Extraction of rubber (*Hevea brasiliensis*) seed oil using soxhlet method

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ABSTRACT

Soxhlet extraction which is also known as solvent extraction refers to the preferential dissolution of oil by contacting oilsseeds with a liquid solvent. This is the most efficient method to recover oil from oilseeds, thus solvent extraction using hexane has been commercialized as a standard practice in today’s industry. In this study, soxhlet extraction had been used to extract the rubber seed oil which contains high percentage of alpha-linolenic acid. In addition, the different solvents will be used for the extraction of rubber seed oil such as petroleum ether, n-hexane, ethanol and water to study the best solvent to extract the rubber seed oil so the maximum oil yield can be obtained. On the other hands, the natural resource, rubber belongs to the family of *Euphorbiaceae*, the genus is *Hevea* while the species of rubber is *brasiliensis*. Rubber (*Hevea brasiliensis*) seeds are abundant and wasted because they had not been used in any industry or applications in daily life. The oil of rubber seeds had been found that contained a significant percentage of long chain polyunsaturated fatty acids especially alpha-linolenic acid (ALA). Alpha-linolenic acid is one of the important elements of omega-3 fatty acids which play important roles in human metabolism, not only playing structural roles in phospholipid bilayers but also acting as precursors to bioactive molecules. Moreover, rubber seed oil also contains a high percentage of oleic acid and linoleic acid, these all are valuable compounds. Thus, rubber seed oil can be regarded as a plant derived oleic-linolenic acid. Rubber seeds can be considered as good sources for human food, animal feed and biofuel with its high content of fat, protein, amino acids and fatty acids. Therefore, it is important to study the method of extraction to extract the valuable components from rubber seeds, purify the extracted seed oil, so that the rubber seeds oil can be utilized into difference industries pharmaceutical, food, oleochemical and cosmetics.

Keywords: Soxhlet extraction, Alpha-linolenic Acid, Rubber Seed Oil, Solvent, Yield

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1. INTRODUCTION

Over the years, Malaysia has been one of the growing producers of natural rubber that involves plantation, processing and manufacturing of rubber trees for local and international customers. There are ongoing researches developed to dismantle this major asset of Malaysia, scientifically known as *Hevea Brasiliensis*, for its maximum utility as it is known to be very beneficial and profitable crop.

Malaysian rubber trees are acknowledged in producing natural rubber for commercialization. The latex obtained from the trees has been widely contributing in the industry for quite a long time now, and continues to serve until now. To date, studies are done for further improvement of the product by modifying the existing latex and convert it into dry rubber, latex concentrate or just as latex intermediates (Malaysian Rubber Board, 2011). Moreover, the rubber tree itself has also been found useful in the industry. In virtue of its uniqueness and durability, furniture manufactured from rubber wood has their own fan around the world. It might seem like the Malaysian rubber trees are utilized completely, but there are not many studies are done on the rubber seed itself. Rubber seed oil has currently been found to also have plenty of industrial potential. In Jerantut, Pahang, rubber seeds has been fermented and are used in condiments and curries. The rubber seeds delicacies are very popular among the people there and are one of the “must have” for tourists. The pleasant bitter taste of it was acknowledged and surprisingly it doesn’t show any adverse effect to the consumers, not even being intoxicated. Therefore, this somehow raises the curiosity of the fact that rubber seed oil might also be edible.

The oil of rubber seeds had been found that contained a significant percentage of long chain polyunsaturated fatty acids especially alpha-linolenic acid (ALA). Alpha-linolenic acid is one of the important elements of omega-3 fatty acids which play important roles in human metabolism. Therefore, it is important to study
the method of extraction to extract the valuable compounds from rubber seed so that it can be utilized in different industries.

Nurhan Dunford, an Agricultural Engineering Associate Professor claimed in his writing that solvent extraction refers to preferential dissolution of oil by contacting oilseeds with a liquid solvent. This is the most efficient method to recover oil from oilseeds, thus solvent extraction using hexane has been commercialized as a standard practice in today’s industry. This method has been used by a number of other studies on RSO and is selected for the high percentage oil (99.5%) extracted from the seeds [1].

Solvent extraction is much related to Soxhlet extraction. Most of this type of extractions is done in the Soxhlet extractor (Figure 1) which was invented in 1879 by Franz von Soxhlet.

![Solvent extractor](image1)

The choice of solvent used is based on solvent extraction capacity, effects of solvent on oil properties, process safety, solvent volatility and stability, and economic considerations [2]. This is because most of the common solvent used; light paraffinic petroleum fraction such as pentane, hexane, heptane and octane are flammable, studies have been developed to use chlorinated hydrocarbons, alcohols, water and etc. as an alternative. According to Devesh et al. [3], aqueous ethanol can be a better alternative solvent to n-hexane as extracting solvent, researchers were very motivated in searching new alternatives. There are also other famous and preferable solvents used, such as petroleum ether, water, alcohols and many more. As reported by Johnson et al. [4], there is quite a number of alternative solvents can be used, each with its own advantages and disadvantages. While water can be the processing aid for aqueous extraction of oilseeds, halogenated hydrocarbons also show tremendous potential in oilseed processing.

2. EXPERIMENTAL

2.1 Reagents and apparatus

The solvents used in solvent extraction were n-hexane, petroleum ether, water and a mixture of ethanol (95% pure) and water (70:30, v:v). Meanwhile, for oil conversion to fatty acid methyl ester (FAME) prior to alpha-linolenic acid (ALA) analysis, sulphuric acid (H₂SO₄), toluene and methanol were utilized. The apparatus of Soxhlet extraction including condenser, tubes, Soxhlet chamber, round bottom flask and heating mantle were set up for solvent extraction. In addition, oil recovery method was done using a rotary vacuum evaporator. The analysis of ALA content in the extracted rubber seed oil was using gas chromatography with helium as the carrier gas. Other equipments used including oven, blender and 0.5mm sieve were used for seed preparation.

2.2 Sampling

Bulk of raw rubber (Hevea Brasiliensis) seeds was de-hulled manually to free kernels from its shell as in Figure 2. Then, the thin outer layer was removed from the kernels (Figure 3) and the cleaned seeds were sliced to approximately the same size for drying.
2.3 Determination of moisture content

The sliced seeds were divided to three portions, 10g for each portion and heated in an oven at 105°C for 4 hours. After the heating was done, the seeds were placed in desiccators, and weighed again. The process was repeated until constant weight was achieved. The Figure 2 and 3 show the rubber kernels and cleaned rubber kernels respectively.

Fig. 2 Rubber kernels

Fig. 3 Cleaned rubber kernels

2.4 Solvent extraction process

10g of 0.5mm rubber seeds powder was transferred into a thimble, and 150 ml of n-hexane (bp: 68°C) was poured into a round-bottomed flask. Then, the Soxhlet's apparatus was heated using a heating mantle for 4 hours. The oil was recovered from the n-hexane solvent used in the extraction by solvent evaporation under reduced pressure in a rotary vacuum evaporator. Besides that, he also reported that easy solvent recovery from the meal is also an important trait of an ideal solvent. Converse from the flammability of n-hexane, the ideal solvent should be nonflammable or has a narrower range of explosive limits to avoid or reduce the possibility of fire or explosion during extraction. The extraction solvent also should be nonreactive to the meal or oil, as well as the extraction equipments. On the other hand, ideal solvents should also have high purity to exhibit more uniform operating characteristics, as well as having low solubility in water for easier separation. Ultimately, an ideal solvent should be easily available at low prices.

In this experiment, three variables were studied which was the choice of solvent, extraction time and solid to solvent ratio. Therefore, the experiment was repeated using each proposed solvent for 4, 6 and 8 hours at solid to solvent ratio of 1:10. After that, the experiment was repeated using solid to solvent ratio of 1:5 and 1:15 using petroleum ether as solvent.

Firstly, the seeds were ground to be extracted. Next, a thimble was placed into the Soxhlet extractor and the ground seeds were added into the extractor. Ensure that the sample and thimble were under the siphon side arm of the extractor. Other than using a thimble, cotton balls are also acceptable. 150 ml of solvent was added into a 250 ml round-bottomed flask, and was fitted to the bottom of the extractor. Then, the condenser was attached to the extractor with water supply tubes to complete the extraction apparatus. Then, the heating mantle was switched on after water was supplied to the condenser. The extraction time started when the first drop of solvent enters the thimble stuffed with the sample. Note to never leave the experiment unattended and ensure that the water bath never runs dry.

2.5 Oil recovery using rotary evaporator

The mixture of oil and excess solvent from the previous extraction process was poured into a weighted round-bottomed flask. The flask was fitted to the rotary evaporator and immersed in the heating medium until all the mixture is totally immersed. The rotary evaporator main supply was switched on and the temperature was set slightly above the boiling point of the solvent in excess. Then, the mixture was rotated slowly to prevent the mixture to evaporate out from the instrument. The vacuum pump was switched on to suck up the evaporated volatile solvent. After 30 minutes of running, the heating system was switched off and left to cool down for another 30 minutes. Finally, the flask was weighed and the concentrated extract was taken out.

2.6 Oil conversion to corresponding Fatty Acids Methyl Ester (FAME)

0.5g oil was treated with 5ml of H2SO4:toluene:methanol with volume ratio of 1:10:20 and reflux for 1 hour. After cooling, the total mixture was diluted with 5ml of water and 7ml of n-hexane respectively. Then, the mixture was separated using separatory funnel and the bottom layer was drained off. The top n-hexane soluble layer was dried with Na2SO4. The dried samples were transferred to sample vials and kept in refrigerator until further required for Gas Chromatography analysis.

According to Kiefer [5], the oil must be converted to a low molecular weight, volatile and non polar derivatives first, in order to analyze its fatty acid compositions. In this context, the rubber (Hevea Brasiliensis) seed oil obtained is converted to fatty acid methyl esters. The conversion process is known as transesterification. According to Schuchardt et al. [6], transesterification is a general description term of important class of organic reactions
where an ester is transformed into another through interchange of the alkoxy moiety.

2.7  **Gas Chromatography-Mass Spectrometry (GC-MS) analysis**

Gas chromatography analysis was performed on a gas chromatograph equipped with flame ionization detector (FID) and capillary column (30 m x 0.25 mm x 0.25 µm films). Helium was used as the carrier gas at a flow rate of 1.0 ml/min and column pressure of 22 kPa. Each sample (0.2 µl) was injected into the injection port using a split ratio of 50:1. The compound was separated following a linear temperature program of 150°C (5 min), 150-250°C (7.5°C/min), 250°C (8 min), total up to 1580 seconds for each run. The percentage composition was calculated using peak normalization method assuming an equal detector response.

3.  **RESULTS & DISCUSSION**

3.1  **Moisture content**

Rubber (*Hevea Brasiliensis*) seed oil should contain approximately 7% of moisture after 4 hours of drying using oven at 105°C. It has been previously reported by other studies that the optimum range of moisture content in oilseed processing is between 5 to 13%. According to Ebewele *et al.* [7], the optimum moisture content of rubber seed oil extraction is 10% at 70°C. He also reported that higher oil yield was observed at a lower moisture content of 7% up to 10 to 13% with exceptional to very low or higher temperature. This is because, during the extraction process, the moisture in the seeds acts as a heat transfer medium and helps in coagulation of protein for oil yield. Therefore, too high or too low temperature may disturb the function of the moisture in oilseed processing.

3.2  **Physical characteristics of rubber (*Hevea Brasiliensis*) seed oil**

Observations have been made to the physical characteristics of the rubber (*Hevea Brasiliensis*) seed oil extracted from all four solvents. The oils from n-hexane and petroleum ether extractions were both golden yellowish as shown in Figure 4, with the original odor of rubber (*Hevea Brasiliensis*) seed. This is because both solvents have low polarity which only extract the glyceride compound in the seeds and are very miscible in the oil. On the other hand, white particles were observed to be extracted together with the oil when using a polar solvents of water and ethanol:water. After the solvents being recovered, the extract was at small yield and it became too viscous and not transparent like normal oil (Figure 5). Hron *et al.* [8] and Ferriera-Dias *et al.* [9] reported that ethanol has the capability to co-extract other compounds in oilseeds which are insoluble in non-polar solvents. Ethanol is a good solvent to extract all three; gossypol, aflatoxin and oil from cottonseed [10].

3.3  **Effect of solvent used on oil yield**

Figure 6 shows the rubber (*Hevea Brasiliensis*) seed oil yield obtained from solvent extraction using four different solvents; n-hexane (b.p 68°C), water (b.p 100°C), petroleum ether (b.p 60-80°C) and ethanol:water mixture (70:30, v:v) (b.p 78.3°C).

Based on Figure 6, it can be obviously seen that using n-hexane and petroleum ether gives higher oil yields than conducting solvent extraction using water and mixture of ethanol:water (70:30, v:v). This obvious gap explains the polarity theory of solvent and oilseeds in solvent extraction. Since polar lipid dissolves and soluble in polar solvent, it can be said that rubber (*Hevea Brasiliensis*) seed oil is non-polar lipid. This is because, water is known to be universal solvent, and ethanol:water have high polarity and are immiscible in oil [9]. This explains the 20% yield of the rubber (*Hevea Brasiliensis*) seed oil.

However, oil is reported to be soluble in ethanol at its boiling point, but decreases with decreasing alcohol concentration with presence of water [8]. Since the mixture of ethanol:water is 70:30 by volume, the solvent gradually inherit the characteristics of water, resulting in low oil yield.
On the other hand, low polarity solvents of n-hexane and petroleum ether extracted the rubber \((\text{Hevea Brasiliensis})\) seed oil with more than 50% yield. Therefore, the nature of the extracted lipid is highly dependent of the solvents’ polarity, which will determine the selectivity of the solvent used [8, 11]. Furthermore, the differences in dielectric constant of solvent used are also the main reason for this result. This is because, solubility of solvent in oil decreases with increasing dielectric constant value, thus increasing its polarity. The dielectric constant of n-hexane, petroleum ether, water and ethanol:water is 1.8, 2.0, 80 and 24.3 respectively.

### 3.4 Effect of extraction time on oil yield

The figure 7 illustrated the trend of oil yield extracted using four different solvents at extraction times of 4, 6 and 8 hours.

Based on Figure 7, it can be obviously seen that the maximum oil yield of all four solvents is at 6 hours extraction time. The oil yield from n-hexane is steadily at all three extraction times, while others are not steady. However, the highest oil yield is shown by petroleum ether extraction at 55%, and the lowest is seen by water extraction. Moreover, there is a slight decrease in oil yield value from extraction of polar solvents at 8 hours.

The amount of oil yield from non-polar solvents did not change significantly after 6 hours, as described by Sayyar et al. [12] on Jatropha seeds extraction. The rubber \((\text{Hevea Brasiliensis})\) seed oil can be said to be fully extracted from the seed after 6 hours although the maximum extracted oil was achieved only after 8 hours. This is because, the concentration of oil increases in the solvent resulting in a decrease in the diffusion rate. Therefore, the oil yield level remains consistent even by extending the reaction time when the maximum amount of extractable oil is obtained.

### 3.5 Effect of solid to solvent ratio on oil yield

The Figure 8 described the effect of oil yield on solid to solvent ratio. The experiment was done using petroleum ether as solvent at 6 hours for its optimum oil yield condition.

Based on Figure 8, the highest oil yield is shown at 1:15 solid to solvent ratio and the lowest is at 1:05 ratio. Therefore, it can be said that increasing the solid to solvent ratio increases the oil yield. This is because the concentration gradient between the solid and the liquid phase becomes greater which favors good mass transfer [12].

### 3.6 Gas Chromatography-Mass Spectrometry (GC-MS) analysis

In the chromatogram (Figure 9), it had showed that the rubber seed oil contain high percentage of alpha-linolenic acid (ALA) which is one of the omega-3 fatty acids. According to Borsonelo and Galduróz [13], DHA has the critical role in the functioning of membranes. Besides this, omega-3 fatty acids can be considered a treatment adjunct for patients displaying aggressive behaviors and substance abuse disorders. During pregnancy, omega-3 fatty acids play an important role because the essential fatty acids requirements are high due to accretion of maternal, placental and fetal tissue occurs. Furthermore, omega-3 fatty acids also can decrease platelet aggregation, blood viscosity, increase bleeding time, hence decreasing the tendency to thrombus formation [14]. ALA is one of the polyunsaturated fatty acids which play
important roles in human metabolism, not only playing structural roles in phospholipid bilayers but also acting as precursors to bioactive molecules [15]. Lastly, Geleijnse, J.M. et al. [16] reported that intakes of n-3 fatty acids above the Adequate Intake (AI) may confer additional health benefits, especially with respect to cardiovascular health noted by Institute of Medicine (IOM).

Fig. 9 The GC-MS chromatogram of alpha-linolenic acid (ALA) in rubber seed oil

4. CONCLUSION

Soxhlet extraction method using petroleum ether and n-hexane as the solvent will gives a higher oil yield compared to ethanol:water because of its high solubility and polarity. Higher efficiency of extraction process will result from longer extraction time with particle size of 0.55 mm while the optimum extraction time was 6 hours. Furthermore, the moisture content in the rubber seed oil was 7% which is in the range of the oilseed processing hereby is not too low to act as the heat transfer agent in favoring the extraction process. From the analysis result, we can observed that the rubber seed oil contain high amount of alpha-linolenic acid (ALA), therefore rubber seed oil has the great potential to be utilize as the new source of omega-3 fatty acids which is the local plant derived omega-3. Last but not least, the rubber seed oil also has the potential to be made into the product of cosmetics, pharmaceutical and so forth.

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