Electronic Supporting Information (ESI)

Protic ionic liquids as cell disrupting agents for the recovery of intracellular carotenoids from yeast *Rhodotorula glutinis* CCT-2186

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PILs synthesis

The PILs were synthesized *via* neutralization reaction of the base with the appropriate acid, according to the procedure previously reported [1]. Briefly; 1 M of the amine was placed in a 100 mL synthesis-flask, which was cooled to 4 °C, under continuous stirring, using an ice water bath. Then, the corresponding carboxylic acid (1 M) was added. Afterwards, the mixture was left overnight under continuous stirring at room temperature using a magnetic stirrer (IKA® model C-MAG, Staufen, Germany). To reduce volatile impurities, all synthesized PILs were washed three times with 100 mL of ethyl acetate, and then dried under constant vacuum stirring at 300 Mbar and 60 °C using a Heidolph (Hei-VAP) rotaevaporator (Schwabach, Germany) coupled with the ultrathermostatic bath Solab-SL 152 (Piracicaba, SP, Brazil).

After the synthesis, the structure and purity of all PILs was confirmed by proton nuclear magnetic resonance (^{1}H NMR), using a Bruker Avance III HD 600 (14.1T) NMR spectrometer (Massachusetts, USA) at 600 MHz. The PILs were previously dissolved in D_2O/DMSO-d*6* purchased from Sigma-Aldrich (St. Louis, MO, USA). The corresponding structures and purities obtained from the NMR analysis and further details about the synthesis are presented in **Table S1**.

Water content and pH measurements of PILs

The residual water content of the PILs was measured by volumetric titration at 25 °C using a Karl Fischer Metrohm® 803 TI-Stand titrator (Herisau, Switzerland), Hydranal- Methanol Rapid (reagent for accelerated volumetric one-component KF titration), and Hydranal-Composite 5 (reagent for volumetric one-component Karl Fischer titration; methanol free), both supplied by Sigma-Aldrich (St. Louis, MO, USA) as titrants.

The pH (± 0.02) of PILs was also determined using a MS Tecnopon® mPA-210 (Piracicaba, SP, Brazil). The calibration of the pH meter was carried out with two buffers (pH values of 4.00 and 7.00). The corresponding pH values obtained are provided in **Table S1**.

Viscosity determination

The viscosity was only measured for the PILs yielding the best extractions, *i.e.* hexanoate-based PILS. For that, the viscosities were determined in a variable temperature range between 25 °C and 70 °C at 5 °C intervals under atmospheric pressure using Anton Paar® SVM 3000 viscometer-densimeter (Graz, Austria). The viscosimeter was previously calibrated using standard solutions.

Inoculum and growing conditions

The inoculum was prepared by the activation of yeast *R. glutinis* CCT-2186 in YPD medium, which has the following composition (w/v): bacteriological peptone (2%); yeast extract (1%); glucose (2%). The inoculum culture was prepared in 100 mL Erlenmeyer® type flasks containing 25 mL of the YPD medium. Cells were grown for 48 h at 30 °C and 150 rpm in the orbital shaker Tecnal, TE- 421 (Piracicaba, SP, Brazil).

Carotenoids production

The growth medium used to produce carotenoids was composed (w/v) of Glucose (2%), KH_2PO_4 (0.052%), $MgSO_4.7H_2O$ (0.052%), KCl (0.052%), $ZnSO_4$ (0.0001%), NH_4NO_3 (0.4%) and Asparagine (1%). For the carotenoids production, Erlenmeyer® type flasks (500 mL) containing 100 mL of autoclaved fresh media were inoculated with 0.2 mg/mL of cell concentration of the 48 h fermented inoculum. The flasks were incubated for 72 h at 30 °C and 150 rpm in the orbital shaker, pH was not adjusted during the process. After 72 h of cultivation a maximum cell biomass concentration of 5.6 g/L was achieved. The fermented medium was then centrifuged in a Hitachi CR-22N (Tokio, Japan) centrifuge, at 2500*xg* for 10 min at 4 °C. The supernatants of all fermented media were then discarded, and the cellular pellets containing carotenoids were collected and stored for the next carotenoids extraction and characterization studies.

To determine the concentration of carotenoids in the original biomass, the pellets containing carotenoid-rich biomass were washed three times with deionized water. 0.2 g of wet cells were resuspended in 1 mL of DMSO and broken using a bead grinder. The suspension was then centrifuged and the supernatant (DMSO phase)

was collected. The process was repeated adding fresh DMSO until the cellular biomass was colourless (3 cycles). DMSO extracts were collected, mixed and the respective carotenoid concentrations quantified using external calibration curves (all details of carotenoids quantification are described below in the section **Carotenoids quantification**). Approximately, the cell disrupting procedure allowed a recovery of 223 μg/mL of β-carotene, 119 μg/mL of torularhodin and 30 μg/mL of torulene from the original biomass.

Scanning electron microscopy (SEM) analysis

Cell samples after the chemical SLE process using DMSO and PILs aqueous solutions were analyzed by SEM. After centrifugation, the samples containing the cell debris were washed 3 times with distilled water to remove the residual solvent content. Washed cell debris were fixed, at 4 ºC for 24 h, with 1 mL of glutaraldehyde aqueous solution (2.5% v/v). The specimens were dehydrated using increasing ethanol solutions (50% to 100% (v/v) of ethanol in water). After the dehydration, samples were coated during 20 seconds with gold layer, at 40mA and under vacuum $2x10^{-1}$ and then examined using a FEG-SEM JEOL scanning electron microscope, model JSM-7500F (Tokyo, Japan), operating at 2.00 kV.

Conventional methodology for separation and characterization of carotenoids

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 firstly qualitatively evaluated by Thin layer chromatography (TLC) on pre-coated TLC sheets ALUGRAM® (silica gel 60, Macherey-Nagel, Germany) to separate carotenoids, and compared with data reported in literature using the above-cited mobile phase as eluent; afterwards, the

extracts were 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 standard 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 [2,3]. The structures of the three purified fractions of carotenoids were confirmed by ^{1}H NMR using a Bruker Avance III HD 600 (14.1T) previously described. The fractions of carotenoids were solubilized in DMSO-*d6* and their corresponding ^{1}H NMR spectra acquired, as listed in **Table S2**.

Carotenoids quantification

After characterization of the carotenoids, the quantification of the three major carotenoids in the samples was performed based on their absorption spectra using Thermo Scientific® (Genesis 10S) UV-Vis spectrophotometer, to obtain the maximum absorption (λ_{max}) of β-carotene, torularhodin and torulene. The quantification of carotenoids by UV-Vis spectroscopy is complex due to the choice of solvent system, as the absorption maxima of extracted carotenoids strongly depend on the polarity of the solvent used. For example, with increasing polarity of the solvent, *i.e.* DMSO, the absorption of isolated carotenoids shows three peaks: 475, 510 and 540 nm; however, when less polar solvents are used, *i.e.* PILs, the peaks shift to 460, 487 and 532 as shown in **Figure S1**. Therefore, in that case, the UV-visible spectra for samples were obtained between 380 and 600 nm, and the chromatograms were standardized and processed at λ_{max}= 450_{nm} for β-carotene, λ_{max}= 480_{nm} for torulene and λ_{max}= 500_{nm} for torularhodin. Then the carotenoids were quantified using external calibration curves. For external calibration curves, the standard stock solutions concentrations were prepared by

dissolving standards in DMSO. The working standard solutions of 0.1, 0.2, 0.4, 0.5 and 1 µg/mL for β-carotene, 0.5,1, 1.5, 2, 2.5 and 3 µg/mL for torulene and 5,10,20,30,40,50 µg/mL for torularhodin were prepared daily using fresh solvent. All solutions were protected against light with aluminium foil and maintained at 4 °C for one month maximum, because longer periods of storage may produce irreproducible results due to degradation or reaction processes.

Table S1. Chemical structure, pH and purity of the studied PILs.

Name	Acronym	Cation	Anion	Residual H_2O (%)	Purity (%)	pH
Propylammonium acetate	[PA][Ac]			3.68	97	6.88
Propylammonium propanoate	[PA][Pro]			4.27	97.12	6.69
Propylammonium butanoate	[PA][But]			5.10	98.5	6.83
Propylammonium hexanoate	[PA][Hex]			2.54	99.06	7.83
3-Dimethylamino-1- propylammonium acetate	[DMAPA][Ac]			4.21	97.20	10.70
3-Dimethylamino-1- propylammonium propanoate	[DMAPA][Pro]			3.95	96.90	10.20
3-Dimethylamino-1- propylammonium butanoate	[DMAPA][But]			4.21	95.70	10.60
3-Dimethylamino-1- propylammonium hexanoate	[DMAPA][Hex]			1.52	99.31	11.11
3-Diethylamino-propylammonium acetate	[DEAPA][Ac]			5.23	98.38	9.05
3-Diethylamino-propylammonium propanoate	[DEAPA][Pro]			4.65	97.02	8.33
3-Diethylamino-propylammonium butanoate	[DEAPA][But]			2.15	96.87	8.76
3-Diethylamino-propylammonium hexanoate	[DEAPA][Hex]			1.24	98.84	9.28

Table S2. Nuclear Magnetic Resonance (^{1}H NMR) of the studied PILs.

Name		
Propylammonium acetate	[PA][Ac]	^{1}H NMR (600 MHz, D_2O) δ ppm: 0.78 (t, 3H, $\mathbf{C}H_3$- COO^-), 1.49 (m, 2H, -$\mathbf{C}H_2$-NH_2), 2.77 (t, 2H, -$\mathbf{C}H_2$-), 1.79 (s, 3H, $\mathbf{C}H_3$-)
Propylammonium propanoate	[PA][Pro]	^{1}H NMR (600 MHz, D_2O) δ ppm: 0.72 (t, 3H, $\mathbf{C}H_3$-), 1.42 (m, 2H, -$\mathbf{C}H_2$- COO^-), 2.70 (t, 2H, -$\mathbf{C}H_2$- NH_2), 0.82 (t, 3H, $\mathbf{C}H_3$-), 2.02 (q, 2H, -$\mathbf{C}H_2$-)
Propylammonium butanoate	[PA][But]	^{1}H NMR (600 MHz, D_2O) δ ppm: 0.56 (t, 3H, $\mathbf{C}H_3$-), 1. 24 (m, 2H, -$\mathbf{C}H_2$-), 1.87 (t, 2H, -$\mathbf{C}H_2$- NH_2), 0.63 (t, 3H, $\mathbf{C}H_3$-), 1.34 (m, 2H, -$\mathbf{C}H_2$-), 1.16 (t, 2H, - $\mathbf{C}H_{2-}$ COO^-)
Propylammonium hexanoate	[PA][Hex]	^{1}H NMR (600 MHz, DMSO-d6) δ ppm: 0.835 (t, 3H, $\mathbf{C}H_3$-), 1.99 (t, 2H, -$\mathbf{C}H_2$-),1.45 (m, 2H, -$\mathbf{C}H_2$- COO^-), 1.23 (m, 4H, -$\mathbf{C}H_2$-), 0.87 (t, 3H, $\mathbf{C}H_3$-), 1.57(m, 2H, -$\mathbf{C}H_2$-), 2.67(t, 2H, -$\mathbf{C}H_2$- NH_2)
3-Dimethylamino-1- propylammonium acetate	[DMAPA][Ac]	^{1}H NMR (600 MHz, D_2O) δ ppm: 2.64 (t, 2H, NH_2-$\mathbf{C}H_2$-), 1.58 (m, 2H, -$\mathbf{C}H_2$-), 2.34 (m, 2H, -$\mathbf{C}H_2$-), 1.73 (s, $\mathbf{C}H_3$- COO^-)
3-Dimethylamino-1- propylammonium propanoate	[DMAPA][Pro]	^{1}H NMR (600 MHz, D_2O) δ ppm: 2.56 (t, 2H, NH_2-$\mathbf{C}H_2$-), 1.53 (m, 2H, -$\mathbf{C}H_2$-), 2.27 (m, 2H, -$\mathbf{C}H_2$-), 0.90 (t, 3H $\mathbf{C}H_3$-, 7.28 Hz), 2.02 (q, 2H, -$\mathbf{C}H_2$- COO^-)
3-Dimethylamino-1- propylammonium butanoate	[DMAPA][But]	^{1}H NMR (600 MHz, D_2O) δ ppm: 2.69 (t, 2H, NH_2-$\mathbf{C}H_2$-), 1.63 (m, 2H, -$\mathbf{C}H_2$-), 2.39 (m, 2H, -$\mathbf{C}H_2$-), 0.75 (t, 3H, $\mathbf{C}H_3$-), 1.42 (m, 2H, -$\mathbf{C}H_2$-), 2.01 (t, 2H -$\mathbf{C}H_{2-}$ COO^-)
3-Dimethylamino-1- propylammonium hexanoate	[DMAPA][Hex]	^{1}H NMR (600 MHz, D_2O) δ ppm: 2.54 (t, 2H, NH_2-$\mathbf{C}H_2$-), 1.51 (m, 2H, -$\mathbf{C}H_2$-, 2.26 (m, 2H, -$\mathbf{C}H_2$-), 0.71 (t, 3H, $\mathbf{C}H_3$-), 1.38 (m, 2H, -$\mathbf{C}H_2$-), 1.13 (m, 4H -$\mathbf{C}H_2$-), 2.01 (t, 2H, -$\mathbf{C}H_2$-COO^-)
3-Diethylamino-propylammonium acetate	[DEAPA][Ac]	^{1}H NMR (600 MHz, D_2O) δ ppm: 0.96 (t, 6H, $\mathbf{C}H_3$-), 2.72 (m, 6H, NH_2-$\mathbf{C}H_2$-), 2.63 (t, 2H, -$\mathbf{C}H_2$-), 1.63 (m, 2H, -$\mathbf{C}H_2$-), 1.69 (s, 3H, $\mathbf{C}H_3$- COO^-)
3-Diethylamino-propylammonium propanoate	[DEAPA][Pro]	^{1}H NMR (600 MHz, DMSO-d6) δ ppm: 0.96 (t, 3H, $\mathbf{C}H_3$-), 2.17(q, 2H, -$\mathbf{C}H_2$-), 3.43 (q, 2H, -$\mathbf{C}H_2$-), 1.03 (m, 2H, -$\mathbf{C}H_2$- NH_2), 1.90 (m, 2H, -$\mathbf{C}H_2$- COO^-), 0.96 (t,6H, $\mathbf{C}H_3$-), 2.17 (q, 4H, $\mathbf{C}H_3$-)
3-Diethylamino-propylammonium butanoate	[DEAPA][But]	^{1}H NMR (600 MHz, D_2O) δ ppm: 1.03 (t, 6H, $\mathbf{C}H_3$-), 2.88 (m, 6H, NH_2-$\mathbf{C}H_2$-), 2.75 (t, 2H, -$\mathbf{C}H_2$-), 1.76 (m, 2H, -$\mathbf{C}H_2$-), 1.03 (t, 3H, $\mathbf{C}H_3$-), 1.55 (m, 3H, -$\mathbf{C}H_2$-), 0.69 (t, 3H, $\mathbf{C}H_3$-), 1.95 (t, 2H, -$\mathbf{C}H_2$- COO^-)
3-Diethylamino-propylammonium hexanoate	[DEAPA][Hex]	^{1}H NMR (600 MHz, D_2O) δ ppm: 1.13 (t, 6H, $\mathbf{C}H_3$-), 2.98 (m, 6H, NH_2-$\mathbf{C}H_2$-), 2.55 (t, 2H, -$\mathbf{C}H_2$-), 1.86 (m, 2H, -$\mathbf{C}H_2$-), 1.05 (t, 3H, $\mathbf{C}H_3$-), 1.65 (m, 3H, -$\mathbf{C}H_2$-), 0.65 (t, 3H, $\mathbf{C}H_3$-), 1.75 (t, 2H, -$\mathbf{C}H_2$- COO^-), 2.21 (t, 2H, -$\mathbf{C}H_2$-)

Table S3. Nuclear Magnetic Resonance (^1H NMR) of the carotenoids from *R.glutinis* CCT-2186

Carotenoids	^1H NMR (600 MHz, DMSO-d6)
β-carotene	δ ppm: 1.23 (s, 6H, CH_3-),1.81 (s, 15H, CH_3-),1.57-2.53 (m, 14H, -CH_2-), 5.61 (t, 1H), 6.51 (s, 14H).
Torulene	δ ppm: 0.90 (s, 6H, CH_3-), 1.79 (s, 15H, CH_3-), 1.50-1.98 (m, 14H, -CH_2-), 5.41 (t, 1H), 6.27 (s, 3H), 6.51 (s, 14H), 9.72 (s, 1H-aldehyde).
Torularhodin	δ ppm:0.90 (s, 6H, CH_3-), 1.79 (s, 15H, CH_3-), 2.40-2.70 (m, 14H, -CH_2-), 5.61 (t, 1H), 6.27 (s, 3H), 6.51 (s 14H), 10.9 (s, 1H-acidic).

Table S4. Recovery of β-carotene, torularhodin and torulene using DMSO and aqueous solution of PILs (90% v/v) at a concentration of 0.2 g/mL of wet cells after 1 h of stirring (30 rpm) at 25 °C. *

Solvent	[PIL] 90 %(v/v)	β- carotene (μg/mL)	Torularhodin (μg/mL)	Torulene (μg/mL)
DMSO	-	28.14 ± 1.86	32.70 ± 1.48	3.33 ± 0.15
[PA]	$[Ac]^-$	10.14 ± 0.62^{r}	9.08 ± 0.26^{rst}	0.30 ± 0.04^{x}
	$[Pro]^-$	30.07 ± 1.02^{o}	27.42 ± 0.87^{pq}	2.05 ± 0.09^{wx}
	$[But]^-$	67.38 ± 0.54^{h}	54.97 ± 3.10^{k}	4.92 ± 1.36^{uv}
	$[Hex]^-$	111.64 ± 1.39^{c}	89.80 ± 0.75^{e}	8.17 ± 0.02^{rst}
[DMAPA]	$[Ac]^-$	34.33 ± 0.53^{n}	24.47 ± 1.35^{q}	1.76 ± 0.09^{wx}
	$[Pro]^-$	37.44 ± 1.14^{lm}	29.05 ± 1.42^{op}	2.88 ± 0.03^{vwx}
	$[But]^-$	71.24 ± 0.61^{g}	57.70 ± 0.94^{j}	6.34 ± 0.46^{tu}
	$[Hex]^-$	120.54 ± 1.76^{b}	102.50 ± 2.36^{d}	9.33 ± 0.32^{rs}
[DEAPA]	$[Ac]^-$	39.16 ± 1.36^{l}	31.150 ± 0.94^{o}	2.22 ± 0.31^{wx}
	$[Pro]^-$	34.85 ± 1.76^{n}	35.08 ± 3.73^{mn}	3.15 ± 0.06^{vw}
	$[But]^-$	76.74 ± 3.09^{f}	63.70 ± 2.07^{i}	7.20 ± 0.14^{stu}
	$[Hex]^-$	128.44 ± 5.10^{a}	111.75 ± 3.94^{c}	10.86 ± 0.16^{r}

*Mean of three independent assays ± 95% confidence levels; means with the same lowercase letter does not present significant difference ($p > 0.05$)

Table S5. Effect of PIL concentration [75%, 80%, 85% and 90% (v/v)] on cell permeability as a function of temperature (25 °C; 45 °C; 65 °C) at a concentration of 0.2 g/mL of wet cells after 1 h of stirring (30 rpm) in the release of β-carotene, torularhodin and torulene.*****

PILs	Temperature	[IL] (v/v)	β- carotene (μg/mL)	Torularhodin (μg/mL)	Torulene (μg/mL)
[PA][Hex]	**25 °C**	75%	81.38 ± 3.73^{stuvwxyzAB}	66.19 ± 2.10^{zABC}	6.28 ± 2.03^{D}
		80%	100.30 ± 2.19^{pqrstuvwx}	80.89 ± 3.99^{tuvwxyzAB}	7.75 ± 2.02^{D}
		85%	124.14 ± 6.19^{ijklmnop}	98.83 ± 4.27^{pqrstuvwxy}	9.27 ± 2.02^{D}
		90%	149.48 ± 4.55^{efghi}	125.42 ± 4.87^{ijklmnop}	12.48 ± 4.77^{D}
	45 °C	75%	109.79 ± 5.68^{lmnopqrs}	82.40 ± 3.34^{stuvwxyzAB}	7.32 ± 0.93^{D}
		80%	122.45 ± 6.80^{ijklmnop}	91.85 ± 5.76^{qrstuvwxyz}	9.00 ± 4.24^{D}
		85%	142.15 ± 2.94^{fghijk}	112.04 ± 4.20^{lmnopqr}	10.50 ± 1.79^{D}
		90%	158.17 ± 4.03^{defg}	129.36 ± 7.31^{hijklmno}	12.92 ± 4.02^{D}
	65 °C	75%	116.22 ± 3.33^{jklmnopq}	84.78 ± 3.09^{rstuvwxyzA}	9.58 ± 0.91^{D}
		80%	147.72 ± 2.96^{efghi}	106.17 ± 2.10^{mnopqrstu}	11.34 ± 1.03^{D}
		85%	158.92 ± 2.07^{defg}	121.63 ± 3.08^{ijklmnop}	12.39 ± 1.13^{D}
		90%	171.31 ± 5.13^{cde}	134.99 ± 0.46^{fghijkl}	14.17 ± 1.81^{D}
[DMAPA][Hex]	**25 °C**	75%	71.04 ± 5.48^{yzABC}	63.85 ± 3.56^{zABC}	7.13 ± 2.51^{D}
		80%	87.95 ± 2.52^{qrstuvwxyz}	75.18 ± 3.40^{wxyzABC}	6.88 ± 0.38^{D}
		85%	111.82 ± 4.14^{lmnopqr}	91.50 ± 2.21^{qrstuvwxyz}	8.65 ± 0.38^{D}
		90%	144.61 ± 4.24^{efghij}	115.57 ± 5.26^{klmnopq}	11.26 ± 2.29^{D}
	45 °C	75%	71.15 ± 3.08^{yzABC}	75.85 ± 2.86^{vwxyzABC}	7.25 ± 0.61^{D}
		80%	104.41 ± 1.70^{nopqrstu}	90.28 ± 5.29^{qrstuvwxyz}	8.68 ± 0.66^{D}
		85%	132.84 ± 5.38^{ghijklmn}	104.04 ± 3.45^{opqrstuv}	9.84 ± 1.33^{D}
		90%	187.85 ± 5.51^{abc}	122.56 ± 2.92^{ijklmnop}	12.86 ±0.32^{D}
	65 °C	75%	105.63 ± 6.00^{mnopqrstu}	50.65 ± 5.38^{C}	10.89 ± 0.09^{D}
		80%	171.59 ± 2.51^{cde}	56.98 ± 3.07^{ABC}	14.57 ± 0.50^{D}
		85%	186.14 ± 2.04^{bcd}	64.79 ± 5.75^{zABC}	15.94 ± 0.11^{D}
		90%	216.13 ± 3.34^{a}	78.54 ± 4.97^{uvwxyzABC}	17.98 ± 2.92^{D}
[DEAPA][Hex]	**25 °C**	75%	69.19 ± 2.00^{zABC}	50.65 ± 2.82^{C}	7.13 ± 0.89^{D}
		80%	87.95 ± 1.14^{qrstuvwxyz}	54.99 ± 1.97^{BC}	7.20 ± 0.12^{D}
		85%	115.83 ± 4.32^{klmnopq}	66.17 ± 4.10^{zABC}	8.62 ± 0.78^{D}
		90%	133.06 ± 9.74^{ghijklm}	78.15 ± 3.83^{uvwxyzABC}	11.25 ± 1.47^{D}
	45 °C	75%	74.69 ± 0.78^{xyzABC}	69.38 ± 2.20^{zABC}	7.61 ± 0.34^{D}
		80%	104.33 ± 7.64^{mnopqrstu}	85.82 ± 5.68^{rstuvwxyz}	8.35 ± 0.56^{D}
		85%	131.05 ± 6.13^{ghijklmno}	103.70 ± 3.08^{opqrstuvw}	10.24 ± 0.58^{D}
		90%	154.42 ± 3.67^{efgh}	122.55 ± 4.99^{ijklmnop}	12.27 ± 1.10^{D}
	65 °C	75%	108.60 ± 5.48^{lmnopqrst}	64.83 ± 2.96^{zABC}	9.84 ± 0.81^{D}
		80%	162.20 ± 4.67^{cdef}	73.44 ± 3.20^{xyzABC}	13.85 ± 1.41^{D}
		85%	185.15 ± 5.48^{bcd}	91.79 ± 5.61^{qrstuvwxyz}	15.51 ± 1.95^{D}
		90%	206.65 ± 10.75^{ab}	112.82 ± 6.09^{lmnopqr}	17.21 ± 1.99^{D}

*Mean of three independent assays ± confidence levels; means with the same lowercase letter present significant difference ($p > 0.05$); means with the same capital letter does not present significant difference ($p > 0.05$).

Table S6. Effect of SLR as a function of the temperature of wet cells after 1 h of stirring (30 rpm) in the release of β-carotene, torularhodin and torulene using different solutions of PILs at 90% (v/v). *

PILs	Temperature	Wet cells (g/mL)	β-carotene (µg/mL)	Torularhodin (µg/mL)	Torulene (µg/mL)
[PA][Hex]	**25 °C**	0.05	40.10 ± 1.03^{vwxyzABCDE}	35.81 ± 3.45^{vwxyzABCDE}	2.60 ± 0.74^{E}
		0.1	66.97 ± 4.93^{nopqrstuvwxyzABCDE}	53.93 ± 1.39^{qrstuvwxyzABCDE}	4.4 ± 0.31^{E}
		0.2	103.19 ± 5.47^{hijklmnopqrstuv}	77.15 ± 1.93^{klmnopqrstuvwxyzABCD}	8.17 ± 1.51^{CDE}
		0.5	114.78 ± 6.59^{ghijklmnopqrst}	94.15 ± 3.71^{ijklmnopqrstuvw}	10.53 ± 0.48^{BCDE}
	45 °C	0.05	45.89 ± 6.08^{stuvwxyzABCDE}	41.57 ± 2.28^{uvwxyzABCDE}	2.07 ± 0.36^{E}
		0.1	72.53 ± 4.52^{lmnopqrstuvwxyzABCDE}	63.25 ± 3.28^{opqrstuvwxyzABCDE}	4.95 ± 0.19^{E}
		0.2	111.64 ± 5.69^{ghijklmnopqrstu}	89.80 ± 3.33^{jklmnopqrstuvwxy}	9.71 ± 1.64^{BCDE}
		0.5	136.24 ± 7.32^{defghijklmn}	118.94 ± 2.91^{fghijklmnopqr}	14.23 ± 1.97^{ABCDE}
	65 °C	0.05	42.27 ± 3.72^{uvwxyzABCDE}	41.94 ± 4.53^{uvwxyzABCDE}	3.16 ± 0.66^{E}
		0.1	89.62 ± 2.23^{jklmnopqrstuvwxy}	65.80 ± 4.11^{nopqrstuvwxyzABCDE}	6.04 ± 0.53^{DE}
		0.2	175.50 ± 7.21^{abcdefg}	134.76 ± 9.84^{defghijklmn}	11.96 ± 0.98^{BCDE}
		0.5	204.78 ± 18.66^{abcd}	171.92 ± 5.00^{abcdefgh}	14.45 ± 0.91^{ABCDE}
[DMAPA][Hex]	**25 °C**	0.05	49.33 ± 2.70^{qrstuvwxyzABCDE}	44.47 ± 2.29^{tuvwxyzABCDE}	3.45 ± 1.02^{E}
		0.1	77.44 ± 2.18^{klmnopqrstuvwxyzABC}	70.20 ± 1.74^{nopqrstuvwxyzABCDE}	6.36 ± 0.73^{CDE}
		0.2	120.54 ± 3.66^{efghijklmnopq}	102.50 ± 6.57^{hijklmnopqrstuv}	9.33 ± 0.78^{BCDE}
		0.5	144.62 ± 5.38^{defghijk}	129.10 ± 3.38^{efghijklmnop}	11.92 ± 2.01^{BCDE}
	45 °C	0.05	49.19 ± 1.53^{qrstuvwxyzABCDE}	44.82 ± 1.16^{stuvwxyzABCDE}	4.13 ± 0.35^{E}
		0.1	93.14 ± 1.86^{ijklmnopqrstuvwx}	71.37 ± 3.20^{mnopqrstuvwxyzABCDE}	6.67 ± 0.45^{CDE}
		0.2	132.22 ± 3.82^{efghijklmno}	115.94 ± 3.15^{fghijklmnopqrs}	12.42 ± 0.68^{BCDE}
		0.5	147.84 ± 5.06^{defghijk}	134.30 ± 3.76^{defghijklmno}	15.41 ± 1.23^{zABCDE}
	65 °C	0.05	55.19 ± 5.47^{qrstuvwxyzABCDE}	45.65 ± 1.85^{stuvwxyzABCDE}	4.39 ± 0.98^{E}
		0.1	97.67 ± 2.61^{ijklmnopqrstuv}	77.66 ± 4.05^{klmnopqrstuvwxyzABC}	7.77 ± 0.27^{CDE}
		0.2	186.80 ± 5.08^{abcdef}	146.49 ± 5.98^{defghijk}	14.25 ± 0.43^{ABCDE}
		0.5	222.12 ± 5.60^{abc}	177.40 ± 5.08^{abcdefg}	19.20 ± 1.49^{yzABCDE}
[DEAPA][Hex]	**25 °C**	0.05	55.84 ± 3.23^{qrstuvwxyzABCDE}	48.89 ± 3.04^{rstuvwxyzABCDE}	4.36 ± 0.86^{E}
		0.1	84.36 ± 2.25^{jklmnopqrstuvwxyzA}	79.96 ± 2.15^{klmnopqrstuvwxyzAB}	7.07 ± .36^{CDE}
		0.2	128.42 ± 1.13^{efghijklmnop}	111.72 ± 3.24^{ghijklmnopqrstu}	10.86 ± 0.73^{BCDE}
		0.5	148.26 ± 5.14^{defghijk}	142.32 ± 4.18^{defghijklm}	13.12 ± 0.76^{ABCDE}
	45 °C	0.05	58.70 ±2.51^{pqrstuvwxyzABCDE}	48.37 ± 4.06^{rstuvwxyzABCDE}	4.84 ± 0.30^{E}
		0.1	98.16 ± 1.97^{ijklmnopqrstuv}	79.74 ± 1.21^{klmnopqrstuvwxyzAB}	7.83 ± 1.34^{CDE}
		0.2	142.86 ± 3.59^{defghijkl}	130.70 ± 7.11^{efghijklmno}	15.80 ± 1.11^{zABCDE}
		0.5	153.24 ± 5.30^{bcdefghij}	152.78 ± 3.47^{cdefghij}	19.75 ± 0.88^{yzABCDE}
	65 °C	0.05	65.41 ± 4.07^{nopqrstuvwxyzABCDE}	50.15 ± 1.93^{qrstuvwxyzABCDE}	5.08 ± 0.34^{E}
		0.1	115.05 ± 3.16^{ghijklmnopqrst}	86.66 ± 4.32^{jklmnopqrstuvwxyz}	9.79 ± 0.31^{BCDE}
		0.2	224.52 ± 9.54^{ab}	162.67 ± 5.58^{abcdefghi}	22.13 ± 0.23^{xyzABCDE}
		0.5	232.79 ± 6.20^{a}	191.14 ± 5.05^{abcde}	23.13 ± 1.93^{wxyzABCDE}

* Mean of three independent assays ± confidence levels; means with the same lowercase does not present significant difference ($p > 0.05$); means with the same capital letter does not present significant difference ($p > 0.05$).

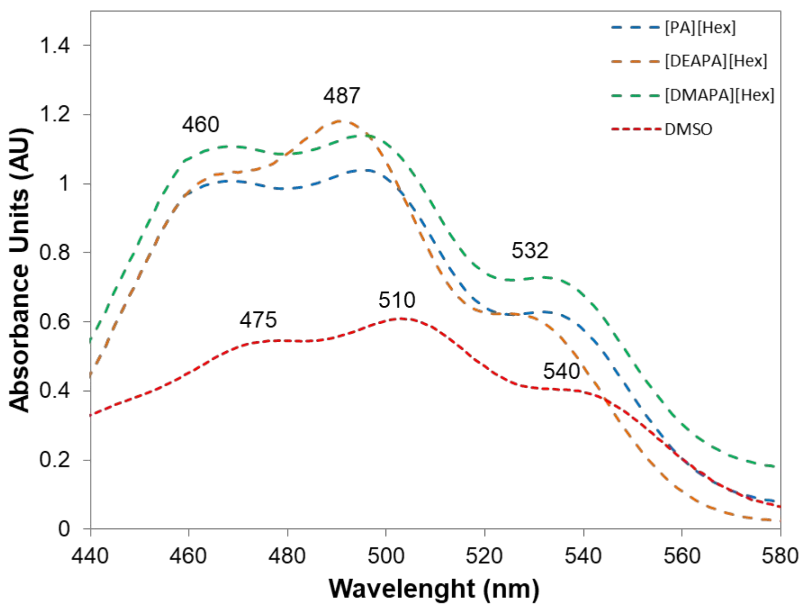

Figure S1. Absorbance spectra of main carotenoids from *R. glutinis* CCT-2186 extracted using PILs and DMSO (control)

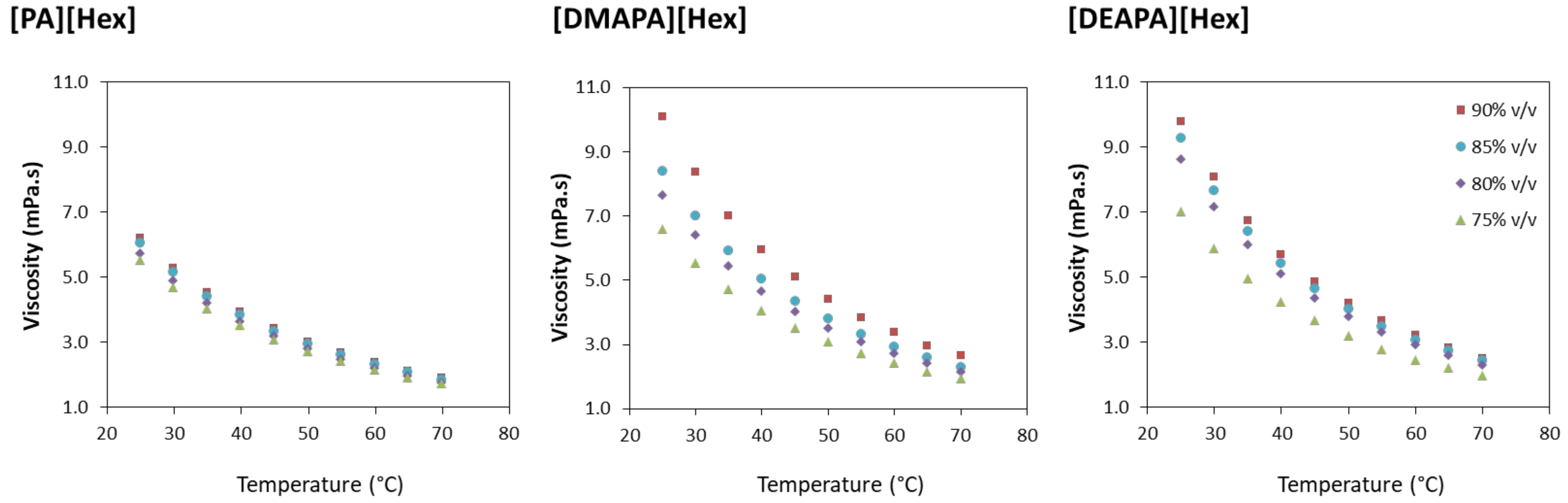

Figure S2. Viscosity (mPa.s) of different aqueous solutions of PILs as a function of temperature. The results represent the mean of three independent trials.

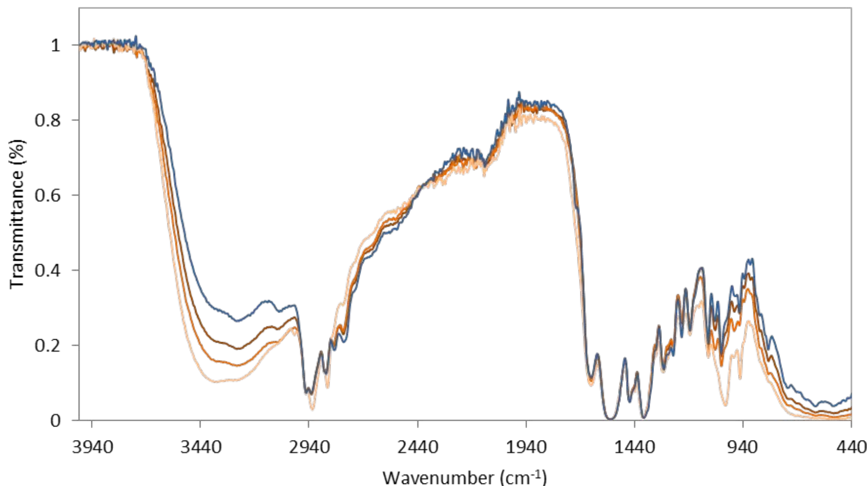

Figure S3. Fourier transform infrared spectroscopy with an attenuated total reflectance (FTIR-ATR) of carotenoids with DMSO extraction (control) (—) and reused [DEAPA][Hex]: 1st reuse (—) 2nd reuse (—) and 3rd reuse (—). Wavenumber (cm^{-1}) in the *x* axis and transmittance (%) in the *y* axis.

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