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EXTRACTION AND CHARACTERIZATION OF RICE BRAN OIL (RBO) FOR POTENTIAL APPLICATION AS BIODIESEL

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Abstract: **Rice bran oil (RBO) is a potential feedstock for biodiesel and biolubricant production among other alternative sources. The rice bran's fat extraction was carried out using maceration with chloroform, solvent recovery as a means of purification was carried out using rotary evaporator. Physicochemical analysis such as percent extraction and Gas Chromatography-Mass Spectrometry (GC-MS), iodine value, peroxide value and saponification values were carried out to ascertain its potential application as Biodiesel. From the GC-MS analysis, over 30 compounds including fatty acids and other phytochemicals were discovered with Oleic acid having the greater percentage of 34.5% having the base peak at m/z of 282.**

Keywords: **Rice bran oil (RBO), biodiesel, biolubricant production.**

1. INTRODUCTION

Rice bran (RB) is a nutrient-rich by-product of the rice milling process. It consists of pericarp, seed coat, nucellus, and aleurone layer. RB is a rich source of a protein, fat, dietary fibers, vitamins, minerals, and phytochemicals (mainly oryzanols and tocopherols), and is currently mostly used as animal feed. Various studies have revealed the beneficial health effects of RB, which result from its functional components including dietary fiber, rice bran protein, and gamma-oryzanol. The health effects of RB including antidiabetic, lipid-lowering, hypotensive, antioxidant, and anti-inflammatory effects, while its consumption also improves bowel function. These health benefits have drawn increasing attention to RB in food applications and as a nutraceutical product to mitigate metabolic risk factors in humans. This review therefore focuses on RB and its health benefits.

Rice (Oryza sativa) is one of the staple foods globally, especially in Asia. Global rice consumption was approximately 490.27 million metric tons in 2019. [1] Rice provides up to 50% of the calories consumed by populations in Asia. [2] Previous studies indicated that rice by-products from the milling process still contain a variety of nutrients and bioactive compounds, which exhibit beneficial health effects.[3] [4]These by-products could be used or added to food products to promote the yield and food sustainability of rice production. [5]

Rice kernels are composed of approximately 70% starchy endosperm (total milled rice), 20% rice husk, and 10% rice bran (RB), depending on the extent of milling and the rice variety.[5],[6] Milled rice is sold as food for humans, while broken rice, rice husk, and RB, considered as by-products, are commonly used for industrial applications and feed for animals.[7],[8] Owing to its high-fat content and nutritional value, RB oil is also extracted for use in cooking. [9]

A substantial number of in vitro and in vivo studies have shown the benefits of RB for certain health parameters, via its antioxidant activity. Moderate consumption of antioxidant-rich foods is important for scavenging the free radicals that cause oxidative stress, premature cell aging, and heart and muscle damage. [10]

Rice bran oil is the oil extracted from the hard outer brown layer of rice called bran. It is known for its high smoke point of 232 °C (450 °F) and mild flavour, making it suitable for high-temperature cooking methods such as stir frying and deep frying. It is popular as a cooking oil in East Asia, the Indian subcontinent, and Southeast Asia including India, Nepal, Bangladesh, Indonesia, Japan, Southern China and Malaysia.[11]

Rice bran oil has a composition similar to that of peanut oil, with 38% monounsaturated, 37% polyunsaturated, and 25% saturated fatty acids.

A component of rice bran oil is the γ-oryzanol, at around 2% of crude oil content. Thought to be a single compound when initially isolated, γ-oryzanol is now known to be a mixture of steryl and other triterpenyl esters of ferulic acids.[11] Also present are tocopherols and tocotrienols (two types of vitamin E) and phytosterols.

With the development of the global economy and increasing environmental pollution problems, the energy crisis caused by increasing global demand for energy becomes steadily more serious. The environmental problems caused by the use of fossil fuels are also of great concern. Because a large amount of carbon dioxide is produced from fossil fuel use and released into the atmosphere, the earth's surface temperature increases, resulting in the melting of ice sheets and a rise in sea levels.

This has prompted many researchers to search for sources of efficient, safe, and renewable green energy. Biodiesel, a monoalkyl ester of fatty acids with 12–24 carbon atoms, has recently gained considerable attention in this context. It was reported that the use of 100% pure biodiesel (B100) could reduce carbon dioxide emissions by 78.5% compared with petroleum-based diesel.

Biodiesel is easy to transport and store because of its high flash point. The cetane number of biodiesel is high and consequently its combustion properties are good. Besides, biodiesel has other advantages such as low sulfur content, low pollution, and good lubrication performance. These factors all lead to biodiesel being considered as a new type of green and renewable energy. The big question arises as to which of the plant material that would be suitable for biodiesel production, several plant products have been used such as groundnut oil, castor oil (*Ricinus communis*), palm kernel oil, soybean oil, neem seed oil, canola oil, rice bran oil and the host of others. Among all the plant materials mentioned above, Rice Bran Oil turns out to be a reliable source. This is due to the fact that it is a waste from Rice Milling factory. Heaps of rice bran are being wasted in the country. Rice bran is not also consumed by humans or other animals. Hence, the choice of rice bran is a good source of oil for the manufacture of biodiesel. Though, this research is solely on the extraction and examination of the oil present in rice bran for its effective use as potential biodiesel.

Nigeria and the world at large have in recent years faced with environmental crisis such as pollution as a result of exhaust fumes from the heavy-duty machines such as trucks, construction equipment, tractors, industries etc. All the aforementioned heavily depend on the use of petroleum-based diesel and they are the essential part of life. Growing concerns have been recorded for an ***alternative*** source of energy (**BIOFUEL**) produced from the transesterification of triglycerides such as vegetable oils (specifically Rice Bran oil) Ahmad Tabish and Reda Fatima (2020).

Rice bran is a byproduct of rice milling, and is left over in large quantity by the rice industry every year. In recent years, most of rice bran is used as animal feed ingredient, fertilizer and fuel. There is an underestimated potential for high- value rice bran production of its high content of fat.

Since biofuels are made from animal and vegetable fat, more demand for these products may raise prices for these products and create food crisis in some countries.

Hence, there is huge advantage in using rice bran compared to other sources. Rice bran (RB) is composed of about 27% fat, Abdul-Hamid and Y.S Energy demand has been on the increase and the need to find alternative source has become paramount. Hence, the need to diversify into the world of biodiesel for so many reasons. Diesel fuels have an important role in the industrial economy.

The high energy demand in the industrialized world and widespread use of fossil fuel is leading to fast depletion of fossil fuel resources as a well as environmental degradation. The degrading air quality due to emissions is the main adverse effect of petroleum based diesel. All these factors necessitate continued search and sustainable development of renewable energy sources that are environmentally friendly. Biomass sources, particularly vegetable oils, have attracted much attention as an alternative source of energy. [1]

They are renewable, non-toxic and can be produced locally from agriculture and plan resources. Their utilization is not associated with adverse effects on the environment because they emit less harmful emissions and green house gases. [2]

The work has been carried out on rice bran oil. RBO is used as raw material for the production of biodiesel.

Rice bran oil is the oil extracted from the germ and inner husk of rice. Rice bran oil is a non-conventional, inexpensive and low-grade vegetable oil.

Rice bran oil contains a range of fats, with 47% of its fats monounsaturated, 33% poly unsaturated and 20% saturated. The fatty acid composition of rice bran oil are shown in the table 1.

Table 1: Fatty acid composition of rice bran oil.

S. No.	Fatty acids	(%)
1	Palmitic C16:0	18.8
2	Stearic C18:0	2.4
3	Oleic C18:1	43.1
4	Linoleic C 18:2	33.2
5	Arachidic C20:0	0.7

Source: Srivastava and Prasad. [2]

Fatty acids (FA) are carboxylic acids with long aliphatic chains, which may be straight or branched, saturated or unsaturated (Mohan et al., 2015). One of the reasons for using biodiesel instead of free fatty acids is to nullify any corrosion that free fatty acids would cause to the metals of engines, production facilities and so forth. Free fatty acids are mildly acidic, but in time can cause cumulative corrosion unlike their esters.

Studies have shown that biodiesel has many advantages over the petroleum-based diesel as stated earlier. There is also a growing need to diversify the economy in Nigeria and the rest of the world, hence the need to synthesize biodiesel from rice bran as the chief source.

Rice bran (RB) is a nutrient-rich by-product of the rice milling process. It consists of pericarp, seed coat, nucellus, and aleurone layer. RB is a rich source of a protein, fat, dietary fibers, vitamins, minerals, and phytochemicals (mainly oryzanols and tocopherols), and is currently mostly used as animal feed. Various studies have revealed the beneficial health effects of RB, which result from its functional components including dietary fiber, rice bran protein, and gamma-oryzanol. The health effects of RB including antidiabetic, lipid-lowering, hypotensive, antioxidant, and anti-inflammatory effects, while its consumption also improves bowel function. These health benefits have drawn increasing attention to RB in food applications and as a nutraceutical product to mitigate metabolic risk factors in humans.

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Rice kernels are composed of approximately 70% starchy endosperm (total milled rice), 20% rice husk, and 10% rice bran (RB), depending on the extent of milling and the rice variety.[5],[6] Milled rice is sold as food for humans, while broken rice, rice husk, and RB, considered as by-products, are commonly used for industrial applications and feed for animals.[7],[8] Owing to its high-fat content and nutritional value, RB oil is also extracted for use in cooking. [9]

Here are different potential feedstocks for biodiesel production. The use of edible vegetable oils or the first generation feed stocks has been of great concern recently; this is because they raise many concerns such as:

- Food versus fuel debate that might cause starvation especially in the developing countries and other environmental problems caused by utilizing much of the available arable land.

- This problem creates serious ecological imbalances as countries around the world began cutting down forests for plantation purposes.

- The use of these feed stocks could cause deforestation and damage to the wildlife.
- Non-edible vegetable oils or the second generation feed stocks have become more attractive for biodiesel production.

These feed stocks are very promising for the sustainable production of biodiesel.

Some examples of *Jatropha curcas*, *Madhuca indica* (mahua), *Pongamia pinnata*

(karanja), *Hevea brasiliensis* (Rubber seed), *Azadirachta indica* (neem), *Oryza sativa* (Rice bran), *Ricinus communis* (castor) ***Azadirachta indica*** **(Neem)** which belongs to the Meliaceae family. It is a multi purpose and an evergreen tree, 12-18m tall, which can grow in almost all kinds of soil including clay, saline, alkaline, dry, stony, shallow soils.

It is native to India, Pakistan, Sri Lanka, Burma, Malaya, Indonesia, Japan, and the tropical regions of Australia. Planting is usually done at a density of 400 plants per hectare. The productivity of Neem oil mainly varies from 2 to 4 t/ha/ yr and mature Neem tree produces 30 - 50 kg fruit. The seed of the fruit contains 20-30 wt% oil and kernels contain 40 - 50% of an acrid green to brown colored oil.

Oryza sativa **(Rice bran)**

Rice bran is the cuticle between the paddy husk and the rice grain and is obtained as a by-product in the production of refined white rice from brown rice and is common in countries such as China and India. The bran is highly nutritious due to the presence of lipids, protein, minerals and vitamins. It is extracted from white rice bran by which the composition of rice bran varies with the rice type, climatic conditions and rice processing methods. The oil content in rice bran varies from 12% to 25%. The estimated potential yield of crude rice bran oil is about 8 million metric tons if all rice bran produced in the world were to be harnessed for oil extraction. Rice bran oil is an underutilized non-edible vegetable oil, which is available in large quantities in rice cultivating countries.

Madhuca indica **(Mahua)**

Madhuca indica is mainly found in India. It belongs to the Sapotaceae family and grows quickly to approximately 20 m in height, possesses evergreen or semi- evergreen foliage, and is adapted to arid environments. The kernel constitutes about 70% of the seed and contains 50% oil. Each tree yields about 20 - 40 kg of seed per year depending up on the maturity and size of the tree and the total oil yield per ha is 2.7 t per year. Its seed contains about 35 - 40% of *Madhuca indica*.

Pongamia pinnata **(Karanja)**

Pongamia pinnata (L.) Pierre (karanja or honge), an arboreal legume is a medium sized evergreen tree belonging to the family (Legumnosae; Pappilonaceae). Which grows in Indian subcontinent and south-east Asia. A single tree is said to yield 9 - 90 kg seeds, indicating a yield potential of 900-9000 kg seed/ha. It is one of the few nitrogen fixing trees (NFTS) that produce seeds with a significant oil content. *Pongamia pinnata* has been recognized as available source of oil for the burgeoning biofuel industry. The oil is reddish brown and rich in unsaponifiable matter and oleic acid.

SELECTION OF FEEDSTOCK FOR BIODIESEL

In general, seeds and nuts should be selected considering all the outcomes and shortcomings; it should be stored in cool and dry conditions, and processed quickly to avoid degradation. The seeds should be processed close to the time when the oil will be processed into biodiesel. Before processing the seeds must be cleaned, screened, and, in some cases, hammered or de-hulled. The meal or cake in some cases must be heated to deactivate toxic components before use. Biodiesel is not the same as straight vegetable oil or animal fat. A normal diesel engine will eventually be damaged through the use of straight vegetable oil or straight animal fat fuel.

Vegetable oils or animal fats must be converted into biodiesel by reacting the oil or fat with an alcohol and a catalyst. This process is referred to as "transesterification".

ALCOHOLS USED IN BIODIESEL

Alcohols that can be used in biodiesel production are those with short chains, including methanol, ethanol, butanol and amylic alcohol. The most widely used alcohols are methanol (CH3OH) and ethanol (C_2H_5OH) because of their low cost and properties. Methanol is often preferred to ethanol in spite of its high toxicity because its use in biodiesel production requires simpler technology; excess alcohol may be recovered at a low cost and higher reaction speeds are reached.

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CATALYSTS USED IN BIODEIESEL

The catalyst used for the transesterification of triglyceride may be classified as basic, acid or enzymatic. Basic catalyst includes Sodium Hydroxide (NaOH),

Potassium hydroxide (KOH), carbonates etc. Acid catalyst includes sulfuric acid, sulfuric acid and hydrochloric acid; there use has been less studied.

Heterogeneous catalyst that have been considered for biodiesel production include enzymes, titanium silicates, and compounds from alkaline earth metals, anion exchange resin guanidine in organic polymers. Lipases are the most frequently used enzymes for biodiesel production.

TRANSESTERIFICATION

Transesterification is defined as the process in which none edible oil is allowed to chemically react with alcohol. In this reaction, methanol and ethanol are the most commonly used alcohols because of their low cost and availability. This reaction has been widely used to reduce the viscosity of none edible oil and for the conversion of triglycerides into ester. Transesterification can be carried out in two ways: Catalytic transesterification and non-catalytic transesterification. It iswidely known that catalytic transesterification faces two problems.

The main problem is the processes are relatively time consuming and need separation of the oil, alcohol, catalyst and saponified impurities mixture from the biodiesel.

Purification of Biodiesel is much easier as no catalyst is required during the supercritical transesterification process, thus preventing soap formation or saponification from occurring. However, the drawbacks of the supercritical alcohol transesterification process are the high temperature and pressure that result in the high cost of the apparatus.

Fatty acid methyl esters (FAME) are a type of fatty acid ester that are derived by transesterification of fats with methanol. The molecules in biodiesel are primarily FAME, usually obtained from vegetable oils by transesterification. It is used to produce biodiesel *Anneken, et al, (2006)*. FAME are typically produced by an alkali-catalyzed reaction between fats and methanol in the presence of base such as sodium hydroxide (NaOH) or potassium hydroxide (KOH).

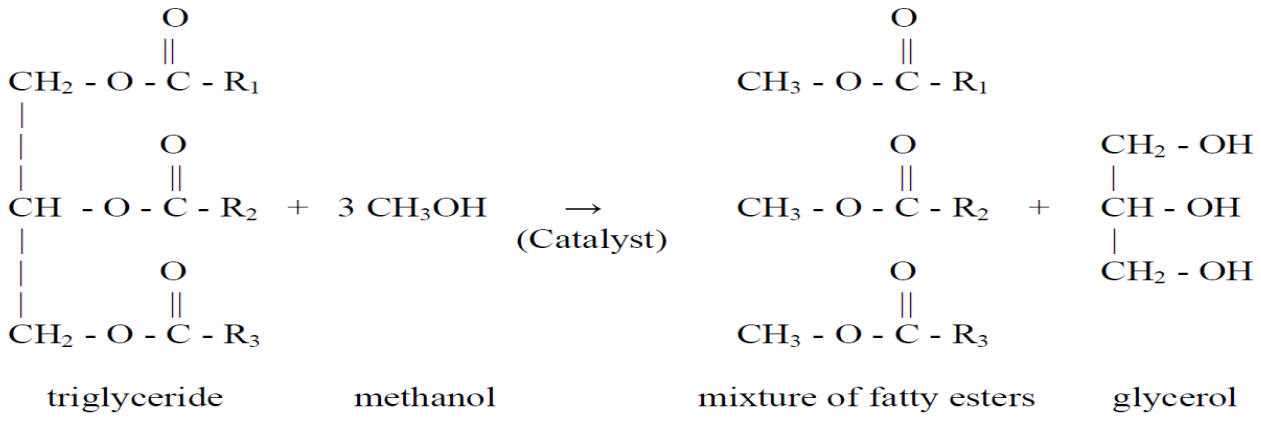

Fig.1: Transesterification reaction for production of fatty acid alkyl ester

2. MATERIALS AND METHOD

PROCEDURE FOR COLD MACERATION EXTRACTION

Exactly 300.0g of the sample of Rice Bran was weighed with analytical balance and placed in a 200ml beaker and 1.5litre of analytical grade chloroform was measured and added unto the sample in the beaker. A glass rod was used to stir the mixture thoroughly. The beaker was then allowed to stand for 24 hours, with intermittent stirring. Afterwards, the mixture was filtered with filtration apparatus. The filtrate, being the solution of oil in chloroform, was placed inside a distillation flask, mounted on a Rotary evaporator, which evaporated the chloroform within 24 hours. The residue is a thick dark brown oil. The weight of the oil was determined, and recorded.

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GC MS ANALYSIS METHOD

The analysis of the fatty acids in the rice bran oil sample was carried out at National Institute of Chemical Technology (NARICT), Zaria, Nigeria, using a Shimadzu QP2010 plus series gas chromatography coupled with Shimadzu QP2010 plus mass spectroscopy detector (GC-MS) system. The temperature programmed was set up from 70ºC to 280ºC. Helium gas was used as carrier gas. The injection volume was 2 µL with injection temperature of 250ºC and a column flow of 1.80 mL/min for the GC. For the mass spectroscopy ACQ mode scanner with scan range of 30-700 amu at the speed of 1478 was used. The mass spectra were compared with the NIST05 mass spectral library (NIST, 2012).

For GC–MS analysis, ~15 mg mL−1 of rice bran oil was methylated. In the preparation of rice bran oil sample, 60 mg of rice bran oil was dissolved in 4 mL of n-hexane (≥97%), and 200 µL of 2 mol L−1 anhydrous KOH/CH_3OH was added, vortexed for 30s and placed at room temperature for 10 min. Then, 1.00 g of NaHSO4 was added, vortexed for 30s for neutralization and centrifuged.

Chromatographic analysis was performed with a QP2010 Ultra GC–MS (Shimadzu, Kyoto, Japan) equipped with an electron impact source. Data were analyzed with GC–MS Solution version 2.6 with NIST08 and NIST08s standard mass spectrometry libraries. This procedure involved a PA-FFAP column (30 m × 0.25 mm i.d., with film thickness of 0.25 µm, Dikma Technologies Inc., Beijing, China) and an MXT-65TG metal capillary column (15 m × 0.25 mm i.d., with film thickness of 0.10 µm and maximum temperature of 370°C; Restek Corp., Bellefonte, PA, USA).

The ion source temperature was set at 250°C. The sample volume injection was 0.8 µL, and the split ratio was 1:40. Helium (≥99.999% purity) was used as a carrier gas with a flow rate of 0.6 mL min−1. Electron energy was 70 eV, and a full-scan mode spectrum was acquired in the m/z range of 35–500 Da.

3. MATERIALS AND METHOD

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SAPONIFICATION VALUE OF RICE BRAN OIL

Saponification occurs when esters are completely hydrolyzed in order to liberate fatty acids, and potassium hydroxide (KOH) is used to neutralise the freed fatty acid present in the fat or oil sample. Oils and fats are fatty acid esters of glycerol, a trihydroxyl alcohol. A sample of oil or fat requires a specific amount of milligrammes of potassium hydroxide to neutralise one gramme of the fatty acids released. Saponification's main byproduct is soap.

$CH_3C_2H_5(C_{17}H_{35}COO)_3 + KOH \longrightarrow CH_3C_2H_5(OH) + 3C_{17}H_{35}COOK$ (potassium stearate, soap).

Principle

The Saponification Value is inversely related to the mean of the fat sample's molecular weights. As a result, esters with smaller molecular weight fatty acids require more alkali to determine Saponification Value.

Reagents Required

a) 0.5 N HCl Standard solution

b) 0.5 N Ethanolic Potassium Hydroxide standard solution

Weight 35 g of KOH dissolve in 20ml distilled water, then make up to the mark with 95% (v/v) ethanol in a 1000 ml volumetric flask.

c) Preparation of Phenolphthalein Indicator

- Weigh 1 g of Phenolphthalein Indicator and place it in a 100 ml beaker
- Add 20 ml 95% ethanol, stir the mixture with a glass rod to completely dissolve the Phenolphthalein.
- Now, transfer into a 100 ml volumetric flask and fill to the mark with the ethanol solution.

Procedure

- Place 0.5 g of the rice bran oil sample inside 100 ml Erlenmeyer flask.
- Add 10 ml of ethanolic 0.5 N KOH solution and shake very well
- Heat at 80 - 85 ^{0}C in a water bath for 5 minutes and cooled to between 30 - 40 ^{0}C,
- Now, add 2 - 3 drops of the Phenolphthalein Indicator and titrate the content of the flask with 0.5 N HCl solution. Record the result.
- Then repeat the procedure on blank (solution of 0.5 N KOH without oil sample).

3.4 PEROXIDE VALUE OF RICE BRAN OIL

Principle

The reactivate oxygen content of fats and oils is reported in milliequivalent (meq) of free iodine per kilogramme of fats and oils. The sample is treated with potassium iodide in an acetic acid-chloroform media during the process. The procedure librates iodine, which is subsequently titrated using a standard solution of sodium thiosulphate.

Preparation of Reagents

Acetic acid: chloroform (3:2)

- ✓ Exactly 90 ml of acetic acid was measured and poured inside a 250 ml beaker,
- ✓ This was followed by pouring 60 ml of chloroform in the same beaker,

✓ The mixture was shaked very well to mix.

✓ The mixture was now ready to be used.

Preparation of 1% Starch Solution

❖ Exactly 50 ml of distilled water was measured and poured inside a 100 ml beaker, the beaker was placed on a hot plate and heated until it boiled.

❖ 0.5 g of Starch was weighed and placed inside the beaker containing the boiling water.

❖ The mixture was continuously stirred to mix and ensure the formation of homogeneous.

❖ When finally a clear and transparent solution was formed, it was then filtered hot.

❖ This gives the required Starch Solution indicator for the titration.

Preparation of 0.01 N Potassium Thiosulphate

i. About 0.25 g of sodium thiosulphate was weighed and placed inside a 100 ml beaker and 50 ml of water was added.

ii. This was followed by the addition of 0.02 g of sodium carbonate and the mixture was stirred very well to mix.

iii. The solution was then quantitatively transferred into a 100 ml volumetric flask and filled to the mark with distilled water.

Saturated Potassium Iodide Solution

i. About 2 ml of distilled water was poured inside a clean test tube.

ii. KI is continuously added inside the test tube with constant shaking until the KI could no longer dissolve.

iii. This gives us the saturated KI solution.

Sample Preparation

a) Exactly 1.0 g of the rice bran oil sample was weighed and placed inside a 250 ml beaker.

b) 3.0 ml of acetic acid: chloroform mixture was added into the sample and mixed very well by shaking the flask.

c) Then 1 ml of saturated KI Solution was added and shaked for about 1 minute to mix very well.

d) About 3 ml of distilled water was added and shaked for another 1 minute.

Titration Procedure

1. A clean burette was filled with the 0.01N sodium thiosulphate and the initial burette reading was recorded.
2. Then 5 drops of the Starch solution indicator was added and mixed.
3. The mixture was then titrated with the 0.01 N sodium thiosulphate with constant shaking.
4. The titration was continued until the black color was discharged (became colourless).
5. The final burette reading was recorded.

3.5. ACID VALUE OF RICE BRAN OIL

Principles

The acid value is defined as the number of milligrams of Potassium hydroxide required to neutralize the free fatty acids present in one gram of fat. It is a relative measure of rancidity as free fatty acids are normally formed during decomposition of triglycerides. The value is also expressed as per cent of free fatty acids calculated as oleic acid, lauric, ricinoleic and palmitic acids. The procedure of determination involves direct titration of alcoholic solution of sodium hydroxide or potassium hydroxide against the oil/fat sample.

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Reagents

1. Phenolphthalein Indicator

2. Alkaline blue 6B Indicator solution:

When testing rice bran oil or rice bran oil based blended oils or fats, which give dark colored soap solution, the observation of the end point of the titration may be facilitated, by using Alkali Blue 6B in place of Phenolphthalein.

Preparation: (2%) Extract 2gm of alkali blue 6B with rectified spirit in a Soxhlet apparatus at reflux temperature. Filter the solution if necessary and dilute to 100Ml with rectified spirit.

Alkali blue 6B indicator to be stored in closed Ambered colored bottle to avoid oxidation of dye.

3. Ethyl alcohol

Standard aqueous solution of potassium hydroxide or sodium hydroxide of 0.5N.

Procedure

a) Exactly 2.5 ml of the oil was weighed and placed inside a 250 ml beaker.

b) Freshly neutralized hot ethyl alcohol (50 ml) was added into the beaker, followed by about 1 ml of Phenolphthalein Indicator.

c) Then 1 ml of alkaline blue 6B Indicator solution was added and the flask was swirled to mix the content very well.

The flask was then heat in a water bath for 5 minutes at a temperature of 75°C.

d) After heating, 1 ml of alkaline blue 6B Indicator was again added.

e) While still hot, the mixture of the beaker was titrated against 0.5 potassium hydroxide solution with vigorous shaking.

f) The end point of the titration was attained when the blue color of the solution disappeared, becoming colorless. The titre value was then calculated.

4. RESULTS AND DISCUSSION

PHYSICOCHEMICAL ANALYSIS

The physicochemical analysis involved are extraction using solvent (chloroform), Gas Spectroscopy Mass Spectrometry, investigation involving acid value, saponification value, peroxide value and percent fat extraction.

4.1 PERCENT FAT EXTRACTION

After a successful extraction, the percent of fat extraction was calculated as follows:

$$\% \text{ fat} = \frac{weight\ of\ the\ extracted\ fat}{weight\ of\ rice\ bran} \text{ x } 100$$

$$= \frac{0.99g}{300g}\ x\ 100$$

= 0.33% of fat extracted.

The yield of 0.33% fat from 150g of rice bran is extremely low, implying that a large amount of rice bran is required to obtain a significant amount of fat. Low yield may be due to poor handling of rice bran, processing method, extraction method, volatility, and a variety of other factors.

4.2. DATA SHEET FROM GC-MS ANALYSIS

After careful observation on the amount of fat extracted, further extractions were carried out to obtain more fats, the following table shows the data obtained from the GC-MS Analysis.

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Table 3: Major fatty acids derived from rice bran oil

PK	RT	Area %	Name of Fatty Acid	Ref	CAS	Qual
1	7.374	1.26	9-Octadecenoic acid, (E)-	142088	000112-79-8	35
2	10.335	2.26	Oxacyclotetradecan-2-one, 14-methyl	89597	027198-63-6	38
3	12.874	2.97	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	138182	000084-69-5	87
4	13.768	0.32	Hexadecanoic acid, methyl ester	130822	000112-39-0	98
5	14.105	1.29	E-2-Methyl-3-tetradecen-1-ol acetate	128678	1000130-81-2-	46
6	16.087	0.07	9,15-Octadecadienoic acid, methyl ester (Z,Z)-	153899	017309-05-6	99
7	16.224	0.27	9-Octadecenoic acid, methyl ester	155758	001937-62-8	99
8	16.900	1.65	8-Hexadecenal, 14-methyl-, (Z)-	113612	060609-53-2	91
9	19.334	3.22	Octacosyl heptafluorobutyrate	273239	1000351-83-6	91
10	20.040	2.46	Octacosyl heptafluorobutyrate	273239	1000351-83-6	90
11	21.203	4.54	1-Nonadecene	126869	018435-45-5	94
12	21.490	2.17	1-Hexacosene	216562	018835-33-1	92
13	22.395	3.21	11,13-Dimethyl-12-tetradecen-1-ol acetate	142133	1000130-81-0	90
14	23.054	3.04	1-Hexacosene	216562	018835-33-1	96
15	23.821	4.33	Tetrapentacontane, 1,54-dibromo-	276082	1000156-09-4	93
16	24.117	3.37	1-Docosene	167463	001599-67-3	97
17	24.553	4.04	Dotriacontyl pentafluoropropionate	273442	1000351-81-4	91
18	24.736	2.59	Tetracosanal	207489	057866-08-7	93
19	25.101	6.51	Docosane	169407	000629-97-0	91
20	25.650	4.14	Dotriacontyl trifluoroacetate	271581	1000351-75-4	93
21	28.803	5.22	Octatriacontyl pentafluoropropionate	275090	1000351-89-1	93
22	26.803	5.22	Cyclotriacontane	247275	000297-35-8	94
23	27.153	2.84	Silane, trichlorooctadecyl-	230698	000112-04-9	78
24	27.695	4.37	Octatriacontyl pentafluoropropionate	275090	1000351-89-1	93
25	28.218	3.36	Cyclotriacontane	247275	000297-35-8	95
26	29.000	3.94	Tetratriacontyl trifluoroacetate	272754	1000351-75-3	84
27	29.648	3.29	Octatriacontyl pentafluoropropionate	275090	1000351-89-1	93
28	30.738	2.24	1-Nonadecene	126869	018435-45-5	89
29	30.882	2.24	1-Nonadecene	126869	018435-45-5	95
30	31.501	2.80	1-Docosene	167463	001599-67-3	89
31	32.429	0.23	.gamma.-Sitostenone	244160	084924-96-9	94
32	33.799	2.62	i-Propyl 9-octadecenoate	182556	1000336-67-1	92
33	34.958	2.60	9-Octadecenoic acid, (E)-	142085	000112-79-8	46
34	36.619	2.78	3-Methylpyrazolobis(diethylboryl)hydroxide	98083	1000159-71-9	37
35	37.475	2.21	Tungsten, tris(.pi.-allyl)(.eta.-3-acetato)-	217086	127649-15-4	53

Operator: Multi-User Science Reasearch Laboratory

Sample: OIL EXTRACT OF RICE BRAN 5ML

4.3 STRUCTURES OF SOME FATTY ACIDS FOUND IN RICE BRAN OIL

Following compounds were confirmed from the GC-MS analyses and the structures are shown below

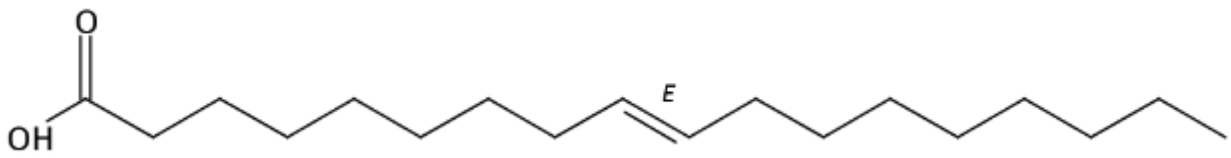

Fig. 1: 9-Octadecenoic acid (Ricinoleic acid).

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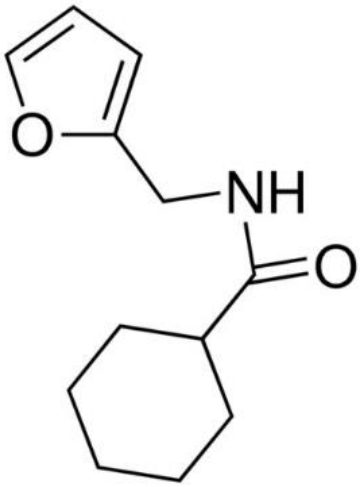

Fig. 2: Cyclohexanecarboxamide, N-furfuryl.

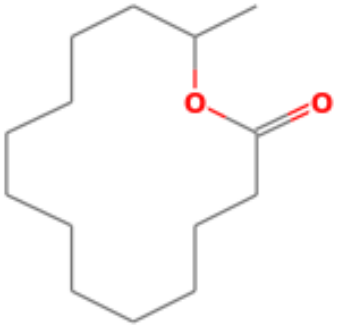

Fig. 3: Oxacyclotetradecan-2-one, 14-methyl

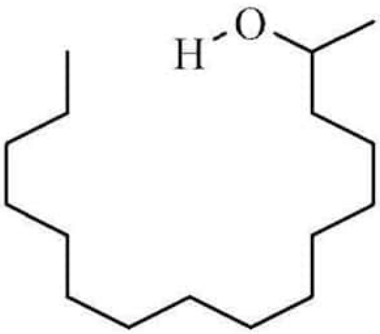

Fig. 4: 2-Hexadecanol

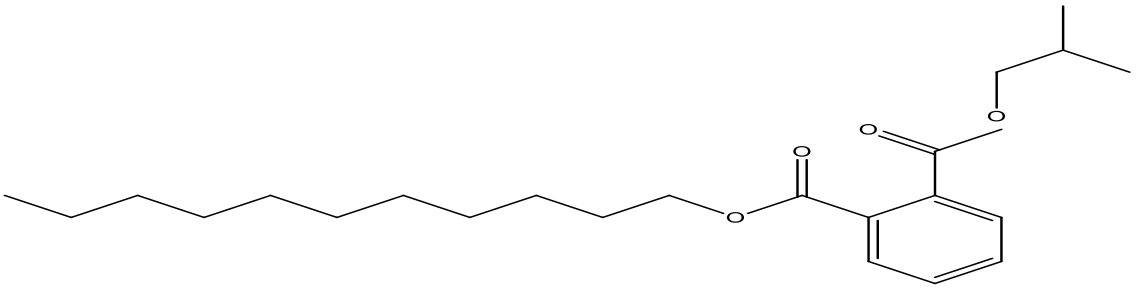

Fig. 5: Phthalic acid, isobutyl undecyl ester

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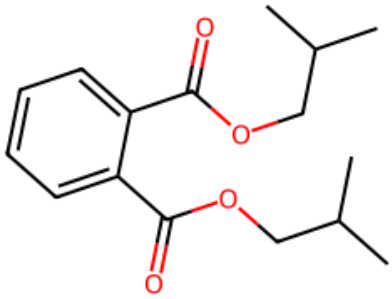

Fig. 6: 1,2-Benzenedicarboxylic-acid-bis-2-methylpropyl-ester

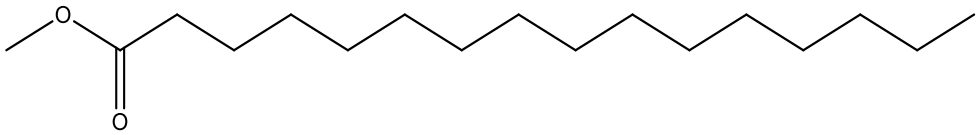

Fig. 7: Hexadecanoic acid, methyl ester

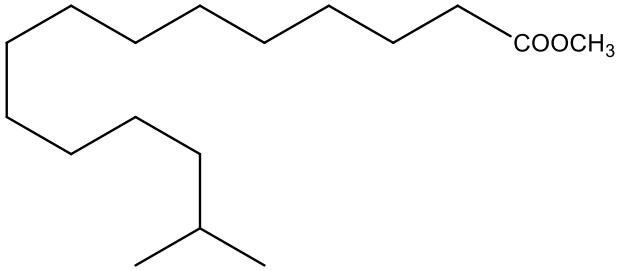

Fig. 8: Pentadecanoic acid, 14-methyl-, methyl ester

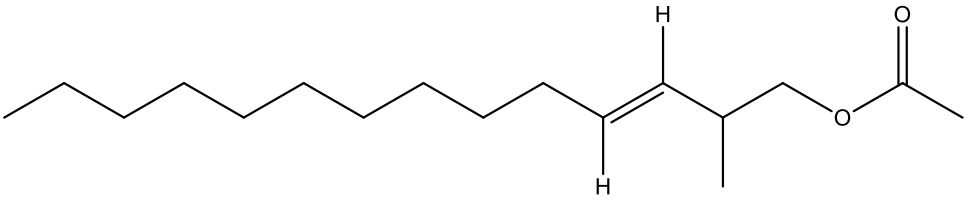

Fig. 9: E-2-Methyl-3-tetradecen-1-ol acetate

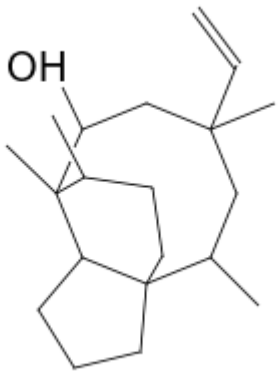

Fig. 10: 2,4,7,14-Tetramethyl-4-vinyl-tricyclo[5.4.3.0(1,8)]tetradecan-6-ol

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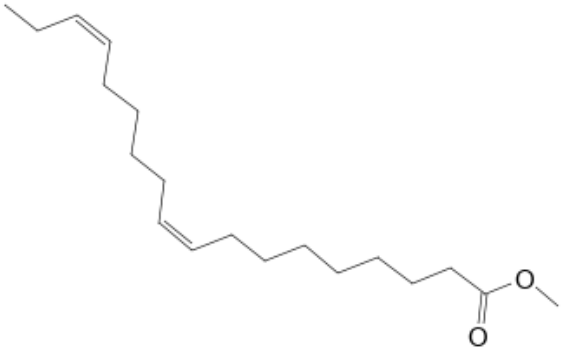

Fig. 11: 9, 15 -Octadecadienoic acid, methyl ester, (Z,Z)-

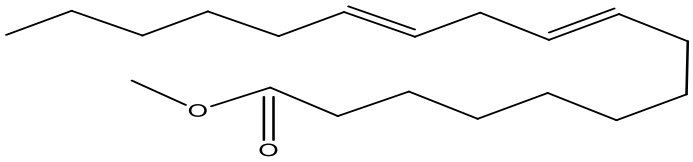

Fig. 12: 9,12-Octadecadienoic acid (Z,Z)-,methyl ester

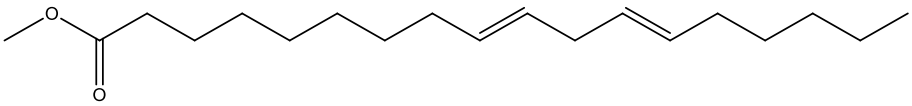

Fig. 13: 9,12-Octadecadienoic acid, methyl ester, (E,E)-

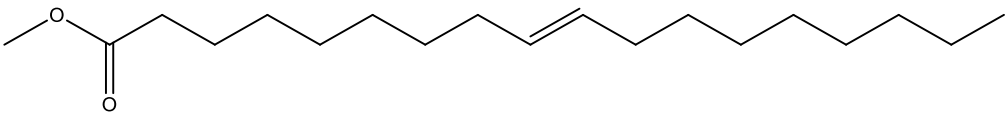

Fig. 14: 9-Octadecenoic acid, methyl ester,(E)-

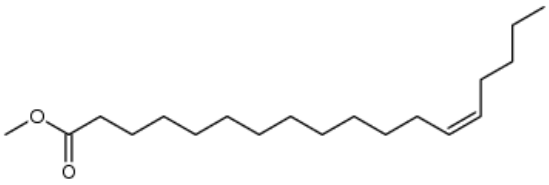

Fig. 15: cis-13-Octadecenoic acid, methyl ester

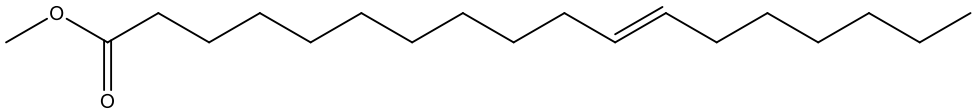

Fig. 16:11-Octadecenoic acid, methyl ester

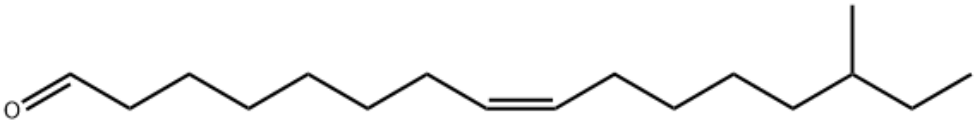

Fig. 17: 8-Hexadecenal, 14-methyl-, (Z)-

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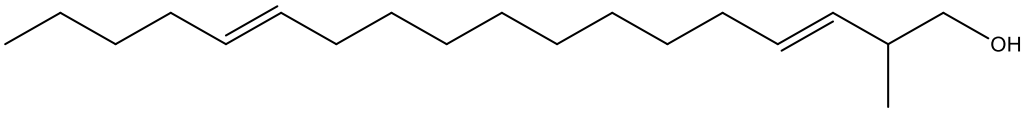
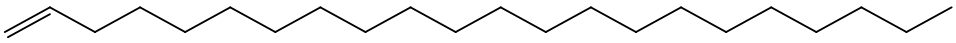


Fig. 18: 2-Methyl-Z,Z-3,13-octadecadienol



1-Docosene

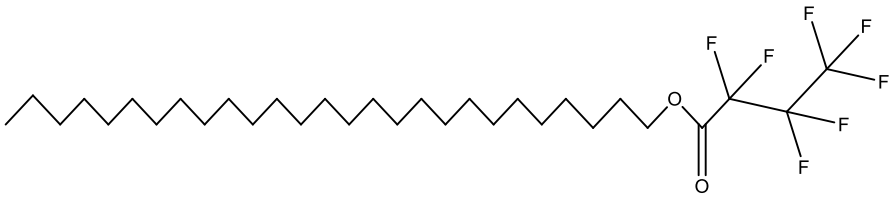


Fig. 19: Octacosyl heptafluorobutyrate

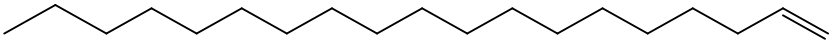


Fig. 20: 1-Nonadecene

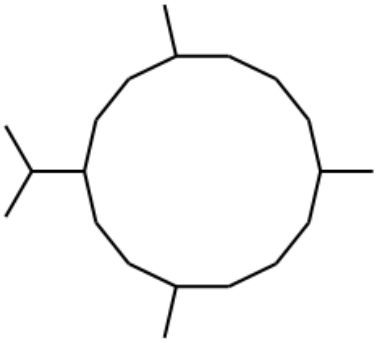


Fig. 21: Cyclotetradecane, 1,7,11-trimethyl-4-(1-methylethyl)-

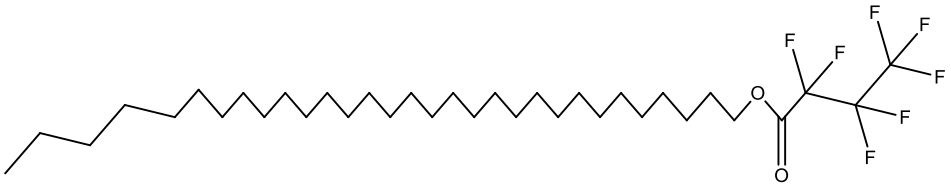


Fig. 22: Dotriacontyl heptafluorobutyrate

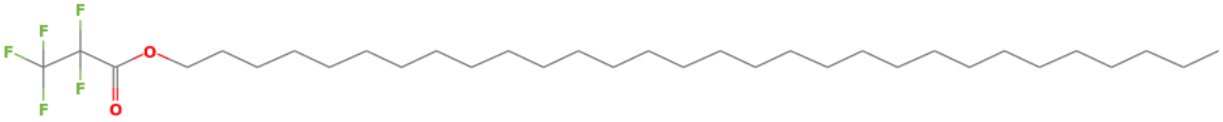


Fig. 23: Triacontyl pentafluoropropionate

Fig. 24: 1-Hexacosene

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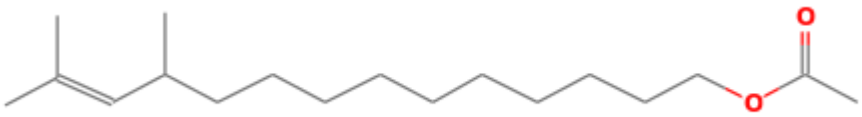

Fig. 25: 11,13-Dimethyl-12-tetradecen-1-ol acetate

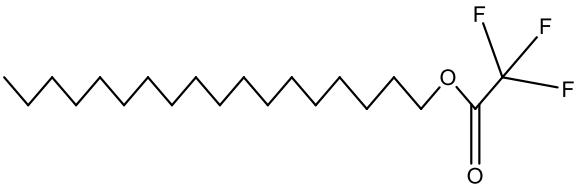

Fig. 26: Octacosyl trifluoroacetate

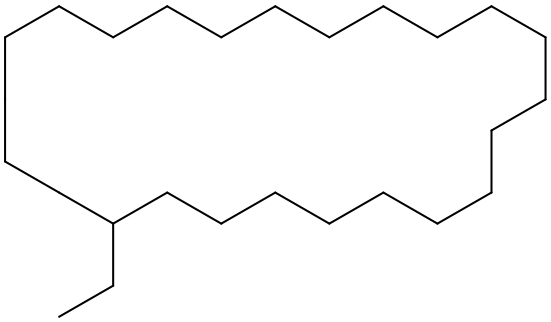

Fig. 27: Cyclodocosane, ethyl-

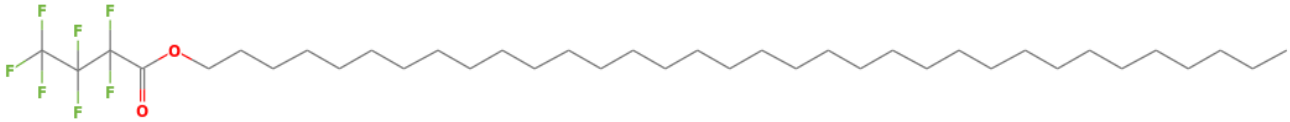

Fig. 28: Tetratriacontyl heptafluorobutyrate

Fig. 29: Tetrapentacontane, 1,54-dibromo-

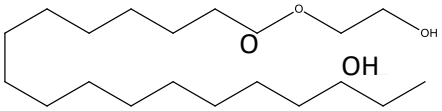

Fig. 30: Ethanol, 2-(octadecyloxy)-

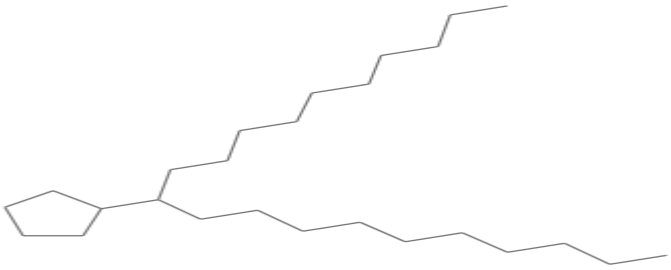

Fig. 31: Heneicosane, 11-cyclopentyl-

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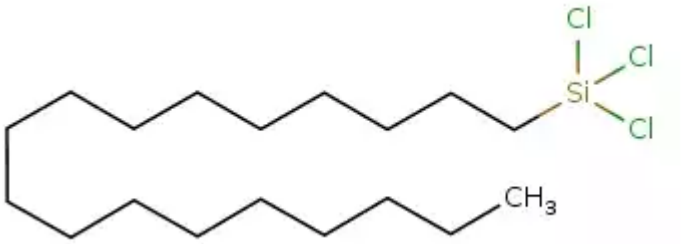

Fig. 32: Silane, trichlorooctadecyl-

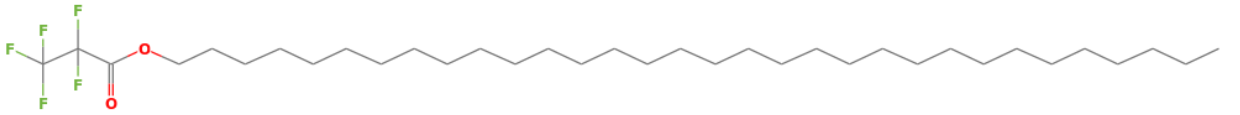

Fig. 33: Dotriacontyl pentafluoropropionate

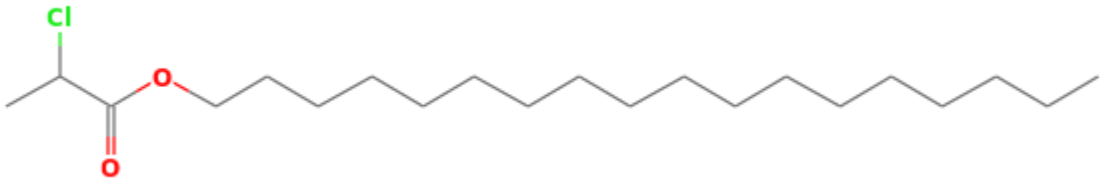

Fig. 34: 2- Chloropropionic acid, octadecyl ester

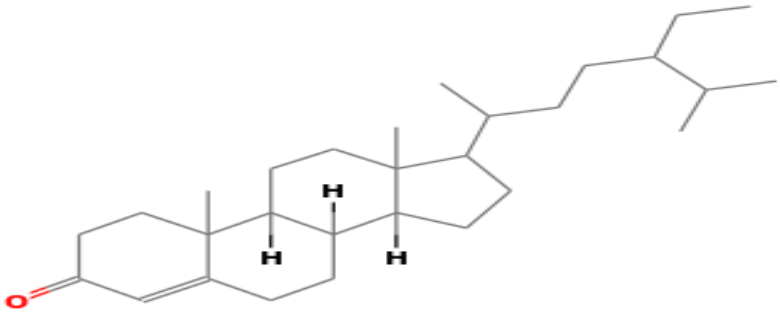

Fig. 35: .gamma.-Sitostenone

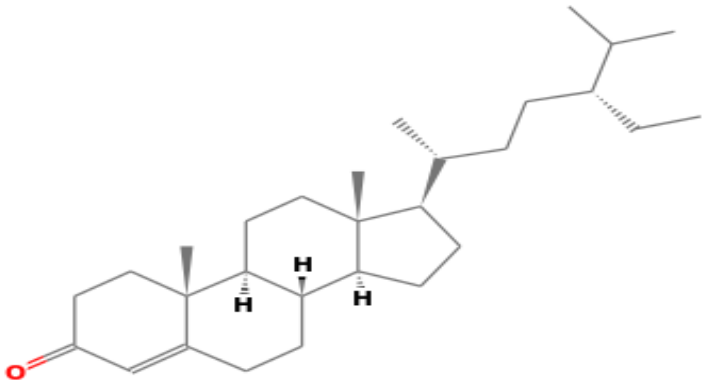

Fig. 36: Stigmast-4-en-3-one

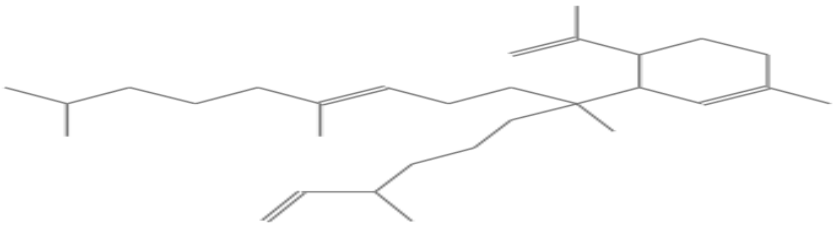

Fig. 37: 1-Methyl-4-(1-methylethenyl)-3-methyl-1-(4-methyl-hex-5-enyl)-5,9 -dimethyloct-4-enyl] cyclohexene

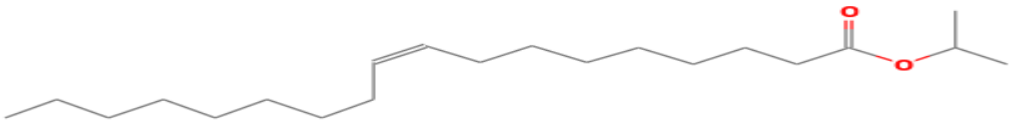

Fig. 38: i-Propyl 9-octadecenoate

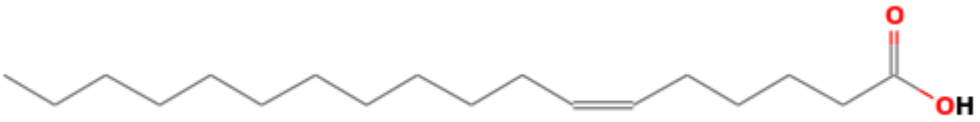

Fig. 39: 6-Octadecenoic acid, (Z)-

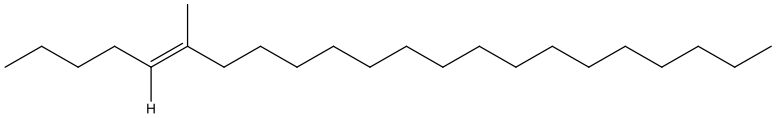

Fig. 40: 5-Methyl-Z-5-docosene

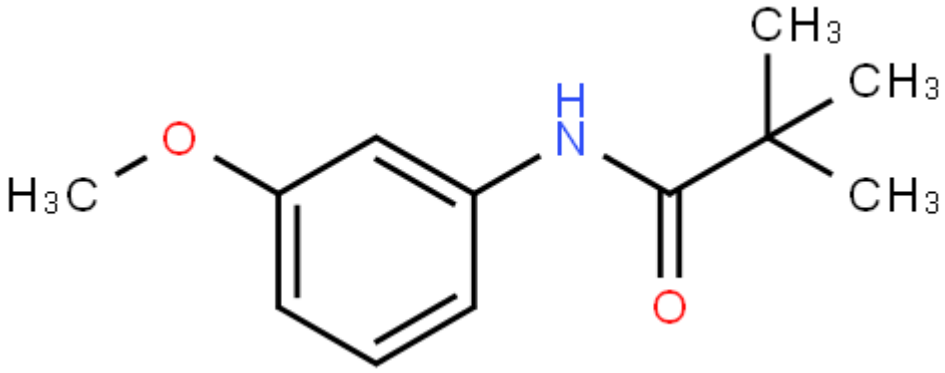

Fig. 41: Propanamide, N-(3-methoxyphenyl)-2,2-dimethyl-

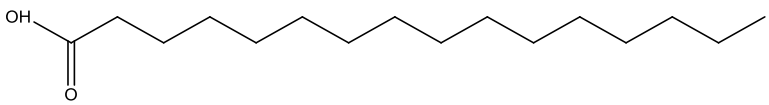

Fig. 42: Hexadecanoic Acid

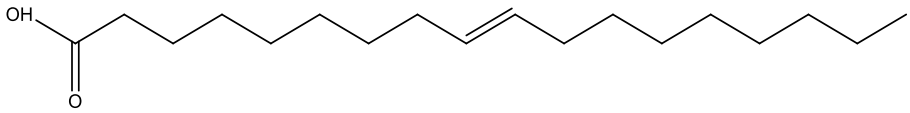

Fig. 43: Oleic acid

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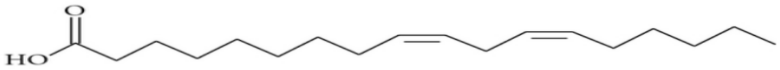


Fig. 44: 9,12-Octadienoic Acid (LINOLEIC ACID)

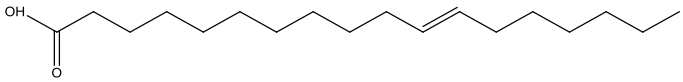


Fig 45: 11-Octadecenoic acid

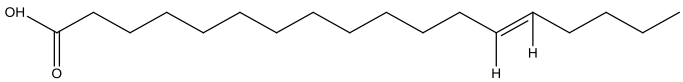
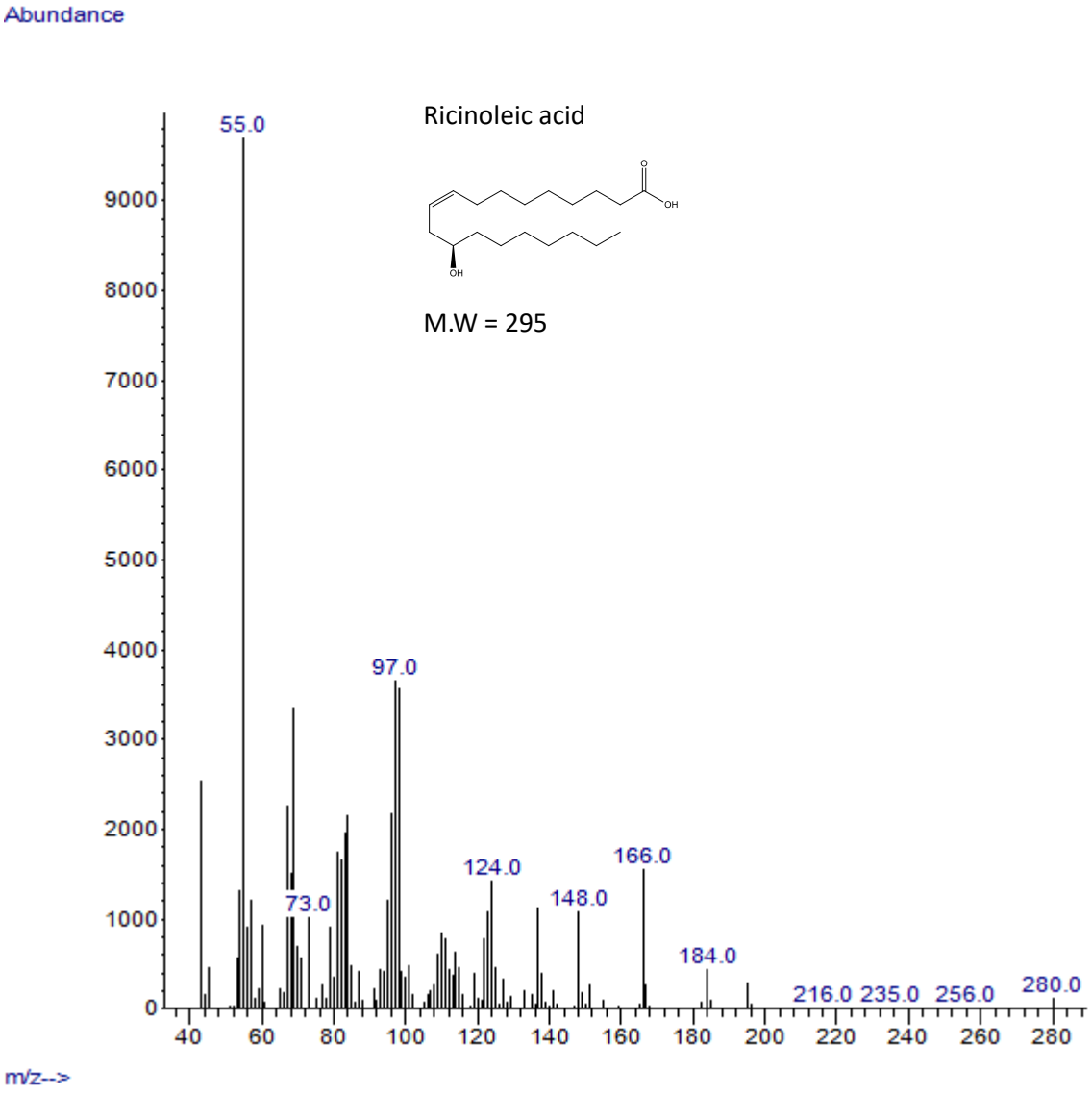


Fig. 46: Cis-13-octadecenoic acid

The above fatty acids and other phytochemicals were discovered in the course of GCMS analysis.

The table below shows the fatty acids, molecular formula, molecular weight and percentage composition in Rice Bran Oil.

4.4 MASS SPECTRA FROM RICE BRAN OIL.



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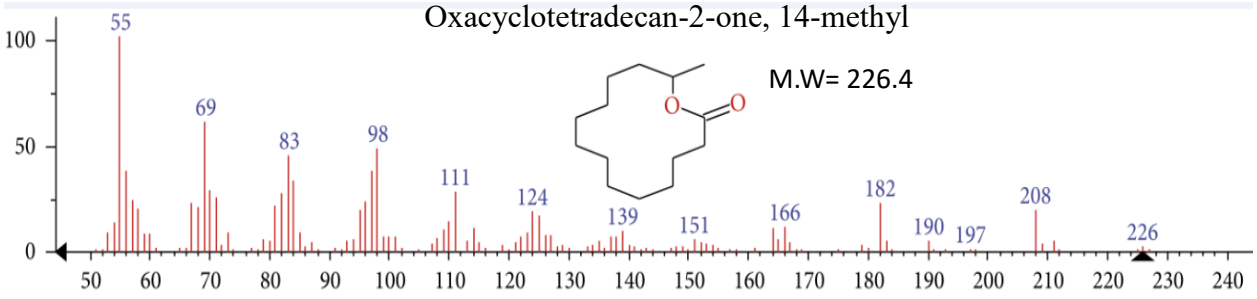
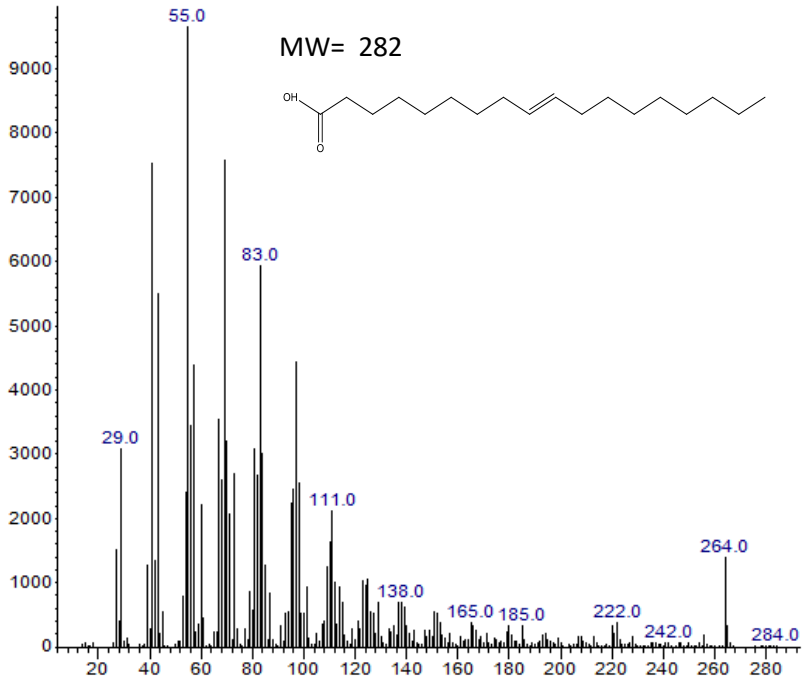
Oxacyclotetradecan-2-one, 14-methyl



Oleic Acid

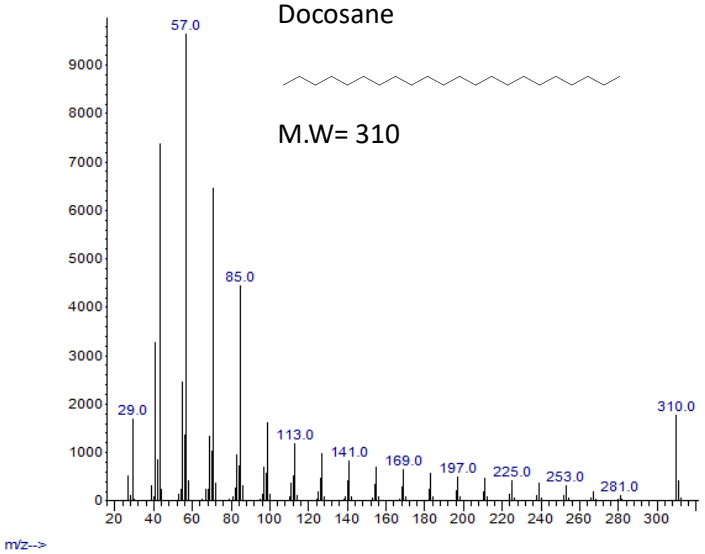
MW= 282



Abundance

Docosane

M.W= 310



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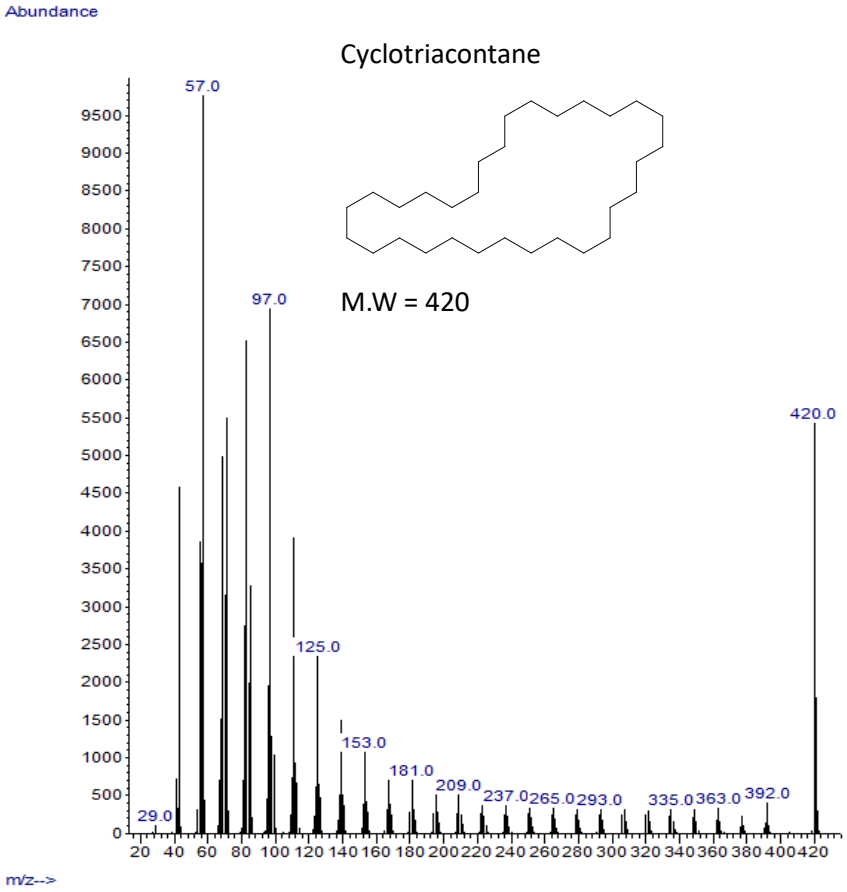
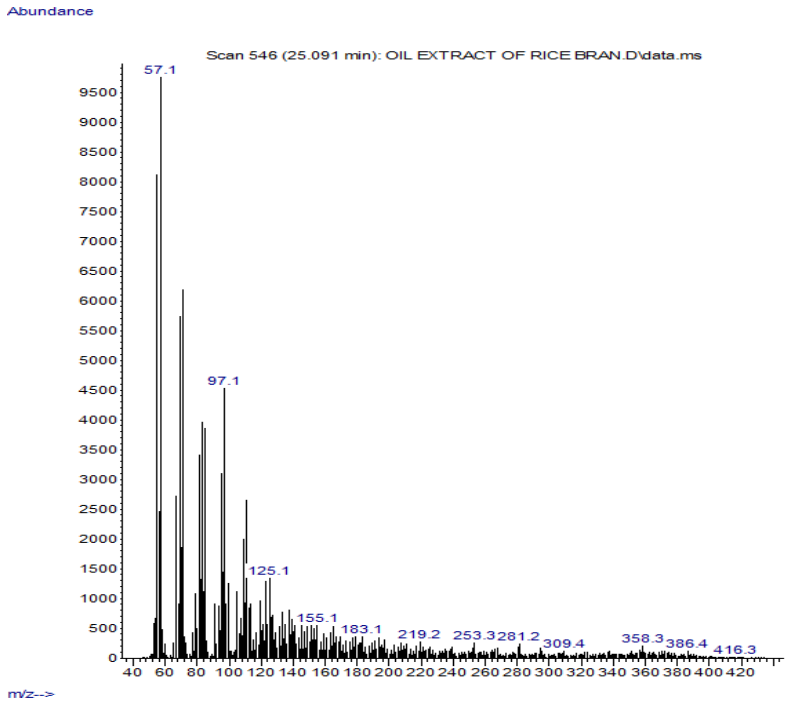
Docosane has a molecular formula of $C_{22}H_{46}$ with a molecular weight of 310g/mol. The base peak is located at m/z of 57 and the fragmentation occurs at $^{+}C_4H_9$ in the structure.



Cylcotriacontane has a molecular formula of $C_{30}H_{60}$ with a molecular weight of 420g/mol. It has the base peak at m/z of 57 which shows that fragmentation occurs at $^{+}C_4H_9$. It appears to be more abundant at 6900.



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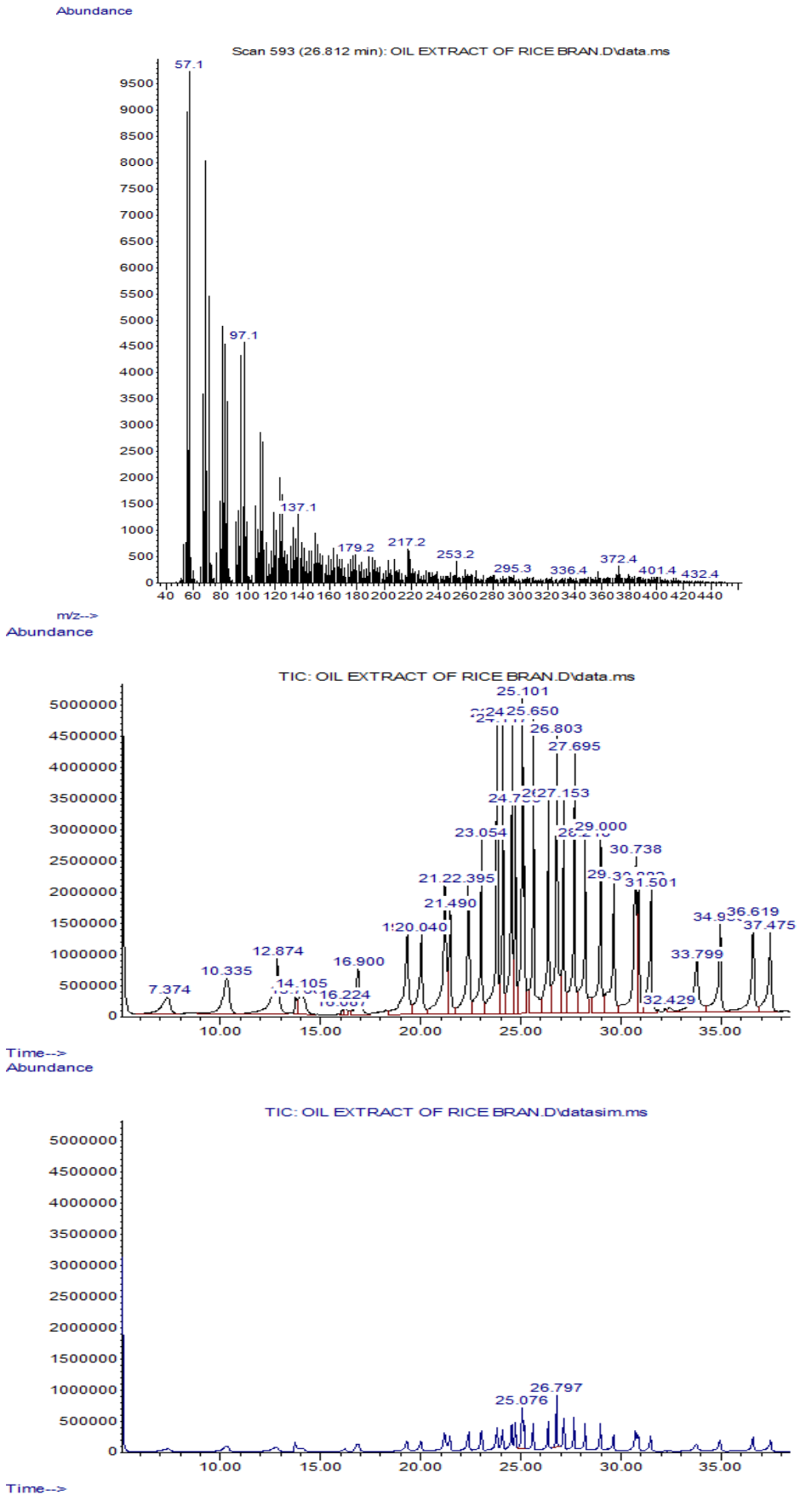

The predominant components identified are Octatriacontyl pentafluoropropionate - (retention time [RT] = 28.803 minutes), Dotriacontyl trifluoroacetate (RT = 25.650 minutes), 9-Octadecenoic acid, methyl ester - (RT = 16.224 minutes), i-Propyl 9-octadecenoate – (RT = 33.799 minutes), 9-Octadecenoic acid, (E)- (RT = 34.958 minutes). Other phytochemical discovered are Cyclotriacontane – (RT = 26.803 minutes), 1-Nonadecene – (RT=30.882 minutes), Docosane - (RT = 25.101 minutes), 1-Docosene (RT= 31.501 minutes), .gamma.-Sitostenone – (RT = 32.429 minutes) and the rest shown above in the table respectively.

SAPONIFICATION VALUE

The following are the records obtained from the determination of saponification value.

Readings

Weight of sample analyzed = 0.5 g

Volume of Ethanolic solution of 0.5 N KOH used = 15 cm^3

Volume of blank used = 15 cm^3

Volume of acid used = 15.5 cm^3

Volume of 0.5 HCl blank titration = 18.5 cm^3.

Calculation

SV = (B - S) × M × *28.05 /Ws.

Where SV = saponification value

B = volume of HCl for blank titration

S = volume of HCl for Sample titration

M = molarity of the acid

W_S = weight of the sample analyzed

* This value is half the molecular weight of HCl

SV = (18.5 - 15.5) × 0.5 × 28.05/ 0.5

= 3 × 0.5 × 28.05/ 0.5

= 84.15 mg/g

Therefore the saponification value of the oil sample of the Rice Bran under investigation is 84.15mg/g.

The saponification value: this value indicates that the saponification value of the rice bran oil utilised is lower than the value given in the literature by Watkins et al 2001, which is 175-195mg/g.

Rice bran oil with a lower saponification value contains a higher amount of high molecular weight fatty acids. This is due to the fact that a lower saponification value requires less potassium hydroxide to saponify the fatty acids, implying that the fatty acids are larger and have more carbon atoms in their chains. Greater molecular weight fatty acid oils are more stable and have greater melting points. They are also less prone to being rancid or oxidised. Rice bran oil with a lower saponification value may be advantageous in particular applications due to its stability.

DETERMINATION OF PEROXIDE VALUE

Calculation

The titre value for 0.01 N sodium thiosulphate = 17.3 ml

Peroxide Value (PV) = titre value × normality of sodium thiosulphate × 100 / weight of sample

PV = 17.3 × 0.01 × 100 / 1

PV = 17.3 meq/kg.

Therefore the Peroxide Value of the rice bran oil sample is 17.3 meq/kg.

As reported in the literature, low peroxide value of between 1-3 meq/kg shows that the oil is of high quality for biodiesel production. In the range of 10 meq/kg, it could be recommended for human consumption. In biodiesel production, the peroxide value (PV) of rice bran oil, is a critical parameter to consider. A high peroxide value in rice bran oil used for biodiesel production can have several negative impacts on the biodiesel and the overall production process:

1. Poor Biodiesel Quality: A high peroxide value in rice bran oil implies accelerated oxidation, indicating that the oil has begun to breakdown. During the transesterification process, oxidised oil can produce free fatty acids, polymers, and other unwanted chemicals. These contaminants can have a negative impact on the quality and performance of biodiesel.

2. Reduced Biodiesel Stability: Biodiesel made from highly oxidised oil is less stable over time. It is more susceptible to further oxidation, which results in the development of extra toxic chemicals, silt, and increased viscosity. This decreased stability might cause storage challenges and diminish the biodiesel's shelf life.

3. Increased Catalyst Deactivation: According to the literature, biodiesel manufacturing entails the use of catalysts such as sodium or potassium hydroxide to enhance the transesterification reaction (Kansedo Jibrail et al). High peroxide levels in the feedstock can hasten catalyst deactivation, resulting in partial conversion of triglycerides to biodiesel and an increase in soap formation. This soap development might cause operational issues and necessitate further purifying operations.

4. Equipment Corrosion: Oxidised biodiesel may contain more corrosive chemicals, which can cause corrosion of equipment and storage tanks throughout the manufacturing and distribution operations.

5. Engine Performance Issues: A high peroxide value in the produced biodiesel may result in poor engine performance, higher exhaust emissions, and increased deposits on engine components. This can have a negative influence on the overall efficiency and longevity of engines that run on biodiesel.

6. To avoid these problems, the feedstock used in biodiesel manufacturing must be of high quality. It is critical to store and handle rice bran oil properly in order to minimise oxidation before converting it into biodiesel. Regular testing of the feedstock's peroxide value and other pertinent characteristics is required to monitor its quality and appropriateness for biodiesel production.

DETERMINATION OF ACID VALUE

Calculation of Acid Value

Acid Value = 28.05 × V × N/ W

Where V = volume of potassium hydroxide used

N = normality of sodium hydroxide

W = weight of sample analyzed.

The titre value of the titration process was 6.5 ml.

Acid Value = 28.05 × 5.2 × 0.5/ 2.5 = 29.17mg KOH/g

Therefore the Acid Value of the Rice Bran oil sample analyzed is 29.17mg KOH/g.

In the context of rice bran oil, an acid value of 29.17 mg KOH/g indicates that there is a significant amount of free fatty acids in the oil.

The implications of a high acid value on biodiesel production are as follows:

1. Transesterification Difficulties: Biodiesel is typically produced through a chemical process called transesterification, where triglycerides (fats/oils) are reacted with an alcohol (e.g., methanol) in the presence of a catalyst to produce biodiesel (fatty acid methyl esters) and glycerol. Oils with a high acid value, like rice bran oil in this case, will have a higher concentration of free fatty acids, which can interfere with the transesterification reaction. These free fatty acids may react with the alcohol and catalyst to form soap instead of biodiesel, reducing the biodiesel yield.

2. Catalyst Consumption: The presence of free fatty acids necessitates the use of a stronger and more expensive catalyst to facilitate the transesterification process. This can increase the cost of biodiesel production.

3. Additional Pre-treatment Steps: High acid value oils may require additional pre-treatment steps, such as acid esterification, to reduce the free fatty acid content before the transesterification process. This adds complexity to the biodiesel production process.

4. Quality of Biodiesel: Biodiesel produced from high acid value oils may have a higher amount of impurities, such as soap, which can negatively affect its quality and performance. It may not meet the required biodiesel standards and could have adverse effects on engine performance and emissions.

To address the challenges posed by the high acid value of rice bran oil, several strategies can be employed:

1. Acid Esterification: Pre-treatment through acid esterification can be employed to reduce the free fatty acid content of the oil before the transesterification process. This step converts free fatty acids into their corresponding esters, making them more amenable to the transesterification process.

2. Enhanced Catalysts: The use of stronger and more efficient catalysts can help overcome the interference of free fatty acids during transesterification.

3. Multiple Transesterification Stages: Multi-stage transesterification processes can be implemented to gradually convert the free fatty acids and triglycerides into biodiesel and glycerol.

4. Blending: Blending high acid value rice bran oil with low acid value oils can help dilute the free fatty acid content and facilitate biodiesel production.

5. Monitoring and Quality Control: Regular monitoring and quality control during the production process are essential to ensure that the biodiesel meets the necessary standards and performance requirements.

5. RECOMMENDATION

Rice bran oil is another source from which biodiesel, an alternative source of energy can be produced. I will recommend that Rice Bran should be used for the production of FAME as it's not a potential feed for animals. It is a rice miller's waste. So, conversion of waste to a useful product is also encouraged. To improve the peroxide value, saponification value and acid value, the process of obtaining the rice bran should be checked. Extraction method of oil from rice bran should also be considered as the oil is volatile,

Another recommendation I have is that, in the process of extraction, solvents are not easily available to students in the school and it's somewhat expensive. There is need to make the different solvents for the process available to students anytime. Apparatus such as rotary evaporator, soxhlet extractor, GC-MS Machine should be provided and renewed once it degrades. I hereby encouraged the system to acquire some sophisticated equipment to boost research in the school.

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