NUTRIENT AND STEROID CONTENTS OF SOME DEEP SEA FISH SPESIES FROM WESTERN SUMATERA, EASTERN INDIAN 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 western Sumatera of the IndianOcean. A number of 11 fish species was examired in this work. Those were species *Diretmoides pauciradiatus, Benthodesmus tenuis, Beryx splendens, Hoplosthethus crasispinus, Hoplothethus* sp., *Ophidiidae* sp1., *Ostracoberyx dorygenis, Godamus colleti,* and *Hyteroglype japonica* were examided. Results show that protein content ranged from 23.0 to 24.8%, fat 1.9 to 4.1%, carbohydrate 0 to 1.75, ash 1.7 to 2.4%, water 70.1 to 72.1%. In amino acid test, 17 amino acids (9 essential amino acids and 8 non essential amino acids) were identified. By using Libermann Burchad test, the steroid contents were found in 8 species of deep sea fish with the greatest concentration occurred in *Diretmoides pauciradiatus, Benthodesmus tenuis, Beryx splendens,* and *Hoplosthethus crasispinus.*

KEYWORDS: **nutrient, steroid content, deep sea fish, Indian Ocean**

INTRODUCTION

Based on the total prediction of Indonesian marine fishery potential which was about 6.6 million tonnes per year, originated from the area of 5 million sq.km territorial waters and 2,1 sq.km EEZ waters. These potentials include all kinds of marine fish, for example the small pelagic fish 3.5 million tonnes, coral fish 0.048 million tonnes per year (Anonymous, 2000).

According to the potential estimation, production and utilization of pelagic fish in Indonesia in 2001, the Malaka Strait and the Java Sea are considered in the state of over fishing (Anonymous, 2001) therefore, in search of new fishing ground instead of the coastal shallow waters area and pelagic area, deep sea region as part of ocean environment provide one of the alternatives area.

Deep sea area is definitely located below the euphotic zone in the open sea deeper than continental shelf (>200 m). These zones provide the widest habitat in the world ocean where rare organisms are found. The water volume is predicted of amount of 85 or 70% of the world surface (Nybakken, 1992).

Some of the rare fishes, however provide an important food source and often looked for by some people in the markets. In Europe, deep sea fish (lunglip) 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 found in restaurant in the form of good quality and unique meat texture. Gold kinglip, red, and black are marketed internationally, but USA preferably the gold and red kinglip (Perkins, 1992). In Australia, deep sea fish (*Beryx splendens*) has been intensively exploited, even nearly over fishing (Anonymous 2004). Soselia & Rustam (1993) reported that *Cubiceps whiteleggi* is one of the economically important fish in the future, eventhough in Indonesia deep sea fish is not utilized optimally yet.

From the result of Baruna Jaya IV expedition hold by the Agency for Marine and Fisheries Research Ministry of Marine Affairs and Fisheries about 529 species of deep sea fish were found. Some of those fishes are probably contain a certain bioactive substances. Based on this information, the current work tried to analyze the content of some bioactive substances of some fish collected from the waters of western Sumatera, Eastern Indian Ocean.

MATERIALS AND METHODS

Fish materials used in this work consisted of 10 species of deep sea fish, such as *Dietmoides pauciradiatus*, *Benthodesmus tenuis, Beryx splendens, Hoplosthethus crassipinus, Hoplothethus* sp., Ophidiidae sp1. *Ostracoberyx dorygenis, Godamus colleti, Myctophidae* sp., and *Hyteroglypne japonica.* The work is divided in 3 parts, by including 1) proximate test; 2) Amino acid test; and 3) Steroid test.

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Proximate Test

Proximate analysis was done to include protein, fat, water, ash, carbohydrate and water level (Apriantono *et al.*, 1989).

Porcelain cup was dried in temperature from 102 to 105°C, for approximately 10 to 12 hours. Then, the cup was put in desiccator (± 30 minutes), and weighted as A gram. The cup with homogenated was weighted again as B gram. The amount of 5 g, was placed in the cup and weighted as C gram.

Measurement:

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

where:
A = cup (g)
B = cup and wet sample (g)
C = dried cup (g)

Ash Level

Ash level was analyzed according to Apriantono *et al.* (1989). Porcelain cup was burned in 650°C for 1 hour, waited until the temperature fall to about 200°C. The cup was put in cool desiccators for 30 minutes and measured (A gram). Then the cup was measured by homogenated sample (B gram), at 5 g. The cup and the sample are put in fireplace under increasing temperature gradually until 650°C. The white ash produced was taken out. Then, the fireplace was cool until 200°C. The cup was cool aerated for 30 minutes and measured the weight (C gram).

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

where:
A = cup (g)
B = cup and wet sample (g)
C = dried cup (g)

Protein Level

Protein level was analyzed following the procedure explain by Apriantono *et al.* (1989).

a. Destruction

The sample was measured of 0.3 g put in a kjeltec tube. A kjeltec tablet was put in the tube, the tablet was used as catalisator.

The chemical reaction will be:

$$\text{Organic nitrogen } \xrightarrow[\text{Catalyst}]{H_2SO4} CO_2 + H_2O + (NH_4)_2SO_4 \quad \ldots. \quad (3$$

Mercury (Hg); Ag and Selenium (Se) were used as a catalyst kjeltec tablet consist of K_2SO_4 and Se. The destruction prcesses was done until the color of liquid changes to clear.

b. Distillation

The cup from the result of destruction was cool aerated and put to the filter cup, than diluted with 200 ml of water, not contain nitrogen, added some boiled stones and 100 ml of NaOH in order the solution became base. The filter cup was put above filter fast. The process went on until nitrogen was caught by H_2SO_4, which is in the erlenmeyer or if 2/3 part of the legend in the cup steamed.

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

$$\%N = \frac{14,01x(A-B)xC}{D} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (4$$

% 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 following Apriantono *et al.* (1989). The sample was measured at 3 g (WI), then covered with filter paper in the upper part while a free fat cotton laid in the bottom. Prepare a fat cup and weighted as (W2) and attached into a soxhlet tube. The tube was put in the extractor of soxhlet tube, then sprayed with fat dissolved (petroleum benzene), follow by the extraction process for 16 hours in 40°C. After the extraction finished, the tube was taken out. The fat dissolver which was in the fat cup distillated until all of the fat dissolver steamed. Then the fat cup was dried in oven at 105°C for 3 to 5 hours. The cool fat cup was weighted in desiccator until constant weight (W3).

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

Carbohydrate Level

Carbohydrate level was analyzed following Winarno, (1992). Carbohydrate level will be obtained by deducting the 100% with the total of the four components of water level, protein, fat, and ash. So that:

% Carbohydrate=100%-(%Water+%Fat+ %Protein+%Ash)

Amino acid Analyses

Amino acid level was analyzed according to Nur & Adijuwana (1989). 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 and used a pico tag amino acids water color. The process used Na-acetate:asetonitril (60:40) as a moving phase and trimetil xylena as quite (non moving) phase.

a. Acid hydrolysis

The sample at ± 0.25 g was measured in a closed reaction tube, added some 5 ml HCl 6N and blown by N2 gas and then closed. Then, the sample was put in oven at 100°C for 18 to 24 hours, the liquid sample was filtered by using filter paper.

b. Drying

The liquid sample resulted from hydrolysis process, was taken at 10 µl to reaction tube and added at 30 µl drying liquid (methanol:Na-acetate:tri etil acetate = 2:2:1). After that, press the matter by vacuum pump at about 50 torr (3x).

c. Derivatisation

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

d. Amino Acid analysis by HPLC

The setting of HPLC when analysis occurred were:

1. Color temperature : 38°C
2. Color : pico tag 3.9 x 150
3. Water speed : 1.5 ml per minutes
4. Pressure : 3,000 psi
5. Program : Gradient
6. Movement phase : - Asetonitril 60%
   - Buffer N
7. Detector : UV
8. The length of wave : 254 nm

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

$$\% \text{ Amino acid} = \frac{\text{The width of sample are} \times \text{standard concentration} \times 5\text{ml} \times \text{BMA} \times 100}{\text{standard area}} \Big/ \text{weight sample} \quad \ldots\ldots (6$$

where:
BMA = the weight of amino acid molecule

Steroid Hormone Test

Steroid extraction was done based on a method, reported by Touchtone & Kasparov (1970) cited by Riris (1994). The deep sea fish flesh of 20 g, homogenized by blender and added with 45 ml of cold acetone, then saved for 24 hours in cold room at 40°C, and 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 twice in etil acetate, chloroform and water (1:1:1) by using separation tube so that become 2 layers. The lower layer of liquid solution is chloroform and the upper layer is etil acetate, then steamed in water heater at 4°C until dry. The extract was used to identify steroid.

Steroid identification was done by Lieberman Burchard Test. Add some acetate anhydrate acid and 0.5 ml chloroform to a little extract of deep sea fish flesh then stirred, and added a drop of sulfate. The green color showed that the extract contained steroid Cook (1958) referred by Riris (1994).

RESULTS AND DISCUSSION

Proximate Test

Result of proximate analysis of deep sea shows that there are some differences of nutrient content for each deep sea fish (Table 1). The differences depend on kind of biota species, age and the condition of living area (Zaitsev *et al.*, 1969 cited by Septarina, 1999).

Table 1. The result of proximate analysis

No	Kode sample	Analisis 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	A8	71.9	1.7	2.1	23.1	1.2
9	A9	72.1	2.1	1.9	23.4	0.5
10	A10	72.1	2.2	2.4	23.3	0

Remarks: A1 = *Dietmoides pauciradiatus*; A2 = *Benthodesmus tenuis*; A3 = *Beryx splendens*; A4 = *Hoplosthethus crassispinus*; A5 = *Hoplothethus* sp.; A6 = Ophidiidae; A7 = *Ostracoberyx dorygenis*; A8 = *Godamus colleti*; A9 = *Myctophidae* sp.; A10 = *Hyteroglypne japonica*

Protein Level

Figure 1, shows the content of deep sea fish flesh protein varied from 23.0 to 24,8%. Based on Stanby & Olcott 1963 cited by Santoso, 1998, deep sea fish's protein content is in higher and low in fat compare with pelagic fish.

As shows in Table 2, *Holoplothetus crassispinus* contain the highest at protein of 27,4% compared with other observed deep sea fish.

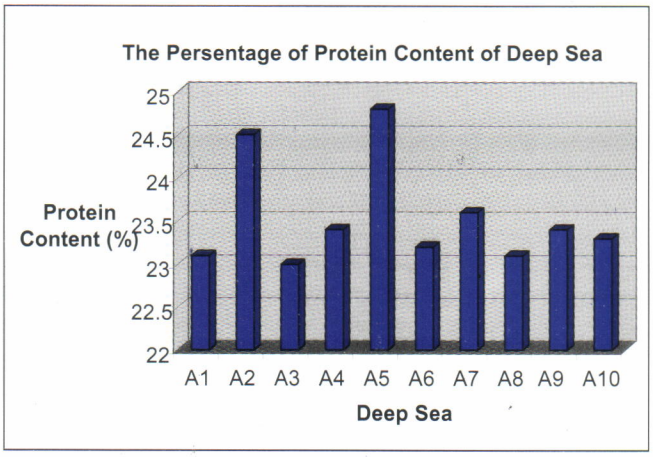

Figure1. Persentage histogram of deep sea protein.

Table 2. Composition nutrient some pelagic fish in 100 g BDD

Name of fish	Protein content (%)	Fat content (%)	Ash content (%)	Water content (%)	Carbohidrate content (%)
Sardines[a)]	20,00	3,00	1,00	76,00	0
Anchoviesi[a)]	16,00	1,00	3,00	80,00	0
Trevallies[b)]	19,90	2,70	1,20	76,20	0
King mackerel[b)]	18,50	2,70	1,40	77,40	5,13
Tuna[c)]	22,00	1,01	1,30	70,56	0,27
Sharks[c)]	20,98	4,51	1,39	72,85	0

Sources: [a)] Hardiansyah & D. Briawan (1994); [b)] FAO (1972); [c)] Riana (2000)

Fat Level

Fat level of some deep sea fish was in the range of 1.9 to 4.1%. These were relatively higher than that of both pelagic fish and fresh water fish. However this difference in fat level is not cleary significant since fat level of the deep sea fish was categorized as low (Stanby & Olcott, 1963) in Santoso, J. 1998. The content of deep sea fish's fat level, was shown in Figure 2.

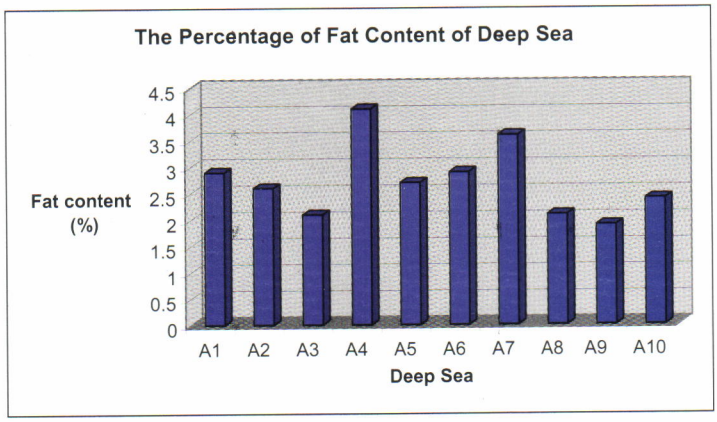

Figure 2. Histogram of percentage of deep sea fish's fat level.

Water Level

Salinity of deep sea water of 34.2$^{0}/_{00}$ was relatively high (Karleskint, 1998). The high rate of inorganic salt cause hypertonic. To maintain the condition of body is isotonic deep sea organism enter solution to their body for adaptation. The condition causes water level of deep sea fish is less than of fresh water fish and pelagic fish.

Amino Acid Test

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 *Diretmoides pauciradiatus* and *Hoplothethus crassispinus* contain the greatest quantity of amino acid in the form of leusin and prolin. *Benthodesmus tenuis* and *Beryx splenden* have the highest content of amino acids in the form of leusin and phenilalanin, followed by *Hoplostethus* sp., *Myctophidae* sp., and *Hyteroglypne japonica* that also have the highest content of amino acid in leusin and phenilalanin. Ophidiidae and *Ostracoberyx dorygenis* have highest contents in glutamat and leusin (Table 3).

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 ketogenic, producing ketone in hearth. Other amino acids included in this catagory are lisin and tripthopan (Lehninger 1994). The function of this amino acid is important biochemical component, needed by body to produced energy, then to stimulate the upper part of brain and keep body for reflection (Anonimous, 2005).

Arginin that having role as *aprodisiach* was in great quantity among some deep sea fish. It has potential as a material of a strong medicine Arginin related with Nitrogen oxidase (NO) enzyme, having role in width of blood vein.

In *Ostracoberyx dorygenis* and Ophidiidae sp.1, amino acids were dominated by glutamic acid. Glutamic acid causes mild flavors and delicious smelt, similar with cod, but it was sweater since that glutamic acid is an amino acids will change to glucose and glycogen by metabolism process called glucogenic (Lehninger, 1994).

Other amino acid including alanin, arginin, asparagin, aspartic acid, sistein, glutamin, glisin, and prolin were dominantly found in *Diretmoides pauciradiatus* and *Hyteroglypne japonica.* These are glucogenic amino acid.

The kinds of amino acids which are combination of ketogenic and glucogenic are fenilalanin and tyrosin. The abundance of fenilalanin were found in *Benthodesmus tenuis, Godamus colleti Beryx splendens, Myctophidae* sp., *Hoplosthethus* sp.,and *Hyteroglypne japonica.*

Table 3. Amino acid content (%) of deep sea fish

No.	Analisis result	Kode sample									
		A1	A2	A3	A4	A5	A6	A7	A8	A9	A10
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

Remarks: A1 = *Diretmoides pauciradiatus*; A2 = *Benthodesmus tenuis*; A3 = *Beryx splendens*; A4 = *Hoplosthethus crassipinus*; A5 = *Hoplothethus* sp.; A6 = Ophidiidae; A7 = *Ostracoberyx dorygenis*; A8 = *Godamus colleti*; A9 = *Myctophidae* sp.; A10 = *Hyteroglypne japonica*

Steroid Hormon Test

Steroid hormone is a hormone containing steroid nucleolus. Aphrodisiac is a stimulating and healthy sexual function. Steroid hormone is one of the biochemical matters having function as aphrodisiac.

Lieberman Burchard Test is used to analyze cholesterol in which sample was diluted in chloroform and mixed with sulfate acid and acetate glacial. The positive result was indicated by the changing of the colour from red to violet and blue to green in some minutes. This test was not only specific for cholesterol, but also for other sterol such as stignasterol and ergosterol which will give positive response (Dence, 1980).

Result of analysis shows that deep sea fishes have steroid content. It was seen in Lieberman Burchard Test, extract of isolation using chloroform gave positive result, changing extract's color to green in ergosterol. The color that giving great effect shown in *Diretmoides pauciradiatus, Benthodesmus tenuis,* and *Hoplosthethus crassispinus.* The changing extract's color to green shows the existence of sterol stigma, and 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 and has some different shapes such as little needle, leaf, plate and amorf. It depends on kind of solution used in crystallization, skill, and the luck of the practition. Since steroid has 17 or more carbon atoms, these component tend to undiluted in water (Wilson & Gisvold, 1982). Ethyl acetate mixed water making the steroid has not restricted possibility, caused the polarity of ethyl acetate is higher than chloroform, 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 Liebermenn Burchad test (Dence, 1980).

The peak which easy to be observed is 6 conjugated ketone ring in 1,670 cm^{-1}, asetilenik C-H close to 3,000 cm^{-1}. From the infra red analyze, it shows that doubled C=C chain appears as the weak peak which close to 2,000 cm^{-1} and vibration C=C is the peak of medium intensity above 1,600 cm^{-1}. The confusion between carbonyl peak and siklohexsane peak in 1,670 cm^{-1} seldom occur, it is caused the carbonyl having one of the strongest spectrum. The strong chain of 1,250 cm^{-1} gives respond to C-O from D ring relation.

From the infra red analysis, it can be seen that *Diretmoides pauciradiatus, Benthodesmus tenuis,* and *Hoplosthethus crassipinus* show positive result toward the test (Table 4). All of the peaks are expected showing nearness to the existence of steroid. While *Beryx splendens, Hoplothethus* sp., *Ophidiidae,* and *Ostracoberyx dorygenis, Godamus colleti,* show negative result to the existence of siklohexsane. It shows

Table 4. Steroid hormone composition

No.	Fish name	Test result (Etil asetat)	Test result (Chloroform)
1	*Diretmoides pauciradiatus*	(-)	(+)(+)
2	*Benthodesmus tenuis*	(-)	(+)(+)
3	*Beryx splendens*	(-)	(+)
4	*Hoplosthethus crassipinus*	(-)	(+)(+)
5	*Hoplothethus* sp.	(-)	(+)
6	Ophidiidae	(-)	(+)
7	*Ostracoberyx dorygenis*	(-)	(+)
8	*Godamus colleti*	(-)	(+)

Remarks: The result of steroid hormone analysis: (+) = available; (-) = not available

the positive correlation between Liebermann Burchad test and Infra red spectrometry test. The strongest existence of steroid was in *Diretmoides pauciradiatus, Benthodesmus tenuis,* and *Hoplosthethus crassipinus.*

Conclusion

Some deep sea fish contained proximate protein between 23.0 and 24.8%. This content was higher than of pelagic fish. The highest content is in *Hoplothethus* sp., at 27.4%. In amino acid test, deep sea fish had 17 amino acid, 9 of which were 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 Liebermen Burchard done to the 8 species of deep sea fish showed that the greatest concentration of steroid content were found in *Diretmoides pauciradiatus* and *Hoplosthethus crassispinus.*

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