Supporting Information for:

Chemo-Enzymatic Cascades for the Sustainable Transformation of Canola Oil into Hydrocarbon Fuels

Lucas, B. Barbosa,[1] Caio M. Pacheco,[1] Isabela G. da Silva, [1] Mauro, R. B. P. Gomez,[1] Alexandre S. França,[1] Gabriela C. Breda, [1] Matheus C. Silva,[1] de Alencar, P. S.,[1] Fernanda A. Lima,[1] Raquel A. C. Leão,[1] Rodrigo V. Almeida,[2] Rodrigo O. M. A. de Souza.[1]*

[1] Biocatalysis and Organic Synthesis Group, Instituto de Química, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil, 21941909;

[2] Laboratório de Microbiologia Molecular e Proteínas, Instituto de Química, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Rio de Janeiro, Brazil, 21941909;

*rodrigosouza@iq.ufrj.br

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Summary

1. Canola oil hydrolysis

1.1. Lipase activity assay

The hydrolysis activity of *Candida rugosa* lipase (CCL) was measured by hydrolysis reaction adapted from Freire et al.[1]

The unit of enzymatic activity (1U) is defined as the amount of the enzyme that catalyzes the conversion of one micromole of fatty acid per minute under specified conditions of the assay method of Chiaradia et al.[2] The esterification activity per gram of support was determined from the following equation:

$$A\left(\frac{U}{g}\right) = \frac{(Va - Vb).C.10^3}{t.m}$$

Where, A = enzymatic activity (U/g)
Va = volume of the NaOH solution spent in titration of the sample collected after 30 min of reaction (mL)
Vb = Volume of the NaOH solution spent in titration of the blank reaction (mL)
m = Mass of sample used in the reaction (g)
t = Reaction time (minutes)
C = Concentration of NaOH solution

[1] Freire, D. M.; Teles, E. M. F.; Bon, E. P. S. & Sant'Anna Jr., G. L. S. Lipase Production by Penicillium restrictum in a Bench-Scale Fermenter: Effect of Carbon and Nitrogen Nutrition, Agitation, and Aeration. *Applied Biochem. Biotechnol*. **63**, 409-421 (1997). doi:10.1007/978-1-4612-2312-2_36
[2] Chiaradia, V.; Soares, N. S.; Valério, A.; de Oliveira, D.; Araújo, P. H. H. & Sayer, C. Immobilization of Candida antarctica Lipase B on Magnetic Poly(Urea-Urethane) *Applied Biochem. Biotechnol*. **180**, 558–575 (2016). doi:10.1007/s12010-016-2116-6

Table S1: Titration volume of NaOH (mL).

Entry	V_{NaOH} (mL)	Average V_{NaOH} (mL)
Va1	50.330	
Va2	52.358	51.175
Va3	50.837	
Vb1	18.358	
Vb2	18.358	18.407
Vb3	18.504	

$$A(U/g) = \frac{(Va - Vb).N.10^3}{t.m}$$

$$A = \frac{(51.175 - 18.407).0{,}029x10^3}{30.0{,}01}$$

$$A = 3167{,}606\ U/g\ ou\ 3{,}17\ U/mg$$

1.2. *Continuous Flow hydrolysis*

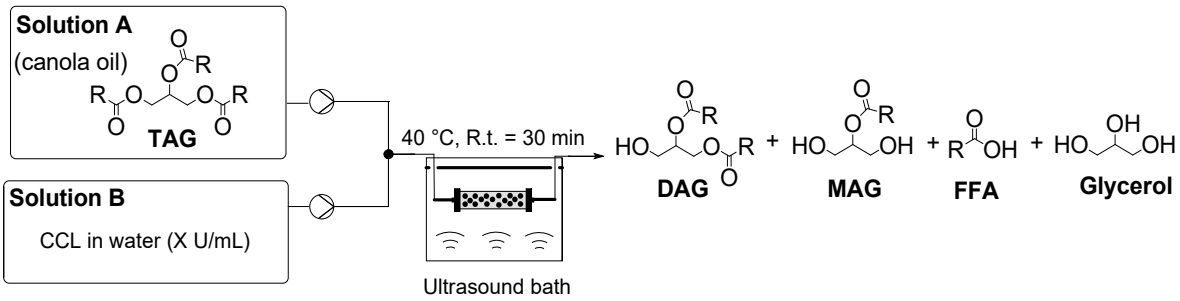


Figure S1: Setup for continuous flow hydrolysis.

2. Hydrogenation of the Canola Oil hydrolyzate

2.1. Batch

Figure S2: Pressure reactor - miniclave drive - Buchiglas (Büchi AG –Switzerland).

Table S2: Hydrogenation of the canola oil hydrolyzate with 10% Pd/C.

Entry	Time (min)	Ethyl stearate (%)*
1	5	16
2	30	29
3	60	33

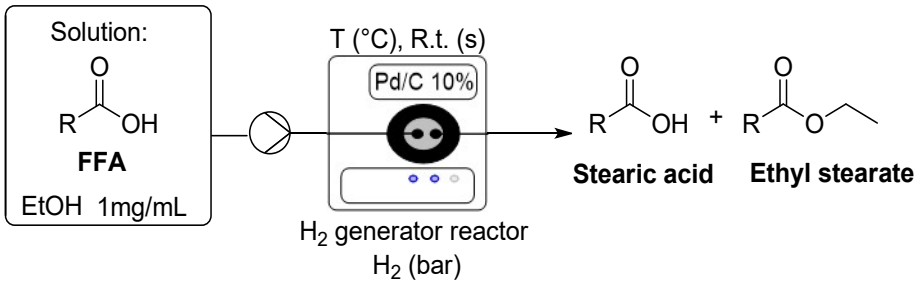
Reaction conditions: solution (100 mL) of canola oil hydrolyzate in ethanol 99.5 % (1 mg mL^{-1}), 5 mg of 10% Pd/C, 10 bar H_2, 25 ºC. *Ethyl stearate (%) values based on GC absolute area.

2.2. Continuous Flow

Figure S3: H-Cube® Mini Plus continuous flow hydrogenation reactor (ThalesNano).

2.2.1. Design of Experiments (DoE) of Continuous Flow Hydrogenation of the Canola Oi l hydrolysate

Table S3: DoE of Continuous-Flow hydrogenation of canola oil hydrolysate with 10 % Pd/C.



Entry	T (°C)	R.T. (s)[a]	Conv. (%)[b]	Stearic acid Sel. (%)[c]	Ethyl stearate Sel. (%)[c]
1	30.0	50.0	75	64	11
2	30.0	100.0	88	69	19
3	50.0	50.0	79	58	21
4	50.0	100.0	95	74	21
5	25.9	75.0	70	66	4
6	54.1	75.0	87	66	21
7	40.0	39.6	75	57	18
8	40.0	110.4	95	75	20
9	40.0	75.0	92	71	21
10	40.0	75.0	92	71	21

Reaction conditions: solution (100 mL) of Canola oil hydrolysate in ethanol 99.5 % (1 mg mL^{-1}), 250 mg of 10 % Pd/C catalyst cartridge (Thales Nano), 15-30 bar H_2. [a]Residence time; [b]Conversion (%) and [c]Selectivity (%) values based on GC absolute area.

The statistical analysis demonstrated that the empirical model (described by the Equation 1; R^2 = 0.95) could be predictive. The factors considered significant (p-value<0.05) by the analysis of variance (ANOVA) were linear temperature and residence time, both with positive effects in the conversion of hydrogenation reaction, and quadratic temperature (Figure S4).

Eq. 1:

$$Conv.\,(\%) = -62.1340 + (5.1325 \times T) + \left(-0.0615 \times T^2\right) + \left(0.8527 \times RT^2\right) + \left(-0.00 \\ (0.0030 \times T \times RT)$$

Conv. (%) = Conversion (%)

T = Temperature (°C)

RT = Residence Time (s)

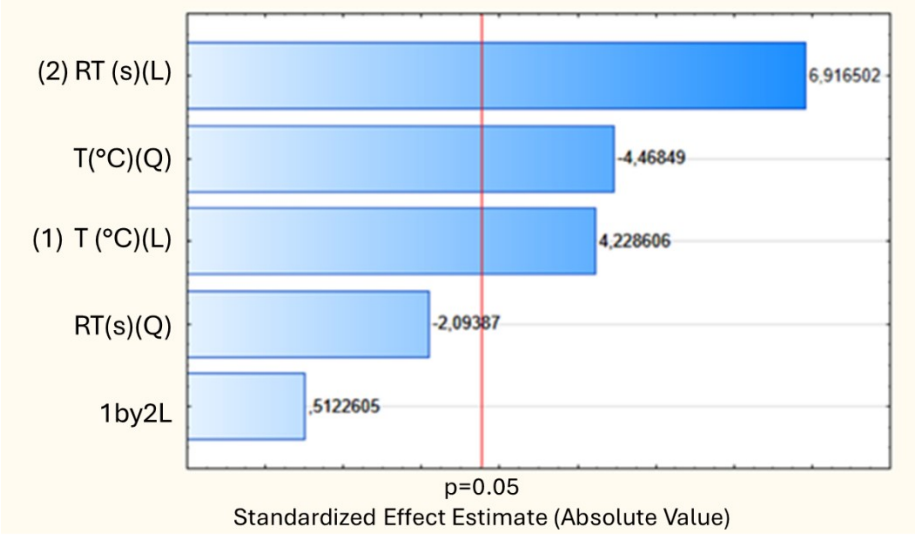


Figure S4: Pareto Chart of standardized effects of canola oil hydrogenation. The factors included in the model are described as: linear effect of temperature ((1) T (°C) (L)); linear effect of residence time ((1) RT (s) (L); quadratic effect of temperature (T(°C)(Q)); quadratic effect of residence time (RT(s)(Q)); and the interaction effect between linear factors (1Lby2L).

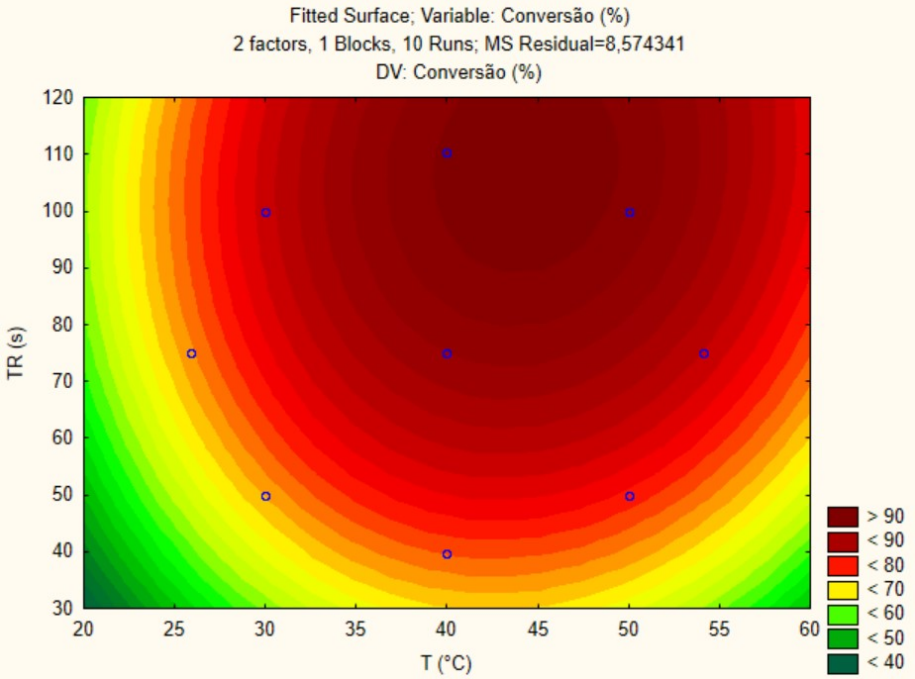
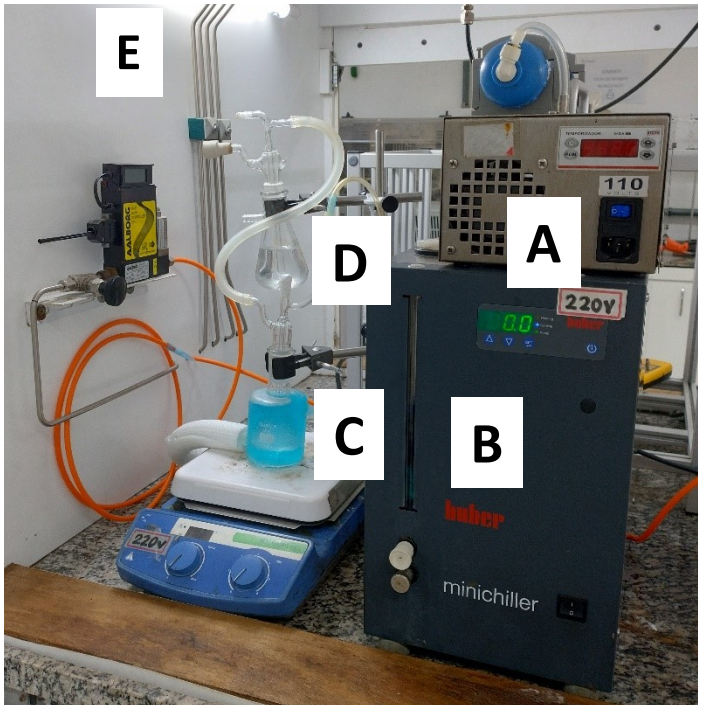


Figure S5: Surface fitting of standardized effects of canola oil hydrogenation.

3. Ozonolysis

3.1. Batch



A: Ozone generator

B: Chiller

C: 50 mL jacketed reaction vessel

D: Quench: KI 5% m/v

E: O_2 Gas Mass Flow Controller

Figure S6: Setup for batch ozonolysis synthesis of azelaic and pelargonic acid from commercial oleic acid.

3.2. Co

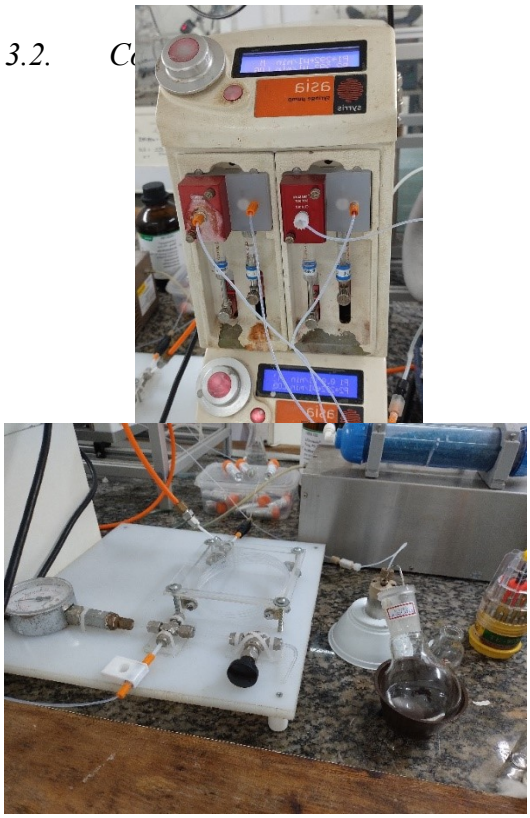


Figure S7: Flow Ozonolysis Setup.

3.3. Product Purification

Figure S8: Azelaic Acid.

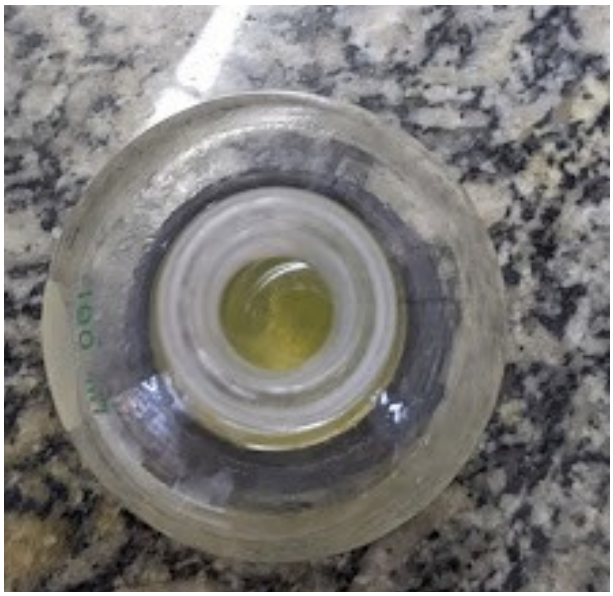


Figure S9: Pelargoic Acid.

4. Enzymatic photodecarboxyl rgonic Acids

Figure S10: ThalesNano Photocube™ and LAUDA Thermocolling.

5. Chromatographic Analysis

5.1. Gas Chromatography-FID Spectrometry (GC-FID):

Method: Injection temperature 350 ºC, injection split ratio 1:10, Hydrogen was used as carrier gas with pressure 112.9 kPa, column flow at 2.60 mL min^{-1}. The oven temperature setting was: 100 ºC for 1 min, then heated at 20 ºC min^{-1} to 150 ºC, then heated at 10 ºC

min^{-1} until 180 ºC for 6 min, then heated at 25 °C min^{-1} to 370 °C for 10 min. Flame temperature 380 ºC. Columns used: DB-1HT or DB-5HT.

5.1.1. Batch Hydrolysis of Canola Oil

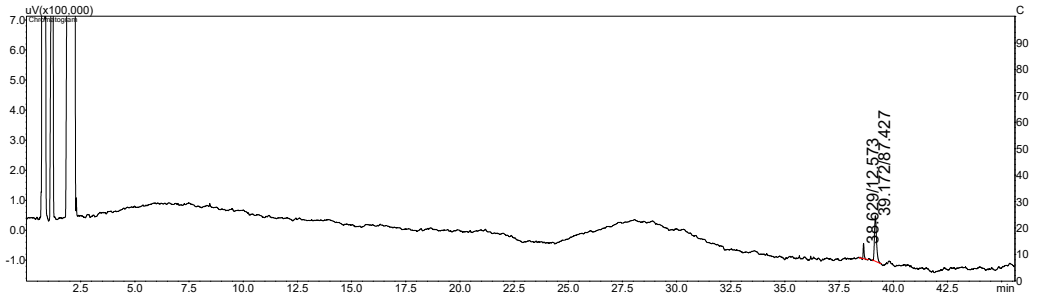


Figure S11: GC−FID Chromatogram of canola oil.

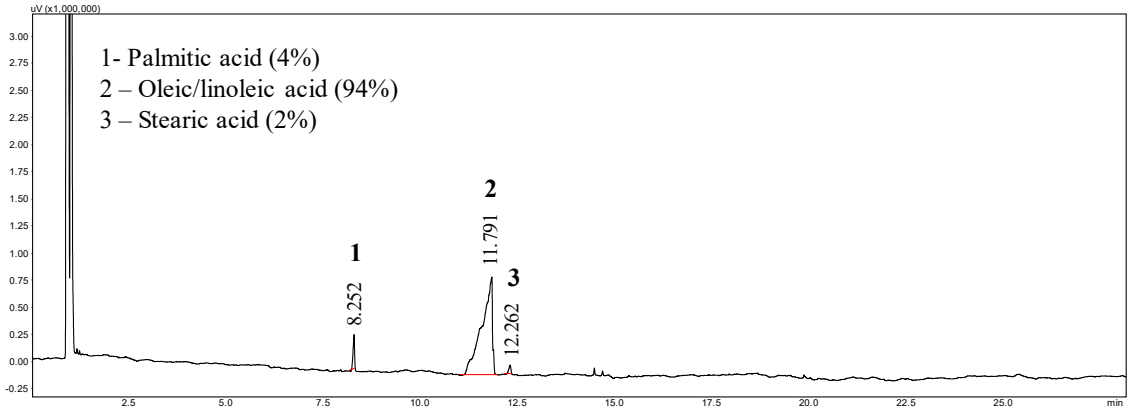


Figure S12: GC−FID Chromatogram of hydrolysis of canola oil under batch mode with oil-water ratio of 1:4, 0.1 % w/v of enzyme for 2 h (Table 1, Entry 4).

5.1.2. Continuous-Flow Hydrolysis of Canola Oil

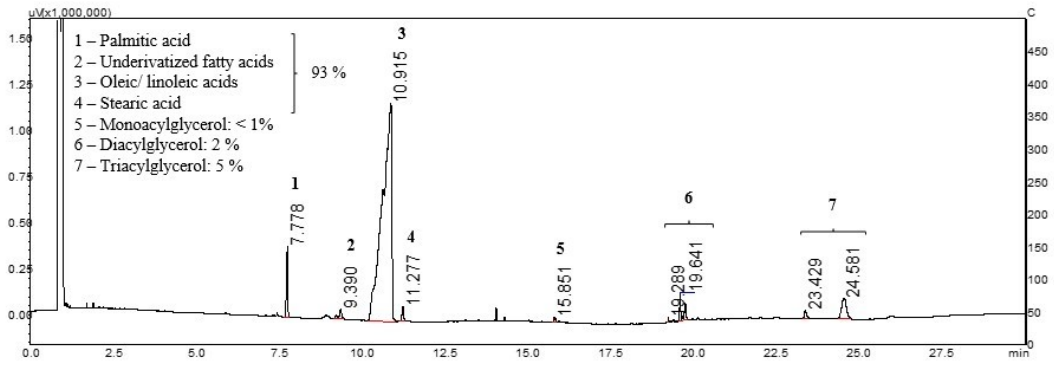


Figure S13: GC−FID Chromatogram of continuous-flow hydrolysis of canola oil under flow mode in configuration C (coil under ultrasound bath) with oil-water ratio of 1:4, 0.5 % w/v of enzyme for 30 min (Table 2, Entry 19).

5.1.3. Ozonolysis

5.1.3.1. Batch ozonolysis of commercial oleic acid

The vanillin stain was prepared by dissolving 1 g of vanillin in 100 mL of ethanol with the addition of 1 mL of concentrated sulfuric acid. After visualization by heating, this reagent provides strong contrast for oxidized compounds such as ozonides.

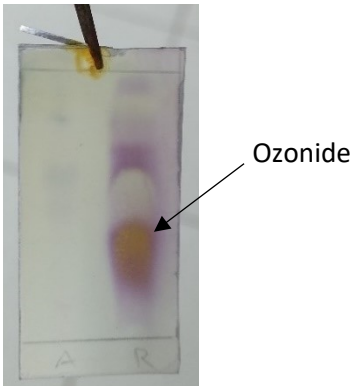


Figure S14: TLC ozonide formation in batch mode 1º Step (Vanilin as dyeing agent, Eluent: ethyl acetate/cyclohexane (8:2).

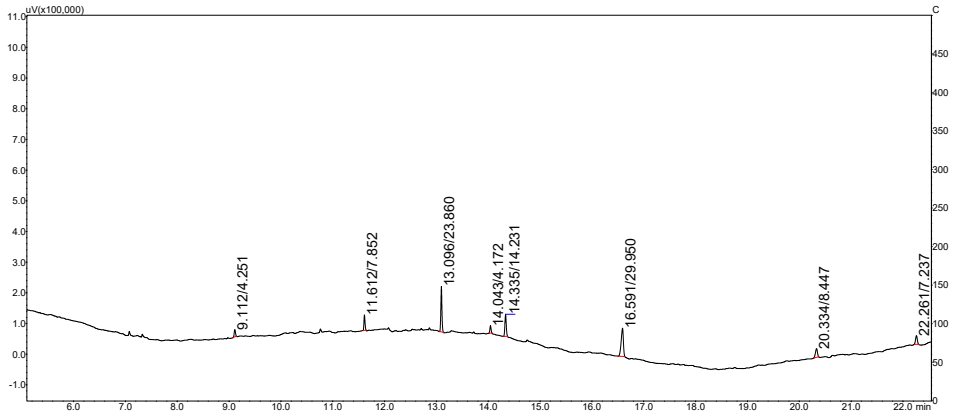


Figure S15: GC−FID Chromatogram of batch ozonolysis of oleic acid after 15 min of O2 (2º step).

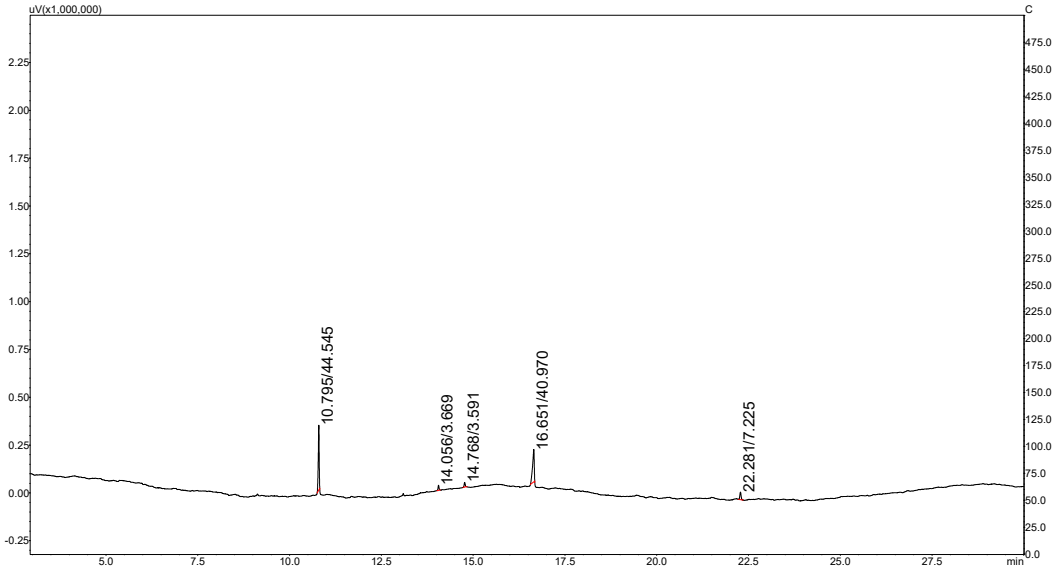
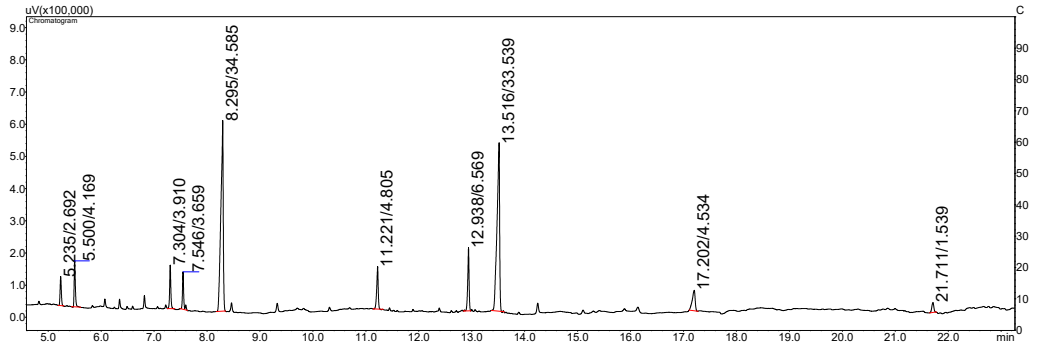


Figure S16: GC−FID Chromatogram of batch ozonolysis of oleic acid after 30 min of O2 (2º step).

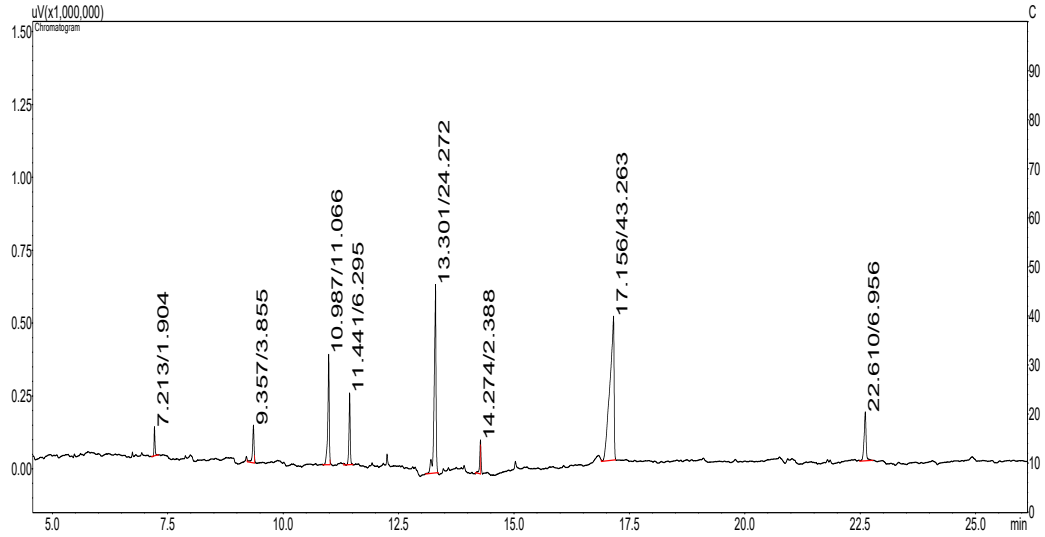
5.1.3.2. Batch hydrolysis and ozonolysis cascade of canola oil



Entry	Retention Time (min)	Substance	(%)
1	5.23	Malonic acid	2.69
2	5.5	Hexanoic acid	4.17
3	8.29	Pelargonic acid	34.58
4	11.22	Tetradecane*	4.8
5	13.52	Azelaic acid	33.54
6	17.20	Palmitic acid	4.53
7	21.71	Stearic acid	1.54

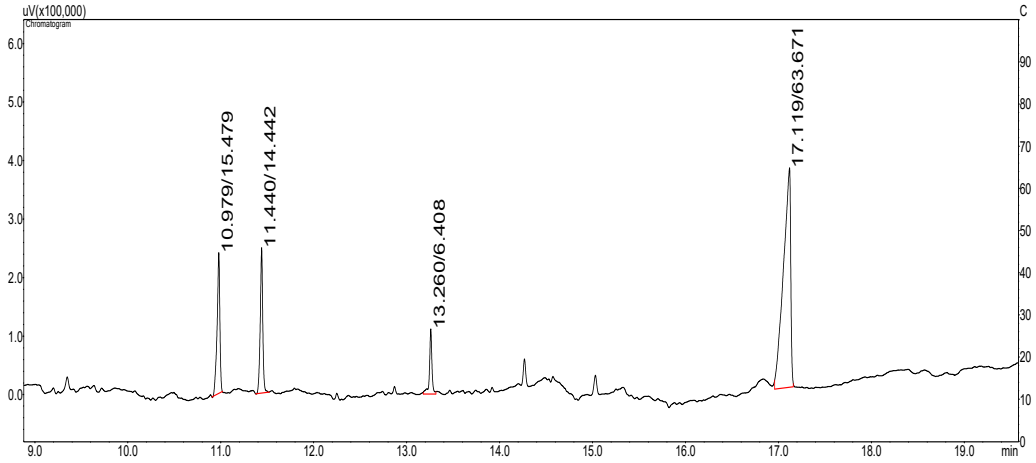
Figure S17: GC−FID Chromatogram of hydrolysis and ozonolysis cascade canola oil under batch mode. * Internal standard

5.1.3.3. Continuous-Flow ozonolysis of commercial oleic acid and canola oil hydrolysate.



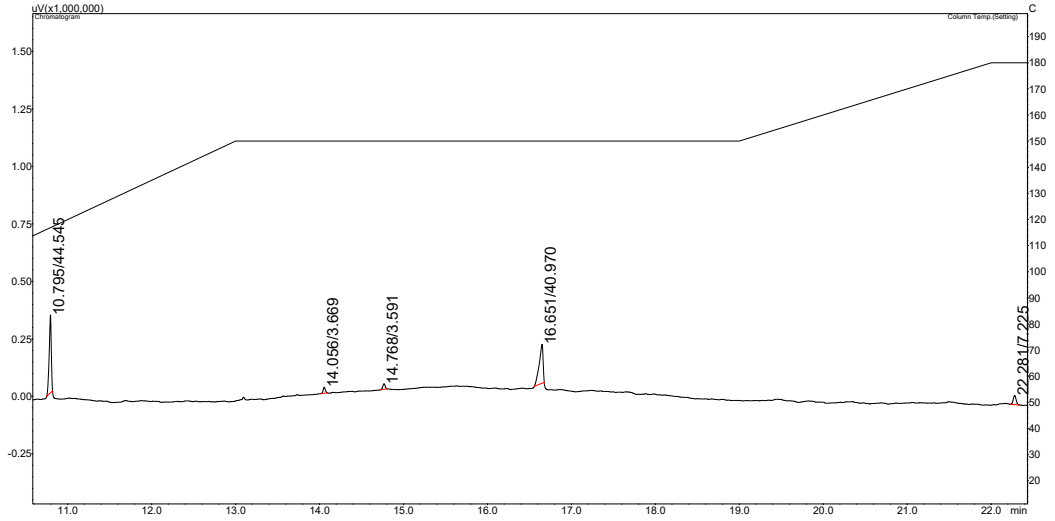
Entry	Retention Time (min)	Substance	(%)
1	10.98	Pelargonic acid	11.07
2	11.44	Tetradecane*	6.29
3	17.17	Azelaic acid	17.15
4	22.61	Palmitic acid	6.95

Figure S18: GC−FID Chromatogram ozonolysis of oleic acid under continuous flow in 30 min R.t. (Table 3, Entry 1). *Internal standart



Entry	Retention Time (min)	Substance	(%)
1	10.98	Pelargonic acid	15.48
2	11.44	Tetradecane*	14.44
3	17.17	Azelaic acid	63.6

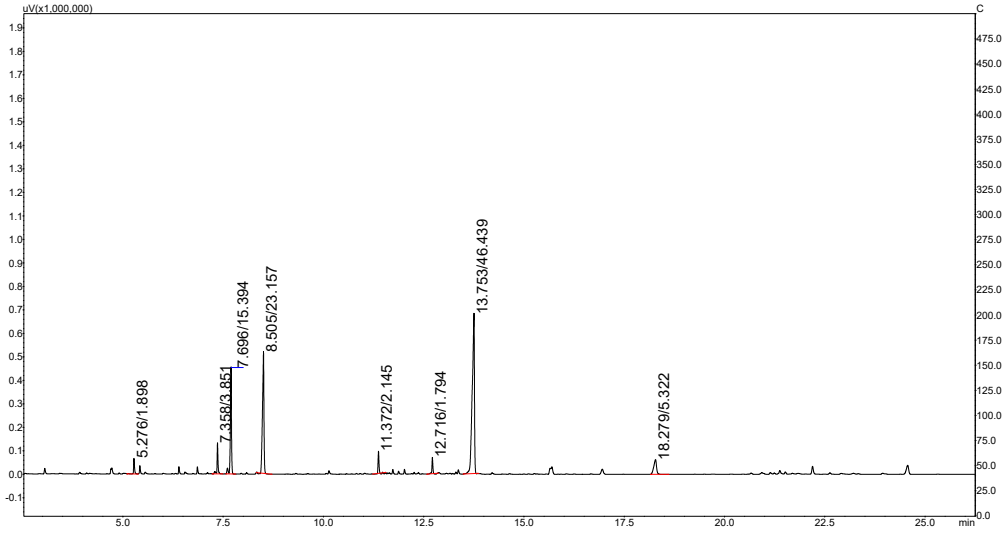
Figure S19: GC−FID Chromatogram ozonolysis of oleic acid under continuous flow in 40 min R.t. (Table 3, Entry 2). *Internal standart



Entry	Retention Time (min)	Substance	(%)
1	10.79	Pelargonic acid	44.45
2	16.65	Azelaic acid	40.97
3	22.61	Palmitic acid	17.22

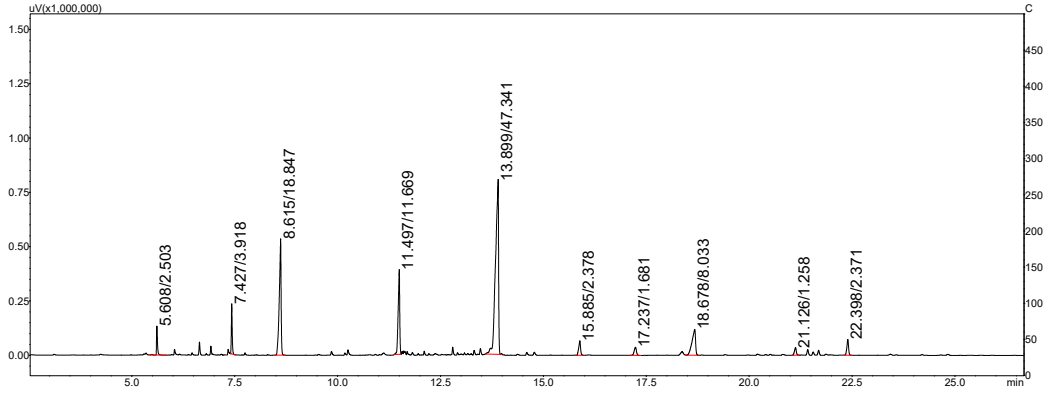
Figure S20: GC−FID Chromatogram ozonolysis of Oleic acid under continuous flow in 50 min R.t. (Table 3, Entry 4).

5.1.3.4. Continuous-Flow hydrolysis and ozonolysis of Canola oil



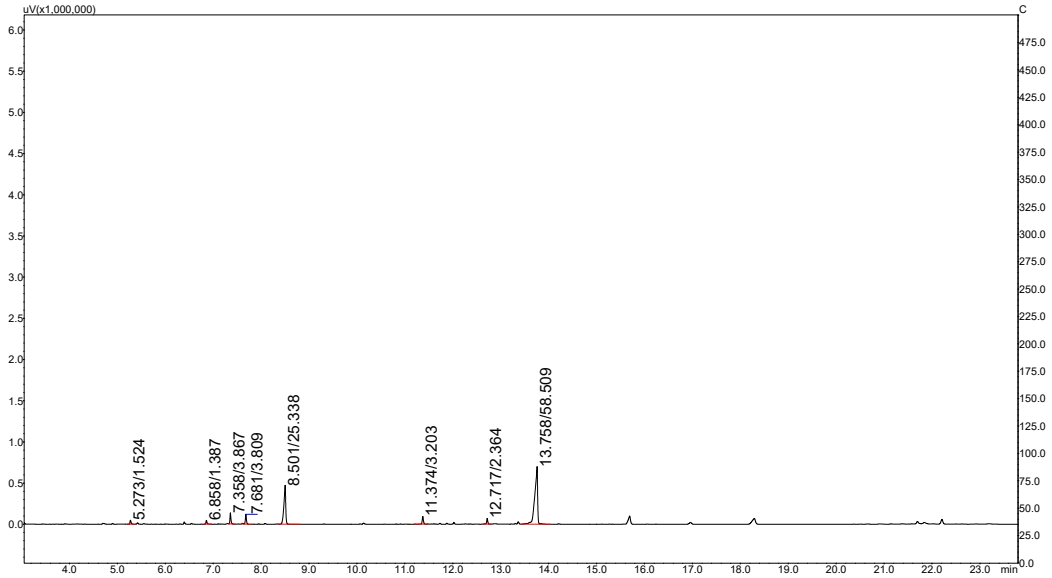
Entry	Retention Time (min)	Substance	(%)
1	8.50	Pelargonic acid	23.2
2	1.7	Lauric acid	1.79
3	13.75	Azelaic acid	46.4
4	18.28	Palmitic acid	5.3

Figure S21: GC−FID Chromatogram ozonolysis of hydrolyzed canola oil under continuous flow in 40 min R.t. (Table 3, Entry 3).



Entry	Retention Time (min)	Substance	(%)
1	5.6	Malonic acid	2.5
3	8.6	Pelargonic acid	18.8
5	13.9	Azelaic acid	47.3
6	18.7	Palmitic acid	8.0
7	22.4	Stearic acid	2.4

Figure S22: GC−FID Chromatogram ozonolysis of hydrolyzed canola oil under continuous flow in 50 min R.t. (Table 3, Entry 5).



Entry	Retention Time (min)	Substance	(%)
1	5.3	Malonic acid	1.5
2	6.8	Hexanoic acid	1.34
3	8.5	Pelargonic acid	25.3
4	11.44	Lauric acid	2.4
5	13.8	Azelaic acid	58.5

Figure S23: GC−FID Chromatogram ozonolysis of hydrolyzed canola oil under continuous flow in 60 min R.t. (Table 3, Entry 6).

5.2. Gas Chromatography-Mass Spectrometry (GC-MS):

5.2.1. Batch hydrogenation of Canola oil hydrolysate

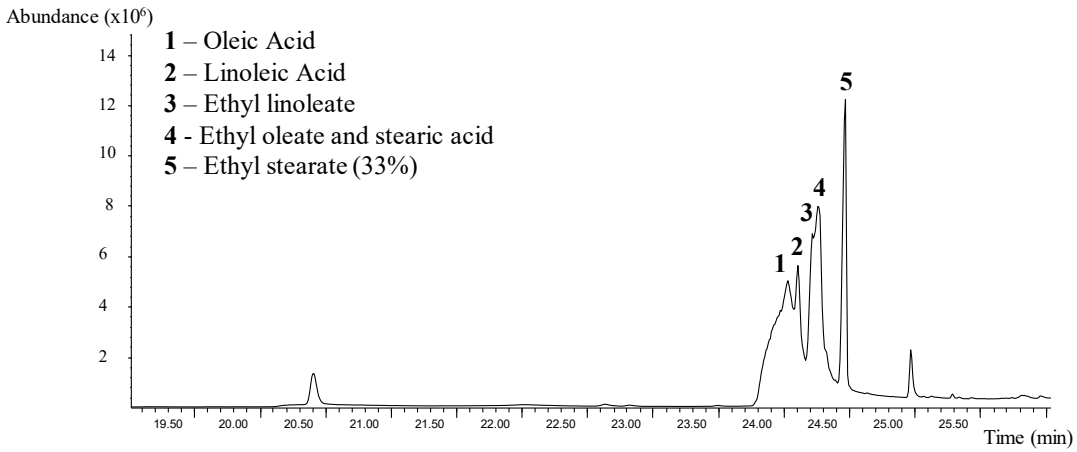


Figure S24: GC−MS Chromatogram of batch hydrogenation of canola oil hydrolysate after 60 min (Table S1, Entry 3).

5.2.2. Continuous-flow hydrogenation of canola oil hydrolysis

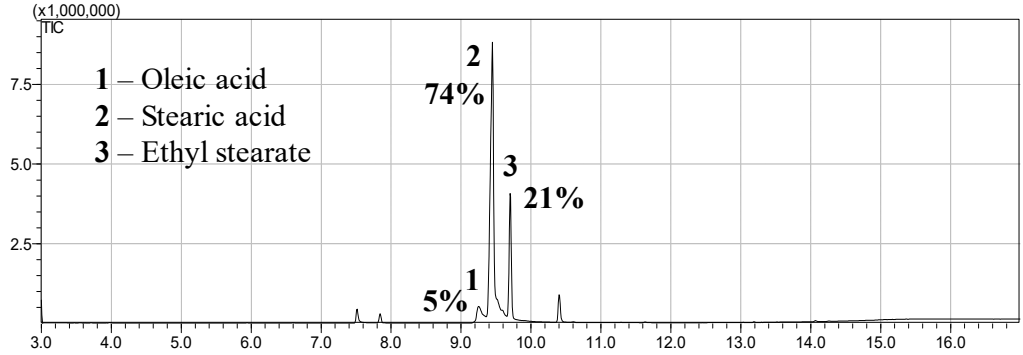


Figure S25: GC−MS Chromatogram of continuous flow hydrogenation of canola oil hydrolyzate using H-cube (DoE, Table S2, Entry 4).

5.2.3 Enzymatic Photodecarboxylation of Stearic and Pelargonic Acids

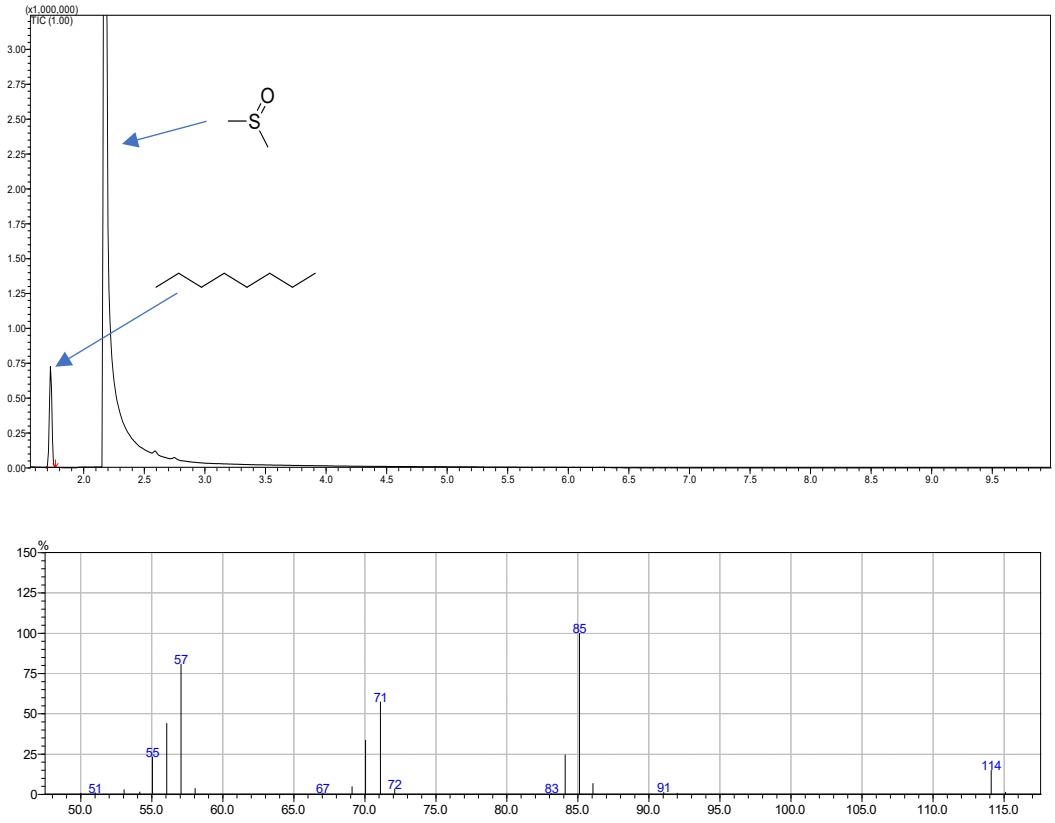
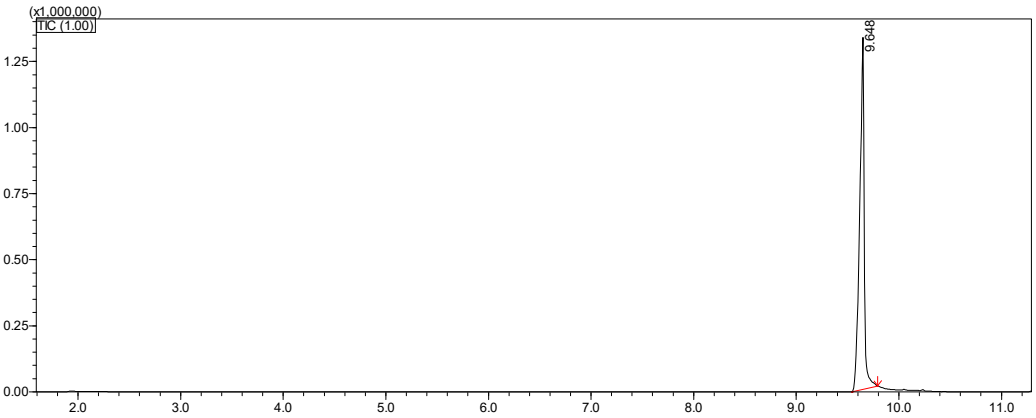


Figure S26: CG-MS Chromatogram of enzymatic photodecarboxylation of pelargonic acid and mass spectrum of octane 1.8 min (> 99 % of conversion).



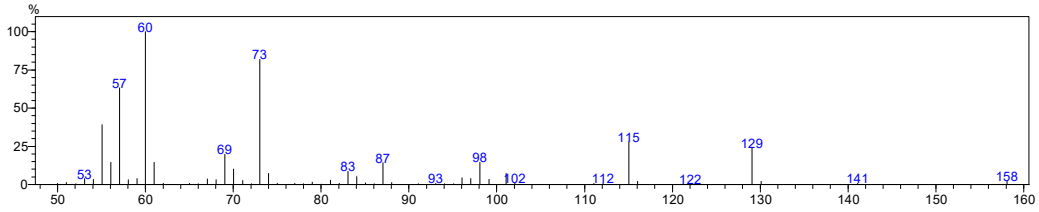


Figure S27: GC-MS Chromatogram and mass spectrum of pelargonic acid (9.6 min).

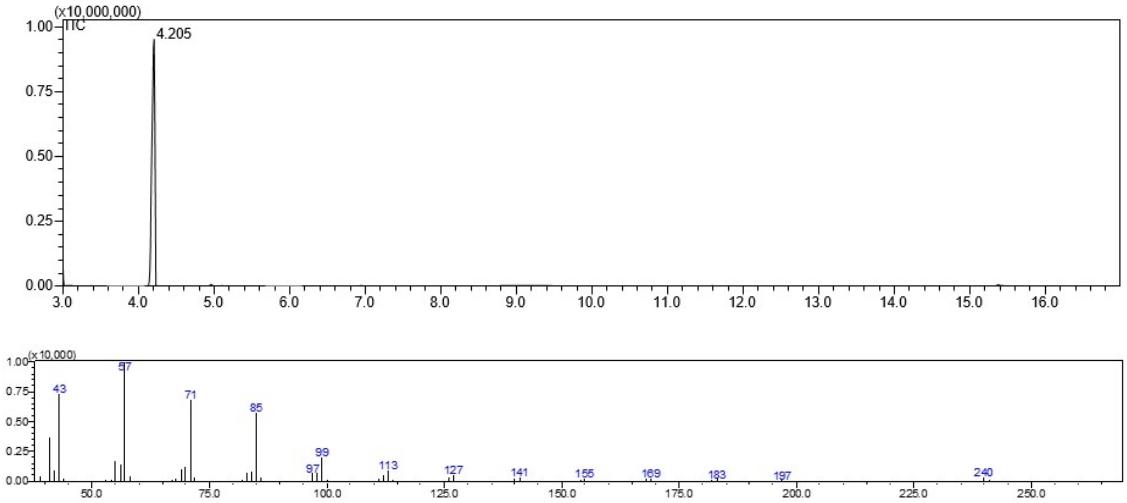


Figure S28: GC-MS Chromatogram and mass spectrum of heptadecane (4.2 min).

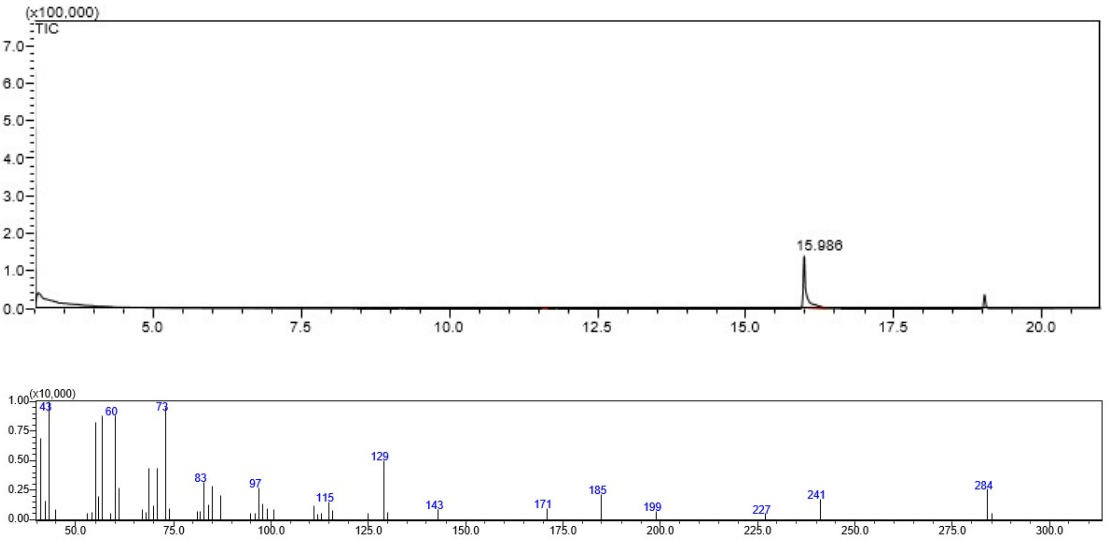


Figure S29: GC-MS Chromatogram and mass spectrum of stearic acid (16 min).

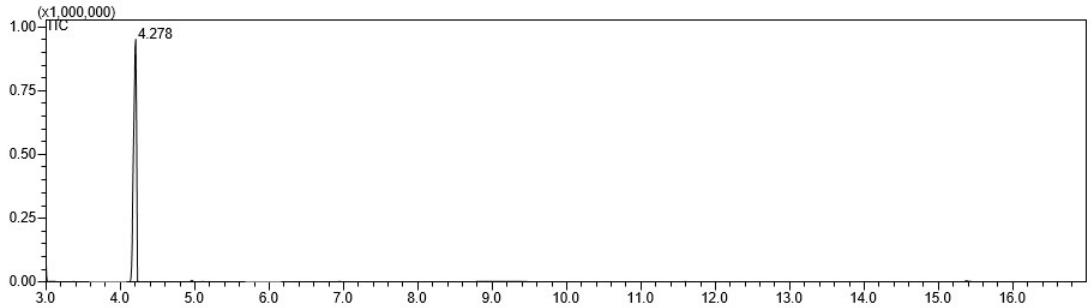


Figure S30: GC-MS Chromatogram of decarboxylation after 5 min with 13 mM of stearic acid (> 99 % of conversion).

5. Spectral data

NMR spectra was recorded on a Bruker Avance III spectrometer operating at 500 MHz (^{1}H and ^{13}C). DMSO-*d6* and $CDCl_3$ (99.9% D, Aldrich, São Paulo, SP, Brazil) were used as a solvent.

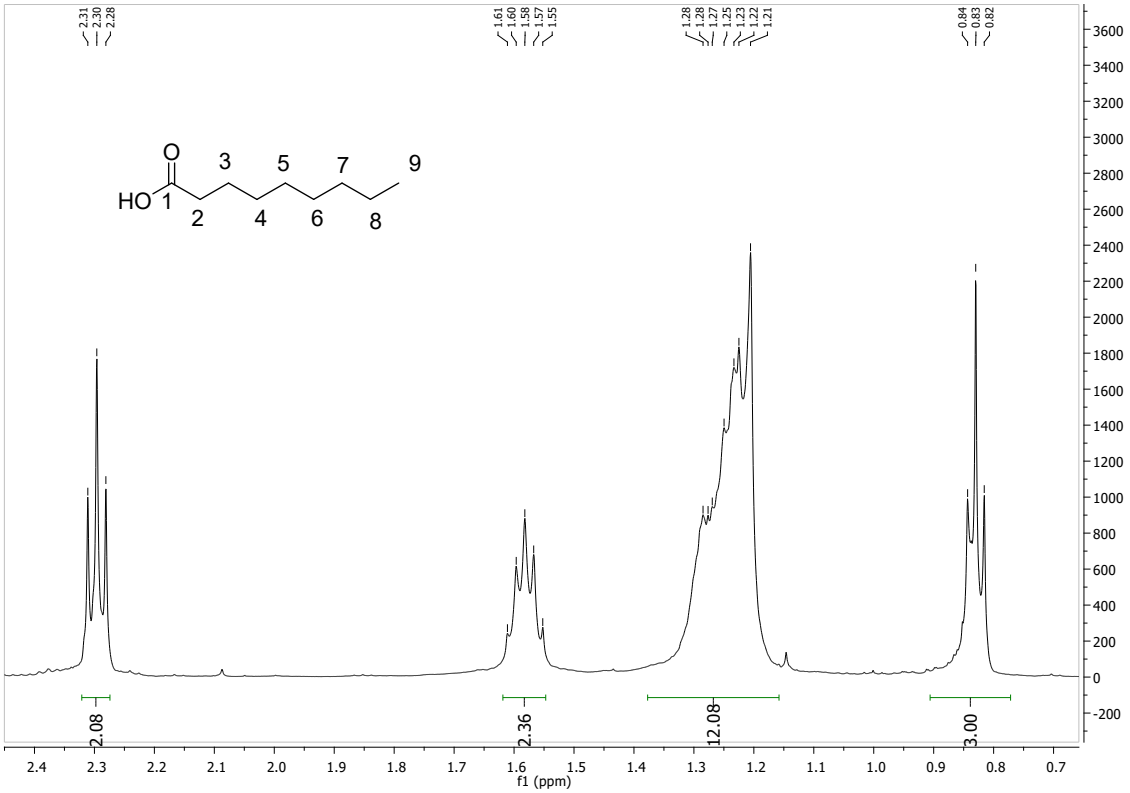


Figure S31: ^{1}H NMR spectrum of pelargonic acid, in $CDCl_3$.

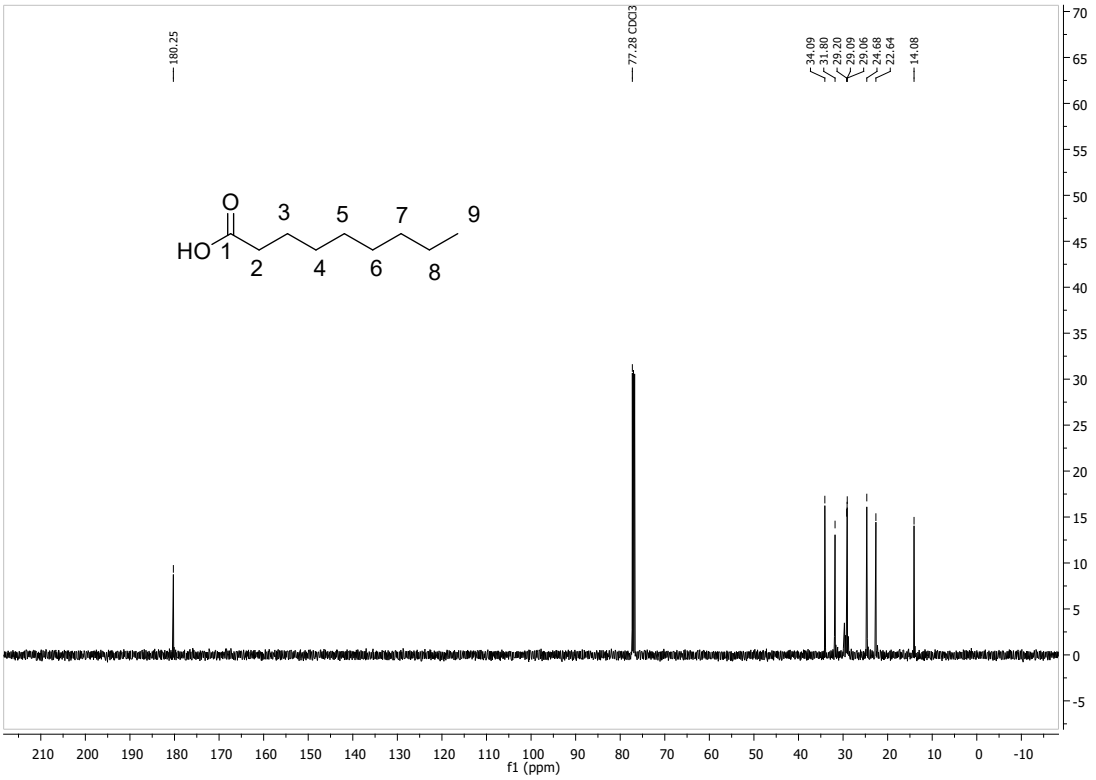


Figure S32: ^{13}C NMR spectrum of pelargonic acid, in $CDCl_3$.

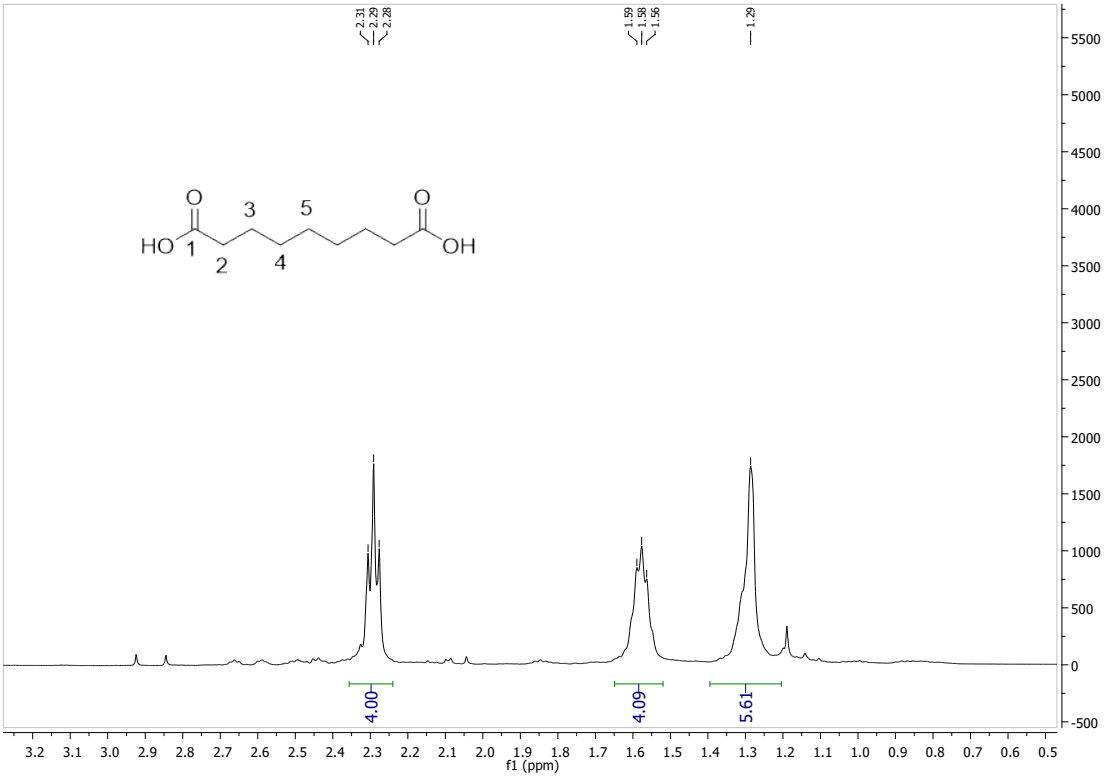


Figure S33: ^{1}H NMR spectrum of azelaic acid, in $CDCl_3$.

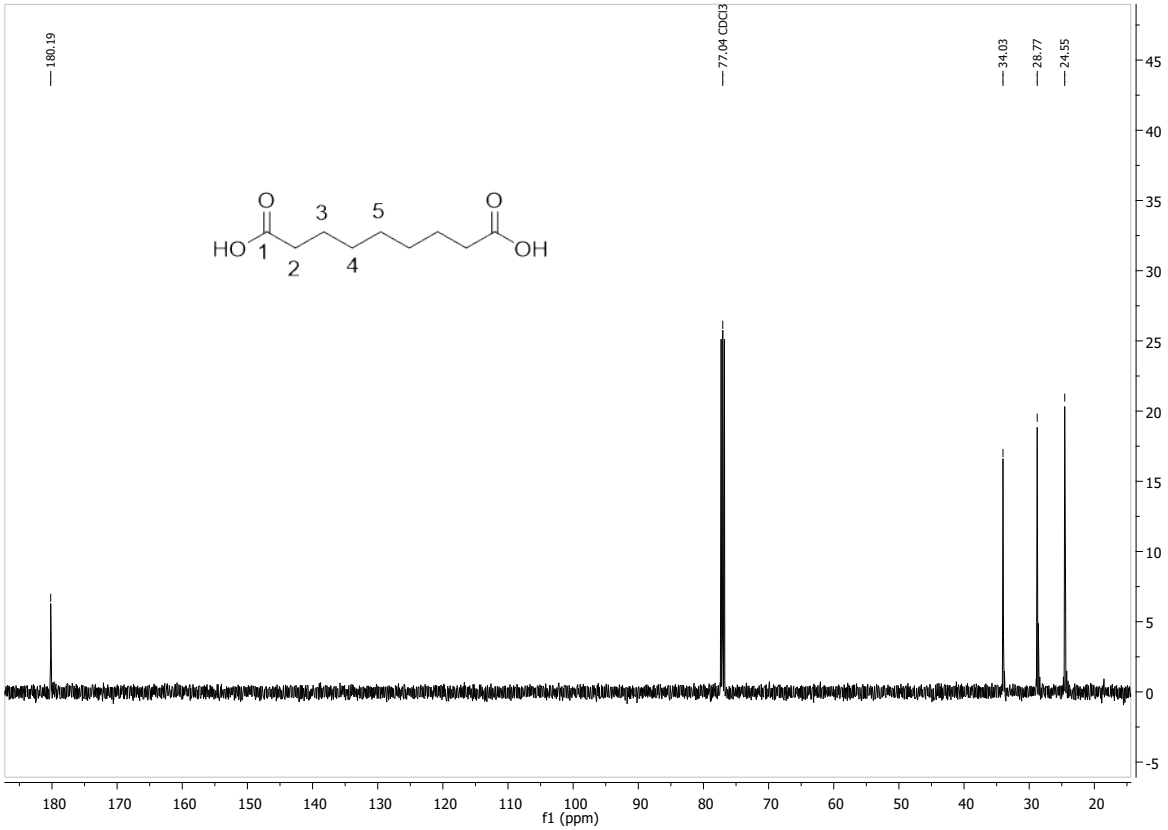


Figure S34: ^{13}C NMR spectrum of Azelaic acid, in $CDCl_3$.