**BIOLOGY, MEDICINE, & NATURAL PRODUCT CHEMISTRY** ISSN 2089-6514 (paper)
Volume 15, Number 2, October 2026 | Pages: 2197-2202 | DOI: 10.14421/biomedich.2026.152.2197-2202 ISSN 2540-9328 (online)

# Soxhlet Extraction of Rubber Seed Oil: Effect of Solvent Selection and Extraction Time on Extraction Yield and Physicochemical Properties

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Manuscript received: 18 August 2026. Revision accepted: 17 November 2026, Published: 19 November 2026.

**Abstract**

Rubber seed (*Hevea brasiliensis*) is an abundant, yet underexploited resource. In fact, the high oil content in its kernel shows potential for a wide range of applications. The effects of solvent polarity and extraction time on the yield of rubber seed oil (RSO) through Soxhlet extraction were investigated. The physicochemical properties of the resulting RSO were then analyzed. The Soxhlet extraction was carried out on the dried rubber seed kernel grounds with n-hexane, ethanol, and methanol for 2, 4, and 6 hours. The yield of RSO was determined and the physical and chemical properties were characterized. It was observed that the yield of RSO increased with extraction time but the increase from 4 hours to 6 hours was relatively small. For all solvents, the maximum yield of RSO was reached after six hours extraction. The highest RSO yield was obtained using n-hexane as solvent, followed by ethanol and methanol. The RSO density ranged from 896.2 to 902.2 g $L^{-1}$, while the kinematic viscosity ranged from 14.5 to 16.2 $mm^2$ $s^{-1}$ at 40 °C and decreased to 3.7–4.3 $mm^2$ $s^{-1}$ at 100 °C. The acid value ranged from 37.2–40.6 mg KOH $g^{-1}$, while the saponification value ranged from 188.1–203.0 mg KOH $g^{-1}$. The RSO obtained showed relatively high acid values, suggesting the need for proper handling and storage of rubber seeds. Valorization of RSO with such high acid values as a biodiesel feedstock requires pretreatment, which affects production costs. Meanwhile, such a high acid value is likely preferable for use as a substrate for biodegradable plastic production.

**Keywords:** Acid number; n-hexane; Rubber seed; Rubber seed oil; Soxhlet extraction.

## INTRODUCTION

Beyond latex production, rubber plants produce large amounts of seeds each year that are still largely underutilized. Annual rubber seed production is estimated between 136–2000 kg $ha^{-1}$ (Zhu et al., 2014), but only about 25% is utilized for nurseries, while the remaining 75% is discarded (Yang et al., 2021). The rubber seed weight varies between 3.10-4.40 g/seed with the kernel contributing 56-60% of the total seed weight. The major components of kernel were lipids (43.20–46.99%), followed by protein (23.17–25.08%), carbohydrates (17.11–20.72%), and fiber (6.27–8.64%) (Okoma et al., 2025). RSO was classified as non-edible oil due to the presence of cyanogenic glycosides in rubber seeds that can release toxic hydrocyanic acid (Gui et al., 2008). However, Salimon et al. showed that, linamarin, a toxic compound present in rubber seed oil, did not release cyanide and did not show a toxicological hazard to rats (Salimon et al., 2012). Rubber seed is a rich source of nutrients, minerals and bioactive compounds, which make it interesting to investigate the potential of rubber seed to be valorized as functional foods for human and animal consumption (Saha et al., 2025).

RSO has received much attention as a raw material for the production of biodiesel. The main components of RSO are unsaturated fatty acids (Table 1), and this gives good cold-flow properties, so that RSO-based biodiesel can be used in countries with cold climate (Gui et al., 2008).

**Table 1.** Fatty acid composition of RSO (%).

| Fatty acid | Yang et al. (2021) | Jisieike et al. (2020) | Onoji et al. (2016) | Roschat et al. (2017) | Ramadhas et al. (2005) |
|---|---|---|---|---|---|
| Myristic (C14:0) | - | 0.09 | - | - | - |
| Palmitic (C16:0) | 10.77 | 0.32 | 13.85 | 9.1 | 10.2 |
| Palmitoleic (C16:1) | - | 0.14 | - | - | - |
| Stearic (C18:0) | 8.05 | 11.42 | 16.82 | 5.6 | 8.7 |
| Oleic (C18:1) | 23.68 | 24.95 | 64.11 | 24.0 | 24.6 |
| Linoleic (C18:2) | 36.16 | 33.41 | - | 46.2 | 39.6 |
| Linolenic (C18:3) | 20.60 | 20.17 | - | 14.2 | 16.3 |
| Arachidic (C20:0) | - | 0.51 | - | - | - |
| Erucic (C22:1) | - | - | 5.22 | - | - |

RSO also has the potential to be a renewable raw material for the production of poly-hydroxyalkanoates (PHAs), bioplastics that can be biodegraded. It was demonstrated that RSO can serve as a feasible carbon source for PHA production with a yield of 1.80 g $L^{-1}$ by *Bacillus cereus* (Kynadi & Suchithra, 2017). The high proportion of unsaturated fatty acids in RSO also makes it a possible raw material for the production of environmentally friendly polyester alkyds. Alkyd resin is an important raw material in the coatings and paint industry and is the main binder in different coatings and paints for decorative applications (Otabor et al., 2019). Previous study revealed that alkyd resins synthesized from a mixture of RSO/SBO via the alcoholysis had comparable properties of coating to commercial polyesters (Ifijen et al., 2021).

Extraction methods can generally be classified into two types, namely mechanical pressing and extraction using solvents. Mechanical pressing, i.e., applying pressure, is a simple way of removing oil from the kernel. Mechanical pressing with a screw press usually has an efficiency of no more than 90%, but the advantage of producing oil without the addition of chemicals. Yang et al. (2021) reported that, solvent extraction can reach an oil recovery of 95.12%, while only 69.01% oil recovery can be achieved through mechanical pressing. Another study reported that, the optimal oil recovery from mechanical pressing can reach 76% (Abduh et al., 2016). Soxhlet extractors were developed by Franz Ritter von Soxhlet in 1879 and are mainly used for quantitative determination of oil content in organic matter (Jensen, 2007). Soxhlet extractors are automatic batch extractors that differ from continuous extractors in that they return extracts to the solvent flask by a siphon mechanism when the solvent level reaches a certain height. Repeated extraction allows a longer contact time between the material and the solvent, therefore the extraction will be more efficient and effective. Soxhlet extraction is a common method used to extract oil from various natural sources, such as *Jatropha curcas* seed (Prabaningrum et al., 2014), *Sapindus mukorossi* seed (Hu et al., 2021), *Crotalaria juncea* (Dutta & Sarkar, 2023), rubber seed (Yang et al., 2021), and spent coffee grounds (Jin et al., 2018). Soxhlet extraction yielded a higher amount of samara oil than supercritical fluid extraction (Mu et al., 2021). Soxhlet extraction can yield a higher RSO than ultrasound-assisted extraction under certain conditions (Mabayo et al., 2018). By improving mass transfer and increasing solvent-material contact, Soxhlet extraction can be scaled-up into a large-scale oil extraction unit (Dutta & Sarkar, 2023).

In this study, Soxhlet extractor was used to extract rubber seed oil (RSO) from kernels using three types of solvents, namely n-hexane, methanol, and ethanol. The use of methanol and ethanol has become interesting along with the development of research on in situ transesterification using both solvents (Abdulkadir et al., 2015; Muhammad et al., 2017).

## MATERIALS AND METHOD

### Materials

Rubber seeds were obtained from a smallholder rubber plantation in Natar, South Lampung, Indonesia. Rubber seeds were selected based on uniform size and good physical condition (Figure 1). Next, the rubber seeds were peeled from the shell, cut into small pieces, then dried in the oven at 80 °C for 4 hours. The dried chopped rubber seed kernels was then mashed using a kitchen blender (HR2115, Philips). Ethanol, methanol, and n-hexane with a technical purity level were used as solvents and are obtained from Bratachem, Bandar Lampung, Indonesia.



**Figure 1.** Rubber seeds.

**Soxhlet extraction**

RSO extraction using the Soxhlet method was performed using a Soxhlet extractor, as illustrated in Figure 2. A sample of 25 g of mashed rubber beans for each treatment was put into the cellulose thimble. Soxhlet extraction was carried out using methanol, ethanol, and n-hexane which have different polarity levels with a seed-to-solvent weight ratio of 1:10. Since the three solvents have different boiling points (Somnuk et al., 2017), the Soxhlet extractor was calibrated so that the duration of each extraction cycle was relatively comparable, i.e. 9 minutes 32 seconds for n-hexane, 9 minutes 49 seconds for methanol, and 9 minutes 42 seconds for ethanol. Extraction was then carried out for 2, 4, and 6 hours. The solvent was separated from the extracted oil using a rotary vacuum evaporator, then the oil was dried at 105 °C for 4 hours. The RSO yield obtained was then calculated using Equation 1:

$$\text{Yield (wt.\%)} = \frac{w_o}{w_s} \times 100 \quad (1)$$



**Figure 2.** Soxhlet extractor.

**Physicochemical characterization**

RSO density was determined using a calibrated 5 mL capacity picnometer (PICNO-M5-C, Iwaki Pyrex) at room temperature. The viscosity of the RSO was determined using a Cannon–Fenske viscometer equipped with a thermostatic water bath in accordance with the ASTM D445 procedure (ASTM, 2019). RSO viscosity wass expressed as kinematic viscosity, which was measured at temperatures of 40 and 100 °C. The number of acids was determined based on SNI 01-3555-1998 (SNI, 1998) and calculated using Equation 2:

$$AV = \frac{V \times N \times 56.1}{w_o} \quad (2)$$

where AV is the acid number (mg KOH $g^{-1}$ of oil), N is the normality of KOH (0.1 N), V is the volume of KOH used for titration (mL), and $w_o$ is the mass of the oil sample (g). The saponification number was determined based on SNI 01-3555-1998 (SNI, 1998) and calculated using Equation 3:

$$SV = \frac{(V_0 - V_1) \times T \times 56.1}{w_o} \quad (3)$$

where SV is saponification number (mg KOH $g^{-1}$ of oil), $V_0$ is the volume of HCl required for blank titration (mL), $V_1$ is the volume of HCl required for sample titration (mL), T is the normality of HCl (0.5 N), and $w_o$ is the mass of RSO (g).

## RESULTS AND DISCUSSION

**RSO yield**

The yield of rubber seed oil (RSO) obtained using n-hexane, ethanol, and methanol at extraction times of 2, 4, and 6 hours is presented in Figure 3. Yang et al. (2021) determined the oil content of rubber seeds with extraction time of 6 hours. Hanif et al. (2019) reported that the difference in yield was only 0.2% when spent coffee grounds from Soxhlet was extracted for 6 hours. Therefore, an extraction time of 6 hours was considered sufficient to obtain an RSO yield that is close to the maximum. As shown in Figure 2, in general, RSO yields increase as the extraction time increases in all three solvents. The increase in yield was related to the increase in the number of extraction cycles, which allows the mass transfer from the solid phase of the rubber kernel to the liquid phase of the solvent to take place further. However, the increase in yield from 4 to 6 hours was relatively small. In the use of n-hexane, the yield increased by only 0.13%, while the increase in methanol and ethanol was 0.17% and 0.62%, respectively. These results indicated that most of the extractable oil was obtained before 6 hours, so extending the extraction time to 6 hours only provides a limited additional yield.

In addition to the extraction time, the type of solvent also showed a clear influence on RSO yield. The maximum yield was obtained by extraction with n-hexane, which is 41.32% in 6 hours. The yields obtained by using ethanol and methanol only reached 17.12% and 13.79%, respectively, at comparable extraction time. The large difference in yield between types of solvent indicated that the polarity of the solvent is one of the important factors in RSO extraction. Hexane has a dielectric constant of 1.89, which is less than that of ethanol, 24.3 and methanol, 33.6. As a non-polar solvent, n-Hexane has a higher affinity to the non-polar lipid components (Reshad et al., 2015).

The result of this study is consistent with the study of Jisieike & Betiku (2020), which obtained the yield of RSO was 62.9 % using n-hexane and 56.7% using a more polar isopropanol, with a solid to solvent ratio of 0.05 g/$mL^{-1}$ and an extraction time of 3 h. A similar trend was also reported for oil extraction from dried spent coffee grounds (DSCG) (Somnuk et al., 2017). The oil

yield was 14.7% with hexane, 13.1% with anhydrous ethanol, 11.8% with aqueous ethanol, and 7.5% with methanol in batch extraction with the weight ratio of DSCG to solvent of 1:25.

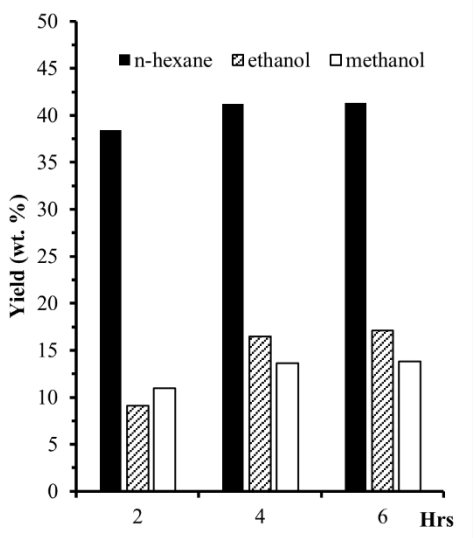



**Figure 3.** Extract yield of crude RSO under various solvents and extraction times.

The RSO yield of 41.32% was the maximum obtained with n-hexane for 6 hours, and it is within the range of reported values for rubber seed oil, about 40–50% (Ikwuagwu et al., 2000). The results indicated that, under the studied conditions, Soxhlet extraction with n-hexane was able to recover most of the oil fraction present in the gum kernel. In contrast, yields obtained with ethanol and methanol were only 17.12% and 13.79%, respectively, indicating that the two solvents were less effective than n-hexane to obtain high RSO yields.

## Physicochemical properties of RSO

The physicochemical properties of RSO obtained through Soxhlet extraction using n-hexane, ethanol, and methanol are presented in Table 2. The density of RSO obtained in this work ranged from 896.2 to 902.2 g $L^{-1}$, which was still within the range of density reported in the previous study (Jisieike & Betiku, 2020; Muhammad et al., 2017; Onoji et al., 2016). The kinematic viscosity of RSO ranged of 14.5–16.2 $mm^2$ $s^{-1}$ at 40 °C and decreases to 3.7–4.3 $mm^2$ $s^{-1}$ at 100 °C, which is typical for liquids with rising temperature. The viscosity values obtained in this study were quite lower than the values reported by previous studies (Jisieike and Betiku, 2020; Muhammad et al., 2017; Onoji et al., 2016).

**Table 2.** Physicochemical properties of RSO.

| Properties | This work | | | Jisieike et al. (2020) | Onoji et al. (2016) | Muhammad et al. (2017) |
|---|---|---|---|---|---|---|
| | Hexane | Ethanol | Methanol | | | |
| Density (g $L^{-1}$) | 902.2 | 899.6 | 896.2 | 869.4 | 886.0 | 920 |
| Kinematic viscosity ($mm^2$ $s^{-1}$): | | | | | | |
| 40 °C | 14.5 | 15.1 | 16.2 | 34.5 | 40.2 | 36.9 |
| 100 °C | 3.7 | 4.1 | 4.3 | - | - | 8.4 |
| Acid value | 37.2 | 39.2 | 40.6 | 13.0 | 18.2 | 17.95 |
| Saponification value | 188.1 | 193.1 | 203.0 | 170.2 | 195.3 | - |

The essential parameter in the present study is the acid value RSO. The acid value was in the range of 37.2-40.6 mg KOH $g^{-1}$. The lowest acid value was found in RSO extracted with n-hexane and the highest acid value was found in RSO extracted with methanol. These values were higher than those reported (Jisieike and Betiku, 2020; Muhammad et al., 2017; Onoji et al., 2016). However, these values were still in the range of the acid values of RSO that have been reported in different studies, which was 15.03–81.6 mg KOH $g^{-1}$ (Zhu et al., 2011).

The high acid value in this study could be related to the condition of the raw materials before extraction. The rubber seeds used were collected from the seeds that fell around the community plantations. Hence, the seeds were exposed to the environment for a certain period before collection. The moisture content of rubber seeds was relatively high (29.92%) and most of the water stored in the kernel (Yang et al., 2021). These conditions allowed biological activity and lipolysis to occur, which caused triglycerides to hydrolyze into free fatty acids and further increase the acid value of RSO (Zhu et al., 2011). Thus, handling raw materials from harvest and shortening the storage time of rubber seeds can be an important step to suppress biological activities that contribute to the increase in the acid value of RSO.

The high acid value is also an important consideration in the use of RSO as a raw material for biodiesel. The RSO is known to have a high acid value, which can be as high as 91.4 mg of KOH $g^{-1}$ (Uemura et al., 2013). The high acid value can interfere with alkaline catalysts in the transesterification and form soap, complicating purification of the products and reducing the biodiesel yields. Therefore, the high acid value of RSO can lead to the need for a pretreatment stage before transesterification, which can ultimately increase the cost of biodiesel production.

Nevertheless, high acid value which results in high FFA levels of RSO can provide an advantage in PHA synthesis. Kynadi & Suchithra (2017) reported that, in a comparison of the various substrates used in PHA synthesis, plant oils are an efficient substrate, with RSO being one of the most efficient substrates and having performance equivalent to waste frying oil. Both RSO and waste frying oil are substrates with high FFA levels. The high levels of FFA in the oil as a substrate can accelerate metabolism, making it easier for biological activities to produce PHA.

The saponification value of RSO obtained in this study was in the range of 188.1–203.0 mg KOH $g^{-1}$, with the highest value obtained from extraction using methanol. This value was still in the range comparable to the results of previous research (Jisieike & Betiku, 2020; Muhammad et al., 2017; Onoji et al., 2016). The difference in the saponification value between the solvent types indicated that there are differences in the characteristics of the oil fraction extracted by n-hexane, ethanol, and methanol. The saponification can vary due to the variations in the composition of the fatty acids composing the oil. A higher saponification value indicates that the oil is composed of fatty acids with shorter chains (Premjet et al., 2021).

## CONCLUSION

Soxhlet extraction was a solvent extraction used to obtain rubber seed oil (RSO) from dried rubber seed kernel grounds using variations in solvents and extraction time, affecting RSO yield. An increase in extraction time from 2 to 6 hours increased the RSO yield in all three solvents, although the increase from 4 to 6 hours was relatively small, so 6 hours could be considered sufficient to obtain a near-maximum yield under the study conditions. The highest extraction yield was achieved using n-hexane as a solvent, 41.32% while the yields obtained when ethanol and methanol were used as solvents were 17.12% and 13.79%, respectively, indicating that n-hexane was more efficient in extracting RSO. The physicochemical properties of RSO were influenced by the solvent type used. The relatively high acid values of RSO (37.2-40.6 mg KOH $g^{-1}$) indicated a high content of FFA. Therefore, valorization of RSO as a raw material for biodiesel production may need a pretreatment step before the transesterification. However, high FFA content also revealed the potential of RSO as a substrate for PHA production.

***Acknowledgements:*** The authors acknowledge the Laboratory of Physics, Department of Chemical Engineering, University of Lampung, and UPT-LTSIT, University of Lampung, for support in conducting the experiments and facilitating data analysis.

***Authors' Contributions:*** Muhammad Hanif: Conceptualization, Supervision, Writing (editing). Siti Apriani: Data acquisition, Formal analysis, Investigation, Writing (original draft). Rico Sinaga: Data acquisition, Formal analysis, Investigation, Writing (original draft). All authors read and approved the final version of the manuscript.

***Competing Interests:*** The authors declare that there are no competing interests.

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