



Applied Chemistry Project

Project title Production of vanillin from *trans*-ferulic acid isolated from rice bran

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Program Bachelor of Science in Applied Chemistry

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Faculty of Science, Chulalongkorn University

**Production of Vanillin from *Trans*-Ferulic Acid
Isolated from Rice Bran**

**by
Mr. Rawin Kunarb**

**In Partial Fulfillment for the Degree of Bachelor of Science
Program in Applied Chemistry (International Program)
Department of Chemistry, Faculty of Science
Chulalongkorn University
Academic Year 2020**

Project Production of Vanillin from *Trans*-Ferulic Acid Isolated from Rice Bran

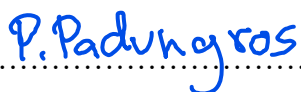
By Mr. Rawin Kunarb

Accepted by Department of Chemistry, Faculty of Science, Chulalongkorn University in Partial Fulfillment of the Requirements for the Degree of Bachelor of Science Program in Applied Chemistry (International Program)

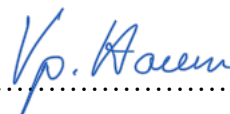
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Abstract

Rice bran is byproduct from rice agricultural industry in Thailand. It constitutes *trans*-ferulic acid as one of major components. *trans*-Ferulic acid is a phenolic compound that can be converted to vanillin by ozonolysis. This transformation would increase value of agricultural byproduct such as rice bran. The objective for this project is to find the most suitable reaction conditions for ozonolysis of *trans*-ferulic acid to yield vanillin. Several participating solvents such as methanol, ethanol, and water were examined as reaction medium for ozonolysis. It was found that using ethanol as solvent for ozonolysis is most efficient since ozonide intermediate was not detected during the reaction thus it avoided the use of dimethyl sulfide for reductive workup. Moreover, ozonolysis using ethanol as solvent provided vanillin in quantitative yield. Next, extraction of *trans*-ferulic acid from rice bran using Soxhlet extraction was carried out. After purification, the result showed that the major products were fatty acids such as palmitic acid, oleic acid, and linoleic acid instead of the desired *trans*-ferulic acid. Further study needed to be carried out in order to achieve the extraction of *trans*-ferulic acid from rice bran.

Keywords: Ozonolysis, Soxhlet Extraction, *trans*-Ferulic acid, Vanillin

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Chapter 1

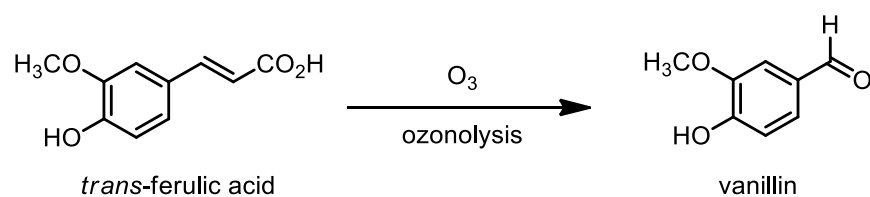
Introduction

1.1 Introduction to the research problem and significance

In Thailand's agriculture export, rice is the agricultural product that export out the most. According to the overview of the rice market in ASEAN, Thailand exports 7.6 million ton of rice in last year (2019).¹ From this data, we can proudly to say that Thailand is one of the major producers of rice, not only in Southeast Asia but in the world. Rice milling is the process to remove husk from the paddy rice and remove bran from rice polishing process.² The rice from this process can be divided into 3 parts which are rice grain (69.5 percent), rice germ (20 percent) and rice bran (10.5 percent).³ Rice bran can be considered as agricultural byproduct which can be processed to increase its value such as extraction of rice bran oil. Moreover, rice bran also contains other valued chemicals such as high levels of vitamin E, gamma-oryzanol and *trans*-ferulic acid.⁴ From the previous reports, the result showed that extraction of rice bran by using Soxhlet extraction yielded *trans*-ferulic acid approximately 0.33 milligrams per 1 gram of dry weight rice bran.⁵ *trans*-Ferulic acid is phenolic compound that can be used in pharmaceutical industry according to its functions.⁶

Natural derived vanilla is extracted from orchid-type plant named *Vanilla planifolia*. There are several chemicals within the vanilla extraction but a major component is vanillin (4-hydroxy-3-methoxybenzaldehyde). Vanilla orchid is main agricultural crop in Madagascar.⁸ The price of original vanillin is about 1,000 USD/kg which is very expensive.⁹ However, only a small fraction of authentic vanilla is used in food industry due to its high cost. In general, majority of vanilla favor in commercial food products is obtained from synthetic vanillin which can be produced in much lower cost (20 USD/kg) from petroleum-based chemicals.

Considering chemical structure of *trans*-ferulic acid, it constitutes of alkene moiety that could be subjected to oxidative cleavage under ozonolysis to yield aldehyde functional group. In this project, the aim of the experiment is to produce vanillin from *trans*-ferulic acid isolated from rice bran by ozonolysis (Scheme 1).



Scheme 1: Synthesis of vanillin from *trans*-ferulic acid by ozonolysis

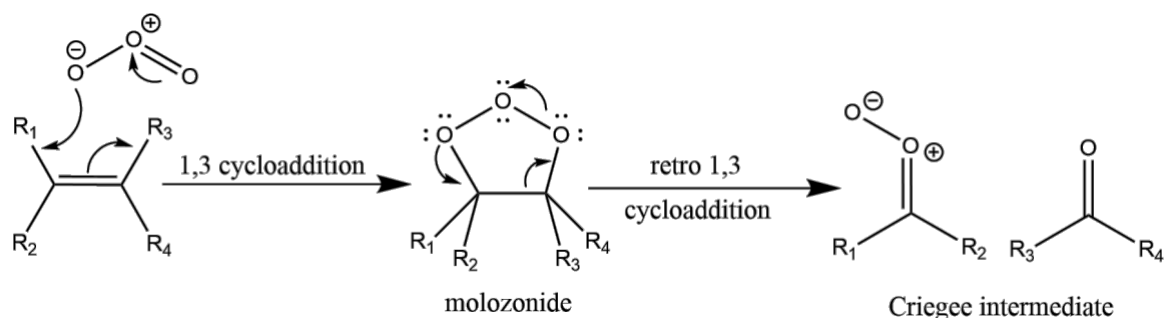
1.2 Research objectives

The objective for this project is to find the most suitable reaction conditions for ozonolysis of *trans*-ferulic acid to yield vanillin.

1.3 Literature review

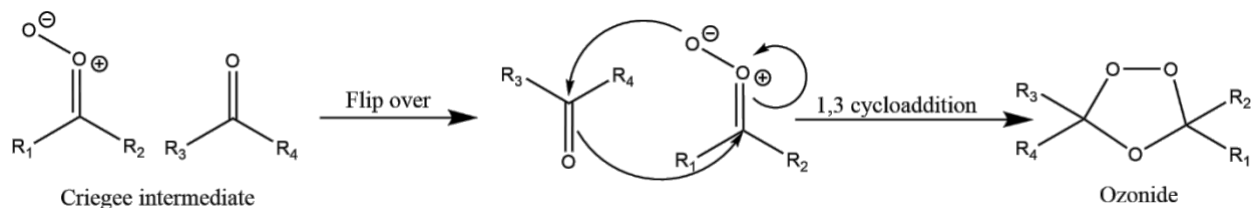
1.3.1 Mechanism of ozonolysis

This project is based on a concept of ozonolysis of olefins. Olefins, also known as alkene, is the compound that have at least one carbon-carbon double bond. The ozonolysis reaction employs ozone, 1,3-dipolar compound, for oxidative cleavage at the unsaturated carbon-carbon bond of olefin.¹⁰ There are 3 steps for mechanism of ozonolysis. First, olefins will react with ozone to form molozonide (or primary ozonide). This process is called “1,3-cycloaddition”. Molozonide will transfer electrons itself through the process of “retro 1,3-cycloaddition” and to form carbonyl oxide (known as Criegee intermediate) and carbonyl compound (Scheme 2).



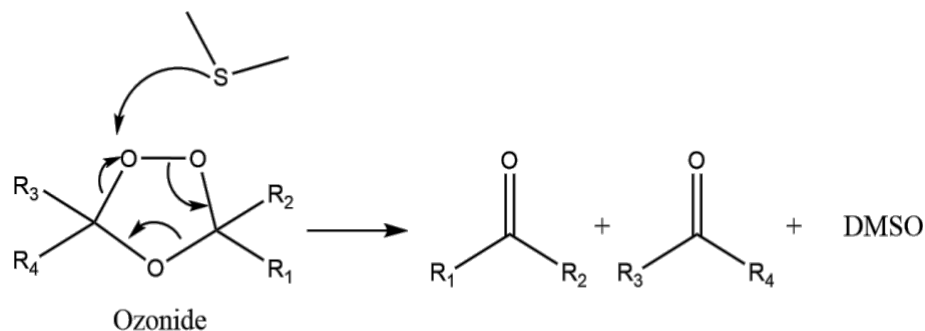
Scheme 2: Mechanism of ozonolysis (olefins to Criegee intermediate)

Since oxygen of both carbonyl oxide and carbonyl compound is rich of electrons, this can cause both of them have partial negative. They will repel each other and one of them will flip over. Then, oxygen of the carbonyl oxide will react with other carbonyl compound through the process of 1,3-cycloaddition and form ozonide (or secondary ozonide) (Scheme 3).



Scheme 3: Mechanism of ozonolysis (Criegee intermediate to ozonide)

Lastly, a reducing agent such as dimethyl sulfide (DMS) will be added to the reaction to quench the ozonide. The lone pair electrons at sulfur from DMS will react with one oxygen from the ozonide intermediate and form dimethyl sulfoxide (DMSO) as a byproduct. The sulfur atom will then transfer a bond to itself, and the two new carbonyl compounds will be formed as the products of the reaction (Scheme 4).



Scheme 4: Mechanism of ozonolysis (ozonide to product)

1.3.2 Natural vanillin

The scientific name for natural vanilla plant is *Vanilla planifolia*. It was originated from Mexican tropical rainforest. It is the main agricultural crop for tropical countries such as Madagascar which is located in Africa⁸. The suitable cultivate condition for vanilla is 20 to 30 degree Celsius because natural vanilla plant can live in temperature condition that not higher than 33 degree Celsius or lower than 10 degree Celsius¹³. Since it mostly found in tropical rainforest, the amount of annual rainfall should be more than 2,000 mm per year and soil in cultivate area must be porous and rich in humus¹⁴.

After three years of planting, vanilla plant is started to flower. The vanilla flowers (Figure 1) will bloom for one month. In each inflorescence of vanilla contains only one flower at one time and age period of flower is only one day. The flowering period of vanillin in Madagascar is between November to January due to the light intensity and temperature of the region are suitable¹⁵.



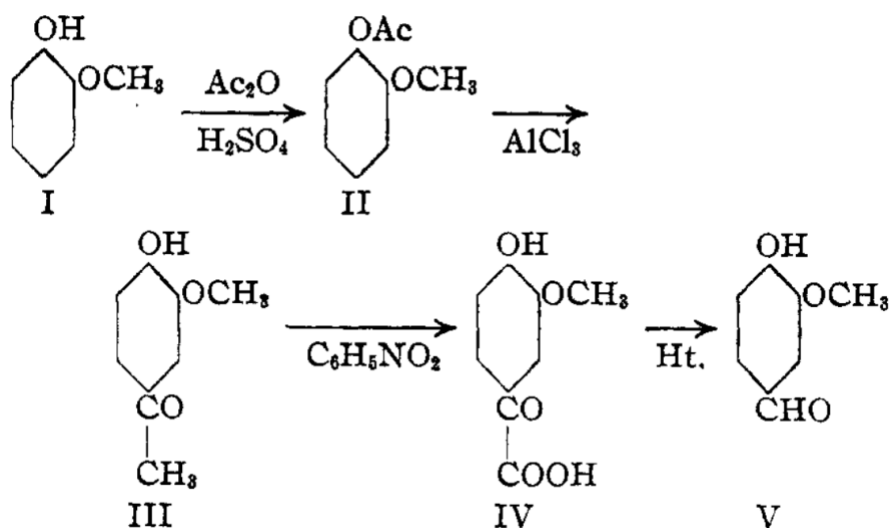
Figure 1: Vanilla orchid¹⁶

As mentioned in the previous paragraph, the flowering time for vanilla flower has affected to pollination process of vanilla bean pods. So, it is quite difficult to harvest due to the farmers have to know the exact time to collect the bean pods. This is the reason that can make the price for authentic vanillin very expensive (1,000 USD/kg)⁹. In conclusion, just a small portion of natural vanillin is used in food industries to reduce the cost of production¹⁷.

1.3.3 Synthetic vanillin

Since small amount of natural vanillin is used in food industries, so the majority of vanillin flavor is from synthetic vanillin which can be synthesized by petroleum-based chemical. The price for synthetic vanillin is 50 times cheaper than the natural vanillin (20 USD/kg).⁹

As shown in scheme 5, the process of synthetic vanillin was used guaiacol as starting reagent. From the scheme, aluminium trichloride (AlCl_3) was added to guaiacol acetate (II) to form apocynin (III). This step of mechanism generated halogenated waste which is not good for environment and it is not sustainable.



Scheme 5: Process for synthesized vanillin from petroleum-based chemical¹⁸

In fact, there are many ways to synthesize vanillin not only from petroleum-based chemical (Figure 2). Rice bran is also one of the ways that can use to synthesized vanillin due to it contain *trans*-ferulic acid which is a phenolic compound that can be used as a precursor to synthesized vanillin by using extraction technique.

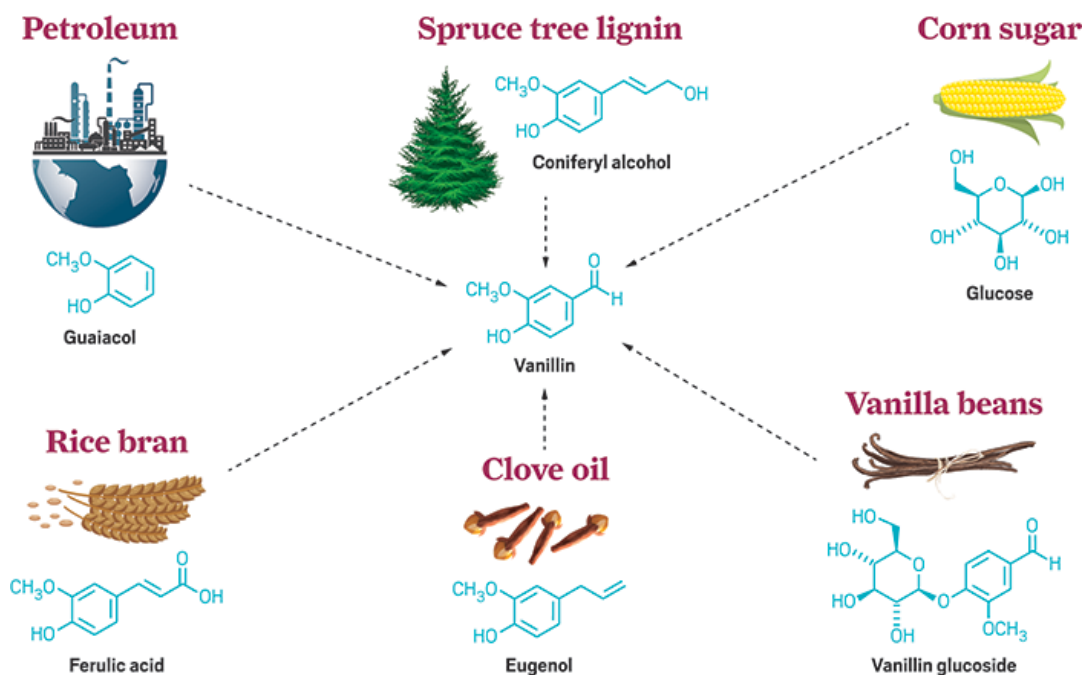


Figure 2: Various ways to synthesized vanillin¹⁹

1.3.4 Extraction of rice bran

The extraction technique that widely used for extraction of rice bran is Soxhlet extraction⁷. (Figure 3)

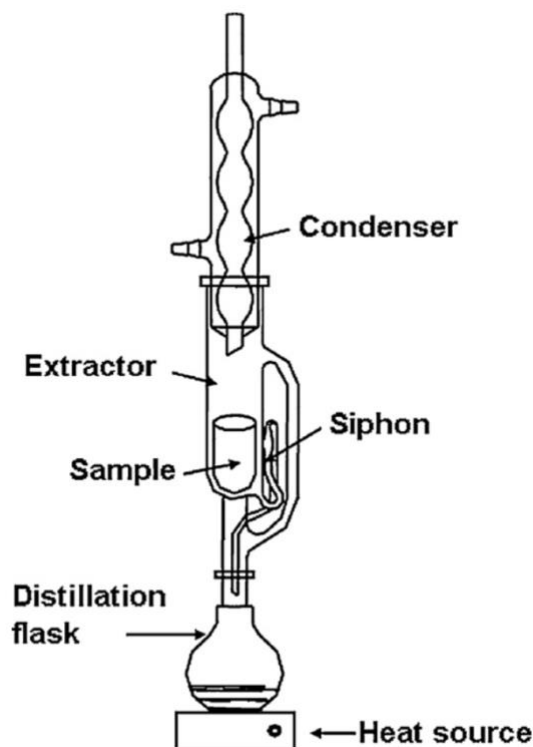


Figure 3: Soxhlet extraction apparatus²⁰

The solvent in distillation flask was heated and evaporated to Siphon tube. When the vapor of solvent is already condensed inside Siphon tube, it will soak the sample. If the solvent is high enough, the pressure in the extractor will increase and make solvent flow back down to distillation flask.













The extraction process in the previous paragraph indicated that the extraction is continuous, saving the amount of solvent, and gives out the high concentration of extract. On the other hand, the apparatus for Soxhlet extraction is complex and the extraction time can be started when the first drop of solvent from extractor to distillation flask was observed²¹.

1.3.5 Principles of green chemistry

Green chemistry is defined as “design of chemical products and processes to reduce or eliminate the use and generation of hazardous substances”²². It is also known as sustainable chemistry which has 12 principles to follow. (Figure 4)

The 12 Principles of GREEN CHEMISTRY

Green chemistry is an approach to chemistry that aims to maximize efficiency and minimize hazardous effects on human health and the environment. While no reaction can be perfectly 'green', the overall negative impact of chemistry research and the chemical industry can be reduced by implementing the 12 Principles of Green Chemistry wherever possible.

<p>1. WASTE PREVENTION</p>  <p>Prioritize the prevention of waste, rather than cleaning up and treating waste after it has been created. Plan ahead to minimize waste at every step.</p>	<p>7. USE OF RENEWABLE FEEDSTOCKS</p>  <p>Use chemicals which are made from renewable (i.e. plant-based) sources, rather than other, equivalent chemicals originating from petrochemical sources.</p>
<p>2. ATOM ECONOMY</p>  <p>Reduce waste at the molecular level by maximizing the number of atoms from all reagents that are incorporated into the final product. Use atom economy to evaluate reaction efficiency.</p>	<p>8. REDUCE DERIVATIVES</p>  <p>Minimize the use of temporary derivatives such as protecting groups. Avoid derivatives to reduce reaction steps, resources required, and waste created.</p>
<p>3. LESS HAZARDOUS CHEMICAL SYNTHESIS</p>  <p>Design chemical reactions and synthetic routes to be as safe as possible. Consider the hazards of all substances handled during the reaction, including waste.</p>	<p>9. CATALYSIS</p>  <p>Use catalytic instead of stoichiometric reagents in reactions. Choose catalysts to help increase selectivity, minimize waste, and reduce reaction times and energy demands.</p>
<p>4. DESIGNING SAFER CHEMICALS</p>  <p>Minimize toxicity directly by molecular design. Predict and evaluate aspects such as physical properties, toxicity, and environmental fate throughout the design process.</p>	<p>10. DESIGN FOR DEGRADATION</p>  <p>Design chemicals that degrade and can be discarded easily. Ensure that both chemicals and their degradation products are not toxic, bioaccumulative, or environmentally persistent.</p>
<p>5. SAFER SOLVENTS & AUXILIARIES</p>  <p>Choose the safest solvent available for any given step. Minimize the total amount of solvents and auxiliary substances used, as these make up a large percentage of the total waste created.</p>	<p>11. REAL-TIME POLLUTION PREVENTION</p>  <p>Monitor chemical reactions in real-time as they occur to prevent the formation and release of any potentially hazardous and polluting substances.</p>
<p>6. DESIGN FOR ENERGY EFFICIENCY</p>  <p>Choose the least energy-intensive chemical route. Avoid heating and cooling, as well as pressurized and vacuum conditions (i.e. ambient temperature & pressure are optimal).</p>	<p>12. SAFER CHEMISTRY FOR ACCIDENT PREVENTION</p>  <p>Choose and develop chemical procedures that are safer and inherently minimize the risk of accidents. Know the possible risks and assess them beforehand.</p>

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Figure 4: 12 principles of green chemistry²³

According to the 12 principles of green chemistry that were explained in Figure 4, here are the experiment protocol in the project that were followed the principles:

- 1. Waste Prevention:** This project doesn't produce any waste that can harmful the environment.
- 2. Atom Economy:** This project uses small scale of pure *trans*-ferulic acid (1 mmol) for the study of ozonolysis to yield vanillin.
- 3. Less Hazardous Chemical Synthesis:** Vanillin which is a desired product that was synthesized in this project is not dangerous to living things and environment.
- 4. Designing Safer Chemicals:** This project doesn't use and produce any toxic substances.
- 5. Safer Solvents and Auxiliaries:** The solvents that were used in this project were methanol, ethanol and water. All of these solvents are not toxic.
- 6. Design for Energy Efficiency:** This project doesn't require overnight heating. Just only Soxhlet extraction that was used electricity for heat source.
- 7. Use of Renewable Feedstocks:** Rice bran that was used in this project is agricultural renewable byproduct of Thailand.
- 8. Reduce Derivatives:** This project doesn't use any protecting group as a derivative in the reaction.
- 9. Catalysis:** The reagent (pure *trans*-ferulic acid) that was used in this project is not in stoichiometric amount.
- 10. Design for Degradation:** The product and byproduct that were carried out in this project are not toxic and harmful to the environment.
- 11. Real-Time Pollution Prevention:** All chemical reactions in this project does not produce any polluting substances.
- 12. Safer Chemistry for Accident Prevention:** The protic solvents (methanol, ethanol and water) were used in the study of ozonolysis can produce alkoxy hydroperoxyl hemiacetal as intermediate of the reaction which is not reactive as secondary ozonide that can be got from using aprotic solvent.

In conclusion, this is the reason that the ozonolysis reaction is chosen to synthesize vanillin from *trans*-ferulic acid in rice bran because this reaction not generates any metal waste and uses renewable feedstocks.

Chapter 2

Experimental

2.1 List of equipment and instrument

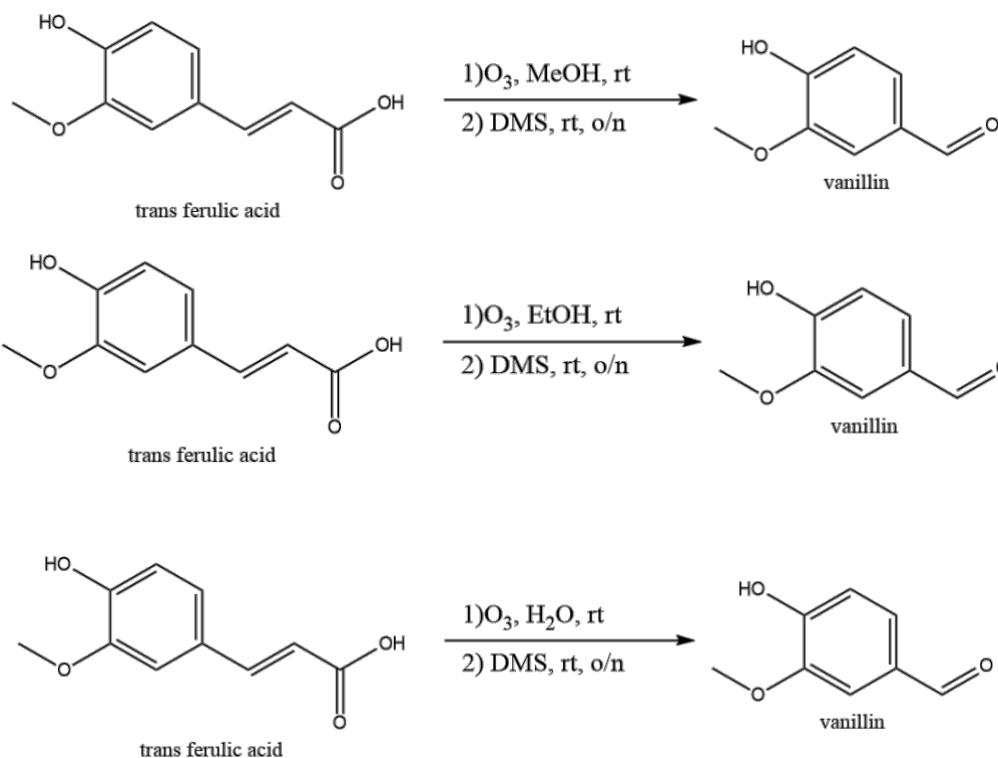
1. Ozone generator model Ozoner-010
2. Thin Layer Chromatography (TLC)
3. Column glassware for chromatography
4. Rotary evaporator model Eyela 1L Rotary Evaporator with water bath, N-1001S-W
5. Soxhlet apparatus glassware
6. NMR instruments model Bruker Avance 400MHz and JEOL 500 MHz
7. Digital scale model Mettler-Toledo model ML204T/00

2.2 List of chemicals and materials

1. Ethanol
2. Ethyl acetate
3. *trans*-Ferulic acid from Tokyo Industrial Chemistry (TCI) with >98% purity
4. Methanol
5. Diethyl ether
6. Hexanes
7. Rice bran purchased from online channel
8. 2,4-DNP (TLC staining agent),
9. Iodine (TLC staining agent),
10. Dimethyl sulfide
11. Sodium sulfate
12. NMR solvents: CDCl₃, DMSO

2.3 Experimental Procedure

2.3.1 Find the most suitable conditions for ozonolysis of *trans*-ferulic acid



Scheme 6: Ozonolysis of *trans*-ferulic acid by using methanol, ethanol and water as solvent

Firstly, approximately 200 milligrams of *trans*-ferulic acid were placed into a round bottom flask. Then, 30 milliliters of solvent (methanol/ethanol/water) was added in the same round bottom flask to make sure that volume of the solvent is excess and must be higher than the connecting tube from ozone generator (Table 1).

Table 1: Amount of *trans*-ferulic acid and dimethyl sulfide used in different types of solvent

Entry	Solvent	<i>trans</i> -Ferulic acid (mg)	Dimethyl sulfide (mL)
1	Methanol	203.5	0.385
2	Ethanol	208.8	0.395
3	Water	205.6	0.389

After that, the round bottom flask was attached to ozone generator and ozonolysis was carried out (Figure 5). The reaction time was fixed at 45 minutes for all three solvents with an oxygen flow rate of 500 mL per minute. After the ozonolysis for 45 minutes, progress of the reaction is checked by thin layer chromatography (mobile phase: 100% ethyl acetate) and stained with 2,4-DNP to check the appearance of the aldehyde product.



Figure 5: setup for ozonolysis reaction

Then, the reaction mixture was treated with dimethyl sulfide and the solution was stirred overnight. Solvent in the reaction mixture (methanol/ethanol/water) was evaporated out by rotary evaporator. The crude mixture was purified by using column chromatography technique. The solvent system of mobile phase for column chromatography was 50:50 ethyl acetate and hexanes (v/v) and the product is collected in the test tubes. After the column chromatography process was finished, appearance of product (vanillin) in each test tube were checked the by TLC and 2,4-DNP. The test tubes that contain vanillin which was observed on TLC under UV light and orange-yellow staining in 2,4-DNP. Then, the product was poured into new round bottom flask to evaporate the solvent out by using rotary evaporator. The product from rotary evaporator was further dried by vacuum pump to remove trace solvent that still remained in the flask. Lastly, characterization of vanillin was confirmed by NMR.

2.3.2 Extraction of rice bran by using Soxhlet extraction

In this part of the experiment, the most suitable solvents were chosen from the previous part (2.3.1) which are methanol and ethanol (Table 2).

Table 2: weight of rice bran that used in each solvent

Entry	Solvent	Amount of rice bran (g)
1	Methanol	100.0
2	Ethanol	100.4

Firstly, approximately 100 grams of rice bran was prepared in the round bottom flask. Then, fill up the round bottom flask with 400 milliliters of solvent (methanol/ethanol) and was attached to the Soxhlet apparatus (Figure 6). The extraction time was fixed at 10 hours and started counting the time when the first drop of solvent was appeared.



Figure 6: Soxhlet extraction setup

After extraction process was finished, the round bottom flask was removed from the Soxhlet apparatus and the solvent (methanol/ethanol) was evaporated out by using rotary evaporator. Then, the appearance of product was checked by using Thin Layer Chromatography (10% ethyl acetate: 90% hexanes) and dipped in iodine. Next, the crude mixture was separated

by column chromatography. The polarity of solvent system was started from 100% hexanes and increased up to 20% ethyl acetate: 80% hexanes. The polarity of solvent system was increased by 5% in every 100 milliliters of eluted solvent and was collected each fragment in a test tube (Table 3). The vanillin in each test tube was checked by using Thin Layer Chromatography (50% ethyl acetate: 50% hexanes) and dipped in iodine. Lastly, the isolated product was confirmed by NMR by using CDCl_3 as solvent.

Table 3: Volume and eluents for column chromatography used to purify crude mixture

Cumulative volume of solvent system (mL)	Ethyl acetate concentration (%)	Solvent system ratio (ethyl acetate: hexanes)
100	0%	0:100
200	5%	5:95
300	10%	10:90
400	15%	15:85
500	20%	20:80

Chapter 3

Results and Discussion

3.1 Extraction of *trans*-ferulic acid from rice bran

After the extraction process had finished, the solvent that was used in the extraction process was removed by the rotary evaporator. The product that obtained was in the form of liquid that was shown in the figure below. (Figure 7 and Figure 8)



Figure 7: Product from the extraction by using ethanol as solvent

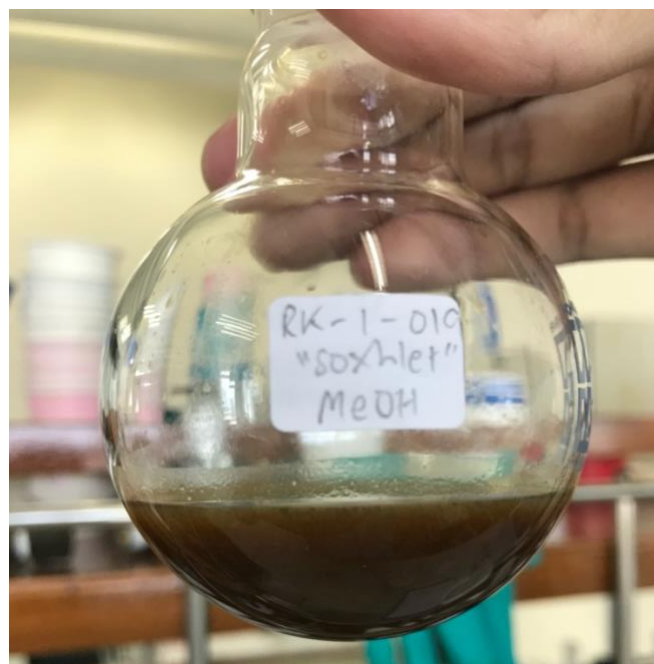


Figure 8: Product from extraction by using methanol as solvent

Then, Thin Layer Chromatography was used to check the amount of the product from extraction of rice bran in both solvents. The mobile phase for Thin Layer Chromatography was 20% Ethyl acetate: 80% Hexane. The product was spotted on Thin Layer Chromatography for 3 columns. First column was the product from the extraction by using ethanol as solvent, labelled as letter "E". The middle column was the co-spot between product from extraction by using methanol and ethanol, labelled as letter "C". Lastly, the third column was the product from the extraction by using methanol as solvent, labelled as letter "M". (Figure 9)

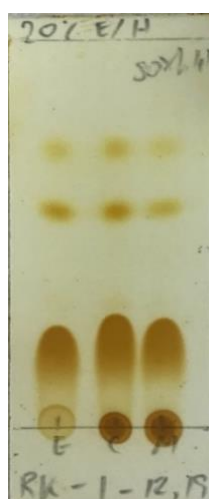


Figure 9: TLC for checking the product from extraction

Thin Layer Chromatography (TLC) shown that the product from the extraction was a mixture that contain 3 compounds as it was shown 3 spots on the TLC. The first thing that can conclude from TLC was product of extraction from both solvents was the same compound due to the height of the spot from TLC was all the same. The spots on TLC cannot be seen under UV light but it was appeared when dipping in iodine (TLC staining agent). This can conclude that all of 3 compounds in the mixture contain unsaturated bond because iodine will react with unsaturated bond and will appear as a brown color spot on the TLC.

After the solvent in column chromatography was removed from the product by rotary evaporator, the isolated product from both extraction in methanol and ethanol that was obtained as solid, shown in the figure 10 below. The figure in the left-hand side is the product from extraction by using ethanol as solvent. The figure in the right-hand side is the product from extraction by using methanol as solvent. (Figure 10)

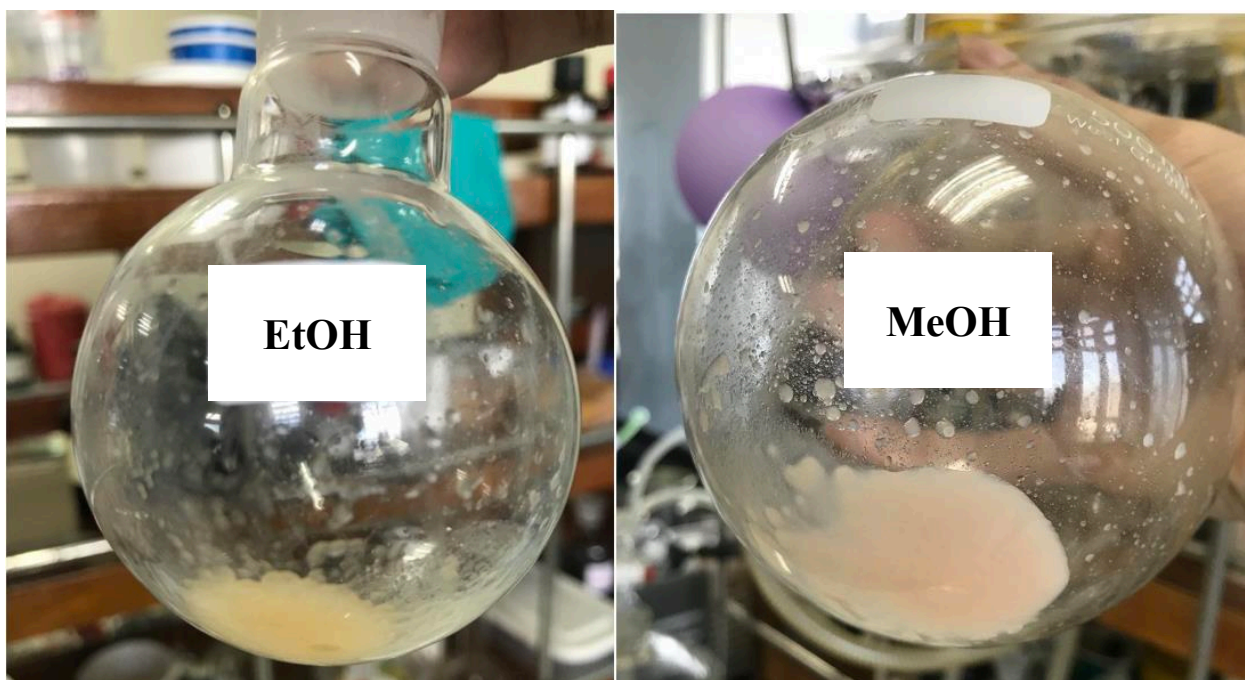


Figure 10: Isolated product from the extraction of rice bran

Table 4: Yield of the isolated product from Soxhlet extraction

Solvent for extraction	Yield of the product Unit: grams per 100 grams of rice bran
Ethanol	Did not weigh the product
Methanol	1.78

Next, chemical structures of the isolated products were characterized by NMR. According to NMR, it shown that the isolated product from the extraction of rice bran was the mixture between oleic acid, linoleic acid and palmitic acid. Oleic acid and linoleic acid are the unsaturated fatty acid with an 18-carbon chain. Only palmitic acid is the saturated fatty acid with a 16-carbon chain (Figure 11).

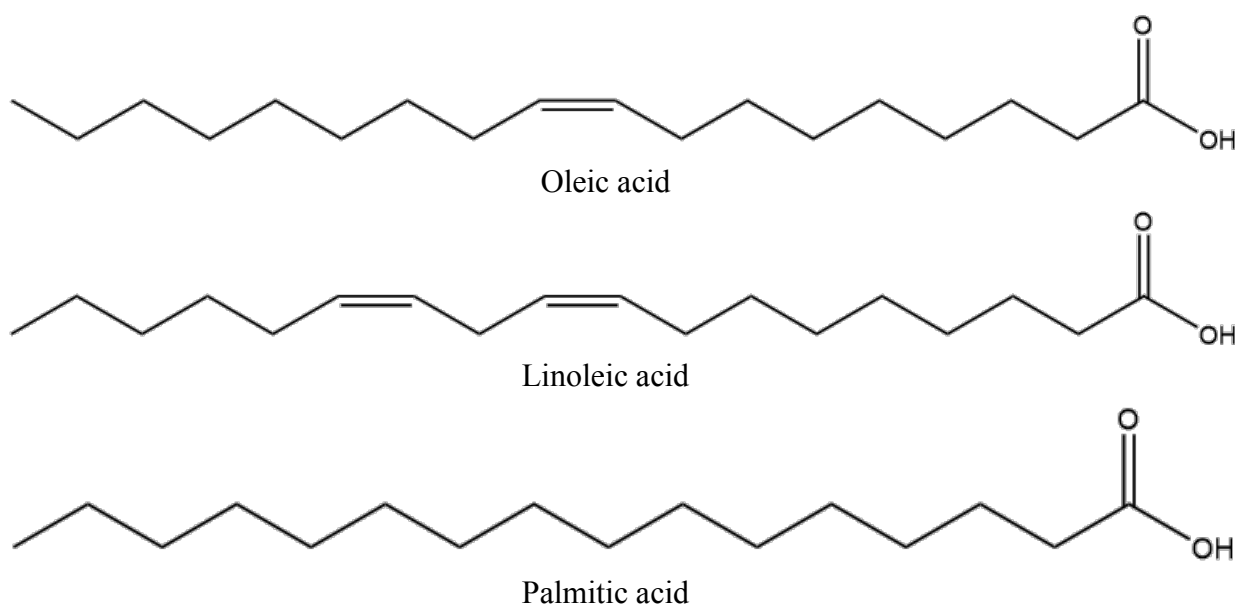


Figure 11: Structure of fatty acid in the product
(From top to bottom: oleic acid, linoleic acid and palmitic acid)

Electrospray Ionization Mass Spectrometry (ESI-MS) negative ion mode was used to confirm the appearance of 3 fatty acids in each isolated product. For the extraction of rice bran by using ethanol (Figure 12), oleic acid ($m/z = 281.15$), linoleic acid ($m/z = 279.29$) and palmitic acid ($m/z = 255.11$) can be all detected under ESI mass spectrometry.

On the other hand, only oleic acid ($m/z = 281.42$) and palmitic acid ($m/z = 255.11$) can be detected by ESI mass spectrometry from the isolated product that used methanol as solvent for extraction. (Figure 13)

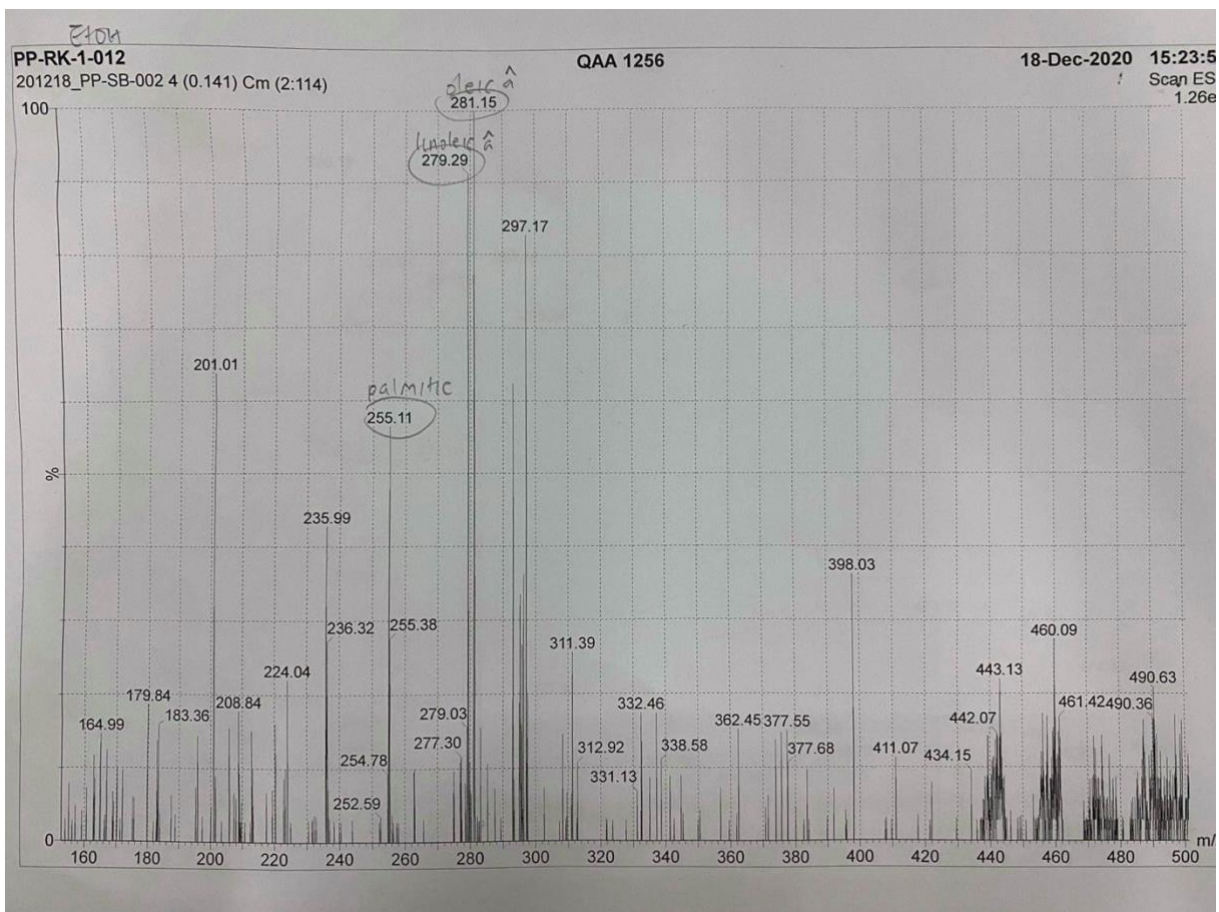


Figure 12: ESI-MS for isolated product by using ethanol as solvent for extraction

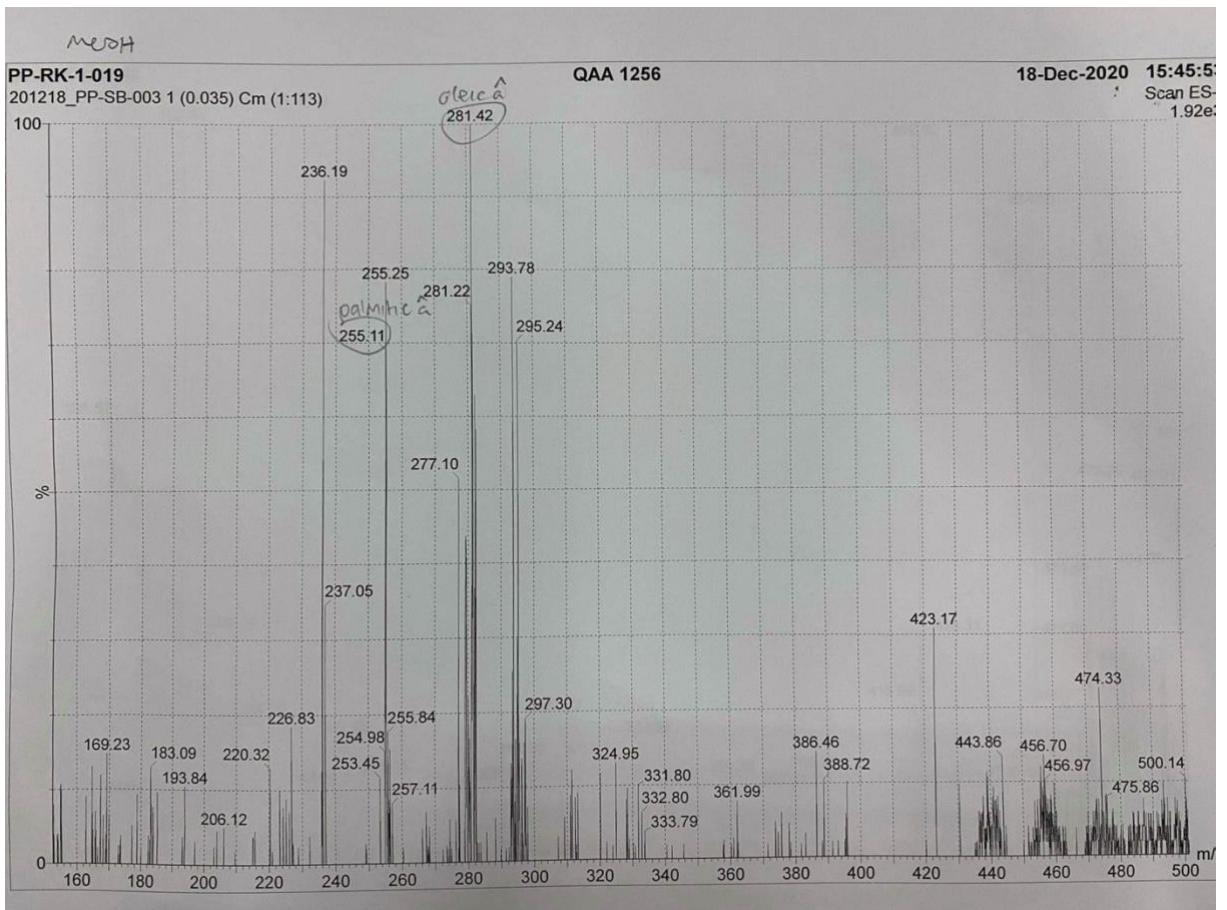


Figure 13: ESI-MS for isolated product by using methanol as solvent for extraction

It was quite difficult to confirm the structure of the product from the NMR due to the peak for long chain fatty acid are almost the same. So, NMR from the experiment was compared to the reference from Magritek¹¹ to confirm the structure that was appeared in the product. (Figure 14)

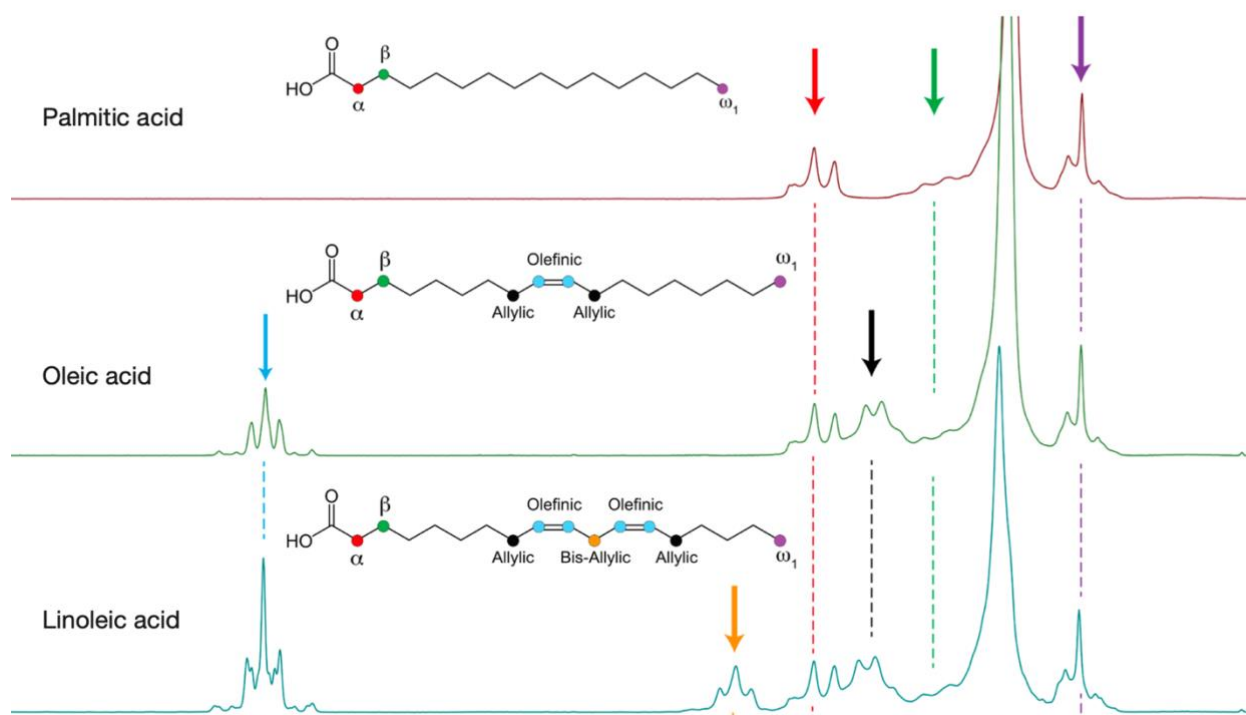


Figure 14: Reference of NMR from Magirtek

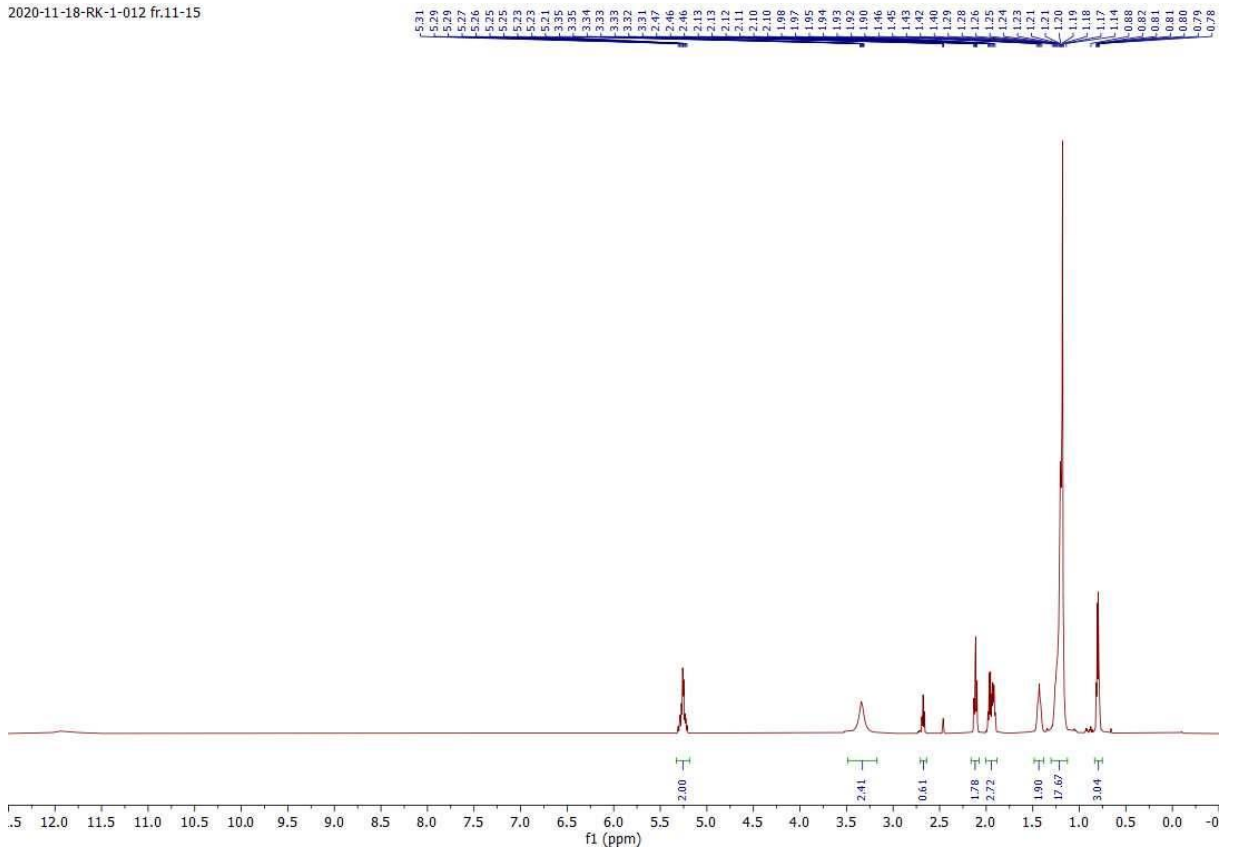


Figure 15: NMR from extraction of rice bran by using ethanol

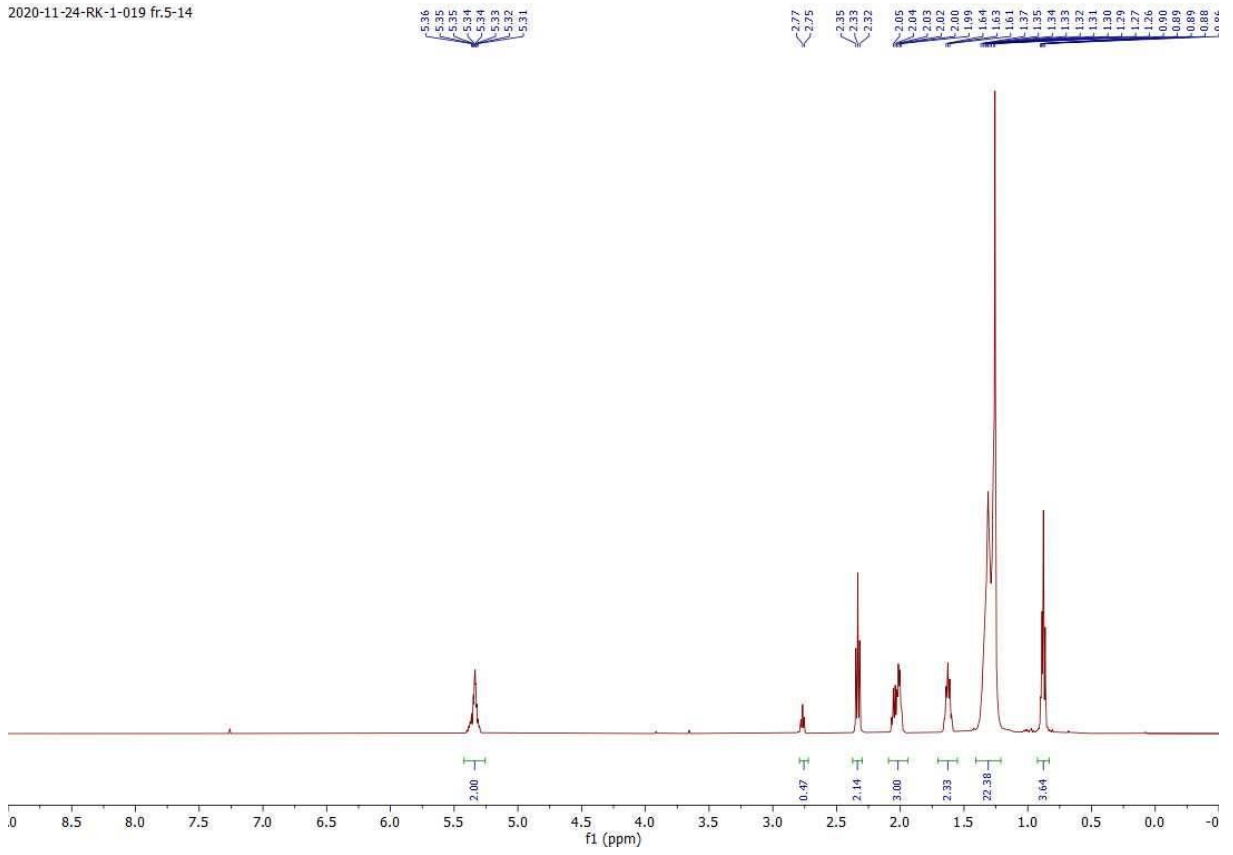


Figure 16: NMR from extraction of rice bran by using methanol

This shown that *trans*-ferulic acid cannot be able to extract out from the isolated rice bran. In this case, commercially available *trans*-ferulic acid was used for the study of ozonolysis to yield vanillin. (Figure 17)

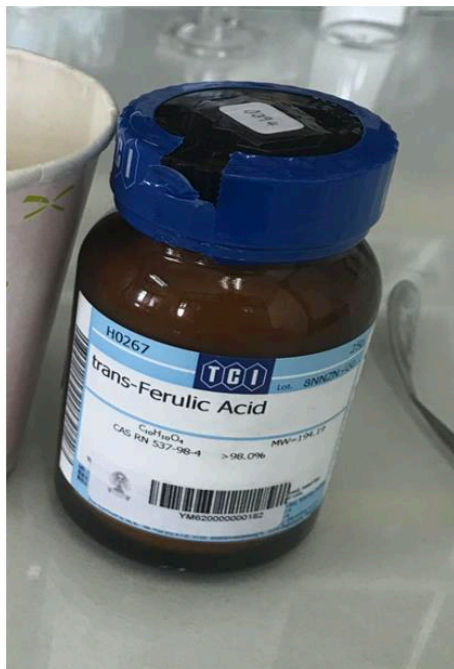
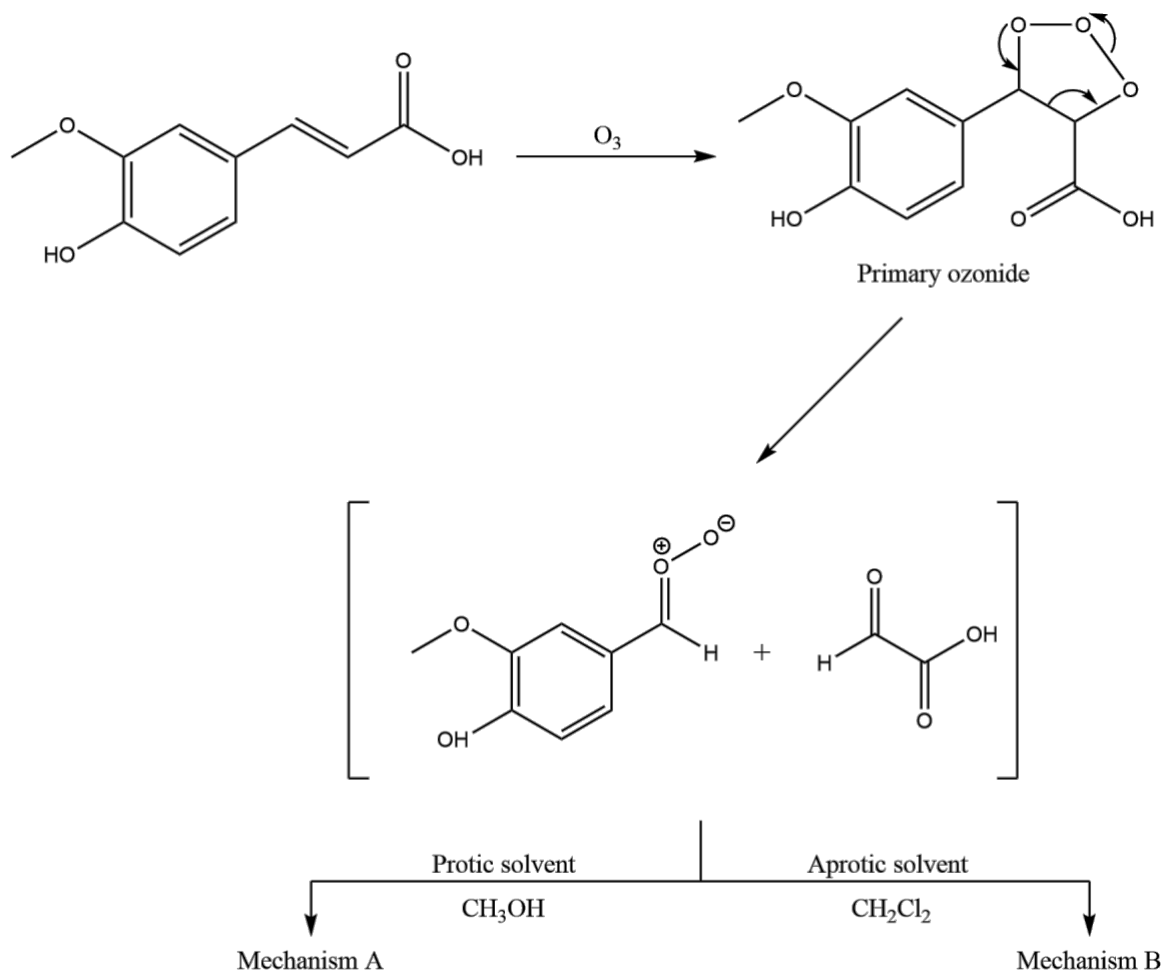


Figure 17: Pure *trans*-ferulic acid for the study of ozonolysis

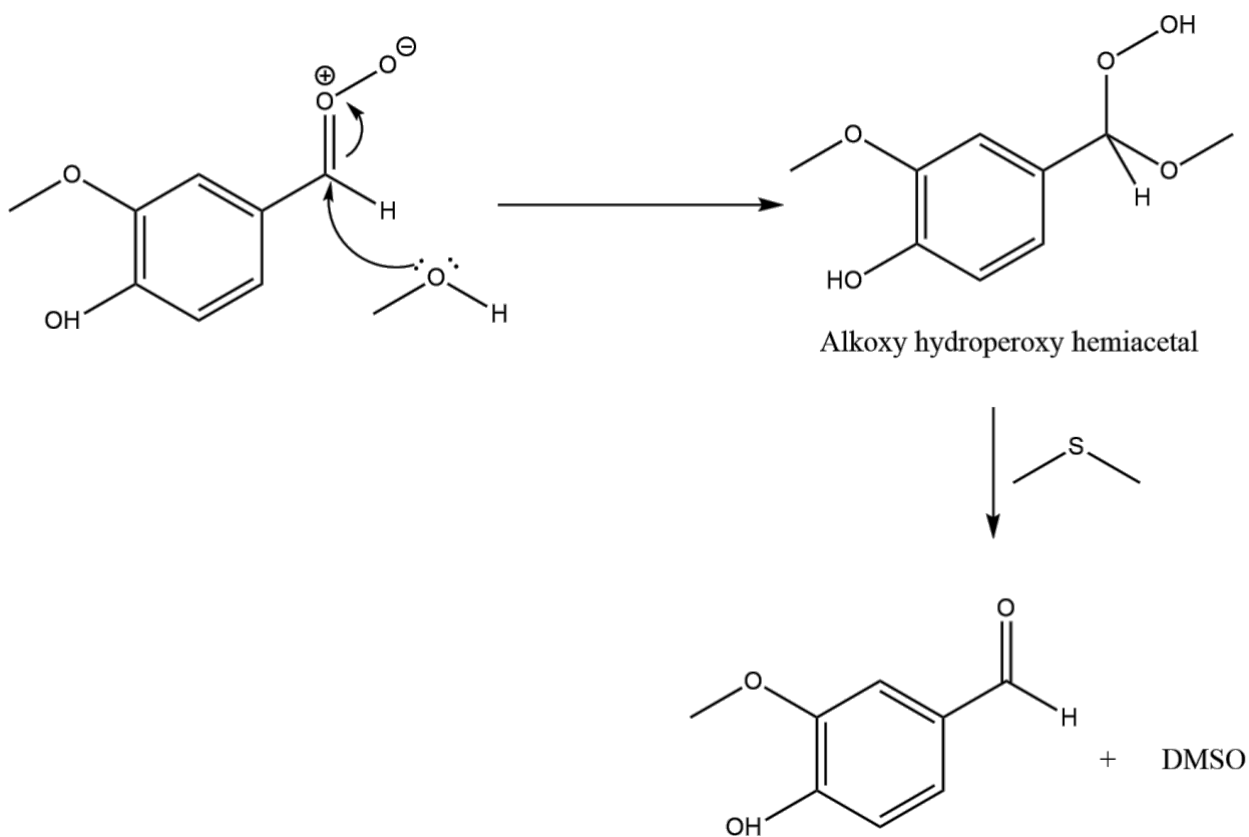
3.2 Ozonolysis of pure *trans*-ferulic acid

First of all, the solvents that were used for the study of ozonolysis were the solvent that can affect the formation of ozonide intermediate. (Scheme 7)



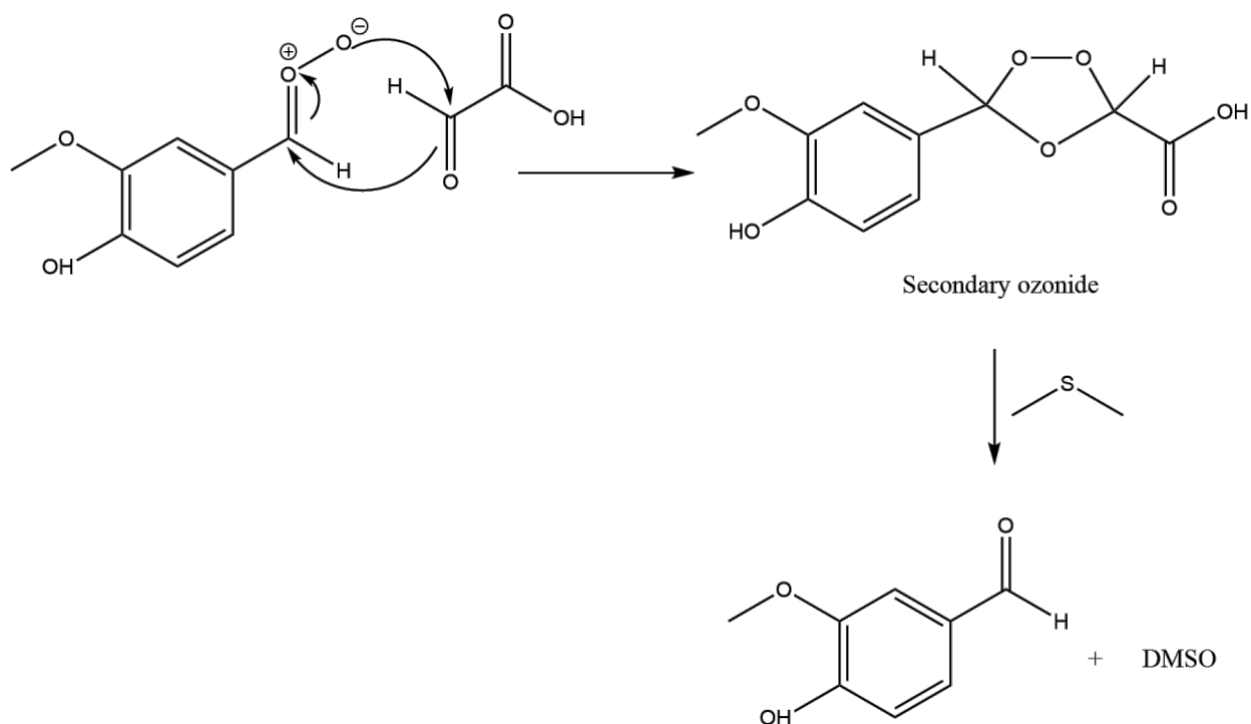
Scheme 7: Choices of solvent for ozonolysis

After Criegee intermediate (carbonyl oxide) carbonyl compound was already formed, the mechanism will be divided into 2 pathways. For mechanism A, the solvent that was used was protic solvent such as methanol as an example. The lone pair of protic solvent will react with carbonyl oxide and alkoxy hydroperoxy hemiacetal will be formed as shown below. (Scheme 8)



Scheme 8: Participation of solvent in ozonolysis (Mechanism A)

On the other hand, aprotic solvent such as dichloromethane (CH_2Cl_2) is added to the reaction as shown in the scheme below (Scheme 9). In this case, there is no participation between the reagent and solvent. So, the Criegee intermediate will react themselves and secondary ozonide will be formed as a reaction intermediate. For both pathways, vanillin is a major product and dimethyl sulfoxide is a byproduct of a reaction.



Scheme 9: No participation of solvent in ozonolysis (Mechanism B)

It is much easier to reduce alkoxy hydroperoxyl hemiacetal than secondary ozonide. So, protic solvent can make the Criegee intermediate converted to vanillin easier than using aprotic solvent. In conclusion, this is the reason that this experiment was focused on using protic solvent.

In this study for ozonolysis of *trans*-ferulic acid to yield vanillin, protic solvents which are methanol, ethanol and water were used to examine the yield of vanillin.

3.2.1 Ozonolysis of pure *trans*-ferulic acid by using methanol as solvent

3.2.1.1 The solubility effect of *trans*-ferulic acid in methanol

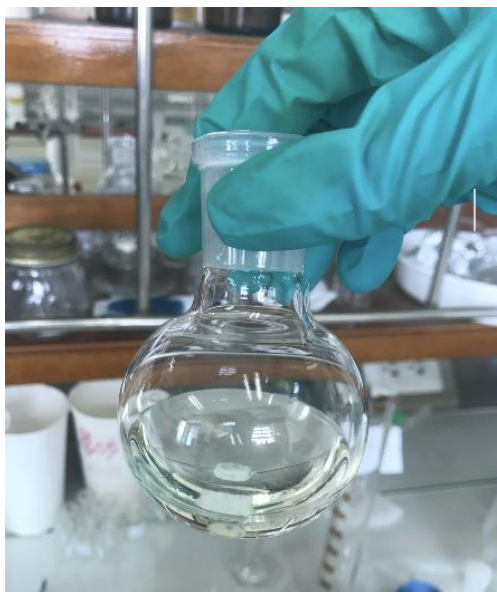


Figure 18: The solubility effect of *trans*-ferulic acid in methanol

As shown in the figure above (Figure 18), all of *trans*-ferulic acid was dissolved in methanol. The color for the solution is colorless and transparent.

3.2.1.2 Formation of the intermediate in the reaction

The formation of the intermediate in the reaction can be observed from TLC. The mobile phase for TLC was developed under 100% ethyl acetate eluent system. The products were spotted on the TLC for 3 columns. The first column was the spot of pure *trans*-ferulic acid. The middle column was the co-spot between pure *trans*-ferulic acid and the product from the reaction and the last column was only the spot from the product. TLC was observed under 2 conditions: under UV light that was shown in the left-hand side of the figure and dipping in 2,4-DNP (TLC staining agent) that was shown in the right-hand side of the figure. The orange spot on TLC under 2,4-DNP staining agent revealed the functional groups as aldehyde in the structure of the compound.

Important points that can be seen from TLC were the vanillin had formed already as the orange spot on the TLC under 2,4-DNP staining agent can be observed and the 4 spots with the lowest R_f value in middle and third column might be intermediate of the reaction. If the hypothesis was true, after reductive workup these 4 spots should be disappeared. (Figure 19)

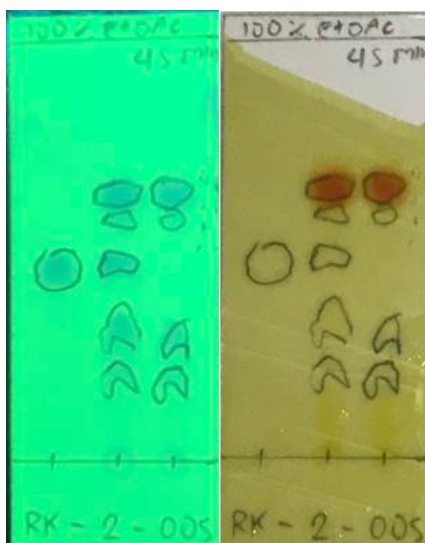


Figure 19: TLC for the product using methanol as solvent

After dimethyl sulfide was added to the reaction to reduce the intermediate, it can be observed that these 4 spots were disappeared. This can conclude that 4 spots on the TLC were the spots of the intermediate. (Figure 20)

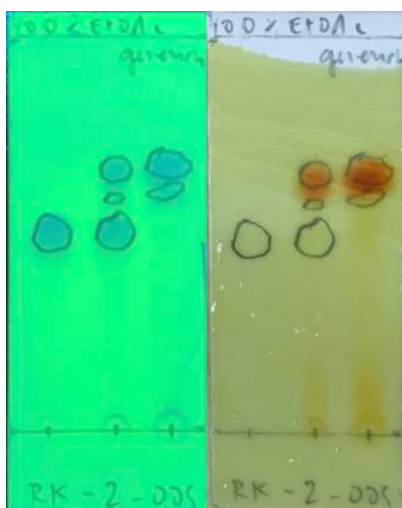


Figure 20: TLC for the product after reductive workup

3.2.1.3 Percentage yield of the product

Vanillin was obtained as yellow liquid (Figure 21). The percentage yield of the final product is 92% and ^1H NMR characterization indicated small amount of impurity in the isolated vanillin.



Figure 21: Vanillin obtained from ozonolysis using methanol as solvent

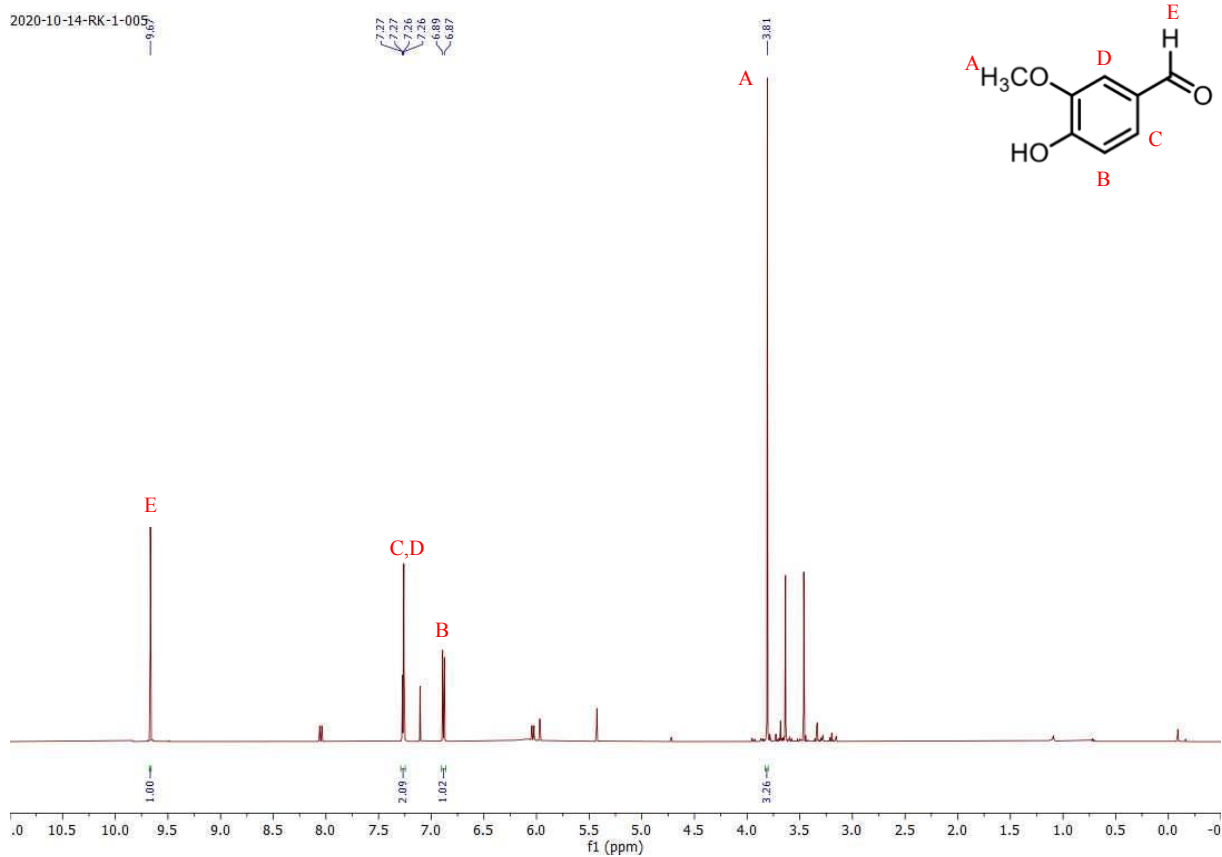


Figure 22: NMR from ozonolysis of pure *trans*-ferulic acid by using methanol

Peak interpretation for vanillin

¹H NMR (400 MHz, CDCl₃) δ 9.67 (s, 1H), 7.26 (d, J = 5.5 Hz, 2H), 6.88 (d, J = 8.5 Hz, 1H), 3.81 (s, 3H).

3.2.2 Ozonolysis of pure *trans*-ferulic acid by using ethanol as solvent

3.2.2.1 The solubility effect of *trans*-ferulic acid in ethanol

As shown in the figure above (Figure 23), all of *trans*-ferulic acid was dissolved in ethanol. The color for the solution is faint yellow and transparent.

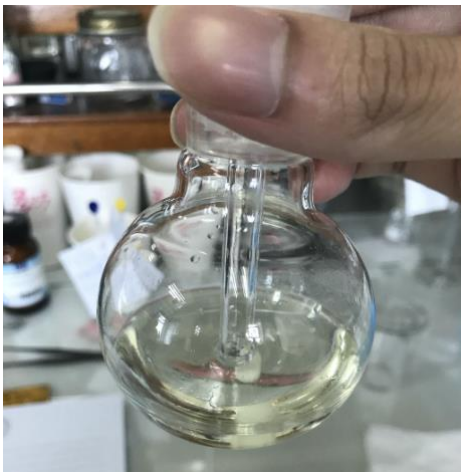


Figure 23: The solubility effect of *trans*-ferulic acid in ethanol

3.2.2.2 Formation of the intermediate in the reaction

The formation of the intermediate in the reaction can be observed from TLC. The mobile phase for TLC was developed under 100% ethyl acetate eluent system. The products were spotted on the TLC for 3 columns. The first column was the spot of pure *trans*-ferulic acid. The middle column was the co-spot between pure *trans*-ferulic acid and the product from the reaction and the last column was only the spot from the product. TLC was observed under 2 conditions: under UV light that was shown in the left-hand side of the figure and dipping in 2,4-DNP (TLC staining agent) that was shown in the right-hand side of the figure. The orange spot on TLC under 2,4-DNP staining agent revealed the functional groups as aldehyde in the structure of the compound

As same as using methanol as solvent, the 2 spots in middle column and third column with the highest R_f value was revealed that the compound which has an aldehyde as functional group was formed already but the different thing that can be observed easily was the 4 spots and second and third column. Just a small spot can be observed. This can conclude that a little amount of intermediate was formed in the reaction. (Figure 24)

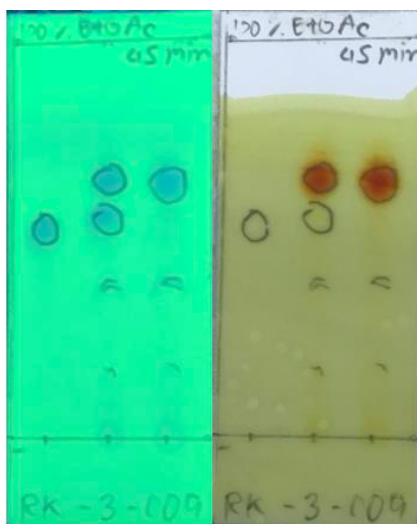


Figure 24: TLC for the product using ethanol as solvent

After dimethyl sulfide was added to the reaction to reduce the intermediate, it can be observed that these 4 spots were disappeared. This can conclude that 4 spots on the TLC were the spots of the intermediate. (Figure 25)

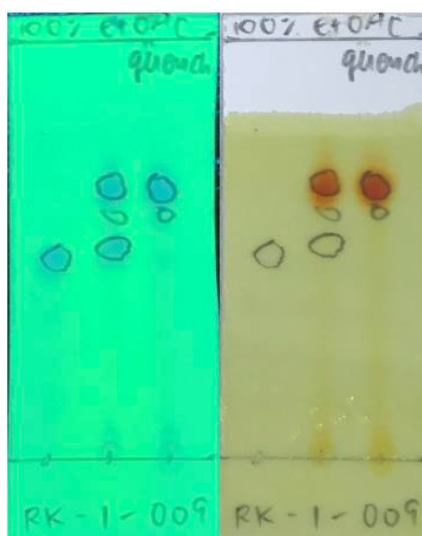


Figure 25: TLC for the product after reductive workup

3.2.2.3 Percentage yield of the product

Vanillin was obtained as yellow liquid (Figure 26). The percentage yield of the final product is quantitative and ^1H NMR characterization indicated small amount of impurity in the isolated vanillin.



Figure 26: Vanillin obtained from ozonolysis using ethanol as solvent

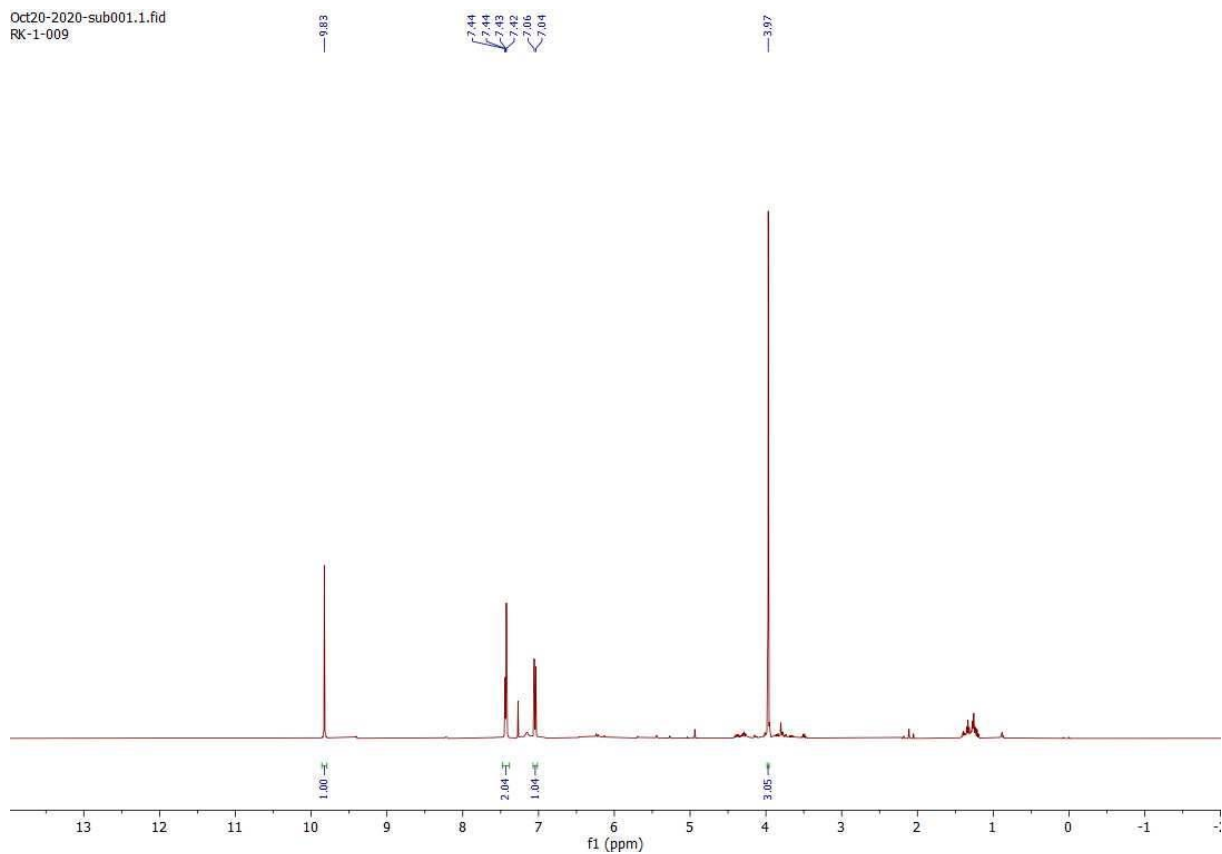


Figure 27: NMR from ozonolysis of pure *trans*-ferulic acid by using ethanol

3.2.3 Ozonolysis of pure *trans*-ferulic acid by using water as solvent

3.2.3.1 The solubility effect of *trans*-ferulic acid in water

Unlike using methanol and ethanol as solvent for ozonolysis, *trans*-ferulic acid did not dissolve well in water despite the presence of carboxylic functional group in the molecule. (Figure 28)



Figure 28: The solubility effect of *trans*-ferulic acid in water

3.2.3.2 Formation of the intermediate in the reaction

As shown on TLC, the left column on TLC was the spot from pure *trans*-ferulic acid. The middle column was the co-spot between pure *trans*-ferulic acid and product, and the third column was the spot from the product of the reaction. The mobile phase was still same as 2 previous experiments.

It was totally different from using methanol and ethanol as solvent due to TLC (Figure 29) had only one spot in each column and there was no orange spot at the highest R_f value in middle and third column of the TLC under 2,4-DNP staining agent. This can conclude that vanillin was not formed by now.

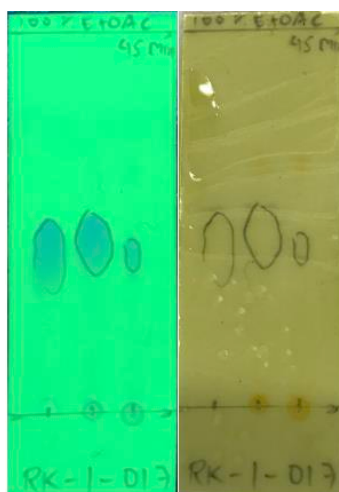


Figure 29: TLC for the product using ethanol as solvent

So, the ozonolysis reaction time was increased to 2 hours. From this point, the orange spot can be observed in TLC under 2,4-DNP staining agent at the base line. However, it cannot be confirmed that the product of the reaction is vanillin. (Figure 30)



Figure 30: TLC of the reaction after ozonolysis for 2 hours under 2,4-DNP staining agent

In this case, the mixture of the reaction was used to reductive workup with dimethyl sulfide. After the process was finished, the color of the mixture was orange brown. This was different from using methanol and ethanol as solvent. (Figure 31)

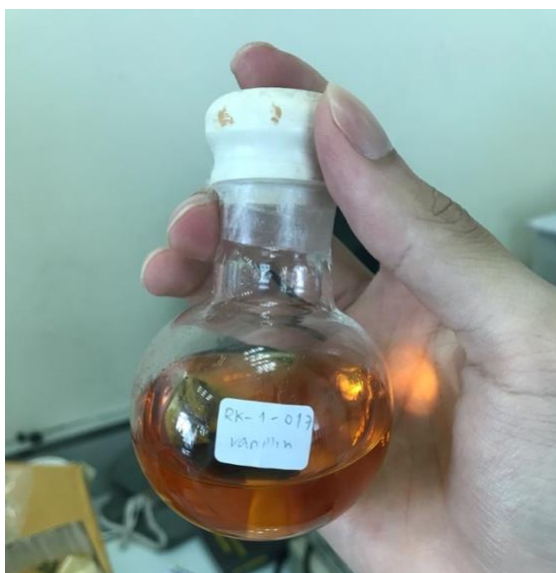


Figure 31: Product after reductive workup by dimethyl sulfide

Since the solvent for this experiment was water, so it was difficult to remove it out by rotary evaporator due to the high boiling point. The water was removed from the mixture by extraction with ethyl acetate (Figure 32). Then, collected the organic phase and use sodium sulfate to remove water out. When extraction is finished, remove the organic solvent in the mixture by rotary evaporator.



Figure 32: Extraction of the mixture by using ethyl acetate

Then, the crude of the mixture was checked by TLC using the same mobile phase condition (100% ethyl acetate) as methanol and ethanol. TLC was still observed under UV light and dipping in 2,4-DNP staining agent. From the figure below, it shown that the vanillin was formed along with another unidentified byproduct. (Figure 33)

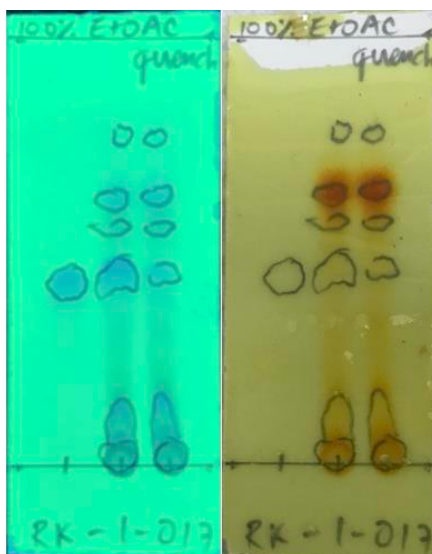


Figure 33: TLC for the product that already quenched by DMS

3.2.3.3 Percentage yield of the product

The final product was vanillin and percent yield of the product was 13% (Figure 32). ^1H NMR characterization indicated large amount of impurity in the isolated vanillin.



Figure 32: Product of ozonolysis of *trans*-ferulic acid by using water as solvent

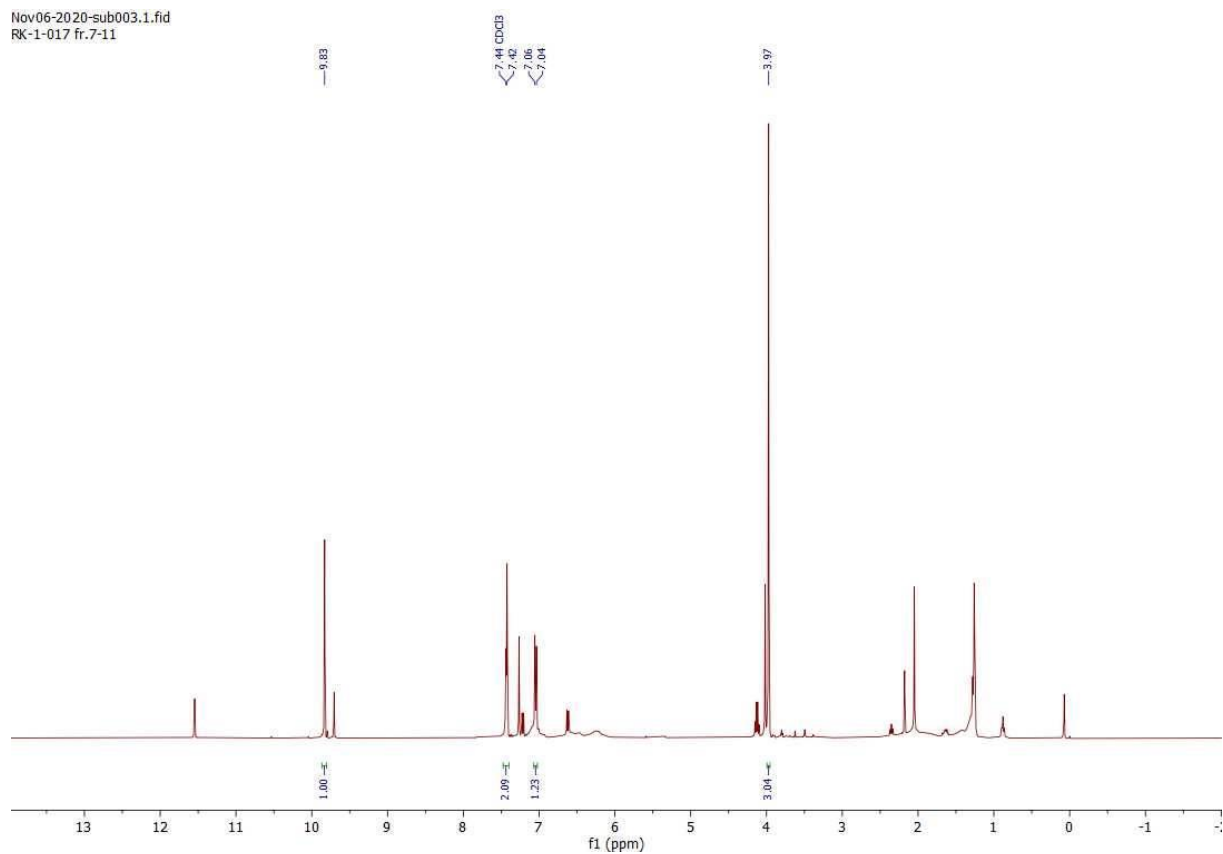


Figure 33: NMR from ozonolysis of pure *trans*-ferulic acid by using water

From the study for ozonolysis of *trans*-ferulic acid to yield vanillin, the results were revealed that ethanol was the most suitable solvent for ozonolysis due to:

1. It gave high yield of the vanillin.
2. Reaction intermediate was generated in a little amount. Reductive workup process might not necessary.
3. Ethanol is a renewable resource which can be produced by fermentation of agricultural products.

Chapter 4

Conclusions

Three organic solvents: methanol, ethanol, and water were examined in ozonolysis of *trans*-ferulic acid. The final product was vanillin and percent yield of the product from each solvent are 92%, quantitative and 13% respectively. The result revealed that ethanol was the most suitable solvent for ozonolysis of *trans*-ferulic acid. For the Soxhlet extraction of rice bran, the solvents for extraction were chosen from the previous experiment which are ethanol and methanol. *trans*-Ferulic acid was not successfully be extracted from rice bran due to the small amount of it. The product from the extraction was a mixture between palmitic acid, oleic acid, and linoleic acid. The weight of the product from the Soxhlet extraction by using ethanol as solvent was not recorded. The weight of the product from Soxhlet extraction of rice bran by using methanol as solvent was 1.78 grams per 100 grams of rice bran. For the further research, since rice bran constitutes small amount of *trans*-ferulic acid, the corn bran is a next agricultural renewable byproduct that will be used in extraction due to the very high amount of *trans*-ferulic acid (2,510 mg per 100 g of corn bran).⁹

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Biography

My name is Rawin Kunarb. My nickname is Jom. I graduated from Saint Gabriel's College (Science-Math) in 2017. Right now, I am fourth year undergraduate student in industrial and management major in Bachelor of Science in Applied Chemistry at Chulalongkorn University. The reason that I choose the project topic that related to organic chemistry is I want to use and apply the knowledge that I've got from the organic chemistry lecture class in second year.

Lastly, I am proudly to say that this project can pull out my potential about organic chemistry and laboratory technique.