FATTY ACID OF *MORINGA OLEIFERA* OIL AT DIFFERENT SAMPLE CONDITION

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ABSTRACT

Moringa oleifera (M.O) is widely known as the miracle tree which has been recognized as one of the possible resources that can produces biodiesel using a transesterification process supported with a base catalyst. Theoretically, most of the research on the fatty acid in M.O oil is based on dry sample seed and there is no information about the fatty acid on M.O oil that can be affected by fungus or still in growth. Hence, by manipulating the sample condition into three parts, the extraction of M.O seed begins by extraction process using thermal soxhlet with hexane as a solvent and all the samples went through transesterification process in order to measure the unknown fatty acid in each sample. The fatty acid is measured using a Gas Chromatography Mass Spectrometer GC-MS. In sample A, B, C, D, E and F the highest percentage area among the two trials is 49.95, 48.87, 54.56, 49.35, 27.32 and 40.05 respectively. Mean while, the retention time for sample A, B, C, D, E and F is at 32.510, 32.510, 32.467, 38.495, 32.478 and 32.483 respectively. It is expected the fatty acid for sample B and E is different as it affected by fungus but the result showed that the fatty acid is the same for the sample dry, wet and effected condition. In the analysis also there is Stearic acid and Palmitic acid that is also commonly find in the vegetables oil. As a conclusion sample A, B, C, D, E and F have a common acid same as the exact fatty acid in the biodiesel. However, the result shows there are additional of unknown fatty acid exist such as Margaric acid and Arachidic acid. Even all the samples have the same fatty acid component, but their oil content is different. The dry sample and the affected has a common oil content while the wet sample shows vice versa because most of it has high moisture content.

ABSTRAK

Moringa oleifera atau dikenali juga sebagai Pokok Kelor dikatakan sebagai pokok ajaib dimana salah satu sebabnya adalah ia boleh menghasilkan biodiesel dengan menggunakan proses transesterification dengan disokong oleh pemangkin alkali. Secara teorinya, kajian biodiesel hanya dilakukan dari kacang M.O yang kering sahaja tetapi tidak untuk kacang M.O yang diserang kulat mahupun kacang M.O yang muda. Oleh itu, pembolehubah adalah pada penyediaan sampel kacang M.O yang dibahagikan kepada 3 keadaan iaitu salah satu diantara 3 keadaan itu dikategorikan sebagai sampel tetap. Experimen bermula dengan pengasingan minyak daripada kacang dengan menggunakan Electro Thermal Soxhlet dan hexane sebagai pelarut. Selepas itu, hexane diasingkan pula dengan menggunakan rotary evaporator. Kemudian, minyak M.O yang diasingkan melalui process transesterification untuk menghasilkan biodiesel (FAME's). FAME yang terhasil dibiarkan tenang selama 1 hari di dalam pemisah corong. Akhirnya, minyak disediakan untuk analisis acid lemak di dalam GC-MS. Di dalam sampel A, B, C, D, E dan F, peratus kawasan tertinggi adalah 49.95, 48.87, 54.56, 49.35, 27.32 dan 40.05. Manakala untuk rentetan masa yang diperlukan adalah 32.510, 32.510, 32.467, 38.495, 32.478 dan 32.483. Dijangka acid lemak yang dihasilkan berbeza bagi sample B dan E namun peratus kenaikan yang pertama ini berkongsi acid lemak yang sama iaitu Oleic acid (sampel A, B, C, D, E dan F). Manakala selebihnya Stearic acid and Palmitic acid yang juga acid lemak yang biasa ditemui di dalam lemak sayur dan haiwan. Walaupun begitu, terdapat pernambahan acid baru iaitu Margaric acid dan Arachidic acid di dalam kajian. Selain itu juga, kajian mendapati kuantiti minyak yang dihasilkan bagi sampel kering dan sampel yang dijangkiti sama tetapi berbeza bagi sampel basah kerana separuh daripada berat kacangnya mengandungi banyak kuantiti air berbanding minyak itu sendiri.

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LIST OF ABBREVIATIONS

FAME	Fatty Acid Methyl Ester
FFA	Free Fatty Acid
GC-MS	Gas Chromatography Mass Spectrometer
GC-FID	Gas Chromatography Flame Ionized Detector
M.O	*Moringa oleifera*
PA	Physical Appearance
TS	Thermal Soxhlet

1 INTRODUCTION

1.1 Motivation and statement

Moringa oleifera(M.O) which known as miracle tree have been recognized as one of the world's most useful trees. Almost every part of *Moringa* tree can be used for food, or has some other beneficial property. According to the Mehnaz (2008), M.O plant have played a significant role in maintaining human health and improving the quality of human life for thousands of years and have served humans well as valuable components of medicines, seasonings, beverages, cosmetics and dyes. Recently, focusing on *Moringa* plant research has been increased because of the oil produced by the seed and a large of evidence has collected to show immense potential of this oil to produces a biodiesel. Despite their use in medicinal, the M.O oil can produce a biodiesel by a simple transesterification process.

Transesterification process is by converting the M.O oil into biodiesel (Fatty Acid Methyl Ester (FAME) with a by product of glycerol, (soap)). This process is supported by addition of catalyst. However, the type of the catalyst depends on the Free Fatty Acid (FFA) in the M.O oil. According to the literature, M.O in Malaysia has low FFA which is below 1%. If the FFA below 1%, base catalyst transesterification is the suitable choice to convert into FAME by Alberta (2012).

The preparation of sample can be changed by implies a different condition of *Moringa* seed which can be affected by fungus and growing seed. This difference expected to produce a different composition and fatty acid in the M.O oil. Theoretically, the biodiesel (FAME) of M.O can be produced by transesterification process using a base catalyst however with a different sample condition and unknown composition of FA in the oil, the result might be different.

Furthermore, most of the studies are focusing on the fatty acid composition in the dry M.O seed. However, these studies are not emphasis on M.O which is still in growth and affected by the fungus. This is important study as the research on biodiesel by M.O is widely developed as the seed oil that can contribute to one of the biodiesel resources in Malaysia and medicinal application. Recently, there are wide plantation of M.O in Sabah State which is 4 major items to be produces such as *Moringa* leaf,

Moringa oil, *Moringa* Bio-Flocculant and *Moringa* feedstock. The *Moringa* oil is being produced with the dry M.O seed. However, with certain circumstances and accidental condition that might be occur to the sample, the oil probably will have a different composition of fatty acid. By that, another pre-treatment and catalyst have to be applied. Therefore, an investigation of composition of fatty acid in M.O seed oil needs to be done for safety on the feedstock sample.

1.2 Objectives

The following are the objectives of this research:

- To investigate experimentally the unknown fatty acid in the M.O oil at different samples collection and storing condition

1.3 Scope of this research

The following are the scope of this research:

i. To compare physical appearance of the oil produced from different sample
ii. To measure oil yield from different sample
iii. To compare different sample with standard FAME

1.4 Main contribution of this work

The main contributions for this work are for the researcher that is using vegetables oil to produces biodiesel. This study is to prove that the free fatty acid in the vegetables oil can be change under certain condition before it can produce a biodiesel. Without a proper treatment the yield of biodiesel produced might be different as it used the same type of catalyst.

1.5 Organisation of this thesis

The structure of the reminder of the thesis is outlined as follow:

Chapter 2 provides a description on the production of Biodiesel using vegetables oil. A general review, characteristic and the benefit of the M.O, as well as the market survey of M.O in Malaysia. In this chapter also brief about the process of producing biodiesel using vegetable oil from collecting raw material until fatty acid analysis. Besides that, it also review about the transesterification process and the types of catalyst used. Lastly, this chapter provide a little view about the fatty acid profile in the M.O oil.

Chapter 3 gives a review about the preparation of raw material and its condition according to the sample. The, a little view about the method grinding and drying process, oil extraction process, separation process, transesterification process and sample preparation for GC-MS analysis.

Chapter 4 is devoted for the result and discussion in comparing the ratio of M.O oil produced and its physical appearance (PA) of each sample. Furthermore, this chapter also discuss about the highest peak and retention time of fatty acid profile using GC-MS analysis for each sample. Lastly, this chapter followed by the comparison of fatty acid profile for each sample of M.O oil.

Chapter 5 gives a summary of the thesis and recommendation for the future work which might be derived from the result found in this work.

2 LITERATURE REVIEW

2.1 Overview

This paper presents the experimental studies on production of biodiesel and its fatty acid by M.O seed oil in a transesterification process supported by base catalyst. The fatty acid composition will be analysed using Gas Chromatography Mass Spectrometer (GC-MS).

2.2 Moringa oleifera(M.O)

M.O is a new plant that has been discovered to have an application for the extracted oil from the seeds besides on its medicine appliance (Foidl, 2001). According to Rizzo et al (2010), M.O best known as the thirteen species of the genus moringae. It was highly valued in the ancient world such as Romans, Greeks and Egyptian as extracted edible oil and used it as perfume and skin lotion (Crist, 2011). In 19th century, plantation of M.O in the West Indies exported the oil to Europe for perfumes and lubricants for machinery (Green Energy Herbal, 2010). For centuries, people used M.O leaves as a medicine for common ailments. Clinical studies have begun to suggest that at least some of this claim is valid. For the time being, some investigation about M.O has been conducted such as the amount and the highest yield of extracted oil that can be produced (Khawaja et al, 2010)

According to the HDRA-Organic Organisation (2010), they discovered the seeds of the M.O seed contains 35% oil by weight and usually used for cooking purposes. Similar to Amaglo (2000), who claimed that the seed yields 38-40% of M.O oil, known as Ben oil. When the seeds are mature, their coating hardens and becomes bitter. This can be pressed for oil extraction. The oil from M.O is extracted by pressing the seed until the oil come out. Otherwise, the seeds can be drowned or roasted and can be separated by boiling process. However, most of the method of oil extraction used is Thermal Soxhlet for essential oil extraction**.** Figure 2.1 (a) (b) (c), shows the tree of M.O, dry M.O pods and the dry M.O seed that have been removed from its pods

(a)

(b)

(c)

Figure 2.1 (a) The tree of M.O, **(b)** The dry M.O pods and **(c)** The dry M.O seed

2.3 Oil Extraction using Electro Thermal Soxhlet

In addition of the fatty acid composition, the oil ratio produced also need to be investigated for different environment of sample. The simplest method to extract oil is using thermal soxhlet (T.S) with hexane as a solvent. Hexane have been recognised as the best solvent to extract oil but the safety of using it have to be alarmed for the user. The concept of T.S is likely a filtration where the desired compound has a limited solubility in a solvent and lastly coupled with evaporation and condensation in order to have a cycle process. Figure 2.2 shows the Electro thermal Soxhlet.

Figure 2.2 The Electro Thermal Soxhlet

2.4 Transesterification process

Biodiesel is produced by changing a long chain triglyceride component in the M.O oil into Fatty Acid Methyl Ester (FAME) and a by product of glycerol (soap). According to the Schuchardt et al (1997), vegetables oil can be directly changed into biodiesel at laboratory by reacting triglyceride with an alcohol in the presence of strong acid or base, producing a mixture of fatty acid alkyl ester and glycerol. **Eq. (1)** below illustrate the reaction occur during transesterification process. The type of catalyst used is depending on the Free Fatty acid in the oil.

$$RCOOR' + R''OH \xrightleftharpoons{catalyst} RCOOR'' + R'OH \quad \text{..... (1)}$$

2.5 Free Fatty Acid (FFA)

Free Fatty Acid (FFA) is an important quality indicator as well as a useful parameter in the method of refining, and choice of catalyst in the transesterification reaction (Berchmans and Hirata, 2008). The FFA and moisture contents of the oil or fat are the main reaction variables which directly affect the choice of catalyst used (Canakci and Van Gerpen, 2001). Another treatment has to be done for removing of the impurities so that the transesterification smoothly converted into methyl ester and not to the yield of glycerol (soap).

2.6 Base catalyst for transesterification

Base Catalyst transesterification are employed in industrial applications because of their higher reaction rates and shorter reaction time (Freedman et al., 1984; Schwab et al., 1987; Alcantara et al., 2000; Oliveira et al., 2008). Base catalyst such as the hydroxides of sodium and potassium (NaOH and KOH), especially NaOH has been widely used (Karmee and Chadha, 2005; Arzamendi et al., 2006) due to its lower cost and high solubility in methanol. Base catalysts are normally used in the concentration range of 0.5-1% (w/w), oil: alcohol molar ratio of 1:6, and near the boiling point of the alcohol to obtain more than 98% of ester yield (Freedman et al., 1984; Ma and Hanna, 1999; Fukuda et al., 2001; Marchetti et al., 2007). The presence of even moderate amount of FFA (1%) will result in soap formation. It is reported that the base-catalyzed

transesteri_cation process is most effective when the level of free fatty acids is less than 1%. As M.O known to have a FFA which is below 1% and it is suitable to a base catalyst transesterification. As the oil produced, the fatty acid profile of the oil can be measured using a Gas Chromatography coupled with Mass Spectrometer.

2.7 Gas Chromatography – Mass Spectrometer (GC-MS)

The formation of FAME will then undergo a GC-MS for Fatty Acid analysis in the M.O oil. The GC-MS is a combined technique in which a mass spectrometer is used as a detector for gas chromatography. The effluent from the gas chromatograph is passed into the inlet of a mass spectrometer, where the molecules of the gas are fragmented, ionized and analyzed using one of a variety of different types of mass analyzers. To perform this method, the sample is vaporized and injected onto the head of the chromatography column. Elution is brought about by the flow of an inert gaseous mobile phase.

2.8 Fatty Acid Profile

According to the (Ashraf, 2007), M.O have high unsaturated fatty acid which is domainly oleic acid above 70%. Meanwhile, there is a saturated fatty acid which is Palmitic acid, Lauric Acid, Stearic Acid, Linoleic Acid and Linolenic acid. As a summary, oleic Acid is dominantly acid in the M.O oil as this acid is important in the medicinal application and biodiesel production. The M.O seed oil contains all the fatty acid in the olive oil except linoleic acid and recognised as an acceptable substitute.

3 MATERIALS AND METHODS

3.1 Materials

The seeds were obtained from the Indian Temple at Gambang, nearby UMP campus. About 5 kg of M.O pods have been taken in dry and wet condition. The raw material obtained directly from the source then kept in laboratory.

3.2 Methodology

3.2.1 Raw material Preparation

There are 3 part of getting the M.O. For the first part, only 500g of M.O seed (Sample A) was able to claim as the season of this tree is nearly at the corner. Mean while for the next season, about 200 g of M.O seed (Sample B) is claimed. As for sample C, the source is easily claimed at the market about 70 g. Different story for sample D and E which is 240 g of M.O seed are able to claimed and separated into half for sample D and E. Lastly for Sample F, about 124 g of M.O seed is claimed . Figure 3.1 (a) (b), shows a dry seed and wet seed. The shell of both M.O seed is dehusked for the nuts.

(a)

(b)

Figure 3.1, (a) Dry seed, **(b)** Wet seed

3.2.2 Grinding and Drying process

All the M.O nuts are being grinded by a blender until it is made into a powder form. However, the smallest size obtained was 2 mm. The smaller the size of the nuts the easier for the solvent to extract the oil because the rate of extraction depends on the

surface area. The higher the surface area the higher extraction rate because contact between solvent and the materials is high. Before that, Sample B and E which half of the dry M.O seed is having an appropriate environment by keeping it in a standard temperature at 25^0C, with no light and additional of wet and dry condition for 7 days long. This specified environment is said suitable for fungus to grow. Then, all sample A, B C, D, E and F is weighted before and after drying at temperature 70^0C for 3 days. This is to ensure no water in the M.O seed. The temperature is set as it is suitable to extract the water and avoid giving damage to the essential oil.

3.2.3 Oil Extraction process

The equipment of the Electro Thermal Soxhlet is set up as shown in the Figure 3.2. The inlet water is set up from the top and exit at the bottom of condenser. The temperature to heat up the hexane is set to 70^0C. The amount of hexane used is 200 ml with 10g of M.O powder. The solvent is changed until 3 cycles were performed. All of the handling regarding this extraction has to be carried out in the fume hood for safety because hexane is dangerous for inhalation and skin irritation. The product of M.O oil then will be collected using rotary evaporator, to evaporate the hexane

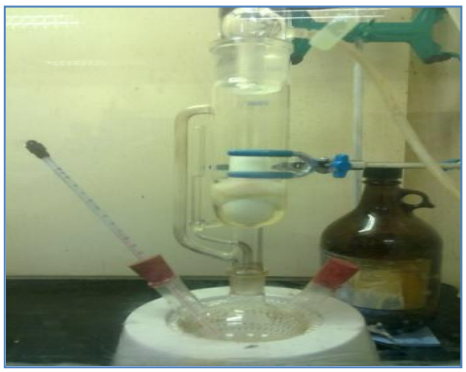

Figure 3.2 Oil Extraction process

3.2.4 Separation process

The hexane was evaporated at rotary evaporator to collect M.O oil as shown in the Figure 3.3. This method is quiet efficient to extract solvent from solution and easily separated. The rotary evaporator is set at temperature 60^0C for 4 hours and the rotation speed was 80 rpm. The product of M.O oil from Sample A, B, C, D, E and F that are able to get is 200, 20, 10, 22, 23 and 10 ml. After the separation the oil produced is prepared for transesterification process.

Figure 3.3 Rotary Evaporator

3.2.5 Sample preparation for transesterification process

Sample then is prepared for transesterification process which required 15 ml of M.O oil for each sample. However, for sample C and F, the M.O oil required only 10 ml as the methanol and Calcium Oxide (CaO) will be changed according to the methanol: oil ratio. This is because the quantity of oil can claimed only 10 ml from the raw sample after extraction. In this process, 2 set of test is prepared which each test contains 3 samples that are different in condition. Practically, Samples A, B and C have the same condition with sample D, E and F. 2 set of test is conducted to decrease the error. Hence 4 samples (A, B, D and E) will use 15 ml of M.O oil each, react with 200 ml methanol and 0.15g of Calcium Oxide (CaO). While another 2 sample(C and F) will only used 10 ml of M.O oil each, react with 133 ml of methanol and 0.10 g of CaO. The transesterification process equipment is set up as shown in the Figure 3.4 (a**)**. The time of reaction is set to 1 hour and 30 minutes with speed was 200 rpm stirring. After that, the FAME produced is being settled out about 1 day using the separating funnel as shown in the Figure 3.4 (b)

(a)

(b)

Figure 3.4, (a) Transesterification process, **(b)** Separating funnel

3.2.6 Sample preparation for vial (GC-MS)

After 1 day of settling, the oil is separated at the bottom of the separating funnel and prepared for GC-MS analysis. The sample vial for GC-MS required only 10µl of FAME and 990 µl of hexane. Micro liters and milliliters pipette is used in order to prepare this sample vial. Furthermore, the FAME's oil and hexane have to be filtered using syringe in order to avoid impurities to Gas Chromatography. Figure 3.5 shows the sampling vials for fatty acid analysis in GC-MS. 2 set of trials samples vial are used in this analysis.

Figure 3.5 sampling vials for fatty acid analysis in GC-MS

3.2.7 GC-MS analysis

A gas chromatography-mass spectrometer (GC/MS) was used to analyze the FAME's oil. The GC-MS can also be used to separate small amounts of materials and determine whether a desired component was present. The fatty acid methyl esters (FAMEs) contents were determined by gas chromatography, model Agilent 7890A type model G3440A, with the GC system of Agilent Technologies coupled with mass spectrometer, model MS−5973 MSD (mass selective detector). The injector type is 7683 B Series, Agilent Technologies with split less inlet injection detector. The columns used are of the J & W Scientific Columns, manufactured by Agilent Technologies, USA type HP-5, with length 30 meters. Separation was performed on a capillary column DB-5MS (30 m ×0.32 mm, 0.25µm of film thickness). The carrier gas was helium with flow rate of 1.5 mL/min. The column temperature was programmed from 120-300 °C at the rate of 10 °C/min. A sample was injected using a split mode, with the split ratio of 1:10. The mass spectrometer was set to scan in the range of m/z 50-550 with electron impact (EI) mode of ionization. Then the reading from the analyzed species monitored using the computer. The sample vial for GCM-MS is prepared for 2 times for accurate reading.

4. RESULTS AND DISCUSSION

The fatty acid profile for the affected seed oil by fungus and growth seed oil is not yet been studied until today. The research on biodiesel is only studied using dry M.O seed but not at other condition. Hence, there are no indicators that can show or compare to the expected result. The only factors that have been considered are only the unknown component of fatty acid in the oil and its composition. By assuming the FAME' oil will have a specific saturated and unsaturated acid, the results is varying for 3 samples with 2 set of each. The control sample(Sample A and D) bottles have the finest quality of FAME's oil while the other 4 sample(Sample B,C,E and F) have a slight different composition of acid and different in ratio of oil produced and physical appearance (PA) of oil.

4.1 Result

4.1.1 Ratio of M.O oil produced and it's (PA)

The result in terms yield of oil produced after the extraction process is tabulated as follows:

Table 4.1 Yield of M.O oil produced and its physical appearance

Sample	Mass of raw M.O seed, g	Moisture content, g	Mass of M.O after extraction, g	Volume of oil produced, ml	Percentage of M.O oil produced %	Physical Appearance, PA
A	500	-	350	200	23.92	Yellow(clear)
B	200	-	188.9	20	5.55	Yellow(cloud)
C	70	25	42.37	10	5.85	Green (Dark)
D	120	-	97.32	22	18.9	Yellow(clear)
E	120	-	84.18	23	29.85	Yellow(clear)
F	124	60	54.40	10	15	Yellow(clear)

According to the Table 4.1, its shows that the oil ratio for all samples varies from 30% theoretical of oil that will be produced by using hexane. This might be from several factors that have been considered for example the cycle of extraction process, the grade of solvent hexane used or the quantity of oil itself inside the seed. For sample A the oil is approximately to theoretical value but different with sample B which produced less.

This probably because the seed for sample B is kept in for too long thus the oil is less. Meanwhile for sample C, the oil is has been expected to get less oil as the quantity of oil itself in the seed is less and start to exist. Furthermore, most of the total weight contain moisture content. Sample D and E can be considered as acceptable oil yield as it is nearly to theoretical value. Lastly, for sample F the oil also produced less oil as the seed is still growing and in a condition to produce oil. In terms of physical appearance of the oil, samples A, D, E and F show an exact color of vegetables oil which is in yellow color (clear). However, sample B and C shows difference by having a yellow cloud and green dark color. Moreover sample B is already achieved to its cloud point where it's frozen. Theoretically, M.O oil has a cloud point range 5 to 10^0C. This could be occurring because of the incorrect preparation of sample before extraction process. The PA of M.O oil for the entire sample A, B, C, D, E and F can be seen at Appendices A.

4.1.2 Gas Chromatography Mass Spectrometer analysis

The characteristics of fatty acid in the M.O oil in the 6 samples showed a same unknown component of fatty acid. In sample A, B, C, D, E and F the highest percentage area among the 1st and 2nd trial is 49.95, 48.87, 54.56, 49.35, 30.17 and 40.05. Mean while the retention time for that percentage area is 32.510, 32.510, 32.467, 38.495, 38.495 and 32.483 min. The data and the graph for the percentage area and the retention time can be seen at Appendices B and Appendices C.

4.2 Discussion

Table 4.2(a) (b) (c**),** shows the comparison between samples on 1st peak and 2nd peak concentration of fatty acid. Theoretically sample A and D should have the same component of fatty acid. The highest fatty acid contain in both sample (A and D) is oleic acid at 49.95% area at 32.510 min retention time and 49.35% area at 38.495 min retention time as shown in Appendices C-A1, C-A2, C-D1 and C-D2. Followed by Palmitic acid and Margaric acid. Palmitic and Oleic acid is the common fatty acid in the dry M.O oil. This comparison acid component sample (A and D) can be seen at Table 4.2 (a)

Mean while for sample (B and E) at Table 4.2 (b), the highest concentration of fatty acid in the 1st peak is Oleic acid 48.87% area at 32.510 min retention time(refer Appendices C-B1, C-E1 and C-E2). For the second peak of concentration, sample (B

and E) have a common acid which is Palmitic acid and Stearic acid. It is expected fatty acid for sample (B and E) is different as it is affected by the fungus. However, the result shows the fatty acid is the same as M.O standard.

Besides that, for sample (C and F) at Table 4.2 (c), the highest concentration of fatty acid contain in M.O oil is also oleic acid at 54.56% at 32.467 min retention time as shown in Appendices C-C1, C-F1 and C-F2. Followed by the 2nd peak of concentration which is containing Palmitic acid and Stearic acid. These both acids are also commonly found in M.O oil.

By this, we can summarize that all the 6 samples produces a common fatty acid in the M.O oil. These samples have same fatty acid even it is dry, wet or affected by fungus. The only factor it is difference is only the yield of M.O oil produced. But, there is an also additional type of acid produce which is Margaric acid. Margaric acid is a trace component of the fat and milk fat of ruminants, but it does not occur in any natural animal or vegetable fat at concentrations over half a percent. It easily be found in cow milk dietary sources and it is rarely found in nature.

Table 4.2 (a) Comparison sample A and D on 1st Peak and 2nd peak

1st Peak	
Sample A	Sample D
9-Octadecenoic acid (Z) (oleic acid)	9-Octadecenoic acid (Z) (oleic acid) 9-Octadecenal Heptadecanoic acid (margaric acid)
2nd Peak	
Sample A	Sample D
Hexadecanoic acid (palmitic acid) 9-Octadecenoic acid (Z) (oleic acid)	Hexadecanoic acid(palmitic acid) Pentadecanoic acid(Stearic acid)

Table 4.2 (b) Comparison sample B and E on 1st Peak and 2nd peak

1st Peak	
Sample B	Sample E
9-Octadecenoic acid (Z) (oleic acid)	9-Octadecenoic acid (Z) (oleic acid)
2nd Peak	
Sample B	Sample E
Pentadecanoic acid (Stearic acid) Hexadecanoic acid(palmitic acid)	9-Octadecenoic acid (Z) (oleic acid) 9-Octadecenal, (Z)

Table 4.2 (c) Comparison samples C and F on 1st Peak and 2nd peak

1st Peak	
Sample C	Sample F
9-Octadecenoic acid (Z) (oleic acid)	9-Octadecenoic acid (Z) (oleic acid) 9-Octadecenal
2nd Peak	
Sample C	Sample F
Hexadecanoic acid (palmitic acid)	Octadecanoic acid (Stearic acid)

5 CONCLUSION AND RECOMMENDATION

5.1 Conclusion

This project focused on the Fatty acid of M.O oil in different sample condition. The component of fatty acid in the M.O oil does not change in the condition of dry, growth and affected by fungus. Meanwhile, the difference is only in terms of oil produced for the growth condition of M.O seed that produce less of oil compare to dry and affected seed that has no change to the oil extraction

5.2 Future work

The research carried in this project is currently defined the unknown fatty acid in the M.O oil. Research on Fatty acid using GC-MS is rarely been used because it is only can detect a fatty acid component at the peak but not at their concentration. Hence, for a better and clear understanding, Gas Chromatography coupled with Flame Ionised Detector (GC-FID) can be used. This equipment is more reliable as the fatty acid in the M.O can be specified with its concentration and easily be compared using the FAME standard. Thus, the fatty acid of M.O oil for each peak easily can be defined.

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