Electronic Supplementary Material (ESI) for Green Chemistry.

Multi-step chemo-enzymatic synthesis of azelaic and pelargonic acids from the soapstock of high-oleic sunflower oil refinement

Beatrice Casali, Elisabetta Brenna*, Fabio Parmeggiani, Francesca Tentori*, and Davide Tessaro

Dipartimento di Chimica, Materiali ed Ingegneria Chimica "Giulio Natta",
Politecnico di Milano, Piazza Leonardo da Vinci 32, 20133, Milano, Italy.
E-mail: mariaelisabetta.brenna@polimi.it; phone: +39 02 23993077
E-mail: francesca.tentori@polimi.it; phone: +39 02 23993173

Electronic Supplementary Information

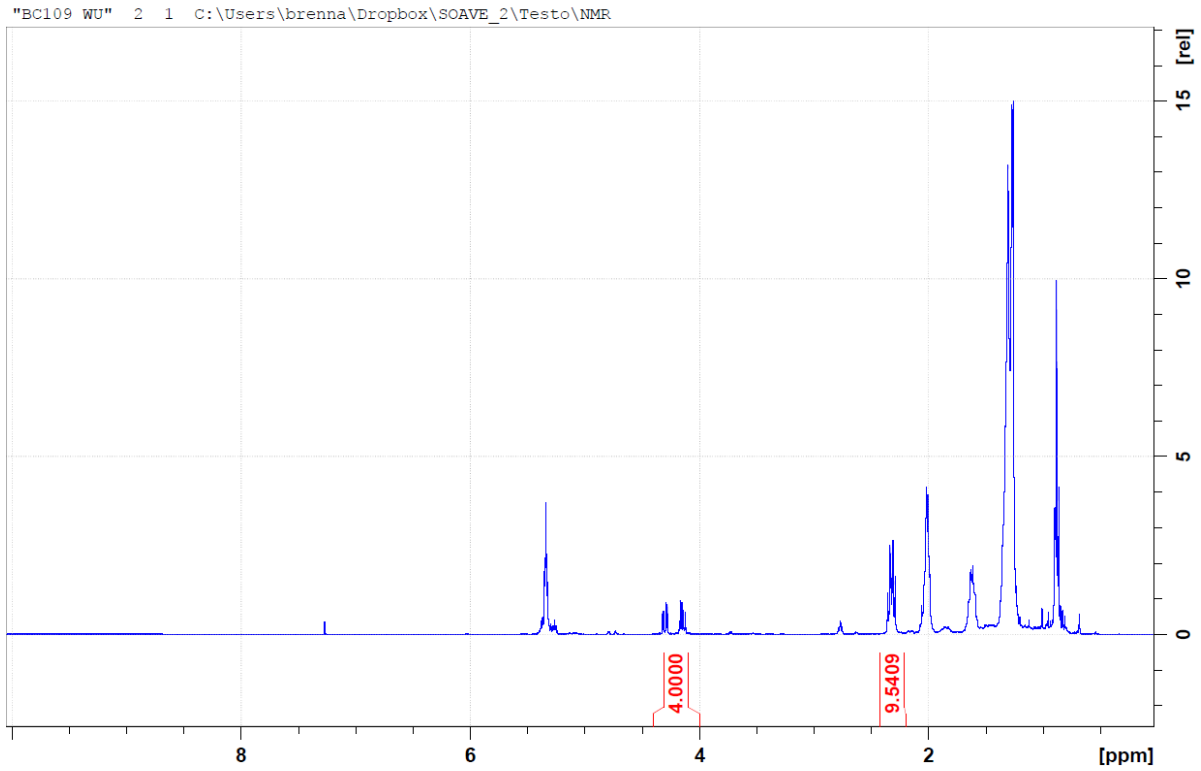
Evaluation of the molar ratio between triglycerides and free fatty acids in samples of high-oleic sunflower oil soapstock

A suitable sample of soapstock (1 g) was dispersed in distilled water (15 mL). The value of pH was lowered from 10 to 6 by adding a diluted solution of H_3PO_4, then the mixture was extracted with EtOAc, dried over Na_2SO_4 and evaporated under vacuum. The molar ratio between triglycerides and free fatty acids was estimated by ^1H NMR by considering the integrals of the following signals:

- 4.40-4.00 ppm, multiplet of the 2 CH_2 units of triglycerides;
- 2.38-2.24 ppm, multiplet of the CH_2 units of triglycerides (3 units) and free fatty acids (1 CH_2 unit) in α position with respect to the carboxylic moiety. The analyzed samples always showed an equimolar quantity of triglycerides and free fatty acids.

The ^{31}P NMR spectrum recorded in $CDCl_3$ showed the absence of phospholipids. A representative ^1H NMR spectrum of a sample of high oleic sunflower oil soapstock is shown below.

Representative ^1H NMR of high-oleic sunflower oil soapstock



Evaluation of the molar distribution of the main fatty acids in the mixtures obtained by enzymatic splitting of samples of high-oleic sunflower oil soapstock

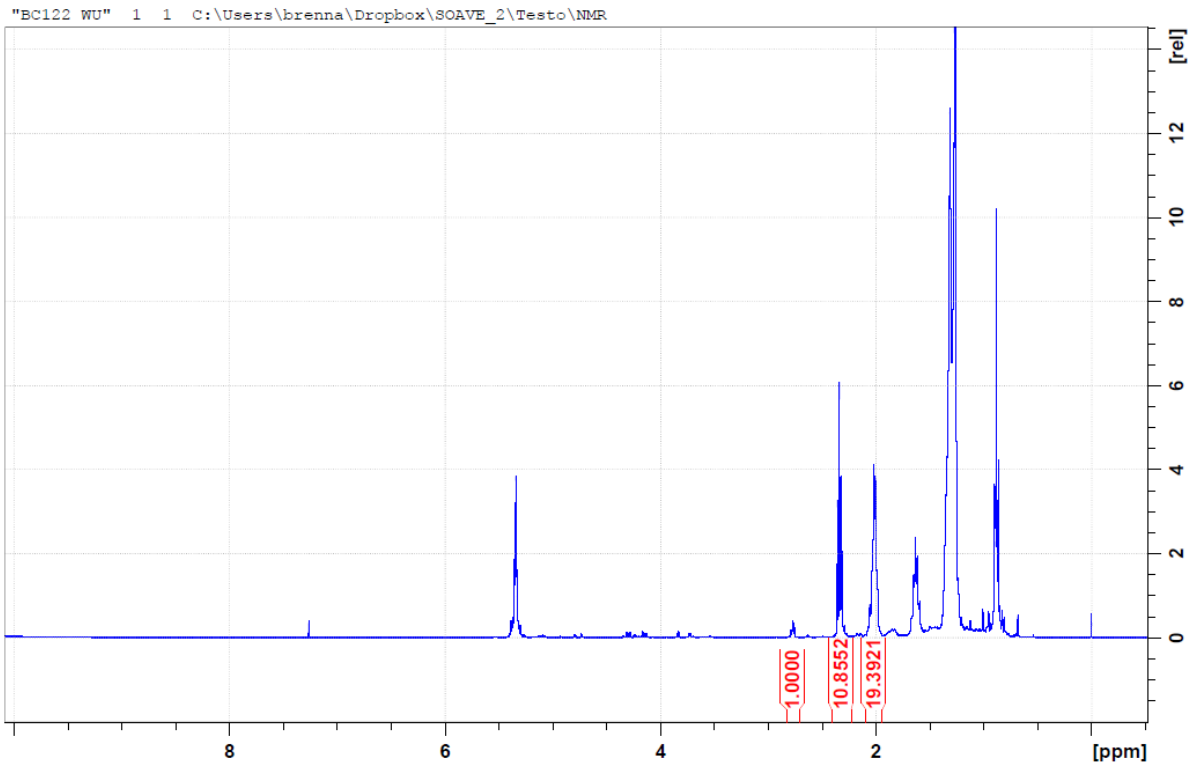
A suitable sample of soapstock was submitted to enzymatic hydrolysis according to the procedure reported in the main text. The molar distribution of the most abundant fatty acids contained in each sample was estimated by ^{1}H NMR by considering the integrals of the following signals:

- 2.83-2.71 ppm, 1 CH_2 unit between the two C=C double bonds of polyunsaturated acids (mainly linoleic acid);
- 2.41-2.23 ppm, 1 CH_2 unit in α position with respect to the carboxylic moiety of all free fatty acids;
- 2.09-2.14 ppm, 2 CH_2 units in allylic position with respect to the C= double bond of monounsaturated fatty acids (mainly oleic acid) and polyunsaturated acids (mainly linoleic acid).

The following distributions were obtained: 80-87% monounsaturated fatty acids (mainly oleic acid), 9% polyunsaturated fatty acids (mainly linoleic acid), 4-11% saturated acids (mainly palmitic and stearic acids). These results were confirmed by GC/MS analysis of the corresponding mixture of methyl esters.

A representative example of ^{1}H NMR spectrum of the mixture of free fatty acids recovered from high oleic sunflower oil soapstock is shown below.

Representative ^{1}H NMR of the mixture of FFAs obtained
by enzymatic hydrolysis of high-oleic sunflower oil soapstock



1. Factorial design – Enzymatic epoxidation of hydrolyzed soapstock

n° run	X1	X2	X3	X4	Y
	Oleic acid [g L^{-1}]	N435 [g L^{-1}]	H_2O_2 [% v/v]	T [°C]	Conversion to oleic acid epoxide [% by GC-MS]
1	10	2	1	30	12
2	10	1	4	30	3
3	50	1	4	30	17
4	50	1	4	30	21
5	10	1	1	30	5
6	10	1	4	50	21
7	10	1	1	50	15
8	10	1	4	30	5
9	10	2	4	50	25
10	50	2	1	50	36
11	50	1	1	50	24
12	10	1	4	50	20
13	50	1	4	50	18
14	10	2	4	50	16
15	50	2	1	50	41
16	10	1	1	50	10
17	10	2	1	30	10
18	50	1	1	50	30
19	10	2	4	30	7
20	50	2	4	30	39
21	50	1	1	30	14
22	50	1	4	50	30
23	10	2	4	30	3
24	50	2	4	50	34
25	10	2	1	50	30
26	50	2	1	30	32
27	50	2	4	30	42
28	10	1	1	30	5
29	50	1	1	30	23
30	50	2	1	30	39
31	10	2	1	50	30
32	50	2	4	50	38

A four-variables (oleic acid and H_2O_2 concentrations, temperature, and enzyme amount) factorial design, with a replicate for each point, was designed and analyzed through Minitab. we considered a range of 10-50 g L^{-1} of oleic acid concentration that corresponded to 12-60 g L^{-1} of hydrolyzed mixture. As for the other conditions, we chose to evaluate system response to temperature (30-50 °C), hydrogen peroxide concentration (1-4 % v/v referred to 35% w/w aq. solution), Novozym® 435 (1-2 g L^{-1}). The final reaction volume was 15 mL. All the 32 reactions were left in a thermoshaker at a certain temperature for 5 h. The reactions were quenched with a saturated solution of $NaHSO_3$, extracted with EtOAc and dried over Na_2SO_4. Conversion of oleic acid into the corresponding epoxide was analyzed as system response and was evaluated by GC-MS analysis after treating samples with MeOH and trimethylsilyldiazomethane.

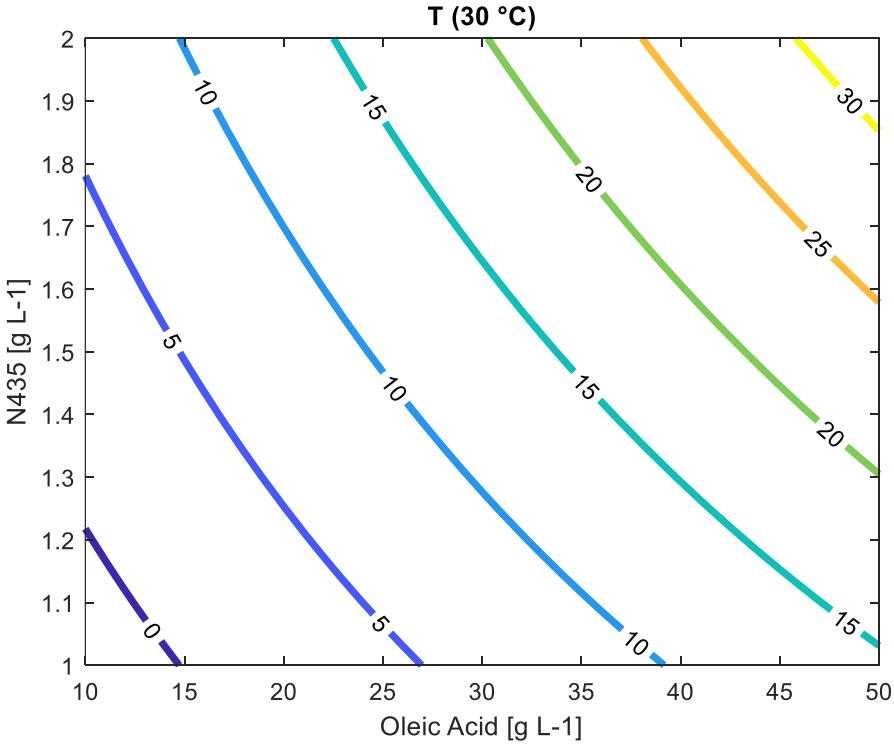


Figure S1. Contour plot of conversion [%] as a function of oleic acid and Novozym 435 concentration [g L^{-1}] evaluated at a temperature of 30°C. Lines refer to constant conversion, and the value is reported in the labels.

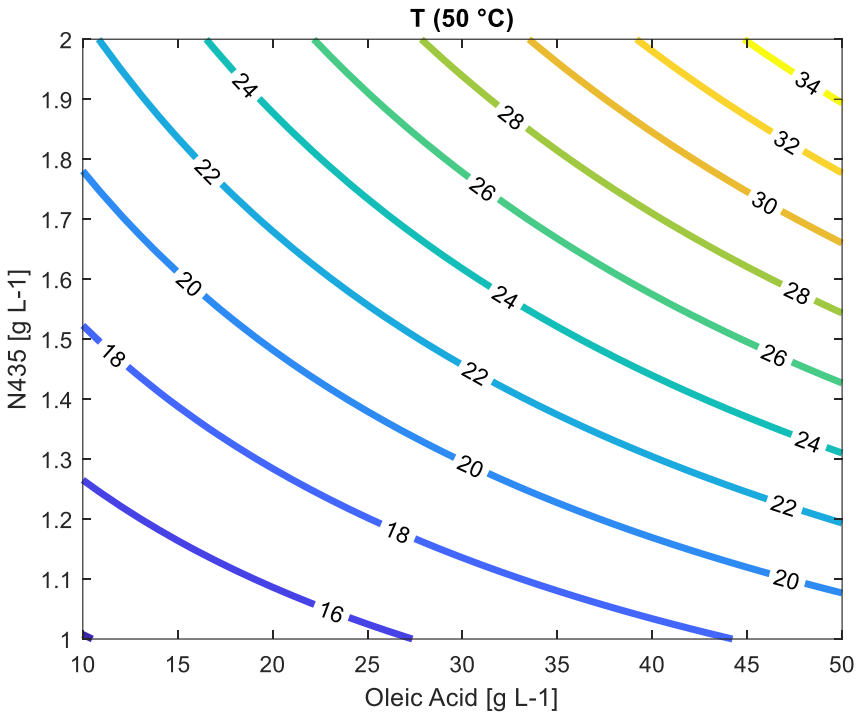


Figure S2. Contour plot of conversion [%] as a function of oleic acid and Novozym 435 concentration [g L^{-1}] evaluated at a temperature of 50°C. Lines refer to constant conversion, and the value is reported in the labels.

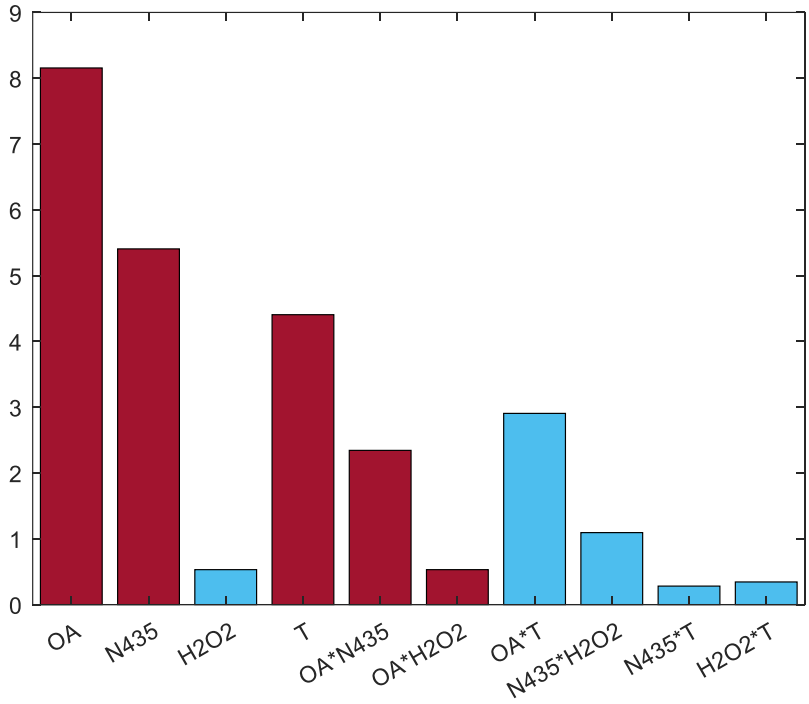


Figure S3. Coefficient plot of variable effects (first and second order); red = positive effect; light blue = negative effect.

2. Factorial design – Oxidative cleavage of oleic acid diol

For all the experiments we submitted 50 mg of oleic acid diol and 1 mg of Triton-X-100 in 5 mL EtOAc. The reactions were run in triplicates. All the 24 reactions were incubated in a thermoshaker (170 rpm) at 45°C or 55°C for 24 h.

	T [°C]	NaClO mol/ mol_{diol}^{-1}	V_{aq} [mL]	Pelargonic acid (%)	Hydroxy-oxo-stearic acid isomers(%)	Total conversion (%)
1	45	5	3	16	11	27
2	45	5	5	5	10	15
3	45	7.5	3	34	10	44
4	55	5	3	14	18	32
5	55	5	5	6	19	25
6	55	7.5	3	30	17	47
7	45	5	3	24	6	30
8	45	5	5	10	6	16
9	55	5	3	16	26	42
10	45	7.5	3	32	22	54
11	45	7.5	5	17	27	44
12	55	7.5	3	31	24	55
13	55	7.5	5	14	40	54
14	55	7.5	3	38	16	54
15	45	7.5	3	47	14	61
16	45	7.5	5	16	17	33
17	45	7.5	5	17	14	31
18	55	5	5	9	27	36
19	55	7.5	5	19	26	45
20	55	5	5	9	37	46
21	55	7.5	5	15	29	44
22	45	5	3	19	22	41
23	45	5	5	11	18	29
24	55	5	3	35	23	58

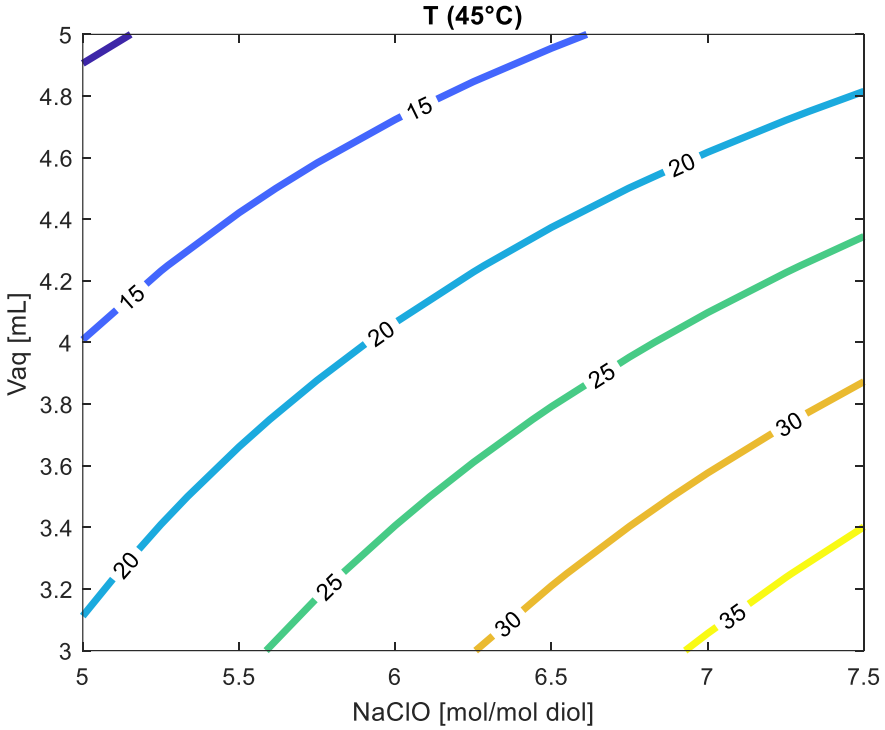


Figure S4. Contour plot at 45 °C of conversion [%] as a function of the total volume [mL] of the aqueous phase (V_{aq}) and the amount of NaClO (NaClO), expressed as the molar ratio [mol/mol diol] between NaClO and diol **4**. Lines refer to constant conversion, and the value is reported in the labels.

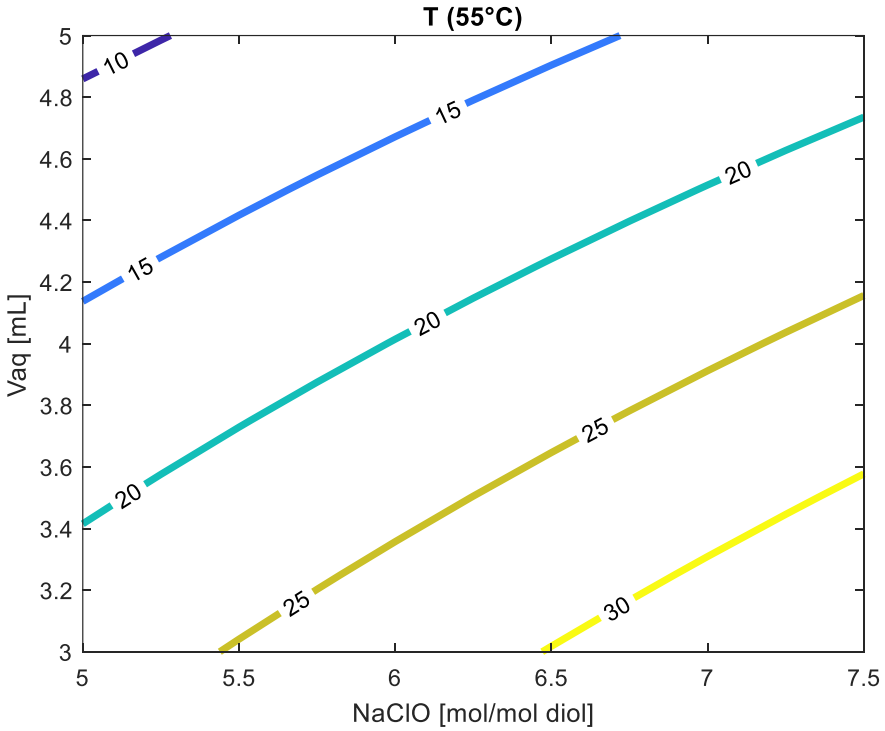


Figure S5. Contour plot at 55 °C of conversion [%] as a function of the total volume [mL] of the aqueous phase (V_{aq}) and the amount of NaClO (NaClO), expressed as the molar ratio [mol/mol diol] between NaClO and diol **4**. Lines refer to constant conversion, and the value is reported in the labels.

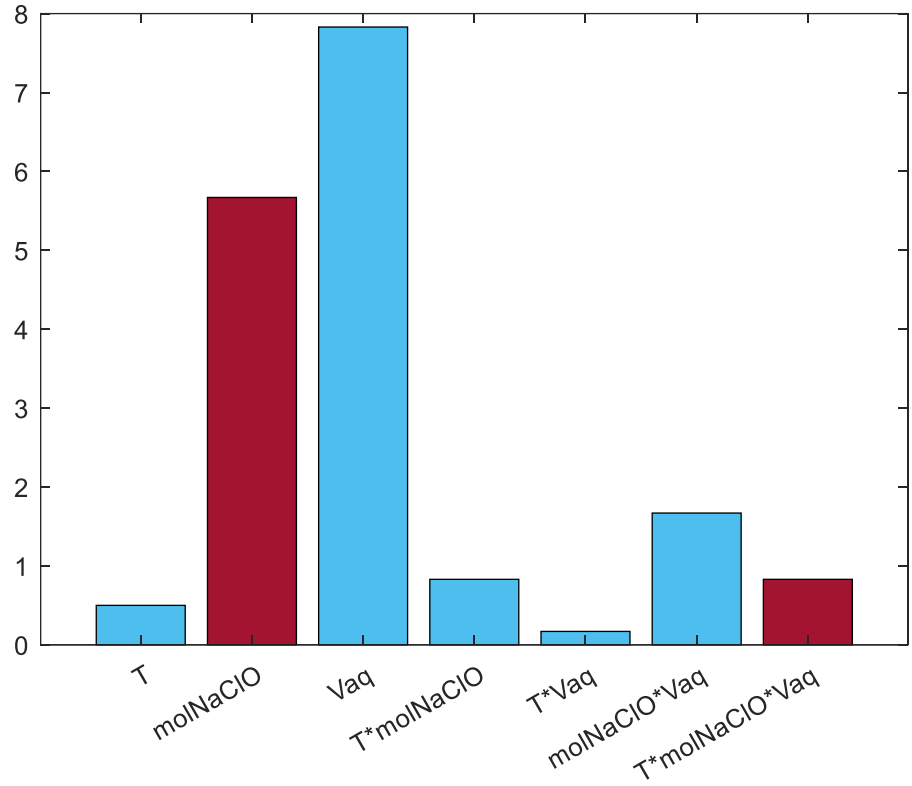


Figure S6. Coefficient plot of variable effects (first, second and third order); red = positive effect; light blue = negative effect.

Calculation of the Simplified Environmental Factors (sE-Factors)

The values of the simplified Environmental Factors (sE-Factors) for the procedure described in this work and for those reported in Scheme 5 were calculated under the hypothesis of completely recycling reaction and post-reaction solvents and water, according to the formula suggested by Roschangar et al. (F. Roschangar, R. A. Sheldon, C. H. Senanayake *Green Chem.*, 2015, **17**, 752 – 768.). The quantities of additives for reaction work-up (acid, base, and reductants) were not reported in the corresponding literature for processes (b)-(e), so the amount of sulfuric acid and sodium bisulfite (the only work-up additives of our procedure) were not considered.

$$\underline{\text{Environmental Impact Factor}} = \frac{\text{mass of total waste}}{\text{mass of target product}}$$

Mass of total waste = sum of masses of raw materials + sum of masses of reactants – mass of target product

Chemoenzymatic process described in this work					**production of 1 kg of the mixture 2+3**	**sE-Factor**
1st step	**raw materials and reactants (g)**				**raw materials and reactants**	3.91
	FFAs from soapstock (g) (83% oleic acid)	mol oleic acid (83%)	MM oleic acid		soapstock (kg)	
	8.300	0.024	282		2.76	
H_2O_2 (mL)	H_2O_2 (g)	H_2O_2 (mol)	MM H_2O_2	d H_2O_2 (g/mL)	H_2O_2 (kg)	
1.600	0.633	0.019	34	1.13	0.21	
	lipase catalyst reused in flow					
	sum of raw materials and reactants (g)				**sum of raw materials and reactants (kg)**	
	8.933				2.97	
	products				**products (kg)**	
	isolated diol **4** (g)	isolated mol diol **4**	MM diol 4		isolated diol **4** (kg)	
	3.300	0.010	316		1.10	
	sum of total waste (g)				**sum of total waste (kg)**	
	5.633				1.87	
2nd step	**raw materials and reactants (g)**				**raw materials and reactants**	
	diol **4** (g)	mmol diol **4**	MM diol 4		diol **4** (kg)	
	0.100	0.316	316		1.10	
NaClO (mL)	NaClO (g)	mmol NaClO	MM NaClO	d (NaClO) g/mL	NaClO (kg)	
1.500	0.177	2.377	74.45	1.18	1.94	
	sum of raw materials and reactants (g)				**sum of raw materials and reactants (kg)**	
	0.277				3.04	
	products (after isolation)					
	acid **2** (g)	mol acid **2**	MM acid **2**			
	0.045		158			
	acid **3** (g)	mol acid 3	MM acid 3			
	0.046		188			
	sum of products (g)				**sum of products (kg)**	
	0.091				1.00	
	sum of total waste (g)				**sum of total waste**	
	0.186				2.04	

Process (b) ref. U.S. Patent 2013/0131379 A1, 23 May 2013					production of 1 kg of the mixture 2+3	sE-Factor
1st step	**raw materials and reactants (g)**				**raw materials and reactants**	2.31
	oleic acid (g) (90%)	mol oleic acid	MM Oleic acid		oleic acid (kg) (90%)	
	50.000	0.177	282		0.77	
	H_2O_2 (g)	H_2O_2 (mol)	MM H_2O_2		H_2O_2 (kg)	
	8.432	0.248	34		0.13	
	HCOOH (g)	mol HCOOH	MM HCOOH		HCOOH (kg)	
	111.320	2.420	46		1.72	
	sum of raw materials and reactants (g)				**sum of raw materials and reactants (kg)**	
	161.320				2.63	
	products				**products (kg)**	
	diol **4** (g) as an oil	isolated mol diol **4**	MM diol **4**		diol **4** (kg) as an oil	
	59.000		316		0.91	
	sum of total waste (g)				**sum of total waste (kg)**	
	102.320				1.71	
2nd step	**raw materials and reactants (g)**				**raw materials and reactants**	
	diol **4** (g)	mmol diol **4**	MM diol 4		diol **4** (kg)	
	1.580	0.005	316		0.91	
	NaClO (g)	mmol NaClO	MM NaClO		NaClO (kg)	
	1.173	0.016	74.45		0.68	
	sum of raw materials and reactants (g)				**sum of raw materials and reactants (kg)**	
	2.753				1.59	
	products (mixture not submitted to separation)					
	acid **2** (g)	mol acid **2**	MM acid **2**			
			158			
	acid **3** (g)	mol acid **3**	MM acid **3**			
			188			
	sum of products (g)				**sum of products (kg)**	
	1.730				1.00	
	sum of total waste (g)				**sum of total waste (kg)**	
	1.023				0.59	

Process (c) ref. J. Am. Oil Chem. Soc. 2015, 9, 1701-1707					production of 1 kg of the mixture 2+3	sE-Factor
	raw materials and reactants (g)					0.99
	oleic acid (g)	mol oleic acid	MM Oleic acid			
	31.866	0.113	282			
	H_2O_2 (g)	H_2O_2 (mol)	MM H_2O_2			
	30.736	0.904	34			
	H_2WO_4 (g) no catalyst recovery					
	0.283					
	sum of raw materials and reactants (g)					
	62.885					
	products (oily mixture, % obtained by GC analysis)					
	acid **2** (g)	mol acid **2**	MM acid **2**			
	12.319	0.078	158			
	acid **3** (g)	mol acid **3**	MM acid **3**			
	19.332	0.103	188			
	sum of products (g)				**sum of products (kg)**	
	31.651				1.00	
	sum of total waste (g)				**sum of total waste (kg)**	
	31.234				0.99	

Process (d) ref. J. Am. Oil Chem. Soc. 2013, 90, 133-140					production of 1 kg of the mixture 2+3	sE-Factor
	raw materials and reactants (g)					0.69
	oleic acid (g)	mol oleic acid	MM Oleic acid			
	18.330	0.065	282			
	H_2O_2 (g)	H_2O_2 (mol)	MM H_2O_2			
	11.050	0.325	34			
	catalyst B (g) recovered, not reused	mol catalyst B	MM catalyst B			
	2.474	0.001	2062			
	sum of raw materials and reactants (g)					
	31.854					
	products (oily mixture, % obtained by GC analysis)					
	acid **2** (g)	mol acid **2**	MM acid **2**			
	8.370	0.053	158			
	acid **3** (g)	mol acid **3**	MM acid **3**			
	10.521	0.056	188			
	sum of products (g)				**sum of products (kg)**	
	18.891				1.00	
	sum of total waste (g)				**sum of total waste (kg)**	
	12.963				0.69	

Process (e) ref. J. Am. Oil Chem. Soc. 2017, 94, 1451-1461					production of 1 kg of the mixture 2+3	sE-Factor
	raw materials and reactants (g)					1.52
	oleic acid (g)	mol oleic acid	MM Oleic acid			
	1.000	0.004	282			
H_2O_2 (mL)	H_2O_2 (g)	H_2O_2 (mol)	MM H_2O_2	d H_2O_2 (g/mL)		
4.000	1.356	0.040	34	1.13		
	catalyst reused 4 times					
	sum of raw materials and reactants (g)					
	2.356					
	products (oily mixture, % obtained by GC analysis)					
	acid **2** (g)	mol acid **2**	MM acid **2**			
	0.381	0.002	158			
	acid **3** (g)	mol acid **3**	MM acid **3**			
	0.553	0.003	188			
	sum of products (g)				**sum of products (kg)**	
	0.934				1.00	
	sum of total waste (g)				**sum of total waste (kg)**	
	1.422				1.52	