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Ultrasonic-assisted Extraction Technique of Fixed Oils from Sudanese Seeds

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Abstract

The extraction of oils from Sudanese seeds is of great economic and traditional significance. This study investigated the use of ultrasonic-assisted extraction to extract oils from five different Sudanese seeds: desert date (*Balanites aegyptiaca* L), baobab (*Adansonia digitata*), peanut (*Arachis hypogaea* L.), watermelon (*Citrullus lanatus*), and roselle (*Hibiscus sabdariffa*). The extraction process used two organic solvents, *n*-hexane and *n*-heptane. The results showed that *n*-heptane was a more effective solvent for extracting oils from Sudanese seeds than *n*-hexane. The highest oil yield was obtained from *B. aegyptiaca* seeds, with a yield of 29%. The optimal seed-to-solvent mass ratio for *B. aegyptiaca* was 1:6, and the optimal extraction time was 1 hour (60 mins). A thermodynamic study was conducted to validate the experimental results. The larger area in the nonpolar region for n-heptane suggests a higher extraction capacity for the seed oils. These results are consistent with the experimental findings of this study. In conclusion, ultrasonic-assisted extraction is a promising method for extracting oils from Sudanese seeds. *N*-heptane is a more effective solvent than *n*-hexane for this purpose, and the optimal seed-to-solvent mass ratio and extraction time are 1:6 and 1 hour (60 mins), respectively. The results of this study could be used to develop new commercial products based on Sudanese seed oils. However, further research is needed to investigate the effects of other factors, such as temperature and pressure, on the extraction of oils from Sudanese seeds.

1. Introduction

The extraction of active compounds is a pivotal initial step in preparing traditional herbal remedies. Historically, the extraction of seed oils has involved roasting seed kernels using a mortar and pestle or between two stones and mixing the crushed mass with water (Olaniyan & Yusuf, 2012). While effective, these traditional methods are often time-consuming and require large quantities of solvents.

In recent years, modern sample-preparation techniques have revolutionised the extraction process. These techniques offer significant advantages over conventional methods, including shorter extraction times, higher yields, optimised solvent volumes, and improved quality of extracts (Gupta *et al.*, 2012). Such advancements are crucial in ensuring the availability of high-quality herbal products to consumers worldwide.

Conventional oil extraction methods, such as solvent extraction and mechanical processing, are commonly used to derive seed oils, leaving minimal residual oil in the cake or meal (Dutta *et al.*, 2015). However, modern techniques like microwave-assisted extraction, ultrasound-assisted extraction, pressurised liquid extraction, and supercritical fluid extraction have been developed to address the major shortcomings of traditional methods like Soxhlet extraction (Kemper, 2005; Pavlić *et al.*, 2018).

Among these modern techniques, ultrasonic-assisted extraction (U.A.E.) has emerged as a green extraction method that reduces energy consumption and extraction time while increasing processing throughput (Kalhor & Ghandi, 2019; Žlabur *et al.*, 2016). The U.A.E. method operates on the principle of processing samples under specific frequencies and amplitudes. The sound waves produced generate vacuum bubbles in the liquid, which collapse upon reaching a critical size, releasing a high amount of energy in the liquid medium.

This energy can be harnessed for mixing, extracting, and grinding (Prabuthas *et al.*, 2011).

The Conductor-like Screening Model for Real Solvents (COSMO-RS) has been utilised in various studies to extract oil from plant seeds. For instance, a study by Hizaddin *et al.* (2022) explored the interaction between Deep Eutectic Solvents (D.E.S.s), phenol, *n*-heptane, and toluene using COSMO-RS. The study suggested that D.E.S.s, considered green solvents, could serve as alternatives to conventional organic solvents and ionic liquids to extract phenolic compounds from pyrolysis oil.

This study aims to determine the yield of oil extracted using U.A.E. from different plant seeds, such as *Hibiscus sabdariffa, Citrullus lanatu, Arachis hypogae, Adansonia digitata,* and *Balanites aegyptiaca*, using different solvents. After identifying the highest extraction yield and the most effective solvent, the factors affecting the extraction, including mass ratio and extraction time, will be investigated. The study will also conduct a thermodynamic analysis to support the selection of solvents. The findings from this study will contribute to the body of knowledge on optimising oil extraction from plant seeds using modern extraction techniques. U.A.E. demonstrated higher yield, less time, lower temperature and less solvent consumption; it was selected to optimise the oil extraction yield from several Sudan seeds. This study aims to determine the yield of oil extracted using U.A.E. from different plant seeds, such as (*Hibiscus sabdariffa, Citrullus lanatu, Arachis hypogae, Adansonia digitata and Balanites aegyptiaca,* using different solvents. After screening for the highest extraction yield and best solvent, factors affecting the extraction, including mass ratio and extraction time, will be investigated. The optimisation, evaluation of the optimum mass ratio and extraction time are also conducted in this study. After that, a thermodynamic analysis was conducted to support the selection of solvents.

2. Methodology

2.1 Preparation of seed

Five different types of dried seeds, Desert date, Peanuts, Baobab, Watermelon, and Roselle, were prepared for extraction. The dried seeds were ground using a mortar and pestle to crush the seeds into granules. This process was undertaken to increase the surface area available for oil extraction. The granularity of the crushed seeds was carefully controlled to ensure a consistent size across all samples. The resulting seed powder was of a fine consistency, optimal for the ultrasonic-assisted extraction process.

2.2 Screening of solvents and seeds

Ten (10) g of seed powder were weighed and placed individually into conical flasks. Following that, 60 g of *n*-heptane was added into each of the flasks to make a sample with a seed-to-solvent mass ratio of 1:6. Each of the conical flasks was then covered with aluminium foil to avoid the evaporation of the solvent. Next, the conical flasks containing the sample solution were placed in the water bath of the sono-reactor for 60 mins at 35°C with 70 rpm agitation speed. After 1 hour, each sample was transferred from conical flasks into centrifuged tubes and centrifuged for 10 mins. At the same time, the weight of the empty beakers (W_1) was measured and labelled respectively. After centrifugation, each sample solution was filtered into the labelled empty beakers to obtain only solvent with extracted oil. Subsequently, the beakers were placed in the fume hood for evaporation of *n*-heptane. The beakers with the extracted oil were weighed and recorded as (W_2). The steps were repeated by using *n*-hexane as the extracting solvent. Lastly, the oil yield (Y) was calculated based on the equation below. (See Figures 1A to 6A in the supplementary file).

$$\%\text{ yield of oil (Y)} = \frac{W_2 - W_1}{W} \times 100 \qquad \text{Eq (1)}$$

Where:
W_2 = weight of beaker with extracted oil after evaporation (g)
W_1 = weight of empty beaker before evaporation (g)
W = initial weight of seeds (g)

Figure 1: Plant seeds powders after grinding.

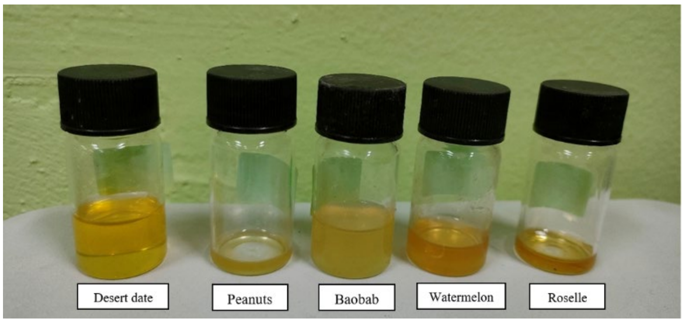


Figure 2: Oil extracted from Desert date, Peanuts, Baobab, Watermelon and Roselle seeds (from left to right) after evaporation.

2.3 Study of factors affecting the extraction

2.3.1 Mass ratio

Firstly, the selected seed powder was added with the solvent into a conical flask with a mass ratio 1:2, where 10 g of seed powder and 20 g of solvent were weighed. The steps were repeated using different mass ratios where the weight for seed powder was kept constant at 10 g, and the weight of the solvent varied from 40 g, 60 g, and 80 g. The steps demonstrated in Section 2.2 were followed to carry out the extraction.

2.3.2 Extraction time

The selected seed powder with the highest yield was added with the solvent into 4 different conical flasks according to a mass ratio of 1:2, where 10 g of seed powder and 20 g of solvent were weighed. The samples were placed into the sono-reactor for various extraction times of 20 mins, 40 mins, 60 mins and 80 mins. Samples were processed using the same steps as demonstrated in Section 2.2

2.4 Thermodynamics of mixing and solubility

This work used COSMO-RS to screen the relative solubility of the fatty acids in hexane and heptane. The geometric optimisation and solvent representation in COSMOtherm were performed according to the methods described in previous work (Hayyan *et al.*, 2023; Salleh *et al.*, 2017).

3. Result and discussion

3.1 Screening of plant seeds and solvents

A preliminary screening of plant seeds and solvents was conducted to select the plant seed and solvent which gives the highest oil yield using an ultrasound-assisted oil extraction method. The result of the preliminary screening is presented in Figure 3.

A graph of oil yield against the type of seeds using *n*-heptane and *n*-hexane as the extracting solvent was plotted to determine the plant seed and solvent producing the highest oil yield. From that, the plant seeds of desert date (*Balanites aegyptiaca*) and solvent (*n*-heptane) were selected for optimisation.

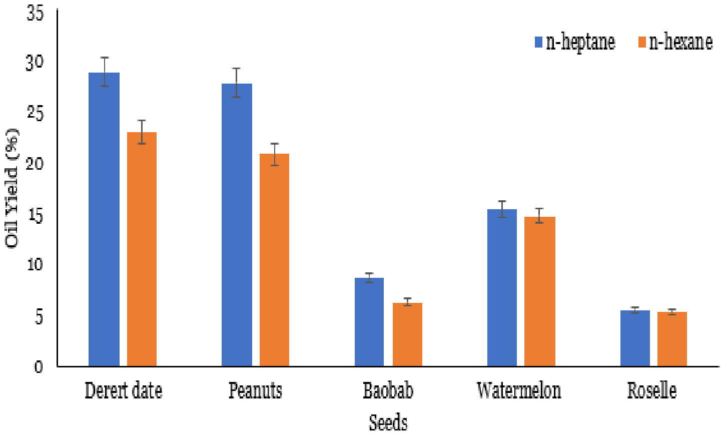


Figure 3: Oil yields from different plant seeds in different solvents (*n*-hexane and *n*-heptane). The error bars represent the standard deviation (S.D.) from replicates of the experiment (n=3).

This study selected edible seeds such as Desert date, Peanut, Watermelon, Baobab and Roselle seeds to evaluate the oil yield. Figure 3 shows that oil desert date seed recorded the highest oil yield, followed by peanut, watermelon, baobab and roselle seeds when using *n*-heptane and *n*-hexane. It also demonstrated that when *n*-heptane is used as the extracting solvent, a higher amount of oil is extracted from the same plant seed than when using *n*-hexane as the extracting solvent. The highest oil yield (29%) was obtained by extracting the oil from *Balanites aegyptiaca* seed using *n*-heptane as the oil-extracting solvent. Therefore, *Balanites aegyptiaca* seed and *n*-heptane were selected for further optimisation.

It was reported by (Alostad *et al.*, 2022) that *n*-heptane could extract a wider mass range of compounds. Heptane was also used to extract oil and bioactive compounds from *Cucumis melo* L. seeds (Mallek-Ayadi *et al.*, 2018). Furthermore, *n*-heptane is less polar and has a higher boiling point than *n*-hexane, making it a better choice for extracting oils that are more soluble in nonpolar solvents. N-heptane is also less volatile and flammable than *n*-hexane, making it a safer choice for some applications. This fact can be significant from an economic perspective in the process sector since it allows for the more effective extraction of fixed oils from plum seeds using nonpolar solvents (*n*-hexane and *n*-heptane) (Savic *et al.*, 2020).

3.2 Effect of mass ratio

The influence of seed-to-solvent mass ratio was investigated by extracting oil from *Balanites aegyptiaca* seed using 20 g, 40 g, 60 g and 80 g of solvent with a constant seed weight of 10 g to obtain seed to solvent mass ratio of 1:2, 1:4, 1:6, and 1:8. Meanwhile, other conditions were maintained constant. The results for the optimisation of seed to solvent mass ratio are shown in Figure 4.

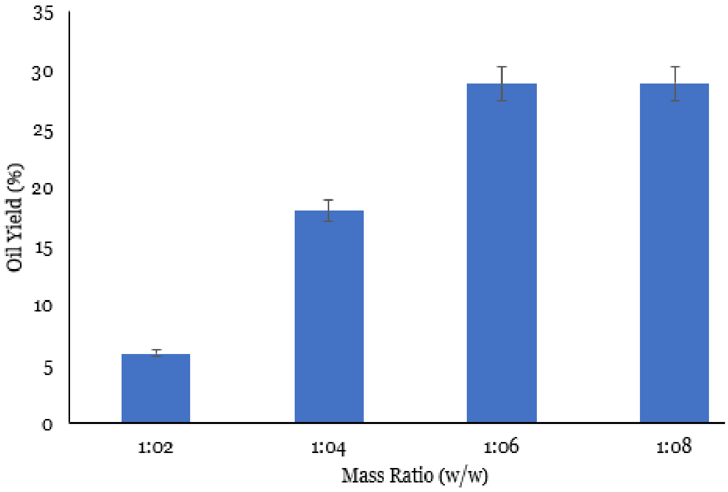


Figure 4: Effect of seed to solvent mass ratio on oil yield at 60 mins extraction time, 35°C extraction temperature, and 75 rpm agitation speed. The error bars represent the standard deviation (S.D.) from replicates of the experiment (n=3).

Figure 4 depicts that oil yield increases with the seed-to-solvent mass ratio until the 1:6 mass ratio. A higher seed-to-solvent mass ratio led to a larger concentration gradient between the solid phase (seed) and liquid phase (solvent), where mass transfer of seed oil from powder to the solvent is favourable (Goula, 2013). Next, by observing Figure 4, beyond the 1:6 mass ratio, no significant improvement in oil yields was observed. This is because the extracting solution, the seed and solvent mixture, has achieved the saturation concentration (Hayyan *et al.*, 2022).

According to the results, a seed-to-solvent mass ratio of 1:6 is sufficient for extracting the maximum oil yield. Since raising the ratio to 1:8 would only result in a minor increase in oil yield, thus, seed to solvent mass ratio of 1:8 is not considered as it would not be economical to use a higher amount of *n*-Heptane for obtaining an insignificant increase in oil yield. Hence, 1:6 (10 g of plant seed to 60 g of solvent) was selected as the optimum seed-to-solvent mass ratio.

3.3 Effect of extraction time

The relationship of extraction time on oil yield was examined by extracting oil from Balanites seed at different extraction times of 20 mins, 40 mins, 60 mins and 80 mins in a sono-reactor with a constant seed-to-solvent mass ratio of 1:6 and extraction temperature of 35°C. With that, the result for optimisation of extraction time is displayed in Figure 5.

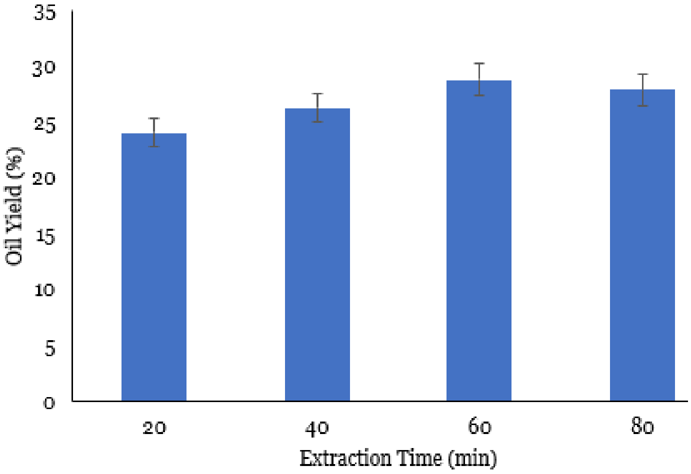


Figure 5: Effect of extraction time on oil yield at 1:6 seed to solvent mass ratio, 35°C extraction temperature and 75 rpm agitation speed. The error bars represent the standard deviation (S.D.) from replicates of the experiment (n=3).

According to Figure 5, it is observed that the yield of oil increases with the extraction time until 60 mins. Increasing the extraction time gives adequate time for the seeds to be exposed to ultrasonic waves. At the same time, the solvent has more time to disrupt and break the cell wall of the seed, allowing a longer penetration time of solvent into the seed to extract the oil content from the seed (Hayyan *et al.*, 2022).

Results show that the maximum extracted oil yield (29%) was achieved at 60 mins. A slight decrease in oil yield after 60 mins was observed. This is likely because a longer extraction time causes oil oxidation and degradation (Torii *et al.*, 2019). In addition, the oil that is dissolved in the solvent tends to reabsorb the seed's granules that have a relatively large surface due to a longer extraction time. Therefore, 60 mins (1 hour) is selected as the optimum time for oil extraction using a sono-reactor as a long time did not result in a significant yield. More prolonged exposure to air can cause the oil to oxidise, leading to the formation of unwanted byproducts and a decrease in oil quality.

Moreover, over-extraction may occur if the extraction process continues too long, removing more than just the desired oil and reducing overall yield (Cai *et al.*, 2021; Lam *et al.*, 2019). Furthermore, some oil components may break down over time, reducing the amount of oil obtained. In addition, longer extraction times may also increase temperature, which can cause thermal degradation of the oil, leading to a reduction in yield (Chemat *et al.*, 2019; Kusuma *et al.*, 2018).

3.4 Thermodynamics of mixing and solubility

Understanding the thermodynamics of mixing and solubility in oil extraction from *Balanites aegyptiaca* seeds is crucial. Solubility, as defined within the framework of Gibb's free energy of mixing, is the ability of solute molecules (in this case, the oils within the seeds) to dissolve and form a homogeneous solution with a solvent, such as *n*-heptane. This solubility is a key determinant of the efficiency of the extraction process.

The composition of *Balanites aegyptiaca* seed oil predominantly includes palmitic acid (16.68%), oleic acid (22.85%), linoleic acid (47.84%), and stearic acid (11.67%) (Murthy *et al.*, 2020). When these oils act as solutes in *n*-heptane, their solubility is governed by the Gibbs free energy of mixing between the solute and solvent. A negative mixing energy signifies that the solute will dissolve and form a homogeneous solution, while a positive mixing energy indicates that the solute will not dissolve in the solvent. As shown in Figure 6, all oil compounds are soluble in *n*-heptane, validating the effectiveness of *n*-heptane in extracting these oils from *Balanites aegyptiaca* seeds.

To further elucidate the interaction between the solute and solvent, we analysed the sigma (σ) profile of each component involved in the mixing process. In COSMO-RS, the σ-profile describes electron density distribution around a solute molecule in a solvent environment, providing insights into the solvation structure and the interaction between the solute and solvent. This information is instrumental in predicting a solution's thermodynamic and transport properties.

The σ-profile is divided into three regions: the polar hydrogen bond donor region when $\sigma < -0.0084$ eA−2, the nonpolar region when σ is between −0.0084 and 0.0084 eA−2, and the polar hydrogen bond acceptor (H.B.A.) region when $\sigma > 0.0084$ eA−2. The presence of peaks in all three regions, as shown in Figure 7(a), suggests that both polar and nonpolar solvents have the potential for extraction. However, nonpolar solvents like *n*-heptane are preferred due to the dominance of peaks in the nonpolar region, indicating higher solubility for these oils.

Figure 7(b) further compares the σ-profiles when the dominant oils in Balanites are combined with the solvents *n*-heptane and *n*-hexane. The larger area under the peak in the nonpolar region for *n*-heptane suggests a higher extraction capacity for the seed oils, aligning with the experimental findings of this study.

The thermodynamics of mixing and solubility play a crucial role in oil extraction from seeds. The solubility of the oil in the solvent determines the efficiency of the extraction process. A study by Mauliza *et al.* (2021) investigated the effect of extraction time on the yield of oil from Amla seeds using hexane as a solvent. They found that the extraction time significantly impacted the oil yield, highlighting the importance of understanding the thermodynamics of the extraction process to the yield.

Similarly, Evangelista *et al.* (2022) evaluated the oil extraction from (*Euphorbia lagascae* Spreng.) by pre-pressing followed by solvent extraction. They assessed the effect of starting seed moisture content and heating temperature on oil extractability and quality. Their findings underscored the importance of understanding the thermodynamics of mixing and solubility in achieving efficient oil extraction and maintaining the quality of the extracted oil. Fetzer *et al.* (2021) reported on extracting oil conditions for maximum yield. A study on the fatty acid profile and anti-Alzheimer's disease activity reported by Wei *et al.* (2022) highlighted the importance of selecting the appropriate extraction method to preserve the beneficial properties of the extracted oil.

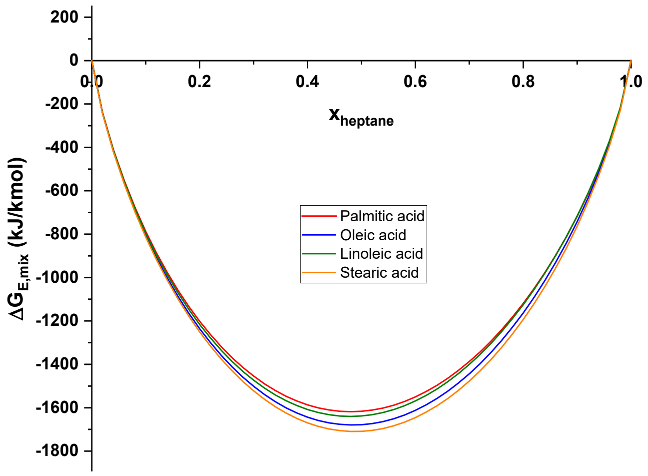
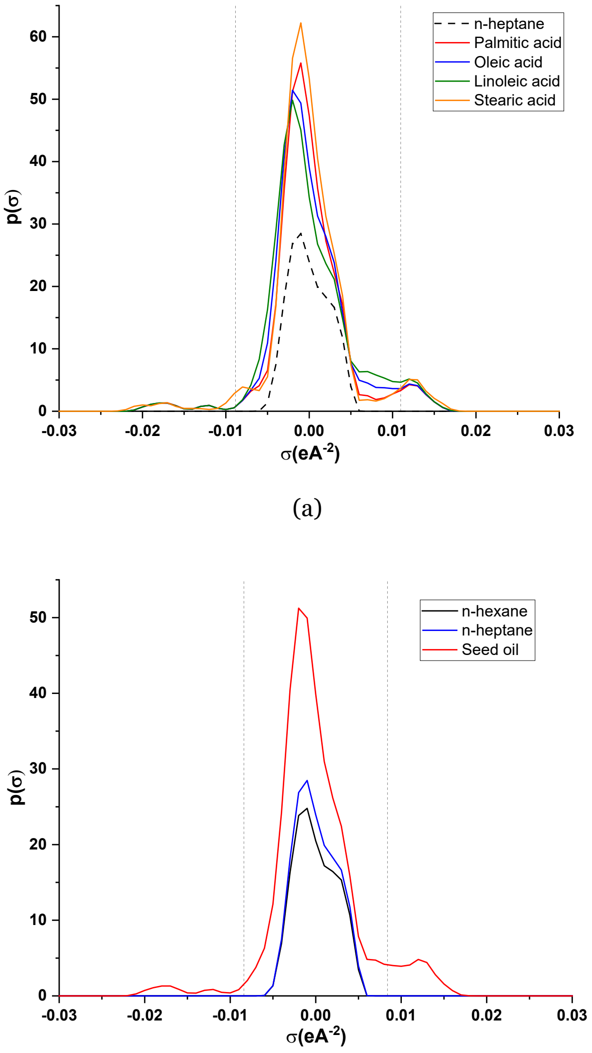


Figure 6: Gibbs free energy of mixing between oil species in *Balanites aegyptiaca* and *n*-heptane.



(a)

(b)

Figure 7: *σ*-profiles of *n*-hexane and/or *n*-heptane in comparison with the (a) individual oil species, and (b) combined oil species.

In conclusion, the thermodynamics of mixing and solubility play a significant role in the extraction process. The choice of solvent, its interaction with the solute, and the solubility of the oils in the solvent all contribute to the efficiency of oil extraction. This study's findings underscore the importance of these factors in optimising the extraction process for *Balanites aegyptiaca* seeds.

4. Conclusion

The study established that *n*-heptane is a more effective solvent for oil extraction from seeds, particularly from desert date (*Balanites aegyptiaca* L.) seeds, yielding up to 29% oil. The optimal extraction conditions were identified as a seed-to-solvent mass ratio of 1:6 and an extraction time of 1 hour. The superior performance of *n*-heptane is attributed to its higher nonpolarity, enhancing its oil solubility. Further research could explore using other nonpolar solvents to verify if they can yield even better results than *n*-heptane. Studies could also be conducted on different types of seeds to broaden the understanding of oil extraction processes. Investigating the environmental impact and economic viability of using *n*-heptane as a solvent on a large scale would be beneficial. Future research could also focus on refining the extraction process to further increase the yield, such as by optimising temperature conditions or exploring enzyme-assisted extraction methods.

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