Sustainable
Food Technology

Accepted Manuscript

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Sustainability Spotlight Statement

View Article Online
DOI: 10.1039/D6FB00012F

This work provides a sustainable food processing by integrating systematic process optimization with solvent recovery and reuse for the extraction of omega-3-rich flaxseed oil. An optimized Soxhlet extraction protocol is developed that reduces the extraction time while incorporating a solvent recovery and solvent reuse strategy. By enabling the reuse of hexane for up to ten cycles without compromising oil yield or fatty acid quality, this protocol drastically reduces solvent consumption and waste. This work aligns with UN Sustainable Development Goal 12 (Responsible Consumption and Production) by making food processing more resource-efficient and secondarily supports SDG 3 (Good Health and Well-being) and SDG 9 (Industry, Innovation and Infrastructure).

DOI: 10.1039/D6FB00012F

Standardization of Extraction Process Parameters and Solvent Reuse for Sustainable Extraction of Omega-3-Rich Flaxseed Oil

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Abstract

Growth emphasizing on health and wellness (in terms of immunity) has highlighted the importance of dietary bioactives like omega-3 fatty acids. Flaxseed is a rich source of omega-3 fatty acids, particularly alpha-linolenic acid (ALA). This work focused on optimization of Soxhlet extraction parameters to maximize oil yield and evaluation of solvent recovery and reusability across multiple extraction cycles. Soxhlet extraction parameters, including solvent type (hexane, ethanol, ethyl acetate), extraction time (8, 12, 16 h), and solid-liquid ratio (1:2.5 to 1:15) were systematically investigated. Hexane proved most effective among the solvents tested, yielding 40.48 ± 2.25% oil at an extraction time of 8 h and a solid-liquid ratio 1:10, followed by ethyl acetate and ethanol. Notably, a yield comparable to that obtained at 16 h with 1:2.5 ratio (43.84 ± 1.51%) was achieved in 8 h by optimizing the solid-liquid ratio to 1:10. Hexane was recovered with an average recovery of ~72% and was reused successfully for up to 10 cycles without significant loss in oil yield or quality. Gas chromatography (GC-FID) confirmed consistent fatty acid composition across all extraction cycles. FT-IR analysis showed no significant changes in functional groups, with only minor variations in peak intensities at later cycles and no new peaks detected. Consistent physicochemical properties, including refractive index, acid value, and free fatty acid content, further confirmed the oil stability. The optimized process provides a sustainable and efficient extraction protocol for omega-3-rich flax oil extraction aligned with industrial cost-efficiency and green chemistry principles.

Keywords: Flax oil, Soxhlet extraction, Free fatty acids, Solvent recycling, Process optimization.

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1. Introduction

Health is the most important element of one's life. The imbalance in today's work and personal life is a growing concern. COVID-19 has also demonstrated the need for and importance of consumption of immunity-boosting foods and supplements such as Omega-3 fatty acids. The health benefits–attributed to the consumption of omega-3 fatty acids include reduced hyperlipidemia[1], a decrease in colon tumor[2] and mammary cancer[34], prevention of cardiovascular disease, hypertension, diabetes, cancer, arthritis, osteoporosis, autoimmune and neurological disorders.[5] Omega-3 fatty acids hold a significant role in anti–ageing[6], improves learning, memory ability, cognitive well-being, and blood flow in the brain[7], helps in proper fetal development, including neuronal, retinal, and immune function and weight management.[8] Therefore, Omega-3 fatty acids such as alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) should be an integral component of the ideal human diet, and adequate amounts should be consumed to meet daily requirements. This is also critical because omega-3 fatty acids cannot be synthesized by the human body.[9] The omega-3 fatty acids can be obtained from both plant (e.g., flaxseed, hemp and peanut) and animal (e.g., fish) sources.[10] Among plant-based sources, flaxseed is exceptionally rich in ALA and has gained attention as a sustainable, vegan alternative to fish oil.

In this work, flaxseed was selected as a source of omega-3 fatty acids due to its high content of ALA (50-60% of its oil fraction) which aids in better nutrition and health.[11] Flaxseeds (*Linum usitatissimum* L.), also called as linseed, belongs to genus *Linum* and the family Linaceae~~)~~. Flax oil, rich in Omega-3 fatty acids, can be obtained from flaxseed by employing conventional techniques such as mechanical screw presses (oil expellers), Soxhlet extraction and organic solvent extraction.[13] However, these methods suffer from bottlenecks, such as long extraction time, low selectivity, and degradation of bioactive compounds.[14] Some of these drawbacks may be overcome by the advanced methods like subcritical fluid extraction[14], pressurized liquid extraction[15] and ultrasound–assisted extraction.[16] Important advantages associated with pressurized liquid extraction are high degree of penetration into the matrix pores, high mass transfer and increased extraction efficiency.[15] The subcritical fluid extraction resulted in shorter extraction time and good selectivity.[14] Ultrasound-assisted extraction enhanced the extraction efficiency through acoustic cavitation by disrupting the cell structure.[17,18] The flax oil extracted using supercritical fluid extraction contained higher PUFAs and phenolics than that obtained from conventional solvent extraction (SE).[11]

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Despite these advancements in various extraction methods, Soxhlet extraction is considered to be the standard test for maximum oil recovery and is commonly adopted by researchers and industries for quick analysis. Also, this method serves as a benchmark for comparing different extraction technologies, while other methods are often employed, focusing on the improvement in oil yield or quality. Therefore, the main aim of our work was to select a convenient, reproducible method (i.e. Soxhlet extraction) for standardization of two key extraction process parameters, such as extraction time and the solid-liquid ratio. Increased extraction time generally results in an increase in oil yield. However, prolonged extraction would lead to an increase in production costs and may degrade the heat-sensitive components.[19] The solid-liquid ratio is yet another parameter of profound importance. A higher solid-liquid ratio increases oil yield due to increased contact area between matrix and solvent, facilitating the leaching of bound oil from the material.[20,21] However, beyond an optimum solid-liquid ratio, the oil yield does not increases further due to reduced concentration gradients because the mass transfer driving force depends on the concentration gradient between the solvent and the matrix. Hence, our work focused on Soxhlet extraction for flax oil aiming to determine the maximum achievable oil yield under optimized conditions, thereby providing a reliable benchmark for comparing process intensified and sustainable green extraction methods. Next critical parameter in Soxhlet extraction is the selection of an appropriate solvent.

The polarity of solvents plays a vital role in oil extraction. Based on the polarities, solvents are broadly categorized as polar (methanol, n-butanol, ethanol, acetone, and isopropanol) and nonpolar(hexane). Hexane was reported as the best solvent by Ekka and Owary (2023) due to the high oil yield (45% w/w) of flaxseed oil employing Soxhlet extraction technique.[22] Ultra-assisted extraction using solvents such as hexane, dichloromethane, acetone, ethanol, methanol and petroleum ether has been employed to obtain flax oil with increased oil recovery and high α-Linolenic acid (ω-3).[23] In another study by Piva G.S. *et. al* (2018) hexane gave the highest yield (36.12%) for Soxhlet extraction when compared to subcritical propane and pressurized ethanol.[13] Hexane is one of the most commonly used organic solvent (exhibits the ability to extract more nonpolar solutes) for oil extraction due to its high efficiency in oil recovery, low cost, recyclability, and low boiling point (~ 68-69 °C) [24,25]. Another solvent, ethyl acetate which is moderately polar has been proposed as a substitute to the conventional hexane because it is cost-effective (33% cheaper when compared to *n*-hexane), less flammable and less hazardous[26]. Ethanol, owing to its high polarity, has the capability to extract more polar compounds such as polyphenols, pigments and soluble sugars.[27] Based on the literature studies,

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hexane, ethyl acetate and ethanol were selected considering the variations in the polarity profile to systematically evaluate their extraction performance. However, despite widespread use of these solvents in extraction process, no previous study has systematically targeted towards standardizing the extraction process parameters (solid-liquid ratio and time) in Soxhlet extraction for maximum oil yields. Therefore, the present work aims to focus on standardization of extraction process parameters and screening the most suitable solvent for extraction of flax oil.

Although organic solvents namely hexane, ethanol, and ethyl acetate are widely used for extraction of oil, their disposal causes serious environmental threat, resulting in pollution and contamination. Moreover, these solvents presents significant health hazards including haematological effect, skin, and eye irritation, liver damage etc..[28] Solvent recovery, reusability and recyclability can prevent immediate disposal, aiding in the reuse of solvents promoting cost-effectiveness and sustainability. To the best of the authors' knowledge, there is scarce information on the reusability and recyclability of solvents for flaxseed oil extraction. Information on the number of extractions cycles a solvent can be reused will help researchers and industrialists to effectively utilize solvent to its full potential. Characterizing the recovered solvent can provide information on the degradative changes. In this work, we assessed the environmental impact and economic feasibility by analyzing solvent quality of recovered solvent and oil collected using the Fourier Transform-Infrared (FT-IR) Spectroscopy and Gas chromatography–flame ionization detector (GC-FID). We estimated the solvent recovery (%) and studied the reusability aspects of solvents recovered over ten successive extraction cycles. Thus, the recovery, recycling and reusability of solvents was explored for the extraction of flax oil in the context of economically viable and sustainable process development. Hence, to provide a comprehensive framework for developing a more sustainable and economically viable flaxseed oil extraction process, this work focusses on an integrated approach by (a) screening of different solvents and standardizing extraction process parameters for flax oil, (b) solvent degradation studies to evaluate its reusability over multiple extraction cycles, and (c) assessing the quality of the extracted oil.

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2. Materials & Methods

2.1 Materials

Flaxseeds were procured from Neuherbs Superfoods Company through Amazon (as per suppliers' declaration: Total fat - 35.67g/100g, Saturated fatty acids – 2.96g/100g, Polyunsaturated fatty acids – 16.14g/100g, Monounsaturated fatty acids – 5.11g/100g). Defatting paper and cellulose thimble employed for Soxhlet extraction were also purchased from Amazon.

Chemicals

The extraction solvents, i.e., hexane (AR grade, purity:99%, CAS: 110-54-3) and ethyl acetate (AR grade, purity:99.5%, CAS: 141-78-6) were purchased from Sisco Research Laboratory (SRL, Mumbai, India). Ethanol (AR grade, purity:99.9%, CAS: 64-17-5) was purchased from MSB Chemical Ltd, (Mumbai, India). The chemicals used for the analysis include potassium hydroxide (KOH, CAS: 1310-58-3), hydrochloric acid (HCl, CAS:7647-01-0) and phenolphthalein solution were purchased from Sisco Research Laboratory (SRL, Mumbai, India), HiMedia Laboratory Pvt Ltd (India) and Qualigens Fine Chemicals (India), respectively. The chemicals used for GC-FID analysis including sodium hydroxide (NaOH, CAS: 1310-73-2), sulfuric acid (H_2SO_4, CAS: 7664-93-9), n-heptane (CAS: 142-82-5), and sodium chloride (NaCl, CAS: 7647-14-5) were also purchased from Sisco Research Laboratory (SRL, Mumbai, India). Methanol (CAS: 67-56-1) employed for fatty acid preparation was purchased from Merck (Mumbai, India). The Supelco 37 component standard mix (Fatty Acid Methyl Ester (FAME), CRM47885) was purchased from Sigma Aldrich (Darmstadt, Germany).

Pre-treatment of Flaxseeds

Flaxseeds were ground in a Philips grinder (750 W) for 3 to 5 mins to disrupt the seed structure and reduce the particle size for the ease of oil extraction. The ground seed material was sieved through a 500 µm mesh to obtain a uniform particle size for all extraction experiments. The sieved material was wrapped in a defatting paper and placed in a cellulose thimble inside the extraction chamber of Soxhlet extraction apparatus.

2.2 Methods

2.2.1 Soxhlet Extraction

Ground flaxseeds (50 g) were wrapped in defatting paper and placed inside a cellulose thimble in the extraction chamber for Soxhlet apparatus. The solvent (Hexane, ethanol and ethyl

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DOI: 10.1039/D6FB00012F

acetate) at 1:2.5 solid-liquid ratio was employed for the extraction of flax oil from flaxseed for 8, 12 and 16 h (**Figure 1)**. The temperature of the heating mantle (LabQuest borosil PRM500, India) was set to 68.7, 78.37 and 77.1°C according to the boiling points of hexane, ethanol and ethyl acetate respectively. After extraction, the oil-solvent mixture was subjected to a rotary evaporator (BUCHI Rotavapor R-300, BÜCHI Labortechnik AG, Flawil, Switzerland) under reduced pressure (hexane:335 mbar, ethanol:175 mbar and ethyl acteate:240 mbar) at 40°C for 90 min to obtain the oil. The oil yield, and extraction efficiency were calculated using **Equation 1** and **Equation 2,** respectively. All the extractions were performed in triplicate to ensure the reproducibility and average values are reported with standard deviations.

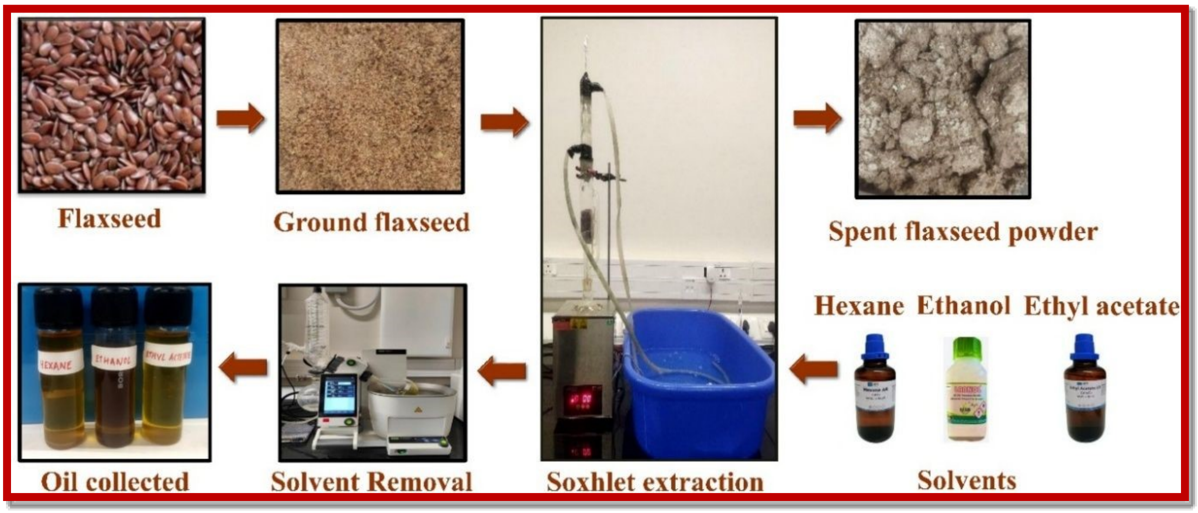

Figure 1: Process protocol followed for flax oil extraction using different solvents

2.2.2 Design of experiments

The experimental plan was designed to optimize the extraction process parameters i.e., extraction time and solid-liquid ratio to maximize the flax oil yield. Initially, Soxhlet extraction of flax oil was conducted with a fixed solid-liquid ratio of 1:2.5 (w/v) using hexane, ethanol, and ethyl acetate as solvents for 8, 12 and 16 h. The solvent that gave highest oil yield (i.e. hexane) at 8 h extraction time was selected for subsequent optimization of solid-liquid ratio. Using a modified extraction protocol from Ishag *et al.,* (2019), solid-liquid ratio was optimized for 1:2.5, 1:5, 1:10 and 1:15..[29] The optimized extraction process parameters (hexane solvent and 8 h extraction time) were used for the solvent reusability studies. The solvent reusability studies aimed at evaluating the purity of solvents recovered and oil extracted at the end of each extraction cycle. These studies were performed for 10 extraction cycles. For each cycle, oil yield and solvent recovery were determined.

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Initially, 10 g of ground flaxseeds and 100 ml of hexane (1:10 of solid-liquid ratio) were used for Soxhlet extraction for 8 h. At the end of the extraction, the volume of solvent was recovered, and oil was collected. Approximately, 3 ml of solvent and oil was retained for further analysis. The volume of the remaining recovered solvent was replenished to 100 ml with fresh solvent (hexane) and used for the next extraction cycle. This procedure is repeated for 10 extraction cycles. Oil yield and solvent recovery (%) were recorded for each cycle. The solvent recovered at the end of each extraction cycle was analyzed to assess their quality in terms of purity, functional groups, and refractive index. The extracted oils were subsequently used for further analysis such as fatty acid composition (GC-FID), functional groups (FT-IR), refractive index, acid value and free fatty acid (FFA) content.

2.2.3 Oil yield (%) and Extraction efficiency (%)

The oil yield (%) of flax oil extracted from flaxseed was calculated using **Equation 1 as:**

$$Yield\ (\%) \frac{\text{Weight of extracted oil } (We)\ in\ g}{\text{weight of initial sample taken for extraction } (Wt)\ in\ g} \times 100 \qquad \textbf{(1)}$$

Extraction efficiency of flax oil extracted with different solvents was calculated using **Equation 2** *as:*

$$Extraction\ Efficiency\ (\%) = \frac{Weight\ of\ the\ extracted\ oil\ (We) in\ g}{Total\ oil\ content\ extracted\ via\ Soxhlet\ using\ hexane (WT)\ in\ g} \times 100 \qquad \textbf{(2)}$$

2.3 Physicochemical properties of oil

2.3.1 Density

Density of the flax oils was estimated using **Equation 3 as:**

$$Density = \frac{\text{Mass of the oil sample (M) g}}{\text{Volume of the oil (V)} in\ ml} \qquad \textbf{(3)}$$

2.3.2 Viscosity

The viscosity of flax oil was measured using a Brookfield rheometer with spindle RCT-50-1 over a shear rate range of 0.06 to 7800 s^{-1} at room temperature (24°C).

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2.3.3 Refractive index

Refractive index of the flax oil and the recovered solvents were determined by using the refractometer (ATAGO, Japan) at room temperature (24 °C). Briefly, the oil or the solvent sample was placed onto the sampling port carefully ensuring that no air bubbles was introduced and the reading was recorded.

2.3.4 Acid value and free fatty acid (FFA) content

Briefly, 1 gm of oil was mixed with 5 ml of neutralized ethanol. Then, 2-3 drops of phenolphthalein (1%) indicator was added to the mixture and the mixture was titrated against 0.1 N KOH solution until the appearance of the first persistent pink color. The acid value was calculated using **Equation 4**. The free fatty acid (FFA) of the oil extracted was determined using the same method as suggested above for acid value and was calculated using **Equation 5.**[30]

$$Acid\ value = \frac{\text{Normality of KOH (N)} \times 56.1 \times \text{Volume of KOH (V)}}{\text{Wt. of Sample in gm (W)}} \quad \textbf{(4)}$$

Where, 56.1 is the molecular weight of KOH.

$$FFA\ \%\ (as\ oleic\ acid) = \frac{\text{Normality of KOH (N) x 28.2 x Volume of KOH(V)}}{\text{Wt. of Sample in gm (W)}} \quad \textbf{(5)}$$

Where, 28.2 is the equivalent factor for oleic acid.

2.3.5 Saponification value

The saponification value was determined using the protocol suggested by Gugale and Mane (2024).[30] Oil sample (2-3 g) was mixed with 25 ml of 0.5 M KOH . The contents were heated under a reflux condenser for 30 - 40 min to ensure that the sample was fully dissolved. Once the sample was cooled, phenolphthalein was added. It was then titrated with 0.5 M of HCl until the disappearance of pink colour, noted as the endpoint. Titrate value was noted for blank with the same time conditions and was calculated using **Equation 6 as:**

$$Saponification\ value = \frac{\text{N} \times 56.1 \times \text{(B−S)}}{\text{W}} \quad \textbf{(6)}$$

Where, B is the volume of HCl for blank sample (ml); S is the volume of HCl for oil sample (ml); N is the normality of HCl; W is the weight of oil sample (g).

View Article Online
DOI: 10.1039/D6FB00012F

2.4 Fatty acid composition by GC-FID

Sample preparation for fatty acid methyl esters (FAME)

Esterification of oil samples to obtain fatty acid methyl esters (FAME) is a crucial step in sample preparation for fatty acid composition using GC-FID. Approximately, 70 to 80 mg of oil sample was mixed with 3 ml of 0.5M NaOH prepared in methanol. The mixture was heated at 90°C for 5 min in a water bath (Stuart SWB Series Biotech, Staffordshire, UK). Subsequently, 4 mL of 2.5 M H_2SO_4 in methanol (prepared to 100 mL) was added, and the mixture was heated at 90 °C for additional 40 min. After cooling, 2 ml of n-heptane and 5 ml of saturated NaCl were added to the mixture followed by centrifugation at 3000 rpm for 5 min. The supernatant containing FAME was collected and used for Gas chromatography (GC) analysis for the fatty acid profile.[31]

Qualitative and quantitative analysis of essential fatty acids

Further, prepared FAME samples were analyzed using gas chromatography-flame ionization detector (GC-FID) (Agilent 8890 GC System, USA) with a polar column (VF-WAXms Column 30m×0.25mm×0.5 µm). The oven temperature was programmed from 100°C to 250°C at 20 °C/min ramp with 2 min hold at final temperature. The injector and detector temperature were 250 °C, and 280 °C respectively with a 5:1 split ratio. The flow rates of air and hydrogen were kept at 400 ml/min and 30 ml/min, respectively. FAME extracts were injected into the GC-FID, and chromatograms were compared with those obtained for a certified FAME standard. The retention times and the % peak area were recorded.

2.5 Fourier-Transform Infrared Spectroscopy Analysis (FT-IR)

FT-IR analysis was carried out using a FT-IR spectrometer (PerkinElmer, Shelton, CT, USA) to identify the functional groups present in the flax oil. Oil samples were placed on the cell plate, and the spectra were collected over the range 500-4000 cm^{-1} with 36 scans at room temperature.[32]

2.6 Statistical analysis

The statistical analysis was performed using OriginPro 2021 software (OriginLab Corporation, USA). One way analysis of variance (ANOVA) was used to evaluate the effects of process parameters, followed by the Tukey's honest significant difference (HSD) test to assess differences among parameters. A p-value < 0.05 was considered statistically significant. All experiments were performed in triplicates ($n = 3$), and data are reported as mean ± standard deviation.

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3. Results and Discussion

3.1 Effect of solvent and extraction time on flax oil yield

Initially, the extraction of oil from flaxseeds was carried out in Soxhlet by employing hexane, ethanol and ethyl acetate to select best solvent based on the oil yield (%). The extracted flax oil exhibited different colour variations based on the solvent used as with hexane light yellow, ethanol gave greenish yellow and ethyl acetate resulted golden yellow colour, as shown in **Figure 2**. Flax oil with hexane exhibits a light-yellow colour due to its ability to extract more nonpolar solutes. [24, 25] Flaxseed oil extracted using ethyl acetate had golden yellow colour due to its semi–polar nature.[33, 34, 26] While, flaxseed oil extracted using ethanol had a greenish yellow colour, due to its capability to co-extract more polar compounds such as polyphenols, pigments and soluble sugars due to its high polarity.[27]

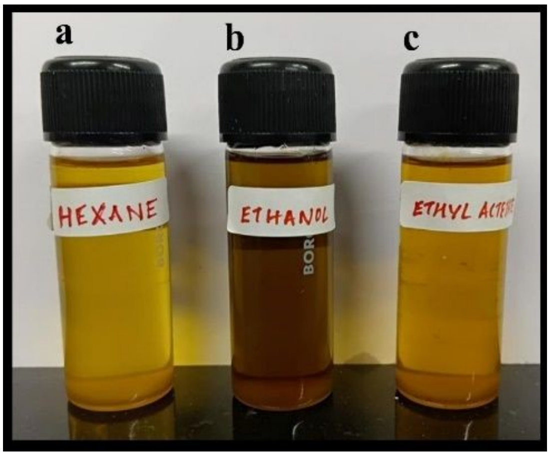

Figure 2. Flax oils extracted by Soxhlet extraction using (a) hexane, (b) ethanol, (c) ethyl acetate

To standardize the extraction time, flaxseed oil was extracted using a Soxhlet apparatus for 8, 12, and 16 h at a fixed solid-liquid ratio of 1:2.5 (w/v) with three solvents. As shown in **Figure 3a**, among the three solvents, hexane resulted in the highest oil yield compared to ethyl acetate and ethanol at 8, 12 and 16 h extraction time. For hexane, the oil yield was increased (39.31 ± 0.91% to 43.44 ± 1.51%) with an increase in extraction time from 8 to 16 h. An increase in oil yield from 39.31 ± 0.91% to 40.25 ± 1.21% was observed when the extraction time increased from 8 to 12 h, with the highest yield (43.44 ± 1.51%) observed at 16 h (**Figure 3a**). This increasing trend is consistent with previous reports by Ghoshal *et al.*(2022) where they reported toil yields of 32.15 ± 0.52%, 37.11 ± 0.58%, and 42.56 ± 0.59% at 6, 8, and 12 h, respectively.[35] This increase in oil yields is attributed to the increased extraction time, which enhances diffusion of lipids and mass transfer from the seed matrix into the extraction solvent.[26]

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The oil yield obtained in this work at 8 h is comparable to the yield of 35.62 ± 1.04% reported for an 8 h Soxhlet extraction using hexane.[26] In comparison, previous studies reported slightly lower oil yield (37.11 ± 0.58%) for Soxhlet extraction using hexane at a 1:10 solid-liquid ratio.[35] A similar oil yield of 36.1% was reported in Soxhlet extraction for 14 h extraction time; however, the solid-liquid ratio was not mentioned.[13] A slightly higher oil yield (42.4 %) was reported for Soxhlet; however, the extraction time was not mentioned.[36] These discrepancies in oil yield are likely attributable to factors such as flaxseed species and variety, extraction process parameters, extraction methods employed and the inherent efficiency and selectivity of the solvents employed.[37] Other studies have reported significantly lower oil yields with different extraction techniques, such as ultrasound-assisted extraction(21.95%)[29], with hull extraction (19.3%)[38] and for accelerated solvent extraction using hexane and ethyl acetate 11.10 ± 1.55% and 19.19 ± 1.29% respectively[26], indicating the effectiveness of Soxhlet extraction in achieving higher oil recovery.

Statistical analysis using ANOVA showed significant differences in oil yields across extraction times (8, 12, and 16 h) for hexane and ethyl acetate ($p < 0.05$). In contrast ethanol exhibited highly significant differences extraction time (8-16 h) (**Table S1, Table S2**). Although a higher yield was obtained at 16 hours (**Figure 3**), prolonged extraction increases energy consumption. Therefore, considering the balance between efficiency, energy consumption, and economic feasibility for scale-up, an extraction time of 8 h was selected for further experiments. A statistical analysis was carried out with a two-way ANOVA to understand the interaction between extraction time and solvent type on the oil yield. The results showed that both extraction time and solvent type had significant effects ($p < 0.001$), with a significant interaction between the two factors ($p < 0.001$), indicating that the effect of extraction time on oil yield depended on the solvent used. Due to the significant interaction effect, one-way ANOVA followed by Tukey's HSD test was performed separately for each solvent to evaluate differences across extraction times. The one-way ANOVA analysis revealed significant differences in oil yield across extraction times (8, 12, and 16 h) for hexane and ethyl acetate ($p < 0.05$), whereas ethanol exhibited highly significant differences ($p < 0.001$) (**Figure 3a**).

3.2 Effect of solid-liquid ratio on oil yield

To further optimize the extraction process, Soxhlet extractions were carried out for 8 h at different solid-liquid ratios (1:2.5,1:5, 1:10, and 1:15) using hexane, ethanol and ethyl acetate. As shown in **Figure 3b,** increasing the solvent volume resulted in higher oil yields for all solvents. At the 1:15 ratio, a higher oil yield obtained for hexane (44.87 ± 1.69%), ethanol

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(41.36 ± 1.76%) and ethyl acetate (43.27 ± 1.20%). Two-way ANOVA revealed that both solid-liquid ratio and solvent type significantly affected oil yield ($p < 0.001$). However, no significant interaction between the two factors was observed ($p > 0.05$), indicating that the effect of solid-liquid ratio on oil yield was consistent across all solvents. One-way ANOVA followed by Tukey's HSD test was performed to compare oil yields for different ratios within each solvent. The results showed that oil yield increased significantly with increasing solid-liquid ratio for all solvents. However, statistical analysis using one-way ANOVA revealed non-significant differences ($p > 0.05$) in oil yields between solid-liquid ratios of 1:10 and 1:15 **(Table S3, Table S4).**

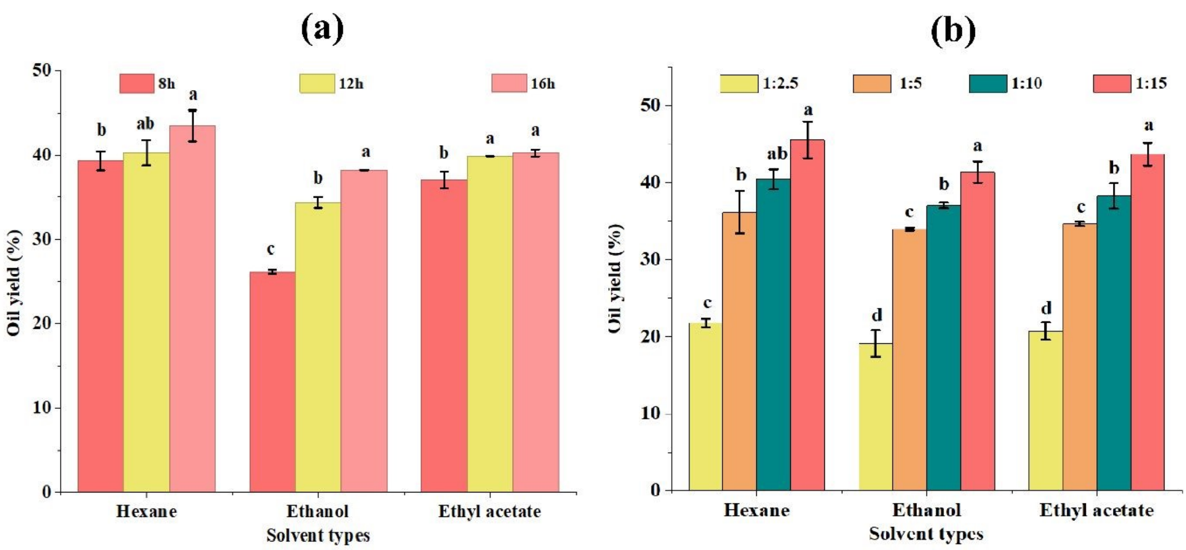

Figure 3. Effect of **(a)** extraction time and **(b)** solid-liquid ratio on oil yields using different solvents. Values are presented as mean ± standard deviation (n = 3). Statistical significance was performed using one-way ANOVA followed by Tukey's post-hoc test. Different letters indicate statistically significant differences ($p < 0.05$) based on Tukey's HSD test. Statistical comparisons were performed within each solvent.

From a sustainability and economic (cost-benefit analysis) perspective, a 1:15 is less favourable choice due to due to higher solvent consumption, increased energy demand for solvent recovery. Therefore, a solid-liquid ratio of 1:10 was selected as the optimal condition, balancing extraction efficiency with economic and environmental considerations. The oil yields obtained in this work compare favourably with those reported in the literature. The oil yields of 35.62 ± 1.04% and 37.11 ± 0.58% reported by Lohani *et al.*(2015) and Ghoshal *et al.* (2022) for flax oil extracted by Soxhlet extraction for 8 h with solid: liquid ratio of 1:15 and

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1:10, respectively.[26,35] A significantly lower oil yield (14.53%) was reported for flax oil extracted using magnetic stirring for 8 h followed by rotary evaporation.[39]

The oil yield obtained in this work with a solid-liquid ratio of 1:10 and 8 h extraction time (40.48 ± 2.25%) was comparable to the oil yield achieved with 1:2.5 solid-liquid ratio and prolonged extraction time of 16 h (43.44 ± 1.51%). This highlights the trade-off between extraction time and solvent volume (i.e. solid-liquid ratio). Given that 16 h extraction doubles the processing time and energy consumption, the 8 h extraction at 1:10 is more efficient. Similar findings highlighting 1:10 and 8 h as optimal conditions for ultrasound-assisted extraction of flax oil, achieving 40.48 ± 2.25% oil yield were reported by Gutte *et al.*(2015).[39] In contrast, Abdi *et al.*(2020) reported an oil yield of 21.95% for Soxhlet extraction (8 h) using hexane at a solid-liquid ratio 1:2.5.[37] Subcritical n-butane extraction has been reported to have a higher yield (28.75%) compared to n-hexane Soxhlet extraction (27.53% at 1:5 for 6 h), and cold-press extraction (19.56%).[19] These oil yields reported are lower than the optimized Soxhlet results of this work.

3.3 Extraction efficiency (%)

At 8 h of extraction time, extraction efficiency increased with increasing solid-liquid ratios for all three solvents (hexane, ethanol and ethyl acetate). Hexane showed the highest extraction efficiency, with values of 75.42 ± 1.27%, 81.64 ± 2.56%, 8%, and 99.47 ± 0.74% at solid-liquid ratios of 1:2.5, 1:5, 1:10, and 1:15, respectively. This superior performance is attributed to its non-polar nature, which enhances solubility and oil recovery. This was followed by ethyl acetate with extraction efficiencies of 66.87 ± 1.46 ,76.2 ± 0.51, 86.80 ± 3.84 and 96.93 ± 2.4% at solid-liquid ratios of 1:2.5, 1:5, 1:10 and 1:15, respectively. Ethanol exhibited comparatively lower extraction efficiency of 60.96 ± 0.74, 74.94 ± 2.2, 81.84 ± 3.86 and 91.95 ± 5.92% at the same solid-liquid ratios. Based on these results, hexane was identified as the most effective solvent, achieving the highest extraction efficiency (99.47 ± 0.74%) at a solid-liquid ratio of 1:15, followed by ethyl acetate and ethanol. Although, the solid-liquid ratio of 1:15 resulted in higher extraction efficiencies for all three solvents increasing the solid-liquid ratio enhanced extraction efficiency for all solvents, likely due to increased solvent availability, improved mass transfer, and a shift in the dissolution equilibrium toward higher oil recovery. Similar extraction efficiency of hexane (97.37% in the third cycle) was reported by Thawornprasert and Somnuk (2024). On repeated cycles of extraction, extraction efficiency further decreased to 84.21% (seventh cycle), limiting its usage till 6th extraction cycle. [40]

DOI: 10.1039/D6FB00012F

Statistical analysis using two-way ANOVA showed that both solvent type and solid-liquid ratio significantly affected the extraction efficiency ($p < 0.001$). However, no significant interaction between the two factors was observed ($p = 0.1819$), indicating that the effect of the solid-liquid ratio on extraction efficiency is independent of the solvent type. One-way ANOVA showed significant statistical differences in extraction efficiency across solid-liquid ratios for all solvents ($p < 0.001$) **(Table S5, Table S6)**. Tukey's HSD test showed that, extraction efficiency increased across all solid-liquid ratios. Based on these analyses, although a 1:15 solid-liquid ratio showed the highest extraction efficiency (99.47 ± 0.74%), considering a practical compromise between extraction efficiency and solvent consumption, a solid-liquid ratio of 1:10 with an extraction time of 8 h was selected for subsequent solvent reusability studies.

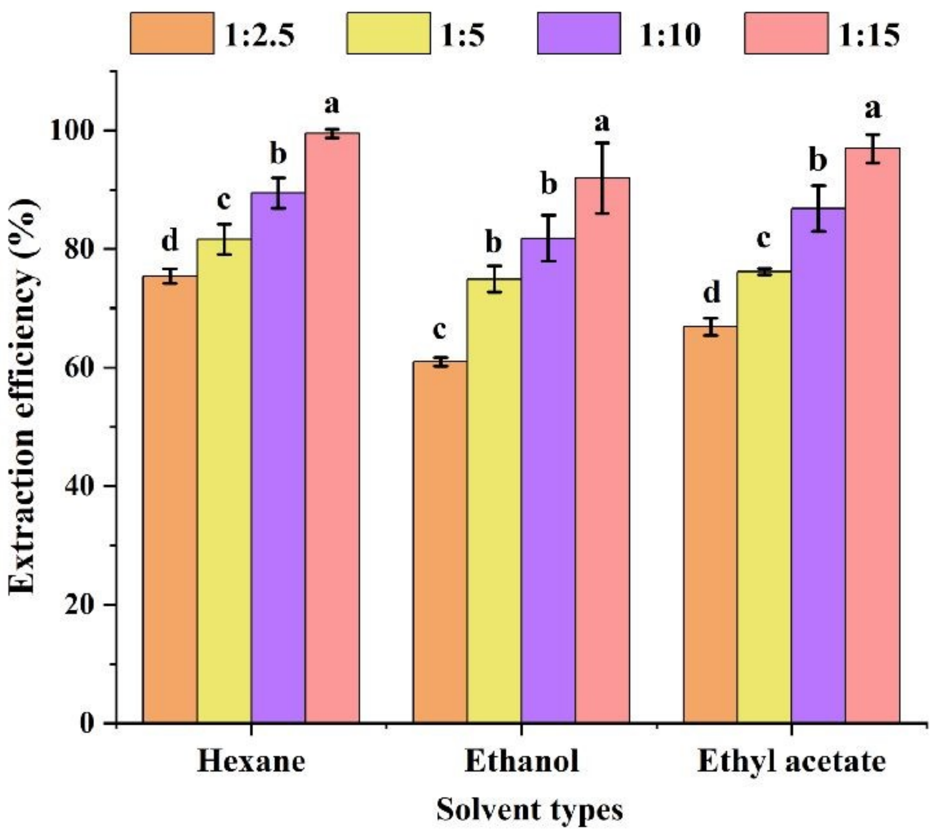

Figure 4: Effect of the solid-liquid ratio on extraction efficiency using different solvents. Values are expressed as mean ± standard deviation ($n = 3$). Different letters indicate statistically significant differences ($p < 0.05$) based on Tukey's HSD test. Statistical comparisons were performed within each solvent.

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3.4 Physicochemical properties of flax oil

The flaxseed oil obtained employing Soxhlet extraction under optimized conditions (Solvent: hexane, time: 8 h, solid-liquid ratio: 1:10) was analyzed for various physicochemical properties **(Table 1)**.

3.4.1 Density

The extracted flax oil had a density value of 0.83 ± 0.05 g/cm^3. This value aligns with the range of 0.83-0.91g/cm^3 and 0.92 g/cm^3 reported by Lohani *et al.,*(2015) and Ishag *et al.*(2019) respectively for Soxhlet-extracted flax oil.[26, 29]

3.4.2 Viscosity

The measured viscosity of the flax oil was 52.44 ± 0.02 mPa.s. This is consistent with the values reported in literature 53 ± 0.01 mPa.s by Calligaris *et al.*(2013) and approximately 54.9 ± 3.5 mPa.s by Ishag *et al.*(2019).[41, 29]

3.4.3 Saponification value (SV)

The saponification value (SV) or saponification number is used to evaluate the average molecular weight of all fatty acids in an oil sample.[42] It is defined as the amount of potassium hydroxide (KOH), usually expressed in milligrams, required to completely saponify 1.0 g of oil.[43] It can be inferred that the larger the saponification value, the smaller the molecular weight of the fatty acids. In this study, the SV for the hexane-extracted flax oil was 204.86 ± 12.68 mg KOH/g oil (**Table 1**). This value is slightly higher than 192.16 and 193.49 mg KOH/g oil for flax oils extracted through Soxhlet extraction.[44, 43] Similarly, other studies reported slightly lower SV values of 185.61 ± 0.56 and 189.79 ± 0.79 mg KOH/g oil for flax oil extracted by Soxhlet.[29, 19] Such variations are likely attributable to differences in the chemical composition of the flaxseed samples, which can be influenced by factors such as seed maturity. For instance, one study documented a decrease in SV from 198.12 to 178 mg KOH/g oil with increasing seed maturity.[38] Additionally, significantly lower SV values 135.21 ± 2.01, 130.23 ± 3.21, and 122.94 ± 3.15 were reported for flax oil extracted using solvent extraction at 8, 12, and 16 h, respectively, at a 1:10 solid-liquid ratio.[35]

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Table 1. Physicochemical properties of flax oil extracted from flaxseed by Soxhlet extraction with hexane under optimized conditions (solid-liquid ratio: 1:10, Extraction time: 8 h).

Physicochemical properties	Value
Density	0.83 ± 0.05 g/cm^3
Viscosity	52.44 ± 0.02 mPa.s
Refractive index	1.47
Free fatty acid value	0.15 ± 0.02 % (as oleic acid)
Acid value	0.30 ± 0.02 mg KOH /g oil
Saponification value	204.86 ± 12.68 mg KOH/g

3.5 Solvent reusability studies

Solvent reusability was assessed by evaluating the solvent recovery (%) and oil yield (%) over ten consecutive Soxhlet extraction cycles under optimized conditions (solvent: hexane, solid-liquid ratio: 1:10, extraction time: 8 h) and the results are depicted in **Figure 5.**

3.5.1 Solvent recovery (%)

After each Soxhlet extraction cycle, the solvent (hexane) was recovered from the solvent-oil mixture using rotary evaporation. The solvent recovery across ten extraction cycles was 68%, 76%, 71%, 75%, 79%, 71%, 71%, 71%, 71% and 71% with an average recovery was 72.4 % (**Figure 5a).** An average solvent loss of approximately 21% to 32% per cycle was observed which may be attributed to the losses occurring during 3 stages: (i) Soxhlet extraction process, (ii) rotary evaporation, and (iii) sampling for analysis. Statistical analysis using one-way ANOVA showed significant differences in solvent (hexane) recovery across Soxhlet extraction cycles ($p < 0.001$) **(Table S7)**. However, despite statistical significance, the variation in solvent recovery was within a relatively narrow range (68–79%), indicating no consistent decline in solvent recovery with increasing number of cycles. Higher solvent recovery values (86.4%, 89.3% and 86%) have been reported for n-hexane, methyl ethyl ketone and chloroform, respectively for the study conducted on the extraction of oil from refinery waste.[45] These differences may be attributed to the variations in the extraction techniques, feedstock, and recovery conditions. Similarly, Thawornprasert and Somnuk (2024) reported recovery of hexane up to six extraction cycles for coffee oil extraction using magnetic stirring followed by evaporation[40]. In contrast, our study demonstrated stable recovery without a downward trend for ten cycles.

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3.5.2 Oil yield (%) across different cycles

The oil yield remained largely consistent across all ten cycles with oil yield values of 42.5%, 40.5%, 42.5%, 40.5%, 42.5%, 40.5%, 42.5%, 42.5%, 42.5%, and 42.5% (w/w) (**Figure 5b**). This stability indicates no significant loss in extraction efficiency with solvent reuse up to 10 extraction cycles. Further, statistical analysis using ANOVA revealed statistically significant differences ($p < 0.001$) in oil yields across extraction cycles. However, the actual variation in oil yield was quite minimal (approximately 2%), indicating that solvent reuse had no practical impact on extraction efficiency. For every 10 g of flaxseed extracted, a residual oil cake of 5 to 6 g was obtained. Detailed oil cake data across extraction cycles are provided in the Supplementary Information (Figure S1). This consistency further supports the stability of the extraction process during repeated solvent reuse. Hence, from an industrial perspective, solvent demonstrates excellent potential for reuse across multiple extraction cycles without substantial loss in extraction performance. In contrast, Thawornprasert and Somnuk (2024) reported an increase in oil yield from 0.76 to 6.20 wt.% for coffee oil extracted by employing magnetic stirring followed by evaporation (oven) on repeated extraction, highlighting that the solvent reuse performance is specific to the extraction method and feedstock. [40]

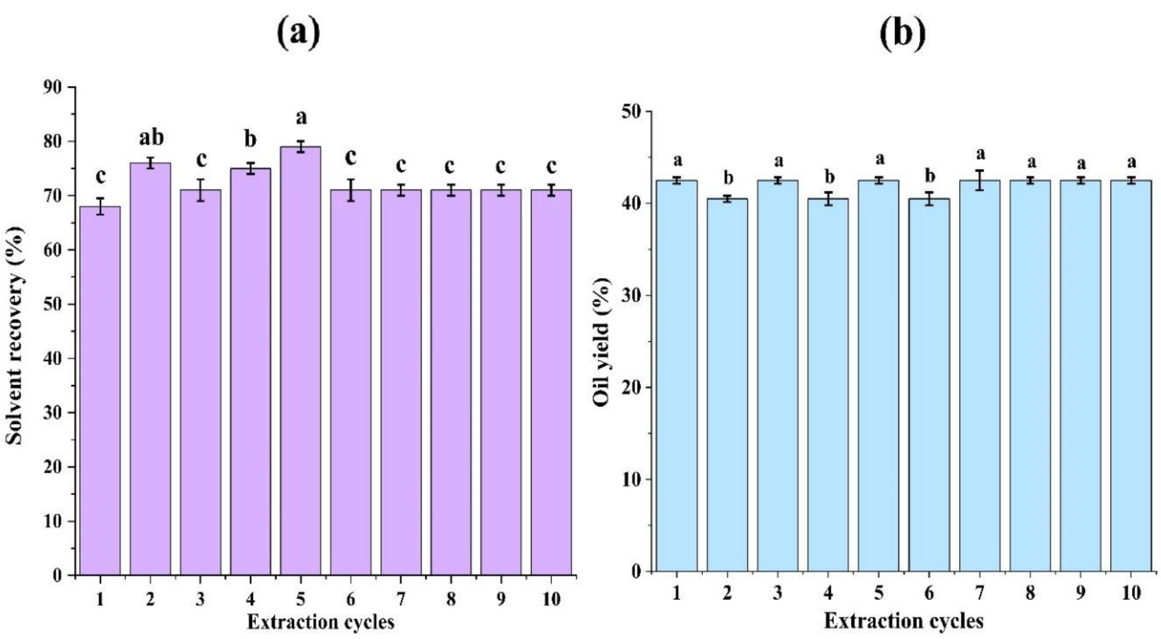

Figure 5. Impact of solvent (hexane) recycling on Soxhlet extraction parameters over ten consecutive cycles: **(a)** solvent recovery (%), and **(b)** oil yield (%). Values are expressed as mean ± standard deviation ($n = 3$). Different lowercase letters indicate statistically significant differences ($p < 0.05$) among extraction cycles, as determined by one-way ANOVA followed by Tukey's post hoc test.

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3.5.3 Visual appearance

The flax oil extracted and recovered solvent from repeated extraction cycles were examined by visual appearance. The yellow colour of the oil remained consistent across all extraction cycles, indicating no apparent degradation. Similarly, no changes were observed in the recovered solvent which remained colourless through the tenth extraction cycle, suggesting no accumulation of pigments or impurities. These qualitative observations confirm the stability of both the extracted oil and the solvent upon reuse (**Figure 6**). These observations further support the potential for reusability of solvents for repeated extractions to have a more economically viable and sustainable process. In contrast, the results of visual observation of coffee oil extracted using magnetic stirring indicated an increase in colour intensity of miscella from 1st to 9th extraction cycles.[40] These differences could be due to the difference in extraction methods, nature of the sample, and the solvent recovery methods employed.

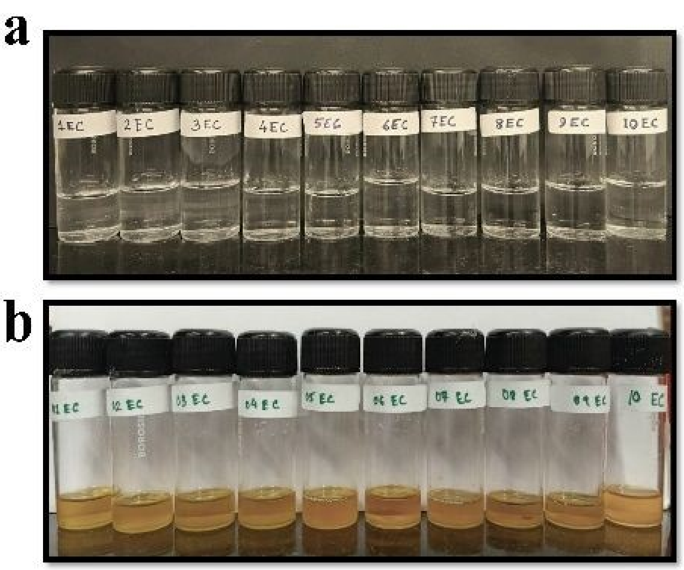

Figure 6. Visual appearance of **(a)** recovered solvent (hexane) colour and (b) extracted oil colour (hexane as solvent) across ten Soxhlet extraction cycles.

3.5.4 Refractive index

The refractive index (RI) of flax oil extracted under optimized conditions through Soxhlet extraction was 1.47 **(Table 1)**. This value aligns with the refractive index of 1.47 reported in the literature for Soxhlet-extracted flax oil and is comparable to the value of 1.478 for oil extracted via subcritical n-butane.[29,19] Slightly lower (1.40) and higher (1.50) refractive index values have been reported for flax oils extracted by mechanical press and Soxhlet extraction,

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respectively.[13] In this work, the refractive index of the oil remained consistent across ten successive extraction cycles, as shown in **Figure 7a**. The RI values remained statistically unchanged ($p > 0.05$) across ten Soxhlet extraction cycles, indicating negligible oxidative degradation or impurity accumulation in the final product supporting the feasibility of solvent reuse.

The refractive index of pure solvent (hexane) was 1.36. Similar values were observed for the solvent recovered from the repeated extractions (**Figure 7b)**. The measured refractive index value of 1.36 was in agreement with the refractive index value of 1.37 reported by Reina and Gonzalez (2010).[46] Non-significant changes observed imply minimal degradation, allowing the usage of recovered hexane for repeated Soxhlet extractions. Hence, the reusability of recovered hexane was validated to ten Soxhlet extraction cycles based on the absence of significant differences in refractive index values.

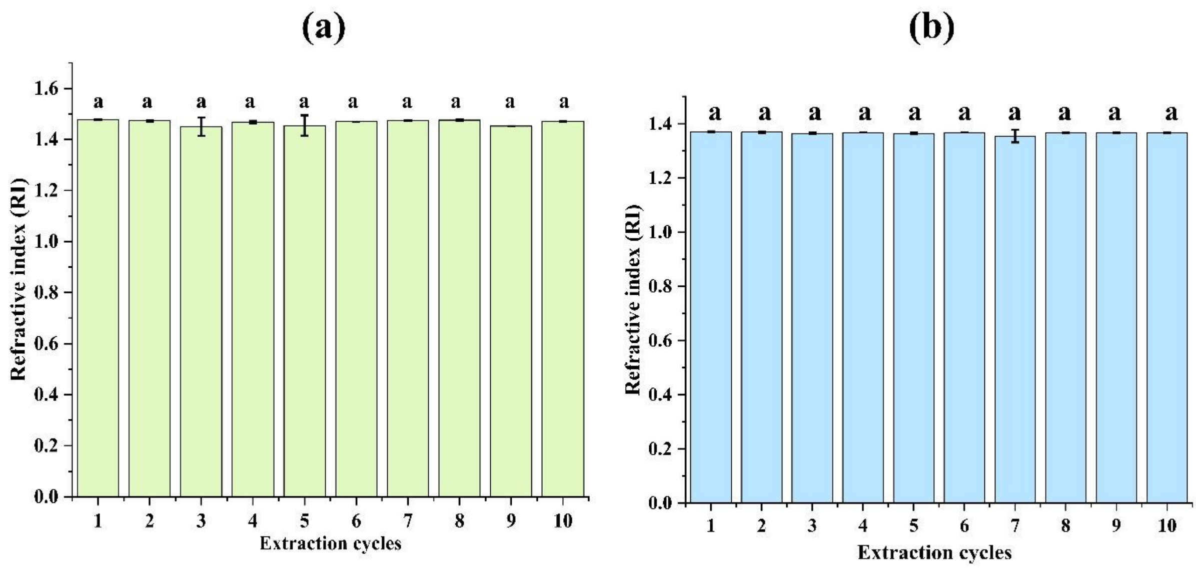

Figure 7 Refractive index stability over ten Soxhlet extraction cycles with solvent reuse for **(a)** hexane-extracted flax oil and **(b)** recovered hexane solvent. Values are expressed as mean ± standard deviation ($n = 3$). Means sharing the same lowercase letter indicate no significant differences ($p > 0.05$) among different cycles of Soxhlet extraction as determined by one-way ANOVA followed by Tukey's post hoc test.

3.5.5 Acid value (AV)

Acid value is defined as the milligrams of potassium hydroxide (KOH) required to neutralize the free fatty acids in 1 g of oil. AV measures the presence of fatty acids not bound to triacylglycerol, i.e. hydrolytic degradation of triglycerides. AV and FFA are interrelated, and the former are directly proportional to the latter. A higher AV indicates greater degradation (poor quality), while a lower AV indicates better oil quality and stability. In this study, the AV of flax oil extracted using hexane under optimized conditions was 0.30 ± 0.02 mg KOH/g oil **(Table1)**. This low AV for flax oil extracted indicated a good quality flax oil with minimal hydrolytic degradation. This suggests that the extraction conditions resulted in good quality oil, aiding Soxhlet extraction as a reliable reference method for maximum oil recovery and high-quality oil. Slightly higher AV values of 0.80 mg KOH/g oil and 0.76 ± 0.10 mg KOH/g oil have been reported in litertaure.[44,37] Much higher AV values (1.68 and 2.23 mg KOH/g oil) have been obtained for flax oils extracted using subcritical n-butane extraction and Soxhlet extraction, respectively.[19, 29] In another study, AV of flax oil decreased **(**3.2 ± 0.10, 2.8 ± 0.15, 1.9 ± 0.10, 1.4 ± 0.08 mg KOH/g oil) with increasing seed maturity.[38] Importantly, in the solvent reusability study, the AV remained statistically unchanged across ten extraction cycles ($p > 0.05$), with values ranging from **0**.2867 to 0.3067 mg KOH/g oil (**Figure 8a)**. One-way ANOVA followed by Tukey's HSD test showed no significant differences between extraction cycles, with all groups assigned the same statistical grouping. These results indicate that the recovered solvent did not cause oil degradation over multiple extraction cycles.

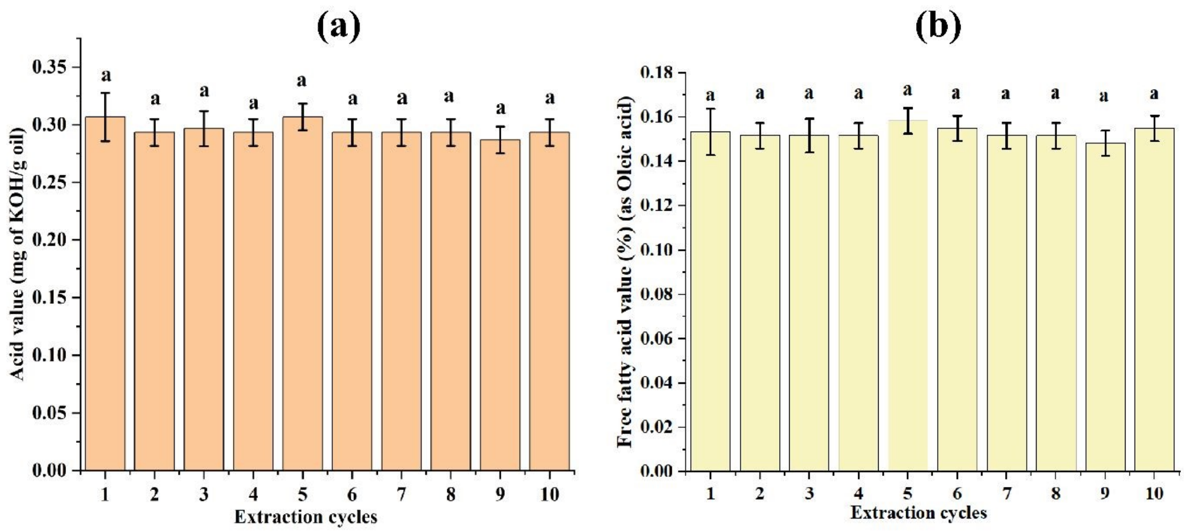

Figure 8 (a) Acid value (AV) and **(b)** Free fatty acid (FFA) value of flax oils extracted using hexane over ten Soxhlet extraction cycles. Values are expressed as mean ± standard deviation ($n = 3$). Means sharing the same lowercase letter indicate no significant differences ($p > 0.05$) among extraction cycles as determined by one-way ANOVA followed by Tukey's post hoc test.

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3.5.6 Free fatty acid (FFA)

Free fatty acid (FFA) value is expressed as the percentage of free fatty acids present in the oil (as oleic acid). The FFA value reported for flax oil extracted using fresh hexane in the first extraction cycle was 0.15 ± 0.01% (as oleic acid) (**Table 1**). A lower FFA value indicates reduced hydrolysis and thus better-quality oil. Thus, flax oil extracted in the first Soxhlet extraction can be considered as good quality oil due to a lower FFA value (0.15%).[47] Slightly higher FFA values of 0.42% and 0.38 ± 0.05% (as oleic acid) have been reported for flax oil extracted using subcritical n-butane extraction and Soxhlet extraction, respectively.[19,29] These differences in FFA values can be attributed to seed variety, seed quality, the maturity index of flax seeds and the extraction methods. The FFA value (0.15 ± 0.01%**)** (as oleic acid) remained statistically unchanged ($p > 0.05$) across 10 extraction cycles (**Figure 8b).** These results confirmed that the solvent reuse did not lead to oil degradation or increased free fatty acid formation across multiple extraction cycles.

3.5.7 Gas Chromatography (GC) Analysis:

GC-FID analysis was performed to evaluate the chemical stability of the recovered hexane during repeated Soxhlet extraction cycles and to determine the fatty acid composition of the extracted flax oil, as well as to screen for potential impurities. The chromatogram of pure hexane showed a single peak at a retention time of 2.09 min, with a total peak area of 99.64 ± 0.02% .The chromatograms for the solvent recovered after all ten extraction cycles were virtually identical, with a major peak area ranging from 99.15 ± 0.01% to 99.76 ± 0.02% at the same retention time (2.09 min) **(Figure S3)**. The absence of additional peaks throughout all 10 cycles indicates that the hexane did not undergo chemical degradation and remained free of detectable impurities. These results confirm that hexane maintains its quality during repeated extraction, supporting its suitability for reuse. The chromatograms of the extracted oil showed the presence of multiple fatty acids such as palmitic acid, palmitoleic acid, cis-heptadecanoic acid, stearic acid, oleic acid, trans-9-elaidic acid, linoleic acid, linolenic acid and gamma-linolenic acid **(Figure S2)**. These components were identified by comparing their retention times and area percentages to those of a FAME standard (**Table S8**). The major fatty acids observed in extracted flax oil were palmitic acid (6.27%), stearic acid (22.73%), linoleic acid (13.64%), and linolenic acid (49.14%). Minor components were cis-heptadecanoic acid (6.63%), oleic acid (0.80%), gamma linolenic acid (0.5%) and trans-9-elaidic acid (2.5%) (**Table S9**). This fatty acid profile is consistent with values reported for flax oil extracted using both Soxhlet and subcritical n-butane methods. For instance, comparable proportions of palmitic (5.96 ± 0.01%), linoleic (12.93 ± 0.01%), and linolenic (43.66 ± 0.02%) acids have been reported for oil obtained via subcritical n-butane extraction.[19] The close agreement demonstrates that repeated use of recovered hexane does not alter the fatty acid composition of the extracted flax oil.

3.5.8 Fourier-Transform Infrared (FT-IR) Spectroscopy

FT-IR analysis was conducted to monitor the chemical stability of both the extracted flax oil and the recovered hexane solvent over ten extraction cycles. The spectra for the flax oil (**Figure S4 ~~2~~**) confirmed the expected functional groups, including carbonyl (C=O) and alkene (C-H) stretches, aligning with standard profiles for flaxseed oil.[32,48] The FT-IR spectra of the oils extracted in repeated Soxhlet extraction cycles remained consistent with respect to functional groups observed (**Figure S4**,**Table S10**). Thus, the functional groups remained intact, and the chemical composition of the oil did not change during successive extractions. The FT-IR spectra of the recovered hexane displayed the characteristic C–H stretching (2940–2880 cm^{-1}), C–H deformation (1480–1365 cm^{-1}), and C–C skeletal vibrations (750–720 cm^{-1}), in agreement with previously reported hexane spectra.[49] Analysis of recovered hexane showed retention of the characteristic functional groups across all ten extraction cycles. However, minor variations in peak intensities began to appear from the third cycle onwards, with more noticeable variations by the tenth cycle (**Figure S5)**. These changes may be attributed to the gradual accumulation of co-extracted compounds.[50, 51]. However, no new functional groups or significant spectral shifts were detected. This suggests that, although minor compositional changes may occur at a trace level, they do not significantly affect the overall chemical integrity of the solvent. Therefore, FT-IR results align with the results obtained from refractive index, free fatty acid, acid value and gas chromatography analyses, confirming that hexane can be effectively reused for up to ten cycles without compromising the quality and yield of the oil under optimized conditions. Further details and spectra are provided in the Supplementary Information.

3.6 SWOT (Strengths, Weaknesses, Opportunities, Threats) analysis of a process

This section highlights the SWOT (Strengths, Weakness, Opportunities, and Threats) analysis based on the results obtained and results of similar works reported in the literature. The integration of Soxhlet extraction coupled with solvent recovery and reusability studies presents a strategic advancement towards a sustainable and efficient oil extraction process. One of the

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major strengths of solvent recovery and reusability emphasizes on its ability to substantially reduce the overall quantity of fresh solvent required per extraction cycle, thereby reducing capital expenditure. This work shows that solvent can be recovered and reused for up to 10 cycles without affecting the quality and yield of the oil. Despite its advantages, certain limitations possessed by Soxhlet extraction are the larger extraction time and high energy consumption. The continued extraction over 10 cycles and the long extraction time may affect the purity and quality of the oil and solvent, which needs further attention while scaling up the process at an industrial scale. Furthermore, Soxhlet extraction is inherently slower and more energy-intensive compared to extraction technologies such as supercritical CO_2 and ultrasonic-assisted extraction, which may limit large-scale applications. Further opportunities include faster extraction with integration of field-assisted based technology for oil extraction to improve the yield, screening of different sustainable solvents such as natural deep-eutectic solvents to improve the solvent recovery. Appropriate use of solvent is a crucial factor while adopting any process or technology. Lack of knowledge and the urge for generating profits by small to medium-scale enterprises, resulting in repeated use of solvents, degrades the quality of oils and bioactives. It may also affect the product quality, safety, sensory attributes, and shelf-life, affecting market acceptance. Further, increased monitoring and restriction on certain solvent types are two ways to avoid the threats and ensure sustainability.

4. Conclusion

This study systematically standardized the extraction process parameters for flax oil and demonstrated the feasibility of solvent recovery and reuse. With hexane as the solvent, a 40.48% yield was achieved at a solid-liquid ratio of 1:10 and an extraction time of 8 h. Statistical analysis confirmed that both extraction time and solid-liquid ratio significantly influenced the oil yield. Also, the absence of significant interaction between solvent type and solid-liquid ratio on oil yield indicates a consistent trend across all three solvents studied in this work. One of the key future contributions of this work includes insights on the recovery of solvent and its reusability across multiple extraction cycles. Solvent reusability studies demonstrated that hexane can be effectively recovered and reused for up to ten Soxhlet extraction cycles without significant changes in oil yield, physicochemical properties, and fatty acid composition. The average solvent recovery per cycle was approximately 72%. FT-IR analysis confirmed that no new peak appeared in the recovered solvent; only minor intensity changes in characteristic peaks appeared from the fourth cycle onward, likely due to co-extracted residues. However, these did not result in measurable changes in oil quality or

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composition. Although Soxhlet extraction has limitations such as longer extraction times and higher energy consumption, reported extraction parameters can serve as a benchmark for future researchers for a comparative study between Soxhlet and other extraction methods. The methodology proposed in this work supports sustainable extraction goals by minimizing solvent consumption, minimizing chemical waste, and lowering operational costs. The findings offer practical guidance for both researchers and industries for balancing extraction efficiency, oil quality and environmental impact of the process.

Abbreviations

FAME	Fatty acid methyl ester
GC	Gas chromatography
FID	Flame ionization detector
ALA	Alpha-linolenic acid
EPA	Eicosapentaenoic acid
DHA	Docosahexaenoic acid

Acknowledgement

NNA acknowledges the Confederation of Indian Industry (CII), Anusandhan National Research Foundation (ANRF), Government of India for the CII-ANRF Prime Minister's Fellowship for Doctoral Research, and Marico Limited, Mumbai, for the industrial collaboration.

CRediT Authorship Statement

Neha N. Areekal: Conceptualization, Investigation, Methodology, Data curation, Formal analysis, Visualization, Validation, Writing – original draft. **Abhishek J Gupta**: Conceptualization, Supervision, Validation, Writing – review & editing, **KSMS Raghavarao:** Conceptualization, Formal analysis, Project administration, Supervision, Validation, Writing – review & editing. **Anil B. Vir:** Conceptualisation, Formal analysis, Project administration, Validation, Resources, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

The data supporting this article have been included as part of the Supplementary Information.

Appendix A. Supplementary data

The Supplementary data can be found online.

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Data Availability Statement

View Article Online
DOI: 10.1039/D6FB00012F

The data supporting this article have been included as part of the Supplementary Information.