ANALYSIS OF SEED OILS FROM SELECTED EDIBLE FRUITS FROM SARAWAK

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27873

Bachelor of Science with Honours

(Program of Resource Chemistry)

2013

ANALYSIS OF SEED OILS FROM SELECTED EDIBLE FRUITS FROM SARAWAK

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A final year project report submitted in fulfillment of the requirement for the award of the degree of Bachelor in Resources Chemistry

(Resources Chemistry)

FACULTY OF RESOURCES SCIENCE AND TECHNOLOGY
UNIVERSITI MALAYSIA SARAWAK
MAY 2013

DECLARATION

"I hereby declare that this final year project report, submitted to Universiti Malaysia Sarawak as a partial fulfillment of the requirement for the degree of Bachelor of Resources Chemistry. I also certify that the work described here is entirely my own except for excerpts and summaries whose resources are appropriate cited in the references."

Signature : ……

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Date : 16 MAY 2013

ACKNOWLEDGEMENT

Thanks to God for His allowed, I have finished my Final Year Project. I express a special appreciation to my supervisor, Prof Zaini Assim, for his patiently guidance, advice and support from the beginning to the completion of this final year project report. Special thanks to my co-supervisor, Prof Fasihudin for his kind on helping solve some problems occur.

I would like to express my gratitude to my Laboratory Assistant, Mr Rajuna Tahir who helps me a lot in the laboratory work of this project especially in preparing enough apparatus and glassware needed. Besides, my thanks to Master students and Mr Leo, Science Officer for their help in giving some idea while doing experiment.

A special thanks also to my lab-mates Rafaizah Jalaludin and Nur Ezzati Mohd Idris for their kindness and support that also help me to handle this project physically and mentally.

Last but not least, a huge of thanks and thousands of love to my parents Mohd Khalid Mohd Nor and Fatimah Shuaib who support by mentally and financial matter.

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LIST OF ABBREVIATIONS

Polyunsaturated Fatty Acids	PUFAs
Unsaturated Fatty Acids	USFAs
High Density Lipoprotein	HDL
Linoleic acid	LA
α-linolenic acid	LNA
γ-linolenic acid	GLA
Gas Chromatography Mass Spectrometry	GC-MS
Atomic Absorption Spectrophotometer	AAS

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Analysis of Seed Oils from Selected Edible Fruits From Sarawak

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ABSTRACT

Shorea Macrophylla, Castanopsis sp. and *Canarium indicum* are exotic fruits of Sarawak. These edible fruits are well known among local people in Sarawak. The fruit samples will be collected from certain areas in Sarawak. Extraction through 'Bligh and Dryer' has been done onto the fruits to obtain oil. In addition, extraction will be the main important step in this project to have a good yield of oil before it will be analyses through gas chromatography. Next, the oil content will be determined using gas chromatography-mass spectrometer (GC-MS). The composition of fatty acids of selected fruits has been identified by respective methods. Besides, minerals content has been determined through a method that has been done by certain researchers. Atomic Absorption Spectrophotometer (AAS) has been used to identify the elements content in seed of fruit.

Keywords: Extraction, Fatty acids, Mineral content, Gas Chromatography-Mass Spectrometer, Atomic Absorption Spectrophotometer (AAS).

ABSTRAK

Shorea macrophylla, Castanopsis sp. dan Canarium indicum adalah buah-buahan eksotik Sarawak. Buah-buahan ini terkenal di kalangan penduduk tempatan di Sarawak. Sampel buah-buahan diambil dari kawasan-kawasan tertentu di Sarawak. Pengekstrakan melalui 'Bligh and Dryer' telah dilakukan ke atas buah-buahan untuk mendapatkan minyak. Di samping itu, pengekstrakan penting bagi memperoleh hasil minyak yang baik sebelum ia dianalisis melalui kromatografi gas. Seterusnya, kandungan minyak akan ditentukan dengan menggunakan kromatografi gas spektrometer jisim (GC-MS). Komposisi asid lemak buah-buahan terpilih telah dikenal pasti dengan kaedah masing-masing. Selain itu, kandungan mineral telah ditentukan melalui kaedah yang telah dilakukan oleh penyelidik tertentu. Spektrometer Serapan Atom (AAS) telah digunakan untuk mengenal pasti kandungan unsur-unsur di dalam benih buah-buahan.

Katakunci: Pengekstrakan, asid lemak, kandungan Mineral, kromatografi gas spektrometer jisim, Spektrometer Serapan Atom (AAS).

1. INTRODUCTION

1.1 General Introduction

Today's people are characterized by having many unhealthy dietary habits. Other than snacking, the insufficient intake of healthy foods triggers a major dietary imbalance, this being a major cause of chronic diseases such as obesity, diabetes mellitus, cardiovascular disease, hypertension, stroke, and several types of cancer (Salas *et al.*, 2010). Fruits are important sources of minerals, fiber and vitamins, which provides essential nutrients for the human health (Aberoumand and Deokule, 2009). Natural antioxidants from fruits and vegetables provide a measure of protection that slows the process of oxidative damage (Einbond *et al*., 2004). According to Deshmukh and Waghmode (2011), edible fruit has nutritional food value, which provides the minerals like sodium, potassium, magnesium, iron, calcium and phosphorus. There are a lot of edible fruits in Sarawak such as *Shorea Macrophylla, Ganua Motleyana, Litsea Garciae, Canarium Odontophyllum Miq., Pangium Edule, Baccaurea Macrocarpa, Artocarpus Odoratissimus, Canarium indicum* and *Dimocarpus Melesianus.*

'Light Red Meranti' is a common name of *Shorea Macrophylla*. However in Sarawak, local people named it as 'Engkabang'. It is a species of plant in the Dipterocarpaceae family. It comes from the kingdom of Plantae, phylum of Tracheophyta, class of Magnoliopsida and order of Theales. This lowland tree is one of the fastest growing species of the genus. In Sarawak, productions of engkabang oils are more important than timber. Local people named it as 'minyak engkabang'. The tree will fruit once or twice a year. Frequently it is in coincidence with durian season. The most important part is the seeds are recognized for its four-wings, which make the seeds drop like a helicopter from the canopy atop. The nut is collected, dried and processed for edible oil. Engkabang is well-known due to its nut called as 'False Illipe Nut', which has moisturising properties that are similar to cocoa butter for skincare and hair care products (Kamal *et al.,* 2010).

Canarium indicum is commonly named as 'Mitus'. It has similar characteristic with nut. The seeds are brown in colour. It is natively growth in Sarawak. According to Orwa et al. (2009), *Canarium indicum* is an evergreen, dioecious, medium-sized to fairly large tree to 40 m tall and a diameter of up to 100 cm. The crown is large, dense crown and buttresses are upto meter high. The bark is grey or brownish-grey to yellow-brown, smooth to scaly and dippled;

inner bark laminated, reddish-brown to pinkish-brown, exuding a milky resin. Canarium has a large genus of trees and the family of Burseraceae has found in the tropics from Malaysia to Melanesia with one species in the West Indies. They could produce edible oily seeds.

Castanopsis sp. is commonly named as ‘Chest Nut’ and the local name Berangan. It is in Genus of Castanopsis and family of Fagaceae. It is an indigenous fruit. It is Berangan species that can be found in Sarawak. The seed is dark brown in colour and the shell of seed is very hard.

1.2 Objectives

The objectives of this project are:

a. to extract the seed oils of selected edible fruits from Sarawak,

b. to identify fatty acids in selected edible fruits from Sarawak through their seed oil by using gas chromatography technique and

c. to determine the mineral content in selected edible fruits from Sarawak.

2. LITERATURE REVIEW

2.1 Edible Fruits

Fruits are important sources of minerals, fibers and vitamins, which provides essential nutrients for the human health (Aberoumand and Deokule, 2009). As stated by Valvi and Rathod (2011), fruits are generally acceptable as good source of nutrient and supplement for food in a world faced with problem of food scarcity. They are known to be excellent source of nutrients such as minerals and vitamins. In fact, living organism require a continuous supply of large number of substances (food) from outside the body to complete their life cycle. This supply is called as nutrition. The mineral nutrition is an important aspect and its pivotal role in human life for healthy growth. Edible fruits have nutritional food value, which provides the minerals like sodium, potassium, magnesium, iron, calcium and phosphorus. They are immune to many diseases and often used in different formulation of 'Ayurveda' in Indian Folk-medicine. They provide fibers which prevent constipation. It is consider that special attention should be paid in order to maintain and improve this important source of food supply (Deshmukh and Waghmode, 2011). Seeds, part of fruits are the primary stage of plant life cycle; they have strong defense mechanism possibly due to the presence of phytoconstituents contributing to antioxidant activity (Kiran *et al.*, 2012).

2.2 Fatty Acids in Fruit

Extraction yield depends on the solvent and method of extraction. Commonly used solvents for extracting various substances from plant material are water, aqueous mixtures of ethanol, methanol and acetone (Jacopic *et al.,* 2009). According Nivas *et al*. (2011), the majority of epidemiologic studies have found that saturated fat or meat intake is associated with markers of insulin resistance or glucose intolerance (type 2 diabetes). Polyunsaturated Fatty Acids (PUFAs) and particularly linoleic acid recognized to prevent inflammatory, arrhythmias and hypertensions. Hence, linoleic acid is one of the most important PUFAs in human food, because of its prevention of distinct heart vascular diseases. The oxidative stability than those containing more Unsaturated Fatty Acids (USFAs) and therefore could be widely used as frying oils. The high degree of unsaturation suggests that the oil maybe used as a drying oil for the manufacture of cosmetics, oil paints and varnishes. It may be used as edible oil for cooking or manufacture of margarine. Further they stated that the oxidative stability can prevent skin irritation caused by oxidation of the oil when used as body cream. The fatty acid profile of edible oils plays an important role in their stability and nutritional value.

Monounsaturates (18:1) and polyunsaturates (18:2) fatty acids have been found to be effective replacements for saturates as part of cholesterol-lowering diets. However, it is also known that the oils with substantial amounts of unsaturation, particularly 18:2 fatty acids, are susceptible to oxidation and may produce products that contribute to arteriosclerosis and carcinogenesis. Some studies with experimental animals indicate that excessive amounts of linoleic acid promote carcinogenesis (Milovanovic and Jovanovic, 2005). Lipid components in fruits, though occurring in minor amounts, are presumed to contribute to the development of characteristic aromas and flavours during ripening as they are considered as precursors for various volatile odorous principles of fruits (Msaada *et al.,* 2009). Major fatty acids were palmitic (34%), oleic (20.85%) and stearic acids (16.20%) respectively. Linoleic (6.07%), eicosatrioneic (3.17%), α-linolenic (1.95%), gamma-linolenic (1.76%), myristic (1.61%), arachidic (1.17%) and lauric acids (1.10%) exhibited the lower concentrations (Ozcan and Baycu, 2008).

2.2.1 Benefits of Essential Fatty Acids

As a result of the change in dietary habits within the past century, the intake of trans fatty acids has increased dramatically. Studies have shown conclusively that trans fatty acids increase total cholesterol levels and diminish the levels of ‘‘good’’ high density lipoprotein (HDL). By supplementing the diet with high levels of unsaturated cis fatty acids, some of these negative effects can be reversed (Leizer *et al.,* 2000). With respect to modern diets, the amount of linoleic acid (LA) consumed compared to the amount of α-linolenic acid (LNA) consumed has increased exceptionally in the past 100-150 years. This disparity has disrupted the proper balance of dietary essential fatty acids that is considered nutritionally optimal. In addition to the lack of these essential fatty acids in the diet, factors such as stress and disease weaken the enzymatic activity that promotes the conversion of LA to γ-linolenic acid (GLA) (Leizer *et al.,* 2000). Therefore, a supplementation of LA can be helpful to alleviate this potential deficiency.

In an ideal diet, the daily consumption of fats should not exceed 15-20% of total caloric intake. Approximately one-third of these fats should be the essential fatty acids in their proper ratio. For a 2500 calorie/day diet, LA intake should be 9-18 grams/day, and LNA intake should be 6-7 g/day. Although these are the ideal amounts to maintain a healthy, balanced diet, certain stresses to the body warrant increased consumption of essential fatty acids, particularly the omega-3 PUFA such as LNA (Leizer *et al.,* 2000).

2.3 Mineral analysis

Mineral ions are of prime importance in determining the fruit nutritional value. Potassium, calcium, and magnesium are the major ones. In the tissue of many fruits, calcium is one of the mineral believed to be an important factor governing fruit storage quality (Valvi and Rathod, 2011). A number of mineral ions are recognized as essential plant nutrients that are directly incorporated into organic compounds synthesized by the plant. Of these, potassium, phosphorus, calcium, magnesium and sodium are the most important quantitatively and are recommended for composition analysis (Ozcan, 2006). According to Adekunle and Adenike (2012), an appreciable amount of zinc, sodium, potassium, phosphorus, and iron is obtained in all the samples making them good for bone and blood building. Magnesium and calcium are essential for healthy bone development and for energy metabolism. Iron is essential to red blood cell production. Red blood cells carry all the nutrients to cells throughout the body (El-Sohaimy and Hafez, 2010).

3. MATERIALS AND METHODS

3.1 Sample collection

The samples were collected from Limbang, Sarawak. The seeds were dried in open air. The samples was them washed and grounded using a mortar and pestle into tiny pieces prior to analysis.

Figure 3.1: Picture of sample (*Canarium indicum*)

Figure 3.2: Picture of sample (*Castanopsis sp.*)

Figure 3.3: Picture of sample (*Shorea macrophylla)*

3.2 Fatty Acids Study

3.2.1 Sample Extraction

The seed oils were extracted by "Bligh and Dyer" method with slight modifications from Zainuddin *et al.* (2011). Samples of seed fruits have been finely grounded by using mortar and pestle. Thirty grams of each grounded sample transferred into 500 mL beaker. Mixture of methanol (60 mL) and chloroform (30 mL) was then added to the seeds and stirred by glass rod. One volume of chloroform (30 mL) was added to the mixture and after stirring for an additional 30 sec, distilled water (30mL) was added. The homogenate was left for 3 hours before filtered under vacuum. The filtrate was transferred to a separating funnel to separate the organic and the water soluble layer. After layers formed, the lower part of layer has been taken and pours back into the separating funnel for once again filtered. The oil layer was then added with 24 mL of sodium sulphate anhydrous. Lastly, the remaining oil has been concentrated with a rotary evaporator with temperature at 60 °C to remove the solvents. The yield of oil remain has been weighed and the percentage has been calculated by using a formula below, as stated in Du (2009).

$$\text{Oil Yield \%} = m / M \times 100 \qquad \text{Equation 3.1}$$

Where

m =weight of extracted oil (g)

M= weight of sample (g)

3.2.1.1 Stock Solution

After undergo rotary evaporator, 2 mL of dichloromethane has been added into the oil remain. Then, it has been transferred into vials and stored in low temperature (fridge) for further activity.

3.2.2 Fatty Acid Methylation

Prior to gas chromatographic analysis, fatty acids were derivative into fatty acid methyl esters in order to improve volatility of the fatty acid compounds. Derivatization has been carried out by adding 1 mL of hexane to 200 µl of lipid extract followed by additional of 0.2 mL of 2M Sodium hydroxide-methanol reagent. The mixture solution was then subjected to 10 seconds shaking by vortex before placing in water bath at 50°C for 20 seconds. After cooling for about 2 minutes, the mixture was subjected to 10 seconds shaking by vortex again. 0.2 mL of 2M Hydrochloride-methanol reagent was finally added. The mixture was vortex for 10 seconds and left to separate into layers. If the layers did not separate well, the mixture solution was subjected to centrifugation and the supernatant was collected.

3.2.2.1 Collecting oil yield

After the supernatant has been transferred into vial, it was blown under nitrogen gas to remove the solvent remain contain in residual oil. The yield remain has been weighed.

3.2.2.2 Dilution activity

Before gas chromatography (GC) analysis, sample has been dilute with dichloromethane at certain amount depends on it concentration. Internal standard of has been added in same concentration with sample. Formula that used for dilution was:

$$M_1V_1 = M_2V_2$$ Equation 3.2

Where,

M = Concentration of sample

V = Volume of solvent added (dichloromethane)

3.2.3 Gas Chromatography Mass Spectrometry (GC-MS) analysis

The GC-MS (Shimadzu) was used to analyze the profile of fatty acid in seed oil. The sample was injected with column BPX-5 (0.25µm thickness × 0.25mm diameter × 29.4 m lengths) in a splitless mode. The injector temperature was 260°C while the column temperature was programmed at an initial temperature 90° C to a final temperature of 300°C. Interface temperature has been set at 320°C. The initial time is 5.00 minutes while final time is 10.00 minutes. Helium has been used as carrier gas.

3.3 Mineral analysis

3.3.1 Preparation of samples for mineral content determination

The mineral content of seeds was determined with slight modifications from Association of Official Analytical Chemists (AOAC, 2000). Samples have been washed with tap water and soaked with 6M HNO_3. After that, it has been rinsed by ultra-water. Four grams of seeds have been weighed and grounded into smaller pieces. Silica crucible has been used for every four grams samples and incinerated into muffle furnace at 450ºC for three hours. Ash has been produced through the incineration and 10 mL of 6M HCL has been added into it. Next, it has been transferred into hot plate for digestion and clean station. After a few minutes, the final residue has been produced. Lastly, 100mL of 0.1M HNO_3 was added into it.

3.3.2 Determination of mineral content

The concentrations were determined with an Atomic Absorption Spectrophotometer (AAS) of Thermo Scientific Ice 3000 Series. Air-acetylene flame has been used to determine the metal content with 1.0 L/min to 4.4 L/min flow fuel prior to type of metal analyze. Concentration of mineral content has been analyzed in mg/kg or ppm. Hence, calculation involve by using formula below.

$$\text{Concentration (mg/kg)} = \frac{\text{concentration (mg/L)}}{\text{weight of seed (kg)}} \times 0.1 \text{ L}$$

Equation 3.3

4. RESULTS AND DISCUSSION

4.1 Yield of Seed Oil

The yield of seed oil for three samples that are *Castanopsis sp*., *Canarium indicum* and *Shorea macrophylla* have been analyzed in triplicate. Table 4.1 shows the percentage of oil yield that produce from samples.

Table 4.1: Percentage of oil yield of *Castanopsis sp*., *Canarium indicum* and *Shorea macrophylla.*

Samples	Percentage of oil yield (%)
Castanopsis sp.	0.44 ± 0.08
C. indicum	31.59 ± 2.24
S. macrophylla	14.60 ± 2.42

The data are mean values ± Standard deviation (SD) of three replicates.

Canarium indicum has produced the highest yield of oil that is 31.59% ± 2.24 while *Castanopsis sp*. produced the lowest of oil yield which is 0.44% ± 0.08. In a meanwhile, *Shorea macrophylla* produced 14.60% ± 2.42 of oil yield. According to research that have been done by Milovanovic and Jovanovic (2005), the oil content of seeds was very high, ranging from 22.1-53.5%. However, yield of oil of these three samples are lower due to technical mistakes may occur in doing experiment.

4.2 Fatty Acids Content in Seed Oils

The chromatogram of Gas chromatography in Figure 4.1 shows the profile of fatty acid contain in *Castanopsis sp.*

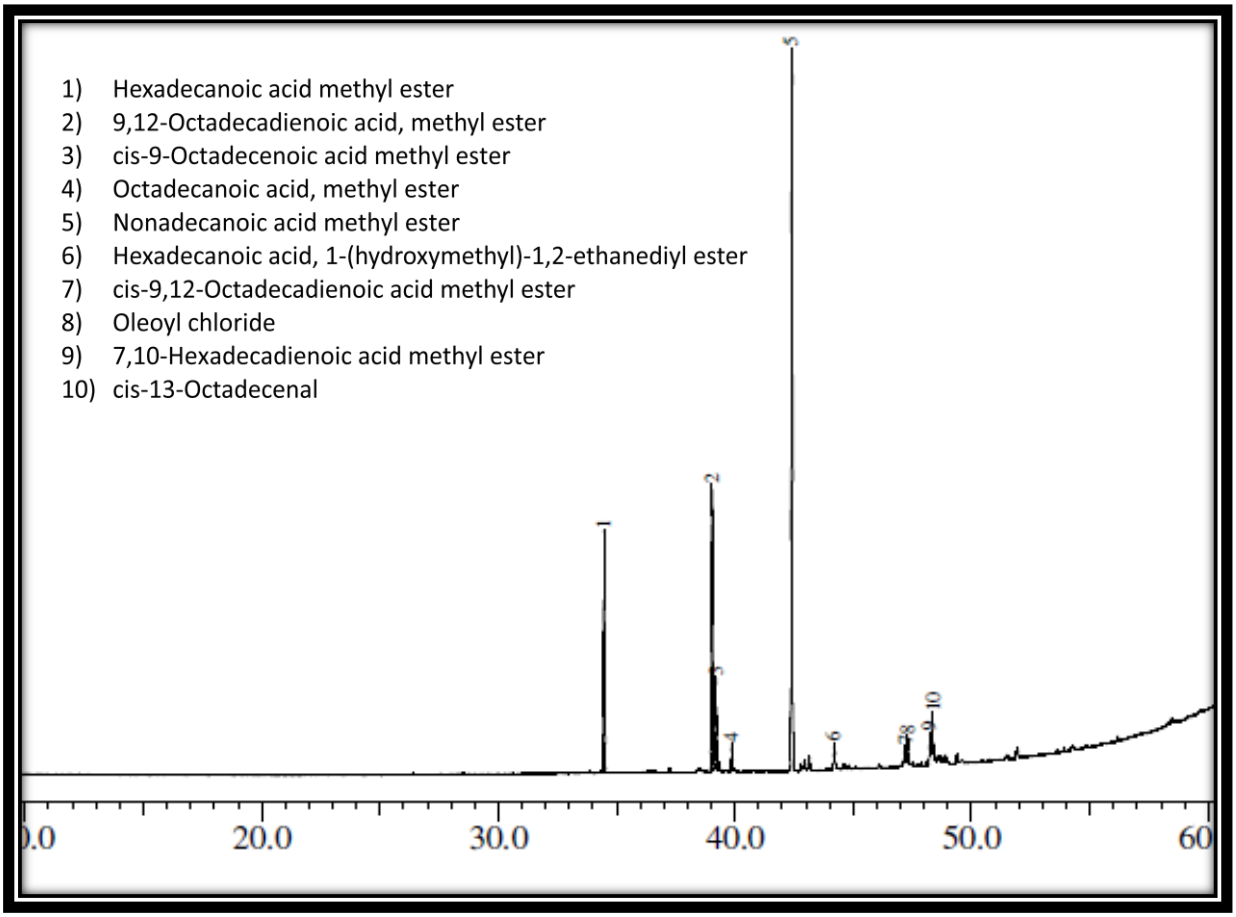

Figure 4.1: Chromatogram of *Castanopsis sp.*

Through the figure, there are a few peaks have been detected. We can see the fatty acids follow with their retention time shown. According to research of a few seed oils by Parashar *et al.* (2010), the pre-dominant fatty acid was linolenic acid (C18:3) followed by linoleic acid, oleic acid, stearic acid and palmitic acid. However, the most dominant fatty acid on this sample is 9,12-octadecadienoic acid (C18:2).