Oilseeds and Seed Oils of *Shorea macrophylla* and *Shorea palembanica*: Evaluation of Proximate, Antinutritive Factors and Chemical Composition

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Shorea macrophylla (*S. macrophylla*) and *Shorea palembanica* (*S. palembanica)* are known as "*Engkabang Jantung*" and "*Engkabang Asu*", respectively, by natives in Sarawak, Malaysia. The oilseeds remain underused due to a lack of scientific approach. This study aimed to determine proximate compositions and antinutritional factors of *S. macrophylla* and *S. palembanica* seeds and compare the fatty acid profiles, chemical properties and antioxidant activity between mechanical extraction (ME) and Soxhlet extraction (SE). The proximate compositions of *S. macrophylla* and *S. palembanica* seeds were 21.47% and 27.25% (moisture); 0.67% and 1.98% (ash); 41.37% and 49.06% (total lipid); 79.44% and 82.37% (total carbohydrate); 15.67% and 7.72% (crude fiber), respectively. Seeds of *S. macrophylla* and *S. palembanica* contained high levels of K (1186.50 and 400.17 mg/100 g), Ca (238.31 and 128.62 mg/100 g), Mg (300.50 and 117.17 mg/100 g), and Na (75.12 and 30.14 mg/100 g). The antinutritional factor phytate was detected in small concentrations in both species. At the same time, oxalate was found at a higher concentration in *S. palembanica* (2.43 mg/100 g) than in *S. macrophylla* (1.91 mg/100 g). The bioavailability of Ca and Zn influenced by antinutritional factors phytate and oxalate was calculated based on their molar ratios. The bioavailability of minerals affected by phytate did not exceed the critical value, suggesting adequate mineral absorption. However, high oxalate content exceeded the critical value of bioavailability (2.5), indicating insufficient mineral availability. SE was more efficient in extracting *Shorea* oils. Stearic, oleic and palmitic acids were the major fatty acids in *S. macrophylla* and *S. palembanica* oils, with no significant difference in fatty acid profiles between types of extraction ($p>0.05$). The acid (AV) and peroxide (PV) values of ME oils (AV: 3.47 to 4.75 mg NaOH/g; PV: 7.96 to 10.62 meq O_2/kg) were lower than SE oils (AV: 4.69 to 8 mg NaOH/g; PV: 9.92 to 14.58 meq O_2/kg). Therefore, mechanical extraction is considered the method of choice to extract *Shorea* oils. The iodine value (IV), AV, and PV of *Shorea* oils do not meet the required standards of the Indonesian National Standard (SNI) of Tengkawang butter and Cocoa Butter standards. Thus, a further refining process is suggested to increase the quality of *S. macrophylla* and *S. palembanica* oils.

Keywords: *Shorea;* mineral content; anti-nutritive; extraction method; antioxidant activity

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Oilseeds are mainly derived from oil-producing plants such as rapeseed-mustard, soybean, sunflower, and oil palm [1]. Oilseeds contain fats, vitamins, minerals, carbohydrates, fiber, and protein [2]. Consuming oilseeds can have health benefits. Oilseeds yielding 20% to 40% oil can be categorized as edible or non-edible depending on the use [2]. In response to population growth, the need for seed oils continues to rise, and food scientists continue to research seed oils' nutritional and functional properties [3]. Several countries have examined underutilized plant resources to produce valuable oils. Morocco's argan oil has been utilized in various culinary, medicinal, and cosmetic uses worldwide [4].

Malaysia is one of the biodiversity hotspots with the richest floral diversity, particularly in Sarawak and Sabah, which house an estimated 12,000 species [5]. Several oil-producing plant species within the genus *Shorea* [6] are underutilized, and research on this genus is still scarce. *Shorea macrophylla (S. macrophylla*) and *Shorea palembanica* (*S. palembanica)* (Fig. 1(a) and (b)) can be found in Borneo (Malaysia, Indonesia and Brunei) [7]. These species, named locally as "*Engkabang Jantung*" [8] and "*Engkabang Asu*" [9], are closely related to *Shorea stenoptera (S. stenoptera)* (Borneo tallow) and *Shorea robusta (S. robusta)* (Sal). *Shorea* seed oils have been utilized as cooking oil and butter in traditional Sarawak cuisine [10]. Despite their popularity among Sarawak locals, the usage of *S. macrophylla* and *S. palembanica* oils on a larger scale has not been explored. Research on oil extraction from these two *Shorea* seeds is currently limited.

Figure 1(a). *S. macrophylla* seed

Figure 1(b). *S. palembanica* seed

Studies on *Shorea* species have been reported, including *S. robusta* as a native species of India [11] and *S. stenoptera* as an Indonesian species [12-13]. *S. macrophylla* and *S. palembanica* seeds from Sarawak, Malaysia, may differ compared to the aforementioned *Shorea* species due to differences in the species, geographical location, and environment affecting the oilseed content [14-18]. Nesaretnam & Ali [10] and Shashi Kumar et al. [11] employed the Soxhlet extraction method, while Gusti & Zulnely [12] and Darmawan et al. [13] extracted *Shorea* oils using the traditional "*apit*" extraction process. Extraction method influences oil yield and types of minor lipids [19], tocopherol content, and antioxidant activity in oil [20]. Antioxidants are significant in oil because they can inhibit oxidation and prevent oil degradation [21]. Reports on antioxidant activity and total phenolic content of *Shorea* oil are still scarce.

Vegetable oil production generates a large number of by-products [22], putting a significant amount of social and environmental pressure on efficient reutilization [23] since oilseed cakes have substantial proportions of carbohydrates, protein, fiber and minerals [22, 24]. The study on proximate composition, minerals, and antinutritive factors on *Shorea* oilseeds from Borneo is minute [10]. Therefore, this study compares oil production, chemical characteristics, and antioxidant activity of *S. macrophylla* and *S. palembanica* oils extracted using Soxhlet and mechanical extraction methods and their proximate compositions, mineral content, and antinutritive factors.

EXPERIMENTAL

Chemicals and Materials

H_2SO_4 (18 M, HmbG), NaOH (EMSURE™, ACS Reagent), ethanol (~99.8% undenatured, R&M Chemicals), desiccant beads (SiO_2, Sigma-Aldrich), D(-)-fructose (MERCK), HNO_3 (69-70%, HmbG), HCl (Mallinckrodt Chemicals, ACS grade), methyl red (R&M Chemicals), concentrated ammonia (25%, PC Laboratory), $CaCl_2$ (anhydrous, UNI CHEM), $KMnO_4$ (UNIVAR, Analytical reagent), NH_4SCN (EMSURE™, ACS, ISO, Reag. Ph Eur), $FeCl_3$ (Bendosen), gallic acid (Sigma Life Science), iodine (resublimed, SYSTEM), chloroform (J.T.Baker, ACS Reagent), Wijs solution (Merck), KI (Bendosen), $Na_2S_2O_3$ (Fischer Scientific, Analytical reagent), starch (China National Chemicals Import), diethyl ether (BDH Analar®), phenolphthalein (UNIVAR, Analytical reagent), glacial acetic acid (J.T.Baker,

ACS Reagent), DPPH (Sigma-Aldrich Chemistry), L(+)-ascorbic acid (HmbG) and Folin-Ciocalteu's phenol reagent (MERCK).

Sample Collection and Preparation

A total of 10 kg of seeds of each of *S. macrophylla* and *S. palembanica* were collected from Kampung Singai, Bau district of Kuching Division, Sarawak, Malaysia (1°25′0″N 110°0′9″E). The samples were immediately stored in a freezer at -4ºC. The wings of the seeds were removed using a knife before extraction. The de-winged seeds were sun-dried for three days before being pulverized with an electric blender. The pulverized samples were immediately transferred into polyethylene ziplock bags and kept in the refrigerator.

Proximate Analysis

Moisture and ash contents of the *Shorea* oilseeds were analyzed according to the method performed by Horwitz & Latimer [25]. Moisture content was determined by heating empty crucibles to 105°C and cooling them for 30 minutes using a furnace (Ney Vulcan, D-550, United States). The crucibles were weighed, and 2 g of seed sample was added to each crucible and then heated for three hours at 105°C. Before weighing, the samples were desiccated for 30 minutes. Moisture content was calculated using Equation 1.

$$\text{Moisture content}=\frac{\text{fresh sample weight-dry sample weight}}{\text{fresh sample weight}}\times 100\% \quad \text{Equation 1}$$

The samples were then dried for three hours at 550ºC. The charred samples were desiccated before weighing. Ash content was calculated using Equation 2.

$$\text{Ash content}=\frac{\text{Weight of ash}}{\text{Weight of sample}}\times 100\% \quad \text{Equation 2}$$

Lipid content was determined according to Akbari et al. [26]. 100 g of dried powdered sample was extracted on the Soxhlet apparatus using 250 mL of *n*-hexane for six hours at 65°C to 70°C. A vacuum rotary evaporator (Buchi Rotavapor R-300, Germany) at 40ºC was employed to evaporate the solvent. Oil content was calculated using Equation 3. Recovered oils were refrigerated at 4ºC until further analysis.

$$\text{Oil content}=\frac{\text{Oil yield (g)}}{\text{Sample (g)}}\times 100\% \quad \text{Equation 3}$$

Crude fiber was determined using the method outlined by Madhu et al. [27]. Briefly, 2 g of defatted material was added to a flask. The flask was filled with anti-bumping chips and 200 mL of 0.25 M H_2SO_4 and boiled for 30 minutes at 80ºC. The solution was filtered through muslin cloth, and the residue washed with hot water (60ºC). The residue was then boiled for 30 minutes in 200 mL of 0.3 M NaOH at 80ºC. The solution was filtered and the residue washed with 25 mL of boiling 1.25% H_2SO_4, three portions of 50 mL of distilled water and 25 mL of ethanol. The residue was weighed in a crucible (W1). The crucible was heated for 30 minutes at 550°C and dried out in a desiccator overnight before being weighed (W2). Crude fiber was estimated using Equation 4.

$$\text{Crude fiber}=\frac{\text{W1-W2 (g)}}{\text{Weight sample (g)}}\times 100\% \quad \text{Equation 4}$$

Carbohydrate was extracted in line with the method used by Smith et al. [28]. Exactly 1 g of powdered sample was boiled with 50 mL of distilled water under reflux for an hour. The mixture was then filtered and the filtrate transferred into a 100 mL volumetric flask. Distilled water was subsequently topped up to the calibrated mark. Total carbohydrate content was determined using the sulfuric acid-UV method [29]. Exactly 15 mL of concentrated H_2SO_4 was added to 5 mL of dissolved fructose. A dark brick red solution (furan solution) formed and vortexed (Labnet, VX-200, USA) for 30 seconds. Standard solutions of 100, 50, 25, 15, 7.5, and 3.75 ppm furan were then established. Subsequently, 5 mL of water extract was mixed with 15 mL of concentrated H_2SO_4. 7 mL of the stock solution was diluted with distilled water in a 100 mL volumetric flask. An exact volume of 20 mL of the resultant solution was added to the mark in a 50 mL volumetric flask. A UV-Vis spectrometer (Agilent, Cary 5000, USA) measured the absorption of the serial dilution of 3.75 to 100 ppm furan standard solutions and the sample solutions at 276 nm. Total carbohydrate was calculated using Equation 6.

$$\%\text{Total carbohydrate}=\frac{142.88\times M\times 0.1}{10} \quad \text{Equation 5}$$

Where, 142.88 is the dilution factor; M is the concentration of the sample in UV-Vis (mg/L); 0.1 is 1 g of sample diluted in 0.1L (L/g) and 10 is the conversion to percentage.

Mineral Analysis

Mineral contents (Bi, Co, Cd, Ba, Fe, B, Cr, Cu, Ag, Al, Ti, Zn, Pb, In, Ni, Mn, Mg, Ca, Sr, Ga, Na, Li and K) of seed samples were analyzed on Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES) (Perkin Elmer, Optima 8000, United States) according to the method described by Morais et al. [30] using ash samples obtained in the total ash assay. An ash sample was initially digested with 5% HNO_3. Then, the mixture was boiled to a homogeneous solution and filtered through 8 µm Whatman filter paper. The clear filtrate was transferred into a 100 ml

volumetric flask and topped up with distilled water to the calibrated mark. The solution was filtered with 0.45 µm membrane filter paper before analysis.

Antinutritive Factor Analysis

Total oxalic acid in oilseed samples was determined using the procedure described by Olawoye et al. [31]. Precisely 2 g of sample was added into a 250 mL conical flask, and then 190 mL of distilled water and 10 mL of 6 M HCl were added into the flask. The mixture was boiled for 1 hour and then filtrated using a filter paper. The filtrate was transferred into a 250 mL volumetric flask and diluted to volume with distilled water. Exactly four drops of methyl red were added to the solution and titrated with concentrated ammonia until the solution turned faint yellow. The solution was heated to 100ºC and allowed to cool for precipitation of ferrous ions. The precipitates were removed and the solution was added with 10 mL of 5% $CaCl_2$ and boiled. The solution was left to rest overnight. The solution was filtered and the residue was kept and washed several times with distilled water. The residue was then dissolved with 5 mL of 25% H_2SO_4. The resultant solution was kept at 80ºC and titrated with 0.02 M $KMnO_4$ until the pink color persisted for approximately 1 minute. A blank was also run for the test sample. Oxalate content was calculated using Equation 6.

1 mL of 0.02 M $KMnO_4$ = 0.000225 g of oxalic acid Equation 6

Phytate in oilseeds was determined according to the procedure outlined by Sarkiyayi & Agar [32]. Briefly, 2 g of oilseed sample was soaked with 100 mL of 2% HCl for 3 hours and filtered through double-layered filter paper. The filtrate was then boiled and transferred into a 50 mL volumetric flask. The solution was top up to the mark with distilled water. The solution was then added with 100 mL of distilled water and 10 mL of 3% ammonium thiocyanate as indicators. The solution was titrated with $FeCl_3$ until the solution retained brownish-yellow color for five minutes. Distilled water was analyzed as blank in the test. The amount of phytate was calculated using Equation 7.

$$\% \text{ Phytate} = [((V_1 - V_b) * 0.00195 * 1.19) / W_0] \times 100 \quad \text{Equation 7}$$

Where 1 mL of $FeCl_3$ solution is 0.00195 g of iron; 1 g of iron = 1.19 of phytin-phosphorous; V_1 is the volume of $FeCl_3$ used for titration; V_b is the volume of $FeCl_3$ consumed by the blank; and W_o is the weight of sample used.

Oil Yield

Mechanical extraction was carried out using a manually operated PITEBA oil expeller [33]. The feeder was loaded with a powdered seed sample (100 g). A pre-weighed empty glass container was used to collect the isolated oil. Oil yield was calculated using the equation for oil content.

Fatty Acid Analysis

Fatty acids in the *Shorea* oils were derivatized into their corresponding fatty acid methyl esters (FAMEs) as outlined by Aldai et al. [34]. Samples were analyzed on a Gas Chromatography-Mass Spectrometer (GC-MS) model QP2010plus (Shimadzu, Japan) equipped with a 30 m x 0.25 mm x 0.25 µm DB5 column using an auto-sampler (AOC-20S, Shimadzu, Japan). The GC-MS was programmed as follows: The initial oven temperature was at 50ºC, held for 10 minutes. The temperature was then ramped to 350°C at the rate of 4.5 ºC/min and held for 10 minutes at the final temperature. The carrier gas was helium at a flow rate of 1.0 mL/min. The sample was injected in a spitless injection mode at 280ºC. The ionization potential was 70 eV and the scanning range of the mass-selective detector was 45-600 m/z.

Chemical Properties

Iodine value (IV) was determined according to AOCS Cd 1-25 method described by Yildiz et al. [35]. In a conical flask, exactly 0.1 g of oil was dissolved in 20 mL of acetic acid and cyclohexane solution (1:4, v/v). The solution was then added with 25 mL of Wijs solution and swirled thoroughly, then let to rest for 30 minutes in the dark. A total volume of 20 mL of 15% KI solution and 100 mL of distilled water were added to the solution and mixed thoroughly. The solution was titrated with 0.1 N $Na_2S_2O_3$ until a pale-yellow colour appeared. Exactly 4 mL of 1% starch solution were added into the solution, resulting in a blue-black color. The titration was continued until the blue-black color disappeared. Distilled water was used as blank. Iodine value was calculated using Equation 8.

$$\text{Iodine value} = \frac{Vb - Vs}{Ws} \times 0.1N \times 12.69 \quad \text{Equation 8}$$

Where V_b is the volume of $Na_2S_2O_3$ titrated with the blank; V_s is the volume of $Na_2S_2O_3$ titrated with the presence of the sample; and W_s is the weight of oil in g.

Acid value (AV) of the oils was determined in line with the official method Ca 5a-40 [36]. 0.1 g of oil was poured into 20 mL of neutral solvent (diethyl ether: ethanol, 1:1). The solution was boiled on a hot plate, and 0.5 mL of 1% phenolphthalein was added as an indicator. The solution was then titrated with 0.1 N NaOH until a light pink color appeared. Acid value was calculated using Equation 9.

$$\text{Acid value} = 40 \times 0.1\ \text{N} \times \frac{\text{Vs - Vb}}{\text{Ws}} \qquad \text{Equation 9}$$

Where 40 is molar mass of NaOH; Vs is the volume of NaOH titrated with the sample; Vb is the volume of NaOH titrated with the blank; and Ws is the weight of the sample in g.

Peroxide value (PV) was determined according to method Cd 8b-90(1) of AOCS [37]. Exactly 0.5 g of oil was dissolved in 50 mL of glacial acetic acid - chloroform (3:2, v/v). The solution was then added with 0.5 mL of saturated KI and swirled for a minute. The solution was then added with 30 mL of distilled water. The solution was titrated with 0.01 N $Na_2S_2O_3$ until the yellow color of iodine disappeared, and 0.5 mL of 1% starch solution was added to form a blue solution. The titration was continued until the blue solution disappeared. Distilled water was used as a blank. Peroxide value was calculated using Equation 10.

$$\text{Peroxide value} = 100 \times \frac{\text{Vs - Vb}}{\text{Ws}} \times 0.01\ \text{N} \qquad \text{Equation 10}$$

Where Vs is the volume of titrated $Na_2S_2O_3$ with the sample; V_b is the volume of $Na_2S_2O_3$ titrated with the blank; and Ws is the weight of the sample in g.

Determination of Antioxidant Activity

Radical scavenging activity of the oils was evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and ascorbic acid as the standard. A series of concentrations (1.427, 2.849, 5.682, 7.092 and 11 ppm) from dissolved oil and ascorbic acid was prepared by adding 0.1 mM DPPH. Absorbance was measured at 517 nm by an Ultraviolet-Visible (UV-Vis) spectrometer as recommended by Wollinger et al. [38]. A triplicate measurement was taken for each concentration. % Inhibition of standard and sample was calculated according to Equation 11. A calibration curve of standard ascorbic acid with y = 5.1326 x + 37.451 in the range of 1.427 ppm to 11 ppm was used to quantify the antioxidant activity and showed good linearity (R^2 = 0.999). The antioxidant activity was expressed in half maximal inhibitory concentration, IC50 µg/ mL.

$$\%\ \text{Inhibition} = (\text{Abs0} - \text{Abs1}) * 100 / \text{Abs0} \qquad \text{Equation 11}$$

Where Abs0 is the absorbance of the blank at 517 nm; Abs1 is the absorbance of the oil and standard at different concentrations at 517 nm.

The extraction of polyphenolic compounds in the oils was carried out according to the method performed by Bouarroudj et al. [39]. Total phenolic content (TPC) was determined by referring to the method described by Kupina et al. [40] using the Folin-Ciocalteu reagent. A series of concentrations of gallic acid (40, 80, 120, 160 and 200 ppm) was prepared as a calibration standard. A calibration curve was developed (y =0.09391 * Conc + 0.03834) using a range of 2.625 to 20.619 mg/L of gallic acid and showed good linearity (R^2 = 0.999). The measurement was carried out in triplicate. Total phenols was calculated using Equation 12.

$$\text{Total phenols} = [(A - b)/m] * [(V * D) / (W)] * 100 \qquad \text{Equation 12}$$

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

Statistical Analysis

All experiments were repeated three times and data are expressed as the mean value of triplicate ± standard deviation. Analysis of variance (ANOVA) was carried out using IBM SPSS (Version 26) to compare the mean difference between the seed oils and oilseeds.

RESULTS AND DISCUSSION

Proximate Composition

Proximate composition of *Shorea* oilseeds was compared against main livestock and poultry feed sources, soybean and cornmeal [41]. As shown in Table 1 *S. palembanica* seed contained higher moisture compared to *S. macrophylla*, at 27.25% and 21.47%, respectively. Moisture content of the *Shorea* seeds was higher than previously reported in *S. macrophylla* [10] and Sal seeds [11]. The discrepancy of moisture content in *S. macrophylla* in this study is because of the different drying methods employed. Nesaretnam & Ali [10] used a controlled chamber temperature at 30ºC with 40% humidity for three days while this study used the sun-drying method. The finding agrees with those reported by Yarahmadi et al. [42]. The post-harvest drying method of Sal seed (*S. robusta*) was similar to this study, where the sun-drying method was employed [11]. Zahran et al. [14] recorded a notable difference in moisture content of seeds among the variants due to genetic variation. In addition, Kim et al. [43] stated the moisture content of seeds collected at different locations is significantly different due to the environmental effects such as soil, water, and weather. Therefore, the variation in moisture content between Sal seed and oilseeds in the present study is due to the differences in genetics and sampling location. Drying reduced the moisture content, enhancing the storage stability and minimizing loss [42]. Thus, oilseeds with low moisture content are preferable because they can be stored for longer periods without damage [11, 44]. Therefore, a longer drying process was conducted before oil extraction.

S. palembanica seed contained a higher ash content than *S. macrophylla,* at 1.98% and 0.67%,

respectively. The ash content in *S. macrophylla* and *S. palembanica* was lower than that reported in *S. robusta*, which was 3.75% [11], due to genetic variation, location, environmental factors and agronomic practices [43, 45]. Tenyang et al. [46] and Cheng et al. [47] reported significant differences in ash content of sesame seeds when using different seed processing methods such as baking, steaming and sun drying, which suggests the processing methods of *Shorea* seeds might affect the ash content. The ash content in *S. macrophylla* was lower than soybean meal [6] and cornmeal [48], which were 4.5% to 6.4% and 1.13%, respectively. The ash content in seeds and tubers must be between 1.5% to 3.5% to be suitable as an animal feed source [49].

The seeds of *S. macrophylla* and *S. palembanica* contained high amounts of lipid, at 41.37% and 49.06%, respectively, as shown in Table 1. Seeds with high fat content are desirable for industrial purposes [11]. The oil content in *S. macrophylla* oilseeds reported by Nesaretnam & Ali [10] was higher (55.9%) compared to this study. The initial hypothesis is the oil content of *S. macrophylla* oilseed in this study will be higher than in the previous report due to the usage of *n*-hexane as extracting solvent instead of petroleum ether which has better extraction efficiency [50-51]. This result might be due to the higher moisture content in this study compared to the previous report. The moisture content in the seeds causes the increase of solvent hydration during oil extraction, which reduces oil solubility in the solvent and decreases extraction efficiency [52]. In addition, the maturity of seeds during harvesting also affects the oil content. Adewusi John et al. [53] reported a significant difference in seed oil content between ripe and unripe *Blighia sapida* seeds ($p<0.001$), where ripe seeds contain higher oil content than unripe seeds. Zahran et al. [14] suggested that the oil content of seeds is affected by the seeds' maturity during harvesting. The oil content of *S. robusta*, which was 30.2% [11], is significantly lower compared to *S. macrophylla* and *S. palembanica* seeds in this study. The significant difference in the oil contents in this study with those reported by Shashi Kumar [11] might be due to genetic diversity, location, and environ- mental factors [43, 54].

Carbohydrates are organic compounds that function as a source of energy for humans and animals [55]. Total carbohydrate of defatted seeds was higher in *S. palembanica* (82.37%) and *S. macrophylla* (79.44%). Both *Shorea* oilseeds have significantly higher total carbohydrates than soybean meal, as shown in Table 1, suggesting that *Shorea* seeds have the potential to be used as a source of carbohydrate in animal feeding. Total carbohydrate in de-oiled cakes in this study was significantly higher compared to the previous report by Shashi Kumar [11], which was 42.11%. Total fiber was substantially higher in *S. macrophylla* (15.67%) compared to *S. palembanica* (7.72%) ($p<0.05$). Total carbohydrate and fiber contents between the *Shorea* species were significantly different ($p<0.05$), which might be due to genetic variation between species [56].

Mineral Content

The concentration of minerals detected in seeds of *S. macrophylla*, *S. palembanica*, sesame [57], soybean [58], and sunflower [59] are shown in Table 2. K was the dominant macro mineral in *S. macrophylla* and *S. palembanica*, at 1186.50 mg/100 g and 400.17 mg/100 g, respectively. *S. macrophylla* had a higher K content compared to sunflower (25 to 103 mg/100 g) and sesame (476.64 to 535.73 mg/100 g), but lower than soybean (2120 to 2320 mg/100 g).

The lowest macro mineral found in the *Shorea* seeds was Na. The amount of Na in the *Shorea* species was lower compared to sunflower, at 160 to 530 mg/100 g. *S. macrophylla* and *S. palembanica* had higher K to Na ratios, at 15.79 and 13.28 mg/100 g, respectively, than sunflower (0.19), indicating more benefits in reducing the risk of hypertension [60]. Mg was the second highest mineral detected in *S. macrophylla*, at 300.5 mg/100 g. Mg is important in regulating osmotic equilibrium, a cofactor in enzyme-catalyzed reactions, prevention of heart diseases, and lowers blood pressure. Insufficient intake of Mg will cause muscle irritability and convulsion, whereas excessive intake will disturb the central nervous system [61]. The amount of Mg in *S. macrophylla* was comparable to sesame and soybean, but higher compared to sunflower.

Table 1. Proximate composition of *Shorea* oilseeds

%	*S. macrophylla*	*S. palembanica*	Soybean meal [6]	Cornmeal [48]
Moisture	21.47±0.05^{a}	27.25±0.04^{b}	5.6-11.5	8.3
Ash	0.67±0.00^{a}	1.98±0.03^{b}	4.5-6.4	1.31
Lipid content	41.37±0.03^{a}	49.06±0.03^{b}	15.5 to 24.7	3.8
Total carbohydrate	79.44±0.00^{a}	82.37±0.00^{b}	31.75-31.85	-
Total fibre content	15.67 ±0.14^{a}	7.72±0.15^{b}	6	-

Means in the same row with the same superscript $^{a/b}$ are not significantly different at 5% level.

Table 2. Mineral composition of *Shorea* oilseeds

Mineral	Species				
	S. macrophylla	*S. palembanica*	Sesame [57]	Soybean [58]	Sunflower [59]
Macromineral					
Mg	300.50±0.00[a]	117.17±0.00[b]	342.78-401.35	308-346	8.6-35
Ca	238.31±0.00[a]	128.62±0.01[b]	1111.61-1786.5	313-416	112-137
Na	75.12±0.00[a]	30.14±0.00[b]	0.94-8.74	29.80-28.30	160-530
K	1186.50±0.00[a]	400.17±0.01[b]	476.64-535.73	2120-2320	25-103
Micromineral					
Zn	6.97±2.23[a]	4.04±3.8[b]	4.96-6.25	4.14-7.7	4.13
Mn	12.43±0.08[a]	0.67±0.60[b]	1.15-1.57	2.97-7.08	0.39
Toxic metal					
Al	n.d.	1.01±0.88[a]	n.d.	n.d.	n.d.

Note: Data are mean value of three replicates ± standard deviation (s.d.) in mg/100 g unit; n.d. is not detected values less than 0.005 mg/100 g; Means in the same row with the same superscript [a/b] are not significantly different at 5% level; Bi, Co, Cd, Ba, Fe, B, Cr, Cu, Ag, Ti, In, Sr, Ni, Pb, Li and Ga were not detected in the *Shorea* seeds.

Ca was the second highest mineral in *S. palembanica* at 128.62 mg/kg, and was found to be comparable to sunflower. Ca is essential for bone formation and neuromuscular functions [62]. The amount of Zn in *S. macrophylla* was higher compared to sesame and sunflower, while the lowest amount of Zn was found in *S. palembanica*. Mn was significantly higher in *S. macrophylla*, at 12.43 mg/100 g, than the listed seeds. Al, a toxic metal, was found in trace amount in *S. palembanica* oilseed at 1.01 mg/100 g. The *Shorea* oilseeds can be considered safe for consumption since heavy metals are in trace amounts. The *Shorea* seeds possibly have nutritional benefits due to the comparable amounts of essential minerals with common seeds listed in Table 2. The mineral contents in the *Shorea* seeds in this study vary significantly between species ($p<0.05$), which is consistent with those reported in Querceus [63] and Chenomeles seeds [64]. This indicates that the mineral profiles might vary based on their genetic factors and ecological conditions [65].

Antinutritive Factors

Antinutritive factors can reduce the availability of essential minerals to be absorbed into the body resulting in stunted growth performance [66]. A minute intake of phytate in the diet has beneficial health effects; such as antioxidative, anticancer, and antidiabetic, and it reduces the risk of cardiovascular diseases. However, a high amount of phytic acid intake will have a noxious effect on health as phytic acid interferes with the digestibility and bioavailability of starch, proteins and minerals such as Ca, Fe and Zn [67]. The screening of antinutritive factors oxalate and phytate are shown in Table 3. The phytate content of the *Shorea* seeds was not significantly different at 5% level. The phytate content of the *Shorea* seeds was distinctively lower compared to high phytic acid oil-seeds such as soybean (1 to 2.2 g/100 g) and mung bean (0.59 to 1.1 g/100 g) [68]. Diarra et al. [69] reported that broilers and egg-type chickens fed diets containing 1.65% and 2% phytate lost 28% and 44% of their body weight, respectively. As a result, our study suggests that feeding chickens with *Shorea* seeds does not significantly reduce chickens' growth rate.

S. palembanica seed had higher oxalate content compared to *S. macrophylla* seed, which were 2.43 g/100 g and 1.91 g/100 g, respectively. The notable difference in oxalate content between the *Shorea* species may be attributed to genetic variation, soil condition, and harvest time [70]. The oxalate content was considered high as the concentration was above 50 mg/100 g [68]. The oxalate level in ruminant feeds must be less than 2% and less than 0.5% in non-ruminant diets [71]. Therefore, this study recommends a further reduction of oxalate before manufacturing as animal feeds [72]. The oxalate content in the *Shorea* seeds was lower than soybean (3.7 g/100 g) [70], but higher than cocoa powder (0.62 g/100 g) [73]. High oxalate diets are a major concern in several countries as the intake enhances kidney stone formation and decreases mineral availability in the body [73].

Table 3. Antinutritional factors of *Shorea* oilseeds

Antinutritional Factor	*S. macrophylla*	*S. palembanica*
Phytate (g/100 g)	0.29±0.06[a]	0.29±0.03[a]
Oxalate (g/100 g)	1.91±0.04[a]	2.43±0.02[b]

Note: Data are mean percentage of three replicates±standard deviations (s.d.); Means in the same row with the same superscript[-b] are not significantly different at 5% level.

Bioavailability is the ratio of an element absorbed from the digestive tract into the systemic circulation of both animals and humans [72]. As mentioned earlier, the antinutritive factors oxalate and phytate bind with minerals to form insoluble compounds that affect absorption and bioavailability and cause health problems such as osteoporosis and anaemia [74]. Minerals' bioavailability is determined based on the molar ratios of anti-nutrient oxalate and phytate with Ca and Zn [75]. Table 4 shows the molar ratios of phytate and oxalate with Ca and Zn in the *Shorea* seeds. The phytate : Ca molar ratios in *S. macrophylla* and *S. palembanica* seeds were 0.07 and 0.14, respectively. The phytate : Ca molar ratios were less than 6, indicating adequate Ca in the *Shorea* seeds [75]. Castro-Alba et al. [75] reported higher molar ratios of phytate to Ca in yellow corn (912.2), oat (4), fava beans (3.94), peanuts (0.79), and flaxseeds (0.32).

The calculated molar ratios of phytate to Zn in *S. macrophylla* and *S. palembanica* seeds were 4.12 and 7.11, respectively. Both of the *Shorea* seeds are in the range of the suggested critical values of molar ratios of phytate to Zn, which is regarded as favorable for Zn absorption [76]. Magallanes-López et al. [77] stated that: phytate : Zn molar ratio higher than 15 is associated with poor bioavailability, between 5 to 15 is moderate, and lower than 5 is considered high bioavailability, corresponding to 15%, 30% and 50% zinc absorption, respectively. The molar ratios of phytate to Zn of the *Shorea* seeds were lower compared to yellow corn (14.6), oat (82.4), fava bean (46.2), peanuts (20.4), and flax seeds (15.8) [75].

High levels of Ca in seeds may aggravate the detrimental impact of phytate on Zn absorption because Ca has a synergistic effect on Zn to form a more stable Ca-phytate-Zn complex at neutral pH [75]. Therefore Alemayehu et al. [72], Castro-Alba et al. [75], and Hailu and Addis [76] suggested that the molar ratio of phytate·Ca : Zn is a better indicator of Zn absorption. The molar ratios of phytate·Ca : Zn in *S. macrophylla* and *S. palembanica* seeds were 0.03 and 0.02, respectively. Considering the molar ratios of phytate : Zn and phytate·Ca : Zn in the *Shorea* seeds do not exceed the critical values of 15 and 1, respectively, Zn may be absorbed sufficiently in the body [76]. The estimated molar ratios of oxalate : Ca in *S. macrophylla* and *S. palembanica* seeds were 3.65 and 8.59, respectively. The molar ratios exceed the critical value of 2.5, indicating poor absorption and may have a negative impact on calcium bioavailability [72]. In this study, we discovered that phytate did not affect mineral absorption since the molar ratios do not reach the limiting threshold. On the other hand, *the Shorea* seeds have relatively high oxalate to calcium molar ratios, which may impact Ca bioavailability. Therefore, further food processing and fortification of the *Shorea* seeds before use as a source of food and animal feed are recommended to reduce the inhibitory impact of the antinutrient oxalate.

Oil Yield

Mechanical expression involves compression of oleaginous material where oil is separated from seeds under the forces from the compressive action caused by a mechanical expeller [78]. The mechanical expeller consists of a horizontal rotating screw in a perforated barrel made of metal bars, as shown in Figure 2 (a) [79]. A cone at the screw head partially blocks the sample, which causes pressure to increase, forcing the separation of oil from the sample. The exuded oil is discharged through slits between the bars of the barrel.

Table 4. Calculated molar ratios of oxalate and phytate to minerals (Ca and Zn) in *Shorea* seeds

Antinutritional factor : mineral	*S. macrophylla*	*S. palembanica*	Critical values [76]
Phytate : Ca[1]	0.07	0.14	6
Phytate : Zn[2]	4.12	7.11	15
Phytate*Ca : Zn[3]	0.03	0.02	1
Oxalate : Ca[4]	3.65	8.59	2.5

Note: [1]mg of phytates/molecular weight of phytates: mg of calcium/molecular weight of calcium; [2]mg of phytates/molecular weight of phytates: mg of zinc/molecular weight of zinc; [3]mg of calcium/molecular weight of calcium) *(mg of phytates/molecular weight of phytates)/ (mg of zinc/molecular weight of zinc); [4]mg of oxalates/molecular weight of oxalate: mg of calcium/molecular weight of calcium

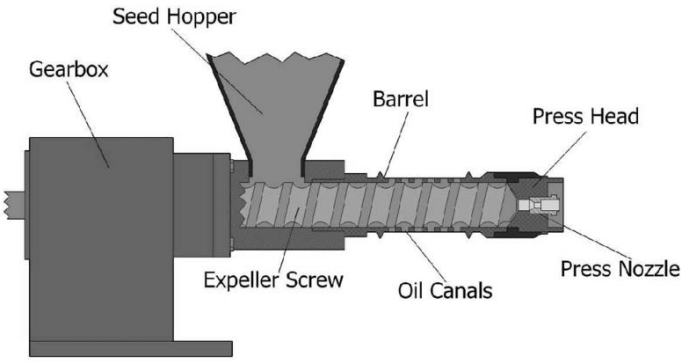

Figure 2 (a). Oil expeller [79]

Soxhlet extraction consists of a solvent distillation flask, a thimble, a syphon, and a condenser. A cellulose thimble is used to contain the sample, which is placed within an extraction thimble. The solvent from the distillation flask evaporates and travels through the thimble, extracting the oil and liquifying it in the condenser. The liquids fall back into the distillation flask as the liquids in the thimble and syphon overflow. The procedure is repeated till extraction is completed [80].

Oil yield is one of the crucial parameters in oil extraction to indicate the efficiency of extraction methods [81]. The oil yields from *S. macrophylla* and *S. palembanica* seeds extracted using two different methods are summarized in Table 5. Soxhlet extraction yielded higher oil compared to mechanical extraction in *S. macrophylla* (41.37% and 37.07%, respectively) and *S. palembanica* (49.06% and 40.82%). Therefore, Soxhlet extraction is more efficient in extracting oil from *S. macrophylla* and *S. palembanica* oilseeds than mechanical extraction. This finding agrees with Gharby et al. [82] and Alrashidi et al. [83], where Soxhlet extraction using hexane had higher oil yields than mechanical extraction.

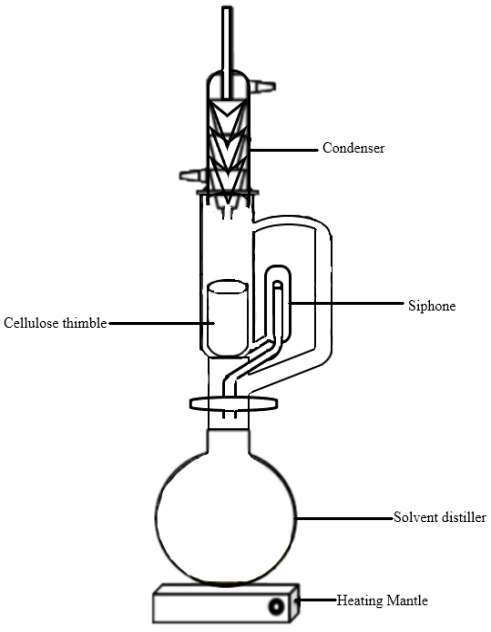

Figure 2 (b). Soxhlet extraction

Table 5. Oil yields of *Shorea* oilseeds using ME and SE methods

Shorea species	Oil yield±s.d (%)	
	Mechanical extraction	Soxhlet extraction
S. macrophylla	37.07±0.85	41.37±0.03
S. palembanica	40.82±0.89	49.06±0.03

Note: s.d – standard deviation of triplicate analysis of oil yield from two types of oil extraction.

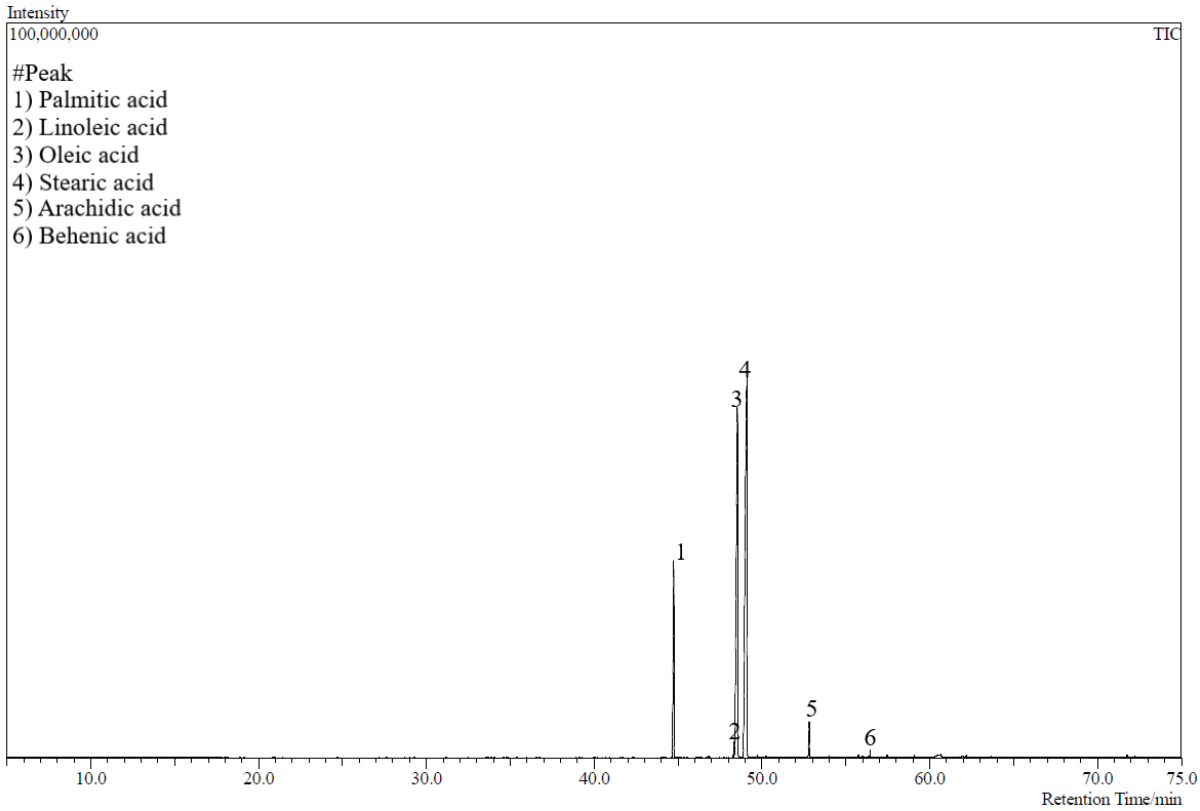

Figure 3. GC-MS chromatogram of *Shorea* seed oils

Fatty Acid Composition

The gas chromatogram and the fatty acid profiles of the extracted *Shorea* oils are shown in Figure 3 and Table 6, respectively. The *Shorea* oils contained palmitic, heptadecanoic, stearic, arachidic, behenic, and lignoceric acids as saturated fatty acids (SFAs). Stearic acid was the dominant SFA in this study, accounting for 39.91% to 44.55% in the *S. macrophylla* oils and 43.6% to 46.71% in the *S. palembanica* oils. The amount of stearic acid found in the *S. macrophylla* oils is comparable to that reported by Nesaretnam and Ali [10], which was 46.7%. In addition, the amounts of stearic acid in the *S. macrophylla and S. palembanica* oils in this study are comparable to other reported *Shorea* oils, viz. *S. robusta* (49.38%) [84] and *S. stenoptera* (40.05% to 46.98%) [85]. Palmitic acid was the second-highest SFA found in the *S. macrophylla* and *S. palembanica* oils, which were 15.57% to 16.93% and 14.12% to 18.12%, respectively. The percentages of palmitic acid present in the *S. macrophylla and S. palembanica* oils in this study are within the range of those reported in other species of *Shorea* seed oils [86]. Oleic acid was the major monounsaturated fatty acid found in this study, which was detected at 29.82% to 34.15% in the *S. macrophylla* oils and 34.34% to 34.23% in the *S. palembanica* oils. The amounts of oleic acid present in the *Shorea* oils in this study are in line with those reported by Hasan et al. [84] and Darmawan et al. [85] in *S. robusta* (34.69%) and *S. stenoptera* (31.14% to 32.74%), respectively.

The effect of extraction methods on the fatty acid profile of the *Shorea* oils is shown in Table 6. The effect is significant when $p<0.05$. The comparison of the composition of fatty acids in the *S. macrophylla* oils extracted using mechanical and Soxhlet extractions did not show a significant difference ($p>0.05$). This indicates that the type of extraction does not affect the fatty acid profile of the *S. macrophylla* oils. Stearic, oleic and palmitic acids are the major fatty acids in the *S. macrophylla* and *S. palembanica* oils. The concentrations of fatty acids were not significantly different between the oils ($p>0.05$), suggesting the type of extraction does not considerably affect the fatty acid profile of the *Shorea* seed oils. The finding agrees with those reported by Gharby et al. [82]. Although the total monounsaturated fatty acids (∑MUFAs) were similar in the *S. palembanica* oils ($p>0.05$), which is in line with Ozcan [87], however, it is worth mentioning that the Soxhlet extracted oil from *S. macrophylla* had a higher ∑MUFA than its respective mechanically extracted oil. This is due to the polarity of *n*-hexane, which has a higher affinity for oleic and [88-89]. Arachidic and behenic acids were the minor fatty acids in the mechanically extracted *S. palembanica* oil and were significantly higher compared to its Soxhlet extracted oil ($p<0.05$). This suggests that the type of extraction affects the concentration of minor fatty acids in the *S. palembanica* oil. A similar result was reported by Zhang et al. [89], where there was a significant difference in the concentration of arachidic and behenic acids in muskmelon oils extracted using mechanical and Soxhlet extraction methods.

Table 6. Percentage of fatty acids in *Shorea* oils extracted using mechanical extraction (ME) and Soxhlet extraction (SE).

Fatty acid	*S. macrophylla*		*S. palembanica*	
	ME	SE	ME	SE
Palmitic acid	16.93±2.02	15.57±2.61	14.12±2.53	18.12±3.69
Heptadecanoic acid	0.11±0.18	n.d.	0.25±0.22	0.20±0.17
Stearic acid	39.91±7.21	44.55±4.94	46.71±7.07	43.60±6.46
Arachidic acid	3.47±1.92	4.00±1.89	4.03±2.06*	3.27±1.13
Behenic acid	4.02±6.77	1.15±1.02	0.31±0.28*	0.25±0.22
Lignoceric acid	4.55±7.88	n.d.	0.09±0.16	0.15±0.13
Oleic acid	29.82±9.03	34.15±1.16	34.34±1.80	34.23±0.98
Gondoic acid	n.d.	n.d.	0.15±0.13	n.d.
Erucic	1.12±1.94	n.d.	n.d.	n.d.
Elaidic acid	0.07±0.13	n.d.	n.d.	0.17±0.15
Linoleic acid	n.d.	0.57±0.65	n.d.	n.d.
ΣSFA	68.97	65.27	65.51	65.59
ΣMUFA	31.01	34.15	34.49	34.40
ΣPUFA	n.d.	0.57	n.d.	n.d.

Note: Data are expressed as mean± standard deviation (n=3); n.d. is undetected; Means with an asterisk (*) within the same row are significantly different at 5% level; PUFA is polyunsaturated fatty acid.

Similarity of the fatty acid composition is one of the main requirements for fats to be used as cocoa butter substitutes (CBSs) or cocoa butter equivalents (CBEs). Palmitic, stearic and oleic acids accounted for 86.66% to 94.27% and 95.17% to 95.95% of the fatty acids in the *S. macrophylla* and *S. palembanica* oils, respectively. The composition of fatty acids in the *Shorea* oils was nearly identical to the fatty acid profile of cocoa butter reported by Akhter et al. [90]. SFAs in the Soxhlet extracted *S. macrophylla* oil (65.27%), mechanical extracted *S. palembanica* oil (65.51%), and Soxhlet extracted *S. palembanica* oil (65.59%) are fairly comparable to cocoa butter (65%) [90]. The Soxhlet extracted *S. macrophylla* oil and the mechanical and Soxhlet extracted *S. palembanica* oils, with 34.15%, 34.49% and 34.40% of total unsaturated fatty acids, respectively, are in the range of cocoa butter (34.22% to 34.70%) reported by Akhter et al. [90]. The fatty acid profiles of the *S. macrophylla* and *S. palembanica* oils are similar to those of cocoa butter, suggesting that the oils have the potential to be developed as cocoa butter equivalents (CBEs). Similarity in the fatty acid profiles between CBEs and cocoa butter is vital as they give similar properties [91].

Chemical Properties

The chemical properties of the extracted oils using Soxhlet and mechanical extractors are shown in Table 7. The iodine value of fats and oils measures the total number of double bonds. Higher iodine values suggest higher unsaturation and lower oxidative stability, making oils and fats softer and more susceptible to oxidation and rancidification [92]. The iodine values of the *S. macrophylla* oils ranged from 27.31 g I_2/100 g to 33.61 g I_2/100 g and the *S. palembanica* oils ranged from 31.53 g I_2/100 g to 31.91 g I_2/100 g. The iodine value of *S. macrophylla* reported by Nesaretnam & Ali [10], which was 30 g I_2/100 g, is comparable with this study. The iodine values of the *Shorea* oils in this study are comparable to other species of *Shorea* oils, which are *S. robusta* (39.44 g I_2/100 g) [84] and *S. stenoptera* (21.72 g I_2/100 g to 32.46 g I_2/100 g) [85]. The level of iodine value of the *Shorea* oils tallies with their amount of unsaturated fatty acid content, as shown in Table 7. The *Shorea* oils are classified as non-drying oil since the iodine values are lower than 100 g I_2/100 g, according to Karak [93], and thus have lower susceptibility to oxidation.

Acid value indicates the degree of rancidity in oil, as it quantifies the amount of free fatty acids generated during lipid hydrolysis [94]. A low level of acid value is required in the food industry because high acid value oil has a bad odor and rancid taste [95]. The acid value in the *S. macrophylla* oils was 3.75 mg NaOH/g to 4.69 mg NaOH/g and in the *S. palembanica* oils 3.47 mg NaOH/g to 8 mg NaOH/g. The acid value

in this study is in line with those reported by Darmawan et al. [13], which was 2.49 to 11 mg NaOH/g.

Peroxide value is one of the critical chemical properties to assess the oil quality because this value suggests the presence of the primary products of lipid oxidation, such as lipid peroxide [92]. The safe limit for consumption and storage is 10 meq O_2/kg [96], and the value needs to be minimized as the presence of primary oxidation from UFA will generate more peroxides and secondary oxidation products such as low molecular weight volatile organic compounds, which contribute to the degradation of oil quality [44]. The *S. macrophylla* oils had lower peroxide values compared to *S. palembanica*, which were 7.96 to 9.92 meq O_2/kg and 10.62 to 14.58 meq O_2/kg, respectively, as shown in Table 7. Differences in the peroxide values between the species are due to the moisture content that is higher in *S. palembanica* seeds compared to *S. macrophylla* seeds (See Table 1). Lipid oxidation favors high temperature, moisture, and oxygen concentration [44]. The peroxide level in this study is comparable to that reported by Darmawan et al. [13], which was 9.45 to 14.03 meq O_2/kg.

The chemical properties of the *Shorea* oils were compared to determine the significant effect of extraction methods on the iodine, acid and peroxide values of the oils. The effect is significant when $p<0.05$, as shown in Table 7. There was no significant effect on iodine value in *S. palembanica* oils ($p>0.05$). *S. macrophylla* oil extracted using a mechanical expeller had a significantly lower iodine value compared to the Soxhlet extracted oil ($p<0.05$). The lower iodine value is due to the lower number of peroxides, which is caused by the lesser degree of unsaturation [96]. There was a significant difference in acid value between mechanical and Soxhlet extracted oils ($p<0.05$). The acid and peroxide values in *Shorea* oils extracted using mechanical extraction were significantly lower compared to Soxhlet extracted oils ($p<0.05$). This suggests that the effect of different extraction methods on acid and peroxide values in the *Shorea* oils is significant. The lower acid and peroxide values in mechanically extracted oils is due to the higher temperature applied during Soxhlet extraction compared to mechanical extraction. Similar findings were reported by Tenyang et al. [46] and Djikeng et al. [92]. Ozcan et al. [96] explained the increasing number of peroxides in Soxhlet oils is caused by the solvent used, heat applied, and the presence of oxygen in the system.

The iodine, acid and peroxide values of the *Shorea* oils in this study were compared against the Indonesian National Standard (SNI) of Tengkawang butter [13] and Cocoa butter Standard [85], as shown in Table 7. The iodine values of the *Shorea* oils in this study (27.31 g I_2/100 g to 33.61 g I_2/100 g) are within the acceptable range for the SNI of Tengkawang butter (25 g I_2/100 g to 38 g I_2/100 g). However, the iodine values are lower than the required range for cocoa butter except for Soxhlet extracted oil from *S. macrophylla* (33.61 g I_2/100 g). The acid value of mechanically extracted *S. palembanica* oil is the lowest acid value reported in this study and does not exceed the maximum limit of the SNI Tengkawang. However, the range of the acid values exceeds the limit of the Cocoa Butter Standard. Therefore, refining process needs to be conducted. The peroxide values of the *S. palembanica* oils exceed the standard limits of the SNI Tengkawang and Cocoa butter standards. Although the peroxide values of the *S. macrophylla* oils are below the maximum standard, the high acid values suggest significant amounts of free fatty acids that are prone to oxidation and can contribute to rancidity [85]. Therefore, refining process of the *Shorea* oils is required to reduce the acid and peroxide values to meet SNI and Cocoa Butter standards.

Table 7. Chemical and antioxidant activities of *Shorea* seed oils

Parameter	Standard Quality (SNI 2903:2016) [13]	Cocoa butter standard [85]	*S. macrophylla*		*S. palembanica*	
			ME	SE	ME	SE
IV (g I_2/100g)	25-38	33-42	27.31±0.82^{a}	33.61±1.75^{b}	31.91±1.25^{b}	31.53±0.33^{b}
AV (mg NaOH/g)	Max 3.5	1.5	3.75±0.21^{a}	4.69±0.05^{b}	3.47±0.02^{c}	8.00±0.01^{d}
PV (meq O_2/kg)	Max 10	10	7.96±0.02^{a}	9.92±1.95^{b}	10.62±1.12^{a}	14.58±1.2^{b}
IC_{50} (µg/mL)	n/a	n/a	244.55±2.28^{a}	360.33±11.52^{a}	813.02±9.01^{b}	238.51±4.5^{a}
TPC (mg GAE/kg oil)	n/a	n/a	8.94±0.63^{a}	3.03±0.17^{b}	2.49±0.1^{b}	0.68±0.03^{c}

Note: SNI is the Indonesian National Standard Quality of *S. stenoptera* oil; Data are expressed as average triplicate±standard deviation; means within the same column with a similar alphabetical superscript $^{(a-c)}$ are not significantly different ($p>0.05$).

Antioxidant Activity

An antioxidant in food is defined as a substance that delays, controls, and prevents oxidation and deterioration of food quality [21]. The antioxidant activity in the *Shorea* oils was determined based on their polar and non-polar fractions. Antioxidant activity in the polar fraction was measured according to total phenolic content (mg GAE/kg oil). In contrast, the non-polar fraction was measured based on free radical scavenging activity (IC_{50}), as shown in Table 7.

Antioxidant activity reduces as the value of IC_{50} increases [97]. The mechanically extracted *S. macrophylla* oil had a lower IC_{50} value (244.55 µg/mL) compared to the Soxhlet extracted oil (360.33 µg/mL). The outcome is expected because antioxidant compounds are unstable at high temperatures [98]. However, the trend in *S. palembanica* contrasts with the initial prediction since the Soxhlet extracted oil had a higher antioxidant activity in the non-polar fraction compared to the mechanical extracted oil, which were 238.51 µg/mL and 813.02 µg/mL, respectively. The lower value of IC_{50} in the Soxhlet extracted *S. palembanica* oil compared to its mechanical extracted oil might be due to the non-polarity of *n*-hexane used during extraction, which facilitates the transfer of non-polar antioxidants such as tocopherol into the oils [99].

Phenolic compounds may function as reducing agents, singlet oxygen quenchers, hydrogen donors and metal chelators which act as antioxidants. TPC in the mechanically extracted oils was significantly higher compared to their respective Soxhlet extracted oils ($p<0.05$). The result is expected because phenolic compounds are easily destroyed at high temperatures during Soxhlet extraction. The high temperature used in cashew oil extraction reduces its antioxidant activity and phenolic concentration [98]. Furthermore, phenols dissolve poorly in non-polar solvents due to their properties. Using *n*-hexane as extracting solvent in SE decreases the phenolic level in oil. Martínez-Ramos et al. [100] explained a relatively higher phenolic content is achieved using acetone-ethanol as solvent compared to *n*-hexane alone because the polarities of the solvent govern the yield of the phenolic compounds. Several studies also recorded similar findings [99, 101]; that is antioxidant activity of mechanically extracted oils is higher than Soxhlet extracted oils. However, a more comprehensive study is required to investigate the effects of extraction under different conditions such as temperature, moisture and solvent used on specific antioxidants such as polyphenols, tocopherols and sterol in each *Shorea* species to determine the optimal extraction method.

CONCLUSION

The proximate and mineral compositions of *S. macrophylla* and *S. palembanica* seeds have been studied, where *Shorea* seeds in this study are comparable with those reported in soybean and cornmeal. Concerning antinutritional factors, oxalate and phytate were not out of the range of values reported for different crops in other literatures. However, the calculated molar ratios of oxalate to Ca in both *Shorea* seeds exceeded the limits, suggesting further seed processing and pre-treatment are needed to ensure adequate Ca absorption. Extraction method affects the efficiency of extraction where SE (*S. macrophylla*: 41.37%; *S. palembanica*: 49.06%) has higher oil yields than ME (*S. macrophylla*: 37.07%; *S. palembanica*: 40.82%). However, the fatty acid composition between the types of extraction methods did not differ significantly ($p>0.05$). The major fatty acids in *Shorea* oils are stearic, oleic and palmitic acids, nearly identical to the fatty acid profiles of *S. stenoptera*, *S. robusta* and cocoa butter. The total saturated fatty acid in Soxhlet extracted *S. macrophylla* oil (65.27%), mechanical extracted *S. palembanica* oil (65.51%), and Soxhlet extracted *S. palembanica* oil (65.59%) were comparable to cocoa butter (65%). The total unsaturated fatty acid in the studied *Shorea* oils (34.14 to 34.49%) was within the range of cocoa butter (34.22 to 34.70%). Similarities in the fatty acid profiles of the *Shorea* oils with cocoa butter indicate the potential of *S. macrophylla* and *S. palembanica* oils as cocoa butter equivalents. Based on the chemical properties of the *Shorea* oils, ME oils have lower acid and peroxide values, indicating a higher quality oil than SE. However, compared to the Indonesian Standard of Tengkawang Butter (SNI), the extracted oils are still unfit for commercialization, suggesting that additional refinement of the *Shorea* oils is required. The determination of antioxidant activity using DPPH shows varied results according to species. Higher antioxidant activity was found in ME oil (244.55 µg/mL) than in SE oil (360.33 µg/mL) of *S. macrophylla*. Whereas in *S. palembanica*, Soxhlet extracted oil (238.51 µg/mL) had a higher antioxidant activity than mechanically extracted oil (813.02 µg/mL). Thus, this study suggests a more in-depth study on the effect of antioxidant activity of different extraction methods under different conditions to fully understand the effect of extraction methods on the antioxidant activity of *Shorea* oils. The total phenolic content (TPC) of mechanically extracted oils was higher than that of Soxhlet extracted oils. Therefore, this study concludes that mechanical extraction produces higher quality *Shorea* oils with respect to chemical properties and TPC.

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