body is isotonic deep sea organism enters solution to their body for adapting. The condition causes water level of deep sea fish is less than of fresh water fish and pelagic fish.

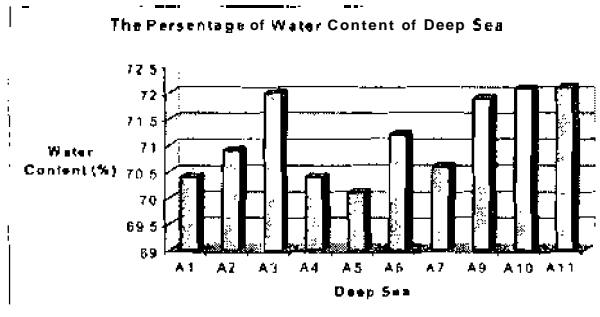


Figure 3. Histogram of percentage of deep sea fish's water level



**2. Amino Acid Test**

**Table 3. Amino acid content (%) of deep Sea Fish**

NO	Analisis Result	Kode Sample									
		A1	A2	A3	A4	A5	A6	A7	A9	A10	A11
1	Aspartat Acid	0,308	0,526	0,348	0,308	0,449	0,467	0,501	0,320	0,289	0,649
2	Glutamat Acid	0,530	0,650	0,692	0,530	0,678	0,795	0,745	0,662	0,515	0,815
3	Serin	0,184	0,208	0,220	0,184	0,339	0,310	0,316	0,284	0,232	0,416
4	Glisin	0,130	0,108	0,150	0,130	0,121	0,171	0,166	0,168	0,156	0,309
5	Histidin	0,146	0,170	0,232	0,146	0,126	0,158	0,158	0,160	0,182	0,284
6	Arginin	0,532	0,470	0,572	0,532	0,461	0,647	0,495	0,508	0,525	0,780
7	Threonin	0,360	0,254	0,328	0,360	0,273	0,247	0,304	0,336	0,241	0,503
8	Alanin	0,380	0,456	0,534	0,380	0,376	0,339	0,355	0,462	0,359	0,512
9	Prolin	0,604	0,408	0,500	0,604	0,506	0,478	0,415	0,574	0,431	0,579
10	Tirosin	0,270	0,384	0,680	0,270	0,550	0,495	0,471	0,518	0,568	0,474
11	Valin	0,420	0,388	0,414	0,420	0,261	0,603	0,363	0,412	0,570	0,534
12	Methionin	0,172	0,212	0,194	0,172	0,175	0,191	0,288	0,186	0,302	0,298
13	Sistein	0,250	0,194	0,238	0,250	0,213	0,494	0,249	0,244	0,152	0,192
14	Isoleusin	0,278	0,220	0,278	0,278	0,245	0,201	0,431	0,300	0,235	0,776
15	Leusin	0,680	0,960	1,068	0,680	1,036	1,174	0,856	0,970	0,812	1,109
16	Fenilalanin	0,260	0,968	0,924	0,260	0,730	0,630	0,437	1,032	0,613	0,899
17	Lisin	0,230	0,360	0,248	0,230	0,161	0,250	0,125	0,236	0,217	0,449

note:

- A1 : *Dietmoides pauciradiatus*
- A2 : *Benthodesmus tenuis*
- A3 : *Beryx splendens*
- A4 : *Hoplostethus crassipinus*
- A5 : *Hoplothethus sp*

- A6 : Ophidiidae
- A7 : *Ostracoberyu dorygenis*
- A9 : *Godamus colleti*
- A10 : Myctophidae sp
- A11 : *Hyteroglypne japonica*

Marine fish have 17 important amino acids which are required by human body. Nine of those are essential amino acid and the other non essential amino acids. Both *Dietinoides pauciradiatus* and *Hoplothethus crassipinus* contain the greatest quantity of amino acid in leusin and prolin. *Benthoides tenuis* and *Beryx splanden* have the highest content of amino acids in leusin and phenilalanin. Mean while, *Holoplethus sp*, *Myctophidae sp* and *Hyteroglypne japonica* have the highest content of amino acid in leusin and phenilalanin. Ophidiidac and *Ostracoberyu dorygenis* have highest in glutamat and leusin.

From all of deep sea fish analyzed, it seems that leusin is dominant in the quantity among all kinds of amino acid found in those fish. Leusin is an essential amino acid and include in hatogenic, producing ketone in hearth. Other amino acids included this category is lisin and tripthopan (Lehninger 1994). The function of this amino acid in as important biochemical component, needed by body - to produced energy, then to stimulate up part of brain and keep body for reflection (<http://www.realtime.net/anr/aminoacd.html>).

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Arginin having role as aprotidisiach was in great quantity among some deep sea fish, moreover it has potential as material of strong medicine Arginin related with Nitrogen oxidize (NO) enzyme, having role in width of blood.

Ostracoberyu dorygenis and family of ophidiidae were dominated by glutamat acid. Glutamat acid gives mild flavors and delicious smelt, the same as cod, but it was sweeter since glutamate acid is a amino acid, change to glucose and glycogen by metabolism current called glucogenic (Lehninger,1994).

Other ainino acid included alanin, arginin, asparagin, aspartat acid, sistein, glutamin, glisin, Prolin dominated in Dietnoides pauciradiatus and Hyteroglypne japonica. They are gloucogenic ainino acid.

The kinds of amino acids which are combination of ketogenic and glucogenic are fenilalanin and tirosin. The domination of fenilalanin was dominate in Benthodesmus tenuis, Godamus colleti Beryx splendens, Myctophidae sp, Hoplothethus sp, Hyteroglypne japonica.

### 3. Steroid Hormone Test

Table 3. Steroid hormone composition

NO	Fish Name	Test Result (Etil Asetat)	Test Result (Chloroform)
1	Dietnoides pauciradiatus	(-)	(+)(+)
2	Benthodesmus tenuis	(-)	(+)(+)
3	Beryx splendens	(-)	(+)
4	Hoplothethus crassipinus	(-)	(+)(+)
5	Hoplothethus sp	(-)	(+)
6	Ophidiidae	(-)	(+)
7	Ostracoberyu dorygenis	(-)	(+)
8	Godamus colleti	(-)	(+)

The result of steroid hormone analysis

(+): There is steroid hormone in deep sea fish

(-): There isn't steroid hormone in deep sea fish

Steroid hormone is hormone containing steroid nucleolus. Aphrodisiac is matter enlarging libido. Steroid hormone is one of the matters having function as aphrodisiac.

Liebmann Burchard Test is used to analyze cholesterol in which sample was deleted in chloroform and mixed by sulfate acid and acetate glacial. The positive result was shown by the changing of the color from red to violet, blue to green in some minutes. This test was not specific for cholesterol, but for other sterol such as stignasterol and ergo sterol which will give positive respond (Dence, 1980).

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The result on the table shows that deep sea fishes analysis has steroid content. It was seen in Liebenmann Burchard Test, extract of isolation using chloroform gave positive result, changing extract's color to green. and ergosterol. The color of Fish giving great effect was shown in *Dietmoides pauciradiatus*, *Benthodesmus tenuis* and *Hoplostethus crassipinus*. The changing extract's color to green shows the existence of sterol-stigma sterol organic indicate the existence of two or more double chain of carbon. While extract isolated by using ethyl acetate did not show the changing of color.

Steroid is white solid crystal. Steroid has some shapes such as little needle, leaf, plate and amorf. It depends on kind of solution used in crystallization, skill and the luck of chemistry expert. Since steroid has 17 or more carbon atoms, so this component tend to undelated in water (Wilson & Gisvold, 1982). Ethyl acetate mixed water has possibility the steroid which is not restricted, caused the polarity of ethyl acetate is higher than chlorofonn, including non polar.

The color of mix which does not change is predicted by the existence of saturated steroid i.e. chonestanol. It is caused the saturated steroid i.e. chonestanol shows negative result toward Liebenmann Burchad test (Dence, 1980).

The peak which easy to be observed is 6 conjugated ketone ring in  $1670\text{ cm}^{-1}$ , asetilenik C-H close to  $3000\text{ cm}^{-1}$ . From the infra red (IR) analyze, it shows that doubled C=C chain appears as the weak peak which close to  $2000\text{ cm}^{-1}$  and vibration C=C is the peak of medium intensity above  $1600\text{ cm}^{-1}$ . The confusion between carbonyl peak and siklohexane peak in  $1670\text{ cm}^{-1}$  seldom occur, it is caused the carbonyl having one of the strongest spectrum. The strong chain of  $1250\text{ cm}^{-1}$  gives respond to C-O from D ring relation.

From the infra red analyze, it can be see that *Dietmoides pauciradiatus*, *Benthodesmus tenuis*, and *Hoplostethus crassipinus* show positive result toward IR test. All of the peak are expected showing nearness to the existence of steroid. Meanwhile *Beryx splendens*, *Hoplothethus sp.*, *Ophidiidae*, *Ostracoberyu doryge* *Godamus colleti nis*, show negative result to the existence of siklohexane. It shows the positive correlation between Liebenmann Rurchad test and Infra red spectrometry test. The strongest existence of steroid is in *Dictmoides pauciradiatus*, *Benthodesmus tenuis* and *Hoplostethus crassipinus*.

## IV. CONCLUSION

Some deep sea fish content proximate, protein between 23,0 and 24,8%. It was higher than pelagic fish. The highest content is in *Hoplothethus sp.*, at 27.4%. In amino acid test, deep sea fish had 17 amino acids, 9 of which essential amino acid and the others were non essential amino acid. Leusin and arginin dominated in the quantity some deep sea fish. Meanwhile steroid hormone test using

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Lieberten Burchard, 8 kinds of deep sea fish content steroid and the greatest concentration is in *Dietmoides pauciradiatus* and *Hoplostethus crassipinus*

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