

**SUPPLEMENTARY INFORMATION**

**Fabrication of nutrient delivery system of docosahexaenoic acid  
nanoemulsions by high energy techniques**

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## 23 **Appendix A. Supplementary data**

24 The following are the supplementary data related to this article:

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26 **Table 1.** Main bands of the FTIR spectra of DHA algae oil.

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| Wavenumber(cm <sup>-1</sup> ) | Functional group                                                 | Mode of vibration      |
|-------------------------------|------------------------------------------------------------------|------------------------|
| 3014.3                        | =C-H ( <i>cis</i> )                                              | Stretching             |
| 2926.8                        | -C-H (CH <sub>2</sub> )                                          | Asym-stretching        |
| 2854.8                        | -C-H (CH <sub>2</sub> )                                          | Sym-stretching         |
| 1751.3                        | -C=O (ester)                                                     | Stretching             |
| 1651.9                        | -C=C- ( <i>cis</i> )                                             | Stretching             |
| 1463.7                        | -C-H (CH <sub>2</sub> , CH <sub>3</sub> )                        | Bending (scissoring)   |
| 1371.3                        | -C-H (CH <sub>3</sub> )                                          | Bending (sym)          |
| 1149.3                        | -C-O and -CH <sub>2</sub>                                        | Stretching and bending |
| 719                           | -(CH <sub>2</sub> ) <sub>n</sub> -and –<br>HC=CH- ( <i>cis</i> ) | Rocking, bending out   |

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36 **Table 2.** Total fatty acid (g/100 g of fatty acid) compositions.

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| Fatty acid composition | Control-DHA<br>algae oil (%) | HSH-DHA-<br>NE (%)      | HPH-DHA-<br>NE (%)       | HSH+HPH-DHA-<br>NE (%) |
|------------------------|------------------------------|-------------------------|--------------------------|------------------------|
| Myristic acid          | 5.660±1.34                   | 5.014±0.49              | 5.210±0.60               | 4.446±0.03             |
| Palmitic acid          | 15.038±1.29                  | 16.765±0.45             | 19.532±0.48 <sup>a</sup> | 17.470±1.60            |
| Stearic acid           | 1.051±0.07                   | 1.224±0.02              | 1.689±0.30               | 1.651±0.50             |
| Oleic acid             | 19.320±1.66                  | 17.954±2.33             | 16.607±0.52              | 17.586±2.30            |
| Linoleic acid          | 1.553±0.01                   | 1.300±0.10 <sup>a</sup> | 1.292±0.12 <sup>b</sup>  | 1.358±0.05             |
| Arachidonic acid       | 0.328±0.06                   | 0.309±0.03              | 0.352±0.08               | 0.271±0.02             |
| Eicosapentaenoic acid  | 0.613±0.18                   | 0.582±0.23              | 0.498±0.03               | 0.568±0.18             |
| Docosapentaenoic acid  | 16.581±0.39                  | 16.357±0.68             | 12.39±2.34 <sup>a</sup>  | 16.522±0.34            |
| Docosahexaenoic acid   | 38.119± 0.07                 | 38.177±0.25             | 36.787±1.20              | 37.502±2.19            |

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39 All the fatty acid composition values are expressed as mean±SD of three  
40 measurements ( $n=3$ ). Different letters are significantly different from control ( $P<0.05$ )  
41 according to the Turkey's test. The lowest value in the same line indicated with a and  
42 the highest value was indicated with b.

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48 **Table 3.** <sup>1</sup>H NMR data of DHA algae oil, HSH, HPH and HSH+HPH-DHA nanoemulsion.

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| Chemical shift value<br>( $\delta$ ) | Multiplicity | Integration value | Position                                           |
|--------------------------------------|--------------|-------------------|----------------------------------------------------|
| 5.32-5.45                            | Multiplet    | 12                | Olefinic proton                                    |
| 2.82-2.89                            | Multiplet    | 10                | five methylene                                     |
| 2.37-2.43                            | Multiplet    | 4                 | two methylene<br>towards carboxylic<br>end         |
| 2.09                                 | Pentet       | 2                 | Methylene between<br>1 and 3 position of<br>C-atom |
| 0.99                                 | Triplet      | 3                 | Terminal methyl                                    |

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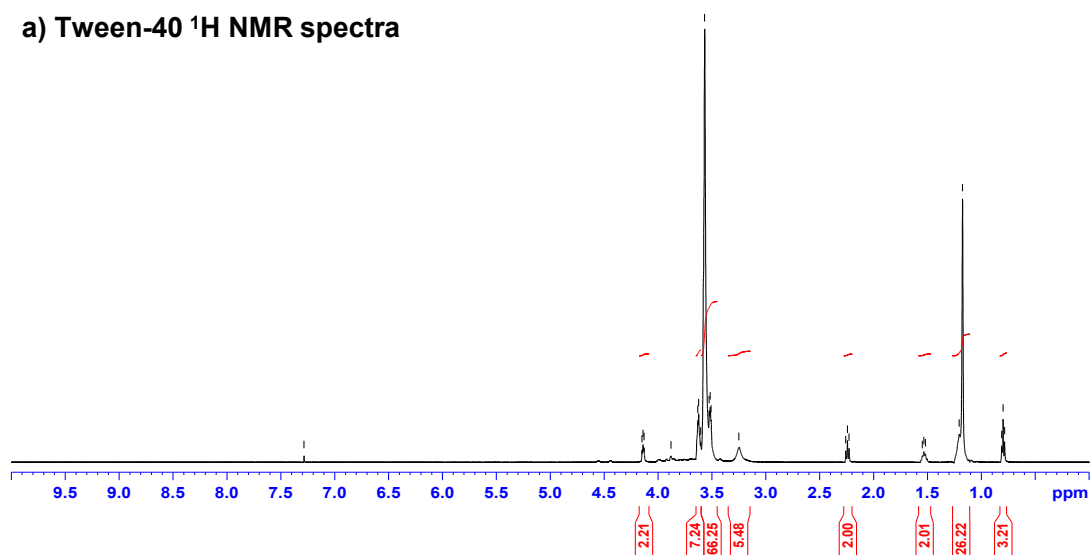
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64 **Appendix B. Supplementary data**

65 **NMR Spectra of Tween-40 emulsifier**

a) Tween-40  $^1\text{H}$  NMR spectra

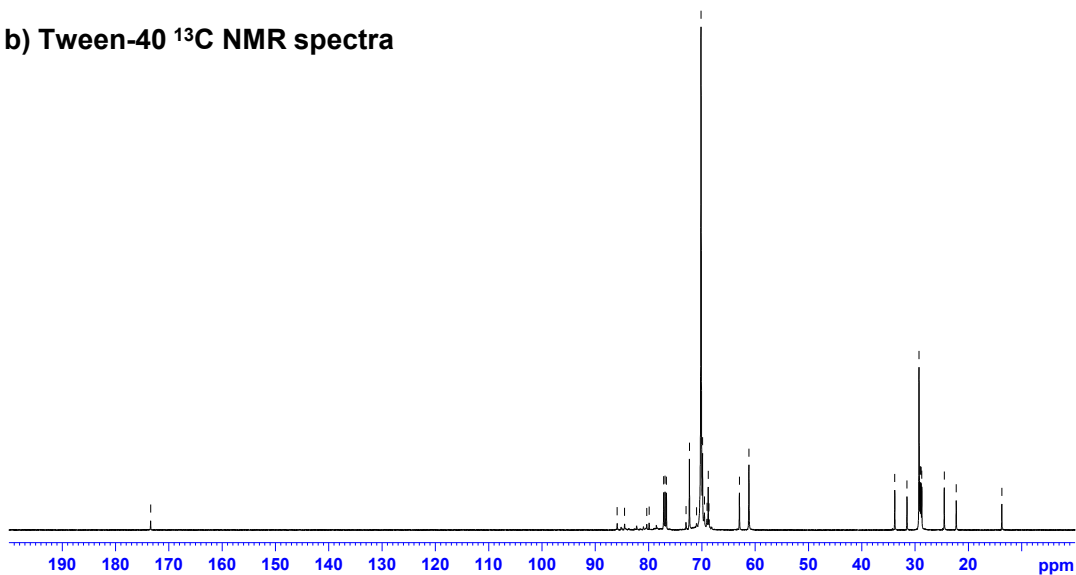


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67 **Fig. 1** Proton NMR spectra of Tween-40 emulsifier

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b) Tween-40  $^{13}\text{C}$  NMR spectra



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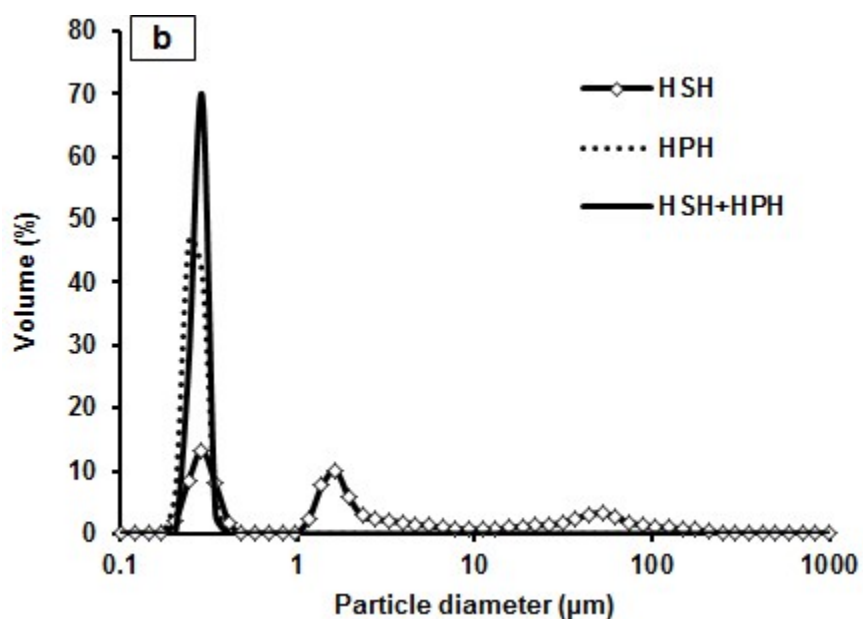
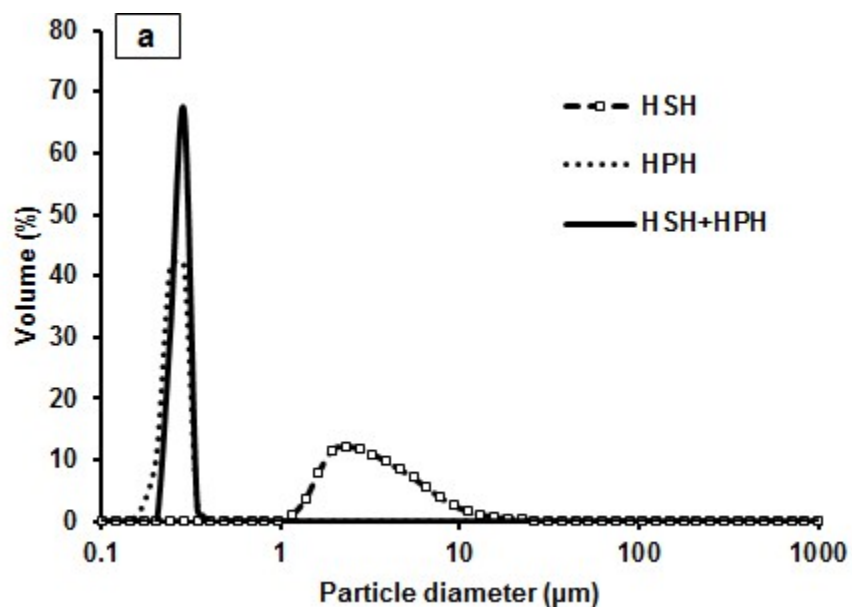
70

71 **Fig. 2** Carbon NMR spectra of Tween-40 emulsifier

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### 73 Appendix C. Supplementary data

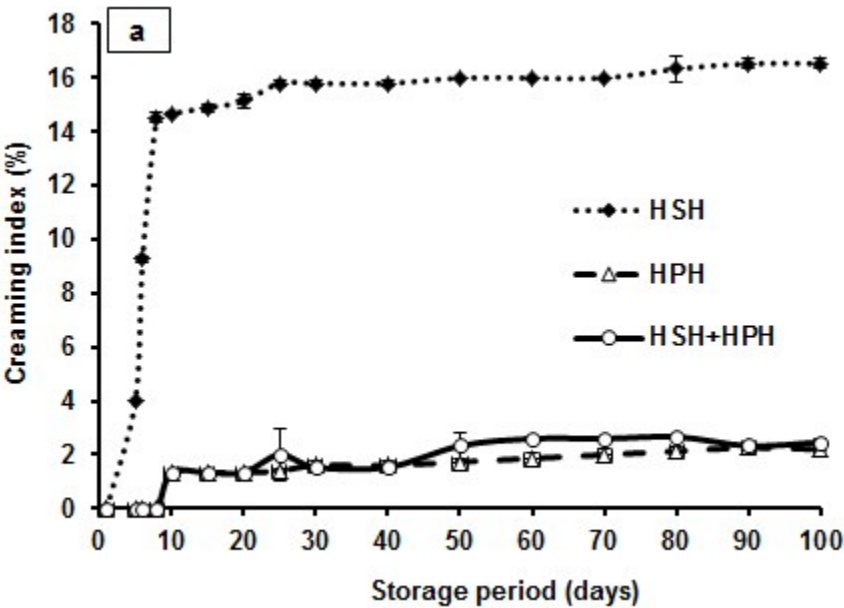
74 Particle size distribution of DHA nanoemulsions at 100th day: a) stored at refrigeration  
75 condition ( $4\pm 1\text{ }^{\circ}\text{C}$ ); b) stored at oven condition ( $40\pm 0.2\text{ }^{\circ}\text{C}$ ).



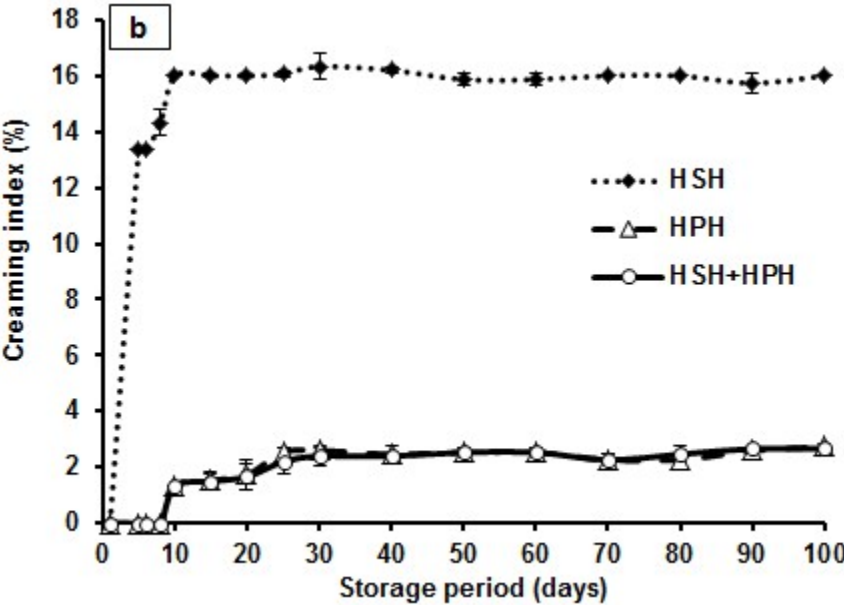
81 **Appendix D. Supplementary data**

82 Creaming stability studies of DHA nanoemulsions: a) stored at refrigeration condition  
83 ( $4\pm1\text{ }^{\circ}\text{C}$ ); b) stored at oven condition ( $40\pm0.2\text{ }^{\circ}\text{C}$ ).

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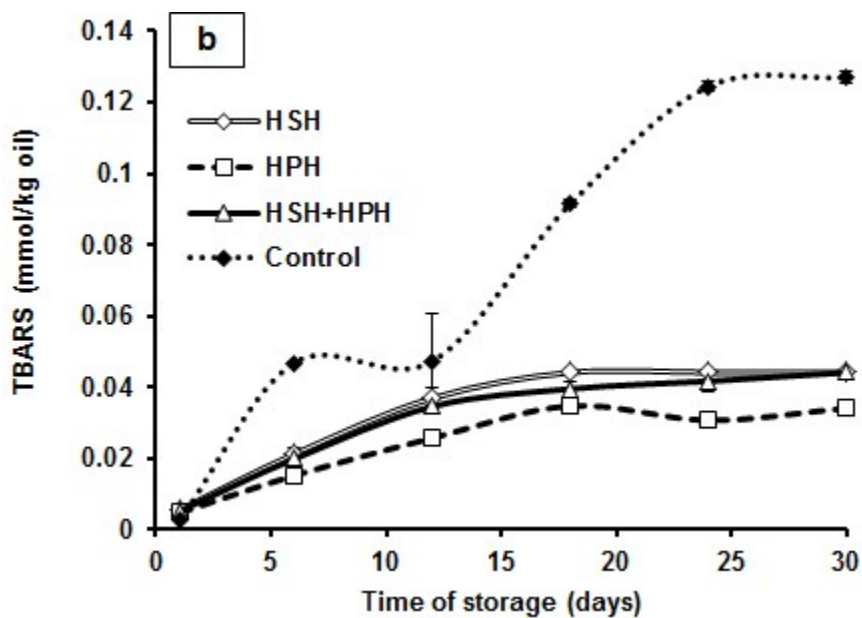
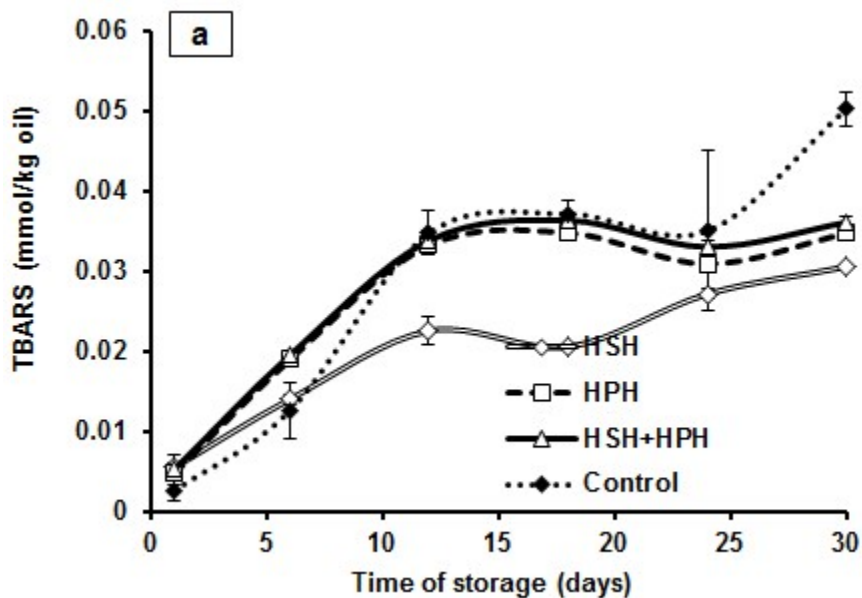


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88 **Appendix E. Supplementary data**

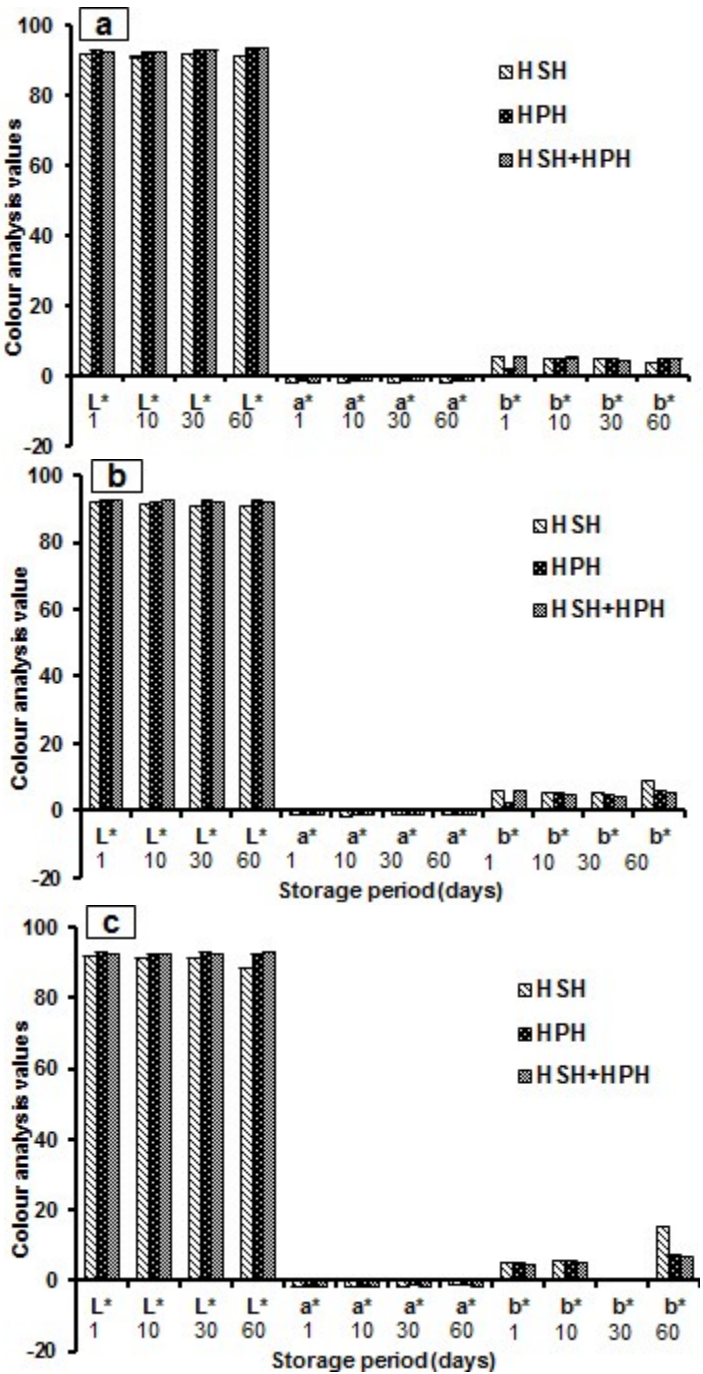
89 Oxidative stability studies of DHA nanoemulsions: a) stored at refrigeration condition  
90 ( $4\pm1$  °C); b) stored at oven condition ( $40\pm0.2$  °C).



96 **Appendix F. Supplementary data**

97 Color stability studies of DHA nanoemulsions: a) stored at refrigeration condition ( $4\pm1$   
98  $^{\circ}\text{C}$ ); b) stored at room condition ( $28\pm1$   $^{\circ}\text{C}$ ); c) stored at oven condition ( $40\pm0.2$   $^{\circ}\text{C}$ ).

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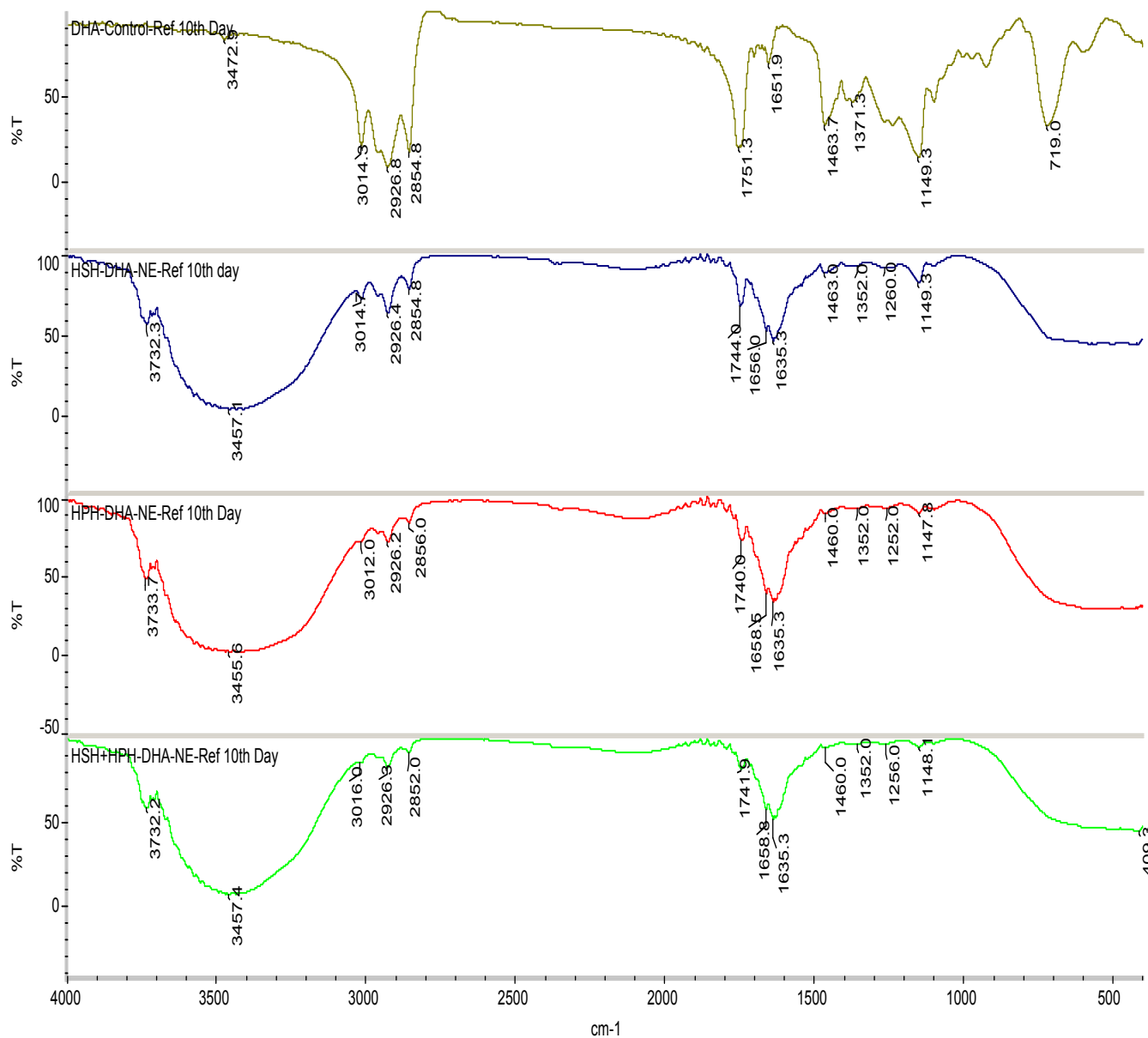


103 **Appendix G. Supplementary data**

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105 FTIR storage studies of DHA algae oil (control) and nanoemulsions during the storage  
106 of 30 days at different temperature ( $4\pm1^\circ\text{C}$ ,  $28\pm1^\circ\text{C}$  and  $40\pm0.2^\circ\text{C}$ ).

107 *a) Stored at refrigeration temperature ( $4\pm1^\circ\text{C}$ ) at 10<sup>th</sup> day*

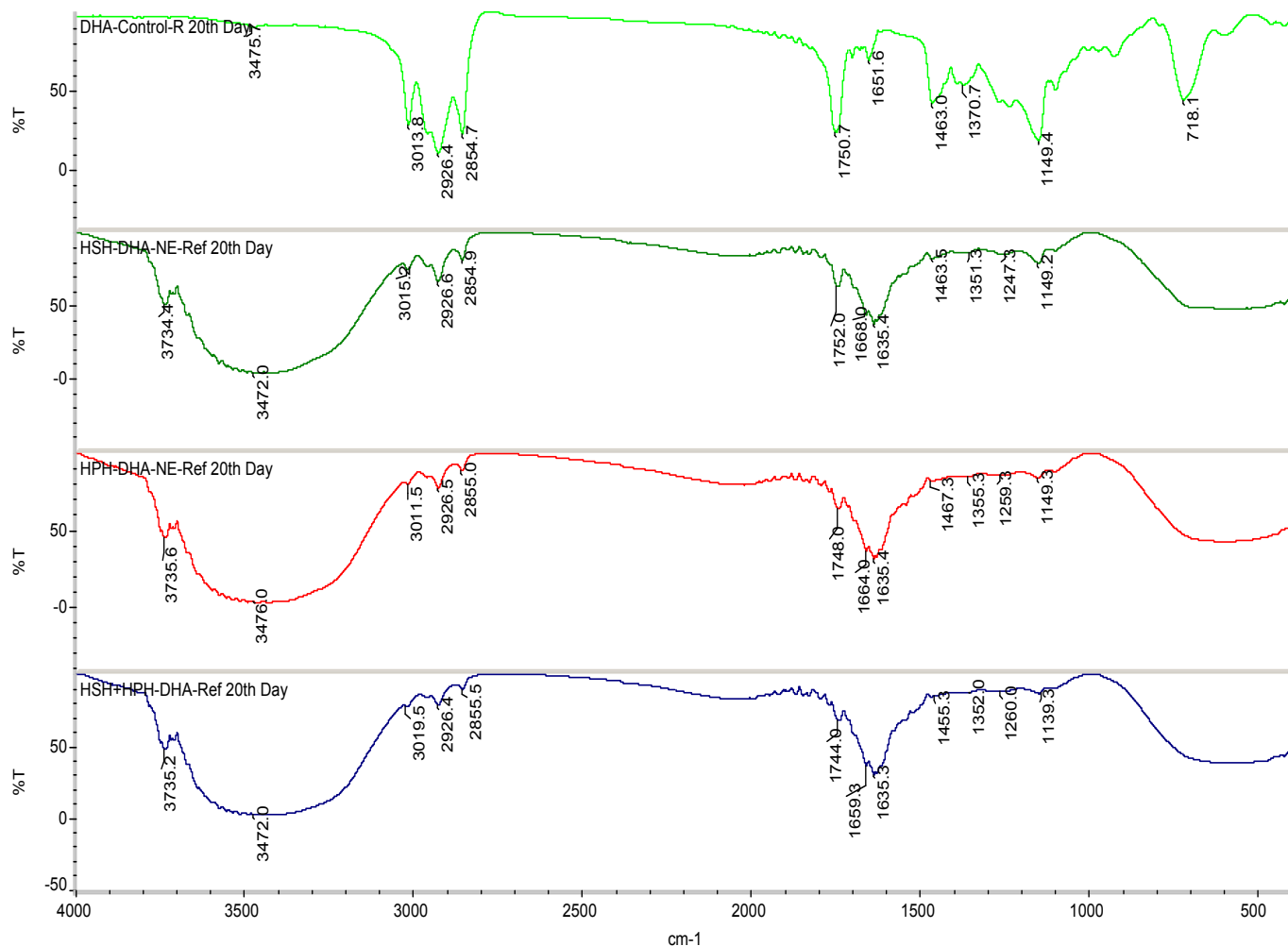


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109 **Fig. 1** FTIR spectra of DHA control, HSH-DHA-NE, HPH-DHA-NE, and HSH+HPH-NE.

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111 *b) Stored at refrigeration temperature ( $4\pm1^{\circ}\text{C}$ ) at 20<sup>th</sup> day*



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114 **Fig. 2** FTIR spectra of DHA control, HSH-DHA-NE, HPH-DHA-NE, and HSH+HPH-NE.

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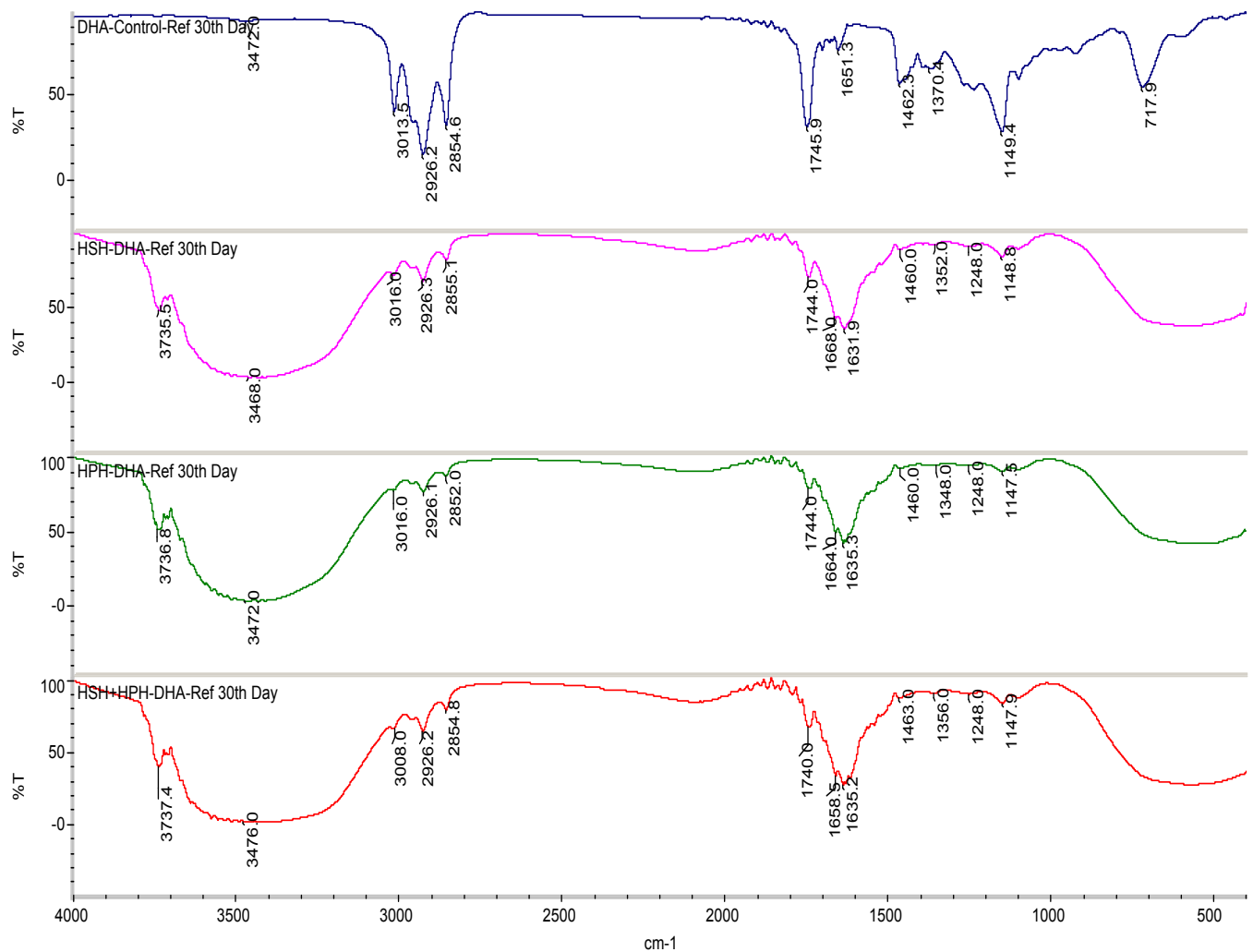
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120 c) Stored at refrigeration temperature ( $4\pm1^{\circ}\text{C}$ ) at 30<sup>th</sup> day



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122 **Fig. 3** FTIR spectra of DHA control, HSH-DHA-NE, HPH-DHA-NE, and HSH+HPH-NE.

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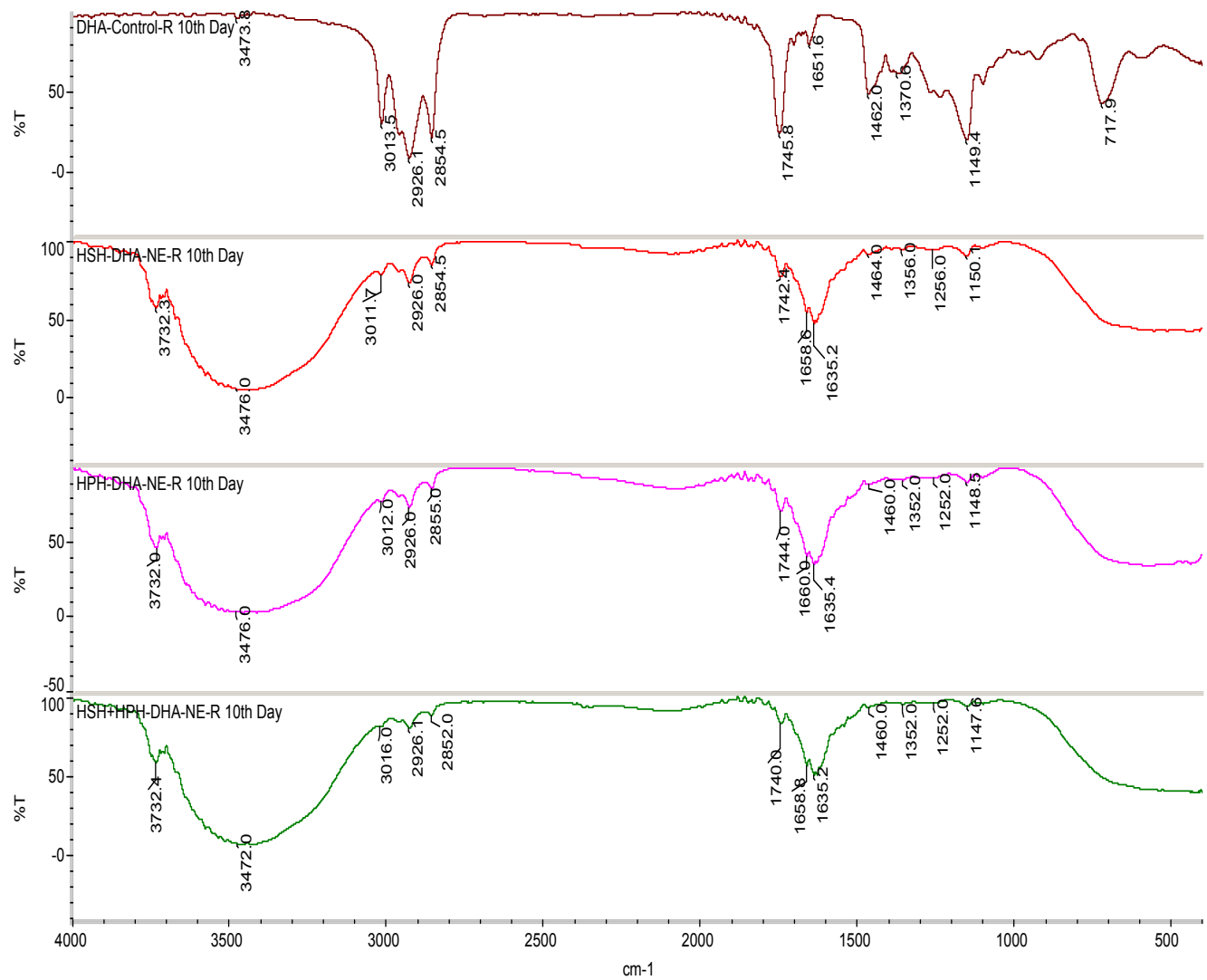
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130 d) Stored at room temperature ( $28\pm1^\circ\text{C}$ ) at 10<sup>th</sup> day



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132 **Fig. 4** FTIR spectra of DHA control, HSH-DHA-NE, HPH-DHA-NE, and HSH+HPH-NE.

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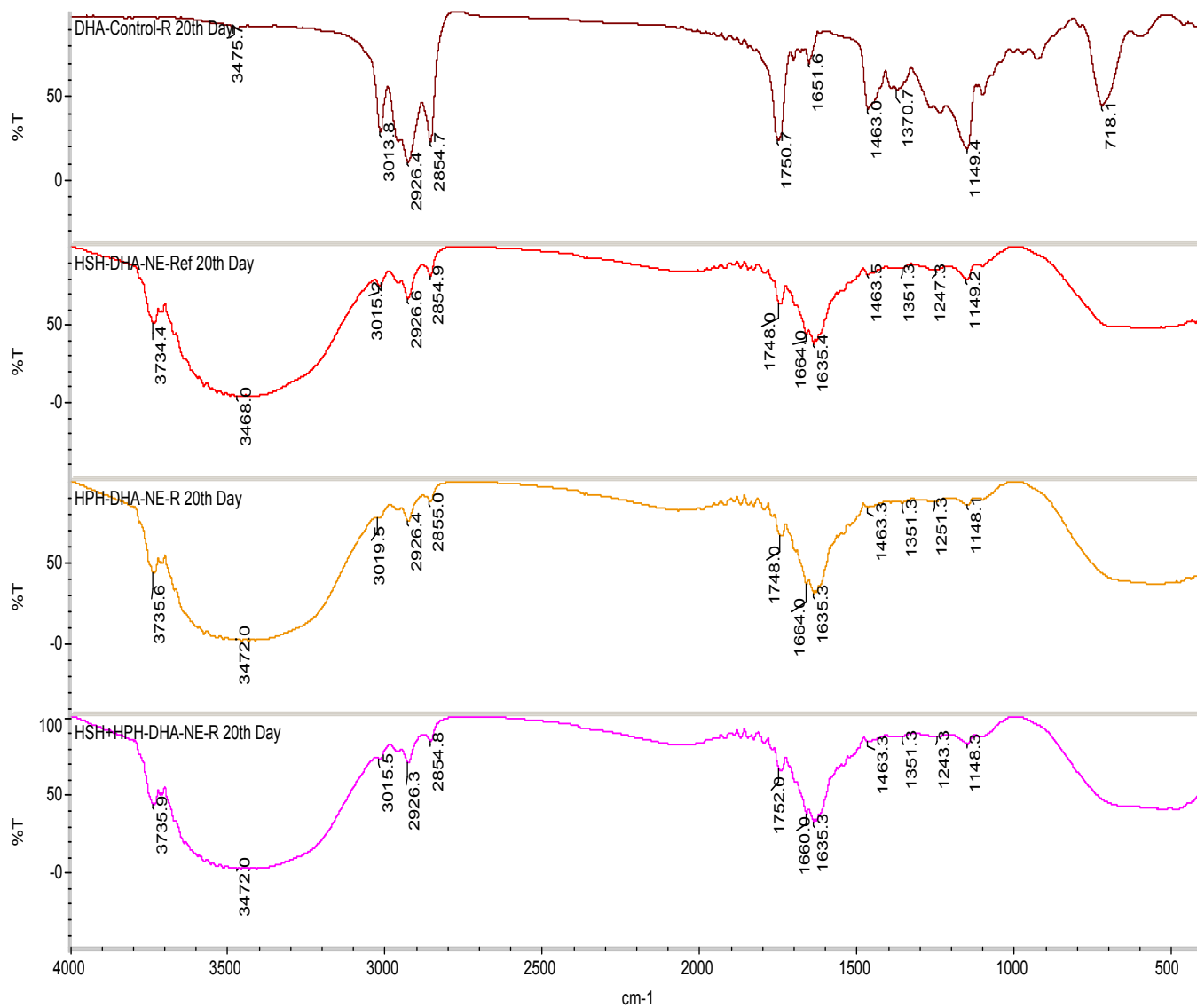
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139 e) Stored at room temperature ( $28\pm1^\circ\text{C}$ ) at 20<sup>th</sup> day

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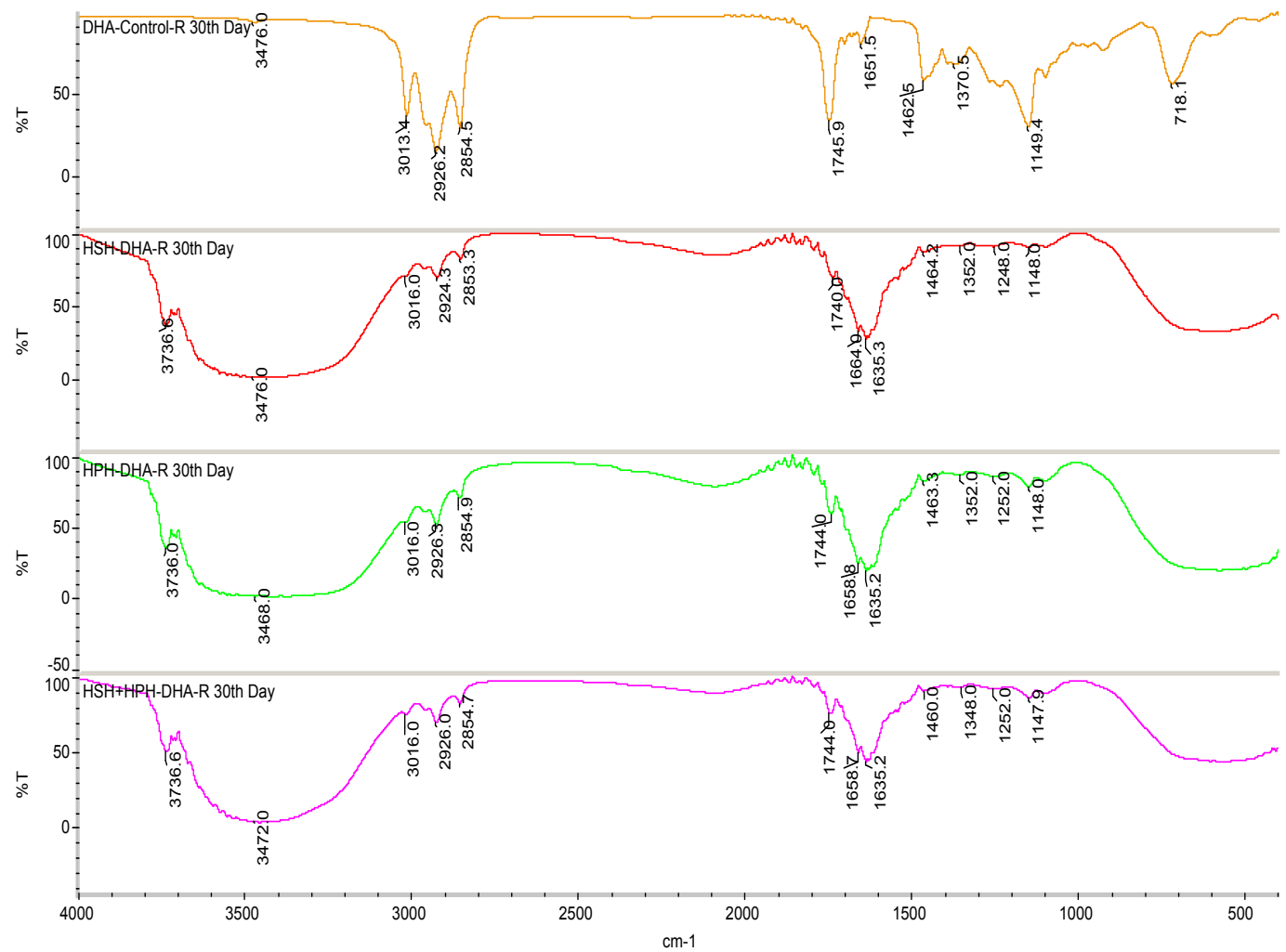
143 **Fig. 5** FTIR spectra of DHA control, HSH-DHA-NE, HPH-DHA-NE, and HSH+HPH-NE.

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147 f) Stored at room temperature ( $28\pm1^{\circ}\text{C}$ ) at 30<sup>th</sup> day



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149 **Fig. 6** FTIR spectra of DHA control, HSH-DHA-NE, HPH-DHA-NE, and HSH+HPH-NE.

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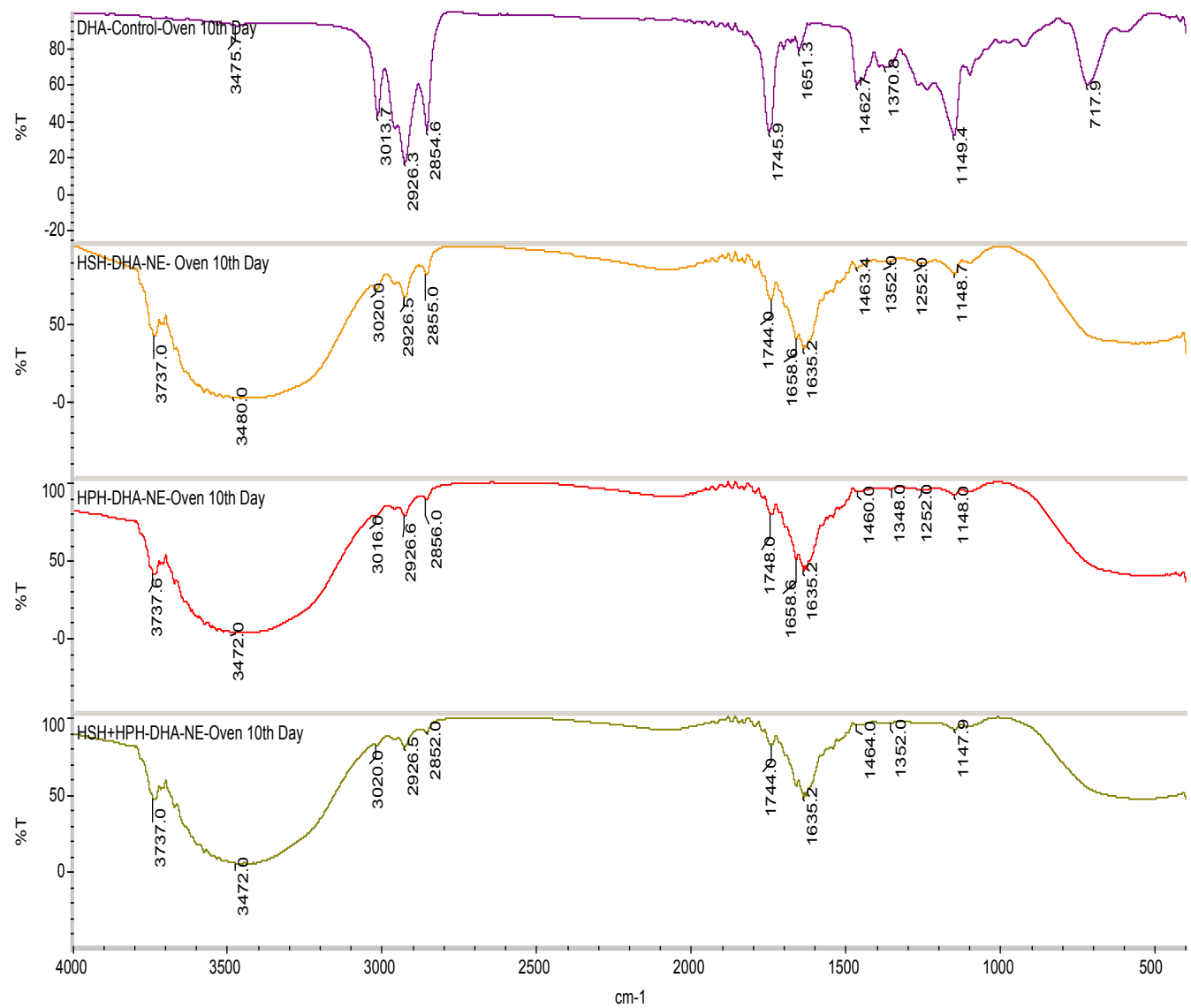
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157 g) Stored at oven temperature (40±0.2°C) at 10<sup>th</sup> day



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159 **Fig. 7** FTIR spectra of DHA control, HSH-DHA-NE, HPH-DHA-NE, and HSH+HPH-NE.

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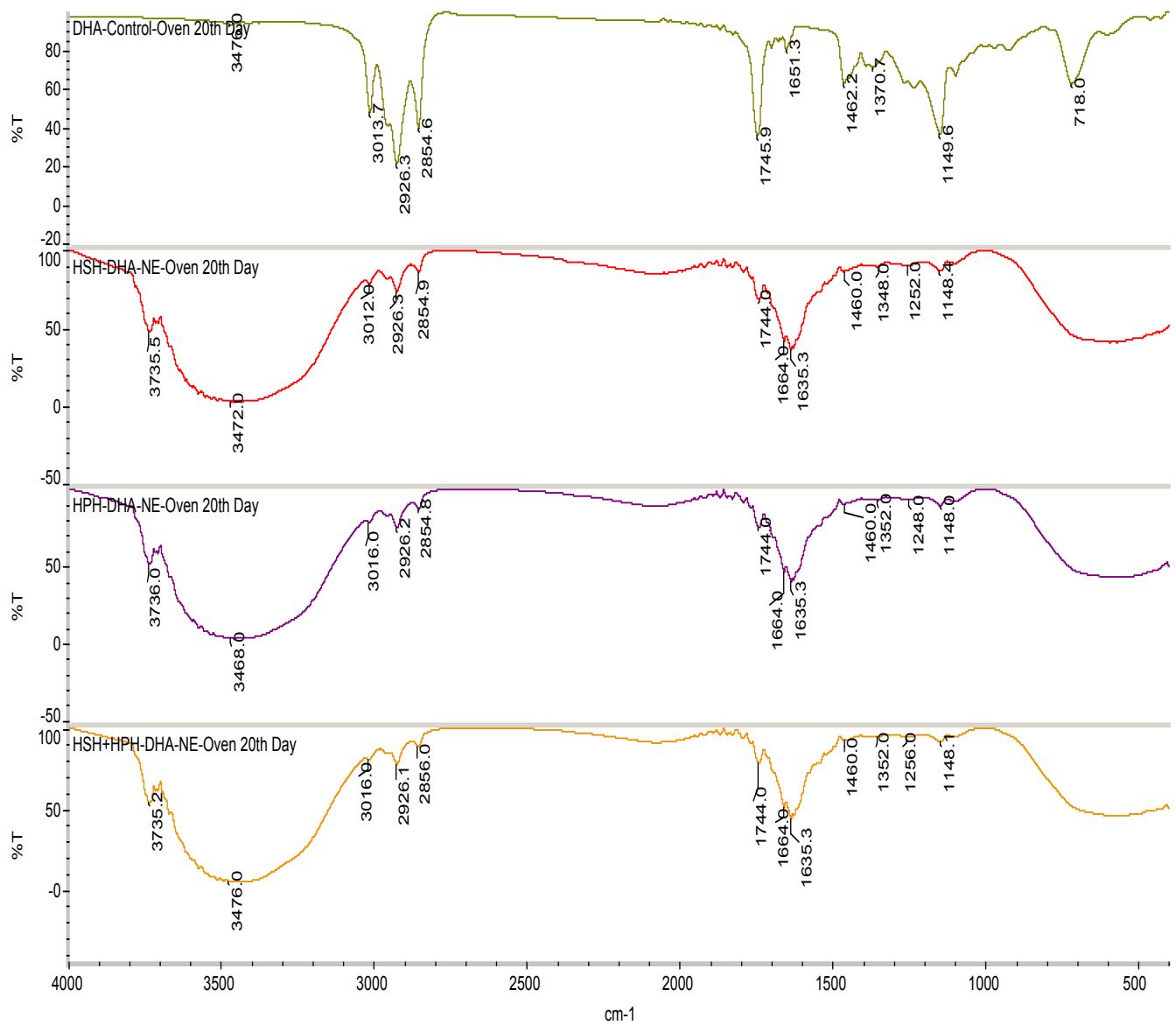
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165 h) Stored at oven temperature ( $40\pm0.2^{\circ}\text{C}$ ) at 20<sup>th</sup> day



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167 **Fig. 8** FTIR spectra of DHA control, HSH-DHA-NE, HPH-DHA-NE, and HSH+HPH-NE.

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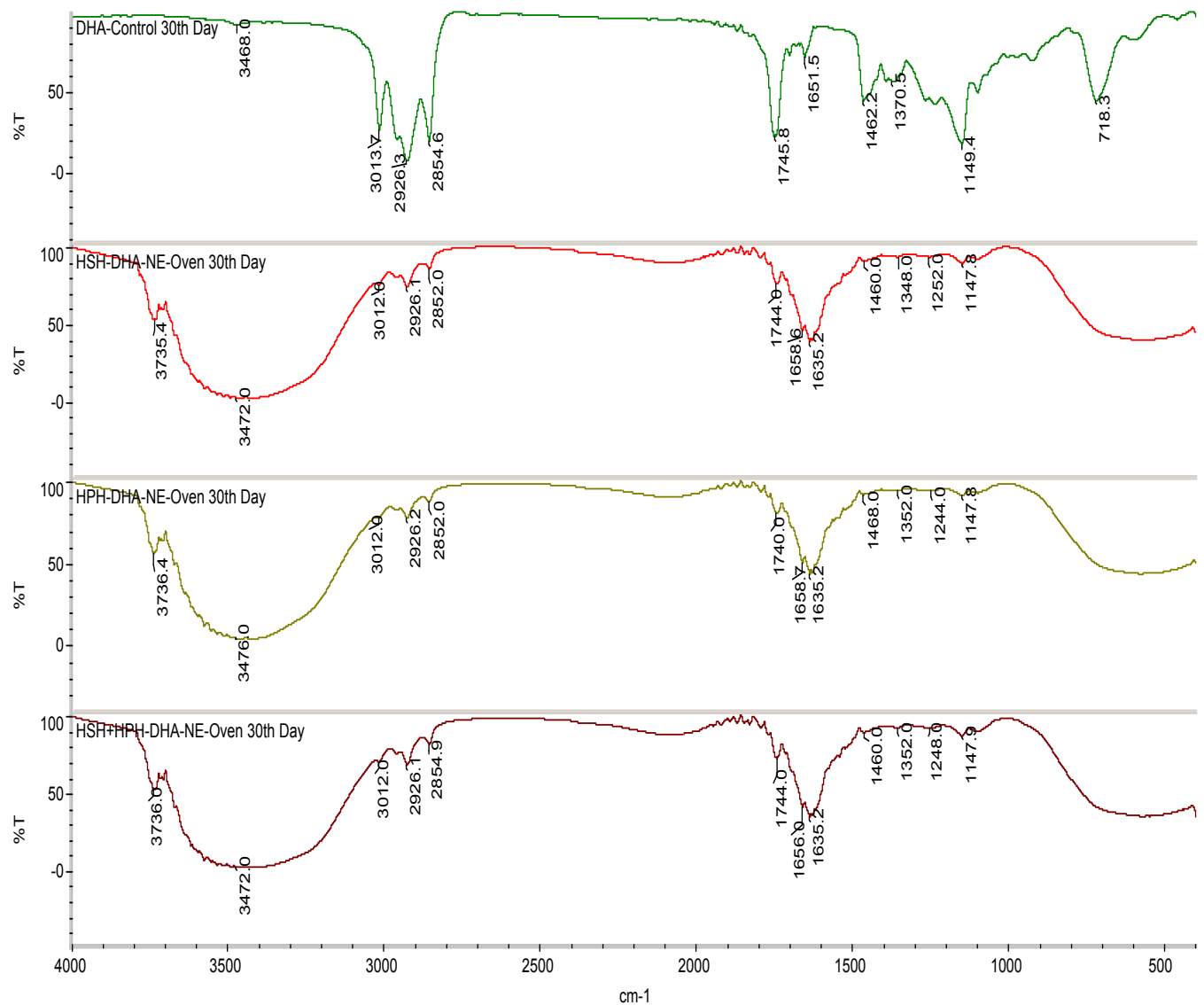
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173 i) Stored at oven temperature ( $40\pm0.2^{\circ}\text{C}$ ) at 30<sup>th</sup> day



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175 **Fig. 9** FTIR spectra of DHA control, HSH-DHA-NE, HPH-DHA-NE, and HSH+HPH-NE.

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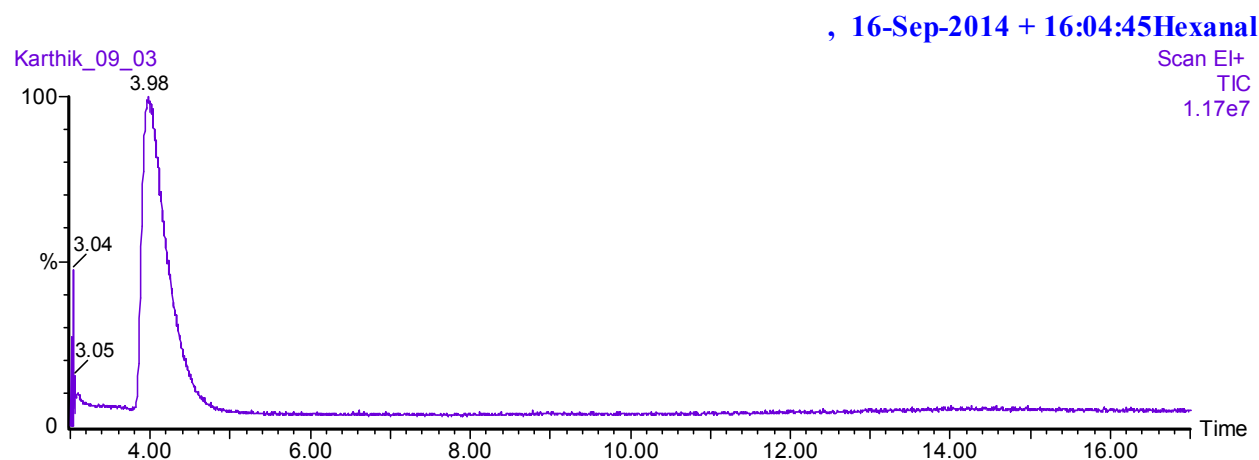
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## Appendix H. Supplementary data

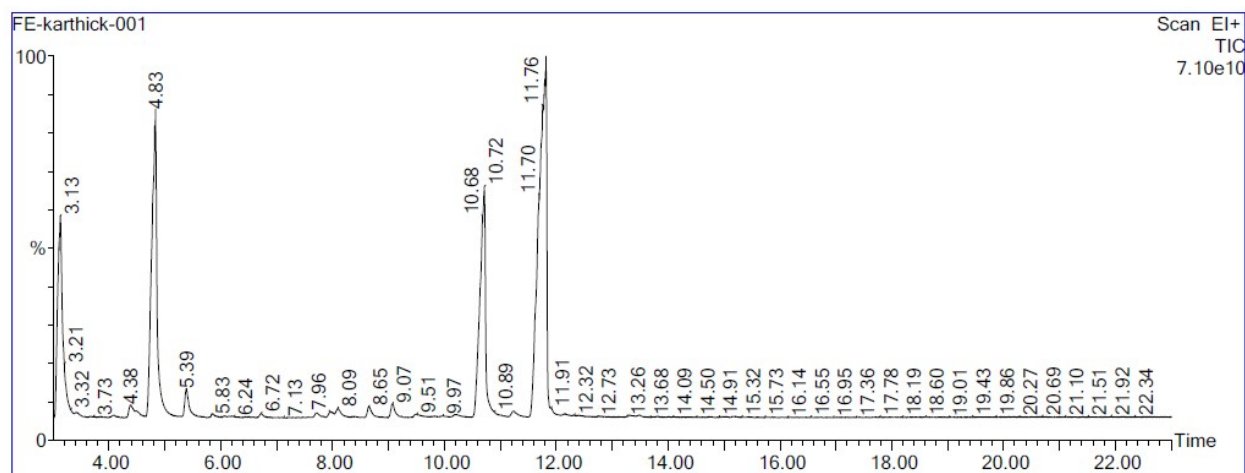
Determination of volatile compounds after formulation of DHA nanoemulsions by comparing the Hexanal with DHA algae oil and nanoemulsified DHA using GC-MS.

### a) Hexanal spectra



**Fig. 1** GC-MS spectra for Hexanal (The peak at RT 3.98 min corresponds to Hexanal).

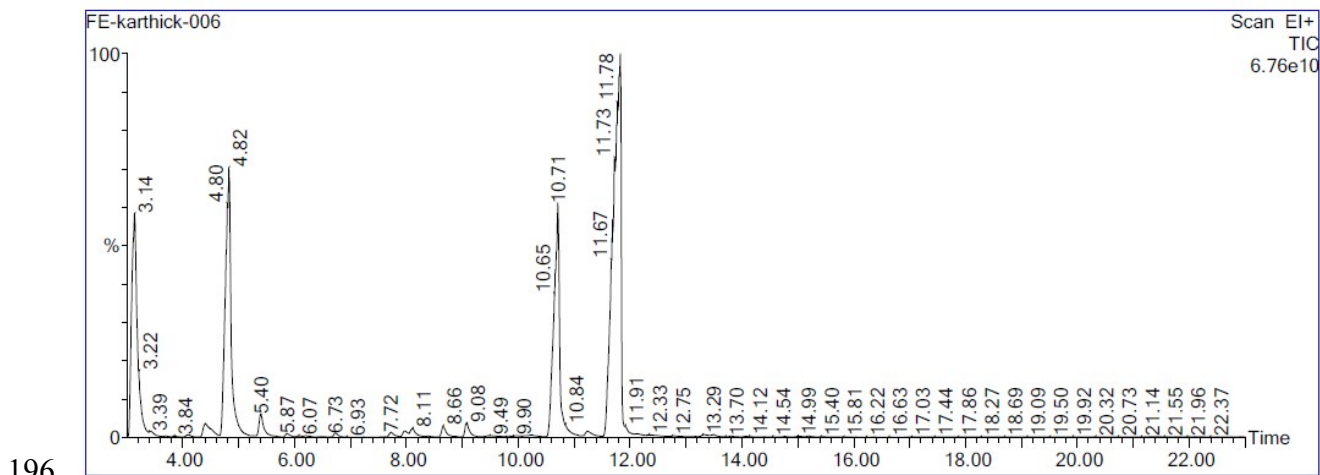
### b) DHA algae oil spectra:



193 **Fig. 2** GC-MS spectra for DHA algae oil (control)

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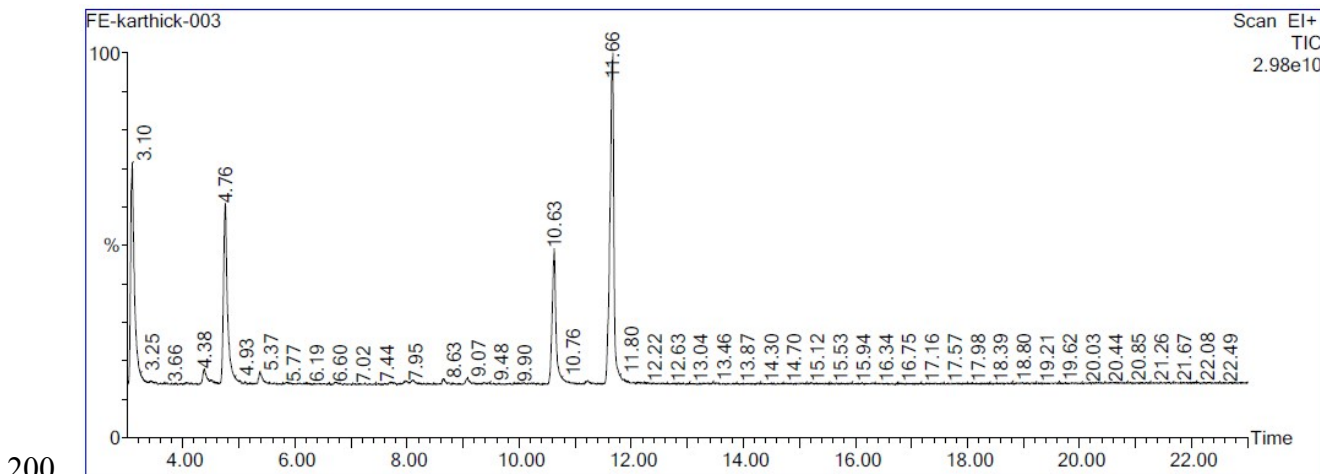
195 *c) HSH DHA Nanoemulsion spectra*



197 **Fig. 3** GC-MS spectra for HPH technique used DHA nanoemulsion.

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199 *d) HPH DHA Nanoemulsion spectra*

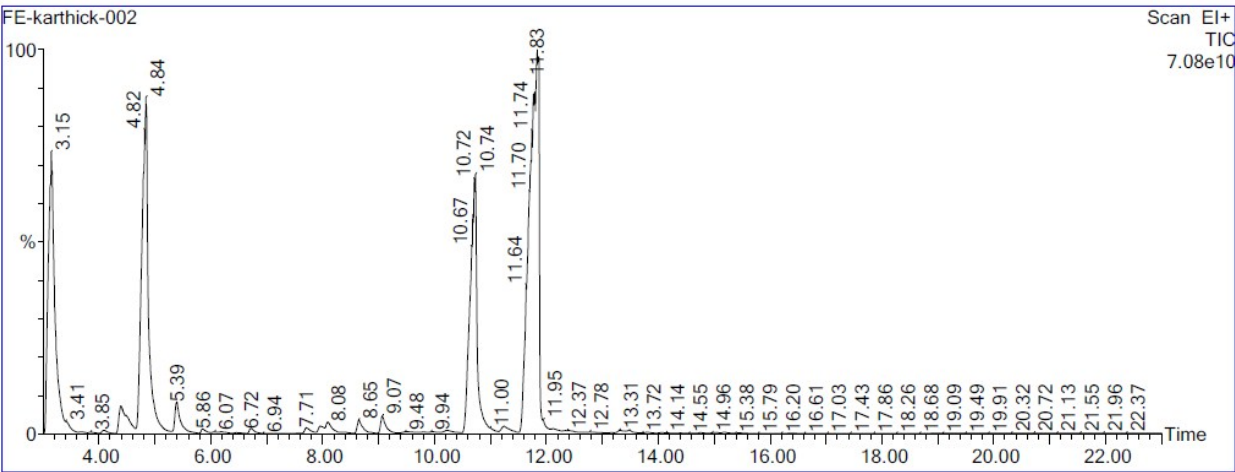


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202 **Fig. 4** GC-MS spectra for HPH technique used DHA nanoemulsion.

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204 e) HSH+HPH DHA Nanoemulsion spectra



207 **Fig. 5** GC-MS spectra for HSH+HPH technique used DHA nanoemulsion.