ELECTRONIC SUPPLEMENTARY INFORMATION

Integrative platform for the selective recovery of intracellular carotenoids and lipids from *Rhodotorula glutinis* CCT-2186 yeast using mixtures of bio-based solvents

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Experimental Section

Chemicals

β-carotene and torularhodin standards were acquired from Carbosynth (San Diego, CA, U.S.A) while pure torulene were obtained from *R. glutinis* cells according the method previously described by us[1]. All chemicals used in the extraction procedures and respective purity and supplier information are listed in **Table S1.**

Solvent	Solvent Acronym.	Incineration[14]	Recycle[14]	Biotreatment[14]	CAS Number	VOC emissions[14]	Purity (%)	Health Hazard[14]	Environmental Impact[14]		Life Cycle Analysis[14]	Density (g/cm³) (25 °C)	Ranking by default (CHEM21 solvent guide)[24]	Boiling point (°C)	Octanol/water partition coefficient (log K_{ow})	Bio-source, *i.e.*, obtained:	Supplier
		Solvent	Formula						Molecular weight (g/mol)								
									Aqueous	Air							
Chloroform	Chlorofor	3	9	5	3			4	7	5	6			61.20	1.80	No bio-sources. Chloroform are obtained from chlorination of methane & chlorination of methyl chloride, produced by the reaction of methanol and hydrogen chloride, are the two common methods for commercial chloroform production.[2]	Fisher Scientific (Portugal)
Cyclohexane (CH)	Cyclohexa												Problematic	80.74	2.67		Sigma-Aldrich (Portugal)
Ethanol (EtOH)	Ethanol													78.37	0.31		Fisher Scientific (Portugal)
	Ethyl acet	10	6	5	4			10	3	5	7		Problematic	77.10	0.73	From lignin degradation compounds over Ni/ZrO_2–SiO_2 catalysts.[3] From oleic acid via either a six- or eight-step enzyme cascade involving hydration, oxidation, hydrolysis and decarboxylation, followed by ring-closing metathesis.[4]	Fisher Scientific (Portugal)
	Isopropar													82.50	0.05		Fisher Scientific (Portugal)
	Methanol													64.70	-0.77		Fisher Scientific (Portugal)
	2-methyltet	5	5	3	4			10	9	5	0.85 - 0.99		Recommended	80.20	0.94	From several feedstocks: beets, corn, molasses, potatoes and other starchy materials such as wheat, barley, oat and rice; or even from lignocellulosic materials such as agricultural wastes and woody materials.[5]	Alfa Aesar (Germany)
	Water													100			
Ethyl acetate																From esterification, acetaldehyde condensation, ethylene adduct and ethanol dehydrogenation. For all	

Table S1. Properties and acronyms of the solvents used in the extraction processes.

										these processes, the primary feedstock is ethanol, which may be obtained from renewable raw materials by fermentation).[6,7]
Isopropanol (IPA)	5	5	3	5	10	8	7	4	Recommended	From anaerobic bacteria (*Clostridia*) fermentation, which convert carbohydrate-containing (waste)streams into IPA, *via* chemical and enzymatic conversion. From the hydrogenolysis of glycerol.[8]
Methanol (MeOH)	4	7	3	3	4	10	7	9	Problematic	From methanotrophic bacteria *Methylosinus trichosporium* fermentation, which produce methanol by the oxidation of methane to methanol.[9,10]
2-methyl-tetrahydrofuran (2-MeTHF)	6	5	3	4	4	7	4	4	Problematic	From bioconversion renewable resources, namely, cellulose, hemicelluloses, and lignin. These raw materials are convenient transformed into levulinic acid or furfural, and both can be converted into 2-MeTHF.[11-13]
Water (H_2O)	4	2	4	6	10	10	8	10	Recommended	-

Few Know Issues **Some Know Issues** **Major Know Issues[14]**

Table S2. Characteristics, nature, and sources of the solvents under study, updated from GSK Solvent Sustainability Guide[14] and CHEM21 solvent guide[24].

Microorganism and growing conditions

Rhodotorula glutinis CCT 2186 yeast was acquired from the Tropical Culture Collection André Tosello (Campinas, SP, Brazil), which was isolated from the leaf of a kaki fruit (Diospyros). The inoculum was prepared by the activation of *R. glutinis* CCT-2186 in Yeast Extract-Peptone-Dextrose (YPD) medium, which has the following composition (g/L in deionized water): peptone bacteriological (20); yeast extract (10); glucose (20). The inoculum culture was prepared in 100 mL Erlenmeyer® type flasks containing 25 mL of the YPD medium. Cells were then grown for 48 h at 30 °C and 150 rpm in an orbital shaker (Tecnal, model TE- 421 (Piracicaba, SP, Brazil)).

For lipids and carotenoids production, culture medium composed of (g/L in deionized water): glucose (10), KH_2PO_4 (0.52), $MgSO_4$ (0.52), NH_4NO_3 (4) and Asparagine (10) was used. Afterwards, 90 mL of inoculum culture at 0.2 mg/mL of cells were transferred to a 5 L stirred-tank bioreactor (Tecnal®, model Tec-Bio-Flex (Piracicaba, SP, Brazil), equipped with disc impeller, oxygen and pH electrodes), and containing 4 L of the culture medium. The initial pH of the medium was adjusted to 5.0 by adding 2 mol/L HCl or NaOH before autoclaving. The *R. glutinis* yeast growing was then conducted at 30 ºC, 300 rpm and 1 vvm (air volume/medium volume/minutes) during 96 h. Antifoam was added, as needed. Since the lipids and carotenoids are intracellular, after the cellular growing, the *R. glutinis* cells were then separated from the supernatant by centrifugation at 2500 x*g* for 10 min at 4º C using a Hitachi CR-22N (Tokio, Japan) centrifuge. The supernatants of all fermented media were then discarded, and the cellular pellets (i.e., wet biomass) containing carotenoids and lipids were collected and stored for the subsequent extraction studies.

Carotenoids isolation and characterization

The *R. glutinis* wet biomass was subject to chemical treatment with successive solvent extractions using DMSO. The DMSO extracts obtained were lyophilized. Then, these extracts were solubilized in acetone and transferred to a liquid chromatography column separation system, with mobile phase hexane/ethyl ether/acetic acid (70:29:1 v/v/v), and major colored fractions of yellow, light red and red were obtained. The yellow, light red and red fractions were collected,

evaporated to dryness and then, the carotenoids were re-suspended again in 1 mL of acetone. These acetone-based extracts were first evaluated qualitatively by Thin layer chromatography (TLC) on pre-coated TLC sheets ALUGRAM® (silica gel 60, Macherey-Nagel, Germany) to separate carotenoids and further comparison with literature data using the above-cited mobile phase as eluent. The extracts were also analyzed by Reverse Phase High Performance Liquid Chromatography (RP-HPLC) to detect the standard peak of absorbance. The homogeneity and the purity of the different fractions were identified by RP-HPLC on a column chromatography Shimadzu® Shim-pack C_{18} (Japan), 4.6×250 mm, using as mobile phase methanol/ acetonitrile/dichloromethane (60:10:30, v/v/v) eluting isocratically for 18 min. The flow rate was 1 mL/min, and the column temperature was set at 30 °C. The corresponding carotenoids were detected using UV-Vis detector at λ_{max} 450 nm. The identification of β-carotene and torularhodin (yellow and red fraction respectively) was done by comparison with the standards retention time found (with high purity level) under the same experimental conditions, and for torulene (light red fraction) were compared with data reported in literature.[15,16] The structures of three purified fractions of carotenoids were finally confirmed by Proton Nuclear Magnetic Resonance (^{1}H NMR) using a Bruker Avance III HD 600 (14.1T).

Lipids isolation and characterization

Lipids of *R. glutinis* wet biomass were extracted using a mixture of ethanol/ethyl acetate/water. Briefly, the cells were harvested by centrifugation (2500 *xg* for 10 min at 10 °C) and mixed with EtOH/EtOAc/H_2O (55:24:21% w/w/w) and homogenized for 1 h at 300 rpm and 65 °C. Afterwards, EtOAc and H_2O were added to reach a final mass ratio (%) of 27:15:58 (EtOH/EtOAc/ H_2O). The ternary mixture was homogenized for 10 min, centrifuged at 2500 *xg* for 5 min. After the phase separation, the top and bottom phases were carefully separated, filtered with a polyethylene membrane (0.22 μm pore size) to remove residual cellular debris.

After the separation, the bottom phase containing EtOH/H_2O and lipids were collected, transferred to rotary evaporator flasks, which were dried under vacuum (at 100 mbar for 30 min) and dried extracts were solubilized in hexane. These hexane-based extracts were first evaluated qualitatively by Thin layer chromatography (TLC) on pre-coated TLC sheets ALUGRAM® (silica gel

60, Macherey-Nagel, Germany) using hexane/ethyl ether/acetic acid (70:29:1 v/v/v) as mobile phase to separate lipids and further comparison with the standards retention time (with high purity level) under the same experimental conditions using the above-cited mobile phase as eluent. The fatty acids were also analyzed in a gas chromatograph (GC). For that, lipids (50 mg) were methyl esterified using 2 mL of a sodium hydroxide methanolic solution (0.2 M) and heated at 80 °C/15min under vigorous stirring. Then, 2 mL of a methanolic solution of sulfuric acid (1 M) was added, repeating the steps of heating and stirring (once time). 1 mL of a saturated NaCl solution and 2 mL of hexane was added. The solution was vortexed and then kept at rest for phase separation. The upper (organic) phase was removed and the extraction procedure with hexane (2 mL) was repeated. The hexane was removed at 40 °C in the rotavapor and the fatty acids methyl esters were stored at 6 °C for further analysis. The fatty acid composition of the structured lipids was determined by gas chromatography using a Shimadzu gas chromatograph (GC-MS-QP2010) equipped with a flame ionization detector. SH-Stabilwax-DA column (30 m length, 0.25 mm diameter, and 0.25 µm film thickness) was used to separate the fatty acid methyl ester at a flow rate of 1.0 mL/min. The injector temperature was set to 200 °C and the transfer line temperature to 280 °C. The GC oven was programmed as follows: after 2 min at 50 °C, the temperature was increased at 30 °C/min to 150 °C, then at 15 °C/min to 230 °C. The total run duration was 25 min. The fatty acids were identified on the basis of the peak areas.

Carotenoids and lipids extraction using biosolvents

The solid-liquid extraction (SLE) of carotenoids and lipids was carried out using pure and mixtures of the following biosolvents: methanol (MeOH), ethanol (EtOH), ethyl acetate (EtOAc), isopropanol (IPA), cyclohexane (CH) and 2-methyl tetrahydrofuran (2-MeTHF). The extraction of carotenoids and lipids was carried out according the following sequence:

1) to remove impurities, the *R. glutinis* wet cells were washed three-times using 10 mL of phosphate buffer (pH 7);

2) after washing, 1 g of wet biomass were added in hermetic Carousel's™ type tubes (50 mL) to prevent solvent evaporation and loss;

3) the tubes were filled with 5 mL of pure or mixed solvents (ratios and acronyms of the different systems are shown in **Table S3**, and the samples were then homogenized using a Carousel Stirring Hotplate 12 Plus Reaction Station™ (with reaction volume of 5 mL) (Radleys, Germany) for 1 h at 65 °C and 300 rpm;

4) after homogenization, all the samples were centrifuged at 2500 *xg* and 25 ºC for 5 min using an Eppendorf® 5804 centrifuge (Willow Springs, NC, USA);

5) after centrifugation, all cell lysate supernatants were filtered using a Millipore® filter membrane (0.22 µm pore size) and the solvents evaporated at 60 °C and 70 mbar using a rotary evaporator Büchi (R-210, Flawil, Switzerland);

6) carotenoids were re-dissolved in acetone and the lipids remained in the flask dried and weighted;

7) carotenoid-rich extracts were filtered using polyethylene membrane (0.22 µm pore size) and quantified using the methodology described in the section **Determination of carotenoids content**, while the lipids were quantified according the methodology described in the section **Determination of total lipids content**.

For the determination of recovery yields (%), the amount of each carotenoid (β-carotene, torularhodin and torulene) in *R. glutinis* cells were determined from the total carotenoids obtained from consecutive extractions using DMSO, while the total lipids were determined after the extraction using standard Bligh and Dyer method. The recovery yields (%) of each carotenoids and lipids were defined as the ratio between the amount (in mass) of each carotenoid or lipids extracted with each biosolvent or mixed biosolvent and the initial amount of each solute accumulated in the *R. glutinis* wet biomass, according to the **Equations S1** and **S2**, respectively:

$$Recovery\ of\ carotenoids\ (\%\ w/w) = \frac{Carotenoids\ extracted}{Carotenoids\ in\ R.\ glutinis\ wet\ biomass} \times\ 100 \quad \text{(S1)}$$

$$Recovery\ of\ lipids\ \%\ (w/w) = \frac{Lipids\ extracted}{Lipids\ in\ R.\ glutinis\ wet\ biomass} \times\ 100 \quad \text{(S2)}$$

Determination of carotenoids content

The carotenoids extraction was carried out according to the method modified by Mussagy et al.[1]. *R. glutinis* wet biomass (1 g) were mixed with 5 mL of DMSO and disrupted by maceration (5 min ON/15 min OFF for a total 1 h). After the procedure, the supernatant was recovered, and the procedure repeated until the cells become fully bleached. The supernatants of the cell lysates were mixed with 10 mL of a NaCl aqueous solution (at 20% (m/v)) and 10 mL of petroleum ether. After the formation of a biphasic system, the non-polar phase was collected and the excess of water removed with sodium sulfate aqueous solutions (Na_2SO_4). The carotenoids-rich extracts were then dissolved in acetone and filtered with a polyethylene membrane (0.22 μm pore size). The quantification of the three carotenoids was obtained from the visible-light absorption spectra using a Microplate reader® UV-Vis spectrophotometer (model Biotek, Synergy HT, Germany). The visible-light spectra from 380 to 600 nm were acquired, and the respective carotenoids calibration curves at 450 nm (β-carotene), 480 nm (torulene), and 500 nm (torularhodin) acquired. The carotenoid concentrations (μg/g) were determined according to pre-established standard curve prepared for the β-carotene, torularhodin and torulene (pure torulene standard was obtained from *R. glutinis* cells using a purification methods previously developed by our group[1]).

Determination of total lipids content

To determine the total lipid content accumulated in the *R. glutinis* cells, the intracellular lipids were extracted, dried and weighed by using a modified Bligh and Dyer procedure[4]. This standard method was also used as control in the subsequent biosolvents-based studies for the recovery of lipids from *R. glutinis* cells. Briefly, the cells were harvested by centrifugation (2500 *xg* for 10 min at 10 °C), washed and dried (50 °C, 24 h) to obtain constant weight. Dried yeast cells were mixed with chloroform, MeOH and H_2O to reach 1:2:1 ratio (v/v/v) and homogenized for 1 h at 300 rpm and 25 °C. Afterwards, chloroform and H_2O were added to reach a final volume ratio of 2:2:2 (chloroform/MeOH /H_2O). The ternary mixture was homogenized for 10 min, centrifuged at 2500 *xg* for 5 min. After the phase separation, coexisting aqueous and organic phases were carefully separated, and filtered to remove residual cellular debris.

After the separation, the bottom phase containing chloroform and lipids was transferred to rotary evaporator flasks (previously weighed, w_1), which were dried under vacuum (at 300

mbar for 30 min) until the organic phase was fully evaporated, and the final weight of the flask measured (w_2). Lipid content, expressed as mg/g dry cell weight *per* initial weight of the pellet (w) was determined according to **Equation S3**:

$$Total\ lipids\ content\ (mg/g) = \frac{W_2 - W_1}{W} \qquad \text{(S3)}$$

Table S3. Solvent compositions (% w/w) used in the solid–liquid extraction (SLE) studies for the recovery of carotenoids and lipids from R. *glutinis* biomass at a wet cell concentration of 0.2 g/mL after 1 h stirring (300 rpm) at 65 °C.

Method	Methanol (MeOH)	Ethanol (EtOH)	Isopropanol (IPA)	Ethyl acetate (EtOAc)	2-methyltetrahydrofuran (2-MeTHF)	Cyclohexane (CH)	Chloroform	Water (H_2O)
Single-step extraction								
MeOH	100	-	-	-	-	-	-	-
EtOH	-	100	-	-	-	-	-	-
IPA	-	-	100	-	-	-	-	-
EtOAc	-	-	-	100	-	-	-	-
2-MeTHF	-	-	-	-	100	-	-	-
CH	-	-	-	-	-	100	-	-
Solvent mixtures								
Control*	50	-	-	-	-	-	25	25
CH/MeOH	50	-	-	-	-	25	-	25
CH/EtOH	-	50	-	-	-	25	-	25
CH/IPA	-	-	50	-	-	25	-	25
CH/2-MeTHF	-	-	-	-	50	25	-	25
EtOAc/MeOH	50	-	-	25	-	-	-	25
EtOAc/EtOH	-	50	-	25	-	-	-	25
EtOAc/IPA	-	-	50	25	-	-	-	25
EtOAc/2-MeTHF	-	-	-	25	50	-	-	25
EtOAc/EtOH/H_2O optimization								
System A	-	0	-	100	-	-	-	0
System B	-	100	-	0	-	-	-	0
System C	-	0	-	0	-	-	-	100
System D	-	44	-	56	-	-	-	0
System E	-	41	-	18	-	-	-	41
System F	-	38	-	12	-	-	-	50
System G	-	27	-	73	-	-	-	0
System H	-	45	-	23	-	-	-	32
System I	-	55	-	24	-	-	-	21
System J	-	62	-	23	-	-	-	15
System K	-	19	-	77	-	-	-	5
System L		39		52				8
System M	-	44	-	0	-	-	-	56
System N	-	23	-	0	-	-	-	77
System O	-	58	-	34	-	-	-	7
System P	-	20	-	74	-	-	-	7
System Q	-	26	-	71	-	-	-	3
System R	-	40	-	25	-	-	-	35

* Reference system Bligh and Dyer method

Conductor-like Screening Model for Real Solvent (COSMO-RS)

The computational modelling was applied following the standard procedure applied in the two steps. In a first step, the molecular geometry of solutes (β-carotene, torularhodin, torulene, margaric acid, trilinolenin, glyceryl-1,3-dilinoleate) and solvents (EtOH, H_2O, and EtOAc) were optimized at the density functional theory level and utilizing the BP functional B88-p86 with a triple- ζ valence polarized basis set (TZVP) and the resolution of identity standard (RI) approximation using a quantum chemical TURBOMOLE V7.3 2018[17] software program package. Subsequently, the obtained COSMO files were used as input in COSMO*therm* software (COSMOlogic, Levekusen, Germany, using parameter BP_TZVP_C30_1801)[18] to obtain the σ-profile and σ-potential.

In addition, COSMO-RS model was also used to estimate the interaction energy between solvent and solute, in term of excess energy.[19] In this context, the interaction energies in pure solvents as well as in their binary mixture of (solvent + solute) were estimated using COSMO-RS at the parameterization. In the molecular approach, COSMO-RS emphases on three specific interaction, namely the electrostatic - misfit energy ($^{H_{\mathrm{MF}}}$), hydrogen bonding energy ($^{H_{\mathrm{HB}}}$), and van der Waals energy ($^{H_{vdW}}$). These energies are described in **Equations S4, S5** and **S6**, respectively:

$$H_{\mathrm{MF}} = a_{\mathrm{eff}}\frac{\alpha}{2}(\sigma + \sigma')^2 \qquad \text{(S4)}$$

$$H_{\mathrm{HB}} = a_{\mathrm{eff}}c_{\mathrm{HB}}\left(0;\min\left(0;\sigma_{\mathrm{donor}} + \sigma_{\mathrm{HB}}\right) \times \max\left(0;\sigma_{\mathrm{acceptor}} - \sigma_{\mathrm{HB}}\right)\right) \qquad \text{(S5)}$$

$$H_{vdW} = a_{\mathrm{eff}}\left(\tau_{\mathrm{vdW}} + \tau_{\mathrm{vdW}}^{'}\right) \qquad \text{(S6)}$$

where $^{a_{\mathrm{eff}}}$ is the effective contact area between two surface segments, α'is the interaction parameter, $^{\sigma_{\mathrm{HB}}}$ is the hydrogen bond strength that the threshold for hydrogen bonding, and the last two $^{\tau_{\mathrm{vdW}}}$ and $^{\tau_{\mathrm{vdW}}^{'}}$ are elements of specific van der Waals interaction parameters.

The interaction energies that present in the binary mixture were also estimated by COSMO-RS using the excess enthalpies as the difference in the enthalpy of the studied EtOH,

EtOAc, H_2O, β-carotene, torularhodin, torulene, margaric acid, trilinolenin and glyceryl-1,3-dilinoleate molecules in its mixture and pure state, according to the **Equation S7**:

$$H_{E,i}(\text{interaction}) = H_{i,mixture}(\text{interaction}) - H_{i,pure}(\text{interaction}) \quad \text{(S7)}$$

The $H_{E,i}$ (interaction) in the COSMO-RS model originates from summing the three specific interaction as described in equations S4-S6. Thus, it can be expressed as **Equation S8**:

$$H_{E,m} = H_{E,MF} + H_{E,HB} + H_{E,vdW} \quad \text{(S8)}$$

Therefore, the COSMO-RS model could provide the information required for the evaluation of molecular interaction occur of solvents in the pure state, as well as in the mixture, as contribution of them in carotenoids and lipids recoveries.

Liquid-liquid extraction of carotenoids and lipids

The liquid-liquid equilibrium (LLE) data for the system with high recovery yields, *i.e.* ternary system composed of EtOAc, EtOH and H_2O, were obtained from previous literature[20]. In order to evaluate the extraction efficiency (EE%) and partition coefficients (*K*) of carotenoids and lipids in different mixture of EtOAc/EtOH/H_2O systems, after the SLE, further amounts of EtOAc and H_2O were added to the samples to reach the solvents concentrations described in **Table S9**, which are needed to induce a phase separation (*i.e.* formation a biphasic regime). After the solvent's addition, each mixture was then homogenized at 100 rpm, 25 ºC for 10 min and left to equilibrate for 1 h at 25 ºC. The formation of EtOAc/EtOH/H_2O biphasic systems was confirmed by the formation of two coexisting phases, namely, a dark orange-(top) phase (rich in EtOAc and carotenoids) and a clear-(bottom) phase (rich in EtOH/H_2O and lipids). The solvent ratio of all biphasic systems are detailed in **Table S9**. After the phase separation, the top and bottom phases were carefully separated, filtered with a polyethylene membrane (0.22 µm pore size), and the content of carotenoids and lipids determined according the methods described in the sections **Determination of carotenoids content** and **Determination of total lipids content**, respectively.

The partition/extraction aptitudes of each system were measured as the extraction efficiency (EE%) and partition coefficient (*K*) of each carotenoid and lipids, which were calculated according the **Equations S9**, **S10** and **S11**, respectively**:**

$$EE\% = \frac{C_{rich\ phase} \times V_{rich\ phase}}{C_{top} \times V_{top} + C_{bottom} \times V_{bottom}} \quad \text{(S9)}$$

where *C* corresponds to the concentration of β-carotene, torularhodin, torulene or lipids, and V to the volume of the coexisting phase in which the solute was preferentially partitioned (*i.e.*, carotenoids in the top phase, and lipids in the bottom phase). The subscripts 'top', 'bottom' refer to the top and bottom phases, respectively.

$$K_{car} = \frac{[car]_{top}}{[car]_{bottom}} \quad \text{(S10)}$$

$$K_{lip} = \frac{[lip]_{bottom}}{[lip]_{top}} \quad \text{(S11)}$$

K was calculated as the ratio between the concentration of each carotenoid (β-carotene, torularhodin and torulene) in the EtOAc-rich phase ($[car]_{top}$) to that in the EtOH/H_2O-rich phase ($[car]_{bottom}$) Eq. (S10) and concentration of lipids in the EtOH/H_2O-rich phase ($[lip]_{bottom}$) to that in the EtOAc-rich phase ($[lip]_{top}$) (Eq. S11).

Environmental assessment by determination of carbon footprint

The carbon footprint corresponds to the sum of greenhouse gas (GHG) emissions expressed as carbon dioxide equivalent ($CO_{2\,eq}$) and is calculated for each scenario studied (g $CO_{2\,eq}.\mu g^{-1}_{carotenoids}$ or g $CO_{2\,eq}.g^{-1}_{lipids}$) according to **Equation S12**.

$$Carbon\ footprint = \frac{\sum (Aj\ x\ GHGj)}{p} \quad \text{(S12)}$$

where, A_j is the amount of each input *j* presented in **Table S4** for each scenario (units: g, mL or Wh), GHG*j* is the GHG emission factor for each input *j* presented in **Table S5** (units: g $CO_{2\ eq}.g^{-1}$, g $CO_{2\ eq}.mL^{-1}$ or g $CO_{2\ eq}.Wh^{-1}$), and p is the amount of extracted carotenoids (β-carotene, torularhodin, and torulene) and lipids in each scenario studied (units: µg for carotenoids and mg for lipids).

The values for A*j* in the case of chemicals and water consist in the real quantities consumed during the experiments. The inputs of electricity were estimated based on the time of operation, nominal power of each equipment and fraction of occupancy over capacity of the equipment. The use of the nominal power can lead to an overestimation of the inputs of electricity because the real power may be lower. The values for GHG*j* were sourced from the Ecoinvent 3.6 database. As data for bacteriological peptone, asparagine and KH_2PO_4 are not provided by the Ecoinvent database, data for a similar chemical were adopted instead. However, these chemicals have a negligible contribution to the total carbon footprint.

Table S4. Inputs of chemicals, water and electricity to obtain extracted carotenoids (β-carotene, torularhodin, torulene) and lipids in the three scenarios under study. Scenario 1- EtOAc and EtOH/H_2O- rich phases were evaporated to reuse, and cold acetone were added to the system to separate proteins from lipids; Scenario 2- EtOAc was evaporated, and EtOH/H_2O- rich phase were submitted to an second LLE procedure to separate proteins from lipids; Scenario 3- Control using conventional Bligh and Dyer method.

	Unit	Scenario 1	Scenario 2	Scenario 3
Inputs				
Biomass Production				
Bacteriological peptone	g	3.48	3.48	3.48
Yeast extract	g	1.74	1.74	1.74
Glucose	g	1.74	1.74	1.74
KH_2PO_4	g	0.09	0.09	0.09
$MgSO_4$	g	0.09	0.09	0.09
NH_4NO_3	g	0.70	0.70	0.70
Asparagine	g	1.74	1.74	1.74
H_2O distilled	mL	0.17	0.17	0.17
Electricity	Wh	6201	6201	6201
Solid-Liquid Extraction				
EtOAc	mL	1.15	1.15	-
EtOH	mL	2.95	2.95	-
H_2O distilled	mL	0.9	0.9	0.8
Chloroform	mL	-	-	1
MeOH		-	-	2
Electricity	Wh	1025	1025	1025
Purification				
EtOAc	mL	3	3	-
EtOH	mL	2	2	-
H_2O - distilled	mL	10	10	1
Chloroform	mL	-	-	1
Electricity	Wh	650		
Polishing				
EtOAc	mL	-	2.5	
Acetone	mL	15	-	15
Electricity	Wh	652.5	652.5	652.5
Outputs				
β-carotene	μg	80.15	78.44	15.56
Torularhodin	μg	62.50	56.20	15.06
Torulene	μg	17.20	15.16	6.13
Lipids	mg	105.91	106.17	70.90

Table S5. GHG emission factors used in the calculation of the carbon footprint and name of the processes in Ecoinvent 3.6.

Input	Reference unit	GHG emissions (g $CO_{2\ eq}$/reference unit)[a]	Name of the process in Ecoinvent
Bacteriological Peptone	g	1.917	Chemical production, organic, global
Yeast extract	g	2.961	Ethanol production from whey, rest of the world
Glucose	g	1.095	Glucose production, Europe
KH_2PO_4	g	0.263	Potassium chloride, Europe
$MgSO_4$	g	0.234	Magnesium sulfate production, Europe
NH_4NO_3	g	2.776	Ammonium nitrate production, Europe
Asparagine	g	1.917	Chemical production, organic, global
EtOAc	g	2.572	Ethyl acetate production, Europe
EtOH	g	1.083	Ethanol, in 95% solution state, from fermentation, rest of the world
Chloroform	g	3.302	Trichloromethane production, Europe
MeOH	g	0.616	Methanol production, global
Acetone	g	2.317	Acetone production, liquid, Europe
H_2O distilled	mL	0.300	Not applicable[b]
Electricity	Wh	0.399	Market for electricity, low voltage, Portugal

[a] Considering global warming potentials for converting the mass of each GHG into mass of $CO_{2\ eq}$ from Myhre *at al*.[21] for a time horizon of 100 years.

[b] GHG emissions are the sum of GHG emissions from tap water production[22] and GHG emissions from electricity consumption during the distillation process.

Table S6. Experimental data obtained for the study of carotenoids (β-carotene, torularhodin and torulene) and lipids recoveries using pure solvents at a wet cell concentration of 0.2 g/mL after 1 h stirring (300 rpm) at 65 °C.

Solvent	Recovery yields ± σ (% w/w)			
	Lipids[1]	β-carotene[2]	Torularhodin[1]	Torulene[1]
MeOH	6.44 ± 0.04^{ab}	18.64 ± 0.19^{a}	24.29 ± 0.20^{b}	28.06 ± 0.35^{ab}
EtOH	4.37 ± 0.11^{ab}	15.85 ± 0.14^{b}	23.18 ± 0.11^{ab}	29.69 ± 0.06^{b}
IPA	8.31 ± 0.26^{ab}	14.39 ± 0.69^{c}	17.98 ± 0.73^{ab}	21.91 ± 0.35^{ab}
EtOAc	14.45 ± 0.20^{a}	2.80 ± 0.03^{e}	9.60 ± 0.20^{ab}	11.82 ± 0.21^{ab}
2-MeTHF	14.66 ± 0.69^{a}	7.41 ± 0.41^{d}	9.25 ± 0.20^{ab}	12.02 ± 0.38^{ab}
CH	2.40 ± 0.04^{b}	1.02 ± 0.19^{f}	3.96 ± 0.01^{a}	11.86 ± 0.50^{a}

[1]Multiple comparison test after Kruskal-Wallis (α = 0.05) and [2]Tukey HSD test (α = 0.05). *Mean of three independent assays ± confidence levels; means with the same lowercase letter does not present significant difference (p > 0.05).

Table S7. Experimental data obtained for the study of carotenoids (β-carotene, torularhodin and torulene) and lipids recoveries using solvents mixtures at a wet cell concentration of 0.2 g/mL after 1 h stirring (300 rpm) at 65 °C.

Solvent	Recovery yields ± σ (% w/w)			
	Lipids[2]	β-carotene[2]	Torularhodin[1]	Torulene[2]
Control	32.16 ± 0.03^{b}	8.20 ± 0.29^{d}	14.43 ± 0.15^{abc}	15.59 ± 0.09^{d}
CH-MeOH	19.78 ± 0.08^{d}	7.15 ± 0.39^{e}	13.12 ± 0.00^{abc}	15.42 ± 0.09^{d}
CH-EtOH	22.18 ± 0.23^{c}	6.43 ± 0.27^{e}	12.48 ± 0.11^{abc}	17.20 ± 0.09^{c}
CH-IPA	2.49 ± 0.08^{i}	10.68 ± 0.48^{c}	16.86 ± 0.11^{abc}	12.90 ± 0.19^{f}
CH-2-MeTHF	14.45 ± 0.15^{f}	2.43 ± 0.01^{f}	7.16 ± 0.11^{ab}	13.76 ± 0.19^{e}
EtOAc-MeOH	18.43 ± 0.08^{e}	24.61 ± 0.54^{b}	22.81 ± 0.19^{bc}	22.10 ± 0.09^{b}
EtOAc-EtOH	35.84 ± 0.23^{a}	32.27 ± 0.08^{a}	38.27 ± 0.79^{c}	23.95 ± 0.17^{a}
EtOAc-IPA	13.81 ± 0.04^{g}	24.81 ± 0.51^{b}	21.80 ± 0.29^{abc}	13.08 ± 0.35^{f}
EtOAc-2-MeTHF	8.72 ± 0.05^{h}	2.08 ± 0.13^{f}	5.45 ± 0.11^{a}	11.71 ± 0.02^{g}

[1]Multiple comparison test after Kruskal-Wallis (α = 0.05) and [2]Tukey HSD test (α = 0.05). *Mean of three independent assays ± confidence levels; means with the same lowercase letter does not present significant difference (p > 0.05).

Table S8. Experimental data obtained for the study of carotenoids (β-carotene, torularhodin and torulene) and lipids recoveries using EtOAc/EtOH/H_2O mixtures at a wet cell concentration of 0.2 g/mL after 1 h stirring (300 rpm) at 65 °C.

System	Recovery yields ± σ (% w/w)			
	Lipids[2]	β-carotene[1]	Torularhodin[1]	Torulene[2]
A	14.45 ± 0.20^{h}	2.80 ± 0.03^{a}	11.43 ± 0.44^{a}	11.53 ± 0.09^{l}
B	4.96 ± 0.38^{j}	15.85 ± 0.14^{abc}	24. 23 ± 0.11^{abc}	29.69 ± 0.06^{e}
C	4.37 ± 0.11^{j}	2.93 ± 0.00^{ab}	10.77 ± 1.63^{ab}	11.69 ± 0.10^{l}
D	17.04 ± 0.56^{g}	14.05 ± 0.06^{abc}	20.84 ± 0.07^{abc}	28.04 ± 0.17^{g}
E	16.27 ± 0.47^{gh}	32.27 ± 0.08^{abc}	38.27 ± 0.79^{abc}	39.14 ± 0.08^{d}
F	30.13 ± 0.42^{e}	9.65 ± 0.08^{abc}	16.43 ± 0.07^{abc}	26.34 ± 0.16^{h}
G	29.84 ± 0.80^{e}	8.84 ± 0.14^{abc}	17.03 ± 0.07^{abc}	24.59 ± 0.11^{i}
H	35.96 ± 0.64^{d}	46.93 ± 1.39^{c}	51.81 ± 0.28^{bc}	47.60 ± 0.19^{b}
I	55.75 ± 1.06^{a}	46.41 ± 0.27^{bc}	59.82 ± 0.11^{c}	50.99 ± 0.08^{a}
J	31.01 ± 0.32^{e}	31.21 ± 0.11^{abc}	40.28 ± 0.31^{abc}	40.07 ± 0.06^{c}
K	14.62 ± 0.36^{h}	4.07 ± 0.06^{abc}	15.59 ± 0.07^{abc}	22.92 ± 0.11^{k}
L	9.33 ± 0.85^{i}	12.21 ± 0.11^{abc}	19.37 ± 0.04^{abc}	26.60 ± 0.02^{h}
M	46.52 ± 0.38^{c}	17.89 ± 0.05^{abc}	23.73 ± 0.07^{abc}	29.29 ± 0.06^{f}
N	50.84 ± 0.51^{b}	17.82 ± 0.20^{abc}	22.53 ± 0.04^{abc}	29.10 ± 0.04^{f}
O	48.07 ± 2.19^{c}	14.65 ± 0.37^{abc}	22.69 ± 0.43^{abc}	28.34 ± 0.10^{g}
P	33.91 ± 0.02^{d}	13.31 ± 0.05^{abc}	21.67 ± 0.07^{abc}	28.26 ± 0.16^{g}
Q	25.61 ± 0.41^{f}	6.11 ± 0.13^{abc}	15.59 ± 0.76^{abc}	23.46 ± 0.17^{j}
R	35.30 ± 0.66^{d}	19.15 ± 0.20^{abc}	20.73 ± 0.04^{abc}	29.76 ± 0.06^{e}

[1]Multiple comparison test after Kruskal-Wallis (α = 0.05) and [2]Tukey HSD test (α = 0.05). *Mean of three independent assays ± confidence levels; means with the same lowercase letter does not present significant difference ($p > 0.05$).

Table S9. Points compositions in the ternary phase diagram and extraction parameters [partition coefficient (K) and extraction efficiency (EE) (%)] of EtOAc/EtOH/H_2O liquid-liquid systems (LLE) used for carotenoids (β-carotene, torularhodin and torulene) and lipids separation at a wet cell concentration of 0.2 g/mL after 1 h stirring (300 rpm) at 65 °C.

System	Points (% w/w)			Volume (mL)		EE% ± σ (%) / *K* ± σ			
	EtOAc	EtOH	H_2O	Top phase	Bottom phase	Lipids[1]	β-carotene[2]	Torularhodin[2]	Torulene[2]
a	15	27	58	5.0 ± 0.01	20.00 ± 1.00	63.60 ± 0.23^{a}/ 3.70 ± 0.60^{a}	84.73 ± 0.26^{a}/ 2.27 ± 0.04^{a}	84.44 ± 0.27^{a}/ 2.21 ± 0.03^{a}	91.66 ± 0.40^{a}/ 2.51 ± 0.21^{a}
b	33	30	37	9.5 ± 0.50	14.90 ± 0.36	81.91 ± 0.05^{ab}/ 3.46 ± 0.00^{a}	60.16 ± 0.38^{c}/ 2.32 ± 0.02^{a}	59.61 ± 0.38^{c}/ 2.26 ± 0.02^{a}	60.41 ± 0.18^{c}/ 2.40 ± 0.11^{a}
c	15	24	61	6.9 ± 0.12	18.83 ± 0.29	60.00 ± 3.99^{a}/ 3.77 ± 0.48^{a}	80.67 ± 0.32^{b}/ 2.31 ± 0.02^{a}	80.31 ± 0.32^{b}/ 2.26 ± 0.02^{a}	82.50 ± 0.67^{b}/ 2.31 ± 0.01^{a}
d	35	30	35	14.5 ± 0.50	9.47 ± 0.15	88.39 ± 0.01^{ab}/ 3.01 ± 0.01^{a}	51.27 ± 0.25^{d}/ 2.09 ± 0.04^{b}	50.70 ± 0.25^{d}/ 2.04 ± 0.04^{b}	52.51 ± 2.05^{d}/ 2.24 ± 0.08^{a}
e	13	15	72	19.2 ± 0.25	19.27 ± 0.25	87.52 ± 0.50^{ab}/ 2.72 ± 0.03^{a}	51.00 ± 0.27^{d}/ 1.22 ± 0.02^{c}	50.43 ± 0.27^{d}/ 1.19 ± 0.02^{c}	50.48 ± 1.04^{e}/ 2.19 ± 0.09^{a}
f	48	24	28	17.0 ± 1.73	22.43 ± 0.81	89.56 ± 0.08^{b}/ 2.82 ± 0.11^{a}	33.32 ± 0.32^{e}/ 1.17 ± 0.02^{c}	32.81 ± 0.32^{e}/ 1.14 ± 0.02^{c}	48.57 ± 0.91^{e}/ 2.32 ± 0.12^{a}

[1]Multiple comparison test after Kruskal-Wallis (α = 0.05) and [2]Tukey HSD test (α = 0.05). *Mean of three independent assays ± confidence levels; means with the same lowercase letter does not present significant difference ($p > 0.05$).

Table S10. Excess enthalpies (J·mol^{-1}) of (EtOH + H_2O + EtOAc + Solute) predicted using COSMO-RS.

	EtOH			H_2O			EtOAc			Solute		
No	H_{MF}	H_{HB}	H_{VdW}	H_{MF}	H_{HB}	H_{VdW}	H_{MF}	H_{HB}	H_{VdW}	H_{MF}	H_{HB}	H_{VdW}
						β-carotene						
1	0.00	0.00	0.00	0.00	0.00	0.00	-0.06	0.00	-0.03	0.08	0.00	0.02
2	-0.16	0.40	-0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.29	-0.00	0.10
3	0.00	0.00	0.00	0.01	0.18	-0.25	0.00	0.00	0.00	0.16	0.00	0.11
4	411.90	1762.20	59.89	0.00	0.00	0.00	-351.81	-1021.79	-51.81	0.33	-0.00	0.10
5	-25.25	-452.28	97.59	106.42	784.75	-334.04	-232.04	-849.45	60.21	0.88	-0.00	0.43
6	-10.40	-382.63	90.88	57.41	497.07	-330.99	-137.00	-572.10	61.83	0.31	0.00	0.17
7	441.61	2060.88	59.08	0.00	0.00	0.00	-361.26	-1151.49	-50.86	0.22	0.00	0.07
8	-78.45	-714.41	119.91	124.22	683.82	-298.47	-167.36	-539.84	20.63	1.11	-0.00	0.52
9	-64.87	-594.63	106.39	133.23	692.51	-235.87	-185.39	-556.79	6.74	1.02	-0.00	0.44
10	-47.06	-470.49	93.47	122.59	609.80	-188.07	-173.08	-498.40	-2.19	0.66	-0.00	0.27
11	249.57	1069.26	56.36	123.34	913.57	-97.21	-400.89	-1411.35	-1.70	0.11	0.00	0.03
12	170.00	520.07	79.76	122.34	765.67	-133.01	-365.83	-1124.35	-9.08	0.29	0.00	0.10
13	-83.69	-871.98	152.06	72.23	298.72	-338.39	0.00	0.00	0.00	0.49	-0.00	0.27
14	0.00	0.00	0.00	10.25	327.83	-226.06	-95.75	-563.62	103.58	0.75	-0.00	0.46
15	110.11	261.46	73.72	91.39	491.22	-106.81	-255.91	-724.76	-17.78	0.32	-0.00	0.11
16	201.62	787.54	59.97	150.44	1107.15	-133.62	-422.91	-1481.19	12.05	0.35	-0.00	0.12
17	306.58	1301.83	64.16	88.71	621.92	-71.26	-396.84	-1306.07	-21.58	0.16	0.00	0.05
18	-28.38	-476.35	100.76	102.88	747.23	-334.89	-217.36	-796.04	57.16	0.52	-0.00	0.26
						Torularhodin						
1	0.00	0.00	0.00	0.00	0.00	0.00	-0.23	-0.17	-0.08	0.20	0.09	0.08
2	-0.09	0.52	-0.09	0.00	0.00	0.00	0.00	0.00	0.00	0.14	-0.19	0.10
3	0.00	0.00	0.00	0.02	0.73	-0.96	0.00	0.00	0.00	0.35	-0.14	0.42
4	411.88	1762.16	59.89	0.00	0.00	0.00	-351.82	-1021.78	-51.81	0.25	-0.08	0.12
5	-25.18	-452.19	97.60	106.40	785.00	-334.02	-231.98	-849.37	60.21	0.43	-0.67	0.45
6	-10.40	-382.57	90.86	57.44	497.37	-331.05	-137.00	-572.06	61.80	0.23	-0.20	0.26
7	441.57	2060.79	59.07	0.00	0.00	0.00	-361.32	-1151.50	-50.88	0.24	-0.02	0.11
8	-78.26	-714.25	119.97	124.19	684.02	-298.44	-167.30	-539.79	20.65	0.46	-0.84	0.50
9	-64.71	-594.32	106.41	133.21	692.74	-235.86	-185.34	-556.73	6.75	0.50	-0.81	0.47

10	-46.95	-470.28	93.49	122.58	609.90	-188.07	-173.05	-498.37	-2.19	0.33	-0.49	0.28
11	249.53	1069.24	56.34	123.34	913.55	-97.21	-401.00	-1411.32	-1.76	0.22	-0.04	0.11
12	170.00	520.11	79.76	122.33	765.68	-133.00	-365.83	-1124.32	-9.08	0.22	-0.15	0.13
13	-83.64	-871.85	152.04	72.24	298.99	-338.39	0.00	0.00	0.00	0.25	-0.38	0.32
14	0.00	0.00	0.00	10.25	328.12	-226.12	-95.73	-563.57	103.56	0.49	-0.38	0.55
15	110.12	261.57	73.71	91.39	491.25	-106.80	-255.91	-724.73	-17.78	0.21	-0.20	0.14
16	201.60	787.52	59.97	150.43	1107.13	-133.62	-422.94	-1481.17	12.03	0.30	-0.10	0.15
17	306.53	1301.81	64.14	88.70	621.91	-71.26	-396.91	-1306.06	-21.61	0.21	-0.05	0.10
18	-28.33	-476.31	100.77	102.87	747.33	-334.88	-217.32	-796.01	57.16	0.23	-0.37	0.25
						Torulene						
1	0.00	0.00	0.00	0.00	0.00	0.00	-0.28	0.00	-0.12	0.30	-0.00	0.10
2	-0.19	0.58	-0.16	0.00	0.00	0.00	0.00	0.00	0.00	0.46	-0.00	0.14
3	0.00	0.00	0.00	0.04	0.81	-1.13	0.00	0.00	0.00	0.41	-0.00	0.45
4	411.79	1762.33	59.83	0.00	0.00	0.00	-351.92	-1021.73	-51.86	0.58	-0.00	0.21
5	-25.26	-452.19	97.55	106.46	784.99	-334.09	-232.06	-849.40	60.17	0.94	-0.00	0.53
6	-10.49	-382.50	90.77	57.54	497.77	-331.21	-137.08	-572.01	61.73	0.74	-0.00	0.47
7	441.51	2060.93	59.03	0.00	0.00	0.00	-361.46	-1151.42	-50.95	0.56	-0.00	0.19
8	-78.40	-714.37	119.91	124.22	683.87	-298.46	-167.35	-539.83	20.63	0.99	-0.00	0.53
9	-64.85	-594.52	106.37	133.23	692.59	-235.87	-185.38	-556.77	6.73	0.98	-0.00	0.48
10	-47.09	-470.29	93.41	122.60	609.89	-188.08	-173.10	-498.38	-2.21	0.74	-0.00	0.35
11	249.48	1069.32	56.31	123.35	913.59	-97.22	-401.16	-1411.24	-1.84	0.54	-0.00	0.19
12	169.90	520.23	79.70	122.35	765.72	-133.01	-365.93	-1124.27	-9.14	0.55	-0.00	0.22
13	-83.77	-871.77	151.93	72.31	299.18	-338.49	0.00	0.00	0.00	0.70	-0.00	0.45
14	0.00	0.00	0.00	10.33	328.52	-226.46	-95.84	-563.55	103.45	0.79	-0.00	0.76
15	110.01	261.70	73.64	91.40	491.27	-106.81	-255.96	-724.71	-17.82	0.54	-0.00	0.22
16	201.55	787.60	59.93	150.45	1107.18	-133.63	-423.10	-1481.09	11.94	0.66	-0.00	0.25
17	306.47	1301.91	64.10	88.71	621.93	-71.26	-397.05	-1305.98	-21.69	0.53	-0.00	0.19
18	-28.43	-476.23	100.69	102.95	747.57	-334.96	-217.41	-795.97	57.09	0.72	-0.00	0.41
						Margaric acid						
1	0.00	0.00	0.00	0.00	0.00	0.00	-0.31	0.00	-0.09	0.42	0.00	0.10
2	-0.03	0.18	-0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.06	-0.07	0.02
3	0.00	0.00	0.00	0.01	0.24	-0.41	0.00	0.00	0.00	0.22	-0.17	0.17

4	411.87	1762.29	59.90	0.00	0.00	0.00	-351.79	-1021.70	-51.79	0.36	-0.14	0.09
5	-25.15	-452.37	97.70	106.34	784.40	-333.92	-231.94	-849.47	60.32	0.36	-0.41	0.20
6	-10.58	-382.42	90.76	57.57	497.91	-331.20	-137.11	-571.91	61.73	0.78	-0.91	0.48
7	441.47	2060.96	59.04	0.00	0.00	0.00	-361.46	-1151.27	-50.91	0.71	-0.18	0.18
8	-78.36	-714.35	120.05	124.20	683.73	-298.38	-167.30	-539.78	20.69	0.65	-0.82	0.33
9	-64.90	-594.03	106.43	133.27	692.75	-235.83	-185.35	-556.61	6.77	0.93	-1.06	0.40
10	-47.02	-470.21	93.53	122.60	609.86	-188.05	-173.05	-498.33	-2.17	0.50	-0.54	0.20
11	249.52	1069.31	56.34	123.34	913.57	-97.21	-401.01	-1411.21	-1.74	0.36	-0.11	0.10
12	170.03	520.09	79.79	122.34	765.66	-133.00	-365.78	-1124.31	-9.05	0.20	-0.12	0.06
13	-84.10	-871.28	151.78	72.42	299.83	-338.56	0.00	0.00	0.00	0.96	-1.41	0.61
14	0.00	0.00	0.00	10.46	329.49	-227.17	-96.15	-563.28	103.22	1.93	-1.78	1.28
15	109.86	262.36	73.60	91.42	491.36	-106.80	-255.97	-724.49	-17.81	0.85	-0.65	0.27
16	201.52	787.68	59.94	150.46	1107.19	-133.62	-423.09	-1480.86	11.99	0.82	-0.31	0.24
17	306.45	1301.95	64.11	88.71	621.93	-71.26	-397.01	-1305.84	-21.64	0.61	-0.21	0.17
18	-28.52	-476.06	100.68	103.00	747.78	-334.93	-217.43	-795.80	57.10	0.76	-0.90	0.42
						Glyceryl-1,3-dilinoleate						
1	0.00	0.00	0.00	0.00	0.00	0.00	-0.28	-0.09	-0.08	0.34	0.05	0.10
2	-0.02	0.17	-0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.05	-0.08	0.02
3	0.00	0.00	0.00	0.01	0.25	-0.38	0.00	0.00	0.00	0.18	-0.19	0.15
4	411.91	1762.29	59.91	0.00	0.00	0.00	-351.79	-1021.76	-51.79	0.28	-0.17	0.09
5	-25.13	-452.39	97.71	106.33	784.43	-333.93	-231.92	-849.51	60.33	0.26	-0.46	0.18
6	-10.53	-382.45	90.78	57.54	497.93	-331.20	-137.08	-571.95	61.74	0.57	-1.01	0.45
7	441.51	2060.99	59.05	0.00	0.00	0.00	-361.44	-1151.41	-50.90	0.57	-0.20	0.17
8	-78.29	-714.43	120.07	124.18	683.76	-298.40	-167.29	-539.83	20.70	0.46	-0.93	0.31
9	-64.79	-594.17	106.46	133.25	692.80	-235.85	-185.34	-556.70	6.77	0.66	-1.22	0.38
10	-46.96	-470.30	93.55	122.59	609.88	-188.06	-173.05	-498.38	-2.17	0.36	-0.62	0.19
11	249.53	1069.31	56.34	123.34	913.59	-97.21	-400.99	-1411.29	-1.74	0.28	-0.12	0.09
12	170.04	520.09	79.79	122.34	765.68	-133.00	-365.78	-1124.34	-9.04	0.15	-0.14	0.06
13	-83.97	-871.39	151.83	72.39	299.83	-338.56	0.00	0.00	0.00	0.67	-1.56	0.57
14	0.00	0.00	0.00	10.42	329.50	-227.05	-96.05	-563.35	103.28	1.48	-1.94	1.19
15	109.95	262.26	73.62	91.42	491.39	-106.81	-255.97	-724.60	-17.81	0.63	-0.76	0.26
16	201.55	787.68	59.95	150.46	1107.24	-133.63	-423.06	-1481.03	12.00	0.64	-0.35	0.23

17	306.48	1301.96	64.12	88.71	621.95	-71.26	-396.99	-1305.96	-21.63	0.48	-0.23	0.16
18	-28.46	-476.11	100.70	102.97	747.80	-334.94	-217.40	-795.87	57.12	0.55	-1.00	0.40
						Trilinolenin						
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-0.41	-0.31	0.00	-0.09	0.42
2	-0.03	0.16	-0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07
3	0.00	0.00	0.00	-0.14	0.01	0.27	-0.42	0.00	0.00	0.00	0.00	0.23
4	411.89	1762.34	59.90	0.00	0.00	0.00	0.00	-1434.54	-351.82	-1021.76	-51.79	0.37
5	-25.15	-452.39	97.70	556.84	106.34	784.45	-333.94	-1031.21	-231.94	-849.52	60.32	0.38
6	-10.56	-382.45	90.76	224.32	57.58	497.98	-331.24	-654.23	-137.11	-571.96	61.73	0.82
7	441.50	2061.08	59.04	0.00	0.00	0.00	0.00	-1574.43	-361.49	-1151.38	-50.91	0.72
8	-78.35	-714.45	120.05	509.56	124.20	683.78	-298.42	-692.61	-167.31	-539.84	20.69	0.70
9	-64.89	-594.19	106.43	590.23	133.28	692.82	-235.87	-741.33	-185.38	-556.71	6.76	1.00
10	-47.02	-470.30	93.53	544.43	122.60	609.89	-188.07	-678.78	-173.06	-498.38	-2.17	0.54
11	249.53	1069.34	56.34	939.73	123.35	913.60	-97.21	-1828.71	-401.02	-1411.27	-1.75	0.37
12	170.03	520.09	79.79	755.01	122.34	765.68	-133.01	-1510.58	-365.79	-1124.35	-9.05	0.21
13	-84.07	-871.39	151.79	33.74	72.44	299.92	-338.61	0.00	0.00	0.00	0.00	1.03
14	0.00	0.00	0.00	112.97	10.48	329.73	-227.23	-562.80	-96.14	-563.36	103.23	1.97
15	109.87	262.30	73.60	476.02	91.43	491.41	-106.81	-1005.47	-256.01	-724.60	-17.82	0.90
16	201.53	787.72	59.94	1124.10	150.47	1107.27	-133.63	-1907.87	-423.14	-1481.01	11.98	0.83
17	306.47	1302.01	64.12	639.42	88.72	621.96	-71.26	-1737.63	-397.04	-1305.94	-21.64	0.62
18	-28.50	-476.12	100.68	515.87	103.00	747.85	-334.97	-965.67	-217.44	-795.89	57.10	0.81

Sigma profile and sigma potential of pure compound

One advantage of using COSMO-RS methodology is the model could estimate thermodynamic properties of both pure fluids and mixtures only using data from electronic structure of individual molecules. In this context, different molecular interactions in the system of interest are formulated from the polarity surfaces of the individual molecules. **Fig. S1** show the sigma profiles for the two data sets of solutes and three solvents, respectively. The solutes are divided into two categories that will be discussed herein. The two vertical dashed lines in **Fig. S1** are the locations of the cut-off values for the hydrogen bond acceptor ($\sigma > 1$ e·nm^{-2}) and hydrogen bond donor ($\sigma < -1$ e·nm^{-2}). The importance of this cut-off value lies in the fact that profile lying on the right side of $\sigma > 1$ e·nm^{-2} will have hydrogen bond acceptor ability whereas left side of $\sigma < -1$ e·nm^{-2} capable to act as hydrogen bond donor. These two regions are known as the polar region. In addition, density distributions of charge around zero, -1 e·nm^{-2} $< \sigma <$ 1 e·nm^{-2}, correspond to the non-polar moiety of the compound. The sigma profiles of β-carotene and torulene are similar in nature (*cf*. **Fig. S1a**). The prominent peaks of β-carotene and torulene lie on the non-polar region of the profile that specify their non-polar character. On the sigma profile of β-carotene, three peaks are observed at -0.3, 0.1, and 0.6 e·nm^{-2} that correspond to carbon atoms, hydrogen atoms, and C=C, respectively. Similar peak location for torulene are also observed, indicates their comparable behavior. Thus, it can be projected that, due to their non-polar character, these two solutes will have high interaction toward non-polar solvent. Indeed, the sigma potential of both β-carotene and torulene, presented in **Fig. S1b**, displays negative values within -0.8 e·nm^{-2} $< \sigma <$ 0.8 e·nm^{-2} that signifies their likeliness to interact with the non-polar moiety of solvents. In addition, due to absence of peak within the polar region in their sigma profile, both β-carotene and torulene show high repulsion toward polar group of solvent. Thus, from their respected sigma profile and potential, it could be projected that suitable solvent for the recovery of β-carotene and torulene must contain appropriate non-polar character in nature.

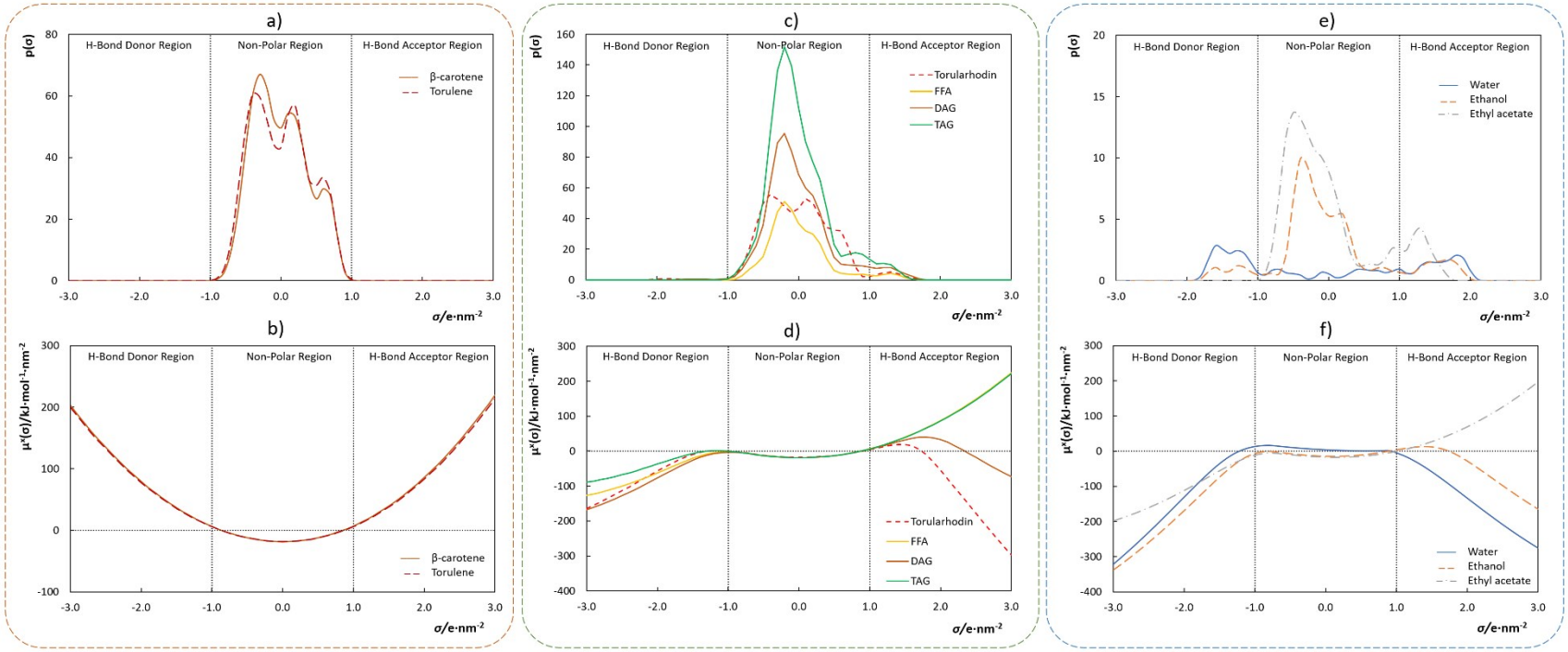

Fig. S1 Sigma profile (a, c, e) and potential (b, d, f) of solutes (β-carotene, torulene, torularhodin, FFA: free fatty acids (margaric acid), TAG: triglyceride (Trilinolenin) and DAG: diglyceride (Glyceryl-1,3-dilinoleate)) and solvents (EtOH, EtOAc and H_2O) at 298.15 K.

The second set of solutes also show peak within the non-polar region (*cf*. **Fig. S1c**), with additional small peaks at both polar regions. For example, peak at circa 1.3 e·nm^{-2} correspond to the oxygen atom of the carboxylic moiety of the compounds that can act as hydrogen bond acceptor. On the other hand, a small peak at circa -1.8 e·nm^{-2} designates to the hydrogen of atom of the carboxylic moiety of the compounds that have the ability as weak hydrogen bond donor. This is predictable as these solutes contain carboxylic acid group. Unlike the first set, the second set of solute shows not only attraction toward non-polar group, but also enticement toward both hydrogen bond donor and acceptor (*cf*. **Fig. S1d**). It should be stressed out that their attraction toward polar group is significantly higher when compare to the non-polar group. Therefore, it is expected that the second set of solutes could interact with solvent through hydrogen bond interaction as well as non-polar moiety of the solvent.

Regarding the solvent, **Fig. S1e** shows sigma profile of H_2O, EtOH, and EtOAc. Sigma profile of H_2O span widely from 2.0 to -1.9 e·nm^{-2}. The peak intensity within the polar region is relatively higher when compare to the non-polar area, indicates the highly polar nature of H_2O molecule. Solid peak 1.9 e·nm^{-2} signifies it strong aptitude of H_2O molecule to act as hydrogen bond donor, whereas peak -1.6 e·nm^{-2} entitles its nature as hydrogen bond donor. Analogous peaks are also observed for the sigma profile of EtOH in the polar region with slightly lower intensity than H_2O molecule. It specifies ability of EtOH as both hydrogen bond donor and acceptor, but weaker when compare to H_2O molecule. In addition, the presence of ethyl group is indicated by the high-intensity peak within the non-polar region that contribute to non-polar character of EtOH. Lastly, the sigma profile of EtOAc only spans within the hydrogen-bond acceptor region to non-polar region only. As consequences, EtOAc may have attraction toward hydrogen bond donor and non-polar moiety.

The aforementioned sigma profile of the solvent lead to different interaction with the solute, as displayed on their sigma potential in **Fig. S1f**. For example, due to polar nature of H_2O molecule, it shows high attraction only toward hydrogen bond donor and acceptor group, while it displays a little repulsion toward non-polar group. Thus, it is expected that H_2O molecule will interact with solute mainly through hydrogen bonding. Similar with H_2O, EtOH also present remarkable attraction toward hydrogen bond donor and acceptor group. In addition, the

presence of ethyl group of EtOH lead to its likeliness to interact with non-polar group of solutes. Finally, EtOAc displays attraction toward hydrogen bond donor as well as non-polar group. The repulsive behavior of EtOAc for the hydrogen bond acceptor group could be addressed due to the existence of ester functional group. It contains two oxygen atoms with highly negative charged that can act as hydrogen bond acceptor, and thus, shows attraction toward hydrogen bond donor.

To conclude, from the sigma profile and potential reveal that β-carotene and torulene are extremely non-polar compound in nature, and thus, they display attraction toward solvent with high non-polar character. Whereas, torularhodin and lipids (represented by FFA: free fatty acids (margaric acid), TAG: triglyceride (trilinolenin) and DAG: diglyceride (glyceryl-1,3-dilinoleate)) are not only attracted toward non-polar solvent, but also toward solvent that have ability to form hydrogen bonding.

Sigma potential for mixture of solvent

As can be observed from their sigma profile, there is enormous difference on the non-polar character between the solute and solvent that lead to low recovery of solute using single solvent. For example, the recovery values of β-carotene are 2.82, 2.93, and 15.80% by using EtOAc, H_2O, and EtOH, respectively. Herein, to improve the recovery of solute, we propose the use of mixture of solvent. In this context, the use of mixture of solvent may alter their non-polar character and consequently, improve the recovery of solute. **Fig. S2** shows sigma potential of pure and representative of mixed solvent studied in this work. Unlike sigma profile, the sigma potential is influenced by the composition and temperature, and thus, can be used to evaluate the likeliness of the mixed solvent. The most notable feature presented in **Fig. S2a** is the sigma potential of EtOAc, particularly within the region of $\sigma > 1$ e·nm^{-2}, where it shows repulsion toward hydrogen bonding acceptor. Adding EtOH into pure EtOAc, as in the case of mixed solvent 4, alter the solvent likeliness, from repulsive to attractive interaction toward hydrogen bond acceptor. In addition, adding EtOH and EtOAc into H_2O, as in the case of mixed solvent 5, shift the repulsive sigma potential to attraction (*cf*. **Fig. S2b**). In general, the obtained mixed solvent in this work, have varied sigma potential when compare to their original solvent (*cf*. **Fig. S2c**). The point that need to be highlighted is that the use of mixed EtOH, H_2O, and EtOAc could produce solvent with

different likeliness when compare to single solvent and ultimately, increase the recovery of solute.

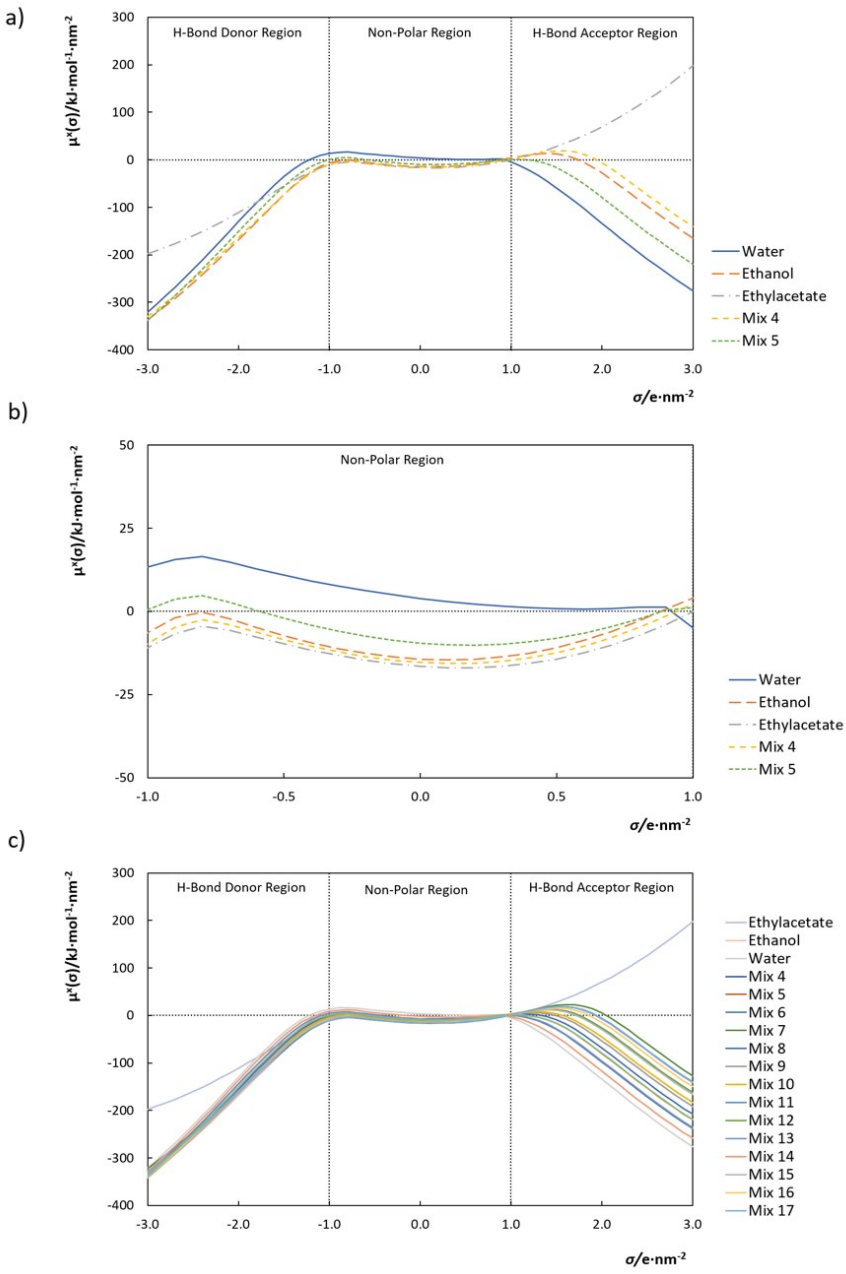

Fig. S2 Sigma potential of pure (EtOAc (A), EtOH (B) and H_2O (C)) and solvent mixtures (Mix 4 (D), Mix 5 (E), Mix 6 (F), Mix 7 (G), Mix 8 (H), Mix 9 (I), Mix 10 (J), Mix 11 (K), Mix 12 (L), Mix 13 (M), Mix 14 (N), Mix 15 (O), Mix 16 (P), Mix 17 (Q)) at 298.15 K. Different capital letters in brackets correspond to mixture points identified in the ternary phase diagram.

Interaction Energies

In order to evaluate the impact of mixed solvent toward %recovery of solute in molecular level, COSMO-RS is then used to estimate the excess enthalpies of mixture. The estimation of excess enthalpies with COSMO-RS was done by taking the sum of the three-contribution associated with electrostatic-misfit, hydrogen bond, and van der Waals forces. Therefore, the model can be used to analyze the significance of the different intermolecular energies that responsible for the recovery of solute using mixed solvent. The estimated excess enthalpies is given in **Table S10**. In general, for the recovery of β-carotene and torulene, favorable (negative values) contribution arise from contribution of both EtOH and EtOAc. Whereas, H_2O molecule contribution is endothermic and is originated from hydrogen bond. In this context, H_2O molecules must break the intermolecular hydrogen bonding between H_2O – H_2O to facilitate the mixing with other solvent and ultimately, the targeted solute. Remarkably, there is good correlation between the %recovery of β-carotene against the electrostatic – misfit of the solute, as depicted in **Fig. 4**. It indicates that the electrostatic – misfit arise from the interaction between non-polar part of solute and non-polar part of mixed solvent, as mentioned previously. The same observation is also observed for the recovery of torulene. This is expected as β-carotene and torulene have similar sigma profile, and thus, have comparable likeliness toward the mixed solvent.

For the second set of solutes, the circumstances are quite different. As previously mentioned, these solutes contain carboxylic group that may form hydrogen bond with the mixed solvent. As consequences, the recovery of lipids is highly governed by the two dominant interaction arise from hydrogen bond of solute in the system, as displayed in **Fig. 4a-d** and electrostatic misfit of solute in the system (**Fig. S4**). Thus, it seems that the electrostatic misfit and hydrogen bond of solute play a subtle balance in the extraction of lipid. For the lipid, the excess enthalpies were calculated against their main constituent, namely margaric acid, trilinolenin and glyceryl-1,3-dilinoleate. Good correlation between the %recovery and hydrogen bonding interaction and electrostatic misfit originate from the solute is observed. One may question if the hydrogen bond control the recovery of solute, H_2O should be the best candidate, as it has the strongest hydrogen bond strength. While it is true, H_2O molecule is repulsive against non-polar

character. Thus, the suitable solvent to enhanced the recovery of solute should have appropriate hydrogen bond strength while possess attraction toward non-polar moiety of the solute.

To conclude, we have showed here that the recovery of valuable solute can be enhanced by using mixed solvent instead of pure one. The improved recovery could be attributed due to alteration of non-polar character of the mixed solvent. In this regard, a mixture of H_2O, EtOH and EtOAc could provide suitable medium to recover the solute.

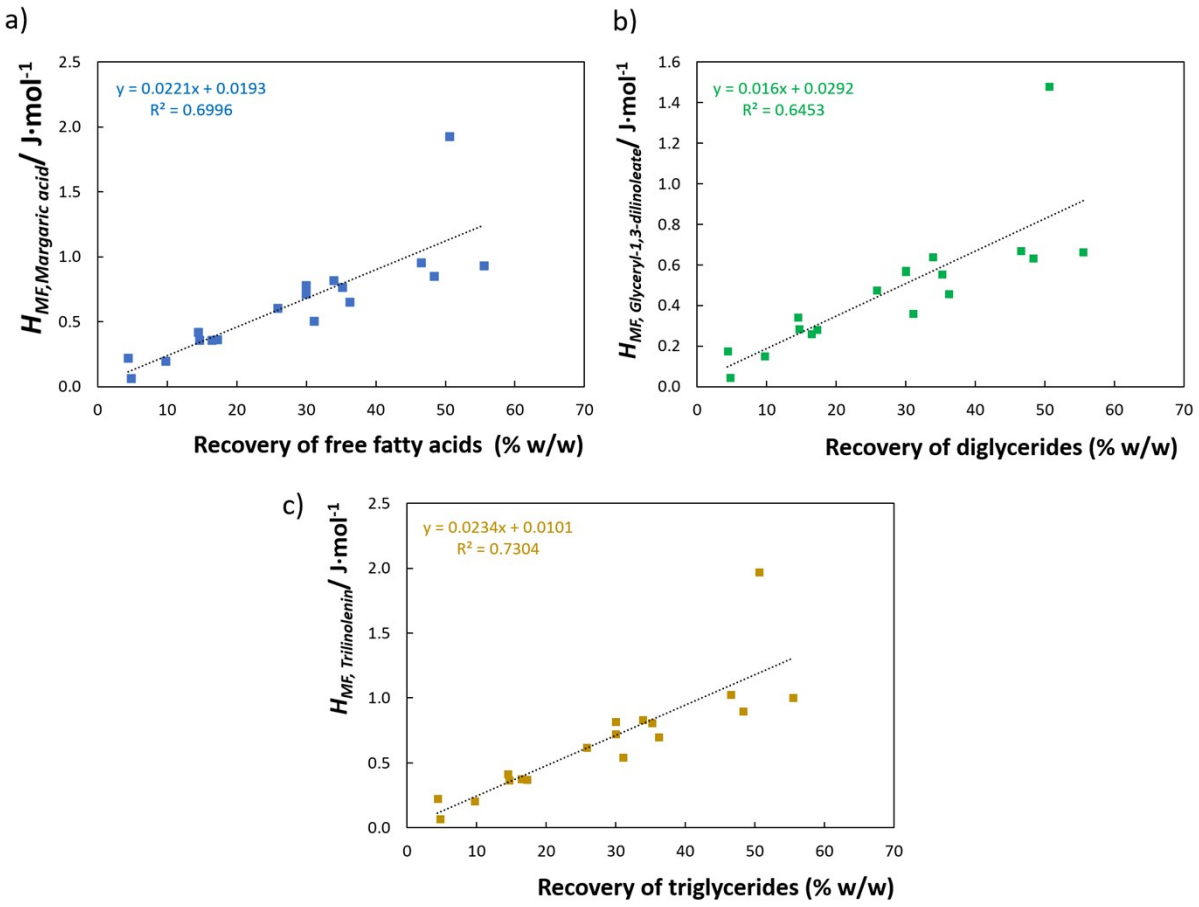

Fig. S3 Correlation plot between the interaction energies (X-axis) of solvents mixtures and the recoveries (% w/w) of a) (■) margaric acid, b) (■) glyceryl-1,3-dilinoleate and c) (■) trilinolein.

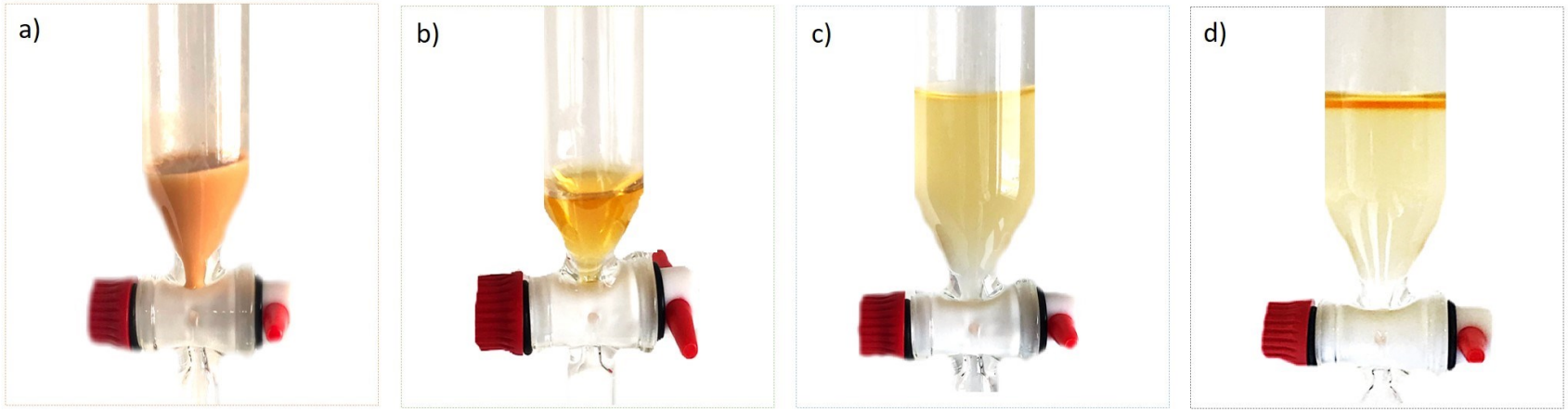

Fig. S4 Carotenoids and lipids polishing at a wet cell concentration of 0.2 g/mL after 1 h stirring (300 rpm) at 65 °C. **a)** *R. glutinis* wet biomass (1 g) in 5 mL of EtOAc/EtOH/H_2O solution (24/55/21% w/w/w). **b)** Initial carotenoids and lipids extracts in 5 mL of EtOAc/EtOH/H_2O solution. **c)** Carotenoids and lipids extracts with an additional of EtOAc (3 mL) and H_2O (10 mL). **d)** Biphasic solution with EtOAc-rich phase containing carotenoids (top phase) and an EtOH-aqueous-rich phase containing lipids (bottom phase).

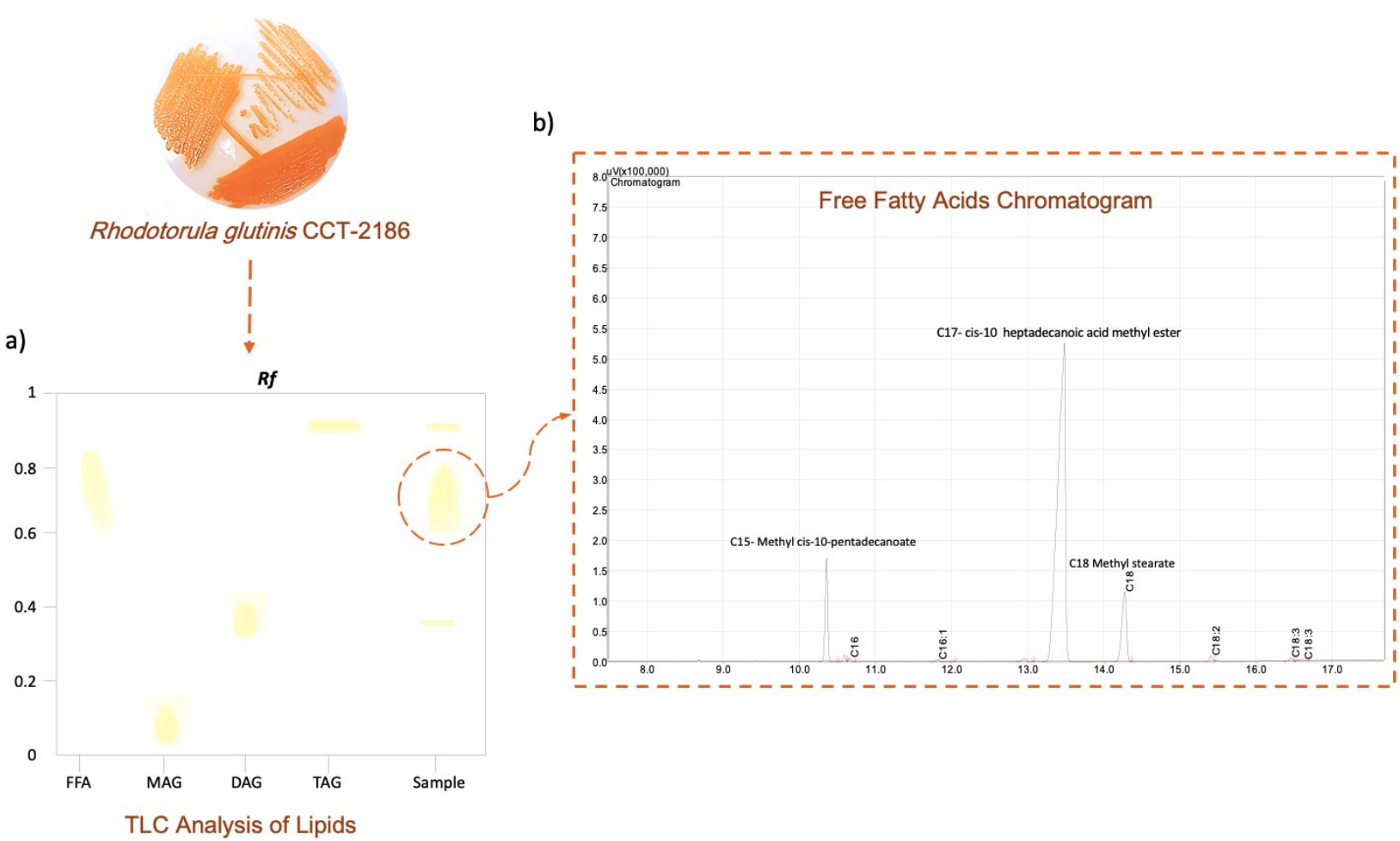

Figure S5. a) TLC analysis of the lipid produced by *R. glutinis* CCT-2186. Symbols: FFA: free fatty acids (oleic acid), TAG: triglyceride (triolein), DAG: diglyceride (diolein), MAG: monoglyceride (monoolein). b) Gas chromatography profiles of the main fatty acid methyl esters (FAMEs) produced by *R. glutinis* CCT-2186.

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