Indonesian Journal of Chemical Research

http://ojs3.unpatti.ac.id/index.php/ijcr Indo. J. Chem. Res., 13 (1), 77-86, 2025

Fatty Acid and Amino Acid Profile of Mancadu Clam (Atrina vexillum) in Waai Village

Meilita Salamony*, Hanoch Julianus Sohilait, Ivonne Telussa

Chemistry Department, Faculty of Science and Engineering, Pattimura University, Jalan Ir. M. Putuhena, Poka, Ambon 97233 Maluku - Indonesia

Corresponding Author: meilitasalamony@gmail.com

Received: January 2025
Received in revised: February 2025
Accepted: May 2025
Available online: May 2025

Abstract

Mancadu clam (Atrina Vexillum) is a food source for Maluku coastel communities with high nutritional value and contains various bioactive components, including fatty acids and amino acids. This study analyzed the content of fatty acids and amino acids in mancadu clams (Atrina Vexillum) in Waai village. Fatty acid analysis was carried out through a maceration extraction process with the Shaking Incubator method and transesterification using the microwave. Amino acid analysis was carried out by hydrolysis in an acidic and alkaline condition for 12 hours. Fatty acid analysis used the GCMS method, while amino acid component analysis used the HPLC method. From the results of the research, the fatty acids of mancadu clams contain 7 fatty acid components, consisting of 2 saturated fatty acids, namely hexadecanoic or methyl palmitate, which is the most significant component (17.61%) and heptadecanoic acid (13.43%). Unsaturated fatty acids are hydrosinnamic acid (15.01%), 5,8,11,14-eicosatetranoic acid/omega-6 fatty acid (7.2%), octadecanoic acid (6.65%), 9-Octadecanoic acid (5.1%) and 8,11-eicosadinoic acid or FUPA (4.68%). The results of amino acid analysis of mancadu clams (Atrina Vexillum) on 18 amino acids obtained 6 types of essential amino acids namely leucine, valine, isoleucine, threonine, methionine and arginine which the largest compound from acid hydrolysis (1150.94 mg/Kg). There are 7 types of nonessential amino acids: glutamic acid, alanine, serine, glycine, aspartic acid, and proline. The highest component is glutamate acid hydrolyzed in an alkaline condition (1805.79 mg/Kg).

Keywords: Amino acids, Atrina Vexillum, Fatty acids, Mancadu clams, Microwave.

INTRODUCTION

The mancadu clams (Atrina vexillum), otherwise known as the pen clams (Audino & Marian, 2020), is an economically valuable bivalve that is widely distributed from New Zealand, Melanesia, and Southeast Africa in the Indo-Pacific region. It lives vertically embedded in bottom sediments, usually mud or silty sand, anchored by a web of byssus threads. It has a triangular clam, slightly rounded posteriorly to axe-shaped. It is mostly black. Otherwise, in some cases, it is reddish or brown, with spines growing on the posterior end of the clams (Ghani & Afsar, 2017). In Indonesia, North Sulawesi, this clam is known as the pearl clam (Tindi et al., 2017). In Maluku, especially in Waai Village, this clam is known as mancadu clam, whose meat is often consumed as a substitute for fish during the bumpy season (Siahainenia & Tuhumury, 2017). But the nutritional content of amino acids and fatty acids of mancadu clams is not yet known by the local community.

The previous research about Analysis of Protein and Fat Content of Bivalvia (Atrina vexillum) in the Coastal Waters of Waai Village**,** was only limited to the analysis of protein and fat content in mancadu clams (Atrina vexillum), with a protein content of 17.6901% and fat content of 0.7017% (Alhamdi et al, 2024). Research on the content of fatty acids and amino acids in mancadu clams is still limited and requires further proof. Therefore, this study aims to analyze the profile of fatty acids and amino acids in mancadu clams using the maceration extraction method with the help of a shaking incubator and microwave transesterification. Fatty acids were analyzed using GC-MS and amino acids were

analyzed through acid hydrolysis and base hydrolysis amino acid analysis using HPLC.

Generally, fatty acid extraction uses soxhlet extraction, which has limitations in efficiency. The heating process during extraction can cause the decomposition of chemical compounds at high temperatures and requires a lot of solvent (Patel et al., 2019; Sari et al., 2020) . This study involves maceration extraction with the help of shaking the incubator with temperature, time and incubator stirring speed/Revolution Per Minute (rpm) settings that are flexible to be adjusted as needed and reduce as well as reducing the time and solvents required (Ahmad, 2018). This method can provide more optimal results in terms of time efficiency, stability of bioactive compounds, and effectiveness of extraction of fatty acid compounds. Amino acid content testing was carried out using acid and base hydrolysis, considering that the most significant change occurred in the concentration of amino acids hydrolyzed in an acidic or alkaline condition (Lumamuly et al., 2019). Thus, this study not only fills biochemical data gaps and nutritional information material of mancadu (Atrina Vexillum) clams in Waai village, which contributes to developing more effective and efficient extraction and hydrolysis methods.

METHODOLOGY

Instrumentals and Materials

The Instruments used were glassware, a magnetic stirrer, a spatula, a separatory funnel, a distillation device, an analytical balance (Adventurer brand), an oven (Memmert brand), a hot plate (Cimarec brand), a shaking incubator (Shel Lab brand), a rotary evaporator (Buchi brand), and a microwave (Sharp 500Watt brand). The fatty acid analysis was performed using a GC-MS instrument (Shimadzu Merck), and the amino acid analysis was done using HPLC brand (Shimadzu).

The materials used were standard p.a. petroleum ether n-hexane, H_2SO_4, $Ba(OH)_2$, BF_3, CH_3OH, Na_2SO_4 anhydrous, Universal indicator, and Aquadest.

Procedure

The Fatty Acid

In the sample preparation stage, mancadu clams were taken from the coast of Waai village and soaked with hot water for 5 minutes, and then the meat from the clams was collected. After that, the meat is brought to the laboratory and dried at 65 ºC in the oven for 1 week, then the sample is mashed.

In the lipid extraction step, a total of 42.79 grams was added with 150 mL of Petroleum Ether macerated with a shaking incubator for two hours with a pressure 32 mmHg, rotation speed of 110 rpm (revolutions per minute), and temperature of 55 ºC. The sample was filtered, the filtrate obtained was evaporated to remove the solvent, then weighed, and the filtrate was uses for fatty acid analysis while the residue was used for amino acid analysis.

In the Fatty Acid Testing step, fatty acid analysis is carried out through transesterification. The lipid extract obtained is added to 12 mL of BF_3 in methanol (BF_3CH_3OH) and then heated in the microwave for 14 seconds at 500 watts. Continued separation was achieved using a separatory funnel by adding n-hexane solvent and water. There was a separation of 2 layers; the top layer was n-hexane and fatty acid methyl esters, while the bottom layer was glycerol and water. Separation with a separatory funnel was carried out until the bottom layer was pH-neutral using a universal indicator. Then, add Na_2SO_4 Anhydrous to remove water. Followed by evaporation to remove n-hexane. The sample was analyzed using GC-MS.

Acid hydrolysis

10 grams of residue from the extraction results were then put into a round bottom flask with 10 mL of H_2SO_4 and 10 mL of water to reflux for 12 hours. Then, neutralize until pH = 7.

Base hydrolysis

10 grams of residue obtained from the extraction results were then put into a round bottom flask and 18.93 grams of $Ba(OH)_2$ 6 M and 10 mL of water refluxed for 12 hours. Then, neutralize until pH = 7. Furthermore, the reflux solution in acidic and alkaline conditions was filtered with filter paper. The filtrate obtained was then analyzed using HPLC.

RESULT AND DISSCUSION

Sampling Location

The sampling of mancadu clams or Atrina Vexillum was carried out when the water was low or by the Waai village community called air meti. The sampling location was in the intertidal zone in the form of a craggy beach (Tristi Indah , 2023). Located between 3.575583 °S 128.325417 °E and 3.569944 °S 128.324111°E Waai, Ambon Island, Maluku (Figure 1).

Lipid Extraction

The dried samples were ground for maceration extraction using a shaking incubator with petroleum ether solvent. Lipids are nonpolar organic compounds that can dissolve in solvents with the same polarity, such as petroleum ether. The resulting lipid extraction results are highly influenced by the polarity of the solvent used (Ghani & Afsar, 2017).

Figure 1. Mancadu Clams Sampling Location

Wendersteyt et al., (2021) state that differences in extract yield depend on the natural conditions of the sample, extraction method, sample particle size, extraction conditions and time, and the ratio of sample to solvent.

Table 1. Mancadu Clam Fat Yield

Sample	Weight (gram)		Yield (%)
	Sample	Oil	
Mancadu Clams	42.79	1.24	2.89

The resulting lipids are continued with the transesterfication process, one of the organic reactions that convert lipids into methyl esters and glycerol (Al Ghifari & Samik, 2023). BF_3 catalyst in methanol is a strong acid catalyst that can accelerate the transesterification reaction rate, helping to break down triglyceride molecules into methyl esters and glycerol more efficiently. The more methanol is used in the transesterification reaction, the more methyl esters produced (Faizal et al., 2013) The use of microwave in the transesterification process involves. Microwave extraction is more efficient in terms of time heating the reactants through dielectric heating, where microwaves cause polar molecules (such as methanol) to oscillate and generate heat (Poerwadi et al., 2019; Fadiyah et al., 2020). The interaction of microwaves with reactants (triglycerides and methanol) results in a significant decrease in activation energy due to an increase in dipolar polarization events (Poerwadi et al., 2019). After the transesterification process, n-hexane is added to the methyl esters and glycerol and continues to separate with a separating funnel. N-hexane will bind methyl esters, which are nonpolar compounds (upper layer), while water will bind glycerides (lower layer) polar compounds. This neutralization process is carried out until the pH of the water is neutral and continued with fatty acid analysis by GC-MS (Cahyaningtyas et al., 2019).

GC-MS Analysis Result

The obtained mancadu clams were analyzed for fatty acids by GC-MC. The results of GC-MS analysis, the chromatogram, show that there are 11 peaks with 7 peaks suspected to be fatty acids, as seen in Figure 2.

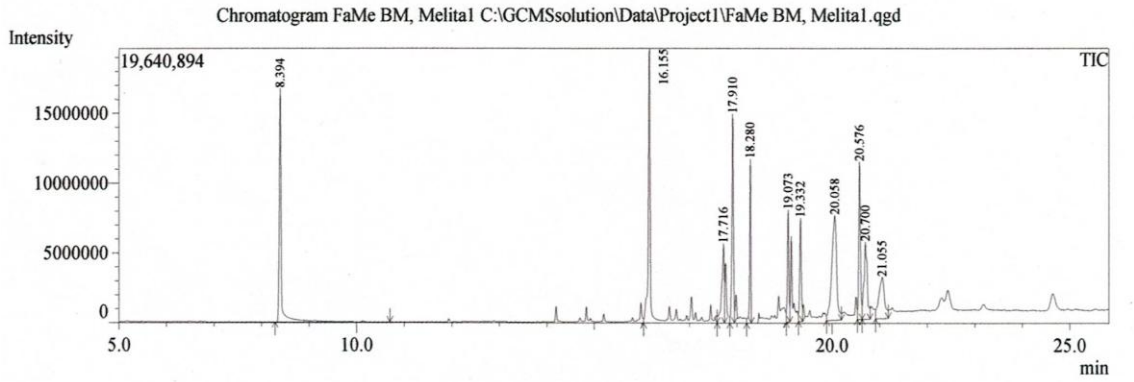

Figure 2. Chromatogram of Mancadu Clams

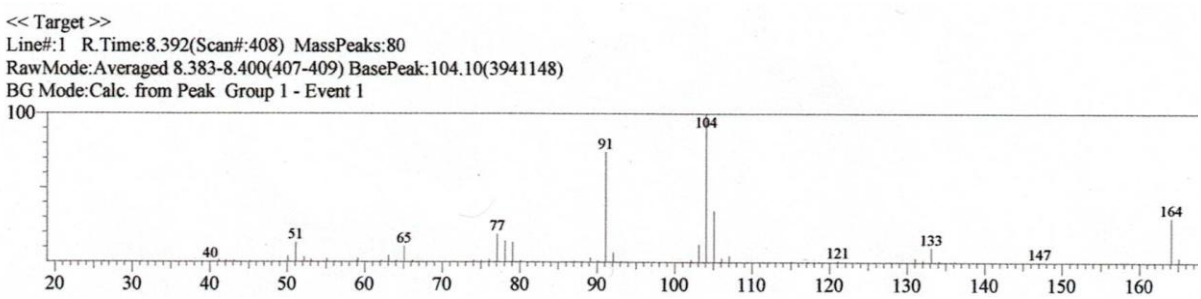

Figure 3. Mass Spectrum Retention Time 8.392 Minutes

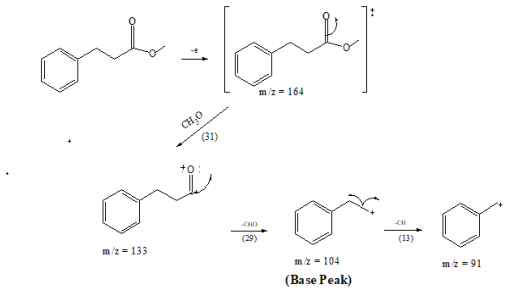

Figure 4. Compound Fragmentation Retention Time 8.392 minutes.

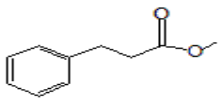

Figure 5. Methyl Hydrosinnamate Molecule Structure

The first peak of the mass spectrum in Figure 3 has a retention time of 8,392 minutes with a composition of 15.01%. After firing with electrons, a molecular ion peak appears at M^+ = m/z = 164. The release of methoxy ion -OCH_3 (M-31), characteristic of methyl ester compounds, produced a fragment with m/z = 133. The basic peak at m/z = 104 was obtained from releasing the carbonyl group -CHO from the fragment with m/z = 133. Followed by the release of -CH ions to produce a fragment with m/z = 91.

Based on the fragmentation of the compound (Figure 4), it can be concluded that the compound with a retention time of 8.392 minutes is methyl hydrosinnamate (1) with the molecular formula $C_{10}H_{12}O_2$ and the structure as shown in Figure 5.

The second chromatogram peak of the mass spectrum in Figure 6, with a retention time of 16.155 minutes is the largest composition in mancadu clams at 17.61%. The molecular ion peak on the fragment with a retention time of 16.155 minutes appears at M^+ = m/z = 270. Release of radical ion profile -C_3H_7 (M-43) to produce a fragment with m/z = 227. This is followed by the release of hexyl group -C_6H_{12} to produce a fragment with m/z = 143 and the release of butyl group -C_4H_8 to produce a fragment at m/z = 87. The basic peak at m/z = 74, wich resulte from the breakdown of ß was followed by McLafferty rearrangement. Based on the fragmentation (Figure 7), it can be proven that the compound with a retention time of 16.155 minutes is hexadecanoic acid or methyl palmitate (2) with the molecular formula $C_{17}H_{34}O_2$ and the structure as shown in Figure 8.

The third chromatogram peak of the mass spectrum in Figure 9 has a retention time of 17.716 minutes and a composition of 5.10%. The molecular ion peak at M^+ = m/z = 296, the release of the methoxy ion -OCH_3 (M-31), was accompanied by the release of H^+ ions to produce a fragment with m/z = 264. A fragment with m/z = 222 was obtained from the release of propylene group -C_3H_6 followed by the release of -C_2H_2O group to produce a fragment with m/z = 180. Fragment with m/z = 137 obtained from

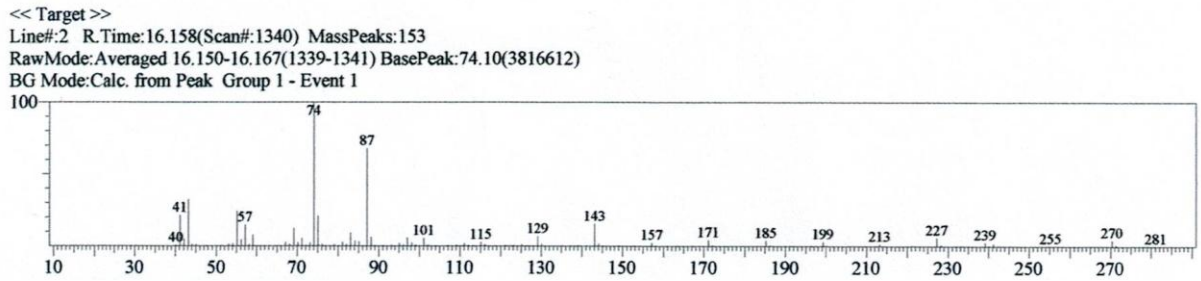

Figure 6. Mass Spectrum Retention Time 16.155 Minutes

the release of the -C_5H_8O group. Fragment with m/z =123 obtained from the release of methylene group -CH2 from fragment m/z = 137. The release of ethenyl group follow this -C_2H_3 to produce fragments

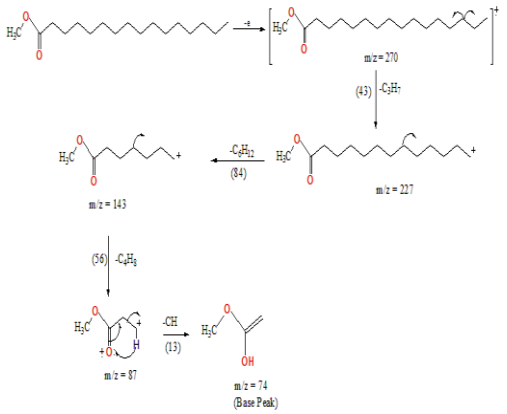

Figure 7. Compound Fragmentation Retention Time 16.155 minutes.

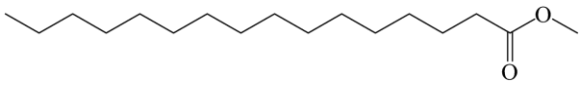

Figure 8. Hexadecanoic Acid or Methyl Palmitate Molecule Structure

m/z= 267. The fragment with m/z = 255 is obtained from releasing the propyl radical ion -C_3H_7 from the molecular ion peak. Successively, there is a release of the alkyl group -C_8H_{16} to produce a fragment with m/z = 143, then continued with the release of the alkyl group -C_4H_8 to produce a fragment with m/z = 87. A **β** splitting, is continued by McLafferty rearrangement (transfer of H atoms to carbonyl oxygen) to produce a basic peak with m/z = 74.

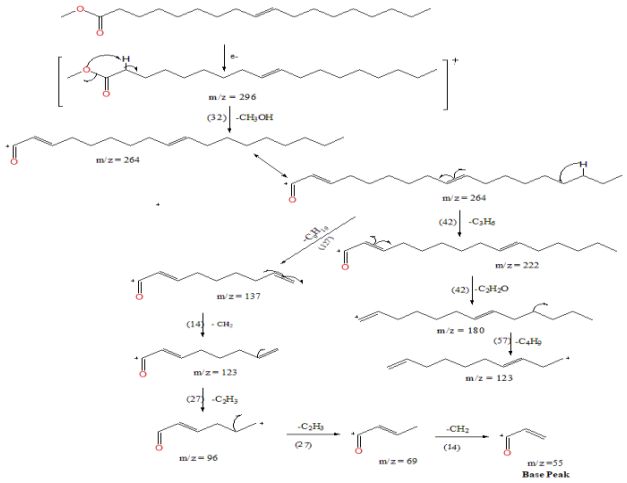

Figure 10. Compound Fragmentation Retention Time 17.716 minutes.

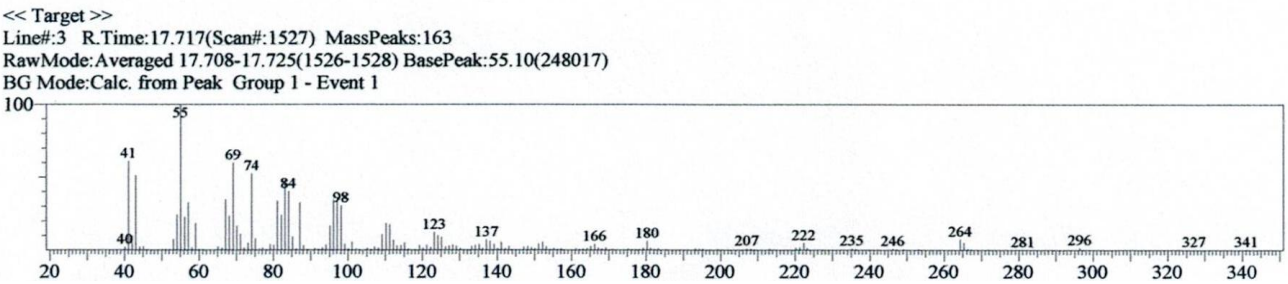

Figure 9. Mass Spectrum Retention Time 17.716 Minutes

m/z = 96 and 69. The basic peak at m/z = 55 was obtained from the release of the methylene group -CH_2.

From the fragmentation of the compound (Figure 10), it is evident that the compound is 9-octadecanoic acid or methyl stearate or MUFA (Monounsatured Fatty Acid) (3) with molecular formula $C_{19}H_{36}O_2$ and structural formula as Figure 11.

The fourth chromatogram peak in the mass spectrum had a retention time of 17.910 minutes and a composition of 13.43%. The mass spectrum in Figure 12. The molecular ion peaks at M^+ = m/z = 298, and there is a release of the methoxy group -OCH_3 (M-31), which is a characteristic of methyl ester compounds, to produce a fragment with

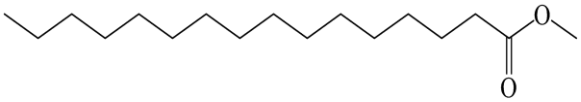

Figure 11. 9-Octadecanoic Acid or Methyl Stearate or MUFA Molecule Structure

Based on the compound fragmentation in Figure 13, the compound with a retention time of 17.910 minutes is octadecanoic acid (4) with the molecular formula $C_{19}H_{38}O_2$ and the structural formula as in Figure 14. The sixth peak of the mass spectrum in the chromatogram of Figure 15 has a retention time of 19.073 minutes and a composition of 7.2%. The fragment with m/z = 203 is obtained from the release of the -$C_7H_{13}O_2$ group from the molecular

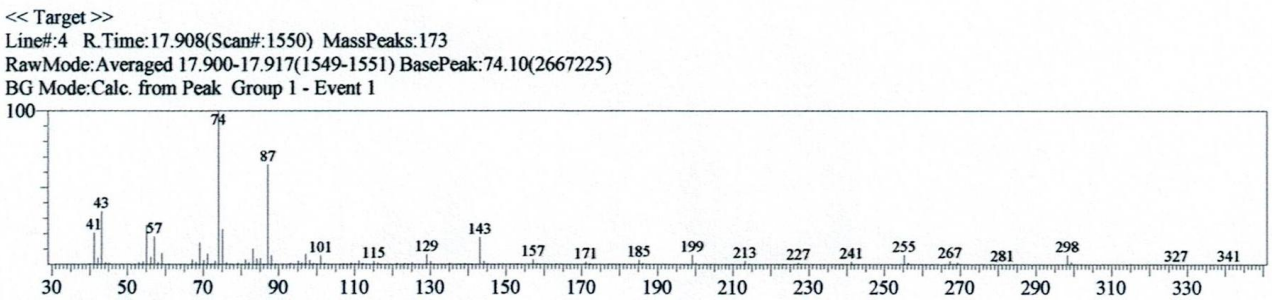

Figure 12. Mass Spectrum Retention Time 17.910 Minutes

ion peak. The fragment m/z = 119 is obtained from the hexene group -C_6H_{12} release, and the fragment m/z = 105 is obtained from the methylene group -CH_2 breakdown. The base peak at m/z = 79 is obtained from releasing the acetylene group -C_2H_2 from the

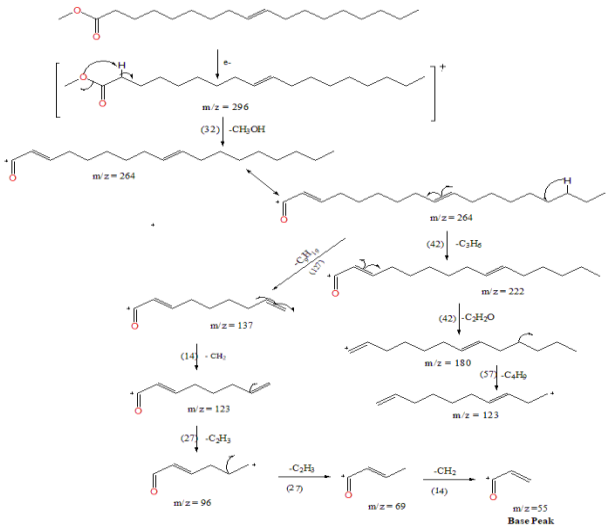

Figure 13. Compound Fragmentation Retention Time 17.910 minutes.

fragment m/z = 105. From the fragmentation of the compound (Figure 16), it can be proven that the compound with a retention time of 19.073 minutes is 5,8,11,14-Eicosatetraenoic acid, which is an omega 6 fatty acid or arachidonic acid (5). The molecular formula is $C_{22}H_{36}O_2$ with a structure seen in

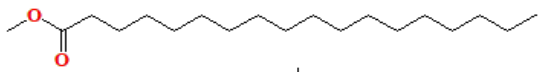

Figure 14. Molecule Structure of octadecanoic acid.

Figure 17.

The seventh peak of the mass spectrum (Figure 18) has retention time of 19.332 minutes with a composition of 6.65%. The molecular ion peak does not appear, but it appears on the fragment (M-2) = m/z = 292, which is one of the characteristics of a less stable ester compound. Strengthened by the release of methoxy ions -OCH_3 (M-31) from the molecular ion peak to produce a peak with m/z = 263.

The fragment at m/z = 208 is obtained from the release of the -C_4H_7 group, followed by the release of -C_8H_{14} to produce a fragment with m/z = 98. Fragments at m/z = 86, 69 and the base peak at m/z = 55 are obtained through the methylene group -CH_2 release. Fragmentation of the compound with a retention time of 19.332 minutes (Figure 19) can be proven to be 8,11-Octadecanoic acid or PUFA (Polyunstarched Fatty Acid) (6), which has the molecular formula $C_{19}H_{34}O_2$ and a structural formula as in Figure 20.

The tenth peak of the mass spectrum (Figure 21) has a retention time of 20.700 minutes and a composition of 4.95%. The molecular ion peak at M+ = m/z = 350 undergoes the release of methoxy ion (M-31) to produce a peak with m/z = 319, which

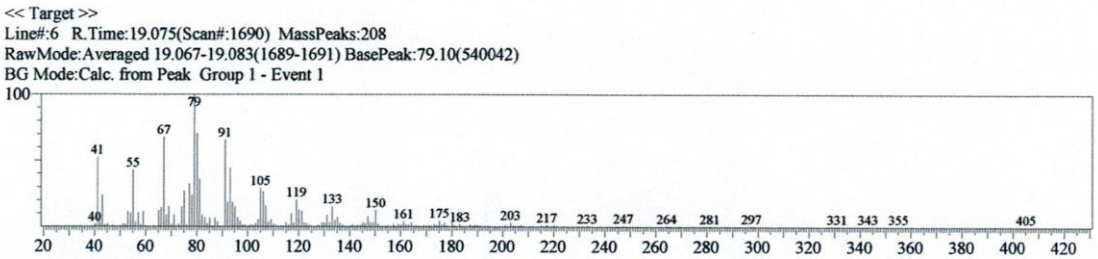

Figure 15. Mass Spectrum Retention Time 19.073 Minutes

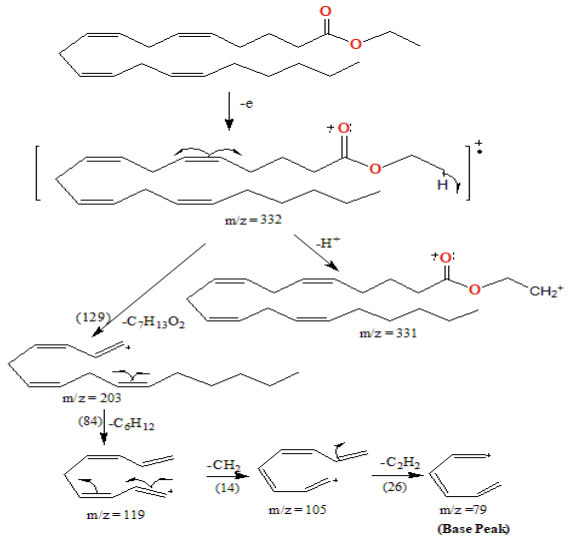

Figure 16. Compound Fragmentation Retention Time 19.073 minutes.

proves that this compound is an ester compound. Followed by the release of alkyl group $-C_8H_{12}$ to obtain a fragment with m/z = 270. The release of carboxyl group $-C_3H_5O$ is by the general formula $C_nH_{2n-1}O$ for the release of the ester group, which produces a fragment with m/z = 150. The fragment at m/z = 109 is obtained through the release of the $-C_3H_5$ group, followed by the release of the methylene group successively to produce a fragment with m/z = 95.81, and the base peak at m/z = 67 through the release of methylene group $-CH_2$.

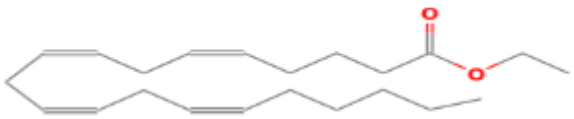

Figure 17. 5,8,11,14 Eicosatetraenoic Acid/Omega-6/Arachidonic Acid Molecule Structure

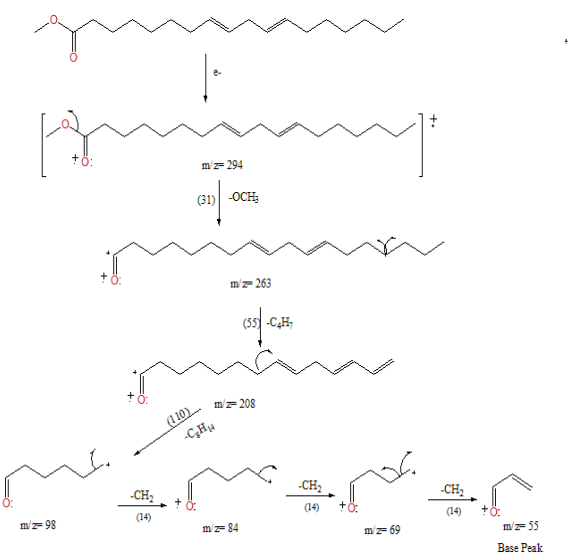

Figure 19. Compound Fragmentation Retention Time 19.332 minutes.

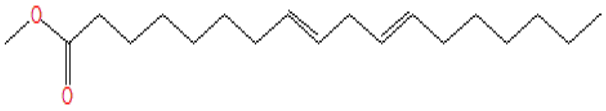

Figure 20. 8,11- Octadecanoic acid/PUFA Molecule Structure

Based on the fragmentation of the compound (Figure 22), it can be concluded that the compound with a retention time of 20.700 minutes is 10,13-Docose Dienoic acid or PUFA (7). It has a molecular structure of $C_{21}H_{38}O_2$, with a structure in Figure 23. The results of the fatty acid mass spectrum in mancadu clams, contain saturated fatty acids and unsaturated fatty acids, namely MUFA, PUFA, and omega 6 fatty acids. The largest composition is in hexadecanoic acid or methyl palmitate of 17.61%. Fatty acid data are presented in Table 2.

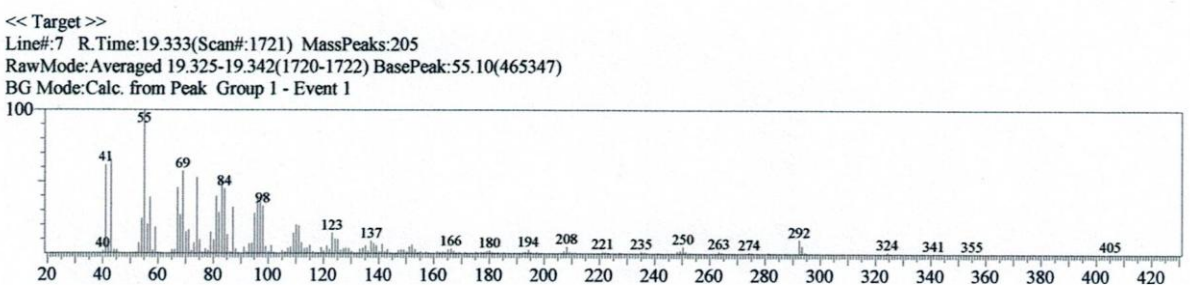

Figure 18. Mass Spectrum Retention Time 19.332 Minutes

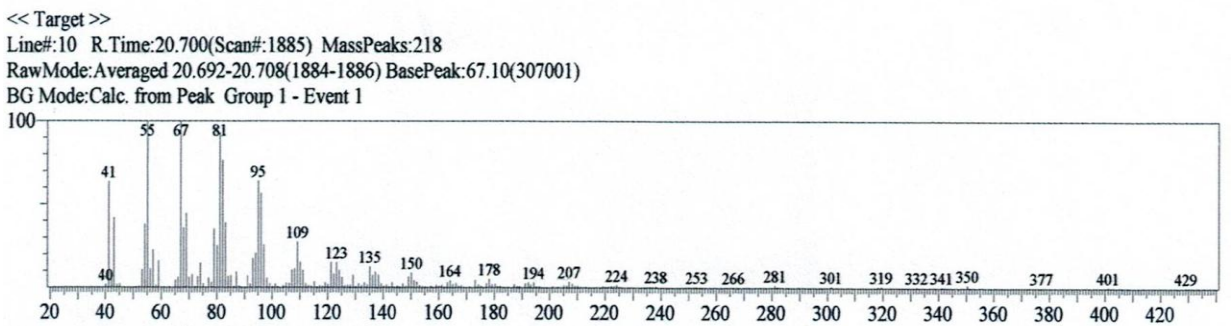

Figure 21. Mass Spectrum Retention Time 20.700 Minutes

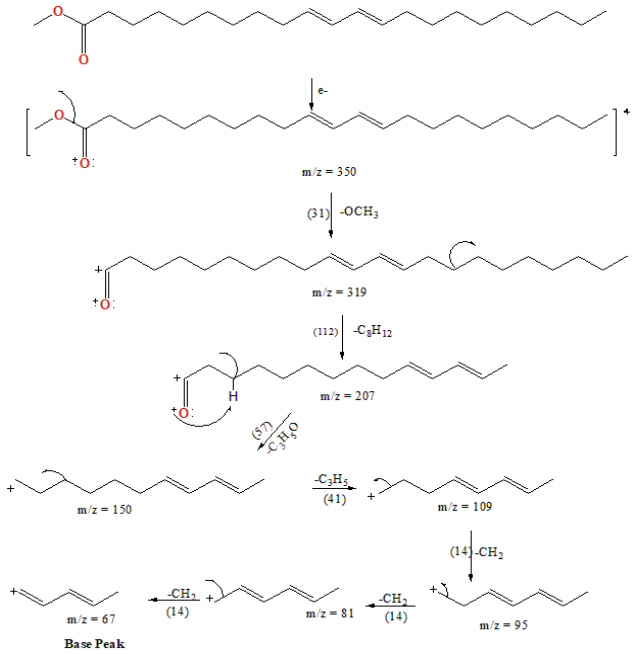

Figure 22. Compound Fragmentation Retention Time 20.700 minutes.

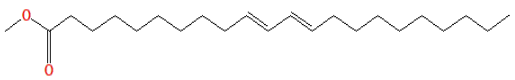

Figure 23. Structure Molecule of 10,13-Docose Dienoic Acid or FUPA

Amino Acids

Amino acids are the building blocks of proteins as monomer units or building blocks of proteins, which are joined together covalently in peptide bonds. There are 20 types of amino acids used in protein synthesis, which are divided into two groups, essential and non-essential amino acids, based on the body's ability to synthesize them (Riski et al., 2020). The body cannot synthesize essential amino acids, so they must be obtained from the food consumed, while non-essential amino acids can be synthesized by the body.

In this study, amino acid hydrolysis was carried out using acid and base. The amino acid determination process used residues that were free of fat. The residue was refluxed for 12 hours using sulfuric acid solvents (acid hydrolysis) and barium hydroxide (base hydrolysis). Sulfuric acid and barium hydroxide function to break down proteins by breaking peptide bonds into free amino acid components. After the reflux process is complete, the neutralization process begins. The solution is then filtered to obtain an analyte solution that is ready to be measured using an HPLC instrument. The amino acids determined in this study consisted of 18 types, of which 9 types are essential amino acids and 9 types are non-essential amino acids. Chromatogram of 18 amino acid standards. The results of HPLC analysis in acidic and basic conditions obtained the amino acid content in mancadu clams (Atrina Vexillum) are presented in table 3.

The amino acid content was hydrolyzed in acidic and basic conditions in mancadu clams in Waai Village, Central Maluku, namely 13 types of amino acids, 7 essential amino acids, and 6 nonessential amino acids (Table 3). The amino acids hydrolyzed in acidic and basic conditions are the largest glutamic acid, lysine, serine, and proline, which have the same amino acid content in both acidic and basic conditions. However, aspartic acid is only found in basic conditions, while in acidic conditions, it cannot be detected. The largest essential amino acid content in mancadu clams is arginine in acidic conditions; conversely, it is not detected in basic conditions,. The types of amino acids that are not detected in acidic or basic conditions are likely to be damaged or deaminated against other amino acids.

Table 2. Fatty acid components of Mancadu mussels

Profile	Peak To-	Retention time (minute)	Molecule mass	Formula Molecule	Methyl Ester Compound	Composition (%)
Saturated Fatty Acids	2	16.155	270	$C_{17}H_{34}O_2$	Hexadecanoic Acid or Methyl Palmitate	17.61
	4	17.910	298	$C_{19}H_{38}O_2$	Octadecanoic Acid	13.43
Unsaturated Fatty Acids	1	8.394	164	$C_{10}H_{12}O_2$	Methyl Hydrocinnamate.	15.01
	3	17.716	296	$C_{19}H_{36}O_2$	9-Octadecanoic Acid or Methyl Stearate or MUFA.	5.1
	6	19.073	332	$C_{22}H_{36}O_2$	5,8,11,14-Eicosatetraenoic Acid / Omega-6 / Arachidonic Acid.	7.2
	7	19.332	294	$C_{19}H_{34}O_2$	8,11-Octadecanoic Acid/ PUFA	6.65
	10	20.700	350	$C_{23}H_{32}O_2$	10,13-Docose Dienoic Acid or FUPA	4.68

Table 3. Amino Acid Content of Mancadu Clams In Acidic and Alkaline Conditions

	Amino Acid	Amino acid content (mg/Kg)			Amino Acid	Amino acid content (mg/Kg)	
		Base	Acid			Base	Acid
Nonessential	L-Glutamic Acid	1805.79	1366.97	Essential	L-Lysine	1051.90	1001.49
	L-Alanin	950.30	661.75		L- Leucine	1061.66	578.81
	L-Serine	468.69	446.23		L-Isoleucine	535.20	509.55
	L-Proline	402.52	382.57		L-Threonine	0	486.07
	Glycine	622.15	360.68		L-Valin	420.45	383.68
					L-Methionine	22.35	11.62
	L-Aspartic Acid	596.48	0		L-Histidine	0	0
					L-Tryptophan	0	0
	L-Cystine	0	0		L-Phenylalanine	0	0
	L- Tyrosine	0	0		L-Arginine	0	1150.94

CONCLUSION

The fatty acids of mancadu clams or Atrina Vexillum contain 7 components. Saturated fatty acids (SFA) 2 components: hexadecanoic acid or methyl palmitate (17.61%) and octadecanoic acid (13.43%). Unsaturated fatty acids were 5 components, namely hydrosinnamic acid (15.01%), 9-Octadecanoic acid/methyl stearate/MUFA (5.1%), 5,8,11,14-eicosatetraenoic acid/omega-6/arachidonic acid (7.2%), 8,11-octadecanoic acid/PUFA (6.65%) and 10,13-docose dienoic acid/FUPA (4.68).

Analysis of 18 amino acids with essential amino acids in mancadu clams resulted in 6 essential amino acids: leucine, valine, isoleucine, threonine, methionine, and arginine. Types of non-essential amino acids in mancadu clams obtained 7 types: glutamic acid, lysine, alanine, glycine, aspartic acid, serine, and proline.

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