Electronic Supporting Information (ESI)

Valorisation of red beet waste: one-step extraction and separation of betalains and chlorophylls using thermoreversible aqueous biphasic systems

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1. Tables

Table S1. Experimental weight fraction data (100 W) for the systems composed of IL (1) + PPG (2) + H_2O (3) at 25 °C and atmospheric pressure (0.1 MPa).[a]

$[N_{1(2OH)(2OH)(2OH)}]Cl$		$[N_{1(2OH)(2OH)(2OH)}]Br$		$[N_{2(2OH)(2OH)(2OH)}]Br$		$[N_{21(2OH)(2OH)}]Br$		$[N_{111(OH)}]Cl$	
100 W_1	100 W_2	100 W_1	100 W_2	100 W_1	100 W_2	100 W_1	100 W_2	100 W_1	100 W_2
5.86	55.96	2.23	88.13	3.92	82.30	1.57	88.48	5.41	56.75
6.65	53.70	6.54	73.30	5.48	77.07	3.71	79.16	6.28	48.71
7.23	51.75	7.67	69.83	7.66	69.79	5.49	70.23	6.95	43.07
7.38	50.45	9.01	65.31	9.27	66.26	10.90	57.50	7.46	39.12
7.91	49.06	10.34	62.70	11.48	60.92	11.68	53.63	8.05	36.28
8.18	48.21	11.45	59.06	13.09	57.75	13.15	48.31	8.44	33.56
8.43	47.34	12.61	56.65	15.09	53.01	14.09	46.58	8.92	31.32
8.63	45.63	13.47	53.49	16.02	51.00	14.60	44.63	9.24	29.62
9.20	44.11	14.44	51.56	17.44	47.44	19.68	36.43	9.84	28.60
9.32	42.91	15.33	49.89	18.94	44.17	22.35	32.48	10.36	27.34
9.47	42.05	15.91	47.58	19.08	43.56	23.33	30.78	10.62	26.22
9.66	41.16	16.62	46.13	19.93	41.78	24.59	28.73	11.22	25.46
9.79	40.09	17.21	44.19	20.88	40.12	26.68	26.31	11.56	24.24
10.19	38.93	17.93	42.97	21.89	38.44	28.05	24.86	12.01	23.57
10.38	38.37	18.27	41.33	23.08	36.01	29.26	23.54	13.01	22.52
10.43	37.33	18.81	40.21	25.46	32.59	31.41	21.89	13.46	21.95
10.69	35.84	19.95	38.22	31.93	24.95	32.77	20.37	13.81	21.21
10.94	33.88	20.29	36.89			34.12	19.00	14.02	20.50
11.57	31.49	21.34	35.15			35.35	18.01	14.34	19.97
12.30	28.53	22.86	32.33			36.72	17.03	14.57	19.33
13.92	24.06	24.18	30.72			37.89	16.14	14.90	18.89
17.28	20.94	25.22	28.95			38.76	15.05	15.24	18.46
19.21	18.19	27.20	26.84			40.05	13.95	15.43	17.95
22.34	15.88	28.65	25.15			42.00	12.82	15.61	17.47
25.40	13.79	31.10	23.32			43.32	11.88	16.01	17.22
27.33	12.18	32.92	21.70			45.55	10.68	16.68	16.79
		34.25	20.33					16.88	16.46
		35.55	19.12					17.55	15.98
		37.59	17.62					17.53	15.48
		39.45	16.28					18.22	15.09
								18.17	14.66

[a] The standard uncertainty for the weight fraction (100 W) is 0.01, the standard uncertainty for the temperature is 1 °C, and the standard uncertainty for pressure is 10 kPa.

Table S2. Experimental weight fraction data (100 W) for the systems composed of IL (1) + PPG (2) + H_2O (3) at 35 °C and atmospheric pressure (0.1 MPa).[a]

$[N_{1(2OH)(2OH)(2OH)}]Cl$		$[N_{1(2OH)(2OH)(2OH)}]Br$		$[N_{2(2OH)(2OH)(2OH)}]Br$		$[N_{21(2OH)(2OH)}]Br$		$[N_{111(OH)}]Cl$	
100 W_1	100 W_2	100 W_1	100 W_2	100 W_1	100 W_2	100 W_1	100 W_2	100 W_1	100 W_2
1.55	72.28	1.51	84.76	2.32	77.87	1.50	84.51	1.25	67.46
2.20	66.27	2.38	80.36	3.19	72.58	2.15	81.17	1.74	60.72
2.67	61.34	2.92	77.21	4.38	69.16	2.80	77.92	1.98	54.89
3.14	57.64	3.66	73.92	4.79	67.23	3.41	74.64	2.59	51.47
3.57	54.43	4.15	71.96	5.32	65.90	3.83	71.50	2.88	46.83
4.31	48.50	4.58	67.53	5.68	64.57	4.33	69.69	3.16	43.75
4.63	45.82	5.70	63.40	5.97	62.78	4.82	67.84	3.33	41.30
4.86	43.10	6.12	61.39	6.27	61.72	5.28	64.89	3.52	38.17
5.33	38.35	6.55	60.01	6.58	60.63	5.88	62.70	3.68	35.22
5.68	34.88	6.91	58.74	6.87	59.61	6.33	60.83	4.05	31.79
6.45	31.55	7.20	57.54	7.14	58.06	6.89	58.92	4.78	28.34
6.93	30.11	7.54	56.43	7.43	57.10	7.19	57.66	5.17	23.86
7.69	28.30	7.74	54.37	7.67	55.69	7.47	56.13	6.91	20.96
8.24	26.61	8.43	52.60	7.88	54.89	7.60	54.38	8.02	18.49
8.85	25.62	8.81	51.91	8.11	54.09	7.95	53.31	9.43	15.68
9.58	24.27	9.09	50.56	8.30	52.84	10.91	34.08	11.48	14.01
10.38	22.91	9.34	49.28	9.00	48.27	11.25	32.62	12.11	12.50
10.95	21.79	9.51	48.11	9.53	45.54	11.87	30.53	13.45	10.89
11.46	21.15	9.72	47.31	9.75	44.95	13.02	28.30	15.40	9.57
12.23	20.18	9.98	46.46	9.89	44.06	14.23	26.30	16.49	8.38
12.84	19.39	10.24	45.64	10.09	43.46	15.81	24.44	18.28	7.28
13.39	18.52	10.36	44.39	10.70	40.85	17.72	22.43		
13.99	17.86	10.39	42.84	10.81	40.12	20.02	20.33		
14.80	17.21	10.60	42.19	10.99	39.63	22.91	18.01		
15.24	16.61	10.83	41.54	11.09	38.95	25.40	16.39		
15.72	16.00	11.46	38.93	11.41	37.81	27.46	14.80		
16.68	15.36	11.55	38.23	12.32	34.63	31.13	11.64		
17.19	14.84	12.10	35.94	12.72	32.91	34.99	10.06		
17.51	14.39	12.81	32.67	13.62	31.28	37.37	8.94		
18.11	13.70	13.17	31.20	14.61	29.50	40.13	7.97		
19.02	12.88	13.61	28.86	15.60	27.98	41.10	7.19		
19.76	11.97	14.75	25.46	16.96	26.12				
21.16	11.09	17.87	21.69	19.82	23.06				
		21.96	18.04						
		26.28	15.40						
		29.15	13.93						
		31.06	12.84						
		33.08	11.65						
		34.83	10.74						
		36.29	10.05						

[a.] The standard uncertainty for the weight fraction (100 W) is 0.01, the standard uncertainty for the temperature is 1 °C, and the standard uncertainty for pressure is 10 kPa.

Table S3. Experimental weight fraction data (100 W) for the systems composed of IL (1) + PPG (2) + H_2O (3) at 45 °C and atmospheric pressure (0.1 MPa).[a]

$[N_{1(2OH)(2OH)(2OH)}]Cl$		$[N_{1(2OH)(2OH)(2OH)}]Br$		$[N_{2(2OH)(2OH)(2OH)}]Br$		$[N_{21(2OH)(2OH)}]Br$		$[N_{111(OH)}]Cl$	
100 W_1	100 W_2	100 W_1	100 W_2	100 W_1	100 W_2	100 W_1	100 W_2	100 W_1	100 W_2
0.59	26.66	1.41	28.20	3.34	21.80	1.83	21.30	0.29	21.05
1.38	24.12	1.85	26.35	5.00	20.50	3.72	18.56	2.70	17.58
1.80	22.98	2.41	24.70	5.58	20.06	7.63	16.00	3.31	16.06
2.22	21.64	4.32	21.97	6.15	19.76	9.99	14.16	4.07	14.35
2.74	20.47	5.48	20.53	7.37	18.71	12.73	12.81	5.27	12.65
3.99	18.15	6.66	18.87	8.62	17.85	14.84	11.81	6.53	10.97
4.30	17.63	8.51	17.17	9.22	17.08	16.14	10.90	7.69	10.03
4.94	17.01	11.35	15.94	10.53	15.89	17.80	9.99	8.52	8.91
5.48	16.33	11.94	15.17	12.07	15.10	20.13	8.96	9.84	8.08
5.94	15.28	13.47	13.72	13.12	14.31	23.28	8.16	11.12	7.42
7.08	14.46	17.13	12.67	14.92	13.74	24.46	7.66	12.11	6.90
7.50	13.70	18.27	12.12	16.23	12.92	26.28	7.07	12.51	6.43
8.14	13.09	19.25	11.59	17.26	11.97	28.46	6.46	13.50	5.89
8.45	12.73	21.59	10.97	21.01	10.39	30.33	5.94	14.09	5.37
9.55	12.22	22.15	10.57			32.37	5.33	15.80	5.02
10.80	11.51	23.33	10.04					15.97	4.70
11.57	10.73	24.38	9.46						
12.14	10.03	26.84	8.80						
13.20	9.39	28.29	8.21						
14.18	8.97								
14.71	8.57								
16.17	8.08								
16.40	7.76								
17.68	7.32								

[a] The standard uncertainty for the weight fraction (100 W) is 0.01, the standard uncertainty for the temperature is 1 °C, and the standard uncertainty for pressure is 10 kPa.

Table S4. Correlation parameters used to describe the experimental binodal data by Equation S1 for all the studied ILs based ABS at 25 °C.

IL	A	± σ	B	± σ	C	± σ	R^2
$[N_{111(OH)}]Cl$	89.08	7.99	0.11	0.01	1,0000	0.02	0.96
$[N_{1(2OH)(2OH)(2OH)}]Cl$	169.94	118.96	-0.35	0.39	0.63	0.28	0.98
$[N_{21(2OH)(2OH)}]Br$	96.77	2.58	-0.06	0.01	0.93	0.04	1.00
$[N_{1(2OH)(2OH)(2OH)}]Br$	97.73	3.23	-0.04	0.01	1.04	0.06	1.00
$[N_{2(2OH)(2OH)(2OH)}]Br$	129.16	7.57	-0.22	0.02	0.00	0.00	1.00

Table S5. Correlation parameters used to describe the experimental binodal data by Equation S1 for all the studied ILs based ABS at 35 °C.

IL	A	± σ	B	± σ	C	± σ	R^2
$[N_{111(OH)}]Cl$	199.06	123.21	-0.95	0.56	0.44	0.16	0.99
$[N_{1(2OH)(2OH)(2OH)}]Cl$	178.54	64.06	-0.70	0.30	0.46	0.11	0.99
$[N_{21(2OH)(2OH)}]Br$	104.45	12.94	-0.12	0.06	0.89	0.16	0.98
$[N_{1(2OH)(2OH)(2OH)}]Br$	100.36	7.82	-0.09	0.03	0.94	0.12	0.99
$[N_{2(2OH)(2OH)(2OH)}]Br$	90.67	6.74	-0.05	0.02	1.17	0.16	0.99

Table S6. Correlation parameters used to describe the experimental binodal data by Equation S1 for all the studied ILs based ABS at 45 °C.

IL	A	± σ	B	± σ	C	± σ	R^2
$[N_{111(OH)}]Cl$	22.06	1.03	-0.11	0.03	0.97	0.10	0.99
$[N_{1(2OH)(2OH)(2OH)}]Cl$	30.92	0.93	-0.21	0.02	0.68	0.04	1.00
$[N_{21(2OH)(2OH)}]Br$	23.20	0.96	-0.06	0.02	0.93	0.08	1.00
$[N_{1(2OH)(2OH)(2OH)}]Br$	36.16	3.22	-0.22	0.07	0.56	0.08	1.00
$[N_{2(2OH)(2OH)(2OH)}]Br$	25.03	1.47	-0.04	0.02	1.04	0.16	1.00

Table S7. Experimental data of TLs (tie lines) and TLLs (tie line length) of ABS composed of PPG + five quaternary ammominum-based ILs at 35 °C. $[IL]_m$ and $[PPG]_m$ stand for both ABS components concentration within the chosen mixture point.

IL	Weight fraction composition (wt%)						TLL	α
	$[IL]_{top\ phase}$	$[IL]_{bot\ phase}$	$[IL]_m$	$[PPG]_m$	$[PPG]_{top\ phase}$	$[PPG]_{botphase}$		
$[N_{111(OH)}]Cl$	0.82	10.50	5.24	51.74	83.48	13.95	70.20	0.54
$[N_{1(2OH)(2OH)(2OH)}]Cl$	1.65	9.78	5.41	51.23	74.04	24.67	50.03	0.54
$[N_{21(2OH)(2OH)}]Br$	2.63	20.05	9.54	56.02	79.63	20.13	61.99	0.60
$[N_{1(2OH)(2OH)(2OH)}]Br$	2.12	23.22	11.45	53.95	83.26	16.97	69.56	0.56
$[N_{2(2OH)(2OH)(2OH)}]Br$	2.12	19.11	10.74	51.81	84.96	19.61	67.52	0.49

Table S8. Extraction efficiencies (*EE*) values for both pigments for all the ILs tested using the following mixture points: for $[N_{1(2OH)(2OH)(2OH)}]Cl$: 5 wt% IL + 52 wt% PPG, for the rest of the systems: 10 wt% IL + 52 wt% PPG was used, with a S/L ratio of 0.1, with (≈6.0) and without pH adjustment.

IL	pH	EE_{chlo} (%)	± σ	EE_{bet} (%)	± σ
$[N_{111(OH)}]Cl$	5.86	93	1	79	3
	5.91	90	4	77	5
$[N_{1(2OH)(2OH)(2OH)}]Cl$	3.78	87	4	86	2
	5.83	79	9	82	9
$[N_{21(2OH)(2OH)}]Br$	7.77	92	2	95	4
	6.02	87	3	93	4
$[N_{1(2OH)(2OH)(2OH)}]Br$	5.27	89	4	87	7
	5.82	88	5	77	2
$[N_{2(2OH)(2OH)(2OH)}]Br$	5.92	81	5	80	9
	5.96	81	5	80	9

Table S9. 2^3 factorial planning.

	X_1	X_2	X_3
1	-1	-1	-1
2	1	-1	-1
3	-1	1	-1
4	1	1	-1
5	-1	-1	1
6	1	-1	1
7	-1	1	1
8	1	1	1
9	-1.68	0	0
10	1.68	0	0
11	0	-1.68	0
12	0	1.68	0
13	0	0	-1.68
14	0	0	1.68
15	0	0	0
16	0	0	0
17	0	0	0
18	0	0	0
19	0	0	0
20	0	0	0

Table S10. Coded levels of dependents variables used in the first and second factorial planning.

		Level				
Parameters	**Symbol**	**Axial**	**Factorial**	**Central**	**Factorial**	**Axial**
		-1.68	**-1.00**	**0.00**	**1.00**	**1.68**
Temperature (°C)	T	10	14	20	26	30
Time (min)	t	20	40	70	100	120
S/L	S/L	0.04	0.07	0.12	0.17	0.20

Table S11. Experimental data and response surface predicted values of the factorial planning considering the betalains' extraction.

Experiment	T (°C)	t (min)	S/L	Y_{bet} observed (%)	Y_{bet} predicted (%)	Residuals
1	14	40	0.07	1.91	2.73	-0.83
2	26	40	0.07	5.42	4.01	1.41
3	14	100	0.07	5.67	4.80	0.87
4	26	100	0.07	2.14	3.98	-1.84
5	14	40	0.17	7.46	6.13	1.33
6	26	40	0.17	4.47	5.85	-1.38
7	14	100	0.17	4.20	6.12	-1.92
8	26	100	0.17	4.06	3.74	0.32
9	10	70	0.12	5.10	5.02	0.08
10	30	70	0.12	4.72	4.09	0.64
11	20	19	0.12	4.58	5.14	-0.56
12	20	120	0.12	6.39	5.11	1.28
13	20	70	0.04	3.33	3.35	-0.02
14	20	70	0.20	6.75	6.01	0.74
15	20	70	0.12	6.96	6.69	0.27
16	20	70	0.12	7.04	6.69	0.35
17	20	70	0.12	7.05	6.69	0.36
18	20	70	0.12	6.12	6.69	-0.57
19	20	70	0.12	6.08	6.69	-0.61
20	20	70	0.12	6.78	6.69	0.09

Table S12. Experimental data and response surface predicted values of the factorial planning considering the chlorophylls' extraction.

Experiment	T (°C)	T (min)	S/L	Y_{chlo} observed (%)	Y_{chlo} predicted (%)	Residuals
1	14	40	0.07	1.17	1.40	-0.23
2	26	40	0.07	1.62	2.13	-0.51
3	14	100	0.07	0.97	1.55	-0.57
4	26	100	0.07	3.10	3.07	0.02
5	14	40	0.17	0.29	0.74	-0.45
6	26	40	0.17	0.40	0.26	0.14
7	14	100	0.17	0.44	0.35	0.08
8	26	100	0.17	0.46	0.67	-0.21
9	10	70	0.12	0.56	0.08	0.49
10	30	70	0.12	1.08	0.96	0.12
11	20	19	0.12	0.83	0.41	0.42
12	20	120	0.12	1.07	0.88	0.19
13	20	70	0.04	4.60	4.04	0.56
14	20	70	0.20	1.52	1.47	0.05
15	20	70	0.12	1.84	1.84	0.00
16	20	70	0.12	1.51	1.84	-0.33
17	20	70	0.12	1.43	1.84	-0.41
18	20	70	0.12	2.59	1.84	0.76
19	20	70	0.12	2.14	1.84	0.31
20	20	70	0.12	1.41	1.84	-0.43

Table S13. Regression coefficients of the predicted second-order polynomial model for the betalains extraction yield (Y_{bet}) obtained from the RSM, R = 0.7979 and adjusted R^2 = 0.6367. Note that the statist results obtained are in terms of the coded values of the factors.

	b*	b* Standard deviation	b	b Standard deviation	t-student	p-value
Intercept	-	-	-1138.4491	335.5968	-3.3923	0.0069
T	37.3891	11.0878	7.6941	2.2817	3.3721	0.0071
t	-5.9012	7.4618	-0.2429	0.3071	-0.7909	0.4474
S/L	10.3847	7.4623	256.4313	184.2693	1.3916	0.1942
T^2	-36.9652	11.0761	-0.0130	0.0039	-3.3374	0.0075
t^2	-1.6362	0.5418	-0.0005	0.0002	-3.0201	0.0129
S/L^2	1.2877	0.5565	129.5071	55.9695	2.3139	0.0432
T*t	7.8818	7.4425	0.0011	0.0010	1.0590	0.3145
T*S/L	-12.0252	7.4430	-1.0116	0.6261	-1.6156	0.1372
t*S/L	-0.3739	0.5224	-0.0896	0.1252	-0.7157	0.4905

Table S14. ANOVA data for the extraction of betalains obtained from the RSM design.

	Sum of squares	Degrees of freedom	Mean square	F-value	P-value
Regression	31.8897	9	3.5433	1.9473	0.1569
Residuals	18.1963	10	1.8196		
Total	50.0860				

Table S15. Regression coefficients of the predicted second-order polynomial model for the chlorophyll's extraction yield (Y_{chlo}) obtained from the RSM, R = 0.9297 and adjusted R^2 = 0.8644.

	b*	b* Standard deviation	b	b Standard deviation	t-student	p-value
Intercept	-	-	-1138.4491	335.5968	-3.3923	0.0069
T	37.3891	11.0878	7.6941	2.2817	3.3721	0.0071
t	-5.9012	7.4618	-0.2429	0.3071	-0.7909	0.4474
S/L	10.3847	7.4623	256.4313	184.2693	1.3916	0.1942
T^2	-36.9652	11.0761	-0.0130	0.0039	-3.3374	0.0075
t^2	-1.6362	0.5418	-0.0005	0.0002	-3.0201	0.0129
S/L^2	1.2877	0.5565	129.5071	55.9695	2.3139	0.0432
T*t	7.8818	7.4425	0.0011	0.0010	1.0590	0.3145
T*S/L	-12.0252	7.4430	-1.0116	0.6261	-1.6156	0.1372
t*S/L	-0.3739	0.5224	-0.0896	0.1252	-0.7157	0.4905

Table S16. ANOVA data for the extraction of chlorophylls obtained from the RSM design.

	Sum of squares	Degrees of freedom	Mean square	F-value	p-value
Regression	17.9978	9	1.9998	7.0848	0.0026
Residuals	2.8226	10	0.2823		
Total	20.8204				

Table S17. Betalains' relative concentration (%) 7, 15 and 30 days after the extraction in the IL-rich phase of the ABS and in 100 wt% water extract. Chlorophylls' relative concentration (%) 7, 15 and 30 days after the extraction in the PPG-rich phase of the ABS and in 100 wt% ethanol extract.

Pigment under analysis	Name of the sample	Betalains' relative concentration (%)		
		Day 7	Day 15	Day 30
Betalains	IL-rich phase	97.48	94.02	29.77
	100 wt% water	85.76	55.81	0.23
Chlorophylls	PPG-rich phase	79.80	63.37	33.52
	100 wt% ethanol	98.29	95.39	62.86

Table S18. Values of average effective concentration (EC_{50}), in $mol \cdot L^{-1}$, obtained after 5, 15, and 30 min of exposure of the marine bacteria *Vibrio fischeri* to the different ILs. For $[N_{21(2OH)(2OH)}]Br$ it was not possible to calculate the EC_{50}. Values for $[N_{111(OH)}]Cl$ were obtained from Ventura *et al.*[1], and the upper limit for each IL was defined considering the Passino classification.[2]

	EC_{50} / $(mol \cdot L^{-1})$			
	5 min	15 min	30 min	Upper Lim
$[N_{111(OH)}]Cl$	0.0046	0.0040	0.0034	0.0072
$[N_{1(2OH)(2OH)(2OH)}]Br$	0.0836	0.0755	0.0909	0.0041
$[N_{2(2OH)(2OH)(2OH)}]Br$	0.1580	0.0847	0.0725	0.0039
$[N_{1(2OH)(2OH)(2OH)}]Cl$	0.2598	0.1264	0.0764	0.0041
$[N_{21(2OH)(2OH)}]Br$	---	---	---	0.0044

2. Figures

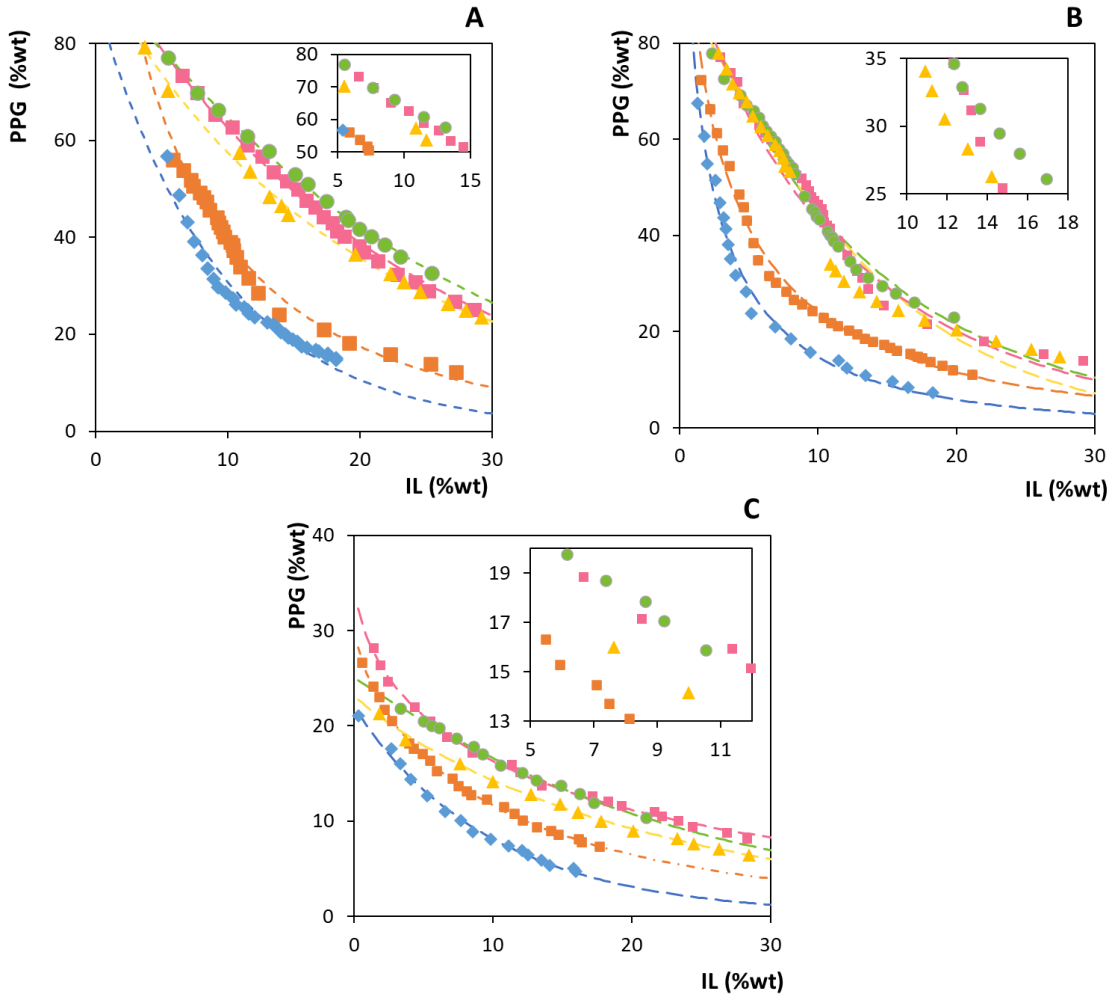

Figure S1. Temperature effect in the phase diagrams of ternary systems composed of $[N_{111(OH)}]Cl$ (♦), $[N_{1(2OH)(2OH)(2OH)}]Cl$ (■), $[N_{21(2OH)(2OH)}]Br$ (▲), $[N_{1(2OH)(2OH)(2OH)}]Br$ (■) or $[N_{2(2OH)(2OH)(2OH)}]Br$ (●)+ PPG + H_2O at 25°C (A), 35°C (B) and 45°C (C).

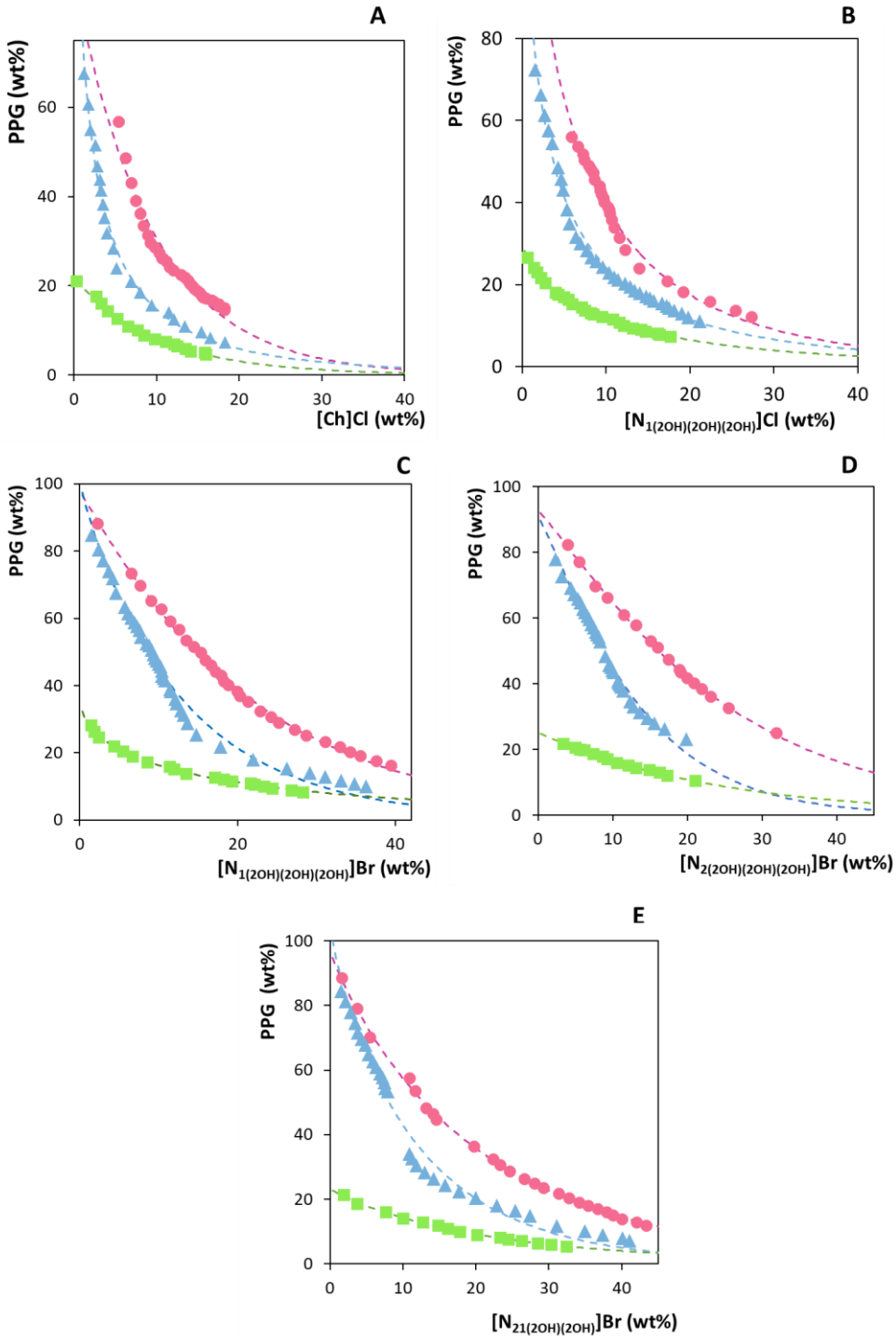

Figure S2. Temperature effect in the phase diagrams of ternary systems composed of $[N_{111(OH)}]Cl$ (A) or $[N_{1(2OH)(2OH)(2OH)}]Cl$ (B), $[N_{1(2OH)(2OH)(2OH)}]Br$ (C) or $[N_{2(2OH)(2OH)(2OH)}]Br$ (D) or $[N_{21(2OH)(2OH)}]Br$ (E) + PPG + H_2O at 25 °C (●), 35 °C (▲) and 45 °C (■).

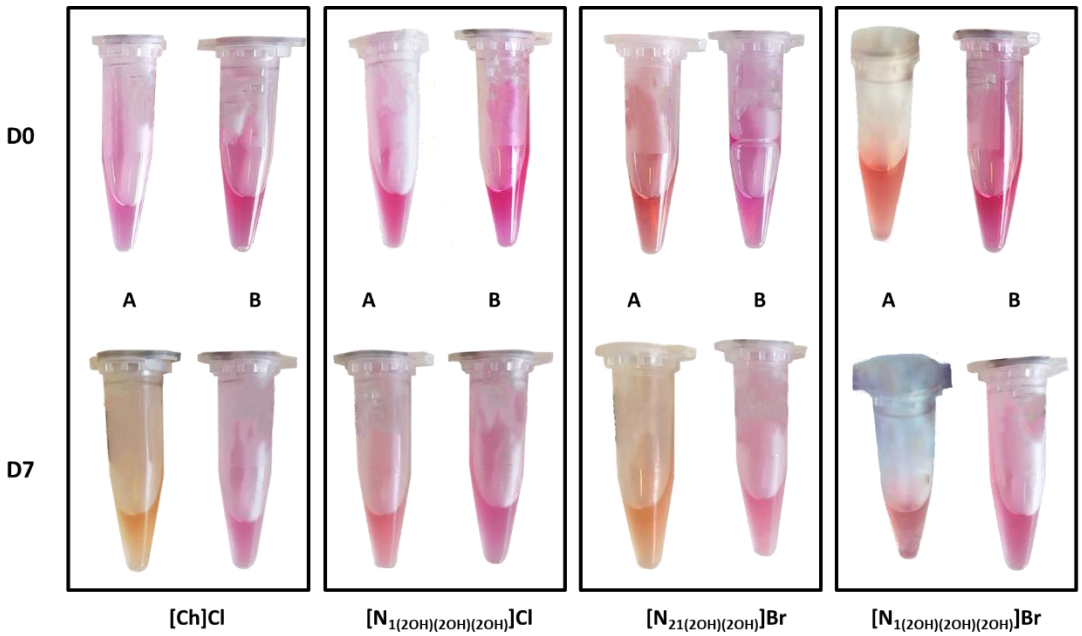

Figure S3. Preliminary stability tests for betalains extracted in the bottom phase of all ABS after 7 days kept in the dark at 4 °C with (B) and without (A) pH adjustment (D0 = Day 0 and D7 = Day 7).

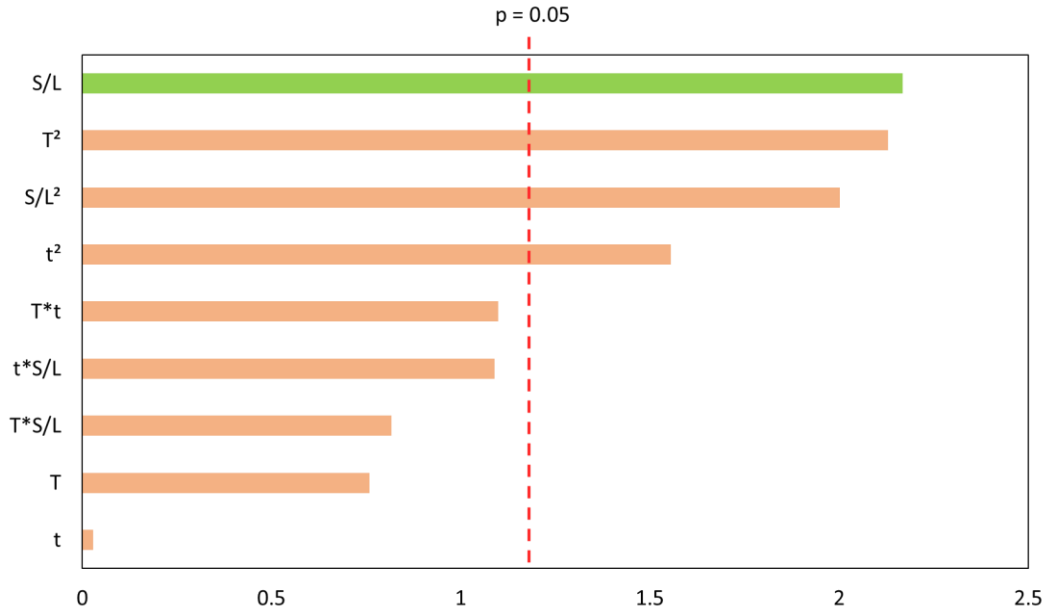

Figure S4. Pareto chart for the standardised main effects (positive (■) and negative (■)) in the factorial planning for the betalains extraction yield. The vertical line indicates the limit of the p-value, where the effects are statistically significant.

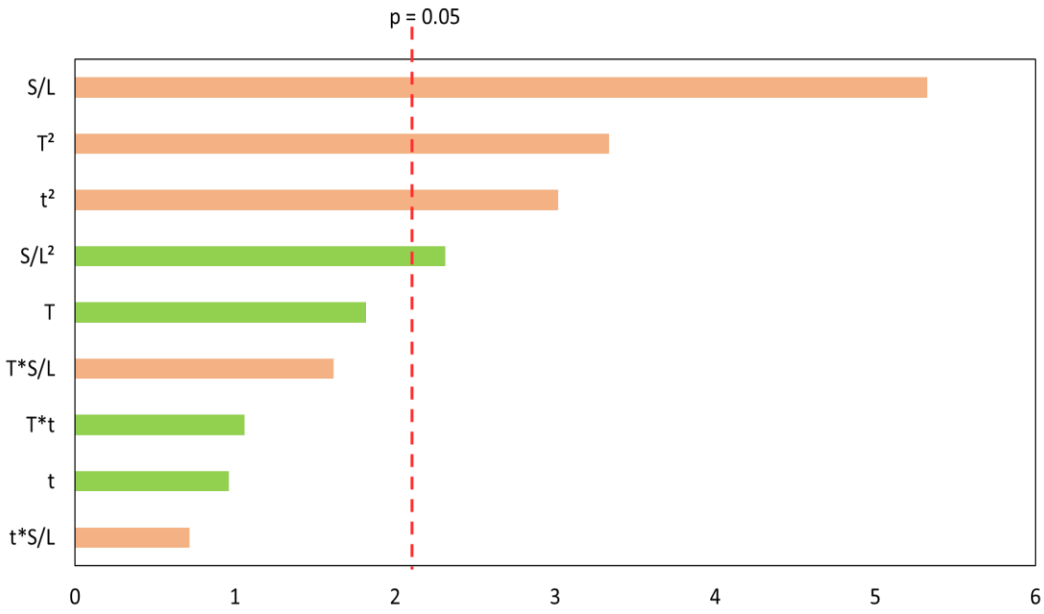

Figure S5. Pareto chart for the standardised main effects (positive (■) and negative (■)) in the factorial planning for the chlorophyll's extraction yield. The vertical line indicates the limit of the p-value, where the effects are statistically significant.

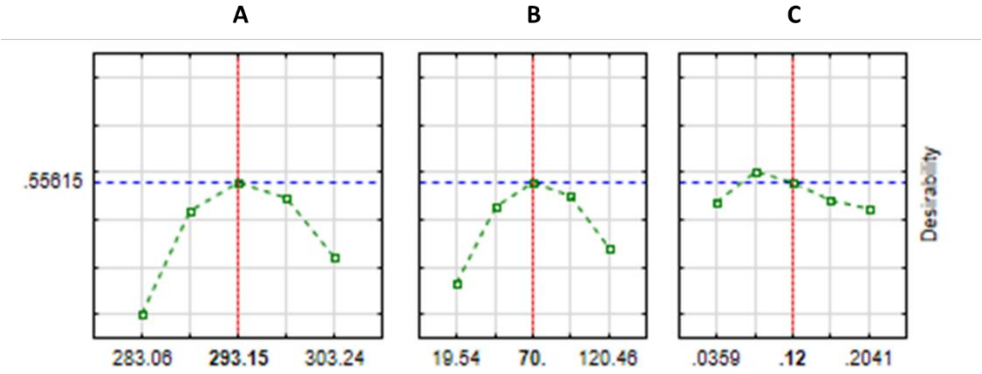

Figure S6. Optimum extraction conditions based on the desirability value of each of the variables: Temperature (A); time (B); S/L (C).

Figure S7. Photograph of resin AmberLite® HPR 1100 when in contact with the IL and betalains rich phase of the ABS.

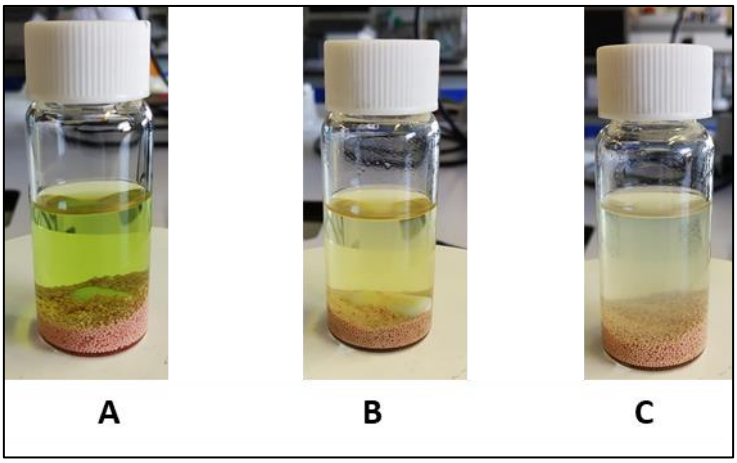

Figure S8. Photographs of the resin Ambersep® 900 OH resin (A) resin when in contact with an acetone sample extract of the chlorophylls and PPG rich phase; (B) 30 min after and (C) 60 min after contact.

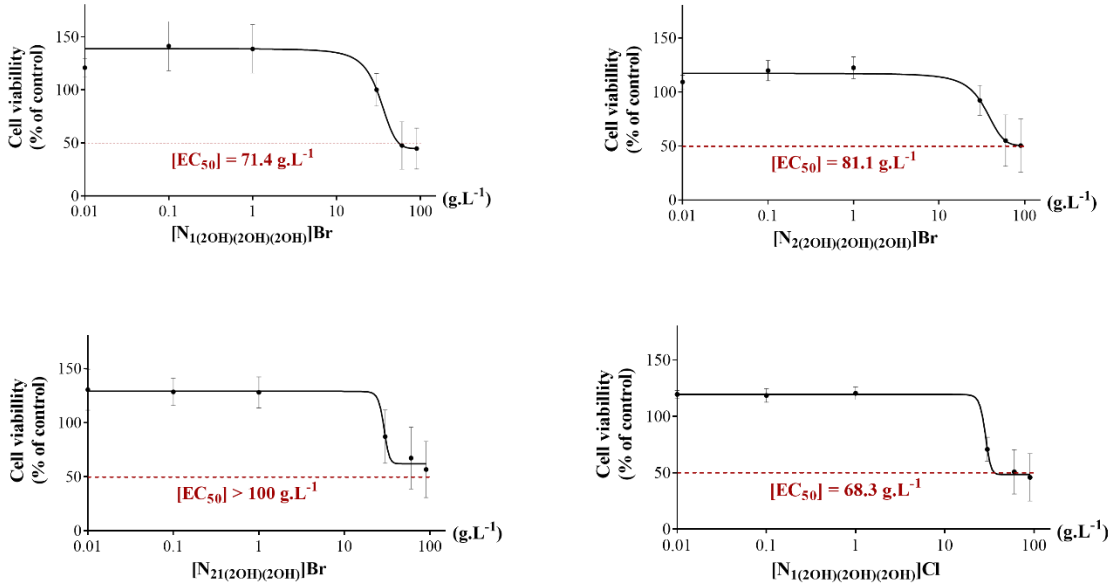

Figure S9. Cytotoxic profile of the synthetized ILs towards Caco-2 cell line. The experimental points in the dose response curves correspond to the average of five replicates of three independent experiments (n = 3) normalized to the control. The vertical lines correspond to the respective standard error of the mean (SEM). The control corresponds to untreated cells. The dash line corresponds to the EC_{50}.

Figure S10. Representation of the biomass preparation process.

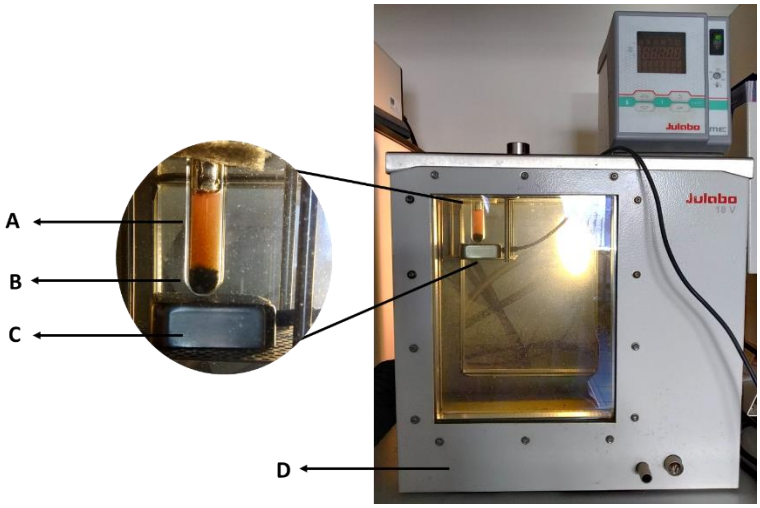

Figure S11. Representation of the extraction process. ABS phase forming components (A); red beets biomass (B); Thermo Scientific™ Cimarec™ i Micro Stirrer (C); Water bath Jubalo ME-18V (D).

3. Procedures/Equations

3.1. Tie line and tie line length determination

Each binodal curve was then fitted to Merchuk[3] equation:

$$[PPG] = A\, e^{(B\times[IL]^C)} \quad \text{(Eq. S1)}$$

where [PPG] and [IL] are weight fractions of the polymer and IL, and A, B, and C are constants obtained by data regression[3] using the TPTDL 2017 Liquid Liquid Equilibrium 2.0.4. program.

The tie line (TL) and respective tie line length (TLL) were determined at 35 °C, the chosen separation temperature. For the TL determinations, the chosen extraction mixture point was prepared for each IL-based ABS, stirred, and then centrifuged at 5000 rpm during 15 min, allowing to reach equilibrium. Each TL and TLL were determined by the application of the leverarm rule to the relationship between the top phase weight and the overall system composition, following a system of five equations (Equations S2 to S6), with four unknown values, namely[3]:

$$[\mathrm{PPG}]_{\mathrm{top\ phase}} = \mathrm{A\ e}^{\left(\mathrm{B}\times[\mathrm{IL}]_{\mathrm{top\ phase}}{}^{\mathrm{C}}\right)} \quad \text{(Eq. S2)}$$

$$[\mathrm{PPG}]_{\mathrm{bot\ phase}} = \mathrm{A\ e}^{\left(\mathrm{B}\times[\mathrm{IL}]_{\mathrm{bot\ phase}}{}^{\mathrm{C}}\right)} \quad \text{(Eq. S3)}$$

$$[\mathrm{PPG}]_{\mathrm{top\ phase}} = \frac{[PPG]_m}{\alpha} \times \frac{1-\alpha}{\alpha}\ [\mathrm{PPG}]_{\mathrm{bot\ phase}} \quad \text{(Eq. S4)}$$

$$[\mathrm{IL}]_{\mathrm{top\ phase}} = \frac{[IL]_m}{\alpha} \times \frac{1-\alpha}{\alpha}\ [\mathrm{IL}]_{\mathrm{bot\ phase}} \quad \text{(Eq. S5)}$$

$$TLL = \sqrt{\left(\left[\left[[IL]\right]\right]_{top\ phase} \times [IL]_{bot\ phase}\right)^2 + \left(\left[\left[[PPG]\right]\right]_{top\ phase} \times [PPG]_{bot\ phase}\right)^2} \quad \text{(Eq. S6)}$$

The subscripts top phase, bot phase and m represent the top, bottom, and the mixture phases, respectively. The parameter α represents the ratio between the top phase weight (PPG rich-phase) and the total weight of the two phases.

3.2. Pigments' extraction

To better compare the results of the extractions using the ABS and the best conditions at extracting the desired pigments, according to the HPLC result from the monophase of the system, the following

calculations were performed (Equations S7 to S10). The extraction efficiencies are defined as the percentage ratio of the total weight of the pigments in one of the phases of the ABS, considering the concentration obtained from the HPLC analysis. The partition coefficients were determined as the ratio between the concentration of each pigments ([chlo] and [bet]) class in the top phase (PPG-rich phase) and the bottom phase (IL-rich phase). Concerning the yields calculated, they provide information about the amount of pigments extracted (g) per biomass weight (g).

$$EE_{\text{bet}} = \frac{[\text{bet}]_{\text{top phase}} * \text{m}_{\text{top phase}}\ (\text{g})}{[\text{bet}]_{\text{bot phase}} * \text{m}_{\text{bot phase}}\ (\text{g}) + [\text{bet}]_{\text{top phase}} * \text{m}_{\text{top phase}}\ (\text{g})} * 100 \quad \text{(Eq. S7)}$$

$$EE_{\text{chlo}} = \frac{[\text{chlo}]_{\text{top phase}} * \text{m}_{\text{top phase}}\ (\text{g})}{[\text{chlo}]_{\text{bot phase}} * \text{m}_{\text{bot phase}}\ (\text{g}) + [\text{chlo}]_{\text{top phase}} * \text{m}_{\text{top phase}}\ (\text{g})} * 100 \quad \text{(Eq. S8)}$$

$$Y_{\text{chlo}} = \frac{\text{m chlorophylls (g)}}{\text{m biomass (g)}} * 100 \quad \text{(Eq. S9)}$$

$$Y_{\text{bet}} = \frac{\text{m betalains (g)}}{\text{m biomass (g)}} * 100 \quad \text{(Eq. S10)}$$

3.3. Optimisation of the operational conditions by a response surface methodology (RSM)

The type of design used is entitled rotatable within central composite with three different levels: central point (zero level), factorial points (1 and -1, level one) and the axial points defined by α (level α) calculated considering the following equation:

$$\alpha = (2^k)^{1/4} \quad \text{(Eq. S11)}$$

where k = 3. The obtained data were treated according to a second order polynomial equation:

$$y = \beta_0 + \sum \beta_i \chi_i + \sum \beta_{ii} {\chi_i}^2 + \sum_{i<j} \beta_{ij} \chi_i \chi_j \quad \text{(Eq. S12)}$$

4. References

1 S. P. M. Ventura, F. A. e Silva, A. M. M. Gonçalves, J. L. Pereira, F. Gonçalves and J. A. P. Coutinho, *Ecotoxicol. Environ. Saf.*, 2014, **102**, 48–54.

2 D. R. M. Passino and S. B. Smith, *Environ. Toxicol. Chem.*, 1987, **6**, 901–907.

3 J. C. Merchuk, B. A. Andrews and J. A. Asenjo, *J. Chromatogr. B Biomed. Sci. Appl.*, 1998, **711**, 285–293.