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# **Alternative solvents for lipid extraction and their effect on protein quality in black soldier fly larvae**

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## **Highlights**

- Comparison of alternative solvents for lipid extraction from black soldier fly.
- Lipid extraction was based on theoretical and experimental data.
- 2-methyloxolane (2-MeO) was found to be the ideal green solvent.
- Defatted BSF flour with 2-MeO had relatively better protein quality parameters.

# **Alternative solvents for lipid extraction and their effect on protein quality in black soldier fly larvae**

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1 **Abstract**

2 Scrutiny of alternative solvents for the extraction of lipid constituents from black soldier fly  
3 (BSF) was the main theme of this research. The present investigation compared a wide array  
4 of solvents for the extraction of desired components theoretically using tools like Hansen  
5 solubility parameters (HSP), conductor-like screening model for real solvents (COSMO-RS)  
6 and technical data (ACD labs) with the application of hurdle technology for solvent  
7 screening. The ideal solvent selected was 2-methyloxolane (2-MeO) which was employed for  
8 conventional and multistage cross-current lipid extraction and the experimental data obtained  
9 was compared with that of n-hexane extract. Fatty acid profile, lipid class, bioactivity of the  
10 oils were analysed and were in good correlation with the theoretical prediction. The kinetics  
11 and diffusion modelling for the extraction system was proposed. The effect of solvent on  
12 protein quality parameters like protein dispersibility index, solubility in alkaline solution,  
13 urease index in the defatted flour was elucidated. The defatting step had no deleterious effect  
14 on the molecular weight distribution of soluble proteins. Overall the study manifested that 2-  
15 MeO had better lipid recovery, enhanced bioactivity in the BSF oil, and relatively better  
16 protein quality in the defatted flour.

17 **Keywords:** Alternative solvents; Black soldier fly; 2-methyloxolane; Lipids; Protein quality.

18

## 19 **1. Introduction**

20 In order to feed the growing population which is roughly estimated to touch 9.6  
21 billion by 2050 (FAO, 2017) and alleviate its negative impact on the food supply chain many  
22 research units, start-ups, think-tanks and other entities are coming up with innovative, greener  
23 alternative solutions to fix the anticipated demand-supply gap. Among the multifarious  
24 options being considered to handle the predicaments, utilisation of insects for food and feed  
25 applications are gaining significant traction. In particular, *Hermetia illucens* or the Black  
26 Soldier Fly larvae (BSFL) belonging to the Diptera order is being contemplated as a  
27 promising substitute to replace the conventional protein sources to an extent. Industrial  
28 rearing, processing, and valorization of BSFL comes with its own challenges, yet their nature  
29 to aggregate the micro, macro-nutrients (Barroso et al., 2017) present in the feeding medium  
30 thereby giving proteins, lipids, chitin derivatives, bioactive peptides, and organic manure  
31 makes them an attractive candidate for numerous applications.

32 *Hermetia illucens* or BSFL for bio-conversion of discarded industrial by-products or  
33 side streams, municipal wastes from urban activities have been extensively studied and the  
34 economic value it imparts makes it a valuable contender among other solutions  
35 recommended. A comprehensive and concise review by Gold et al., 2018 on the  
36 decomposition of biowaste types such as human, animal manures, fruits, vegetable wastes,  
37 etc. was reviewed recently. Manure management system for laying hens treated with BSFL  
38 diminished the manure accumulation by 50% and yielded 42% protein, 35% fat feedstuff is  
39 one such example (Sheppard et al., 1994).

40 Due to the higher lipid concentration (St-Hilaire et al., 2007; Liland et al., 2017),  
41 defatting BSFL should be the primary processing step in the downstream valorization of the  
42 insect biomass. For the production of protein, fat/oil and chitin, to be used in animal feed, the  
43 raw insect materials must undergo a heat treatment process as described in the legislation on  
44 animal-by-products (Regulation (EC) No 1069/2009) (EFSA scientific committee., 2015).  
45 There is a sharp increase in the number of insect companies foraying into the feed market  
46 across continental Europe and North America capitalizing on the new regulation on Novel  
47 Foods (EU 2015/2283) passed by the European Parliament. The fortification or replacement  
48 of conventional feed with BSFL to augment the protein content has been a time tested idea.  
49 The efficacy of BSFL as a feed additive for poultry and a sustainable aquafeed ingredient has  
50 been widely advocated (Bondari & Sheppard., 1981; Kroeckel et al., 2012; Vargas-Abundez

51 [et al., 2019](#)). The benefits of BSFL as a feed component is not limited to its protein content  
52 and quality, its oil as a potential replacement for soybean oil in Jian Carp diets without any  
53 negative effect on growth, feed efficiency in fish fillets was suggested ([Li et al., 2016](#)).

54 In the case of oilseeds, the solvent extraction of oil with n-hexane is preferred as it  
55 promotes easier oil recovery and has a narrow boiling point (69 °C). But, n-hexane reacts  
56 with free pollutants to form ozone, photo chemicals and is said to affect the neural system  
57 when inhaled by humans ([Kumar et al., 2017](#)). Though the idea of complete replacement of  
58 petrochemical solvents for oil extraction is far-fetched, it is imperative that efforts to reduce  
59 the dependency on them and find suitable, economically feasible alternatives for the same  
60 must be given due consideration. Several research articles have articulated the effectiveness  
61 of green solvents for oil extraction from various biomass. Bio-based solvents for the  
62 extraction of oil from rapeseed ([Sicaire et al., 2015](#)), the green extraction of lipids from  
63 oleaginous yeast biomass ([Breil et al., 2016](#)), the green extraction of *Litsea Cubeba* kernel  
64 oil using alternative solvents ([Zhuang et al., 2018](#)) are few examples where a green and eco-  
65 friendly approach for the extraction of oil was proposed.

66 The objective of this work was to probe and identify an optimal green solvent from a  
67 wide array of solvents including alcohols, ethers, esters and terpenes for defatting the BSFL  
68 matrix by combining theoretical and experimental methods. Theoretical data for determining  
69 the interactions between the solute and solvent were realized with tools such as Hansen  
70 solubility parameters, consequently supported with relatively precise data obtained from  
71 conductor-like screening model for real solvent (COSMO-RS) and finally with the  
72 application of hurdle technology for decisive selection of suitable solvent based on the  
73 absolute theoretical data retrieved. It was within the purview of this study to establish an  
74 industrial scale simulation for oil extraction and to develop kinetic modelling for a better  
75 understanding of lipid diffusivity in the solvent medium. Furthermore, to elucidate the lipid  
76 composition data by identifying the fatty acid profile, lipid class constituents, the bioactivity  
77 of BSF oil and compare the effect of solvent on the protein quality parameters in the defatted  
78 BSF flour.

79

## 80 **2. Materials and methods**

### 81 2.1. Larvae harvesting conditions

82 Black Soldier Fly Larvae (BSFL) was provided by a local insect rearing plant based in  
83 Avignon region. The larvae were freeze-dried, milled (<1mm), and stored at -18 °C until  
84 further analysis. The proximate values of the freeze-dried BSFL were crude nitrogen:  
85 40.27±0.62; crude lipid: 36.41±1.29; ash content: 9.01±0.13; moisture: < 3% (AOAC, 1990)

### 86 2.2. Solvents, Standards and Reagents

87 All solvents for extraction and chromatographic analysis were of analytical grade and  
88 purchased from VWR international (Darmstadt, Germany). The solvent 2-methyloxolane also  
89 known as 2-methyl tetrahydrofuran (CAS: 96-47-9) was sourced from Honeywell, Sigma-  
90 Aldrich Co, St. Louis (MO, USA). Standards: Supelco 37 FAME mix, DL- $\alpha$ -palmitin,  
91 glyceryl 1,3-dipalmitate, glyceryl tripalmitate, palmitic acid, phospholipid mixture were  
92 purchased from Sigma-Aldrich (USA). Ergosterol, 98% was procured from Acros organics  
93 (Germany). Milli-Q water was used for electrophoresis and the protein ladder was sourced  
94 from Bio-Rad.

## 95 **2.3. Extraction**

### 96 2.3.1. Conventional Soxhlet

97 The freeze-dried BSFL powder 25 g was taken in a cellulose thimble and subjected to  
98 exhaustive Soxhlet extraction for a period of 6 h with 250 mL of n-hexane and 2-MeO  
99 solvents respectively. To achieve complete lipid removal the reflux was temporarily stopped  
100 every two hours and the sample inside the thimble were mixed thoroughly to facilitate  
101 percolation and reduce agglomeration in the matrix. The solvents were collected after 6 – 7 h  
102 to establish approximately similar extraction cycles and then evaporated under reduced  
103 pressure in a rotavapor. The yield was calculated gravimetrically and extractions were carried  
104 out in triplicates.

### 105 2.3.2. Multistage cross-current

106 To mimic the industrial scale oil extraction a multistage cross-current extraction  
107 system was set up, wherein three stages of sequential conventional extraction (Fig. 3) was  
108 used for maximum oil solubilization in the solvents. The powdered matrix 10 g was mixed  
109 with 100 mL solvent for 1 h under constant agitation (150 rpm). Later the solvent was



110 collected and the residue matrix was subjected to another stage of extraction by infusing 100  
111 mL of fresh solvents with both n-hexane and 2-MeO respectively. This above-mentioned step  
112 was repeated again, where the solvent was collected and the residual matrix was extracted  
113 again with fresh solvents. The extractions were performed at 55 °C and temperature was  
114 maintained using a Huber Pilot system. Therefore in total three stages of conventional  
115 extraction was executed and the yield at every stage was noted and the cumulative yield at the  
116 end of three stages was compared to conventional Soxhlet extraction results.

### 117 2.3.3. Kinetics

118 The kinetics study comparison to find the solubilization efficacy of both the solvents  
119 (n-hexane and 2-MeO) was established by placing 10 g of freeze-dried powder in 100 mL  
120 solvent at 55 °C. To understand the lipid extraction with respect to time in both solvents  
121 approximately 1 mL of clear solvent was collected from the extraction flask within specific  
122 intervals (1,3,5,10,15,20,30,60,90,120,150, and 180 min) and evaporated at 40 °C under  
123 nitrogen using a block heater. The yield was calculated and extrapolated to get results for  
124 100g of dry powder.

### 125 2.3.4. Diffusion model

126 During extraction the solvent system was perfectly agitated, therefore, the major  
127 mass resistance during extraction was the internal diffusion of lipid within larvae powders  
128 (Baümler et al., 2017). In this case, the extraction process can be theorized by the diffusion  
129 model based on Fick's second law. Several hypotheses were given prior to simulation (Tao et  
130 al., 2017):

131 a) Larvae powders were regarded as spherical geometry and lipids were initially  
132 homogeneously distributed within larvae powders. The radius of the larvae powders was 0.5  
133 mm.

134 b) The diffusion coefficient of lipid did not change throughout the extraction process. c)  
135 Lipid content in larvae particles changed with time and position.

136 d) No external mass resistance was taken into consideration due to external agitation.

137 The diffusion equation for spherical geometry is written as (Tao et al., 2017):

$$138 \frac{\partial c_s}{\partial t} = D_e \left( \frac{1}{x^2} \frac{\partial}{\partial x} \left( x^2 \frac{\partial c_s}{\partial x} \right) \right) \quad (1)$$

139 where  $C_S$  is lipid concentration within larvae particle ( $\text{g}/\text{m}^3$ ),  $D_e$  is effective diffusion  
 140 coefficient for lipid ( $\text{m}^2/\text{s}$ ),  $x$  is the radial distance in the diffusion direction (m), and  $t$  is time  
 141 (s).

142 The initial conditions are  $C_S = C_{S,0}$  for the solid phase and  $C_L = 0$  for the liquid phase.  
 143  $C_{S,0}$  is the initial concentration of lipid in larvae particles ( $\text{g}/\text{m}^3$ ) and  $C_L$  is the concentration of  
 144 lipid in solvent ( $\text{g}/\text{m}^3$ ).

145 The boundary conditions for Eq. 1 are:

$$146 \left( \frac{\partial C_S}{\partial x} \right)_{x=0} = 0 \quad (2)$$

$$147 -D_e A \left[ \frac{\partial C_S(x,t)}{\partial x} \right]_{x=r} = V \frac{dC_L(t)}{dt} \quad (3)$$

148 where  $A$  is the surface area of larvae particles ( $\text{m}^2$ ),  $V$  is the volume of solvent used for  
 149 extraction ( $\text{m}^3$ ),  $r$  is the radius of larvae particles (m).

150 The “*pdepe*” function in Matlab, R2010a (The MathWorks, Inc., MA, USA) was used to  
 151 solve the aforementioned parabolic partial differential equation (Tao et al., 2019). To be  
 152 exact, the original partial differential equation (Eq. 1) was first discretized spatially. After  
 153 that, the resulting ordinary differential equations were integrated in time and solved by the  
 154 *pdepe* solver. The  $D_e$  value was adjusted iteratively to fit the experimental data, thus  
 155 minimizing the root mean square error (*RMSE*) between experimental and predicted the  
 156 content of lipid in larvae particles:

$$157 RMSE(\text{g}/\text{m}^3) = \sqrt{\frac{1}{n} \sum_{i=1}^n [C_{S,p}(t) - C_{S,e}(t)]^2} \quad (4)$$

158 where  $C_{S,e}$  and  $C_{S,p}$  is the experimental and predicted values of lipid content in larvae  
 159 particles ( $\text{g}/\text{m}^3$ ), respectively.  $n$  is the number of experimental points.

160 Once the optimized  $D_e$  value was obtained, three statistical indicators, including  $R^2$   
 161 (coefficient of determination), *RMSE* and absolute average deviation (*AAD*) were calculated  
 162 using the data about lipid extraction yield to test the predictive accuracy of the diffusion:

$$163 R^2 = 1 - \frac{\sum_{i=1}^n (Y_e - Y_p)^2}{\sum_{i=1}^n (Y_e - Y_m)^2} \quad (5)$$

$$164 RMSE(\text{mg}/100\text{g DM}) = \sqrt{\frac{1}{n} \sum_{i=1}^n [Y_p(t) - Y_e(t)]^2} \quad (6)$$

165 
$$AAD(\%) = \left[ \frac{\sum_{i=1}^n (|Y_e - Y_p|) / Y_{S,e}}{n} \right] \times 100 \quad (7)$$

166 where  $Y_e$ ,  $Y_p$  and  $Y_m$  are the experimental, predicted values of lipid extraction yield,  
167 respectively (mg/100g DM).  $Y_m$  is the average lipid extraction yield (mg/g DM).

168 Following the numerical simulation results, the distributions of lipid content within  
169 larvae powders at different stages of extraction were visualized by programming in Matlab.

## 170 **2.4. BSFL oil analyses**

### 171 2.4.1. Fatty acid profile

172 Fatty acid methyl esters (FAMES) were prepared from BSF oil samples by acid-  
173 catalyzed transmethylation (Breil et al., 2016). Ten to fifteen milligram of oil sample was  
174 taken to which 1 mL of methanolic sulfuric acid was added, the mixture was heated in a  
175 heating block to facilitate transmethylation and 1.5 mL of 0.9% NaCl solution, and 1 mL of  
176 GC-FID grade hexane was added after it reached room temperature. The organic layer was  
177 collected and subjected to further analysis. Triheptadecanoin (C17:0; TAG) was used as  
178 internal standard and FAME mix was used to calibrate the system. An Agilent (Kyoto, Japan)  
179 gas chromatography coupled with a flame ionization detector (GC-FID) system was used to  
180 determine the fatty acid profiles of BSF oils. The system was equipped with a BD-EN14103  
181 capillary column with dimensions 30 m × 320 μm × 0.25 μm. The carrier gas (He) velocity  
182 was set at 33 cm.s<sup>-1</sup>. The sample injection volume was 2 μL and two washes with n-hexane  
183 were executed to avoid carryovers after every sample run. The detection was enabled in split  
184 mode (split ratio 1:20), the injection temperature was set at 250 °C. The oven temperature  
185 gradient was initially 50 °C for 1 min and then increased at a constant rate of 20 °C/min from  
186 50 °C to 180 °C and then raised from 180 °C to 220 °C at a rate of 2 °C/min. Once it reached  
187 230 °C the temperature was maintained for a period of 10 min and the fatty acids were  
188 identified based on retention time and standards used for calibration.

### 189 2.4.2. Polar and neutral lipids quantitation

190 High-performance thin-layer chromatography (ATS 5 automatic TLC sampler, ADC  
191 2 automatic developing chamber, CAMAG 3 TLC scanning densitometer) was used for  
192 separation, identification and relative quantitation of the lipid classes of BSF oil extracted  
193 with n-hexane and 2-MeO. All stock solutions were prepared in chloroform and 10 mg of  
194 sample lipid fraction was used for quantitation. A known volume of lipid extract was loaded

195 on 20 × 10 cm Silica gel 60 F254 HPTLC plates. Polar lipids were separated with eluent A, a  
196 mixture of methyl acetate/isopropanol/chloroform/methanol/KCl (0.25%) in a ratio of  
197 25:25:25:10:9 (v/v/v/v/v). Neutral lipids were eluted with eluent B, a mixture of n-  
198 hexane/diethyl ether/glacial acetic acid in a ratio of 70:30:2 (v/v/v). Both plates were allowed  
199 to reach a height of 7 cm from the origin, then dried and was dipped in a primuline dye  
200 reagent (10 mg of primuline, 160 mL of acetone, 40 mL of distilled water) for better  
201 visualization of the lipid classes. Lipid standards were used to identify and quantify the lipid  
202 classes in BSF oil.

### 203 2.4.3. Total polyphenol content (TPC) and radical scavenging capacity (RSC)

204 Twenty microliters of appropriate dilutions of the lipid samples or gallic acid  
205 (standard) in methanol were taken in a 96-well microplate and 80 µL of 7.5 % Na<sub>2</sub>CO<sub>3</sub>  
206 solution was added and allowed to equilibrate at room temperature for 10 mins. Rapid  
207 addition of 100 µL of 1N Folin-Ciocalteu reagent was completed and absorbance was read at  
208 750 nm for every 5 min over a period of 60 min. Distilled water was used as blank and results  
209 were calculated as gallic acid equivalents (GAE).

210 Similarly, for RSC, 50 µL of samples in the methanolic phase was allowed to react  
211 with 0.5 mM methanolic DPPH<sup>\*</sup> radical for 60 mins and the absorbance was measured at 520  
212 nm. Methanol was used as blank, trolox was used for generating the standard curve and  
213 results were expressed in trolox equivalent (TE).

## 214 **2.5. Defatted BSFL flour analyses**

### 215 2.5.1. Protein molecular weight distribution

216 Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) was used  
217 to determine the molecular weight distribution of soluble proteins from BSFL. The proteins  
218 were extracted according to [Janssen et al., 2017](#) with few modifications. The dry BSFL  
219 powder was placed in citric acid – disodium phosphate buffer (1:10; w/v) and was gently  
220 mixed with a magnetic stirrer. After 60 min, the mixture was centrifuged at 9000 rpm for 20  
221 min and the supernatant collected was dialyzed at 18 °C with a dialysis tubing having a cut-  
222 off value of 12- 14 kDa and then the dialyzed fraction was subjected to lyophilization to  
223 obtain the soluble proteins. The soluble protein 2 mg/ml in milli-Q water was denatured by  
224 addition of equal volume of Laemmli sample buffer (65.8mM Tris-HCl at pH 6.8, 26.3%  
225 (w/v) glycerol, 2.1% SDS, 0.01% bromophenol blue, 5% 2-mercaptoethanol) at 90 °C for 5

226 mins and the electrophoresis was run in a Bio-Rad mini-PROTEAN system using TGX pre-  
227 cast gels (4-15%).

#### 228 2.5.2. Protein quantification, dispersibility index, solubility

229 The nitrogen content in different BSFL solid fractions were analysed by Kjeldahl  
230 (Buchi speed digester K-425 system) and the protein content in liquid fractions was  
231 calculated by Lowry method with BSA as standard. The defatted BSFL flour protein quality  
232 was evaluated according to the quality analyses manual for soybean products in the feed  
233 industry ([Van Eys et al., 2004](#)) where the protein dispersibility index (PDI), protein solubility  
234 in 0.2% potassium hydroxide solution, urease index, and the absorbance at 420 nm were  
235 identified.

### 236 **2.6. Theoretical predictions**

237 For an effective understanding of theoretical data that are generated by tools such as  
238 Hansen, COSMO-RS and ACD labs it is essential to establish a local solute solvent database  
239 which is specific to the sample chosen for analysis (BSF) and chemical constituents of  
240 interest present in them. In this case solvents of different polarity (n-hexane, ethanol, iso-  
241 propanol, methyl acetate, ethyl acetate, ethyl lactate, dimethyl carbonate, 2-methyl  
242 tetrahydrofuran, cyclopentyl methyl ether,  $\alpha$ -pinene, d-limonene, p-cymene) and solutes  
243 generally present in the lipid fraction of BSF were chosen after careful literature review.  
244 Preliminary parameters was set up with solutes belonging to various classes of the lipid  
245 fraction such as free fatty acids (FFA– Lauric Acid), monoglycerides (MAG– Glycerol 1-  
246 laurate), diglycerides (DAG– Glycerol 1,2-dipalmitate), triglycerides (TAG- Lauric  
247 triglyceride), Vitamin E (VE1–  $\alpha$ -tocopherol; VE2–  $\gamma$ -tocotrienol), sterols (ST1-  $\beta$ -sitosterol;  
248 ST2- Cholesterol) , and pigments (CA1-  $\beta$ -carotene). The solutes were selected based on  
249 previously reported data ([Liland et al., 2017](#); [Ushakova et al., 2016](#); [Caligiani et al., 2018](#)) on  
250 the lipid fraction of BSF.

251

### 252 2.6.1. Hansen Solubility Parameters (HSP)

253 HSP helps in understanding the solubility of two compounds, more precisely the  
254 miscibility of two components in a medium, it is based on a simple, yet classical principle  
255 “like dissolves like” phenomenon. This principle serves as the rule of thumb in characterizing  
256 the solute-solvent interactions, theoretically the total cohesive energy density ( $\delta_{\text{total}}$ ) is equal  
257 to the square root of sum of the energy densities required to overcome atomic dispersion  
258 forces ( $\delta d^2$ ), molecular polar forces due to dipole moments ( $\delta p^2$ ) and hydrogen bonds  
259 (loss/gain of proton and exchange of electrons) between molecules ( $\delta h^2$ ) and mathematically  
260 represented by the following equation:

$$261 \quad \delta_{\text{total}} = \sqrt{(\delta d^2 + \delta p^2 + \delta h^2)} \quad (8)$$

262 The magnitude of affinity between solute and solvent is directly proportional to the  
263  $\delta_{\text{total}}$  value, higher the  $\delta_{\text{total}}$  greater the affinity. The relative energy difference (RED) is  
264 another parameter that indicates the miscibility of solute in solvents and is calculated:

$$265 \quad RED = \frac{R_a}{R_b} \quad (9)$$

266 where  $R_a$  is the distance of a solvent located inside the Hansen solubility sphere and  $R_b$  is the  
267 radius of the Hansen solubility sphere. The chemical structures and simplified molecular  
268 input line entry syntax (SMILES) notations were fabricated using ACD/ChemSketch  
269 (Toronto, Canada).

### 270 2.6.2. Conductor-Like Screening Model for Real Solvents (COSMO-RS)

271 COSMO-RS is used for the calculation of the thermodynamic properties for solvation,  
272 without any experimental data. It is the best tool for molecular description and solvent  
273 screening based on quantum-chemical approach. The usability of COSMO-RS for  
274 determining the relative solubility index  $\log_{10}(X\text{-solub})$  for sample-specific solutes and  
275 solvents has been well documented in our previous work (Ravi et al., 2018). The sheer  
276 amount of data generated based on the parameters like  $\sigma$ -surface,  $\sigma$ -profile,  $\sigma$ -potential can be  
277 used to predict the compatibility of the solvent for the solubilization of solutes (Fig. 2.). The  
278 calculations were executed in a COSMOthermX'17 program (version C30 release 13.01).  
279 The standard  
280 quantum chemical method triple-valence polarized basis set (TZVP) was used in this study

281 and 55 °C was the temperature used for the solubility prediction to draw parallels with  
282 industrial processing conditions

283

$$284 \quad \log_{10}(x_j) = \log_{10} \left[ \frac{\exp(\mu_j^{pure} - \mu_j^{solvent} - \Delta G_{j,fusion})}{RT} \right] \quad (10)$$

285  $\mu_j^{pure}$ : chemical potential of pure compound j (Joule/mol)

286  $\mu_j^{solvent}$ : the chemical potential of j at infinite dilution (Joule/mol)

287  $\Delta G_{j, fusion}$ : free energy of fusion of j (Joule/mol)

288  $x_j$  : solubility of j (g/g solvent).

289  $\alpha$ -tocopherol ( $\sigma$ -surface) was the solute used in the representative image and for its  
290 solubilization in respective solvents ( $\sigma$  -profile,  $\sigma$ -potential) were chosen for theoretical  
291 calculations. This process is repeated for all potential solutes, thereby generating the  $\log_{10}(x$ -  
292 solub) values which eventually was compared and tabulated (Table 2.)

### 293 2.6.3. Hurdle technology for solvent screening

294 Application of the hurdle concept has been predominantly used for food preservation,  
295 food quality and safety assessment (Khan et al., 2016). It is an excellent decision-making tool  
296 that can assist in numerous applications once the factual hurdles are established. For instance,  
297 Fig. 1. clearly depicts the impediments that are associated with the solvent selection and how  
298 the technical parameters of the solvents are used for solvent screening purposes. A list of  
299 candidate solvents are considered and the technical properties such as Log P, boiling point,  
300 toxicity index and solvent origin were chosen as appropriate hurdles in this work. These  
301 parameters are consolidated (Table 3.) and paves way for taking an informed decision based  
302 on the theoretical data available.

303

### 304 3. Results and discussion

#### 305 3.1. Solvent selection based on theoretical studies

306 Relative energy difference (RED) is the empirical value that denotes the ability of a  
307 solvent to dissolve the solutes of interest present in the sample matrix. The major constituents  
308 of BSFL lipid fraction were selected based on literature review, a generalized approach was  
309 taken for the solute scrutiny, this way a broader class of compounds can be analysed for their  
310 solubility based on Hansen solubility parameters. A representative compound for each class  
311 of solutes: lauric acid for fatty acids, glyceryl 1-laurate for monoglycerides, glyceryl 1,2-  
312 dipalmitate for diglycerides, lauric triglyceride for triglycerides,  $\alpha$ -tocopherol and  $\gamma$ -  
313 tocotrienol for vitamin E, cholesterol and  $\beta$ -sitosterol for sterols and finally  $\beta$ -carotene for  
314 pigments were selected. It is important to understand that the dietary components of the  
315 BSFL heavily influence its chemical composition and is subject to vary drastically based on  
316 rearing conditions.

317 The solvent n-hexane was chosen as a reference and [table 1](#) summarizes the RED  
318 scores that were colour coded for easy identification of solvents better than reference. Among  
319 the overall class of solvents chosen, ethers and terpenes outperformed the rest. Ultimately, 2-  
320 methyloxolane and cyclopentyl methyl ether had the best RED scores for all the solutes  
321 considered indicating their theoretical ability to solubilize a wide range of chemical  
322 constituents. In case of 2-MeO the RED score ranged between 0.73 for TAG and 1.75 for  
323 MAG, similarly, CPME had 0.56 for TAG and 1.76 for MAG as its boundary values. These  
324 values symbolise the solvents relative capacity to solubilize the solutes and n-hexane had  
325 better theoretical solvation than alcohols and esters collectively. If we look at the data to  
326 identify solvents for better solubilization of solutes individually no trend can be found for  
327 example  $\alpha$ -tocopherol had the best solvation in d-limonene and so did cholesterol which in  
328 some cases may not be desirable, hence the model works perfectly for a collectively similar  
329 class of compounds (polar or non-polar).

330 The relative solubility of the lipid contents ([Table 2](#)) in different solvents was given  
331 by COSMO-RS software which uses quantum calculations from sequential, iterative  
332 integration of data generated in the conductor like environment where initially the  $\sigma$ -surface  
333 was generated, then  $\sigma$ -profile and  $\sigma$ -potential were used to calculate  $\log_{10}(x_{\text{solub}})$  value.  
334 The solubilization of solute  $\alpha$ -tocopherol as indicated in the example ([Fig. 2](#)) was compared  
335 with the solvent list wherein the above-mentioned calculations are executed iteratively to



336 generate the relative value. The ideal solvents had a value of 0 meaning theoretically they  
337 were the best solvents for better solubilization of their respective solutes. Again, 2-MeO and  
338 CPME proved to be relatively better than other solvents considered. Thus, the collective  
339 theoretical result with regard to the solvation power of each solvent for select solutes using  
340 Hansen and COSMO-RS determined that 2-MeO and CPME were the best solvents for  
341 extraction of lipid-based chemical constituents from BSFL.

342 Hurdle technology was employed to screen the solvents according to their technical  
343 properties (Table 3) such as Log P, boiling point, toxicity index, enthalpy of vaporization, the  
344 energy required to evaporate 1 metric ton of solvent, and the nature of solvent (petroleum  
345 based, bio-based etc.). These parameters guide in assessing the ecological footprint  
346 associated with usage of solvent for extraction purposes. In the scope of food preservation,  
347 hurdle technologies provide a framework for combining a number of preservation techniques  
348 for achieving an enhanced level of product safety and stability (Gupta et al., 2012). Similarly,  
349 the same concept can be retrieved and applied for solvent screening, the parameters  
350 considered were clustered together as hurdles (Fig. 1) and the solvents were assorted based  
351 on their classes to observe if there was any trend exhibited by any particular class of solvent.  
352 Interestingly, ethers had the upper hand in this scrutiny as well, they were able to surpass all  
353 the hurdles showing the versatility and advantages of employing them for extraction. With all  
354 parameters and theoretical data considered, CPME and 2-MeO were deduced to be the best  
355 suitable solvents and out of the two only 2-MeO is truly a bio-based solvent and produced  
356 from lignocellulosic biomass. CPME manufacturing involves the methylation of  
357 cyclopentanol or the addition of methanol to readily available cyclopentane (Wanatabe et al.,  
358 2007). The mission was to find a green solvent that could potentially replace n-hexane and  
359 can be used industrially for oil extraction and 2-methyloxolane met all the criteria put forth  
360 and therefore was used for further experimental analyses.

### 361 3.2. Solvent performance comparison: yield, fatty acid profile, lipid class

362 Conventional Soxhlet extraction with n-hexane, 2-MeO recovered  $32.51 \pm 0.39\%$  and  
363  $35.83 \pm 1.12\%$  of lipids respectively. The lipid fraction of BSFL comprises of a complex set  
364 of substances, the main reason behind such lipid accumulation is that the adult larvae don't  
365 feed after the pupal instar ends. This is because of the lack of development of functional  
366 mouthparts, rendering them to rely only on the reserves accumulated during larval stages  
367 (Gobbi et al., 2013).

368 The fatty acid profile of oils extracted using n-hexane and 2-MeO were relatively  
369 similar (Table 4), lauric acid (C12) was the major fatty acid with 42.29 %, followed by  
370 linoleic acid (C18:2n6) with 13.91%, palmitic acid (C16) with 13.83%, oleic acid (C18:1n9)  
371 with 11.43%, myristic acid (C14) with 9.36% in decreasing order. These five acids combined  
372 made up almost 90% of the fatty acid profile of BSF oils in both cases. The saturated fatty  
373 acids were the largest class of fatty acids accounting for 69.13%, followed by  
374 polyunsaturated fatty acids responsible for 15.44 % and closely succeeded by  
375 monounsaturated fatty acids taking up 14.34%. The data were consistent with previously  
376 published results (Liland et al., 2017) with a similar trend of fatty acids being reported.  
377 Across all previous research articles that probed the fatty acid profile of BSF, lauric acid was  
378 found to be the principal fatty acid with almost 35-40% present in BSF fed average diets,  
379 without any extreme feed formulations (Caligiani et al., 2018; Ushakova et al., 2016).

380 The neutral and polar lipid classes of BSF oils were identified and quantified using  
381 HPTLC. Monoglycerides, diglycerides, triglycerides, ergosterol and fatty acid classes were  
382 relatively quantified. Free fatty acids were the largest neutral lipid class among the two oils  
383 compared, accounting for almost 62% in the 2-MeO lipid fraction and 48% in the n-hexane  
384 lipid fraction. In BSF oil extracted with 2-MeO, the sequence was as follows FFA > DAG >  
385 ERGO > TAG > MAG, whereas in n-hexane it was FFA > ERGO > DAG > TAG > MAG.  
386 The relative quantity of each class is detailed (Fig. 5) with the HPTLC plate of neutral lipids  
387 that shows the migration distance of each lipid class. Polar lipids in particular phospholipids  
388 were quantified in the 2-MeO BSF oil and were not present in the oil extracted by n-hexane,  
389 the solvent polarity plays a major part in extracting polar lipids, sterols, pigments and waxes.  
390 This explains the increased oil yield when using 2-MeO as a solvent for defatting as it  
391 enhances the overall oil profile by eluting other non-polar constituents present in the matrix.  
392 Phosphatidylethanolamine was the primary polar lipid (42%), followed by  
393 phosphatidylinositol and phosphatidylcholine present in the 2-MeO BSF oil. This was the  
394 first time polar lipids were identified in the lipid fraction of BSF. The absence of polar lipids  
395 in n-hexane BSF oil was anticipated. Authors, Ushakova et al., 2016 presented the  
396 composition of glycerides in BSFL and attempted to identify other components of the lipid  
397 fraction. Dodecanoic ethyl ester, stigmaterol and cholesterol are few of the compounds they  
398 quantified, reiterating the importance of understanding lipid prospects of larval biomass and  
399 its importance in black soldier fly artificial breeding. Likewise, authors (Liland et al., 2017)  
400 vividly elucidated the dietary modulation of BSF with seaweed-enriched media to alter the

401 lipid profile of BSF, where the total vitamin-E content increased by four folds in the 100%  
402 seaweed feed based diet fed to BSF when compared to that of the control. Thus making BSF  
403 a unique one-of-a-kind bioreactor like system which aggregates the desired components  
404 present in the feed. This particular aspect of BSF as an enriching system is underexplored and  
405 can be properly exploited in all field of life sciences and more.

### 406 3.3. Oil diffusion kinetics and industrial modelling

407 While comparing the solvents efficacy to elute lipids from biomass its kinetics aid in  
408 understanding the diffusion mechanism with respect to time under predetermined conditions  
409 like temperature (55 °C), pressure, agitation (200 rpm). In solid-liquid extraction, the solute  
410 of interest in this case oil is readily available and at the very instant that it comes in contact  
411 with the solvent it is freely dispersed in the solvent medium. The readily solubilized oil at  $t =$   
412 0-5 min is termed starting accessibility ( $\delta X_s$ ; g of extract / g of dry matter) signifying the  
413 amount of solute solubilized in a limited time frame via the convection of solvent interacting  
414 with the surface of the biomass. The oil yield in solvents n-hexane and 2-MeO were plotted  
415 against time (Fig. 4a) and the data was extrapolated to 100 g of dry material. The individual  
416 oil yield using the solvents n-hexane and 2-MeO indicate the effective diffusivity achieved,  
417 exhaustive oil recovery was not accomplished with this setup as it was only meant to give  
418 data of oil solubilized as a function of time.

419 In mass transfer terms, solvent extraction occurs in two stages a) solvent-surface  
420 interaction transpires for a short duration, followed by the main mass transfer mechanisms  
421 mediated by various b) penetration processes in the solvent-solute system (capillary forces,  
422 molecular diffusivity, etc.).

423 A multistage cross-current system was incorporated to witness the oil recovery  
424 efficacy of both the solvents in an industrially scalable system. Three stages of extraction  
425 with each stage lasting 1 hour and the solvent replenished at the beginning of every new stage  
426 was executed. Incidentally, the cumulative oil yield extracted with this system was precisely  
427 the same as the results obtained by conventional Soxhlet system which acknowledges the  
428 efficiency and robustness of such system for enhanced oil recovery in a short period of time.  
429 After each stage the oil yield was calculated (Fig. 4b) and 2-MeO had relatively higher yield  
430 gravimetrically in every stage but when the percentage oil recovery was considered n-hexane  
431 (70.2%) had better oil solubilization in stage 1 when compared to that of 2-MeO (66.7%), in  
432 subsequent stages 2-MeO exhibited better recovery than n-hexane. The number of stages for

433 exhaustive lipid recovery can be effectively reduced by supplementing the system with other  
434 intensification techniques like ultrasonication, microwave. Ultrasonication in synergy with  
435 this system amplified the oil recovery from rapeseed cake (Sicaire A.G. et al., 2016).

#### 436 3.4. Diffusion modelling and numerical simulation for lipid extraction

437 The experimental and simulated results of lipid extraction kinetics are illustrated in  
438 [Fig. 6a](#). Evidently, the diffusion model provided a satisfactory description of the evolution of  
439 lipid yield during extraction, although there were certain divergences in some areas, especial  
440 in the early stage of extraction, the overall model was justified for the extraction system.  
441 Meanwhile,  $R^2$  values for both cases exceeded 0.96, and  $AAD$  values were also quite low  
442 ([Table 5](#)). Accordingly, the diffusion model was qualified to model the extraction process and  
443 investigate the mass mechanism using different solvents. The extraction yield at  $t=0$  min was  
444 set at 0, so as to enhance the predictive accuracy.

445 The distributions of lipid content during extraction at 5 and 20 min were visualized in  
446 [Fig. 6b](#). There was a relatively high concentration gradient of lipid within larvae powders at  
447 the early stage of extraction using n-hexane as a solvent. Along with the increase of  
448 extraction time, the lipid concentration gradient decreased. When 2-MeO was used as  
449 extraction solvent, the distribution of lipid within larvae powder was more homogenous than  
450 that using n-hexane as solvent at the beginning of extraction, probably due to the high  
451 extraction rate and fast movement of lipid within the powders.

#### 452 3.5. Bioactivity of BSF oil

453 The total polyphenol content (TPC) and radical scavenging capacity (RSC) of the oils  
454 were examined ([Table 6](#)). BSF oil extracted with 2-MeO had a polyphenol content of  
455  $19.03 \pm 1.11$  mg GAE which was 2.5 times higher than what was present in BSF oil extracted  
456 using n-hexane. Comparable results were found in the RSC activity as well, BSF oil (2-MeO)  
457 had higher DPPH radical scavenging capacity. The enhanced bioactivity in BSF oil obtained  
458 using 2-MeO might facilitate lowered lipid peroxidation at ambient and elevated  
459 temperatures and could be used as a functional oil for various dietary supplements in animal  
460 feed applications. The vitamin E content contributes to the bioactivity in the lipid fraction,  
461 pigments like carotenoids can boost the overall crude oil profile of BSF. The results obtained  
462 were compared with refined sunflower oil to establish a reference. Recently, a skincare  
463 product with sterilized BSF oil in their formulation was patented ([US 2018/0256483 A1](#)).  
464 Using alternative solvents to extract oil with enhanced functional properties from insect

465 biomasses can lead to more innovations and its incorporation in cosmetic and therapeutic  
466 formulations.

### 467 3.6. Effect of solvent on protein quality in defatted flour

468 Protein dispersibility index (PDI), is a parameter mostly used to ascertain soybean  
469 meal protein quality. It measures the amount of protein dispersed in water after high-speed  
470 blending of the biomass. Measuring the PDI for BSF meal (defatted flour) gives a rough idea  
471 of protein solubility in water after lipid removal. In this study, the PDI value suggested that  
472 31.6% of the protein in 2-MeO defatted flour and 29.1% of the protein in n-hexane defatted  
473 flour was soluble in water indicating a reduced availability of protein in the n-hexane defatted  
474 meal.

475 The presence of non-protein nitrogen in insects specifically in BSFL results in the  
476 overestimation of true protein available, therefore a nitrogen-to-protein conversion factor  
477 (Kp) value of  $4.76 \pm 0.09$  and  $5.6 \pm 0.39$  was proposed for larvae as such and after protein  
478 extraction respectively (Janssen et al., 2017). This might explain the low PDI values in the  
479 defatted flours as the crude protein quantitation includes the chitin, glucosamine nitrogen as  
480 well, and a Kp value of 6.25 was used for protein assessment. This hypothesis was not probed  
481 further as it was out of the scope of this study.

482 Similarly, protein solubility in 0.2% potassium hydroxide (KOH), urease index (UI),  
483 and the absorbance at 420 nm were quantified (Table 7) as these are the generic parameters  
484 considered for soybean products in the feed industry. The protein solubility in alkaline  
485 conditions for 2-MeO BSF meal was higher than its n-hexane counterpart. As the extraction  
486 and analytical conditions were similar the variation in values can be attributed to the nature of  
487 the solvent. The crude protein content in 2-MeO BSF and n-hexane meal was  $62.16 \pm 0.62\%$   
488 and  $59.09 \pm 0.62\%$  respectively, these results are concurrent, as the lipid recovery was higher  
489 in case of 2-MeO thereby concentrating protein content in its corresponding defatted meal.  
490 These values combined help determine the protein quality, the state of anti-nutritional factors,  
491 and further processing techniques to be employed in soybean meal. The presence of anti-  
492 nutritional factors in BSF might depend on the feed substrate, for now very little is known  
493 about its presence in BSF reared for feed purposes. Effectively, establishing these values for  
494 insect biomass will help potential feed manufacturers relate to the properties of the defatted  
495 flour of their respective insect products and compare them to existing industry standards.  
496 Insects particularly BSF as a dietary feed for livestock, arctic salmon, turbot, crustaceans were

497 adequately reviewed (Barroso et al., 2013; Makkar et al., 2014; Wang & Shelomi., 2017)  
498 projecting the inclusion and infiltration of BSF in feed formulations.

499 The molecular weight distribution of soluble proteins extracted from 2-MeO defatted  
500 BSF flour, n-hexane defatted BSF flour were compared to that of freeze-dried BSF flour (Fig.  
501 7). Almost all proteins belonging to the soluble fractions were within the 25 and 75 kDa  
502 range. The most abundant protein band was close to 75 kDa followed by a sharp band next to  
503 50 kDa, these proteins can be enzymes, muscle proteins, exoskeleton proteins or other  
504 proteins like melanisation-inhibiting proteins as reported by Yi et al., 2013 for *Tenebrio*  
505 *molitor* larvae. Ideally, since there was no visible change in the protein bands observed by  
506 electrophoretic separation it can be assumed that no deleterious effects were imparted during  
507 the defatting step at least in the soluble protein fraction. Further protein extraction,  
508 fractionation, purification might help throw some light on the elusive protein profile of BSF.  
509 The techno-functionality properties of flours and proteins from mealworm and BSFL was  
510 compared by Bubler S. et al., 2016 who also found two soluble proteins from BSFL of which  
511 the most abundant was identified to have a molecular weight of 80.5 kDa and the other 14.3  
512 kDa. Only one protein was designated in the reviewed section of the UniProt database, it was  
513 cecropin-like peptide 1 with accession number: L7VIN3 and molecular mass of 4.84 kDa,  
514 supposedly an antimicrobial peptide active against gram-negative bacteria.

515 The solvent 2-methyloxolane was efficient in lipid recovery and had improved the  
516 protein quality in defatted BSF flour while considering it to that of n-hexane defatted flour,  
517 making it a better solvent than n-hexane in all technical aspects. Addition of intensification  
518 techniques, pre-treatment of the biomass will enhance the extractability and should also be  
519 considered to maximise efficiency. Microwave, ultrasound, thermal treatments were  
520 effectively used for lipid extraction from yeast (Meullemiestre et al., 2016). A novel  
521 Simultaneous Distillation and Extraction Process (SDEP) with alternative terpene solvents for  
522 lipid extraction was successfully demonstrated (Tanzi et al., 2013) proving the growing need  
523 to find alternative green bio-based solvents to replace toxic petrochemical solvents.

#### 524 **4. Conclusion**

525 Numerous solvents were studied for the extraction of lipid constituents from BSFL  
526 biomass. The solvent 2-methyloxolane (2-MeO) was found to be the ideal solvent based on  
527 theoretical prediction and experimental results obtained. Extraction kinetics, diffusivity  
528 modelling, and industrial simulation for lipid recovery were established. The yield was

529 significantly higher in 2-MeO BSF oil in conventional as well as the industrial system.  
530 Lauric, linoleic, palmitic, oleic, and myristic acids were the major fatty acids. Among the  
531 lipid classes, free fatty acids were the major component, phospholipids were identified and  
532 quantified in BSF oil (2-MeO) and were absent in BSF oil extracted with n-hexane.

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## Figure caption.

**Figure 1.** Application of hurdle technology for solvent screening.

**Figure 2.** Representative image for theoretical solubility prediction using COSMO-RS.

**Figure 3.** Graphical representation of the design of experiment.

**Figure 4. a)** BSF oil extraction kinetic curve **b)** Oil yield in multistage cross-current extraction system.

**Figure 5. a)** Relative content of neutral lipids in BSF oil **b)** Relative content of polar lipids in BSF oil **c)** HPTLC plate of neutral lipids.

**Figure 6. a)** Experimental versus predicted values of lipid extraction yields. ●: n-hexane; ●: 2-MeO; The solid lines represent the diffusion model. **b)** Visuals of lipid content distribution within larvae powders during extraction using the numerical simulation results.

**Figure 7.** Molecular weight distribution (kDa) of soluble proteins of freeze dried and defatted flour, FDBSF – Freeze Dried Black Soldier Fly; HR – n-hexane residue; 2-MeO – 2-methyloxolane residue.

**Table 1.** Solvent selection based on RED scores of Hansen Solubility parameters

Solute	FFA	MAG	DAG	TAG	VE1	VE2	ST1	ST2	CA1
Solvent	Relative Energy Difference : RED score								
n-hexane	2.24	3.21	2	1.24	1.4	2.03	1.5	1.6	1.49
Ethanol	3.22	2.23	3.42	4.39	4.39	4.25	4.42	4.28	4.9
Iso-propanol	2.3	1.34	2.47	3.48	3.44	3.33	3.49	3.35	3.97
Methyl acetate	0.89	1.09	1.15	1.69	1.88	2.07	1.91	1.79	2.41
Ethyl acetate	0.42	1.05	0.64	1.28	1.42	1.62	1.47	1.35	1.98
Ethyl lactate	1.55	0.65	1.81	2.69	2.73	2.69	2.76	2.62	3.26
DMC	1.34	0.97	1.62	2.29	2.44	2.54	2.47	2.34	2.97
2-MeO	0.89	1.75	0.98	0.73	0.89	1.09	0.84	0.75	1.27
CPME	0.83	1.76	0.85	0.56	0.73	1.02	0.71	0.61	1.17
$\alpha$ -pinene	1.56	2.55	1.35	0.46	0.49	1.11	0.57	0.68	0.67
d-limonene	1.07	2	0.92	0.55	0.24	0.48	0.23	0.13	0.7
p-cymene	1.45	2.39	1.34	0.53	0.43	0.72	0.29	0.38	0.42

Gray - Reference solvent; Green - Equivalent or better than reference; Red - Worse than reference

\*FFA- Lauric acid; MAG- Glycerol 1-laurate; DAG- Glycerol 1,2-dipalmitate; TAG- Lauric triglyceride;

VE1-  $\alpha$ -tocopherol; VE2-  $\gamma$ -tocotrienol; ST1-  $\beta$ -sitosterol; ST2- cholesterol; CA1-  $\beta$ -carotene

\*\*DMC- Dimethyl carbonate; 2-MeO- 2-methyloxolane; CPME- Cyclo pentyl methyl ether

**Table 2.** Solvent selection based on the solubility index  $\log_{10}(x_{\text{solub}})$  of COSMO-RS

Solute*	FFA	MAG	DAG	TAG	VE1	VE2	ST1	ST2	CA1
Solvent**	Solubility index : $\log_{10}(x_{\text{solub}})$								
n-hexane	-1.0307	-2.1617	-0.6419	-0.1963	0	-0.345	-0.4479	-0.1823	0
Ethanol	0	0	-0.5865	-1.0797	-0.8543	-0.0836	-0.5312	-0.7166	-1.7267
Iso-propanol	0	0	-0.1874	-0.7162	-0.5073	0	-0.2727	-0.4068	-1.3477
Methyl acetate	0	0	0	-0.0394	0	0	-0.2694	-0.255	0
Ethyl acetate	0	0	0	0	0	0	-0.0307	0	0
Ethyl lactate	0	0	0	0	0	0	-0.0798	-0.0743	-0.1278
DMC	-0.2469	-0.3211	-0.7839	-0.6539	-0.5882	0	-0.8265	-0.8554	-0.5794
2-MO	0	0	0	0	0	0	0	0	0
CPME	0	0	0	0	0	0	0	0	0
$\alpha$ -pinene	-0.9093	-1.9464	-0.5993	-0.1173	-0.0119	-0.2729	-0.4623	-0.232	0
d-limonene	-0.7317	-1.6106	-0.3792	0	0	-0.0869	-0.3972	-0.1979	0
p-cymene	-0.7266	-1.5291	-0.4172	0	0	-0.0739	-0.4638	-0.2939	0

Gray - Reference solvent; Green - Ideal solvent; Yellow - Equivalent or better than reference; Red - Worse than reference

\*FFA- Lauric acid; MAG- Glycerol 1-laurate; DAG- Glycerol 1,2-dipalmitate; TAG- Lauric triglyceride; VE1-  $\alpha$ -tocopherol; VE2-  $\gamma$ -tocotrienol; ST1-  $\beta$ -sitosterol; ST2- cholesterol; CA1-  $\beta$ -carotene

\*\*DMC- Dimethyl carbonate; 2-MO- 2-methyloxolane; CPME- Cyclopentyl methyl ether

**Table 3.** Technical parameters for solvent screening

Solvents / Parameters	Log P	Boiling point	Toxicity index	Energy required to evaporate 1 metric ton of solvent (kW.h)	Enthalpy of vaporization [ $\Delta_{\text{vap}}H(T_{\text{bp}})$ ]; kJ/mol
n-hexane	3.9	68	6	120.1	28.85
Ethanol	-0.2	78.37	5	268.6	38.56
Iso-propanol	0.2	82.5	5	225.8	39.85
Methyl acetate	0.2	57.1	5	130.8	30.32
Ethyl acetate	0.7	77.1	5	128.7	31.94
Ethyl lactate	-0.2	154	5	171.0	45.57
DMC	0.2	90	5	134.8	33.05
2-MeO	0.8	80.2	4	126.1	30.74
CPME	1.4	106	4	132.4	33.00
$\alpha$ -pinene	4.4	155	4	142.5	37.83
d-limonene	4.4	176	5	153.8	37.83
p-cymene	4.0	177	5	155.8	39.34

Green – Good score; Yellow – Average score; Red – Poor score (relative comparison).

DMC- Dimethyl carbonate; 2-MO- 2-methyloxolane; CPME- Cyclopentyl methyl ether.

**Table 4.** Relative percentage of fatty acid profiles of BSF oil

Fatty acids	n-hexane (%)	2-MeO (%)
C10	1.02 ± 0.01	1.02 ± 0.00
C12	42.27 ± 0.13	42.29 ± 0.18
C14	9.41 ± 0.01	9.36 ± 0.01
C14:1	0.19 ± 0.01	0.18 ± 0.00
C15	0.18 ± 0.01	0.18 ± 0.00
C15:1	0.06 ± 0.01	0.06 ± 0.00
C16	13.91 ± 0.05	13.83 ± 0.00
C16:1	2.73 ± 0.02	2.67 ± 0.00
C18	2.28 ± 0.01	2.23 ± 0.01
C18:1n9	11.84 ± 0.05	11.43 ± 0.12
C18:2 n6 trans	14.29 ± 0.09	13.91 ± 0.03
C18:3n3	1.41 ± 0.01	1.37 ± 0.01
C20	0.16 ± 0.01	0.16 ± 0.00
C22	0.06 ± 0.00	0.06 ± 0.00
C22:2 n6	0.17 ± 0.01	0.16 ± 0.00
Σ SFAs	69.29	69.13
Σ MUFAs	14.82	14.34
Σ PUFAs	15.87	15.44
Others	0.02	1.09

SFA - Saturated Fatty acids

MUFA - Mono Unsaturated Fatty Acids

PUFA - Poly Unsaturated Fatty Acids



**Table 5.** Effective diffusion coefficients of lipids at different extraction conditions and accuracy of the diffusion model

Solvent	$D_e$ (m <sup>2</sup> /s)	$R^2$	RMSE (mg/100g DM)	AAD (%)
n-hexane	$2.17 \times 10^{-9}$	0.99	0.438	3.27
2-MeO	$6.67 \times 10^{-10}$	0.97	1.013	4.75

$D_e$  – Effective diffusion coefficient for lipid;  $R^2$  – Coefficient of determination; RMSE – Root Mean Square Error; AAD – Absolute Average Deviation

**Table 6.** Total Polyphenol Content and Radical Scavenging Capacity of BSF oil

Description	TPC (mg GAE/g of oil)	RSC (mg TE/g of oil)
Refined sunflower oil (reference)	3.60 ± 0.09	0.79 ± 0.17
BSF oil (n-hexane)	7.42 ± 0.51	0.41 ± 0.03
BSF oil (2-MeO)	19.03 ± 1.11	5.42 ± 0.76

GAE - Gallic Acid Equivalent; TE - Trolox Equivalent.

**Table 7.** Protein quality evaluation of defatted BSF flour

Protein quality parameters	Defatted BSF (n-hexane)	Defatted BSF (2-MeO)
Crude protein; %	59.09 ± 0.62	62.16 ± 0.62
PDI; %	29.09	31.55
PS - KOH; %	78.29	80.6
UI; Δ pH units	0.13	0.15
Abs @ 420nm	0.063	0.071

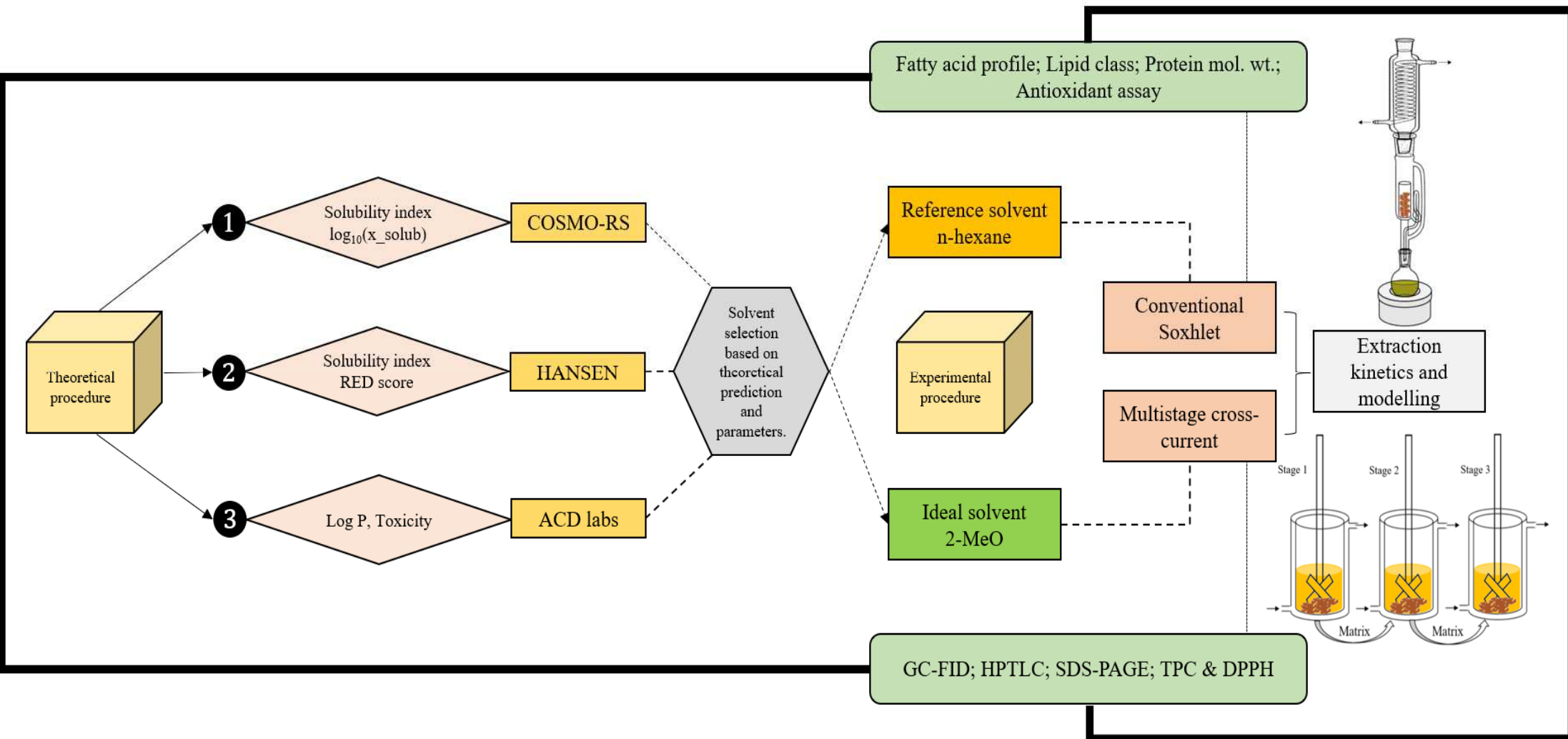
PDI -Protein Dispersibility Index; PS - Protein solubility in 0.2 % KOH.

UI - Urease Index; Abs - Absorbance at 420 nm.

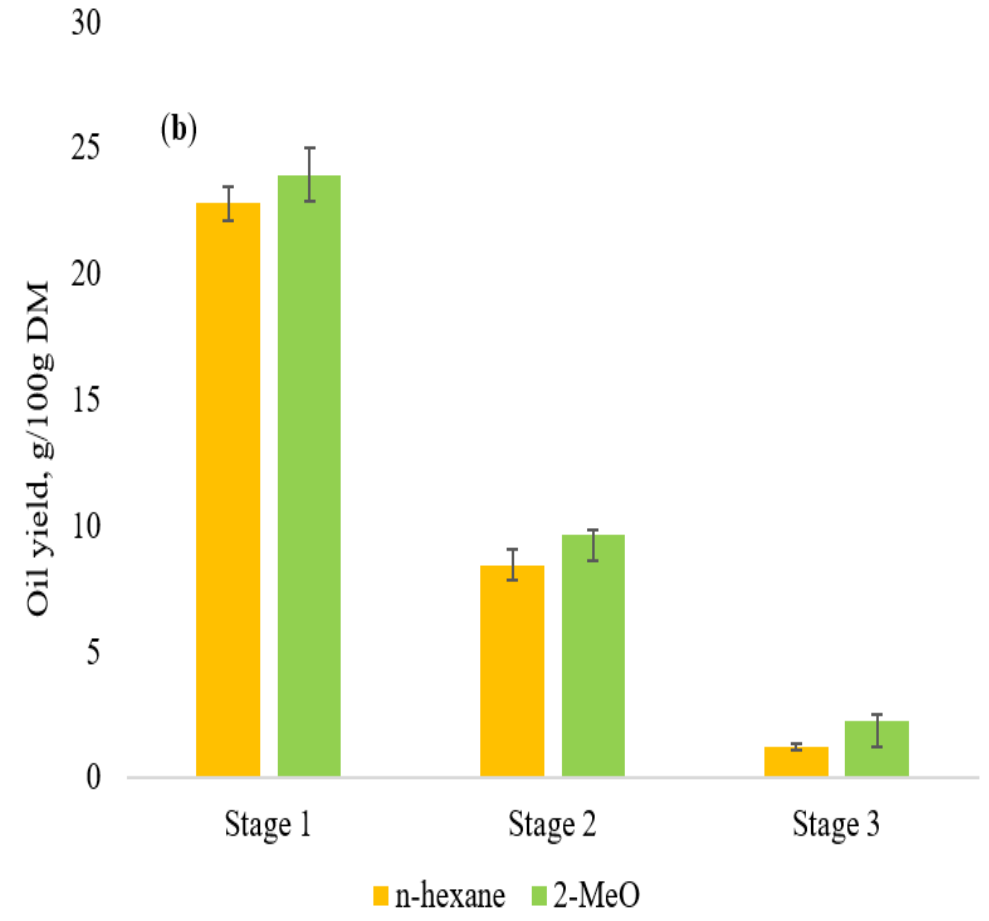
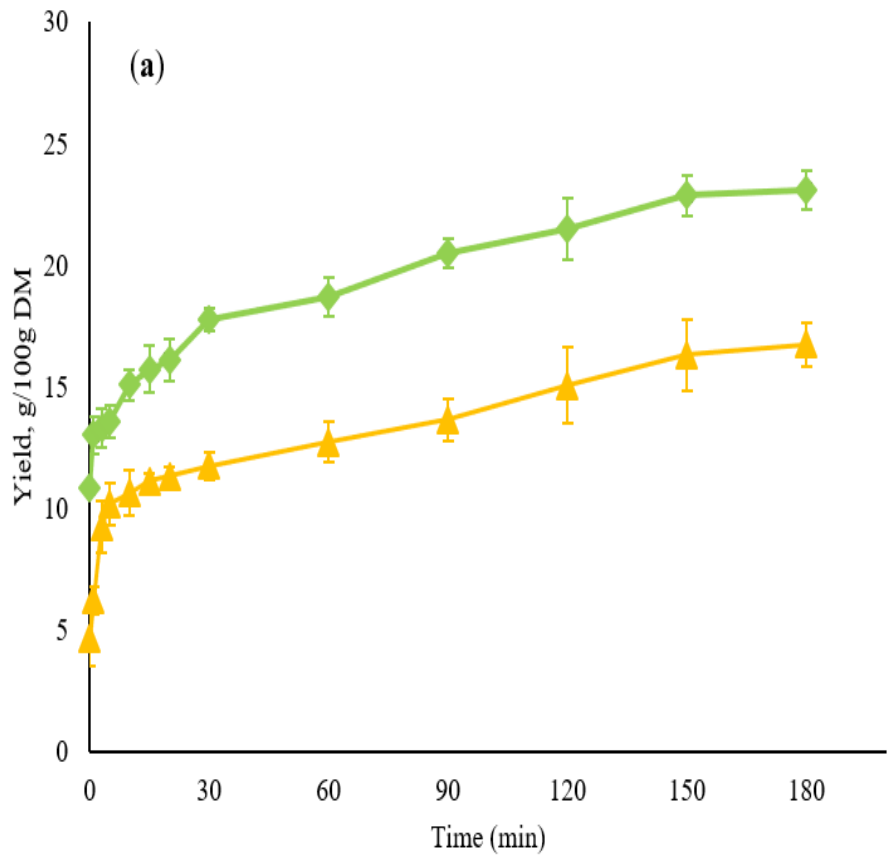




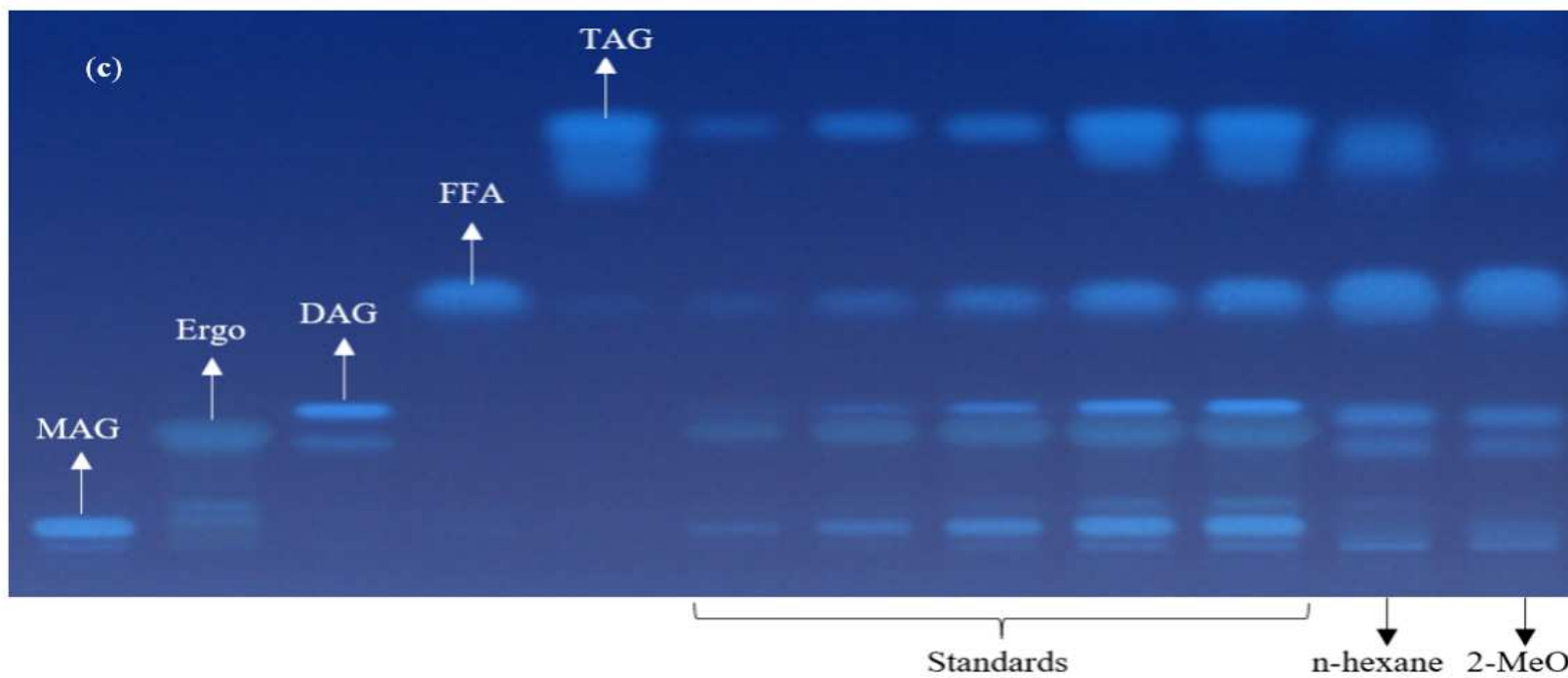
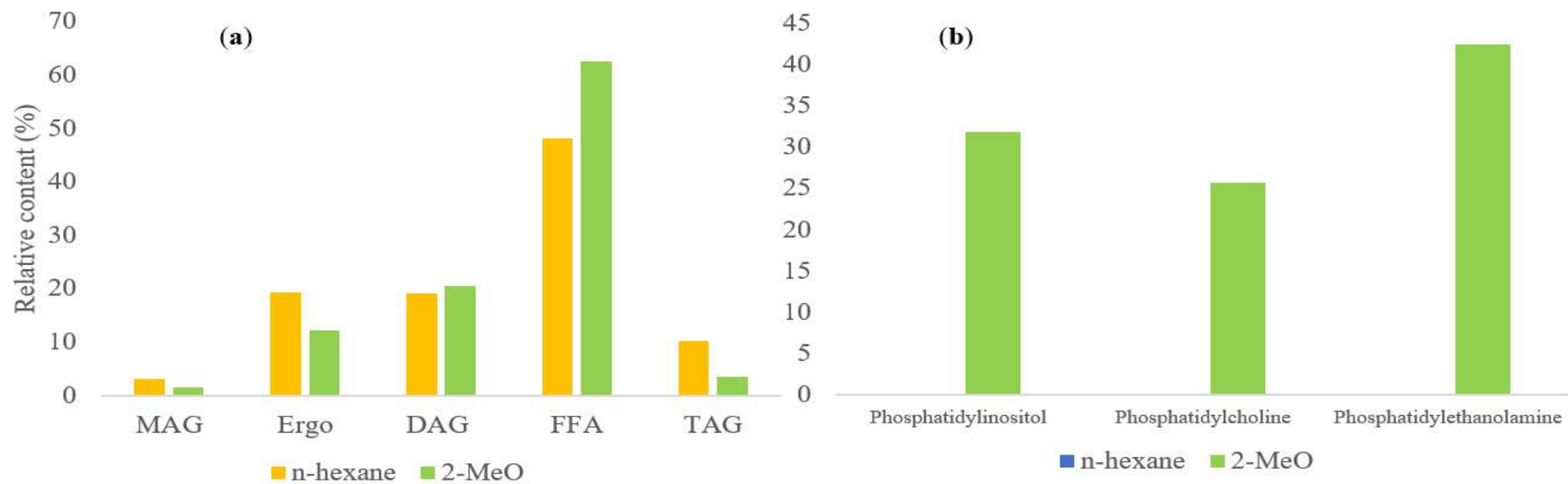
Figure 3.



**Figure 4.**

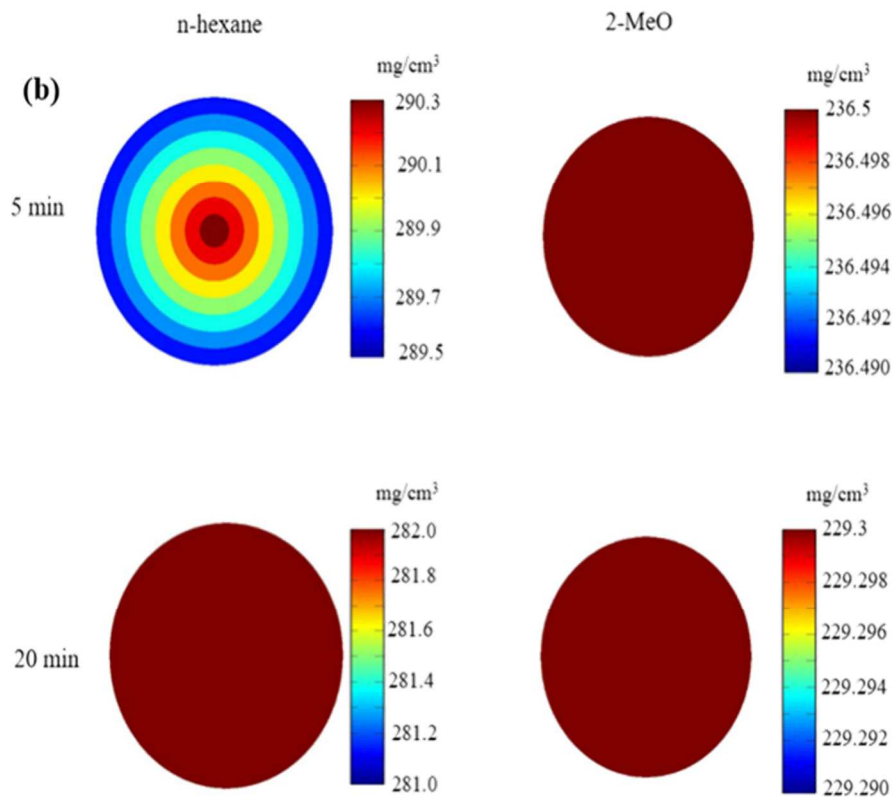
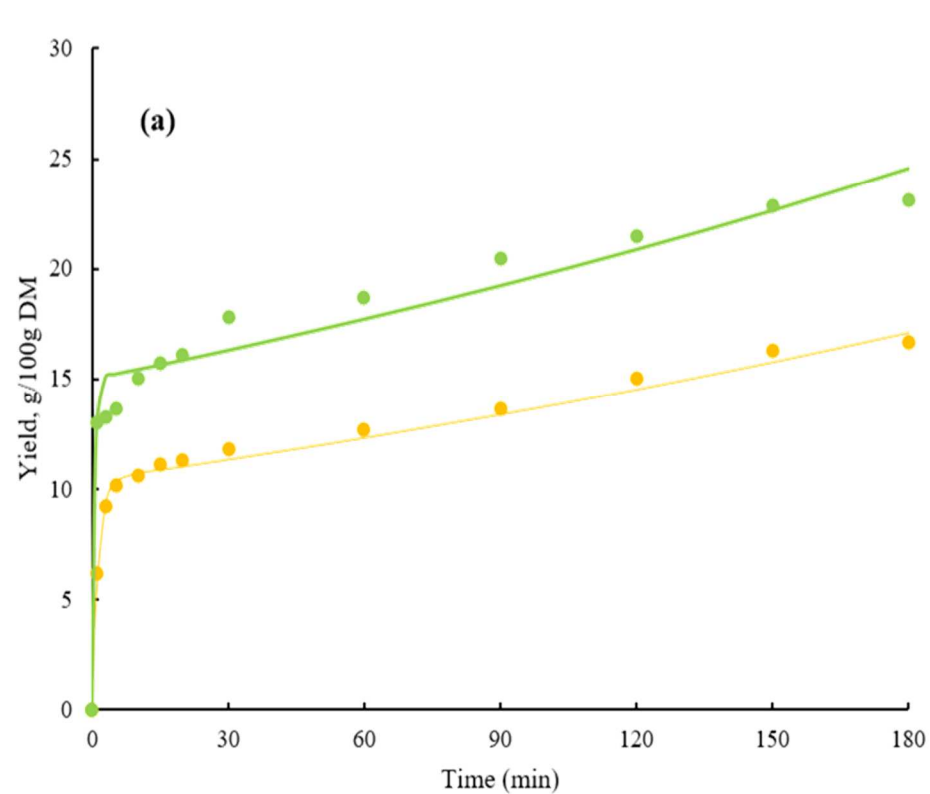


**Figure 5.**





**Figure 6.**



**Figure 7.**

