Lipase-catalysed acidolysis for enrichment of medium-chain triacylglycerols in virgin coconut oil and its functional properties

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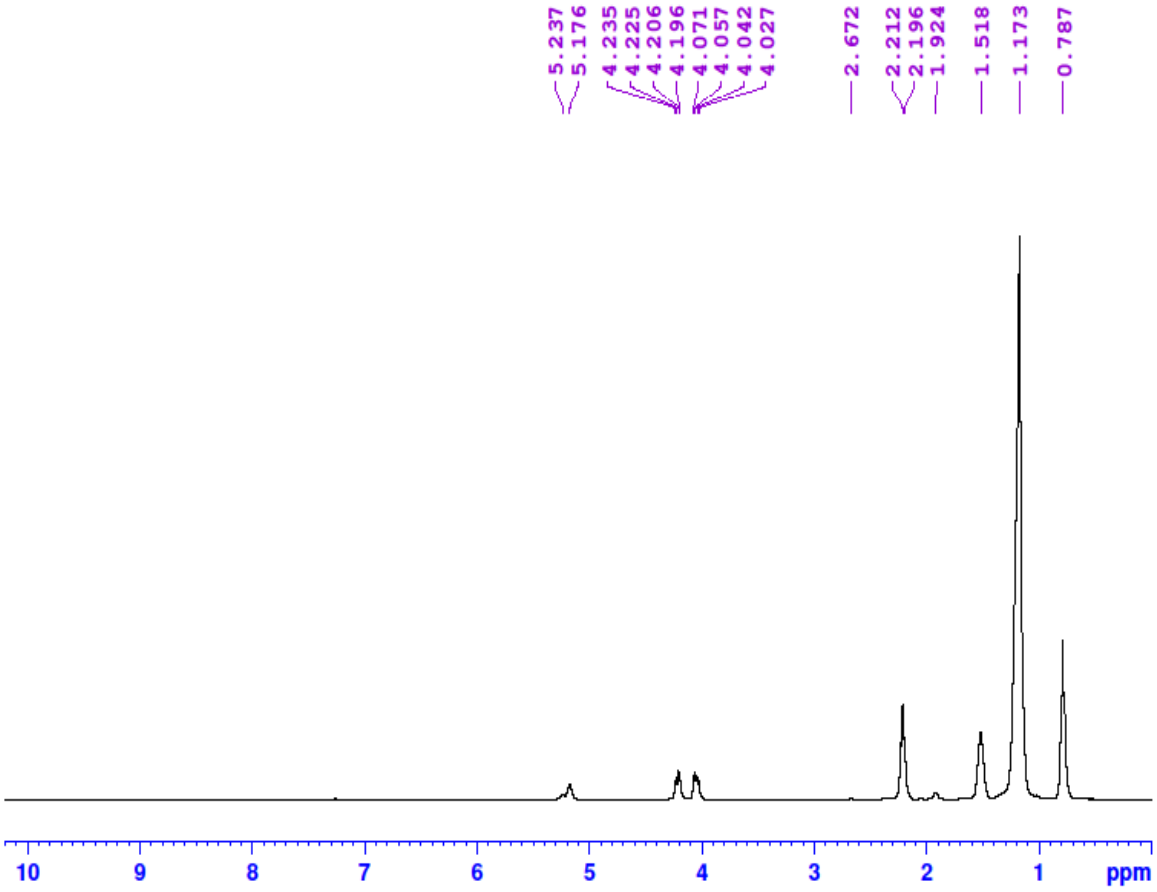

Fig S1: ^{1}H NMR spectrum of VCO

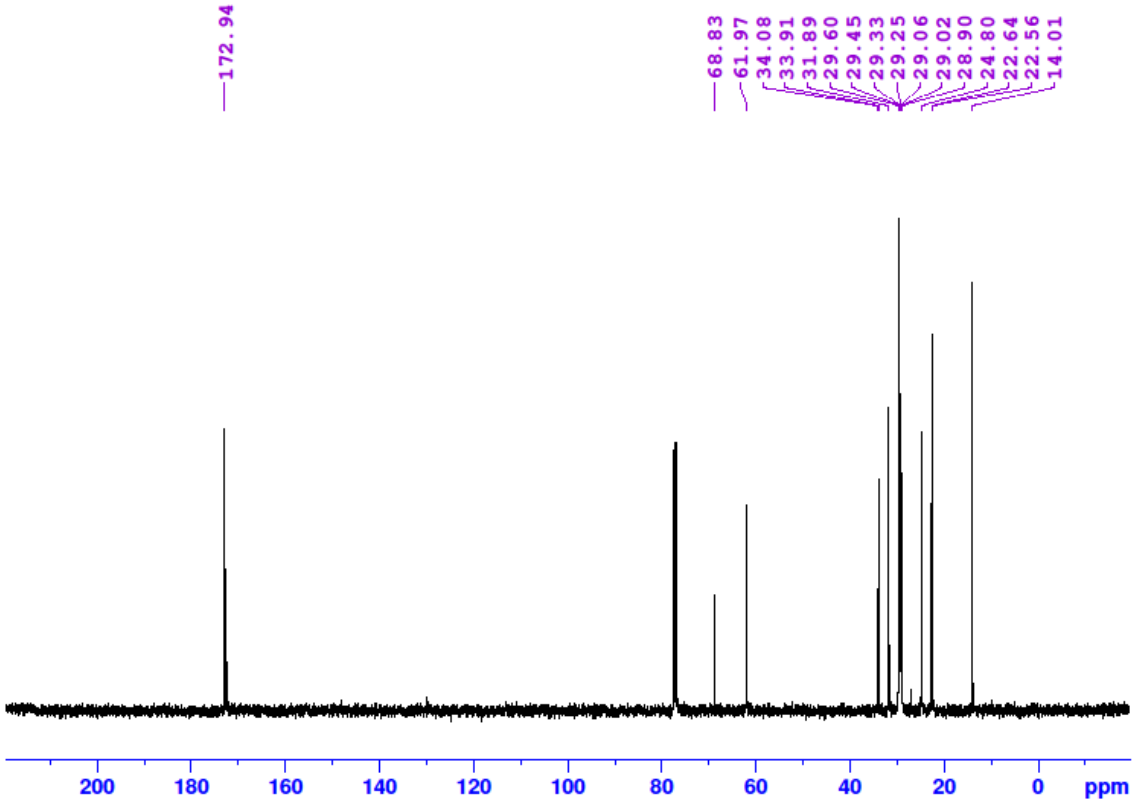

Fig S2: $^{13}C\{^{1}H\}$ NMR spectrum of VCO

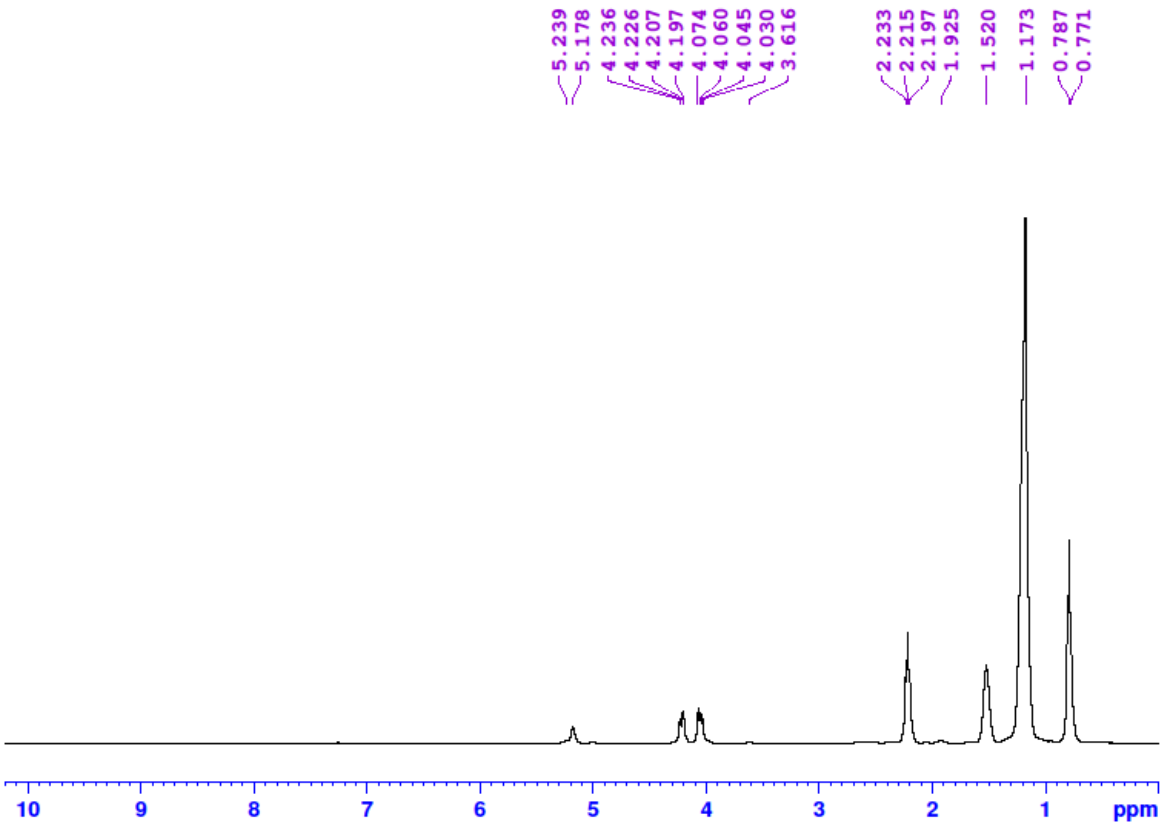

Fig S3: ^{1}H spectrum of MCT enriched VCO

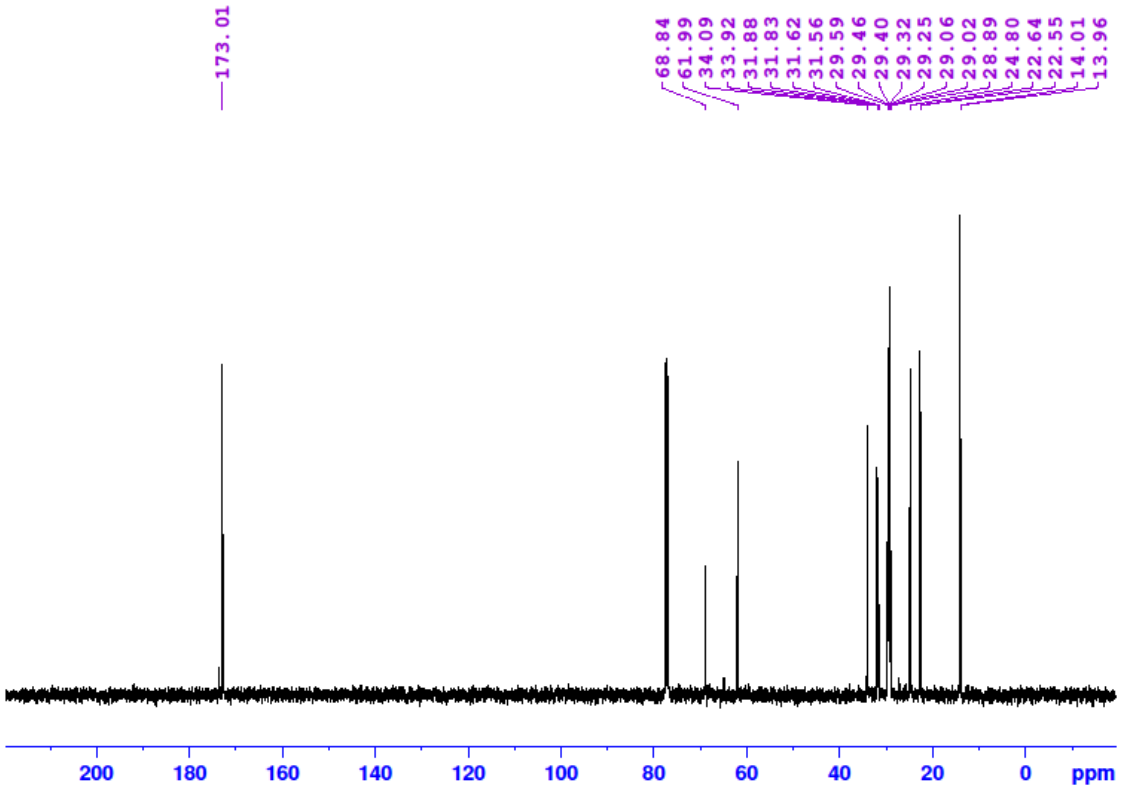

Fig S4: $^{13}C\{^{1}H\}$ NMR spectrum of MCT enriched VCO

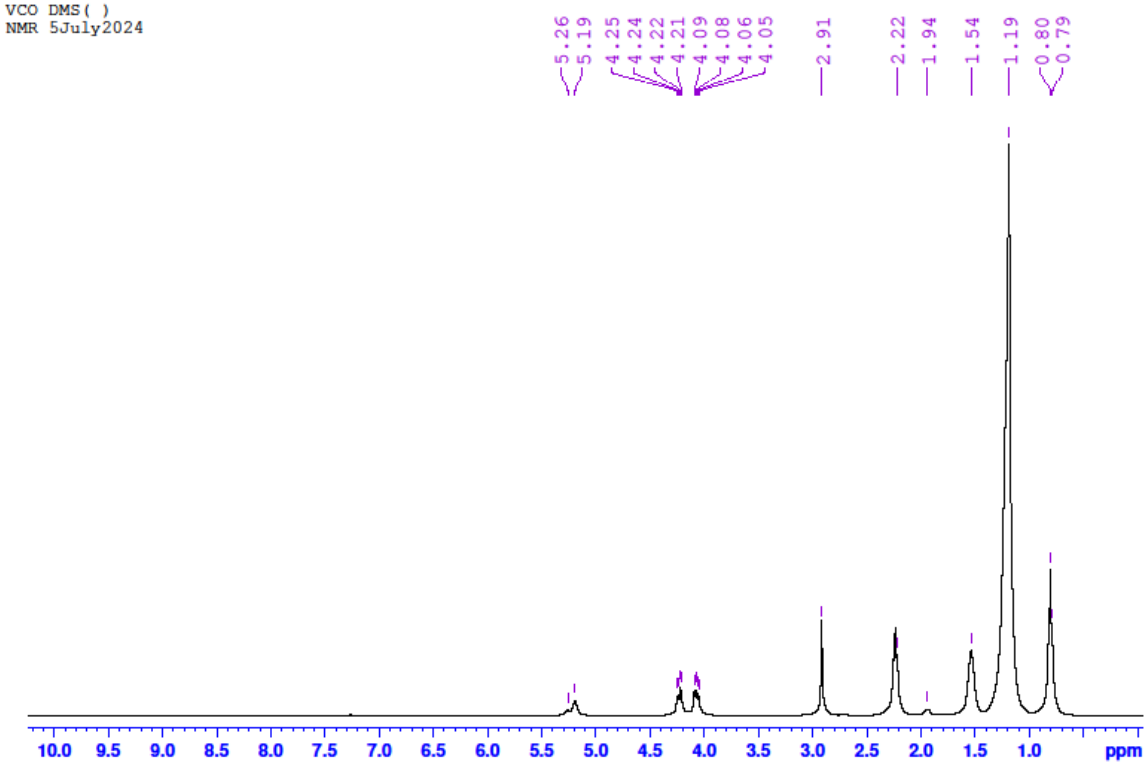

Fig S5: ^{1}H NMR spectrum of VCO (DMS added)

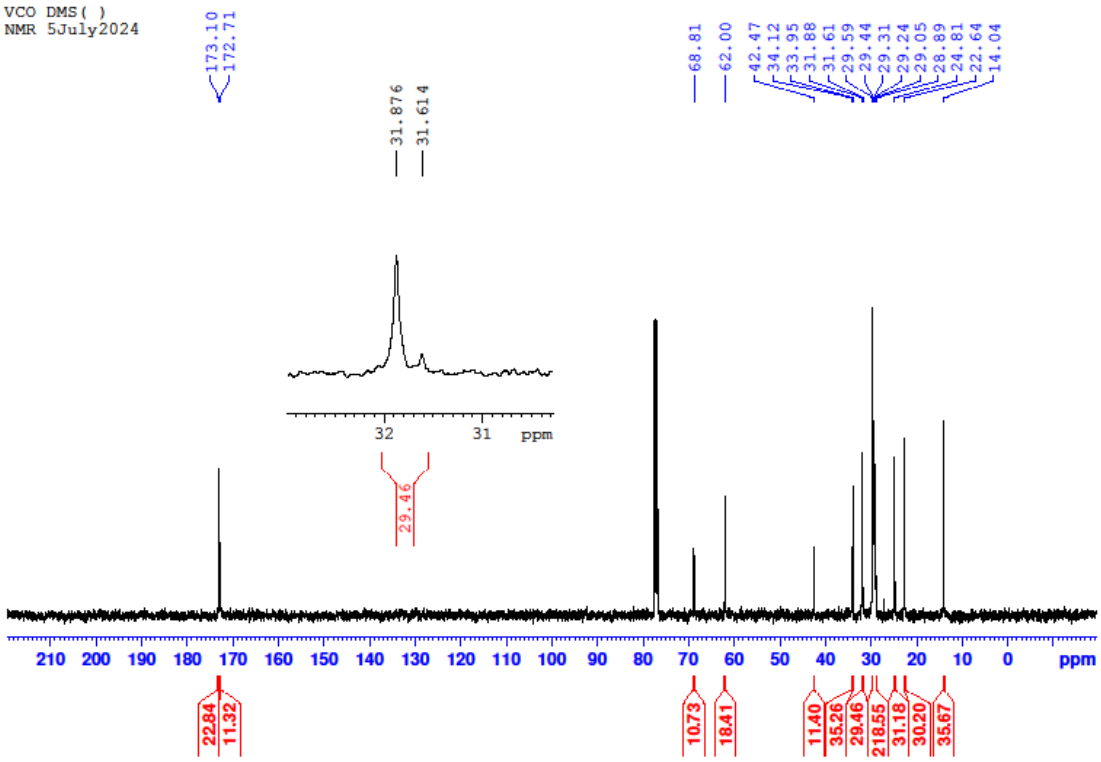

Fig S6: ^{13}C NMR with inverse gated ^{1}H-decoupling spectrum of VCO (DMS added)

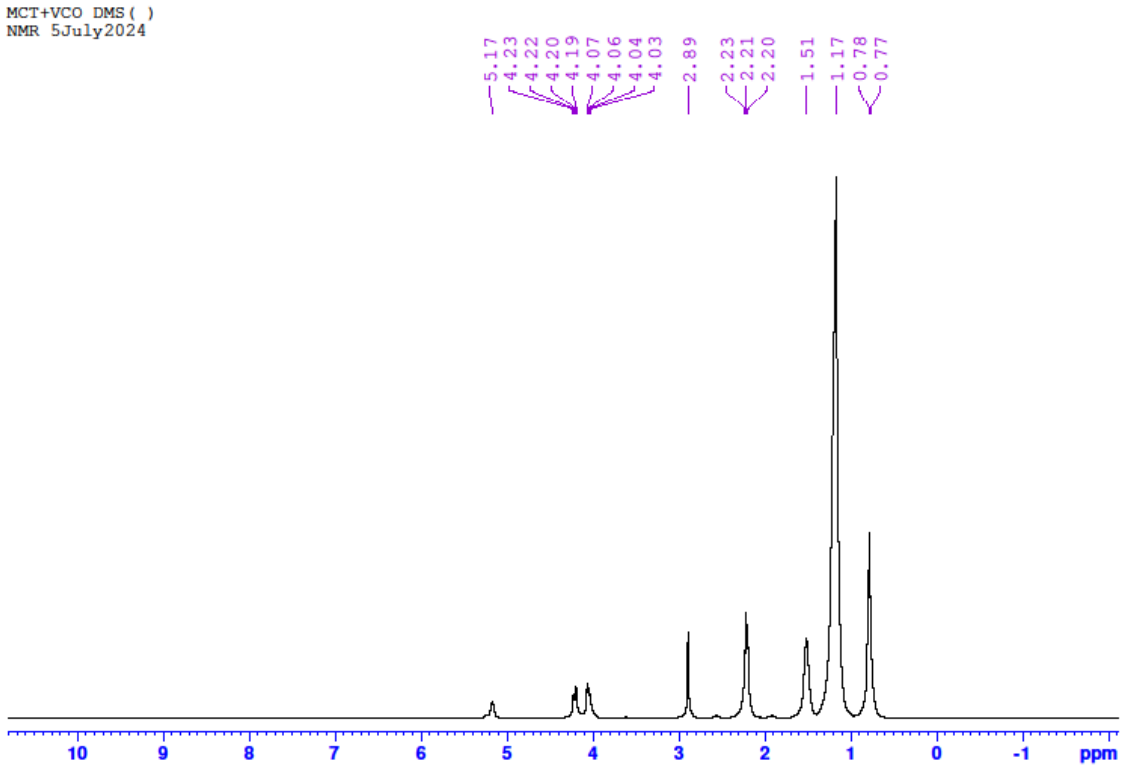

Fig S7: ^{1}H spectra of MCT enriched VCO (DMS added)

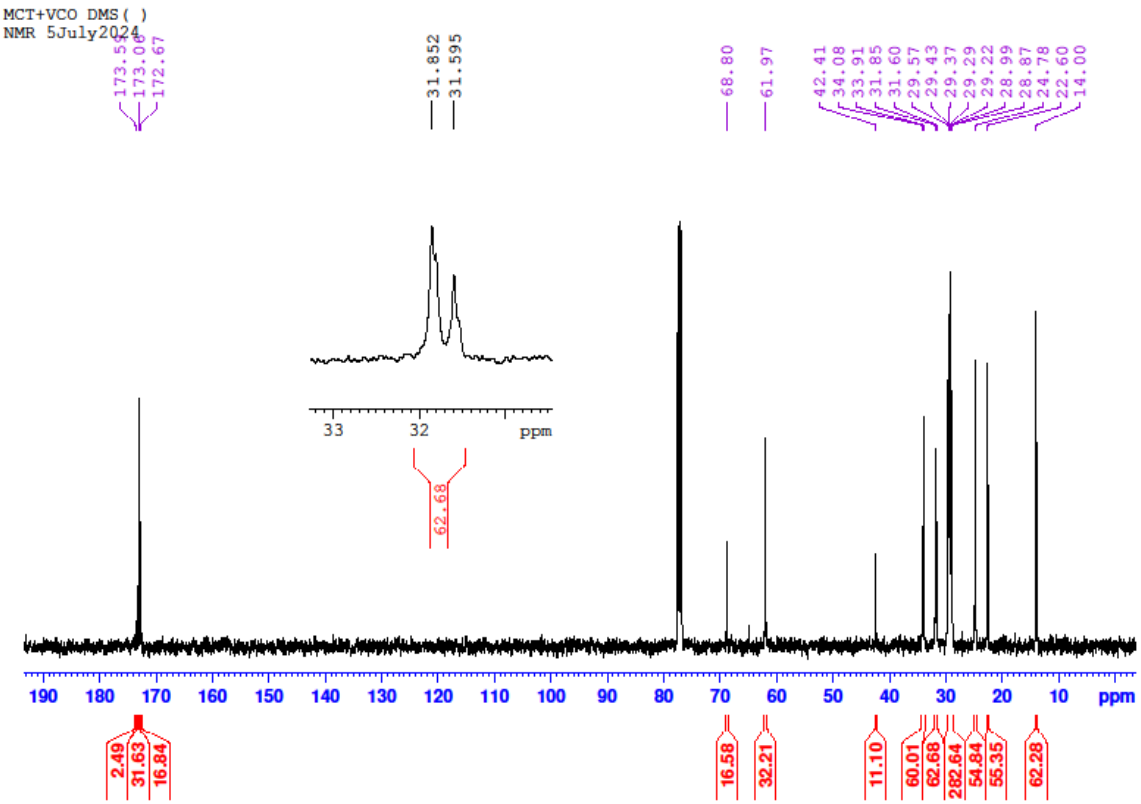

Fig S8: ^{13}C NMR with inverse gated ^{1}H-decoupling spectrum MCT enriched VCO (DMS added)

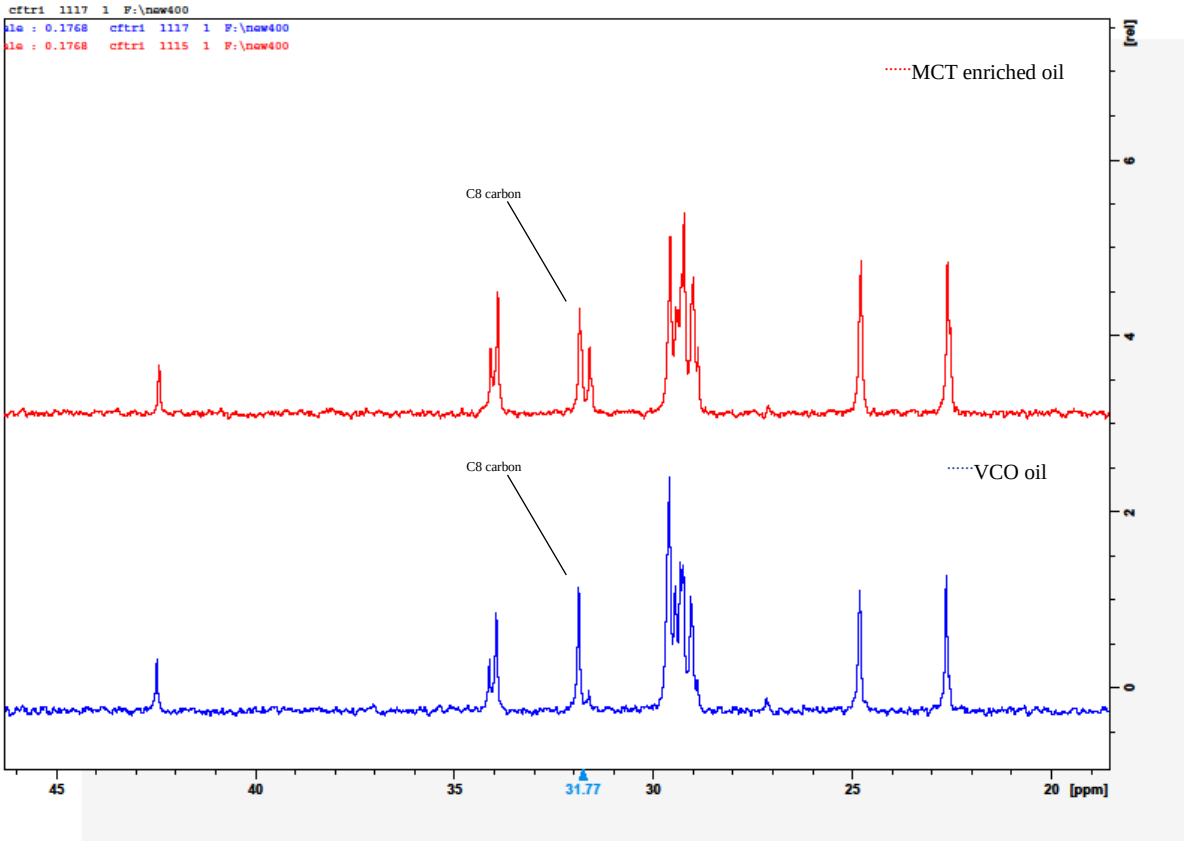

Fig S9: ^{13}C NMR with inverse gated ^{1}H-decoupling spectra: Red- MCT enriched VCO (DMS added), Blue- VCO (DMS added)

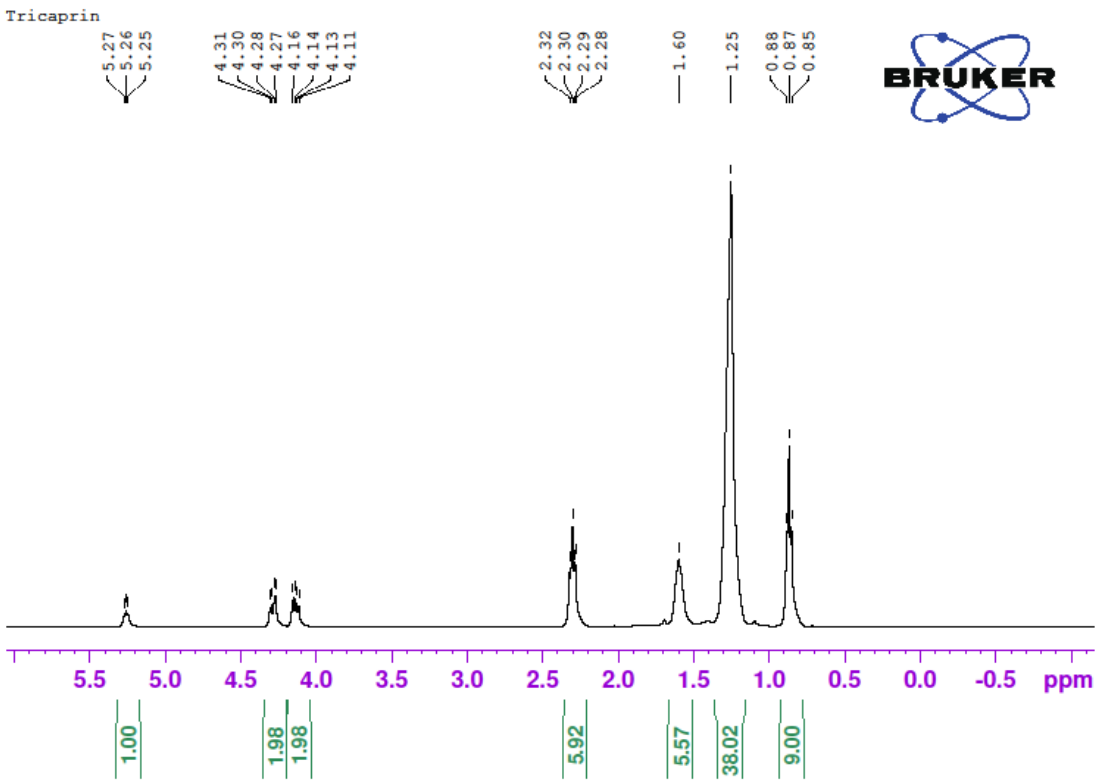

Fig S10: ^{1}H NMR of Tricaprin

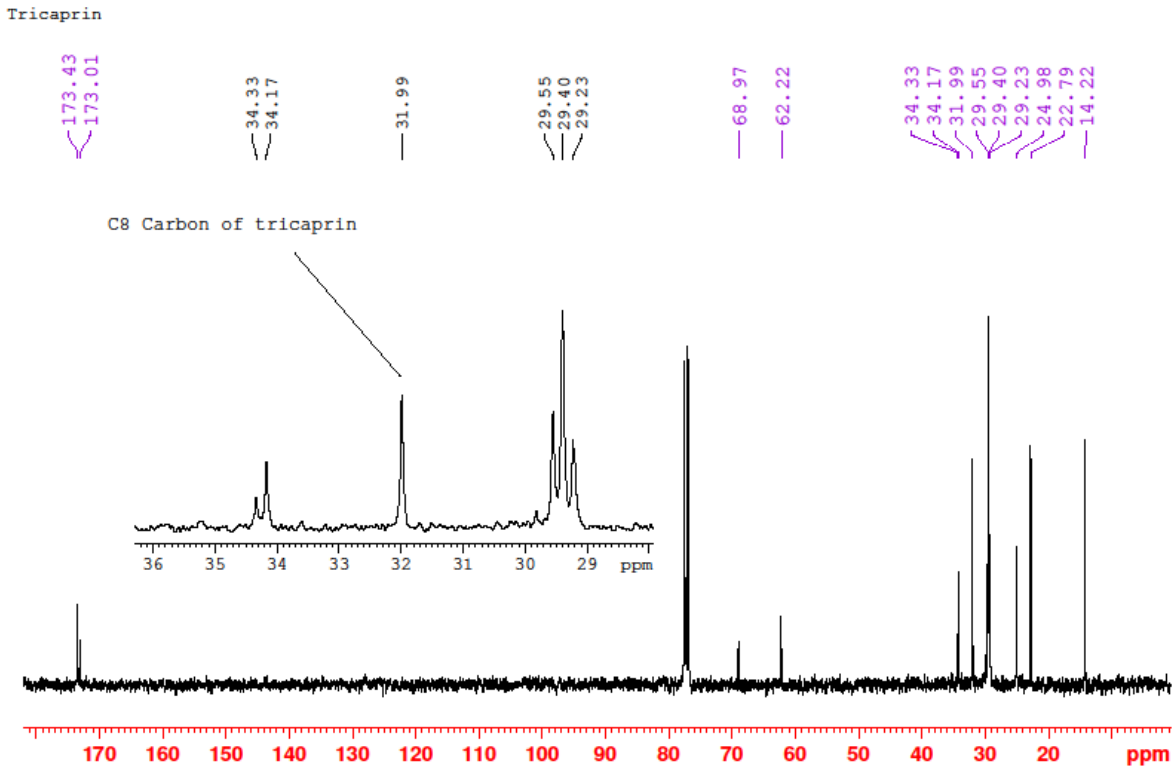

Fig S11: ^{13}C NMR of Tricaprin

NMR methodology and detailed calculations of MCT enhancement

The ^{13}C NMR data interpretation for triacyl glycerol is in reported by Mayokha et al 2025[1]. In the work reported by Mayokha et al 2025 has been demonstrated that C8 carbons of C10 fatty acid (capric acid) attached to glyceryl moiety in triacyl glycerol occur at 31. 75 and 31.55 ppm and C8 carbon of fatty acid (capric acid) attached in the tricaprin occurs at 31.99 ppm.

In our work, these C8 carbon peak related to C10 fatty acid (capric acid) was selected for gauging the enhancement of MCT via ^{13}C NMR(Inverse gated decouple spectra) for quantification methodology.

Step 1:

Initially known quantity of virgin coconut ol VCO (0.01683 g) with known amount of NMR internal standard dimethyl sulphone DMS (0.0114 g) was taken and both were dissolved in 0.6 mL of $CDCl_3$, subsequently ^{1}H and ^{13}C NMR(Inverse gated decouple spectra) spectra were recorded as reported in the material and method section.

Subsequently. a known quantity of MCT enriched VCO oil (0.01988 g) with known among of NMR internal standard DMS (0.0111 g) was taken and dissolved 0.6 mL of $CDCl_3$, ^{1}H and ^{13}C NMR(Inverse gated decoupled spectra) spectra were recorded as reported in the material and method section.

Step 2: NMR integrations were performed for all the peaks in ^{13}C NMR(Inverse gated decoupled spectra) *figure S3 and figure S4), the values are tabulated in the table

Table S1: NMR integrations details

SL No	**Peaks considered for integration**	Integral values (VCO oil)	MCT enriched VCO oil
1	31. 75 and 31.55 ppm.(both peaks are integrated together as slight overlap is there and as both corresponds to C8 carbons of C10 fatty acids attached to triacylglycerols)	29 .46	62.8
2	42.1 ppm peak (Dimethyl sulfone carbon peak)	11.4	11.1

Step 3: Calculations related to NMR quantification

The following formula was used to asses the quantity of enhancement MLCT. As explained in the above paragraphs, the enhancement of C10 fatty acids related to MLCT was probed via

enhancement C8 carbon peaks corresponding to C10 fatty acid,

In the NMR formula,[2] as the C8 carbon is a methylene functional group in the following discussion it is represented as CH_2 group

$$GCH2 = Gdms\ \frac{FCH2\ NdmsMCH2}{Fdms\ NCH2\ Mdms} \qquad Eq\ 1$$

Wherein ,

NCH_2 = 1 (number of carbon atoms)

Ndms = 2 (number of carbon atoms in dms)

Mdms = 94.13 (Molecular mass of dimethyl sulfone)

MCH_2 = 14.043 (Molecular mass of functional group CH_2)

Fdms = Area of dimethyl sulfone from NMR integration

FCH_2 = Area of functional group CH_2 from NMR integration

Gdms = Amount of dms employed for quantification (in mg)

Substituting the integral values from table S1 in equation 1 and remembering that VCO oil employed for NMR recording 0.01683 g

VCO oil used for recording NMR = 0.01683 g

GCH2 of VCO (0.0168 g) = 0.0087 g

Hence,

GCH2 (in 0.0199 mg) of VCO oil = 0.0103 g

Substituting the integral values from table S1 in equation 1 and remembering that MCT enriched VCO oil employed for NMR recording 0.0199 g

GCH2 of MCT enriched VCO oil (0.0199 g) = 0.0187 g

$$\%\ Enchancement\ of\ C10\ fatty\ acid = \frac{GCH2\ of\ MCT\ enriched\ VCO - GCH2\ ofVCO}{GCH2\ of\ VCO}\ x100$$

% enhancement of C10 fatty acid = 0.0084/0.103 x 100 = 81 %

% enhancement of C10 fatty acid ~ 81 %

Table S2:

Triacylglycerol	Enhancement percentage (%) observed from HPLC	Net C10 fatty acid Enhancement
TAG (C10-10-10)	18	54 %
TAG (C8-10-10)	11	22 *%
TAG (C8-8-10)	3	3
Total C10 fatty acid enhancement		79%

^{13}C NMR quantification demonstrated that there is C8 carbon enhancement of 81 % , as only C10 fatty acid attached to triacylglycerol can have C8 carbon chemical shift in the ^{13}C NMR. This clearly indicates via enhance of C8 carbon that there is ~ 81 % enhancement of C10 fatty acid attached to triacylglycerol which correlates with 79 % of enhancement of C10 fatty acid as determined by HPLC.

References:

1. V.P. Mayookha, U. Raksha. V.H. Nanishankar, G. Suresh Kumar, Solvent-free synthesis of medium chain triacylglycerols by esterification of capric, caprylic acids with 1,3-specific and non-specific lipaces. *J.Am.Oil Chem.Soc* 2025, **102**, 657–667.

2. D.A.L. Otte, D.E. Borchamann,C.Lin, M. Weck, K.A. Woerpel 13C NMR spectroscopy for the quantitative determination of compound ratios and polymer end groups. *Org. Lett.2014,16(6).* 1566–1569