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Project title Determination of mineral oil hydrocarbon in rice bran oil using liquid chromatography with gas chromatography flame ionization detector

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Determination of Mineral Oil Hydrocarbon in Rice Bran Oil using Liquid Chromatography with Gas Chromatography Flame Ionization Detector

by
Mr. Jetaphat Sathirachawal

In Partial Fulfillment for the Degree of Bachelor of Science
Program in Applied Chemistry (International Program)
Department of Chemistry, Faculty of Science

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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 oil is an edible oil which is used in food preparation. In Thailand, standard conditions and methods in detecting mineral oil hydrocarbons (MOH) were not regulated. Therefore all rice bran oil quality and safety control was done abroad. This research proposed determination conditions in detecting mineral oil hydrocarbon using an off-line liquid chromatography with gas chromatography flame ionization detector. This method was adapted from ISO 17780 : animal and vegetable fats and oils-determination of aliphatic hydrocarbons in vegetable oil. Silver nitrate impregnated with silica gel was used to purify and separate mineral oil saturated hydrocarbon (MOSH). GC-FID using pulse splitless injection system combining with pulse time at 0.5 minute, inlet temperature at 250°C, and inlet pressure at 30 psi were considered to be the best conditions due to the largest area under the graph. The hump in the chromatogram represents the mass fraction of MOSH. This fraction is only presented in an eluent fraction of 55 mL hexane. Three crude rice bran oil samples were analysed, RBACTG25A, RBACTF27A, and RBACTF22A. Different levels of MOSH were detected with the highest being RBACTF27A (164.2 mg/kg) followed by RBACTG25A (90.5 mg/kg) and RBACTF22A (34.1 mg/kg). The comparison between the result obtained and reference data showed a slight difference due to the differences in sampling and injection method used. However, it gives out the same trend as the reference data. The amount of C25-C35 MOSH which is the target hydrocarbon is approximately 90% which is considered to be high and can not be refined to edible oil.

Keyword: Rice bran oil, Mineral oil saturated hydrocarbon (MOSH), gas chromatography with flame ionization detector (GC-FID), liquid chromatography with gas chromatography flame ionization detector (LC-GC-FID)

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List of Abbreviations

MOH	Mineral oil hydrocarbon
MOSH	Mineral oil saturated hydrocarbon
MOAH	Mineral oil aromatic hydrocarbon
LC	Liquid chromatography
GC	Gas chromatography
FID	Flame ionization detector
g	gram
mg	miligram
mL	milliliter
µL	microliter
min	minute
cm	centimeter
mm	millimeter
kPa	kilopascal
°C	degree celsius
psi	pound-force per square inch
W_{HC}	MOSH mass fraction
A_i	signal area attributed to MOSH
A_{is}	peak area of the internal standard.
m_{is}	mass of the internal standard added to the sample in mg.
m	mass of the test portion in gram.

Chapter 1
Introduction

1.1 Introduction to the research problem and significance

Agriculture is one of the main occupations of Thai. Around 50% of the land was used in the agricultural sector.[1] Rice has been recognized as the most widely grown agricultural product across the country. Rice bran is a by - product of the rice milling process obtained by scrubbing the membrane part, causing the rice to have a beautiful white color. Rice bran is also high in nutrients, including protein, fat, dietary fiber, ash, vitamins, and minerals. Due to this fact, many brands of rice bran oil were launched into the market. Moreover, the higher demand of rice bran oil was found with greater health concerns. Rice bran oil was an alternative oil used for cooking and was an essential component in both cosmetics and pharmaceuticals industries. Rice bran oil is extracted from the hard outer brown layer of rice. It has a high burning point of 232 °C with mild flavor, making it suitable for high-temperature cooking methods such as stir frying. Rice bran oil is mainly composed of long chain hydrocarbons and triacylglycerols. It contains several health benefits, due to the unique fatty acid composition. Gamma oryzanol, tocopherols, and tocotrienols are antioxidants produced in rice bran oil that provide not only higher nutraceutical value but also improving the nervous system, preventing stomach problems, lowering cholesterol, minimizing cancer risk, and increasing immunity.[2] Thailand produces more than 54,500 tons of rice bran oil annually. This high quality oil was exported throughout the world, mainly to the United States, Australia, and South Korea. Thai customs statistics[3] showed a total export volume of refined rice bran oil of 34,458 metric tons in 2019 with 1,592,304,253 Baht of the total amount of FOB (Free on board) in Bangkok. The global rice bran oil market demand was expected to reach 1.78 million tons or around $5.6 billions by 2025. The historical trends for the rice bran oil industry were recorded and shown in Fig. 1.1. The International Council of Rice bran oil (ICRBO) was formed in Thailand in order to establish a scientific standard focusing on value added products of rice derivatives and promoting the commercial trade among the members of ICRBO. This will enhance the rice bran oil production of Thailand. Even though there is mass production of rice bran oil, only a small quantity of rice bran oil was used in edible oil production. Since the degradation level was high due to the contact between lipase enzymes and oil which causes the hydrolysis and the release of free fatty acids and glycerols. This reduces the quality of rice bran oils and shelf life[4]. Moreover, rice bran oil contains contaminants such as mineral oil hydrocarbon (MOH) which is harmful to health during processing, transportation, storage, and sources. Therefore, they must be removed before releasing to the market.

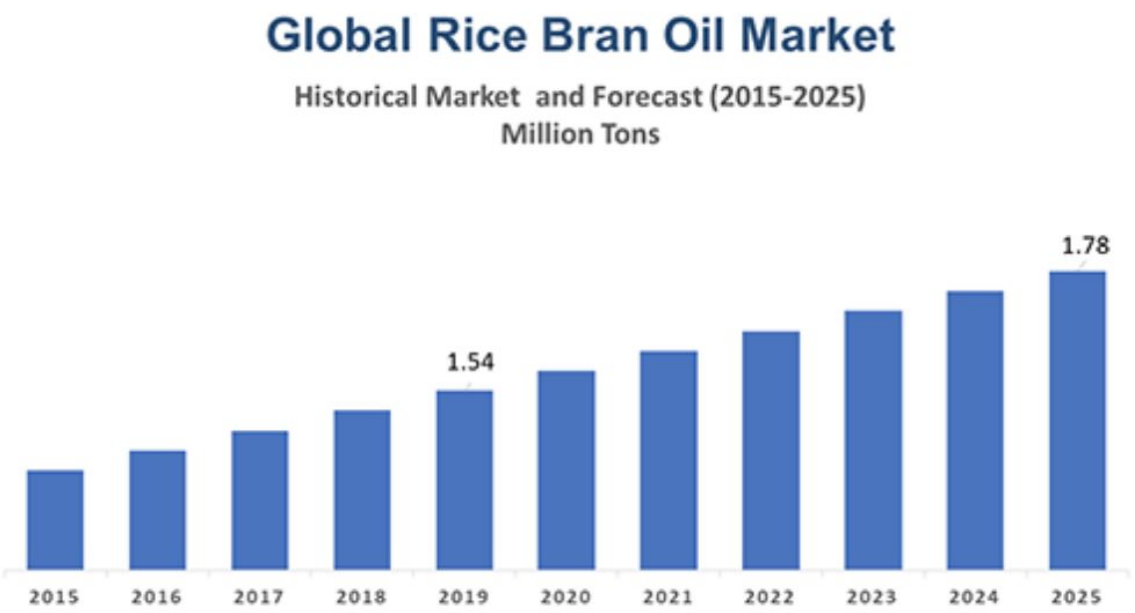

Figure. 1.1 Historical market and forecast of Rice bran oil[5]

In mineral oil hydrocarbon determination, liquid chromatography coupled with gas chromatography and flame ionization detector (LC-GC-FID) has become the preferred method in analysing[6]. FID is a nonselective detector which is sensitive to all hydrocarbons, the signals of which roughly depend on the number of carbon atoms making it a preferred detector for quantifications. However, due to lack of selectivity, additional sample preparation techniques were required to eliminate interference. Bingning et al. (2017)[6] reported the use of GC-FID for detecting contamination of mineral oil saturated hydrocarbon (MOSH) in vegetable oil. The experiment was performed to ensure the quality of vegetable oils. This has proven the potential of GC-FID in hydrocarbon determination. According to JRC EU 29666, European commission regulates the requirements for analysis of mineral oil hydrocarbon[7]. In Thailand, all rice bran oil quality and safety control was performed on board which results in both money and time consuming. The procedure was complex due to the eliminating process of olefin fraction present in the samples. This research purpose is to provide suitable conditions for GC-FID in hydrocarbon determination in rice bran oil for our country.

1.2 Research objectives

To optimize conditions for analyzing mineral oil saturated hydrocarbons in rice bran oil by off-line LC-GC-FID.

1.3 Literature search

1.3.1 Rice bran oil

Vegetable oil is mainly composed of long chain hydrocarbons with individual fatty acid composition. They are extracted from seeds such as soybean oil and also from other parts of vegetables such as rice oil. Rice bran oil is one of the healthiest types of vegetable oil. Rice bran oil was produced from the brown outer layer of rice coating (bran). This brown layer is removed during the milling process resulting in white rice.

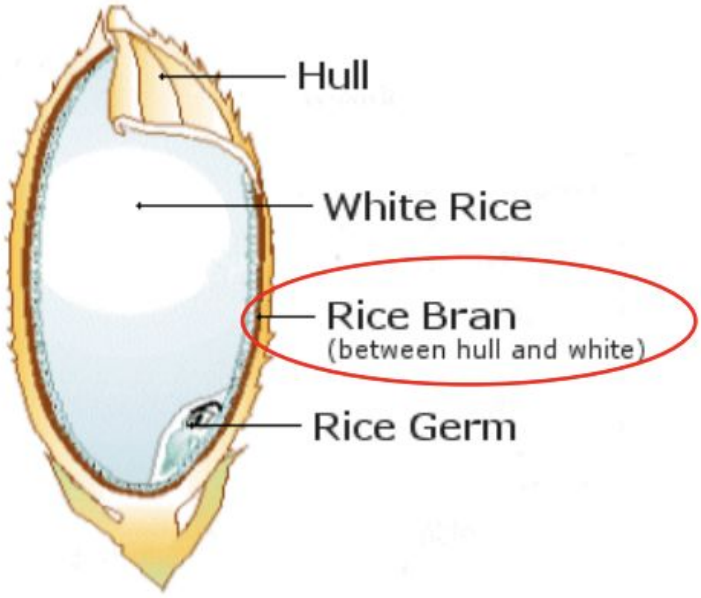

Figure. 1.2 Structure of rice[13]

The chemical and nutrient composition of rice bran depends on the variety of rice, treatment of grain, and the manufacturing process. Nutrient composition of rice bran was described in Table 1.1.

Table 1.1 : Nutrients Composition of Rice Bran[14-15]

Nutrients	Units	Raw rice bran (per 100g)
Moisture	g	4.80
Protein	g	18.37

Fat	g	15.15
Insoluble dietary fibre	g	17.13
Soluble dietary fibre	g	1.98
Carbohydrate	g	50.44
Calcium	mg	51.11
Phosphorus	mg	1185.30
Iron	mg	27.90
Zinc	mg	5.95
Antioxidants activity	mg	67.00

In the food industry, vegetable oils usually consist of hydrocarbons, triaclyglycerols or complex mixtures, and diacylglycerols[15]. The hydrocarbons can be categorized into two categories, which are mineral oil saturated hydrocarbon (MOSH) and mineral oil aromatic hydrocarbon (MOAH). MOSH can be used to distinguish the presence quality of mineral oils. Rice bran oil normally contains around 20% of oil content, or approximately 19% of fatty acids. The major fatty acid units of the lipids included linoleic acid or omega 6, alpha-linoleic acid or omega 3, palmitic acid, and oleic acid as shown in Table 1.2.

Table 1.2 : Fatty acids composition[16]

Fatty acid	Content (%) in total of lipids
Linoleic acid	29 - 45
Alpha linoleic acid	≤ 3
Palmitic acid	12 - 22
Oleic acid	35 - 50

Moreover, rice bran oil contains bioactive minor components such as gamma oryzanol, tocopherols, and tocotrienols. Gamma oryzanol was a mixture of 10 or more steryl ferulates[17]. Tocopherols and tocotrienols are different forms of vitamin E. All of them were considered to be good antioxidants with LDL-cholesterol-lowering activities.

1.3.2 Mineral Oil Hydrocarbon (MOH)

Mineral oil hydrocarbon consists of a group of hydrocarbons with different structures and sizes, obtained mainly from crude oil. MOH is classified into mineral oil saturated hydrocarbon (MOSH) and mineral oil aromatic hydrocarbon (MOAH). The composition of MOH mixture determines its toxicity and strongly depends on MOAH due to its mutagenic and carcinogenic properties. MOSH are less toxic, but accumulated in human tissues further forming microgranulomas.

1.3.2.1 Mineral Oil Saturated Hydrocarbon (MOSH)

Mineral oil saturated hydrocarbons are straight or branched open chain alkane (paraffins) and largely alkylated cycloalkane (naphthenes) that is synthesized from crude oil or coal. These paraffins and naphthenes were mainly MOSH fractions. Short exposure to MOSH will result in no health effect. However, daily intake of MOSH will cause bioaccumulation which leads to the formation of micro-granulomas in the liver and mesenteric lymph nodes. MOSH can be contaminated in rice bran oil due to the contamination of packaging, food addictive, and transporting processes[18]. MOSH can be accumulated inside the human body via inhalation, food, and physical contact.

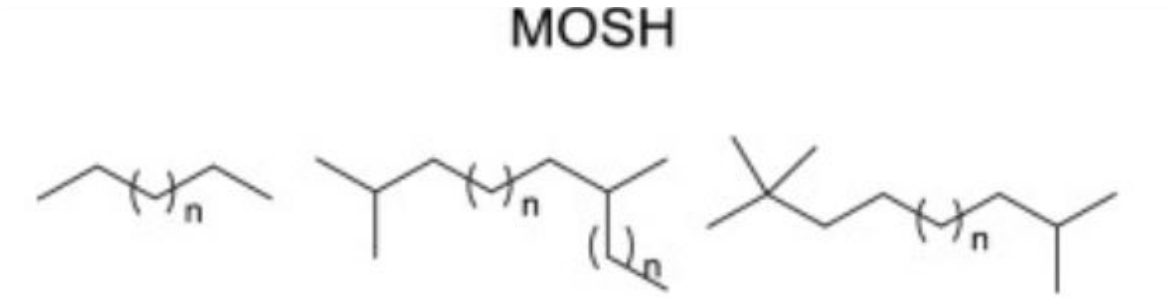

Figure. 1.3 Examples of MOSH[19]

1.3.2.2 Mineral Oil Aromatic Hydrocarbon (MOAH)

Mineral oil aromatic hydrocarbons are alkylated mono/poly-cyclic aromatic hydrocarbons with one or more ring systems. MOAH are considered as carcinogenic substances especially for polycyclic aromatic hydrocarbons (PAH)[20]. MOAH sources are usually from the recycle packaging materials. Since the materials were made from recycled paperboard, some may have mineral oil ink printed on. This mineral oil ink has genotoxic potential.

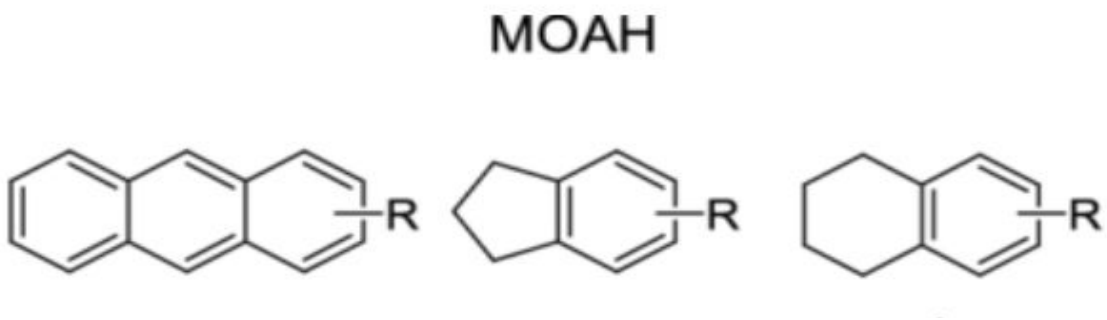

Figure. 1.4 Examples of MOAH[19]

1.3.3 Liquid Chromatography coupled with Gas Chromatography and Flame ionization detector (LC-GC-FID)

GC-FID has been used in various sectors including the food industry. In 2009, the GC-FID method was used to determine the mineral oil paraffins in food. Combining it with high performance liquid chromatography enhances the GC-FID potential[8]. Later in 2010, Florini et al reported that GC-FID was a suitable determination method to detect mineral paraffins in food. This research was developed on vegetable oils and have been adjusted and applied to dried fruit[9]. Moret et al (2011) reported that off-line SPE-GC-FID based on the use of silver-silica gel can determine only MOSH which showed good performance to retain fat and retention of interfering olefin[10]. Alternatively, pure off-line mode could also be used in separating the MOSH and MOAH, which requires an addition of silica/silver nitrate ($AgNO_3$) inside the glass column and manual sample separation/collection which is very time consuming[6]. On-line LC-GC-FID was developed and used by Mondello et al (2012) to detect saturated hydrocarbon contamination in baby food[11]. Moreover, Weber et al (2018) reviewed the use of on-line LC-GC-FID on determination of MOSH and MOAH. On-line LC-GC offers many advantages, such as high separation efficiency, low solvent consumption and reduced sample manipulation and contamination during sample preparation. LC-GC-FID will detect 2 fractions which are MOSH and MOAH which give out a regular hump[12]. Off-line mode requires a larger volume of samples and manual sample transfer to be injected in the GC system compared with the on-line coupling mode.Therefore, all evidence has proven the LC-GC-FID potential in MOSH determination.

1.3.3.1 Liquid chromatography on silica gel impregnated with silver nitrate

The LC column was prepared in order to separate the complex mixture of alkanes or other compounds such as MOSH and MOAH. Silver-ion chromatography method is normally used for the separation of unsaturated compounds such as alkenes and prepared

by impregnating silver nitrate on silica gel. This technique is based on the principle that unsaturated organic molecules react reversibly with transition metals such as silver to form polar charge-transfer complexes. A sigma bond is formed between the occupied 2p π* electrons of the double bond and the free 5s and 5p orbitals of the silver ion. A π acceptor back bond between the free antibonding 2p π* electrons of the double bond and the occupied 4d orbitals of the silver ion is also involved. The strength of the complex is determined by accessibility of the electrons in the filled orbitals and steric hindrance of the orbitals[21]. The chromatographic retention and separation of unsaturated organic compounds is based on the formation of weak and reversible complexes formed between the silver ions impregnated on the silica gel surface and the double-bonds in unsaturated compounds due to double bond are the most common functional group in organic compounds and silver ions are highly selective to double bonds.

1.3.3.2 Gas chromatography

Separation in GC relies on different boiling points of the volatile compounds and their partition between stationary phase and mobile phase. The stationary phase remains static when a gas or liquid moves over its surface and separates out into its various components. The mobile gas phase which is an inert gas moves the sample through the system. The sample is injected into the inlet through a septum which enables the injection of the sample mixture without leaking. The analytical column is held in the column oven which is heated during the analysis to elute the less volatile components. The outlet of the column is inserted into the detector which responds to the chemical components eluting from the column to produce a signal. The signal is recorded by the acquisition software in a computer to produce a chromatogram. Fig. 1.5 illustrates the overall structure of GC[22]. Injection system of gas chromatography is the first appearance of chromatographic analysis to induce samples onto the column. The amount injected must not be overloaded with the applied column and the width of the injected plug has to be small compared to the spreading from the process otherwise the separation capability will reduce. There are two common types of injection that are used for capillary columns; split and splitless injection. Split injection is the most widely used because of uniform flow profile, sharpening peak shapes and removal of solvent. The process of split injection is critical and must take place almost instantaneously, accurately, repeatedly and without loss of flow efficiency, which allows only a small part of the vapor to enter the column in a permanently hot vaporizing chamber. Splitless injection is a variation of split injection and was designed particularly for trace analyses in highly diluted samples because nearly all of the sample vapor is transferred from the injector into the column for a certain period of injection. The amount of solvent injected onto the column is many times larger compared to split injection[23]. In most split/splitless GC injection systems, there is a finite volume within the liner in which the gaseous sample occupies. If the gaseous volume of the

sample exceeds the liner volume, then backflash occurs. Subsequent backflash injections could involve re-solubilising some of this deposited material, pushing it back into the inlet and ultimately onto the column. This contaminates the injected sample vapour. In order to improve sensitivity and reproducibility in the analysis, pulsed pressure can be applied during the injection by increasing the pressure of the inlet, which constrains the expansion of the sample. The increased pressure applied within the inlet is typically reduced prior to the end of the splitless injection period to re-establish the required pressure and flow through the column for the separation[24].

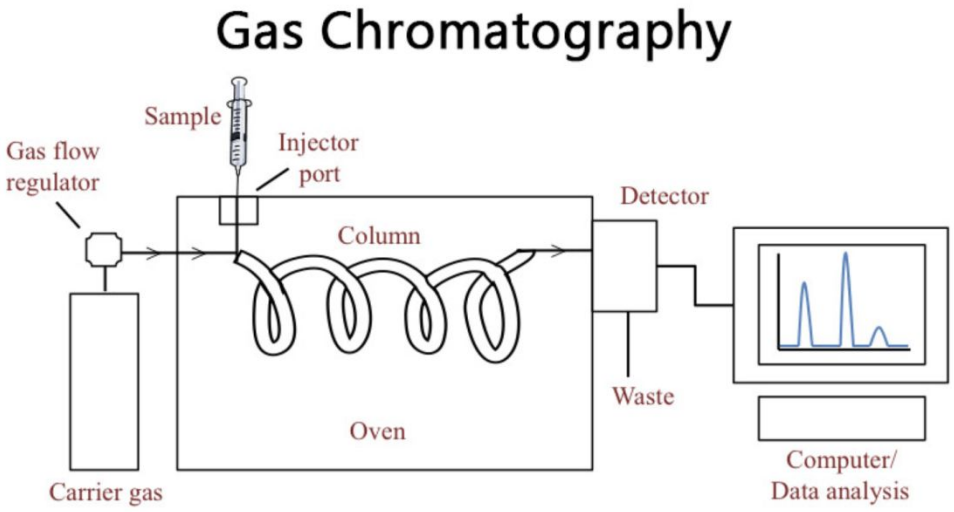

Figure. 1.5 Gas Chromatography system

1.3.3.3 Flame ionization detector

The flame ionisation detector (FID) is the automotive emissions industry standard method of measuring hydrocarbon (HC) concentration[25]. It is frequently used as a detector in GC. Detector sensor works on the principle of detecting ions formed according to combustion of organic compounds in the sample, producing charged molecules that cause electrical conduction between two electrodes. The ions are attracted to a collector plate and induce a current. The sample gas is introduced into a hydrogen flame inside the FID. Any hydrocarbons in the sample will produce ions when they are burnt. Ions are detected using a metal collector which is applied with a high DC voltage. The current across this collector is thus proportional to the rate of ionisation which in turn depends upon the concentration of HC in the sample gas. The ionisation process is very rapid, so the slow time response of conventional FIDs is mainly due to sample handling. The overall structure of FID was shown in Fig. 1.6

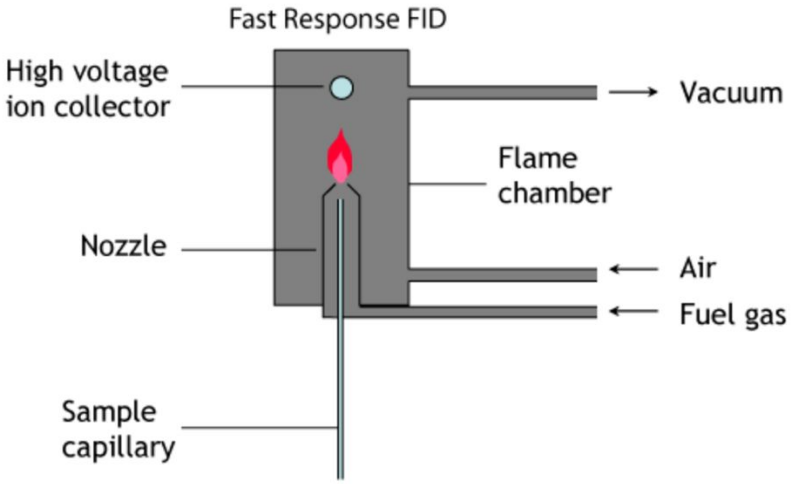

Figure. 1.6 Flame ionized detector components[24]

1.3.4 Interpret and calculate FID chromatogram

The signal area in the FID chromatogram attributed to MOSH is calculated by integration of the chromatogram covering the range of C10 -C35, taking the baseline of the blank into account. The calculation of the MOSH mass fraction[26] (W_{HC}) are performed by using the following equation:

$$W_{HC} = \frac{A_i \, x \, m_{is} \, x \, 1000}{A_{is} \, x \, m}$$, where

A_i is the signal area attributed to MOSH and MOAH(total of C-fraction) after the elimination of the identified sharp peaks above the hump;

A_{is} is the peak area of the internal standard.

m_{is} is the mass of the internal standard added to the sample in mg.

m is the mass of the test portion in gram.

The content of hydrocarbons (W_{MOSH}) is expressed as a mass fraction in mg/kg and referred to the internal standard by using the following equation:

$$W_{MOSH} = W_{HC1} - W_{HC2}$$, where

W_{HC1} integrate manually the total signal composed of the hump and sharp peak.

W_{HC2} integrate tracing manually the valley-to-valley baseline over the hump profile for all the sharp peaks.

Chapter 2
Experimental

2.1 List of equipment and instrument

(1) Glass column for chromatography
(2) Glass rods
(3) 250mL and 500 mL Round-bottomed flasks
(4) Rotary evaporator
(5) Automatic evaporator
(6) 10 mL Conical glass sample vials
(7) Gas Chromatograph
(8) Data acquisition system
(9) Capillary column
(10) Analytical balance
(11) Pasteur pipette.

2.2 List of chemicals and reagents

(1) Silica gel 60
(2) Water
(3) Anhydrous sodium sulfate
(4) n-Hexane
(5) Internal standard : n-Octadecane C18
(6) n-Decane C10
(7) Ocatatetracontane C48
(8) Silver nitrate
(9) Carrier gas for gas chromatography
(10) Auxiliary gases for flame ionization detector
(11) Alkane standard mixture C10-C40
(12) Viscous paraffin and highly liquid paraffin.

2.3 Experimental and Procedure

Sample preparation was developed from ISO 17780 animal and vegetable fats and oils-determination of aliphatic hydrocarbons in vegetable oils. The saturated aliphatic hydrocarbons of the sample were isolated by LC using a glass column containing silica gel impregnated with silver nitrate.

2.3.1 Chromatography column preparation

2.3.1.1 Preparation of silver nitrate impregnated silica gel

Chromatography columns were prepared by weighting 45 g of silica gel in a 500 mL round bottomed flask using analytical balance then protected by aluminium foil. The silver nitrate solution (4.5 g of silver nitrate with 6 mL distilled water) was added drop by drop into the flask then continuously shaken the mixture for 30 min until being homogenized by using an automatic shaker. The mixture was kept at room temperature and covered with aluminium foil for 12 hours before use.

2.3.1.2 Column packing

Each of the Column was packed using 18.5 g of silver nitrate impregnated silica gel in 40 mL of n-hexane. Tapping the column gently using a glass rod then add 0.5 cm to 1.0 cm of sodium sulfate on top of it and rinse using 60 mL of n-hexane to remove impurities. The columns were covered by aluminum foil to protect oxidation of silver nitrate. The column of silver nitrate was shown in Fig. 2.1

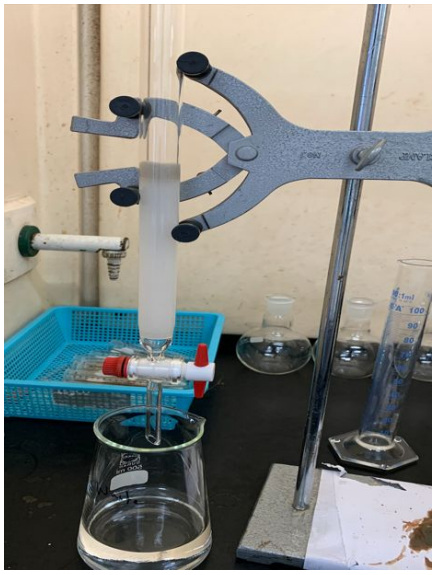

Figure. 2.1 Chromatography column packed with silver nitrate impregnated with silica gel

2.3.1.3 Elution of the hydrocarbon fraction

The internal standard solution (C18) was prepared by dissolving 50 mg of *n*-octadecane in 25 mL of *n*-hexane, then 1 ml of this solution was diluted to 5 mL by *n*-hexane. The rice bran oil sample (1 g) was weighted and added into 1 mL of the internal standard solution. Transferred this mix solution into a chromatographic column and then washed with 2 portions of 1 mL *n*-hexane. The hydrocarbon fraction was diluted with 55 mL, 50 mL, 10 mL and 10 mL of *n*-hexane, respectively, with the rate of 15 drops within

every 10 seconds. Each fraction was collected in a 250 mL flask. The solvent was evaporated to result in 2 mL of the fraction with a rotary evaporator equipped with a water bath at 35 ℃ and transferred into a 10 mL conical tube. The solvent was further concentrated to 0.5 mL under a stream of nitrogen using an automatic evaporator. Lastly, the dilution was done by adding 0.5 mL of paraffin into the prepared *n*-hexane solution.

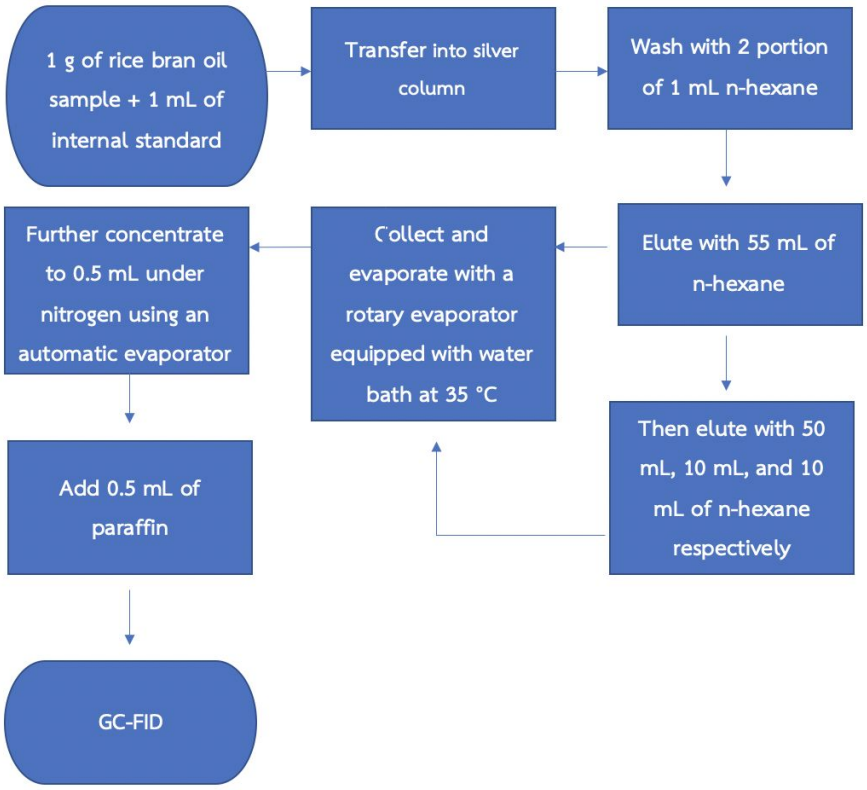

Figure. 2.2 Flow chart of experiment

2.3.1.4 Procedural blank

A procedural blank sample which contains only internal standard was analysed in order to test the purity of the reagents and other possible sources of contamination. The blank solution was prepared with the same procedures which was done on the rice bran oil samples, 1 mL of internal standard was used without mixing of any oil sample.

2.3.2 GC-FID Setup

The saturated aliphatic hydrocarbons of the sample are determined by GC-FID using an internal standard. The injection end of the DB5-HT column and detection end

were cut approximately 12 mm and 3-5 mm, respectively. The analysis was carried out by injecting the sample and setting the initial oven temperature at 60℃ held for 3 min. The oven temperature was increased 350 ℃ with the rate of 12℃/min held for 10 min. The carrier gas hydrogen head pressure was set at 100 kPa while the detector temperature was set at 370 ℃ with the injection volume of 1 µL. The result was compared using split, splitless, and pulsed splitless injection systems. Gas chromatography conditions were all varied to obtain the best result based on total area in the chromatograms. The inlet pressure was varied from 30, 45, until 60 psi. The inlet temperature was varied at 250, 270, 290, and 300℃. Lastly, pulse times of 0.1, 0.2, 0.5, and 1.0 min were applied.

2.3.3 Determination of MOSH in crude rice bran oil sample

The best conditions for analysing were used to analyse the crude rice bran oil samples (batch number RBACTG25A, RBACTF27A, and RBACTF22A. The characteristic of crude vegetable oil is a dark brown color, limpid with sediment, and heterogeneous solution without specific odour or taste. The whole analysis was done 4 times on each sample. The result obtained was compared with the reference result received from the foreign country.

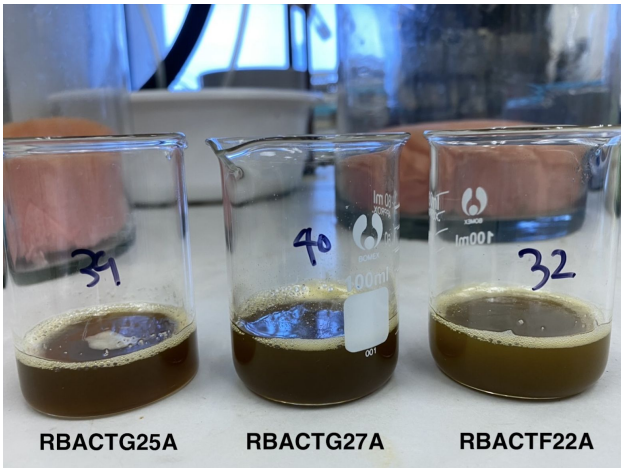

Figure. 2.3 Crude rice bran oil samples

2.3.3 Calculation of MOSH in sample

To determine mineral oil hydrocarbon of sample the two following equations were used :

$$W_{MOSH} = W_{HC1} - W_{HC2}$$

$$W_{HC} = \frac{A_i \, x \, m_{is} \, x \, 1000}{A_{is} \, x \, m}$$

Both equations were used to calculate signal area attributed to MOSH from C10 to C35. W_{HC1} by integrating the total peak area composed of the hump and the sharp peaks above the baseline (range of retention time 14- 24min).

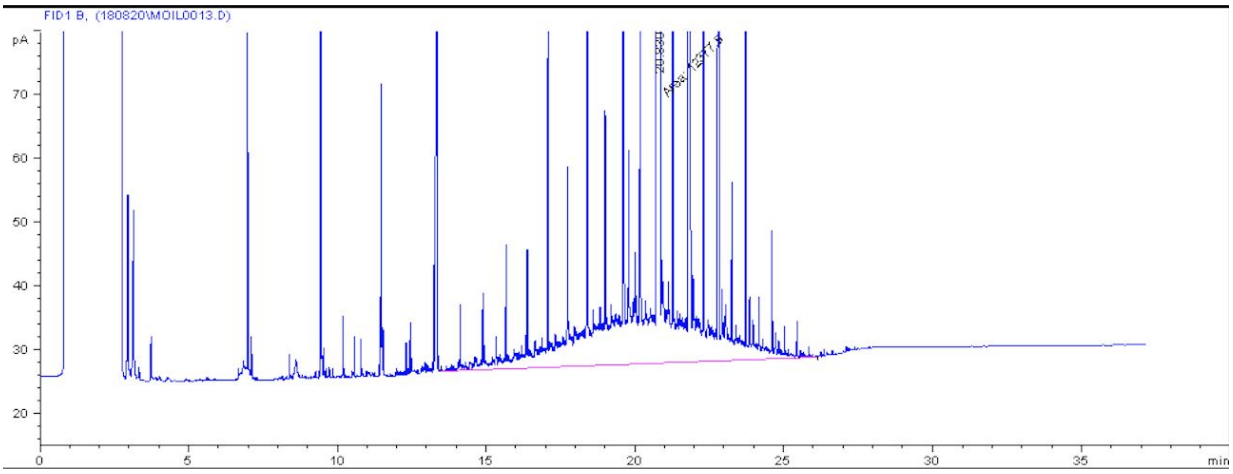

Figure. 2.4 Chromatogram of W_{HC1} integration

For W_{HC2} , the signal was integrated from one of the valleys to the next valley baseline which was over the hump for all the sharp peaks. The value of A_i and A_{is} were obtained from the chromatogram.

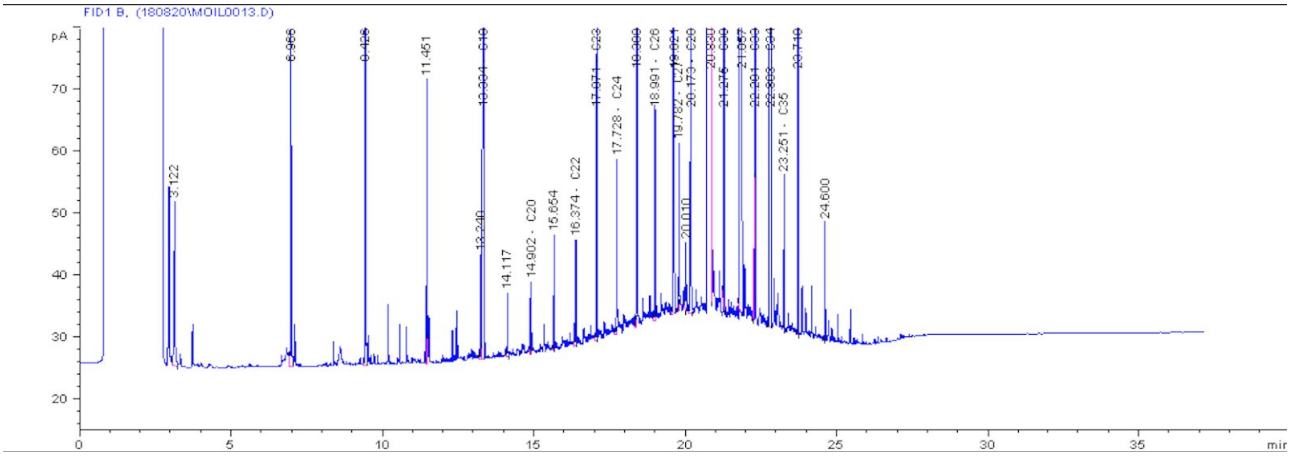

Figure. 2.5 Chromatogram of W_{HC2} integration

The mass of the sample (*m*) was weighted around 1 g and the mass internal standard : C18 (m_{is}) was 0.04 mg/L. Data acquisition system was used manually to integrate the peak area. The sum of all calibrated peaks obtained from the system represented the A_i in the equation.

Table 2.1 : Integration value of each peak from W_{HC2}

Peak	Retention time	Area	Name
1	0.000	0.000	C8
2	0.000	0.000	C10
3	0.000	0.000	C11
4	6.930	90.941	
5	0.000	0.000	C12
6	0.000	0.000	C13
7	9.422	117.479	
8	0.000	0.000	C14
9	10.167	18.566	
10	0.000	0.000	C15
11	11.448	57.371	
12	11.517	13.384	
13	0.000	0.000	C16
14	12.428	12.920	
15	0.000	0.000	C17
16	13.235	24.581	
17	13.333	1154.665	C18
18	14.112	14.809	
19	0.000	0.000	C19
20	14.896	17.793	C20
21	15.649	25.688	
22	0.000	0.000	C21
23	16.368	24.634	C22
24	17.067	125.452	C23
25	17.724	49.673	C24

Peak	Retention time	Area	Name
26	0.000	0.000	C25
27	18.385	344.986	
28	18.986	53.407	C26
29	19.621	909.538	
30	19.776	39.391	C27
31	20.168	236.004	C28
32	0.000	0.000	C29
33	20.823	8.368	
34	21.270	189.040	C30
35	0.000	0.000	C31
36	21.847	2181.283	
37	0.000	0.000	C32
38	22.286	82.136	C33
39	22.798	509.764	C34
40	23.244	34.183	C35
41	23.711	96.026	
42	0.000	0.000	C36
43	24.591	28.399	
44	0.000	0.000	C37
45	0.000	0.000	C38
46	0.000	0.000	C39
47	0.000	0.000	C40
48	0.000	0.000	C48

Chapter 3
Result and Discussion

3.1 Chromatography column

After transferring the solution of sample mixed with internal standard to the chromatographic column, the sample penetrates into the stationary phase slower than flow rate of 15 drops every 10 seconds that setting from the beginning of the experiment resulting in more time consuming and may affect all fractions. Aluminum foils are used to avoid oxidation of the silver nitrate. After elution, Silver columns separate and turn into dark layers.

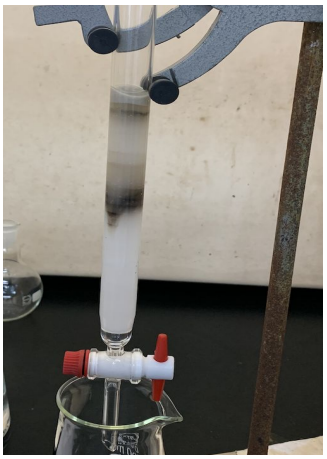

Figure. 3.1 Chromatography column after elution step

3.2 Quantitative of each elution hydrocarbon fraction

Chromatograms obtained from fraction 2, 3, and 4 elute with 50 mL, 10 mL, and 10 ml of hexane had extremely low humps area which cannot be used to analyse MOSH because the value of W_{MOSH} close to 0. The hydrocarbons contain in fraction 1 elute with 55 mL of hexane because the sample separated through the silver column first. Therefore, in this research only fraction 1 is used for MOSH analysis. The chromatograms shown in Fig. 3.2 illustrated the influence of different fractions. However, If A_{is} was presented in any fraction of a chromatogram, all A_{is} values have to be summed up and calculated.

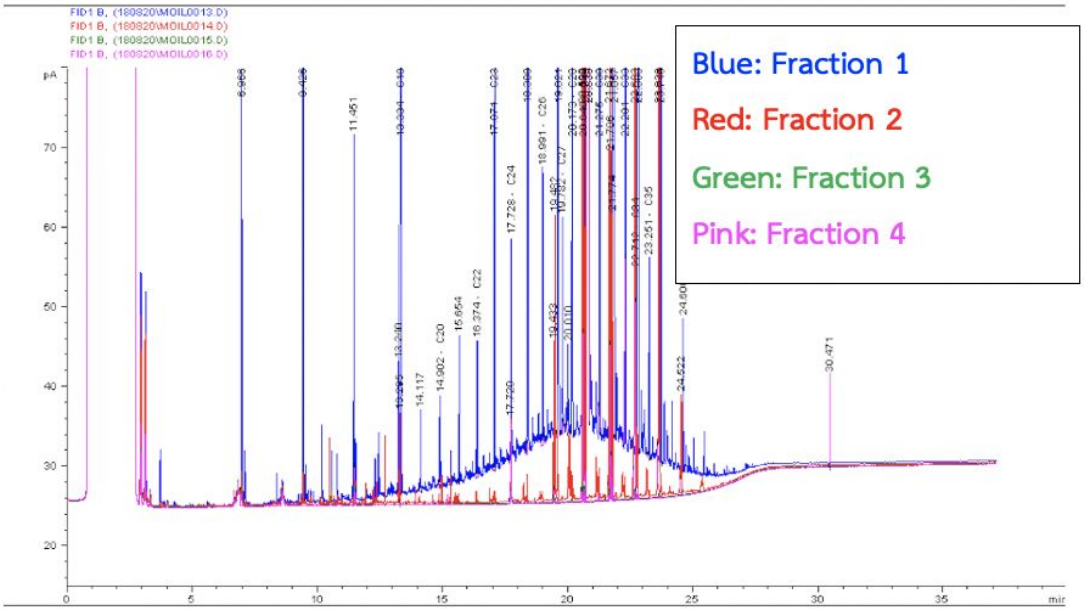

Figure. 3.2 Chromatogram of each elution hydrocarbon fraction

3.3 Purity test

The mineral oil content of the blank solution was lower than 10 mg/kg which was an acceptable contamination level of mineral oil content. Only the internal standards peak are presented in the chromatogram. There were no impurities present since all glassware used in the determination process were cleaned and rinsed with n-hexane before all the procedures were done.

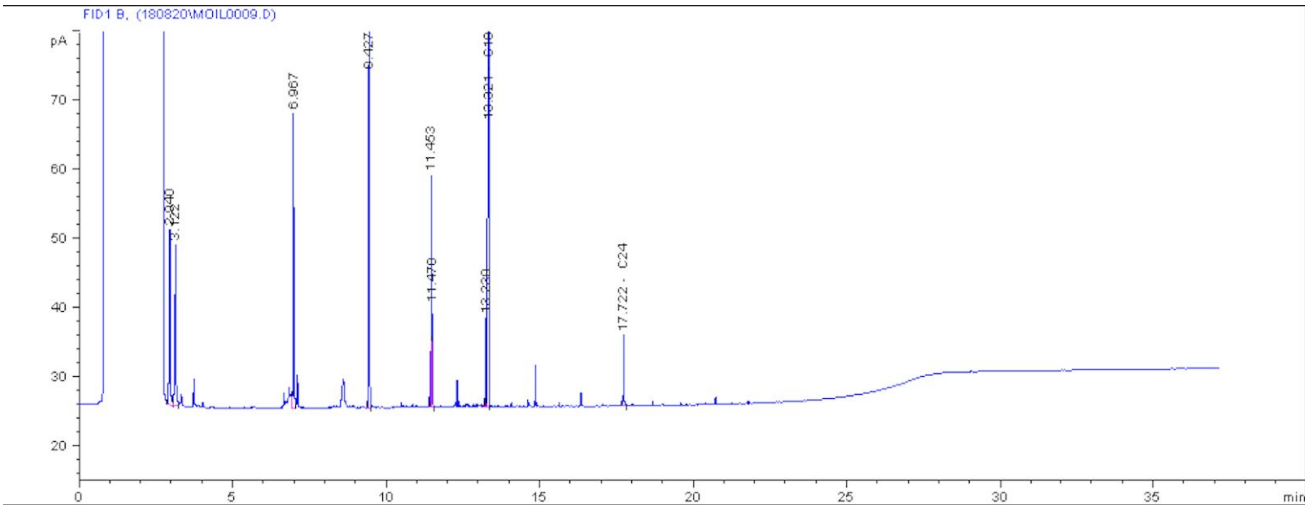

Figure. 3.3 Chromatogram of blank portion

3.4 GC-FID Conditions evaluation

Different conditions of GC-FID were varied which included the injection modes, inlet temperature, inlet pressure, and pulse time. The evaluation was performed to obtain the suitable analysis conditions on detecting MOSH.

3.4.1 Different injection mode analysis

The chromatograms of different injection approaches used in analysing rice bran oil were shown in Fig. 3.4 where the injection approaches were varied with fixed inlet pressure, inlet temperature, and pulse time. The blue chromatogram represents split mode, red represents the splitless mode, and green represents pulse-splitless mode. The analysis showed that each injection mode influences the chromatogram. Different modes resulted in different chromatograms with different total areas. The hump represents the MOSH present in the sample. The area under each graph was compared. As a result, the pulse splitless injection mode was considered to be the best injection mode due to providing the largest area under the graph. This can be explained by the pulse splitless injection mode applied to the higher pressure introducing a larger amount of sample onto the column. In addition, the high pressure significantly shortened retention times of the peaks eluting before 5 min due to the high column flow during the pulse injection.

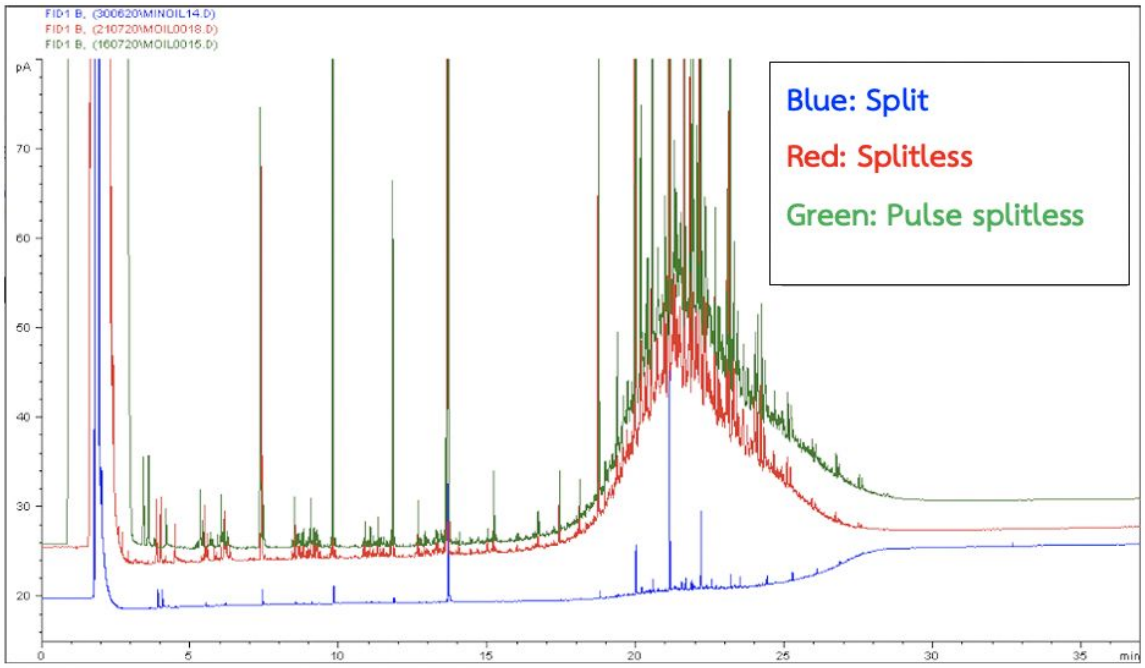

Figure. 3.4 Chromatogram of Rice bran oil using different injection mode

3.4.2 Inlet pressure analysis

The pulsed splitless conditions were further investigated. The inlet pulse pressure was varied while the inlet temperature and pulse time were fixed. Fig. 3.5 illustrated the chromatogram of pulsed splitless injection mode with different inlet pulse pressure varied from 30 - 60 psi. The blue chromatogram represents inlet pressure at 30 psi, red represents the inlet pressure at 45 psi, and green represents inlet pressure at 60 psi. The inlet pressure at 30 psi was proven to provide the greatest performance as indicated by the highest total peak area of the sample.

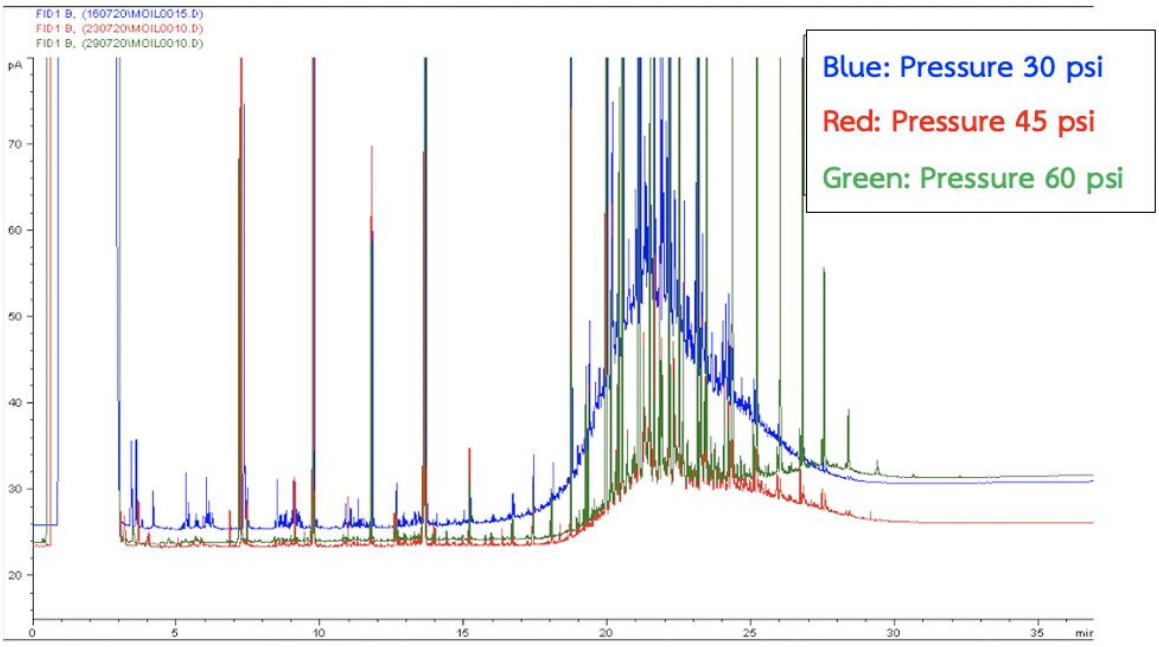

Figure. 3.5 Chromatogram of pulse splitless injection mode with different inlet pressure

3.4.3 Inlet temperature analysis

Pulsed splitless injection mode with inlet pressure at 30 psi was used to analyse the MOSH in rice bran oil. Inlet temperatures varied from 250 - 300℃ while pressure and pulse time were constant. The chromatograms shown in Fig. 3.6 illustrated the influence of different temperatures on the sample. The blue chromatogram was inlet temperature at 300℃, red at 250℃, green at 270℃, and pink was inlet temperature at 290℃. Due to the chromatogram presented, the red line or inlet temperature at 250℃ has shown the largest area under the graph compared to others. Therefore, inlet temperature at 250℃ could be concluded as the most suitable inlet temperature. This indicated the sample loss during the injection at higher temperature (*e.g.* via backflash caused by the greater gas expansion). However, application of temperature of <250 °C is not recommended due to the sample containing high boiling point components such as hydrocarbons above C30 and triacylglycerols.

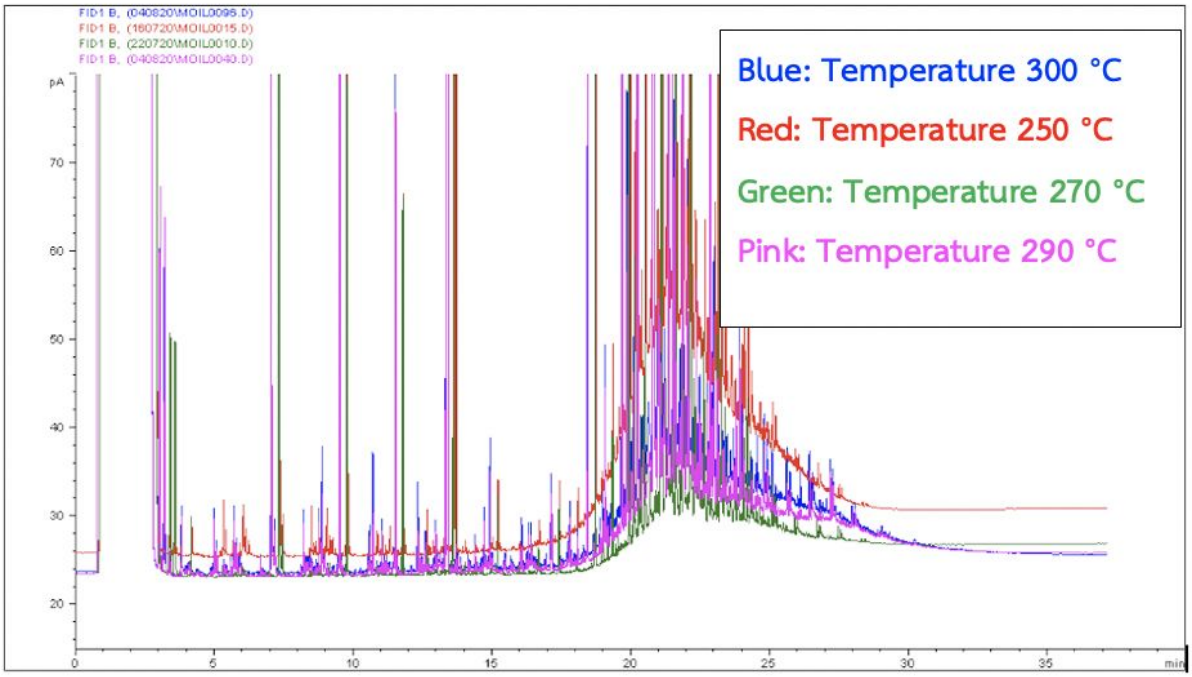

Figure. 3.6 Chromatogram of pulse splitless injection mode with different inlet temperature

3.4.4 Chromatogram of pulsed splitless varies on pulse time

Effect of different pulse time on detecting MOSH was investigated, with the pulse time varied from 0.1 - 1 min. The pulse time of 0.5 min (green chromatogram) is considered to be the best since it resulted in the largest total area under the graph as shown in Fig. 3.7. The blue, red, and pink chromatograms represent pulse time at 0.1, 0.2, and 1.0 min, respectively. It should be noted that too long pulse time of 1.0 min is expected to result in a similar or higher total peak area compared with the 0.5 min pulse. However, the 1.0 min condition showed significantly lower total peak area which can be explained by the error or sample evaporation/aggregation during the repeated injections. In addition, too long pulse time can cause peak retention time shift and decrease separation efficiency caused by the high column flow which should be avoided in GC.

In summary, the pulse time at 0.5-minute with pulse splitless injection mode, inlet temperature at 250℃ and inlet pressure of 30 psi would give out the best condition for MOSH performance.

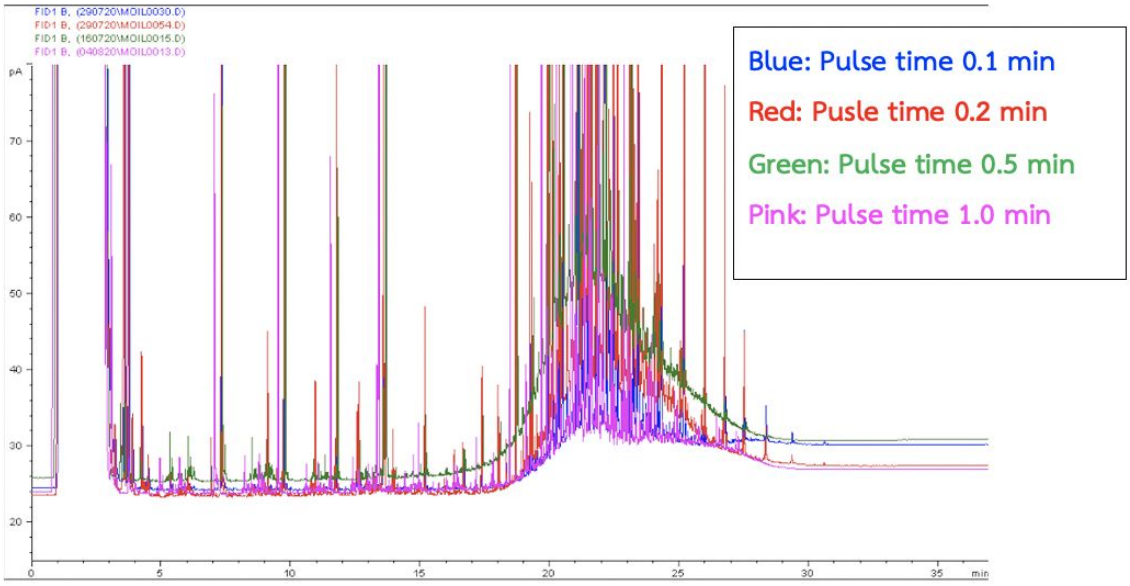

Figure. 3.7 Chromatogram of pulse splitless injection mode with different pulse time

3.5 Analysis of MOSH in crude rice oil samples

The parameters from the equation for W_{MOSH} determination was shown in Table 3.1 These parameters were obtained from a chromatogram of fraction 1 with 55 mL hexane from each sample. Note that the error in value may occur from the sampling step because the characteristic of crude rice bran oil is heterogenous with sediment.

Table 3.1 : Data presented in each sample.

			A_{is}	A_i for W_{HC1}	W_{HC1}	A_i for W_{HC2}	W_{HC2}	W_{MOSH}
RBACTG25A	Method A	1	1051.305	12190.534	410.675	5094.423	171.621	239.1
		2	1062.585	11442.23	409.706	4713.009	168.756	240.9
	Method B	3	2440.499	11919.369	193.274	7569.274	122.737	70.5
		4	1793.61	11692.947	256.806	6662.033	146.315	110.5
RBACTF27A	Method A	1	903.732	10864.476	392.404	4343.112	156.865	235.5
		2	1163.32	12836.609	388.77	5173.023	156.67	232.1
	Method B	3	1819.742	17520.406	378.722	6774.237	146.432	232.3
		4	1222.762	10961.941	350.041	5821.300	185.888	164.2
RBACTF22A	Method A	1	921.683	4688.162	195.183	3726.153	155.132	40.1
		2	2661.313	7202.417	103.416	4596.266	65.995	37.4

	Method B	3	1260.306	4892.553	154.803	3905.010	123.457	31.3
		4	1370.179	4787.698	139.432	3827.747	111.934	27.5

Three crude rice bran oil samples with a batch number of RBACTG25A, RBACTF27A, and RBACTF22A received from the rice bran oil export company in Thailand were analysed. Because the crude oil is not homogeneous, rather cloudy and sediment. Each sample therefore was homogenized either by centrifugation (method A) or sonication (method B) before sampling to further separate by Ag-silica gel column. The test of each method was repeated to find the average value. The first two tests, the sampling were taken from crude oil obtained by method A while the last two tests were sampling from crude oil obtained by method B. Only the results from method B which gives a more homogeneous substance than method A are shown in Table 3.1 and compared with the ones obtained from Chemiservice Laboratory (reference values). It is found that the average values of MOSH from the third and the fourth test of each batch are comparable to the reference ones shown in Table 3.2.

Table 3.2 : MOSH quantity present in the crude rice bran oils samples

		Batch number(Method B)			Batch number (reference)		
		RBACTG25A	RBACTF27A	RBACTF22A	RBACTG25A	RBACTF27A	RBACTF22A
Result MOSH (mg/kg)	MOSH C10-C16	3.9	6.7	2.0	1	1.4	< 1
	MOSH C17-C24	3.1	6.9	1.5	44.5	37.9	6.5
	MOSH C25-C35	83.5(92%)	150.6(92%)	30.6(90%)	81.6(64%)	100.5(72%)	23(78%)
	MOSH total	90.5	164.2	34.1	127.1	139.8	29.5

It is interesting to note that the more of MOSH C25-C35, the less of the edible oil can be produced. The contamination of target C25-C35 analyzed in this work for all four tests, of each batch, are approximately 90% of the total values, while the reference values are approximately 70%. One of the reasons that MOH of test number 1 and 2 was different from test number 3 and 4 is the homogenized oils, another reason is the injection method used. The injection method performed by the chemiservice laboratory, Italy is on-column injection on-line LC-GC-FID which nowadays is accepted to be the best

analysing method for determination of mineral oil hydrocarbons. However, there is no such expensive equipment available in Thai laboratories. Usually, C10-C25 MOSH contents in crude rice bran oil can be evaporated during the processing and further used to produce edible oil, and C25-C35 MOSH contents are considered as waste that left in crude oil is still cannot be eliminated without affecting nutrients and some antioxidants and therefore used for biodiesel purposes.

To obtain better results, more samples and more tests including the validation needed to be performed to reach better statistical data.

Chapter 4
Conclusion

Rice bran oil is widely used due to health benefits. The increase in consumption requires quality and safety control to ensure the safety of rice bran oil. Mineral oil saturated hydrocarbon (MOSH) is a contaminant found in vegetable oil during processing, transportation, storage, and sources. In our research, an off-line Liquid chromatography with gas chromatography flame ionization detector (LC- GC-FID) is a standard method modified from ISO 17780 (animal and vegetable fats and oils-determination of aliphatic hydrocarbons in vegetable oil) for determination MOSH in rice bran oil. LC was done to reduce interference in the analysis. LC analysis was conducted using 18.5 g silica gel impregnated with silver nitrate in a glass column. Silica gel impregnated with silver nitrate separates the complex mixture of MOSH. Gas chromatography with flame ionization detector (GC-FID) was done to determine the mass fraction of MOSH from FID chromatogram. GC-FID condition was optimized resulting in a pulse splitless injection system combining with pulse time at 0.5-minute, inlet temperature at 250℃, and the inlet pressure of 30 psi which are the best conditions for GC-FID performance in hydrocarbon analysis. These best conditions were selected by the chromatogram that gives out the largest area under the graph since the largest area represents the highest MOSH content. Only the eluted MOSH fractions of 55 mL with hexane were used to calculate MOSH content because it gives out the highest hump and largest area under the graph. The content of MOSH in crude rice bran oil RBACTF27A, RBACTG25A and RBACTF22A samples were analysed using the optimized conditions. The averaging MOSH content of RBACTF27A, RBACTG25A and RBACTF22A are 164.2 mg/kg, 90.5 mg/kg, and 34.1 mg/kg,respectively. The amount of MOSH detected in this research is slightly different from chemiservice laboratory, Italy, due to the differences in sampling and injection method used. However, the MOSH content in the obtained results showed the same trend as the reference MOSH content. The amount of C25-C35 MOSH which is the target hydrocarbon is approximately 90% which is considered to be high and can not be refined to edible oil. Therefore, it was more suitable to be used for biodiesel purposes.

Suggestion for future work

More samples should be tested to get better statistic data. LOD, LOQ must be determined. Using comprehensive two-dimensional gas chromatography (GCxGC) for the determination of both MOSH and MOAH is suggested.

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Biography

Mister Jetaphat Sathirachawal was born on the 14th of July, 1998 in Bangkok, Thailand. He graduated from Bangkok Christian College class of 2017 and continued studying bachelor degree at Chulalongkorn University, Faculty of Science, Bachelor of Science in Applied Chemistry (BSAC), Bangkok, Thailand. The current address is 165/37 Soi Bangkae 10, Bangkae road, Bangkae, Bangkae, Bangkok, Thailand 10160.

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