



Faculty of Resource Science and Technology

**Evaluation of Physicochemical Properties, Fatty Acid Composition and
Antioxidant Activity of Engkabang (*Shorea* spp.) Seed Oils**

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Master of Science

2022

Evaluation of Physicochemical Properties, Fatty Acid Profile and
Antioxidant Activity of Engkabang (*Shorea* spp.) Seed Oils

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A thesis submitted
In fulfillment of the requirements for the degree of Master of Science
(Chemistry)

Faculty of Resource Science and Technology
UNIVERSITI MALAYSIA SARAWAK
2022

DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Malaysia Sarawak. Except where due acknowledgements have been made, the work is that of the author alone. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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ACKNOWLEDGEMENT

Alhamdulillah, all praise Allah for his strength and blessing in completing this thesis. A special appreciation goes to my supervisor, Prof. Dr Zaini Bin Assim and co-supervisor, Prof. Dr Fasihuddin Badruddin Ahmad, for their supervision and constant support. Their invaluable constructive comments, suggestions and continuous push during experimental and thesis writing have contributed to the completion of this study. I would also like to thank the Malaysian Public Service Department for providing financial support through the Excellent Student Programme and the Sarawak Forestry Corporation for granting research permission (SFC.810-4/6/1(2021)-003). I want to thank Mr Benedict Samling, for assistance in operating the GC-MS instrument and Mrs Dayang Fatimawati for her help during laboratory work.

My sincere appreciation goes out to my Mamak Mrs. Saniah, Bapak Mr. Abdul Rakman, both of my aunts and my siblings for their unwavering support both financially and morally and their unceasing prayers hoping I can complete my studies. Additionally, I want to express my gratitude to my extraordinary friends: Umi and Fenie for frequently driving me to the faculty. Thanks to Lisa, Bella and Easter for making my time at Dahlia College enjoyable. Collecting mangosteen around the FSTS is hard to forget. Allysha is always willing to assist me, especially during the MCO, even though she had to drive and climb the back gate to deliver the necessities. Nisa, a wonderful housemate, is willing to go with me to the hospital despite her busy schedule, not to mention the night she had to wait until three in the morning in the emergency room and cook delicious hot meals for me. Finally, Nazmi every day never fails to encourage and treat me to food, especially during hectic lab work and writing. Without them, earning a Master's degree would be a difficult path for me.

ABSTRACT

The seeds from the *Shorea* genus are popular among Sarawak locals and known as ‘*Buah Engkabang*’. However, the information on *Shorea* seed and oil is still scarce. Thus, the objectives of this study were to collect the *Shorea* oilseeds (*Shorea macrophylla*, *Shorea palembanica*, *Shorea stenoptera* and *Shorea pinanga*) and then to gather their proximate, minerals and anti-nutritive factors data, as well as the effects of extraction methods and oil refining on the physical and chemical properties, fatty acid composition, antioxidant activity and total phenolic content (TPC). The proximate composition are significantly different ($p < 0.05$) between the seeds of *S. macrophylla* (moisture: 21.47%; ash: 0.67%; total lipid: 41.37%; total fibre: 15.67%; total carbohydrate: 79.44%), *S. palembanica* (moisture: 27.25%; ash: 1.98%; total lipid: 49.06%; total fibre: 7.72%; total carbohydrate: 82.37%), *S. stenoptera* (moisture: 16.06%; ash: 4.42%; total lipid: 38.80%; total fibre: 30.687%; total carbohydrate: 66.45%) and *S. pinanga* (moisture: 35.56%; ash: 2.23%; total lipid: 46.91%; total fibre: 8.09%; total carbohydrate: 79.64%). K and Zn are the predominant macro and microminerals, respectively. The evaluation of antinutrients disclosed low phytate but high oxalate content in *S. macrophylla* (0.29% and 1.91%), *S. palembanica* (0.29% and 2.43%), *S. stenoptera* (0.09% and 2.65%) and *S. pinanga* (0.06% and 3.61%). The mechanical extraction method is the choice of *Shorea* seed oil extraction because the mechanically extracted *S. palembanica* oil (*cis*-MUFAs: 34.49%; acid value: 3.47 mg NaOH/kg; peroxide value: 10.62 meq O₂/kg; TPC: 2.49 mg GAE/kg) is of higher quality than the Soxhlet extracted oil (*cis*-MUFAs: 34.23%; acid value: 8.00 mg NaOH/kg; peroxide value: 14.58 meq O₂/kg; TPC: 0.68 mg GAE/kg) ($p < 0.05$). The fatty acid composition in *Shorea* oils dominantly consists of stearic, oleic and palmitic acids similar to the fatty acid profile of

cocoa butter, indicating the potential of *Shorea* seed oils to be utilised in the chocolate industry. Point-Biserial Correlation (r_{pb}) was used to investigate the impact of oil refining on physical and chemical characteristics, fatty acid composition, antioxidant activity and TPC. A significant negative correlation between oil refining and acid and peroxide values ($r_{pb} = -1.00$, $p < 0.05$) demonstrates that oil refining effectively reduces acid and peroxide values, resulting in improved oil quality. The refining process has no significant impact on the fatty acid content according to oil analysis using Gas Chromatography-Mass Spectrometer (GC-MS) and Attenuated Total Reflectance-Fourier Transform Infrared Spectrometer (ATR-FTIR) ($p > 0.05$). However, there is an increase in essential fatty acid of linoleic acid in *S. macrophylla* oil from undetected to 1.01% and *S. pinanga* oil from 0.34% to 0.41% with the elimination of harmful fatty acids such as elaidic and erucic acids in *S. macrophylla* oil. Refining increased the antioxidant activity of *S. palembanica* (crude: 813.02 $\mu\text{g/mL}$; refined: 239.88 $\mu\text{g/mL}$) and *S. pinanga* (crude: 214.11 $\mu\text{g/mL}$; refined: 56.77 $\mu\text{g/mL}$) oils, whilst refining *S. macrophylla* (crude: 244.55 $\mu\text{g/mL}$; refined: 471.36 $\mu\text{g/mL}$) and *S. stenoptera* (crude: 152.22 $\mu\text{g/mL}$; refined: 249.49 $\mu\text{g/mL}$) oils reduced their antioxidant activity.

Keywords: *Shorea* oilseed, oil refining, fatty acids, gas chromatography-mass spectrometry, chemical properties.

Penilaian Sifat Fisikokimia, Profil Asid Lemak dan Aktiviti Antioksidan Minyak dari Biji Benih Engkabang (Shorea spp.)

ABSTRAK

Biji benih daripada genus Shorea popular di kalangan penduduk tempatan Sarawak dan dikenali sebagai 'Buah Engkabang'. Walau bagaimanapun, maklumat mengenai biji benih dan minyak Shorea masih terhad. Oleh itu, objektif kajian ini adalah untuk mengumpul biji minyak Shorea (Shorea macrophylla, Shorea palembanica, Shorea stenoptera dan Shorea pinanga) dan kemudian untuk menghimpunkan data proksimat, mineral dan faktor anti-pemakanan, serta kesan kaedah pengekstrakan dan penapisan minyak ke atas sifat fizikal dan kimia, komposisi asid lemak, aktiviti anti-oksidan dan jumlah kandungan fenolik (TPC). Komposisi proksimat antara biji benih S. macrophylla (kelembapan: 21.47%; abu: 0.67%; jumlah lipid: 41.37%; jumlah serat: 15.67%; jumlah karbohidrat: 79.44%), S. palembanica (kelembapan: 27.25%; abu: 1.98%; jumlah lipid: 49.06%; jumlah serat: 7.72%; jumlah karbohidrat: 82.37%), S. stenoptera (kelembapan: 16.06%; abu: 4.42%; jumlah lipid: 38.80%; jumlah serat: 30.687%; jumlah karbohidrat: 66.45%) dan S. pinanga (kelembapan: 35.56%; abu: 2.23%; jumlah lipid: 46.91%; jumlah serat: 8.09%; jumlah karbohidrat: 79.64%) adalah berbeza dengan ketara ($p < 0.05$). K dan Zn masing-masing adalah makro dan mikromineral yang dominan. Penilaian anti-pemakanan mendedahkan kandungan fitat rendah tetapi tinggi oksalat dalam S. macrophylla (0.29% dan 1.91%), S. palembanica (0.29% dan 2.43%), S. stenoptera (0.09% dan 2.65%) dan S. pinanga (0.06% dan 3.61%). Kaedah pengekstrakan mekanikal adalah pilihan pengekstrakan minyak biji benih Shorea kerana minyak S. palembanica yang diekstrak secara mekanikal (cis-MUFAs: 34.49%; nilai asid: 3.47 mg NaOH/kg; nilai peroksida: 10.62 meq O₂/kg; TPC: 2.49 mg

GAE/kg) adalah lebih berkualiti daripada minyak yang diekstrak secara Soxhlet (cis-MUFAs: 34.23%; nilai asid: 8.00 mg NaOH/kg; nilai peroksida: 14.58 meq O₂/kg; TPC: 0.68 mg GAE/kg) ($p < 0.05$). Komposisi asid lemak dalam minyak Shorea secara dominan terdiri daripada asid stearik, oleik dan palmitik mirip profil asid lemak mentega koko, menunjukkan potensi minyak biji benih Shorea boleh digunakan dalam industri coklat. Korelasi Takat Biserial (r_{pb}) digunakan untuk menyiasat kesan penapisan minyak ke atas sifat fizikal dan kimia, komposisi asid lemak, aktiviti antioksidan dan TPC. Korelasi negatif yang ketara antara penapisan minyak dan nilai asid dan peroksida ($r_{pb} = -1.00$, $p < 0.05$) menunjukkan penapisan minyak berkesan mengurangkan nilai asid dan peroksida yang menghasilkan kualiti minyak yang lebih baik. Proses penapisan tidak mempunyai kesan yang signifikan terhadap kandungan asid lemak berdasarkan analisis minyak menggunakan Kromatografi Gas- Spektrometri Jisim (GC-MS) dan Jumlah Pantulan yang Dilemahkan-Inframerah Transformasi Fourier (ATR-FTIR) ($p > 0.05$). Walau bagaimanapun, terdapat peningkatan dalam asid lemak penting asid linoleik dalam minyak S. macrophylla daripada tidak dapat dikesan kepada 1.01% dan minyak S. pinanga daripada 0.34% kepada 0.41% dengan penyingkiran asid lemak berbahaya seperti asid elaidik dan erusik dalam minyak S. macrophylla. Penapisan minyak meningkatkan aktiviti anti-oksidan S. palembanica (mentah: 813.02 µg/mL; ditapis: 239.88 µg/mL) dan S. pinanga (mentah: 214.11 µg/mL; ditapis: 56.77 µg/mL), manakala penapisan minyak biji benih S. macrophylla (mentah: 244.55 µg/m; ditapis: 471.36 µg/mL) dan S. stenoptera (mentah: 152.22 µg/mL; ditapis: 249.49 µg/mL) mengurangkan aktiviti anti-oksidannya.

Kata kunci: Biji benih minyak Shorea, penapisan minyak, asid lemak, kromatografi gas-spektrometri jisim, sifat kimia.

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LIST OF ABBREVIATIONS

ABTS	2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
AOCS	American Oil Chemists' Society
ATR-FTIR	Attenuated Total Reflectance–Fourier Transform Infrared
CAD	Coronary artery disease
CBE	Cocoa butter equivalence
CHD	Coronary heart disease
CVD	Cardiovascular disease
DHA	Docosahexaenoic acid
DPPH	2,2-diphenyl-1-picrylhydrazyl
EPA	Eicosapentaenoic acid
ECR	European Commission Regulation
FAME	Fatty acid methyl ester
FDA	Food and Drugs Administration
FFA	Free fatty acid
FNB	Food Nutrition Board
GC-MS	Gas chromatography-mass spectrometry
GRAS	Generally Recognized as Safe
HDL	High density lipoprotein
HDL-C	High density lipoprotein cholesterol
IUCN	International Union for Conservation of Nature

LDL-C	Low density lipoprotein cholesterol
MUFA	Monounsaturated fatty acid
MyBIS	Malaysian Biodiversity Information System
NTFP	Non-timber forest product
ORAC	Oxygen radical absorbance capacity
PUFA	Polyunsaturated fatty acid
ROS	Reactive oxygen species
SFA	Saturated fatty acid
SNI	Indonesian National Standard
TAG	Triacylglyceride
TPC	Total phenolic content
UFA	Unsaturated fatty acid
USFDA	United States Food and Drugs Administration
UV-Vis	Ultraviolet-visible spectroscopy
VOC	Volatile organic compound
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

1.1 General Introduction

The exponential growth of the human population and the consumption rate per capita raises one major crisis: food security. Vegetable oil, which can be extracted from oilseeds is considered one of the main components in our food. In addition, the shifting consumer preferences from animal fats to vegetable oils for consumption accelerates the demand for vegetable oils. Therefore, it is necessary to search for various oilseed sources that can yield edible oils.

The fatty acid composition of seed oils is critical when examining the purpose of oils since it impacts the nutritional and health risks associated with their use. Seed oil, which mainly comprises saturated fatty acids (SFAs) is stable at high temperatures and is suitable for deep frying. However, several studies have linked high SFA oils, particularly lauric acid to an increased risk of cardiovascular disease (CVD) (Bauer et al., 2015; Orsavova et al., 2015; Liu et al., 2017a). In contrast to SFAs, researchers suggested increasing the intake of cis-monounsaturated fatty acids (MUFAs) such as oleic acid and essential polyunsaturated fatty acids (PUFAs) such as linoleic and α -linolenic acids due to health benefits such as lower incidence of cancer (González-Fernández et al., 2017) and CVDs (Froyen & Burns-Whitmore, 2020). Nevertheless, the application of high unsaturated fatty acid (UFA) oils is not recommended due to their instability at high temperatures.

Edible vegetable oils are susceptible to deterioration through oxidation and microbial degradation resulting from improper extraction processes, handling and storage which cause rancidity and hence loss of nutritional values and flavour (Negash et al., 2019). Thus, it is vital to consider several key parameters such as acid, peroxide and iodine values when considering the oil for consumption, as they determine the oil's shelf-life quality and economic value. The acid value indicates the amount of free fatty acid (FFA) released from triacylglycerides (TAGs). Oils containing high acid value are unsuitable for consumption as they have an off-flavour and an off-odour. A high peroxide value in oil indicates a high content of oxidised products with toxic and carcinogenic properties (Cai et al., 2021). One of the possible reasons for oil instability is the lack of antioxidants. Antioxidants are compounds that can postpone, retard or inhibit oxidation processes. Antioxidants enhance oxidative stability in various ways, including scavenging free radicals and reactive oxygen species (ROS), deactivating peroxides, acting as metal chelators and quenching secondary oxidation products (Mishra et al., 2021). Therefore, higher antioxidant content in oils increases the economic value of the oils.

The increased oil production from oilseeds also increases the waste, which causes environmental problems and economic losses. Oilseed cakes are the major by-product of oil extraction and contain remaining fibre, carbohydrates, protein and oil. Despite the vast quantities of residual oilseed cakes and meals from oil extraction, many are underutilised, probably due to a lack of adequate information on the nutritional value of defatted seed oils (Duodu et al., 2018). Thus, the proximate composition is essential to determining oilseeds' nutritional value.

'Engkabang' fruit is a general term referring to oilseeds from the *Shorea* genus used by Sarawak locals. Seeds from *Shorea macrophylla* and *Shorea stenoptera* are traditionally

extracted for traditional delicacies such as flavouring in rice, butter and cooking oil. However, *Shorea* oilseed remains underutilised due to a lack of scientific approaches and extraction technologies. There are 54 species of *Shorea* in Borneo that have been listed as critical, endangered, or vulnerable in the International Union for Conservation of Nature (IUCN) Red List, where critical possesses the highest risk of extinction (Purwaningsih & Kintamani, 2018). The declination of *Shorea* species is due to their popularity as a source of timber which attracts loggers. The destruction of habitat due to land development for plantations is also the main reason for the declination of *Shorea* species. Both of these reasons are caused by a lack of awareness and limited information regarding other products by *Shorea*. The approach of forest products as NTFPs is better than timber products in achieving conservation goals, which could save the declination of *Shorea* species (Dao & Hölscher, 2018). Therefore, this study aims to provide scientific information that could be used as a platform for utilising *Shorea* oilseed as an NTFP product.

1.2 Problem Statement

Shorea oilseeds have been collected and extracted traditionally by natives of Sarawak as a flavouring in rice and cooking oil and have the potential to be utilised in the food industry. However, there is scarce information regarding scientific research and extraction technologies on *Shorea* oilseed in Sarawak. Although one study has outlined the proximate data of *Shorea* oilseeds from Sarawak (Nesaretnam & Ali, 1992), the mineral composition and antinutritive factors in *Shorea* seeds have not been recorded. Analysing mineral composition and antinutritive factors is essential when considering the oilseed for consumption.

Maharani et al. (2016) described the traditional *Shorea* oil extraction method named ‘*Apit*’ as tedious, laborious and low yield. Therefore, a study on the new method which is high yield and less strenuous is needed. Oil extraction methods affect oil yield and influence the concentration of minor lipids (Cai et al., 2021), as well as the fatty acid profile, tocopherol content and antioxidant activity of the oil (Dong et al., 2017). The Soxhlet extraction of *Shorea* seed has been performed in previous studies (Nesaretnam & Ali, 1992; Shashi et al., 2016). However, there is no literature on oil extraction using mechanical extraction to extract *Shorea* oil has been delineated. In addition, the effect of extraction methods on chemical properties, fatty acid composition, antioxidant activity and total phenolic content (TPC) is limited.

Oil refining aims to eliminate undesirable minor molecules such as phospholipids, FFAs, pigments, metal contaminants and moisture, which affect the quality and stability of oil over time (Mishra et al., 2021). Hidayat et al. (2019) and Darmawan et al. (2020) disclosed a significant reduction in acid and peroxide value of refined *S. stenoptera* oils from Indonesia. Still, the relationship between oil refining with physicochemical properties, fatty acid composition, antioxidant activity and TPC has not been recorded.

1.3 Objectives of the Study

The objectives of this study were:

- i. to collect the oilseeds of *Shorea* species and to investigate nutritional and anti-nutritional factors of *Shorea* oilseeds
- ii. to determine the choice of extraction methods for *Shorea* seed oil

- iii. to compare physicochemical properties, fatty acid composition and antioxidant activity of crude and refined oil
- iv. to statistically assess the relationship between oil refining towards physicochemical properties and antioxidant activity

1.4 Chapter Summary

This chapter provides a general overview of the importance and parameters for determining oil quality and the utilisation of oilseed extraction by-products. Problem statements have been provided to address the knowledge gap from the previous studies regarding *Shorea* seeds.

CHAPTER 2

LITERATURE REVIEW

2.1 Botanical Aspects of Dipterocarpaceae

There are 308,312 vascular plant species described which consist of angiosperms (95.80%), ferns (3.43%), lycopods (0.42%), and gymnosperms (0.35%) (Christenhusz & Byng, 2016). Angiosperms from the Dipterocarpaceae family are angiosperms from the Malvales order. Angiosperm is a flowering plant which has extensive uses in human existence. All crops plants such as wheat, rice and corn are included as flowering plants, forming the basic food supply as staple foods for the human population. Angiosperms are classified into monocotyledons (one cotyledon) and dicotyledons (more than two cotyledons).

The family of Dipterocarpaceae is named after one of its genera, *Dipterocarpus*, which means two-winged fruit. The Dipterocarpaceae family includes 16 genera and 695 species (Christenhusz & Byng, 2016). The family members are primarily large emergent trees with heights that can reach up to 40 to 70 meters. The plant grows in red or yellow podzolic soil types with an altitude lower than 1300 metres above sea level. Dipterocarpaceae is found worldwide, from South America to Africa, Seychelles, Sri Lanka, the Philippines, India, China, Thailand, Indonesia and Malaysia, with the most significant proportion in Western Malaysia.

Borneo Island is diversified with Dipterocarpaceae, which account for more than 300 species. The flora of Borneo has close affinities with Malaya as it was connected during the

Pleistocene era by the Sunda shelf (Smythies, 1958). The tropical rainforest in the Western Malaysia region is dominated by the Dipterocarpaceae family, which are essential trees in the tropics and have their vegetation zone (Ashton, 1964). Dipterocarps occupy many habitats such as coastal, inland, riverine, swampy and dry land areas (Symington, 1943). Table 2.1 shows the altitudinal zonation of dipterocarps in Peninsular Malaysia. This altitudinal zonation, however, does not apply to Borneo since habitat zones differ based on height and natural barriers such as big rivers and watersheds. Compared to peat swamp, limestone and riverine fringe, a greater variety of species can be found in the dry area of the freshwater marsh (Symington, 1943). As a result, the northeast of Kalimantan, Sabah and Sarawak have a far greater species diversity than the rest of Kalimantan (Appanah & Turnbull, 1998).

Table 2.1: Ranges of Dipterocarps Habitat in Peninsular Malaysia (Appanah and Turnbull, 1998)

0-300 m	Low undulating Dipterocarp Forest
300-750 m	Hill Dipterocarp Forest
750-1200 m	Upper Dipterocarp Forest

Dipterocarps are the most common species in the international tropical timber trade, where their wood is highly prized and plays an integral part in the economies of most Southeast Asian countries. Borneo has been put under more significant pressure as the demand for timber increases, especially for *Shorea*. *Shorea* is known as Red Meranti, one of the major export products of Sarawak, Sabah and Kalimantan. However, due to deforestation and timber extraction, the trees are now facing scarcity (Blicher-Mathiesen, 1994). The

dipterocarp forest also provides a variety of forest products that have become a source of income for the locals such as Damar and Camphor, oilseed kernels and traditional medicines.

2.2 Botanical aspects of *Shorea*

Shorea, often known as meranti, is the most widespread Dipterocarpaceae genus in Malaysia and Indonesia. *Shorea* has buttressed roots, 5-winged fruit (two rudimentary and three big wings), and a dome-shaped canopy. *Shorea* grows in a variety of habitats, according to Purwaningsih and Kintamani (2018), including lowland dipterocarp forest, peat swamp forest, hill forest, heath forest, swamp forest, riparian forest, coastal forest and limestone forest, with varied characteristics for each species.

Shorea flowers and seeds at irregular intervals, with mass fruiting known as the "great season," occurring every three to four years (Ashton, 1964). The irregularity in fruiting, fast germination and infestation of seed predators cause significant problems in fruit production, such as price fluctuations over the years and the collection of seeds is harder to plan. Borneo natives near their settlement have planted *Shorea* but most of the sources are still obtained from the forest. *Shorea* trees have an abundance of fruits but only a few can survive and germinate as they are recalcitrant and contain fats favoured by wild boars (Purwaningsih & Kintamani, 2018). Meranti trees grow slowly and their wood is commonly used for building materials and furnishings. The drastic reduction of tree population numbers in this genus is due to the continuous exploitation for its timber and takes a long time to recover. Several species in this genus are classified as endangered on the IUCN Red List (Red Data Book) (Ashton, 1998). Besides its timber, this genus is also exploited for its oil,

resin and camphor which have an economic impact on the local community (Shiva & Jantan, 1998).

Approximately 160 species of *Shorea* have been recorded in Malaysia, where 102 species (81.6%) were explicitly recorded in Borneo. *Shorea* population have been declined rapidly due to illegal logging and land development for plantations. *Shorea* habitat is not suitable for mountain forests at altitudes of 1500 m above sea level (Purwaningsih & Kintamani, 2018). The IUCN red list includes approximately 54 *Shorea* species that are critical, endangered and vulnerable (Purwaningsih & Kintamani, 2018). The list determined 40 species in critical, 12 in endanger and 2 in vulnerable criteria. Species within critical criteria possess a high risk of extinction. The scarcity level is established by the limited range of species and their endemism. According to Purwaningsih and Kintamani (2018), *Shorea* has the most endemic species compared to other genera such as *Dipterocarps*, *Vatica*, *Hopea* and *Dryobalanops*.

Wong (1988) described Borneo illepe is referred to the oil-bearing nuts of some *Shorea* species. Illepe known as engkabang, abang or kawang in Sarawak, while sengkawang or sinkawang in Kalimantan. The Iban community calls these illepe nuts based on their size; engkabang is for a bigger size, while lelanggai is for smaller ones. Illepe nuts have been one of the most important export products for Sarawak between 1953 to 1962. The highest export recorded in 1959 accounts for 22000 because of the increase in cocoa butter production. A total of 10 sub-species of *Shorea* have been collected in the forest, of which approximately 90% of the collected *Shorea* seeds are *S. macrophylla* and *S. stenoptera*. The selection of species largely depends on the ease of collecting, oil content and size of the individual seed (Connell, 1974).

The Sarawak Government had protected Engkabang since 1918, when Sir James Brooke prohibited the cutting or damaging of engkabang (Smythies, 1958). The protection was further strengthened due to the listing under the Sarawak Wildlife Protection Ordinance 1998 where the collection and cultivation of species of *Shorea* were prohibited except under and following the Protection Ordinance 1998 except for the traditional collection of engkabang nuts as NTFP (Coolen, 2013). Borneo has approximately 200 species of *Shorea* and most of these species bear nuts, but only a few species have sufficient lipid content for commercialisation.

2.2.1 *Shorea macrophylla* de Vriese

S. macrophylla was initially named *S. gysbertsiana* Burck and then changed into *S. macrophylla* (de Vriese) in 1962 (Randi et al., 2019b). *S. macrophylla* is known as Engkabang Jantong or Kawang Jantung in Sarawak which refers to the heart shape of the winged nuts. This species also known as Tengkawang Majan or Melebekan in Indonesia (Rudjiman & Adriyanti, 2002).

S. macrophylla is an endemic species to Borneo and is frequently found in the Northern and central parts of Borneo and lesser in the East part (Coolen, 2013). This species is native to Sabah and Sarawak (Malaysia), Kalimantan (Indonesia) and Brunei (Randi et al., 2019b). This species is grown abundantly on deep clay alluvium-type soil and riverbank (Rudjiman & Adriyanti, 2002). It is usually planted near settlements such as long houses and traditional villages for its nuts. The timber logging and forest conversion to oil palm plantations outside Borneo's protected areas have threatened this species. However, *S.*

macrophylla is only listed as Least Concern on IUCN Red List as the decline rate is not suspected to reach the threshold amount to be included in the threatened category.

Randi et al. (2019b) reported that the tree of *S. macrophylla* is valuable for local communities as timber and oil sources. *S. macrophylla* is the most important species of Shorea for illepe nut export as it contributes to 85-90% of the production (Smythies, 1958; Blicher-Mathiesen, 1994). *S. macrophylla* produces a huge and good quality nut, heart rot resistance timber and the collection of nuts is more accessible as the species is riverine (Coolen, 2013).

Rudjiman and Adriyanti (2002) described the tree of *S. macrophylla* would grow up to 50 m tall with a crown large, oblong or hemispherical shape. The leaf blade is oval with 19-48 cm x 6-21.5 cm in size and coriaceous. Approximately 13 to 18 pairs of secondary veins on each side arching near the margin as shown in Figure 2.1. The flower has pale pink petals with 15 stamens. The fruit has three long and two shorter wings. Longer wing is approximately 6.3 to 10 cm long and 3 to 4 cm wide; shorter wing is approximately 4 to 7.3 cm long and 1.8 to 2.1 cm wide. The nut size is approximately 5.5 to 6 cm long and 2.9 to 3.2 cm wide.

(a)



(b)



(c)



Figure 2.1: (a) Upper Leaf, (b) Lower Leaf, and (c) *S. macrophylla* Fruit

2.2.2 *Shorea stenoptera* Burck

Shorea stenoptera is locally known in Sarawak as Engkabang Rusa and this name refers to the size of deer's testicle with the size of *S. stenoptera* nuts (Coolen, 2013). This species is also known as Engkabang Kerangas by the Iban as it is the only *Shorea* species that grows in the heath forest. *S. stenoptera* is native to Sarawak (Malaysia) and Kalimantan Indonesia (see Figure 2.2) where it is found frequently on groundwater podzols in heath forests, terraces and plateaus and on periodically flooded sandy alluvium (Coolen, 2013). *S. stenoptera* is known as Tengkawang Tajau or Tenkawang Tungkul in West Kalimantan, Indonesia (Rudjiman & Adriyanti, 2002).

S. stenoptera has been planted for its nuts as sources of illepe butter and further developed as ingredients in cosmetics and substitutes for cocoa butter in chocolate production (Randi et al., 2019a). The fat is easily absorbed by the skin which causes the butter of *S. stenoptera* to be favoured in cosmetic industries. The timber of *S. stenoptera* is used for several purposes such as panel and joint, light carpentry, boxes and crates but the timber quality of this species is lower compared to the timbers from other *Shorea* species. Because of habitat destruction and timber extraction, this species is classed as Near Threatened on the IUCN Red List. The loss of habitat is caused by increased agriculture, which includes shifting cultivation and industrial oil palm plantations (Randi et al., 2019a).

Botanical description of *S. stenoptera* has been given in detail by (Rudjiman & Adriyanti, 2002). *S. stenoptera* is a crown conical-shaped tree with a height can reach up to 50 m tall. The leaf is oval 21 cm to 44 cm long and 9.5 cm to 25.5 cm wide. The leaf has 12-19 pairs of prominent secondary veins arching near the margin (see Figure 2.3a), while the tertiary veins are distinct, and perpendicular. *S. stenoptera* has small flowers with pink or red petals. The nut is surrounded by three longer and two shorter wings as shown in Figure 2.3b. The measurement of a longer wing is approximately 3.5 cm to 7.5 cm long and 1.5 cm to 2.5 cm wide. The shorter wing is approximately 2 cm to 5.5 cm long and 0.5 cm to 1 cm wide. The nut size is approximately 4 cm to 6 cm long and 3 cm to 2.5 cm wide.

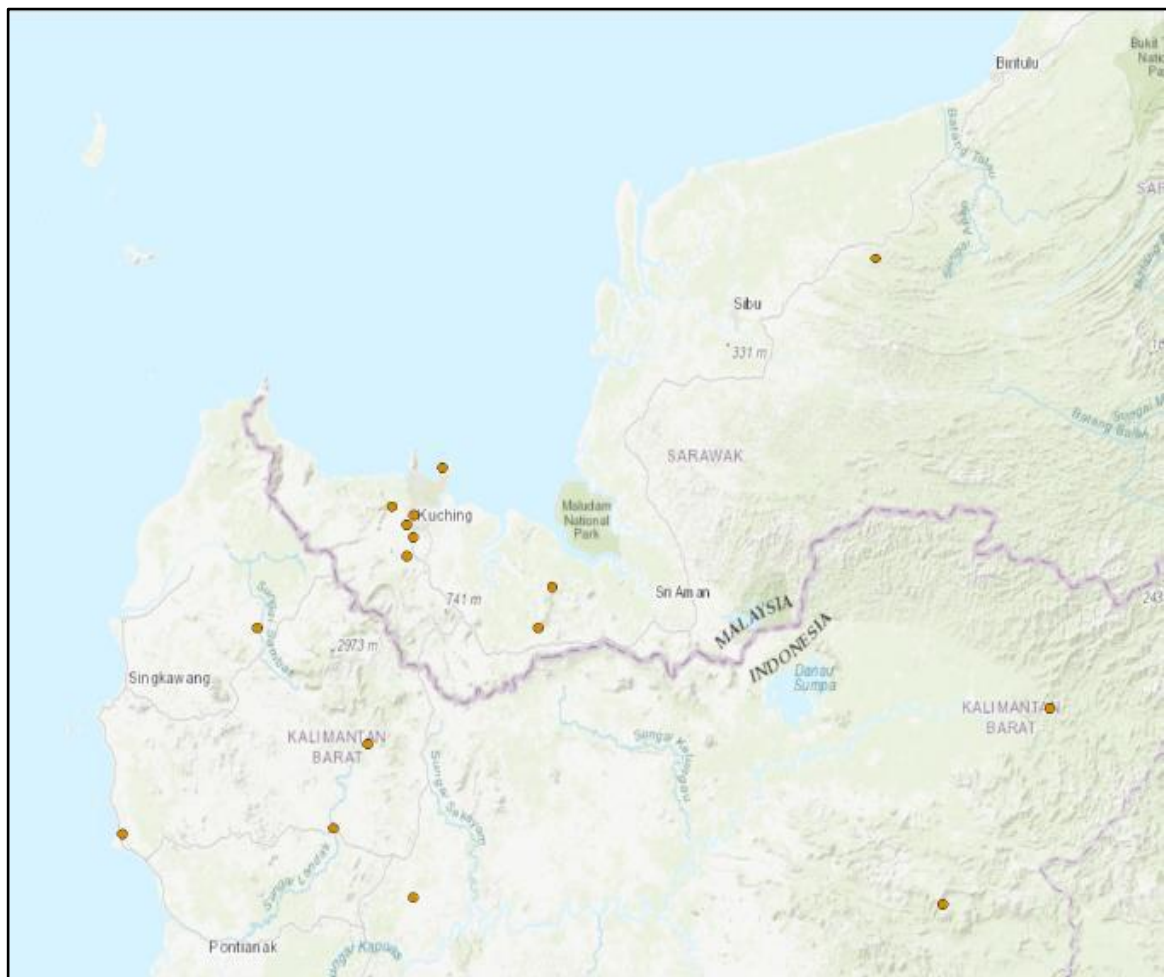
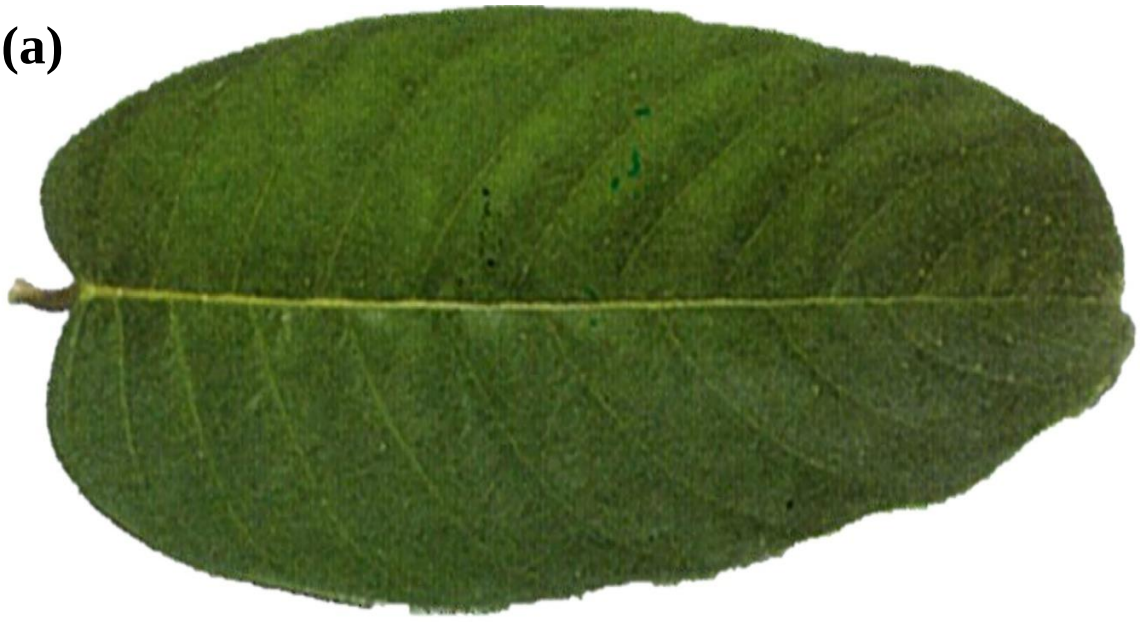


Figure 2.2: Distribution of *S. stenoptera* in Sarawak and Kalimantan (Indonesia) (Randi et al., 2019a)

(a)



(b)



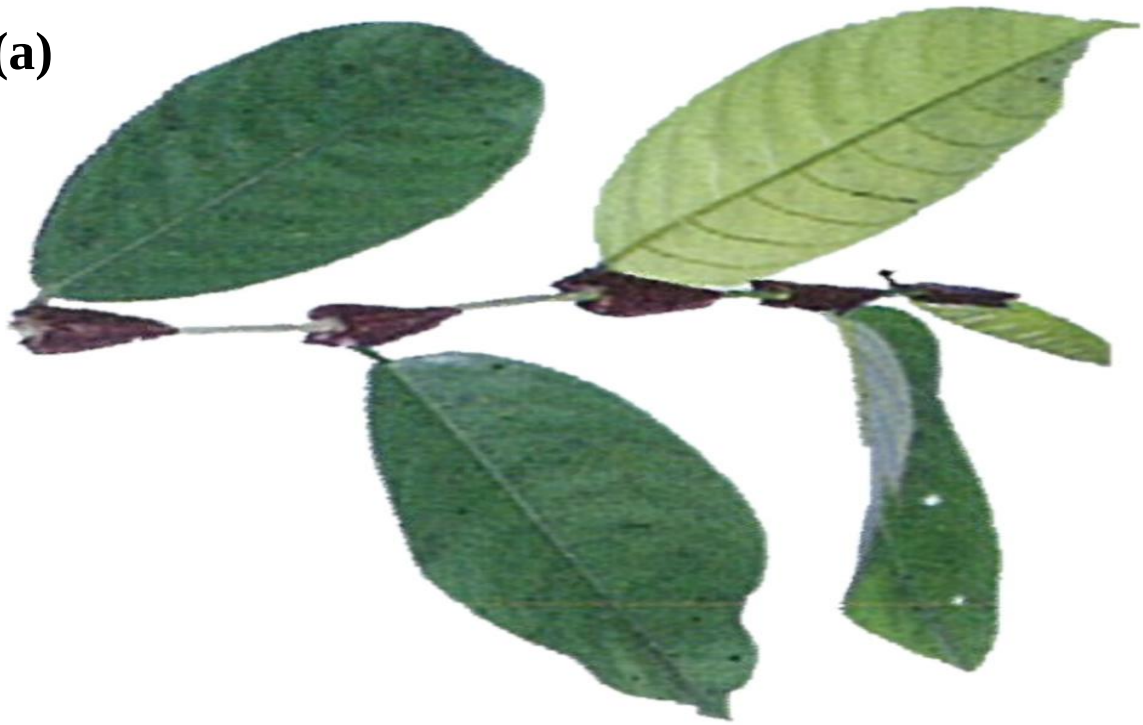
Figure 2.3: (a) Leaf and (b) Fruit of *S. stenoptera*

2.2.3 *Shorea splendida* (De Vriese) Ashton

Shorea splendida was originally named *Shorea martiniana* Scheff and was also known as *Hopea splendida* de Vriese (Browne, 1955; Randi et al., 2019c). This species is known locally in Sarawak as Engkabang Bintang which refers to the spherical shape of the nuts. In contrast, *S. splendida* is known as Tengkawang Rambai or Tengkawang Goncang in Kalimantan, Indonesia (Rudjiman & Adriyanti, 2002). *S. splendida* is endemic species to Borneo (Randi et al., 2019c). This species grows well in moist deep clay or loam alluvial soils and is widely distributed in the West Sarawak and West Kalimantan of Borneo Island. *S. splendida* is closely related to *S. macrophylla* as both species share similar habitat (Browne, 1955). *S. splendida* is considered an essential source of illepe nuts and oil as it has large nuts, but the tree has not been utilised as timber due to the protection by traditional rights (Smythies, 1958; Randi et al., 2019c). Randi et al. (2019c) considered *S. splendida* as endangered species because of habitat conversion for oil palm and tree plantation and several anthropogenic activities such as settlement and industry.

This species is a medium-sized tree that can reach a height of 40 metres and has a dense, rectangular crown (Rudjiman & Adriyanti, 2002). The leaf is round with 9.5 cm to 28.5 cm in length and 3.5 cm to 13 cm wide as shown in Figure 2.4a and the lower surface of the leaf is shiny. There are 13 pairs of secondary vein arching near the margin while tertiary nerves are distinct and perpendicular. The fruit part consists of nuts covered with three longer wings and two shorter wings as shown in Figure 2.4b. The size of the nut ranges from 3.7 cm to 5.5 cm long and 2.1 cm to 3.0 cm wide; longer wings range from 5 cm to 7.5 cm long with 1.3 cm to 2.3 cm wide; shorter wings range from 3.8 cm to 6.5 cm long with 0.7 cm to 1.2 cm wide.

(a)



(b)



Figure 2.4: (a) Leaves and (b) Fruit of *S. splendida*

2.2.4 *Shorea pinanga* Scheff

The common name for *S. pinanga* is light red meranti which is known as Engkabang Langai Bukit in Sarawak, while local people of West Kalimantan Indonesia called Tengkawang Rambai, Tengkawang Telur or Tengkawang Bunga (Rudjiman and Adriyanti, 2002). This species grows in lowland mixed dipterocarp forest on ridges and low hills (Ashton, 2004). Robiansyah et al. (2019) stated that *S. pinanga* is endemic and well-distributed across Sabah, Sarawak, Kalimantan and Brunei. The wood of *S. pinanga* can be exploited as a construction material due to its low percentage of heart rot (Ashton, 2004; Robiansyah et al., 2019). The medium size nut is considered an essential source of vegetable oil in Sarawak. The species can produce flowers and fruits after six years of plantation. The reduction of the *S. splendida* population occurred because of habitat loss and the construction of new roads for the logging industry and agriculture; however, the species is listed as Least Concern on IUCN Red List because this species remained abundant (Robiansyah et al., 2019).

Rudjiman and Adriyanti (2002) described *S. pinanga* as medium-sized to large trees up to 60 m tall. The leaf petiole is grey-brown coloured and 1.1 cm to 2.2 cm long. The leaf is round 11.5 cm to 21 cm long and 4.9 cm to 9.1 cm wide (see Figure 2.5a). The upper surface of the leaf is glabrous with a yellow-brown indumentum on the midrib. The lower surface of the leaf is dull or shiny or with sparse brown short indumentum on the surface and vein. The secondary vein has 10 to 16 pairs on each side arching along the length. The intermediate veins might present while tertiary nerves are conspicuous and perpendicular. The fruit part of this species consists of nuts with an average size ranging from 3.4 cm to 5.2 cm long and 2.5 to 2.8 cm wide covered with three long and two shorter wings as shown in

Figure 2.5b. The long wing ranges from 20 cm to 28 cm long with 2.5 cm to 3.5 cm wide, while the short wing is from 8 cm to 17 cm with 0.8 to 1.4 wide.

(a)



(b)



Figure 2.5: (a) Leaf and (b) Fruit of *S. pinanga*

2.2.5 *Shorea mecistopteryx* Ridl

Malaysian Biodiversity Information System (MyBIS) (2020a) stated that *S. mecistopteryx* Ridl. synonym to *Sophora chrysophylla* which is known locally as Engkabang Burung, Engkabang Larai and Kawang Tikus in Sarawak, while in Sabah is known as kawang burung. *S. mecistopteryx* is known as Tengawang Layar in Kalimantan Barat, Indonesia (Rudjiman & Adriyanti, 2002). Kusumadewi et al. (2019) noted the species is native to all states in Borneo as it is widely spread and relatively common except in North Kalimantan, Indonesia as shown in Figure 2.6. Approximately 80% of species reduction in Kalimantan, Indonesia is due to the conversion of lowland habitats to oil palm plantations, other agricultural purposes, construction and extension of logging roads (Gaveau et al., 2016).

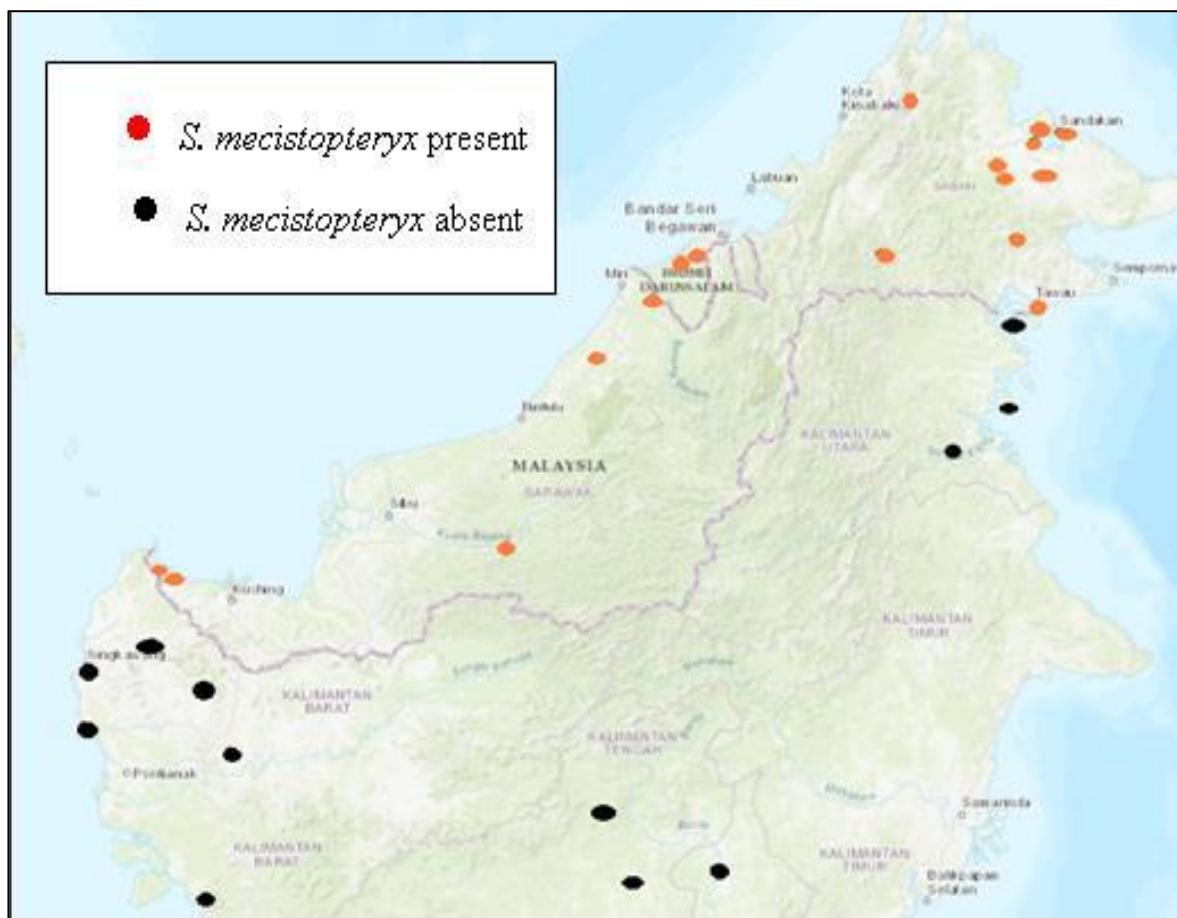
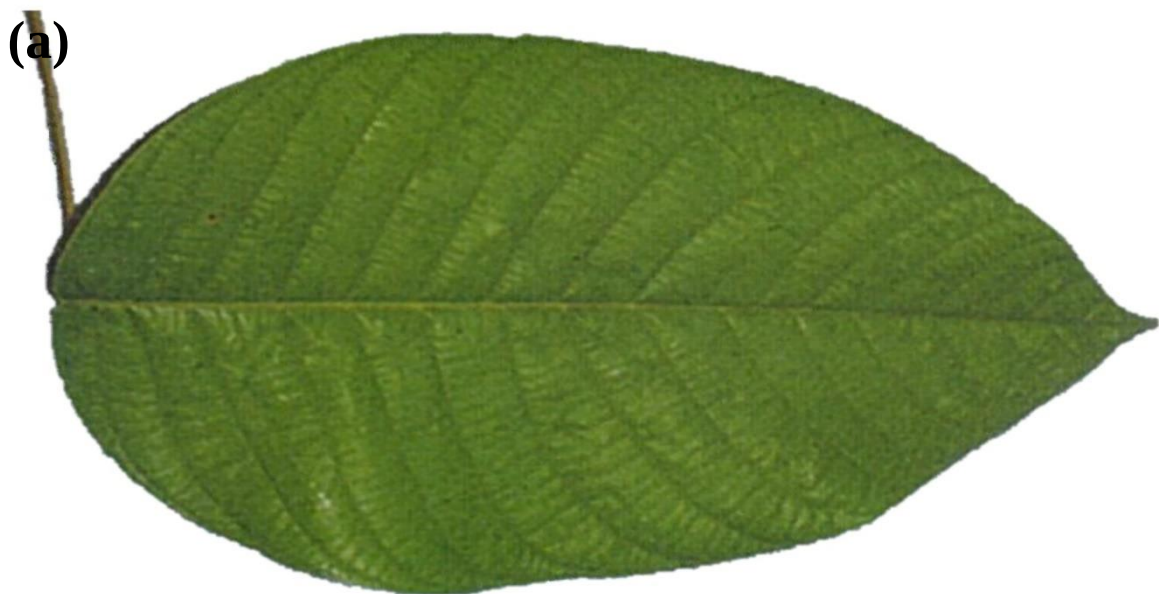


Figure 2.6: Distribution of *S. mecistopteryx* in Borneo (Kusumadewi et al., 2019)

Kusumadewi et al. (2019) described that *S. mecistopteryx* grows on igneous rocks in lowland, mixed dipterocarp forests and is mainly restricted to the coastal area. This species is exploited for its timber, damar and edible nuts as a source for illipe nuts (Sosef, 2017; Kusumadewi et al., 2019). Rudjiman and Adriyanti (2002) described *S. mecistopteryx* as a medium-sized to large tree which can reach up to 60 m in height with irregular crowns and brownish green-coloured branches. The leaf blade is an oblong shape 14 cm to 40 cm long and 6.4 cm to 19.5 cm wide. The upper surface of the leaf is smooth with sparse or dense brown indumentum on the midrib as shown in Figure 2.7a. The leaf's lower surface is dull and light yellow-brown has 16 to 23 pairs of secondary veins on each side arching near the margin while tertiary veins are conspicuous and perpendicular. The fruit part has 15 stamens where the nut is incorporated with three long and two shorter wings as shown in Figure 2.7b. The nut size is 3.8 cm to 4.4 cm long and 2.5 cm to 2.8 cm wide. The longer wing is 15 cm to 31 cm long and 3 cm to 4 cm, while the shorter wing is 7 cm to 12 cm long and 0.9 cm to 1.5 cm wide.



(b)



Figure 2.7: (a) Leaf and (b) Fruit of *S. mecistopteryx*

2.2.6 *Shorea palembanica* Miq

Shorea palembanica is known locally as Meranti Tengkawang Air in Peninsular Malaysia, Engkabang Asu or Kawang Asu by Sarawak's local and Tengkawang Majan in Indonesia (Rudjiman & Adriyanti, 2002; MyBIS, 2020b). 'Air' means water referring to the type of habitat of the *S. palembanica* which is along banks of streams, swamp areas, silty floodplains and banks of sluggish rivers (Chua et al., 2010). 'Asu' means a dog which refers to the size of the *Shorea* nuts in comparison to the dog's testicles.

Chua et al. (2010) reported *S. palembanica* is distributed in Malaysia, Sumatera and Kalimantan, Indonesia. This species can be found in Johor, Kelantan, Pahang, Perak and Terengganu of Peninsular Malaysia as shown in Figure 2.8. *S. palembanica* is found

abundant in Sabah such as Beluran, Pitas, Sandakan, Semporna and Tawau, and also Sarawak such as Belaga, Bintulu, Kapit, Kuching, Lubok Antu, Lundu, Miri, Samarahan, Sibuan, Simunjan and Tatau as shown in Figure 2.9. *S. palembanica* is a vital source of illepe nut and light red Meranti timber. However, this species has been categorised as vulnerable due to land conversion resulting in population reduction.

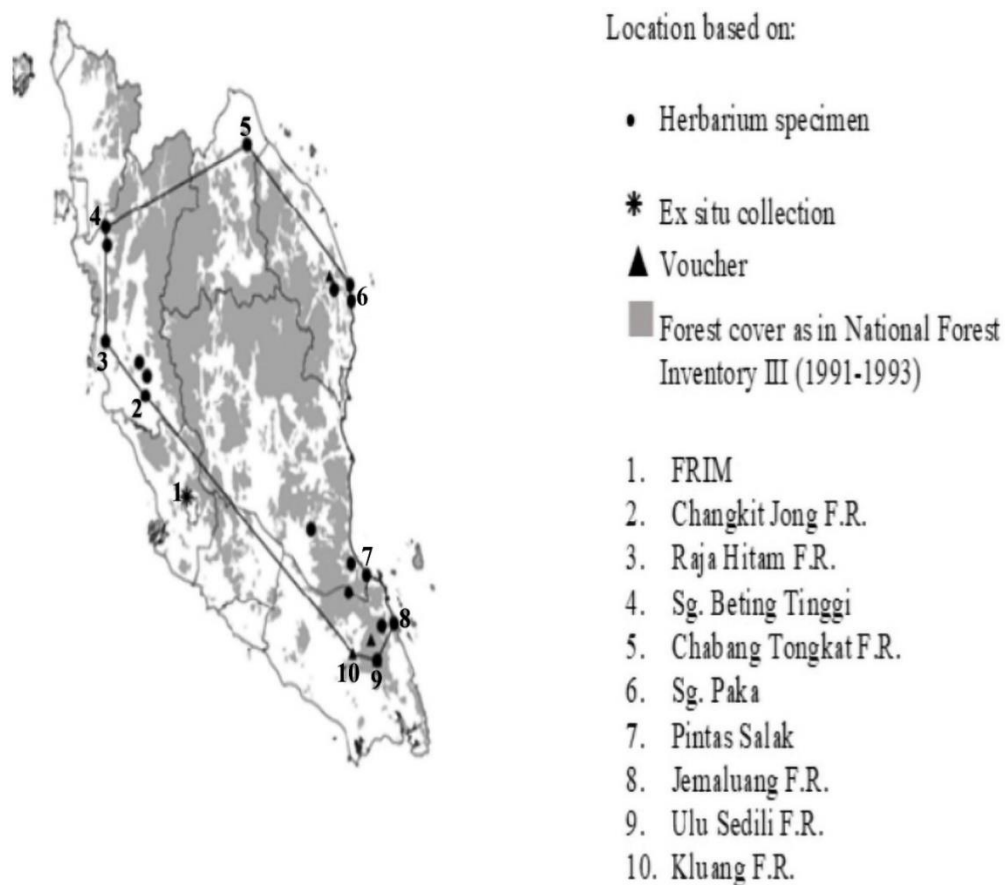


Figure 2.8: Distribution of *S. palembanica* in Peninsular Malaysia (Chua et al., 2010)

S. palembanica tree can grow up to 45 m tall with a large, dark green crown and the buttresses are twisted or gnarled (SimeDarby, 2017). The bole is greyish or dark coloured, fissured to scaly and sometimes with star-shaped lenticels. The flowers have yellow petals with 15 stamens. The nut size ranges from 2.2 cm to 2.4 cm long and 1.7 cm to 2.2 cm wide

covered with three longer wings which are 2.5 cm to 5 cm long and 0.6 cm to 0.7 cm wide and two shorter wings which are 1.7 cm to 3 cm long and 0.3 cm wide (Rudjiman & Adriyanti, 2002).

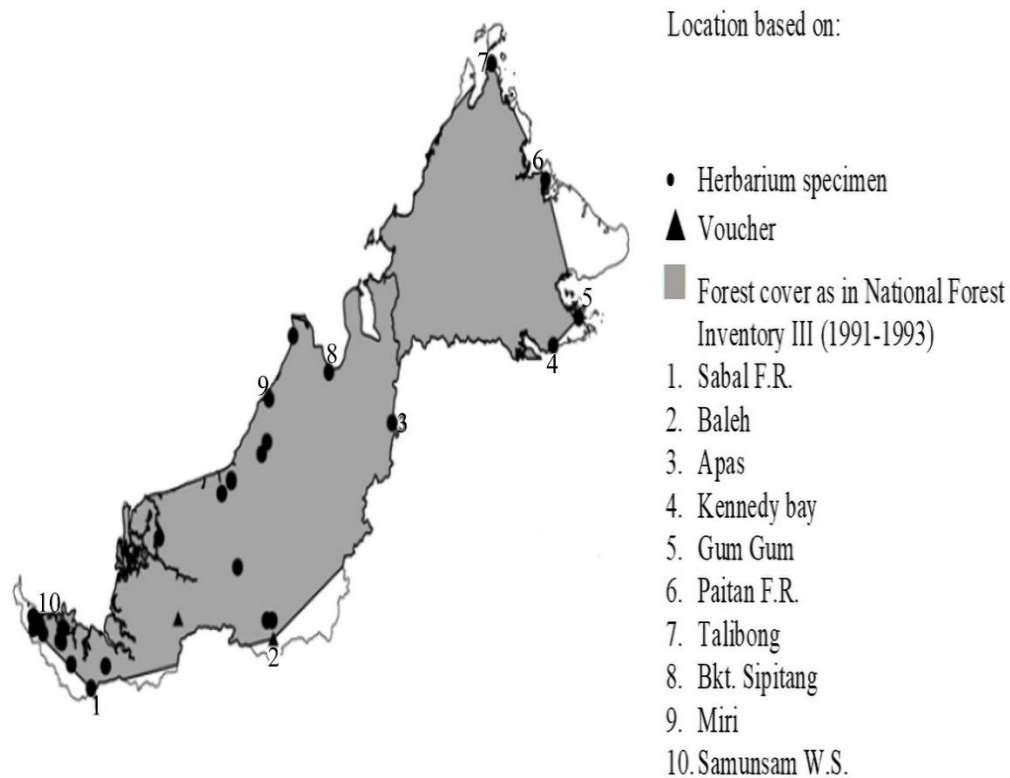


Figure 2.9: Distribution of *S. palembanica* in Sabah and Sarawak (Chua et al., 2010)

2.3 Collection of *Shorea* oilseed

Local inhabitants originally collected *Shorea seed* in Sarawak with minimal government influence as one of their sources of income (Coolen, 2013)(Coolen, 2013). However, the government influenced the collection of *Shorea* seeds between 1908 to 1998 due to an increase in demand that impacted Sarawak's economy (Coolen, 2013). The plantation of *Shorea* in forest orchards was usually together with durian (*Durio zibenthinus*) and rambutan (*Nephelium lappaceum*). Still, the farmer did not give much attention to the

seedlings after the plantation until the tree started to yield fruits (Coolen, 2013). The longhouses folk in Sarawak collected matured *Shorea* fruits during the windy weather. The fruits of *Shorea* grown near riverbank are usually collected in the river water by using the fishing net, temporary fence or bamboo to trap the fallen fruits (Coolen, 2013). The collectors also used boat engines to access remote areas (Anderson, 1975). The seeds of *Shorea* grown far from rivers are collected from the forest floors.

2.4 Traditional Method of Oil Processing from *Shorea* Oilseed

Traditional methods of processing *Shorea* oilseed are varied according to their place. Sarawak (Malaysia), Kalimantan (Indonesia) and India have their conventional processing methods of the oilseed from *Shorea*.

2.4.1 Borneo (Sarawak, Malaysia and Kalimantan, Indonesia)

Two traditional methods are usually used to process *Shorea* oilseed in Sarawak (Coolen, 2013). The immersion method is the most preferred because it produces a high percentage of oil. The seeds are packed into bamboo crates and immersed in rivers or streams for several weeks. This immersion step kills insects and facilitates the deshelling action as the nuts will germinate and burst out of their shells (Anderson, 1975). Immersion for longer than 30 to 40 days will cause the seeds to decay. The seeds are then sundried for five to seven days (four to six hours each day) after the immersion step, performed on an external or elevated platform of the longhouse that produces black nuts. The seeds are then shelled and broken into halves.

The second method is the kiln drying method (Anderson, 1975). The seeds are placed on a rattan mat on top of a constructed platform to roast the seeds with a slow-burning fire until dry which takes up to 36 hours. This method produced the least preferred brown nuts. The seeds are flipped over and over while roasting to ensure equal drying. A roasting temperature of less than 70°C is required, as a higher temperature will reduce the quality of the oil. The process requires constant roasting for 36 hours. A sun-drying step is then conducted after the deshelling process is conducted.

Coolen (2013) described oil extraction from *Shorea* seeds in Sarawak after drying the dried seeds, which are pounded into powder and fried for 30 minutes. The hot fried powder is poured into a rattan bag between two wooden planks (wooden press) to squeeze out the oil. The oil is then poured into a bamboo tube. The Iban community in Ulu Krian, Saratok, Sarawak has their own *Shorea* oil extraction method (Utusan.Online, 2010). The flesh of sundried *Shorea* seeds is boiled until the texture becomes softer which usually takes up to 18 hours. The flesh is then crushed using a wooden mortar. The crushed flesh was placed into the cotton bag and squeezed until the oil came out.

According to Maharani et al. (2016), the ‘*Apit*’ method was used in the traditional extraction of *Shorea* oil in Kalimantan, Indonesia, where it varies depending on its origin. *Shorea* seeds were dried and ground into powder, then placed in a sack and steamed for an hour. The oil is extracted using a wooden plank known as an *Apit*. *Shorea* oil was extracted and stored in bamboo. *Apit* produced 25 to 30% oil. The melting point and free fatty acid content of oil produced in various districts differ significantly, suggesting that each *Apit* method has a distinct fat grade.

2.4.2 India

In India, the seed of *S. robusta* is used as a source of fat, and the plant is known as Sal or illipe (Talbot, 2015). *S. robusta* covers 1/8 of India's total forest area, playing a crucial part in the economics of various Indian states (Orissa, Jharkhand, and Madhya Pradesh), where around 30 million forest inhabitants rely on Sal seeds as a source of income. Furthermore, Buddhists and Hindus regard the *S. robusta* tree as having immense cultural significance because it is believed that Buddha was born and died under the *S. robusta* tree. Sal seed harvesting begins in the second to the third week of May (Southwest monsoon) when the windy weather facilitates the fall of *Shorea* seeds (Shashi et al., 2015).

Three ways have been used in India to process the seed (Shashi et al., 2015). One approach involves scattering the seeds on plain hard ground and beating Sal with a wooden stick to remove the wings. This approach is chosen since the kernels' quality and nature remain unchanged. The second way is to spread the seeds on the ground and then burn them to remove the wings. This procedure is not recommended since the high temperature has a negative impact on the seed and oil content. According to Sanjoy (2015), the seeds should not be burned to extract the dry kernel because the high temperature would impact the oil quality by increasing the number of FFAs, rendering the oil unacceptable for the export market. The third method is to spread the seeds on the ground and then crush them with a wooden or stone roller. The kernel was separated from the wings during this operation. Natural or artificial air blowing is used to separate the kernels and wings. The seeds are ground into powder after decortication, and the usual extraction method is water rendering (Shashi et al., 2015). However, no detailed instructions describing this procedure have been

recorded. The traditional method of extracting *Shorea* seed oil is utilised for cooking, lighting oil, soap production, and vanaspati (non-dairy ghee).

2.5 Modern Oilseeds Processing

Modern oilseed processing includes several steps such as post-harvest, pre-treatments, oil extraction and purification.

2.5.1 Post-harvest Technology

Proper post-harvest process of oilseed is vital to minimise the loss of oil during handling, processing and preservation of oilseeds and products. The efficient method reduces the cost of oilseed processing and thus increases oil yield (Muhammad et al., 2013). The oilseeds are susceptible to auto-catalytic deteriorative processes, enzyme action and microbial spoilage (McClements & Decker, 2009; Shashi et al., 2015). The impurities containing moisture, such as weeds, leaves, stems, dirt and sand contribute more humidity to the immature, high moisture content and damage seeds faster. PUFAs are oxidised as oxygen intake increases, reducing the nutraceutical values and the quality of the oilseeds. High moisture content in oilseed can cause the breakdown of TAG into FFAs whereas a high level of FFAs would cause the seed oil to be unfitted for human consumption (Ebongue et al., 2008). Thus, all the oilseeds must be cleaned and dried until an acceptable moisture level is obtained before storage.

Sun drying is the best method for drying *Citrullus lanatus* seed oil compared to oven drying (70°C) (Idris et al., 2017). There is no discernible variation in the oil yield and colour

obtained from the two drying techniques. However, the moisture level of the sun-dried product was lower than that of the oven-dried product, which was 2.2% and 4.6%, respectively. The linoleic, linolenic, heptadecanoic, and oleic acids are present in sundried seed oil while only linoleic acid was found in oven-dried seed oil. Thus, the authors suggest sun-drying method is the best method for drying seeds for oil extraction.

Adenuga et al. (2021) studied the effects of drying using the sun, air conditioner assisted and oven (with and without husks, at 45, 75 and 105°C) drying methods on oil yield, chemical properties and fatty acid composition of *Calophyllum inophyllum* kernel oil. The oil yield of sundried seeds is comparable with other drying methods and seeds dried with husks has a lower yield than oven-dried seeds without husk. The sun-dried kernel seed without husks contained a high acid value of 46.32 mg KOH/g compared to air conditioner-assisted drying of 28.81 mg KOH/g. The rapid oven dried seeds at a temperature higher than 75°C have the acid value of 50% lower than sundried seeds. According to the authors, fast drying slowed the microbiological and enzymatic activity rate within the kernel and its oil. As a result, decreasing the breakdown of TAGs to FFA.

The iodine value in sundried (68.67 g I₂/100 g) is significantly lower than air conditioner assisted (80.93 g I₂/100 g) and oven drying (up to 97.33 g I₂/100 g). The reduction in iodine value from oven dried to sundried is because the rapid drying (temperature higher than 75°C) took 24 hours upon completion. In contrast, the other drying method took almost two weeks. Thus, reducing the exposure oxidation and humidity for a longer time. The author proposed the significant difference in iodine value between oven drying with and without husk is because the husk protects the kernel from the free radical attack which is usually initiated by Ultra-Violet (UV) radiation from sunlight and humidity. The peroxide value in air conditioner-assisted drying is higher than sundried and oven-dried.

The authors also noted the increased peroxide value from drying seeds with husk to without husk. The author explained the decorticated seeds were exposed to light, humidity and air. Thus, drying with husks produced oil that is more resistant to oxidation and breakdown of TAGs to FFA. Sundried seeds had a total of 24 fatty acids, while oven-dried seed oils contained 21 fatty acids. The sundried oils have higher levels of essential fatty acids PUFAs than oven-dried oils.

In their investigation of the effects of drying methods on the compositional characterisation and functional features of *Blighia sapida aril* oil, Oluba et al. (2021) concluded that sun-drying is better suited for both nutritional and health-promoting purposes than oven drying. The sundried seed has considerably greater total phenolic content (TPC) and antioxidant activity assay (DPPH) than oven-dried seed ($p < 0.05$). Significantly higher oil yield was observed in sundried seeds compared to oven dried which is 38.5% and 31.3%, respectively. The refractive index, specific gravity, peroxide, acid, iodine and saponification values of aril seed oil dried using sun drying and oven drying procedures were not significantly different ($p > 0.05$).

There is no significant difference in the chemical characteristics and lipid profiles of soursop (*Annona muricata*) seed oil ($p > 0.05$) when using different drying methods which are the sun (drying for four days, average temperature 33°C with relative humidity 67%) and oven (50°C for 30 hours) (Tiencheu et al., 2021).

2.5.2 Oil Extraction from Oilseeds

The oilseed can be extracted or pressed to obtain seed oil. The cold or hot expression methods were utilised traditionally. Solvent extraction has superseded these processes, resulting in higher oil output. The *n*-hexane solvent is used to extract the oil from the oilseed in solvent extraction. Subsequently, the *n*-hexane is separated from the oil, recovered and repurposed. *n*-hexane can be easily eliminated by evaporation because of its low boiling point (Venkatesh & Raghavan, 2004). There are four primary methods to extract oil from seed as shown in Figure 2.10 (Sari, 2006). Mechanical expression is a mechanical compression of oleaginous material, where oil can be separated from the seeds under the forces from compressive action that were caused by a machine called an expeller. This low-cost method produces low contaminants, rich in protein and low-fat cake (Lavenburg et al., 2021). However, this processing is inefficient because 15-20% of oils remain in the pressed cake.

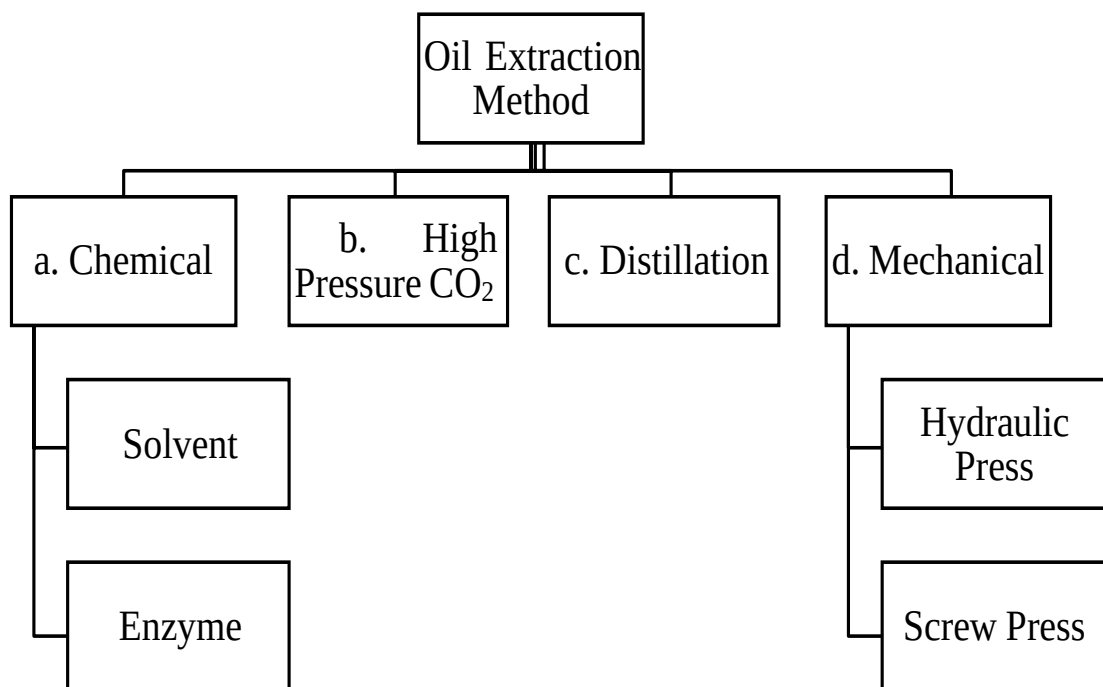


Figure 2.10: Type of Extraction Methods (adapted from Sari, 2006)

Venkatesh and Raghavan (2004) stated that mechanical pressing is a simple and safe technique but has a lower oil yield compared to solvent extraction. This is because the high amount of oil is still trapped in the waste. The other type of mechanical pressing is cold pressing which does not require heat. However, the oilseeds must be very clean, uniform in size and have appropriate moisture levels. The oil yield is lower than hot pressing and solvent extraction. However, cold-pressed oil is clean, safe and of excellent quality which can be ingested immediately. Cold pressed oil is a highly desirable product for food, cosmetics and pharmaceuticals.

The mechanical expression method is the oldest method used to extract oil from the seeds and the most common method for producing edible oil (Lavenburg et al., 2021). The seeds are placed between permeable barriers, and when the volume of the seeds decreases, the pressure on the seeds increases. The oil is then squeezed from the seeds. Oil extracted using mechanical extraction produced high-quality oil but low oil yield.

Solvent extraction is a modern method that separates the liquid from a liquid-solid system using solvent (Khan & Hanna, 1983). This process is reported to give a higher oil yield and drier oilcake than mechanical expression. Solvent extraction can remove all the available oil from the seed. Thus, the higher yield obtained, the higher the completion of oil removal and the higher quantity and quality of protein produced.

In addition, solvent extraction is one of the modern methods of extracting oil from seeds (Lavenburg et al., 2021). This is the most efficient technique for extracting oils. The method is predicted to have less than 1% oil remaining in the oil cake. Solvent extraction is the separation process of liquid from a liquid-solid system with the use of solvent. There are some disadvantages to recovering the oilseed using this method. Some chemical solvents are

quite harmful to human health and highly flammable, which fires and explosions might happen.

Johnson and Lusas (1983) narrated those seeds with low oil content like soya beans (20%) are subjected to direct solvent extraction. Solvent extraction removes soluble material from an insoluble solid phase by dissolving it in a liquid solvent. Soya beans like other oilseeds must be pre-treated before being extracted. Pre-treatment activities include dehulling, cracking, and flaking. *n*-hexane is generated from petroleum and has been widely utilised as a solvent in the oil extraction from oilseeds. This is due to the low boiling point of *n*-hexane (63°C to 69°C), good stability, low corrosiveness, minimal greasy residual effect and improved odour.

Wan and Wakelyn (1997) stated in solvent extraction, hexane is generally used as the solvent. The oil in the seeds is dissolved in the solvent, which will be separated from the solids. Then, the solvent-oil mixture is sent to the recovery operation where the solvent is removed by evaporation. During extraction, oil and solids are subjected to heat treatment, causing a reduction in the quality of oil and solids. The coextraction of undesired components further reduces the quality of the oil.

Gharby et al. (2015) studied the chemical characteristics of *Nigella sativa* seed oil extracted in Morocco using the cold press and Soxhlet extraction (hexane as solvent) methods. Higher oil yield using Soxhlet extraction (37%) than cold press mechanical extraction (27%). The high yield in Soxhlet extraction is due to hexane's high solvent efficiency in extracting oleo resin compared to cold pressing. FFA content in oil extracted via cold press (0.9%) method is lower than Soxhlet oil (2.3%). The author suggested the increase in FFA is due to the saponification reaction favours solvent extraction associated with heat. Essential PUFAs linoleic and linolenic acids in seed oil extracted via cold press

mechanical extraction were reported higher (58.55% and 0.5%, respectively) than Soxhlet extraction (56.50% and 0.2%, respectively). Total SFA is lower in the cold press than in Soxhlet extraction which is 16.8% and 25.5%, respectively. There is no significant difference recorded in fatty acid composition between cold press and Soxhlet extracted oils.

The effect of extraction methods on the physicochemical properties of *N. sativa* seed oil from Al-Qassim has been investigated (Alrashidi et al., 2020). Cold press mechanical extraction and Soxhlet extraction methods with different solvents were used (hexane, tetrahydrofuran, ethanol, chloroform, methanol and methanol-water mixture). The Soxhlet extraction method generally has a higher oil yield compared to cold press mechanical extraction (21.73%) except methanol-water mixture (18.4%) where the highest is ethanol (40.1%) and hexane (32.90%). The cold press mechanically extracted oil (7.84%) is reported to have the lowest FFA followed by Soxhlet extracted oil using hexane (13.18%) and ethanol (13.92%), where the highest is methanol (19.28%). Alrashidi et al. (2020) explained the high FFA content in the polar solvent because polar solvent facilitates the liberation of FFA in vegetable oils as it induces the hydrolysis of TAGs and saponification reaction. Apart from solvents, higher heat and temperature were needed in the Soxhlet reaction triggered lipase enzyme which assists in the liberation of FFA from TAGs. The lowest peroxide value was recorded in Soxhlet extraction using hexane (8.14 meq O₂/kg), ethanol (9.17 meq O₂/kg) and cold press (9.42 meq O₂/kg). A higher composition of individual fatty acids was detected in cold press oil and Soxhlet extracted oil using hexane. Total UFA was found higher in the cold press than in Soxhlet extraction.

The findings from Bhuiya et al. (2020) disagree with Gharby et al. (2015) and Alrashidi et al. (2020). The former authors stated that solvent extraction using n-hexane produces a higher yield, quality oil and is oxidatively stable than oil extracted from

mechanical extraction. The AV of *Calophyllum inophyllum* oil extracted via mechanical extraction (39.22 mg KOH/g) has been reported higher than solvent extraction (38.86 mg KOH/g). The oil from mechanical extractor has a larger amount of FFA than solvent extraction which is 20.23% and 19.54%. The solvent extracted oil has recorded higher oxidative stability than mechanical extraction which is 0.28 hours and 0.27 hours, respectively. Total UFAs were found lower in Solvent extracted oil compared to mechanical extraction, which is 67.2% and 67.6%, respectively. Keneni et al. (2021) stated that hexane solvent is the most efficient solvent in extracting seed oil because most oils are soluble in hexane, which contributes to the superior ability resulting in higher yield. However, solvent extraction using hexane requires high production costs and is time-consuming. Therefore, Bhuiya et al. (2020) suggested recovering the hexane solvent after extraction to get full benefits and reduce production costs.

Sey et al. (2020) studied the effect of solvent extraction (solvent mixture: methanol, chloroform and water) and single and double press mechanical expeller on canola oil produced under boreal climatic. The extraction method exhibits significant differences in the levels of SFA, MUFA and PUFA. Solvent extraction oil has a higher proportion of MUFA, linolenic acid and SFA while single and double-pressed extractor oils have higher levels of linoleic acids. Double pressed expeller methods are more effective than single pressed mechanical extraction methods, with only 8% to 11% residual oils in pressed cake compared to the single pressed cake which contained 10% to 15% remaining oil. However, the high temperature used in the mechanical extractor method which was 104°C, can affect the oil quality.

Several researchers found the significant effects of different extraction methods on chemical composition, fatty acid profile and oxidative stability in seed oils (Gharby et al.,

2015; Taghizadeh et al., 2018; Alrashidi et al., 2020; Bhuiya et al., 2020; Sey et al., 2020; Keneni et al., 2021).

Oil extraction methods affect oil yield and influence the concentration and type of minor lipid components in oil (Cai et al., 2021), as well as the fatty acid profile, tocopherol content and antioxidant activity of the oil (Dong et al., 2017). Soxhlet extraction of *Shorea* seed has been carried out in previous studies (Nesaretnam & Ali, 1992; Shashi et al., 2016). The differences between the two traditional extraction methods in extracting *S. stenoptera* seed oil from Nanga Yen and Sahan villages have been conducted (Maharani et al., 2016). They found a higher melting point, saponification value and FFA in oil extracted via the Nanga Yen method compared to Sahan. In addition, there is no literature on oil extraction using mechanical extraction to extract *Shorea* oil has ever been studied.

2.5.3 Oil Refining

Oil refining is essential to reduce FFA value and improve oil quality. Figure 2.11 shows the stages in oil refining. Zahrina et al. (2018) stated degumming process is to remove the sap or mucus without decreasing the amount of FFA. Phosphoric acid is a polybasic organic acid was used in degumming process to remove phospholipids as it aids in emulsifying oil. Hidayat et al. (2019) stated that after degumming process, clear oil is produced. However, the amount of FFA increased due to remaining phosphoric acid which reacted with fats and produced more FFA.

Neutralisation decreases the amount of FFA by reacting them with an alkaline solution to form soap (Zahrina et al., 2018). Hidayat et al. (2019) suggested NaOH be used in neutralization to break the FFA's ester. During the stirring and addition of NaOH, the soap

will begin to produce and float on the surface. The longer the period of neutralisation higher the amount of soap produced as the FFA started to accumulate. The concentration of NaOH affects the efficiency of the neutralisation process where 10% of NaOH of the weight of degummed oil is considered optimum condition (Hidayat et al., 2019). After the neutralisation was completed, the soap was separated at the top layer and the oil was washed with water to remove the remaining soap. Hidayat et al. (2019) found that the oil will be clearer, and the amount of acid number decreased, indicating improved oil quality and longer shelf life. Gusti and Zulnely (2015) recorded there is an increase in iodine number after the neutralisation process, representing more C=C bonds present in the oil.

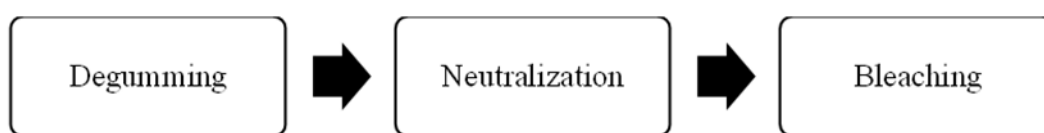


Figure 2.11: Oil Refining Process

The bleaching process is the removal of other impurities and unwanted substances in oil (Hidayat et al., 2019). This process is mainly conducted by mixing the oil with a neutral adsorbent such as porous soil, active clay and activated carbon. The use of activated carbon in the bleaching process adsorbs unwanted dyes and impurities without oxidising the oil (Hidayat et al., 2019).

Pal et al. (2015) investigated the impact of refining on the quality and composition of sunflower oil, and found that the refining reduced FFA (38.80% to 36.3%) and peroxide value (22.5 meq O₂/kg oil to 7.9 meq O₂/kg oil) significantly ($p < 0.05$). Neutralisation using alkali treatment eliminated FFA as soap. Pal et al. (2015) recommended the consumption of refined sunflower oil due to the removal of rancid taste and odour. The reduction of peroxide value might be due to absorbance in bleaching earth. The author reported no significant differences in fatty acids composition between crude and refined sunflower oils. However,

it is worth noting that the percentage of oleic acid reduced after refining from 52.30% to 43.3%.

Chew et al. (2017) recommended the optimum condition for neutralisation is 3.75% NaOH for 20 minutes at 40°C in refining kenaf seed oils. The author reported a significant reduction in specific gravity after the alkali treatment, from 0.90 to 0.88 ($p < 0.05$). The weight of oil was affected by the removal of gums and pigments which resulted in lower specific gravity from crude oil to neutralised oil. There is a significant decrease in peroxide value from crude to neutralised oil (2.90 meq O₂/kg to 1.57 meq O₂/kg) recorded. However, the result was in disagreement with Farhoosh et al. (2009) where the authors recorded a significant increase in peroxide value in neutralized soybean oil. Chew et al. (2017) explained the increase of peroxide value in previously mentioned soybean oil was because the high temperature was used in the neutralisation stage which was up to 90°C. A significant reduction of FFA from crude to neutralized oil (1.94% to 0.12%) was observed. The neutralisation process did not affect iodine value and fatty acid composition significantly; however, it is important to consider that neutralisation reduced total SFA (23.97% to 23.7%) and increased total PUFA (34.76% to 34.97%) significantly. Significant increase ($p < 0.05$) was noted in heptadecanoic (0.22% to 0.24%), γ -linolenic (3.22% to 3.54%), α -linolenic (0.69% to 0.73%) and arachidic acid (0.60% to 0.66%) and significant decrease ($p < 0.05$) in stearic acid which was from 3.67% to 3.62%.

Pan et al. (2019) investigated the relationship between the chemical refining process with the composition and oxidative stability of perilla seed oil, where a notable reduction of acid value (2.17 mg KOH/g to 0.37 mg KOH/g) and peroxide value (4.26 meq O₂/kg to 1.32 meq O₂/kg) was detected. The iodine value represents the degree of unsaturation and solidification in samples with no significant difference in crude and refined perilla seed oils.

Oil refining on perilla seed oil did not affect fatty acid composition however individual fatty acids such as oleic increased slightly from 0.03% to 0.18%, γ -linolenic from undetected to 0.06%, slightly decreased in gondoic and erucic acid which were 0.2% to 0.19% and 0.16% to undetected, respectively. A significant decrease in TPC was observed from 663.09 mg GAE/kg oil to 335.43 mg GAE/kg oil.

Wu et al. (2019) highlighted the slight increase of peroxide value in rapeseed oil after bleaching caused by the ester hydrolysis of bleaching earth. Then, a significant reduction of FFA and peroxide values found in refined oil after deodorisation might be attributed to the volatilisation and further decomposition at high temperatures. Up to 42.16% of tocopherol and 61.19% of TPC were lost during refining.

Liu et al. (2019a) reported chemical refinement caused a significant reduction ($p < 0.05$) in FFA, peroxide value, total tocopherols and total sterol content in coconut oil. The composition of fatty acids between crude and refined coconut oil does not differ significantly. However, it is worth noting that the SFA was increased considerably and oleic and linoleic acids decreased significantly after oil refining. This is due to the isomerisation of both oleic and linoleic acids which lowered the relative levels of both acids and increased the proportion of SFA such as caprylic, capric and stearic acid especially at high temperatures. The investigation of the effect on chemical refinement on rice bran oil reported the reduction in squalene and a huge loss of total polyphenols was noted which is up to 83.96% (Liu et al., 2019b). This is due to the easy removal of polyphenols using an aqueous solution such as NaOH which both of them have polar properties. Significant reduction in antioxidant activity assays such as ABTS (2,2'-azino- bis(3-ethylbenzothiazoline-6-sulfonic acid)), ORAC (oxygen radical absorbance capacity) and DPPH (2,2-diphenyl-1-picrylhydrazyl) or refined rice bran oil have also been observed.

Pan et al. (2020) reported the chemical refining of evening primrose oil to reduce peroxide value (7.35 meq O₂/kg to 0.79 meq O₂/kg) and acid value (1.88 mg KOH/g to 0.37 mg KOH/g). However, the appreciable amount of α , γ , δ tocopherols, squalene and TPC also reduced significantly due to the high temperature applied during oil refining. The fatty acid composition in evening primrose oil was not significantly different ($p>0.05$), a slight reduction in percentage of γ -linolenic and linoleic acid was noted which was 12.91% to 12.58% and 61.24% to 61.04%, respectively. A slight increase in total SFA and MUFA and a decrease in total PUFA were observed in refined oil. Özcan et al. (2021) reported a similar finding on the effect of chemical refinement in Canola oil where the reduction of FFA (1.09% to 0.06%) and peroxide value (3 meq O₂/kg to 0.15 3 meq O₂/kg) were observed. However refining Canola oil significantly affects the composition of fatty acids where total SFA increased from 6.398% to 7.155% ($p<0.05$), increased of total PUFA from 29.771% to 31.93% ($p<0.05$) and reduction in MUFA from 63.844% to 60.916% ($p<0.05$). Harmful fatty acid which is erucic acid significantly reduced up to 50% in refined Canola oil.

Liu et al. (2019ab), Pan et al. (2019) and Wu et al. (2019) reported the reduction of tocopherols in refined oils. However Lucci et al. (2020) reported the trend of tocopherols reduction in nine samples of olive oils due to the refining process was unclear. This is because there is significant increase of total tocopherols in four (sample number 4, 6, 7 and 8) out of 9 bleached oil samples and a significant increase in 1 (sample number 3) out of 9 deodorised oils. The author explained that the increase of tocopherol could be interpreted as concentration effect: higher initial FFA in crude oil resulted in greater mass oil. The author suggested that the loss of oil during removal of fatty acids concentrate the amount of tocopherol in oils.

Zarei Jelyani et al. (2021) found a similar finding to Lucci et al. (2020) where there is a significant increase in the amount of total tocopherol in refined Baneh oil. The increase of total tocopherol was observed during degummed, neutralized where the highest during bleaching which was up to 32% and reduced 694 mg/kg which are still higher compared to its crude oil (572 mg/kg). The regeneration of tocopherols by breaking dimeric bonds between tocopherols or breaking the ester bond between tocopherols and other compounds was related to the increase in tocopherols (Chew et al., 2016). The amount of TPC from crude to degumming and neutralisation shows decrease in phenolic and remained constant during bleaching and then increased during deodorisation. This might be due to the phenolic might bonded with other compound when the phenolic was released during bleaching resulted in a significant increase in TPC.

The refining of *Shorea* seed oils has been studied by Hidayat et al. (2019) and Darmawan et al. (2020). Previous research only focused on reducing the acid value and peroxide value at different refining stages under other conditions (temperature, concentration and thermal/acid activated clay), which was significantly reduced after refining. However, the effects of refining on fatty acid content or the antioxidant activity of seed oils are unknown.

2.6 Proximate Composition of Studied *Shorea* Oilseed

S. robusta oilseed has the highest moisture content compared to other species which is 12.14 while *S. macrophylla* oilseed (5.2%) has the lowest moisture content as shown in Table 2.2. Oilseeds with low moisture content are beneficial due to a longer period of storage without rotting (McClements & Decker, 2009; Shashi et al., 2016). Soltani Firouz et al.

(2014) emphasized that determination of moisture content is crucial in all post-harvest operations such as handling, storage, milling and oil extraction. Moisture content in oilseeds has to be limited to lower the rate of deterioration and improves oil extraction efficiency (Pérez et al., 2019).

Table 2.2: Proximate Composition of *Shorea* oilseeds

Parameter	Species				
	SAL ^a	SING ^b	MEC ^b	MAC ^b	STE ^b
Moisture (%)	12.14	7	7.3	5.2	5.9
Ash (%)	3.75	n/a	n/a	n/a	n/a
Crude Protein(%)	9.39	5	6.5	5.4	5.2
Crude Fibre (%)	2.41	n/a	n/a	n/a	n/a
Total carbohydrate (%)	42.11	n/a	n/a	n/a	n/a
Fat (%)	30.20	38.7	59.3	55.9	52

SAL is *Shorea robusta*; SING is *Shorea singkawang*; MEC is *S. mecistopteryx*; MAC is *S. macrophylla*; STE is *S. stenoptera*; ^a Shashi et al. (2016); ^b Nesaretnam and Ali (1992).

Akinoso et al. (2006) recorded that the optimum moisture content for expression by oil expeller in extracting sesame oil is 4.6%. Singh et al. (2002) described moisture content of seed as an important factor the in the screw or hydraulic presses where 5.9% of moisture content Crambe seed is optimal for oil extraction. The oil yield decreases as the moisture content increases because moisture behaved as a lubricant which reduced the resistance during pressing and cause reduced in oil yield. Moisture content less than 4.1% was recorded to be inefficient and resulted in increased brittleness.

A study by Pérez et al. (2019) on the effect of sunflower collets moisture on extraction yield and oil quality suggested the presence of free water is important in oil

extraction. The increase in adsorbed water in monolayer region which are mainly build from carbohydrates and protein will cause cell structures to swell and disrupt. The disruption of cell structure will improve the adsorption and capillary condensation of hexane which increased solvent accessibility into oilseed. Free water will fill in the pores of oilseed with a moisture content greater than 10% which interferes the solvent penetration and lowering extraction rates. Shashi et al. (2016) described higher moisture content of *S. robusta*, higher energy required by the expeller to rupture the seed in oil pressing. Table 2.2 shows the moisture content of *Shorea* oilseeds is below 10% except for *S. robusta* where further drying process is needed prior extraction process.

The term "ash content" refers to the quantity of inorganic residue that remains after the combustion or oxidation of organic matter. This is critical for nutritional evaluation and is the first step to prepare the sample for particular elemental analysis (Marshall, 2011). The highest ash content and crude protein was reported in *S. robusta* oilseed which were 12.14% and 9.39% compared to other *Shorea* species as listed in Table 2.2 but was still lower than soybean seed (37.67%) as reported by Etiosa et al. (2017). Mansouri et al. (2018) stated the protein intake is important for animal growth and breeding as it contributes to the formation of hormones which controls growth, repair and maintenance. Protein content in meal is important parameter in livestock feed such as cattle, fish and poultry feeds where the quality of amino acids in protein meal determines its nutritional values (Mansouri et al., 2018).

Only *S. robusta* has been recorded its crude fibre which is 2.41% (Shashi et al., 2016). Fibre is known to have little to none nutritional value and cannot be broken down in intestinal tract but still can be metabolised by bacteria in the lower gut (Ajani et al., 2016). Fibre is necessary for sustaining adequate peristaltic movement in the intestine. Lack of fibre in diet will cause constipation lead to colon diseases (Akinhanmi et al., 2008). Anderson et al.

(2009) added individual with high fibre in diet has lower risk to develop coronary heart disease (CHD) stroke, hypertension and gastrointestinal diseases. However, Mansouri et al. (2018) reported, extremely high intake of fibre also may lower nutritional value which results in delay of growth and reproduction. Fat percentage is one of the most important economic factors in growing and processing oilseeds (Balbino, 2017). Fat percentage in *Shorea* oilseeds range from 30.20% to 88.67% as shown in Table 2.2 which is favourable in oil industries.

2.7 Antinutritive Factors in Oilseed

Seeds and nuts have gained attention in recent years because of the high content of nutraceutical and therapeutic value of their bioactive constituent (Meru et al., 2018; Rezig et al., 2019; Yang et al., 2019; Dotto & Chacha, 2020). Alternative protein sources for chickens, pigs and aquatic animals have been investigated using unrefined residual oilseed cakes and meal for oil extraction as feed ingredients to replace a fish meal (Duodu et al., 2018). A study on Nile Tilapia fed with diets based on shea nut meal (Agbo et al., 2015) revealed poor growth performance due to poor digestibility and lower feed intake. The availability of antinutrients such as oxalate, phytate, tannins, trypsin inhibitors, lectins, gossypol, saponin, antivitamin and mycotoxin limit the application of oilseed cake (Duodu et al., 2018). These compounds are responsible for reducing the growth performance by restricting the bioavailability of essential nutrients in the body (Gibson et al., 2018; Petroski & Minich, 2020).

Oxalate is a substance that can form insoluble salts with minerals such as Na, K, Ca, Fe and Mg (Petroski & Minich, 2020). This compound is produced in plants and mammals.

Oxalate is manufactured in plants and acts as calcium regulator, plant protector and detoxification agent of heavy metals. The formation of oxalate in mammals results from the breakdown of ascorbate, glyoxylate, hydroxyproline and glycine. Oxalate generated from plants is found in two forms: soluble in water oxalate (oxalic acid, potassium, sodium and ammonium oxalate) and insoluble oxalate salts (calcium oxalate) (Savage et al., 2000). Soluble oxalate chelates minerals and is absorbed through the intestine and colon which contribute to the formation of calcium oxalate kidney stones (Savage et al., 2000) while insoluble oxalate is excreted in the faeces (Chai & Liebman, 2005a). Petroski and Minich (2020) regarded oxalates as antinutrients because they reduce mineral absorption and may be responsible for kidney stones. According to Chai and Liebman (2005a), soluble oxalate has a greater effect on absorption and kidney stone development compared to insoluble oxalate. Nuts and beans are reported to be rich in oxalates such as cashews (262 mg/100g) and almonds (469 mg/100g) and soybean (370 mg/100g) (Chai & Liebman, 2005b; Shi et al., 2018).

Phytate functions as energy storage of phosphate and antioxidant for germinating seeds (Buades Fuster et al., 2017; Petroski & Minich, 2020). Phytate was synthesised during the seed growth and contributed to total phosphorus content between 60 to 90% in legumes, cereal, nuts, grains and seeds (Bohn et al., 2008). Phytate binds with 12 protons because the compound structure consists of six phosphate groups attached to the inositol ring. Phytate is considered a potent chelator due to its phosphate groups, which readily bind with mineral cations, especially Cu^{2+} , Ca^{2+} , Zn^{2+} and Fe^{3+} to form complexes (Castro-Alba et al., 2019). Mineral bioavailability may be decreased as the complexes are insoluble at neutral pH and cannot be digested by human enzymes (Gibson et al., 2018). To prevent adverse effects on

mineral absorption, such as mineral insufficiency, the intake of seeds containing high phytate must be limited (Buades Fuster et al., 2017).

Cereals, legumes, nuts, seeds and pseudo-cereals have the highest phytate concentration (Castro-Alba et al., 2019). Vashishth et al. (2017) found that vegetarians and rural residents in underdeveloped nations consume between 2000 and 2600 mg of phytate per day, while mixed diets may contain as little as 650 mg. The ability of phytate to bind with minerals depends on the phytate to metal ion ratio and pH (Silva & Bracarense, 2016). There must be a phytate/iron ratio of less than 0.4, a phytate/zinc ratio of less than 5, and a phytate/calcium ratio of less than 0.17 for the minerals to be adequately absorbed by the human body. Miller et al. (2015) found that zinc absorption was reduced by 0.04 mg/day by increasing dietary phytate intake by 500 mg. For this reason, Armah (2016) recommended that consumers consume more minerals and ascorbic acid-rich supplementary foods to get the full health benefits of phytate-rich diets.

The information regarding antinutrients in *Shorea* seed is limited. The composition of tannin in *S. robusta* seed meal for poultry is the only antinutrient study conducted for the *Shorea* seed, which is 24.2 g/kg (Mahmood et al., 2006). The micronutrient deficiencies such as vitamins and minerals are among developing countries' most significant health issues (Black et al., 2013). Therefore, the assessment of anti-nutritional factors is a primary goal to ensure the supply of micronutrients is sufficient.

2.8 Fatty Acid

The fatty acid is a bioactive chemical that is generated when lipids are broken down and plays a critical role in many signalling pathways and the structure of cell membranes in humans (Orsavova et al., 2015). Changes in lipid metabolism alter membrane composition, which alters cell membrane permeability and disturbs signalling networks, resulting in detrimental outcomes such as cancer, cardiovascular, neurodegenerative, metabolic and inflammatory issues (Huang & Freter, 2015; Orsavova et al., 2015). There are two types of fatty acids: essential and non-essential. In contrast to non-essential fatty acids, which the body is able to synthesise, essential fatty acids are those that cannot be produced by humans. The amphipathic properties of FFA and its salts have been used in the manufacturing of detergents and soaps (Rustan & Drevon, 2005).

Rustan and Drevon (2005) described fatty acid as composed of carbon chains that attach with a methyl group (ω) and carboxylic acid (COOH). The carbon atom that was labelled as α is the nearest carbon to the carbonyl group, whereas the carbon labelled as β was referred to as the second nearest carbon to the carbonyl group. The n is referred to the number of carbons in between the methyl group and β carbon as shown in Figure 2.12. Fatty acids can be classified into two categories which is saturated fats and unsaturated fats.

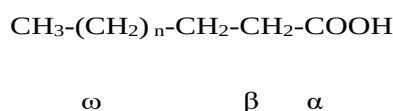


Figure 2.12: Structure of Fatty Acids (Rustan and Drevon, 2005)

The physical and chemical properties of the oil are influenced by its fatty acid makeup, which differs according to species, ecology and climate (Akkaya, 2018). Thus, knowledge of the various fatty acid profiles of the seed oil is important to determine its quality and make oil production possible for intended purposes. Liu et al. (2017a) stated the current recommendation for total fat consumption based on the World Health Organization (WHO) and Food and Nutrition Board (FNB) is somewhere between 20% and 35%. Low-fat consumption can lead to atherogenic dyslipidemia, high triglyceride-rich lipoproteins, and other health problems; hence, consuming at least 20% of one's calories from fat is recommended. A diet with a higher than maximum recommendation was observed to cause weight gain. Indelicato et al. (2017) suggested the increased consumption of vegetable oils due to their high content of PUFAs. However, a higher level of UFAs will promote oxidation, which affects oil stability (Konuskan et al., 2019). Unstable oil is undesirable as it contains unpleasant taste and flavour, has reduced nutritional value and may produce toxic compounds (Redondo Cuevas et al., 2017).

2.8.1 Saturated Fatty Acids (SFAs)

An SFA chain is filled with hydrogen and is a straight-chain hydrocarbon with an even number of carbon atoms found in plants and animals. Ratnayake and Galli (2009) stated SFAs are classified into four subclasses based on their chain length: short-chain SFA (3 to 7 carbon), medium-chain SFA (8 to 13 carbon), long chain SFA (14 to 20 carbon) and very long-chain SFA (more than 21 carbon). Kenar et al. (2017) mentioned that SFAs containing more than 18 carbon alkyl chains are often present as waxes and are often negligible in most seed oils. Oil or fat with a higher percentage of saturated acids will have a higher melting

point than oil with a higher content of UFAs. The fatty acid profile affects the viscosity of oil; the higher the amount of SFA, the higher the viscosity of oil; and the activation energy of SFA is found to be higher than unsaturated fatty acids, which makes the oil thermally stable (Ajala et al., 2016; Abdul-Hammed et al., 2020). Table 2.3 listed the fatty acid composition of nine seed oils.

Table 2.3: Fatty Acid Composition of Seed Oil from Nine Different Types of Oilseeds

No. Carbon	RP ^b	SES ^c	FIC ^d	SNF ^b	SHE ^e	SAL ^f	STE ^h	PIN ^g	MEC ^g
C12:0	-	-	-	-	-	-	0.15	-	-
C14:0	-	0.9	-	-	-	-	0.08	-	-
C15:0	-	-	0.02	-	-	-	-	-	-
C16:1	0.17	0.4	0.09	0.95	-	-	-	-	-
C16:0	3.97	5.6	8.75	5.94	4.87	5.5	20.2	11.7	14.5
							2	8	1
C17:1	-	-	0.05	-	-	-	-	-	-
C17:0	-	-	0.05	-	-	-	-	-	-
C18:3n6	6.75	-	41.26	-	-	-	-	-	-
C18:2n6	17.4	46.1	31.69	21.5	2.91	1.7	0.45	22.0	27.0
c	3			8				4	5
C18:3n3	-	0.4	-	-	-	-	-	-	-
C18:1n9	63.6	40.3	14.95	68.8	47.8	41	31.2	42.7	31.2
c	8			8	1		9	9	8
C18:0	2.12	5.3	3.0	2.53	40.0	43.2	42.6	1.56	0.8
					9		0		
C20:1n9	2.82	-	0.11	0.23	-	-	-	-	-
C20:0	0.7	0.05	0.09	0.28	-	7.3	3.53	-	-
C22:1n9	1.93	-	-	-	-	-	-	-	-
C22:0	0.37	0.5	-	0.49	-	-	-	-	-
C24:0	-	-	-	0.31	-	0.5	-	-	-

^a is standard deviation less than 0.05; ^b (Konuskan et al., 2019); ^c (Hama, 2017); ^d (Hssaini et al., 2020); ^e (Abdul-Hammed et al., 2020); ^f (Kamalakar et al., 2015); ^g (Gusti et al., 2012); ^h (Darmawan et al., 2020); RP is rapeseed (*Brassica napus*); SES is sesame (*Sesamum indicum* L.); FIC is *Ficarus carica*; SNF is sunflower (*Helianthus annuus*); SHE is shea (*Vitellaria paradoxa*, C.F. Gaertn.); SAL is *Shorea robusta*; STE is *S. stenoptera*; PIN is *S. pinanga*; MEC is *S. mecistopteryx*.

The odd-numbered fatty acid chain is rare and only present in a trace amount in animal fats and plant lipids (Kenar et al., 2017). As shown in Table 2.3, Hssaini et al. (2020)

reported the presence of an odd chain number, which was pentadecanoic acid (0.02%) in *Ficarus carica* seed oil. Table 2.3 lists palmitic (C16:0) and stearic (C18:0) acids as the two most prevalent saturated acids in seed oil and Orsavova et al. (2015) found a similar trend. *S. stenoptera* was recorded as having the highest total SFA, which was 66.58% (Darmawan et al., 2020) followed by *S. robusta* seed oil which was 56.50%. The amount of long fatty acids, such as behenic and lignoceric, is less than 0.55% in rapeseed oil, sesame seed oil, sunflower seed oil and sal oil.

Liu et al. (2017a) recommended reducing dietary SFA to minimise cardiovascular disease (CVD) risk. Limiting SFA intake to less than 10% is recommended by the WHO's Dietary Guidelines for Americans 2015–2020 (Liu et al., 2017a). (Orsavova et al., 2015) found various effects of SFAs on human health. The total cholesterol to high-density lipoprotein (HDL) cholesterol ratio can be considered a marker for coronary artery disease CADs. CAD risk increases when the total cholesterol to HDL-C ratio is reduced (Lawrence, 2013). Lauric acid (C12:0) was found in *S. stenoptera* at 0.15% by Darmawan et al. (2020) While myristic and palmitic acids have a more substantial influence on lowering the total cholesterol to HDL cholesterol ratio, stearic acid has essentially little effect on reducing it (Lawrence, 2013; Bauer et al., 2015).

2.8.2 Unsaturated Fatty Acids (UFAs)

MUFA and PUFA are two subcategories of UFA (Bozza & Viola, 2010). It is typical for fatty acids found in nature to be in the *cis* configuration. In contrast to MUFAs, which have only one carbon-carbon double bond, PUFAs have multiple. Non- conjugated double bonds in PUFAs are commonly separated by a methylene group. Although these are

widespread in nature, *trans*-configuration, unique double-bond placement and conjugated double bonds are also present but in small amounts (Kenar et al., 2017).

2.8.2.1 Monounsaturated Fatty Acids (MUFAs)

Cis-MUFAs are common components of commodity oils, with over 100 identified MUFAs (Kenar et al., 2017). Bozza and Viola (2010) stated that hydrogenation produces *trans*-unsaturated fatty acids. A *cis*-unsaturated fatty acid is a potent inducer of adiposomes, lipid droplets in cell signalling, regulation of lipid metabolism and control of the synthesis and secretion of inflammatory mediators (Kenar et al., 2017). MUFAs can be synthesised *de novo* through biosynthetic pathways involving desaturation and/or elongation. For instance, palmitic acid is desaturated to become palmitoleate (C16:1n9c) and then elongated to *cis*-vaccenic acid (C18:1n11c). The melting points of MUFAs are affected by the number of carbons, so the longer the chain, the higher the melting points; *trans*-MUFAs' melting points are higher than *cis*-MUFAs', while they are almost the same as SFAs.

Liu et al. (2017a) stated that industrial *trans* fats derived from partially hydrogenated oils increase the risk of CHD more than any other micronutrient because they raise LDL-C (Low-density lipoprotein cholesterol), triglycerides, inflammation, endothelial dysfunction and hepatic fat synthesis while decreasing HDL-C (high-density lipoprotein cholesterol). Consequently, it is advised to reduce one's daily intake of *trans* fat to as little as possible. Beginning in the year of 2003, Denmark was the first country to prohibit the sale of items containing *trans* fats. As a result, the European Union began to limit *trans* fats in the food supply. The Food and Drug Administration (FDA, 2015) decided to remove partially hydrogenated oils from the foods that are Generally Recognized as Safe (GRAS) list.

The highest MUFA contents are found in sunflower seed oil (70.06%), rapeseed oil (68.6%) and shea butter (47.81%), as shown in Table 2.3. MUFA content in *Shorea* species was considered medium to high which included *S. pinanga* (42.79%), *S. robusta* (41%), *S. stenoptera* (31.29%) and *S. mecistopteryx* (31.28%), where the only MUFA that has been detected is oleic acid (Gusti et al., 2012). However, studies on fatty acid composition in *S. stenoptera* oil from Sarawak have reported the presence of palmitoleic acid (C16:1) in *S. stenoptera* oil of less than 0.6% (Kershaw, 1987). The difference in oil composition might be due to ecological conditions and variation in species (Konuskan et al., 2017). No recent studies have been done on the fatty acid composition of *Shorea* oil seed in Sarawak in the last ten years.

Oleic acid (C18:1n7) is the most abundant MUFA in the listed seed oil, with *F. carica* oil having the least oleic acid content at 14.95%. Oleic acid would decrease LDL-C but does not have much effect on affecting HDL-C levels (González-Fernández et al., 2017). The consumption of MUFAs such as oleic acid causes a low incidence of CHD, colon, breast and skin cancer in the Mediterranean region. Orsavova et al. (2015) stated that MUFAs might lower LDL-C while increasing HDL-C. Oleic acid may also promote insulin resistance in contrast to PUFAs, which prevent insulin resistance. Anti-apoptotic and anti-inflammatory effects of oleic acid have also been reported, leading to the activation of downstream inflammatory mediators (Kim et al., 2015).

Erucic acid (C22:1n7) is only found in rapeseed oil where high consumption would be harmful to humans (Food Standards Australia New Zealand, 2003). Konuskan et al. (2017) stated erucic acid had been reported to cause lipidosis and increase blood cholesterol levels, which is why some countries restrict the intake of erucic acid to no more than 5% of total fatty acid daily intake. Sabikhi and Sathish Kumar (2012) reported that erucic acid

would cause an accumulation of fat in the heart and skeletal muscles and disrupt growth. Canadian plant breeders bred new rapeseed variants, known as Canola, to reduce the content of glucosinolates (anti-nutritional) and erucic acid. Rapeseed which has less than 2% of erucic acid and 30 $\mu\text{mol/g}$ glucosinolates is regarded as canola (Bauer et al., 2015).

2.8.2.2 Poly-Unsaturated Fatty Acids (PUFAs)

Plant oil has an abundance of PUFA chains containing less than three double bonds, as shown in Table 2.3, while PUFAs exceeding six double bonds in animals and algae occur frequently. PUFAs are liquid at room temperature due to their unsaturated chain. α -linolenic and linoleic acids are common fatty acids present in a wide variety of plant and commodity oils. Animals and humans, on the other hand, are unable to produce the aforementioned FAs on their own; they must be acquired from food. PUFAs that are longer than 20 carbons are biosynthetically produced from α -linolenic and linoleic acids and are vital in supporting human health (Choque et al., 2014).

Kenar et al. (2017) reported linoleic acid is the shortest PUFA and is commonly found in plant oils. Sesame oil contains the highest percentage of linoleic acid, which is 46.1%, followed by *F. carica* (31.69%) and *S. mecistopteryx* (27.05%). The percentage of linoleic acid among the species of the *Shorea* genus is varied as *S. pinanga* contains almost the same as *S. mecistopteryx*. Still, the other two species which are *S. robusta* and *S. stenoptera*, have very low linoleic acid. Linoleic acid will be converted in the body by desaturase and elongate to become a longer-chain n-6 PUFA such as arachidonic acid and docosapentaenoic acid. γ -linolenic acid an isomer of α -linolenic is found in small amounts in animal fats. GLA is the most abundant fatty acid in *F. carica* oil, accounting for 46.10%.

Kenar et al. (2017) described the linoleic acid-to-arachidonic-acid transition involving γ -linolenic acid as an intermediate. Due to health and nutritional claims that γ -linolenic acid can prevent or treat a wide range of human disorders, γ -linolenic acid has been extensively studied.

Kenar et al. (2017) reported that α -linolenic is found in most plant oils and is an essential fatty acid. In contrast, only sesame oil contains α -linolenic, as shown in Table 2.3, but the amount is minimal. α -linolenic is the precursor of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are popular due to their beneficial role in preventing various diseases. Although PUFAs contain myriad health benefits for human health, their higher rate of unsaturation promotes oxidation which lowers the stability of oil (Konuskan et al., 2019). Unstable oil has an undesirable taste and flavour, has lost its nutritional value and might produce toxic effects (Redondo Cuevas et al., 2017).

2.9 Chemical Properties of Studied *Shorea* Seed Oils

Oxidative stability in oil determines the suitable application, quality and shelf life of products since the fats and oils degrade over time (de Camargo et al., 2016; Maszewska et al., 2018). The degradation of oil is primarily due to lipid oxidation and hydrolytic rancidity (McClements & Decker, 2009). The breakdown of fatty acids will produce primary and secondary products that mostly have off-odours and off-flavour volatile organic compounds (VOCs) (Syed, 2016). The oxidative stability of oil can be determined by assessing acid, peroxide and iodine values.

Acid value measures the amount of FFA liberated from TAGs during the hydrolysis reaction by lipase. FFA is susceptible to oxidation rancidity as it is less stable than neutral

oil. The amount of FFA in oil varies depending on the amount of time, temperature and moisture the oil is exposed to during storage, processing, heating and frying (Mahesar et al., 2014). FFA is responsible for oil off-flavours, reducing consumer preferences toward the oil (Pal et al., 2015). The FFA concentration of edible oils is an important parameter as it is related to their quality and economic value. Colorimetric-phenolphthalein based standard methods have been recommended for the determination of acid value by the American Oil Chemists' Society (AOCS), the Association of Official Analytical Chemists (AOAC) and the European Commission Regulation (ECR) (Mahesar et al., 2014). Oil is dissolved in hot ethanol or diethyl ether and titrated against a strong base and phenolphthalein as an indicator. *S. robusta* and crude *S. stenoptera* were recorded as the highest acid value which were 45.09 mg NaOH/g and 15.48 mg NaOH/g as shown in Table 2.4. The lowest content of acid value was reported in *S. mecistopteryx* (0.58 mg NaOH/g) and *S. macrophylla* (0.38 mg NaOH/g). Darmawan et al. (2020) reported a significant reduction in acid value after refining using thermally activated clay from 15.48 mg NaOH/g to 4.78 mg NaOH/g.

Table 2.4: The Chemical Properties of Studied *Shorea* Oils

Oil	AV (mg NaOH/g)	IV (g I ₂ /100 g)	PV (meq O ₂ /kg oil)
<i>S. mecistopteryx</i> ^a	0.58	36.3	-
<i>S. macrophylla</i> ^a	0.38	30.0	-
<i>S. robusta</i> ^b	45.09	n/a	3.80
<i>S. stenoptera</i> ^c	15.48	31.5	9.45
<i>S. stenoptera</i> ^d	4.78	30.75	4.84

Note: ^a (Nesaretnam & Ali, 1992); ^b (Shashi et al., 2016); ^c crude *S. stenoptera* oil (Darmawan et al., 2020); ^d Thermal activated-bleached *S. stenoptera* oil (Darmawan et al., 2020); AV is acid value; IV is iodine value; PV is peroxide value.

The iodine value measures unsaturation based on the uptake of halogen by carbon-carbon double bonds in the fatty acid chain. The higher the iodine value, the higher the unsaturation and the higher the susceptibility of the oil to undergo oxidation (Bhuiya et al., 2020). An increase in iodine value also reduces the firmness and shelf life of the oil (Boler, 2014). According to (Karak, 2012) oils are classified based on their iodine value: drying oil (iodine value greater than 150 g I₂/100g), semi-drying oil (iodine value greater between 100 and 150 g I₂/100g) and non-drying oil (iodine value less than 100 g I₂/100g). The classification is important to determine the suitable application of oils in various industries. Drying oil such as linseed oil is used as a primer and binder for structural coatings. Soybean oil is a semi-drying oil blended with other oil classes in manufacturing house paints, varnishes and baking finishers for automobiles. Non-drying oils for example, castor oil is used as plasticisers for lacquers and leather dressing dopes. Wij's method is widely used in the oil industry, such as in palm oil to determine iodine value (da Silva Oliveira et al., 2019). The procedure requires iodine chloride to saturate double bonds in oil. Iodine consumed is measured by titration against sodium thiosulfate solution. The lowest iodine value was recorded in *S. macrophylla* at 3 g I₂/100g (Nesaretnam & Ali, 1992). Darmawan et al. (2020) found that oil refining reduced the iodine value from 36.3 g I₂/100g to 30.0 g I₂/100g.

Peroxide value indicates the presence of lipid oxidation and is expressed as the reactive oxygen content in milliequivalents per 1 kg of fat. Maszewska et al. (2018) reported oil containing less than 10 meq O₂/kg is suitable for consumption and storage. Peroxide value in oils must be limited as peroxides such as hydrogen peroxide and lipid peroxide react with UFAs to generate more peroxides. McClements and Decker (2009) reported lipid hydroperoxide does not contribute to rancidity; however, decomposition of peroxides produces low molecular weight VOCs responsible for off-flavours and aromas. The titration

method has been widely used in the determination of peroxide value in oil, which relies on the ability of peroxides to oxidise indicator compounds (McClements & Decker, 2009). The peroxide value of *Shorea* listed in Table 2.4 ranges from 3.80 10 meq O₂/kg to 9.45 10 meq O₂/kg is fit for consumption.

2.10 Antioxidant Activity and Total Phenolic Content (TPC)

McClements and Decker (2009) reported the kinetics of lipid oxidation is comprised of a lag phase followed by an exponential increase as shown in Figure 2.13. The lag phase is vital in manufacturing oil and food containing oil as oxidation during this phase is undetected. The higher the oxidative stability of the oil, the longer the lag phase and shelf life. Figure 2.13 shows that the presence of antioxidants prolongs a product's lag phase and shelf life. Oxidation and degradation of food quality can be prevented or delayed by the presence of antioxidants (Shahidi, 2015). Antioxidants in the body are substances that lessen the risk of oxidative stress-induced degenerative illnesses. There are two types of antioxidants: primary and secondary antioxidants. The primary antioxidants are those that donate hydrogen atoms or transfer single electrons in order to neutralise free radicals (McClements & Decker, 2009). Secondary antioxidants neutralise the prooxidant catalyst, including prooxidant metals like Fe and Cu (Shahidi, 2015).

As Shahidi and Zhong (2015) mentioned, antioxidant activity assessment has evolved in recent years using diverse chemical assays and sensitive and automated detection methods. In radical scavenging assays, the capacity of an antioxidant to donate hydrogen or transfer electrons is measured. The DPPH radical scavenging assay is the most widely used method in evaluating antioxidant activity. DPPH is a commercially available, stable deep

purple colour chromogen that does not need to be produced prior to the experiment. The DPPH test is an electron transfer-based approach that determines the antioxidant's scavenging capacity against the DPPH radical by measuring the rate of signal intensity reduction at the endpoint. The DPPH assay is a straightforward procedure requiring a 517 nm UV spectrometer. Antioxidant activity in the DPPH assay is expressed as EC_{50} or IC_{50} . The effective antioxidant concentration (EC_{50}) is the concentration of antioxidants required to reduce the initial DPPH concentration by 50%. In contrast, the inhibitor concentration (IC_{50}) is the concentration of antioxidants necessary to reduce half of the DPPH signal response.

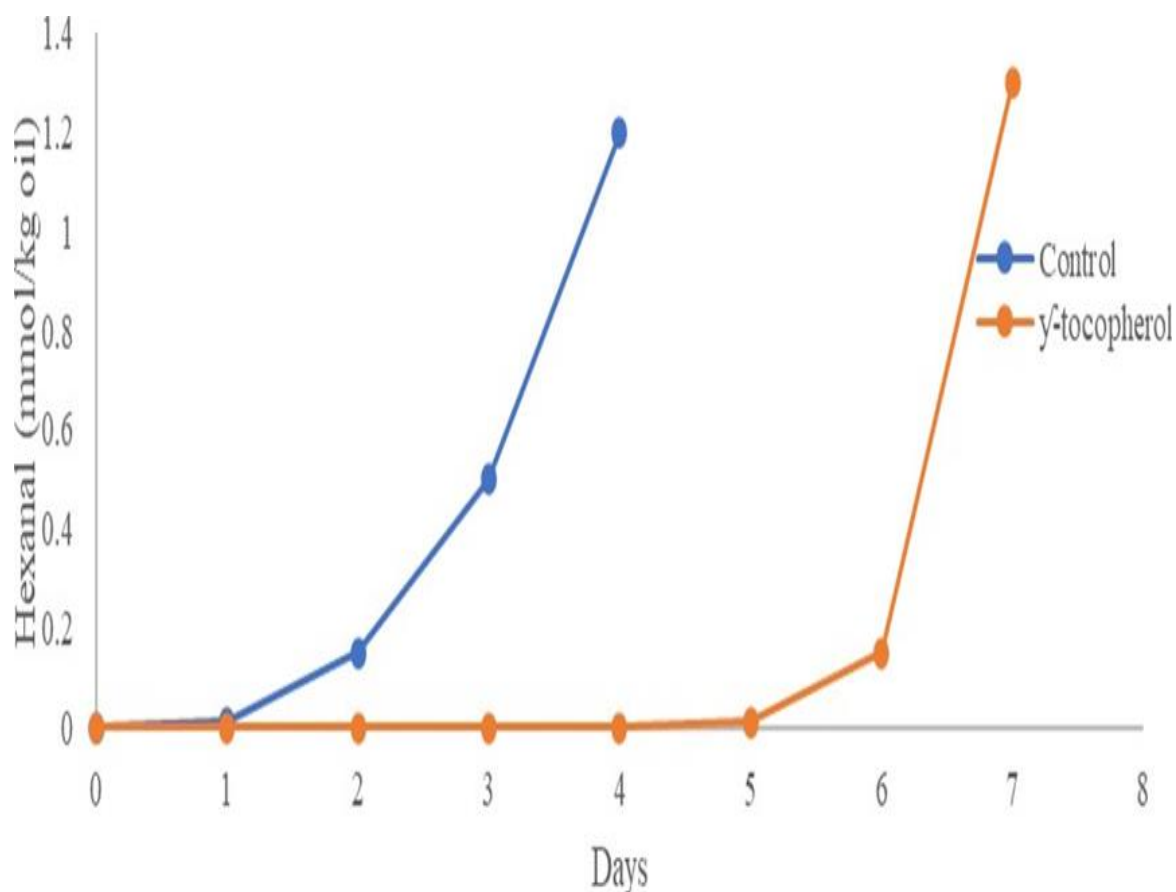


Figure 2.13: Influence of γ -tocopherol in the Lag Phase of Oxidation of Corn Oil in Water Emulsion (Adapted from McClement and Decker, 2009)

Zhou et al. (2019) stated phenolic compounds are widely distributed in plants as secondary metabolites which have vital roles in both plant and human health. Phenolics can lower CVD and hypertension, mainly attributed to their antioxidant properties (Alu'datt et al., 2017). Phenolics typically present in plants as free, esterified and insoluble phenolics are bound to macromolecules (Liu et al., 2017b). Degon et al. (2021) reported that TPC and total tocopherol in peanut butter are from 35.20 to 31.59 mg GAE/g and 19.05 mg/g, respectively. Suri et al. (2022) recorded %Radical scavenging assay (DPPH) and TPC in *N. sativa* oil were 60.21% and 100.02 µg GAE/mL, respectively.

2.11 Gas Chromatography-Mass Spectrometry (GC-MS)

Gas chromatography (GC) is a separation technique applied to determine the molecular composition of a gas-phase volatile sample. A chromatogram, heights and areas of isolated peaks and their chemical identification are among the data acquired (Blumberg, 2021). The fatty acid is derivatised into fatty acid methyl ester (FAMES) before analysis because it has a higher boiling point than FAMES.

The derivatisation of fatty acid to fatty acid methyl ester (FAME) is usually needed for the study of oil which comprises fatty acids with a carbon number larger than 10 (Chiu & Kuo, 2020). The derivatisation methods are classified into two types which are acid and basic derivatisations. The basic derivatisation methods required short times, no double bond isomerisation problem, easy operation and less aggressive reagents. However, this method is unsuitable for derivatising free fatty acids (Kramer et al., 1997; Murrieta et al., 2003; Petrović et al., 2010; KoohiKamali et al., 2012; Salimon et al., 2014). Sodium methoxide is typically used where it is reacted with lipid sample at 45°C for five minutes. Then the mixture

was neutralised with sodium bisulfate, and the FAMES were extracted using an organic solvent (Lankinen et al., 2009; Glaser et al., 2010). Next, potassium methoxide can be used to substitute sodium methoxide whereas the derivatisation using potassium methoxide generally requires condition at 50°C for a few minutes (Ostermann et al., 2014).

The HCl derivatisation method is the most common method used in acid derivatisation. The first step is to add methanolic HCl to the dried lipid, which is heated at a certain temperature for a certain period (Masood et al., 2005; Ichihara & Fukubayashi, 2010; Amusquivar et al., 2011). However, the major drawback is only particular lipid is soluble in HCl. Thus a few additions of solvent are needed (Ichihara & Fukubayashi, 2010). Next, the acetyl chloride method is by adding the acetyl chloride to the mixture of sample and methanol and is heated at 95°C for 60 minutes. The problem with this method is the inaccuracy of PUFA detection due to the instability at high temperatures from the exothermic reaction and the requirement of high temperature (Xu et al., 2010). Finally, sulphuric acid's derivatisation method is not recommended since it is a potent oxidising agent (Schlechtriem et al., 2008; Or-Rashid et al., 2010).

Gas chromatography has two phases: mobile and stationary. According to Nielsen and Qian (2017), the mobile phase is a chemically inert gas that acts as a carrier gas, transporting the analytes through the GC column. High purity gas is required, as are all regulators, gas lines and fittings of the highest quality. Nitrogen, helium and hydrogen are common carrier gases used in GC. A solid adsorbent or a liquid on inert support is used as the stationary phase.

Capillary column is typically used in GC due to having more advantages than pack column as most FAMES compound can be separated using capillary column such as DB1 or DB5. The selection of column is based on the properties of FAMES. I.e., a non-polar FAMES

compound is separated by non-polar column. The non-polar DB1 column isolated FAMES according to their boiling points, where lower boiling point compound will be eluted first (Mjos & Petterson, 2001). In addition, the DB5 column is an intermediate polar column which has very wide temperature range (60°C to 325°C) and very stable. Mjos and Petterson (2001) recorded the elution order of DB5 column is the unsaturated fatty acid will be eluted first than saturated fatty acid.

The mass spectrometer (MS) will ionise and fragment the separated analyte that exited the GC column at different retention times (Sneddon et al., 2007). Then the molecules and fragments are then accelerated using a mass analyser where the ions are classified according to their mass to charge ratio (m/z). The peak regions indicate the number of molecules in a complex compound. The separation of complexes by GC-MS produces many peaks in GC, where each peak has a unique mass spectrum that may be used to determine the molecule. The quality and quantity of fatty acids in oil samples can be determined using large, commercially accessible mass spectra libraries.

2.12 Fourier Transform Infrared Spectroscopy – Attenuated Total Reflectance (FTIR–ATR)

The FTIR is an effective, reliable and quick technique (Mohamed Shameer & Mohamed Nishath, 2019) used to obtain the infrared spectrum of absorption, emission and photoconductivity of solid, liquid and gas. It is used to detect different functional groups. The FTIR spectrum was recorded between 4000 and 400 cm^{-1} (Sindhu et al., 2015). FTIR can identify unknown materials, determine the sample's quality and the number of components in the mixture. The basic theory is that in infrared spectroscopy, IR radiation is

passed through a sample, some of the infrared radiation is absorbed by the sample, and some is passed through (transmitted). The resulting spectrum represents the molecular absorption and transmission, creating a molecular fingerprint or unique. Hasan et al. (2020) identified various functional group that present in *S. robusta* oil from India using FTIR. The significant peak, corresponding wave number and possible functional group are listed in Table 2.5.

ATR is one of the sampling methods for FTIR, which can acquire spectra from the surface of the original sample without preparation (Dong et al., 2020) by contact with a window containing ATR crystal, commonly germanium or diamond. Hind et al. (2001) explained in FTIR-ATR spectroscopy, the sample is in contact with the ATR crystal. As the radiation travels through the crystal, the radiation will interact with the sample. The total internal reflection referred to as the ‘Evanescent Wave’ occurs due to the differences in the refractive indices in the crystal where the wave extends into the sample. A small part of the infrared light is absorbed when the evanescent wave interacts with the sample producing a slightly attenuated total reflection.

Table 2.5: IR peaks, Corresponding Wave Numbers and Possible Functional Groups (Hasan et al., 2020)

Region of functional group	Wave number cm^{-1}	Functional group	Compound class
Hydrogen stretching	2924.09	Stretching of	Alkane
	2854.65	aliphatic CH_2 group	
Double bond stretching	1745.58	$\text{C}=\text{O}$ stretching ester carbonyl of TAGs	Aldehyde, ketone, carboxylic acid and α , β -unsaturated esters.
Bond deformation and bending	1462.04	Bending of aliphatic CH_2 and CH_3	Alkane
	1377.17		
Fingerprint region	1163.08	Stretching of C-O group	Alcohol and esters
	723.31	Out of plane vibration of <i>cis</i> - disubstituted olefins	Alkane

Ozulku et al. (2017) investigated the classification of pure sesame oils with adulterated hazelnut, canola and sunflower using a combination of ATR-FTIR and chemometric analysis. They recorded the spectra of pure sesame oils and vegetable oils such as hazelnut, canola and sunflower oil used for adulteration are very similar. A peak at 1744 cm^{-1} is the broad absorption band of triacylglycerides. A small peak at 3008 cm^{-1} (3009 to 3006 cm^{-1}) is the result of stretching of *cis* double bond oils ($=\text{CH}$), and the band's position depends on oil composition. Peaks detected at 3008 , 2923 and 2853 cm^{-1} are attributed to the stretching of hydrogen bonds. The most significant peaks at 2923 and 2865 cm^{-1} result from the asymmetric and symmetric stretching vibration of the aliphatic $-\text{CH}_2$ groups. Oils had peaks around 1462 cm^{-1} with weak intensity from bending vibrations of CH_2 and CH_3 groups and 1377 cm^{-1} with medium intensity from rocking vibration of *cis*-disubstituted olefins. A peak with 1098 cm^{-1} corresponds with the stretching of the C-O ester group. Finally, the peak at 722 cm^{-1} is from the rocking vibration of methylene $-\text{CH}_2$ and out of the plane of disubstituted olefins. However, the absorbance units were found to be different from each other depending on the functional group in oils. The classification of vegetable oils sesame, adulterated with sunflower, hazelnut and canola oils at 1% to 50% is determined using the functional group absorbance (peak) at 1267 - 1209 cm^{-1} and 1121 to 1045 and 896 - 814 cm^{-1} . The classification is conducted utilising a dendrogram (HCA) and score plot using the first and third principal components analysis. The author stated the variation in fatty acid concentration led to the discrepancy in FTIR data, which can play a significant role in oil clustering.

Dong et al. (2020) proposed an indirect analytical method to determine the FFA content in oil using ATR-FTIR spectroscopy. The FFA was converted to carboxylate species via reaction with phthalimide potassium salt, where they found a linear relationship between

FFA content with the peak areas at 1541-1616 cm^{-1} . They suggested that the proposed (ATR-FTIR spectroscopy) and American Oil Chemists' Society (AOCS) (titration method) have a reasonable correlation of $R^2 > 0.99$. In addition, they reported the proposed method has more advantages than the AOCS titration method as ATR-FTIR is of excellent reproducibility, has high exactitude, simple operation, easy instrument cleaning, covers a wide range of oil and has strong anti-disturbance.

2.13 Chapter Summary

This chapter summarises the botanical aspects of the Dipterocarpaceae family and *Shorea* genus. The species identification and distribution of several species of *Shorea* in Borneo have been compiled. Previous studies found that extraction methods and oil refining affect the chemical properties of oils. This chapter provides the studies conducted on *Shorea* oilseeds and seed oils. There are a few study gaps regarding *Shorea* oilseeds and seed oils that have been addressed: Limited information on *Shorea* oilseeds from Sarawak, the approach of modern oil extraction method is minute and the effect of refining on fatty acid composition and antioxidant activity in *Shorea* seed oils has not been recorded.

CHAPTER 3

METHODOLOGY

3.1 Sample Collection and Preparation

Approximately 10 kg of each species of *S. macrophylla*, *S. palembanica*, *S. stenoptera* and *S. pinanga* seeds were collected from Singai Village, Bau District, Sarawak, Malaysia and kept in the freezer (-4°C). Prior to extraction, the seed's wings were removed using a knife. The de-winged seeds were then sun-dried for three days and grinded into powder using an electric blender.

3.2 Proximate Analysis

Shorea oilseeds were subjected to proximate analysis and the determination of moisture and ash contents were conducted using method outlined by AOAC (2005). Moisture content was determined by heating empty crucible at 105°C in a furnace (Ney Vulcan, D- 550, United States) for 30 minutes and allowed to cool. The weight of the empty crucible was recorded and 2 g of seed sample was added into the empty crucible. The sample was heated in the furnace at 105°C for three hours. The sample was cooled in the desiccator for 30 minutes prior weighing. The moisture content of the sample was calculated by using Equation 3.1. Ash content was measured by burning the dried sample at 550°C for three hours. The burnt sample was cooled in the desiccator prior weighing. The percentage of ash content were then determined using Equations 3.2.

$$\% \text{ Moisture} = ((W_1 - W_2) / (W_1 - W_0)) * 100 \quad \text{Equation 3.1}$$

$$\% \text{ Ash} = ((W_3 - W_1) / (W_2 - W_1)) * 100 \quad \text{Equation 3.2}$$

where, W_0 is the weight of heated empty crucible; W_1 is the weight of sample and crucible before drying (g); W_2 is the weight of sample and crucible after drying (g); and W_3 is the weight of crucible (g).

The lipid content was determined according to procedure as proposed by Akbari et al. (2019). Approximately 100 g of dried *Shorea* oilseed was poured into a cellulose thimble and extracted using *n*-hexane and a Soxhlet extraction equipped with heating mantle (MTOPS, MS-EAM9202-03, South Korea) for six hours at 65°C to 70°C. Then, the solvent-oil mixture was subjected to a rotary evaporator (Buchi, Rotavapor R- 300, Germany) at 40°C. Oil yield was calculated using Equation 3.3. The obtained oil was stored at 4°C in a refrigerator to prevent oil degradation for further analysis.

$$\% \text{ Lipid} = (W_{\text{oil}} / W_s) * 100 \quad \text{Equation 3.3}$$

where, W_{oil} is the weight of oil yielded; W_s is the weight of seed sample.

The analysis of crude fibre was carried out by referring to the method performed by Madhu et al. (2017). Approximately, 2 g of samples were added into a 500 mL round-bottom flask. The anti-bumping chips and 200 mL of 0.25 M H_2SO_4 were poured into the flask. The mixture was boiled for 30 minutes at 80°C. The sample was filtered through muslin cloth and washed with boiling water until the wash was no longer acidic. The residue was then transferred into a round bottom flask and boiled with 200 mL of 0.3 M NaOH for 30 minutes. The residue was filtered through a muslin cloth and washed with 25 mL of boiling 1.25% H_2SO_4 , three 50 mL portions of water and 25 mL of alcohol. The residue was transferred

into a pre-weighed crucible (W_1). The crucible was then burned at 550°C for 30 minutes. The crucible was kept in a desiccator overnight. The weight of the crucible (W_2) was measured and the crude fibre content was calculated using Equation 3.4.

$$\% \text{Crude fibre} = ((W_2 - W_1) / \text{Weight of sample}) * 100 \quad \text{Equation 3.4}$$

The total carbohydrate of *Shorea* oilseeds was extracted according to the water extraction method as described by Smith et al. (1964). Exactly 1 g of dried defatted sample was transferred into a 200 mL round bottom flask containing 50 mL of distilled water. The mixture was then boiled under reflux for 1 hour. The mixture was filtered using Whatman No. 42 filter paper (pore size: 2.5µm) and the residue was washed with 25 mL of distilled water. The total resulting solution was transferred into a 100 mL volumetric flask and distilled water was used to top-up the solution to the calibrated mark.

The total carbohydrate content in water extracts was determined using the sulfuric acid-UV method as delineated by Albalasmeh et al. (2013). Exactly 1 g of fructose was dissolved using 100 mL of distilled water. Subsequently, a 15 mL of concentrated H_2SO_4 was transferred into a test tube containing 5 mL of dissolved fructose. The dark brick red solution (furan solution) formed and was immediately vortexed (Labnet, VX-200, United States) for 30 s. A serial dilution of 100, 50, 25, 15, 7.5 and 3.75 ppm of furan as a standard was established. Then, 5 mL of water extract was reacted with 15 mL of H_2SO_4 and kept as stock solution. Exactly 7 mL of stock solution was diluted into a 100 mL volumetric flask, where the solution was further diluted using distilled water. Exactly 20 mL of the resulted solution was transferred into a 50 mL volumetric flask and added with distilled water to the mark. The serial dilution of 100 to 3.75 ppm of standard and sample solution was analysed using a UV-Vis spectrometer (Agilent, Cary 5000, United States) at 276 nm. The total carbohydrate was calculated using Equation 3.5.

$$\% \text{Total carbohydrate} = ((142.88 * M * 0.1) / 10) \quad \text{Equation 3.5}$$

Where, 142.88 is the dilution factor; M is the concentration of sample in UV-Vis (mg/L); 0.1 is the weight of sample diluted in 0.1L (L/g) and 10 is the conversion to percentage.

3.3 Mineral Analysis

The minerals (Bi, Co, Cd, Ba, Fe, B, Cr, Cu, Ag, Al, Ti, Zn, Pb, In, Ni, Mn, Mg, Ca, Sr, Ga, Na, Li and K) content in seed samples were analysed on an Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES) (Perkin Elmer, Optima 8000, United States) according to a method employed from Morais et al. (2016). The ash samples produced from total ash determination were digested with 10 mL of 5% HNO₃. The mixture was then heated until a homogenous solution was formed. The solution was filtered using Whatman filter paper No. 40 (pore size: 8 µm) into a 100 mL volumetric flask. The filtrate is then diluted until the mark with distilled water. Prior to analysis, the solution was filtered with membrane filter paper (pore size: 0.45 µm).

3.4 Antinutritive Factors

The total oxalic acid in oilseeds samples was determined by following the procedure as proposed by Agboola and Adebayo (2019). Approximately 2 g of sample was added into 250 mL conical flask, then 190 mL of distilled water and 10 mL of 6 M HCl were filled into the flask. The mixture was boiled for 1 hour and then filtered with Whatman filter paper grade 4 (pore size: 25µm). The filtrate was transferred into a 250 mL volumetric flask and diluted to volume with distilled water. Subsequently, four drops of methyl red were added

into the solution and titrated with concentrated ammonia until the solution turned faint yellow. The solution was then heated to 100°C and allowed to cool for precipitation of ferrous ions. The precipitates were removed and the solution was added to 10 mL of 5% CaCl₂ and boiled. The solution was left to rest overnight. The solution was filtered using Whatman filter paper grade 1 (pore size: 11 µm) and the residues were kept and washed several times with distilled water. The residue was then dissolved with 5 mL of 25% H₂SO₄. The resultant solution was kept at 80°C and titrated against 0.02 M potassium permanganate until a pink colour persisted for approximately 1 minute. A blank was also run for the test sample. The oxalate content was calculated using Equation 3.6.

$$1 \text{ mL of } 0.02 \text{ M KMnO}_4 = 0.000225 \text{ g of oxalic acid} \quad \text{Equation 3.6}$$

Phytate levels in oilseeds were determined according to the procedure used by Sarkiyayi and Agar (2010). Briefly, 2 g of oil seed sample was soaked with 100 mL of 2% HCl for 3 hours and filtered through double layered filter paper (Whatman filter paper grade 4, pore size: 25 µm). The filtrate was then boiled and transferred into a 50 mL volumetric flask. The solution was topped up to the calibrated mark with distilled water. The solution was then added to 100 mL of distilled water and 10 mL of 3% ammonium thiocyanate as an indicator. The solution was titrated with FeCl₃ until the solution retained a brownish- yellow colour for five minutes. Distilled water as a blank was run in the test. The amount of phytate was calculated using Equation 3.7.

$$\% \text{ Phytate} = (((V_1 - V_b) * 0.00195 * 1.19) / W_0) * 100 \quad \text{Equation 3.7}$$

where, 1 mL of FeCl₃ solution is the 0.00195 grams of iron; 1 gram of iron = 1.19 of phytin-phosphorous; V₁ is the volume of FeCl₃ used for titration; V_b is the volume of FeCl₃ consumed by blank; and, W₀ is the weight of sample used.

3.5 Mechanical Extraction via PITEBA Oil Expeller

The oil was extracted using mechanical extraction method by oil expeller (Piteba, Holland) as shown in Figure 3.1, according to the method of (Popoola et al., 2016). The powdered oilseed sample will be filled into the feeder. An empty glass bottle was weighed to collect the extracted oil. The percentage yield will be calculated by using Equation 3.3.

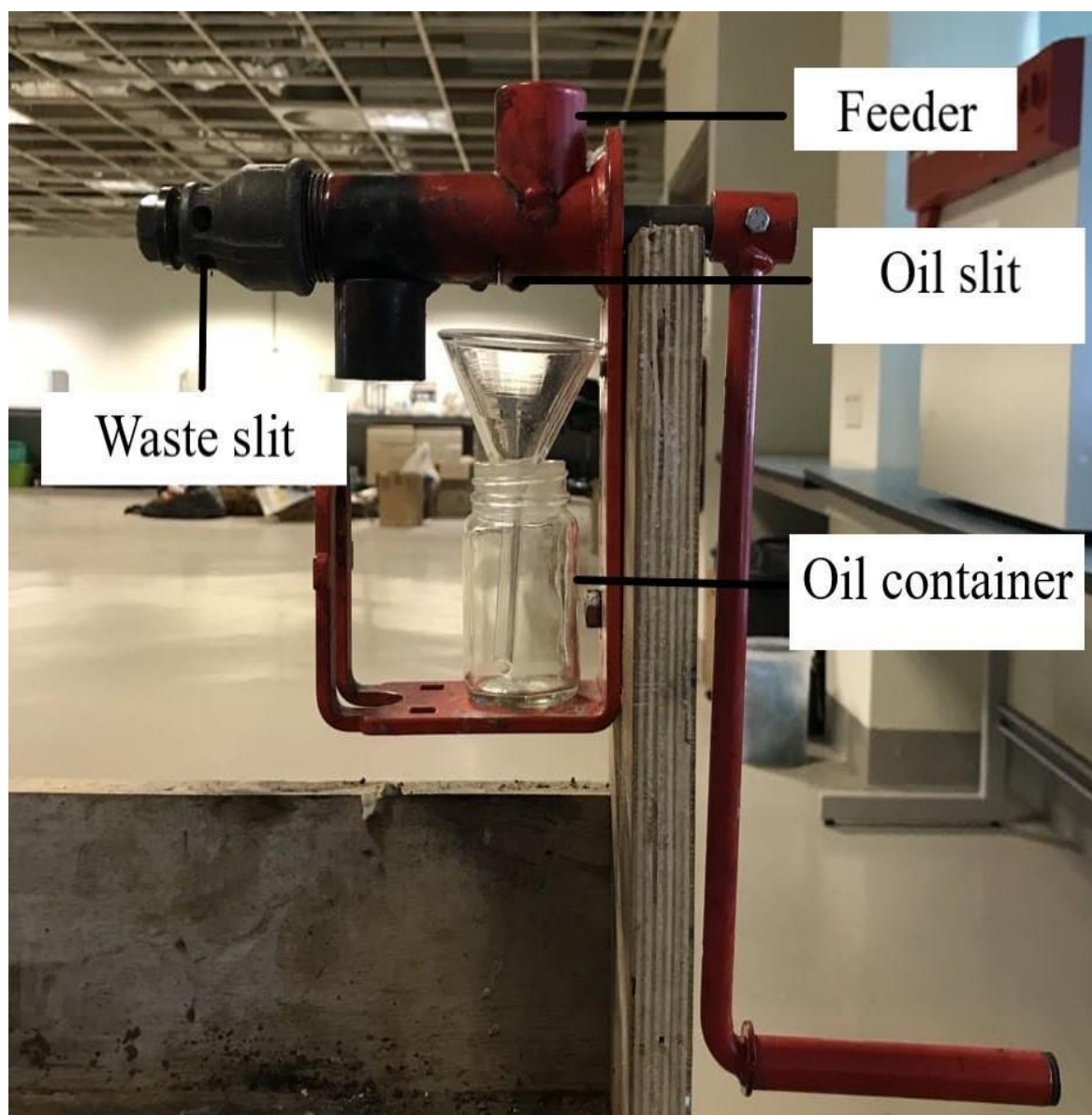


Figure 3.1: Piteba Oil Expeller

3.6 Oil Refining

The refining process of fat/oil was carried out according to the method outlined by Hidayat et al. (2019) with slight modification. Approximately 150 g of extracted fat was added into a 300 mL beaker and melted at a temperature 60°C to 70°C by double boiling. About 20% of phosphoric acid by 1% (w/w) was poured into melted fat and stirred at 400 rpm (rotation per minute) for 10 minutes using a magnetic stirrer. The mixture was poured into a separating funnel where 5% of hot water was added into the solution to wash the mixture. The solution was mixed and let rest until two layers were obtained. The upper layer is fat, which was then transferred into a beaker. Briefly, 1 M of NaOH was poured into the fat. The amount of NaOH added was equal to 7.5% of the degummed fat weight. The mixture was stirred with a magnetic stirrer for 15 minutes at 400 rpm at a temperature (60°C to 70°C). After the soap phase separated from the fat, the mixture was transferred to a separating funnel and 5% hot water was added. The lower part was discarded. The weight of the fat was measured, and 1% of the weight of the fat was added, which was then heated at 70°C for 30 minutes at 400 rpm. The oil was filtered from activated carbon using filter paper and a Buchner funnel.

3.7 Physical Properties of *Shorea* Seed Oils

The slip melting point (SMP) of *Shorea* seed oils was determined using the method outlined by Syafira et al. (2021). Solid fat from *Shorea* was inserted into a single open-end capillary tube until 1/3 of the tube was filled with fat. The melting point metre (Stuart, SMP 20, United Kingdom) was set at 27°C and increased by 0.5°C per minute. The SMP was

recorded in the beginning of the fat starting to melt and completely melt. The density of *Shorea* oil was measured manually by pipetting 1 mL of oil using a micropipette onto a four decimal point analytical balance (BIOBASE, BA1104B, China). The density was calculated using Equation 3.8.

$$\text{Density} = \text{mass (g)}/\text{volume (cm}^3\text{)} \quad \text{Equation 3.8}$$

3.8 Chemical Properties of *Shorea* Seed Oils

The acid value in oil was determined as outlined by official method Özogul et al. (2016). A neutral solvent containing a mixture of 25 mL of diethyl ether and 25 mL of ethanol was prepared. Approximately 0.1 g of oil was poured into a conical flask and added along with 20 mL of neutral solvent. The solution was boiled on a hot plate and added with 0.5 mL of 1% phenolphthalein indicator. The solution was then titrated with 0.1 N of NaOH until a light pink appeared. The acid value was calculated using Equation 3.9.

$$\text{Acid value (mg NaOH/kg oil)} = 40 \cdot N \cdot (V/W_0) \quad \text{Equation 3.9}$$

where N is the normality of NaOH; V is the volume of NaOH titrated; and W_0 is the weight of oil sample.

The determination of peroxide value from extracted oil was carried out by using method Cd 8b-90(1) of American Oil Chemists' Society as explained by Ayyildiz et al. (2015). Exactly 0.5 g of oil was poured into a 250 mL conical flask. Approximately 50 mL of a solution mixture of glacial acetic acid-chloroform (60:40, v/v) was added to the sample and swirled until a homogenous solution was formed. About 0.5 mL of saturated KI was pipetted and swirled for a minute. About 30 mL of distilled water was poured into the

solution. The solution was then titrated with 0.01 Na₂S₂O₃ until the yellow colour of the iodine solution almost disappeared and 0.5 mL of starch solution was pipetted to form a blue starch solution. The titration with Na₂S₂O₃ is continued until the blue solution disappeared. A blank determination was conducted using distilled water. The peroxide value of oil was calculated using Equation 3.10.

$$\text{Peroxide value (meq O}_2\text{/kg oil)} = 100 * ((V_s - V_b) / W_0) * M \quad \text{Equation 3.10}$$

where V_s is the volume of Na₂S₂O₃ titrated for determination; V_b is the volume titrated Na₂S₂O₃ for blank; M is the molarity of Na₂S₂O₃; and W_0 is the weight of oil sample.

The iodine value was determined according to the procedure outlined by Kabbashi et al. (2017). Exactly 0.1 g of oil sample was mixed with 4 mL of chloroform in a conical flask and added with 12 mL of Hanus solution. The flask was then closed using parafilm. The mixture was shaken continuously in dark place for an hour and then added with 4 mL of 15% KI solution and then 40 mL of distilled water. The solution was sonicated for 2 minutes and followed by titration with 0.1 N Na₂S₂O₃ until a yellow colour appeared. Approximately two to three drops of starch were dropped into the solution to produce a blue colour and the titration was continued until the blue colour disappeared. The iodine value was determined using Equation 3.11.

$$\text{Iodine value (g I}_2\text{/100 g oil)} = ((V_b - V_s) / W_0) * 0.1 \text{ N of Na}_2\text{S}_2\text{O}_3 * 12.69 \quad \text{Equation 3.11}$$

where, V_b is the volume of Na₂S₂O₃ titrated with blank; V_s is the volume of Na₂S₂O₃ titrated with presence of sample; and W_0 is the weight of oil.

3.9 Fatty Acid Characterisation of *Shorea* Seed Oils

The derivatisation of fatty acid into fatty acid methyl ester (FAME) was carried out according to the method explained by Aldai et al. (2005) with slight modification. Exactly 10 μL was pipetted instead of 100 μL of oil sample into a 6 mL vial. The oil was diluted with 1 mL of *n*-hexane and followed by the addition of 1 mL of 2 M NaOH. The mixture was sonicated for 5 minutes at 50°C. Then, 100 μL of 2M HCl was added to the solution. The solution was sonicated for 5 minutes at 50°C and subjected to centrifugation at 6000 rpm for 3 minutes. The solution was then incubated at 50°C for 24 hours. The upper layer, which contains FAMEs was separated from the aqueous solution and transferred into a clean vial.

Exactly 1 μL of FAMEs sample was injected into the gas chromatography-mass spectrometer (GC-MS, QP2010plus, Shimadzu, Japan) equipped with DB5 (30 m X 0.25 mm X 0.25 μm) column using auto-sampler (Shimadzu, AOC-20S, Japan). Operating conditions of GC-MS were as follows: The initial oven temperature was 50°C, held for 10 minutes. The temperature was then ramped at a rate of 4.5°C/min to 350°C and held for 10 minutes. The carrier gas was He at a flow rate of 1.0 mL/min and the spitless injection temperature was set at 280°C. The ionisation potential was 70 eV and the scanning range of the mass-selective detector was 45-600 m/z . Analyses were performed in triplicate ($n = 3$) and expressed as the mean average $\pm$ standard deviation. The peaks of FAME were identified by comparing the mass spectra with the MS library and standard Supelco 37 FAMEs mix (C4-C24) in dichloromethane.

The attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy was used to characterise the fatty acid profile in *Shorea* seed oil without further

preparation. The spectra of each sample were recorded in transmittance mode from 4000 to 400 cm^{-1} at a resolution of 4 cm^{-1} using FTIR spectrometer (Perkin Elmer, FT-IR 1605, United States). The background spectrum was first obtained prior to analysing samples to correct background noise. A small amount of fat (5 μL) was placed onto the surface of the crystal and analysed. The spectra were baseline corrected by using Omnic software. The determination was carried out in triplicate.

3.10 Determination of Antioxidant Activity and Total Phenolic Content (TPC) in Oil

The antioxidant activity was determined based on the radical scavenging activity of extracted oil using 2,2-diphenyl-1-picrylhydrazyl (DPPH) method and ascorbic acid as standards according to the method outlined by Wollinger et al. (2016) with slight modification. Exactly 0.005 g of DPPH was dissolved into 10 mL of methanol and then topped up with 40 mL of ethyl acetate. Approximately 1 mg of oil was dissolved into 1 mL of ethyl acetate. The concentration of 1.427, 2.849, 5.682, 7.092 and 11 ppm of dissolved oil and ascorbic acid were established by adding 0.1 mM DPPH. The absorbance was measured at 517 nm by UV-Vis (Agilent, Cary 500, United States). Triplicate measurements were taken for each concentration. IC_{50} values were calculated by using the IC_{50} graph prism.

The polyphenolic compounds were extracted according to the method Bouarroudj et al. (2016) for TPC assay. Exactly 0.5 g of oil was poured into the centrifuge tube followed by the addition of 10 mL of 80% methanol. The solution was centrifuged for 1 hour at 6000 rpm. The supernatant was transferred into a round bottle flask. The residue was reextracted with 10 mL of 80% methanol and then subjected to centrifugation for 1 hour at 6000 rpm. The supernatant was collected and mixed with the first supernatant. The resulting

supernatant was subjected to evaporation until dry. The dried sample was transferred into vial and was added with 3 mL methanol and refrigerate before analysis.

The total phenolic assay was carried out using Folin & Ciocalteu method as described by Kupina et al. (2018). A series of concentrations of gallic acid (40, 80, 120, 160 and 200 ppm) were prepared as calibration standards. Seven series of test tubes labelled A, B, C, D, E, F and G were prepared. Each test tube containing 15 mL of water and 1 mL of Folin & Ciocalteu's phenol reagent. Exactly 1 mL of gallic acid with concentration of 40, 80, 120, 160 and 200 ppm were pipetted into test tubes A, B, C, D and E respectively. While test tube F was added with 1 mL of polyphenolic extract, test tube G was added with 1 mL of distilled water, which served as a blank. The contents were mixed and allowed to rest for 6 minutes. Exactly, 3 mL of 20% sodium carbonate solution was added into each test tube and mixed well. The solution was then left to rest at room temperature for 2 hours. During measurement, 1 mL from each test tube was transferred into a cuvette and absorbance was recorded at 765 nm after zeroing the spectrophotometer (Agilent, UV-Vis Cary 500, United States). The calibration standard solutions were measured first, followed by the sample test solutions. The measurement was carried out in triplicate. Total phenols were calculated using Equation 3.12.

$$\text{Total phenols} = ((A - b)/m) * [(V*D)/(W)] * 100 \quad \text{Equation 3.12}$$

Where A is the absorbance of sample test solution at 765 nm; b is y intercept of calibration curve; m is the slope of calibration curve; W is the weight of test material (mg); V is the volume of sample test solution (1 mL); D is the dilution factor. Note that values are expressed as gallic acid equivalents (mg GAE/ kg oil).

3.11 Statistical Analysis

All experiments were repeated three times and the data are expressed as the mean value of triplicate $\pm$ standard deviation. The analysis of variance (ANOVA) was carried out using the Tukey test and the mean differences were considered significantly different if the p-value is ≤ 0.05 . The correlation between the effects of type of extraction and refining on chemical properties, fatty acid composition, antioxidant activity and total phenolic content were statistically measured using Point-Biserial Correlation Coefficient (r_{pb}). All statistical analysis were performed using IBM SPSS (Version 26).

3.12 Chapter Summary

This chapter describes the materials and methods used. The methodology consisted of sample collection, preparation, proximate composition, oil extraction, oil refining, physicochemical properties, antioxidant activity and total phenolic content of *Shorea* oils.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Proximate Composition of *Shorea* Seeds

The moisture, ash, lipid and fibre content of four *Shorea* oilseeds were determined and are shown in Table 4.1. The moisture content in oilseeds is significantly different ($p<0.05$) between species, although the oilseeds have been dried using a similar method. This is might due to genetic variation (Deme et al., 2017). The oilseed of *S. pinanga* has the highest moisture content, approximately 35.56%, while *S. stenoptera* has the lowest moisture content, 16.06%. Nesaretnam and Ali (1992) reported moisture content in *S. macrophylla* and *S. stenoptera* oilseeds was 5.2% and 5.9%, respectively. These amounts were lower compared to the amounts detected in this study with 21.47% and 16.06% for *S. macrophylla* and *S. stenoptera*, respectively. Variation in moisture content within species can be caused by different pre-treatment methods with temperature and duration affecting moisture content in seed (Mohamed Ahmed et al., 2021).

Table 4.1: Proximate Data of Four *Shorea* Oilseeds

Species	<i>S. macrophylla</i>	<i>S. palembanica</i>	<i>S. stenoptera</i>	<i>S. pinanga</i>
Moisture (%)	21.47±0.05 ^a	27.25±0.04 ^b	16.06±0.02 ^c	35.56±0.05 ^d
Ash (%)	0.67±0.01 ^a	1.98±0.03 ^b	4.42±0.06 ^c	2.23±0.06 ^d
Total lipid	41.37±0.03 ^a	49.06±0.03 ^b	38.80±0.81 ^a	46.91±0.78 ^c
Total Fibre (%)	15.67±0.14 ^a	7.72±0.15 ^b	30.68±0.02 ^c	8.09±0.01 ^d
Carbohydrate (%)	79.44±0.00 ^a	82.37±0.00 ^b	66.45±0.00 ^c	79.64±0.00 ^a

Note: Data are expressed in average of triplicates with standard deviation; Value within each row with different superscripts (^{a-d}) are significantly different ($p<0.05$). Defatted sample was used in the analyses of total fibre and carbohydrate.

Nesaretnam and Ali (1992) used a controlled chamber temperature at 30°C with 40% humidity for three days to dry *S. macrophylla* and *S. stenoptera* seeds and the moisture content was decreased below 10% using an oven. The seeds that were sundried for three days have higher moisture content. Reducing moisture content in oilseed is required to slow the rate of deterioration and thus improve oil extraction efficiency (Pérez et al., 2019). Mwithiga and Moriasi (2007) recommended the moisture content of the seed be kept between 5.0% and 9.3% to increase the yield of the expeller method. Thus, further drying processes in all *Shorea* oilseeds have been carried out to reduce the moisture content and temperatures below 4°C are required during storage to minimise oil seed deterioration. Ash content is the amount of unignited inorganic residues that indicates the total metal content in oilseed. As shown in Table 4.1, the ash content of *Shorea* oilseed varies significantly between species ($p < 0.05$), with *S. stenoptera* oilseed having the lowest ash content compared to other species. The ash content of *S. stenoptera* oilseed (4.42%) is comparable to the ash content of *S. robusta* oilseed (6%) as reported by (Singh et al., 2014).

Seeds with high oil content are preferable industrially and considered worth extracting (Shashi et al., 2016). Soxhlet extraction with *n*-hexane as extracting solvent was used to determine the total lipid content in *Shorea* oilseeds. The highest oil yield is from *S. palembanica* (49.06%) followed by *S. pinanga* (46.91%). Oil content does not differ significantly ($p > 0.05$) between *S. macrophylla* (41.37%) and *S. stenoptera* (38.80%). Nesaretnam and Ali (1992) reported a higher oil content extracted with Soxhlet extractor using petroleum ether as a solvent in *S. macrophylla* (55.90%) and *S. stenoptera* (52%) than in this study.

The variation in oil content might be due to differences in extracting solvent (Keneni et al., 2021) used the drying method (Fawole et al., 2022) and seeds maturity (Zahran et al.,

2020; Keneni et al., 2021). Theoretically, *n*-hexane is more efficient in extracting oil from seeds than petroleum ether (Banat et al., 2013). However, the difference in moisture content in seeds due to different drying methods (Fawole et al., 2022) increased the water in the solvent, leading to solvent hydration. Solvent hydration is the amount of water content in the solvent that reduces the oil yield during extraction as water reduces the solubility in the solvent at a certain temperature (Capellini et al., 2017). This resulted in lesser amount of oil yielded. In addition, the maturity of the seeds affects the oil content in the seed where the ripe seed has higher oil content than the unripe seed (Zahran et al., 2020; Keneni et al., 2021).

The crude fibre of *S. stenoptera* oilseed (30.68%) is significantly higher ($p < 0.05$) than *S. macrophylla* (15.67%), *S. pinanga* (8.09%) and *S. palembanica* (7.72%). The amount of crude fibre in *Shorea* oilseeds is comparable with soybean seed (Li et al., 2015), sesame seed (Dravie et al., 2020) and sunflower seed (Zajac et al., 2021) which were 3.6 to 9.9%, 8.22% and 17.1%, respectively. Adequate dietary fibre consumption can help to prevent constipation, promote satiety and maintain stable reproductive performance (Li et al., 2021).

The calibration curve of standard fructose was constructed at 276 nm wavelength using UV-vis and shows good linearity ($R^2 = 0.995$) shown in Appendix 1. The highest total carbohydrate was detected in *S. palembanica* (82.37%) followed by *S. pinanga* (79.64%). In this study, the total carbohydrate amount in *Shorea* oilseed was higher than in *S. robusta* (42.11%) (Shashi et al., 2016).

4.2 Mineral Contents of *Shorea* Seeds

The calibration curves of standards constructed for Na, K, Mg and Ca shows good linearity as shown in Figure 4.1. The calibration curves for other elements are presented in Appendices 2 to 6. Table 4.2 presents ultra-trace, micro and macro mineral composition and antinutritive factors in *Shorea* oilseeds. The macro minerals between *Shorea* oilseeds are significantly different ($p < 0.05$). K is the predominant macro mineral in *Shorea* which is *S. palembanica* (400.17 mg/100 g), *S. stenoptera* (419.23 mg/100 g), *S. pinanga* (507.92 mg/100 g) and *S. macrophylla* (1186.50 mg/100 g). Mg is the second most abundant macro mineral in *S. macrophylla* (300.50 mg/100 g) and *S. stenoptera* (78.66 mg/100 g), while Ca is the most abundant in *S. palembanica* (117.17 mg/100 g). Na in *S. pinanga* is significantly higher compared to other *Shorea* species with 356.22 mg/100 g. Diets with a high K to Na ratio can lower the risk of hypertension (Millena & Sagum, 2018). This suggests that *Shorea* oilseeds can be considered a good source of macro minerals.

Shorea oilseeds also contain microminerals such as Zn, Mn and Ga have also been detected. There is significant variation in microminerals between species ($p < 0.05$). Zn is the dominant micromineral in *S. stenoptera* (13.00 mg/100 g), followed by *S. macrophylla* (6.97 mg/100 g). Mn is dominant in *S. macrophylla* (12.43 mg/100 g), followed by *S. pinanga* (2.69 mg/100 g). The amount of Zn and Mn in *Shorea* is comparable with other common oil seeds such as sesame seed (4.96 to 6.25 mg/100 g and 1.15 to 1.57 mg/100 g) (Deme et al., 2017), soybean seed (4.14 to 7.7 mg/ 100 g and 2.97 to 7.08 mg/100 g) (Ibáñez et al., 2020) and sunflower seed (4.13 and 0.39 mg/100 g) (Subaşı et al., 2020).

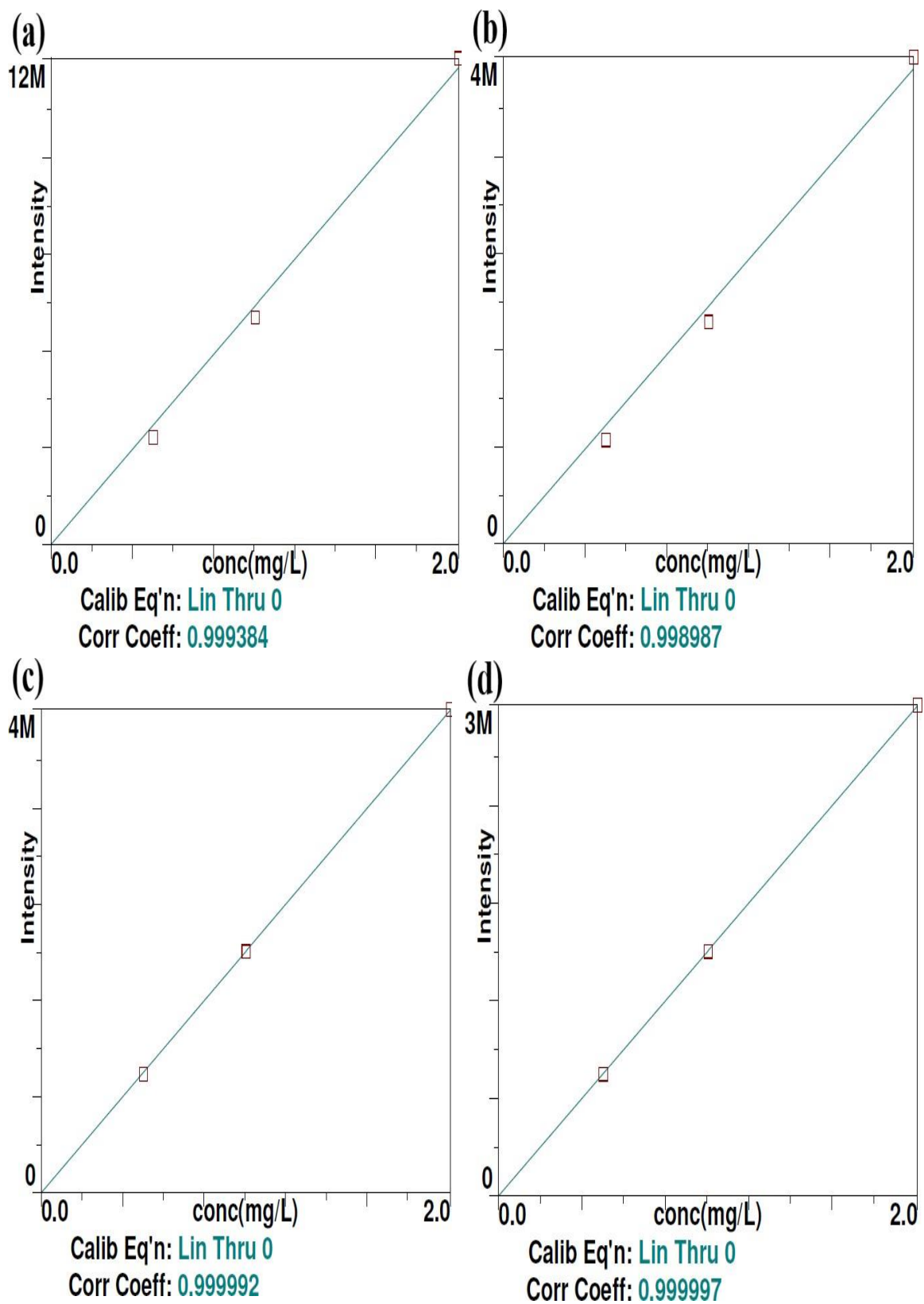


Figure 4.1: Calibration Curve of Standard Minerals Na, K, Mg and Ca

Table 4.2: Mineral Composition in *Shorea* oilseeds

Mineral	Species			
	<i>S. macrophylla</i>	<i>S. palembanica</i>	<i>S. stenoptera</i>	<i>S. pinanga</i>
Ba	n.d.	n.d.	0.91±0.00 ^a	0.90±0.00 ^a
Al	n.d.	1.01±0.88 ^a	0.75±0.13 ^a	0.90±0.40 ^a
Zn	6.97±2.23 ^a	4.04±3.8 ^a	13.00±0.80 ^b	4.04±0.08 ^a
Mn	12.43±0.80 ^a	0.67±0.60 ^b	1.36±0.00 ^{bc}	2.69±0.00 ^c
Ga	n.d.	n.d.	0.23±0.00 ^a	0.75±0.00 ^b
Mg	300.50±0.00 ^a	117.17±0.04 ^b	78.66±0.00 ^c	77.43±0.00 ^c
Ca	238.31±0.00 ^a	128.62±0.00 ^b	24.81±0.00 ^c	51.42±0.00 ^d
Na	75.12±0.00 ^a	30.14±0.00 ^b	4.22±0.00 ^c	356.22±0.50 ^d
K	1186.50±0.00 ^a	400.17±0.01 ^b	419.23±0.02 ^c	507.92±0.03 ^d

Note: Mineral data are expressed in mean average of triplicates± standard deviation mg/100 g, respectively; Mean within row using same superscript letters are not significantly different ($p>0.05$). Pb, Cd, Fe, Ag, Li, Cr, Co, Bi, Cu, In, Sr, Ni and Ti are undetected in samples.

Al and Ba are in trace amounts in *Shorea* oilseeds, except *S. macrophylla* which is undetected. The variation in mineral composition among *Shorea* oilseeds might be due to the ability of plants to accumulate different types of minerals due to genetic variation (Moreda-Piñeiro et al., 2016). The content of toxic heavy metals such as Pb, Cd, Cr, Li and Ni are below detection in the studied sample. Therefore, *Shorea* seeds may be considered safe for consumption.

4.3 Antinutritive Factors in *Shorea* Seeds

The bioavailability of macro and micronutrients in humans and animals is limited by anti-nutritional found in food and plant sources (Deme et al., 2017; Millena & Sagum, 2018). High intakes of anti-nutritional factors can adversely affect the health and growth of humans and animals. Table 4.3 presents the compositions of anti-nutritive in *Shorea* oilseeds. The amount of oxalate varied between species ($p<0.05$), with the highest amount being detected

in *S. pinanga* seed (3.61%) followed by *S. stenoptera* seed (2.65%). The amount of oxalate in *Shorea* oilseeds is higher compared to other seeds reported by other researchers, such as cashews (0.26%), and almonds (0.47%) (Chai & Liebman, 2005b) and soybeans (0.37%) (Shi et al., 2018). Petroski and Minich (2020) suggested oxalate can reduce the bioavailability of minerals such as Ca, Na, K, Fe and Mg by forming insoluble complexes which cannot be absorbed by the body and lead to kidney stone formation. Shi et al. (2018) stated that 5 g of oxalate in diets could cause death in humans due to its corrosive gastroenteritis characteristics, convulsions and renal damage. Thus, pre-treatment such as soaking and cooking is suggested before consumption to reduce the intake of oxalate (Chai & Liebman, 2005a; Alemayehu et al., 2021).

Table 4.3: Antinutritive Factors in *Shorea* Oilseeds

Antinutritive factors	Species			
	<i>S. macrophylla</i>	<i>S. palembanica</i>	<i>S. stenoptera</i>	<i>S. pinanga</i>
Oxalate%	1.91±0.04 ^a	2.43±0.02 ^b	2.65±0.01 ^c	3.61±0.03 ^d
Phytate%	0.29±0.06 ^a	0.29±0.03 ^a	0.09±0.01 ^b	0.06±0.01 ^b

Note: Antinutritive data are expressed in mean average of triplicates± standard deviation (%), respectively; Mean within row using same superscript letters are not significantly different (p>0.05).

The phytate level in *S. macrophylla* and *S. palembanica* seeds is not significantly different (p>0.05) which was 0.29%. The amount of phytate present in *S. macrophylla* and *S. palembanica* is considerably higher (p<0.05) compared to *S. stenoptera* (0.09%) and *S. pinanga* (0.06%). Phytate content in *Shorea* oilseeds is comparable to other common seeds such as Niger seed (0.35%), linseed (0.10%) and sesame seed (0.29%) (Deme et al., 2017). Phytate is produced during seed development and is the primary phosphorous storage in the plant. Phytate readily binds with mineral cations which are insoluble and cannot be digested

by the human body as humans lack phytase enzymes (Gibson et al., 2018). Millena and Sagum (2018) have reported the presence of phytate or phytic acid in Philippine Pili nuts (*Canarium ovatum* Engl.) which reduce the availability of Fe and Zn minerals in the human body. Despite the negative effect on the consumption of diets incorporating phytates towards mineral availability, several studies have highlighted the roles of phytates in diets such as antioxidant, anticancer, hypocholesterolemic and hypolipidemic (Vucenik & Shamsuddin, 2006; Cocato et al., 2019).

The differences in proximate composition, mineral and antinutritive factors between *Shorea* oilseeds of different species may be due to genetic, location and environmental factors such as type of soil and water (Deme et al., 2017). Data of proximate composition, mineral concentration and anti-nutritive aspects of *Shorea* seeds gives essential information on nutritional value and serves as a reference for diet formulation in which *Shorea* seeds can be considered alternative feeds.

4.4 Oil Extraction Methods of *Shorea* Seed

The optimal extraction method was determined based on oil yield, fatty acid profile, chemical properties, antioxidant activity and TPC of *S. palembanica* oils extracted using mechanical expeller and Soxhlet extraction, as shown in Table 4.4. Soxhlet extraction yielded a significantly high amount of oil from *S. palembanica* seeds compared to mechanical extraction with 49.06% and 40.82% ($p < 0.05$), respectively. Kemerli-Kalbaran and Ozdemir (2019) reported that Soxhlet extraction was more efficient than mechanical extraction. The fatty acid profile of *S. palembanica* oils is listed in Table 4.4, with seven identified individual fatty acids.

A higher percentage of MUFAs was detected in mechanical extraction oils compared to Soxhlet extracted oils. *Cis*-MUFAs such as gondoic acid were identified in mechanical extraction oils, while harmful *trans*-MUFAs like elaidic acid were detected in Soxhlet extracted oils. Song et al. (2015) stated the formation of *trans*-fat in oil is due to high-temperature treatment, which is supported by findings from Żyżelewicz et al. (2014) who found an increase in the formation of *trans*-fatty acid in cocoa butter due to the high temperature used during the roasting process. The intake of *trans*-MUFA is not recommended as it contributes to the accumulation of cholesterol in arteries and increases the risk of hypertension (Eckel et al., 2014; Lottenberg et al., 2018).

The acid and peroxide values are important parameters in determining the oil quality. The acid value shows the degree of rancidity in oil hydrolysis since the acid value measures the level of FFAs formed after lipid hydrolysis (Cong et al., 2020). Oil with low acid value is desirable in the food industry because high FFAs lower the oil odour and taste quality (Li et al., 2013). The acid value in Soxhlet extracted oil is significantly higher ($p < 0.05$) compared to mechanical extraction oil. The increase in acid value is due to the high temperature used during Soxhlet extraction. This is consistent with those reported by other researchers (Tenyang et al., 2017; Djikeng et al., 2018).

Table 4.4: Quality Parameters for *S. palembanica* Oils Extracted Using Mechanical Extraction and Soxhlet Extraction

Parameter	<i>S. palembanica</i>	
	Mechanical extraction	Soxhlet extraction
Oil yield	40.82±0.89 ^a	49.06±0.03 ^b
Fatty acid (%)		
Palmitic acid	14.12±2.53 ^a	18.12±3.69 ^b
Heptadecanoic acid	0.25±0.22 ^a	0.20±0.17 ^a
Stearic acid	46.71±7.07 ^a	43.60±6.46 ^a
Arachidic acid	4.03±2.06 ^a	3.27±1.13 ^a
Behenic acid	0.31±0.28 ^a	0.25±0.22 ^a
Lignoceric acid	0.09±0.16 ^a	0.15±0.13 ^a
ΣSFA	65.51 ^a	65.59 ^a
Oleic acid	34.34±1.80 ^a	34.23±0.98 ^a
Gondoic	0.15±0.13	n.d.
Elaidic acid	n.d.	0.17±0.15
ΣMUFA	34.49 ^a	34.40 ^a
Linoleic acid	n.d.	n.d.
ΣPUFA	n.d.	n.d.
Chemical Properties		
Acid value (mg NaOH/ kg oil)	3.47±0.19 ^a	8.00±0.00 ^b
Peroxide value (meq O ₂ /kg oil)	10.62±1.12 ^a	14.58±1.20 ^b
Free radical scavenging activity (IC ₅₀ µg/mL)	813.02±9.01 ^a	238.51±4.50 ^b
Total phenolic content (mg GAE/kg oil)	2.49±0.01 ^a	0.68±0.03 ^b

Note: Data are expressed in average of triplicates with standard deviation; The difference between ME and SE oil was tested using Tukey HSD test. The mean within each row with different superscript^(a-d) are significantly different (p<0.05)

The peroxide value indicates the level of oil deterioration by measuring the main products of primary lipid oxidation which are lipid peroxide and hydroperoxide in oil. The peroxide value must be limited as the decomposition of lipid hydroperoxides will produce additional radicals which contribute to an exponential increase in oxidation and the decomposition of lipid hydroperoxide will also produce volatile compounds that produce off-flavours known as rancidity (McClements & Decker, 2009; Syed, 2016). Soxhlet extracted oil has a higher peroxide value than mechanically extracted oil, which are 14.58 meq O₂/kg and 10.62 meq O₂/kg, respectively. Mohamed Ahmed et al. (2021) reported that increased temperature during roasting (pre-treatment) before oil extraction increases the

peroxide value in sesame oil. Djikeng et al. (2018) recorded a high level of peroxide value in cocoa butter roasted at 200 to 220°C (traditional method) compared to 180°C (temperature-controlled oven). Temperature, light and oxygen induce lipid oxidation (Grosshagauer et al., 2019; Fruehwirth et al., 2020). Thus, high peroxide value in Soxhlet extracted oil compared to mechanically oil due to high temperature used during extraction.

The determination of the free radical scavenging activity of DPPH for the non-polar extract of *S. palembanica* oils is shown in Table 4.4. A low IC₅₀ value indicates high radical scavenging activity, implying that oils have high antioxidant activities. Non-polar extract from *S. palembanica* oils possesses weak antioxidant activity compared to ascorbic acid (IC₅₀ 2.44 µg/mL). The higher IC₅₀ value in mechanically extracted oil (813.02 µg/mL) compared to Soxhlet extracted oil (238.51 µg/mL) might be due to non-polar solvent usage *n*-hexane as extracting solvent during extraction which increases the solubility of non-polar antioxidants into the oils.

Phenolics are important bioactive components in plant oils and function as reducing agents, singlet oxygen quenchers, hydrogen donors and metal chelators (Grajzer et al., 2020). Phenolic compounds from *S. palembanica* oils were extracted using 80% methanol, and the total phenolic content (TPC) is presented in Table 4.4. The mechanically extracted oil has higher TPC than Soxhlet extracted oil with 2.49 mg GAE/kg oil and 0.68 mg GAE/kg oil, respectively. A similar result was reported by Can-Cauich et al. (2019) for oils extracted using mechanical extraction with an extraction temperature of 45°C has higher TPC than those extracted using Soxhlet extraction with an extraction temperature of 75°C. High temperatures during extraction stimulate lipid hydroperoxide breakdown, which can oxidise the oils' beneficial components (Papuc et al., 2017). Non-polar solvents during Soxhlet extraction lower the phenolic content in oils because phenolic compounds are polar. Thus,

phenolics dissolve poorly in *n*-hexane solvent, reducing the phenol content in oils. Similar findings were reported by other researchers, Mokrani and Madani (2016), Taghizadeh et al. (2018) and Martínez-Ramos et al. (2020).

Statistical analysis of Point-Biserial correlation (r_{pb}) was conducted to test whether types of extraction have a significant effect on the chemical properties of *S. palembanica* oil as shown in Table 4.5. There was a negative correlation between the type of extraction and total UFA and was statistically significant ($r_{pb} = -1.0$, $n = 6$, $p < 0.01$) which indicates the significant effect of type of extraction on total UFA which lowers UFA was yielded using Soxhlet extraction compared to mechanical extraction. A positive correlation was recorded between the types of extraction, acid and peroxide values which was significant ($r_{pb} = 1.0$, $n = 6$, $p < 0.01$) and ($r_{pb} = 0.90$, $n = 6$, $p < 0.05$). The positive correlation indicates higher acid and peroxide values in Soxhlet extracted oil compared to mechanically extracted oil. There was a negative significant correlation between the type of extraction and IC_{50} value ($r_{pb} = -1.0$, $n = 6$, $p < 0.01$) which entails a higher amount of non-polar antioxidant in Soxhlet extracted oil compared to mechanically extracted oil. The significant negative correlation between the type of extraction and TPC ($r_{pb} = -1.0$, $n = 6$, $p < 0.01$) indicates lower TPC in Soxhlet extracted oil compared to mechanically extracted oil. The Soxhlet extraction method produces high yields of oil but the mechanical extraction method produces high quality oils. Thus, the mechanical extraction method was selected for *Shorea* at the later stage of the study.

Table 4.5: Point-Biserial Correlation Analysis (r_{pb}) on Type of Extraction towards Chemical Properties, Antioxidant Activity and TPC

	UFA	AV	PV	DPPH	TPC
Type of extraction	-1.000**	1.000**	.902*	-1.000**	-1.000**

** . Correlation is significant at the 0.01 level (2-tailed)

* . Correlation is significant at the 0.05 level (2-tailed)

4.5 Physical Properties of *Shorea* Oils

Figure 4.2 shows the colour of the crude and refined *Shorea* oils extracted using mechanical extraction. Visual observation found that refined *Shorea* seed oils have a lighter colour than their crude counterparts. Refining at the bleaching stage using activated carbon adsorbed dark pigmentation from the oils has been reported by Pal et al. (2015). The density of crude and refined *Shorea* oils is presented in Table 4.6. Crude *S. palembanica* oil has the highest density with 0.969 cm^3 , followed by *S. macrophylla* crude oil, which is 0.925 cm^3 . Refined oils have a lower density than their crude oils because of the removal of impurities. Abdullahi et al. (2021) reported that the degumming process of *Acacia nilotica* seed oil reduced oil impurities and the report is in lined with Chew et al. (2017).

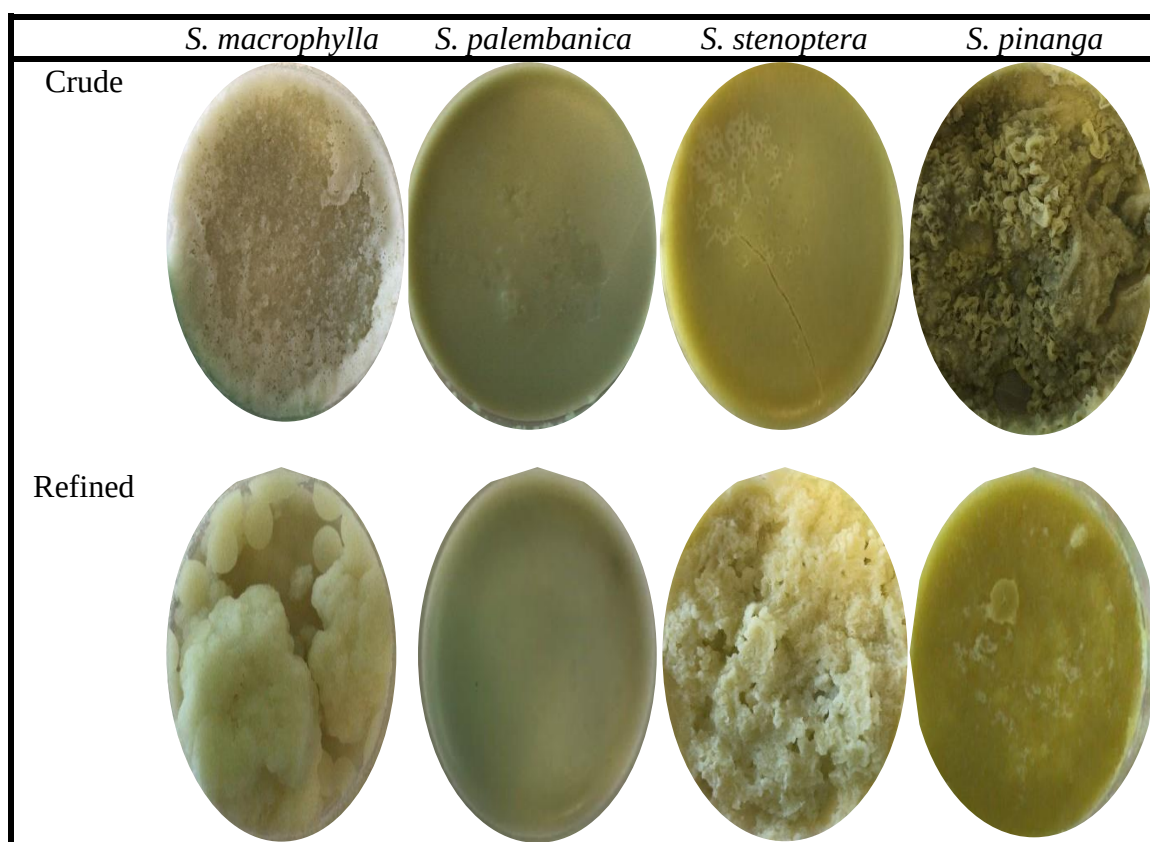


Figure 4.2: Crude and Refined *Shorea* Oils

The highest slip melting point (SMP) is crude *S. stenoptera* oil which is 35.0 to 62.8°C followed by its refined oil which is 34.1 to 62.0°C. The lowest SMP is the refined oils from *S. stenoptera* and *S. pinanga* which are 29.0 to 41.1°C and 30.0 to 41.90°C, linked to the low content of SFA in refined oils than their respective crude. Norazlina et al. (2022) reported a decrease in the SMP of Bambang (Mangifera pajang) kernel lipids when combined with cocoa butter due to the increased amount of UFAs from cocoa butter. Shin et al. (2021) reported that the blending of duck fat (high SFA content) with soybean oil (low SFA content) increased the melting point of margarine.

Table 4.6: Slip Melting Point (SMP) and Density of *Shorea* Oils

Oil	Density (g/cm ³)	SMP (°C)
<i>S. macrophylla</i>		
Crude	0.925	38.0-50.0
Refined	0.917	31.0-56.0
<i>S. palembanica</i>		
Crude	0.969	30.0-42.0
Refined	0.903	34.1-62.0
<i>S. stenoptera</i>		
Crude	0.912	35.0-62.8
Refined	0.908	29.0-41.1
<i>S. pinanga</i>		
Crude	0.906	34.0-43.0
Refined	0.901	30.0-41.90

The SMP of cocoa butter is 25 to 37°C, ideal for chocolate and confectionery industries. Still, its usage is limited due to fat blooms (Bahari & Akoh, 2018b) and softening problems in subtropical and tropical countries (Zhang et al., 2020). Butter with a high melting point can increase fat bloom resistance (Bahari & Akoh, 2018b) and reduce softening problems (Norazlina et al., 2022). Therefore, this study indicates that *Shorea* oils have potential as cocoa butter replacers or blends in the chocolate industry.

4.6 Chemical Properties of *Shorea* Oils

The acid value in *S. macrophylla* crude oil (3.75 mg NaOH/g) is significantly higher than the crude oils of *S. palembanica* (3.47 mg NaOH/g), *S. pinanga* (2.93 mg NaOH/g) and *S. stenoptera* (1.67 mg NaOH/g) as shown in Table 4.7. Contrary to our expectation that mechanically extracted oil contains a lower acid value compared to Soxhlet extracted oil in Section 4.4, we found a higher acid value in crude *S. macrophylla* extracted via mechanical extraction in this study compared to *S. macrophylla* oil extracted using Soxhlet extraction in the previous study reported by Nesaretnam and Ali (1992) which was 0.38 mg NaOH/g (equivalent to 0.27% FFA oleic acid). The primary cause of the discrepancy is the variation in drying methods. Nesaretnam and Ali (1992) dried samples using an environmental control chamber at 30°C and 40% humidity for three days and dried them further in an oven until the moisture content was reduced to approximately 10%, while the samples in the present study were sundried for three days and the moisture content was further reduced to less than 10%.

The high acid value recorded in current crude *S. macrophylla* oil was also due to UV radiation from sunlight which facilitates the breakdown of TAGs into FFA. This is in line with Adenuga et al. (2021). They reported a higher acid value in *Calophyllum inophyllum* in oil extracted from sundried seed compared to air-conditioned and oven-dried samples (Adenuga et al., 2021). Crude *Shorea* oils in the present study are lower compared to *S. robusta* (45.09 mg NaOH/g) and *S. stenoptera* (15.48 mg NaOH/g), as reported by Shashi et al. (2016) and Darmawan et al. (2020), respectively due to the difference in sampling location (Haile et al., 2019).

Table 4.7: Chemical Properties of Crude and Refined *Shorea* Seed Oils Extracted Using Mechanical Extraction

Species	Oil	AV (mg NaOH/g)	PV (meq O ₂ /kg)	IV (g I ₂ /100g)
<i>S. macrophylla</i>	Crude	3.75±0.21 ^g	7.96±0.02 ^{def}	27.31±0.82 ^{bcd}
	Refined	1.97±0.20 ^{cd}	5.96±0.01 ^{bcd}	31.61±1.25 ^{efg}
<i>S. palembanica</i>	Crude	3.47±0.19 ^{fg}	10.62±1.12 ^g	31.91±1.25 ^{efg}
	Refined	1.11±0.08 ^a	5.59±0.01 ^{bcd}	30.15±0.26 ^{def}
<i>S. stenoptera</i>	Crude	1.67±0.13 ^{bcd}	8.91±0.09 ^{efg}	26.34±0.52 ^{bc}
	Refined	1.47±0.23 ^{abcd}	3.92±0.10 ^{ab}	36.79±0.76 ^a
<i>S. pinanga</i>	Crude	2.93±0.23 ^{ef}	3.90±0.10 ^{ab}	26.18±1.94 ^{bc}
	Refined	1.94±0.10 ^{cd}	2.57±0.51 ^{da}	30.33±2.08 ^{defg}

Note: Data are the mean percentage of three replicates ± standard deviation (s.d); Means in the same column with the same superscript are not significantly different at 5% level.

Darmawan et al. (2020) stated that the Indonesian National Standard (SNI) for Illepe butter (*S. stenoptera*) must be lower than 3.5 mg NaOH/g oil. Thus, oil refining for *Shorea* oils is needed. A significant reduction in acid value was observed after refining as shown in Table 4.7. The decline in acid value is due to the neutralisation process in which FFAs react with NaOH to form soap (Gusti & Zulnely, 2015; Zahrina et al., 2018; Darmawan et al., 2020). Codex Alimentarius (1999) sets the limit of acid value for edible oil at 4 mg KOH/g oil (2.85 mg NaOH/g oil). All refined oils studied are within the permissible limit.

S. palembanica crude oil (10.62 meq O₂/ kg oil) has the highest peroxide value compared to other crude oil *S. stenoptera* (8.91 meq O₂/ kg oil), *S. macrophylla* (7.96 meq O₂/ kg oil) and *S. pinanga* (3.92 meq O₂/ kg oil). The peroxide value of crude *S. stenoptera* oil in this study is significantly lower than that of those reported from Kalimantan which is 9.05 to 14.03 meq O₂/ kg oil (Darmawan et al., 2020). The peroxide value in edible oil must be below 15 meq O₂/kg oil (Codex Alimentarius, 2017; Mohamed Ahmed et al., 2021) and all the extracted oils are below the limit. However, a lower peroxide value is desirable to increase the oil shelf life. Significant reductions in peroxide value after refining are observed in Table 4.7 except for *S. stenoptera* oil. The bleaching stage in oil refining uses adsorbents

such as activated carbon, active clay and bentonite to remove excess dyes and impurities from oil (Foletto et al., 2011). Hidayat et al. (2019) and Darmawan et al. (2020) found similar results.

Iodine value indicates the degree of unsaturation in oil and higher unsaturation suggests the oil is more susceptible to lipid oxidation (Djikeng et al., 2018). The highest iodine value in crude oil is in *S. palembanica* (31.91 g I₂/100 g oil), followed by *S. macrophylla* (27.31 g I₂/100 g oil). The level of iodine value in *Shorea* oils presented in Table 4.7 is expected because crude *S. palembanica* and *S. macrophylla* have the highest unsaturation according to their fatty acid profile. Table 4.7 shows that the iodine value of *S. stenoptera* increases after refining due to an increase in oleic acid composition in refined *S. stenoptera*. However, Darmawan et al. (2020) reported that the iodine value in *S. stenoptera* oil decreases after refining. *Shorea* oil presented in Table 4.7 can be classified as non-drying oil because the iodine value is lower than 100 g I₂/100 g oil (Karak, 2012).

4.7 Fatty Acids Profile Characterisation

The profiles of crude and refined *Shorea* seed oils were determined through their derivatised oil using GC-MS while the melted oil using ATR-FTIR spectroscopy.

4.7.1 GC-MS Analysis of FAMES Standard

The GC-MS chromatogram for a mixture of FAME standards is presented in Figure 4.3. Fatty acids in extracted oils have been characterised by comparing the mass spectra with the MS library data system and confirmed by comparing their retention times of the

respective FAMES standards solution. Fatty acids were named according to systematic or common nomenclature where the letter *n* entails the double bond position closest to the methyl end. The letter *c* indicates the double bond with the *cis* configuration. Finally, *t* denotes the carbon-carbon double bond in *trans* configuration. Table 4.8 lists the retention times of 37 FAMES in the standard solution analysed on the DB-5 column of GC-MS. Numbers in the chromatogram in Figure 4.3 are referred to in the list of FAMES in Table 4.8.

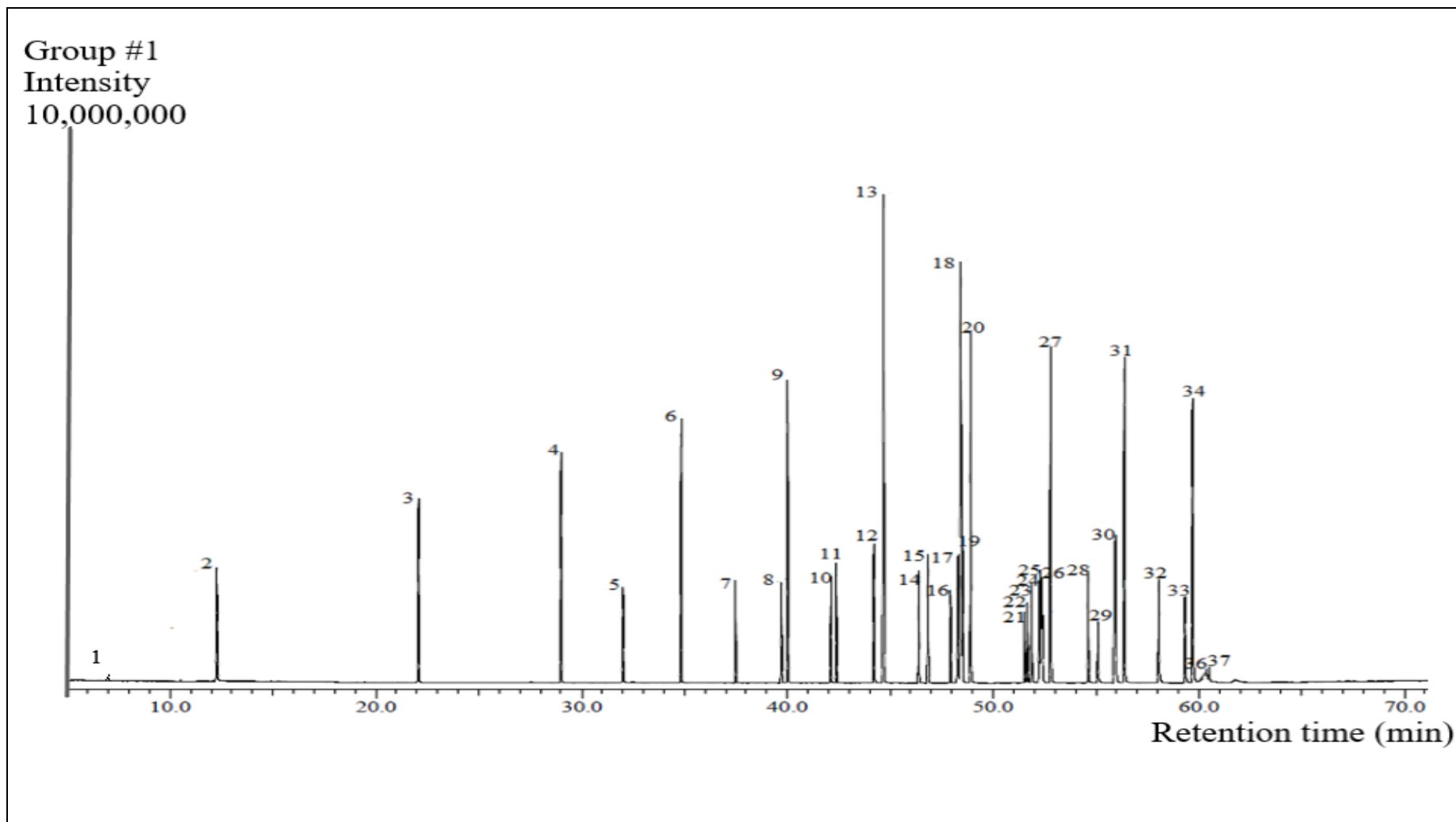


Figure 4.3: GC-MS Chromatogram of Standard Solution Containing 37 FAMES Standard

Table 4.8: Retention Times of FAMES Standard Solution Separated by DB-5 Column on GC-MS

Peak Number	FAMES	Carbon No.	Retention Time (min) (mean±s.d.)
1	Butyric acid methyl ester	C4:0	7.23±0.01
2	Caproic acid methyl ester	C6:0	12.28±0.01
3	Caprylic acid methyl ester	C8:0	22.08±0.01
4	Capric acid methyl ester	C10:0	29.01±0.01
5	Undecanoic acid methyl ester	C11:0	32.02±0.01
6	Methyl laurate	C12:0	34.84±0.01
7	Methyl tridecanoate	C13:0	37.49±0.01
8	Myristoleic acid methyl ester	C14:1	39.71±0.01
9	Myristic acid methyl ester	C14:0	40.01±0.01
10	<i>cis</i> -10-pentadecenoate methyl ester	C15:1n5c	42.12±0.01
11	Methyl pentadecanoate	C15:0	42.39±0.01
12	Methyl palmitoleate	C16:1n7c	44.21±0.01
13	Methyl palmitate	C16:0	44.68±0.01
14	Methyl <i>cis</i> -10-heptadecenoate	C17:1n7c	46.40±0.01
15	Methyl Heptadecanoate	C17:0	46.83±0.01
16	Methyl- γ -linolenate	C18:3n6c	47.94±0.01
17	Methyl linoleate	C18:2n6c	48.27±0.01
18	Methyl oleate	C18:1n9c	48.42±0.01
19	Methyl <i>trans</i> -9-eladiate	C18:1n9t	48.52±0.01
20	Methyl stearate	C18:0	48.92±0.01
21	Arachidonic acid methyl ester	C20:4n6c	51.53±0.01
22	Methyl <i>cis</i> -5,8,11,14,17 eicosapentaenoate	C20:5n3c	51.67±0.01
23	Methyl- <i>cis</i> -8,11,14-eicosatrienoic acid	C20:3n6c	51.88±0.01
24	Methyl linolelaidate	C18:2n6t	52.24±0.01
25	Gondoic acid methyl ester	C20:1n9c	52.33±0.01
26	α -linolenic acid methyl ester	C18:3n3c	52.39±0.01
27	Methyl arachidiate	C20:0	52.80±0.01
28	Methyl heneicosanoate	C21:0	54.62±0.01
29	Methyl <i>cis</i> -4,7,10,13,16,19-docosahexanoate	C22:6n3c	55.10±0.01
30	Methyl- <i>cis</i> -11,14,17-eicosatrienoate	C20:3nc	55.90±0.01
31	Erucic acid methyl ester	C22:1n9c	55.96±0.01
32	Methyl behenate	C22:0	56.38±0.01

Table 4.8 continued

33	Methyl tricosanoate	C23:0	58.06±0.01
34	Nervonic acid methyl ester	C24:1nc	59.32±0.01
35	Methyl lignocerate	C24:0	59.70±0.01
36	Methyl <i>cis</i> -11,14-eicosadienoate	C20:2n6c	60.30±0.01
37	Methyl <i>cis</i> -13,16-docosadienoate	C22:2	60.41±0.01

Note: Retention time data are the mean of retention time of three replicates ± s.d.

4.7.2 Fatty Acid Composition in *S. macrophylla* Oils

The fatty acids chromatograms of crude and refined *S. macrophylla* seed oils are shown in Figure 4.4 and the composition are summarised in Table 4.9. Total SFA in crude in *S. macrophylla* is higher compared to refined oil which is 68.99% and 65.66%, respectively. Dominant SFA in *S. macrophylla* in both crude and refined oils is stearic acid 39.91% and 43.65%, respectively, followed by palmitic acid (16.93% and 18.03%, respectively). The refining process does not alter the composition of SFA of *S. macrophylla* oil as there are no significant differences in the amounts of total SFAs. The finding agrees with Pal et al. (2015), Chew et al. (2017) and Pan et al. (2019). However, it is worth noting that refining of *S. macrophylla* oil resulted in the absence of minor SFA which is heptadecanoic and lignoceric acids as presented in Table 4.9. The amount of SFA in *S. macrophylla* oil reported by Nesaretnam and Ali (1992) is comparable (63.1%).

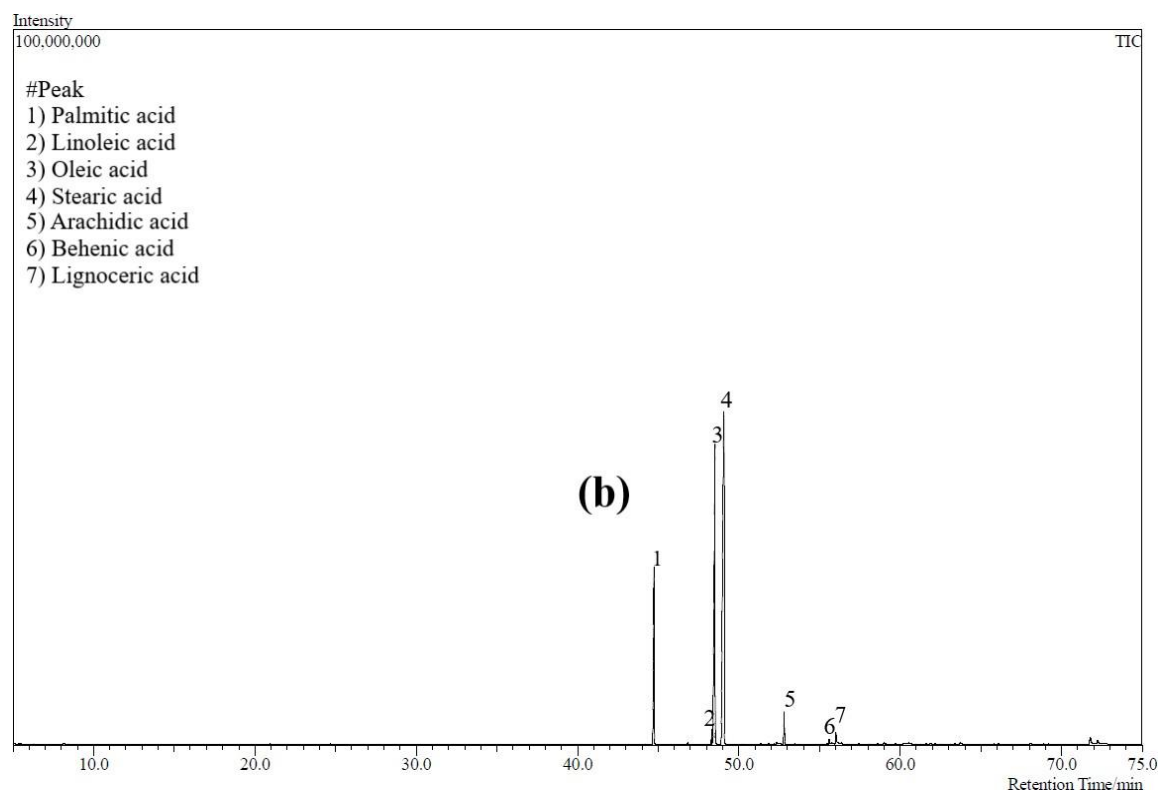
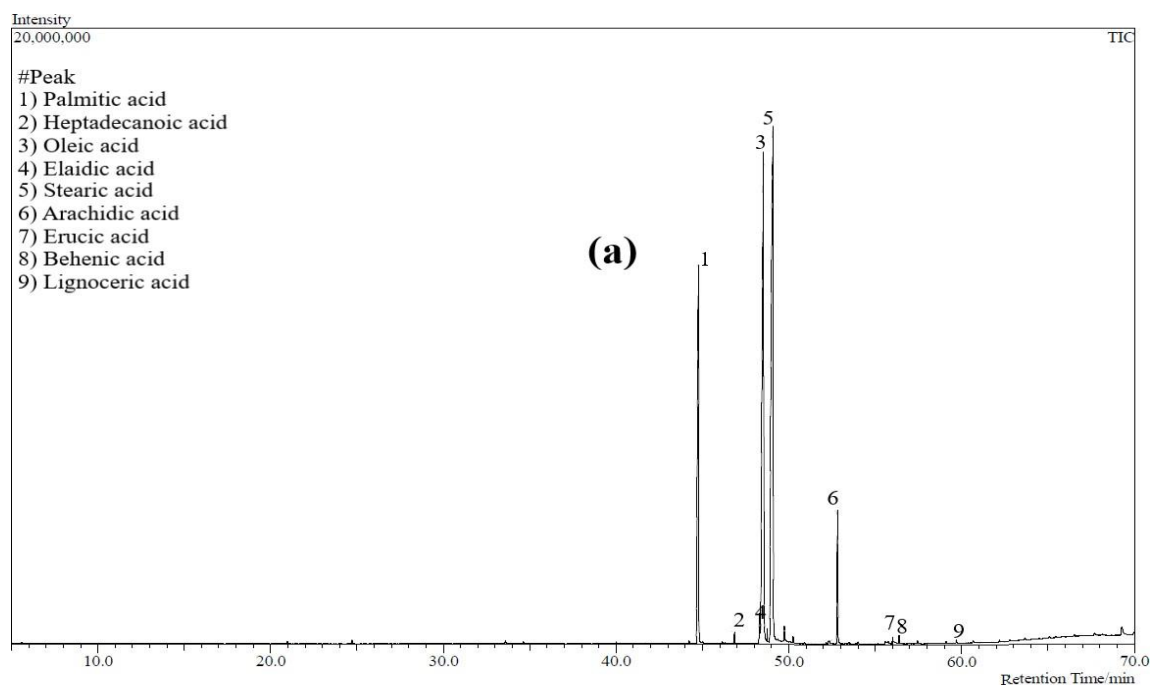


Figure 4.4: GC-MS Chromatogram of (a) Crude and (b) Refined *S. macrophylla* Oils

Table 4.9: Fatty Acid Composition in *S. macrophylla* Seed Oils

Fatty acid	<i>S. macrophylla</i> seed oil	
	Crude	Refined
Palmitic acid	16.93±2.02 ^a	18.03±2.66 ^a
Heptadecanoic acid	0.11±0.18 ^a	n.d.
Stearic acid	39.91±7.20 ^a	43.65±3.74 ^a
Arachidic acid	3.47±1.92 ^a	3.57±2.19 ^a
Behenic acid	4.02±6.77 ^a	0.41±0.70 ^a
Lignoceric acid	4.55±7.88 ^a	n.d.
ΣSFA	68.99 ^a	65.66 ^a
Oleic acid	29.82±9.03 ^a	33.82±0.97 ^a
Erucic acid	1.12±1.94 ^a	n.d.
Elaidic acid	0.07±0.13 ^a	n.d.
ΣMUFA	31.01 ^a	33.82 ^a
Linoleic acid	n.d.	1.01±0.45 ^a
ΣPUFA	n.d.	1.01 ^a

Note: Data are the mean percentage of three replicates ± standard deviation (s.d); n.d. is not detected; Means in the same column with the same superscript are not significantly different at 5% level.

MUFAs in *S. macrophylla* crude and refined oils are 31.01% and 33.82%, respectively. Oleic acid is the dominant MUFA in *S. macrophylla* crude and refined oils with 29.82% and 33.82%, respectively. Oleic acid is an Omega-9 fatty acid commonly found in seed oils, particularly olive oil (Romano et al., 2021). Oleic acid has received significant attention worldwide for its beneficial role in health (Sales-Campos et al., 2012) and the United States Food and Drugs Administration (US-FDA, 2018) claimed that oleic acid helps to reduce the risk of CHD. Initially, it was expected that mechanically extracted oil does not contain elaidic acid but the acid was detected in crude oil. The first possibility is the variation in fatty acid composition between species. However, Nesaretnam and Ali (1992) did not detect erucic and elaidic acids in the fatty acid profile of *S. macrophylla* oil. As mentioned

by Song et al. (2015) the formation of elaidic acid is due to the high-temperature treatment. To assure the most suited approach for generating high quality oil for each *Shorea* species, more research is needed to understand the impacts of drying, pre-treatment and oil extraction under various variables such as temperature and humidity.

The refining process enhances beneficial MUFA in *S. macrophylla* oil while decreasing the amount of toxic MUFA such as *trans*-MUFA (elaidic acid) and erucic acid. Erucic acid is present in SMEC oil with 1.12% undetected after refining. Pan et al. (2019) reported a 100% reduction while Özcan et al. (2021) reported a 50% reduction of erucic acid after oil refining. The consumption of erucic acid is restricted to a 5% level in daily intake as it causes lipidosis, increases blood cholesterol and disrupts growth (Sabikhi & Sathish Kumar, 2012; Konuskan et al., 2017). Nesaretnam and Ali (1992) reported a low amount of MUFA (33.40%) in *S. macrophylla* seed oil comprised of oleic acid (33.20%) and palmitoleic acid (0.2%).

Linoleic acid is the only PUFA detected in *S. macrophylla* refined oil with 1.01% in refined oil. The amount of linoleic acid in *S. macrophylla* reported by Nesaretnam and Ali (1992) was comparable with the current study. Linoleic acid consumption helps to lower the risk of CVD (Marangoni et al., 2020). Oil refining is advantageous for *S. macrophylla* oil because it reduces the total amount of SFAs, eliminates harmful MUFAs and increases the level of important oleic and essential PUFAs. The discrepancies in the fatty acid composition of *S. macrophylla* oil reported by other researchers in this study could be attributed to the solvent employed and the post-harvest procedure.

The infrared (IR) spectra of crude and refined *S. macrophylla* oils acquired in the mid-infrared region of 3500 to 500 cm^{-1} are shown in Figure 4.5. Two regions in the spectra can be used to confirm the presence of functional groups in the FTIR spectra (Hasan et al.,

2020). The spectra of *S. macrophylla* in crude and refined oils are almost similar suggesting no significant differences in fatty acid composition due to refining. The identified peaks are summarised in Table 4.10. The peak at 1729.88 to 1734.81 cm^{-1} indicates the presence of functional group C=O from TAGs, carboxylic acids, aldehydes and ketones as reported by Arslan and Çağlar (2019).

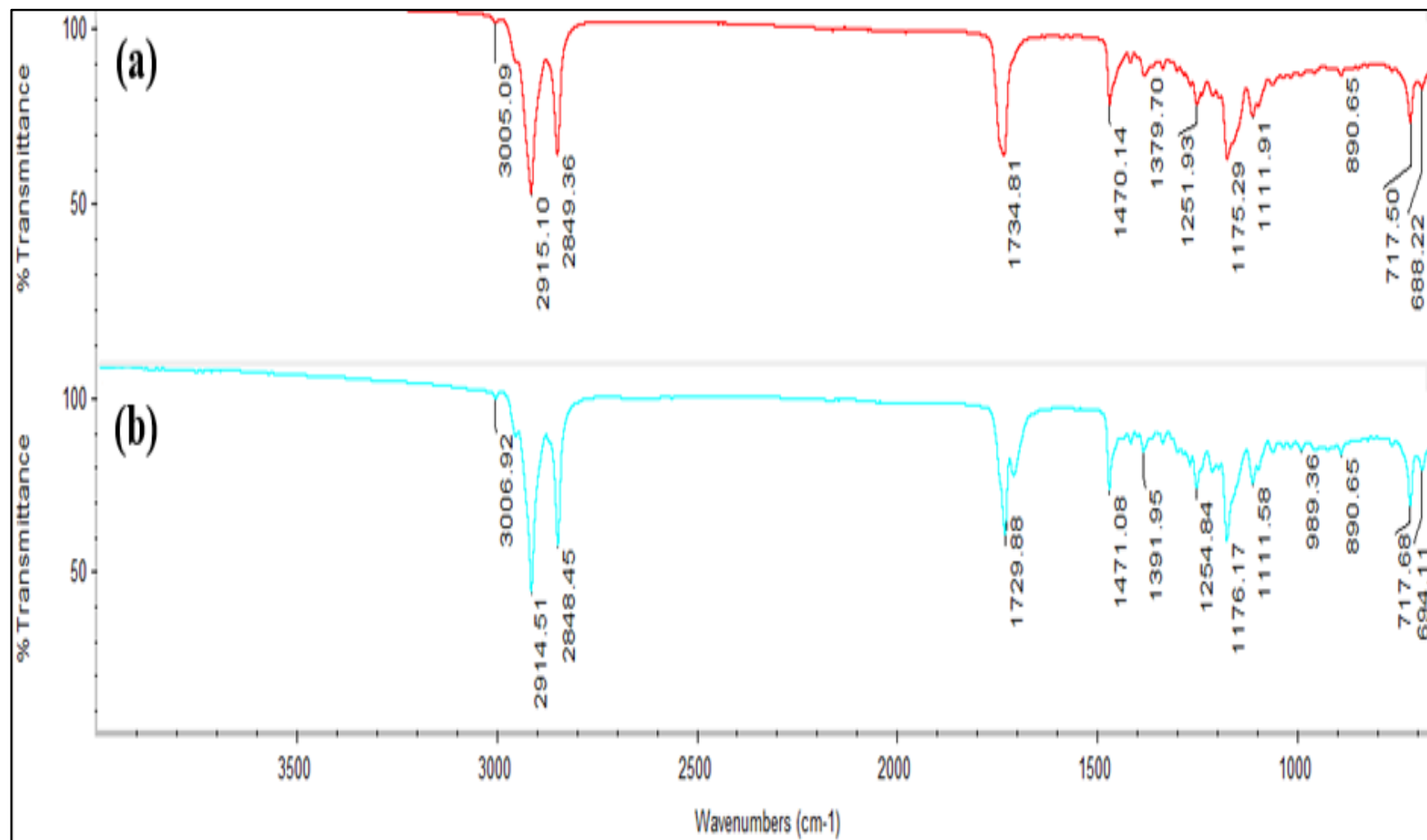


Figure 4.5: ATR-FTIR Spectra of (a) Crude and (b) Refined *S. macrophylla* Oils

Table 4.10: List of Detected IR Peaks with Corresponding Wave Number and Possible Functional Group Present in *S. macrophylla* Oils

Wave Number (cm ⁻¹)	Functional groups	Vibration
3005.09-3006.92	=C—H- (<i>cis</i>)	Stretching
2914.51-2915.10	-C—H- (CH ₂)	Stretching (asym)
2848.45 -2849.36	-C—H (CH ₂)	Stretching (sym)
1729.88-1734.81	-C=O (TAGs, carboxylic acids, aldehydes and ketones)	Stretching
1470.14-1471.08	-C—H (CH ₂)	Bending (scissoring)
1379.70-1391.95	-C—H (CH ₃)	Bending (sym)
1251.29-1254.84	-C—H- (CH ₂)	Bending
1175.29-1176.17	-C—H- (CH ₂)	Bending
1111.58-1111.91	-C—O	Stretching
989.36	-HC=CH- (<i>trans</i>)	Bending (out of plane)
890.65	-HC=CH- (<i>cis</i>)	Bending (out of plane)
717.50-717.68	-HC-CH- (<i>cis</i>)	Bending (out of plane)
688.22-694.11	C=C	Bending (out of plane)

Note: Table was constituted according to references (Arslan and Caglar, 2019).

4.7.3 Fatty Acid Composition in *S. palembanica* Oils

The fatty acid chromatograms of crude and refined *S. palembanica* oils are shown in Figure 4.6 and the compositions were summarised in Table 4.11. Total SFAs in crude and refined oils are 65.51% and 65.38%, respectively. Stearic acid is the dominant SFA in *S. palembanica*, where crude oil has the highest amount of stearic acid at 46.71%. Stearic acid-rich fats are favourable for consumption because they do not alter plasma cholesterol levels and have a neutral effect on inducing CHDs (Salas et al., 2011). Palmitic acid is the second highest SFA in *S. palembanica* crude oil (14.12%) and refined oils (14.20%). Liu et al. (2017c) suggested the usage of palm oil which contained up to 50% palmitic acid and can be used in baking and processed food to replace hydrogenated oils. Odd chain SFA, heptadecanoic acid is detected as a trace fatty acid in crude oil (0.25%) and refined oil (0.09%). Odd chain SFA can lower the risk of cardiometabolic disorders and higher dietary intake of this fatty acid has been linked to a lower mortality rate (Venn-Watson et al., 2020).

Total MUFAs in *S. palembanica* oils ranged from 34.40% to 35.23% where oleic acid is the dominant MUFA. The oleic acid level is higher in refined oil (34.57%) compared to crude oil (34.34%). There are no significant differences in oleic acid levels among *S. palembanica* seed oils. Gondoic acid is detected in trace amounts in crude and refined oils with 0.15% and 0.66%, respectively. The refining process in *S. palembanica* oils does not alter the quality of fatty acids but decreases the total SFAs and increases the total MUFAs. Similar findings were reported by Pal et al. (2015), Chew et al. (2017) and Pan et al. (2019).

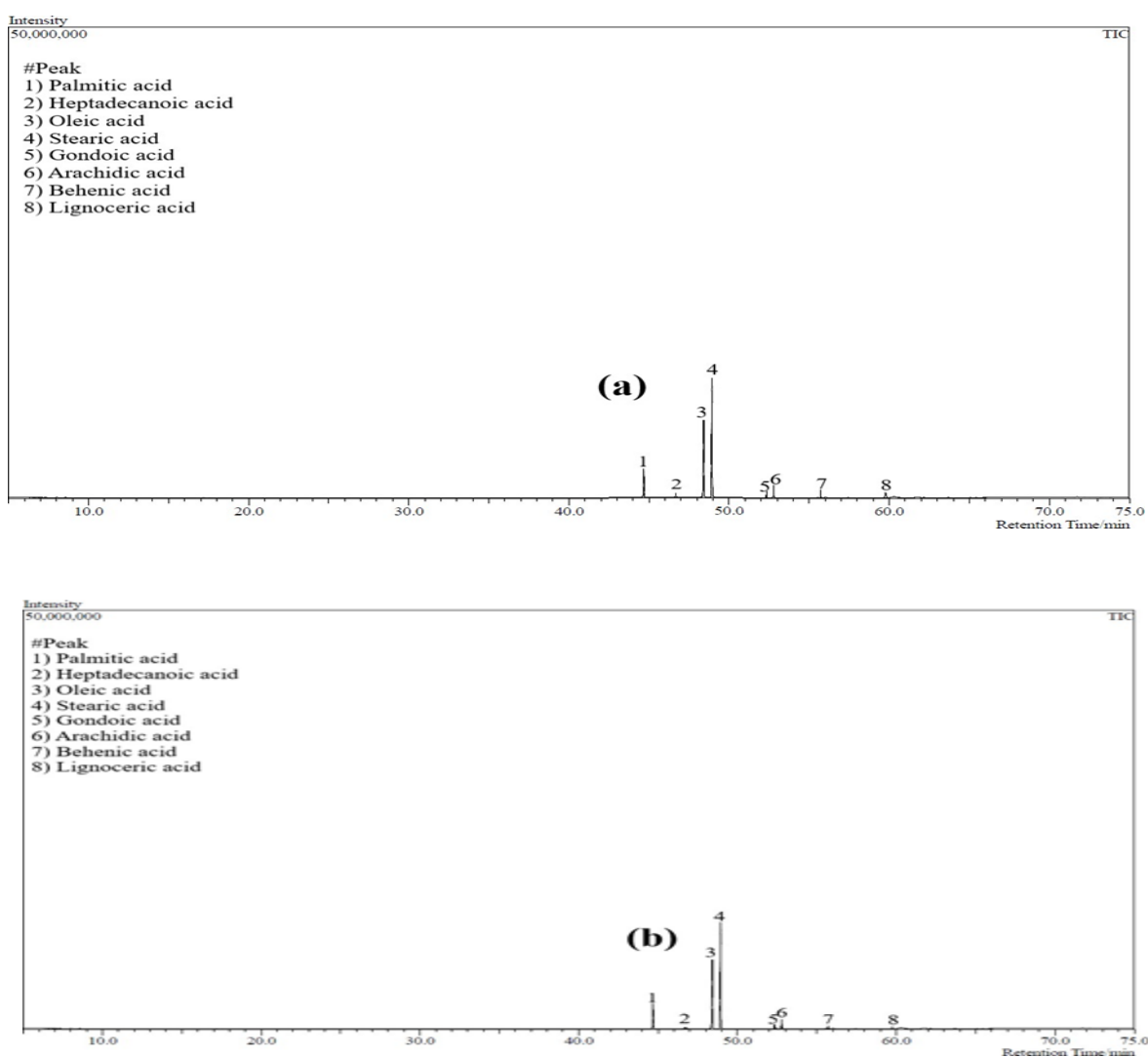


Figure 4.6: GC-MS Chromatogram of (a) Crude and (b) Refined *S. palembanica* Oils

Table 4.11: Fatty Acid Composition in *S. palembanica* Oils

Fatty acid	<i>S. palembanica</i> seed oil	
	Crude	Refined
Palmitic acid	14.12±2.53 ^a	14.12±2.59 ^a
Heptadecanoic acid	0.25±0.22 ^a	0.09±0.15 ^a
Stearic acid	46.71±7.07 ^a	46.61±7.16 ^a
Arachidic acid	4.03±2.06 ^a	4.12±2.13 ^a
Behenic acid	0.31±0.28 ^a	0.28±0.24 ^a
Lignoceric acid	0.09±0.16 ^a	0.08±0.13 ^a
ΣSFA	65.51 ^a	65.38 ^a
Oleic acid	34.34±1.80 ^a	34.57±1.99 ^a
Gondoic	0.15±0.13 ^a	0.66±0.11 ^a
ΣMUFA	34.49 ^a	35.23 ^a
Linoleic acid	n.d.	n.d.
ΣPUFA	n.d.	n.d.

Note: Data are the mean percentage of three replicates ± standard deviation (s.d); Means in the same column with the same superscript are not significantly different ($p>0.05$).

IR spectra of *S. palembanica* oils obtained using ATR-FTIR in the mid-infrared region of 3500-500 cm^{-1} are shown in Figure 4.7. There are two regions in the spectra of *S. palembanica*: the first region contains three peaks and the second region comprises nine peaks. The IR spectra of crude and refined *S. palembanica* oils show no significant differences in the pattern, indicating that oil refining does not significantly affect fatty acid composition in *S. palembanica* oils. The identified peaks are listed in Table 4.12.

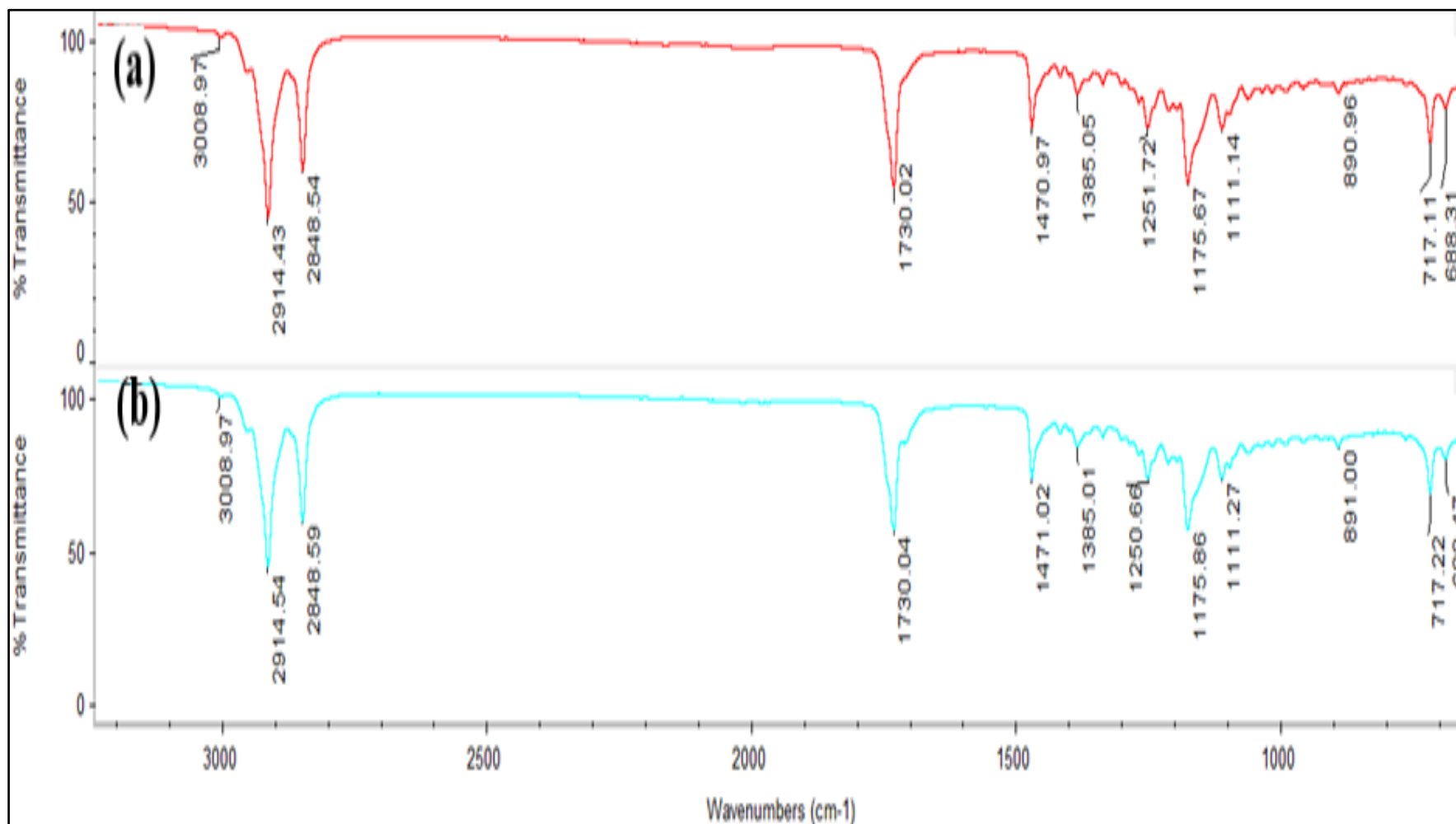


Figure 4.7: ATR-FTIR Spectra of (a) Crude and (b) Refined *S. palembanica* Oils

Table 4.12: IR Peaks Detected with Corresponding Wave Numbers and Possible Functional Group Present in *S. palembanica* Oils

Wave Number (cm ⁻¹)	Functional groups	Vibration
3008.97	=C—H- (<i>cis</i>)	Stretching
2914.43 to 2914.54	-C—H- (CH ₂)	Stretching (asym)
2848.54 to 2848.59	-C—H (CH ₂)	Stretching (sym)
1730.02 to 1730.04	-C=O (TAGs, carboxylic acids, aldehydes and ketones)	Stretching
1470.97 to 1471.02	-C—H (CH ₂)	Bending (scissoring)
1385.01 to 1385.05	-C—H (CH ₃)	Bending (sym)
1250.66 to 1251.72	-C—H- (CH ₂)	Bending
1175.67 to 1175.86	-C—H- (CH ₂)	Bending
1111.14 to 1111.27	-C—O	Stretching
890.96 to 891.00	-HC=CH- (<i>cis</i>)	Bending (out of plane)
717.11 to 717.22	-HC-CH- (<i>cis</i>)	Bending (out of plane)
688.31 to 688.47	C=C	Bending (out of plane)

Note: Table was constituted according to references (Arslan & Çağlar, 2019)

4.7.4 Fatty Acid Composition in *S. stenoptera* Oils

The fatty acids chromatograms of crude and refined oils of *S. stenoptera* oils are presented in Figure 4.8. Total SFA in *S. stenoptera* crude oil is significantly higher than in refined oil which is 70.97% and 58.28%, respectively as shown in Table 4.13. Significant reduction of total SFA in *S. stenoptera* because of the significant decrease of stearic acid from 45.03% to 35.92%. Palmitic acid is the second most dominant SFA after stearic acid and the level of stearic acid in crude oil is not significantly different from refined oil. Arachidic acid is present in a trace amount in *S. stenoptera* oils. The amount of oleic acid in refined oil (42.82%) is significantly higher than in crude oil (29.04%). Refining of *S. stenoptera* oil resulted in a significant increase in the amount of oleic acid which is more beneficial for consumption than their crude oil. The fatty acid content of *S. stenoptera* in this study is similar to that reported by Darmawan et al. (2020), with the exception of the presence of linoleic acid at 0.45%. The variation in fatty acid composition might be due to

the different types of extraction methods and geographical factors (Solà Marsiñach & Cuenca, 2019).

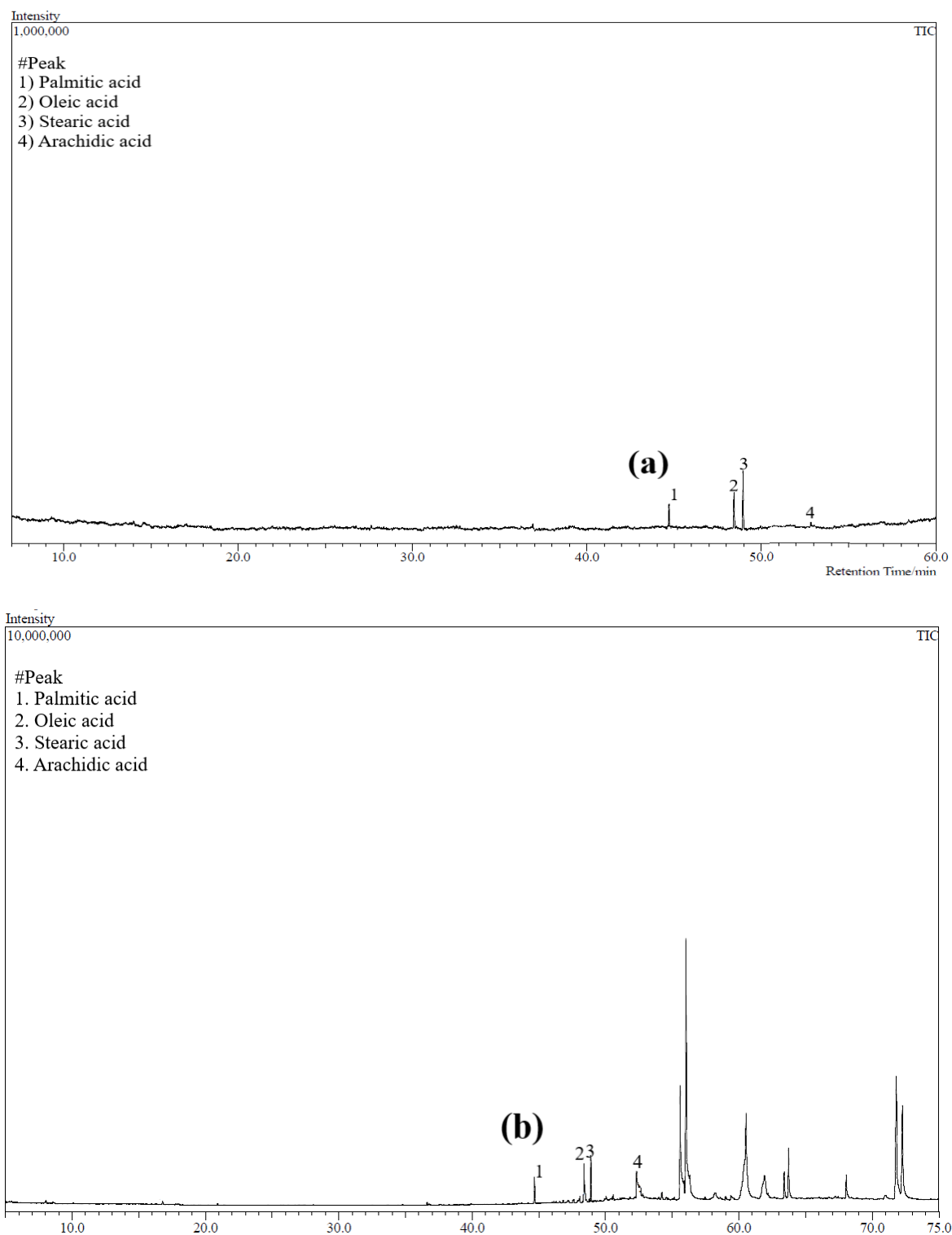


Figure 4.8: GC-MS Chromatogram of (a) Crude and (b) Refined *S. stenoptera* Oils

Table 4.13: Fatty Acid Composition in *S. stenoptera* Oils

Fatty acid	<i>S. stenoptera</i> seed oil	
	Crude	Refined
Palmitic acid	23.92±0.88 ^c	19.05±0.84 ^{abc}
Stearic acid	45.03±0.92 ^a	35.92±2.86 ^b
Arachidic acid	2.02±3.50 ^a	3.31±0.53 ^a
ΣSFA	70.97 ^a	58.28 ^b
Oleic acid	29.04±1.88 ^a	42.82±3.84 ^b
ΣMUFA	29.04 ^a	42.82 ^b
Linoleic acid	n.d.	n.d.
ΣPUFA	n.d.	n.d.

Note: Data are the mean percentage of three replicates ± standard deviation (s.d); Means in the same column with the same superscript are not significantly different at 5% level.

IR spectra of *S. stenoptera* oils have been established using ATR-FTIR in the mid-IR region are shown in Figure 4.9. The IR spectra can be divided into two regions, which are from 3005.80 cm⁻¹ to 2848.32 cm⁻¹ and 1729.79 cm⁻¹ to 688.32 cm⁻¹. A total of 12 peaks have been identified and are summarised in Table 4.14. The comparison of spectra crude and refined oils found no significant difference in the pattern indicating the refining process does not affect the fatty acid composition of *S. stenoptera* oils.

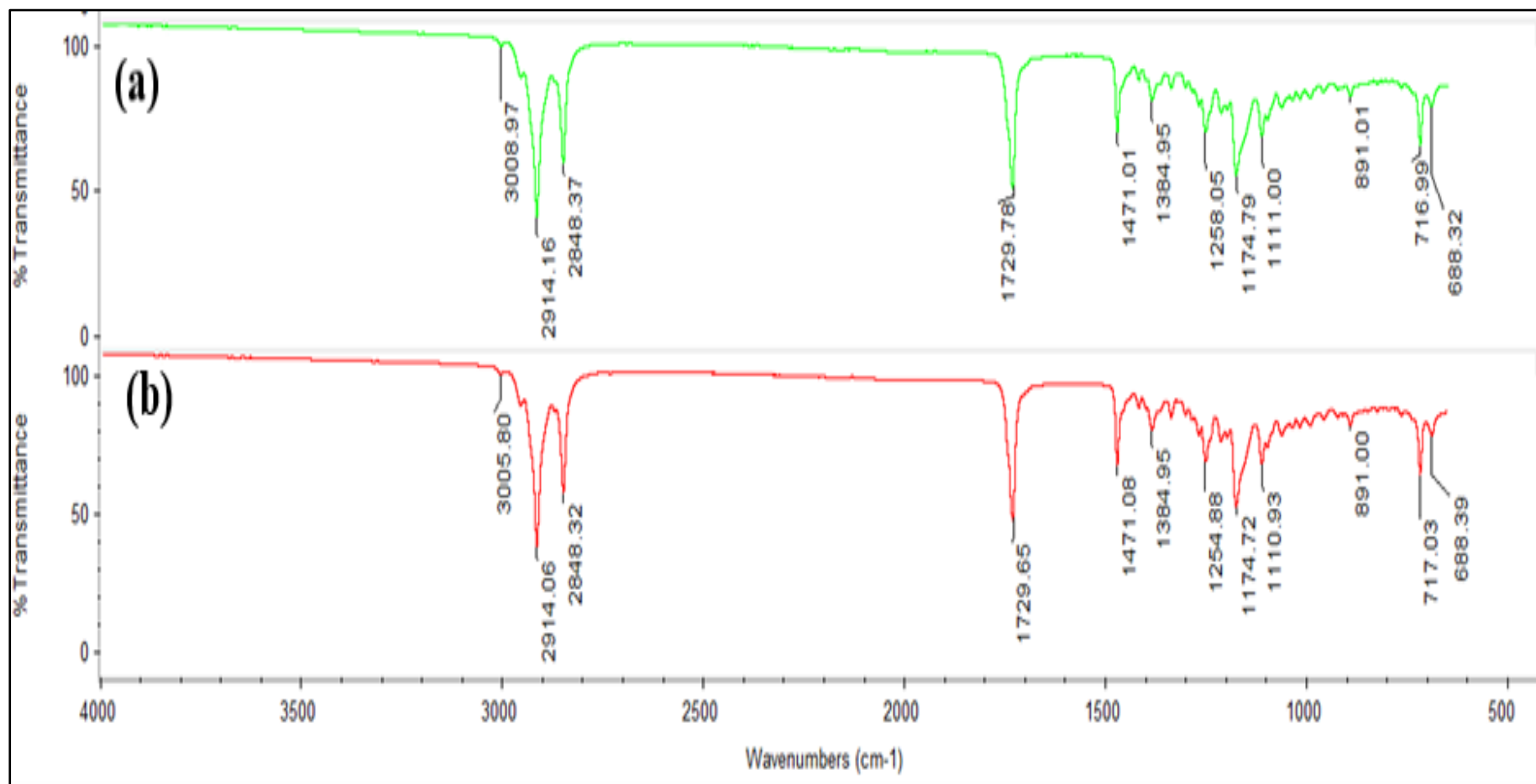


Figure 4.9: ATR-FTIR Spectra of (a) Crude and (b) Refined *S. stenoptera* Oils

Table 4.14: IR Peaks Detected with Corresponding Wave Numbers and Possible Functional Group Present in *S. stenoptera* Oils

Wave Number (cm ⁻¹)	Functional groups	Vibration
3005.80 to 3008.97	=C—H- (<i>cis</i>)	Stretching
2914.06 to 2914.16	-C—H- (CH ₂)	Stretching (asym)
2848.32 to 2848.37	-C—H (CH ₂)	Stretching (sym)
1729.65 to 1729.78	-C=O (TAGs, carboxylic acids, aldehydes and ketones)	Stretching
1471.01 to 1471.08	-C—H (CH ₂)	Bending (scissoring)
1384.95	-C—H (CH ₃)	Bending (sym)
1254.88 to 1258.05	-C—H- (CH ₂)	Bending
1174.72 to 1174.79	-C—H- (CH ₂)	Bending
1110.93 to 1111.00	-C—O	Stretching
891.00 to 891.01	-HC=CH- (<i>cis</i>)	Bending (out of plane)
716.99 to 717.03	-HC-CH- (<i>cis</i>)	Bending (out of plane)
688.32 to 688.39	C=C	Bending (out of plane)

Note: Table was constituted according to references (Arslan & Çağlar, 2019)

4.7.5 Fatty Acids Composition in *S. pinanga* Oils

The fatty acid composition of crude and refined *S. pinanga* oils is shown in GC- MS chromatograms in Figure 4.10. The identified fatty acids in *S. pinanga* were summarised in Table 4.15. Crude *S. pinanga* oil contains higher total SFAs compared to its refined oil which is 67.58% and 65.03%, respectively. Stearic acid is the major SFA in *S. pinanga* oils which ranges from 44.41% to 46.13%, followed by palmitic acid which is 17.04% to 18.57%, respectively. Arachidic acid is detected as a minor SFA in *S. pinanga* crude and refined oils which is 2.57% and 3.58%, respectively. Behenic acid is only detected in crude *S. pinanga* oil at 0.31%. The amount of total SFAs in *S. pinanga* oil decreased after refining. Oleic acid is the only MUFA detected in *S. pinanga* oils. The fatty acid content in refined oil is higher compared to crude oil which is 34.96% and 32.09%, respectively. Linoleic acid is present as minor fatty acid in crude and refined *S. pinanga* oils with a higher concentration of linoleic acid found in refined oil which is 0.41%.

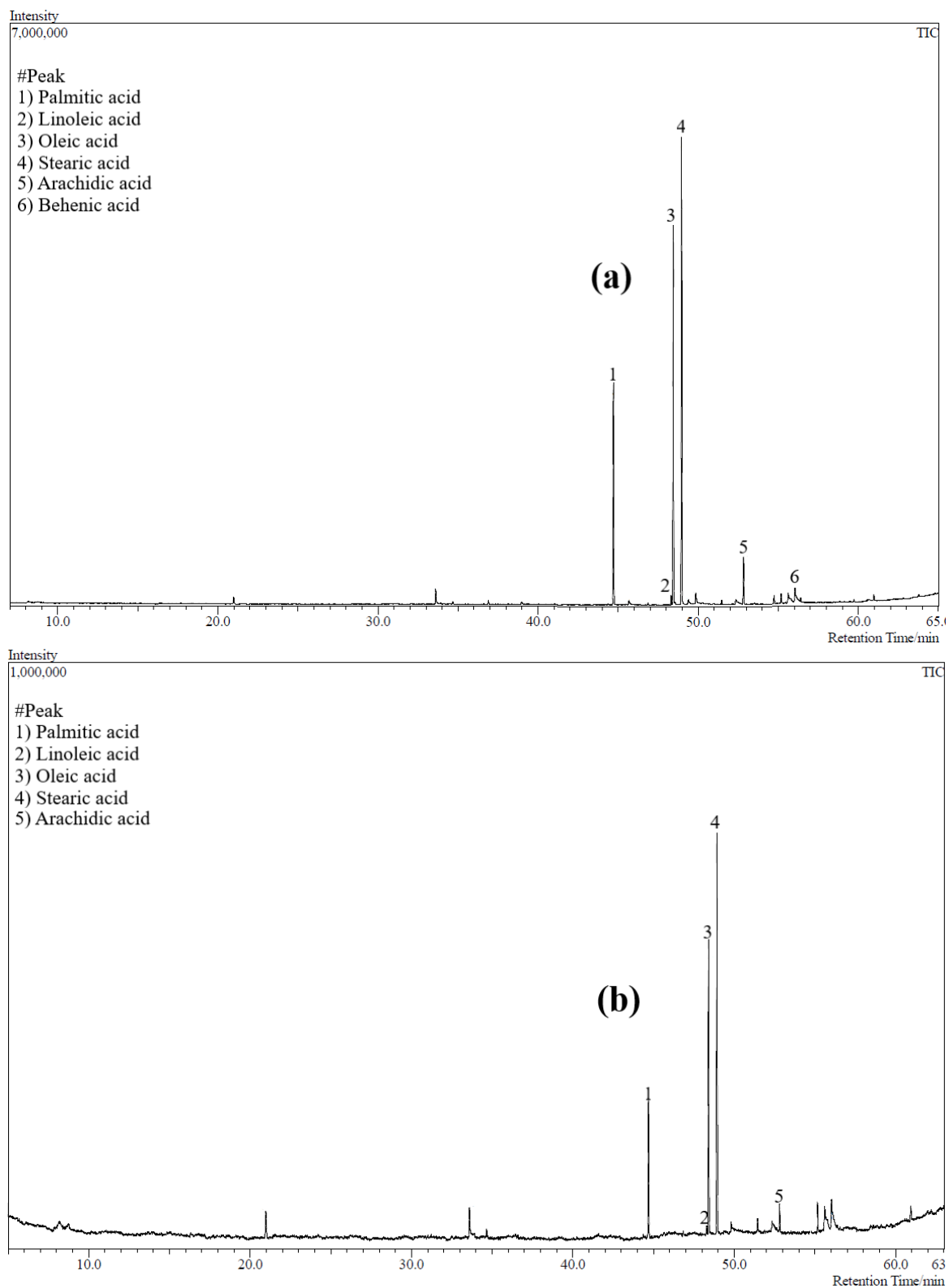


Figure 4.10: GC-MS Chromatogram of (a) Crude and (b) Refined *S. pinanga* Oils

Table 4.15: Fatty Acid Composition in Crude and Refined *S. pinanga* Oils

Fatty acid	<i>S. pinanga</i> seed oil	
	Crude	Refined
Palmitic acid	18.57±2.40 ^a	17.04±0.47 ^a
Stearic acid	46.13±3.73 ^a	44.41±0.90 ^a
Arachidic acid	2.57±2.23 ^a	3.58±0.03 ^a
Behenic acid	0.31±0.27 ^a	n.d.
ΣSFA	67.58 ^a	65.03 ^a
Oleic acid	32.09±3.31 ^a	34.96±0.81 ^a
ΣMUFA	32.09 ^a	34.96 ^a
Linoleic acid	0.34±0.59 ^a	0.41±0.71 ^a
ΣPUFA	0.34 ^a	0.41 ^a

Note: Data are the mean percentage of three replicates ± standard deviation (s.d); Means in the same column with the same superscript are not significantly different at 5%.

IR spectra of *S. pinanga* oils were obtained in the mid-infrared region of 3500 to 500 cm⁻¹, as shown in Figure 4.11. Two regions have been detected: the first is from 3008.97 cm⁻¹ to 2847.49 cm⁻¹, and the second is from 1732.98 cm⁻¹ to 688.13 cm⁻¹, with four and nine peaks, respectively. No significant difference in the pattern of *S. pinanga* oils between crude and refined suggests similar fatty acid composition. A total of 12 identified peaks in *S. pinanga* oils are listed in Table 4.16.

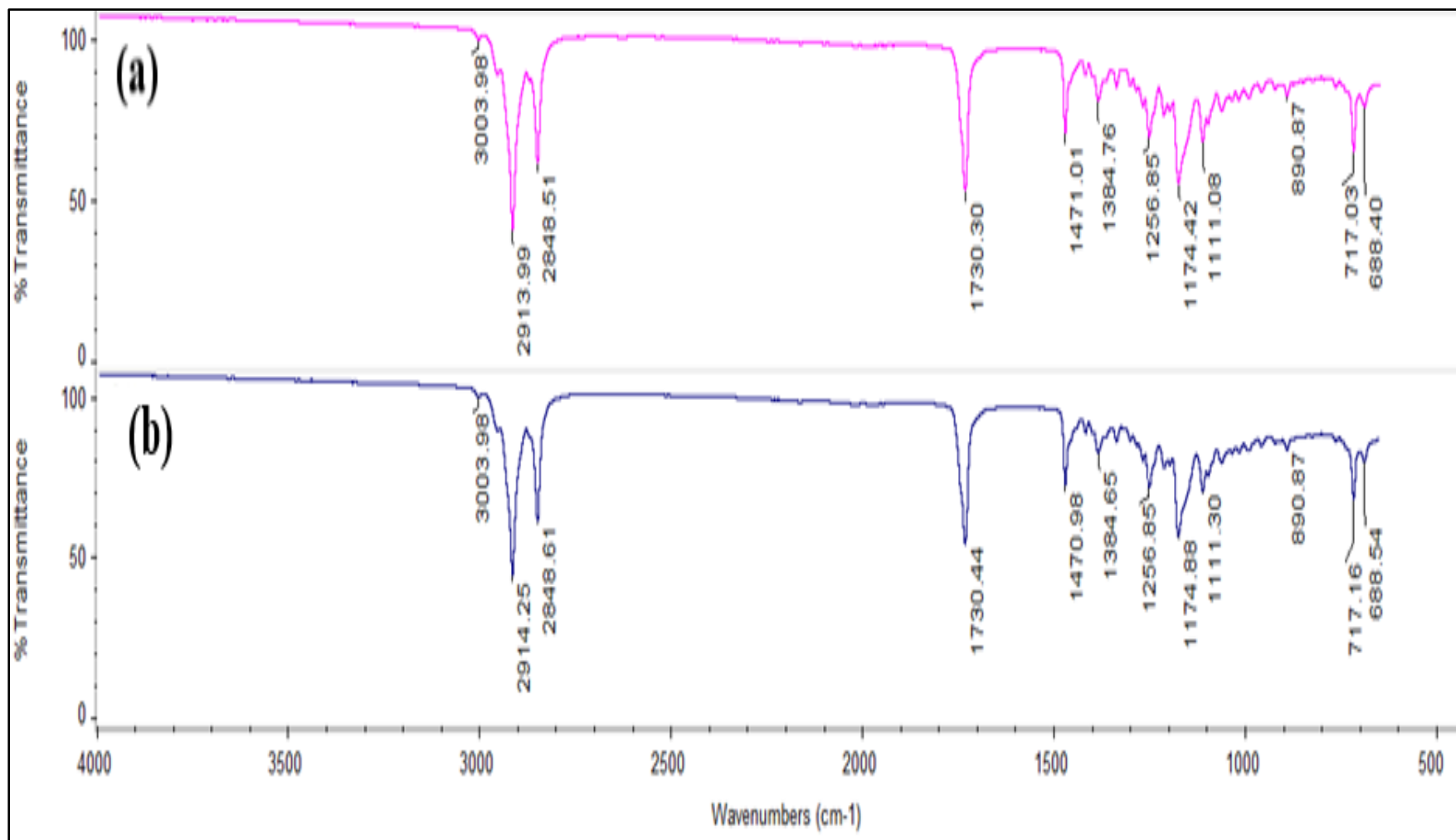


Figure 4.11: IR Spectra of (a) Crude and (b) Refined *S. pinanga* Oils Using ATR-FTIR

Table 4.16: IR Peaks Detected with Corresponding Wave Numbers and Possible Functional Group Present in *S. pinanga* Oils

Wave Number (cm ⁻¹)	Functional groups	Vibration
3003.98	=C—H- (<i>cis</i>)	Stretching
2913.99 to 2914.25	-C—H- (CH ₂)	Stretching (asym)
2848.51 to 2848.61	-C—H (CH ₂)	Stretching (sym)
1730.30 to 1730.44	-C=O (TAGs, carboxylic acids, aldehydes and ketones)	Stretching
1470.98 to 1471.01	-C—H (CH ₂)	Bending (scissoring)
1384.65 to 1384.76	-C—H (CH ₃)	Bending (sym)
1256.85	-C—H- (CH ₂)	Bending
1174.42 to 1174.88	-C—H- (CH ₂)	Bending
1110.08 to 1111.30	-C—O	Stretching
890.87	-HC=CH- (<i>cis</i>)	Bending (out of plane)
717.03 to 717.16	-HC-CH- (<i>cis</i>)	Bending (out of plane)
688.40 to 688.54	C=C	Bending (out of plane)

Note: Table was constituted according to references (Arslan & Çağlar, 2019)

The major fatty acids in *S. robusta* oil from India are stearic, oleic and arachidic acids as reported by Kamalakar et al. (2015) which is significantly different from *Shorea* oil obtained from Sarawak. Palmitic acid is detected as a minor oil and is present at 5.5%. Solà Marsiñach and Cuenca (2019) suggest the differences in fatty acid composition might be due to sampling origin (environmental factor; type of soil and climate), post-harvesting method and species.

Shorea oils in this study mainly comprise SFAs up to 70.97%. Oils with a high amount of SFA have a higher melting point and are more stable thermally than high UFA oils (Kenar et al., 2017; Abdul-Hammed et al., 2020). Despite the advantages of *Shorea* oils containing SFA, the consumption must be controlled to be less than 10% of total energy intake as suggested by FAO/WHO (Darmawan et al., 2020). There is a slight reduction in the total SFA in *Shorea* oils except for *S. stenoptera* oil ($p < 0.05$). Several researchers reported similar findings (Pal et al., 2015; Chew et al., 2017; Liu et al., 2019ab]. The primary fatty acids in *Shorea* oils are stearic, oleic and palmitic, constituting up to 97.99% of the

total. The major fatty acids in this study are comparable to the cocoa butter fatty acid profile (Bahari & Akoh, 2018a). This indicates *Shorea* oils have the potential to be developed as cocoa butter equivalents (CBE). The similarities in chemical composition, such as the fatty acid profile between CBE and cocoa butter are essential as they give comparable properties such as melting points (Ewens et al., 2021).

4.8 Antioxidant Activity and Total Phenolic Content of *Shorea* Oils

The antioxidant activity of *Shorea* seed oils was determined using the free radical scavenging activity of DPPH. A lower IC₅₀ value indicates a greater capacity for scavenging free radicals. There is a significant decrease in the antioxidant activity of *S. macrophylla* (244.55 to 471.36 µg/mL) and *S. stenoptera* (152.22 to 249.49 µg/mL) after the refining process as shown in Table 4.17. Similar results were reported for palm oil (Szydłowska-Czerniak et al., 2011), rice bran oil (Liu et al., 2019b) and perilla seed oil (Pan et al., 2019) and rapeseed oil (Wu et al., 2019). The changes in antioxidant activity of *S. palembanica* and *S. pinanga* oils differed from other *Shorea* oils. Refining of *S. palembanica* and *S. pinanga* oils increased their antioxidant activity. Lucci et al. (2020) explained the significant increase in the total amount of tocopherol in refined Baneh oil might be due to a concentration effect. I.e., the initial FFA in crude oil resulted in greater mass in oil. Hence the removal of FFA after refining concentrates the amount of tocopherol in oil (higher antioxidant activity). Zarei Jelyani et al. (2021) reported the increase in tocopherol was caused by breaking the dimeric bonds between tocopherol and other compounds or by breaking the ester bond and other compounds.

Table 4.17: Antioxidant Activity and TPC for *Shorea* Seed Oils

Species	Radical scavenger (IC ₅₀ µg/mL)	TPC (mg GAE/kg oil)
<i>S. macrophylla</i>		
Crude	244.55±2.28 ^{cd}	8.94±0.63 ^f
Refined	471.36±11.39 ^e	1.44±0.09 ^{bc}
<i>S. palembanica</i>		
Crude	813.02±9.01 ^f	2.49±0.01 ^e
Refined	239.88±10.66 ^{cd}	2.34±0.04 ^{de}
<i>S. stenoptera</i>		
Crude	152.22±8.04 ^b	1.56±0.00 ^{cd}
Refined	249.49±15.67 ^d	0.15±0.00 ^a
<i>S. pinanga</i>		
Crude	214.11±4.26 ^c	2.39±0.00 ^e
Refined	56.77±0.71 ^a	0.26±0.00 ^a

Note: Radical scavenging activity was determined using lipophilic fraction; while TPC was determined using hydrophilic fraction; Results are presented as the mean value of three replicates±standard deviations (s.d.); n.d. is not detected value less than 0.005 %; Means in the same column with the same superscript ^a to ⁿ are not significantly different at 5% level.

There is a significant reduction in TPC content after the refining process in *S. macrophylla* (8.94 mg GAE/kg oil to 1.44 mg GAE/kg oil), *S. stenoptera* (1.56 mg GAE/kg oil to 0.15 mg GAE/kg oil) and *S. pinanga* (2.39 mg GAE/kg oil to 0.26 mg GAE/kg oil). The loss of TPC after refining is 83.89% in *S. macrophylla*, 20% in *S. palembanica*, 90.38% in *S. stenoptera* and 89.12% in *S. pinanga*. Our findings in this study are in agreement with palm oil (Szydłowska-Czerniak et al., 2011), rapeseed oil (Szydłowska-Czerniak & Łaszewska, 2015) and rice bran oil (Liu et al., 2019b). The reduction in TPC occurred during neutralisation steps. Phenolic compounds are mainly weak acids which can be easily removed, particularly with aqueous solutions especially NaOH (Szydłowska-Czerniak et al., 2008). The amount of TPC in *Shorea* oils is comparable to that of shea butter which is 1.62 to 7.64 mg GAE/kg oil (Tchobo et al., 2018). *Shorea* oils listed in Table 4.17 show weak

antioxidant activity compared to sesame oils with IC₅₀: 8.3 to 16.88 mg/g and TPC: 2950 to 6950 mg GAE/kg (Agidew et al., 2021) and olive oils with TPC: 52 to 315 mg GAE/kg (Pedan et al., 2019). Despite having weak antioxidant activity, *Shorea* oils have exceptional high oxidative stability due to high saturation in fatty acids.

4.9 Effects of Oil Refining on Chemical Properties, Fatty Acid Composition, Antioxidant Activity and TPC in *Shorea* Oils

The influence of oil refining on chemical properties, fatty acid composition and antioxidant activity in *Shorea* oils were determined using the Point-Biserial Correlation Coefficient (r_{pb}). Table 4.18 shows that there is a strong correlation between oil refining and the amount of total SFA (r_{pb} : -1.00, $p < 0.01$) and total UFA (r_{pb} : 1.00, $p < 0.01$) in *S. macrophylla* oils. The results are in tally with the reduction in total SFA and the increment of total UFA in *S. macrophylla* (see Table 4.9). Significant correlations are observed at 5% and 1% between oil refining with acid value (r_{pb} : -0.0903) and peroxide value (r_{pb} : -1.000), which indicates the reduction of acid and peroxide values after the refining process. These agree with the level of acid and peroxide values of *S. macrophylla* crude and refined oils (see Table 4.7). These findings suggest refining increases the oil quality of *S. macrophylla* oil. The positive value of r between oil refining and IC₅₀ value (r_{pb} : 0.998, $p < 0.05$) and the negative value with TPC (r_{pb} : -0.995, $p < 0.05$) suggests the reduction in antioxidant activity and TPC of *S. macrophylla* oils.

Table 4.18: Point-Biserial Correlation between Refining and Chemical Properties, Fatty Acid Composition, Antioxidant Activity and TPC of *Shorea* Oils

Species	Σ SFA	Σ UFA	AV	PV	IC ₅₀	TPC
<i>S. macrophylla</i>	-1.000**	1.000**	-.903*	-1.000**	.998**	-.995**
<i>S. palembanica</i>	-1.000**	1.000**	-.999**	-.963**	-1.000**	-.953**
<i>S. stenoptera</i>	-1.000**	1.000**	-.561	-1.000**	.978**	-1.000**
<i>S. pinanga</i>	-1.000**	1.000**	-.959**	-.910*	-.999**	-1.000**

**Correlation is significant at 0.01 level (2-tailed)

*Correlation is significant at 0.05 level (2-tailed)

Strong negative correlation between total SFA (r_{pb} : -1.000, $p < 0.01$) after refining indicates the reduction of total SFAs after the refining process, while the strong positive correlation between refining process with total UFAs (r_{pb} : 1.000, $p < 0.01$) indicates the enhancement of total UFAs content in *S. palembanica* oils as shown in Table 4.18. This concurs well with the fatty acid composition of crude and refined oils of *S. palembanica* as shown in Table 4.11. The acid value (r_{pb} : -0.999, $p < 0.01$) and peroxide value (r_{pb} : -0.963, $p < 0.01$) are highly correlated with the oil refining process which suggests the oil refining process is necessary to reduce the acid and peroxide values for *S. palembanica* oils. The negative correlation between IC₅₀ and oil refining indicates the increased lipophilic antioxidants of *S. palembanica* oils. In contrast, the negative correlation in TPC suggested the reduction in TPC of a hydrophilic extract of *S. palembanica* oil after the refining process.

The correlation coefficient between oil refining on chemical properties, fatty acid composition, antioxidant activity and TPC of *S. stenoptera* oil are presented in Table 4.18. The effects of oil refining on the total SFA content (r_{pb} : -1.000, $p < 0.01$) and total UFA content (r_{pb} : 1.00, $p < 0.001$) are highly significant which indicates the substantial reduction in total SFA and increment of total UFA after refining *S. stenoptera* oil. A weak relationship

between oil refining and acid value is observed indicating the insignificant effects of the oil refining process on the reduction of acid value ($p>0.05$). The refining process highly affects the peroxide value (r_{pb} : -1.000, $p<0.01$) for *S. stenoptera* oil suggesting the oil refining process efficiently reduces peroxide levels. The positive correlation in IC_{50} (r_{pb} : 0.978, $p<0.01$) and the negative correlation of TPC (r_{pb} : -1.000, $p<0.01$) between oil refining shows the reduction in the antioxidant activity and TPC of refined *S. stenoptera* oil.

The effects of oil refining on the chemical properties, fatty acid composition, antioxidant activity and TPC of *S. pinanga* oils are shown in Table 4.18. The negative and positive Point-Biserial Correlation Coefficient (r_{pb}) between total SFA (r_{pb} : -1.000, $p<0.01$) and total UFA (r_{pb} : 1.000, $p<0.01$) respectively, indicates the reduction of total SFA content and increase total UFA content in *S. pinanga* after the refining process. This is inline with the fatty acid composition in crude and refined *S. pinanga* oils is shown in Table 4.15. A strong negative correlation between oil refining with acid value (r_{pb} : -0.959, $p<0.01$) and peroxide value (r_{pb} : -0.910, $p<0.01$) shows a significant reduction in acid and peroxide levels in *S. pinanga* oil after the refining process. The correlation between IC_{50} (r_{pb} : -0.999, $p<0.01$) value and oil refining show a significant increment in lipophilic antioxidants in *S. pinanga* oil. The strong negative correlation of TPC with oil refining indicates the significant reduction of hydrophilic antioxidants in *S. pinanga* oil after refining.

4.10 Chapter Summary

This chapter discusses the findings based on the results obtained. The data on proximate analysis and antinutritive value of *Shorea* oilseeds have been collected and the data shows the potential for alternative feeds. The comparison of the mechanical extraction and Soxhlet extraction methods in extracting *S. palembanica* oil shows that mechanical extracted oil has a higher quality compared to Soxhlet extracted oil. Oil refining improves the quality of *Shorea* seed oils significantly.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

A total of four species of *Shorea* oilseeds have been collected from Singai Village, Bau, Sarawak: *S. macrophylla*, *S. palembanica*, *S. stenoptera* and *S. pinanga*. The oilseeds have been analysed for their proximate composition. Relatively high moisture content in *Shorea* seed is recorded in *S. pinanga* seed (35.56%). The total lipid in *S. macrophylla* (41.37%) and *S. stenoptera* (49.06%) is lower compared to the previous study. *Shorea* seeds are abundant in K and other minerals such as Na, Ca, Mg, Zn and Mn. In addition, toxic heavy metals such as Pb, Cd, Cr, Li, and Ni are absent in *Shorea* seeds. The proximate and mineral composition in *Shorea* species is significantly different ($p < 0.05$) due to genetic variation between species. The analysis of antinutritive factors oxalate and phytate have been evaluated, where *S. macrophylla*, *S. palembanica*, *S. stenoptera* and *S. pinanga* contain low phytate content, which are 0.29%, 0.29%, 0.09% and 0.06%, respectively. However, the oxalate content is recorded at a high concentration in all *Shorea* seeds (*S. macrophylla*: 1.91%; *S. palembanica*: 2.43%; *S. stenoptera*: 2.65%; *S. pinanga*: 3.61%).

S. palembanica seed was subjected to oil extraction using mechanical extraction and Soxhlet extraction. The mechanically extracted oil contains higher total *cis*-MUFAs (34.49%), lower acid (3.47 mg NaOH/kg) and peroxide (10.62 meq O₂/kg oil) values and higher TPC (2.49 mg GAE/kg oil) than Soxhlet extracted oil (total *cis*-MUFAs: 34.23%;

acid value: 8 mg NaOH/kg oil; peroxide value: 14.58 meq O₂/kg oil; TPC: 0.68 mg GAE/kg oil). Therefore, mechanical extraction was selected for the later stage of the study.

Mechanically extracted oils from four *Shorea* species were subjected to refining. Physical characteristics, chemical properties, fatty acid composition and antioxidant activity of crude and refined oils were compared. The fatty acid profiling of *Shorea* oils using GC-MS has found that stearic, oleic and palmitic acid are the predominant fatty acids. Refining effects on chemical characteristics, fatty acid content, antioxidant activity and TPC were evaluated using Point-Biserial correlation analysis (r_{pb}). Oil refining significantly affects the chemical properties, antioxidant activity and TPC of *Shorea* oils ($p < 0.05$). However, the evaluation of fatty acid composition using GC-MS and FTIR-ATR of crude and refined *Shorea* oils indicates that refining weakly affects the fatty acid composition ($p > 0.05$).

5.2 Recommendations

These are several recommendations for further study. The study of *Shorea* oilseed should include the utilisation of defatted oilseed cake as animal feeds such as cattle and fish feeds. The study should also analyse other nutritive and antinutritive factors such as total protein, vitamin, tannin, glucosinolate and saponin. The study on efficiency in other types of extraction methods, such as green extraction, should be conducted to increase the oil yield and quality and reduce the usage of chemical solvents which could adversely affect the environment. Studies on utilising *Shorea* oils as CBEs should be conducted and parameters such as TAG profile, sensory properties and chemometric analysis should be further analysed.

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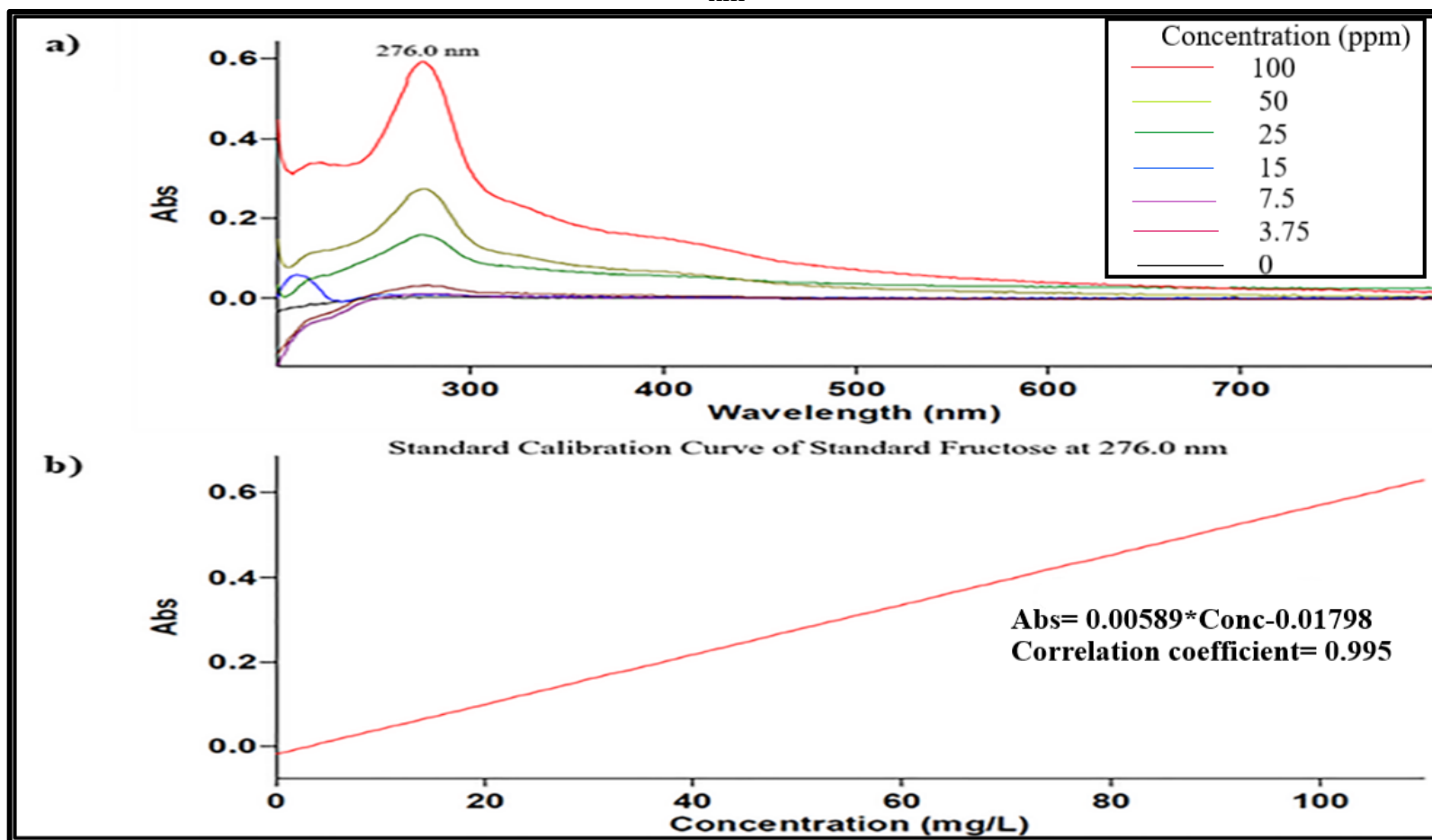
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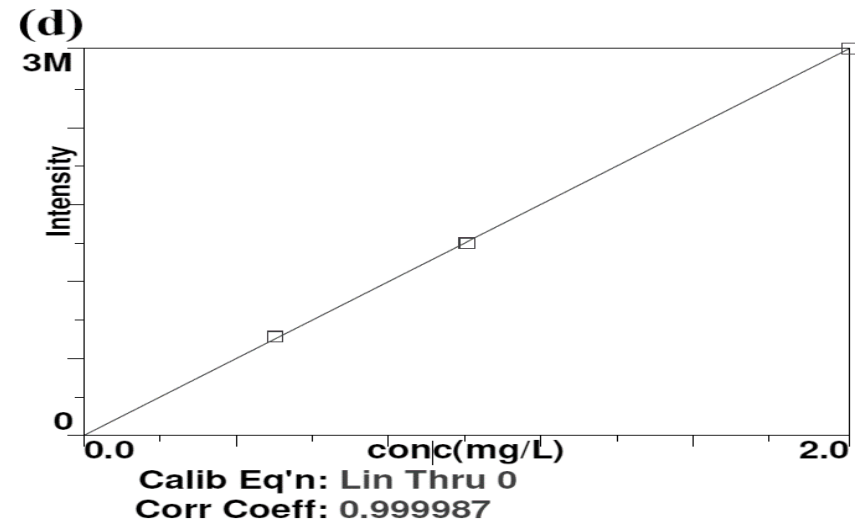
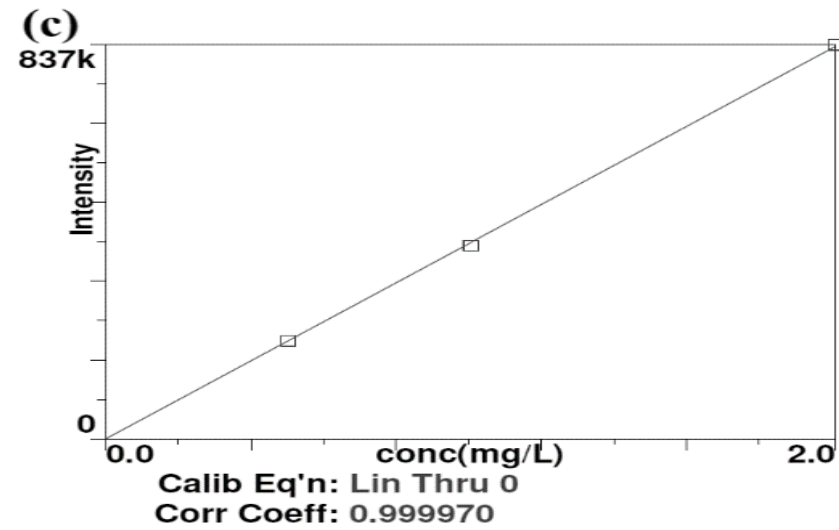
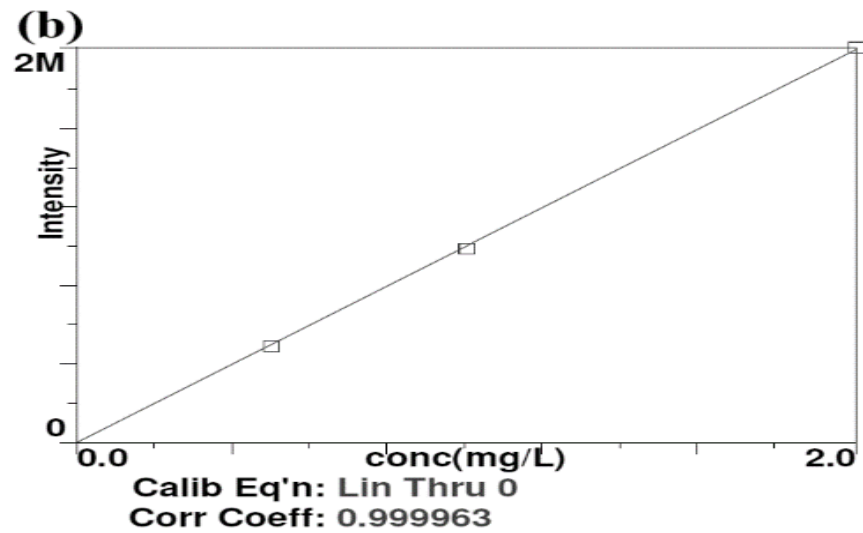
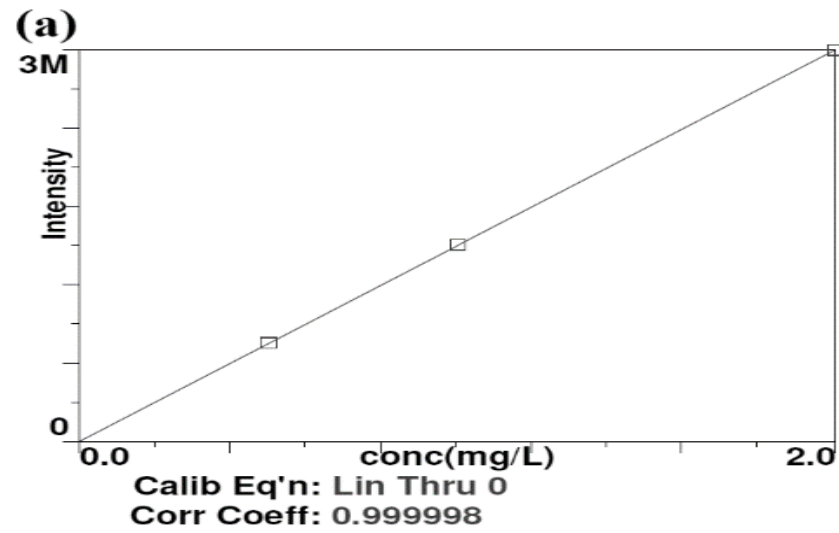
Żyżelewicz, D., Budryn, G., Krysiak, W., Oracz, J., Nebesny, E., & Bojczuk, M. (2014). Influence of roasting conditions on fatty acid composition and oxidative changes of cocoa butter extracted from cocoa bean of Forastero variety cultivated in Togo. *Food Research International*, 63, 328-343.

APPENDICES

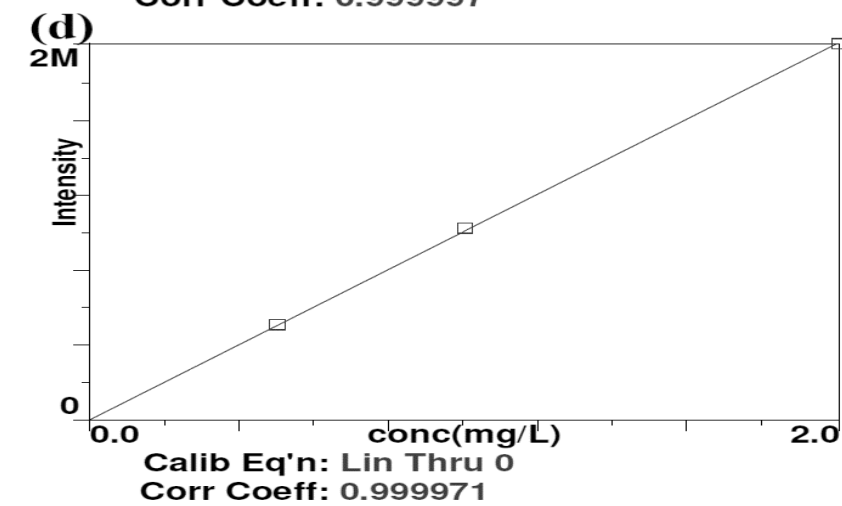
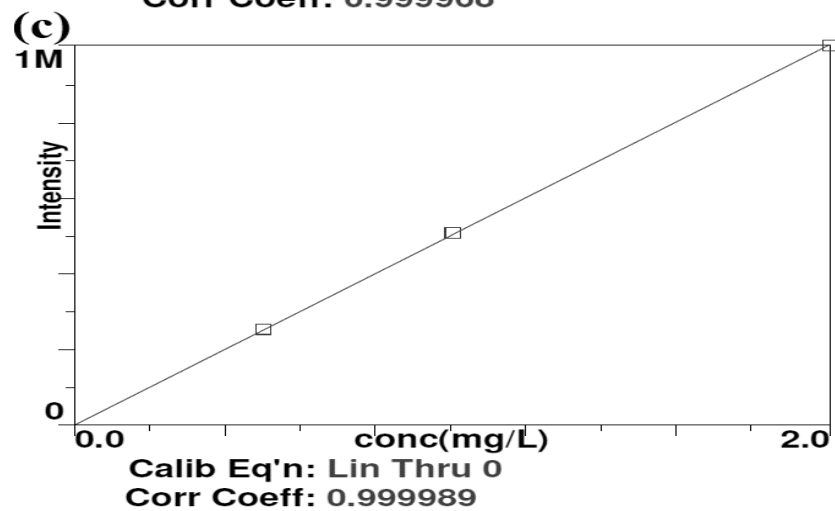
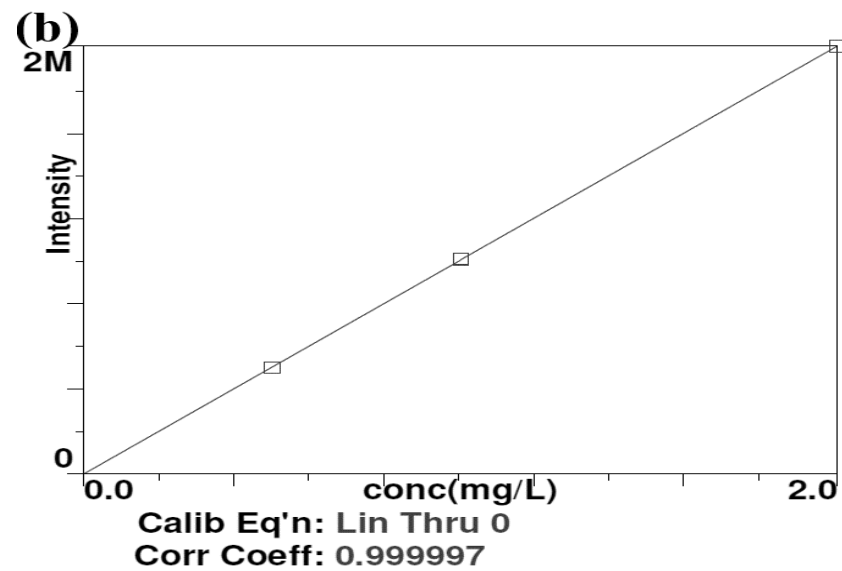
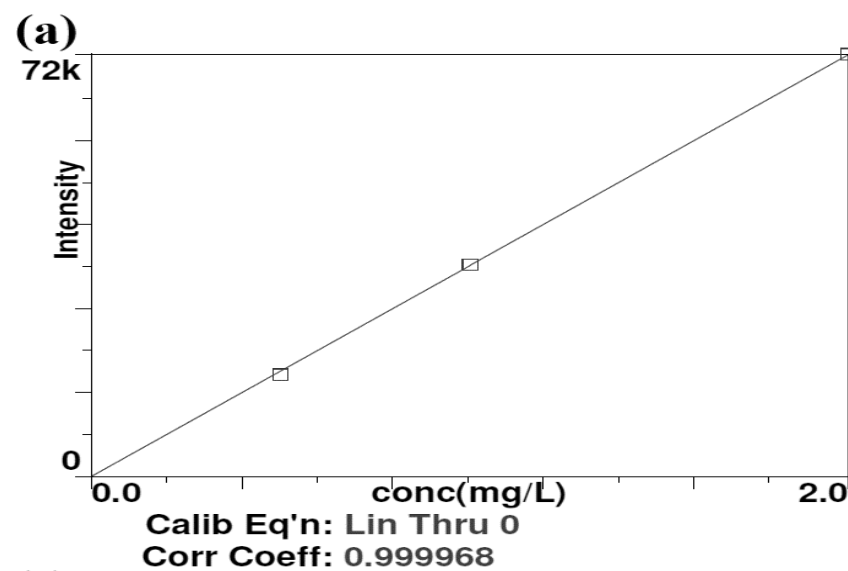
Appendix 1: (a) UV-vis Spectra of Different Concentration of Fructose Standard and (b) Standard Calibration Curve of Fructose at 276.0 nm



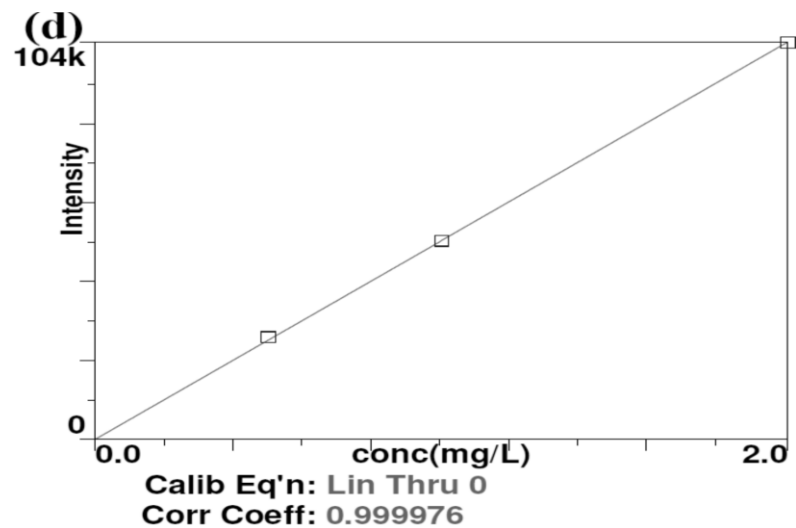
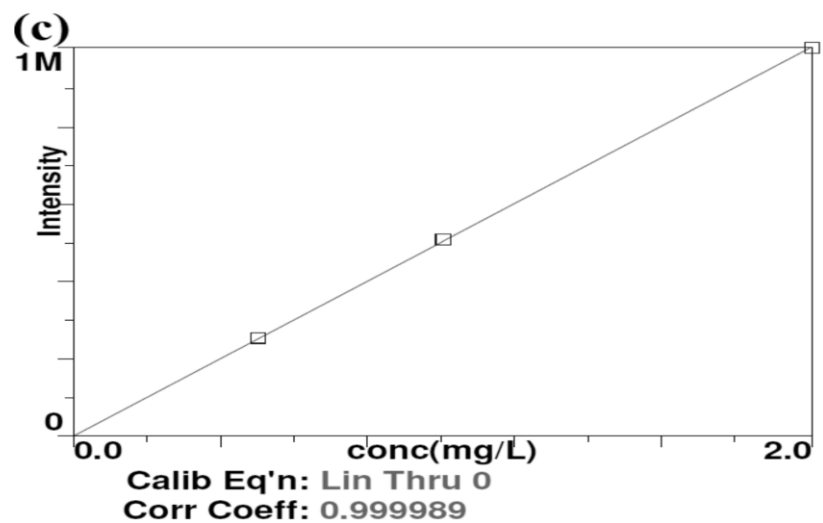
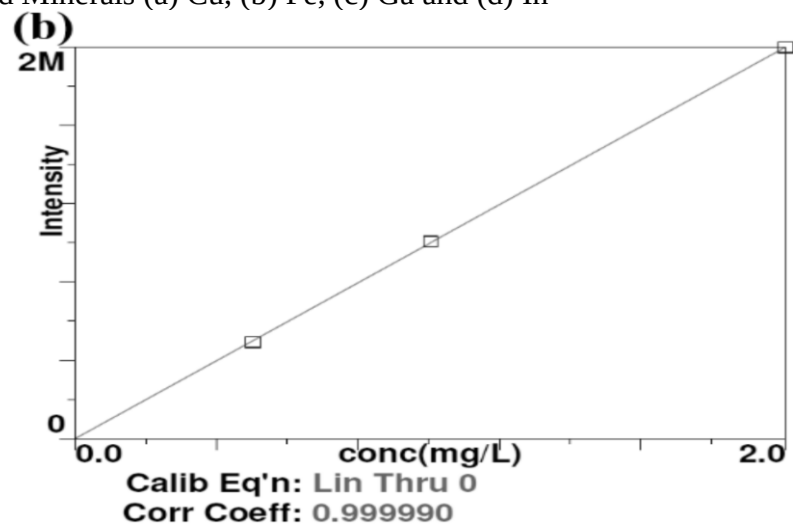
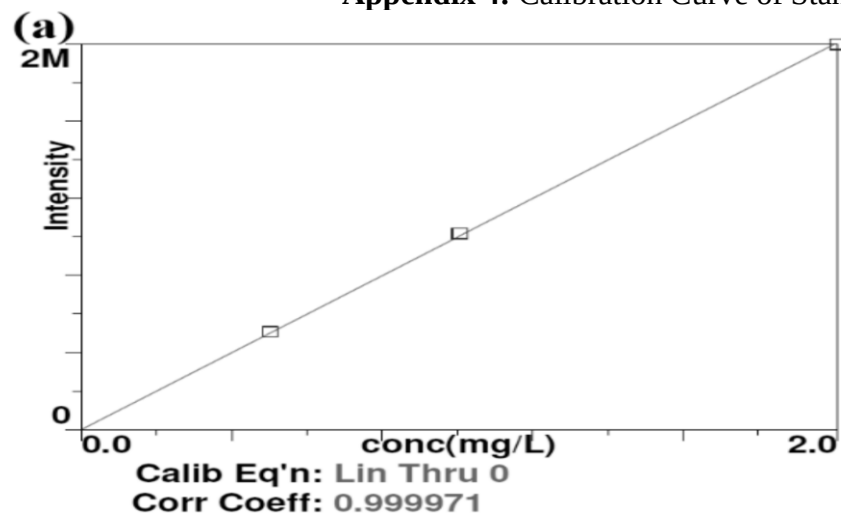
Appendix 2: Calibration Curve of Standard Minerals (a) Ag, (b) Al, (c) B and (d) Ba



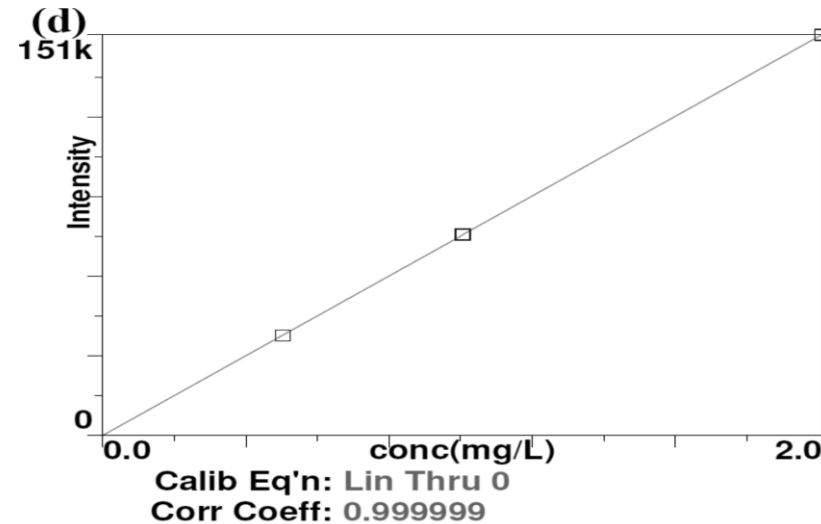
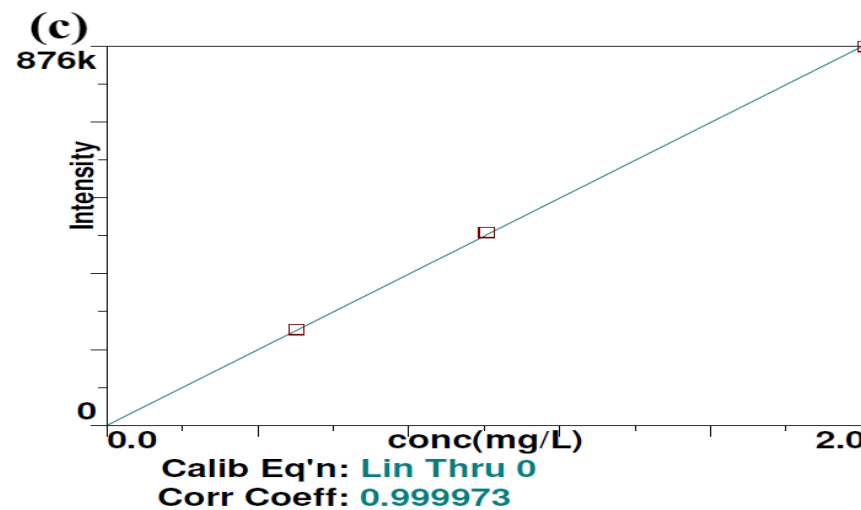
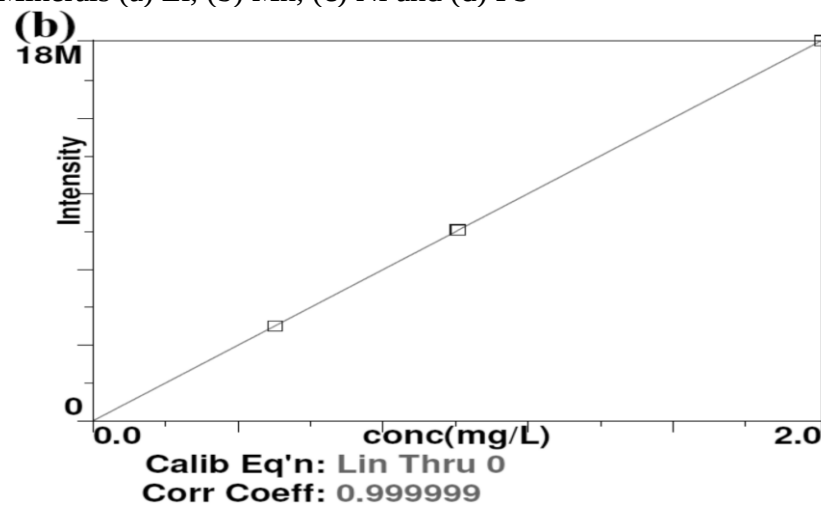
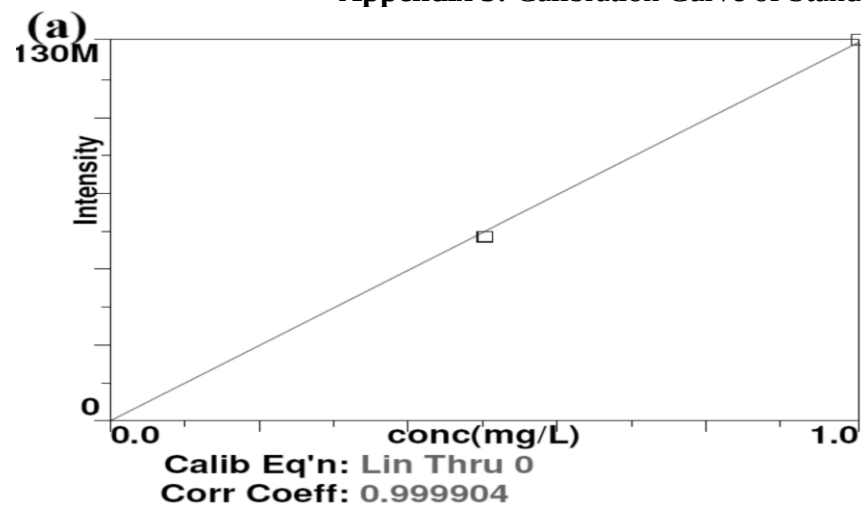
Appendix 3: Calibration Curve of Standard Minerals (a) Bi, (b) Cd, (c) Co and (d) Cr



Appendix 4: Calibration Curve of Standard Minerals (a) Cu, (b) Fe, (c) Ga and (d) In



Appendix 5: Calibration Curve of Standard Minerals (a) Li, (b) Mn, (c) Ni and (d) Pb



Appendix 6: Calibration Curve of Standard Minerals (a) Sr, (b) Ti, and (c) Zn

