RJC

**RASĀYAN** *J. Chem.*
Vol. 9 | No. 4 |673 - 679 | October - December | 2016
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

# ANALYSIS OF FATTY ACID AND AMINO ACID PROFILE OF "METI" MUSSELS (*Batissa violacea* L. von Lamarck, 1818) IN LA'A RIVER OF PETASIA DISTRICT NORTH MOROWALI REGENCY

**Jamaluddin[1*], Mappiratu[2], Septiawan[1] and Yonelian Yuyun[1]**

[1]Department of Pharmacy, Faculty of Mathematic and Natural Science, Tadulako University, Palu, Indonesia.

[2]Department of Chemistry, Faculty of Mathematic and Natural Science, Tadulako University, Palu, Indonesia.

*E-mail: jamal_farmasi02@yahoo.co.id

**ABSTRACT**

Freshwater mussel, "meti" (*Batissa violacea* L. von Lamarck, 1818) is Sulawesi endemic mussel coming from the Petasia District North Morowali Regency of Central Sulawesi. "Meti" mussel is consumed by people around the river, but it is not known about the nutritional content. This study aims to determine the amount of the fatty acid and amino acid profile and the nutritional content of Freshwater "Meti" Mussels (*Batissa violacea* L. von Lamarck, 1818). Sampling was taken by touching the bottom of the river and then put the mussels into a container which had been filled with water from the habitat. The method use in testing the moisture content is using the oven method, ash content is using a dry ashing method or the direct use of furnaces, fat content using soxhlet method, and protein content using Kjeldhal method. Testing Profile fatty acids is using gas chromatography and amino acid profile testing is using High Performance Liquid Chromatography (HPLC) method. The results showed that "meti", freshwater mussels' oil contains 17 kinds of unsaturated fatty acids by the amount of 45.16% and 7 types of saturated fatty acids with a number of 54.98%. Meti Protein contains 18 types of amino acids, 10 types of essential amino acids and eight kinds of non essential amino acids. Meti Mussel is containing 69.18% water, ash 1.67%, 10.66% fat, protein and carbohydrates 13.31% to 5.18%.

**Keyword:** Molluscs, proximate analysis, nutrient content



## INTRODUCTION

Meti is a species of mussels (*Batissa violacea* L. von Lamarck, 1818) that is found quite abundant and serve as a source of income or the majority of people who live around the riverbanks and far from the riverbanks. These mussels are very popular with the public because it tastes delicious and is very plentiful existence in La'a river, affordable and easily obtained.

Molluscs or soft animals whose presence is in the freshwater and seawater are called mussel, and are believed to have the content of omega-3 and omega-6 which are beneficial for brain development and for preventing heart disease. Comparison of fatty acids omega-3 and omega-6 in shellfish are generally the same with the fish that is 2: 1.[1]

Hartono (2007) reported that nutrient content of freshwater mussels called kijing (sp Anodonta.) consists of 87% water, 7.37% protein, 0.78% fat, 3.3% carbohydrate and 1.6% ash.[2] The researchers found the protein of freshwater mussels, kijing, contains essential amino acid which is complete and contains monounsaturated fatty acids and plural including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). EPA and DHA contribute to improve intelligence, reduce blood cholesterol levels and prevent various kinds of diseases caused by cholesterol.[3] With these roles, it is necessary to do research about its existence on freshwater mussel, meti.

## EXPERIMENTAL

### Materials

Meti Mussel, distilled water, selenium, sulfuric acid ($H_2SO_4$) acid, boric acid ($H_3BO_3$), methyl red indicator, hexane ($C_6H_{14}$), hydrochloric acid (HCl), 0.1 N, 6 N and 25%, methanol ($CH_3OH$) , boron trifluoride ($BF_3$), sodium chloride (NaCl) saturated, sodium hydroxide (NaOH) 40%, 0.5 M and 1 M, AccQ-Fluor Borate, Fluor Reagent A, a standard solution of fatty acid and amino acid standard solutions.

### Wet Sample Preparation

Meti freshwater mussels are washed and then separated between the flesh and the shell. The flesh is taken then washed thoroughly and drained, then stored in a refrigerator.

### Dry Sample Preparation

The flesh is taken then washed thoroughly and drained, then dried in an oven at 105°C for 2 hours. Then the samples blended to a powder and stored at room temperature (20-25°C) in a steel container stainleess and ready to be tested.[4]

### Analysis of water content[5]

The first empty cup dried in an oven for 30 minutes at a temperature of 105oC, then cool in a desiccator for 15 minutes and weighed. A total of 5,041 gram sample is inserted into the cup, then dried in an oven for 2 hours 100-105oC. Grail is cooled in a desiccator for 30 minutes and weighed again. Heating and cooling is repeated to obtain a fixed weight (constant).

### Analysis of ash content[5]

Heated porcelain dish in the oven and then cooled in a desiccator and weighed. A total of 5,040 gram sample is inserted in the cup previously dried. Porcelain and burned until no smoke, then ashed at a temperature of 600°C until it is getting white (all sample ashes) and constant weight. After it is cooled in a desiccator and weighed.

### Analysis of fat content[5]

First flask to be used dried in an oven, then cooled in a desiccator and weighed. A sample of 10.05 g included in a paper sleeve (Hulls). Samples contained in a paper sleeve extracted with hexane solvent method soxhletasi using for 5 hours. After the subsequent extraction results included in the round-bottom flask to dirotavapor at 55°C until the residual solvent evaporates and falls in the round bottom flask. Then heated in an oven at a temperature of 105°C to achieve permanent weight and then cooled in a desiccator. Furthermore flask along with fat in it is weighed and the weight of the fat can be known.

### Analysis of protein content[5]

Measurements of protein made by micro-Kjeldahl method. As much as 0.5014 gram sample is weighed, then put in a 100 ml Kjeldahl flask, then added 0.25 grams of selenium and 3 ml of concentrated H2SO4. Samples are destructed until the solution is clear then cooled. Once cool, add to the Kjeldahl flask 50 ml of distilled water and 20 ml of 40% NaOH, then made the process of distillation. Distilled accommodated in 250 ml Erlenmeyer flask containing 10 ml of a mixture of boric acid ($H_3BO_3$) 2% and 3 drops of methyl red indicator pink. Once the volume of distillate reached 40 ml and bluish-green, the distillation process is stopped. The resulting distillated with 0.1N HCl until it changes color into pink. The volume of titrant is read and recorded. The blank solution as the sample analyzed.

### Analysis of fatty acids[6]

### Extraction of oil scallops with methods soxhletation

The samples of freshwater mussels in the form of dried, weighed 50 grams entered in a paper sleeve (Hulls). Samples contained in a paper sleeve extracted with hexane solvent method soxhletasi using for 5

hours. After the subsequent extraction results included in the round-bottom flask to dirotavapor at 55°C. Then heated in an oven at a temperature of 105°C to achieve permanent weight and then cooled in a desiccator, condensed extract vial stored in a jar.

**Testing of samples**

Samples obtained from the previous stage derivatized into fatty acid methyl esters. Results were weighed 0.0375 grams extract oil and add 2 ml of 0.5 M NaOH (approximately 0.5 grams of NaOH dissolved in methanol and 25 ml) and heated in a water bath at 100°C for 20 minutes. Subsequently cooled and then added a solution of 14% BF3 in methanol (14 g BF3 methanol was added to 100 ml) and reheated to a temperature of 100°C for 20 minutes. Cool and shake until at 30°C and add 2.0 ml of saturated NaCl. After that vortexed for ± 2 minutes and added n-hexane, then restoring vortexed for 2 minutes. Furthermore, it is silenced at room temperature. Hexane, methyl ester layer is taken, then transferred to a 10 ml measuring flask, diluted and attached with n-hexane.

Before injecting layer of n-hexane, methyl ester into a gas chromatography to analyze the fatty acid composition and the sample, the first solution of FAME (Faty Acid Methyl Ester) mix containing AA / EPA / DHA diluted and attached on n-hexane into the flask 10 ml containing 500 mL standard solution. Furthermore, 1 mL of the standard solution is injected into gas chromatography then injected 1 mL of sample solution.

**Analysis of amino acid[7]**

**Sample solution**

The sample is weighed 0.2095 grams was then added 5 ml of HCl 6 and vortexed, after hydrolysis for 22 hours at a temperature of 110°C and let cool, then transfer to a 50 ml flask. Then, it is added aquabidest to mark boundaries and then filtered with a 0.45 μm filter and pipette 500 mL, then 40 mL of a-amino-N-butyric acid (AABA), then added aquabidest approximately 460 mL, then pipette 10 mL of the solution, then added 70 mL AccQ-Fluor Borate, then vortex. After the fluorine added 20 mL reagent A, then vortex then let stand for 1 minute and then incubated at 55°C for 10 minutes, after it was injected on HPLC.

**Standard solution**

Pipette 40 mL of the standard mix of amino acids were then added 40 mL of internal standard a-amino-n-butyric acid (AABA) then added 920 mL homogen aquabidest afterwards. Taken standard 10 mL and then added 70 mL AccQ-Fluor Borate and vortex. Then add 20 mL reagent fluorine-A, and then diamakan vortex for 1 minute. After it was incubated at 55 ° C for 10 minutes and then injected on the HPLC.

## RESULTS AND DISCUSSION

This study was conducted to analyze and determine the content of nutrients, as well as fatty acid profile and amino acids of freshwater mussel, meti, (*Batissa violacea* L. von Lamarck, 1818) from La'a river of North Morowali district.

The samples are freshwater mussel freshly taken from the La'a river stored in water styrofoam from their habitat in order to survive prior to further processing. Freshwater mussel' shell has a large and thick, rounded shape is somewhat elongated. In this research, the proximate analysis that includes moisture content, ash content, fat content and protein content.

Based on the results of studies were shown in Table- 1., it is known that the water content contained in meti about 69.18% of this value is quite high, in contrast to water content under study by Hartono (2007) is 87%, but inversely when compared with sea scallops (Atactodea striata) studied by Waransalembun (2007) which only have a value of 7.84% (dry basis).[2,8]

Based on the results shown in Table- 1., it is known that the ash content contained in meti ie 1.67% of this value is not much different from the value of Taiwanese barnacle mussels' ash content (Anadonta sp.) which were investigated by Hartono (2007), in which levels ash on the study 1.6%. Value ash mussels

meti not much different from the value of the levels studied by Ghifari (2011) on the snow clam 1.19%, 1.4% tiger snails, clams out 1.33%.[2,9]

Based on the results shown in Table- 1., it is known that the fat content found in meti mussel ie 10.66% of this value is quite high compared to the results of testing levels of fat in Taiwanese scallops, kijing, studied by Hartono (2007) is 0.78%. Meti mussels content value is quite high when compared with the research Ghifari (2011) to snow mussel by 0.11%, tiger snail is only 0.33%, and mussels out only by 0.24%.[2,9]

Meti mussel protein analysis results are presented in Table-1. Meti mussel protein content is the second largest after the value of water. Results of the analysis showed that meti mussel's protein content is the highest value of 43.20% (dry basis), the amount of content owned by meti mussel is very high and far different from Ghifari study (2011) on the mussels out only amounted to 9.39%, tiger snail, 17.38%, 11.37% and snow mussel. If seen from the calculation of protein of mussels wet basis, meti has a content value of 13.31%, this value is also quite high when compared with mussels and clams know snow studied by Ghifari (2011).[9]

The results of carbohydrate content is obtained by calculation by difference, known as carbohydrate by difference that is by calculating the difference of the number 100 with the number of components of other materials (moisture content, ash content, protein content and fat content). The results of carbohydrate found in meti mussel's flesh presented in Table-1. is equal to 5.18% of the value is quite large when compared with the research Ghifari (2013) in snow mussel only has a value of 3.55% and at the tiger snail only has a value of 2, 65%.[9]

The profile of fatty acids contained in freshwater mussels (*Batissa violacea* L. von Lamarck, 1818) containing 17 types of fatty acids consist of 7 types of saturated fatty acids with a number of 52.546%, monounsaturated fatty acids amounted to 23.438%, and unsaturated fatty acids plural of 22.111 %. Types of fatty acids owned freshwater mussels and complete meti enough when compared with scallops out, snow mussels, and tiger snail studied by Ghifari (2011) only has 11 kinds of fatty acids. Levels of saturated fatty acids contained in meti mussel is quite high when compared to snow mussel, snails and tiger snail knows only ranges of 36.1%. According to Osman, *et al* (2007) saturated fatty acid most commonly found in food that is 15-15% of the total fatty acids contained namely palmitate. The highest levels of saturated fatty acids of the analytical results is sour mussels meti palmitic (C16: 0) is 20.384% of the total amount of saturated fatty acids. Palmitic acid contained in meti quite large when compared to snow clams, snails tiger, and mussels out, only around 11.22%.[9,10]

Levels of monounsaturated fatty acids found in freshwater mussels meti amounted to 23.438%, this number is very large compared to snow clams, snails tiger, and scallops know researched by Ghifari (2011) with a total of 12.98%, fatty acids monounsaturated contained in meti mussel consists of oleic acid (C18: 1 / ω9) of 7.991%, palmitoleic acid (C16: 1) amounted to 14.479% and eicosenoic acid (C20: 1) of 0.968. Seen in Figure 4.2 fatty acids monounsaturated dominated by palmitoleic acid (C16: 1) amounted to 14.479, the result is quite large when compared with the research Ghifari (2011) type of palmitoleic acid which is owned by snow mussel, tiger snail, and mussels out only by 7,5% of the total species.[9]

Unsaturated fatty acids plural found in meti freshwater mussel consisting of linoleic acid (C18: 2) amounted to 5,047%, linolenic acid (C18: 3) 6.332%, eicosenoic acid (C20: 2) 0.817%, arachidonic acid (C20: 4) 5.29%, eicosapentaenoic acid (EPA) (C20: 5) 2.234% and docosahexaenoic acid (DHA) (C22: 6) 2.291%. In Table-2. showed the difference unsaturated fatty acid compound, higher levels found in linolenic acid which is 6.332% these results indicate freshwater mussels meti have high levels of linolenic acid which is quite high when compared with the research Ghifari (2011) on the snow mussel, tiger snail, and scallops know that is equal to 0.34% of the total mussel, different numbers of levels which is owned by meti mussel way much different from the three mussels.[9]

Meti, freshwater mussel has fairly complete amino acid profile consists of 10 types of essential amino acids and eight kinds of non-essential amino acids, if added together result 18 amino acid building blocks of protein. Result analysis of meti freshwater mussels can be seen in Table- 3 contains essential amino acids numbered 10 essential amino acids that phenylalanine by 1.21%, 1.34% isoleucine, arginine 2.05%,

1.36% valine, lysine 2, 37%, leucine 2.34%, 1.51% thereonin, histidine 0.61%, 0.52% methionine, tryptophan 0.29%. So that freshwater mussels, meti, have the essential amino acids that are complete enough compared with scallops know researched by Chairunisah (2011) only has 9 essential amino acids.[11]

Table-1: The results of proximate analysis

| Nutrition content | Sampel weight (gram) | | Concentration (%) | | Mean of concentration (%) |
|---|---|---|---|---|---|
| | I | II | I | II | |
| Water | 5,08 | 5,08 | 69,09% | 69,27% | 69,18% |
| Ash | 5,08 | 5,04 | 1,72% | 1,62% | 1,67% |
| Lipid | 10,05 | 10,02 | 10,70% | 10,62% | 10,66% |
| Protein | 0,5014 | 0,5125 | 13,32% | 13,30% | 13,31% |
| Carbohydrate | - | - | 5,17% | 5,19% | 5,18% |

Table-2: The results of fatty acid analysis

| Fatty acid | Area | Composition (%) |
|---|---|---|
| C 14:0 (miristic acid) | 15.993 | 6.726 |
| C 15:0 (pentadecanoic acid) | 4.510 | 1.896 |
| C 15:1 (pentadecanoic acid) | 1.018 | 0.428 |
| C 16:0 (palmitic acid) | 72.239 | 30.384 |
| C 16:1 (palmitoleic acid) | 34.425 | 14.479 |
| C 17:0 (heptadecanoic acid) | 11.213 | 4.716 |
| C 18:0 (stearic acid) | 19.421 | 8.168 |
| C 18:1 (oleic acid /ω9c) | 18.999 | 7.991 |
| C 18:2 (linoleic acid /ω6c) | 12.000 | 5.047 |
| C 20:0 (arachidat acid) | 3.856 | 1.621 |
| C 20:1 (eicosenoic acid) | 2.302 | 0.986 |
| C 20:3 (linoleic acid / ω3) | 15.055 | 6.332 |
| C 20:2 (eicosadienoic acid) | 1.943 | 0.817 |
| C 20:0 (behenic acid) | 1.196 | 0.503 |
| C 22:4 AA (arachidonic acid / ω6) | 12.579 | 5.290 |
| C 20:5 EPA (eicosapentaenoic acid /ω3) | 5.313 | 2.234 |
| C 20:6 DHA (docosahexaenoic acid / ω6) | 5.689 | 2.391 |

We can saw in Table-3 that lysin had the highest levels found in meti mussel reaching to 2.37% while the lowest is the essential amino acid tryptophan. The value of amino acids lysine contained in meti mussel high enough when compared with Chairunisah study (2011) on the mussels out only by 0.98%, 0.79% and snow mussel leopard slug 1.20%.[11]

Table-3: The results of amino acid analysis

| Amino acid | Concentration | |
|---|---|---|
| | (mg/kg) | (%) |
| Phenylalanine | 12128.79 | 1.21 |
| Isoleucine | 13365.49 | 1.34 |
| Proline | 11738.58 | 1.17 |
| Arginine | 20537.20 | 2.05 |
| Glycine | 16424.32 | 1.64 |
| Glutamate | 36886.98 | 3.68 |
| Valine | 13619.74 | 1.36 |
| Lysine | 23766.48 | 2.37 |
| Leucine | 22401.47 | 2.24 |

| | | |
|---|---|---|
| Tyrosine | 7874.14 | 0.78 |
| Threonine | 15187.65 | 1.51 |
| Histidine | 6089.88 | 0.61 |
| Serine | 15590.34 | 1.55 |
| Aspartic | 22796.24 | 2.27 |
| Alanine | 17144.62 | 1.71 |
| Methionine | 5293.76 | 0.52 |
| Cystine | 774.05 | 0.08 |
| Tryptophan | 2985.20 | 0.29 |

From the analysis can be seen in Table-3. that meti mussel has eight types of non-essential amino acid proline consisted of 1.17%, 1.64% glycine, glutamic 3.68%, 0.78% tyrosine, serine 1.55%, aspartate 2.17%, alanine 1.71% and 0.08% cystine, these results indicate a non-essential amino acids contained in freshwater mussels meti quite a lot when compared with the scallops out, clams and snails snow leopard studied by Chairunisah (2011) only has 6 kinds of essential amino acids. Non-essential amino acid is an amino acid that can be made in the body also called endogenous amino acids.[11,12]

Glutamic acid is a non-essential amino acid of the highest points on the scallops meti (*Batissa violacea* L. von Lamarck, 1818) is 3.68%, while a low of 0.08%. Glutamic acid content value-owned meti mussel quite high when compared with the kind of glutamic acid contained in snow mussel only by 2.14% and 2.24% scallops.

## CONCLUSION

Results of analysis of fatty acid profiles of meti, freshwater mussels, from the La'a river Petasia District of Morowali Regency has saturated fatty acids (52.546%) consisting of myristic acid, palmitic acid, acid pentadekanoat, heptadekanoat acid, arachidat acid, behenic acid, stearic acid, monounsaturated fatty acids (23.438%) consisting of palmitoleic acid, oleic acid, eikosenoat acids, polyunsaturated fatty acids (22.111%) consisting of linoleic acid, linolenic acid, eikosenoat acid, arachidonic acid, eicosapentaenoic acid, docosahexaenoic acid.

The results of the analysis of the amino acid profile of freshwater mussels from the La'a river Petasia District of North Morowali Regency has 18 kinds of amino acids making up the protein consists of 10 kinds of essential amino acids (phenilalanin 1.21%, 1.34% isoleucine, arginine 2.05 %, 1.36% valine, threonine 1.51%, 2.24% leucine, histidine 0.61%, 2.37% lysine, 0.52% methionine, tryptophan 0.29%) and non-essential amino acids (1.17%, 1.64% glycine, glutamic 3.36%, 0.78% tyrosine, serine 1.55%, 2.17% aspartate, alanine 1.71%, cystine 0.08%).

The results of the analysis of the nutritional content of freshwater mussels from the La'a river Petasia District of North Morowali district includes moisture 69.18%, ash content of 1.67%, 10.66% fat content, protein content and carbohydrate 13.31% 5.18%.

## ACKNOWLEDGEMENT

The authors thank to PT. Saraswanti Indo Genetech (SIG), Bogor, Indonesia for analysis of fatty acid and amino acid profile.

## REFERENCES

1. Anonim, 2000, *Natural food-seafood and freshwater food. http:/%www.naturalhu6.-com/*; (Online Mar 31th, 2015).
2. N. Hartono, 2007, Pengaruh Berbagai Metode Pemaaakan Terhadap Kelarutan Mineral Kijing Taiwan (Anodonta woodiana Lea) [Skripsi]. Departemen Teknologi Hasil Perairan, Fakultas Perikanan dan Ilmu Kelautan, IPB.
3. P. Suwignyo, J. Basmi, DTFL, Batu. R. Affandi, 1981, Studi Biologi Kijing Taiwan (*Anodonta woodiana Lea)*, Fakultas Perikanan, Institut Pertanian Bogor. Bogor
4. Mustar, 2013, Studi Pembuatan Abon Ran Gabus *(Ophiocephalus Striatus)* sebagai makanan suplemen (Food Soplement) Study Of Making Snakehead Shedded *(Ophiochpalus Striatus)* As Food

Suplement. Skripsi. Program Studi Ilmu dan Teknologi Pangan, Jurusan Teknologi Pertanian, Fakultas Pertanian Universitas Hasanuddin Makassar.
5. AOAC, 2005, Official Methods of Analysis of The Association of Official Analytical Chemistry. 14th Ed. Virginia : AOC, Inc.
6. SIG, Saraswanti IndoGenetech, 2013. Diagram Alir Uji Profil Asam Lemak Dengan Gas Kromatografi.PT Saraswanti Indo Genetech.Bogor.
7. SIG, Saraswanti Indo Genetech. 2013, Diagram Alir Pengujian Asam Amino Metode KCKT.PT Saraswanti Indo Genetech, Bogor.
8. C. Waranmaselembun, 2007, Komposisi kimia dan aktivitas inhibitor topoisomerase I dari kerang Mas Ngur *(Atactodea striata)*, [tesis]. Sekolah Pascasarjana, Institut Pertanian Bogor, Bogor.
9. A. Ghifari, 2011, Karakteristik Asam Lemak Daging Keong Macan *(Babylonia spirata)*, Kerang Tahu *(Meretrix meretrix)*, Dan Kerang Salju *(Pholas dactylus)* [skripsi], Departemen Teknologi Hasil Perairan Fakultas Perikanan Dan Ilmu Kelautan Institut Pertanian Bogor, Bogor.
10. F. Osman. I. Jaswir. H. Khaza'ai. R. Hashim, *J. Oleo. Science.,* **56**,107(2007) http://doi.org/10.5650/jos.56.107
11. R. Chairunisah, 2011, Karakteristik Asam Amino Daging Kerang Tahu (Meretrix meretrix), Kerang Salju *(Pholas dactylus)* Dan Keong Macan *(Babylonia spirata)* [Skripsi]. Departemen Teknologi Hasil Perairan Fakultas Perikanan Dan Ilmu Kelautan Institut Pertanian Bogor, Bogor .
12. F.G. Winarno, 2008, Kimia Pangan dan Gizi, Gramedia. Pustaka Utama, Jakarta.

[RJC-1499/2016]