

# Sustainable Food Technology

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## Sustainability Spotlight Statement

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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).



# 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  $40.48 \pm 2.25\%$  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 ( $43.84 \pm 1.51\%$ ) was achieved in 8 h by optimizing the solid-liquid ratio to 1:10. Hexane was recovered with an average recovery of  $\sim 72\%$  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.



## 31 1. Introduction

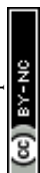
32 Health is the most important element of one's life. The imbalance in today's work and personal  
33 life is a growing concern. COVID-19 has also demonstrated the need for and importance of  
34 consumption of immunity-boosting foods and supplements such as Omega-3 fatty acids. The  
35 health benefits—attributed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

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



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

82  
83 The polarity of solvents plays a vital role in oil extraction. Based on the polarities, solvents are  
84 broadly categorized as polar (methanol, n-butanol, ethanol, acetone, and isopropanol) and  
85 nonpolar (hexane). Hexane was reported as the best solvent by Ekka and Ovary (2023) due to  
86 the high oil yield (45% w/w) of flaxseed oil employing Soxhlet extraction technique.<sup>22</sup> Ultra-  
87 assisted extraction using solvents such as hexane, dichloromethane, acetone, ethanol, methanol  
88 and petroleum ether has been employed to obtain flax oil with increased oil recovery and high  
89  $\alpha$ -Linolenic acid ( $\omega$ -3).<sup>23</sup> In another study by Piva G.S. *et. al* (2018) hexane gave the highest  
90 yield (36.12%) for Soxhlet extraction when compared to subcritical propane and pressurized  
91 ethanol.<sup>13</sup> Hexane is one of the most commonly used organic solvent (exhibits the ability to  
92 extract more nonpolar solutes) for oil extraction due to its high efficiency in oil recovery, low  
93 cost, recyclability, and low boiling point ( $\sim$  68-69 °C)<sup>24,25</sup>. Another solvent, ethyl acetate which  
94 is moderately polar has been proposed as a substitute to the conventional hexane because it is  
95 cost-effective (33% cheaper when compared to *n*-hexane), less flammable and less  
96 hazardous<sup>26</sup>. Ethanol, owing to its high polarity, has the capability to extract more polar  
97 compounds such as polyphenols, pigments and soluble sugars.<sup>27</sup> Based on the literature studies,



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

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

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## 130 2. Materials & Methods

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### 131 2.1 Materials

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

### 137 Chemicals

138 The extraction solvents, i.e., hexane (AR grade, purity:99%, CAS: 110-54-3) and ethyl acetate  
139 (AR grade, purity:99.5%, CAS: 141-78-6) were purchased from Sisco Research Laboratory  
140 (SRL, Mumbai, India). Ethanol (AR grade, purity:99.9%, CAS: 64-17-5) was purchased from  
141 MSB Chemical Ltd, (Mumbai, India). The chemicals used for the analysis include potassium  
142 hydroxide (KOH, CAS: 1310-58-3), hydrochloric acid (HCl, CAS:7647-01-0) and  
143 phenolphthalein solution were purchased from Sisco Research Laboratory (SRL, Mumbai,  
144 India), HiMedia Laboratory Pvt Ltd (India) and Qualigens Fine Chemicals (India),  
145 respectively. The chemicals used for GC-FID analysis including sodium hydroxide (NaOH,  
146 CAS: 1310-73-2), sulfuric acid (H<sub>2</sub>SO<sub>4</sub>, CAS: 7664-93-9), n-heptane (CAS: 142-82-5), and  
147 sodium chloride (NaCl, CAS: 7647-14-5) were also purchased from Sisco Research Laboratory  
148 (SRL, Mumbai, India). Methanol (CAS: 67-56-1) employed for fatty acid preparation was  
149 purchased from Merck (Mumbai, India). The Supelco 37 component standard mix (Fatty Acid  
150 Methyl Ester (FAME), CRM47885) was purchased from Sigma Aldrich (Darmstadt,  
151 Germany).

### 152 Pre-treatment of Flaxseeds

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

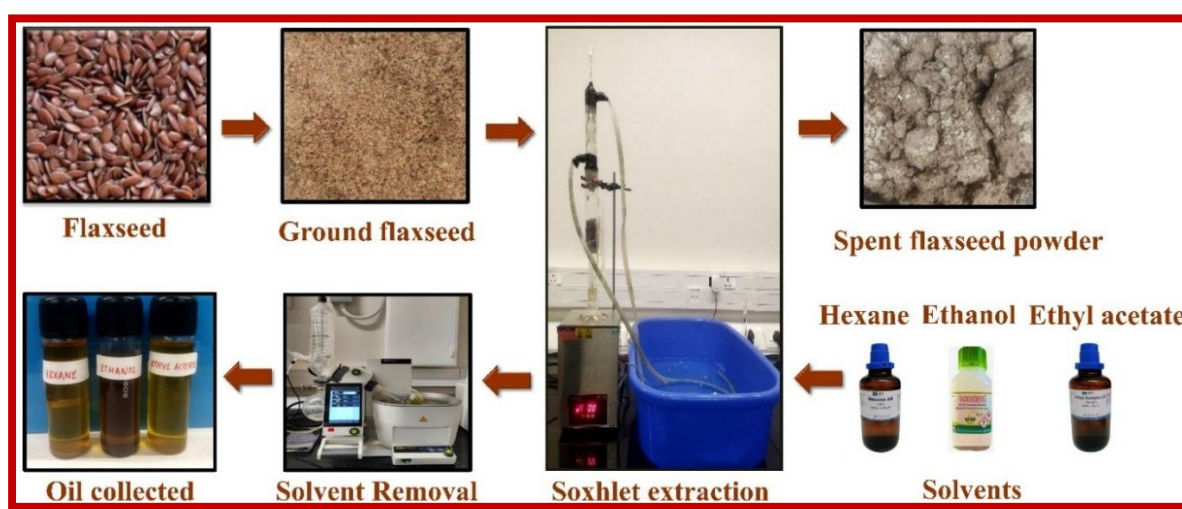
## 158 2.2 Methods

### 159 2.2.1 Soxhlet Extraction

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



162 acetate) at 1:2.5 solid-liquid ratio was employed for the extraction of flax oil from flaxseed for  
 163 8, 12 and 16 h (**Figure 1**). The temperature of the heating mantle (LabQuest borosil PRM500,  
 164 India) was set to 68.7, 78.37 and 77.1°C according to the boiling points of hexane, ethanol and  
 165 ethyl acetate respectively. After extraction, the oil-solvent mixture was subjected to a rotary  
 166 evaporator (BUCHI Rotavapor R-300, BÜCHI Labortechnik AG, Flawil, Switzerland) under  
 167 reduced pressure (hexane:335 mbar, ethanol:175 mbar and ethyl acetate:240 mbar) at 40°C for  
 168 90 min to obtain the oil. The oil yield, and extraction efficiency were calculated using **Equation**  
 169 **1** and **Equation 2**, respectively. All the extractions were performed in triplicate to ensure the  
 170 reproducibility and average values are reported with standard deviations.



171  
 172 **Figure 1:** Process protocol followed for flax oil extraction using different solvents

### 173 2.2.2 Design of experiments

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



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

### 195 2.2.3 Oil yield (%) and Extraction efficiency (%)

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

$$197 \text{ Yield (\%)} = \frac{\text{Weight of extracted oil (We) in g}}{\text{weight of initial sample taken for extraction (Wt) in g}} \times 100 \quad (1)$$

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

$$200 \text{ Extraction Efficiency (\%)} = \frac{\text{Weight of the extracted oil (We) in g}}{\text{Total oil content extracted via Soxhlet using hexane (WT) in g}} \times 100 \quad (2)$$

## 202 2.3 Physicochemical properties of oil

### 203 2.3.1 Density

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

$$205 \text{ Density} = \frac{\text{Mass of the oil sample (M) g}}{\text{Volume of the oil (V) in ml}} \quad (3)$$

### 206 2.3.2 Viscosity

207 The viscosity of flax oil was measured using a Brookfield rheometer with spindle RCT-50-1  
 208 over a shear rate range of 0.06 to 7800 s<sup>-1</sup> at room temperature (24°C).

209

210

211



212 **2.3.3 Refractive index**

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

217 **2.3.4 Acid value and free fatty acid (FFA) content**

218 Briefly, 1 gm of oil was mixed with 5 ml of neutralized ethanol. Then, 2-3 drops of  
219 phenolphthalein (1%) indicator was added to the mixture and the mixture was titrated against  
220 0.1 N KOH solution until the appearance of the first persistent pink color. The acid value was  
221 calculated using **Equation 4**. The free fatty acid (FFA) of the oil extracted was determined  
222 using the same method as suggested above for acid value and was calculated using **Equation**  
223 **5**.<sup>30</sup>

$$224 \text{ Acid value} = \frac{\text{Normality of KOH (N)} \times 56.1 \times \text{Volume of KOH (V)}}{\text{Wt. of Sample in gm (W)}} \quad (4)$$

225 Where, 56.1 is the molecular weight of KOH.

$$226 \text{ FFA \% (as oleic acid)} = \frac{\text{Normality of KOH (N)} \times 28.2 \times \text{Volume of KOH (V)}}{\text{Wt. of Sample in gm (W)}} \quad (5)$$

227 Where, 28.2 is the equivalent factor for oleic acid.

228 **2.3.5 Saponification value**

229 The saponification value was determined using the protocol suggested by Gugale and Mane  
230 (2024).<sup>30</sup> Oil sample (2-3 g) was mixed with 25 ml of 0.5 M KOH . The contents were heated  
231 under a reflux condenser for 30 - 40 min to ensure that the sample was fully dissolved. Once  
232 the sample was cooled, phenolphthalein was added. It was then titrated with 0.5 M of HCl until  
233 the disappearance of pink colour, noted as the endpoint. Titrate value was noted for blank with  
234 the same time conditions and was calculated using **Equation 6 as:**

$$235 \text{ Saponification value} = \frac{N \times 56.1 \times (B-S)}{W} \quad (6)$$

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

238

239



## 240 **2.4 Fatty acid composition by GC-FID**

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### 241 **Sample preparation for fatty acid methyl esters (FAME)**

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

### 251 **Qualitative and quantitative analysis of essential fatty acids**

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

## 260 **2.5 Fourier-Transform Infrared Spectroscopy Analysis (FT-IR)**

261 FT-IR analysis was carried out using a FT-IR spectrometer (PerkinElmer, Shelton, CT, USA)  
262 to identify the functional groups present in the flax oil. Oil samples were placed on the cell  
263 plate, and the spectra were collected over the range 500-4000 cm<sup>-1</sup> with 36 scans at room  
264 temperature.<sup>32</sup>

## 265 **2.6 Statistical analysis**

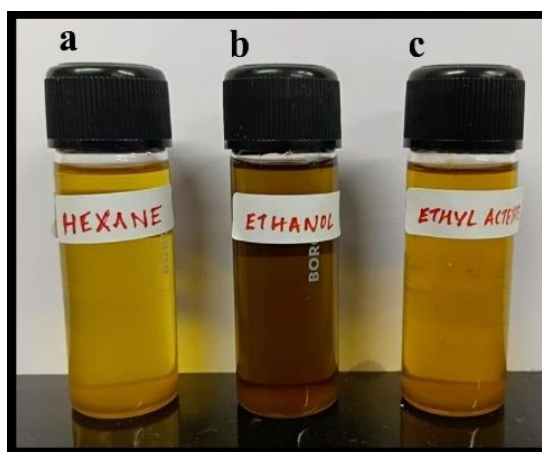
266 The statistical analysis was performed using OriginPro 2021 software (OriginLab Corporation,  
267 USA). One way analysis of variance (ANOVA) was used to evaluate the effects of process  
268 parameters, followed by the Tukey's honest significant difference (HSD) test to assess  
269 differences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



### 272 3. Results and Discussion

#### 273 3.1 Effect of solvent and extraction time on flax oil yield

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



283  
284 **Figure 2.** Flax oils extracted by Soxhlet extraction using (a) hexane, (b) ethanol, (c) ethyl  
285 acetate

286 To standardize the extraction time, flaxseed oil was extracted using a Soxhlet apparatus for 8,  
287 12, and 16 h at a fixed solid-liquid ratio of 1:2.5 (w/v) with three solvents. As shown in **Figure**  
288 **3a**, among the three solvents, hexane resulted in the highest oil yield compared to ethyl acetate  
289 and ethanol at 8, 12 and 16 h extraction time. For hexane, the oil yield was increased ( $39.31 \pm$   
290  $0.91\%$  to  $43.44 \pm 1.51\%$ ) with an increase in extraction time from 8 to 16 h. An increase in oil  
291 yield from  $39.31 \pm 0.91\%$  to  $40.25 \pm 1.21\%$  was observed when the extraction time increased  
292 from 8 to 12 h, with the highest yield ( $43.44 \pm 1.51\%$ ) observed at 16 h (**Figure 3a**). This  
293 increasing trend is consistent with previous reports by Ghoshal *et al.* (2022) where they reported  
294 oil yields of  $32.15 \pm 0.52\%$ ,  $37.11 \pm 0.58\%$ , and  $42.56 \pm 0.59\%$  at 6, 8, and 12 h, respectively.<sup>35</sup>  
295 This increase in oil yields is attributed to the increased extraction time, which enhances  
296 diffusion of lipids and mass transfer from the seed matrix into the extraction solvent.<sup>26</sup>



297 The oil yield obtained in this work at 8 h is comparable to the yield of  $35.62 \pm 1.04\%$  reported  
298 for an 8 h Soxhlet extraction using hexane.<sup>26</sup> In comparison, previous studies reported slightly  
299 lower oil yield ( $37.11 \pm 0.58\%$ ) for Soxhlet extraction using hexane at a 1:10 solid-liquid  
300 ratio.<sup>35</sup> A similar oil yield of 36.1% was reported in Soxhlet extraction for 14 h extraction time;  
301 however, the solid-liquid ratio was not mentioned.<sup>13</sup> A slightly higher oil yield (42.4 %) was  
302 reported for Soxhlet; however, the extraction time was not mentioned.<sup>36</sup> These discrepancies  
303 in oil yield are likely attributable to factors such as flaxseed species and variety, extraction  
304 process parameters, extraction methods employed and the inherent efficiency and selectivity  
305 of the solvents employed.<sup>37</sup> Other studies have reported significantly lower oil yields with  
306 different extraction techniques, such as ultrasound-assisted extraction( 21.95%)<sup>29</sup>, with hull  
307 extraction (19.3%)<sup>38</sup> and for accelerated solvent extraction using hexane and ethyl acetate  
308  $11.10 \pm 1.55\%$  and  $19.19 \pm 1.29\%$  respectively<sup>26</sup>, indicating the effectiveness of Soxhlet  
309 extraction in achieving higher oil recovery.

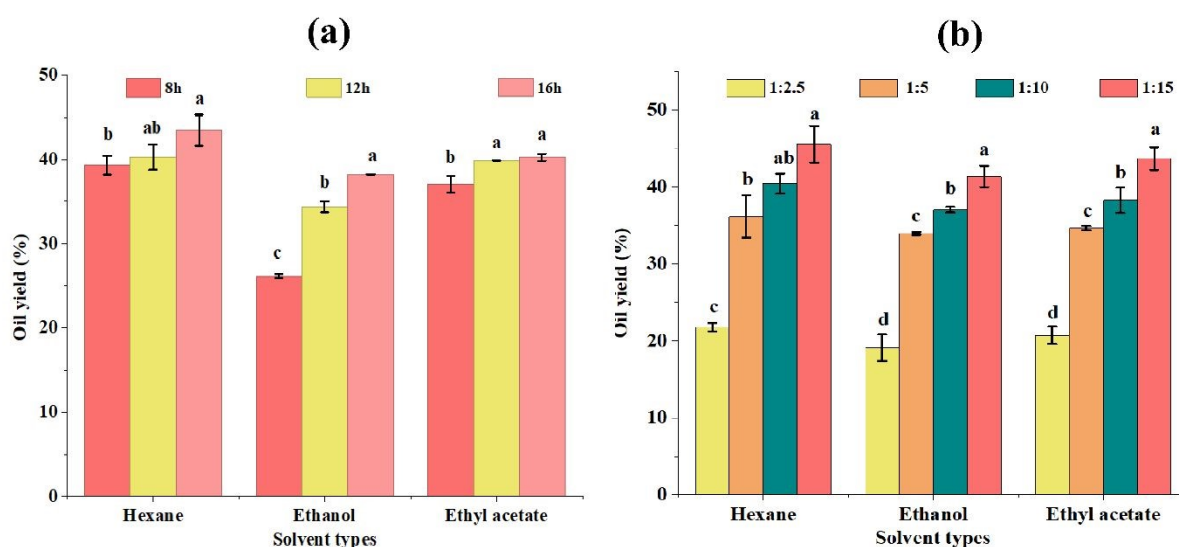
310 Statistical analysis using ANOVA showed significant differences in oil yields across extraction  
311 times (8, 12, and 16 h) for hexane and ethyl acetate ( $p < 0.05$ ). In contrast ethanol exhibited  
312 highly significant differences extraction time (8-16 h) (**Table S1, Table S2**). Although a higher  
313 yield was obtained at 16 hours (**Figure 3**), prolonged extraction increases energy consumption.  
314 Therefore, considering the balance between efficiency, energy consumption, and economic  
315 feasibility for scale-up, an extraction time of 8 h was selected for further experiments. A  
316 statistical analysis was carried out with a two-way ANOVA to understand the interaction  
317 between extraction time and solvent type on the oil yield. The results showed that both  
318 extraction time and solvent type had significant effects ( $p < 0.001$ ), with a significant  
319 interaction between the two factors ( $p < 0.001$ ), indicating that the effect of extraction time on  
320 oil yield depended on the solvent used. Due to the significant interaction effect, one-way  
321 ANOVA followed by Tukey's HSD test was performed separately for each solvent to evaluate  
322 differences across extraction times. The one-way ANOVA analysis revealed significant  
323 differences in oil yield across extraction times (8, 12, and 16 h) for hexane and ethyl acetate ( $p$   
324  $< 0.05$ ), whereas ethanol exhibited highly significant differences ( $p < 0.001$ ) (**Figure 3a**).

### 325 3.2 Effect of solid-liquid ratio on oil yield

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



330 (41.36 ± 1.76%) and ethyl acetate (43.27 ± 1.20%). Two-way ANOVA revealed that both solid-  
 331 liquid ratio and solvent type significantly affected oil yield ( $p < 0.001$ ). However, no significant  
 332 interaction between the two factors was observed ( $p > 0.05$ ), indicating that the effect of solid-  
 333 liquid ratio on oil yield was consistent across all solvents. One-way ANOVA followed by  
 334 Tukey's HSD test was performed to compare oil yields for different ratios within each solvent.  
 335 The results showed that oil yield increased significantly with increasing solid-liquid ratio for  
 336 all solvents. However, statistical analysis using one-way ANOVA revealed non-significant  
 337 differences ( $p > 0.05$ ) in oil yields between solid-liquid ratios of 1:10 and 1:15 (**Table S3**,  
 338 **Table S4**).



339 **Figure 3.** Effect of (a) extraction time and (b) solid-liquid ratio on oil yields using different  
 340 solvents. Values are presented as mean ± standard deviation (n = 3). Statistical significance  
 341 was performed using one-way ANOVA followed by Tukey's post-hoc test. Different letters  
 342 indicate statistically significant differences ( $p < 0.05$ ) based on Tukey's HSD test. Statistical  
 343 comparisons were performed within each solvent.  
 344

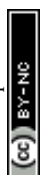
345  
 346 From a sustainability and economic (cost-benefit analysis) perspective, a 1:15 is less favourable  
 347 choice due to due to higher solvent consumption, increased energy demand for solvent  
 348 recovery. Therefore, a solid-liquid ratio of 1:10 was selected as the optimal condition,  
 349 balancing extraction efficiency with economic and environmental considerations. The oil  
 350 yields obtained in this work compare favourably with those reported in the literature. The oil  
 351 yields of 35.62 ± 1.04% and 37.11 ± 0.58% reported by Lohani *et al.*(2015) and Ghoshal *et al.*  
 352 (2022) for flax oil extracted by Soxhlet extraction for 8 h with solid: liquid ratio of 1:15 and



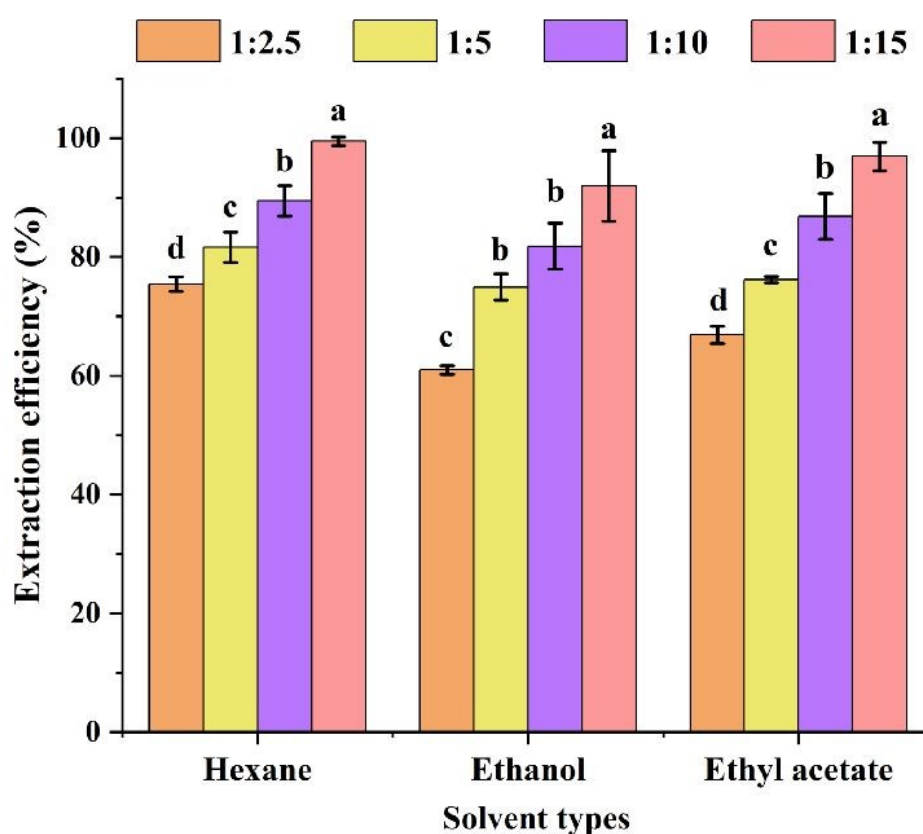
353 1:10, respectively.<sup>26,35</sup> A significantly lower oil yield (14.53%) was reported for flax oil  
354 extracted using magnetic stirring for 8 h followed by rotary evaporation.<sup>39</sup>  
355 The oil yield obtained in this work with a solid-liquid ratio of 1:10 and 8 h extraction time  
356 ( $40.48 \pm 2.25\%$ ) was comparable to the oil yield achieved with 1:2.5 solid-liquid ratio and  
357 prolonged extraction time of 16 h ( $43.44 \pm 1.51\%$ ). This highlights the trade-off between  
358 extraction time and solvent volume (i.e. solid-liquid ratio). Given that 16 h extraction doubles  
359 the processing time and energy consumption, the 8 h extraction at 1:10 is more efficient.  
360 Similar findings highlighting 1:10 and 8 h as optimal conditions for ultrasound-assisted  
361 extraction of flax oil, achieving  $40.48 \pm 2.25\%$  oil yield were reported by Gutte *et al.*(2015).<sup>39</sup>  
362 In contrast, Abdi *et al.*(2020) reported an oil yield of 21.95% for Soxhlet extraction (8 h) using  
363 hexane at a solid-liquid ratio 1:2.5.<sup>37</sup> Subcritical n-butane extraction has been reported to have  
364 a higher yield (28.75%) compared to n-hexane Soxhlet extraction (27.53% at 1:5 for 6 h), and  
365 cold-press extraction (19.56%).<sup>19</sup> These oil yields reported are lower than the optimized  
366 Soxhlet results of this work.

### 367 3.3 Extraction efficiency (%)

368 At 8 h of extraction time, extraction efficiency increased with increasing solid-liquid ratios for  
369 all three solvents (hexane, ethanol and ethyl acetate). Hexane showed the highest extraction  
370 efficiency, with values of  $75.42 \pm 1.27\%$ ,  $81.64 \pm 2.56\%$ , 8%, and  $99.47 \pm 0.74\%$  at solid-  
371 liquid ratios of 1:2.5, 1:5, 1:10, and 1:15, respectively. This superior performance is attributed  
372 to its non-polar nature, which enhances solubility and oil recovery. This was followed by ethyl  
373 acetate with extraction efficiencies of  $66.87 \pm 1.46$ ,  $76.2 \pm 0.51$ ,  $86.80 \pm 3.84$  and  $96.93 \pm 2.4\%$   
374 at solid-liquid ratios of 1:2.5, 1:5, 1:10 and 1:15, respectively. Ethanol exhibited comparatively  
375 lower extraction efficiency of  $60.96 \pm 0.74$ ,  $74.94 \pm 2.2$ ,  $81.84 \pm 3.86$  and  $91.95 \pm 5.92\%$  at the  
376 same solid-liquid ratios. Based on these results, hexane was identified as the most effective  
377 solvent, achieving the highest extraction efficiency ( $99.47 \pm 0.74\%$ ) at a solid-liquid ratio of  
378 1:15, followed by ethyl acetate and ethanol. Although, the solid-liquid ratio of 1:15 resulted  
379 in higher extraction efficiencies for all three solvents increasing the solid-liquid ratio enhanced  
380 extraction efficiency for all solvents, likely due to increased solvent availability, improved  
381 mass transfer, and a shift in the dissolution equilibrium toward higher oil recovery. Similar  
382 extraction efficiency of hexane (97.37% in the third cycle) was reported by Thawornprasert  
383 and Somnuk (2024). On repeated cycles of extraction, extraction efficiency further decreased  
384 to 84.21% (seventh cycle), limiting its usage till 6<sup>th</sup> extraction cycle.<sup>40</sup>



385 Statistical analysis using two-way ANOVA showed that both solvent type and solid-liquid ratio  
 386 significantly affected the extraction efficiency ( $p < 0.001$ ). However, no significant interaction  
 387 between the two factors was observed ( $p = 0.1819$ ), indicating that the effect of the solid-liquid  
 388 ratio on extraction efficiency is independent of the solvent type. One-way ANOVA showed  
 389 significant statistical differences in extraction efficiency across solid-liquid ratios for all  
 390 solvents ( $p < 0.001$ ) (Table S5, Table S6). Tukey's HSD test showed that, extraction efficiency  
 391 increased across all solid-liquid ratios. Based on these analyses, although a 1:15 solid-liquid  
 392 ratio showed the highest extraction efficiency ( $99.47 \pm 0.74\%$ ), considering a practical  
 393 compromise between extraction efficiency and solvent consumption, a solid-liquid ratio  
 394 of 1:10 with an extraction time of 8 h was selected for subsequent solvent reusability studies.  
 395



396  
 397 **Figure 4:** Effect of the solid-liquid ratio on extraction efficiency using different solvents.  
 398 Values are expressed as mean  $\pm$  standard deviation ( $n = 3$ ). Different letters indicate statistically  
 399 significant differences ( $p < 0.05$ ) based on Tukey's HSD test. Statistical comparisons were  
 400 performed within each solvent.  
 401

402



### 403 **3.4 Physicochemical properties of flax oil**

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

#### 407 **3.4.1 Density**

408 The extracted flax oil had a density value of  $0.83 \pm 0.05 \text{ g/cm}^3$ . This value aligns with the range  
409 of  $0.83\text{-}0.91\text{g/cm}^3$  and  $0.92 \text{ g/cm}^3$  reported by Lohani *et al.*,(2015) and Ishag *et al.*( 2019)  
410 respectively for Soxhlet-extracted flax oil.<sup>26, 29</sup>

#### 411 **3.4.2 Viscosity**

412 The measured viscosity of the flax oil was  $52.44 \pm 0.02 \text{ mPa.s}$ . This is consistent with the  
413 values reported in literature  $53 \pm 0.01 \text{ mPa.s}$  by Calligaris *et al.*(2013) and approximately  $54.9$   
414  $\pm 3.5 \text{ mPa.s}$  by Ishag *et al.*(2019).<sup>41, 29</sup>

#### 415 **3.4.3 Saponification value (SV)**

416 The saponification value (SV) or saponification number is used to evaluate the average  
417 molecular weight of all fatty acids in an oil sample.<sup>42</sup> It is defined as the amount of potassium  
418 hydroxide (KOH), usually expressed in milligrams, required to completely saponify 1.0 g of  
419 oil.<sup>43</sup> It can be inferred that the larger the saponification value, the smaller the molecular weight  
420 of the fatty acids. In this study, the SV for the hexane-extracted flax oil was  $204.86 \pm 12.68$   
421 mg KOH/g oil **(Table 1)**. This value is slightly higher than 192.16 and 193.49 mg KOH/g oil  
422 for flax oils extracted through Soxhlet extraction.<sup>44, 43</sup> Similarly, other studies reported slightly  
423 lower SV values of  $185.61 \pm 0.56$  and  $189.79 \pm 0.79 \text{ mg KOH/g oil}$  for flax oil extracted by  
424 Soxhlet.<sup>29, 19</sup> Such variations are likely attributable to differences in the chemical composition  
425 of the flaxseed samples, which can be influenced by factors such as seed maturity. For instance,  
426 one study documented a decrease in SV from 198.12 to 178 mg KOH/g oil with increasing  
427 seed maturity.<sup>38</sup> Additionally, significantly lower SV values  $135.21 \pm 2.01$ ,  $130.23 \pm 3.21$ , and  
428  $122.94 \pm 3.15$  were reported for flax oil extracted using solvent extraction at 8, 12, and 16 h,  
429 respectively, at a 1:10 solid-liquid ratio.<sup>35</sup>

430

431



432 **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).  
 433

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

434

### 435 3.5 Solvent reusability studies

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

#### 439 3.5.1 Solvent recovery (%)

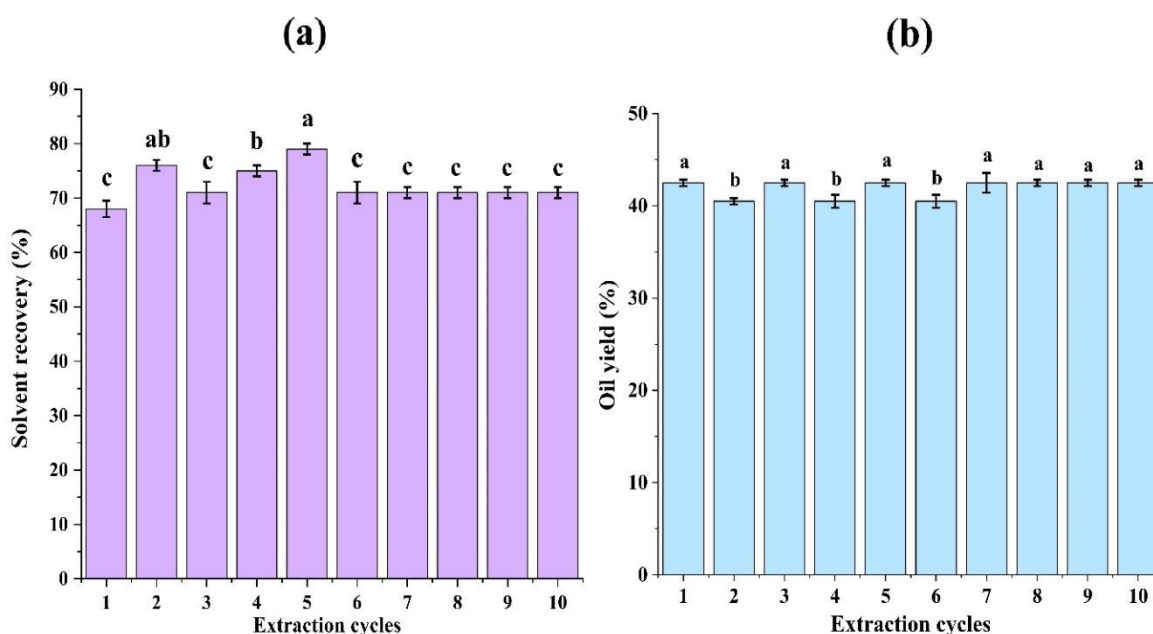
440 After each Soxhlet extraction cycle, the solvent (hexane) was recovered from the solvent-oil  
 441 mixture using rotary evaporation. The solvent recovery across ten extraction cycles was 68%,  
 442 76%, 71%, 75%, 79%, 71%, 71%, 71%, 71% and 71% with an average recovery was 72.4 %  
 443 (**Figure 5a**). An average solvent loss of approximately 21% to 32% per cycle was observed  
 444 which may be attributed to the losses occurring during 3 stages: (i) Soxhlet extraction process,  
 445 (ii) rotary evaporation, and (iii) sampling for analysis. Statistical analysis using one-way  
 446 ANOVA showed significant differences in solvent (hexane) recovery across Soxhlet extraction  
 447 cycles ( $p < 0.001$ ) (**Table S7**). However, despite statistical significance, the variation in solvent  
 448 recovery was within a relatively narrow range (68–79%), indicating no consistent decline in  
 449 solvent recovery with increasing number of cycles. Higher solvent recovery values ( 86.4%,  
 450 89.3% and 86%) have been reported for n-hexane, methyl ethyl ketone and chloroform,  
 451 respectively for the study conducted on the extraction of oil from refinery waste.<sup>45</sup> These  
 452 differences may be attributed to the variations in the extraction techniques, feedstock, and  
 453 recovery conditions. Similarly, Thawornprasert and Somnuk (2024) reported recovery of  
 454 hexane up to six extraction cycles for coffee oil extraction using magnetic stirring followed by  
 455 evaporation<sup>40</sup>. In contrast, our study demonstrated stable recovery without a downward trend  
 456 for ten cycles.



### 457 3.5.2 Oil yield (%) across different cycles

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458 The oil yield remained largely consistent across all ten cycles with oil yield values of 42.5%,  
459 40.5%, 42.5%, 40.5%, 42.5%, 40.5%, 42.5%, 42.5%, 42.5%, and 42.5% (w/w) (**Figure 5b**).  
460 This stability indicates no significant loss in extraction efficiency with solvent reuse up to 10  
461 extraction cycles. Further, statistical analysis using ANOVA revealed statistically significant  
462 differences ( $p < 0.001$ ) in oil yields across extraction cycles. However, the actual variation in  
463 oil yield was quite minimal (approximately 2%), indicating that solvent reuse had no practical  
464 impact on extraction efficiency. For every 10 g of flaxseed extracted, a residual oil cake of 5  
465 to 6 g was obtained. Detailed oil cake data across extraction cycles are provided in the  
466 Supplementary Information (Figure S1). This consistency further supports the stability of the  
467 extraction process during repeated solvent reuse. Hence, from an industrial perspective, solvent  
468 demonstrates excellent potential for reuse across multiple extraction cycles without substantial  
469 loss in extraction performance. In contrast, Thawornprasert and Somnuk (2024) reported an  
470 increase in oil yield from 0.76 to 6.20 wt.% for coffee oil extracted by employing magnetic  
471 stirring followed by evaporation (oven) on repeated extraction, highlighting that the solvent  
472 reuse performance is specific to the extraction method and feedstock.<sup>40</sup>

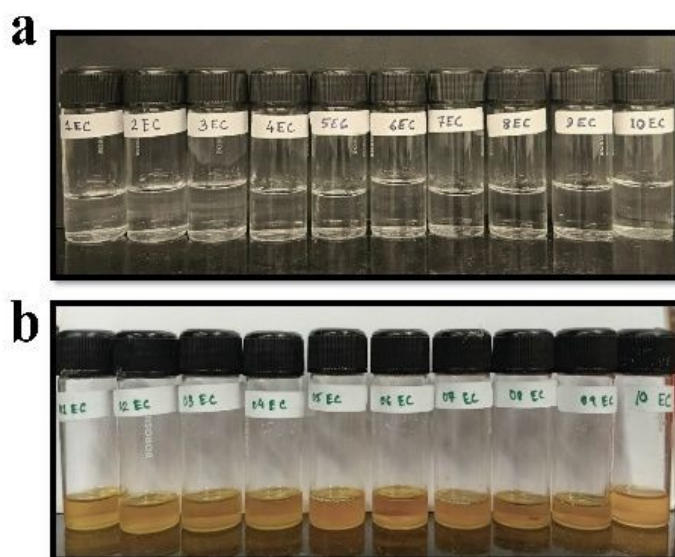


473  
474 **Figure 5.** Impact of solvent (hexane) recycling on Soxhlet extraction parameters over ten  
475 consecutive cycles: **(a)** solvent recovery (%), and **(b)** oil yield (%). Values are expressed as  
476 mean  $\pm$  standard deviation ( $n = 3$ ). Different lowercase letters indicate statistically significant  
477 differences ( $p < 0.05$ ) among extraction cycles, as determined by one-way ANOVA followed  
478 by Tukey's post hoc test.



### 479 3.5.3 Visual appearance

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



491  
 492 **Figure 6.** Visual appearance of (a) recovered solvent (hexane) colour and (b) extracted oil  
 493 colour (hexane as solvent) across ten Soxhlet extraction cycles.

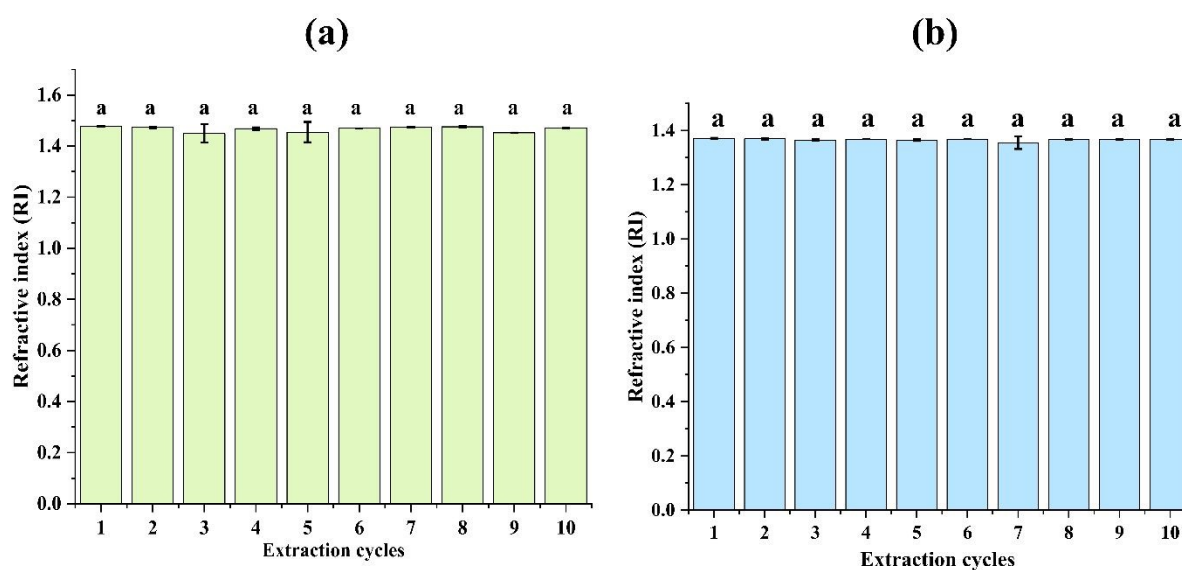
### 494 3.5.4 Refractive index

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



500 respectively.<sup>13</sup> 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.

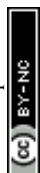
505 The refractive index of pure solvent (hexane) was 1.36. Similar values were observed for the solvent recovered from the repeated extractions (**Figure 7b**). The measured refractive index value of 1.36 was in agreement with the refractive index value of 1.37 reported by Reina and Gonzalez (2010).<sup>46</sup> 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.



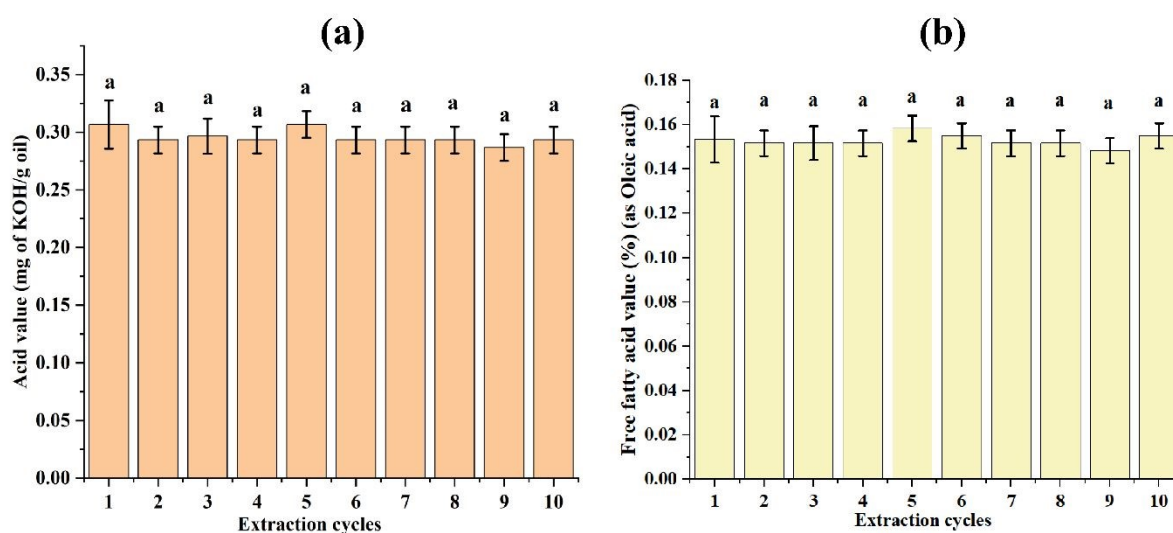
513 **Figure 7** Refractive index stability over ten Soxhlet extraction cycles with solvent reuse for  
 514 **(a)** hexane-extracted flax oil and **(b)** recovered hexane solvent. Values are expressed as mean  
 515  $\pm$  standard deviation ( $n = 3$ ). Means sharing the same lowercase letter indicate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

### 519 3.5.5 Acid value (AV)

520 Acid value is defined as the milligrams of potassium hydroxide (KOH) required to neutralize  
 521 the free fatty acids in 1 g of oil. AV measures the presence of fatty acids not bound to



522 triacylglycerol, i.e. hydrolytic degradation of triglycerides. AV and FFA are interrelated and  
 523 the former are directly proportional to the latter. A higher AV indicates greater degradation  
 524 (poor quality), while a lower AV indicates better oil quality and stability. In this study, the AV  
 525 of flax oil extracted using hexane under optimized conditions was  $0.30 \pm 0.02$  mg KOH/g oil  
 526 (**Table1**). This low AV for flax oil extracted indicated a good quality flax oil with minimal  
 527 hydrolytic degradation. This suggests that the extraction conditions resulted in good quality  
 528 oil, aiding Soxhlet extraction as a reliable reference method for maximum oil recovery and  
 529 high-quality oil. Slightly higher AV values of 0.80 mg KOH/g oil and  $0.76 \pm 0.10$  mg KOH/g  
 530 oil have been reported in literature.<sup>44,37</sup> Much higher AV values (1.68 and 2.23 mg KOH/g oil)  
 531 have been obtained for flax oils extracted using subcritical n-butane extraction and Soxhlet  
 532 extraction, respectively.<sup>19, 29</sup> In another study, AV of flax oil decreased ( $3.2 \pm 0.10$ ,  $2.8 \pm 0.15$ ,  
 533  $1.9 \pm 0.10$ ,  $1.4 \pm 0.08$  mg KOH/g oil) with increasing seed maturity.<sup>38</sup> Importantly, in the  
 534 solvent reusability study, the AV remained statistically unchanged across ten extraction cycles  
 535 ( $p > 0.05$ ), with values ranging from 0.2867 to 0.3067 mg KOH/g oil (**Figure 8a**). One-way  
 536 ANOVA followed by Tukey's HSD test showed no significant differences between extraction  
 537 cycles, with all groups assigned the same statistical grouping. These results indicate that the  
 538 recovered solvent did not cause oil degradation over multiple extraction cycles.



539

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



### 545 3.5.6 Free fatty acid (FFA)

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

### 558 3.5.7 Gas Chromatography (GC) Analysis:

559 GC-FID analysis was performed to evaluate the chemical stability of the recovered hexane  
560 during repeated Soxhlet extraction cycles and to determine the fatty acid composition of the  
561 extracted flax oil, as well as to screen for potential impurities. The chromatogram of pure  
562 hexane showed a single peak at a retention time of 2.09 min, with a total peak area of  $99.64 \pm$   
563  $0.02\%$ . The chromatograms for the solvent recovered after all ten extraction cycles were  
564 virtually identical, with a major peak area ranging from  $99.15 \pm 0.01\%$  to  $99.76 \pm 0.02\%$  at the  
565 same retention time (2.09 min) (**Figure S3**). The absence of additional peaks throughout all 10  
566 cycles indicates that the hexane did not undergo chemical degradation and remained free of  
567 detectable impurities. These results confirm that hexane maintains its quality during repeated  
568 extraction, supporting its suitability for reuse. The chromatograms of the extracted oil showed  
569 the presence of multiple fatty acids such as palmitic acid, palmitoleic acid, cis-heptadecanoic  
570 acid, stearic acid, oleic acid, trans-9-elaidic acid, linoleic acid, linolenic acid and gamma-  
571 linolenic acid (**Figure S2**). These components were identified by comparing their retention  
572 times and area percentages to those of a FAME standard (**Table S8**). The major fatty acids  
573 observed in extracted flax oil were palmitic acid (6.27%), stearic acid (22.73%), linoleic acid  
574 (13.64%), and linolenic acid (49.14%). Minor components were cis-heptadecanoic acid  
575 (6.63%), oleic acid (0.80%), gamma linolenic acid (0.5%) and trans-9-elaidic acid (2.5%)  
576 (**Table S9**). This fatty acid profile is consistent with values reported for flax oil extracted using



577 both Soxhlet and subcritical n-butane methods. For instance, comparable proportions of  
578 palmitic ( $5.96 \pm 0.01\%$ ), linoleic ( $12.93 \pm 0.01\%$ ), and linolenic ( $43.66 \pm 0.02\%$ ) acids have  
579 been reported for oil obtained via subcritical n-butane extraction.<sup>19</sup> The close agreement  
580 demonstrates that repeated use of recovered hexane does not alter the fatty acid composition of  
581 the extracted flax oil.

### 582 **3.5.8 Fourier-Transform Infrared (FT-IR) Spectroscopy**

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

### 604 **3.6 SWOT (Strengths, Weaknesses, Opportunities, Threats) analysis of a process**

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



609 major strengths of solvent recovery and reusability emphasizes on its ability to substantially  
610 reduce the overall quantity of fresh solvent required per extraction cycle, thereby reducing  
611 capital expenditure. This work shows that solvent can be recovered and reused for up to 10  
612 cycles without affecting the quality and yield of the oil. Despite its advantages, certain  
613 limitations possessed by Soxhlet extraction are the larger extraction time and high energy  
614 consumption. The continued extraction over 10 cycles and the long extraction time may affect  
615 the purity and quality of the oil and solvent, which needs further attention while scaling up the  
616 process at an industrial scale. Furthermore, Soxhlet extraction is inherently slower and more  
617 energy-intensive compared to extraction technologies such as supercritical CO<sub>2</sub> and ultrasonic-  
618 assisted extraction, which may limit large-scale applications. Further opportunities include  
619 faster extraction with integration of field-assisted based technology for oil extraction to  
620 improve the yield, screening of different sustainable solvents such as natural deep-eutectic  
621 solvents to improve the solvent recovery. Appropriate use of solvent is a crucial factor while  
622 adopting any process or technology. Lack of knowledge and the urge for generating profits by  
623 small to medium-scale enterprises, resulting in repeated use of solvents, degrades the quality  
624 of oils and bioactives. It may also affect the product quality, safety, sensory attributes, and  
625 shelf-life, affecting market acceptance. Further, increased monitoring and restriction on certain  
626 solvent types are two ways to avoid the threats and ensure sustainability.

#### 627 4. Conclusion

628 This study systematically standardized the extraction process parameters for flax oil and  
629 demonstrated the feasibility of solvent recovery and reuse. With hexane as the solvent, a  
630 40.48% yield was achieved at a solid-liquid ratio of 1:10 and an extraction time of 8 h.  
631 Statistical analysis confirmed that both extraction time and solid-liquid ratio significantly  
632 influenced the oil yield. Also, the absence of significant interaction between solvent type and  
633 solid-liquid ratio on oil yield indicates a consistent trend across all three solvents studied in this  
634 work. One of the key future contributions of this work includes insights on the recovery of  
635 solvent and its reusability across multiple extraction cycles. Solvent reusability studies  
636 demonstrated that hexane can be effectively recovered and reused for up to ten Soxhlet  
637 extraction cycles without significant changes in oil yield, physicochemical properties, and fatty  
638 acid composition. The average solvent recovery per cycle was approximately 72%. FT-IR  
639 analysis confirmed that no new peak appeared in the recovered solvent; only minor intensity  
640 changes in characteristic peaks appeared from the fourth cycle onward, likely due to  
641 co-extracted residues. However, these did not result in measurable changes in oil quality or



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

## 649 Abbreviations

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

650

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## 656 CRediT Authorship Statement

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

## 663 Declaration of competing interest

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



666 **Data availability**View Article Online  
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667 The data supporting this article have been included as part of the Supplementary Information.

668 **Appendix A. Supplementary data**

669 The Supplementary data can be found online.

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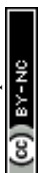
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## **Data Availability Statement**

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

