Supporting Information

Integrated biocatalytic platform based on aqueous biphasic systems for the sustainable oligomerization of rutin

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Experimental section

Chemicals

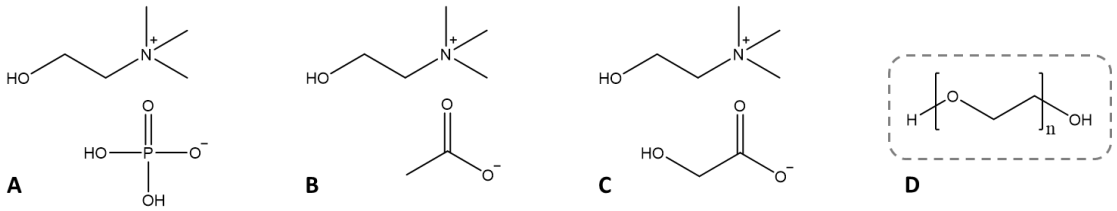


Figure S1. Chemical structure of the investigated ILs and PEG: (**A**)[Ch][DHph], (**B**) [Ch][Ac], (**C**) [Ch][Gly] and (**D**) PEG.

Selection of the most promising ABS as a reaction medium

Preliminary studies to determine the most promising ABS to be used as reaction medium for the laccase-catalyzed production of rutin oligomers were performed. The separation performance of the ABS composed of aqueous combinations of PEG 600 and either [Ch][Ac], [Ch][Gly] or [Ch][DHph] was evaluated at 25˚C by determining the extraction efficiency (*%EE*) of each compound to same or opposite phases. The composition of the ABS tested was picked in accordance to phase diagrams previously determined by Pereira *et al.*,[1] selecting mixture points comprised in the biphasic region of the diagram. Briefly, 1 g of each ABS was prepared in 1.5 mL Eppendorf tubes and both oligorutin and laccase from T. versicolor were supplied to each system. After vigorously homogenizing the ABS in a vortex mixer, phase separation was achieved through centrifugation (VWR MICRO STAR centrifuge, 10000 rpm, 5 min) and laccase activity in each phase was recorded using a Shimadzu UV-1800 spectrophotometer, following the protocol developed by Zimmerman *et al.*,[2] allowing the calculation of the laccase activity extraction efficiency ($\%EE_{laccase\ activity}$) to each phase defined as the ratio between laccase units present in that specific phase and the total amount of laccase units present in the complete ABS. Since oligorutin was the only brown-colored compound involved in these assays, the oligorutin extraction efficiency ($\%EE_{oligorutin}$) was visually estimated in this preliminary assay.

HPLC protocol for rutin measurement

HPLC (Shimadzu, PROMINENCE) analyses were performed with an analytical reversed-phase column (250 × 4.60 mm), Kinetex, 5 µm, C18 100-Å, from Phenomenex. The mobile phase used was a gradient system of 0.5% acetic acid-ultra-pure water (phase A) and acetonitrile (phase B), previously degassed by ultrasonication. The separation was conducted using the following gradient mode: 0 min 18% of B, 9 min 35% of B, 11 min of B, and then

returned to initial conditions during 20 min to ensure the column stabilization. The flow rate used was 1 mL/min, with an injection volume of 10 µL. DAD was set at 355 nm. Each sample was analyzed at least in duplicate. The column oven was operated at a controlled temperature of 25 °C. Under these conditions, rutin displayed a retention time of 3.7 min.

Rutin conversion at a given time i, X_i, also referred in this work as oligomerization yield, was calculated based on the depletion of rutin observed through HPLC analysis as follows (equation S1),

$$X_i(\%) = \frac{Rutin\ concentration_{t=0} - Rutin\ concentration_{t=i}}{Rutin\ concentration_{t=0}} \cdot 100 \qquad \text{(S1)}$$

Spectrophotometric measurement of oligorutin concentration

Oligorutin concentration was measured spectrophotometrically (Shimadzu UV-1800) by recording the absorbance at 490 nm; since oligorutin and rutin presented maximum absorbances at values close to each other (~355 nm), 490 nm was selected as a wavelength in which oligorutin still displayed absorbance but rutin did not, to avoid the effect of the unreacted rutin in this measurement, and a concentration-absorbance calibration curve was obtained for each pH of reaction studied.

Determination of the ABS phase diagrams and tie-lines

The ABS phase diagrams comprise the binodal curve and respective tie-lines (TLs). The binodal curve represents the borderline between the monophasic and the biphasic regions, whereas TLs describe the compositions of the two phases in equilibrium for given mixture compositions. The ternary phase diagram of the ABS composed by PEG 600 and [Ch][DHph] at 25˚C used in this work was taken from the literature.[1] For this same ABS, the binodal curve at 40˚C with no pH alteration, alongside with the binodal curve at 25˚C at a pH adjusted to 6.5, were obtained through the cloud point titration method,[3] at atmospheric pressure in all cases. Briefly, aqueous solutions of [Ch][DHph] were added drop by drop over aqueous solutions of PEG under continuous stirring until the formation of a cloudy solution was observed; ultrapure water was then added drop wise until the formation of a limpid solution. When necessary, the pH of ultrapure water, PEG and [Ch][DHph] aqueous solutions were previously adjusted to obtain the solubility curves at the desired pH values, adding drop by drop NaOH at 10 M. The ternary system compositions were then determined by weight quantification (± 10^{-3} g). Data from the obtained binodal curves was successfully fit to the commonly known as “Merchuk equation” (equation S2),[4]

$$[PEG] = A\exp[(B \cdot [IL]^{0.5} - C[IL]^3)] \quad \text{(S2)}$$

where [PEG] and [IL] are the PEG and IL weight percentages, respectively, and A, B and C constants obtained by regression.

Tie-lines (TLs) of each phase diagram were determined by a gravimetric method firstly developed by Merchuk *et al.*[4] with slight modifications. A mixture at the biphasic region is prepared, each component weighted (PEG + IL + water), then actively stirred and centrifuged (Heraeus Megafuge 16R, 5000 rpm) at the desired temperature for 30 min to reach the complete separation and equilibria of the two aqueous phases. When needed, the pH of the mixture was adjusted by the addition of NaOH 10 M. After the separation step, both top and bottom phases were weighted. Each TL and respective tie-line length (TLL) was determined by the application of the lever-arm rule to the relationship between the top phase weight and the overall system composition, as reported in previous works.[3]

Recovery and laccase reuse

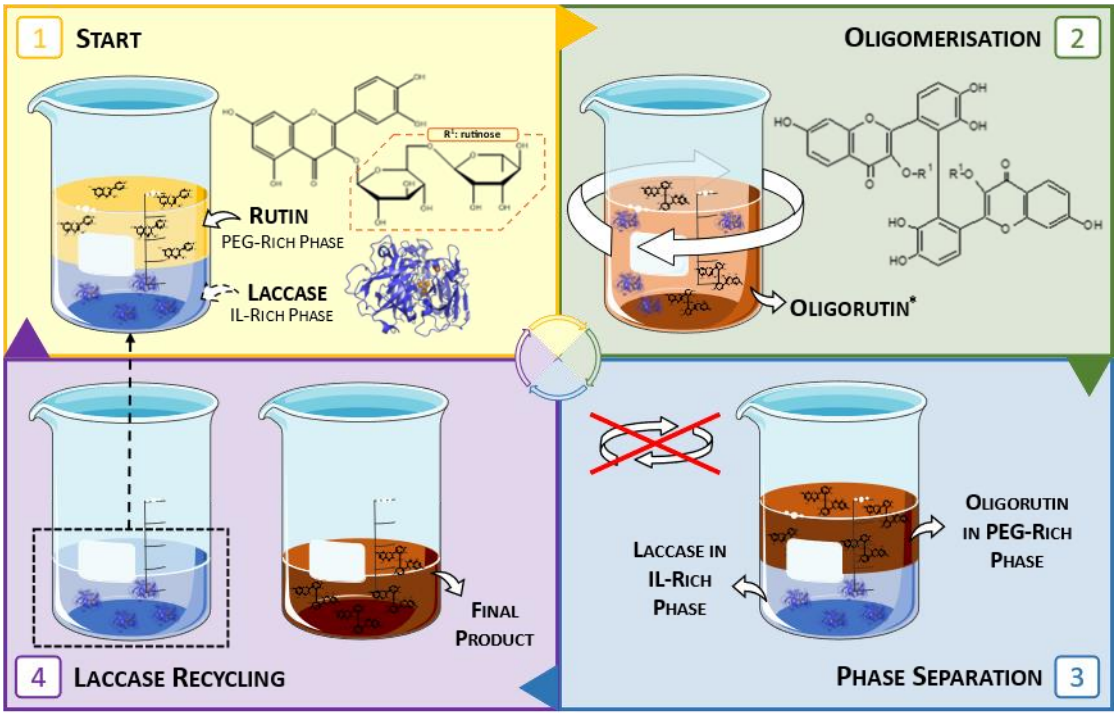


Figure S2. Integrated reaction-separation platform for the reuse of the laccase-enriched bottom phase in continuous cycles of oligorutin production in ABS: (■) rutin-enriched phase, (■) oligorutin synthesis, (■) oligorutin-enriched phase, (■) laccase-enriched phase. *Oligorutin (degree of polymerisation: 2) structure proposed by Anthoni *et al.*[5]

Oligorutin characterization

Mass spectra of retentate and permeate products after ultrafiltration were determined by MALDI-TOF (Matrix-assisted laser desorption/ionization – time of flight) based on the protocol described by Anthoni *et al.*,[6] with slight modifications. Briefly, lyophilized samples were dissolved 10 g/L in acetonitrile/water (30:70 v/v) with 0.1% TFA and mixed at a 1:1 ratio with 2,5-dihydroxybenzoic acid (DHB) at 20 mg/L in acetonitrile/water (30:70 v/v); then 1 µL of this dilution was spotted on the MALDI plate and crystallized at room temperature. MALDI-TOF analysis was performed on an Ultraflex III TOF/TOF mass spectrometer equipped with a Smartbeam® laser (Bruker Daltonics) in linear operation mode and negative polarity. The acceleration voltage was set to 25 kV and a total of 1200 laser shots per spot were automatically acquired. Data acquisition and data processing were performed by the Flex Analysis software (Bruker Daltonics).

The antioxidant activity of the oligorutin produced in the 3 successive cycles comprising laccase reuse was measured following the Trolox Equivalent Antioxidant Capacity (TEAC) protocol as described by Gullón *et al.*,[7]. Briefly, the decrease in the absorbance (λ: 734 nm) of 1 mL $ABTS^{\cdot+}$ solution mixture in contact with 10 µL sample was recorded after 6 min incubation at 25˚C. $ABTS^{\cdot+}$ solution was previously prepared in a 12-16 h reaction of 7 mM ABTS with 2.45 mM potassium persulfate at room temperature and protected from light exposure, then diluting the resulting product with distilled water until an absorbance ~0.70 was achieved. Results were expressed in Trolox equivalents by means of a calibration curve (0 – 500 mg Trolox /L, R^2: 0.995).

Results

Selection of the most promising ABS as a reaction medium

Table S1. Screening of biocompatible ABS as separation platforms for laccase and oligorutin: weight fraction (wt%) of the mixture points tested and extraction efficiencies (%EE) for both laccase activity and oligorutin to enriched.

ABS	Mixture point		$\%EE_{laccase\ activity}$	$\%EE_{oligorutin}$
	wt% PEG 600	wt% [Ch][DHph]	to bottom phase	to top phase
[Ch][Ac] - PEG 600	40	40	100	>90
[Ch][Gly] - PEG 600	40	35	100	<10
[Ch][DHph] - PEG 600	30	30	96	~70

ABS phase diagrams and tie-lines and laccase-catalyzed rutin oligomerization using ABS as reaction medium

In the first approach for recovering and reusing laccase, we studied the synthesis of oligorutin in a homogeneous medium (at 25˚C) to take advantage of the thermoreversible behavior of the selected ABS, previously described by Pereira *et al.*[1] when switching temperature from 25 to 50˚C. These authors observed that an increase in temperature enhanced the biphasic region due to a decrease in the binary salt-PEG hydrogen-bonding interactions, which play a dominant role in this kind of systems. In this approach, after the reaction, a mild increase in temperature was used to induce phase formation, thus leading to oligorutin and laccase separation to opposite phases. Since a temperature of 50˚C would negatively affect laccase stability, this ABS was here studied at 40˚C, taking into account the optimum temperature range of laccase to avoid its activity loss.[8] As depicted for 25 – 40˚C curve comparison (Figure S3), the effect of temperature upon this system was maintained in this shorter temperature interval. To fully characterize this system and determine the composition of the immiscible aqueous phases, a tie-line was gravimetrically obtained for a mixture point comprised between the solubility curves displayed at both temperatures, namely 26.75 wt% [Ch][DHph] and 26.08 wt% PEG 600 (data presented in Figure S4). This mixture point would fall on the monophasic region at 25˚C, but already on the biphasic region when temperature increases up to 40˚C. Therefore, in such ABS, the reaction can be carried out at in a homogenous medium, after which an increase in temperature up to 40˚C leads to the creation of two phases and the simultaneous separation of the enzyme from the product. Thus, the oligomerization of rutin in [Ch][DHph]-PEG 600 ABS was performed using this mixture point as composition for the reaction medium, since the mixture point used in the initial screening would fall in the biphasic region at 25˚C. However, this approach did not lead to a high oligomerization yield (calculated based on equation S1) with a good enzyme activity performance (rutin conversion of 29%, and relative enzyme activity loss of 28%, as reported in Table S3).

Table S2. Experimental weight fraction data for the system composed of [Ch][DHph] (1) + PEG 600 (2) + H_2O at 40˚C and atmospheric pressure.

$wt\%_1$	$wt\%_2$	$wt\%_1$	$wt\%_2$
2.53	67.31	17.92	35.54
3.20	64.93	18.96	34.16
3.64	63.48	20.63	32.48
4.57	61.60	26.09	25.85
4.95	59.89	26.48	25.28
5.37	59.00	27.51	24.03
5.76	57.67	27.82	23.63
6.18	56.89	28.22	23.13
6.46	55.75	29.38	21.64
6.92	54.53	30.25	20.67
7.34	53.85	30.65	20.23
7.60	53.28	31.03	19.74
7.90	52.29	32.08	18.42
8.55	51.12	32.79	17.65
9.03	50.13	32.92	17.47
9.19	49.11	33.37	17.03
9.41	48.61	33.71	16.58
9.71	48.04	33.97	16.30
10.16	47.24	34.11	16.14
10.67	46.64	34.93	15.23
10.87	46.20	35.58	14.45
11.04	45.83	36.29	13.70
11.37	45.09	36.91	13.04
11.84	44.51	37.04	12.88
12.06	44.13	37.25	12.61
13.36	41.94	38.11	11.74
13.71	41.48		
17.09	36.66		

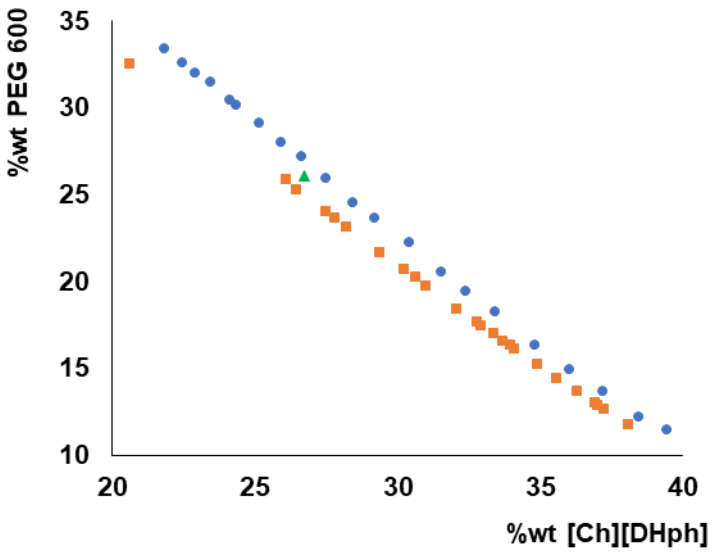
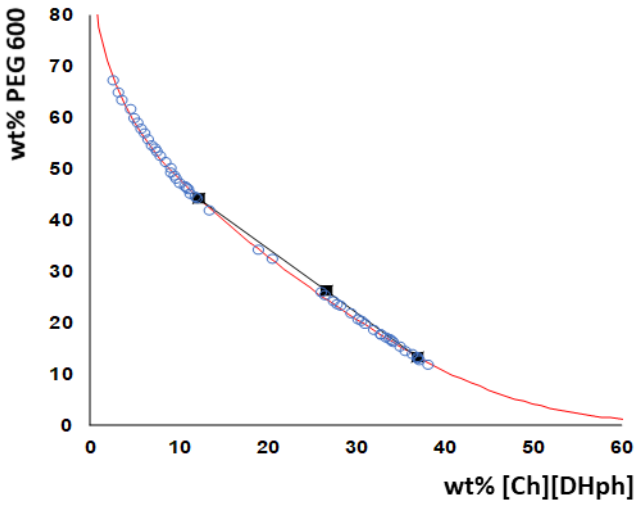


Figure S3. Thermoreversibility of [Ch][DHph]-PEG 600 ABS at the 25[1] (●) – 40 (■)˚C temperature interval obtained at atmospheric pressure. Mixture point chosen as reaction medium for rutin oligomerization (▲).



Mixture point			Top phase			Bottom phase		
wt% IL	wt% PEG	wt% H_2O	wt% IL	wt% PEG	wt% H_2O	wt% IL	wt% PEG	wt% H_2O
26.75	26.08	47.17	12.19	44.25	43.56	37.03	13.25	49.71

Tie-line lenght	Merchuk equation parameters			
	A	B	C	R^2
37.76	96.34 ± 1.28	-2.16E-01 ± 4.84E-03	1.32E-05 ± 5.45E-07	0.99945

Figure S4. Tie-line determination for [Ch][DHph]-PEG 600 ABS at 40˚C: experimental solubility curve (○), fit of the experimental data to the Merchuk equation (—) and composition of the mixture point and of the top/bottom phases (■).

Table S3. Rutin conversion (%), relative laccase activity drop (%) and $\%EE_{laccase\ activity}$ achieved during the oligomerization of rutin using the thermoreversible ABS composed of [Ch][DHph]-PEG 600 mixture at a monophasic regime as reaction medium.

Mixture point		Rutin conversion (%)	Relative laccase activity drop (%)	$\%EE_{laccase\ activity}$
wt% $_{PEG\ 600}$	wt% $_{[Ch][DHph]}$			
26.08	26.75	28.45 ± 3.17	28.39 ± 0.04	86.64 ± 0.30

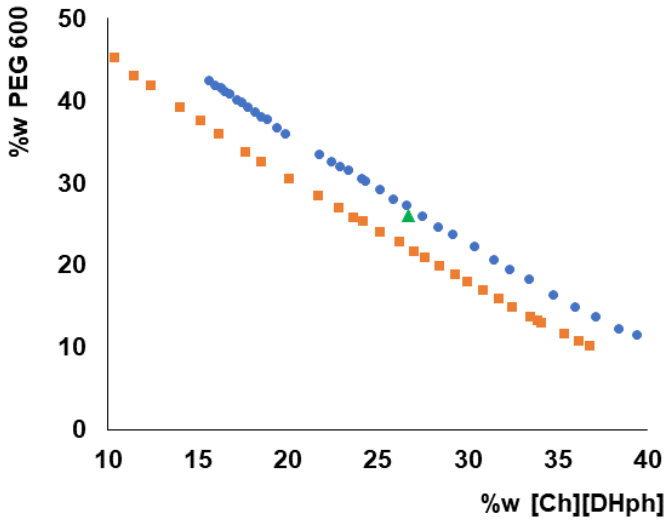


Figure S5. pH-reversibility of [Ch][DHph]-PEG 600 ABS at pH 4.3 (●)[1] and pH 6.5 (■) at 25˚C. Mixture point chosen for ABS formulation as reaction medium for rutin oligomerization (▲).

Table S4. Rutin conversion, relative laccase activity and rutin concentration in controls (%) over time at pH 4.5, 5.5 and 6.5, using [Ch][DHph]-PEG 600 on a biphasic regime as reaction media (26.08 wt% PEG + 26.75 wt% [Ch][DHph]) at 25˚C.

	Rutin conversion (%)			Relative laccase activity (%)			Rutin concentration in control experiments (%)		
t (h)	pH 4.5	pH 5.5	pH 6.5	pH 4.5	pH 5.5	pH 6.5	pH 4.5	pH 5.5	pH 6.5
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
2	21.57 ± 3.48	23.22 ± 4.46	50.93 ± 7.71	89.05 ± 4.95	76.38 ± 1.78	83.98 ± 4.40	-	-	-
4	25.07 ± 0.51	42.90 ± 1.13	72.84 ± 2.72	81.47 ± 3.01	78.36 ± 4.53	86.14 ± 0.96	-	-	-
6	24.66 ± 5.00	53.24 ± 1.81	82.87 ± 0.76	87.81 ± 0.70	77.53 ± 4.89	88.83 ± 3.38	-	-	-
24	49.27 ± 8.72	85.05 ± 1.36	97.31 ± 0.54	68.77 ± 4.78	67.39 ± 8.95	82.68 ± 4.15	95.91 ± 6.04	92.04 ± 10.66	92.94 ± 8.04

Table S5. Extraction efficiencies (*%EE*) of laccase activity, oligorutin and rutin in the [Ch][DHph]-PEG 600 ABS at pH values of 4.5, 5.5 and 6.5.

		PEG 600-rich phase	[Ch][DHph]-rich phase
$\%EE_{Laccase\ activity}$	**pH: 4.5**	13.19 ± 0.95	86.81 ± 0.95
	pH: 5.5	18.67 ± 2.00	81.33 ± 2.00
	pH: 6.5	9.29 ± 1.04	90.71 ± 1.04
$\%EE_{Oligorutin}$	**pH: 4.5**	62.58 ± 6.19	37.42 ± 6.19
	pH: 5.5	72.70 ± 6.40	27.30 ± 6.40
	pH: 6.5	68.72 ± 7.13	31.28 ± 7.13
$\%EE_{rutin}$	**pH: 4.5**	64.03 ± 1.58	35.97 ± 1.58
	pH: 5.5	77.95 ± 1.09	22.05 ± 1.09
	pH: 6.5	88.80 ± 2.38	12.53 ± 2.38

Table S6. Rutin conversion (%) and relative laccase activity (%) for oligorutin synthesis using the separated phases of [Ch][DHph]-PEG 600 ABS as reaction media at pH 6.5 and 25˚C.

	Rutin conversion (%)		Relative laccase activity (%)	
t (h)	**PEG-rich phase**	**IL-rich phase**	**PEG-rich phase**	**IL-rich phase**
0	00.00 ± 0.00	00.00 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
2	20.13 ± 0.62	44.49 ± 23.49	99.68 ± 8.67	92.41 ± 4.93
4	31.08 ± 4.94	65.91 ± 2.69	97.18 ± 5.02	87.34 ± 7.82
6	40.41 ± 10.48	84.58 ± 1.64	89.57 ± 2.30	90.46 ± 9.52
24	80.72 ± 1.47	99.54 ± 0.14	87.35 ± 8.85	97.36 ± 9.90

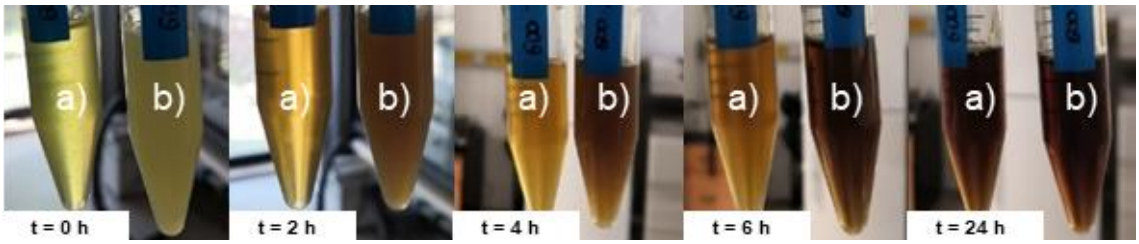
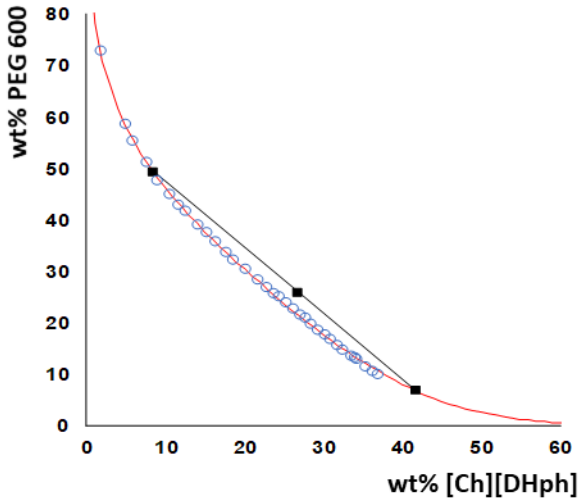


Figure S6. Oligorutin synthesis using the separated phases of [Ch][DHph]-PEG 600 ABS as reaction media at pH 6.5 and 25 ˚C: a) TOP PEG 600-rich phase and b) BOTTOM [Ch][DHph]-rich phase.



Mixture point			Top phase			Bottom phase		
wt% IL	wt% PEG	wt% H_2O	wt% IL	wt% PEG	wt% H_2O	wt% IL	wt% PEG	wt% H_2O
26.70	26.08	47.21	8.47	49.49	42.04	41.68	6.86	51.46

Tie-line lenght	Merchuk equation parameters			
	A	B	C	R^2
54.04	98.99 ± 1.52	-2.35E-01 ± 5.33E-03	1.59E-05 ± 7.26E-07	0.99942

Figure S7. Tie-line determination for [Ch][DHph]-PEG 600 aqueous biphasic systems at pH 6.5 and 25˚C: experimental solubility curve (○), fit of the experimental data to the Merchuk equation (—) and composition of the mixture point and of the top/bottom phases (■).

Table S7. Experimental weight fraction data for the systems composed of [CH][DHph] (1) + PEG 600 (2) + H_2O at pH 6.5 and 25˚C and atmospheric pressure.

wt%$_1$	wt%$_2$	wt%$_1$	wt%$_2$
1.79	73.16	24.23	25.26
4.92	58.69	25.19	24.07
5.75	55.53	26.22	22.80
7.55	51.28	27.06	21.68
8.90	47.93	27.66	20.95
10.44	45.24	28.46	19.87
11.50	43.07	29.36	18.81
12.46	41.76	30.05	17.89
14.07	39.18	30.91	16.88
15.20	37.60	31.74	15.83
16.23	35.89	32.49	14.85
17.69	33.77	33.56	13.65
18.56	32.48	33.95	13.21
20.10	30.51	34.13	12.96
21.75	28.42	35.39	11.64
22.87	26.93	36.22	10.74
23.68	25.70	36.81	10.10

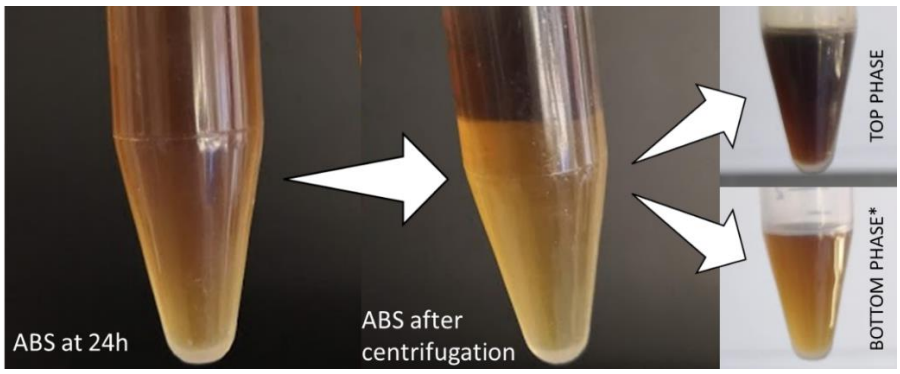


Figure S8. Example of phase formation induced by centrifugation at 25˚C after 24 h of oligorutin synthesis at pH 6.5 in the [Ch][DHph]-PEG 600 ABS in a biphasic regime, obtaining a TOP oligorutin-enriched phase and a BOTTOM laccase-enriched phase.

*Since $\%EE_{oligorutin}$ to bottom phases was ~33%, laccase-enriched bottom phases still possessed a product-related brown colour, remarkably lighter than top oligorutin-enriched phases, thus reflecting that the separation of products from enzyme, despite not being perfect, resulted successful.

3.1. Recovery and laccase reuse

Table S8. Rutin conversion and relative laccase activity (%) for the consecutive reaction-separation cycles performed using [Ch][DHph]-PEG 600 ABS pH 6.5 at 25˚C in a biphasic regime as reaction medium.

	Cycle 1	Cycle 2	Cycle 3
Rutin conversion (%)	95.20 ± 3.10	90.50 ± 1.70	89.20 ± 1.60
Relative laccase activity (%)	71.60 ± 1.50	79.70 ± 4.90	70.40 ± 0.80

Table S9. Extraction efficiencies (*%EE*) of laccase activity and oligorutin for the consecutive reaction-separation cycles performed using [Ch][DHph]-PEG 600 ABS pH 6.5 at 25˚C as reaction medium.

		PEG 600-rich phase	[Ch][DHph]-rich phase
$\%EE_{laccase\ activity}$	**Cycle 1**	5.13 ± 2.62	94.87 ± 2.62
	Cycle 2	5.51 ± 2.37	94.49 ± 2.37
	Cycle 3	6.71 ± 5.80	93.29 ± 5.80
$\%EE_{oligorutin}$	**Cycle 1**	67.09 ± 3.43	32.91 ± 3.43
	Cycle 2	67.23 ± 8.16	32.77 ± 8.16
	Cycle 3	65.76 ± 3.74	34.24 ± 3.74

3.2. Oligorutin characterization

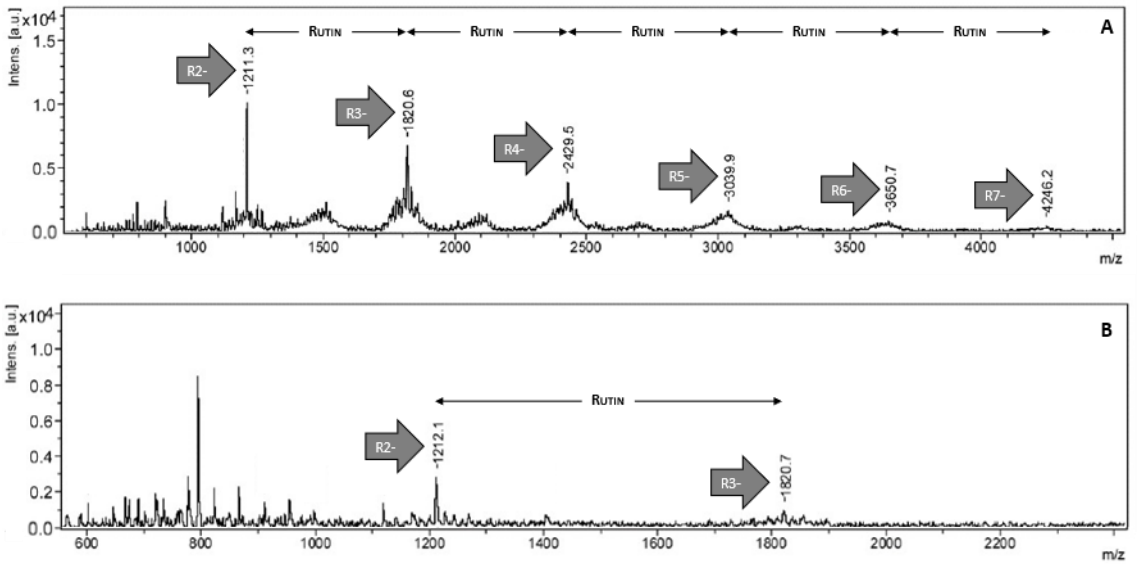


Figure S9. MALDI-TOF analysis of retentate (A) and permeate (B) derived from top phase fractions after ultrafiltration step.

Table S10. TEAC of rutin and oligorutin produced in three successive syntheses involving laccase reuse.

	mg Trolox eq. / mg
Cycle 1 - Top phase	451.82 ± 54.75
Cycle 2 - Top phase	412.93 ± 20.68
Cycle 3 - Top phase	447.68 ± 21.93
Rutin	955.22 ± 36.84

3.3. Oligorutin synthesis at higher concentration

Table S11. Rutin conversion (%), $\%EE_{oligorutin}$, $\%EE_{laccase\ activity}$ and relative laccase activity (%) after 24 h synthesis of oligorutin starting at 10 g/L rutin in ABS [Ch][DHph]-PEG 600 at pH 6.5 at 25˚C.

	Rutin conversion (%)	$\%EE_{oligorutin}$ to top PEG-rich phase	$\%EE_{laccase\ activity}$ to bottom IL-rich phase	Relative laccase activity (%)
24 h	93.34 ± 0.63	64.53 ± 0.76	91.50 ± 1.35	55.67 ± 4.40

3.4. Recovery of PEG 600 and [Ch][DHph] in top phase

Table S12. Composition of retentate and permeate streams in the ultrafiltration study for PEG 600 and [Ch][DHph] recovery.

Top phase dilution	wt% PEG 600 + [Ch][DHph]			$\%EE_{oligorutin}$		% PEG 600 + [Ch][DHph] recovered	Oligorutin purity fold
	Input	Retentate	Permeate	Retentate	Permeate		
1:2	30.32	29.92 ± 0.03	27.84 ± 0.25	97.27 ± 0.41	2.73 ± 0.41	48.20 ± 0.20	1.87
1:4	15.91	17.70 ± 0.15	12.99 ± 0.07	93.18 ± 0.65	6.82 ± 0.65	68.76 ± 0.07	3.04

3.5. Environmental assessment

Figure S10. Main steps, inputs, outputs, and energy requirements of the different scenarios considered for LCA study of oligorutin production allowing laccase reuse.

Table S13. Summary of primary inventory data per gram of final product for LCA study.

Inputs					
Materials	*Unit*	*Sc1*	*Sc2*	*Sc3*	*Sc4*
Water	g	0.712	7.14	87.2	124.2
Chemicals: salts, ethanol, NaOH, N_2, etc.	g	0.02	0.17	1.22	0.93
Chemicals: PEG 600	g	0.84	0.83	--	--
Rutin	g	$8.63 \cdot 10^{-3}$	$1.66 \cdot 10^{-2}$	$5.23 \cdot 10^{-1}$	$7.10 \cdot 10^{-2}$
Laccase	mg	$6.15 \cdot 10^{-3}$	$3.35 \cdot 10^{-2}$	$4.57 \cdot 10^{-2}$	118.26*
[Ch][DHph]	g	0.14	0.14	--	--
Nanoparticles	mg	--	--	--	44.46
Energy	*Unit*	*Sc1*	*Sc2*	*Sc3*	*Sc4*
Electricity	kWh	$8.70 \cdot 10^{-3}$	$3.96 \cdot 10^{-2}$	3.78	7.67
Outputs					
Product	*Unit*	*Sc1*	*Sc2*	*Sc3*	*Sc4*
Freeze-dried product	g	1.00	1.00	1.00	1.00
Oligorutin	g	$5.56 \cdot 10^{-3}$	$9.88 \cdot 10^{-3}$	0.46	0.07
Emissions into air	*Unit*	*Sc1*	*Sc2*	*Sc3*	*Sc4*
Steam	g	0.71	2.10	78.47	124.17
Ethanol	g	--	--	--	41.99
Nitrogen	g	--	0.14	0.64	--
Liquid emissions	*Unit*	*Sc1*	*Sc2*	*Sc3*	*Sc4*
Wastewater to treatment	g	--	5.13	8.72	

* Value provided in Units of immobilized laccase

Table S14. LCA characterization results per g of oligorutin produced.

Impact category	Acronym	Unit	Sc1	Sc2	Sc3	Sc4
Acidification	AC	g SO_2 eq	5.41	15.23	21.5	302.26
Eutrophication	EU	g PO_4^{3-} eq	1.30	3.55	4.94	69.91
Global Warming	GW	kg CO_2 eq	0.80	1.94	2.63	37.36
Ozone Layer Depletion	OD	mg CFC-11 eq	0.06	0.13	0.15	2.14
Photochemical oxidation	PO	g C_2H_4 eq	0.39	0.65	0.76	246.83

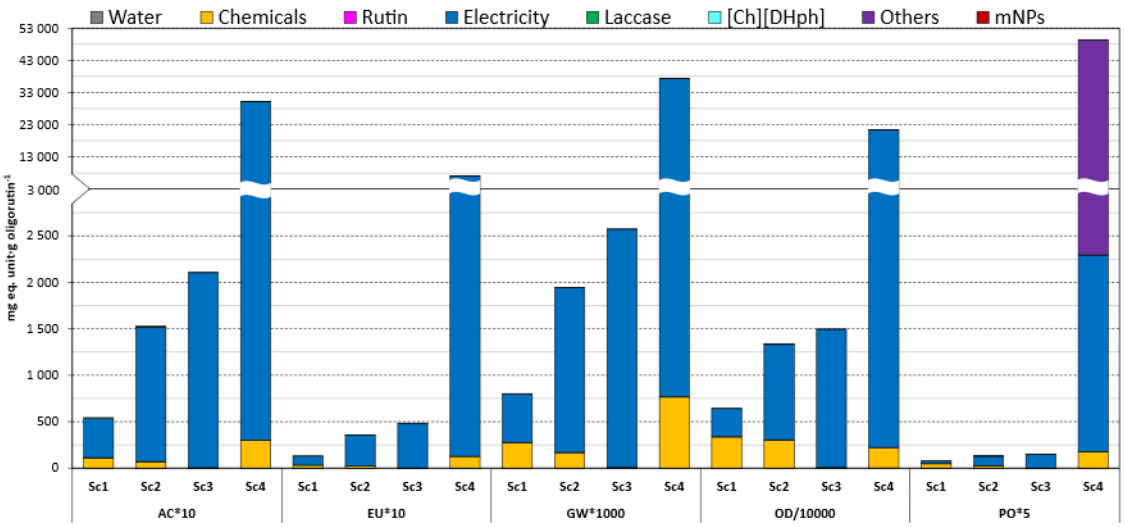


Figure S11. Distribution of impacts between contributing factors and impact categories for the scenarios under study.

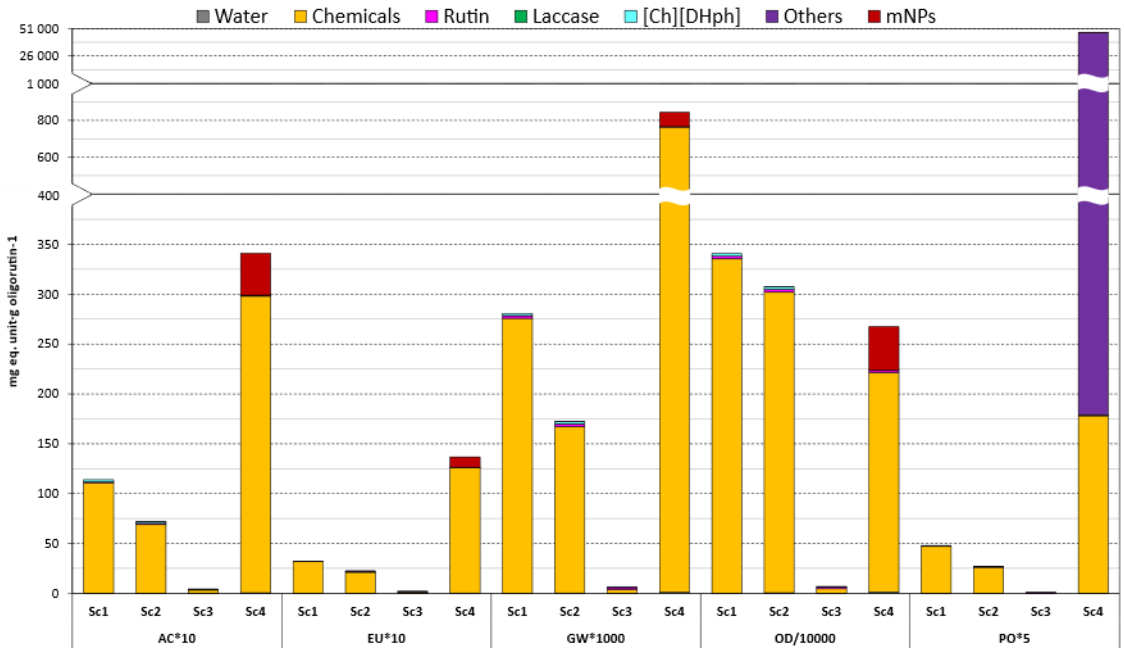


Figure S12. Distribution of impacts between contributing factors and impact categories for the scenarios under study after withdrawing electricity-related impacts.

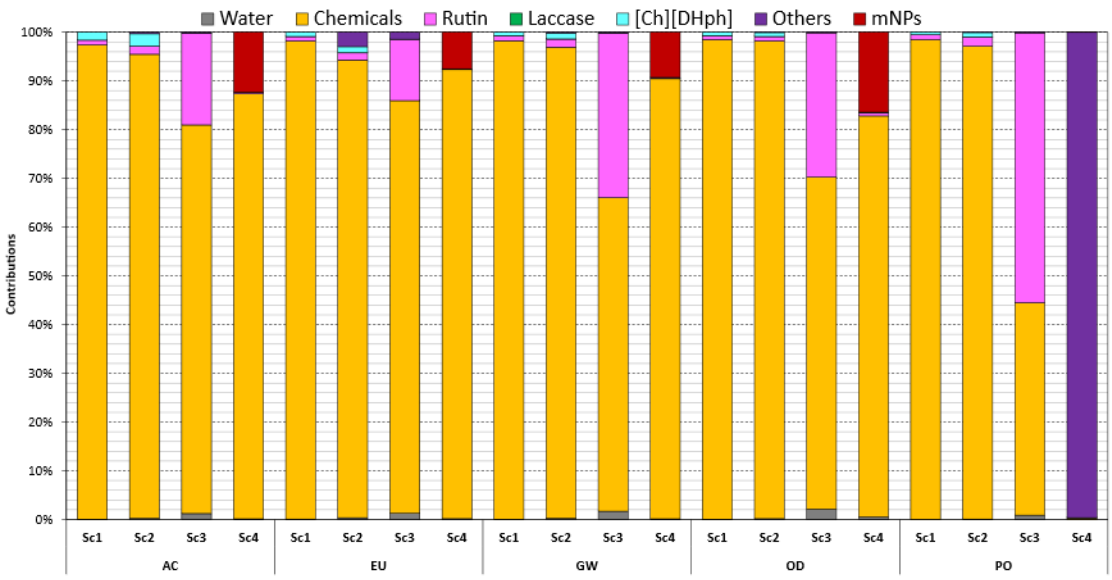


Figure S13. Percental contribution of each factor upon all category impact for the scenarios under study after withdrawing electricity-related impacts.

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