Synthesis of the Fatty Acid Ethyl Esters (FAEE) from Krabok (*Irvingia malayana*) Seed Oil for Use as the Main Ingredient in the Production of Herbal Massage Oil

Amonrat Thangthong[1,2], Wuttichai Roschat[2,3,4,*], Chonlapha Hachai[2,3], Kittisak Inthikhot[2,3], Kanthida Kiinti[2,3], Aekkaphon Thammayod[2,3,4], Sunti Phewphong[2,4], Tappagorn Leelatam[2,4,5], Sumana Tawil[3,4], Preecha Moonsin[6] and Vinich Promarak[7]

[1]*Program of Environment Science, Faculty of Science and Technology, Sakon Nakhon Rajabhat University, Sakon Nakhon 47000, Thailand*

[2]*Biomass Energy Research Laboratory, Center of Excellence on Alternative Energy, Research and Development Institution, Sakon Nakhon Rajabhat University, Sakon Nakhon 47000, Thailand*

[3]*Program of Chemistry, Faculty of Science and Technology, Sakon Nakhon Rajabhat University, Sakon Nakhon 47000, Thailand*

[4]*Innovation in Chemistry for Community Research Unit, Faculty of Science and Technology, Sakon Nakhon Rajabhat University, Sakon Nakhon 47000, Thailand*

[5]*Appropriated Technology Center, Faculty of Science and Technology, Sakon Nakhon Rajabhat University, Sakon Nakhon 47000, Thailand*

[6]*Program of Chemistry, Faculty of Science, Ubon Ratchathani Rajabhat University, Ubon Ratchathani 34000, Thailand*

[7]*Department of Material Science and Engineering, School of Molecular Science & Engineering, Vidyasirimedhi Institute of Science and Technology, Rayong 21210, Thailand*

(*Corresponding author's e-mail: roschat1@gmail.com)

Abstract

This study focused on synthesizing fatty acid ethyl esters (FAEE) from Krabok (*Irvingia malayana*) seed oil via transesterification for use as the primary ingredient in herbal massage oil production. Optimal conditions included 5 wt.% of KOH catalyst compared to the oil weight, 15:1 molar ratio of ethanol to Krabok seed oil, 60 min for reaction time and reaction temperature of 78 ± 3 °C, resulting in FAEE yields as high as 99.83 ± 0.17 %. Analysis using FT-IR, ^{1}H and ^{13}C-NMR, and GC techniques revealed FAEE as ester-type compounds, primarily comprising lauric acid ($C_{12:0}$) and myristic acid ($C_{14:0}$), constituting 97 wt.% saturated fatty acids and 3 wt.% unsaturated fatty acids. Physicochemical properties indicated clear yellow liquids with viscosity of 1.73 ± 0.03 cSt/s, density of 0.8199 ± 0.0049 g/cm^3, cloud point of +4 °C, and pour point of −1 °C. Acid value, free fatty acid content, iodine value, water content and oxidation stability were measured at 0.703 ± 0.001 mg KOH/g of oil, 0.354 ± 0.001 wt.%, 0.59 ± 0.04 g I$_2$/100 g of oil, 1482.3 ± 6.15 ppm, and greater than 12 h, respectively. When the synthesized FAEE was used to produce herbal massage oil products, the results showed that the herbal massage oil products were characterized as a clear yellow liquid with no sediment, low viscosity, and pH in the range of 5 - 6, meeting

standard criteria. Thus, FAEE derived from Krabok seed oil is suitable as a primary ingredient for herbal massage oil production, enhancing the value of local resources within the community.

Introduction

The herbal massage oil or spa massage oil, a product renowned for its healing properties and stress-relief benefits on the muscular system, incorporates various herbal ingredients like wintergreen oil, camphor, essential oils (such as Eucalyptus oil, peppermint oil, rosemary oil and lemon oil) and Plai rhizome oil (*Zingiber montanum*). In contemporary times, herbal massage oil has gained significant popularity and attention. Utilized during massage sessions, it serves to lubricate, enhancing comfort and reducing friction while dissipating heat generated from rubbing. Additionally, it nourishes the skin, leaving it soft and supple. Widely available in the market, herbal massage oil aids in alleviating various symptoms. Ideally, it should consist of oils extracted through natural methods such as coconut, sesame, olive, or palm oil, possibly blended with essential oils or herbal extracts, for an indulgent spa experience [1-5]. Numerous research reports have explored the effectiveness of herbal massage oil in treating and alleviating symptoms in patients, employing various treatment methods. The findings consistently indicate that massage with herbal oils effectively reduces muscle pain across different body areas and enhances patients' overall quality of life [6-8].

Thailand actively promotes tourism as a key economic driver, aiming to boost national income. Central to this effort is the promotion of cultural values, known as Soft Power. Traditional Thai massage, a branch of Thai traditional medicine, is a significant component of Thai culture, further elevating its cultural influence. During the 14th meeting of the Convention Committee in Bogotá, Colombia, UNESCO announced the registration of Thai traditional massage. It was registered under the English title "Nuad Thai, traditional Thai massage" as an intangible cultural heritage item in the category of the "Representative List of the Intangible Cultural Heritage of Humanity". This marks the 2nd registration of intangible cultural heritage in Thailand, on December 13, 2019 [9]. Hence, it is evident that businesses associated with Thai massage offer an intriguing avenue for economic development and income enhancement, particularly in community areas that serve as tourist attractions. Nevertheless, enhancing and adding value to Thai massage oil or spa massage oil derived from local herbs is another crucial aspect that can be promoted. This support would contribute to elevating Thailand's tourism sector to a higher level of excellence [10-12].

Body massage oil for spas or massages typically consists of oils derived from natural or synthetic sources, such as palm oil, coconut oil, sesame oil, sunflower oil, olive oil and mineral oil. These oils can be used individually or in various combinations, sometimes blended with essential oils or herbal extracts. They are employed to massage and nourish the body during spa treatments [1,5,13]. Moreover, certain formulations may incorporate beeswax to enhance the viscosity of spa massage oil products. Based on the provided information, it is evident that the primary constituents of spa massage oil products are vegetable oils or animal fats. These oils consist of ester compounds ($R_1-COO-R_2$) commonly referred to as triglycerides. While each type of vegetable oil or animal fat shares the same triglyceride compound, the variation in fatty acid composition results in distinct chemical structures for each oil or fat. Consequently, this diversity directly impacts a range of physicochemical properties such as viscosity, density, acid value, cloud point, pour point and oxidation stability [14-16]. From the chemical structure of vegetable oils and animal fats which are triglycerides with large molecules, they exhibit high viscosity. When incorporated as ingredients in the production of spa massage oil products, the resulting product may possess a high viscosity. Due to its substantial chemical structure, it may struggle to penetrate the skin effectively. This

limitation affects the absorption of various crucial substances present in the massage oil product, resulting in reduced efficacy in alleviating muscle pain.

Converting the structure of triglycerides into monoglycerides, which have smaller molecular sizes, is a commonly employed technique, especially in the biodiesel production process. The resulting monoglycerides undergo changes in physicochemical properties, notably viscosity. Typically, the transesterification process is utilized, where triglycerides react with a small molecule alcohol such as methanol or ethanol in the presence of a catalyst to convert the large molecular structure of triglycerides into monoalkyl esters (methyl esters or ethyl esters) as smaller molecules [17,18]. Utilizing methanol as a reagent which results in the production of fatty acid methyl esters (FAME), is not suitable for producing spa massage oil due to the methanol relatively toxic nature. However, selecting ethanol as a reagent in the transesterification reaction to convert the triglyceride structure to FAEE makes it appropriate for use as the main ingredient in making spa massage oil. This is because ethanol is a less toxic reagent and is commonly utilized in the production of various cosmeceutical products. This aligns with numerous studies that have explored and identified FAEE as another intriguing option. This substance serves as an excellent solvent for extracting essential compounds from certain plant sources. Notably, FAEE boasts robust environmentally friendly properties, being renewable from natural resources and non-toxic [19,20]. An example of research is the report by Diacon *et al*. [21], who investigated FAEE as a new, green and renewable solvent for extracting carotenoids from tomato waste products. They discovered a novel green solvent in FAEE, which proved significantly more effective than sunflower oil and hexane for extracting lycopene and beta-carotene from tomato waste. FAEE being a non-toxic renewable resource is environmentally friendly and to their knowledge has never been utilized as a vegetal extraction fluid before. Moreover, the efficiency of FAEE extraction was notably enhanced under ultrasound-assisted extraction (UAE) conditions compared to both sunflower oil and hexane. An additional significant advantage of FAEE is that once enriched with the extracted nutraceuticals and they can be directly utilized as a food additive.
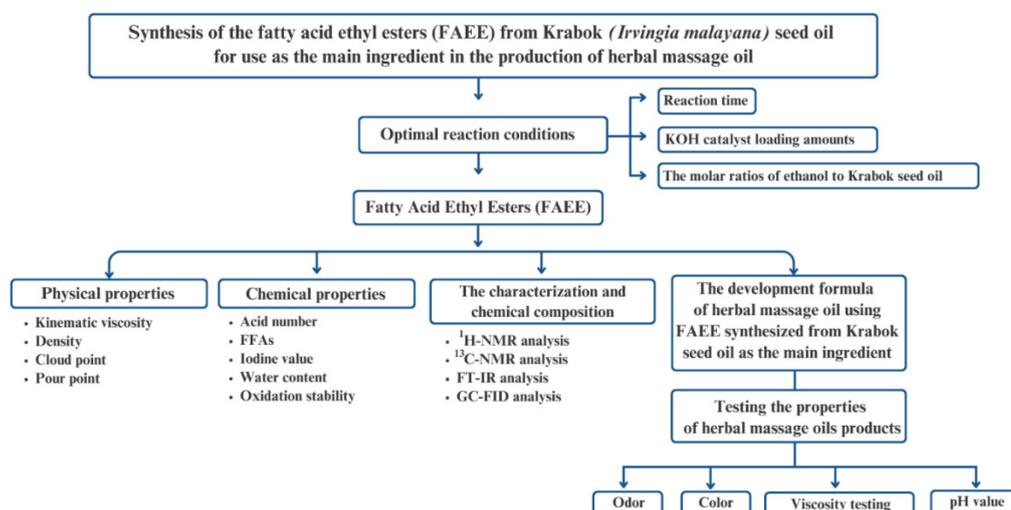

Figure 1 Flow diagram of the process for synthesis of the FAEE from Krabok seed oil to use as the main ingredient in the production of the herbal massage oil.

From our previous research reports, we identified oil from Krabok seeds as a new raw material in Thailand. The oil content of the seed flesh of Krabok was found to be as high as 63.58 wt%. The primary

fatty acid compositions of the extracted Krabok seed flesh oil were lauric acid ($C_{12:0}$) and myristic acid ($C_{14:0}$), constituting approximately 52 and 35 % by weight, respectively. Moreover, the analysis revealed that over 95 % of the extracted Krabok seed flesh oil comprised saturated fatty acid compounds [16]. This aligns with the research findings of Preecharram *et al.* [22], who investigated the nutritional values and potential applications of Krabok seed. They discovered that Krabok seed had an oil content as high as 75.42 % by weight. Hence, the concept of synthesizing and investigating the physicochemical properties of FAEE derived from Krabok seed oil, a small molecule triglyceride compound, is indeed intriguing. The obtained FAEE can be utilized for the production of herbal massage oil products, presenting promising potential and representing a novel area of study that has not been explored previously. Therefore, the objective of this research was to explore the synthesis of FAEE from Krabok (*Irvingia malayana*) seed oil for use as the primary ingredient in herbal massage oil products. In **Figure 1**, a schematic diagram illustrates the process for synthesizing FAEE from Krabok seed oil, which serves as the primary ingredient in the production of herbal massage oil. Furthermore, by adding value to locally available Krabok seeds, we can not only increase income for farmers but also contribute to the conservation of native plants. Processing Krabok seeds and various herbs into a variety of products presents an opportunity to utilize local resources sustainably.

Materials and methods
Materials

The study utilized chemicals such as hexane (C_6H_{14}) of commercial grade, ethanol (C_2H_5OH) of analytical grade and calcium oxide (CaO) of analytical grade, which were supplied by Fluka and Kemaus. Additionally, various chemicals and ingredients employed in formulating herbal massage oils, including Mok flower (*Wrightia religiosa Benth.*) essential oil, Plai oil, esldpagpon oil, wintergreen oils or methyl salicylate ($C_6H_4(HO)COOCH_3$), eucalyptus oil, camphor ($C_{10}H_{16}O$), borneol camphor ($C_{10}H_{18}O$) and menthol ($C_{10}H_{20}O$), were procured from a chemical store in Mueang District, Sakon Nakhon, Thailand. Additionally, this research also supplied samples of 2 commercially available brands of massage oil products to examine their various properties in comparison with samples of herbal massage oils developed from FAEE substances.

The Krabok seed oil used in this study was obtained by extracting the seed flesh of Krabok using a hexane solvent and the Soxhlet extraction method, as described in the research report by Roschat *et al*. [16]. The Krabok seeds were collected from Sakon Nakhon province in the Northeastern of Thailand. To begin extracting oil from Krabok seeds, peels the seeds and removes only the seed pulp. The pulp was dried using a hot air oven at 110 °C for 6 h, then grind it finely using a blender. Install the Soxhlet extraction equipment and initiate oil extraction by weighing 100 g of finely crushed Krabok seeds. Place the seeds into the Trimble inlet of the extraction apparatus and add 250 mL of hexane into a 500 mL round bottom flask equipped with an extraction chamber and a condenser. The Soxhlet extractor was then heated to the boiling point of hexane, allowing it to condense and soak the sample, thereby initiating the siphoning phenomenon to repeat the extraction process. The Soxhlet extraction method was carried out for 2 h and repeated twice using the original residue of dried Krabok seed flesh powder. The final step involved evaporating the extracted solution with hexane using a rotary evaporator. The experiment was repeated 3 more times to calculate the percentage yield of Krabok seed oil (% yield) compared to the dry weight of Krabok seeds.

Synthesis of fatty acid ethyl esters (FAEE) from Krabok seed oil as raw materials

The synthesis of FAEE products involved utilizing Krabok seed oil as a reactant under varying reaction conditions with KOH catalyst amounts ranging from 1 to 6 % by weight compared to the oil. The

molar ratios of ethanol to Krabok seed oil were 6:1, 9:1, 12:1, 15:1 and 18:1, with a reaction temperature of 80 ± 2 °C. The reaction times were 30, 45, 60, 75 and 90 h, respectively. The progress of the transesterification reaction of Krabok seed oil to be FAEE was monitored using the thin-layer chromatography (TLC) method, as described in the reports by Supamathanon *et al*. [23], Phewphong *et al*. [17,24] and Roschat *et al*. [25].

The reaction process begins by weighing the specified amount of KOH catalyst into a round-bottomed flask, served as the reactor. Ethanol is then measured according to the specified ratio and added to the flask to mix with KOH. Throughout this process, the temperature is maintained at 80 ± 2 °C and the reaction is timed for 30 min. Meanwhile, 50 mL of warm Krabok seed oil is measured under the same temperature conditions as the mixture of potassium hydroxide and ethanol. After 30 min, the warm Krabok seed oil is poured into the reactor and mixed with the KOH-ethanol mixture, with the reaction timed as specified. The reaction rate is monitored using the TLC technique employing a solvent mixture composed of petroleum ether, diethyl ether and acetic acid in a ratio of 75:5:1. After the specified reaction time has elapsed, lower the temperature of the reactor and pour out the mixture. The resulting mixture is then subjected to evaporation to remove the remaining ethanol using a rotary evaporator. Subsequently, the ethanol-removed mixture is transferred to a separation funnel to separate the FAEE product from glycerol which is a by-product. The FAEE product will form the top layer, while glycerol will settle at the bottom. Finally, the resulting FAEE product is separated, and the progress of the reaction is monitored by assessing the percentage of FAEE using the ^{1}H-NMR technique, as described in Eq. (1), **Figure 2**, and **Figure 4** (^{1}H-NMR):

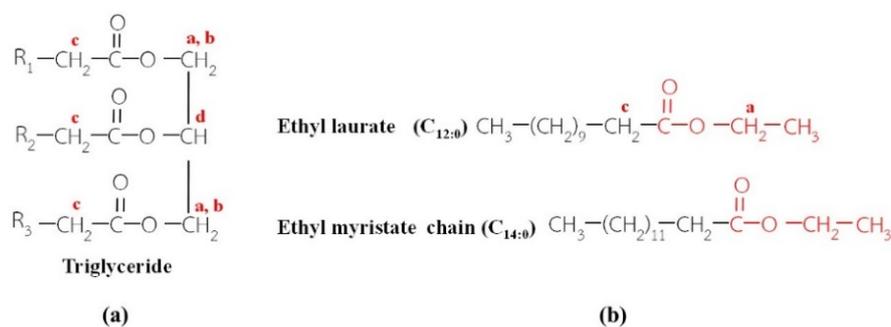$$\%\text{FAEE} = \frac{(a_{\text{-O-CH}_2}) - (b_{\text{-O-CH}_2})}{c_{\alpha\text{-CH}_2}} \tag{1}$$

Figure 2 The chemical structure of (a) the obtained extract of Krabok seed flesh oil sample (triglyceride) and (b) FAEE of ethyl laurate (C12:0) and ethyl myristate (C14:0) which is the major fatty acid composition of Krabok seed flesh oil.

Where %FAEE represents the conversion percentage of Krabok seed oil to FAEE yield. $(a_{\text{-O-CH}_2})$ is the integration value of the methylene ethoxy proton of the FAEE at a chemical shift of approximately 4.10 - 4.14 ppm (proton at peak a). The $(b_{\text{-O-CH}_2})$ is the integration value of the methylene ethoxy proton groups in Krabok seed oil (triglyceride) at a chemical shift of about 4.28 - 4.30 ppm (proton at peak b). The $(c_{\alpha\text{-CH}_2})$ denotes the area under the peak of α-methylene proton (proton at peak c) of both the FAEE product and Krabok seed oil appearing at a chemical shift of around 2.27 - 2.30 ppm. This occurs because the area under the peak of the methylene ethoxy proton (peak a) is in a 1:1 ratio to the area under the peak of the α-

methylene proton (proton at peak c). Therefore, if the transesterification reaction of Krabok seed oil incompletely forms the methylene ethoxy proton peak (proton at peak a), which belongs to the FAEE, the ratio of peak a to peak c (α-methylene proton in both Krabok seed oil and FAEE) will not be equal to 1:1. This reduction ratio is directly proportional to the decrease in %FAEE, respectively [26].

The FAEE synthesized under the optimal conditions was analyzed to determine the percentage of ethyl ester content using Gas Chromatography (GC-2010, Shimadzu). The FAEE produced under these conditions was further investigated to study their chemical structure using Infrared Spectroscopy (FT-IR; Perkin-Elmer) and Proton and Carbon Nuclear Magnetic Resonance spectroscopy (^{1}H and ^{13}C-NMR; Bruker AVANCE 600 MHz) techniques.

Study of physicochemical properties of FAEE synthesized from Krabok seed oil

An example of FAEE synthesized from Krabok seed oil was analyzed for its physical properties, including kinematic viscosity at 40 °C, density at 15 °C, cloud point and pour point. Additionally, the chemical properties of the synthesized FAEE were studied namely acid value, free fatty acids (%FFAs), oxidation stability, iodine value and water content. All parameters for evaluating the physicochemical properties of the FAEE sample were tested at the Clean Fuel Technology and Advanced Chemistry Research Laboratory, National Energy Technology Center (ENTEC), under the National Science and Technology Development Agency (NSTDA) in Thailand. In addition, the experimental methods used to test the physicochemical properties in this study were referenced from the reports of Rabie *et al*. [27], Roschat *et al*. [16,28], Moonsin *et al*. [29] and Taghipour [30]. It is important to highlight that the experiment was repeated 3 times to explore the physicochemical properties of FAEE synthesized from Krabok seed oil under each condition. All data presented in this study were averaged from 3 measurements with deviations within 3 %.

The development formula of herbal massage oil using FAEE synthesized from Krabok seed oil as the main ingredient

This research focuses on developing a herbal massage oil formula utilizing FAEE synthesized from Krabok seed oil as the primary ingredient. The development of herbal massage oils is divided into 2 distinct formulas. Formula 1 begins with the preparation of the Phase A mixture, comprising 20 g of FAEE product combined with 5 g of Mok flower essential oil, 10 g of Plai oil, 10 g of esldpagpon oil and 25 g of methyl salicylate. Next, the Phase B mixture is prepared comprising 5 g of camphor mixed with 5 g of borneol camphor and 10 g of menthol which is dissolved by heating. Subsequently, the Phase A mixture is combined with the Phase B mixture and heated until it melts into a uniform blend. The mixture is then allowed to cool until the temperature drops below 50 °C. Finally, 30 g of eucalyptus oil is added, stirred thoroughly and left to cool at room temperature before being filled into a container.

For Formula 2, the preparation steps are identical to Formula 1, with the only difference lying in the preparation of the Phase A mixture. Formula 2 is prepared by weighing the Phase A mixture which includes 20 g of FAEE product mixed with 5 g of Mok flower essential oil, 10 g of Plai oil, 10 g of esldpagpon oil, 25 g of methyl salicylate, 15 g of Krabok seed oil and 15 g of coconut oil. These ingredients are mixed into a paste and then gently heated using a stirrer and stove. Next, the Phase B mixture was prepared in a manner similar to the development of herbal massage oil Formula 1. Then, the Phase A mixture and Phase B mixture was mixed together following the method described above as outlined in Formula 1. The herbal massage oil products obtained from both formulas were stored in sealed containers for use in testing their properties in the next step.

Preliminary testing of product properties of herbal massage oils

Physical property testing involves examining general characteristics such as color and odor, as well as specific traits like oil separation and sedimentation. These observations are made with the naked eye in accordance with the industry standards outlined in the "Body Massage Oil for Spa" regulations set by the Thai Industrial Standards Institute (TISI), Ministry of Industry, Thailand, as per Industrial Standards Institute Announcement No. 29, 2018 [1]. The color testing procedure mandates consistency in color. This involves visual observation by 20 testers divided into 2 groups: Group 1 comprised spa massage staff, while group 2 consisted of the general public. Each group conducted separate assessments and provided independent ratings. A sample of commercial massage oil and massage oil of this research was poured into a white porcelain dish for examination. Color assessment was conducted through visual observation, with scoring criteria outlined in **Table 1**.

Table 1 The scoring criteria for color testing [1].

Inspected properties	Assessment level	Points awarded
Color	The color is consistent and satisfactory	3
	The color is sufficiently consistent	2
	Inconsistent color	1

Table 2 The scoring criteria for scent testing [1].

Inspected properties	Assessment level	Points awarded
Scent	The aroma is pleasing and in line with the ingredients utilized.	3
	The aroma is acceptable, considering the ingredients used.	2
	There is an abnormal or unwanted odor, such as musty, rancid, or spoiled smell.	1

Additionally, this research evaluated the color of herbal massage oil products alongside commercial massage oils by utilizing the CIE Hunter L*, a*, b* colorimeter system for measurement. L*, a* and b* are 3-dimensional descriptions of color. L* represents brightness, while a* and b* indicate the direction of the color. A positive value of a* indicates the red direction, whereas a negative value denotes the green direction. Similarly, a positive value of b* points towards the yellow direction, while a negative value signifies the blue direction. The odor test must exhibit a pleasant aroma consistent with the ingredients utilized, devoid of any undesirable odors such as musty, rancid, or spoiled smells, in accordance with industry standards. This evaluation involves 20 testers, akin to the color testing procedure. A sample of both commercial massage oil and the massage oil under research is poured into a white porcelain dish, and the smell is assessed through olfactory examination. Scoring criteria are outlined in **Table 2**.

Viscosity analysis was conducted using Ostwald-viscometers installed in a temperature-controlled bath with a stirrer heated to 45 °C. A volume of 10 mL of the sample massage oil was added to the Ostwald-viscometers. The massage oil was drawn above the upper volume mark using a rubber pipette bulb, then allowed to flow down to the upper volume mark while the timer was started. Once the massage oil reached the lower volume limit, the timer was stopped and the time in seconds was recorded. This time value was

then multiplied by the Ostwald-viscometers constant to obtain the viscosity unit in centistokes (cSt). The final step involves measuring the pH of both commercial massage oil and the research massage oil samples using paper universal indicators.

Results and discussion

Study of oil extraction from Krabok seed

This study focuses on synthesizing FAEE from Krabok seed oil, intended for use as a primary ingredient in herbal massage oil production. The moisture content of Krabok seeds was analyzed by initially drying the seed pulp, followed by grinding and subsequent baking. The findings revealed an average moisture content of Krabok seed pulp to be 0.49 ± 0.02 % (%RSD equal to 4.10 %). However, it is noted that the moisture content of Krabok seeds may vary depending on the harvesting season, with seeds collected during the rainy season potentially containing higher moisture levels. Additionally, the geographical location of Krabok plants may influence seed moisture content. Nevertheless, the moisture content observed in this study was relatively low. Prior research by Ellis *et al*. [31], as well as Nyam *et al*. [32], suggests that seed moisture content should ideally remain within 8 % to facilitate longer storage and prevent microbial activity.

The study investigates the effectiveness of extracting oil from Krabok seeds by employing hexane as a solvent and utilizing the Soxhlet extraction method. Results demonstrate that employing the flow extraction method with a circulation duration of 3 h yields significantly high extraction efficiency, reaching up to 70.83 ± 1.92 %. Each extraction method underwent 5 repetitions, with the experimental error (%RSD) matching the percentage, measuring at 2.71 %. Consequently, the experiment suggests that dried Krabok seeds (processed to remove moisture) contain a relatively high oil content. When comparing the results of our experiment with the research report by Roschat *et al*. [16], which focused on oil extraction from kale seeds using hexane solvent and various extraction methods such as maceration, reflux, ultrasonic and Soxhlet extraction, it was found that the reflux extraction method could achieve an oil extraction efficiency of up to 63.58 %. While the research report by Preecharram *et al*. [22] indicates that dried Krabok seeds possess a high oil content ranging from 74 to 76 % by weight. These findings suggest that the amount of oil in Krabok seeds may vary depending on factors such as the season of harvest, the age of the trees and the specific species of Krabok trees, as well as the geographical location where they are grown. Consequently, the synthesis process of essential compounds from the Krabok plant may differ accordingly.
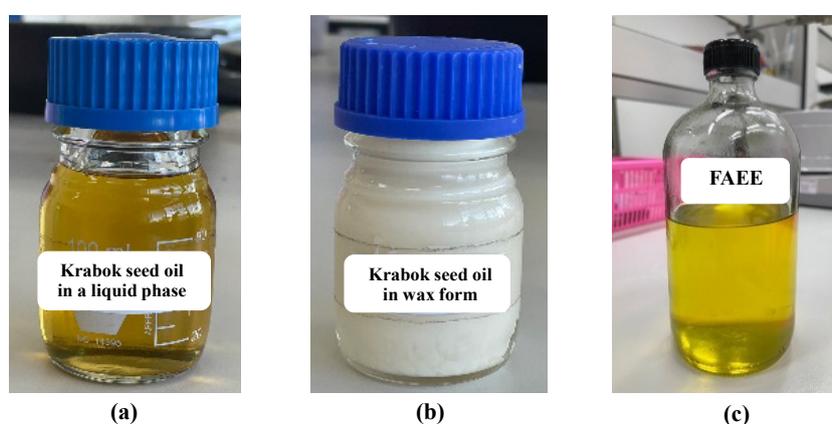

| (a) | (b) | (c) |

Figure 3 The obtained extract of Krabok seed flesh oil sample displaying (a) in a liquid phase, (b) in wax form upon complete cooling at room temperature and (c) the FAEE product derived from Krabok seed flesh oil.

The physical properties of Krabok seed oil were visually represented in **Figure 3**, showcasing the obtained extract of Krabok seed flesh oil sample in both its liquid phase and as it solidifies into wax form upon complete cooling at room temperature. The color characteristics of Krabok oil indicate that the extracted seeds are initially clear yellow. However, upon cooling at room temperature, they transform into a solid resembling beeswax, exhibiting a soft white hue. In a previous research report by Roschat *et al.* [16], it was demonstrated that the primary component of Krabok seed oil consists of small molecules of saturated fatty acids, constituting approximately 95.73 % by weight. Consequently, when left at room temperature, the oil solidifies resembling beeswax in its consistency. Indeed, the physical property of Krabok seed oil, resembling beeswax when solidified at room temperature, presents a promising alternative raw material for producing various cosmeceuticals such as soaps, creams, lotions and herbal massage oils. Given that these cosmeceuticals often utilize beeswax as a main ingredient, predominantly synthetic due to the rarity and costliness of natural beeswax, Krabok seed oil could offer a more accessible and potentially superior substitute. This could lead to the development of natural and sustainable alternatives for the cosmetics industry. However, the direct use of Krabok seed oil which possesses a waxy nature may present limitations in the production process of various products. For instance, it may require heating to melt the Krabok seed oil wax into a liquid before use. Additionally, mixing various ingredients with Krabok seed oil too slowly could lead to poor mixing as the oil may harden back into wax before achieving thorough incorporation with other components. Furthermore, the products produced using Krabok seed oil may exhibit excessive hardness, which might not align with the preferences of some consumers who favor softer textures, such as those found in lotions or herbal massage oils. For this reason, the chemical structure of Krabok seed oil, being a triglyceride, has been altered to FAEE, a monoglyceride with a smaller molecular size. This modification aims to enhance the physical properties of the FAEE precursor, making it more suitable for producing various products, particularly herbal massage oils.

Synthesis of FAEE from Krabok seed flesh oil raw materials

This research investigated optimal conditions for synthesizing FAEE from Krabok seed flesh oil. The study explored catalyst quantity, the ratio of Krabok seed flesh oil to ethanol and reaction time. The findings are presented in **Table 3**. The results regarding the optimal conditions for synthesizing FAEE from Krabok seed flesh oil raw materials revealed that the quantity of KOH catalyst used was suitable for the synthesis, constituting 5 wt.% by weight compared to the Krabok seed oil raw material. Additionally, the reaction can be catalyzed to obtain a FAEE product of up to 99.83 ± 0.17 %, evaluated by measuring the %FAEE using the ^{1}H-NMR technique. An example of calculating the %FAEE value can be done by using the integration values of the methylene ethoxy proton ($a_{\text{-O-CH}_2}$) of the FAEE, the methylene ethoxy proton groups ($b_{\text{-O-CH}_2}$) in Krabok seed oil (triglyceride), and α-methylene proton ($c_{\alpha\text{-CH}_2}$) of both the FAEE product and Krabok seed oil and substituting these values into Eq. (2) as follows:

$$\%\text{FAEE} = \frac{4.11 - 2.68}{9.85} \times 100 = 14.51 \ \% \tag{2}$$

The transesterification conditions consist of a KOH catalyst loading amount of 1 wt.%, an ethanol to oil molar ratio of 15:1, a reaction temperature of 80 ± 2 °C and a reaction time of 60 min (experimental conditions 1). It should be noted that triplicate experiments were carried out for each condition, and all the data in this work were averaged from 3 measurements, with the deviation within 3 %. This aligns with the findings of Roschat [26], who determined the optimal reaction conditions included a ratio of palm kernel oil to ethanol of 15:1 mol, a KOH catalyst amount of approximately 6 wt.% by weight compared to the raw

material oil and a reaction time of 1 h. The study on the amount of KOH catalyst used to catalyze the said reaction revealed that if the amount of catalyst used was less than 5 wt.%, the %FAEE obtained would be quite low (less than 70 %). This is attributed to the low catalytic efficiency of catalyst amounts less than 5 wt.%, although it may take more than 1 h to complete the reaction under these conditions. On the other hand, when using more than 5 wt.% of catalyst, the FAEE product obtained was only 74.64 % under the same reaction conditions. These results suggest that an excess amount of KOH catalyst could lead to the formation of soap through a side reaction, known as the saponification reaction, consequently resulting in fewer FAEE products [17,29,33,34].

A study on the ratio of Krabok seed flesh oil to ethanol found that the optimum ratio was 15:1 mol, with a lower ratio resulting in a FAEE product less than 70 % at a reaction time of 1 h and catalyst loading amount of 5 wt.%. This could be attributed to the limited amount of the reaction reagent (ethanol), leading to a low reaction rate. However, it may be necessary to increase the reaction time to more than 1 h for the reaction to be complete (%FAEE > 95 %). While increasing the ratio of Krabok seed to ethanol to 18:1 mol, the FAEE product was found to be 85.39 %. These results suggest that excess ethanol may dissolve the glycerol, which is a by-product in the reaction, and hinder the bonding between ethanol and the Krabok seed oil as a raw material. This is consistent with the research report of Takase *et al*. [35], Keera *et al*. [36] and Roschat *et al*. [37], which found that the amount of reagent entering the reaction (alcohol) in the transesterification reaction will have a ratio of approximately 15:1 mol, which is the most appropriate ratio for the reaction. In the case where the amount of reagent entering the reaction is too small, the reaction will be slow. Conversely, if the amount of reagent entering the reaction is too much, it may greatly hinder the reaction as well.

Table 3 Study of the optimal conditions for synthesizing FAEE from Krabok seed flesh oil raw materials.

Experimental conditions	KOH Catalyst (wt.%)	Ethanol:oil (mol)	Reaction time (min)	$\%\text{FAEE} = \dfrac{(a_{\text{-O-CH}_2}) - (b_{\text{-O-CH}_2})}{c_{\alpha\text{-CH}_2}}$
1	1	15:1	60	14.51
2	2	15:1	60	38.48
3	3	15:1	60	40.12
4	4	15:1	60	68.23
5*	5	15:1	60	100.05
6	6	15:1	60	74.64
7	5	6:1	60	46.73
8	5	9:1	60	57.19
9	5	12:1	60	67.29
10*	5	15:1	60	99.86
11	5	18:1	60	85.39

Experimental conditions	KOH Catalyst (wt.%)	Ethanol:oil (mol)	Reaction time (min)	$\%FAEE = \dfrac{(a_{-O-CH_2}) - (b_{-O-CH_2})}{c_{\alpha-CH_2}}$
12	5	15:1	15	72.77
13	5	15:1	30	72.90
14	5	15:1	45	86.51
15*	5	15:1	60	99.63
16	5	15:1	75	96.24
17	5	15:1	90	62.29

Note: 5*, 10* and 15* are the same experimental conditions, and all of the results in **Table 3** were averaged from the repeats analytical 3 times and the standard deviation was within 3 %.

Studying the appropriate reaction time for FAEE product synthesis revealed that at a reaction time of 60 min, the %FAEE value reached its peak average of 99.83 %. However, for reaction times shorter than 60 min, the %FAEE value dropped to less than 90 %. This decline is attributed to incomplete reaction due to insufficient time. Inadequate reaction time results in some of the Krabok seed oil precursor and ethanol remaining unreacted, leading to reactants being present even after the allotted time. However, the results also showed that with more than enough time (more than 60 min), the %FAEE value of the product tended to decrease. This result can be explained by the excessive time required for the reaction to completely form FAEE products. If the reaction continues beyond the optimum time, the remaining reactant (ethanol) and catalyst (such as KOH) may undergo hydrolysis reactions, converting FAEE products into other compounds such as soap or carboxylic acids. Additionally, the transesterification reaction of Krabok seed oil can be reversible. Therefore, the ideal time for synthesizing FAEE products in this research is approximately 60 min. These results align with the research report of Roschat [26], who found that the optimal reaction time was approximately 60 min, as exceeding this time frame decreased the percentage of products from the transesterification reaction.

From the results of the experiment, the optimum conditions for the synthesis process of FAEE products from Krabok seed oil as raw material through transesterification reaction were determined. It was found that the optimum condition consisted of the amount of KOH catalyst being equal to 5 wt.% compared to the weight of Krabok seed oil. The molar ratio of ethanol as a reagent to Krabok seed oil as a reactant was set at 15:1 mol, and the reaction time was set to 60 min. The temperature for the reaction was maintained at approximately 78 ± 3 °C, which was identified as the most suitable temperature based on the research report by Roschat *et al*. [38,39]. The research report explains that the optimum temperature for FAEE synthesis from vegetable oil is approximately 78 ± 3 °C, corresponding to the boiling temperature range of ethanol as a reagent. This temperature range provides the ethanol molecules with sufficient energy to react with the vegetable oil precursor, which consists of triglyceride compounds. Consequently, this facilitates the breaking of old bonds and the formation of new bonds, resulting in the production of FAEE.

The characterization of the extracted Krabok seed oil as raw materials versus FAEE product

^{1}H-NMR analysis

Upon studying the chemical structure of Krabok seed oil in comparison to the FAEE product synthesized using ^{1}H-NMR spectral techniques as demonstrated in **Figure 4(a)**, it was observed that the peak corresponding to olein protons (double bond position H−C=C−H) in the triglyceride appeared at chemical shifts of approximately 5.27 ppm, albeit minimally. This observation can be attributed to the predominant presence of saturated fatty acid compounds in Krabok seed oil, the main component of the FAEE product. The main structure observed is the triglyceride structure, with peaks appearing at chemical shifts of positions 4.07 - 4.23 ppm (doublet) corresponding to R−(C=O)−O−CH_2−R (peak a) above and below the triglyceride structure. Additionally, the peak corresponding to R−(C=O)−O−CH−R (peak b) within the triglyceride structure is clearly visible at the position of 5.20 ppm. The chemical shifts at 2.24 ppm, the peak corresponds to the proton of the methylene group (α-CH_2−) (triplet), which constitutes the hydrocarbon chain (peak c). Furthermore, protons from the aliphatic proton hydrocarbon group were identified at peak positions below 1.65 ppm. These consistent findings were reported and elucidated in the studies conducted by Roschat *et al*. [16], who previously investigated the synthesis of a high-quality biodiesel product derived from Krabok seed oil as a novel raw material in Thailand.

During the synthesis process of FAEE products from Krabok seed oil as a precursor, it was observed that a transesterification reaction occurred. However, the formation was not yet complete as indicated by the presence of unreacted components detectable in the ^{1}H-NMR spectrum of the entire FAEE product. The main structure identified was a monoglyceride compound along with traces of precursor triglyceride structures, as depicted in **Figure 4(b)**. Once Krabok seed oil has been fully converted into the FAEE product, a distinct main peak emerges, differing from that of the Krabok seed oil precursor, as displayed in **Figure 4(c)**. The peak R−(C=O)−O−CH_2−CH_3 corresponding to the methylene ethoxy proton (peak a) of FAEE was only depicted within the chemical shift range of 4.10 to 4.14 ppm. Notably, there is an absence of a methine proton peak at 5.26 ppm (R−(C=O)−O−CH−R; peak d) and the methylene ethoxy proton peak of triglyceride within the chemical shift range of 4.27 to 4.30 ppm (R−(C=O)−O−CH_2−R; peak b). Moreover, an α-methylene proton peak manifests within the range of 2.28 to 2.31 ppm (α-CH_2−; peak c) [16,26].

^{13}C-NMR analysis

In the study of the chemical structure of Krabok seed oil using the ^{13}C-NMR technique, as depicted in **Figure 5(a)**, the results revealed specific peaks indicative of the carbonyl group of carbon in the R−(C=O)−O−CH_2−R moiety. The peaks of the carbonyl group situated at the top and bottom of the triglyceride structure appeared at a chemical shift of δ173.27 ppm (peak b), while the carbonyl group in the middle of the triglyceride structure appeared at δ172.86 ppm (peak a). Additionally, the carbon group of R−(C=O)−O−CH_2−R groups located above and below the triglyceride structure manifested peaks at δ62.11 ppm (peak c), whereas the carbon of the R−(C=O)−O−CH−$(CH_2)_2$− group in the middle of the triglyceride structure (peak d) appeared as a peak at δ68.90 ppm, respectively. In contrast, in the FAEE product derived from Krabok seed oil as illustrated in **Figure 4(b)**, the carbon peak of the carbonyl group R−(C=O)−O−CH_2−CH_3 within the structure exhibited a single peak at the chemical shift of δ173.95 ppm (peak a), while the carbon of the R−(C=O)−O−CH_2−CH_3 group appeared as a peak at position δ60.16 ppm. However, in the ^{13}C-NMR spectrum of FAEE synthesized from Krabok seed oil, the main structure observed is a monoglyceride compound. Nonetheless, a small amount of the precursor triglyceride structure is still detectable indicating that the reaction may not have been fully completed [16,26,40,41].
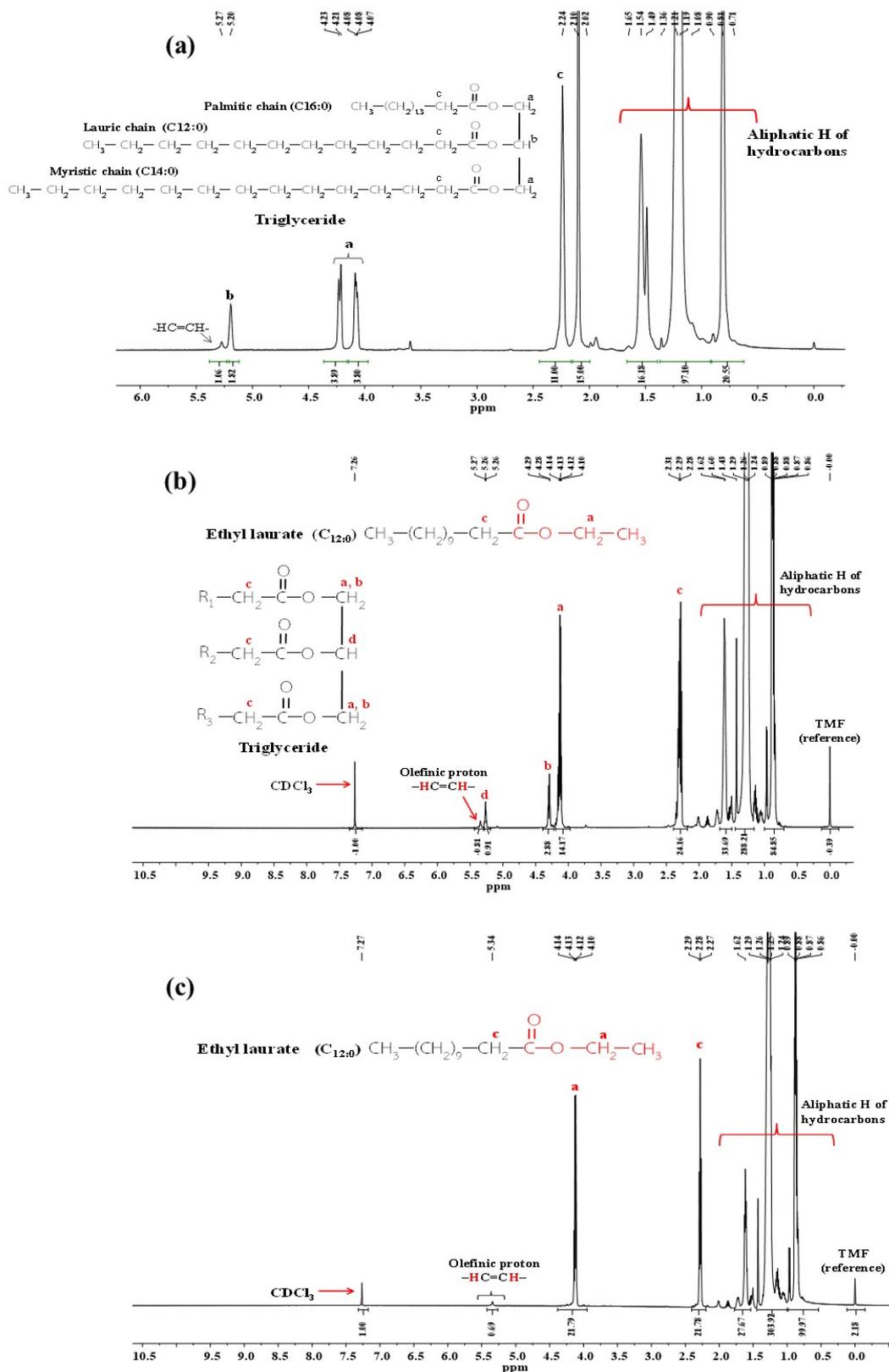
Figure 4 ^{1}H-NMR spectrum of (a) the obtained Krabok seed oil as raw material, (b) uncompleted transesterification reaction to produce FAEE product and (c) completed transesterification reaction to produce FAEE product from Krabok seed oil.
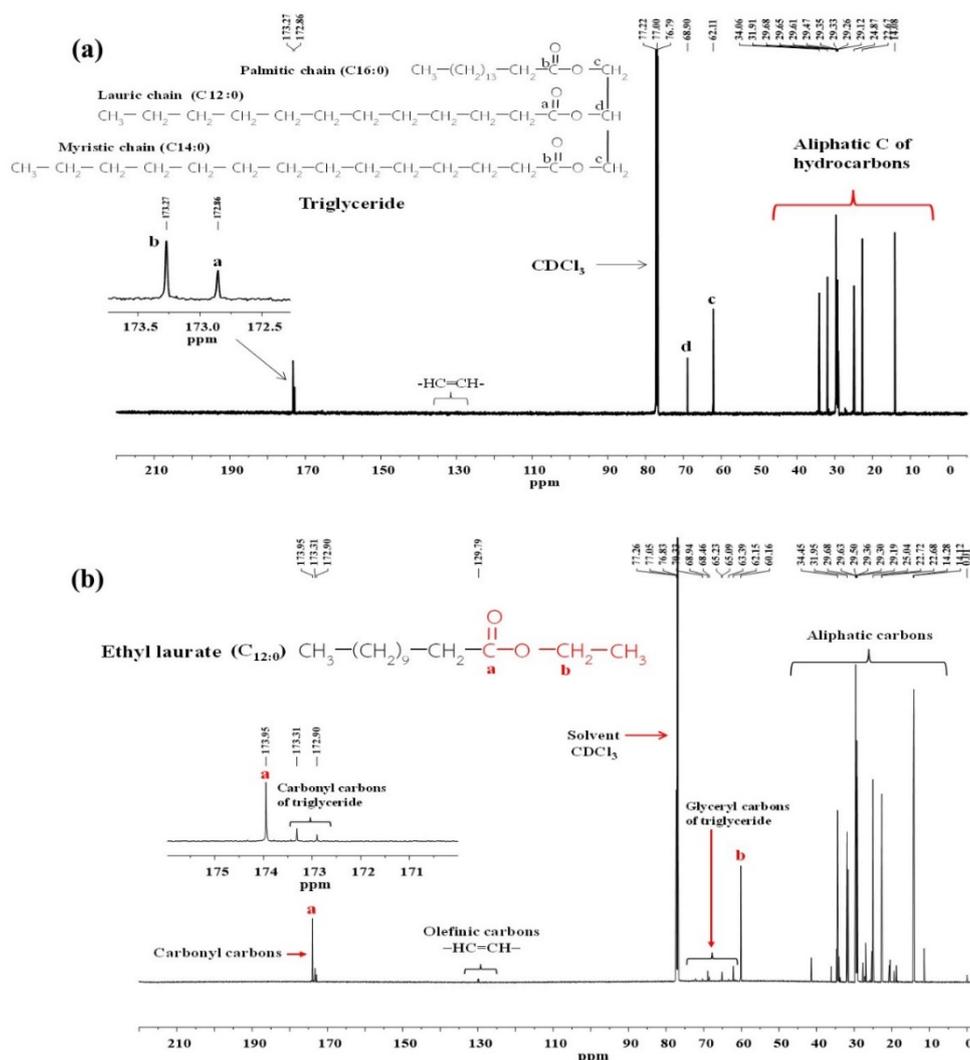
Figure 5 ^{13}C-NMR spectrum of (a) the obtained Krabok seed oil as raw material and (b) ^{13}C-NMR spectrum of completed transesterification reaction to produce FAEE product from Krabok seed oil.

FT-IR analysis

In this research, the study of the chemical functional groups of FAEE products was compared with that of the precursor Krabok seed oil using the FT-IR technique. This comparison aimed to confirm the changed chemical structure of FAEE compounds, providing additional information in the identification process, as depicted in **Figure 6(a)**. The information provided suggests that both Krabok seed oil and the FAEE product contain ester functional groups R_1−(C=O)−O−R_2 as their main structural components. The specific functional groups identified include: C=O group stretching vibrations at around 1737 cm^{-1}, C−H group stretching vibrations for −CH_2− and −CH_3 at around 2916 – 2850 cm^{-1}. The functional groups exhibiting −C−O−C− stretching vibrations bending appeared in the spectral range of 1172 – 1029 cm^{-1}. The C−H, −CH_2− and −CH_3 groups exhibit bending and rocking vibrations of the (−CH_2−)$_n$ functional group at around 1464 – 1373 cm^{-1} and 717 cm^{-1}, respectively. These vibrational frequencies and bending modes are characteristic of ester functional groups, which are commonly found in both natural oils like Krabok seed oil and in synthetic FAEE [16,28,37,42,43].

However, the analysis of the FAEE precursor and product Krabok seed oil samples using the FT-IR technique revealed distinct peaks that differed between these 2 samples as shown in **Figure 6(b)**. In the

Krabok seed oil sample, acting as a precursor, only 1 bending vibration peak of the −C−O−C− functional group was identified at around $1172 − 1029$ cm^{-1}. This is attributed to its chemical structure as a rigid triglyceride group, where the bonds vibrate predominantly in the same plane or direction. While in the FAEE product, the peak of the −C−O−C− functional group was observed to be split into 2 peaks at around 1177 and 1162 cm^{-1}. This split is attributed to the vibration of ethoxide −O−CH$_2$−CH$_3$ (from ethanol), indicative of the chemical structure as a monoglyceride group of substances. Consequently, the bond vibrates in the same plane, exhibiting both rocking and up-and-down vibrations, as depicted in **Figure 6(b)**. Therefore, through the comparative analysis of Krabok seed oil as the raw material and the FAEE product using the FT-IR technique, it is evident that a qualitative analysis was conducted. Initially, the differences in the chemical structure of the 2 samples can be elucidated. Based on the provided information, it can be inferred that the transesterification reaction of Krabok seed oil with ethanol, catalyzed by KOH solution, resulted in the formation of the FAEE product [16,28,37,42,43].
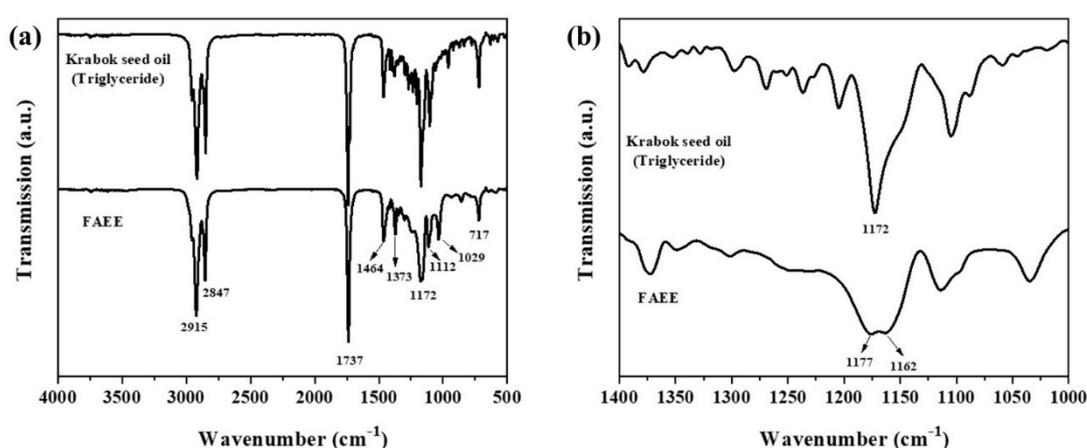

Figure 6 FT-IR spectrum of (a) the obtained Krabok seed oil as raw material and FAEE product and (b) the enlarged image of the FT-IR spectrum in the range wave number of 1100 - 1400 cm^{-1}.
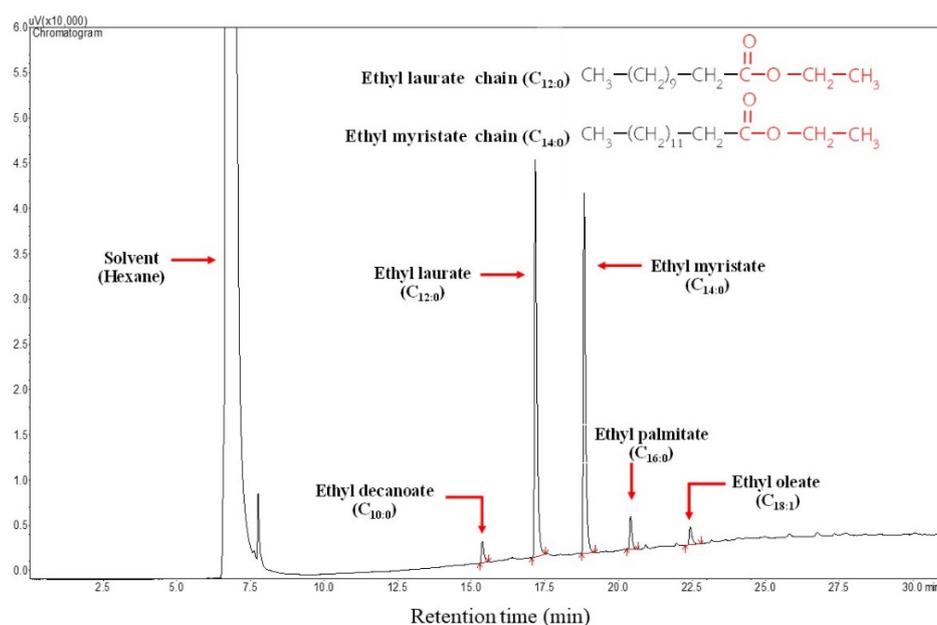

Figure 7 GC-chromatogram of FAEE product derived from Krabok seed oil.

GC-FID analysis

The results of studying the chemical composition of FAEE products using gas chromatography techniques (GC-FID) revealed that the FAEE products obtained from extracted Krabok seed oil predominantly consisted of lauric acid ($C_{12:0}$) comprising approximately 52 wt.% of the total composition. This was followed by maristic fatty acid ($C_{14:0}$), constituting around 42 wt.%. Additionally, there were trace amounts of other fatty acids such as decanoic acid ($C_{10:0}$), palmitic acid ($C_{16:0}$) and oleic acid ($C_{18:1}$), as illustrated in **Figure 7**. When separated by the type of saturated fatty acids and unsaturated fatty acids, it was discovered that the FAEE products obtained from Krabok seed oil exhibited a saturation composition of approximately 97 wt.%, with unsaturated fatty acids constituting around 3 wt.%. These findings align with the research report by Roschat *et al*. [16], which previously documented the fatty acid compositions of Krabok seed oil.

Physicochemical properties of FAEE product derived from Krabok seed oil

Analysis of the physical and chemical properties of the FAEE products synthesized from Krabok seed oil precursor revealed that the fatty acid composition of Krabok seed oil mainly consisted of 3 wt.% unsaturated fatty acids and 97 wt.% saturated fatty acids. Consequently, the precursor, Krabok seed oil (with a triglyceride structure), would solidify or become waxy when left at room temperature due to its predominantly saturated fatty acid content, as depicted in **Figure 3(b)**. However, after modifying the structure of Krabok seed oil, the resulting FAEE product, characterized by a mono-glyceride structure, remained in a liquid state when kept at room temperature, as depicted in **Figure 3(c)**. When Krabok seed oil was extracted and synthesized into FAEE products, the physical properties of the obtained FAEE products were studied, as shown in **Table 4**. The results indicated that the FAEE products had an average viscosity value of 1.73 ± 0.03 cSt/s and an average density value of 0.8199 ± 0.0049 g/cm^3. In comparison, the viscosity and density values of the Krabok seed oil precursor could not be measured since such reactants are solid or waxy at room temperature. The cloud point and pour point values of the FAEE products were determined to be +4 and –1 °C, respectively, aligning with the trend observed in the viscosity and density measurements. However, measurements for the cloud point and pour point values of Krabok seed oil could not be obtained. This is attributed to its solid or waxy appearance at room temperature, primarily due to its composition of triglycerides, a type of saturated fatty acid.

A study examining the chemical properties of FAEE products extracted from Krabok seed oil revealed average acid value and FFAs values of 0.703 ± 0.001 mgKOH/g of oil and 0.354 ± 0.001 wt.%, respectively. In comparison with the report of Roschat *et al*. [16], which found that the free fatty acids of extracted Krabok seed oil were equal to 0.210 ± 0.002 mg KOH/g of oil, indicating lower values than those observed for the FAEE products in this research. This discrepancy may be attributed to variations in the seasons and geographical regions where Krabok seeds are harvested for oil extraction, potentially affecting the quality, chemical composition and presence of various important substances. However, despite these differences, the acid value and FFAs levels of the FAEE products synthesized from Krabok seed oil in this study still comply with ASTM - D6751 and EN - 14214 standards, which mandate that they should not exceed 3 mgKOH/g of oil and 1.5 wt.%, respectively. Nonetheless, the acid value and FFAs levels surpass this threshold, indicating a potential risk of ester compound conversion to soap upon reacting with a base [16,17,20,28,36]. The analytical test for the iodine value of the FAEE product synthesized from Krabok seed oil yielded an average value of 0.59 ± 0.04 g I$_2$/g of oil. The iodine value is a measure of the unsaturated fatty acid composition in vegetable oils and animal fats as triglyceride compounds (total unsaturated fatty acid content). Iodine combines with the structure of triglycerides containing double bonds; thus, the number of such bonds can be inferred from the amount of iodine that bleaches vegetable oils and animal fats. After

the reaction, the remaining iodine was analyzed by adding starch water to form a dark blue compound, which was then titrated with a sodium thiosulfate solution of known exact concentration. Iodine values are typically expressed in grams of iodine used per 100 g of vegetable oil or animal fat. Unsaturated triglycerides exhibit higher iodine values, indicating a higher content of double bonds in their fatty acid chains. Many vegetable oils, such as sunflower oil, are rich in unsaturated triglycerides, with iodine values ranging from 110 to 143 g I_2/100 g of oil, whereas typical animal fats have lower iodine values, approximately 35 to 48 g I_2/100 g of oil. In contrast, coconut oil, highly saturated, has an iodine value of only 6 to 11 g I_2/100 g of oil. From this information, it's evident that FAEE products from kale seed oil exhibit minimal iodine values [44-46]. This aligns with findings from fat composition analysis using GC technique, revealing that FAEE products from Krabok seed oil consist of 97 wt.% saturated fatty acids, with unsaturated fatty acids (those containing double bonds in their chemical structure) constituting only 3 wt.%, significantly lower than the levels found in typical vegetable oils or animal fats.

Furthermore, the investigation into the oxidation stability of the FAEE product synthesized from Krabok seed oil revealed a duration exceeding 12 h, aligning with the results from the GC analysis of fatty acid composition and the iodine value discussed earlier. This correlation stems from the fact that vegetable oils or animal fats rich in unsaturated bonds (double bonds) are prone to oxidation reactions with oxygen in the air and moisture. Conversely, those containing saturated fatty acids exhibit greater resistance to oxidation [16,44,45]. In the case of FAEE obtained from Krabok oil, its exceptionally high saturation value (over 97 wt.%) contributes to its remarkable oxidation stability. This attribute is advantageous for FAEE products derived from Krabok seed oil, as they remain stable against air and humidity. Consequently, they can be stored for extended periods without developing a rancid odor, making them suitable for use as ingredients in herbal massage oils and other applications.

Table 4 Physicochemical properties of FAEE product derived from Krabok seed oil.

Properties	The results of the experiment					
	Repeat 1st	Repeat 2nd	Repeat 3rd	Average	SD	%RSD
Physical properties						
Kinematic viscosity at 40 °C (cSt/s)	1.71	1.73	1.76	1.73	0.03	1.45
Density at 15 °C (g/cm³)	0.8157	0.8188	0.8253	0.8199	0.0049	0.60
Cloud point (°C)	+4	+4	+4	+4	0	0
Pour point (°C)	−1	−1	−1	−1	0	0
Chemical properties						
Acid number (mg KOH/g of oil)	0.702	0.703	0.703	0.703	0.001	0.08
FFAs (wt.%)	0.353	0.354	0.354	0.354	0.001	0.16
Iodine value (g I_2/100 g of oil)	0.63	0.59	0.55	0.59	0.04	6.78
Water content (ppm)	1479.6	1489.3	1477.9	1482.3	6.15	0.41
Oxidation stability (h)	> 12	> 12	> 12	> 12	0	0

Testing the properties of herbal massage oils products
Viscosity testing

This research focused on producing herbal massage oils using FAEE precursors derived from Krabok seed oil as the primary ingredient. Two herbal massage oil formulations with distinct ingredients were developed and studied. The properties of the herbal massage oil products made from FAEE precursors were compared with a commercial massage oil product sourced from the community in Sakon Nakhon, Thailand. As shown in **Table 5**, the results indicated that the FAEE precursor displayed the lowest viscosity, with a value of 1.73 ± 0.03 cSt/s. In contrast, herbal massage oil products Formula 1 and Formula 2 exhibited viscosity values of 4.37 ± 0.05 and 5.30 ± 0.11 cSt/s, respectively. Indeed, the observed differences in viscosity values highlight the impact of various ingredients and mixing ratios. Both herbal massage oil Formulas, 1 and Formulas 2, incorporate different types of ingredients and mixing proportions, which include coconut oil and Krabok seed oil. These variations in formulation likely contribute to the distinct viscosity values observed for each formula. Based on the experimental results, it is observed that the viscosity of herbal massage oil products Formula 1 was low, accompanied by reduced slipperiness and oiliness. Consequently, this product is well-suited for conversion into herbal massage spray. Its key advantage lies in its non-sticky nature, facilitating easy absorption of the product into the skin. The 2nd formula of the herbal massage oil product incorporates coconut oil and Krabok seed oil to enhance slipperiness and oiliness, rendering it suitable for use as a spa massage oil. Despite a slight increase in viscosity compared to the 1st formula, the 2nd formula remains suitable for packaging in pump bottles. Both herbal massage oil formulas possess desirable properties, being slippery and oily without being sticky, facilitating easy absorption into the skin. Their suitability for portability is evident, with options for spray and pump packaging. When compared to commercial herbal massage oil products sourced from the community, significant disparities in viscosity values are noticeable. These differences arise from variations in ingredient compositions and mixing ratios among the products produced by different communities. As a result, a wide range of viscosity values is observed for the end products, reflecting the diversity in formulations and manufacturing practices within the community.

Evaluation of the color, odor and pH value of herbal massage oil products from FAEE in comparison with commercial herbal massage oil products

Evaluating the color and odor profiles of herbal massage oil products derived from FAEE involves employing a total of 20 testers. This evaluation utilizes color tone measurements facilitated by a CIE Hunter system color meter, alongside pH value assessments using paper universal indicators. Each tester contributes to the assessment, providing valuable insights into both the visual and olfactory aspects of the oils. Through the color meter, precise measurements are obtained, allowing for objective analysis of color properties such as luminance and hue. Simultaneously, the pH value measurements offer an understanding of the chemical composition of the oils. By combining these methodologies, a comprehensive evaluation of the oils quality and characteristics is achieved, facilitating informed decision-making in herbal massage oil product selection. According to the data in **Table 5**, all formulas of the herbal massage oil were found to be homogeneous before undergoing stability testing. They exhibited a clear yellow appearance and possessed a consistent texture. The aroma was deemed satisfactory based on the ingredients used. There was no observed separation of layers or precipitation. The acidity-alkaline value fell within the pH range of approximately 5 to 6, meeting the industry standard criteria outlined in TISI No. 29, 2018 [1], for massage oils used in spas.

Table 5 The measures the viscosity, color measurement value using the CIE Hunter and pH value of herbal massage oil products produced with FAEE precursors compared to FAEE precursors and a commercial massage oil product sourced from the community in Sakon Nakhon, Thailand.

Sample	Viscosity (cSt/s)	Sample product images	Color measurement value using the CIE Hunter			pH
			L*	a*	b*	
FAEE precursor	1.73 ± 0.025		-	-	-	6 - 7
Herbal massage oil products Formula 1	4.37 ± 0.047		58.72	–4.62	79.44	5 - 6
Herbal massage oil products Formula 2	5.30 ± 0.11		57.14	–3.19	75.65	5 - 6
Commercial herbal massage oil products 1	22.09 ± 0.27		70.48	–2.60	98.57	5 - 6
Commercial herbal massage oil products 2	3.95 ± 0.5		60.58	29.17	103.80	5 - 6

Additionally, the CIE Hunter color values L*, a* and b* were measured using a CIE Hunter colorimeter as presented in **Figure 8** and **Table 5**. Here, L* represents brightness, while a* and b* indicate the direction of light. Positive values for a* denote redness, while negative values signify greenness. Similarly, positive values for b* indicate yellowness, whereas negative values denote blueness. The study results revealed that the 2 formulations of herbal massage oil products exhibited slightly differing color values, specifically in terms of L*, a* and b*. Both formulations had an L* value exceeding 50, indicating a tendency towards brightness. The a* value was negative, indicating a color leaning towards the green spectrum, while the b* value was positive, suggesting a yellowish hue. Consequently, both herbal massage oil products appeared clear yellow upon color measurement. Notably, Formula 1 exhibited a slightly brighter yellow color compared to Formula 2. This disparity can be attributed to variations in ingredients and mixing ratios. Formula 2, with its higher viscosity, resulted from a higher proportion of coconut oil and Krabok seed oil, aimed at enhancing the slipperiness and oiliness of the product. In contrast, commercial massage oil products sourced from the Sakon Nakhon community in Thailand may exhibit a similar yellow

hue. However, notable differences in characteristics exist, namely clear yellow and dark yellow variants. These distinctions could arise from varying mixtures and mixing ratios utilized during production, leading to divergent properties and color characteristics.

Table 6 The results of qualification testing and satisfaction assessment regarding color and odor of herbal massage oil products from FAEE in comparison with commercial herbal massage oil products.

Sample	Color		Odor	
	Observation results	**Points awarded**	**Results of smelling**	**Points awarded**
Herbal massage oil products Formula 1(a)	The color characteristics are consistent.	2.95[i]	The smells good according to the ingredients used.	3.00[i]
Herbal massage oil products Formula 2(b)	The color characteristics are consistent.	3.00[i]	The smells good according to the ingredients used.	3.00[i]
Commercial herbal massage oil products 1(c)	The color characteristics are consistent.	2.90[i]	The smells good according to the ingredients used.	2.85[i]
Commercial herbal massage oil products 2(d)	The color characteristics are consistent.	2.95[i]	The smells good according to the ingredients used.	2.95[i]

Note: (1) The letter 'i' indicates the statistical test results that are not significantly different at the 95 % confidence level. (2) The criteria for scoring in the color test of Herbal massage oil products by observation, with 20 testers, 3 = consistent color, 2 = consistent color with acceptable variation and 1 = inconsistent color. (3) The criteria for scoring in the odor test of spa massage oils by observation, with 20 testers, 3 = pleasant aroma according to the components used, 2 = acceptable aroma according to the components used and 1 = abnormal scent or undesirable scent.
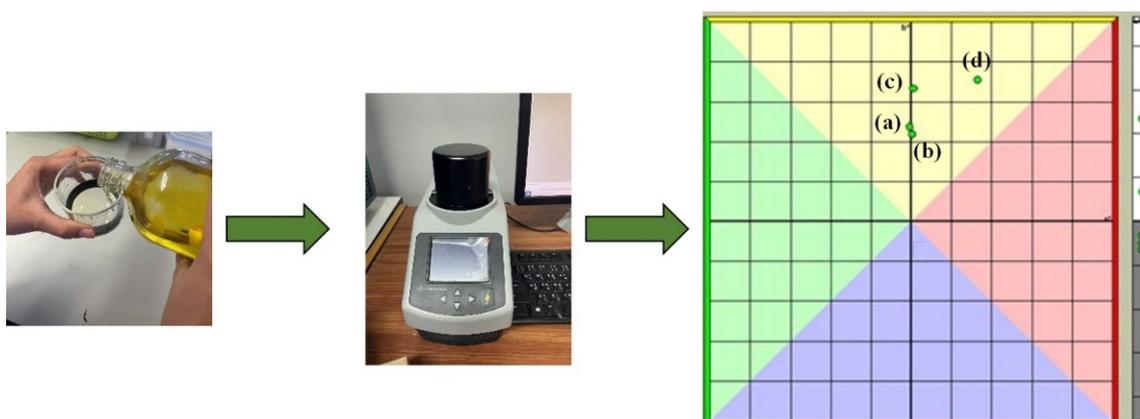

Figure 8 The evaluation color tone value using the CIE Hunter system of herbal massage oil products from FAEE in comparison with commercial herbal massage oil products: (a) Herbal massage oil products Formula 1, (b) herbal massage oil products Formula 2, (c) commercial herbal massage oil products 1 and (d) commercial herbal massage oil products 2.

Upon visually assessing the color appearance satisfaction of 20 testers, the study yielded significant findings as presented in **Table 6**. Evaluators of herbal massage oil products Formula 1 assigned a color evaluation score of 2.95 out of a perfect score of 3.00, indicating consistent color characteristics. Similarly, Formula 2 of herbal massage oil products received a score of 3.00, denoting consistent color as well. Commercial herbal massage oil products 1 and 2 were also evaluated, with scores of 2.90 and 2.95, respectively, indicating consistent color for both. Notably, the consistency in satisfaction scores across all products suggests comparable levels of satisfaction among evaluators. However, it is essential to acknowledge that individual preferences may influence these visual evaluations. Nonetheless, this research juxtaposed the herbal massage oil products with both commercial variants, revealing similar satisfaction scores among evaluators. In evaluating the satisfaction regarding odor characteristics through olfactory assessment with 20 test subjects, significant findings emerged as shown in **Table 6**. Herbal massage oil products Formula 1 received a perfect score of 3.00 out of 3.00 points, indicating consistent and excellent odor characteristics, akin to herbal massage oil products Formula 2, which also scored 3.00 points for its good smell according to the ingredients used. However, commercial herbal massage oil products 1 achieved a score of 2.85 points, while commercial herbal massage oil products 2 scored 2.95 points, both denoting a good smell in accordance with the ingredients used. Notably, commercial herbal massage oil products tended to exhibit a somewhat stronger odor compared to the 2 formulas developed in this research. It is essential to note that individual satisfaction may influence the results of olfactory evaluations. Nonetheless, this research compared the olfactory characteristics of the herbal massage oil products with those of commercial variants, revealing nuanced differences in scent satisfaction among evaluators. Hence, the herbal massage oil products underwent initial testing and evaluation by a sample of real users, yielding notably high satisfaction scores. Building on this positive reception, the synthesis of FAEE substances from Krabok seed oil was employed in the production of these herbal massage oil products. This initiative not only contributes to the advancement of knowledge in innovative health products but also adds value to local plants like Krabok trees. By utilizing such resources for various purposes, this endeavor aligns with the goal of conserving local resources while promoting sustainable practices and economic development within the community.

Conclusions

In summary, this study focused on synthesizing FAEE from Krabok seed oil for use in herbal massage oil products. Employing a circulation extraction method yielded an impressive oil extraction rate of 70.83 ± 1.92 %. Optimal synthesis conditions included using KOH as a catalyst at 5 wt.% loading relative to the Krabok seed oil weight, with an ethanol to oil molar ratio of 15:1, a reaction time of 60 min and a temperature of 78 ± 3 °C. FAEE yield under these conditions was 99.83 %, as assessed by ^{1}H-NMR analysis. The FAEE products exhibited favorable physical and chemical characteristics, including viscosity, density, cloud point, pour point, acid value, FFAs content, iodine value, water content and oxidation stability. Analysis revealed a predominance of mono-glyceride esters rich in lauric acid and myristic acid. Using the synthesized FAEE, herbal massage oil products were produced with consistent quality meeting industry standards, albeit with variations based on different mixtures and ratios. Overall, this study successfully achieved its objectives, with the resulting products comparable to commercial alternatives from Sakon Nakhon, Thailand. Preliminary user testing revealed high satisfaction scores, underscoring the potential of these products in innovation and the utilization of local resources such as Krabok trees. Leveraging these resources for multiple purposes aligns with the aim of conserving local assets and fostering sustainable practices, thereby contributing to economic development within the community.

Acknowledgements

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