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Fatty Acid and Amino Acid Composition in *Mene maculata* in The Sea of Maluku

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***Abstract*—**Fish is a kind of food that contains many nutritions, one of those is the long chain of unsaturated fatty acids as omega-3 and omega-6 fatty acids and essential amino acid in enough amount for the necessity of our body. Like pelagic fish that found in the sea of Maluku. This research was done to identify fatty acids and amino acids composition in Moonfish (*M. maculata*) using transesterification reaction steps and Gas Chromatograph-Mass Spectrophotometer (GC-MS) and High-Performance Liquid Chromatography (HPLC). The result showed that fatty acids composition in Moonfish (*M. maculata*) contained tridecanoic acid (2.84%); palmitoleic acid (2.65%); palmitic acid (35.24%); oleic acid (6.2%); stearic acid (14.20%); and 5,8,11,14-eicosatetraenoic acid (1.29%) and 12 amino acids composition that consist of 7 essential amino acids, were leucine, isoleucine, valine, phenylalanine, methionine, lysine, and histidine, and also 5 non-essential amino acid, were tyrosine, glycine, alanine, glutamic acid, and arginine.Thus, these fishes can be used by the people to complete the necessity of essential fatty acid and amino acid.

***Keywords*—**Moonfish (*M. maculata*), fatty acid, amino acid, GC-MS, and HPLC.

I. Introduction

MALUKU is one of the provinces with an area of ocean about 90% has large fishery resources. However, most of the marine resources have not been used optimally by the community, especially for nutrition and health care quality improvement. Marine natural products have long been used as foods, fragrances, pigments, insecticides, medicines etc., knowledge on biochemical composition of any edible organisms is extremely important since the nutritive value is reflected in its biochemical contents [1]. Marine Fish is one of food that contains various nutrients such as amino acid and protein, vitamins, essential minerals, fats, and coenzyme Q_{10}. Amino acids are building blocks for protein. On the basis of needs from the diet for growth, the amino acid was traditionally classified as nutritionally essential (indispensable) or non-essential (indispensable) for fish. Essential amino acid is those that either cannot be synthesized or are inadequately synthesized de novo by animals relative to needs. The amino acids content varies not only from species to species but also from specimen to the specimen and between different tissues. This variation depends on the environmental conditions and the size of the individual species. In addition to, fatty acids in fish oil have a very distinctive character compared to fatty acids from other sources. They consist not only essential fatty acids, but also a significant source of omega-3 fatty acids-especially eicosapentaenoic acid (EPA, C20:5n3) and docosahexaenoic acid (DHA, C22:6n3). There are reports that EPA can prevent heart disease, since it decreases triglycerides and very low-density lipoprotein cholesterol [2], whereas DHA is a primary component of membranes in the brain and possibly delays the onset of Alzheimer's disease [3].Moonfish (*M. maculata*) is a one of pelagic fish that found in the sea of Maluku, but it is less to be consumed because of its little of flesh quantity and also there is a few of nutrition information especially about fatty acid and amino acids component. Hence, the present work was planned to study the proximate composition of M. *maculata* through estimating their major biochemical components such as fatty acid and amino acid.

II. Materials and Methods

The moonfish, *M. maculata* were collected from the landing center of Ambon City, Maluku Province. M. *maculate* is purely marine fish, they were brought to the laboratory; the fish tissue was collected and dried at 55 °C in an oven and used for biochemical analysis. The compositions of the experimental fish samples were determined by using standard methods.

A. Isolation of Oil

The meat of dried fish weighed as much as 100 grams and then wrapped with filter paper shaped sleeve, the top of the sleeve covered with cotton, put in the extractor, and put 300 mL petroleum benzene. The extraction is done until the mixture inside the extractor becomes clear. The extract obtained was then poured into a weighted erlenmeyer, vaporized solvent using a vacuum pump, covered with filter paper, and stored until the remainder of the solvent evaporated and then weighed.

B. Transesterification

For fatty acid analysis, the samples (body tissue) were homogenized with chloroform: methanol (2:1 v/v) mixture and the samples were extracted using the method [5]. Into two three-neck flasks equipped with a magnetic stirrer and a ball cooler were introduced 1.12 grams of M. *maculata* oil, added 20 mL of BF3 15% in methanol, then stirred, and refluxed for 90 min over water bath at 65 ^{o}C. The reflux results were cooled and after cooling added 25 mL of aquades then inserted into the separating funnel, then 25 mL of n-hexane was added, after which it was extracted. After forming two layers, the top layer (methyl ester) (A1) is separated and the bottom layer (B1) is extracted using 20 mL of n-hexane twice to obtain the upper (methyl ester) (A2) and bottom layer (B2) layers. The upper layers (A1 and A2) are combined and then washed with aquades until the pH is neutral and separated. Furthermore, the methyl ester coating is added with anhydrous Na2SO4. The mixture was filtered and the n-hexane was evaporated using a Buchi evaporator, then the result of the methyl ester mixture was analyzed using Gas Chromatography-Mass Spectrometer (Hewlett Packard 5890, USA).

C. Analysis of Amino Acid Components in Acid Hydrolysis

The fat-free M. *maculata* sample was refluxed at 110 ℃ for 12 hours with concentrated H_2SO_4, after which the solution was neutralized by added $Ba(OH)_2$ 4 M slowly, then the solution filtered to a 50 mL limit on the pumpkin measures

D. Analysis of Amino Acid Components in Base Hydrolysis

One gram of fat-free M. *maculata* meat was refluxed at 110 ° C for 12 hours with 10 mL ($Ba(OH)_2$) 4 M, then slowly added H_2SO_4 slowly, until the mixture was neutral (pH = 7) and filtered. The filtrate is accommodated in Erlenmeyer. Further hydrolysis results were used to identify amino acids using HPLC (Merck Hitachi L-7400) [4].

III. Results and Discussion

A. Fatty Acid Analysis

Isolation of Mene *maculata* was done by Soxhlet extraction method using benzene petroleum solvent. After the water content in M. *maculata* is removed which is done before the extraction process, the moisture content is obtained 74,50%. The results obtained from the extraction of 100 g of dried fish meat were 13.94 g (13.94%) dark brown fish oil. Then, 1,12 g of fish oil was used for transesterification using a 15% BF--Methanol catalyst to obtain fatty acid methyl ester and analyzed using Gas Chromatography–Mass Spectroscopy (GC-MS).The result of transesterification of M. *maculata* oil analyzed using GC-MS was obtained by eleven chromatogram peaks as in Fig. 1.

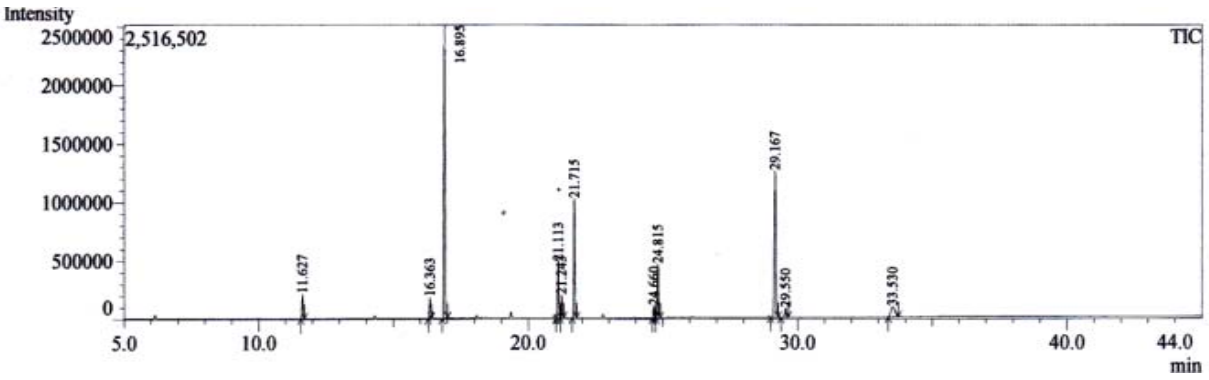


Fig. 1 Chromatogram of transesterification of M. *maculata*

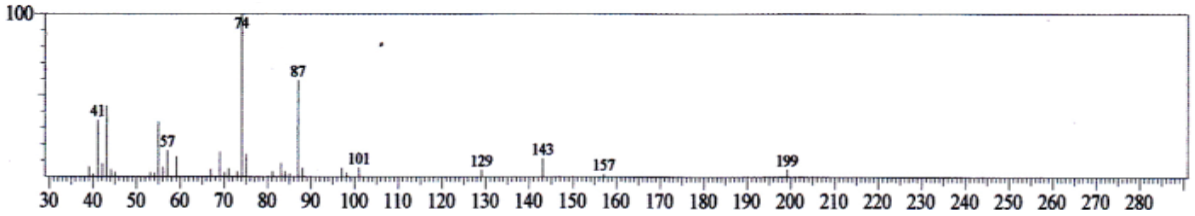


Fig. 2 The mass spectrum of the compound with a retention time of 11.625 minutes

After the identification of the mass spectrum of the dominant peaks, there are six identifiable compounds as presented in Table I.

TABLE I
Transesterification Result of M.Maculata Analyzed Using GC-MS

No	Retention Time (minutes)	Methyl Esters Compounds	Composition (%)
1	11,625	Tridecanoic	2,84
2	16,367	Palmitoleic	2,65
3	16,892	Palmitic	35,24
4	21,117	Oleic	6,82
5	21,717	Stearic	14,20
6	24,658	Methyl-5,8,11,14-eikosatetraenoat	1,29

The peak chromatogram with a retention time of 11.625 minutes gives the mass spectrum as in Fig. 2.

Based on the mass spectrum in Fig. 2, it can be made fragmented as in Fig. 3.

In Figure 3, it is seen that the molecular ion of the methyl ester compound is not present. The presence of saturated bonds is indicated by the appearance of peaks on m/z = 43, 57, 71, and 85, which is an ion series of $CnH_2n + 1+$. While the ion series of CnH_2n-1O2+ for the aliphatic ester appears to peak at m/z = 101, 129, 143, 157, and 199. The peak with m/z = 197 comes from the release of the methoxy group (a) of molecular ions (m/z = 228) indicating the presence of a methyl ester compound. The peak with m/z = 185 comes from the release of the propyl group (b) from the molecular ion. The peak at m/z = 87 comes from the escape of $–C_7H_{14}$ by m/z = 185. The base peak appears at m/z = 74 coming from the $C_3H_6O_2^+$ ion through the McLafferty rearrangement of the fragment β which is the result of breaking of the fragment with m / z = 87 Figure 3 shows that the fragmentation and comparison with the standard

compound, it can be assumed that the compound with a retention time of 11.625 is methyl tridecanoate.

Next will be shown peak chromatogram with a retention time of 11.638 minutes gives the mass spectrum as in Fig. 3.

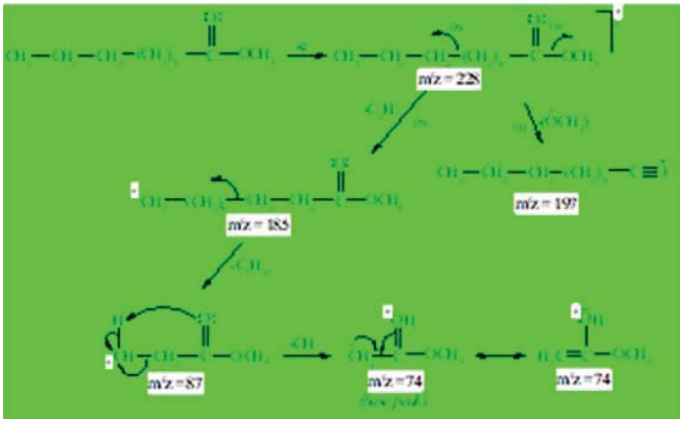


Fig. 3 Fragmentation of the compound with a retention time of 11.625 minutes

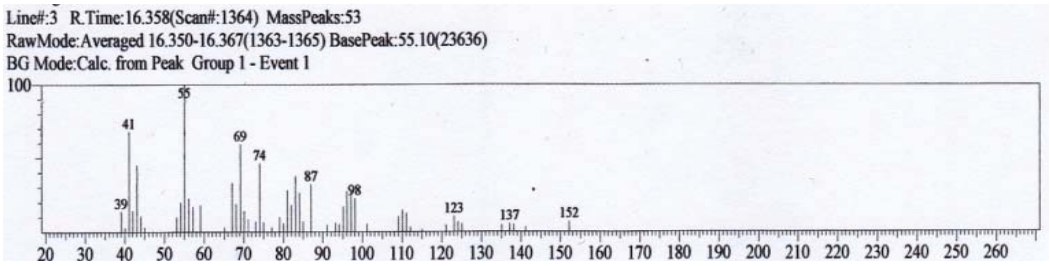


Fig. 4 The mass spectrum of the compound with a retention time of 11.638 minutes

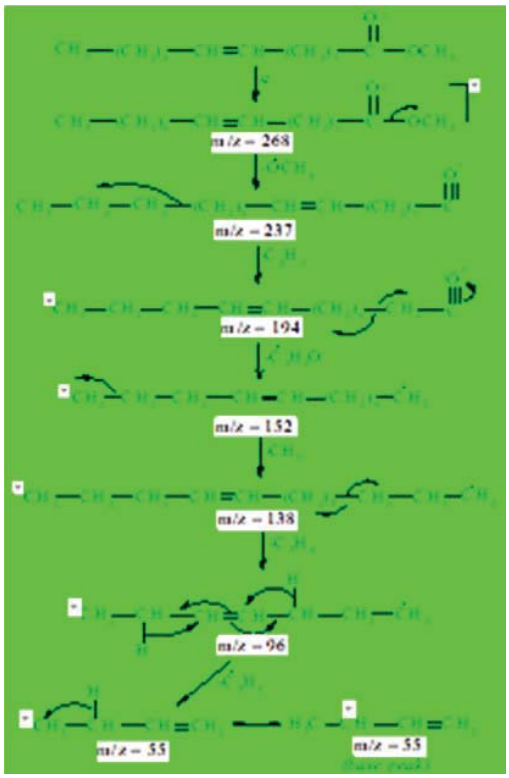


Fig. 5 Fragmentation of the compound with a retention time of 11.638 minutes

Based on this chromatogram in 3, it can be made fragmented as in Fig. 4.

The peak chromatogram with a retention time of 16.367 minutes is methyl palmitoleate. The presence of C=C bond is indicated by the emergence of the CnH2n-1$^+$ a C ion series at the peak of m/z = 41, 55, 69, 83, 97, 111, and 125, followed by a small spill of the CnH2n+1$^+$ peak C=C bond m/z = 43, 57, 71, and 85. In addition to, the presence of one C is also indicated by the appearance of the CnH2n$^+$ ion series at the peak of m / z = 42, 56, 70, 84, 98, and 112. with m / z = 237 derived from $C_{16}H_{29}O^+$ ions resulting from the release of methoxy ($\bullet OCH_3$) groups from molecular ions (m/z = 268) indicating the presence of a methyl ester compound. Fragments with m / z = 194 are derived from the release of the propyl group from the molecular ion. Fragments with m/z = 152 are derived from the release of C_2H_2O radicals from -CH_2- group of–fragments with m / z = 194. With the release of the fragments m/z = 152 yields m/z = 138 and the release of group radicals $\bullet C_3H_6$ of the m/z = 138 produces fragments with m/z = 96. Fragments with m/z = 96 are rearranged and release radicals $\bullet C_3H_5$ resulting in fragments with m/z = 55 which is the base peak.

The results show that six different fatty acids were found. They are three saturated fatty acids (SFA), two monounsaturated fatty acids (MUFA) and one polyunsaturated fatty acids (PUFA) in M. *maculate*. The percentage (%) composition of a fatty acid of *M. maculata* are given in Table II.

TABLE II
THE PERCENTAGE (%) COMPOSITION OF FATTY ACID OF *M. MACULATA*

Fatty Acids		Carbon Atom (n)	% of fatty acid
SFA	Tridecanoic acid	C13:0	02,84
	Palmitic acid	C16:0	35,24
	Stearic acid	C17:0	14,20
MUFA	Palmitoleic acid	C16:1	02,64
	Oleic acid	C18:1	06,82
PUFA	Asam-5,8,11,14 eikosatetraenoat	C:18:1	01,29

Saturated fatty acids (SFA) contained in the M. *maculata* is tridecanoic acid, palmitic acid, and stearic acid. The content of saturated fatty acids is due to the same relative fatty acids are a basic component of the system is the formation of fat in living organisms. The consumption of saturated fatty acids may increase the risk of cancer and disorders of the immune system. Some research also suggests that the dominant effect of the consumption of saturated fatty acids, especially long-chain saturated fatty acids (atom C> 10) is an increase in levels of total cholesterol and LDL-cholesterol (Low-Density Lipoprotein). Consumption of saturated fats causes the liver to produce large amounts of LDL are associated with heart disease and improve cholesterol levels thus allowing atherosclerosis [7]. Besides SFA, M. *maculata* also contains monounsaturated fatty acids (MUFA) i.e.palmitoleic acid and oleic acid. In general, MUFA can lower blood cholesterol levels, especially when used as a substitute for saturated fatty acids. MUFA is more stable and more effective for lowering blood cholesterol levels than plural unsaturated fatty acids (PUFA) so it is more popularly used for processing foodstuffs. MUFA can lower LDL cholesterol and increase HDL-cholesterol [8]. In addition to SFA and MUFA, M. *maculata* also contains plural unsaturated fatty acids (PUFA), namely acid-5,8,11,14-eicosatetraenoate (arachidonic acid). PUFA is found in fish is not a result of synthesis in the body but comes from the food chain that includes phytoplankton, zooplankton, algae, and shellfish (shellfish). The content of PUFA in phytoplankton, zooplankton, and algae, respectively from 15.5 to 26.2%; 0.4 to 14.3%; and 54.8 to 85.8% of the dry weight [9]. In Table III, there is a comparison of the composition of fatty acids in M. *maculata* with results of previous studies that have been done by Edirisinghe, obtained from the waters of Sri Lanka.

TABLE III
COMPARISON OF FATTY ACID COMPOSITION OF M. *MACULATA* WITH RESULTS OF PREVIOUS STUDIES BY EDIRISINGHE

No.	Fatty Acids	% fatty acid	
1.	Tetradecanoic acid	2.84[a]	–
2.	Myristic acid	–	6,00[b]
3.	Pentadekanoat acid	–	0,50[b]
4.	Palmitic acid	35,24[a]	23,9[b]
5.	Heptadekanoat acid	–	1,20[b]
6.	Stearic Acid	14,20[a]	8,00[b]
	Σ SFA	**52,28**	**39.60**
7.	Palmitoleic acid	2,65[a]	6,40[b]
8.	Oleic acid	6,82[a]	12,50[b]
	Σ MUFA	**9,47**	**18,90**
9.	9,12- oktadekadienoat acid	–	0,80[b]
10.	6,9,12,15-oktadekatetraenoat acid	–	0,20[b]
11.	5,8,11,14-eikosatetraenoat acid	1,29[a]	0,60[b]
12.	5,8,11,14,17-eikosapentaenoatacid	–	8,80[b]
13.	7,10,13,16,-dokosatetraenoat acid	–	1,10[b]
14.	7,10,13,16,19-dokosapentaenoat acid	–	2.80
15.	4,7,10,13,16,19-dokosaheksaenoat acid	–	18,30[b]
	Σ PUFA	**1,29**	**28,70**

[a] S Unwakoly
[b]Edirisinghe, *et al* (2004)

In Table III shows that there are some components of fatty acids in the same *M.maculata* namely palmitic acid, stearic acid, palmitoleic acid, oleic acid, and the acid-5,8,11,14-eicosatetraenoate. Meanwhile, myristic acid; pentadecanoic acid; heptadecanoic acid; acid-9,12-octadecadienoate acid-6,9,12,15-oktadecatetranoate; 5,8,11,14,17-eicosapentaenoic acid and docosahexaenoic acid-4,7,10,13,16,19-fatty acids are not obtained from the sea of Maluku.

B. Amino Acid Analysis

The amino acid analysis used HPLC method for acid hydrolysis (Fig. 5) with H_2SO_4 4 M at a 55ºC for 24 hours and bases hydrolysis (Fig. 6) using $Ba(OH)_2$ 4 M with a sample weight of 1 gram.

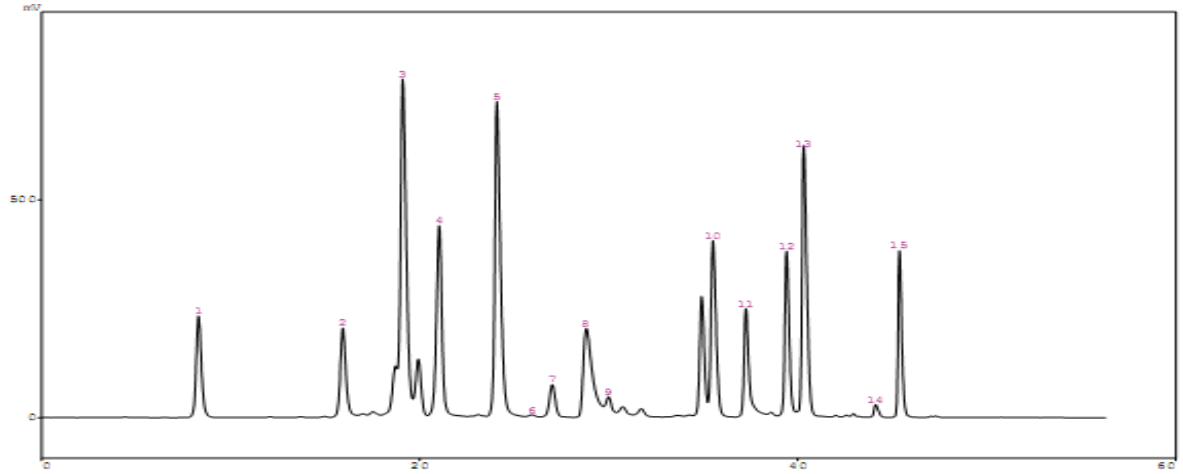


Fig. 6 Chromatogram analyzes the amino acid compositions of acid hydrolysis

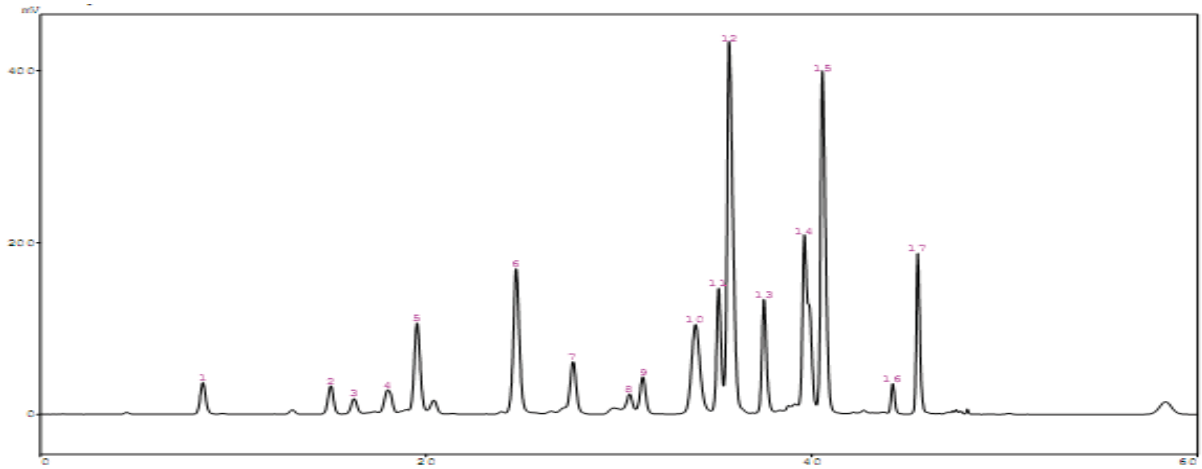


Fig. 7 Chromatogram analyzes the amino acid compositions of bases hydrolysis

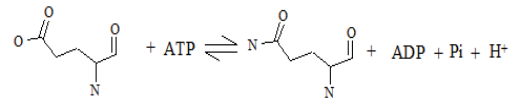
The percentage compositions of essential and non-essential amino acids are presented in Table IV. The total amino acids obtained by acid hydrolysis was 45.0% and 50,0% by base hydrolysis, among which essential amino acids were found to be as 33.3% and nonessential amino acids were 55.6% from acid hydrolysis, while the base hydrolysis obtained 58.3% of the essential amino acids and 30.0% nonessential amino acids.

TABLE IV
THE PERCENTAGE (%) OF AMINO ACID FOUND IN M. *MACULATA*.

Amino Acid	% of amino acid	
	Acid Hydrolysis	Base Hydrolysis
Glutamic acid	4,44	-
Histidine*	-	0,83
Glycine	20,38	5,02
Arginine	8,56	-
Alanine	14,65	8,36
Tyrosine	1,60	3,25
Methionine*	-	5,57
Valine*	11,99	21,06
Phenilalanine*	4,84	5,22
Isoleucine*	10,64	12,79
Leucine*	-	16,27
Lysine *	5,06	5,63

*essential amino acid

The results of amino acid components analysis in table 4, shows that there is a difference between amino acids produced by the acid hydrolysis and base hydrolysis. Of twelve types of amino acids contained in M. *maculata*, glutamic acid and arginine are an amino acid found only in acid hydrolysis, whereas histidine, leucine, and methionine are found only in base hydrolysis. Glutamic acid on acid hydrolysis can be produced glutamine, as in the following reaction:



Glutamic acid Glutamin

Fig. 7 Reaction of produced glutamine from glutamic acid

Acid hydrolysis resulted in some components amino acid such as the tryptophan, glutamine and other amino acids will be damaged and needed further hydrolysis to restore all of the amino acids. In addition, acid hydrolysis also lead to the

formation of humin complex which can separate the amino acids of the hydrolyzed. Therefore, base hydrolysis is required to identify the amino acids that were damaged by acid hydrolysis and that can be identified by base hydrolysis. In general, the M. *maculate* have a balanced distribution of all essential amino acids required for an adult per day. In the present study the essential amino acids in M. *maculata*, valine (21,06%) was maximum and the minimum histidine (0,83%); and non-essential amino acid, glycine (20,38%) was maximum and minimum as glutamic acid (4,44%). In general, from the above observation, it is clear that the tissue of the M. *maculata* with rich nutritive value can be used for alternate source as a regular sea food which supplies nutrients for the growing children, pregnant women and people suffering from malnutrition

IV. Conclusion

Based on research results obtained it can be concluded that fatty acids composition in *M. maculata* contained tridecanoic acid, palmitoleic acid, palmitic acid, oleic acid, stearic acid and 5,8,11,14-eicosatetraenoic acid and 12 amino acids composition that consists of 7 essential amino acids, were leucine, isoleucine, valine, phenylalanine, methionine, lysine,and histidine, and also 5 non-essential amino acid, were tyrosine, glycine, alanine, glutamic acid, and arginine..

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